TAXONOMIC GUIDE TO INFECTIOUS DISEASES

Understanding the Biologic Classes of Pathogenic Organisms

SECOND EDITION

Jules J. Berman

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Second Edition

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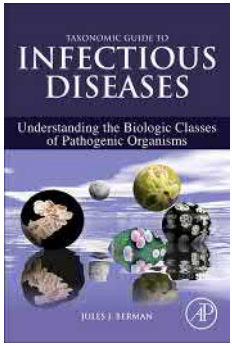

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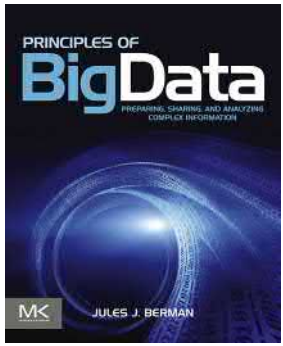

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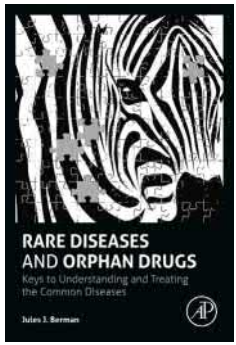

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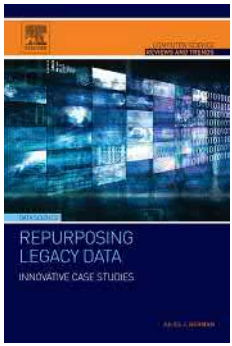

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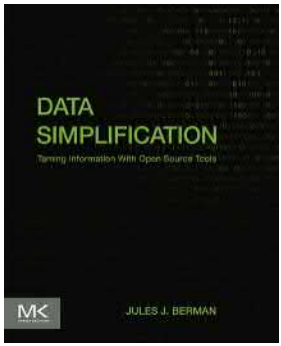

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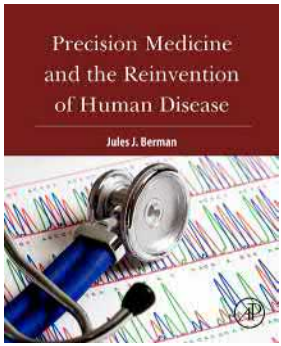

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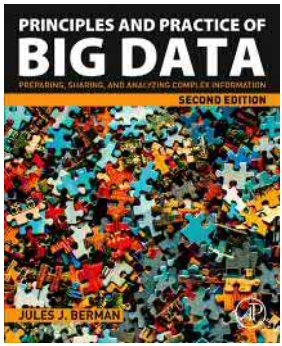

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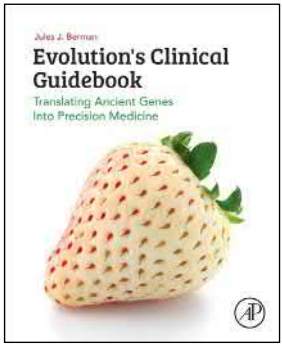

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About the author

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Preface to second edition

Everything has been said before, but since nobody listens we have to keep going back and beginning all over again.

Andre Gide

This second edition of the *Taxonomic Guide to Infectious Diseases*, like the first edition, confronts the impossibility of mastering all the human infections. There are just too many of them. Instead, we take the easy way out by learning the basic biology of the 40 or so different classes of organisms that contain infectious species. Within each class of infectious organisms, the member species have traits in common with one another. If we understand the characteristic biological properties and identify features of one prototypical species from each class of organisms, we can pretty well guess how the other species from the same class will behave. We will learn that if we can confidently assign a suspected pathogen to a well-described genus (i.e., a class of related species), we can often determine how to treat the infection and prevent the occurrence of additional infections in the at-risk community.

As in the first edition, we abandon the ranking system employed by classic taxonomists (e.g., Kingdom, Phylum, Order, Family, Genus, Species) and their subcategories (e.g., Superphylum, Phylum, Subphylum, Infraphylum, and Microphylum). In this book, all ranks will simply be referred to as "Class." The direct father class is the superclass, and the direct child class is the subclass. The use of "Class," "Superclass," and "Subclass" conforms to nomenclature standards developed by the metadata community (i.e., uses a standard terminology employed by the computational field that deals with the description of data). The terms "genus" (plural "genera") and "species" will preserve the binomial assignment of organism names.

In the prior edition, viruses were considered to be nonliving biological agents; little more than nucleic acid wrapped in a capsule. The present edition argues that viruses are living organisms, with their own phylogenetic histories. The role that viruses play in the evolution of the organisms they infect will be discussed. Also, in the prior edition, various classes of living organisms were described, but there was scant discussion of the evolutionary developments that account for the different classes of living organisms. This edition rectifies the oversight, and provides a plausible explanation as to how ancient classes of organisms arose, and how new species of organisms arise.

The first edition included an appendix listing the class lineages for most of the known infectious organisms in humans; an inclusion that added a great deal to the mass of data contained in the book, without adding much light on the subject. The second edition dispenses with the listing and replaces it with a collection of images, inserted into the chapters, intended to highlight the physical traits of the classes of organisms that infect humans. The images serve as visual reminders of the prototypical features that characterize taxonomic classes and their subclasses. Finally, the first edition of the *Taxonomic Guide to Infectious Diseases* was published in 2012, and this edition provides an opportunity to catch up with new developments in the field.

Today, health-care workers, medical researchers, students, and curious laypersons have ample access to a wealth of detailed information concerning the thousands of organisms that are potential human pathogens. None of us lack data, but all of us lack a resource that makes sense of the data at hand. This book organizes, simplifies, and provides meaning to the rapidly growing field of medical microbiology.

Preface to first edition

Order and simplification are the first steps toward the mastery of a subject.

Thomas Mann

This book explains the biological properties of infectious organisms in terms of the properties they inherit from their ancestral classes. For example, the class of organisms known as Apicomplexa contains the organisms responsible for malaria, babesiosis, cryptosporidiosis, cyclosporan gastroenteritis, isosporiasis, sarcocystosis, and toxoplasmosis. When you learn the class properties of the apicomplexans, you'll gain a basic understanding of the biological features that characterize every infectious organism in the class.

If you are a student of microbiology, or a health-care professional, you need to be familiar with hundreds of infectious organisms. There are many resources, web-based and paper-based, that describe all of these diseases in great detail, but how can you be expected to integrate volumes of information when you are confronted by a sick patient? It is not humanly possible. A much better strategy is to learn the basic biology of the 40 classes of organisms that account for all of the infectious diseases that occur in humans. After reading this book, you will be able to fit newly acquired facts, pertaining to individual infectious species, onto an intellectual scaffold that provides a simple way of understanding their clinically relevant properties.

Biological taxonomy is the scientific field dealing with the classification of living organisms. Nonbiologists, who give any thought to taxonomy, may think that the field is the dullest of the sciences. To the uninitiated, there is little difference between the life of a taxonomist and the life of a stamp collector. Nothing could be further from the truth. Taxonomy has become the grand unifying theory of the biological sciences. Efforts to sequence the genomes of prokaryotic, eukaryotic, and viral species, thereby comparing the genomes of different classes of organisms, have revitalized the field of evolutionary taxonomy (phylogenetics). The analysis of normal and abnormal homologous genes in related classes of organisms have inspired new disease treatments targeted against specific molecules and pathways characteristic of species, classes, or organisms. Students who do not understand the principles of modern taxonomy have little chance of perceiving the connections between medicine, genetics, pharmacology, and pathology, to say nothing of clinical microbiology.

Here are some of the specific advantages of learning the taxonomy of infectious diseases.

1. As a method to drive down the complexity of medical microbiology

Learning all the infectious diseases of humans is an impossible task. As the number of chronically ill and immune-compromised patients has increased, so have the number of opportunistic pathogens. As global transportation has become commonplace, the number of exotic infections spread worldwide has also increased. A few decades ago, infectious disease experts were expected to learn a few hundred infectious diseases. Today, there are over 1400 organisms that can cause diseases in humans, and the number is climbing rapidly, while the techniques to diagnose and treat these organisms are constantly improving. Textbooks cannot cover all these organisms in sufficient detail to provide health-care workers with the expertise to provide adequate care to their patients.

How can any clinician learn all that is needed to provide competent care to patients? The first step in understanding infectious diseases is to understand the classification of pathogenic organisms. Every known disease-causing organisms has been assigned to one of 40 well-defined classes of organisms, and each class fits within a simple ancestral lineage. This means that every known pathogenic organism inherits certain properties from its ancestral classes and shares these properties with the other members of its own class. When you learn the class properties, along with some basic information about the infectious members of the classes, you gain a comprehensive understanding of medical microbiology.

2. Taxonomy as web companion

Getting information off the Internet is like taking a drink from a fire hydrant.

Mitchell Kapor

The web is a great resource. You can find a lot of facts, and if you encounter an unfamiliar word or a term, the web will provide a concise definition, in a jiffy. The web cannot, however, provide an understanding of the related concepts that form the framework of a scientific discipline. The web supplies facts, but books tell you what the facts mean.

Before the web, scientific texts needed to contain narrative material as well as the detailed, raw information pertaining to the field. For example, a microbiology text would be expected to contain long descriptions of each infectious organism, the laboratory procedures required to identify the organism, its clinical presentation, and its treatment. As a result, authors were caught between writing enormous texts that contained much more information than any student could possibly absorb, or they wrote short works covering a narrow topic in microbiology, or they wrote review books that hinted at many different topics. Today, authors have the opportunity to create in-depth and comprehensive works that are quite short, without sacrificing conceptual clarity. The informational details can be deferred to the web! This book concentrates on its primary goal; describing all pathogenic organisms in relation to their taxonomic assignments. All of the ancestral classes and every genus is explained in some detail, with

every species listed, but the details are left to the web. You will notice that for a relatively short text, the Taxonomic Guide to Infectious Diseases has a large index. The index was designed as a way to connect terms and concepts that appear on multiple places within the text, and as a key to information on the web. Most of the index terms have excellent discussion in Wikipedia. You will find that the material retrieved from Wikipedia will make much more sense to you, and will have much more relevance to your own professional activities, after you have read this book.

3. As protection against professional obsolescence

There seems to be so much occurring in the biological sciences, it is just impossible to keep on top of things. With each passing day, you feel less in tune with modern science, and you wish you could return to a time when a few fundamental principles grounded your chosen discipline. You will be happy to learn that science is all about finding generalizations among data or among connected systems (i.e., reducing the complexity of data or finding simple explanations for systems of irreducible complexity). Much, if not all, of the perceived complexity of the biological sciences derives from the growing interconnectedness of once-separate disciplines: cell biology, ecology, evolution, climatology, molecular biology, pharmacology, genetics, computer sciences, paleontology, pathology, statistics, and so on. Scientists today must understand many different fields, and must be willing and able to absorb additional disciplines, throughout their careers. As each field of science becomes entangled with others the seemingly arcane field of biological taxonomy has gained prominence because it occupies the intellectual core of virtually every biological field.

Modern biology seems to be data-driven. A deluge of organism-based genomic, proteomic, metabolomic, and other "omic" data is flooding our data banks and drowning our scientists. This data will have limited scientific value if we cannot find a way to generalize the data collected for each organism to the data collected on other organisms. Taxonomy is the scientific method that reveals how different organisms are related. Without taxonomy, data has no biological meaning.

The discoveries that scientists make in the future will come from questions that arise during the construction and refinement of biological taxonomy. In the case of infectious diseases, when we find a trait that informs us that what we thought was a single species is actually two species, it permits us to develop treatments optimized for each species, and to develop new methods to monitor and control the spread of both organisms. When we correctly group organisms within a common class, we can test and develop new drugs that are effective against all of the organisms within the class, particularly if those organisms are characterized by a molecule, pathway, or trait that is specifically targeted by a drug. Terms used in diverse sciences, such as homology, metabolic pathway, target molecule, acquired resistance, developmental stage, cladistics, monophyly,

model organism, class property, phylogeny, all derive their meaning and their utility from biological taxonomy. When you grasp the general organization of living organisms, you will understand how different scientific fields relate to each other, thus avoiding professional obsolescence.

How the text is organized

If you are reading *Taxonomic Guide to Infectious Diseases* to gain a general understanding of taxonomy, as it applies to human diseases, you may choose to read the introductory chapters, followed by reading the front sections of each subsequent chapter. You can defer reading the genera and disease lists until you need to relate general knowledge of a class of organisms to specific information on pathogenic species. If you are a health-care professional, you will find that when you use the index to find the chapter that lists a particular organism or infectious disease, you can quickly grasp the fundamental biological properties of the disease. This deep knowledge will help you when you use other resources to collect detailed pathologic, clinical, and pharmacologic information.

Though about 334 living organisms account for virtually all of the infectious diseases occurring in humans, about 1000 additional organisms account for "case report" incidents, involving one or several people, an isolated geographic region, or otherwise-harmless organisms that cause disease under special circumstances. The book Appendix lists just about every infectious organism (about 1400 species), and the taxonomic hierarchy for each genus. When you encounter the name of an organism, and you just can't remember anything about its taxonomic lineage (i.e., the class of the organism and the ancestral classes), you can find it quickly in the appendix. With this information, you can open the chapter that describes the class properties that apply to the species.

Some clinical concepts are taxonomically promiscuous. For example, the hepatitis viruses (A through G) are dispersed under several different classes of viruses. Moreover, the A through G list of hepatitis viruses excludes some of the most important viruses that target the liver (e.g., yellow fever virus, dengue virus, Epstein-Barr virus). Topics that cross class boundaries, such as hepatitis viruses, long-branch attraction, virulence factors, vectors, zoonoses, and many others, are included in the Glossary.

Nota Bene

Biological nomenclature has changed a great deal in the past few decades. If you learned medical microbiology in the preceding millennium, you may be surprised to learn that kingdoms have fallen (the once mighty kingdom of the protozoans has been largely abandoned), phyla have moved from one kingdom to another (the microsporidians, formerly protozoans, are now fungi), and numerous species have changed their names (Pneumocystis carinii is now Pneumocystis jirovecii). Most striking is the expansion of the existing ranks.

Formerly, it was sufficient to divide the classification into a neat handful of divisions: Kingdom, Phylum, class, Order, Family, Genus, and Species. Today, the list of divisions has nearly quadrupled. For example, Phylum has been split into the following divisions: Superphylum, Phylum, Subphylum, Infraphylum, and Microphylum. The other divisions are likewise split. The subdivisions often have a legitimate scientific purpose. Nonetheless, current taxonomic order is simply too detailed for readers to memorize. Taxonomists referring to a class of any rank will sometimes use the word "taxon." I find this term somewhat lacking because it cannot be modified to refer to a direct parent or child taxon. In this book, all ranks will simply be referred to as "Class." The direct father class is the superclass, and the direct child class is the subclass. The terms "genus" (plural "genera") and "species" will preserve the binomial assignment of organism names. In the case of viruses, Baltimore Classification is used, which places every virus into one of seven Groups. Since "Group" is applied universally and consistently by virologists who employ the Baltimore Classification, its use is preserved here. Subdivisions of the Baltimore Group viruses are referred to herein as classes.

The use of "Class," "Superclass," and "Subclass" conforms to nomenclature standards developed by the metadata community (i.e., uses a standard terminology employed by the computational field dealing with the description of data). This simplified terminology avoids the complexities endured by traditional taxonomists. Regarding the use of upper and lower case terminology, when referring to a formal taxonomic class, positioned within the hierarchy, the uppercase letters and Latin plural forms are used (e.g., Class Eukaryota). When referring to the noun and adjectival forms, lowercase characters and the English pluralized form are used (e.g., an eukaryote, the eukaryotes, or eukaryotic organisms).

Each chapter contains a hierarchical listing of organisms, roughly indicating the ordered rank of the infectious genera covered in each chapter. Classes that do not contain infectious organisms are omitted from the schema. Traditionally, the class rank would be listed in the hierarchy (e.g., Order, Suborder, Infraorder). In this book, the relative descent through the hierarchy is indicated by indentation. The lowest subclass in each taxonomic list is "genus," which is marked throughout with an asterisk. This visual method of ranking the classification produces an uncluttered, disease-only taxonomy and provides an approximate hierarchical rank for each class and species.

Chapter 1

Principles of taxonomy

Section 1.1 The consequence of evolution is diversity

There can be only one

Motto of the immortals in the fictional Highlander epic

Most readers are familiar with the premise of the "Highlander" movies and television shows, which depict a "survival-of-the-fittest" struggle among a population of immortal humans. In the end, there must be only one surviving immortal. Of course, the most casual glance at our surroundings informs us that we live in an "Anti-Highlander" world wherein evolution pushes us to ever-increasing species diversity [Glossary Survival of the fittest].

Introductory courses in evolution stress the notion that evolution leads to improved species, through natural selection [1]. If evolution served the single purpose of improving species, then we would live in a Highlander world, where a small number of the most successful species would prevail, and the others would perish. One of the recurring themes discussed in this book is that the primary consequence of evolution is speciation, the biological process that accounts for the enormous diversity of species that inhabit our planet. When we understand speciation, we can fully grasp the phylogenetic classification of organisms (i.e., the classification of species by their ancestral lineages). When we understand classification, we can simplify the task of understanding the biological properties of the thousands of species that are potential pathogens in humans. Furthermore, we can discover general methods of prevention or treatment that apply to whole classes of related organisms [Glossary Human ancestral lineage, Organism].

How many species live on earth today? A large number of species comes from the prokaryotes (i.e., cells with no nuclei, consisting of Class Bacteria plus Class Archaea), which are estimated to have between 100 thousand and 10 million species. These numbers almost certainly underestimate the true number of prokaryotic species, as they are based on molecular techniques that would exclude valid species that happen to have sequence similarities with other species [2]. As an example of how methodology impacts numbers, samples of soil yield a few hundred different species per gram, based on culturing. If the species are counted on the basis of 16s RNA gene sequencing, we find a few thousand different species of bacteria in each gram of soil. If we base the count on DNA-DNA reassociation kinetics, the number of different bacterial species, per gram of soil, rises to several million [3].

Taxonomic Guide to Infectious Diseases. https://doi.org/10.1016/B978-0-12-817576-7.00001-8

The eukaryotes (i.e., organisms whose cells contain a nucleus) are estimated to have about 9 million species [4]. As for the viruses, we really don't have any good estimate for the number of their species, although it is claimed that viruses account for the greatest number of organisms, species, and classes of species on the planet [5–7]. If we confine ourselves to counting just those viruses that infect mammals, we have an estimate of about 320,000 [8]. Adding up the estimates for prokaryotes, eukaryotes, and viruses, we get a rough and conservative 10-20 million living species.

In addition to the individual species of organisms that live on earth, there are numerous combinations of organisms whose lives are entangled with one another. Perhaps the best known examples of which are the lichens. Formerly known as the Mycophycophyta, lichens are now recognized to be aggregate organisms wherein each component has its own phylogenetic lineage. Lichens independently emerged from fungi associating with algae and cyanobacteria multiple times throughout history [9].

It is worth noting that species counts, even among the most closely scrutinized classes of organisms, are always subject to revision. In the past, the rational basis for splitting a group of organisms into differently named species required, at the very least, heritable functional or morphologic differences among the members of the group. Gene sequencing has changed the rules for assigning new species. For example, various organisms with subtle differences from Bacteroides fragilis have been elevated to the level of species based on DNA homology studies. These include Bacteroides distasonis, Bacteroides ovatus, Bacteroides thetaiotaomicron, and Bacteroides vulgatus [10]. Accounting for underestimation, it should come as no surprise that one study has suggested that there are at least a trillion species of organisms on earth [11].

Of course, the number of living species is a tiny fraction of all the species that have lived and died through the course of earth's history. It is estimated that 5–50 billion species have lived on earth, and more than 99% of them have met with extinction, leaving a relatively scant 10–100 million living species [12]. If the purpose of every species were to ensure its own survival, then they are all doing a very bad job of it, insofar as nearly all species become extinct. In Section 2.2, "The Biological Process of Speciation," we shall see that the determinant of biological success, for any species, is to produce new species. It is the production of descendant classes of species that confers inherited cellular properties that we observe in all living organisms, and that we now use to construct classifications of organisms.

Although there are millions of species on this planet, we should be grateful that only a tiny fraction is infectious to humans. Nobody knows the exact number of living species, but for the sake of discussion, let us accept that there are 50 million species of organisms on earth (a gross underestimate by some accounts). There have been about 1400 pathogenic organisms reported

in the medical literature. This means that if you should stumble randomly upon a member of one of the species of life on earth, the probability that it is an infectious pathogen is about 0.000028 [Glossary Burden of infectious diseases, Incidence, Infectious disease].

With the all the different species of organisms on earth today, numbering perhaps in the hundreds of millions, how can we hope to understand the biosphere? It's all done with classification. Infectious agents fall into a scant 40 biological classes (32 classes of living organisms plus 7 classes of viruses plus 1 current class of prions). When we have learned the basic biology of the major taxonomic divisions that contain the infectious organisms, we will understand the fundamental biological features that characterize every clinically relevant organism.

Section 1.2 What is a classification?

Deus creavit, Linnaeus disposuit, Latin for "God Creates, Linnaeus organizes."

Carolus Linnaeus

The human brain is constantly processing visual and other sensory information collected from the environment. When we walk down the street, we see images of concrete, asphalt, grass, other persons, birds, and so on. Every step we take conveys another world of sensory input. How can we process it all? The mathematician and philosopher Karl Pearson (1857–1936) has likened the human mind to a "sorting machine" [13]. We take a stream of sensory information and sort it into objects, and then we collectively put the individual objects into general classes. The green stuff on the ground is classified as "grass," and the grass is subclassified under some larger groups such as "plants." Flat stretches of asphalt and concrete may be classified under "road" and the road might be subclassified under "man-made constructions." If we did not have a culturally determined classification of objects in the world, we would have no languages, no ability to communicate ideas, no way to remember what we see, and no way to draw general inferences about anything at all. Simply put, without classification, we would not be human.

Every culture has some particular way to impose a uniform perception of the environment. In English-speaking cultures, the term "hat" denotes a universally recognized object. Hats may be composed of many different types of materials, and they may vary greatly in size, weight, and shape. Nonetheless, we can almost always identify a hat when we see one, and we have no trouble distinguishing a hat from all other types of objects. An object is not classified as a hat simply because it shares a few structural similarities with other hats. A hat is classified as a hat because it has a relationship with every other hat, as an item of clothing that fits over the head.

Taxonomists search for relationships, not similarities, among different species and classes of organisms [14]. But isn't a similarity a type of

relationship? Actually, no. To better understand the difference, imagine the following scenario. You look up at the clouds, and you begin to see the shape of a lion. The cloud has a tail, like a lion's tale, and a fluffy head, like a lion's mane. With a little imagination, the mouth of the lion seems to roar down from the sky. You have succeeded in finding similarities between the cloud and a lion. When you look at a cloud and you imagine a tea kettle producing a head of steam, you may recognize that the physical forces that create a cloud from the ocean's water vapor and the physical forces that produce steam from the water in a heated kettle are the same. At this moment, you have found a relationship. The act of searching for and finding relationships lies at the heart of science; it's how we make sense of reality. Finding similarities is an aesthetic joy, but it is not science.

General principles of classification

Oddly enough, despite the importance of classification in our lives, few humans have a firm understanding of the process of classification; it's all done for us on a subconscious level. Consequently, when we need to build and explain a formal classification, it can be difficult to know where to begin. As an example, how might we go about creating a classification of toys? Would we arrange the toys by color (red toys, blue toys, etc.), by size (big toys and medium-sized toys), or by composition (metal toys, plastic toys, cotton toys). How could we be certain that when other people create a classification for toys, their classification will be equivalent to ours?

For modern biologists, the key to the classification of living organisms is evolutionary descent (i.e., phylogeny). The hierarchy of classes corresponds to the succession of organisms that evolved from the earliest living organism to the current set of extant species. Historically, pre-Darwinian biologists, who knew nothing about evolution, somehow produced a classification that looked much like the classification we use today. Before the discovery of the Burgess shale (discovered in 1909 by Charles Walcott), taxonomists could not conduct systematic reviews of organisms in rock strata; hence, they could not determine the epoch in which classes of organisms first came into existence, nor could they determine which fossil species preceded other species. Until late in the 20th century, taxonomists could not sequence nucleic acids; hence, they could not follow the divergence of shared genes in different organisms. Yet they managed to produce a fairly accurate and modern taxonomy. A 19th-century taxonomist would have no trouble in adjusting to the classification used in this book [Glossary Taxonomy, Clade, Cladistics, Class, Monophyletic class, Synapomorphy].

How did the early taxonomists arrive so close to our modern taxonomy, without the benefit of the principles of evolution, geobiology, modern paleontological discoveries, or molecular biology? For example, how was it possible for Aristotle to know, about 2000 years ago, that a dolphin is a mammal, not a fish? Aristotle studied the anatomy and the developmental biology of many different types of animals. One large group of animals was distinguished by a

gestational period in which a developing embryo is nourished by a placenta, and the offspring are delivered into the world as formed, but small versions of the adult animals (i.e., not as eggs or larvae), and the newborn animals feed from milk secreted from nipples, overlying specialized glandular organs (mammae). Aristotle knew that these were features that specifically characterized one group of animals and distinguished this group from all the other groups of animals. He also knew that dolphins had all these features; fish did not. He correctly reasoned that dolphins were a type of mammal, not a type of fish. Aristotle was ridiculed by his contemporaries for whom it was obvious that dolphins were a type of fish. Unlike Aristotle, they based their classification on similarities, not on relationships. They saw that dolphins looked like fish and dolphins swam in the ocean like fish, and this was all the proof they needed. For about 2000 years following the death of Aristotle, biologists persisted in their belief that dolphins were a type of fish. For the past several hundred years, biologists have acknowledged that Aristotle was correct after all; dolphins are mammals.

Aristotle, and legions of taxonomists who followed him, understood that taxonomy is all about finding the key properties that characterize entire classes and subclasses of organisms. Selecting the defining properties from a large number of morphologic, developmental and physiologic features in many different species requires attention to detail, and occasional moments of intellectual brilliance. To build a classification, the taxonomist must perform the following: (1) define classes (i.e., find the properties that define a class and extend to the subclasses of the class); (2) assign species to classes; (3) position classes within the hierarchy; and (4) test and validate all the above. These tasks require enormous patience and humility.

A classification is a hierarchy of objects that conforms to the following principles:

–1. The classes (groups with members) of the hierarchy have a set of properties or rules that extend to every member of the class and to all of the subclasses of the class, to the exclusion of all other classes. A subclass is itself a type of class wherein the members have the defining class properties of the parent class plus some additional property(ies) specific for the subclass [Glossary Parent class].

–2. In a hierarchical classification, each subclass may have no more than one parent class. The root (top) class has no parent class. The biological classification of living organisms is a hierarchical classification.

–3. In the classification of living organisms, the species is the collection of all the organisms of the same type (e.g., every squirrel belongs to a species of “squirrel”).

–4. Classes and species are intransitive. For example, a horse never becomes a sheep, and Class Bikonta never transforms into Class Unikonta.

–5. The members of classes may be highly similar to each other, but their similarities result from their membership in the same class (i.e.,

conforming to class properties), and not the other way around (i.e., similarity alone cannot define class inclusion).

When we look at a schematic that represents a classification, we are typically shown a tree of nodes, with each class occupying a node, and the branches to lower nodes represent the connections of a class to its subclasses. A taxonomy is a classification that has all of its members assigned to their respective classes. In the case of the classification of living organisms, the classes are assigned according to their ancestry (i.e., by their phylogenetic relationships).

It is essential to distinguish a classification system from an identification system. An identification system matches an individual organism with its assigned species name. Identification is based on finding several features that, taken together, can help determine the name of an organism. For example, if you have a list of identifiers: large, hairy, strong, African, jungle-dwelling, knuckle-walking; you might correctly identify the organisms as a gorilla. These identifiers are different from the phylogenetic features that were used to classify gorillas within the hierarchy of organisms (Animalia: Chordata: Mammalia: Primates: Hominidae: Homininae: Gorillini: Gorilla). Specifically, you can identify an animal as a gorilla without knowing that a gorilla is a type of mammal. You can classify a gorilla as a member of Class Gorillini without knowing that a gorilla happens to be large. One of the most common mistakes in biology is to confuse an identification system with a classification system. The former simply provides a handy way to associate an object with a name; the latter is a system of relationships among organisms [Glossary Classification versus ontology].

Section 1.3 The tree of life

Individuals do not belong in the same taxon because they are similar, but they are similar because they belong to the same taxon.

George Gaylord Simpson (1902–84) [15]

Taxonomy is the science of classifying the elements of a knowledge domain. In the case of terrestrial life forms, taxonomy involves assigning a name and a class to every species of life. Biologists presume that there are at least 50 million living species on earth, so the task of building a biological taxonomy is likely to continue for as long as science persists. Not all scientists are suited, intellectually or emotionally, to be taxonomists. Nonetheless, every thoughtful scientist understands that taxonomy is essential to the advancement of science and to the preservation of life on earth.

Most biologists would agree to the following:

– Statement 1. Every organism on earth belongs to a class of organisms with a set of shared biological features that was inherited through an ancestral lineage.

– Statement 2. All organisms on earth have a genome consisting of DNA or RNA. DNA is a highly stable nucleic acid that is transcribed into RNA, and RNA is translated into proteins.
– Statement 3. Every organism on earth belongs to one of the four classes (to be described in later chapters):

```
Class Archaea
Class Bacteria
Class Eukaryota
Class Viridae
```

Questions of precedence (i.e., "Which class arose first?") and parentage (i.e., "Which class served as the progenote for which other class?") is a matter for lively debate [16, 17]. It is generally accepted that the prokaryotes (i.e., Class Archaea plus Class Bacteria) preceded the emergence of Class Eukaryota, insofar as the root eukaryote seems to have been constructed from biological components extracted from prokaryotes and possibly viruses [18–21].

In addition, the rightful inclusion of Class Viridae (viruses) is disputed, insofar as many biologists consider viruses to be little more than nonliving packets of infective genetic material. In Chapter 7, "Viruses," we will examine the controversial status of viruses as living organisms.

– Statement 4. Every eukaryotic organism that lives today is a descendant of a single eukaryotic ancestor [18].
– Statement 5. Every organism belongs to a species that has a set of features that characterizes every member of the species and that distinguishes the members of the species from organisms belonging to any other species.

Of course, it is difficult to garner unanimous agreement by scientists, and every fundamental principle of taxonomy has been challenged at one time or another. For those who would include prions among the living organisms, statements 1–3 are debatable (as will be discussed in Section 8.2, "Prion Diseases: Fulfilling Koch's Postulates, but Without an Organism").

Because each class of organisms has exactly one parent class, we can use the classification of living organisms to construct a simple, unbranched ancestral lineage, for each and every included class or species. For example, here is the ancestral lineage for mosquitoes (scientific name, Culicidae), a species of Class Diptera (flies):

```
Culicidae (mosquitoes)
Culicoidea
Culicomorpha
Nematocera
Diptera (class of flies)
Holometabola
Neoptera
Pterygota
Dicondylia
Insecta
Hexapoda
Pancrustacea
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Mandibulata
Arthropoda
Panarthropoda
Ecdysozoa
Protostomia
Bilateria
Eumetazoa
Metazoa
Opisthokonta
Eukaryota
cellular organisms
```

Statement 5. Introduces the concept of "species," which has a long and disputatious history. It has been argued that nature produces individuals, not species; the concept of species being a mere figment of the human imagination, created for the convenience of taxonomists who need to group similar organisms. There are those who would use computational methods to group organisms into various species. If you start with a set of feature data on a collection of organisms, you can write a computer program that will cluster the organisms into species, according to their similarities. In theory, one computer program, executing over a large dataset containing measurements for every earthly organism, could create a complete biological classification. The status of a species is thereby reduced from a fundamental biological entity to a mathematical construction.

This view is anathema to classic taxonomists, who have long held that a species is a natural unit of biological life, and that the nature of a species is revealed through the intellectual process of building a consistent taxonomy [22]. There are a host of problems consequent to computational methods for classification. First, there are many different mathematical algorithms that cluster objects by similarity. Depending on the chosen algorithm, the assignment of organisms to one species or another would change. Secondly, mathematical algorithms do not cope well with species convergence. Convergence occurs when two species independently acquire identical or similar traits through adaptation; not through inheritance from a shared ancestor. Examples are: the wing of a bat and the wing of a bird; the opposable thumb of opossums and primates; and the beak of a platypus and the beak of a bird. Unrelated species frequently converge upon similar morphologic solutions to common environmental conditions or shared physiological imperatives. Algorithms that cluster organisms based on similarity may group divergent organisms under one class.

It is often assumed that computational classification, based on morphologic feature similarities, will improve when we acquire whole-genome sequence data for many different species. Imagine an experiment wherein you take DNA samples from every organism you encounter: bacterial colonies cultured from a river, unicellular nonbacterial organisms found in a pond, small multicellular organisms found in soil, crawling creatures dwelling under rocks, and so on. You own a powerful sequencing machine, which produces the full-length sequence

for each sampled organism, and you have a powerful computer that sorts and clusters every sequence. At the end, the computer prints out a huge graph, wherein all the samples are ordered. Groups with the greatest sequence similarities are clustered together. You may think you've created a useful classification, but you haven't really, because you don't know anything about the organisms that are clustered together. You don't know whether each cluster represents a species, or a class (a collection of related species), or whether a cluster may be contaminated by organisms that share some of the same gene sequences, but are phylogenetically unrelated (i.e., the sequence similarities result from chance or from convergence, but not by descent from a common ancestor). The sequences do not tell you very much about the biological properties of specific organisms, and you cannot infer which biological properties characterize the classes of clustered organisms. You have no certain knowledge whether the members of any given cluster of organisms can be characterized by any particular gene sequence (i.e., you do not know the characterizing gene sequences for classes of organisms). You do not know the genus or species names of the organisms included in the clusters, because you began your experiment without a presumptive taxonomy. Basically, you simply know what you knew before you started; that individual organisms have unique gene sequences that can be grouped by sequence similarity. A strictly molecular approach to classification has its limitations, but we shall see that thoughtful biologists can use molecular data to draw profound conclusions about the classification of living organisms [23, 24] [Glossary Convergence, LUCA].

Taxonomists are constantly engaged in an intellectual battle over the principles of biological classification. They all know that the stakes are high. When unrelated organisms are mixed together in the same class, and when related organisms are separated into unrelated classes, the value of the classification is lost, perhaps forever. To understand why this is true, we need to understand that a classification is a hypothesis-generating machine. Species within a class share genes, metabolic pathways, and structural anatomy. Shared properties allow scientists to form general hypotheses that may apply to all the members of a class. Without an accurate classification of living organisms, it would be impossible to make significant progress in the diagnosis, prevention, or treatment of classes of infectious organisms [Glossary Blended class, Pathway, Pathway-driven disease].

James Joyce is credited with saying that “there are two sides to every argument; unfortunately, I can only occupy one of them.” Students of the life sciences simply cannot hope to understand terrestrial organisms without accepting, at least tentatively, statements 1–5. After they have mastered the principles and practice of modern taxonomy, as described herein, they can reassess the value of contrarian arguments.

Glossary

Blended class Class blending refers to a mistake in proper classification, in which members are assigned to the wrong classes, or in which a class is created whose members are unrelated. These kinds of mistakes often arise when taxonomists are unaware of the differences among the members assigned to a class, or when taxonomists create an untenable class. For example, if you were to make a Mouse class, and you included Mickey Mouse as one of the instances of the class, you would be blending a cartoon with an animal, and this would be a mistake. If you were to create a Flying Animal class, you would be blending birds and houseflies and flying squirrels, none of which are biologically related.

After reading the preceding paragraph, you might be thinking that class blending is the kind of careless mistake that you will be smart enough to avoid. Not so. Class blending is a pervasive and costly sin that is committed by virtually every biological scientist at some point in his or her career. One error can easily set your research back a decade, if you're not mentally focused on this subtle issue.

When you read old texts, written before we knew anything about microorganisms, it's clear that the causes of historical plagues are largely unknown. We recognize today that one of the plague bacteria is Yersinia pestis. But, in fact, we do not know with certainty the specific causes of any of the major plagues in ancient Greece and medieval Europe. Typhus may have been involved. Measles and smallpox are the likely causes of past plagues. Malarial outbreaks should not be overlooked.

Now suppose you are a statistician and are magically ported to Southern Italy, in 1640, where people are dying in great number, of the plague, and you are a doctor trying to cope with the situation. You're not a microbiologist, but you know something about designing clinical trials, and one of the local cognoscenti has just given you an herb that he insists is a cure for the plague. “Take this drug today, and your fever will be gone by the next morning,” he tells you. As it happens, the herb is an extract of bark from the Cinchona tree, recently imported from Brazil. It is a sure-fire cure for malaria, a disease endemic to the region. But you don't know any of this. Before you start treating your patients, you'll want to conduct a clinical trial.

At this time, physicians knew nothing about the pathogenesis of malaria. Current thinking was that it was a disease caused by breathing insalubrious swamp vapors; hence the word roots “mal” meaning bad, and “aria” meaning air. You have just been handed a substance derived from the Cinchona tree, but you do not trust the herbalist. Insisting on a rational approach to the practice of medicine, you design a clinical trial, using 100 patients, all of whom have the same symptoms (delirium and fever) and all of whom carry the diagnosis of plague. You administer the cinchona powder, also known as quinine, to all the patients. A few improve, but most don't. You call the trial a washout. You decide not to administer quinine to your patients.

What happened? We know that quinine arrived as a miracle cure for malaria. It should have been effective in a population of 100 patients. The problem with this hypothetical clinical trial is that the patients under study were assembled based on their mutual symptoms: fever and delirium. These same symptoms could have been accounted for by any of hundreds of other diseases that were prevalent at the time. The criteria employed to render a diagnosis of plague were imprecise, and the trial population was diluted with nonmalarial patients who were guaranteed to be nonresponders. Consequently, the trial

failed, and you missed a golden opportunity to treat your malaria patients with quinine, a new, highly effective, miracle drug.

It isn't hard to imagine present-day dilemmas not unlike our fictitious quinine trial. If you are testing the effectiveness of an antibiotic on a class of people with bacterial pneumonia, the accuracy of your results will be jeopardized if your study population includes subjects with viral pneumonia, or smoking-related lung damage. The consequences of class blending are forever with us. It is impossible to conduct rational trials for appropriate targeted therapies when the trial groups are composed of blended classes of individuals [25]. The medical literature is rife with research of dubious quality, based on poorly designed classifications and blended classes.

One caveat, efforts to reduce class blending can be counterproductive if undertaken with excess zeal. For example, in an effort to reduce class blending, a researcher may choose groups of subjects who are uniform with respect to every known observable property. For example, suppose you want to actually compare apples with oranges. To avoid class blending, you might want to make very sure that your apples do not include any kumquats or persimmons. You should be certain that your oranges do not include any limes or grapefruits. Imagine that you go even further, choosing only apples and oranges of one variety (e.g., Macintosh apples and Navel oranges), size (e.g., 10 cm), and origin (e.g., California). How will your comparisons apply to the varieties of apples and oranges that you have excluded from your study? You may actually reach conclusions that are invalid and irreproducible for more generalized populations within each class. In this case, you have succeeded in eliminating class blending, at the expense of losing representative populations of the classes.

Burden of infectious diseases Each year, 50–60 million people die worldwide. How many of these deaths can be attributed to infectious diseases? According to World Health Organization, in 1996, "Infectious diseases remain the world's leading cause of death, accounting for at least 17 million (about 33%) of the 52 million people who die each year" [26]. Of course, only a small fraction of infections result in death, and it is impossible to determine the total incidence of infectious diseases that occur each year, for all organisms combined. Still, it is useful to consider some of the damage inflicted by just a few of the organisms that infect humans.

Malaria infects 500 million people. About 2 million people die each year from malaria [26].

About 2 billion people have been infected with Mycobacterium tuberculosis. Tuberculosis kills about 3 million people each year [26].

Each year, about 4 million children die from lung infections, and about 3 million children die from infectious diarrheal diseases [26]. Rotaviruses are one of many causes of diarrheal disease (Group III Viruses). In 2004, rotaviruses were responsible for about half a million deaths, mostly in developing countries [27].

Worldwide, about 350 million people are chronic carriers of Hepatitis B, and about 100 million people are chronic carriers of Hepatitis C. In aggregate, about one quarter (25 million) of these chronic carriers will eventually die from ensuing liver diseases [26].

Infectious organisms can kill individuals through mechanisms other than the direct pathologic effects of growth, invasion, and inflammation. Infectious organisms have been implicated in vascular disease. Organisms implicated in coronary artery disease and stroke include Chlamydia pneumoniae and Cytomegalovirus [28].

Infections caused by a wide variety of infectious organisms can result in cancer. About 7.2 million deaths occur each year from cancer, worldwide. About one-fifth of these cancer deaths are caused by infectious organisms [29]. Hepatitis B alone accounts

for about 700,000 cancer deaths each year, from hepatocellular carcinoma [30]. Organisms contributing to cancer deaths include bacteria (Helicobacter pylori), animal parasites (schistosomes and liver flukes), and viruses (Herpesviruses, Papillomaviruses, Hepadnaviruses, Flaviviruses, Retroviruses, Polyomaviruses). Though fungal and plant organisms do not seem to cause cancer through human infection, they produce a multitude of biologically active secondary metabolites (i.e., synthesized molecules that are not directly involved in the growth of the organism), some of which are potent carcinogens. For example, aflatoxin produced by Aspergillus flavus is possibly the most powerful carcinogen ever studied [31].

In aggregate, infectious diseases are the number one killer of humans worldwide, and contribute to vascular disease and cancer, the two leading causes of death in the most developed countries. These observations clearly indicate that every health-care professional, not just infectious disease specialists, must understand the biology of infectious organisms.

Clade A clade consists of a monophyletic class and all of its descendant monophyletic classes. A clade should be distinguished from a lineage, the latter being the list of a class's ascendant superclasses. Because a class can have more than one child class, a pictogram of a clade will often look like a branching tree. In a classification, where each class is restricted to one parent class, ascending lineages are represented as a nonbranching line of ancestors, leading to the root (i.e., top class) of the classification.

Cladistics The technique of producing a hierarchy of clades, wherein each clade is a monophyletic class. In this book, we define a classification so that it conforms to the rules of cladistics. Hence, a classification is cladistic and clades are equivalent to classes and subclasses. The terms "cladistics" and "clade," enjoyed by taxonomists, are omitted from the text. Instead, we employ the "class" terminology (e.g., class, subclass, child class, parent class) that is preferred by bioinformaticians and computer scientists who rely upon object-oriented programming languages [32].

Class A defined group within a taxonomy. The most familiar classes in biological taxonomy are the classes that form the ranked hierarchy of living organisms: Kingdom, Phylum, Class, Order, Family, Genus, and Species. It is somewhat confusing that one of the classes of organisms is "Class," and another of the classes is named "Order." This means that when the terms "Class" or "Order" appear in a sentence, the reader must somehow distinguish between the general term and the specific term. In this book, classes are unranked. The word "class," lowercase, is used as a general term. The word "Class," uppercase, followed by an uppercase animal division (e.g., Class Animalia), represents a group within the taxonomy. In the biological hierarchy, each class has exactly one direct ancestor class (also called parent class or superclass), though an ancestor class can have more than one direct descendant class (also called child class, or subclass).

Classification versus ontology A classification is a system in which every object in a knowledge domain is assigned to a class within a hierarchy of classes. The properties of superclasses are inherited by the subclasses. Every class has one immediate superclass (i.e., parent class), although a parent class may have more than one immediate subclass (i.e., child class). Objects do not change their class assignment in a classification, unless there was a mistake in the assignment. For example, a rabbit is always a rabbit, and does not change into a tiger.

A classification should be distinguished from an ontology. In an ontology, a class may have more than one parent class and an object may be a member of more than one class. A classification can be considered a restrictive and simplified form of ontology wherein

each class is limited to a single parent class and each object has membership in one and only one class [33].

Convergence When two species independently acquire an identical or similar trait through adaptation; not through inheritance from a shared ancestor. Examples are: the wing of a bat and the wing of a bird; the opposable thumb of opossums and primates; and the beak of a platypus and the beak of a bird.

Human ancestral lineage Here is the ancestral lineage of human beings, beginning with the earliest indication of life on earth. Wherever possible, the major classes of organisms are annotated with a very approximate chronology. It is useful to have some notion of the time interval between classes of ancestral organisms, even if it is somewhat inaccurate.

```
Earliest indication of life 4100 mya
Prokaryota (3900 mya)
Eukaryota (2100–1000 mya)
Podiata
Unikonta (Amorphea)
Obazoa
Opisthokonta
Holozoa (1300 mya)
Apoikozoa (950 mya)
Metazoa (760 mya)
Eumetazoa (Diploblasts, Histozoa, Epitheliozoa) (635 mya)
ParaHoxozoa
Planulozoa
Bilateria (Triploblasts) (555 mya)
Nephrozoa (555 mya)
Deuterostomia (Enterocoelomates) (540 mya)
Chordata (530 mya)
Craniata (480 mya)
Vertebrata (500 mya)
Gnathostomata (419 mya)
Euteleostomi
Sarcopterygii (419 mya)
Dipnotetrapodomorpha
Tetrapodomorpha (390 mya)
Tetrapoda (367 mya)
Amniota (340 mya)
Synapsida (308 mya)
Mammalia (220 mya)
Theriiformis
Theria (160 mya)
Eutheria (160–125 mya)
Boreoeutheria (124–101 mya)
Euarchontoglires (100 mya)
Euarchonta (99–80 mya)
Primatomorpha (79.6 mya)
Primates (75 mya)
Haplorrhini (63 mya)
Simiiformes (40 mya)
Catarrhini (30 mya)
Hominoidea (28 mya)
Hominidae (15 mya)
```

```
Homininae (8 mya)
Hominini (5.8 mya)
Hominina (4 mya)
Homo (2.5 mya)
Homo sapiens (0.3 mya)
Homo sapiens (modern) (0.07 mya)
```

Incidence The number of new cases of a disease occurring in a time interval (e.g., 1 year), expressed as a fraction of a predetermined population size (e.g., 100,000 people). For example, if there were 11 new cases of a rare disease occurring in a period of 1 year, in a population of 50,000 people, then the incidence would be 22 cases per 100,000 persons per year.

Infectious disease A disease caused by an organism that enters the human body. The term "infectious disease" is sometimes used in a way that excludes diseases caused by parasites. In this book, the parasitic diseases of humans are included among the infectious disease. The term "infectious disease" is often used interchangeably with "infection," but the two terms are quite different. It is quite possible to be infected with an organism, even a pathogenic organism, without developing a disease. In point of fact, the typical human carries many, perhaps dozens, of endogenous pathogenic organisms that lie dormant under most circumstances. Examples are: Pneumocystis jiroveci (the fungus that causes pneumonia in immunodeficient individuals), Varicella (the virus that may, when the opportunity arises, erupt as shingles), Aspergillus species (which not uncommonly colonize the respiratory tract, producing pneumonia in a minority of infected individuals), Candida (a ubiquitous fungus that lives on skin and in mucosal linings and produces diseases of varying severity in a minority of infected individuals).

LUCA Abbreviation for Last Universal Common Ancestor, also known as the cenancestor. Assuming that all organisms on earth descend from a common ancestor, then LUCA is the most recent population of organisms from which all organisms now living on Earth have a common descent. LUCA is thought to have lived 3.5–3.8 billion years ago [34].

Monophyletic class A class of organisms that includes a parent organism and all its descendants, while excluding any organisms that did not descend from the parent. If a subclass of a parent class omits any of the descendants of the parent class, then the parent class is paraphyletic. If a subclass of a parent class includes organisms that did not descend from the parent, then the parent class is polyphyletic. A class can be paraphyletic and polyphyletic, if it excludes organisms that were descendants of the parent and if it includes organisms that did not descend from the parent. The goal of cladistics is to create a hierarchical classification that consists exclusively of monophyletic classes (i.e., no paraphyly, no polyphyly).

Organism A living entity that is composed of identifiable parts that act in concert to perform some measurable action(s). This definition permits us to think of organisms as biological systems confined to a defined structure.

This definition runs into some trouble when we observe organisms that are composed of other organisms. For example, all animals are composed of cells. Both the animal and its component cells satisfy the definition of an organism. By convention, we allow the composite organism (e.g., a human) to subsume the component organisms (e.g., the liver cell). It's worth remembering that we can preserve the life of individual cells of an organism long after the composite organism has died (e.g., tissue culture or freezing). It

is currently well within the realm of our imaginations that we can reconstruct a composite organism (more precisely, its genetic equivalent) from stored individual cells that have been induced to become totipotent stem cells.

Parent class The immediate ancestor or the next-higher class (i.e., the direct superclass) of a class. For example, in the classification of living organisms, Class Vertebrata is the parent class of Class Gnathostomata. Class Gnathostomata is the parent class of Class Teleostomi, and so on.

Pathway According to traditional thinking, a pathway is a sequence of biochemical reactions, involving a specific set of enzymes and substrates that produces a chemical product or that fulfills a particular function. The classic pathway is the Krebs cycle. It was common for students to be required to calculate the output of the cycle (in moles of ATP) based on stoichiometric equations employing known amounts of substrate. As we learn more and more about cellular biology, the term "pathway" acquires a broader meaning. One pathway may intersect or subsume other pathways. Furthermore, a pathway may not be constrained to an anatomically sequestered area of the cell, and the activity of a pathway may change from cell type to cell type or may change within one cell depending on the cell's physiologic status. The individual enzymes that participate in a pathway may have different functions, in alternate pathways. New pathways evolve by recruiting enzymes from various preexisting pathways [35].

The many ways in which the component parts of a pathway can be assigned has led to an inflation of pathway networks. When we assume that a published pathway represents a specific and uniform cellular process, we may easily draw false inferences, leading to unverifiable claims [36]. In general, the term "pathway" is best used as a convenient conceptual device to organize classes of molecules that interact with a generally defined set of partner molecules to produce a somewhat consistent range of biological actions.

Pathway-driven disease Refers to disorders whose clinical phenotype is largely the result of a single, identifiable pathway. Diseases with similar clinical phenotypes can often be grouped together if they share a common, disease-driving pathway. Examples include the channelopathies (driven by malfunctions of pathways involving the transport of ions through membrane channels), ciliopathies (driven by malfunctions of cilia), and lipid receptor mutations (driven by any of the mutations involving lipid receptors).

Certain types of conditions do not fall easily into the "pathway-driven" paradigm. For example, it is difficult to speak of a class of diseases all driven by errors in transcription factor pathways. A single transcription factor may regulate pathways in a variety of cell types with differing functions and embryologic origins. Hence, the syndromes resulting from a mutation in a transcription factor may involve multiple pathways and multiple tissues and will not have any single, identifiable pathway that drives the clinical phenotype.

At this point, our ability to sensibly assign diseases to pathways is limited because the effects of a mutation in a single gene may indirectly affect many different pathways, and those pathways may vary from cell-type to cell-type. There is some hope that as more cell-based data becomes available, modern data analysis techniques will reliably match specific diseases with specific pathways [37].

Survival of the fittest Phrase was first used by Herbert Spencer, a contemporary of Darwin's, in his Principles of Biology (1864), who referred to natural selection as a process that favored the survival of the fittest "races" (Spencer's terminology). The term was not intended to refer to the survival of the fittest individuals of a species. Moreover, fitness, as it applies to species, refers to the ability of the species to speciate, to produce a diverse

class of descendant species over time. There is nothing in the theory of evolution through natural selection that specifically addresses the issue of the survival of individuals in the species.

Synapomorphy A trait found in the members of a class and its subclasses (i.e., shared by the species descending from the ancestral species in which the trait first appeared).

Taxonomy When we write of "taxonomy" as an area of study, we refer to the methods and concepts related to the science of classification, derived from the ancient Greek taxis, "arrangement," and nomia, "method." When we write of "a taxonomy," as a construction within a classification, we are referring to the collection of named instances (class members) in the classification. To appreciate the difference between a taxonomy and a classification, it helps to think of taxonomy as the scientific field that determines how the different members within the classification are named. Classification is the scientific field that determines how related named members are assigned to classes, and how the different classes are related to one another. A taxonomy is similar to a nomenclature; the difference is that in a taxonomy, every named instance must have an assigned class.

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Chapter 2

Species and speciation

Section 2.1 A species is a biological entity

The purpose of narrative is to present us with complexity and ambiguity.

Scott Turow

It has been argued that nature produces individuals, not species; the concept of species being a mere figment of the human imagination, created for the convenience of taxonomists who need to group together similar organisms. In point of fact, there are many excellent reasons to believe that species are biological entities, on equal or better scientific footing than individual organisms. The justification for the species concept follows.

1. **Species are well defined, and membership within a species is immutable.**

Early definitions of species were fashioned to exclude most organisms, including all bacteria, all unicellular eukaryotes, and all fungi. One long-held definition for a species was that it was a class of animals that shared main characteristics and that could breed with one another. Aside from excluding the vast majority of living organisms, this early definition didn't do much to help explain how species came into existence, and did not inform us how to choose the main characteristics that determined membership in a species.

The modern definition of species can be expressed in three words: "evolving gene pool" [1]. This elegant definition is a simple concept to comprehend and to defend and serves to explain how new species come into existence (i.e., by collecting a new gene pool) [1–3]. Because each member of a species has a genome constructed from its unique species gene pool, it is clear that membership within a species is immutable (e.g., a fish cannot become a cat and a cat cannot become a goat because their genomes come from the unique gene pools of their respective species).

2. **Membership within a species is biologically determined for every living organism.**

It is interesting to note that we humans have no trouble grasping the idea that individual organisms (such as ourselves) are distinct biological entities, while

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we tend to think of our species as belonging to some sort of life continuum, with no sharp boundaries between one species and another species of the same class (e.g., two species of similar-looking frogs). In point of fact, each of us is a chimeric organism, with a diploid part (what we see as our functioning bodies), and a haploid part (our germ cells that can potentially recombine with other germ cells to produce endless generations of diploid/haploid chimeric organisms). Furthermore, each of us is composed of billions of cells that can live and replicate independently (e.g., in tissue culture, or as a transplant). We are individuals only in the sense that we choose to think of ourselves as such. We can assert that a species has a real biological domain, inasmuch as if we eliminate a species, then every member of the species must perish.

3. **Species respond biologically to natural selection.**

Natural selection operates on the gene pool of a species, changing the balance of available genes. Hence, species, not individuals, are influenced biologically, by natural selection.

4. **Species live and die and have a primary biological purpose: speciation.**

Millions of species occupy the biosphere [4]. The observed diversity of species suggests that the purpose and destiny of a species is to speciate; to produce new offspring species. Hence, the success of a species is not determined by whether it has produced lots of individuals of the species, or whether it has persisted for a long time, but whether it has produced a descendant lineage of new species.

In summary, species have the properties associated with every living entity: uniqueness, life, death, and the issuance of progeny.

Section 2.2 The biological process of speciation

One of the most fundamental goals of modern biological research is comprehension of the way in which species arise.

George Gaylord Simpson (1902–84), in 1945 [5]

George Gaylord Simpson, a mid-20th century evolutionary biologist and taxonomist, had a gift for posing some of the best and enduring questions in his field. It is intriguing that one year after Simpson explained the importance of solving the mystery of how species arise, the science fiction author, Ray Bradbury, inadvertently found the answer. In "The Million-Year Picnic," one of the stories in "The Martian Chronicles," Bradbury relates how a man takes his family from our planet, to live permanently on Mars. Once there, they burn the rocket that transported them, so that they can never return to earth. One evening, the father tells his children that he is going to take them to see the Martians. They walk together to a canal, and the father shows them their own reflections in the water.

The family had become, by virtue of leaving the earth behind, a new species; a species of Martians. The family depicted by Ray Bradbury had separated itself from the gene pool of earthlings, and had established, along with the other Martian colonists, a gene pool that would evolve in its own manner, to produce a species that would, over time, become obviously different from its parent species: Homo sapiens. The Martians might develop a horny coat to protect against cosmic radiation, or hypertrophied tear glands to protect against dust storms, or any number of modifications to cope with the toxic Martian atmosphere. Regardless, we can be certain that the gene pool available to the colonizing Martians would evolve differently than the gene pool of earthlings.

1. Species speciate. Individuals do not.

On the subject of science fiction, you doubtless recall the 25th episode of the third season of Star Trek: The Next Generation, titled, "Transfigurations." In this episode, a Zalkonian named "John" takes refuge aboard the Enterprise to escape pursuit by Captain Sunad, a zealous Zalkonian determined to capture John and return him to Zalkon, where John will be punished for crimes unspecified.

During the ensuing drama, and between commercial breaks, John acquires strange powers, including the power to heal. In the last moment, John evolves into a being of pure energy, and flies under his own power through space. Presumably, he is headed back to Zalkon, where he will be the father of a new species of energetic Zalkonians, like himself.

Of course, this book on taxonomy is focused on terrestrial entities, but it should be apparent that evolution on the planet Zalkon is very different from evolution here on earth. For starters, earthling organisms never speciate, the reason being that a species is an evolving gene pool. Individuals contribute only one set of genes to a pool composed of the genes of many individuals. Hence, a single individual cannot evolve to become a new species.

Can a single organism account for a diverse collection of subspecies? Yes, and we suspect that it happens all the time on islands or in geographic areas isolated by mountains, rivers, or habitat barriers. The key point to remember is that the founder of a new species is not itself a new species; it is simply the first contributor to a gene pool that will grow and evolve, to produce new species.

To understand how gene pools speciate, imagine a female primate of Class Strepsirrhini, the parent class of Class Lemuriformes, desperately clinging to a tree limb and washed out to sea, only to turn up on the shore of Madagascar. After a few days, she assesses her situation and determines that she is the only bona fide member of Class Strepsirrhini on the island. Adding to the drama, she discovers that she is pregnant. She hopes it's a boy, maybe twins.

If she and her brood survive, and if the children successfully breed, they will establish a new gene pool from which species will evolve. In fact, a scenario something like the one described has been suggested for the origin of all the species of lemur on Madagascar. The reasoning is simple: lemurs are found only on the island of Madagascar and on the Comoros Islands, northwest of Madagascar. Hence, they must have arisen on the island from an ancestral species that lived

only on the island. The nonlemur members of Class Lemuriformes, the galagos and lorisids, live in Africa and Asia. Hence, the founding parent of the lemurs of Madagascar most likely came from Africa.

The important point here is that the member of Class Lemuriformes who landed in Madagascar was nothing special. Unlike John the Zalkonian, she didn't transform into another species. Her only claim to fame was that she contributed to a gene pool. The gene pool evolved, and the process of evolution required nothing more than natural selection of pooled genes. Eventually, all the different species of lemur now living in Madagascar came to be. Should we give credit to the original Madagascar primate for all the species of lemur living on earth today? Technically, no. We owe credit to the gene pool that she helped fill. Had there been a different pregnant primate, arriving under the same set of circumstances, we would have no lemurs, but we would have a different collection of species in their place.

Evolutionary biologists suspect that just as one strepsirrhine primate may account for all the different types of lemur on the island of Madagascar, one caviate rodent may have been responsible for all the extant rodents in South America.

2. Species can only speciate into something that they already are.

In this book, we stress the point that we, as humans, are members of every class of organisms in our ancestral lineage. Hence, we are eukaryotes, we are deuterostomes, we are craniates, and so on. Furthermore, the assertion that humans are eukaryotes must not be interpreted as a didactic device, intended to remind us of our early roots. In point of fact, we actually are eukaryotes, the reason being that the gametes in every organism represent the true progeny of the gametes in the organism's ancestry. Skeptics are counseled to follow the history of their own gametes backwards through evolution. The gametes in our bodies are the progeny of the fusion of two parental gametes. This process can be followed iteratively up through the chain of ancestors within a species, and up through the gametes of the parent species, and on and on, until reaching the root eukaryote, a single-celled organism that was, for all practical purposes, "all gamete." This explains why we have hundreds of the same core genes found in all eukaryotic organisms, and why we are dues-paying members of Class Eukaryota, Class Deuterostomia, and Class Craniata, down the line to Class Homo sapiens.

3. Speciation is inevitable.

All species that survive will speciate, given time. Eventually, something will happen that isolates a subpopulation (e.g., migration to an island, change in the habitat in a geographic region, even a new highway that splits a population). New species begin at the moment when a population is split, even if there are no outward signs of change. The horseshoe crab is often touted as a species that never speciates, insofar as the horseshoe crabs living today look much like the

horseshoe crabs that lived 100 million years ago. Nevertheless, the living horseshoe crabs belong to at least four modern species, with their own phylogenies determined by molecular analysis [6] (Fig. 2.1).

Over time, and after a population has split off and isolated itself, small variations in the separate gene pools will produce subtle differences in the respective members of each population [7]. We can guess that several million years ago, some horseshoe crabs split from the herd, and started their own species.

4. We can learn about class properties by studying divergent sister classes.

There are many instances in which we have lost the living representatives of ancestral sister classes through extinction. In some cases, we risk losing sister classes through inattention and negligence. Currently, there is one living species that represents the sister class to all other angiosperms (i.e., flowering plants). This species, Amborella trichopoda, is an unassuming shrub found only on the small Pacific island of New Caledonia. It is feared that Amborella trichopoda is on the brink of extinction. A botanist wrote, in a 2008 PhD thesis (translated from the French), "The disappearance of Amborella trichopoda would imply the disappearance of a genus, a family and an entire order, as well as the only witness to at least 140 million years of evolutionary history," [8] [Glossary Sister class and cousin class].

5. Speciation does not require new genetic mutations.

As remarked previously, when a group of individuals, each representing a sampling from the species' gene pool, wander off somewhere and mate exclusively with one another, they are creating their own evolving gene pool; hence their own species. The initial gene pool of the new species is a subset of the gene pool of the parent species, and the members of the new species are indistinguishable,

FIG. 2.1 Horseshoe crabs (Limulus polyphemus), an example of an animal that has evolved, but slowly. *(Source, Wikipedia, and released into the public domain by its author, Breese Greg, of the US Fish and Wildlife Service.)*

as a group, from the members of the parent species. Hence, the creation of the new species did not require the acquisition of any new genetic mutations.

It's reasonable to ask, "If the new species is indistinguishable from the parent species, and has a gene pool that was present in the parent species, then how can we possibly assert that a new species was created?" Gradually the gene pool of the new species will evolve, accumulating new variants of genes, and serving as the genetic material for individuals who will look less and less like the parent species, over time. The greatest threat to the survival of a new species is cross-mating between members of the parent species and the child species; this would result in a mixing of the genes, and we would no longer have two separately evolving gene pools.

Just for fun, let's assume that we are wrong, and that each new species arises from a new gene that evolved from the parent species, rendering the offspring sufficiently different to earn themselves a place in the list of terrestrial species. If this were the case, then we should be able to identify the "squirrel" gene, the "tulip" gene, and the "mosquito" gene that distinguishes each of these species from its parent species and from every other species on earth. Of course, this is an impossibility. We cannot identify the defining "species gene" or the set of genes that is characteristic for any species on earth. We cannot even find the gene that separates human from gorilla. If our species were defined by the acquisition of one new gene, then a loss-of-function mutation in our "species gene" would cause affected individuals to regress back to our ancestral species. We would have Neanderthals walking among us. Of course, this is nonsensical. There simply is no such thing as the "human gene." What, then, distinguishes one species from another? The answer is "the gene pool." Humans have their own gene pool. Squirrels have their gene pool. Tulips have their gene pool. It is as simple as that.

Section 2.3 Diverse forms of diversity

> *I always wanted to be somebody, but now I realize I should have been more specific.*
>
> Lily Tomlin

When we think about biological diversity, there is a tendency for each of us to contemplate the issue in relation to our own chosen field of interest. A zoologist is likely to think of diversity in the number and behavior of living animal species. A geneticist will consider the totality of different functional genes available to the biosphere. A chemist might think in terms of all the different molecular species synthesized by living organisms. Of course, the different modes of diversity are biologically related. For example, a species is basically an evolving gene pool, and each species will eventually contribute its gene pool to the number of genes cataloged in a large genomic databases. Due to the overwhelming complexity

of trying to think in terms of all the types of biological diversities, as they relate to one another, it is probably best to focus on the most familiar concepts, one by one: species diversity, genetic and proteomic diversity, regulatory diversity, structural (i.e., anatomic and cytologic) diversity, and chemical diversity [9].

Diversity of Species

As discussed in Section 1.1, "The Consequence of Evolution is Diversity," there are many millions of species of organisms that live on our planet. The estimates vary from a low number of 10 million to a high number of 1 trillion [10]. From this staggeringly large number of terrestrial species, we can infer that speciation is a relatively easy, almost inevitable, process.

We can see several reasons why species tend to diversify over time:

–1. Species need other species. Aside from the many carnivorous organisms that prey on other living organisms, there are many organisms that live off dead, decaying, or fully decayed organisms. Even plants that live off of sunlight and water and carbon dioxide rely on soil nutrients containing the decomposed detritis of formerly living species.

–2. The term "fittest" has no absolute meaning. An organism that is more fit for survival under one set of conditions may be totally unfit under another set of circumstances (i.e., extremes of weather, susceptibility to infection, diminished food supplies). The best way of providing a fit organism, for any environmental condition, is to produce lots and lots of species, expecting that a few of them will be suited to the new environment [Glossary Susceptibility].

–3. Third, and most important, species speciate. A species will always produce another species if the genetic and environmental conditions permit (as discussed in the earlier sections of this chapter).

Speciation is something that some classes of organisms seem to do much better than others. For example, there are over 350,000 species of beetle, exceeding the combined number of plant species (250,000) plus roundworm species (12,000) plus mammals (4000). Vertebrates are biological underachievers, compared with beetles, when it comes to speciation. It would seem that the ability to produce other species is itself a trait held by species, and this trait is referred to by the term "evolvability."

One of the most evolvable vertebrates is the cichlid (Class Cichlidae), many species of which are found in home aquaria. There are about 2000 known species of cichlid, and hundreds of different species can be found swimming together in African lakes. The hundreds of cichlid species in Lake Victoria took an estimated 15,000–100,000 years to radiate (i.e., to diversify from a single founder species), a very short time span by evolutionary standards [11]. It is impossible to determine any specific factor that renders a species capable of diversifying. In the case of cichlids, nearly every variable examined in the geographic

and ecological history of this fish would seem to encourage diversity (populations that expand and contract, lakes that swell up and dry out, changes in flora and fauna cohabiting the lake, etc.). On a molecular level, cichlids are endowed with a genome containing a large number of gene duplications, an abundance of noncoding elements, and many novel microRNAs [11]. Any of these factors may have played an important role in the adaptive radiation of cichlid species. This serves as an example of the relationship between genetic diversity and species diversity.

Gene diversity

The earth's proteome consists of all the different protein-coding genes on the planet. The estimates of the planetary proteome vary widely. The lowest number seems to be 5 million [12]. Elsewhere, we read that the human intestine contains about 40,000 species of bacteria producing a whopping 9 million unique bacterial genes [13–15]. It seems plausible that before all the counts come in, we'll find that there are billions of genes in the total collection.

The human genome contributes a meager 20–25 thousand genes to the proteome. Other, seemingly less complex, animals have a genetic repertoire larger than humans. For example, the nearly microscopic crustacean Daphnia pulex (the water flea) has 31,000 genes. Plants tend to have way more genes than animals. For example, rice contributes an estimated 46–56 thousand genes to Earth's proteome [16].

Chemical diversity

We must begin by confessing that the metazoans (animals), as a class, are metabolic underachievers in terms of the chemical and metabolic diversity [17]. The prokaryotes are far more advanced [18]. Among the eukaryotes, only Class Archaeplastida (i.e., plants) and Class Fungi seem to be making any effort to impress [19, 20]. The eukaryotes largely rely on endosymbiotic relationships with current or former prokaryotes to perform complex biosyntheses (e.g., mitochondria and chloroplasts captured from former bacteria). Otherwise, eukaryotes are saddled with the rather hum-drum tasks of synthesizing organelles and membranes and manipulating the various ingredients for cellular life (carbohydrates, structural and enzymatic proteins, lipids, and nucleic acids). These fundamental chemical constituents of living organisms were established about 3 or 4 billion years ago, and haven't changed much since.

Some estimates suggest that the first fungi appeared as early as 1.3 billion years ago, while the first land plants may have evolved 700 million years ago. The first fossils of vascular land plants are dated to about 480 million years ago, just after the end of the Cambrian explosion (about 500 million years ago). Regardless of the timing, we can surmise that following the Cambrian explosion, plants, fungi, and metazoans were obliged to evolve their own coping mechanisms for cohabitation [Glossary Cambrian explosion].

Whereas animals rely on their body structure for both aggressive and defensive activities (e.g., running after prey and running away from predators), plants and fungi rely on their ability to synthesize bioactive chemicals that act as respiratory poisons (e.g., cyanide), neuro-muscular agents (e.g., nicotine), irritants (e.g., capsaicin), and a host of other chemical warfare tactics. When we eat plants and mushrooms, we can expect to ingest some of the chemicals that were created with the intention of killing us. For example, cycasin is a toxin and carcinogen found in the seeds and the pollen of every class of cycad tree [21]. Among the fungi, Aspergillus flavus, a ubiquitous fungus found growing on peanuts and other crop plants, synthesizes aflatoxin, one of the most powerful liver carcinogens known [22]. Peanut butter manufacturers take great pains to insure that peanuts are harvested under conditions that minimize their contamination with aflatoxin, carefully monitoring the amounts of aflatoxin in manufactured peanut butter to ensure that batches exceeding an allowed level will never reach the market [Glossary Carcinogen, Carcinogenesis].

Fungi produce alpha-amanitin, a strong, often fatal toxin, produced by the mushroom Amanita phalloides. Another fungal product is gyromitrin, a hydrazine compound present in most members of the common False Morel genus. Nobody is quite sure what effects gyromitrin and other related hydrazine molecules may have on human consumers.

Weaponized molecules play no role in the primary functions of plant and fungal cells (i.e., do not participate in cellular physiologic processes), and are referred to as secondary metabolites or as idiolites [19]. The terminology conveys the idea that if all the secondary metabolites in a plant cell or a fungal cell were to disappear, then the cells would survive happily, provided that no predatory organisms spoiled the fun.

Secondary metabolites account for a large portion of the chemical diversity in bacteria, plants, and fungi [20]. Plants devote 15%–25% of their genes to producing enzymes involved in the synthesis of secondary metabolites; of which several hundred thousands have been reported [23]. We presume that every secondary metabolite is bioactive under some set of circumstances; otherwise, the synthetic method for creating the chemical would not have evolved. In point of fact, a good bit of medicinal chemistry, as it was pursued in the 20th century, consisted of finding appropriate secondary metabolites from bacteria, fungi, or plants that would have some utility in the prevention or treatment of human diseases.

Structural diversity

The one area of diversity wherein animals seem to take the lead is structural diversity. Structural diversity really took off in the Cambrian explosion, when nearly all the extant metazoan body plans were established. Although there were multicellular animals that lived prior to the Cambrian, it would seem that the dominant eukaryotes were standard issue unicellular organisms. These organisms varied in terms of size, shape, and external structures (e.g., wavy

membranes, pseudopods, undulipodia, and cilia), but couldn't compete with the diversity of structures that arose in the Cambrian explosion.

The key event that propelled the attainment of structural diversity in metazoans was almost certainly the evolution of specialized junctions, particularly the desmosome, that uniquely characterize animal cells. The desmosomes act like rivets, and soft animal cells serve as somewhat modular building blocks that can be assembled into almost any shape and size imaginable (e.g., ducts, glands, acini, cavities, membranes). The application of cuticles and other hardened tissue, from collagen, keratin, or chitin; and the synthesis of bone from hydroxyapatite deposited into proteinaceous matrices, allowed the animal kingdom to produce a multitude of species with variable shaped outer and inner structures. The field of paleontology has, until recently, been devoted to understanding the characteristic design of hard structures that distinguish one animal from another.

Among the animals, the holometabolous insects probably take the prize when it comes to structural diversity, insofar as a single organism may pass through multiple stages of life, each having a its own structural morphology [Glossary Holometabolism].

Fungi and plants display a fair amount of structural diversity; usually simple variations on common themes (e.g., stalk, leaves, flowers). With few exceptions, the plant kingdom does a much better job with colors than does the animal kingdom. Plants rely on flavonoids (particularly anthocyanins) and carotenoids to produce their wonderful and vivid colors. For the most part, animals have a single pigment molecule, melanin, as their primary source of coloration. An assortment of colors can be coaxed from the melanin molecule by controlling the concentration and spatial distribution of melanin within cells, and by making small modifications to the base molecule. Other colors, such as the red of hemoglobin, are produced with iron and other metal cofactors bound to proteins [Glossary Cofactor].

Regulatory diversity

Relatively early in eukaryotic phylogeny, cells evolved a diverse methodology for regulating their genomes. This would include the evolution of the epigenome, wherein the DNA of genetic material is modified by base methylations, and these modifications are themselves modified at every step of cell-type development.

Chromatin, the structural backbone of the genome, is modified by the attachment of proteins (histones and nonhistone varieties), and by the wrapping of units of DNA into tight nucleosomes. There are numerous ways in which chromatin is modified, including remodeling factors, histone deacetylases, heterochromatin-binding proteins, and topoisomerases [24].

Aside from the complexities of the epigenome, there are a host of genome modifiers that micromanage every aspect of gene expression, including transcription (e.g., transcription factors, promoters, enhancers, silencers, pseudogenes, siRNA, miRNA, competitive endogenous RNAs), posttranscription

(splicing, RNA silencing, RNA polyadenylation, mRNA stabilizers), translation (e.g., translation initiation factors, ribosomal processing), and posttranslational protein modifications (e.g., chaperones in mammals, protein trafficking). Disruptions of any of these regulatory processes may produce disease in humans and other metazoans [25–35]. These genomic regulators will not be discussed further here except to remark that some level of gene regulation is found in every class of organisms (i.e., prokaryotes, single-celled eukaryotes, animals, plants, fungi, and viruses). Many of these regulatory systems are common to all eukaryotes, while others seem to be specific for particular subclasses. For example, imprinting among animals seems to be confined to Class Eutheria (i.e., placental mammals). Similarly, chaperone proteins seem to be something exclusive to Class Mammalia.

The value of diversification

Diversity affords us the opportunity to find new antibiotics, new anticancer agents, and new methods to control and modify just about any metabolic pathway or regulatory mechanism we choose to study. It is due to genetic diversity among diverse species that we have found the thermophilic taq polymerase (from Thermus aquaticus bacteria) used in PCR (polymerase chain reactions) and the gene-editing enzymes used in CRISPR/Cas9 (prokaryotic species) and Cre-LoxP (bacteriophage P1), and CAR-T (lentiviral and gammaretroviral vectors) [36–39] [Glossary CAR T-cell therapy].

Section 2.4 The species paradox

A horse is a horse of course of course

Mr. Ed television show, theme music, 1961–66

In the introductory section of this chapter, we defined species as an evolving gene pool. Does this simple definition account for what we see when we try to find a species? Not really. Whenever we want to study a species, we always end up collecting a bunch of individual organisms that are members of the species. From these organisms, we try to find the features that characterize all the members of the species and that distinguish one species from every other species. When doing so, we always make the same observation: that every individual member of a species is a unique organism and that every offspring of every organism is uniquely different from either of its parents.

Here is the apparent paradox. We observe that every species is a collection of organisms that are all different from one another; and the differences among the organisms are constantly reassorted into new and unique individuals, with every generation. With its members constantly changing, how can we ever have a species that has stable characteristics that distinguish one species from other species? How can we uphold the intransitive property of species that forbids

individuals to change their membership from one species to another, when new species are constantly evolving from the existing species? It doesn't seem to make any sense! [Glossary Intransitive property].

The solution to the paradox is very simple. Every species is defined by its ancestral lineage; not by the collective diversity of the individuals within the species. Two individuals belong to the same species if they share the same ancestry; regardless of differences in their genomes.

If we were to sequence the genome of an apple tree in an orchard, we will not find an "apple" gene that establishes its species. There is no such thing as an "apple" gene, but there is such a thing as an ancestral lineage for the species known as apple (i.e., Malus domestica). We will find that any particular apple tree has a unique genome that is different from the genome of every other apple tree on the planet (with the exception of the trees that were cloned from the same founder). If we look a bit harder, we would probably find a set of sequences that, considered together, does a good job at identifying a sample as a member of the apple species.

We can think of each species as having a collective gene pool, with the genome of each unique individual representing a sampling from the pool. Changes in a single gene introduced to the species gene pool may account for startling phenotypic variants within the species, but they never account for the birth of a new species.

For example, anyone who visits the produce department of a well-stocked grocery store will encounter varieties of Brassica oleracea, each producing a different menu item:

- Brassica oleracea Acephala Group—kale and collard greens
- Brassica oleracea Alboglabra Group—kai-lan (Chinese broccoli)
- Brassica oleracea Botrytis Group—cauliflower, Romanesco broccoli, and broccoflower
- Brassica oleracea Capitata Group—cabbage
- Brassica oleracea Gemmifera Group—brussels sprouts
- Brassica oleracea Gongylodes Group—kohlrabi
- Brassica oleracea Italica Group—broccoli

The Acephala group (from the root meaning without a head), represented by kale and collard greens, is phenotypically most like the wild cabbage. Cauliflower differs from wild cabbage because of mutation in a single gene (the CAL gene) which produces an inflorescence. This means that the stem cells (of the meristem) grow into a mass of undifferentiated cells; basically a Brassica hamartoma [Glossary Hamartoma, Wild type].

One of the many cultivars of Brassica oleracea, known as Jersey cabbage, grows up to 3 m tall. These giant-sized cabbages have woody stalks that look like tree limbs. Hence, a single species of plant can provide nearly every common green vegetable that appears on American dinner plates, as well as stalks suitable as walking canes. (Fig. 2.2)

FIG. 2.2 Jersey cabbage walking sticks are another member of the Brassica oleracea species. *(Source, Wikipedia, from a photograph by Man Vyi and entered into the public domain.)*

The different cultivars of Brassica oleracea are analogous to serotypes of bacteria or breeds of animals. They all represent variants of the same species. Although the cultivars may be distinguished from one another by simple genetic variations, sometimes involving a single gene, they all belong to the same species because they all share the same ancestry and the same gene pool.

We can glimpse some of the enormous genetic variation within the members of a species by focusing our attention on SNPs (single-nucleotide polymorphisms), which are easy to detect, and for which much data has been collected. A SNP is a variation between members of a species that occurs in at least 1% of the population. To get an accurate determination of all the SNPs in the human population, we would need to sequence everyone's genome. Sequencing everyone's genome is impossible, at present, but we can do our best to get a fair sampling of the human population. The current rough estimate of the number of SNPs in the human population is 10–30 million. This number may increase substantially, as we improve the gene sampling process, and it is fair to assume that the number would be much higher if we counted rare polymorphisms occurring in <1% of the population. It is startling to acknowledge that despite the enormous genetic variations among individuals, we all belong to the same species, we all descended from the same parent species, and we all share the same evolving gene pool [Glossary Codon, Codon synonymy, Polymorphism, Single nucleotide polymorphism].

Glossary

CAR T-cell therapy In August, 2017, a gene therapy for the treatment of children and young adults with B-cell acute lymphoblastic leukemia was approved by the US Food and Drug Administration [40]. The therapy is centered on several stunning refinements of an old and nearly defunct approach, using a patient's own immune system to destroy cancer cells. The successful methodology that was developed is known as CAR-T (Chimeric Antigen Receptor for T cells) [39, 41–43].

Cambrian explosion Studies of shale strata indicate that something very special happened, in the history of terrestrial animals, in a relatively short period, stretching from about 550–500 million years ago. In this 50 million year span, nearly all of the major phyla of animals that exist today came into existence. We call this era the Cambrian explosion. The word "Cambrian" is a latinized form of the Welsh language word for Wales, where shale deposits of the Cambrian age were first studied. The word "explosion" tells us that paleontologists have come to think of a span of 50 million years as a blinding flash in earth's history. Our understanding of the major classes of animals is based almost entirely on fossils found in shale. Animals certainly preceded the Cambrian period, but such animals were soft and uncalcified and would be underrepresented in the fossil record [44, 45]. Furthermore, our reliance on body plans, as the only measure of a phyla's emergence, is somewhat presumptuous. It may very well be that the defining expression of animal phyla may have developed before or after the Cambrian explosion [46–48]. In particular, the bryozoans (tiny invertebrate aquatics that filter food particles from water) seem to have arisen sometime after the Cambrian.

Carcinogen The term "carcinogen" refers to agents that cause cancer, but there is considerable controversy over how to apply the term. Some people use the term "carcinogen" to mean a chemical, biological, or physical agent that produces cancer in animals, without the addition of any other agents or processes. Sometimes, the term "complete carcinogen" is used to emphasize the self-sufficiency of the agent as the primary underlying cause of a cancer. Others in the field use the term "carcinogen" to include any agent that will increase the likelihood of tumor development. This definition would apply to agents that must be followed or preceded with another agent for tumors to occur, or agents that increase the number of cancers occurring in a population known to be at high risk of cancer due to an inherited condition.

Carcinogenesis The cellular events leading to cancer. Equivalent to the pathogenesis of cancer. Carcinogenesis in adults is a long process that involves the accumulation of genetic and epigenetic alterations that eventually produce a growing clones of malignant cells. The conjectured sequence of events that comprise carcinogenesis begins with initiation, wherein a carcinogen damages the DNA of a cell, producing a mutant clonal founder cell that yield a group of cells that have one or more subtle (i.e., morphologically invisible) differences from the surrounding cells (e.g., less likely to senesce and die, more likely divide, less genetically stable, better able to survive in an hypoxic environment). After a time, which could easily extend into years, subclones of the original clone emerge that have additional properties that confer growth or survival advantages (e.g., superior growth in hypoxic conditions). The process of continual subclonal selection continues, usually for a period of years, until a morphologically distinguishable group of cells appear: the precancer cells. Subclonal cells from the precancer eventually emerge, having the full malignant phenotype (i.e., the ability to invade surrounding tissues and metastasize to distant sites). The entire process may take decades.

Codon Messenger RNA is translated into protein by successively matching sequential triplets of mRNA against the amino acids for which they code. Each RNA coding triplet is known as a codon.

As the mRNA sequence is being processed, successive triplets are attached to a triplet-specific tRNA (transfer RNA) molecule in the ribosome that binds to the amino acid matching the triplet, and the amino acid is then added to the protein sequence that is being built. The process of reading the codons proceeds as the amino acid sequence of its encoded protein is assembled.

Codon synonymy Refers to codons (nucleotide triples) that have different sequences but which produce an equivalent transcriptional result due to redundancy in the genetic code. For example, guu, guc, gua, and gug all code for the amino acid valine and are therefore synonymous with one another.

Cofactor When biochemists use the term "cofactors," they are referring to chemicals that bind to enzymes, to activate the enzyme or to enhance the activity of the enzyme. Some enzymes or enzyme complexes need several cofactors (e.g., the pyruvate dehydrogenase complex which has five organic cofactors and one metal ion). Vitamins are often cofactors for enzymes.

Hamartoma Hamartomas are benign growths that occupy a peculiar zone lying between neoplasia (i.e., a clonal expansion of an abnormal cell) and hyperplasia (i.e., the localized overgrowth of a tissue). Some hamartomas are composed of tissues derived from several embryonic lineages (e.g., ectodermal tissues mixed with mesenchymal tissue). This is almost never the case in cancers, which are clonally derived neoplasms wherein every cell is derived from a single cell type.

Hamartomas occasionally occur in abundance in inherited syndromes; as in tuberous sclerosis. The characteristic lesion in tuberous sclerosis is the brain tuber, the hamartoma from which the syndrome takes its name. Tubers of the brain consist of localized but poorly demarcated malformations of neuronal and glial cells. Like other hamartoma syndromes, the germline mutation in tuberous sclerosis produces benign hamartomas as well as carcinomas; indicating that hamartomas and cancers are biologically related. Hamartomas and cancers associated with tuberous sclerosis include cortical tubers of brain, retinal astrocytoma, cardiac rhabdomyoma, lymphangiomyomatosis (very rarely), facial angiofibroma, white ash leaf-shaped macules, subcutaneous nodules, cafe-au-lait spots, subungual fibromata, myocardial rhabdomyoma, multiple bilateral renal angiomyolipoma, ependymoma, renal carcinoma, and subependymal giant cell astrocytoma [49].

Another genetic condition associated with hamartomas is Cowden syndrome. Cowden syndrome is associated with a loss of function mutation in PTEN, a tumor suppressor gene [50]. Features that may be encountered are macrocephaly, intestinal hamartomatous polyps, benign hamartomatous skin tumors (multiple trichilemmomas, papillomatous papules, and acral keratoses), dysplastic gangliocytoma of the cerebellum, and a predisposition to cancers of the breast, thyroid, and endometrium [51–53].

Holometabolism Complete metamorphosis, as observed in all insect species of Class Endopterygota, involving four developmental stages: egg, larva, pupa, and imago or adult. The term "holometabolism" is reserved for insects, but we see complete multistage metamorphosis in other animals, such as the European eel, a type of fish.

Intransitive property One of the criteria for a classification is that every member belongs to exactly one class. From this criterion comes the intransitive property of classifications; namely, an object cannot change its class. Otherwise, over time, an object would belong to more than one class. It is easy to apply the intransitive rule under most circumstances. A cat cannot become a dog and a horse cannot become a sheep. What do we do when a caterpillar becomes a butterfly? In this case, we must recognize that caterpillar and butterfly represent phases in the development of one particular member of a species, and we do not create separate caterpillar classes or butterfly classes.

Polymorphism The term "polymorphism" can have several somewhat different meanings in various fields of biology. Herein, polymorphism refers to genetic polymorphism, indicating that variants of a gene occur in the general population. A polymorphism is usually restricted to variants that occur with an occurrence frequency of 1% or higher. If a variant occurs at a frequency of <1%, it is considered to be sufficiently uncommon that it is

probably not steadily maintained within the general population. All commonly occurring polymorphisms are assumed to be benign or, at worst, of low pathogenicity; the reasoning being that natural selection would eliminate frequently occurring polymorphisms that reduced the fitness of individuals within the population. Nonetheless, different polymorphisms may code for proteins with some deficits in functionality.

Single nucleotide polymorphism Abbreviation: SNP. Locations in the genome wherein different individuals are known to have single base differences in DNA sequence. It is currently estimated that there are more than 35 million SNPs in the human population, and a SNP occurs about once in every 300 nucleotides [54]. By general acquiescence, the term SNP is reserved for variations that are found in at least 1% of the general population. The term SNV (single nucleotide variant) is applied to any observed variations among individuals, regardless of its frequency of occurrence in the general population. As DNA samples are sequenced for more and more individuals, the number of single nucleotide variants increases. Presumably, SNVs may occur anywhere along the 3 billion nucleotides that sequentially line the genome. Assuming that there is, at each nucleotide location, one particular nucleotide that dominates in the general population, there would be three possible nucleotide variants at each location, producing a theoretical limit of 9 billion SNV location/nucleotide pairs. SNPs are just one form of genetic polymorphism among individuals that include alterations in chromosome number, small alterations within a chromosome, deletions of stretches of DNA, insertions of DNA, and a host of subtle complex variations wherein parts of chromosomes are translocated elsewhere within the same chromosomes or to other chromosomes.

Sister class and cousin class When a class splits into two or more subclasses, each of the subclasses is known as a sister class of the parent class. It's nonsense to assign gender to a class, but there you have it. Two classes are cousin classes if they have a common grandparent class. Because taxonomists don't generally distinguish levels of "cousinality," the term cousin class is informally extended to any two classes that share any ancestral class above the parent class. Of course, this means that, technically, every class is a sister or a cousin to every other class. By convention, we reserve the "cousin class" term for nonsiblings who share a relatively close ancestor (e.g., a grandparent or great-grandparent in common).

We can watch the evolutionary pathways followed by two classes that diverge at a point in time. We do this by studying sister classes and their descendant subclasses. That is why it is so very important to preserve sister classes, whenever possible. Currently, there is one living species that represents the sister class to all other angiosperms (i.e., flowering plants). This species, Amborella trichopoda, is an unassuming shrub found only on the small Pacific island of New Caledonia. It is feared that Amborella trichopoda is on the brink of extinction. A botanist wrote, in a 2008 PhD thesis (translated from the French), "The disappearance of Amborella trichopoda would imply the disappearance of a genus, a family, and an entire order, as well as the only witness to at least 140 million years of evolutionary history," [8].

Having a verifiable member of a sister class has a number of uses. By comparing genes, metabolic pathways, and genetic diseases in sister species, we can sometimes:

- Verify phylogenetic relationships and taxonomic trees.
- Distinguish acquired traits from inherited ancestral traits.
- Improve our understanding of embryologic development [55].

- Use sister classes as the comparison species for a molecular clock analysis to determine the time at which the sister classes diverged.
- Determine the pathways operative in diseases.

Susceptibility Refers to a state of increased risk of harm. The frequently encountered term "susceptibility gene" would imply that individuals with the gene have an increased susceptibility to a particular disease. From the point of view of understanding pathogenesis, "susceptibility" is not a helpful concept, in that it doesn't signify a specific biological process. Is "susceptibility" an event, is it a pathway, or is it a disease? Is it simply a constitutive condition of the individual that heightens risk of disease? We say that something increases our susceptibility to a disease if it increases the probability that the disease will occur. Shouldn't we be asking ourselves, "How does susceptibility influence a pathologic process?"

Confusion around the term "susceptibility" is demonstrated in the following example. Caucasians have a higher risk of developing skin cancers than African-Americans. We say that being Caucasian increases susceptibility to skin cancer, but we never say that being Caucasian is the underlying cause of skin cancer. If a native of Nigeria is born with albinism, then that individual is highly susceptible to skin cancer, and we might say that albinism is the underlying cause of skin cancer in these cases. In these two instances, the word "susceptibility" does not tell us much about pathogenesis and does not help us resolve issues of causality with any consistency.

Wild type The common, nonmutated version of a gene occurring naturally in a population.

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Chapter 3

Bacteria

Section 3.1 Overview of Class Bacteria

The beginnings and endings of all human undertakings are untidy.

John Galsworthy

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Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
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To understand the classification of bacteria, let us look at the controversy at the root of the tree of life. Three major groups of organisms account for all life on earth: bacteria, archaea, and eukaryotes. When you compare species of Class Bacteria with species of Class Archaea, you're not likely to notice any big differences. The bacteria have the same shapes and sizes as the archaea. All species of bacteria and all species of archaea are single-celled organisms, and they all have a typical prokaryotic structure (i.e., lacking a membrane-bound nucleus to compartmentalize their genetic material). As it happens, Class Bacteria contains all of the prokaryotic organisms (i.e., cellular organisms lacking a nucleus) that are known to be pathogenic to humans. The archaeans are nonpathogenic; many are extremophiles, capable of living in hostile environments (e.g., hot springs, salt lakes), but some archaean species can occupy less demanding biological niches (e.g., marshland, soil, human colon). Class Archaea does not hold a monopoly on extremophilic prokaryotes; some members of Class Bacteria live in extreme environments (e.g., the alkaliphilic Bacillus halodurans). The third major class of organisms, the Eukaryotes, is distinguished by the presence of a membrane-bound nucleus.

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For decades, archaean species were considered just another class of bacteria. This changed in 1977. Woese and Fox had been studying ribosomal RNA. Because ribosomal RNA is a fundamental constituent of all cellular organisms, sequence comparisons in the genes coding for ribosomal RNA are considered a reliable way to estimate the degree of relatedness among organisms. In 1977, Woese and Fox surprised biologists when they demonstrated profound differences in the sequence of ribosomal RNA that distinguished archaean species from bacteria [1]. Much more shocking was their finding that the sequence of archaean ribosomal RNA was more closely related to eukaryotic cells than to other bacterial cells. Other sources have since shown that the archaeans share with the eukaryotes a variety of features that are lacking in the bacteria. These include the presence of histones in some archaeans, the manner in which DNA is replicated and organized, and the finding that archaeans and eukaryotes employ several similar transcription factors (proteins that bind to DNA and control the transcription of DNA to RNA) [2]. Woese and Fox proposed that the archaeans (then called archaebacteria) comprised a kingdom, separate but equal to the bacteria. That paper, and the many contributions of Woese and colleagues that followed, sharpened our understanding of terrestrial biology and sparked a controversy that shook the foundations of taxonomic orthodoxy [3, 4] [Glossary Molecular clock, Taxonomic order].

Today, there are numerous theories that try to provide a convincing account of the origin of the major classes of organisms that populate this planet. All these theories attempt to provide an answer to several overriding questions:

- How did life begin on earth?
- Which class of organism came first?
- What was the relationship between the first class of organisms on earth and the subsequent classes?

In this book, we will not delve into arguments for or against any of the "origin of life" hypotheses. Readers can simply accept that every serious evolutionary biologist is obsessed with the question of how cellular life originated on earth [5]. Many biologists believe that the cell is a living document, holding the record of biological life on our planet, and that it is our job to correctly interpret its contents [6].

Woese extended his earlier observations by using ribosomal RNA sequence variations as a biologic chronometer. By observing changes in the sequences of ribosomal RNA, he determined the branchings, over time, that occurred during the evolution of organisms. Though he was looking at sequence similarities, his observations were focused on those similarities that revealed the sequential phylogenetic steps of bacterial evolution [7]. From these observations, Woese built a phylogenetic classification of bacteria upon a preexisting nomenclature that defined organisms on a species level.

Prior to the work of Woese and others, in the 1990s, there was little hope that the bacteria could be classified sensibly into a phylogenetic hierarchy. It was

known that bacteria exchange genetic material horizontally, from one species of organism to another. During the several billion years of bacterial evolution, it was likely that primitive organisms merged with one another. There is every reason to believe that early viruses pulled and pushed fragments of DNA among many different bacterial organisms. It is even possible that native molecules of DNA, formed in the primordial soup of ancient earth, were acquired by members of different branches of evolving organisms. Biologists expected that these promiscuous exchanges of genetic material would have created a collection of bacterial organisms for which a strict geneologic classification was impossible to construct. Prior to Woese, generations of microbiologists had tried and failed to produce a credible classification for bacteria. Because most bacteria fall into a narrow range of form and size, taxonomists lacked a sufficient set of morphologic features from which they could define and distinguish classes of organisms. They settled for a systematic grouping of bacteria based on a few morphologic features, dichotomizing staining properties (Gram positivity and Gram negativity) and a set of growth characteristics (e.g., nutrient requirements and the expression of traits expressed by colonies grown on various substrates) [Glossary Horizontal gene transfer].

It came as a great surprise to many when Woese and others developed a sensible classification of bacteria based on their analyses of a single molecule, rRNA. It remains to be seen whether Woese's one-molecule classification will withstand further scrutiny, employing whole genome methods [8] [Glossary Next-generation sequencing].

In the past decade, taxonomists have acquired access to the full genome sequences of many different organisms. The genome size of most bacteria fall in the range of 0.5 million base pairs up to about 10 million base pairs. This is a tiny fraction of the size of the human genome, which is about 3 billion base pairs in length. The organism with the largest genome is currently thought to be Polychaos dubium (Class Amoebozoa), with a genome length of 670 billion base pairs. Because the bacterial genome is small, many of the first genomes to be fully sequenced belong to bacterial species, and numerous full-length sequences are currently available to taxonomists.

It was hoped that comparisons between whole-genome sequences, obtained on many different bacterial species, would solve many of the mysteries and controversies of bacterial taxonomy. These expectations were overly optimistic, due, in no small part, to an analytic phenomenon now known as “nonphylogenetic signal” [9]. When gene sequence data is analyzed, and two organisms share the same sequence in a stretch of DNA, it can be very tempting to infer that the two organisms belong to the same class (i.e., they inherited the identical sequence from a common ancestor). This inference is not necessarily correct. Because DNA mutations arise stochastically over time (i.e., at random locations in the gene, and at random times), two organisms having different ancestors may achieve the same sequence in a chosen stretch of DNA. Conversely, if two organisms are closely related to an ancient ancestor,

then we may find that the ancestor shares the same DNA sequence found in one of the two organisms, that is not found in the other organism. When mathematical phylogeneticists began modeling inferences drawn from analyses of genomic data, they assumed that most class assignment errors would occur when the branches between sister taxa were long (i.e., when a long time elapsed between evolutionary divergences, allowing for many random substitutions in base pairs). They called this phenomenon, wherein nonsister taxa were mistakenly assigned the same ancient ancestor class, "long branch attraction." In practice, errors of this type can occur regardless of whether the branches are long, short, or in-between. Over the years, the accepted usage of the term "long branch attraction" has been extended to just about any error in phylogenetic grouping due to gene similarities acquired through any mechanism other than inheritance from a shared ancestor. This would include random mutational and adaptive convergence [10]. The moral here is that powerful data-intensive analytic techniques are sometimes more confusing than they are clarifying [Glossary Long branch attraction, Nonphylogenetic signal, Taxa, Taxon].

Though the field of computational taxonomy is imperfect, readers must understand that the field of classical taxonomy suffers from a self-referential paradox known as bootstrapping. Classical taxonomists need to have a classification of organisms before they can clearly see the relationships among classes (that is the purpose of a classification). Furthermore, taxonomists must see the relationships among classes before they can create the classification. Basically, a classification cannot be built without the assistance of a finished classification. In practical terms, taxonomists are continually constructing classes from their own scientific biases related to the essential features of organisms. Classical taxonomists tend to be happy with class assignments that reinforce their original bias. Computational taxonomists insist that a mathematical approach overcomes the self-referential paradox by clustering similar organisms into classes based on an unbiased (though arbitrary) set of objectively measured features.

The wise taxonomist understands that new classifications are built upon old classifications, and that the value of any classification stems from our persistence in testing and revising tentative class assignments; not in our ability to recompute a classification from a blank slate.

Computational taxonomy, based on sequence analyses, seems best suited for the following purposes:

–1. To provide some objective method by which newly discovered species can be added to the classification.
–2. To accommodate methods by which the classification can be continually tested; and corrected when necessary.
–3. To provide informative biological markers not found by morphologic examination, permitting more accurate assignment of species.
–4. To serve as a phylogenetic chronometer to determine when subclasses may have first appeared.

The schema for the classification of bacteria that are infectious to humans appears at the beginning of this chapter. It corresponds closely to the general classification of bacteria proposed by Woese and Fox [7]. Each bacterial subclass will be covered in separate chapters, but there are two divisions of the bacteria that must be discussed here, because they cross taxonomic barriers. These are Gram stain reaction and G+C content.

A cell wall is a chemical structure that lies outside the membrane that encloses the cytoplasm (i.e., the insides) of bacteria. Some cell walls contain peptidoglycan, and it is this molecule that reacts with the Gram stain to produce a blue color. Some bacteria lack a cell wall, and some bacteria have cell walls that do not contain peptidoglycan. These cells do not react with the Gram stain. Other bacteria contain peptidoglycan in their cell walls, but they have a second, outer membrane that covers the cell wall. Gram stain cannot easily penetrate the outer cell membranes, thus decreasing the Gram stain reaction in these cells. Because there are many different ways by which an organism's Gram reaction is determined, you might guess that Gram staining is a taxonomically perverse property: you would be correct.

With a few exceptions, the Gram-positive cells have a single membrane, and the Gram negative cells have an inner plus an outer membrane. The Gram positive cells that are round or rod-shaped almost all fall into Class Bacilli or Class Clostridia. The mollicutes are bacteria that have no cell wall to absorb or exclude the Gram stain; hence, members of Class Mollicute are neither Gram positive nor Gram negative, but they are traditionally counted among the Gram-positive bacteria because they lack an outer membrane and share a close ancestor with Class Bacilli and Class Clostridia. Among the filamentous bacteria, Class Actinobacteria is also Gram positive.

All bacterial species with an outer membrane are Gram negative. All members of the Class Proteobacteria have an inner and outer membrane and are Gram negative. Class Proteobacteria accounts for the majority of Gram-negative pathogens. Other Gram-negative classes are Class Chlamydiae, Class Spirochaetes, Class Fusobacteria, and Class Bacteroidetes.

Endotoxins are toxins produced by bacteria that are part of the structure of the organism (i.e., not an excreted molecule as found in exotoxins). Most of the endotoxins are cell-wall lipopolysaccharides. Gram-negative bacteria have an outer membrane, and it is not surprising that most endotoxins come from Gram-negative bacteria. Endotoxins can produce a wide range of generalized inflammatory reactions when injected into humans. A classic example of an endotoxin is lipooligosaccharide, produced by Neisseria meningitidis (Class Beta Proteobacteria).

The G+C ratio is a measurement of the proportion of Guanine and Cytosine nucleotide bases in an organism's genome. Because all four nucleotide bases (Guanine, Cytosine, Adenine, and Thymidine) are essential constituents of the genetic code, the G+C ratio of most organisms lies in a somewhat restricted range, close to 50%. For certain classes of Gram-positive bacteria, the G+C

ratio is used as a useful taxonomic feature. Members of Class Actinobacteria (filamentous cells) have a high G+C ratio, with the ratio of some species exceeding 70%. Members of other Gram-positive classes (Class Bacilli and Class Clostridia) tend to have low G+C ratios (under 50%), though exceptional species of Class Bacilli have G+C ratios exceeding 50% (e.g., Bacillus thermocatenulatus). Isolated DNA with a high G+C ratio happens to be more stable than low G+C DNA, and is more resistant to high temperatures. The significance of high G+C content to living organisms, however, is not understood at this time.

Section 3.2 Alpha Proteobacteria

dark matter of the biological world.

Edward O. Wilson, referring to small organisms, invisible to the eye

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
      Rhizobiales
        Bartonellaceae
          Bartonella (genus)
        Brucellaceae
          Brucella (genus)
      Rickettsiales
        Anaplasmataceae
          Neorickettsia (genus)
          Ehrlichia (genus)
          Anaplasma (genus)
          Wolbachia (genus)
        Rickettsiaceae
          Rickettsia (genus)
          Orientia (genus)
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
```

Class Proteobacteria contains more human pathogens than any other bacterial class. The human pathogens fall into four biologically distinctive, phylogenetic subclasses (alpha, beta, gamma, and epsilon), discovered through ribosomal RNA sequence analyses [7]. Woese had his own name for Class Proteobacteria; purple bacteria, based on their descent from a common ancestor, and capable of a photochemical reaction that yielded a photochrome that conferred a purple tinge to bacterial colonies. In addition, all members of Class Proteobacteria are Gram negative. Class Proteobacteria accounts for the majority of the Gram-negative bacteria.

The Alpha Proteobacteria are characterized by their small size, and their intimate associations with eukaryotic cells. The Alpha Proteobacteria live as symbionts, as endosymbionts, or as intracellular parasites. This close relationship between Alpha Proteobacteria and Class Eukaryota may extend back to the very first eukaryotic cell. Based on sequence similarities between the Alpha Proteobacteria and eukaryotic mitochondria, it has been proposed that eukaryotic mitochondria evolved from an endosymbiotic member of Class Alpha Proteobacteria [Glossary Parasite].

There are two major subclasses of Class Alpha Proteobacteria: Rhizobiales and Rickettsiales. Class Rhizobiales contains nitrogen-fixing bacteria that live in a symbiotic relationship with plant roots. Without class Rhizobiales, life on earth, as we know it, would cease to exist. Class Rhizobiales contains two human pathogenic genera: Bartonella and Brucella.

```
Alpha Proteobacteria
  Rhizobiales
    Bartonellaceae
      Bartonella (genus)
    Brucellaceae
      Brucella (genus)
```

Genus Bartonella, formerly known as Rochalimaea, is a group of facultative intracellular organisms that produce a wide range of diseases, but which seem to share a common life cycle. The pathogenic species of Genus Bartonella are injected into humans from the bite of a blood-feeding vector: fleas, lice, sandflies, and possibly ticks. The bartonella organism infects the endothelial cells that line blood vessels. Later, the organism leaves the endothelial cell and infects erythrocytes. A blood-feeding vector extracts infected red blood cells from an infected human or from an animal reservoir. The cycle repeats.

The diseases and symptoms caused by Bartonella species vary, to some extent, on the most favored host cell (endothelial cell, erythrocyte, lymphocyte, monocyte, or granulocyte), on the numbers of infectious organisms, on the chronicity of the infection, and on the pathogenic properties of individual species of the organism (e.g., does it cause red blood cell lysis?). Though Bartonella species can infect healthy persons, most infections arise in immunocompromised patients and in children.

Several of the diseases produced by members of Genus Bartonella are pathologically distinctive: Carrion disease, cat-scratch fever, peliosis hepatis, and bacillary angiomatosis.

Carrion disease, named for Daniel Alcides Carrion, is caused by Bartonella bacilliformis, is endemic in Peru and only occurs in South and Central America. It produces an acute phase characterized by fever, hepatomegaly, splenomegaly, lymphadenopathy, and hemolysis. A chronic phase, known as Verruga Peruana (Peruvian wart), presents as a skin rash characterized histologically by marked proliferation of endothelial cells [Glossary Hemolytic syndromes].

Cat-scratch disease is caused by Bartonella henselae, and possibly Bartonella clarridgeiae, and has a cat reservoir. The mode of infection is somewhat controversial. Despite the name given to the disease, implying a scratch inoculation, it is likely that fleas carry the bacteria from cats to humans. The disease produces a localized, somewhat persistent, lymphadenopathy, often accompanied by systemic complaints.

Immunocompromised individuals, especially AIDS patients, may form an exaggerated endothelial growth reaction in the skin (bacillary angiomatosis) and in the liver (peliosis hepatis, characterized by localized areas of vascular dilatation and blood pooling). In the skin, the lesions mimic Kaposi sarcoma (also seen in immunocompromised individuals). In the case of bacillary angiomatosis, Bartonella organisms can be histologically identified in the proliferating endothelial cells. Bacillary angiomatosis can be caused by Bartonella henselae, the same organism that causes cat-scratch fever, transmitted by cat scratch, cat bite, and possibly ticks and fleas. Bacillary angiomatosis is also caused by Bartonella quintana, transmitted by lice.

Various species of Bartonella have recently been associated with bacteremias with or without endocarditis, myocarditis, and retinitis. It is likely that additional pathogenic Bartonella species will be identified, as the techniques for analyzing blood samples continue to improve.

Readers should be careful not to confuse Bartonella with the similar-sounding Bordetella (Beta Proteobacteria).

Genus Brucella contains facultative intracellular organisms transmitted from an animal reservoir, usually via drinking unsterilized milk, or through direct contact with animals or their secretions. Human to human transmission may occur, rarely. Infection is usually accompanied by fever, arthralgia, myalgia, and bacteremia. Brucella species causing brucellosis include: Brucella abortus (cattle reservoir), Brucella canis (dog reservoir, rare in humans), Brucella melitensis (sheep and cattle reservoir), and Brucella suis (pig reservoir, occurring in human handlers).

Because Brucella is a facultative intracellular pathogen, it can live for some time outside of cells; hence its mode of transmission, via milk and secretions. The ability to survive outside of cells is unusual among members of Class Alpha Proteobacteria, and has been exploited, in the past, to weaponize strains of brucella.

Readers should be aware that brucellosis has been known by a great number of different names, including undulant fever (from the undulating, wave-like, progression of fevers), Malta fever, and Mediterranean fever. Mediterranean fever, an arcane synonym for brucellosis, should not be confused with Familial Mediterranean fever (a gene disorder characterized by fever and abdominal pain) or with Mediterranean anemia (a synonym for thalassemia).

```
Alpha Proteobacteria
  Rickettsiales
    Anaplasmataceae
      Neorickettsia (genus)
      Ehrlichia (genus)
      Anaplasma (genus)
      Wolbachia (genus)
    Rickettsiaceae
      Rickettsia (genus)
      Orientia (genus)
```

Class Anaplasmataceae includes four genera containing human pathogens: Neorickettsia, Ehrlichia, Wolbachia, and Anaplasma. All the pathogenic species are intracellular, morphologically similar to one another, and produce similar diseases.

The disease Ehrlichiosis is actually a collection of different infections, caused by organisms in Genus Ehrlichia, Genus Anaplasma, and Genus Neorickettsia, and are vectored from animal reservoirs, by any of several species of ticks (with one exception, Neorickettsia sennetsu, transmitted by trematodes). After inoculation into humans, the organisms that cause Ehrlichiosis invade and inhabit white blood cells, wherein they can be visualized by microscopic examination. As you would expect from a disease of white cells, the symptoms of Ehrlichiosis are systemic, and include headache, fatigue, and muscle aches.

The species producing Ehrlichioses include: Ehrlichia ewingii (human ewingii ehrlichiosis), Ehrlichia chaffeensis (human monocytic ehrlichiosis), and Ehrlichia canis (Rickettsia-like infection), Neorickettsia sennetsu (Sennetsu ehrlichiosis), and Anaplasma phagocytophilum [11]. The last-listed Ehrlichiosis pathogen, Anaplasma phagocytophilum, causes human granulocytic anaplasmosis. Anaplasma phagocytophilum is the name given to one organism formerly assigned, erroneously, to two different species: Ehrlichia phagocytophilum and Ehrlichia equi [12]. Anaplasma phagocytophilum is endemic to New England and to north-central and north-west United States. Ehrlichia ewingii is primarily an infection of deer and dogs. It occurs in humans most commonly in the south central and southeastern states. Ehrlichia chaffeensis is most common in the south central and southeastern states. Ehrlichia canis is disease of dogs, with sporadic human cases occurring in the Southeast United States [Glossary Sporadic].

Neorickettsia contains one pathogenic species, Neorickettsia sennetsu, formerly Ehrlichia sennetsu, the cause of Sennetsu ehrlichiosis. The disease

is said to mimic a mild case of infectious mononucleosis; both diseases produce mononucleosis and generalized systemic symptoms. Different from the Ehrlichioses, Neorickettsia sennetsu is transmitted by trematodes (flukes), harbored within fish. Human infection follows ingestion of undercooked or uncooked infected fish. Readers should be aware that Neorickettsia, despite its name, is not a species of Class Rickettsiaceae. Neorickettsia is a member of Class Anaplasmataceae. Hence, Neorickettsia sennetsu infection is neither an Ehrlichiosis nor a Rickettsiosis.

Wolbachia contains one, somewhat indirect human pathogen: Wolbachia pipientis. Wolbachia pipientis happens to be an endosymbiont that infects most members of the filarial Class Onchocercidae [13]. Onchocerca volvulus is the filarial nematode (roundworm) that migrates to the eyes and causes river blindness, the second most common infectious cause of blindness worldwide [14]. Wolbachia pipientis lives within Onchocerca volvulus, and it is the Wolbachia organism that is responsible for the inflammatory reaction that leads to blindness.

Members of Class Rickettsiaceae are obligate intracellular organisms. Diseases are caused by species in Genus Rickettsia, transmitted by insects, from a reservoir in animals or other humans. The various species of pathogenic rickettsia are split into two groups: the typhus group and the spotted fever group. In the typhus group, disease is louse-borne or flea-borne. In the spotted fever group, disease is usually tick-borne, though some species are transmitted by mites or fleas [Glossary Facultative intracellular organism, Obligate intracellular organism, Tick].

The typhus group contains two pathogenic organisms: Rickettsia typhi, the cause of murine typhus or endemic typhus, which is transmitted by fleas that feed on infected rats; and Rickettsia prowazekii, the so-called epidemic typhus or Brill-Zinsser disease and carried by the human body louse (Pediculus humanus), feeding on infected humans. Typhus fever of either type can occur worldwide.

Like many of the diseases caused by members of Class Alpha Proteobacteria, symptoms are systemic. Typhus is characterized by a high fever. Endemic typhus is usually a milder disease than epidemic typhus. Between 1918 and 1922, epidemic, louse-borne, typhus (Rickettsia prowazekii) infected 30 million people, in Eastern Europe and Russia, accounting for about 3 million deaths [15].

The second group of infections, the so-called spotted fever group, is caused by many different rickettsial species. The diseases can be divided into groups based on the transmission vector (i.e., tick, mite, or flea).
Tick-borne diseases:

- Rickettsia rickettsii (Rocky Mountain spotted fever, found in Western continents, and not confined to the Rocky Mountains)
- Rickettsia conorii (Boutonneuse fever, found in Mediterranean region, Africa, Asia, India)
- Rickettsia japonica (Japanese spotted fever, found in Japan)
- Rickettsia sibirica (North Asian tick typhus, found in Siberia and China)

- Rickettsia australis (Queensland tick typhus, found in Australia)
- Rickettsia honei (Flinders Island spotted fever)
- Rickettsia africae (African tick bite fever, found in South Africa)
- Rickettsia parkeri (American tick bite fever)
- Rickettsia aeschlimannii (Rickettsia aeschlimannii infection)

Mite-borne diseases:

- Rickettsia akari (Rickettsialpox, found in the United States and Russia)
- Orientia tsutsugamushi (the so-called scrub typhus)

Flea-borne diseases:

- Rickettsia felis (Flea-borne spotted fever, found in the Americas, Southern Europe, and Australia) (Fig. 3.1)

As the name suggests, the spotted fevers are characterized by rash and fever. A favorite host cell of these organisms is the endothelial cells lining small and medium blood vessels, accounting for the typical rash. Readers should not be confused by the term "scrub typhus" for infection by Orientia tsutsugamushi (also known as Rickettsia tsutsugamushi, but now recognized as belonging to the closely related Genus Orientia). This disease is grouped as a "spotted fever," and is most certainly not a form of typhus. As an interesting note, prior infection by Orientia tsutsugamushi has been reported to retard the progression of HIV infection (in terms of viral load) [16]. It seems that the HIV virus shares homologous genes with Orientia tsutsugamushi, and antigens coded by these genes elicit antibodies that are somewhat protective against the virus [16].

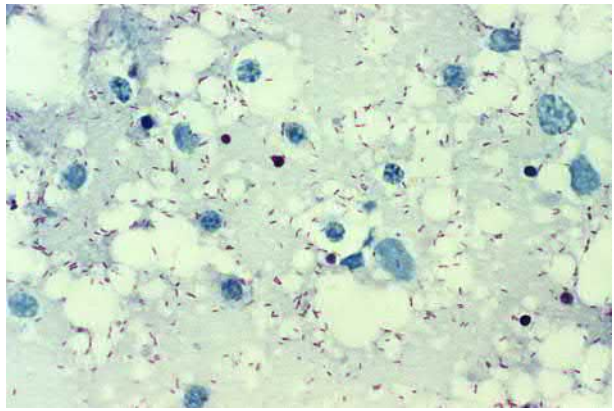

FIG. 3.1 Photomicrograph of Rickettsia rickettsii, the cause of Rocky Mountain spotted fever. In the human body, these bacteria would appear primarily as intracytoplasmic organisms inhabiting the endothelium of small- to medium-sized vessels. In this image, the bacteria are extracellular, and growing within the fluid of their particular culture medium (yolk sac). *(Source, a public domain image from the US Center for Disease Control and Prevention, Billie Ruth Bird, photographer.)*

Infectious Genera

Bartonella (formerly Rochalimaea)

- **Lineage**. Alpha Proteobacteria: Rhizobiales: Bartonellaceae: Bartonella
- **Infection**. Bartonella bacilliformis (Carrion disease or Oroya fever or Verruga peruana or Peruvian wart, bartonellosis)
- **Infection**. Bartonella quintana, formerly Rochalimaea quintana (trench fever, bacteremia, bacillary angiomatosis, endocarditis)
- **Infection**. Bartonella species, formerly Rochalimaea species (bacteremia)
- **Infection**. Bartonella henselae (cat scratch disease, bacillary angiomatosis, bacillary peliosis, peliosis hepatis, bacteremia, endocarditis)
- **Infection**. Bartonella elizabethae (endocarditis)
- **Infection**. Bartonella grahamii (retinitis)
- **Infection**. Bartonella vinsoni (endocarditis, bacteremia)
- **Infection**. Bartonella washonsis (myocarditis)
- **Infection**. Bartonella clarridgiae (bacteremia)
- **Infection**. Bartonella rochalimae (Carrion's disease like syndrome)

Brucella

- **Lineage**. Alpha Proteobacteria: Rhizobiales: Brucellaceae: Brucella
- **Infection**. Brucella abortus (brucellosis or Malta fever)
- **Infection**. Brucella canis (dog brucellosis, rarely infecting humans)
- **Infection**. Brucella melitensis (sheep and cattle brucellosis, sometimes infecting humans)
- **Infection**. Brucella suis (pig brucellosis, transferrable to human handlers)

Neorickettsia

- **Lineage**. Alpha Proteobacteria: Rickettsiales: Anaplasmataceae: Neorickettsia
- **Infection**. Neorickettsia sennetsu, formerly Ehrlichia sennetsu, formerly Rickettsia sennetsu (Sennetsu ehrlichiosis)

Anaplasma

- **Lineage**. Alphaproteobacteria: Rickettsiales: Anaplasmataceae: Anaplasma
- **Infection**. Anaplasma phagocytophilum, formerly Ehrlichia phagocytophilum and Ehrlichia equi (human granulocytic anaplasmosis, formerly known as human granulocytic ehrlichiosis)

Ehrlichia

- **Lineage**. Alphaproteobacteria: Rickettsiales: Anaplasmataceae: Ehrlichia
- **Infection**. Ehrlichia ewingii (ewingii ehrlichiosis)
- **Infection**. Ehrlichia chaffeensis (which causes human monocytic ehrlichiosis)
- **Infection**. Ehrlichia canis (Rickettsia-like infection) [11]

Orientia

- **Lineage**. Alphaproteobacteria: Rickettsiales: Rickettsiaceae: Orientia
- **Infection**. Orientia tsutsugamushi, alternately Rickettsia tsutsugamushi (scrub typhus)

Rickettsia

- **Lineage**. Alpha Proteobacteria: Rickettsiales: Rickettsiaceae: Rickettsia
- **Infection**. Rickettsia typhi, alternately Rickettsia mooseri (endemic typhus, murine typhus)
- **Infection**. Rickettsia prowazekii (Epidemic typhus, Brill-Zinsser disease, Flying squirrel typhus)
- **Infection**. Rickettsia rickettsii (Rocky Mountain spotted fever)
- **Infection**. Rickettsia conorii (Boutonneuse fever)
- **Infection**. Rickettsia japonica (Japanese spotted fever)
- **Infection**. Rickettsia sibirica (North Asian tick typhus)
- **Infection**. Rickettsia australis (Queensland tick typhus)
- **Infection**. Rickettsia honei (Flinders Island spotted fever)
- **Infection**. Rickettsia africae (African tick bite fever)
- **Infection**. Rickettsia parkeri (American tick bite fever)
- **Infection**. Rickettsia aeschlimannii (Rickettsia aeschlimannii infection)
- **Infection**. Rickettsia akari (Rickettsialpox)
- **Infection**. Rickettsia felis (Flea-borne spotted fever)

Wolbachia

- **Lineage**. Alpha Proteobacteria: Rickettsiales: Rickettsiaceae: Wolbachia
- **Infection**. Wolbachia pipientis, the endosymbiont of Onchocerca volvulus (river blindness)

Section 3.3 Beta Proteobacteria

Bacteria will no longer be conceptualized mainly in terms of their morphologies and biochemistries; their relationships to other bacteria will be central to the concept as well.

Carl R. Woese [7]

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
      Burkholderiales
        Alcaligenaceae
          Alcaligenes (genus)
          Bordetella (genus)
        Burkholderiaceae
```

```
          Burkholderia (genus)
      Neisseriales
        Neisseriaceae
          Eikenella (genus)
          Neisseria (genus)
          Kingella (genus)
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
```

Members of Class Beta Proteobacteria are Gram-negative organisms that are either aerobic or facultative anaerobic (i.e., employing respiratory metabolism when oxygen is present and fermentative metabolism when oxygen is absent). Class Beta Proteobacteria has two subclasses containing organisms that infect humans: Class Burkholderiales and Class Neisseriales.

```
Beta Proteobacteria
  Burkholderiales
    Alcaligenaceae
      Alcaligenes (genus)
      Bordetella (genus)
    Burkholderiaceae
      Burkholderia (genus)
```

Class Burkholderiales contains class Alcaligenaceae and Class Burkholderiaceae.

Members of Class Alcaligenaceae are found in water and soil and can infect a range of animals. Class Alcaligenaceae contains two genera with organisms infectious to humans: Alcaligenes and Bordetella.

In humans, Genus Alcaligenes contains opportunistic pathogens that cause disease in immunocompromised patients. Alcaligenes xylosoxidans has been found in the respiratory tract of patients with cystic fibrosis, but its role in respiratory disease is unsettled at this time. Alcaligenes xylosoxidans can cause corneal keratitis in patients who use contact lenses [17]. Alcaligenes faecalis has been found in some urinary tract infections [Glossary Opportunistic infection].

Bordetella species can infect the respiratory tract of healthy individuals. Pertussis, also known as whooping cough, is caused by Bordetella parapertussis and Bordetella pertussis. Bordetella bronchiseptica infects small animals, and may rarely infect humans, producing bronchitis.

Class Burkholderiaceae contains the genus Burkholderia, which includes Burkholderia mallei, the cause of glanders, and Burkholderia pseudomallei, the cause of melioidosis. Glanders is a serious disease, with a high fatality rate, that is endemic to Africa, Asia, the Middle East, and South America. It is a zoonosis, with a reservoir in horses and other mammals, spread through contaminated water. An active surveillance system has eliminated the disease from North America. Infected patients develop lung nodules, upper airway ulcerations, and, eventually systemic symptoms. Human to human transmission occurs, and infection can be transmitted from nasal discharge. Survivors of the infection may become carriers [Glossary Zoonosis, Carrier].

Melioidosis, also known as pseudoglanders, is caused by Burkholderia pseudomallei. Like glanders, it can have a high mortality. The disease is endemic in Asia and occurs sporadically throughout much of the world. The organism contaminates soil and water; humans are exposed to the bacteria through a break in the skin. As you might expect, rice paddy farmers, who continuously submerge their hands in groundwater, are particularly at risk of exposure. Most humans exposed to the bacteria do not develop disease, but diabetic patients, and individuals with chronic diseases, are highly susceptible. Incubation may be short (1 day) or long (decades). Symptoms and the severity of disease vary widely: fever, pneumonia, and joint pain may occur. Abscesses arising in various organs are a common finding in melioidosis.

The Burkholderia cepacia complex is a collection of more than a half-dozen related species. Burkholderia cepacia is found in soil and water, and produces pneumonia in individuals who have an underlying lung disease (such as cystic fibrosis), or who have weakened immune systems. Infection can be passed from person to person. Burkholderia cepacia organisms are difficult to treat (i.e., demonstrate antibiotic resistance) and difficult to remove from the environment (i.e., withstand common disinfectant procedures.

Genus Burkholderia was previously known as Genus Pseudomonas.

```
Beta Proteobacteria
  Neisseriales
    Neisseriaceae
      Eikenella (genus)
      Neisseria (genus)
      Kingella (genus)
```

Class Neisseriaceae are strictly aerobic and grow chiefly in pairs (i.e., diplococci) or small clusters.

Eikenella corrodens is a normal inhabitant of the mouth, but can produce a bacteremia if mechanically forced into the blood stream (e.g., chewing bite). It can also act opportunistically in diabetics and immunocompromised individuals. Eikenella corrodens can cause cellulitis as well as endocarditis and is included with the HACEK group of endocarditis-producing organisms [Glossary HACEK].

Later, we will describe another oral inhabitant, Prevotella dentalis (Class Bacteroidetes), that can cause a bite bacteremia.

Genus Neisseria includes two important pathogens: Neisseria gonorrhoeae, the cause of gonorrhea; and Neisseria meningitidis, a cause of meningitis. Gonorrhea is a sexually transmitted disease. Approximately 1.5 million new cases of gonorrhea occur annually in North America, where gonorrhea is the third most common sexually transmitted disease [18]. The second most common sexually transmitted disease is chlamydia (Class Chlamydiae), with about 4 million new cases each year in North America [18]. The most common sexually transmitted disease is trichomoniasis (Class Metamonada), with about 8 million new cases each year in North America [18] (Fig. 3.2).

Neisseria meningitidis is sometimes referred to as meningococcal meningitis, and the organism is often called by an abbreviated form, meningococcus. The bacteremic form of the disease is known as meningococcemia. Neisseria meningitidis accounts for about 3000 cases of meningitis in the United States, annually. The organism is only found in humans; hence, all infections are thought to be due to human to human transmission. Most cases occur in children. The organism is found in a significant percentage of healthy adults (about 10%). Other bacteria cause meningitis in children, including Haemophilus influenzae (Gamma Proteobacteria) and Streptococcus pneumoniae (Class Bacilli); but Neisseria meningitidis is the only bacterial organism known to produce epidemics of the disease.

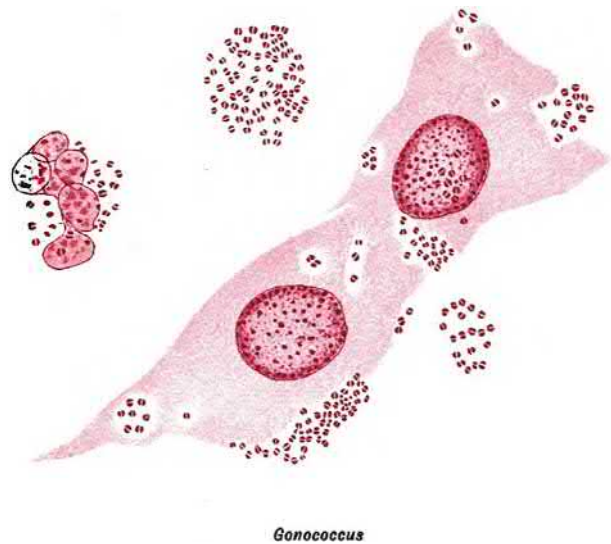

FIG. 3.2 Graphic depiction of Neisseria gonorrhoeae. Numerous paired gonococci (the so-called diplococci) organisms are found within vacuoles in an epithelial cell (center of image), phygocytosed by white blood cells (left), or as extracellular organisms. *(Source, from a plate in Shattock's* An Atlas of the Bacteria Pathogenic in Man*, published in 1899 and now in the public domain [19].)*

Kingella kingae causes a variety of infectious diseases in children. The organism is commonly found in the throats of children. Diseases include septic arthritis and osteomyelitis. Bacteremia can lead to endocarditis, and Kingella kingae is included in the HACEK group of endocarditis-producing organisms.

Infectious Genera

Alcaligenes

- **Lineage**. Beta Proteobacteria: Burkholderiales: Alcaligenaceae: Alcaligenes
- **Infection**. Alcaligenes species (corneal keratitis)

Bordetella

- **Lineage**. Beta Proteobacteria: Burkholderiales: Alcaligenaceae: Bordetella
- **Infection**. Bordetella bronchiseptica (bronchitis)
- **Infection**. Bordetella parapertussis (pertussis, also called whooping cough)
- **Infection**. Bordetella pertussis (pertussis, also called whooping cough)

Burkholderia

- **Lineage**. Beta Proteobacteria: Burkholderiales: Burkholderiaceae: Burkholderia
- **Infection**. Burkholderia cepacia complex (pneumonia in immunodeficient individuals with concurrent lung disease)
- **Infection**. Burkholderia mallei, formerly Pseudomonas mallei (glanders)
- **Infection**. Burkholderia pseudomallei, formerly Pseudomonas pseudomallei (melioidosis)

Eikenella

- **Lineage**. Beta Proteobacteria: Neisseriales: Neisseriaceae: Eikenella
- **Infection**. Eikenella corrodens, previously Bacteroides corrodens (bite bacteremia, HACEK endocarditis)

Neisseria

- **Lineage**. Beta Proteobacteria: Neisseriales: Neisseriaceae: Neisseria
- **Infection**. Neisseria gonorrhoeae (gonorrhea)
- **Infection**. Neisseria meningitidis (meningitis)

Kingella

- **Lineage**. Beta Proteobacteria: Neisseriales: Neisseriaceae: Kingella
- **Infection**. Kingella kingae (childhood septic arthritis, childhood osteomyelitis, childhood spondylodiscitis, childhood bacteraemia, childhood endocarditis, childhood pneumonia, childhood meningitis, HACEK endocarditis).

Section 3.4 Gamma Proteobacteria

We are just "a volume of diseases bound together."

John Donne

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
      Enterobacteriales
        Enterobacteriaceae
          Edwardsiella (genus)
          Enterobacter (genus)
          Escherichia (genus)
          Klebsiella (genus)
          Morganella (genus)
          Proteus (genus)
          Providencia (genus)
          Salmonella (genus)
          Shigella (genus)
          Yersinia (genus)
        Plesiomonaceae
          Plesiomonas (genus)
      Cardiobacteriales
        Cardiobacteriaceae
          Cardiobacterium (genus)
      Legionellales
        Coxiellaceae
          Coxiella (genus)
        Legionellaceae
          Fluoribacter (genus)
          Legionella (genus)
      Pasteurellales
        Pasteurellaceae
          Aggregatibacter (genus)
          Haemophilus (genus)
          Pasteurella (genus)
      Pseudomonadales
        Moraxellaceae
          Acinetobacter (genus)
          Moraxella (genus)
        Pseudomonadaceae
          Pseudomonas (genus)
      Thiotrichales
```

```
        Francisellaceae
          Francisella (genus)
      Vibrionales
        Vibrionaceae
          Vibrio (genus)
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
```

Members of Class Gamma Proteobacteria share a similarity in their ribosomal RNA, and, like all subclasses of Class Proteobacteria, they are Gram negative [20]. Otherwise, the Gamma proteobacteria have widely divergent properties: some are aerobic, others not; some are rods, some are cocci; some have a short curve. Others are spiral-shaped, some cells are small, and others are large.

Class Gamma Proteobacteria holds in excess of 20 genera containing about four dozen species that infect humans. It would be unproductive to describe each species in this large and heterogeneous group of organisms. The infectious Gamma proteobacteria are best understood by understanding the characteristic features of the major subclasses and their genera. The diseases and clinical conditions associated with pathogenic species of the Gamma Proteobacteria are listed at the end of the section.

```
Beta Proteobacteria
  Enterobacteriales
    Enterobacteriaceae
      Edwardsiella (genus)
      Enterobacter (genus)
      Escherichia (genus)
      Klebsiella (genus)
      Morganella (genus)
      Proteus (genus)
      Providencia (genus)
      Salmonella (genus)
      Shigella (genus)
      Yersinia (genus)
    Plesiomonaceae
      Plesiomonas (genus)
```

Class Enterobacteriales contains organisms that live in the intestinal tracts of humans or other organisms. Species in Class Enterobacteriales are commonly

known as enterobacteria, and they fall into one of the two subclasses: Enterobacteriaceae and Plesiomonaceae.

Class Enterobacteriaceae contains many organisms: some are aerobic, others are anaerobic; most are facultative anaerobes. Many members of Class Enterobacteriaceae are nonpathogenic commensals, which live in the human intestines, becoming pathogenic under abnormal circumstances. Other enterobacteria are always pathogenic. The enterobacteriaceae are rod-shaped bacteria. Most are about 1–5 μm in length [Glossary Commensal].

Edwardsiella contains several species that live in a variety of animals, including snakes, seals, and fish. A rare disease Edwardsiellosis typically occurs after ingestion of insufficiently cooked freshwater fish (which harbors the organism in its gastrointestinal (GI) tract). Most cases occur in tropical climates (e.g., Southeast Asia, India, Cuba). A case possibly contracted from ornamental (aquarium) fish has been reported [21]. The disease usually presents in humans as a gastroenteritis, which may be severe, and occasionally fatal [Glossary Rare disease].

Enterobacter contains rod-shaped species that live in the human intestines. Several enterobacters are opportunistic pathogens, including Enterobacter aerogenes and Enterobacter cloacae. Disease caused by Enterobacter species usually involves the urinary tract and lung.

Escherichia is another rod-shaped facultatively anaerobic genus with species that inhabit the human intestine. Most species of Escherichia are nonpathogenic commensals. Other species cause enteritis or urinary tract infections. Escherichia coli has pathogenic and nonpathogenic strains, and is the Escherichia species most commonly responsible for human disease. One strain of Escherichia coli (strain O157:H7) produces a toxin (shiga-like toxin) that confers heightened pathogenicity, producing a hemorrhagic enteritis. This dangerous strain of

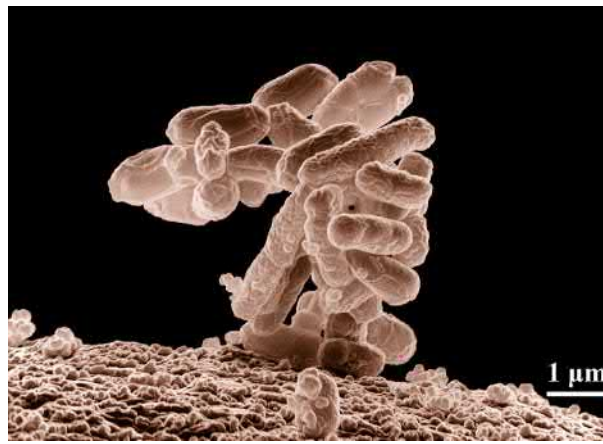

FIG. 3.3 Electron micrograph of a clump of Escherichia coli organisms. E. coli organisms are Gram-negative rods (Gram stain not shown). *(Source, Wikipedia, from a public domain photograph prepared by the US Department of Agriculture, Agricultural Research Service image number K11077-1.)*

Escherichia coli is transmitted to humans, often by ingesting contaminated beef, water, or vegetables that have not been properly sanitized (Fig. 3.3).

Genus Klebsiella contains several species of rod-shaped bacteria with thick polysaccharide capsules. Species are found throughout the environment. Klebsiella pneumoniae, the most common Klebsiella pathogen in humans, inhabits the GI tracts of a wide diversity of animals, either chronically or transiently, and is found in sewage and soil [22]. Klebsiella pneumoniae, as its name suggests, causes pneumonia in humans. Klebsiella pneumoniae and its closely related relative, Klebsiella oxytoca, are capable of producing a wide range of conditions in humans, particularly sepsis and urinary tract infections.

Klebsiella granulomatis, formerly Calymmatobacterium granulomatis, formerly Donovania granulomatis, is the cause of granuloma inguinale, also known as donovanosis, also known as granuloma venereum. Infection produces genital ulcers, and can be mistaken clinically with two other diseases that are characterized by genital ulcers: syphilis (Class Spirochaetae) and chancroid (vida infra). In addition, granuloma venereum, caused by Klebsiella granulomatis, must not be confused with lymphogranuloma venereum, caused by Chlamydia trachomatis (Class Chlamydiae).

Rhinoscleroma is a granulomatous disease, endemic to Africa, Southeast Asia, South America, and some parts of Eastern Europe. Cases may occur in the United States. In rhinoscleroma, the nose is affected in over 95% of cases [Glossary Granuloma].

Readers should not confuse rhinoscleroma, caused by Klebsiella rhinoscleromatis (Class Gamma Proteobacteria), with rhinosporidiosis, caused by Rhinosporidium seeberi (Choanozoa).

Morganella is a genus of anaerobic organisms, of which only one is infectious to humans: Morganella morganii. This organism lives in the intestines of animals (including humans). It can cause a wide variety of diseases, including enteritis (causing the so-called summer diarrhea), sepsis, and organ infections. The bacterial genus Morganella should not be confused with the fungal genus of the same name.

Genus Proteus contains rod-shaped bacteria that inhabit soil and water. Species in the genus produce urease. The genus contains three opportunistic pathogens that are sometimes found in the human intestinal tract: Proteus mirabilis, Proteus vulgaris, and Proteus penneri. All of the Proteus species are capable of causing urinary tract infections. The species most commonly found in Proteus-caused infections is Proteus Mirabilis.

Proteus species have a particular association with struvite stones of the kidney. Because Proteus species produce urease, the resulting high levels of ammonia in urine leads to alkalinity, which in turn favors the crystallization of struvite (an ammonia-containing mineral) in urine. Kidney stones passed through urine, or surgically extracted, are routinely analyzed for chemical composition. Stones composed of struvite are uncommon. Most stones contain calcium oxalate, calcium phosphate, or uric acid. A struvite stone should alert clinicians that the patient may have a Proteus urinary tract infection.

Genus Providencia contains species that are found in soil, water, and sewage. In humans, Providencia species are opportunistic pathogens, superinfecting burns or causing urinary tract infections in patients with indwelling catheters. Providencia stuartii is a common cause of the so-called purple urine bag syndrome, a condition associated with various infectious members of Class Proteobacteria that cause urinary tract infections in catheterized patients (i.e., patients whose urine is caught in a plastic bag). An early name given to members of Class Proteobacteria was "purple bacteria," based on their purple tinge. Urine contaminated with purple bacteria causes purple urine bag syndrome, and the most common proteobacteria causing the syndrome is Providencia stuartii.

Members of Genus Salmonella are found in a wide variety of animals, including reptiles, and pathogenic species produce enteritis, often transmitted through contaminated food (i.e., the so-called salmonellosis food poisoning).

Salmonella enterica contains a number of disease-causing serovars (subtypes). Subtypes S. typhi and S. typhimurium produce typhoid. Subtype S. paratyphi produces paratyphoid. Typhoid, also known as typhoid fever, is a severe gastroenteritis, usually accompanied by a high fever. Paratyphoid is a similar, but generally less severe disease. Neither of these diseases should be confused with typhus fever, caused by Rickettsia typhi and Rickettsia prowazekii, and transmitted by fleas and body lice, respectively. Both diseases (typhoid and typhus) take their root from a Greek word meaning stupor, referring to the neurologic manifestations of the diseases (Fig. 3.4). Another subtype of S. enterica is S. enteritidis which, as its name suggests, causes enteritis.

Species of Genus Shigella cause bacillary dysentery, also known as shigellosis. Despite its disease name, readers should not assume that the cause of bacillary

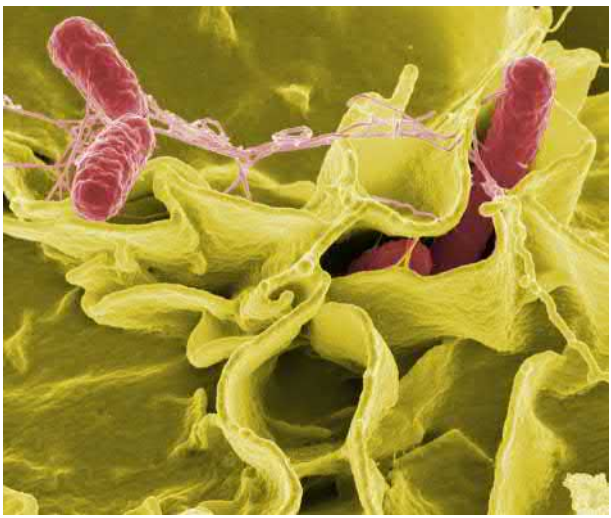

FIG. 3.4 Color-enhanced scanning electron micrograph of Salmonella typhimurium bacilli (red rods) growing on cultured human cells. *(Source, Wikipedia, from a public domain image produced by the US National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories.)*

dysentery is a member of Class Bacilli. The exclusive cause of bacillary dysentery is Shigella species, belonging to Class Gamma Proteobacteria.

Shigella boydii (Gamma Proteobacteria), one of the causes of shigellosis, should not be confused with Pseudallescheria boydii, a fungus in Class Ascomycota, one of many fungal organisms associated with the skin infection maduromycosis.

Yersinia enterocolitica is the cause of yersiniosis, a type of enteritis [23]. It is a zoonotic infection found in a wide variety of animals, including cattle. Humans are most often infected by ingesting contaminated food or water. Genus Yersinia contains species that are siderophilic (iron-loving). Consequently Yersinia enterocolitica seems to have a high infection rate in patients who have a high blood heme content (e.g., hemochromatosis patients). In addition, Yersinia enterocolitica seems to thrive in stored blood and is occasionally found as a contaminant of blood products.

Plague, caused by Yersinia pestis, is not an extinct disease. Each year, several thousand cases of plague occur worldwide, resulting in several hundred deaths. Virtually all of the cases occur in Africa. Its reservoir is small animals (e.g., rodents), and its most common route of spread is from animal to flea to animal. Humans can be infected by flea bite, by direct or indirect contact with infected animals, or by aerosolized droplets produced by infected humans. The clinical manifestation of plague is influenced by the mode of infection: flea bites lead to the bubonic form, characterized by large, necrotic lymph nodes (buboes); entry through skin abrasions, leading to blood infection, may cause a septicemic form of plague; and infection by aerosolized droplets may produce the pneumonic form of plague (Fig. 3.5).

Class Plesiomonaceae is the second subclass contained in Class Enterobacteriales. It contains one genus, with one currently recognized human pathogenic species: Plesiomonas shigelloides, an organism found in diverse animals, including fish and shellfish. Plesiomonas shigelloides was thought to be an exclusively opportunistic pathogen, producing a gastroenteritis, capable of developing into sepsis, in immunocompromised patients. It has now been found to be associated with a significant number of cases of mild enteritis in otherwise healthy individuals [24].

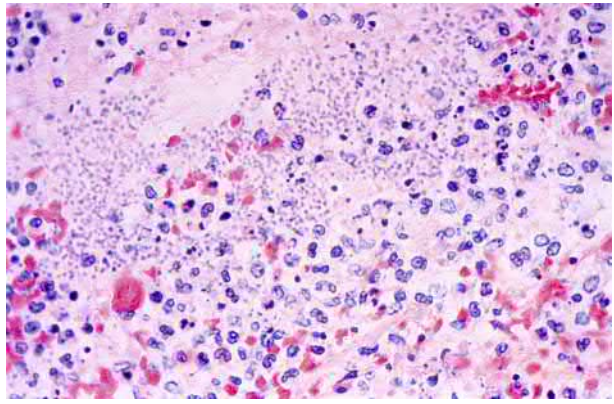

FIG. 3.5 Giemsa-stained section of lung tissue demonstrating numerous Yersinia pestis organisms (tiny, light blue bacteria) in an acute inflammatory background. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Readers should not confuse Plesiomonas shigelloides, containing the species epithet "shigelloides," with the genus name "Shigella" (vida supra).

```
Beta Propriobacteria
  Cardiobacteriales
    Cardiobacteriaceae
      Cardiobacterium (genus)
```

Genus Cardiobacterium contains one pathogenic species: Cardiobacterium hominis, a pleomorphic but often rod-shaped bacteria that causes some cases of endocarditis. It is included with the HACEK organisms.

```
Beta Propriobacteria
  Legionellales
    Coxiellaceae
      Coxiella (genus)
    Legionellaceae
      Legionella (genus)
      Fluoribacter (genus)
```

Whereas most subclasses of the Gamma Proteobacteria live extracellular lives, the members of Class Legionellales that infect humans live inside cells.

Genus Coxiella contains one human pathogen: Coxiella burnetii, the cause of Q fever. Coxiella burnetii is an obligate intracellular organism that is capable of reverting to a small cell variant of itself, that is resistant to intracellular degradation by lysosomes. This highly infectious zoonosis is transmitted by inhaling aerosolized respiratory material, or from direct contact with secretions from an infected animal (including cattle, sheep, goats, dogs, and cats). A flu-like syndrome results, which may progress to chronic pneumonia, ARDS (acute respiratory distress syndrome), and various systemic sequelae, including endocarditis [Glossary Syndrome].

Genera of Class Legionellaceae are facultative intracellular organisms and include Legionella and Fluoribacter [25].

Legionella species live within amoeba in the environment. Infection occurs after inhalation of the bacteria, and epidemics have been linked to contaminated sources of aerosolized water, from water holding systems. The name of the disease and of the organism derive from the first diagnosed epidemic, occurring in members of an American Legion who attended a bicentennial convention in Philadelphia, in July, 1976. Direct person to person spread has not been established. Disease most often occurs in immunocompromised individuals and the elderly. Infection is usually pulmonary and can be fatal. Pontiac fever is a milder form of Legionnaires disease. Between 10,000 and 50,000 cases of Legionnaire disease occur each year in the United States. The bacteria can be visualized in tissue sections with a silver stain. Aside from Legionella pneumophila, there are more than 50 species of Legionella, some of which have been shown to produce Legionnaires disease.

DNA analysis of species of Legionella demonstrated that the many species of Legionella have sequence dissimilarities, suggesting that Genus Legionella could be assigned several genera [26]. Genus Fluoribacter was created as a new genus in Class Legionellaceae, and this genus was assigned Fluoribacter bozemanae, formerly Legionella bozemanae. The species in Genus Fluoribacter behave clinically like Legionella species.

```
Beta Propriobacteria
  Pasteurellales
    Pasteurellaceae
      Aggregatibacter (genus)
      Haemophilus (genus)
      Pasteurella (genus)
```

Class Pasteurellaceae contains numerous species of facultative anaerobic bacteria, that are, in most cases, rod-shaped. Most species live as commensals in vertebrates, and are found primarily in the upper respiratory tracts, reproductive tracts, and, in some cases, the GI tract. Three genera contain species pathogenic to humans: Aggregatibacter, Haemophilus, and Pasteurella.

Genus Aggregatibacter contains the oral commensal Aggregatibacter actinomycetemcomitans, formerly Actinobacillus actinomycetemcomitans, which causes an aggressive form of periodontitis.

Genus Haemophilus contains the pathogenic species: Haemophilus influenzae, Haemophilus parainfluenzae, and Haemophilus ducreyi.

Haemophilus influenzae is found in the upper respiratory tract of normal individuals. It causes pneumonitis, meningitis, and bacteremia, in infants and young children. Its species name, influenzae, was assigned when the bacteria was mistakenly thought to be the cause of influenza. Influenza, also known as the flu, is caused exclusively by the influenza virus, a Group V orthomyxovirus.

Haemophilus parainfluenzae causes some cases of endocarditis. It is included among the HACEK organisms. Readers should remember that despite its name, Haemophilus parainfluenzae is not the cause of the disease known as parainfluenza. Parainfluenza is a type of croup (laryngotracheobronchitis), and about 75% of the cases of croup are caused by the parainfluenza virus, a Group V virus.

Haemophilus ducreyi is the cause of chancroid, a sexually transmitted disease. Chancroid produces painful genital ulcers. It must not be confused with other diseases that cause genital ulcers: syphilis and granuloma inguinale. Klebsiella granulomatis, in Class Enterobacteriaceae, causes granuloma inguinale, and was described previously in this chapter. Syphilis is caused by a spirochete. Adding to the confusion, the syphilitic genital ulcer is known as a chancre (Fig. 3.6).

Genus Pasteurella contains Pasteurella multocida, the cause of pasteurellosis, a zoonosis. Pasteurella multocida lives as a commensal or as a pathogen in a variety of animals. Infection in humans usually results from close contact

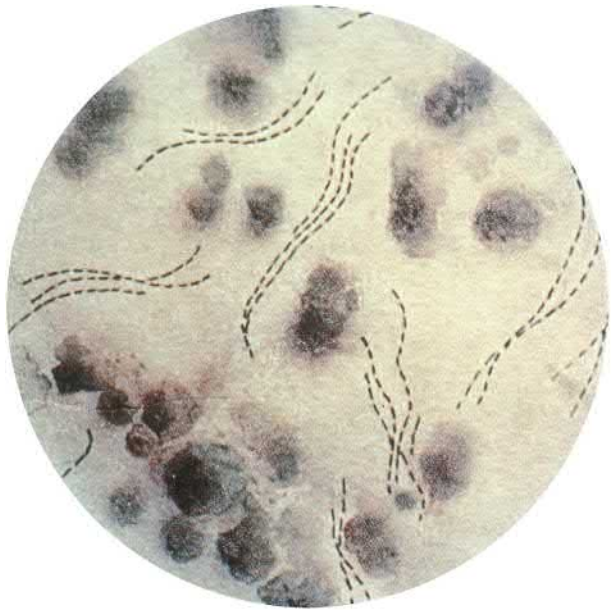

FIG. 3.6 Haemophilus ducreyi. Gentian violet stained smear demonstrating long chains of intact coccobacilli among scattered degenerating inflammatory cells. *(Source, Wikipedia, from a public domain image produced by the US Centers for Disease Control and Prevention.)*

with infected animals. Infections may arise through bites or scratches from pets, particularly cats. Infection can result in sepsis, pneumonia, or skin infections. Skin infections often occur at the site of a cat scratch.

```
Beta Propriobacteria
  Pseudomonadales
    Moraxellaceae
      Acinetobacter (genus)
      Moraxella (genus)
    Pseudomonadaceae
      Pseudomonas (genus)
```

Class Pseudomonadales contains organisms that cause opportunistic infections. Several grow as surface biofilms, making them hard to disinfect from contaminated medical devices, and thus potential sources of nosocomial (hospital-acquired) infections. Biofilm infections in hospitals will be described again when we discuss the gliding bacteria.

Members of Genus Acinetobacter are found in soil. The organisms grow as coccobacillary forms, sometimes clumped in pairs. Species of Acinetobacter are found in hospitals, particularly as surface colonies. Though Acinetobacter species are generally nonpathogenic in healthy individuals, serious infections may

occur in weakened individuals in intensive care units, on ventilatory support, or recovering from wounds. Outbreaks of Acinetobacter infections have occurred in military hospitals. Acinetobacter baumanii is often associated with pneumonia in hospital patients, particularly in patients receiving ventilator support. Infections may lead to bacteremia, meningitis, and skin infections. Bacteria and disseminated infections can be associated with another species, Acinetobacter lwoffi.

Readers should not confuse Genus Acinetobacter (Gamma Proteobacteria) with Class Actinobacteria.

Moraxella catarrhalis is an opportunistic pathogen that often targets young children, the elderly, and immunocompromised individuals. It can cause a wide range of infections. In adults it tends to cause pneumonia, bronchitis, sinusitis, and can lead to sepsis. In young children, it can cause otitis media. The organism seems to live in humans exclusively, colonizing the respiratory tracts of infected individuals [27].

Much like Genus Acinetobacter, members of Genus Pseudomonas are opportunistic pathogens that are often nosocomial (i.e., hospital-acquired) infections. Pseudomonas species can grow as biofilms. The biofilms contain an excreted exopolysaccharide that makes interferes with disinfection of colonized surfaces. Patients, in a weakened condition, are particularly vulnerable to infections. The most common Pseudomonas species involved in human infections is Pseudomonas aeruginosa, which causes some of the same kinds of infections observed with Acinetobacter species (pneumonia, bacteremia, urinary tract infections, and infections occurring in burn sites) (Fig. 3.7).

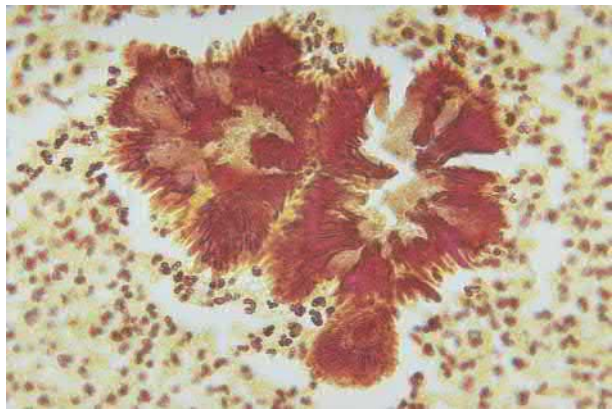

FIG. 3.7 Brown and Brenn stain of tissue section of a botryomycotic lesion draining through the skin, from the lower abdomen. In the center of the photomicrograph is a granule composed of multiple Pseudomonas aeruginosa organisms. The periphery contains numerous white blood cells. *(Source, public domain image from the US Center for Disease Control and Prevention, photographed by Dr. Lucille K. George, in 1973.)*

Several species, formerly assigned to Genus Pseudomonas, are now assigned to Genus Burkholderia (Beta Proteobacteria). These include species now known as Burkholderia mallei and Burkholderia pseudomallei.

```
Beta Propriobacteria
  Thiotrichales
    Francisellaceae
      Francisella (genus)
```

Class Thiotrichales has received scientific attention as it contains the largest known species of bacteria, Thiomargarita namibiensis. This ocean-dwelling nonpathogen can reach a size of 0.75 mm, visible to the unaided eye [Glossary Largest species].

In contrast, Genus Francisella contains small organisms, under a micron in size. Francisella tularensis is the cause of tularemia, also known as rabbit fever. The species occurs in two serotypes (A and B), with Type B producing milder disease. The organism's reservoir is infected members of Class Glires (including rabbits and beavers), and is usually transmitted to humans via arthropod bites, particularly tick bites. Human infections can also follow ingestion of contaminated water, soil, or by handling infected animals. Tularemia is a serious disease that can produce a wide variety of symptoms. A common presentation involves lymph nodes (glandular form) and infection of the facial skin. The enlargement of lymph nodes is similar to the buboes (ulcerating enlarged lymph nodes) associated with plague. Other species of Genus Francisella, Francisella novicida and Francisella philomiragia, previously assigned to Genus Yersinia, can produce sepsis.

```
Beta Propriobacteria
  Vibrionales
    Vibrionaceae
      Vibrio (genus)
```

Genus Vibrio contains motile species characterized by a curved shape and a distinctive polar flagellum. Many vibrio species are pathogens for fish and crustaceans. Vibrio cholerae is the cause of cholera, a particularly aggressive enteritis. Sporadic cholera cases, in developed countries, most often occur after ingesting undercooked seafood. Large outbreaks of cholera usually result from poor sanitary conditions, with infected humans contaminating the water supply. Worldwide, about 3–5 million people contract cholera, annually. Other vibrio species that are pathogenic to humans are Vibrio parahaemolyticus and Vibrio vulnificus, both causing enteritis.

Infectious Genera

Edwardsiella

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Edwardsiella
- **Infection**. Edwardsiella tarda (Edwardsiellosis)

Enterobacter

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Enterobacter
- **Infection**. Enterobacter aerogenes (opportunistic sepsis)
- **Infection**. Enterobacter cloacae (urinary tract infections, respiratory tract infections)

Escherichia

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Escherichia
- **Infection**. Escherichia coli (food poisoning, enteritis)

Klebsiella

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Klebsiella
- **Infection**. Klebsiella oxytoca (colitis, sepsis)
- **Infection**. Klebsiella pneumoniae (pneumonia)
- **Infection**. Klebsiella rhinoscleromatis (rhinoscleroma)
- **Infection**. Klebsiella granulomatis, formerly Calymmatobacterium granulomatis, formerly Donovania granulomatis (granuloma inguinale, donovanosis, granuloma venereum)

Morganella

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Morganella
- **Infection**. Morganella morganii (a wide variety of organ infections, and sepsis)

Proteus

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Proteus
- **Infection**. Proteus mirabilis (struvite renal stones)
- **Infection**. Proteus penneri, formerly a substrain of Proteus vulgaris (nosocomial urinary tract infections and sepsis)
- **Infection**. Proteus vulgaris (nosocomial urinary tract infections and sepsis)

Providencia

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Providencia

Providencia species (urinary tract infections, gastroenteritis and bacteremia)

- **Infection**. Providencia stuartii (purple urine bag syndrome)

Salmonella

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Salmonella

- **Infection**. Salmonella arizonae (salmonellosis)
- **Infection**. Salmonella enteritidis (gastroenteritis)
- **Infection**. Salmonella paratyphi A,B,C (paratyphoid fever)
- **Infection**. Salmonella typhi (typhoid fever)
- **Infection**. Salmonella typhimurium (paratyphoid fever, food poisoning)
- **Infection**. Salmonella enterica, formerly Salmonella choleraesuis (food poisoning)

Shigella

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Shigella
- **Infection**. Shigella boydii (shigellosis, bacillary dysentery)
- **Infection**. Shigella dysenteriae (shigellosis, bacillary dysentery)
- **Infection**. Shigella flexneri (shigellosis, bacillary dysentery)
- **Infection**. Shigella sonnei (shigellosis, bacillary dysentery)

Yersinia

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Enterobacteriaceae: Yersinia
- **Infection**. Yersinia pestis, variously known as Pasteurella pestis, Bacillus pestis, Pesticella pestis, Bacterium pestis, and Yersinia pseudotuberculosis subspecies pestis (Plague)
- **Infection**. Yersinia enterocolitica (yersiniosis, occasionally included with Shigella species as a cause of bacillary dysentery)
- **Infection**. Yersinia pseudotuberculosis (pseudotuberculosis)

Plesiomonas

- **Lineage**. Gamma Proteobacteria: Enterobacteriales: Plesiomonaceae: Plesiomonas
- **Infection**. Plesiomonas shigelloides (gastroenteritis, developing into sepsis, in immune compromised humans)

Cardiobacterium

- **Lineage**. Gamma Proteobacteria: Cardiobacteriales: Cardiobacteriaceae: Cardiobacterium
- **Infection**. Cardiobacterium hominis (endocarditis)

Coxiella

- **Lineage**. Gamma Proteobacteria: Legionellales: Coxiellaceae: Coxiella
- **Infection**. Coxiella burnetii (Q fever)

Fluoribacter (formerly Legionella)

- **Lineage**. Gamma Proteobacteria: Legionellales: Legionellaceae: Fluoribacter
- **Infection**. Fluoribacter bozemanae, formerly Legionella bozemanae (pneumonia)

Legionella

- **Lineage**. Gamma Proteobacteria: Legionellales: Legionellaceae: Legionella
- **Infection**. Legionella pneumophila (Legionellosis, Legionnaires disease, Legion fever, Pontiac fever)
- **Infection**. Legionella species (Legionellosis, Legionnaires disease, Legion fever, Pontiac fever)

Aggregatibacter

- **Lineage**. Gamma Proteobacteria: Pasteurellales: Pasteurellaceae: Aggregatibacter
- **Infection**. Aggregatibacter actinomycetemcomitans, formerly Actinobacillus actinomycetemcomitans (periodontal disease)

Haemophilus

- **Lineage**. Gamma Proteobacteria: Pasteurellales: Pasteurellaceae: Haemophilus
- **Infection**. Haemophilus ducreyi (chancroid)
- **Infection**. Haemophilus influenzae (meningitis)
- **Infection**. Haemophilus parainfluenzae (endocarditis)

Pasteurella

- **Lineage**. Gamma Proteobacteria: Pasteurellales: Pasteurellaceae: Pasteurella
- **Infection**. Pasteurella multocida (pasteurellosis)

Acinetobacter

- **Lineage**. Gamma Proteobacteria: Pseudomonadales: Moraxellaceae: Acinetobacter
- **Infection**. Acinetobacter baumannii (nosocomial pneumonia, ventilator-associated pneumonia)
- **Infection**. Acinetobacter lwoffi (nosocomial pneumonia, nosocomial bacteremia, nosocomial meningitis, nosocomial wound infections)

Moraxella

- **Lineage**. Gamma Proteobacteria: Pseudomonadales: Moraxellaceae: Moraxella
- **Infection**. Moraxella catarrhalis (respiratory tract infections, sepsis, especially in immunocompromised individuals)

Pseudomonas

- **Lineage**. Gamma Proteobacteria: Pseudomonadales: Pseudomonadaceae: Pseudomonas
- **Infection**. Pseudomonas aeruginosa (multiorgan infections, botryomycotic skin and internal abscesses, sepsis in immunocompromised individuals, hot tub rash)

Francisella

- **Lineage**. Gamma Proteobacteria: Thiotrichales: Francisellaceae: Francisella
- **Infection**. Francisella tularensis (tularemia, rabbit fever)

Vibrio

- **Lineage**. Gamma Proteobacteria: Vibrionales: Vibrionaceae: Vibrio
- **Infection**. Vibrio cholerae, including El Tor (cholera)
- **Infection**. Vibrio parahaemolyticus (watery diarrhea)
- **Infection**. Vibrio vulnificus (watery diarrhea)

Section 3.5 Epsilon Proteobacteria

Names are an important key to what a society values. Anthropologists recognize naming as one of the chief methods for imposing order on perception.

David S. Slawson

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
      Campylobacterales
        Helicobacteraceae
          Helicobacter (genus)
        Campylobacteraceae
          Campylobacter (genus)
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
```

Astute readers may notice that there is no chapter for the Delta Proteobacteria. This is because the Delta Proteobacteria contain no organisms that are pathogenic to humans. Circumstances may change. Class Delta Proteobacteria, Genus Desulfovibrio has been associated with an opportunistic human infection [28]. Until more definitive studies come to light, we can skip directly to Class Epsilon Proteobacteria.

Epsilon Proteobacteria, like all other proteobacteria are Gram-negative bacteria, with an inner and outer membrane enclosing the cell wall. These bacteria are curved or spiral-shaped and most genera live in the digestive tracts of animals. Two genera are pathogenic to humans: Campylobacter and Helicobacter. Helicobacter species live in the stomach. Campylobacter species live in the duodenum.

```
Epsilon Proteobacteria
  Campylobacterales
    Helicobacteraceae
      Helicobacter (genus)
    Campylobacteraceae
      Campylobacter (genus)
```

More than half of the world's human population is infected by H. pylori. Infection rates are highest in developing countries. Helicobacter pylori lives in the stomach, an organ that, for many years, was thought to be sterile: no bacteria could possibly survive the acidic environment of the gastric lining! In 1983, Robin Warren and Barry Marshall described what they called "unidentified curved bacilli" in patients with gastritis, by direct visualization under a microscope. These structures were later proven to be Helicobacter pylori. To demonstrate the viability and the pathogenicity of these organisms, Marshall experimented on himself, by ingesting gastric juice from a "dyspeptic" man. About 10 days later, he developed gastritis, suggesting that an agent in stomach contents from patients with gastritis, could transmit the disease to other people. Marshall and Warren submitted their findings in a scientific abstract, which was rejected by the Australian Gastroenterological Association [29]. Luckily for us, these two stalwart scientists persevered. In 2005, the Nobel prize for medicine was awarded to Robin Warren and Barry Marshall for discovering the role of Helicobacter pylori in gastric disease. In retrospect, we now know that these bacteria were observed in histologic examination of gastritis specimens as early as 1875 [30]. Until Warren and Marshall, nobody made the cognitive leap, connecting the visualized organisms to a human disease; pathologists were intellectually invested in the false belief that the stomach is a sterile organ.

The mechanism of transmission of Helicobacter has not been conclusively determined, but the organism has been isolated from feces, saliva, and tooth plaque of infected individuals, suggesting a direct human-to-human spread [31]. Infections tend to be persistent. Helicobacter pylori is believed to be a common cause of peptic ulcers, chronic gastritis, duodenitis, and stomach cancers; both adenocarcinoma of stomach and MALT (mucosa-associated lymphoid tissue) lymphoma. Since the discovery of Helicobacter pylori, a variety of additional Helicobacter species have been isolated from animals and humans; many are enteropathogenic. Examples are Helicobacter suis, Helicobacter felis, Helicobacter bizzozeronii, and Helicobacter salomonis.

Some of these newly discovered strains seem to have greater disease-causing potential than Helicobacter pylori, with more organs involved. Helicobacter cinaedi causes some cases of cellulitis in immunocompromised individuals [32]. A role for Helicobacter species in Crohn disease and other enteric and hepatic inflammatory conditions is currently being studied. Numerous strains have been detected by PCR (polymerase chain reaction), but not isolated from tissues.

The second genus of Class Epsilon Proteobacteria is Campylobacter, the cause of campylobacteriosis. About a dozen species of Campylobacter are currently under study as possible causes of human disease. Campylobacter jejuni lives in the small intestine and colon, and infections typically cause an acute, self-limited enteritis. Campylobacter fetus is an opportunistic pathogen that causes sepsis in newborns (Fig. 3.8).

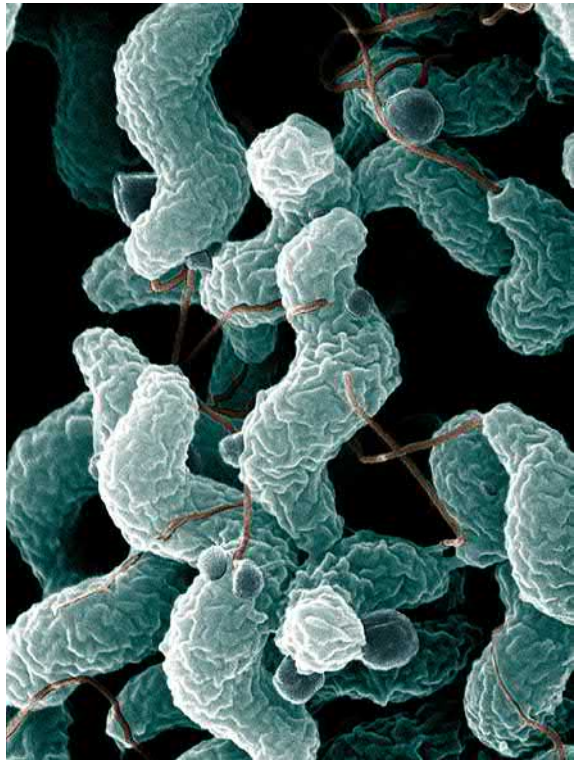

FIG. 3.8 Colorized scanning electron microscope image of Campylobacter jejuni. Note the s-shaped organisms (Campylobacter means "curved bacteria"). Each bacterium has a long flagellum extending from one or both poles. *(Source, Wikipedia, from a public domain image provided by the US Department of Agriculture's Agricultural Research Service, and prepared by De Wood and Pooley.)*

Infectious Genera

Campylobacter

- **Lineage**. Epsilon Proteobacteria: Campylobacterales: Campylobacteraceae: Campylobacter
- **Infection**. Campylobacter fetus (sepsis in newborns)
- **Infection**. Campylobacter jejuni (enteritis)

Helicobacter

- **Lineage**. Epsilon Proteobacteria: Campylobacterales: Helicobacteraceae: Helicobacter
- **Infection**. Helicobacter pylori, formerly Campylobacter pylori, formerly Campylobacter pyloridis (gastritis, increased risk of gastric cancer, and gastric MALT lymphoma)
- **Infection**. Helicobacter cinaedi (cellulitis) [32].

Section 3.6 Spirochaetes

And what physicians say about disease is applicable here: that at the beginning a disease is easy to cure but difficult to diagnose; but as time passes, not having been treated or recognized at the outset, it becomes easy to diagnose but difficult to cure.

Niccolo Machiavelli

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
    Spirochaetales
      Leptospiraceae
        Leptospira (genus)
      Spirochaetaceae
        Borrelia (genus)
        Treponema (genus)
      Brachyspiraceae (formerly Serpulinaceae)
        Serpulina (genus)
        Brachyspira (genus)
      Spirillaceae
        Spirillum (genus)
  Bacteroidetes
  Fusobacteria
```

```
Firmicutes
  Bacilli
  Clostridia
  Mollicutes
Chlamydiae
Actinobacteria
```

It was not too long ago that microbiologists classified spirochetes among the single-celled eukaryotes [33]. We now know that the spirochetes are bacteria with a peculiar morphologic feature that is characteristic of every member of the class. Class Spirochaetes are Gram-negative organisms, and have an inner and outer membrane. Spirochetes are long, helical organisms with an axial flagellum that runs between the inner and the outer membrane. In this location, the motion of the flagellum twists the entire organism, and this twisting motion accounts for the motility of the organism.

Most of the members of Class Spirochaetes require direct transmission from an animal reservoir, either through a bite of an louse (Class Hexapoda), tick (Class Chelicerata), rat (Class Craniata), through intimate sexual contact with an infected human, or through close skin-to-skin contact with another human (e.g., yaws). The exception is Genus Leptospira.

```
Spirochaetes
  Spirochaetales
    Leptospiraceae
      Leptospira (genus)
    Spirochaetaceae
      Borrelia (genus)
      Treponema (genus)
    Brachyspiraceae (formerly Serpulinaceae)
      Serpulina (genus)
      Brachyspira (genus)
    Spirillaceae
      Spirillum (genus)
```

Numerous species of Genus Leptospira can cause Leptospirosis; also known as Weil disease, Weil syndrome, canicola fever, canefield fever, nanukayami fever, 7-day fever, rat catcher's yellows, Fort Bragg fever, and pretibial fever. The disease produces a bacteremia, with consequent splenitis and infection of multiple organs. Jaundice is a common feature of the disease. Leptospirosis is transmitted to humans through contact with the body fluids of infected animals. Drinking water or eating food that has been contaminated with infected urine is a common route of transmission. A wide range of animals may carry the infection, but rats and mice are usually considered the most important hosts. Though leptospirosis is a rare disease, it can occur just about anywhere in the world, often as localized outbreaks. Nepal has been a frequent site of leptospirosis outbreaks [Glossary Host].

Borrelia burgdorferi causes Lyme disease. It is transmitted by the bite of infected deer ticks. The rates of Lyme disease infections are increasing in the United States. Though the disease my occur anywhere, it is found most often in the Northern hemisphere. In Europe, most cases of Lyme disease are caused by Borrelia afzelii and Borrelia garinii. It is likely that newly identified species of Borrelia, capable of producing Lyme disease, will be encountered in the near future. Lyme disease has been known to produce a bewildering array of symptoms, particularly when it enters its chronic stage. Thus, it has been confused with various chronic ailments and neurodegenerative disorders of unknown etiology (Fig. 3.9).

Relapsing fever is an infection characterized by recurring fevers often accompanied by other systemic symptoms including fever, nausea, and rash. It is caused by any of several genera of class Borrelia and is transmitted by a tick or a louse. Tick-borne relapsing fever (TBRF) is transmitted by the Ornithodoros tick, a genus in Class Argasidae, whose members are all soft bodied. It occurs in many areas of the world and is found in the United States and Canada. Species associated with TBRF are: Borrelia duttoni, Borrelia parkeri, and Borrelia hermsii. Louse-borne relapsing fever is caused by Borrelia recurrentis and occurs most often in Africa, Asia, and Latin America.

Borrelia lonestari (Southern tick-associated rash illness or STARI) is probably the most redundant name in taxonomy, as it encompasses the abbreviation STARI (loneSTARI) and its common geographic location (Texas, the Lone Star State). Though the disease occurs in Texas, it can also be found in every state between Texas and Maine (Fig. 3.10).

Brachyspira pilosicoli is a colonizing spirochete in the large intestine of pigs, and is occasionally found in humans. It can produce diarrhea and rectal bleeding [34]. Brachyspira aalborgi accounts for a small fraction (less than 1%) of cases of acute appendicitis.

FIG. 3.9 Darkfield photomicrograph of Borrelia burgdorferi. Organisms have corkscrew (helical) shape, typical of members of Class Spirochaetes. *(Source, Wikipedia, from a public domain image provided by the US Centers for Disease Control and Prevention.)*

FIG. 3.10 An engorged female lone-star tick, Amblyomma americanum. This tick is known to transmit Borrelia lonestari, the pathogen causing Southern tick-associated rash illness (STARI), a condition that is clinically similar to Lyme disease. *(Source, a public domain image provided by the US Center for Disease Control and Prevention, prepared by James Gathany.)*

Subspecies of Treponema pallidum are responsible for four diseases: syphilis, pinta, bejel, and yaws. Syphilis, caused by Treponema pallidum (a pallidum variant), is transmitted sexually. It can also be transmitted from mother to infant via transplacental infection or through physical exposure to the neonate during its passage through the birth canal. Humans are the only animal reservoir for syphilis. In adults, syphilis is a multistage disease, first appearing as a primary skin lesion (chancre), followed weeks later by a rash and various systemic symptoms (secondary syphilis), followed by a long latency period (years), followed by a systemic illness affecting the brain, cardiovascular system, and other organs (tertiary syphilis). The tertiary phase is noninfectious. Today, syphilis is most common in developing countries [35].

Pinta, bejel, and yaws are nonvenereal infections caused by subspecies of Treponema pallidum (variants carateum, endemicum, and pertenue, respectively). Each is primarily a skin disease that is spread by direct skin-to-skin contact. Lesions beginning in the skin can spread to joints and bones. Pinta occurs primarily in Central and South America. Yaws occurs primarily in Asia, Africa, and South America. Most cases of bejel occur in the Mediterranean region and Northern Africa and often involve the mouth and oral mucosa, with mouth-to-mouth transmission (Fig. 3.11).

Treponema denticola is a major cause of periodontitis.

The taxonomic assignment of genus Spirillum is currently unstable, being placed in either Class Spirochaetes or Class Beta Proteobacteria. Adding to the

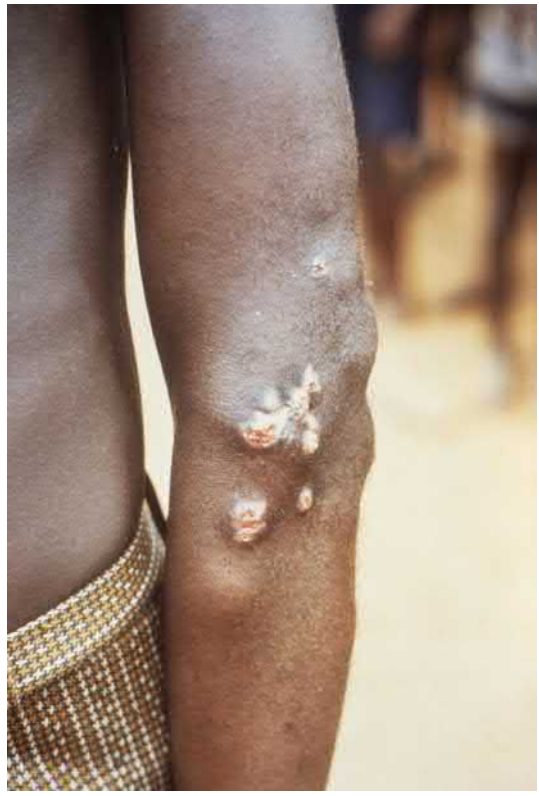

FIG. 3.11 Nodules on the elbow, typical of yaws, an infection produced by Treponema pertenue. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

confusion, the only human pathogen in the genus, produces a disease that is attributed to two separate classes of organisms. Rat-bite fever is caused by either Spirillum minus or Streptobacillus moniliformis (Class Fusobacteria) [36]. Evidently, diseases do not read taxonomic textbooks. Regardless of the causative organism, or the phylogenetic classes to which the organisms are assigned, the clinical symptoms are similar, as is the treatment.

Infectious Genera

Leptospira

- **Lineage**. Spirochaetes: Spirochaetales: Leptospiraceae: Leptospira
- **Infection**. Leptospira species, including Leptospira interrogans (leptospirosis, Weil disease, Weil syndrome, canicola fever, canefield fever, nanukayami fever, 7-day fever, rat catcher's yellows, Fort Bragg fever, and pretibial fever)

Borrelia

- **Lineage**. Spirochaetes: Spirochaetales: Spirochaetaceae: Borrelia
- **Infection**. Borrelia burgdorferi (Lyme disease in the United States)
- **Infection**. Borrelia afzelii (Lyme disease in Europe)
- **Infection**. Borrelia garinii (Lyme disease in Europe)
- **Infection**. Borrelia duttoni (TBRF, typhinia)
- **Infection**. Borrelia recurrentis (louse-borne relapsing fever, typhinia)
- **Infection**. Borrelia hermsii (TBRF, typhinia)
- **Infection**. Borrelia parkerii (TBRF, typhinia)
- **Infection**. Borrelia lonestari (STARI)

Brachyspira

- **Lineage**. Spirochaetes: Spirochaetales: Brachyspiraceae (formerly Serpulinaceae): Brachyspira
- **Infection**. Brachyspira pilosicoli, formerly Serpulina pilosicoli (diarrhea, spirochetosis)
- **Infection**. Brachyspira aalborgi (appendicitis)

Treponema

- **Lineage**. Spirochaetes: Spirochaetales: Spirochaetaceae: Treponema
- **Infection**. Treponema carateum, alternately known as Treponema pallidum var carateum (pinta)
- **Infection**. Treponema pallidum, alternately known as Treponema pallidum var pallidum (syphilis, congenital syphilis)
- **Infection**. Treponema endemicum, alternately known as Treponema pallidum var endemicum (bejel)
- **Infection**. Treponema pertenue, alternately known as Treponema pallidum var pertenue (Yaws, frambesia tropica, Pian)
- **Infection**. Treponema denticola (periodontal disease and opportunistic infections)

Spirillum

- **Lineage**. Spirochaetes: Spirochaetes: Spirochaetales: Spirillaceae: Spirillum
- **Infection**. Spirillum minus (rat-bite fever)

Section 3.7 Bacteroidetes and Fusobacteria

The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge.

Stephen Hawking

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
```

```
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
    Bacteroidales
      Bacteroidaceae
        Bacteroides (genus)
      Porphyromonadaceae
        Porphyromonas (genus)
      Prevotellaceae
        Prevotella (genus)
    Flavobacteria
      Flavobacteriales
        Flavobacteriaceae
          Elizabethkingia (genus)
  Fusobacteria
    Fusobacteriales
      Fusobacteriaceae
        Fusobacterium (genus)
        Streptobacillus (genus)
  Firmicutes (low G+C Gram+)
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
  Actinobacteria
```

The two phyla, Bacteroidetes and Fusobacteria, are grouped in this chapter, because they were both formerly assigned to the same (now obsolete) class, Saprospirae, the gliding, fermenting bacteria [37]. Fermentation is a metabolic process wherein organic molecules yield energy. Bacterial gliding is a process in which cells on a flat surface move through excreted slime (polysaccharides). The process of gliding is different from the process of swimming, and does not require flagella. Aside from organisms in Class Bacteroidetes and Class Fusobacteria, gliding is also seen in some members of Class Gamma Proteobacteria, particularly Class Pseudomonadales.

```
Bacteroidetes
  Bacteroidales
    Bacteroidaceae
      Bacteroides (genus)
    Porphyromonadaceae
      Porphyromonas (genus)
    Prevotellaceae
      Prevotella (genus)
```

```
Flavobacteria
  Flavobacteriales
    Flavobacteriaceae
      Elizabethkingia (genus)
```

Class Bacteroidetes has two major subdivisions, Class Bacteroidales and Class Flavobacteria. The two divisions lack morphologic resemblance to one another and are physiologically distinct (Class Bacteroidales is anaerobic, Class Flavobacteria is aerobic). Nonetheless, their genotypes are similar, and some species from either group produce sphingolipids, a chemical that is seldom encountered in other bacterial classes [7].

Members of Genus Bacteroides are mostly nonpathogenic commensals, living in the human GI tract; some are opportunistic pathogens. Bacteroides organisms account for much of the material that composes feces. The high concentration of Bacteroides species in fecal matter is a reminder that not all intestinal bacteria belong to Class Enterobacteriaceae (Gamma Proteobacteria) (Fig. 3.12).

Peritonitis may occur when Bacteroides species leak into the normally sterile peritoneal cavity. Bacteroides fragilis causes the vast majority of peritonitis cases in humans.

Members of Genus Porphyromonas live as commensals or as opportunistic pathogens in the human oral cavity. Porphyromonas gingivalis causes a clinically aggressive gingivitis that can lead to acute necrotizing ulcerative gingivitis (ANUG) or extend to the tissues of the mouth and face, a condition known as noma or cancrum oris. Currently under investigation is the theory that Porphyromonas gingivalis plays a causal role in the pathogenesis of Alzheimer disease [38, 39]. This topic is discussed further in Section 8.4, "Discovering New Infections Among the Diseases of Unknown Origin."

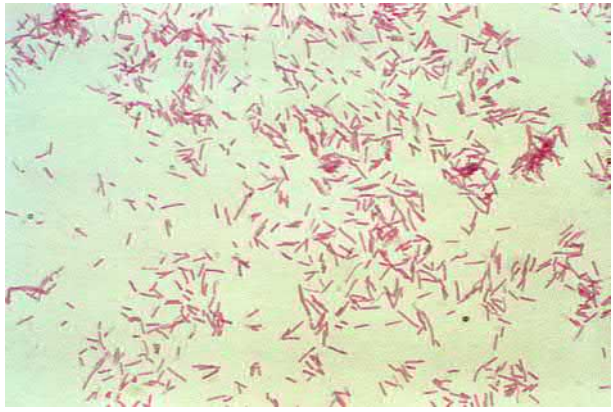

FIG. 3.12 Gram stain of Bacteroides fragilis, a Gram-negative rod-shaped bacteria. Various bacteroides species are found as normal inhabitants of the human intestine. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Genus Prevotella, like Genus Porphyromonas, contains oral inhabitants that can produce plaque, halitosis, and periodontal disease. Prevotella dentalis produces so-called bite infections, wherein oral bacteria are inoculated, by a bite or abrasion, into adjacent tissues, producing abscesses, wound infections, or bacteremia. Prevotella dentalis bacteremia can lead to disseminated infections. Readers should remember that Eikenella corrodens (Class Beta Proteobacteria) is another oral "bite" organism that can produce a bacteremia if mechanically forced into the blood stream.

Members of Genus Elizabethkingia are opportunistic pathogens that grow as surface biofilms. Like other organisms that grow on surfaces (see Class Pseudomonadales, Gamma Proteobacteria), Elizabethkingia species cause nosocomial infections, including bacteremia associated with indwelling intravascular devices and pneumonia associated with ventilatory support [40]. Elizabethkingia meningoseptica, formerly Chryseobacterium meningosepticum, formerly Flavobacterium meningosepticum, has been associated with meningitis outbreaks in neonatal intensive care units. It can also cause soft tissue infections in immune-competent individuals.

```
Fusobacteria
  Fusobacteriales
    Fusobacteriaceae
      Fusobacterium (genus)
      Streptobacillus (genus)
```

Class Fusobacteria contains organisms that are very similar to those in Class Bacteroidales. Genus Fusobacterium contains active pathogens that can colonize the oropharynx [41]. Fusobacterium necrophorum causes a significant percentage of acute and recurring sore throats in humans. The other significant causes of sore throats are Streptococcus species (Class Bacilli) and viruses (e.g., Influenza virus, Rhinovirus, and Coxsackie virus). Fusobacterium necrophorum may also cause tonsillar abscesses and bacteremia, and is the agent responsible for Lemierre disease (bacteraemia with disseminated abscesses, following a sore throat).

Genus Streptobacillus contains the pathogen Streptobacillus moniliformis. Rat-bite fever, also known as Haverhill fever, is caused by either Streptobacillus moniliformus or Spirillum minus (Class Spirochaetes) [36]. Regardless of the causative organism, or the phylogenetic classes to which the organisms are assigned, the clinical symptoms are somewhat similar, with fever and generalized complaints followed by a rash and arthritis. Deaths sometimes occur if endocarditis develops. The disease is transmitted by rodents, either by bite, close contact, or through contaminated water or food.

Infectious Genera

Bacteroides

- **Lineage**. Bacteroidetes: Bacteroidales: Bacteroidaceae: Bacteroides
- **Infection**. Bacteroides fragilis (peritonitis)

Elizabethkingia

- **Lineage**. Bacteroidetes: Flavobacteria: Flavobacteriales: Flavobacteriaceae: Elizabethkingia
- **Infection**. Elizabethkingia meningoseptica, formerly Chryseobacterium meningosepticum, formerly Flavobacterium meningosepticum (nosocomial infections, including pneumonia, meningitis, and sepsis; cellulitis)

Streptobacillus

- **Lineage**. Streptobacillus: Fusobacteria: Fusobacteriales: Fusobacteriaceae: Streptobacillus
- **Infection**. Streptobacillus moniliformis (rat-bite fever, Haverhill fever)

Fusobacterium

- **Lineage**. Fusobacterium: Fusobacteria: Fusobacteriales: Fusobacteriaceae: Fusobacterium
- **Infection**. Fusobacterium necrophorum (Lemierre syndrome, sore throat, peritonsillar abscess)

Porphyromonas

- **Lineage**. Bacteroidetes: Bacteroidales: Porphyromonadaceae: Porphyromonas
- **Infection**. Porphyromonas gingivalis (gingivitis, sometimes leading to ANUG and noma, or cancrum oris)

Prevotella

- **Lineage**. Bacteroidetes: Bacteroidales: Prevotellaceae: Prevotella
- **Infection**. Prevotella dentalis (abscesses, bacteraemia, wound infection, bite infections, genital tract infections, and periodontitis)
- **Infection**. Prevotella species (supragingival plaque and halitosis in children)

Section 3.8 Mollicutes

The old idea that wall-less bacteria, mycoplasmas, are phylogenetically remote from other (eu)bacteria is incorrect; the true mycoplasmas are merely "degenerate" clostridia.

Carl Woese [7]

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
```

```
  Epsilon Proteobacteria
Spirochaetes
Bacteroidetes
Fusobacteria
Firmicutes (low G+C Gram+)
  Bacilli
  Clostridia
  Mollicutes
    Anaeroplasmatales
      Erysipelothrix (genus)
    Mycoplasmataceae
      Mycoplasma (genus)
      Ureaplasma (genus)
Chlamydiae
Actinobacteria
```

Class Mollicutes is essentially synonymous with several other classes that appear in the literature: Class Aphragmabacteria, Class Tenericutes, and Class Mycoplasmas. Mollicutes (Latin "mollis," soft and "cutis," skin) are intracellular parasitic organisms that lack cell walls. Like many other parasitic organisms, they have jettisoned many of their inherited functionalities, preferring to live on the largesse of their hosts. Aside from shedding their cell walls (and the genes coding for cell walls), mollicutes have shrunk to about 0.2 μm; and they have a remarkably small genome (under 1000 kbases).

The mollicutes, with no cell wall to absorb the Gram stain, are technically not Gram positive. Nonetheless, the mollicutes are usually counted among the Gram-positive organisms, as they lack the outer membrane that is characteristic of Gram-negative organisms. Sequence similarities for ribosomal RNA and a low G+C ratio (about 35%) suggest that the mollicutes are close relatives of Class Bacilli and Class Clostridia. For this reason, Class Mollicutes is often included, along with Class Bacilli and Class Clostridia, as a subclass of Class Firmicutes, the Gram-positive low G+C bacteria.

The mollicutes have exempted themselves from the strict, universal code that controls the translation of RNA into protein. Virtually every organism on earth uses the triplet codon UGA to code for "stop," thus signaling an interruption in RNA translation. Mollicutes are the exception, using UGA as a codon for tryptophan. It has been suggested that the low G+C content of mollicutes encourages the evolution of synonymous codons containing the overly abundant A (Adenine) or U (Uracil). In cells other than mollicutes, there are three synonymous "stop" codons: UAA, UAG, and UGA; while there is only one tryptophan codon: UGG. Mollicutes supplement UGG, the normal codon for tryptophan, with a codon that contains adenine and uracil, UGA.

Though it is relatively easy to detect the presence of mollicute species in human tissues, using PCR techniques, these organisms cannot be cultured with

any regular success. Consequently, medical scientists have implicated numerous members of Class Mollicutes as human pathogens, without fulfilling all of the rigorous studies that fully establish disease causation (i.e., they have not produced disease in humans or animals by inoculating the cultured organisms). In this chapter, we cover some of the less controversial mollicute pathogens. There are three genera of Class Mollicutes that cause diseases in humans: Erysipelothrix, Mycoplasma, and Ureoplasma.

```
Mollicutes
  Anaeroplasmatales
    Erysipelothrix (genus)
```

Genus Erysipelothrix contains one infectious species; Erysipelothrix rhusiopathiae, the cause of erysipeloid, a type of cellulitis (subcutaneous infection). Erysipeloid is usually a mild condition, typically occurring on the hands of workers who are exposed to the bacteria when they handle infected fish or meat. Students should not confuse erysipeloid with the similar-sounding disease, erysipelas. Both erysipeloid and erysipelas are types of cellulitis. Erysipelas is caused by members of Genus Streptococcus (Class Bacilli) and is a more common and, potentially, more serious disease than erysipeloid. Two additional similar-sounding skin conditions are erythrasma, characterized by brown scaly skin patches; caused by Corynebacterium minutissimum (Class Actinobacteria), and erythema infectiosum, caused by Parvovirus B19. All four skin conditions are associated with reddened skin, and all three diseases take their root from the Greek, "erusi," meaning red.

```
Mollicutes
  Mycoplasmataceae
    Mycoplasma (genus)
    Ureaplasma (genus)
```

Genus Mycoplasma contains two accepted human pathogens: Mycoplasma pneumoniae and Mycoplasma genitalium. Mycoplasma pneumoniae, as its name suggests, causes pneumonia. It is the most common cause of pneumonia in young adults. The pneumonia produced tends to be somewhat mild and chronic (the so-called walking pneumonia), unlike the acute and fulminant pneumonias produced by other bacteria.

Mycoplasma genitalium is a common cause of sexually transmitted urethritis.

Mycoplasma hominis inhabits the human genital tract and is a suspected cause of some cases of pelvic inflammatory disease in women.

Mycoplasma fermentans, Mycoplasma pirum, and Mycoplasma penetrans are additional Mycoplasma species that are being studied as potential human pathogens.

Genus Ureoplasma contains two putative infectious species: Ureoplasma urealyticum and the closely related Ureoplasma parvum. They are found in

the genital tracts of a very high percentage of sexually active, healthy humans (about 70%); thus, their role as pathogens in genital and perinatal diseases is somewhat controversial. It is suspected that Ureaplasma species account for some cases of urethritis.

Infectious Genera

Erysipelothrix

- **Lineage**. Mollicutes: Anaeroplasmatales: Erysipelothrix
- **Infection**. Erysipelothrix rhusiopathiae (erysipeloid, a cellulitis)

Mycoplasma

- **Lineage**. Mollicutes: Mycoplasmataceae: Mycoplasma
- **Infection**. Mycoplasma genitalium (urethritis)
- **Infection**. Mycoplasma pneumoniae (mycoplasma pneumonia)

Ureoplasma

- **Lineage**. Mollicutes: Mycoplasmataceae: Ureaplasma
- **Infection**. Ureaplasma urealyticum (urethritis)
- **Infection**. Ureaplasma urealyticum (urethritis)

Section 3.9 Class Bacilli plus Class Clostridia

We need above all to know about changes; no one wants or needs to be reminded 16 hours a day that his shoes are on.

David Hubel

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes (low G+C Gram+ bacteria)
    Mollicutes
    Bacilli
      Bacillales (catalase positive)
        Listeriaceae
          Listeria (genus)
        Staphylococcaceae
          Staphylococcus (genus)
```

```
        Bacillaceae
          Bacillus (genus)
      Lactobacillales (catalase negative)
        Enterococcaceae
          Enterococcus (genus)
        Streptococcaceae
          Streptococcus (genus)
  Clostridia
    Clostridiales
      Clostridiaceae
        Clostridium (genus)
        Peptostreptococcus (genus)
    Veillonellaceae
      Veillonella (genus)
Chlamydiae
Actinobacteria
```

Class Bacilli and Class Clostridia constitute a group of bacteria that, together, are sometimes called Class Firmicutes. In this book, the class name "Firmicutes" is abandoned because it has been used, at various times, to include Class Mollicutes. Still, Class Bacilli and Class Clostridia are sister classes and share a number of important phylogenetic properties that are best discussed together. The class name "Bacilli" is somewhat confusing inasmuch as the word "bacillus" (small "b") merely indicates that an organism is rod-shaped, and does not qualify an organism for inclusion in Class Bacilli. There are many different rod-shaped (hence, bacillary) organisms that are unrelated to members of Class Bacilli. Furthermore, there are members of Class Bacilli that are not bacilli (i.e., not rod-shaped). Most prominently, Staphylococcus and Streptococcus are coccoid and nonbacillary genera of Class Bacilli.

Class Clostridia and Class Bacilli are characterized by Gram-positive species that have a low G+C ratio (a feature that distinguishes this group from Class Actinobacteria, whose members are Gram positive, with a high G+C ratio). Bacteria in Class Clostridia and Class Bacilli have a propensity for synthesizing biologically active chemicals, accounting for several of the most potent toxins in biology (e.g., botulinum toxin, tetanospasmin). Members of Class Bacilli and Class Clostridia tend to be short rods (bacilli) or round (cocci), anaerobic, and capable of forming endospores. Endospore formation, though not present in all members of Class Bacilli and Class Clostridia, is never seen outside these classes.

Bacterial endospores, often referred to by the shortened form, "spores," are fundamentally different from the spores produced by eukaryotes (i.e., endospores are not the equivalent of the germinative cell of a multistage life cycle). Bacterial endospores are simplified forms of the bacteria, consisting of the DNA genome, some small amount of cytoplasm, and a specialized coating

that confers resistance to heat, radiation, and other harsh external conditions. Endospores are virtually immortal, and can be reactivated, under favorable growth conditions, after lying dormant for hundreds or perhaps millions of years [42]. Due to their ability to grow under anaerobic conditions, and to lay dormant for long periods, it can be nearly impossible to prevent infections caused by spore-forming pathogenic species of Class Bacilli and Class Clostridia.

```
Bacilli
  Bacillales (catalase positive)
    Listeriaceae
      Listeria (genus)
    Staphylococcaceae
      Staphylococcus (genus)
    Bacillaceae
      Bacillus (genus)
  Lactobacillales (catalase negative)
    Enterococcaceae
      Enterococcus (genus)
    Streptococcaceae
      Streptococcus (genus)
```

The human pathogens in Class Bacilli are split into two groups: Class Bacillales, the catalase positive genera; and Class Lactobacillales, the catalase negative genera.

```
Bacilli
  Bacillales (catalase positive)
    Listeriaceae
      Listeria (genus)
```

Listeria monoctyogenes is the organism that causes listeriosis. Though Listeria monocytogenes is widely known as a cause of food-borne outbreaks, readers should understand that it is an opportunistic infection that rarely causes disease when it infects healthy adults. Disease, when it occurs, often manifests as sepsis or meningitis, and can be fulminant, with a high mortality (25%). Listeria is one of the few bacterial organism that produce meningoencephalitis. Other bacterial causes of meningitis include Neisseria meningitidis (Class Beta Proteobacteria), and Elizabethkingia meningoseptica (Class Bacteroidetes). Listeriosis should not be confused with the similar-sounding disease, leptospirosis (Class Spirochaetae).

Another species of Genus Listeria is Listeria ivanovii, a pathogen for nonhuman animals, particularly ruminants. Like Listeria monocytogenes, it is opportunistic, and is a rare cause of disease in immunocompromised individuals [43]. It can produce an enteritis with bacteremia.

```
Bacilli
  Bacillales (catalase positive)
    Staphylococcaceae
      Staphylococcus (genus)
```

Members of Genus Staphylococcus are round cocci (from the Greek, "kokkos" meaning berry-like) despite their inclusion in Class Bacilli (from the Latin, "bacillum," meaning small rod). The two most prevalent pathogenic species are Staphylococcus aureus and Staphylococcus epidermidis (Fig. 3.13).

Staphylococcus aureus lives as a nonpathogenic commensal on the skin and nasal mucosa of a significant portion of the human population (about 20%). It can cause skin disease (acne, skin abscesses, cellulitis, staphylococcal scalded skin syndrome) and is a common source of wound infections. If sepsis occurs, it can produce multiorgan disease, and is a cause of toxic shock syndrome in women. It is a common cause of nosocomial (hospital-acquired) infections, and strains of Staphylococcus aureus have emerged that are resistant to standard antibiotic treatment. The bacteria produces a toxin that causes enteritis when ingested with food that has been colonized by the bacteria.

Staphylococcus epidermidis is a commensal that lives on human skin. It is nonpathogenic in most circumstances. Chronically ill patients with indwelling catheters are prone to urinary tract infections caused by Staphylococcus epidermidis. This organism can grow as a biofilm, enhancing its ability to glide over surfaces (such as catheters). Nosocomial opportunistic organisms, that glide into tissues via an invasive instrumented portal (e.g., catheter, intravenous line, pulmonary assistance tubing) were described earlier with Class

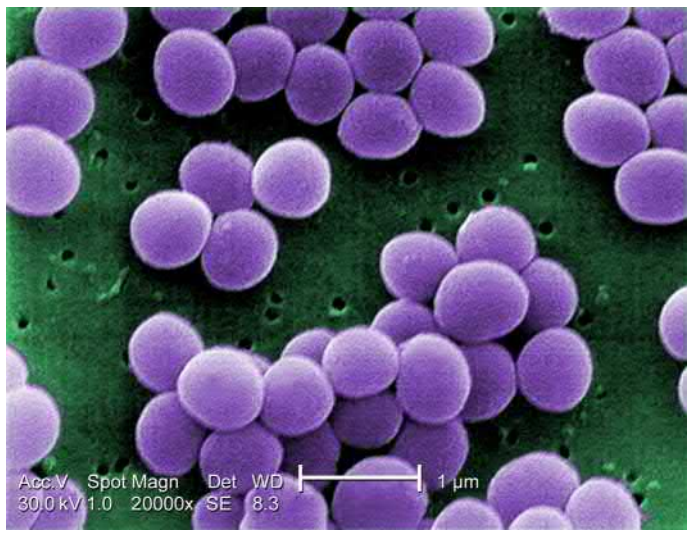

FIG. 3.13 Photomicrograph of clumps of uniform, round Staphylococcus aureus organisms. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Pseudomonadales (Class Gamma Proteobacteria) and again with the gliding bacteria (Class Bacteroidetes and Class Fusobacteria).

```
Bacilli
  Bacillales (catalase positive)
    Bacillaceae
      Bacillus (genus)
```

Bacillus cereus causes food-borne enteritis. Bacillus cereus endospores can survive conditions that would kill other bacterial forms. Consequently, food poisoning due to Bacillus cereus occurs under similar conditions as food poisoning due to Clostridium perfringens (vida infra) or Staphylococcus aureus (vida supra). Depending on the strain of Bacillus cereus and the conditions of its growth, an enterotoxin may accumulate in contaminated food. If preformed enterotoxin is present in contaminated food, emesis often results, within a few hours, and the condition may simulate Staphylococcus aureus food poisoning (vida supra). If no enterotoxin is present in food contaminated with Bacillus cereus, diarrhea usually begins after about 10 h, and the condition may simulate infections with Clostridium perfringens.

Bacillus anthracis is the cause of anthrax, an acute disease that is often fatal if not treated quickly and aggressively. The disease is transmitted by endospores (not by active bacteria) that are, in most cases, spread by infected animals. Animals become infected by grazing on plants and soil containing long-dormant spores, or by eating an actively infected animal. Humans become infected by inhaling endospores emanating from the carcass of a dead infected animal (leading to pulmonary anthrax), by eating undercooked infected animals (leading to enteric anthrax), or by handling infected animals, with spores entering the skin through abrasions (leading to cutaneous anthrax). Anthrax has been weaponized by various combatant entities over the decades, but its long dormancy and the difficulty in containing spores within a specified target location have made this weapon a double-edged sword [44] (Fig. 3.14).

```
Bacilli
  Lactobacillales (catalase negative)
    Enterococcaceae
      Enterococcus (genus)
```

Species of Genus Enterococcus are normal inhabitants of the GI tract. Disease most often occurs in a hospital setting, in weakened patients who have had surgery, or who have indwelling devices (e.g., urinary catheters). Urinary tract infections or bacteremia and its sequelae (e.g., endocarditis, meningitis) have been associated with Enterococcus faecalis and Enterococcus faecium species.

```
Bacilli
  Lactobacillales (catalase negative)
    Streptococcaceae
      Streptococcus (genus)
```

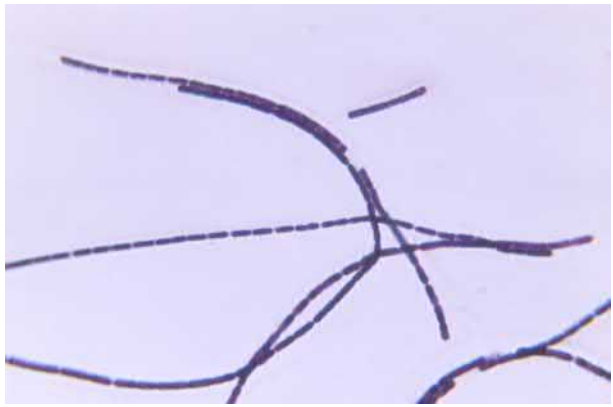

FIG. 3.14 Gram-stained photomicrograph Bacillus anthracis. Numerous Gram-positive rods (bacilli) grouped end to end to form long strands. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Genus Streptococcus contains numerous pathogenic and nonpathogenic species (too many to describe here).

Streptococcus pneumoniae, as its name suggests, causes pneumonia. It may also cause disease via nasopharyngeal spread (e.g., otitis media, sinusitis, meningitis).

Streptococcus pyogenes is a very common cause of human infection, producing pharyngitis (strep throat) and skin infections (streptococcal impetigo). Toxin-producing strains can produce scarlet fever (systemic symptoms plus rash). Bacteremia may lead to toxic shock syndrome. Following infection with streptococcus pyogenes, an immune response cross-reacting between bacterial antigens and normal host proteins (e.g., muscle proteins, glomerular basement membranes) may lead to rheumatic fever or glomerulonephritis.

```
Clostridia
  Clostridiales
    Clostridiaceae
      Clostridium (genus)
      Peptostreptococcus (genus)
```

Class Clostridia is composed of obligate anaerobic organisms (unlike Class Bacilli, which includes facultative anaerobes). Class Clostridia has two genera that contain human pathogens: Clostridium and Peptostreptococcus.

Genus Clostridium contains four species that commonly produce disease in humans: Clostridium botulinum, Clostridium difficile, Clostridium perfringens, and Clostridium tetani.

Clostridium botulinum produces botulinum toxin, one of the most powerful poisons in existence. Disease in adults is caused by ingesting the preformed toxin produced by bacteria growing in contaminated food. Because all members

of Class Clostridia are obligate anaerobes, contamination occurs in foods stored in anaerobic conditions (e.g., cans), without first killing all the bacteria and spores. When adults ingest Clostridium bacilli, the bacteria are usually killed by competing organisms in the intestinal tract. The ingested toxin causes the disease known as botulism. In infants, ingested bacteria may survive in the intestinal tract, actively producing toxin. Spores of Clostridium botulinum in honey have been known to produce active Clostridium botulinum infection in children.

Clostridium difficile inhabits human intestine and is nonpathogenic under normal circumstances. After long-term antibiotic use, when many of the normal gut bacteria are reduced in number, an overgrowth of Clostridium difficile may cause severe GI disease (the so-called pseudomembranous colitis). Colon ulcerations have an overlying pseudomembrane (i.e., a membrane not formed as a biological construction by living organisms). The pseudomembrane, consisting of a layer of necrotic mucosal cells admixed with inflammatory cells, is the hallmark of this disease.

Clostridium perfringens is a ubiquitous organism that is sometimes found in the human GI tract, without causing disease. It is a common cause of food poisoning. When contaminated food is ingested, diarrhea often follows, in about 10 hours, in susceptible individuals (some individuals are resistant to enteric disease). Clostridium perfringens is a common infection in necrotic tissue, due to the anoxic conditions therein. The organism causes the so-called gas gangrene (tissue necrosis accompanied by the liberation of bacterial-produced gas). Clostridia perfringens also causes emphysematous gangrenous cholecystitis, a condition occurring with gallbladder necrosis, in which the necrotic gallbladder tissue is infiltrated by gas produced by the organism. The ability of Clostridium perfringens to produce gas has a beneficial purpose: used as a leavening agent in baked goods.

Clostridium tetani is the cause of tetanus. Spores live in soil, and human infection usually follows the mechanical introduction of soil-borne spores into a wound. The organism produces a potent neurotoxin that manifests clinically as muscle rigidity: risus sardonicus (rigid smile), trismus (also known as lock-jaw, rigid jaw), and opisthotonus (rigid, arched back).

Though only members of Class Bacilli and Class Clostridia have the ability to form endospores, not all members of these two classes are spore-forming. Genus Peptostreptococcus is an exception. Species of genus Peptostreptococcus are found as commensals in virtually every type of mucosa that lines humans. They have pathogenic potential when they are traumatically introduced deep into tissues, when the host becomes weakened from concurrent chronic infections, or when the host becomes immunodeficient. Under these circumstances, they can produce sepsis, with abscesses occurring in multiple organs (Fig. 3.15).

```
Clostridia
  Veillonellaceae
    Veillonella (genus)
```

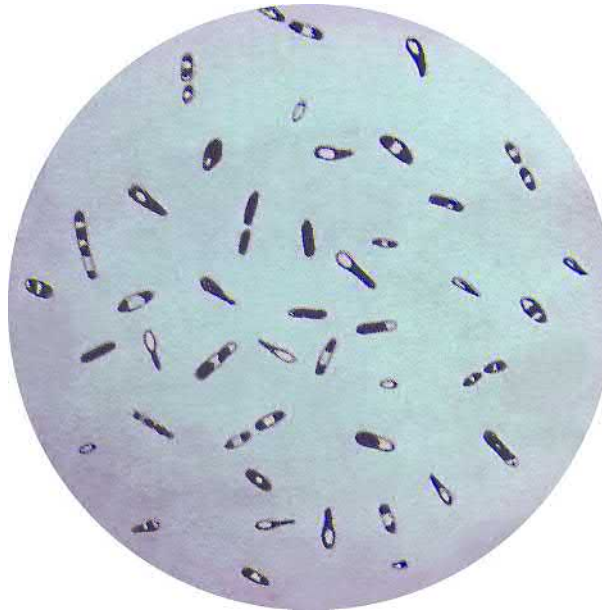

FIG. 3.15 Photomicrograph of a Gram-stained culture of Clostridium feseri, bacteria (blue). The poorly stained round structures are endospores. *(Source, a public domain image from the US Center for Disease Control and Prevention.)*

Species in Genus Veillonella are normal inhabitants of the GI tract of humans and other mammals. As with infections from Peptostreptococcus species, Veillonella species may cause sepsis, with multiorgan disease in predisposed individuals. Veillonella infections are rare.

Infectious Genera

Listeria

- **Lineage**. Bacilli: Bacillales: Listeriaceae: Listeria
- **Infection**. Listeria ivanovii (in immune deficient hosts)
- **Infection**. Listeria monocytogenes (listeriosis)

Staphylococcus

- **Lineage**. Bacilli: Bacillales: Staphylococcaceae: Staphylococcus
- **Infection**. Staphylococcus aureus (Staphylococcal scalded skin syndrome, Toxic shock syndrome, acne, skin abscesses, cellulitis, sepsis, food poisoning)
- **Infection**. Staphylococcus epidermidis (catheter-transmitted urinary tract infections)

Bacillus

- **Lineage**. Bacilli: Bacillales: Bacillaceae: Bacillus

- **Infection**. Bacillus cereus (fried rice syndrome, small fraction of food-borne illnesses)
- **Infection**. Bacillus anthracis (anthrax)

Enterococcus

- **Lineage**. Bacilli: Lactobacillales: Enterococcaceae: Enterococcus
- **Infection**. Enterococcus species (bacteremia, diverticulitis, endocarditis, meningitis, urinary tract infections)

Streptococcus

- **Lineage**. Bacilli: Lactobacillales: Streptococcaceae: Streptococcus
- **Infection**. Streptococcus pneumoniae (pneumococcal pneumonia, childhood meningitis)
- **Infection**. Streptococcus agalactiae (bacterial septicemia of the newborn) [45]
- **Infection**. Streptococcus iniae (bacteremic cellulitis, sepsis, in immunodeficient individuals)
- **Infection**. Streptococcus pyogenes (Scarlet fever, Erysipelas, Rheumatic fever, Streptococcal pharyngitis, poststreptococcal glomerulonephritis)
- **Infection**. Streptococcus suis (multiorgan infections)

Clostridium

- **Lineage**. Clostridia: Clostridiales: Clostridiaceae: Clostridium
- **Infection**. Clostridium botulinum (botulism)
- **Infection**. Clostridium difficile (pseudomembranous colitis)
- **Infection**. Clostridium perfringens (gas gangrene, clostridial necrotizing enteritis, food poisoning, emphysematous cholecystitis)
- **Infection**. Clostridium tetani (tetanus)

Peptostreptococcus

- **Lineage**. Clostridia: Clostridiales: Clostridiaceae: Peptostreptococcus
- **Infection**. Peptostreptococcus species, formerly Peptococcus species, including Peptostreptococcus magnus (septicemia, organ abscesses, cellulitis, particularly in immunocompromised patients)

Section 3.10 Chlamydiae

Simplicity, carried to the extreme, becomes elegance.

Jon Franklin

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
```

```
    Beta Proteobacteria
    Gamma Proteobacteria
    Epsilon Proteobacteria
  Spirochaetes
  Bacteroidetes
  Fusobacteria
  Firmicutes
    Bacilli
    Clostridia
    Mollicutes
  Chlamydiae
    Chlamydiae
      Chlamydiales
        Chlamydiaceae
          Chlamydophila (genus)
          Chlamydia (genus)
  Actinobacteria
```

All members of Class Chlamydiae are obligate intracellular pathogens (like the Rickettsia, members of Alpha Proteobacteria). All Chlamydiaceae are Gram negative, and express the same lipopolysaccharide epitope that has only been observed in Class Chlamydiaceae. Members of Class Chlamydiaceae are extremely small, less than 1 μm in size. These organisms grow exclusively within eukaryotic cells. Two genera of Chlamydiaceae contain human pathogens: Chlamydia and Chlamydophila. Genus Chlamydia and Genus Chlamydophila are closely related and, prior to 1999, all of the group species were assigned to Genus Chlamydia. Molecular studies indicated that these two genera can be cleanly distinguished from one another, based on genome size, DNA reassociation, and sequence dissimilarities.

Most obligate intracellular infectious agents require a vector to transfer themselves from one host to another. For example, rickettsia are transmitted via fleas, ticks, or lice. Malaria and babesiosis, caused by obligate intracellular members of Class Apicomplexa, are transmitted by arthropods. Surprisingly, the human pathogens in Class Chlamydiaceae manage to move from one host to another, without the aid of a vector. How do they do it? These tiny organisms create even smaller, infective forms, known as elementary bodies. Elementary bodies have a rigid outer membrane and are resistant to environmental conditions outside their hosts. They travel in expelled droplets, in the case of a pneumonic infection, or in secretions, in the case of a venereal infection or an eye infection. The elementary bodies attach to host cells membranes, and are internalized within host cell endosomes, where they remain. Elementary bodies transform into reticulate bodies, the metabolically active form of the organism. Chlamydia and Chlamydophila organisms inhibit the fusion of host endosomes with host lysosomes, and thus escape the normal cellular mechanism by which phagocytozed bacteria are killed by eukaryotes. This bacterial

survival trick is similar to that employed by Coxiella burnetii, another obligate intracellular bacteria that is resistant to degradation by host lysosomes. When the endosome is filled with organisms of Class Chlamydiaceae, the enlarged endosome becomes a cytoplasmic inclusion body, visible under the light microscope. Active infections are characterized by eukaryotic cell lysis. Most cases of infection with Chlamydia or Chlamydophila are asymptomatic, indicating that cytopathic effects are often minimal. Immune deficiency exacerbates the clinical virulence of infections caused by pathogenic members of Class Chlamydiae.

```
Chlamydiae
  Chlamydiae
    Chlamydiales
      Chlamydiaceae
        Chlamydophila (genus)
        Chlamydia (genus)
```

In terms of documented infections and disease, the most clinically important species in Class Chlamydiaceae is Chlamydia trachomatis. This species can be divided into several biological types, and the biological types can be subdivided into distinct serologic variants (serovars). The different diseases caused by Chlamydia trachomatis are each associated with their own variants of the species. Estimates suggest that worldwide, more than half a billion people are infected with one or another subtypes of Chlamydia trachomatis.

Infection by Chlamydia trachomatis is the second most common sexually transmitted disease, with about 4 million new cases occurring annually in North America [18]. The disease name for this infection is somewhat confusingly called “Chlamydia,” perhaps the only disease that takes the name of a genus, without modification. The proper disease name “chlamydiosis” is reserved for infections by another species, Chlamydophila psittaci (vida infra). This is an etymologic disaster, as Chlamydophila psittaci infection is known by most clinicians as psittacosis, and would more accurately be called chlamydophilosis, in any event. In men and women, chlamydia can produce urethritis and rectal inflammation. Chlamydia can also produce prostatitis in men. In women, infections that ascend the genital tract can yield endometritis, salpingitis, and pelvic inflammatory disease. Infants born to infected mothers may develop inclusion conjunctivitis (named for the cytoplasmic inclusion bodies produced by chlamydial organisms), and chlamydial pneumonia (Fig. 3.16).

The high infection rate in the population is made possible, in part, by the high prevalence of asymptomatic carriers: about one-third of infected men and women have no clinical symptoms. In addition, infection does not confer immunity, and re-infections are common.

According to the World Health Organization, there are about 37 million blind persons, worldwide. Trachoma, caused by Chlamydia trachomatis, is

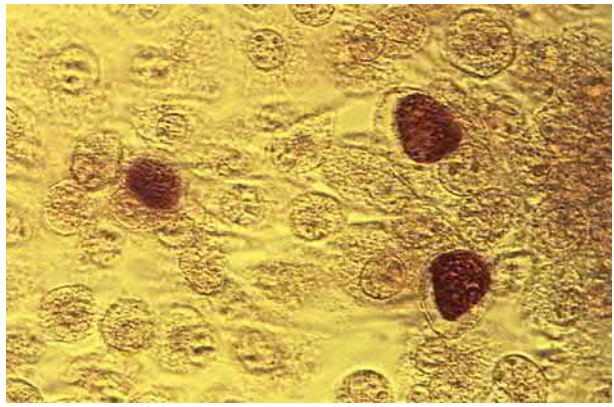

FIG. 3.16 Chlamydia trachomatis growing in tissue culture monolayer of McCoy cells (mouse fibroblast origin). Large round endosomes containing C trachomatis reticulate bodies are visible by phase contrast microscopy. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, photography by Drs. E. Arum and N. Jacobs.)*

the number one infectious cause of blindness and accounts for about 4% of all cases. The second most common infectious cause of blindness worldwide is Onchocerca volvulus (Class Nematoda), accounting for about 1% of all cases [14]. The number one cause of blindness worldwide is cataracts, causing nearly half of all cases. Trachoma is spread by direct or indirect contact with eye secretions. Infection causes intense inflammation of the conjunctiva.

Readers should not confuse trachoma with inclusion conjunctivitis, as each disease is caused by distinct variants of the same species (Chlamydia trachomatis). Trachoma is contracted by exposure to eye secretions from people with trachoma. Inclusion conjunctivitis is caused by ocular exposure to secretions from the sexually transmitted infection.

Chlamydia trachomatis may also cause lymphogranuloma venereum, a disease that usually presents as swollen lymph nodes in the groin. The lymph nodes often have draining abscesses. The disease is rare, with only a few hundred cases occurring in the United States each year. Lymphogranuloma venereum must not be confused with granuloma inguinale, also known as granuloma venereum, caused by the bacterium Klebsiella granulomatis.

Chlamydophila psittaci is the cause of psittacosis, a disease that takes its name from birds of Class Psittaciformes (i.e., parrots) the organism's animal reservoir. Chlamydophila psittaci infects the lungs of birds. The birds pass the infection to human through droplet secretions.

Chlamydophila pneumoniae is a significant cause of pneumonia in the United States. The disease is often mild. In addition, Chlamydophila pneumoniae has been associated with coronary artery disease and stroke [46]. The organism can infect the endothelial cells of coronary arteries, and has been found in atherosclerotic plaques. At this time, a causative role in atherogenesis has not been demonstrated (Fig. 3.17).

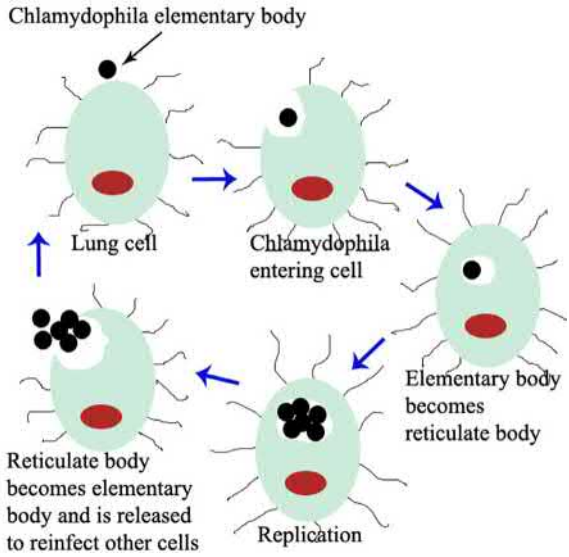

FIG. 3.17 Graphic depicting the life cycle of Chlamydophila pneumoniae. Infectious elementary bodies grow within vacuoles in human cells to become reticulate bodies. *(Source, Wikipedia, and placed into the public domain by its author, InvictaHOG.)*

Infectious Genera

Chlamydophila

- **Lineage**. Chlamydiae: Chlamydiae: Chlamydiales: Chlamydiaceae: Chlamydophila
- **Infection**. Chlamydophila psittaci (psittacosis)
- **Infection**. Chlamydophila pneumoniae, formerly TWAR serovar, TWAR agent, or Chlamydia pneumoniae (pneumonia)

Chlamydia

- **Lineage**. Chlamydiae: Chlamydiae: Chlamydiales: Chlamydiaceae: Chlamydia
- **Infection**. Chlamydia trachomatis (trachoma, genital infection)

Section 3.11 Actinobacteria

Before I speak, I have something important to say.

Groucho Marx

```
Bacteria
  Proteobacteria
    Alpha Proteobacteria
```

```
  Beta Proteobacteria
  Gamma Proteobacteria
  Epsilon Proteobacteria
Spirochaetes
Bacteroidetes
Fusobacteria
Firmicutes, low G+C Gram+
  Bacilli
  Clostridia
  Mollicutes
Chlamydiae
Actinobacteria, high G+C Gram+
  Actinomycetales
    Actinomycetaceae
      Actinomyces (genus)
      Arcanobacterium (genus)
    Corynebacterineae
      Corynebacteriaceae
    Corynebacterium (genus)
      Dermatophilaceae
    Dermatophilus (genus)
      Mycobacteriaceae
    Mycobacterium (genus)
      Nocardiaceae
        Nocardia (genus)
        Rhodococcus (genus)
    Cellulomonadaceae
        Tropheryma (genus)
    Propionibacteriaceae
      Propionibacterium (genus)
    Streptosporangineae
      Thermomonosporaceae
    Actinomadura (genus)
  Bifidobacteriales
      Bifidobacteriaceae
        Gardnerella (genus)
```

There are two large classes within the Gram-positive group (not all of which are actually Gram positive). These are The Firmicutes (containing Class Bacilli, Class Clostridia, and Class Mollicutes) and the Actinobacteria. The Firmicutes are characterized by low G+C DNA. The Actinobacteria are characterized by high G+C (Guanine plus Cytosine) DNA.

Members of Class Actinobacteria tend to be filamentous, and this morphologic feature has led to great confusion. In the past, these filamentous bacteria were mistaken for fungal hyphae, and many of the diseases caused by members

of Class Actinobacteria were mistakenly assigned fungal names (e.g., actinomycosis, mycetoma, maduromycosis).

Molecular analysis of the actinobacteria indicates that they are bacteria that share a high degree of sequence similarity among the subclasses [7]. High sequence similarity among sister subclasses is generally interpreted to mean that the classes are young (i.e., descended from a common ancestor relatively recently, before their genomes had an opportunity to diverge).

```
Actinobacteria
  Actinomycetales
    Actinomycetaceae
      Actinomyces (genus)
      Arcanobacterium (genus)
```

Actinomyces is the cause of the so-called actinomycosis (Greek "actino," ray and "myco," fungus), a suppurative condition arising in the oral cavity and nasopharynx, characterized by acute and chronic inflammation and the discharge of the so-called sulfur granules. Despite its deceptive name, actinomycosis is a bacterial infection, not a fungal (i.e., mycotic) infection. Actinomycotic abscesses can be caused by any of several species of Genus Actinomyces (e.g., Actinomyces israelii or Actinomyces gerencseriae), as well as closely related species (e.g., Propionibacterium propionicus). Sulfur granules are yellow flecks found mixed with inflammation and extruded in exudate, consisting of numerous bacterial filaments, often radiating from a core (hence the prefix "actino") and resembling fungal hyphae (hence the suffix "mycosis"). Actinomyces species inhabit normal mouths, and sulfur granules are frequently found in the tonsillar crypts of healthy individuals (Fig. 3.18).

Several species closely related to Genus Actinomyces can produce an allergic disease of the lung after chronic inhalation (e.g., Micropolyspora faeni and less commonly Saccharopolyspora rectivirgula). This disease, which is not a true infection, is known by a number of different names, including farmer's lung.

Genus Arcanobacterium contains Arcanobacterium haemolyticum, formerly assigned to a different genus, as Corynebacterium haemolyticum. Arcanobacterium haemolyticum is a cause of pharyngitis.

```
Actinobacteria
  Actinomycetales
    Corynebacterineae
      Corynebacteriaceae
        Corynebacterium (genus)
      Dermatophilaceae
        Dermatophilus (genus)
      Mycobacteriaceae
        Mycobacterium (genus)
      Nocardiaceae
        Nocardia (genus)
        Rhodococcus (genus)
```

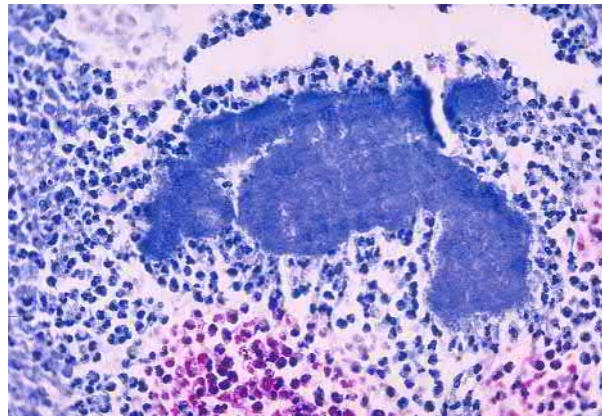

FIG. 3.18 Biopsy of an abscess produced by infection with Actinomyces israelii, in this case. In the center of the image is the so-called sulfur granule, which is composed largely of dead Actinomyces organisms and other cellular debris; not sulfur. Around the sulfur granules is a dense collection of white blood cells, predominantly granulocytes, characteristic of an abscess. Abscesses caused by Actinomyces infection typically occur in the oral cavity, often in or near the tonsils. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Members of Class Corynebacterineae share a distinctive and complex cell-wall composition. Most species contain mycolic acids in their cells walls, linked to peptidoglycans. Class Corynebacterineae, like all the members of Class Actinobacteria, are Gram positive. Class Corynebacterineae (particularly Genus Mycobacteria and Genus Nocardia) resist decolorization with acid. The term "acid fast" refers to this property, in which the Gram stain is "fastened" to the cell wall. Mycolic acid contributes to "acid-fastness." Pathologists employ the so-called acid-fast stains (technically, the organism is acid-fast, not the stains) to identify pathogenic organisms in this group.

Class Corynebacterium, a subclass of Class Corynebacterineae, contains several pathogenic species.

Corynebacterium minutissimum causes erythrasma, a skin rash. Corynebacterium jeikeium, which is normally confined to skin, tracks into blood via indwelling devices, causing opportunistic nosocomial infections (e.g., sepsis, endocarditis) in immunocompromised patients, particularly those who have received bone marrow transplants [47].

Corynebacterium diphtheriae is the cause of diphtheria, a disease that occurs where vaccination is underutilized. The disease usually presents as a sore throat, covered with a characteristic membrane. The term diphtheria has its root in the Greek work, "diphthera" meaning two leather scrolls, referring to the thick, double membrane. The most aggressive strains of the organism produce a toxin, encoded by a phage (bacterial virus) in the genome. The toxin contributes to tissue necrosis. Some cases of diphtheria involve the skin (Fig. 3.19).

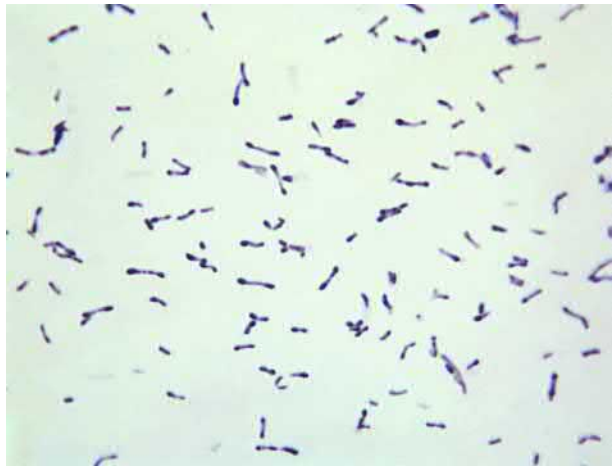

FIG. 3.19 Gram stain of Corynebacterium diphtheriae, a club-shaped Gram-positive bacteria. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Readers should be aware of the highly confusing term, "diphtheroid," commonly applied to all the nonpathogenic species within Genus Corynebacterium. As nonpathogens, the diphtheroids do not cause diphtheria. Diphtheria is caused by Corynebacterium diphtheriae (i.e., a nondiphtheroid).

Species in Genus Dermatophilus (meaning "skin loving") produce skin lesions in a variety of animals, including humans [48]. Pitted keratolysis is a skin condition characterized by the appearance of discoloration on the palms or the plantar surfaces, with superficial craters. It may arise from various species of Genus Dermatophilus, Dermatophilus congolensis among them [49]. Human infections may occur following exposure to infected animals. Human-to-human contagion does not seem to be a mode of transmission (Fig. 3.20).

About 2 billion people (of the world's 7 billion population) have been infected with Mycobacterium tuberculosis. Most infected individuals never develop overt disease. Nonetheless, tuberculosis kills about 3 million people each year [50]. Disease is transmitted from humans who have active, untreated disease, often via aerosolized droplets. The disease usually presents as a granulomatous process in the lungs. Multiorgan involvement may occur if the lung disease is not adequately treated. Aside from Mycobacteria tuberculosis, several other species may cause a tuberculosis-like disease. These species include: Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canettii, and Mycobacterium microti (Fig. 3.21).

Mycobacterium xenopi produces a chronic pulmonary disease simulating tuberculosis. M. xenopi does not seem to be transmitted from person to person. The organism has been isolated from water and soil, and the presumed mode of transmission is through environmental exposure. Though the disease

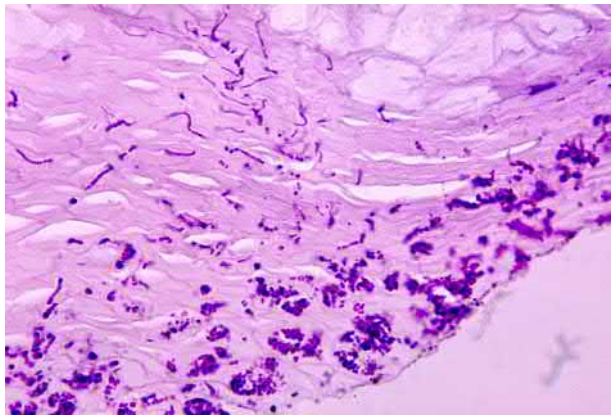

FIG. 3.20 Tissue biopsy demonstrating pitted keratolysis due to a Dermatophilus species (PAS stain). Numerous small bacteria forming filaments of organisms are observed in the superficial layers of the epidermis. Pitted keratolysis most frequently occurs on the soles of the feet or on the palms. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

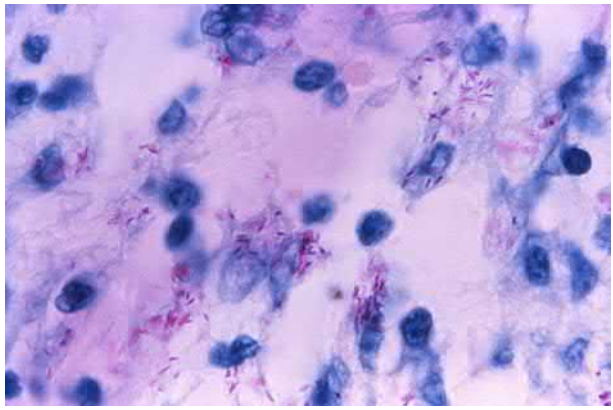

FIG. 3.21 Skin biopsy showing Mycobacterium, species unidentified. The mycobacteria are visualized as purple rods, many inside cells. In extremely heavy infections, the rods may appear bundled in packets. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

is currently rare in the United States, cases are not unusual in England, Europe, and Canada.

Leprosy, also known as Hansen disease, is caused by Mycobacterium leprae and Mycobacterium lepromatosis. The disease produces granulomas in the skin, including the nerves of the skin, and the respiratory tract. Untreated leprosy is slowly progressive. Most human cases are transmitted by aerosolized droplets produced by actively infected and untreated patients. It is believed that animals, particularly the armadillo, are potential reservoirs. In 2005, there were about 300,000 new cases reported, worldwide [51].

Mycobacterium ulcerans causes Buruli ulcer, a skin condition seen in the tropics. Mycobacterium ulcerans produces a toxin, mycolactone, that is responsible for most of the tissue destruction observed clinically. Other mycobacteria that produce mycolactone include Mycobacterium liflandii, Mycobacterium pseudoshottsii and strains of Mycobacterium marinum. The disease does not appear to spread from human to human; aquatic insects are the suspected vectors.

The atypical mycobacteria are a group of about 13 organisms that are found in soil and water, as pathogens in animals, or even as commensals growing in the pharynx of humans. Diseases, when they occur in otherwise healthy individuals, tend to be mild, involving skin, lungs, or lymph nodes. The atypical mycobacteria pose a serious problem in immunocompromised patients, particularly those with AIDS. Organisms in the Mycobacterium-avium-intracellulare complex, found throughout the environment, are a particular threat for patients with advanced AIDS.

Genus Nocardia contains dozens of species that cause disease in animals. In humans, Nocardia organisms are opportunistic pathogens affecting children, the elderly, immunocompromised individuals, and patients with a preexisting serious disease. Nocardia asteroides is the most common cause of human nocardiosis. Pneumonia is a common presentation of nocardiosis. Encephalitis, endocarditis, abscess formation, and sepsis are also seen. Nocardiosis brasiliensis has been implicated in some cases of mycetoma. A full discussion of mycetoma is found under Genus Neotestudina in Section 6.4, "Ascomycota" (Fig. 3.22).

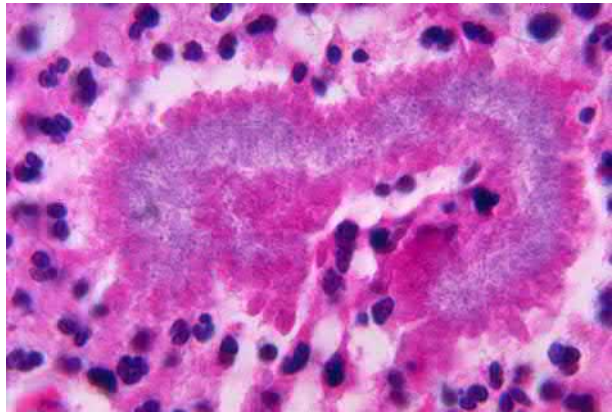

FIG. 3.22 Chronic inflammation due to infection with Nocardia brasiliensis. In the center of the lesion is a granule ringed by an intensely eosinophilic outer border. The center of the granule is composed predominantly of dead or degenerate bacteria mixed with necrotic cellular debris. The eosinophilic material is presumed to be largely inflammatory proteins (e.g., immunoglobulins and other immune-reactants) that had been attracted to the site of intense, long-standing bacterial growth. Around the granule are chronic inflammatory white blood cells, primarily eosinophils and lymphocytes. The eosinophilic-ringed granule is characteristic of the Splendore-Hoeppli phenomenon, and can be seen with a variety of infections and inflammatory conditions. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Readers should not be confused by the plethora of organisms with "brasiliensis" as the species of the binomen. These include Nocardia brasiliensis, Leishmania brasiliensis (alternately spelled Leishmania braziliensis), Paracoccidioides brasiliensis, and Borrelia brasiliensis. Brazil has inspired another infectious organism, spelled similarly to the others: the nematode Ancylostoma brasiliense.

Rhodococcus equi is the cause of foal pneumonia, or rattles, in young horses. It can infect immunocompromised humans, causing a disease that closely simulates tuberculosis.

```
Actinobacteria
  Actinomycetales
    Cellulomonadaceae
      Tropheryma (genus)
```

The only pathogenic species in Class Cellulomonadacea is Tropheryma whipplei, the cause of Whipple disease. As a general rule, bacteria in the human body are eaten by macrophages, wherein they are degraded. In the case of Tropheryma whipplei, certain susceptible individuals have a problem with the degradation of the organism within macrophages. Consequently, the organisms multiply within macrophages. When organisms are released from dying macrophages, additional macrophages arrive to feed, but this only results in the local accumulation of macrophages bloated by bacteria. Whipple disease is characterized by the infiltration of organs by foamy macrophages containing Tropheryma whipplei. The organ most often compromised is the small intestine, where infiltration of infected macrophages in the lamina propria (the connective tissue underlying the epithelial lining of the small intestine) causes malabsorption. Whipple disease is quite rare. It occurs most often in farmers and gardeners who work with soil, in which the organism lives. As recently as 1992, the cause of Whipple disease was unknown [52]. We will discuss Whipple disease in greater detail in Section 8.1, "Abandoning Koch's Postulates" [Glossary Koch's postulates].

Readers should not confuse the bacterial genus Tropheryma with the similar-sounding term Taphrinomycotina, the fungal genus that includes Pneumocystis (Class Ascomycota).

```
Actinobacteria
  Actinomycetales
    Propionibacteriaceae
      Propionibacterium (genus)
```

Class Propionibacteriaceae is named for a particular metabolic talent, propionic acid synthesis. Species in the class live in the intestinal tracts of various animals. In humans, species of Genus Propionibacterium are commensals that live within sweat glands. Some cases of acne and other skin conditions have been attributed to propionibacteria. As previously mentioned, Propionibacterium propionicus is another agent capable of causing the so-called actinomycotic abscess of the oral cavity.

```
Actinobacteria
  Actinomycetales
    Streptosporangineae
      Thermomonosporaceae
        Actinomadura (genus)
```

Genus Actinomadura contains species found in soil. These filamentous organisms were previously mistaken for fungal hyphae. The inflammatory skin condition, most commonly occurring on the foot (i.e., from the tissue most directly exposed to soil), and caused by bacterial species of the Actinomadura genus were given the fungal misnomers actinomycetoma and maduromycosis. Actinomadura madurae, like Nocardiosis brasiliensis (vida supra), has been implicated in some cases of mycetoma. A full discussion of mycetoma is under Genus Neotestudina in Section 6.4, "Ascomycota."

Persons who are immunocompromised may have heightened susceptibility to systemic infections with Actinomadura species [53] (Fig. 3.23).

```
Actinobacteria
  Bifidobacteriales
    Bifidobacteriaceae
      Gardnerella (genus)
```

Gardnerella vaginalis is the only species of Genus Gardnerella that is associated with a pathologic condition. G. vaginalis is a normal inhabitant of the vagina. Massive overgrowth of G. vaginalis, along with other vaginal bacteria produces a condition known by the generic and nondescriptive name of "vaginosis" (meaning condition of the vagina). Vaginosis raises the pH of the vaginal fluid and produces

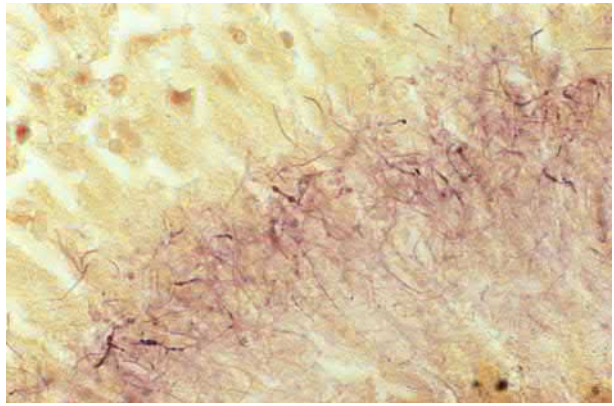

FIG. 3.23 Tissue section of the so-called Actinomycotic mycetoma (Brown and Brenn stain). Despite the name of this condition, which incorrectly indicates a fungal (i.e., mycotic) origin, this lesion is caused by a bacterial species. A band stretching from the lower left to the upper right of the image demonstrates filamentous bacteria consistent with Actinomadura madurae. The upper left of the section contains granulation tissue, and the lower right contains mostly necrotic debris. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

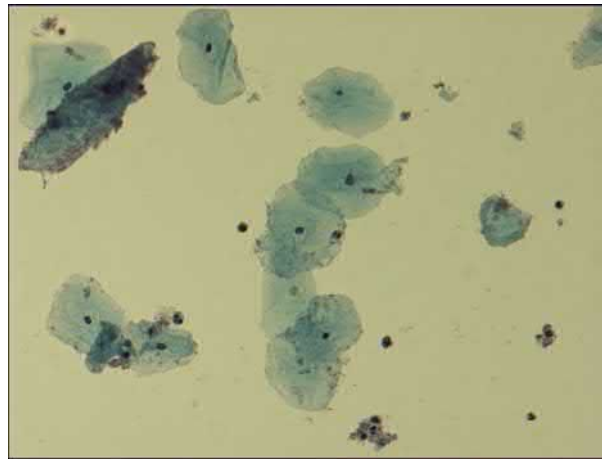

FIG. 3.24 Clue cells of Gardnerella vaginalis overgrowth. Gardnerella overgrowth can be observed in routine Pap smears as tiny organisms matting the surface of flattened squamous epithelial cells (the so-called "clue cells"). A dark clue cell, matted by small coccobacilli hugging its surface, is seen in the upper left corner of the image. *(Source, Wikipedia, from an image donated to the public domain by its author, Dr. F.C. Turner.)*

a malodorous discharge. The underlying causes of vaginosis are many (antibiotic use, douching, sexual activity), but all causes seem to lead to an overgrowth of G. vaginalis, which can be visualized on Pap smear preparations (Fig. 3.24).

Infectious Genera

Actinomyces

- **Lineage**. Actinobacteria: Actinomycetales: Actinomycetaceae: Actinomyces
- **Infection**. Actinomyces gerencseriae (dental plaque)
- **Infection**. Actinomyces israelii (formerly actinomycosis)

Arcanobacterium

- **Lineage**. Actinobacteria: Actinomycetales: Actinomycetaceae: Arcanobacterium
- **Infection**. Arcanobacterium haemolyticum, formerly Corynebacterium haemolyticum (pharyngitis)

Corynebacterium

- **Lineage**. Actinobacteria: Actinomycetales: Corynebacterineae: Corynebacteriaceae: Corynebacterium
- **Infection**. Corynebacterium diphtheriae (diphtheria)
- **Infection**. Corynebacterium minutissimum (erythrasma)
- **Infection**. Corynebacterium pseudotuberculosis (ulcerative lymphangitis in horses and cattle)
- **Infection**. Corynebacterium jeikeium (sepsis)

Dermatophilus

- **Lineage**. Actinobacteria: Actinomycetales: Dermatophilaceae: Dermatophilus
- **Infection**. Dermatophilus Dermatophilus sp. (dermatophilosis, pitted keratolysis)
- **Infection**. Dermatophilus congolensis (dermatophilosis, mud fever, pitted keratolysis)

Mycobacterium

- **Lineage**. Actinobacteria: Actinomycetales: Corynebacterineae: Mycobacteriaceae: Mycobacterium
- **Infection**. Mycobacterium abscessus (chronic pulmonary disease, wound infections, in immunocompromised patients)
- **Infection**. Mycobacterium avium (persistent cough, can cause disseminated disease, including bone marrow infection, in immunocompromised individuals, collarstud abscess of neck lymph node in children)
- **Infection**. Mycobacterium haemophilum (collarstud abscess of neck lymph node in children)
- **Infection**. Mycobacterium intracellulare (persistent cough, can cause disseminated disease, including bone marrow infection, in immunocompromised individuals)
- **Infection**. Mycobacterium scrofulaceum (cervical lymphadenitis in children)
- **Infection**. Mycobacterium chelonae (granulomatous and acute inflammatory infections of skin and soft tissues)
- **Infection**. Mycobacterium fortuitum (pulmonary diseases, postsurgical wound abscesses, sepsis with multiorgan involvement)
- **Infection**. Mycobacterium kansasiim (aquarium granuloma)
- **Infection**. Mycobacterium leprae (leprosy, Hansen disease)
- **Infection**. Mycobacterium lepromatosis (leprosy, Hansen disease)
- **Infection**. Mycobacterium malmoense (cervical lymphadenitis in children, pulmonary disease in adults with preexisting lung conditions)
- **Infection**. Mycobacterium marinum (aquarium granuloma)
- **Infection**. Mycobacterium paratuberculosis (suspected to cause some cases of Crohn disease)
- **Infection**. Mycobacterium simiae (granulomatous lung disease)
- **Infection**. Mycobacterium szulgai (tuberculosis-like pulmonary infection, disseminated disease in immunocompromised individuals)
- **Infection**. Mycobacterium tuberculosis complex, including Mycobacterium caprae, Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium bovis BCG
- **Infection**. Mycobacterium microti, Mycobacterium canettii, Mycobacterium pinnipedii, Mycobacterium mungi (tuberculosis)
- **Infection**. Mycobacterium tuberculosis (tuberculosis)

- **Infection**. Mycobacterium ulcerans (Buruli ulcer)
- **Infection**. Mycobacterium xenopi (Mycobacterium xenopi pneumonia)

Nocardia

- **Lineage**. Actinobacteria: Actinomycetales: Corynebacterineae: Nocardiaceae: Nocardia
- **Infection**. Nocardia asteroides (nocardiosis)
- **Infection**. Nocardia brasiliensis (nocardiosis)
- **Infection**. Nocardia caviae (nocardiosis)
- **Infection**. Nocardia farcinica (nocardiosis)
- **Infection**. Nocardia nova (nocardiosis)
- **Infection**. Nocardia otitidiscaviarum (nocardiosis)

Rhodococcus

- **Lineage**. Actinobacteria: Actinomycetales: Corynebacterineae: Nocardiaceae: Rhodococcus
- **Infection**. Rhodococcus equi, formerly Corynebacterium equi, formerly Bacillus hoagii, formerly Corynebacterium purulentus, formerly Mycobacterium equi, formerly Mycobacterium restrictum, formerly Nocardia restricta, formerly Proactinomyces restrictus (chronic pulmonary infection simulating tuberculosis)

Tropheryma

- **Lineage**. Actinobacteria: Actinobacteridae: Actinomycetales: Cellulomonadaceae: Tropheryma
- **Infection**. Tropheryma whipplei, formerly Tropheryma whippelii (Whipple disease)

Propionibacteriaceae

- **Lineage**. Actinobacteria: Actinobacteridae: Actinomycetales: Propionibacteriaceae: Propionibacterium
- **Infection**. Propionibacterium propionicus (another agent capable of causing the so-called actinomycotic abscess of oral cavity)

Actinomadura

- **Lineage**. Actinobacteria: Actinobacteridae: Actinomycetales: Streptosporangineae: Thermomonosporaceae: Actinomadura
- **Infection**. Actinomadura madurae (mycetoma, maduromycosis, madura foot)
- **Infection**. Actinomadura pelletieri (mycetoma)

Gardnerella

- **Lineage**. Actinobacteria: Bifidobacteriales: Bifidobacteriaceae: Gardnerella
- **Infection**. Gardnerella vaginalis, formerly Corynebacterium vaginalis, formerly Haemophilus (vaginosis)

Glossary

Carrier In the field of genetics, a carrier is an individual who has a disease-causing gene that does not happen to cause disease in the individual. For example, individuals with one sickle cell gene are typically not affected by sickle cell disease, which usually requires homozygosity (i.e., both alleles having the sickle cell gene mutation) for disease expression. When two carriers mate, the offspring has a 25% chance of inheriting both alleles (i.e., being homozygous for the sickle cell mutation) and developing sickle cell disease.

In the field of infectious diseases, a carrier is an individual who harbors an infectious organism, but who suffers no observable clinical consequences. If the carrier state is prolonged, and if infectious organisms cross to other individuals, a single carrier can cause an epidemic.

Commensal A symbiotic relationship in which one of the organisms benefits and the other is unaffected, under normal conditions. An opportunistic commensal is an organism that does not produce disease in its host, unless the host provides a physiologic opportunity for disease, such as malnutrition, advanced age, immunodeficiency, overgrowth of the organism (e.g., after antibiotic usage), or some mechanical portal that introduces the organism to a part of the body that is particularly susceptible to the pathologic expression of the organism, such as an indwelling catheter, or an intravenous line).

Facultative intracellular organism An organism that is capable of living and reproducing inside or outside of cells. The term may apply to any organism, but most of the facultative intracellular organisms are bacteria. Examples of genera include: Brucella, Francisella, Histoplasma, Listeria, Legionella, Mycobacterium, Neisseria, and Yersinia.

Granuloma A small nidus of chronic inflammation usually containing a mixture of fibroblasts, lymphocytes, and macrophages. Depending on the cause of the granuloma, there may be areas of necrosis, eosinophils, and acute inflammation. The presence of a granuloma may indicate the presence of an organism (e.g., mycobacterium) or foreign body (e.g., insect skeleton) that cannot be effectively cleared by the normal activities of the immune system. In some cases, granulomas can be felt (e.g., if they are in the skin), or, if sufficiently large, visualized radiographically (e.g., tuberculosis, sarcoidosis).

HACEK A group of proteobacteria, found in otherwise healthy individuals, that are known to cause some cases of endocarditis, especially in children, and which do not grow well from cultured blood (due primarily to their slow growth rates). The term HACEK is created from the initials of the organisms of the group:

- Haemophilus, particularly Haemophilus parainfluenzae Gamma Proteobacteria (Gamma Proteobacteria)
- Aggregatibacter, including Aggregatibacter actinomycetemcomitans and Aggregatibacter aphrophilus (Gamma Proteobacteria)
- Cardiobacterium hominis (Gamma Proteobacteria)
- Eikenella corrodens (Beta Proteobacteria)
- Kingella, particularly Kingella kingae (Beta Proteobacteria)

Hemolytic syndromes Hemolytic syndromes are characterized by the destruction of red blood cells. Red cell destruction may be caused by organisms that invade and rupture the cells (e.g., Plasmodium species Babesia species), that release chemicals that lyse red cells (e.g., hemolysins, listeriolysin O, rhamnolipid), or that induce an antigenic response

against the patient's own red cells (poststreptococcal autoimmune hemolytic anemia). Hemolytic syndromes (red cell lysis) should be distinguished from hemorrhagic syndromes resulting from vascular rupture or leakage.

Horizontal gene transfer The direct transfer of genetic material between organisms, by mechanisms other than reproduction (i.e., other than the transfer of DNA from parents to offspring). The very first eukaryotic ancestors derived their genetic material by horizontal gene transfer from prokaryotes (bacteria and archaean organisms), viruses, and possibly from other now-extinct organisms that might have preceded the eukaryotes. The early eukaryotes almost certainly exchanged DNA between one another, and we see evidence of such exchanges in modern single-celled eukaryotes and fungi [54, 55].

To an unknown extent horizontal gene transfer occurs throughout the animal kingdom. For example, tardigrades, a microscopic animal, has a genome one-sixth of which was derived from bacteria, archaeans, plants, and fungi [56].

It should be noted that many of the most significant evolutionary advances came from interspecies gene acquisitions. The primordial mitochondrion that helped to create the first eukaryotic cell was an acquisition from a bacteria. The very first chloroplast in the most primitive precursor of the plant kingdom was a pilfered cyanobacteria. The big jump in adaptive immunology came with the acquisition of the RAG1 gene. This gene enabled the DNA that encodes a segment of the immunoglobulin molecule to rearrange, thus producing a vast array of protein variants [57]. The RAG1 gene, which kicked off adaptive immunity in animals, was derived from a transposon, an ancient DNA element that was acquired through horizontal gene transfer or through infection from another living organism or from a virus; not by random base mutations.

Host The animal in which an infectious organism resides.

Koch's postulates Koch's postulates are a set of observations and experimental requirements proposed by Heinrich Hermann Robert Koch in the late 1800s, intended to prove that a particular organism causes a particular infectious disease. Koch's postulates require that the suspected causal organism be extracted from the infected lesion (i.e., from diseased, infected tissue), the isolation; that the organism be cultured in a laboratory; and that the lesion be reproduced in animals after inoculation with the cultured organism.

Modern diagnostic techniques have made Koch's postulates somewhat irrelevant, insofar as the causes of diseases are now being discovered without growth in culture, and without replication of the disease in an animal model [58]. Whipple disease serves as an example. Whipple disease was first described in 1907 [59], but its cause was unknown until 1992, when researchers isolated and amplified, from Whipple disease tissues, a 16s ribosomal RNA sequence that could only have a bacterial origin [52]. Based on molecular features of the ribosomal RNA molecule, the researchers assigned it to Class Cellulomonadacea, and named the species Tropheryma whipplei, after the man who first described the disease, George Hoyt Whipple.

Particularly noteworthy, in the case of Whipple disease, is that Koch's postulates never came close to being satisfied. The putative pathogen has never been isolated and grown in culture. There is no animal model for Whipple disease. The resolution of Whipple disease was an achievement of molecular biologists and human geneticists [52, 60].

Side-stepping Koch's postulates, including its reliance on animal models, has become de rigueur in modern medicine. For example, the United States has experienced a recent increase in cases of acute flaccid myelitis, a rare disease of children [61]. Diagnosis is based on a metagenomic analysis (i.e., culture-independent sequence searches conducted

on an assemblage of microbial gene sequences in a biologic sample) of DNA obtained from nasopharyngeal swabs. The organism that is present in most of the examined cases is enterovirus-D68, and this virus is the presumed causal organism of acute flaccid myelitis, until proven otherwise.

Largest species For some time, the largest known bacterium was Epulopiscium fishelsoni, which grows to about 600 μm × 80 μm, much larger than the typical animal epithelial cell (about 35 μm in diameter) [62]. This record has since been exceeded by Thiomargarita namibiensis. This ocean-dwelling nonpathogen can reach a size of 0.75 mm, visible to the unaided eye. The largest single-celled organisms are deep-sea protists of Class Xenophyophorea, a subclass of Class Rhizaria. These organisms can exceed 4 in. in length. Class Mimiviridae was thought to contain the largest viruses. Class Mimiviridae viruses can exceed 0.8 μm in length, thus exceeding the size of some bacteria (e.g., Mycoplasma species may be as small as 0.3 μm). The genome of species in Class Mimiviridae can exceed a million base pairs, encoding upwards of 1000 proteins. Class Megaviridae is a newly reported class of viruses, related to Class Mimiviridae, but bigger [63]. The organism with the largest genome is currently thought to be Polychaos dubium (Class Amoebozoa), with a genome length of 670 billion base pairs; humans have a puny 3 billion base pair genome.

Long branch attraction When gene sequence data is analyzed, and two organisms share the same sequence in a stretch of DNA, it can be very tempting to infer that the two organisms belong to the same class (i.e., that they inherited the identical sequence from a common ancestor). This inference is not necessarily correct. Because DNA mutations arise stochastically over time, two species with different ancestors may achieve the same sequence in a chosen stretch of DNA. Inheritance is not involved. There may be an identifiable ancestor with the same DNA sequence for one of the two organisms, but not for the other. When mathematical phylogeneticists began modeling inferences for gene data sets, they assumed that most of class assignment errors based on DNA sequence similarity would occur when the branches between sister taxa were long (i.e., when a long time elapsed between evolutionary divergences, allowing for many random substitutions in base pairs). They called this phenomenon, wherein nonsister taxa were assigned the same ancient ancestor class, "long branch attraction." In practice, errors of this type can occur whether the branches are long, short, or in-between. Over the years, the accepted usage of the term "long branch attraction" has been extended to just about any error in phylogenetic grouping due to gene similarities acquired through any mechanism other than inheritance from a shared ancestor. This would include random mutational and adaptive convergence [10].

Molecular clock The molecular clock is a metaphor describing an analytic method by which the age of phylogenetic divergence of two species can be estimated by comparing the differences in sequence between two homologous genes or proteins. The name "molecular clock" and the basic theory underlying the method were described in the early years of the 1960s, when the amino acid sequence of hemoglobin molecule was determined for humans and other hominids [64]. It seemed clear enough at the time that if the number of amino acid substitutions in the hemoglobin sequence, when compared among two species, was large, then a very great time must have elapsed since the phylogenetic divergence of the two species. The reason being that sequence changes occur randomly over time, and as more time passes, more substitutions will occur. Conversely, if the differences in amino acid sequence between species is very small, then the time elapsed between the species divergence must have been small.

As with all simple and elegant theories in the biological sciences, the devil lies in the details. Today, we know that analyses must take into account the presence or absence of conserved regions (whose sequences will not change very much over time). Indeed analysts must apply a host of adjustments before they can claim to have a fairly calibrated molecular clock [65, 66]. At the end of the process, biologists have additional information related to the timing of species divergences, possibly corroborating the prior chronology, or tentatively establishing new timelines.

Next-generation sequencing Refers to a variety of new technologies that sequence genomes quickly and cheaply [67].

Nonphylogenetic signal DNA sequences that cannot yield any useful conclusions related to the evolutionary position of an organism. Because DNA mutations arise stochastically over time (i.e., at random locations in the gene, and at random times), two organisms having different ancestors may achieve the same sequence in a chosen stretch of DNA. Long-branch attraction, mutational convergence, and adaptive convergence account for many of the errors that occur when nonphylogenetic signal is incorrectly assumed to have phylogenetic value [10].

Obligate intracellular organism An obligate intracellular organism can only reproduce within a host cell. Obligate intracellular organisms can include any type of organism, but the term aptly describes all viruses and all members of Class Chlamydia. Examples of genera that contain obligate intracellular species include: Coxiella, Leishmania, Plasmodia, Rickettsia, Toxoplasma, and Trypanosoma.

Opportunistic infection Opportunistic infections are diseases that do not typically occur in healthy individuals, but which can occur in individuals who have a physiologic status favoring the growth of the organisms (e.g., diabetes, malnutrition). Sometimes, opportunistic infections occur in patients who are very old, or very young. Most often, opportunistic infections occur in immunocompromised patients. Specific diseases may increase susceptibility to specific types of organisms. For example, diabetics are more likely to contract systemic fungal diseases than are nondiabetic individuals. Some opportunistic infections arise from the population of organisms that live within most humans, without causing disease under normal circumstances (i.e., commensals).

The concept of an opportunistic organism is, at best, a gray area of medicine, as virtually all of the organisms that arise in immunocompromised patients will, occasionally, cause disease in immune-competent patients (e.g., Cryptococcus neoformans). Moreover, the so-called primary infectious organisms, that produce disease in normal individuals, will tend to produce a more virulent version of the disease in immunosuppressed individuals (e.g., Coccidioides immitis). Examples of organisms that cause opportunistic infections are: Acinetobacter baumanni, Aspergillus sp., Candida sp., Clostridium difficile, Cryptococcus neoformans, Cryptosporidium parvum, Cytomegalovirus, Herpes zoster, Histoplasma capsulatum, Human herpesvirus 8, Pneumocystis jirovecii, Polyomavirus JC, Proteus sp., Pseudomonas aeruginosa, Streptococcus pyogenes, and Toxoplasma gondii.

Recent observations would suggest that many seemingly immunocompetent individuals who contract opportunistic infections have identifiable genetic abnormalities that account, specifically, for their heightened susceptibilities [68–72].

Parasite A parasite is an organism that lives and feeds in or on its host. In common usage, the term "parasite" is often reserved for animals that are parasitic in humans and other animals, and this has historically included the so-called one-celled animals (i.e., members of Class Protoctista). We now know that one-celled organisms are not animals (i.e., not member of Class Metazoa) and include a wide range of members of Class Eukaryota.

As we learn more and more about classes of organisms, the term "parasite" seems to have diminishing biologic specificity. In this book, the term "parasite" refers to any infectious organism.

Rare disease As written in Public Law 107-280, the Rare Diseases Act of 2002, "Rare diseases and disorders are those which affect small patient populations, typically populations smaller than 200,000 individuals in the United States" [73]. This translates to a prevalence of less than 1 case for every 1570 persons. This is not too far from the definition recommended by the European Commission on Public Health; a prevalence less than 1 in 2000 people.

Sporadic Refers to a disease or a specific case occurrence of a disease with no known cause, and without any discernible pattern of occurrence (e.g., genetic, environmental).

Syndrome A syndrome is a constellation of physical findings that occur together. The symptoms of a syndrome may or may not all have the same pathogenesis; and, hence, may or may not constitute a disease.

The common cold is a disease, with a specific cause, that produces a syndromic pattern of headache, sniffles, cough, and malaise. All these symptoms arise after a viral infection at about the same time, and all the symptoms clear at about the same time. Multiple expressions of physical disorder, occurring in multiple body systems are more likely to be syndromes than diseases, when the different conditions are inconsistently present or separated by intervals of time. Contrariwise, a localized pathological condition that is restricted to a particular set of cells is almost always a disease; not a syndrome.

There are exceptions. It is possible to imagine some highly unlikely syndromes that are restricted to one cell-type. Clinorchis sinensis is a species of trematode that causes biliary tract disease when the fluke take up permanent residence therein [74]. Localized reaction to the fluke infection results in chronic cholangitis (i.e., inflammation of the cells lining the bile ducts). In some cases, cholangiocarcinoma (i.e., cancer of the bile duct) develops. Cholangitis and cholangiocarcinoma arise through different biological pathways, despite having the same root cause (i.e., clinorchiasis). This being the case, it seems reasonable to count cholangitis and cholangiocarcinoma following Clinorchis sinensis infection as a syndrome, not as a disease; thus breaking the general rule that a localized pathologic condition is not a syndrome.

There are no hard or fast rules, but the distinction between a disease and a syndrome has therapeutic and diagnostic consequences. A true disease has one pathogenesis, despite its multiorgan manifestations and can be potentially prevented, diagnosed, or treated by targeting events and pathways involved in the development of the disease. A syndrome is a confluence of clinical findings that may or may not share the same pathogenesis.

Taxa Plural of taxon.

Taxon A taxon is a class. The common usage of "taxon" is somewhat inconsistent, as it sometimes refers to the class name, and at other times refers to the instances (i.e., members) of the class. In this book, the term "taxon" is abandoned in favor of "class," the term preferred by computer scientists.

Taxonomic order In traditional taxonomy, the hierarchical lineage of organisms are divided into a descending list of named orders: Kingdom, Phylum (Division), Class, Order, Family, Genus, and Species. In recent times, taxonomists have deemed it necessary to add many more formal categories (e.g., supraphylum, subphylum, suborder, infraclass, etc.). In some cases, where no named subdivision exists, a new class is created and given an "unranked" order. In many cases, the reason for these additional divisions relates to the need to impose monophyly on classes. A good example is the unranked eukaryotic class, Discoba. Molecular phylogenetic evidence has come to light suggesting that Class Jakobid is closely related to

Class Percolozoa and Class Euglenozoa. Because these classes (Jakobid, Percolozoa, and Euglenozoa) seem to have a common direct ancestor, they need to be assigned a common superclass. In this particular case, a superclass for these three sister classes was invented: Class Discoba. Because no named subdivision (i.e., rank) was available for Class Discoba, it is considered an "unranked" class. Aside from being an undue burden upon students who are trying to understand the practical aspects of biological taxonomy, it seems somewhat absurd to have a system larded with orders given an official label of "unranked." In this book, all classes are simply referred to as order "Class," followed by the name of the class. Each class has one named parent class. When you know the name of the parent for each class, you can determine the complete ancestral lineage for every class and species within the classification.

Tick Ticks are members of Class Chelicerata and should not be confused with insects. The hierarchy for the tick, Ixodes, is: Ixodes: Ixodinae: Ixodidae: Ixodoidea: Ixodida: Parasitiformes: Acari: Arachnida: Chelicerata: Arthropoda. Species Ixodes scapularis transmits Babesia microti (babesiosis), Borrelia burgdorferi (Lyme disease), and Anaplasma phagocytophilum (human granulocytic anaplasmosis) [75]. Tick-borne viruses include: Crimean-Congo hemorrhagic fever, Tick-borne encephalitis, Powassan encephalitis, Deer tick virus encephalitis, Omsk hemorrhagic fever, Kyasanur forest disease (Alkhurma virus), Langat virus, and Colorado tick fever.

Zoonosis A infectious disease of humans that is acquired from a nonhuman animal reservoir. The method of infection (e.g., vector) does not determine whether a disease is considered to be zoonotic. Most fungal diseases are nonzoonotic, because fungi typically grow in soil or water, and are delivered to humans as airborne spores. For example, malaria, passed by a mosquito vector, is not a zoonosis, because the reservoir for the malarial species that cause human malaria is usually another human. If there were no human carriers of malaria, the incidence of human malaria would drop to insignificance. The same is true for schistosomiasis, river blindness, and elephantiasis. Though these diseases are transmitted by nonhuman vectors, their typical reservoir is human.

Examples of zoonotic diseases are as follows:

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Anthrax
Babesiosis
Balantidiasis
Barmah Forest virus disease
Bartonellosis
Bilharzia
Bolivian hemorrhagic fever
Borna virus infection
Borreliosis (Lyme disease and others)
Bovine tuberculosis
Brucellosis
Campylobacteriosis
Cat Scratch Disease
Chagas disease
Cholera
Cowpox
Creutzfeldt-Jakob disease (vCJD)
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Crimean-Congo hemorrhagic fever
Cryptosporidiosis
Cutaneous larva migrans
Dengue fever
Ebola fever
Echinococcosis
Erysipeloid
Escherichia coli O157:H7
Giardiasis
Glanders
H1N1 flu
Hantavirus infection
Helminthic infections
Hendra virus infection
Henipavirus infection
Korean hemorrhagic fever
Kyasanur forest disease
Lassa fever
Leishmaniasis
Leptospirosis
Listeriosis
Lyme disease
Lymphocytic choriomeningitis
Marburg fever
Mediterranean spotted fever
Monkey B
Mycobacterium marinum
Nipah fever
Ocular larva migrans
Omsk hemorrhagic fever
Orf (animal disease)
Oropouche fever
Pasteurellosis
Plague
Psittacosis
Puumala virus infection
Q-Fever
Rabies
Rift Valley fever
Ringworm from Microsporum canis
Rotavirus (when transmitted by dogs, cats and other animals)
Salmonellosis
SARS
Sodoku

Sparganosis
Streptococcus suis infection
Toxocariasis
Toxoplasmosis
Trichinosis
Tularemia
Typhus
Venezuelan hemorrhagic fever
Visceral larva migrans
West Nile fever
Western equine encephalitis virus
Yellow fever (sylvatic cycle)
Yersiniosis

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Chapter 4

Eukaryotes

Section 4.1 Overview of Class Eukaryota

The evolution of sex is the hardest problem in evolutionary biology.

John M. Smith

If we exclude viruses, all cellular life can be divided into two forms: the eukaryotes, which have a membrane-bound organelle, known as the nucleus, that contains the cell's genetic material; and the prokaryotic life forms (bacteria and archaeans) that have no nucleus.

Nobody knows when the first eukaryotes appeared on earth. One school of thought estimates that eukaryotes developed 2.7 billion years ago, about 1 billion years after the first appearance of prokaryotic life forms. This theory is based on finding sterane molecules in shale rocks, dating back nearly 3 billion years. Eukaryotic cells are the only known source of naturally occurring sterane molecules. Their presence in shale is held as evidence that the first eukaryotic organisms must have existed no later than 2.7 billion years ago [1]. Other biologists tie their estimate of the beginning or eukaryotic life to the epoch in which the first eukaryotic fossil remains are found, about 1.7 billion years ago. This leaves a 1 billion year gap between estimates for the origin of the eukaryotes (i.e., 1.7–2.7 billion years ago).

Although there is an enormous range between the smallest and the largest eukaryotic cells, it is reassuring to note that most eukaryotic cells look very similar to one another and are of about the same size (i.e., 25–50 μm in diameter). The largest single-celled eukaryotic organism is 20 cm in length. This is Syringammina fragilissima, a member of Class Foraminifera, found off the coast of Scotland. The heaviest eukaryotic cell is the egg of the ostrich (Struthio camelus), which typically weighs between 3.5 and 5 pounds (Fig. 4.1)

The smallest eukaryotes are picoplankton that have a diameter as small as 0.2–2 μm [2]. There are many eukaryotic species that have never been adequately studied, and these include pico- and nano-sized stramenopiles [3].

The eukaryotes have a number of chemical and metabolic features in common. For example, actin and closely related filamentous molecules are components of the cytoskeleton of every eukaryote, and are only found in eukaryotes [4]. The defining features of eukaryotes are their membrane-delimited organelles.

Taxonomic Guide to Infectious Diseases. https://doi.org/10.1016/B978-0-12-817576-7.00004-3

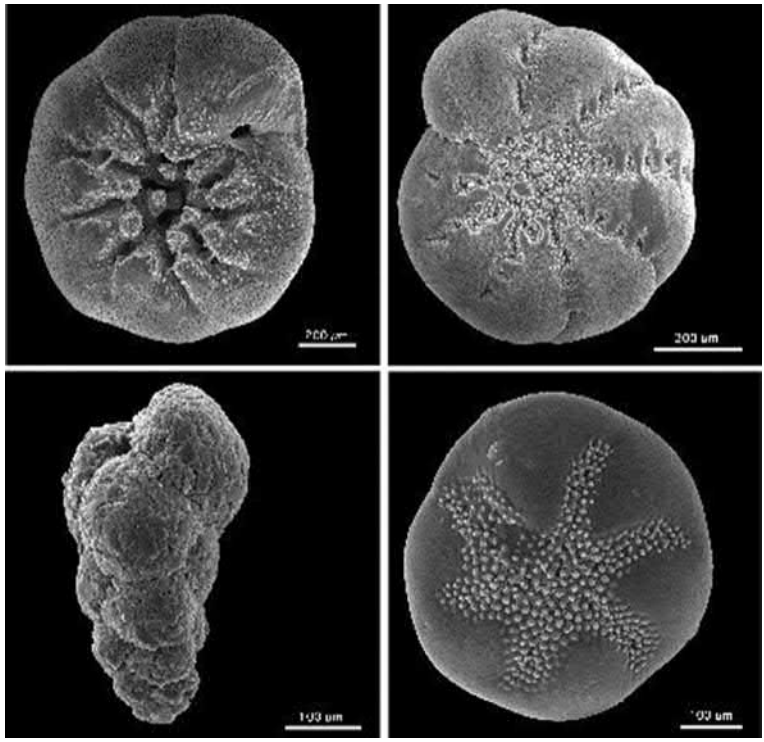

FIG. 4.1 Single-celled eukaryotes of Class Foraminifera. Clockwise starting from the top left: Ammonia beccarii, Elphidium excavatum clavatum, Buccella frigida, and Eggerella advena. Foraminifera are among the largest known single-celled organisms. *(Source, Wikipedia, a public domain image produced by the US Geological Survey.)*

The earliest eukaryotes, and all of their living descendants, came equipped with three structures that separate them from prokaryotes: mitochondria, at least one undulipodium, and a nucleus.

–1. Mitochondria

Mitochondria are membrane-delimited organelles, with their own genome, that proliferate within the eukaryotic cell. Current theory holds that mitochondria developed as an obligate intracellular endosymbiont from an ancestor of Class Rickettsia. All existing eukaryotic organisms descended from early eukaryotes that contained mitochondria. Furthermore, all existing eukaryotic organisms, even the so-called amitochondriate classes (i.e., organisms without mitochondria), contain vestigial forms of mitochondria (i.e., hydrogenosomes and mitosomes) [5–8].

The mitochondria provide eukaryotes with a source of internal energy, via oxidative phosphorylation. If fuel is abundant, then the amount of energy produced by a cell is proportional to the number of mitochondria, and the cells

that utilize the most energy contain the greatest number of mitochondria. For example, nearly half the cytoplasmic volume of heart muscle cells is composed of mitochondria, with several thousand mitochondria per cell.

Mitochondria provide eukaryotic cells with lots of evolutionary options. Eukaryotic cells can get bigger and bigger, depending on their mitochondria to provide sufficient energy to maintain the complex set of physiologic processes necessary to maintain a large organism. Cells can carry a ridiculous amount of junk DNA, and the energy costs of replicating the enlarged genome will be paid by the mitochondria. Cells that can create their own energy can afford to specialize, producing muscle cells that aid in movement and sensory cells that aid in finding food. Basically, it was the mitochondria that permitted the eukaryote to evolve into multicellular animals.

Of course, with the mitochondria came the mitochondriopathies; diseases whose underlying cause is mitochondrial pathology (i.e., dysfunctional mitochondria, or an abnormal number of mitochondria). Mitochondriopathies can be genetic or acquired. Most of the genetic mitochondriopathies are caused by nuclear, not mitochondrial, gene mutations. Though mitochondria live outside the nucleus and have their own genomes, mitochondrial DNA codes for only 13 proteins of the respiratory chain. All the other proteins and structural components of mitochondria are coded in the nucleus.

As we might expect, mitochondriopathies affect the cells that are most dependent on their mitochondria for their functionality. It is not surprising that most of the mitochondriopathies produce multisystem disorders that produce muscle weakness, cardiomyopathy, and ataxia. Additional features of mitochondriopathies may include: pigmentary retinopathy, ocular atrophy, deafness, gut motility disorder, and sideroblastic anemia, among many others. A mitochondriopathy should be in the differential workup for any unexplained multisystem disorder, especially those arising in childhood [9].

Isolated deafness (i.e., deafness as the only symptom) is observed in some forms of inherited cardiomyopathy in humans [10]. Sometimes, isolated deafness is present in acquired conditions, such as seen with deafness following antibiotic usage (e.g., aminoglycosides). Why would an antibiotic produce a mitochondriopathy? We must remember that mitochondria evolved from a captured bacteria that adapted to an intracellular existence within eukaryotes. Our mitochondria, true to their bacterial origins, are susceptible to toxicity induced by some antibacterial agents [11]. Why do such agents produce an isolated form of deafness? Presumably, the mitochondriopathic effect in these cases is systemic, affecting every cell of the body, to some extent. The cells involved in hearing happen to be the most sensitive. It has been observed that about a quarter of individuals receiving aminoglycoside therapy have some loss in hearing, as measured by audiometry testing.

–2. Undulipodia

Prokaryotes and eukaryotes have flagella, rods that protrude from the organism; their back-and-forth motion propel cells forward through water.

Aside from a superficial resemblance, the flagella of eukaryotes have no relationship with the flagella of prokaryotes [3]. Eukaryotic flagella are orders of magnitude larger than the prokaryotic flagella, contain hundreds of species of proteins not present in the flagella of prokaryotes, have a completely different internal structure, anchor to a different cellular location, and do not descend phylogenetically from prokaryotic flagella [12]. Specifically, flagella are composed primarily of flagellin. Undulipodia are composed primarily of tubulin and contain more than 100 other identified proteins, including dynein. Flagella have a diameter of 0.01–0.025 μm. Undulipodia have a much larger diameter (0.25 μm). Biologists provided the eukaryotic flagellum with its own name: undulipodium. Perhaps they chose a term with a few too many syllables. Most biologists continue to apply the misleading term "flagellum" (plural "flagella") to eukaryotes. Regardless, every existing eukaryote descended from an organism with an undulipodium. The undulipodium is a highly conserved feature of eukaryotes, and all descendant eukaryotic classes contain undulipodia or structures that evolved as modified forms of undulipodia. For example, we humans have cilia on the surface of mucosal lining cells, which are a shortened type of undulipodium. Human spermatocytes have long, undulipodial tails that undulate their way to their intended target (i.e., the oocyte).

A variety of structures in eukaryotic organisms have evolved from the unulipodium and their homologous derivatives, all of which are composed of tubulins [13]. These include pericentriolar bodies, centrioles, kinetids, specialized receptors, haptonemes of coccolithophorids, and undulating membranes of trypanosomes. Such structures are found in all the descendant classes of the eukaryotes. The primary cilium, a derivative of the undulipodium, is found exclusively in vertebrates. Disorders of the primary cilium are a newly characterized family of human diseases known as the ciliopathies.

–3. Nucleus

As far as anyone knows, the very first eukaryote came fully equipped with a nucleus. Theories abound, but nobody really knows where the nucleus came from. There are many commonalities between the eukaryotic nucleus and archaean cells, in terms of the structure and organization of DNA, RNA, and ribosomes. Here are few examples.

- Only eukaryotes and archaeans have a TATA box (a sequence of thymidine-adenine-thymidine-adenine that specifies where RNA transcription can begin). Bacteria have the so-called Pribnow box, consisting of a TATAAT sequence.
- Eukaryotes and archaeans have histone proteins attached to their DNA [14] [Glossary Histone].
- The RNA polymerase and ribosomes of eukaryotes and archaeans are very similar.

Based on close similarities between the archaean and eukaryotic genome, it has been hypothesized that the eukaryotic nucleus was derived from an archaean organism [1].

Recently, following the discovery of giant viruses that rival eukaryotes in terms of genome size and complexity, and the observation that genes of giant virus origin have been found in eukaryotes, a viral origin of the eukaryotic nucleus has risen as a possibility [15–17].

Along with the nucleus came eukaryote-specific methods of transcribing DNA into RNA. In eukaryotes, DNA sequences are not transcribed directly into full-length RNA molecules, ready for translation into a final protein. There is a pretranslational process wherein transcribed sections of DNA, so-called introns, are spliced together, and a single gene can be assembled into alternative spliced products. Alternative splicing is one method whereby more than one protein form can be produced by a single gene [18, 19]. Cellular proteins that coordinate the splicing process are referred to, in aggregate, as the spliceosome. All eukaryotes have a spliceosome [20].

Errors in normal splicing can produce inherited disease, and it estimated that 15% of disease-causing mutations involve splicing [21, 22]. One might expect that mutations in spliceosomes would cause deficiencies in diverse cell types, with multiorgan and multisystem disease (e.g., syndromic disease). This is not the case. For example, spliceosome mutations account for a form of retinitis pigmentosa and a form of spinal muscular atrophy [18]. In both diseases, pathology is limited to a specific type of cell; retinal cells and their pigment layer in retinitis pigmentosa, and motor neuron cells in the spinal muscular atrophy. Today, nobody can adequately predict the cell-type specificity of diseases that arise from a constitutive loss of function of essential splicing factors.

– The first subclasses of the eukaryotes: bikonts and unikonts.

The very top division of Class Eukaryota has been the subject of intense interest over the past few decades, and there is as yet no general consensus as to how the split should be drawn. Formerly, it was thought that all eukaryotes had either one undulipodium or two undulipodia, and the two major subdivisions of eukaryotes were Class Unikonta and Class Bikonta [23]. The wisdom of this simple morphologic division was bolstered by genetic findings that three fused genes (carbamoyl phosphate synthase, dihydroorotase, and aspartate carbamoyltransferase) are uniquely characteristic of Class Unikonta. Two fused genes (thymidylate synthase and dihydrofolate reductase) characterize Class Bikonta. Hence, the morphologic property dividing Class Eukaryota into unikonts and bikonts was shadowed by a genetic property that draws the equivalent taxonomic division.

Further studies indicated that this simple split did not achieve monophyletic subclasses (i.e., could not ensure that all members of either division had the features that defined its assigned division and lacked the features that defined its sister division). Rather than entering into controversy, we use a somewhat outmoded

schema for the top classes of the eukaryotic tree, because it conforms to most textbooks, makes it easy to identify the subclasses of pathogenic species, and is no less stable than any competing high-level schema.

```
Eukaryota (organisms that have nucleated cells)
  Bikonta (2-undulipodia)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida, from which Kingdom Plantae derives
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-undulipodium)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
```

A quick glance at the eukaryotic schema indicates that the very first division in the classification of the eukaryotes is based on the number of undulipodia: Class Bikonta (from the Greek "kontos," meaning pole) consists of all organisms with two undulipodia; and Class Unikonta consists of all organisms with one undulipodia [24].

The value of the eukaryotic undulipodium as a taxonomic divider is demonstrated in Class Opisthokonta. Class Opisthokonta is a subclass of Class Unikonta that contains Class Choanozoa, Class Animalia, and Class Fungi, among others. The opisthokonts all descend from an organism with its undulipodium extending from the rear (from the Greek "opisthios," meaning rear and "kontos" meaning pole). The rear-ended undulipodium distinguishes the members of Class Opisthokonta from unikonts that have an undulipodium extending from anterior (pole near nucleus) or lateral (smaller width) edges.

Section 4.2 Metamonada

Our greatest responsibility is to be good ancestors.

Jonas Salk

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
        Trichozoa
```

```
          Parabasalidea
            Trichomonadida
              Trichomonadidae
                Trichomonas (genus)
              Monocercomonadidae
                Dientamoeba (genus)
        Fornicata
          Diplomonadida
            Hexamitidae
              Giardia (genus)
    Discoba
      Euglenozoa
      Percolozoa
  Archaeplastida
  Chromalveolata
    Alveolata
      Apicomplexa
      Ciliophora (ciliates)
    Heterokonta
Unikonta (1-flagellum)
  Amoebozoa
  Opisthokonta
    Choanozoa
    Animalia
    Fungi
```

Class Metamonada is a class of anaerobic eukaryotes that lack mitochondria. The first eukaryotes possessed three defining anatomic structures: a nucleus, mitochondria (one or more), and undulipodium (one or more). Evolution can be an intolerant process. Structures that serve no important biological purpose cannot justify the resources required to maintain their continued existence. In particular, mitochondria may be a great way for deriving energy from oxygen, but these complex organelles may have little value when conditions are anaerobic.

As we will see again and again throughout this book, phylogenetic traits are seldom lost, without leaving some trace of their heritage. The anaerobic members of Class Metamonada maintain a relict organelle, derived from an ancestral mitochondrium. The relict is usually referred to as a mitosome, though a specific term, hydrogenosome, is used to refer to mitochondrial relicts that use iron-sulfide proteins to yield molecular hydrogen and ATP. Various so-called amitochondriate eukaryotic classes that have lost classic mitochondria have retained mitosomes or hydrogenosomes that form molecular hydrogen: Class Metamonada [6], Class Amoebozoa [7], and Class Microsporidia [8].

```
Metamonada
  Trichozoa
    Parabasalidea
      Trichomonadida
        Trichomonadidae
          Trichomonas (genus)
        Monocercomonadidae
          Dientamoeba (genus)
```

Members of Class Trichomonadida live as commensals with a single morphologic form: trophozoites. Cysts are not formed. The absence of a cyst form is significant because cyst forms resist adverse environmental conditions, permitting the organism to live outside the host for varying lengths of time, and to infect organisms without direct contact with the infected host. Since they have no cyst forms, infection by members of Class Trhichomonadida involves direct transmission between infected host organisms.

The two human pathogens in Class Trichomonadida are: Trichomonas vaginalis and Dientamoeba fragilis.

Trichomonas vaginalis is the most common sexually transmitted disease, with about 8 million new cases occurring annually in North American [25, 26]. Its reservoir is humans. Its role as a causative agent of urethritis, vaginitis, and cervicitis, in women, is well known. Fewer people seem to be aware that both men and women are commonly infected. Though many individuals infected with Trichomonas vaginalis are asymptomatic, the organism can cause urethritis in a significant percentage of infected men [27]. Like all members of Class Trichomonadida, it exists only in the trophozoite form, and transmission is typically caused by the exchange of infected secretions during sexual intercourse. The trophozoites can be easily visualized in cervical Pap smears by light microscopic examination (Fig. 4.2).

It is important not to confuse trichomonas with the similar-sounding name trachoma. Trachoma is a bacterial infection of the eyes caused the Chlamydia trachomatis.

Dientamoeba fragilis causes diarrhea and other gastrointestinal (GI) symptoms. It causes disease worldwide. Its reservoir seems to be restricted to humans and other primates. Like the other members of Class Trichomonadida, it is found only in the trophozoite form. The organism lives in the large intestine. Though it can be found in stools, it degenerates quickly (hence the “fragilis” in Dientamoeba fragilis), and the organism can be difficult to demonstrate. A 2005 study employed a sensitive and specific PCR (polymerase chain reaction) test on stool specimens from 6750 patients with GI symptoms and on a control set of 900 asymptomatic individuals. The study found that about 1% of the symptomatic patients tested positive for Dientamoeba fragilis. None of the asymptomatic patients demonstrated infection [28]. This indicates that the organism, when present, causes

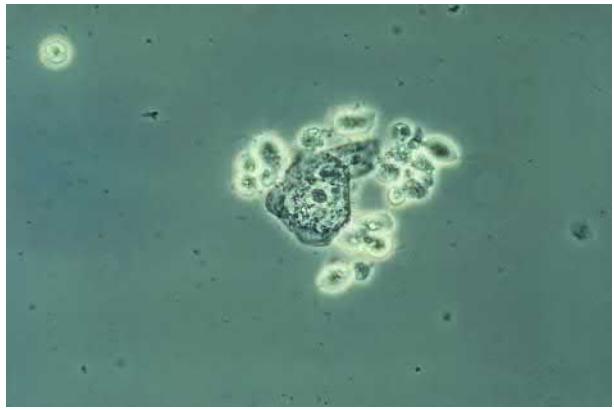

FIG. 4.2 Phase contrast photomicrograph of two overlapping squamous cells, closely surrounded by about 20 Trichomonas vaginalis organisms (center). A few very thin undulipodia are seen protruding from several organisms. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

symptoms; and that Dientamoeba is a significant cause (on the order of 1%) of GI complaints. Because the trophozoite is passed in stools, and because the organism has no cyst stage, the presumed method of transmission is through fecal-oral transfer.

```
Metamonada
  Fornicata
    Diplomonadida
      Hexamitidae
        Giardia (genus)
```

Members of Class Diplomonadida have so-called mirror morphology, with two sets of nuclei, undulipodia and cytoplasm, symmetrically arranged about a central axis [3]. There is only one human pathogen in Class Diplomonada: Giardia lamblia (alternately known as Giardia intestinalis or Giardia duodenalis, and formerly known as Lamblia intestinalis). Giardia lamblia can be found worldwide and infects a wide variety of animals, including cats, dogs, and birds. Its infection in beavers has inspired the rhyming couplet, beaver fever (Fig. 4.3).

Unlike the pathogens in Class Trichomonadida, Giardia grows in two forms: trophozoite and cyst. The organism lives in the small intestine, in contrast with Entamoeba histolytica (Class Amoebozoa), which lives in the large intestine. Entamoeba histolytica, and Giardia lamblia both have cyst forms, but the two organisms can be distinguished by the morphology of their trophozoites: Giardia trophozoites have flagella; Entamoeba

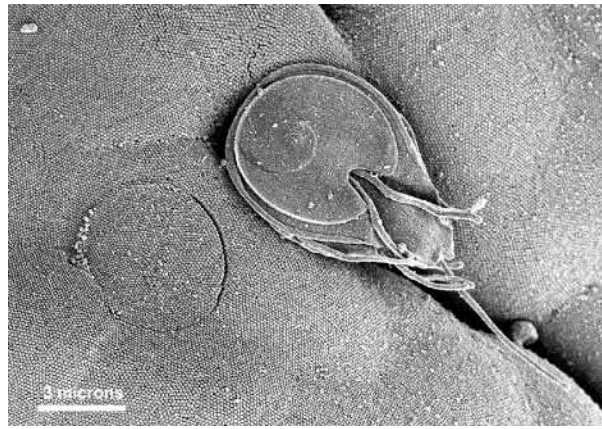

FIG. 4.3 Scanning electron micrograph of a single organism of Giardia species, sitting atop the intestinal mucosal surface. The Giardia organism is approximately the same size as the apical surface of an intestinal epithelial cell, and the polygonal surface borders of individual epithelial cells can be barely discerned as furrowed lines. The tiny bumps on the epithelial cells are microvilli. In this image, the trophozoite is resting upside down, on its dorsal surface, revealing the ventral adhesive disk that would normally attach the organism to the epithelial surface. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, photographed by Dr. Stand Erlandsen.)*

trophozoites do not. The cyst form supports the long-term survival of the organism in contaminated water supplies. Transmission can occur from the fecal-oral route or from ingesting contaminated water. Giardiasis is characterized by diarrhea and associated GI complaints.

Infectious Genera

Dientamoeba

- **Lineage**. Metamonada: Trichozoa: Parabasalia: Trichomonadida: Monocercomonadidae: Dientamoeba
- **Infection**. Dientamoeba fragilis (dientamoebiasis)

Trichomonas

- **Lineage**. Metamonada: Trichozoa: Parabasalia: Trichomonadida: Trichomonadidae: Trichomonas
- **Infection**. Trichomonas vaginalis (trichomoniasis)

Giardia

- **Lineage**. Metamonada: Fornicata: Diplomonadida: Hexamitidae: Giardia
- **Infection**. Giardia lamblia, same as Giardia intestinalis, Giardia duodenalis, and Lamblia intestinalis (giardiasis, beaver fever)

Section 4.3 Euglenozoa

Each organism's environment, for the most part, consists of other organisms.

Kevin Kelly

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Discicristata
          Euglenozoa
            Kinetoplastida
              Trypanosomatida
                Leishmania (genus)
                Trypanosoma (genus)
          Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
```

The Euglenozoa are single-cell organisms that are closely related to the Percolozoans. Class Euglenozoa plus Class Perocolozoa constitute the only subclasses of Class Discicristata that contain human pathogens. As such, euglenozoans and percolozoans have disc-shaped mitochondria, characteristic of their superclass. All members of Class Euglenozoa that are pathogenic to humans fall under one class: Class Trypanisomatida. Class Trypanisomatida contains two infectious genera: Leishmania and Trypanosoma, and these two genera account for three of the most debilitating, widespread, and prevalent diseases of humans: leishmaniasis, Chagas disease, and sleeping sickness.

```
Euglenozoa
  Kinetoplastida
    Trypanosomatida
      Leishmania (genus)
      Trypanosoma (genus)
```

Class Trypanisomatida is a subclass of Class Kinetoplastida. As members of a subclass of Class Kinetoplastida, members of Class Trypanisomatida contain a unique and perplexing structure known as a kinetoplast. A kinetoplast is a clump of DNA composed of multiple copies of the mitochondrial genome, tucked inside a mitochondrion. All the members of Class Trypanisomatida are parasitic (i.e., they spend much of their lives inside a host organism). The members of class Trypanisomatida that are pathogenic to humans all have a primary host (humans) and an insect serving as an intermediate host [Glossary Primary host, Intermediate host, Secondary host].

Members of Class Trypanisomatida have features that are either unique to these organisms or that are seldom found in other organisms:

–1. Cell division is neither mitotic nor meiotic in members of Trypanisomatida. The organelle in which the division process is focused is the kinetoplast (a condensed mitochondrial genome). Each cell has one mitochondrion containing one kinetoplast, and the time at which division of the organism occurs is regulated by the cell cycle of the kinetoplast. The kinetoplast divides, while the undulipodium, anchored to the kinetoplast at the basal body, replicates, permitting the cell to bifurcate into separate cells. The nucleus replicates its DNA but never condenses into chromosomes; no mitotic spindle is formed. Nuclear DNA migrates to the new cell when the replicated kinetoplast and undulipodium are formed. This feature is completely unique to Class Trypanisomatida.

–2. Replication occurs in primary and intermediate hosts. In most cases, wherein a parasite has multiple hosts, reproduction occurs in the primary host, and maturation occurs in the secondary host. In the case of members of Class Trypanisomatida, parasites replicate in the primary (animal) and the secondary (insect) host.

–3. Intracellular forms have undulipodia. Most parasites that retain their undulipodia live extracellular lives. Presumably, the undulipodia make it hard to live inside a cell. It would be like trying to open your umbrella in a phone booth. Most members of Class Trypanisomatida have a so-called amastigote phase characterized by a very small cell with a tiny, virtually invisible undulipodia. Amastigotes are the intracellular form of the organism. The only disease organism in Class Trypanisomatida that does not have an amastigote phase is Trypanosoma brucei, the cause of African trypanosomiasis. This organism has no intracellular phase and is found as a swimming (so-called flagellate) form in blood and body fluids.

–4. Organisms have novel methods to control or avoid the host immune response. For example, Leishmania organisms that pass from insect vector (female sandfly) to human host are quickly phagocytosed by neutrophils. The neutrophils serve as Trojan horses for the leishmania. Normally, after a neutrophil engulfs foreign organisms, the neutrophil soon dies, along with the contained organisms. Leishmania actively stabilizes neutrophils for several days, until macrophages arrive to eat

the neutrophil and the leishmania within. The leishmanial organisms live as intracellular organisms within macrophages [Glossary Sandfly].

The two infectious genera in Class Trypanisomida are Leishmania and Trypanosoma. Leishmania species cause leishmaniasis, a disease that infects about 12 million people worldwide. Each year, about 60,000 people die from the visceral form of the disease. It is a tropical disease that occurs most often in India, Africa, and Brazil (in the order of decreasing incidence) (Fig. 4.4).

Leishmaniasis is transmitted to humans by the sandfly species of Genus Phlebotominae (Fig. 4.5).

Many different Leishmania species infect a wide range of animals, with about 21 different species infecting humans. In most cases, the species that are infective in humans have a nonhuman animal reservoir.

The target organs, and the subsequent clinical syndrome, vary with the species of Leishmania. All infective species are transmitted by the bite of a female sandfly. There are many species of sandfly, but all of the species that transmit leishmaniasis seem to belong to the Phlebotominae family.

The clinical forms of disease, which are dependent on the infective species of Leishmania, are: cutaneous (localized skin lesion), diffuse cutaneous, mucocutaneous, and visceral. The visceral form of the disease is the most severe form and often results in death, if not treated.

Trypanosoma brucei is the cause of African trypanosomiasis (sleeping sickness). Two subspecies cause infections in humans: Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense. The disease occurs almost exclusively in sub-Saharan Africa. The reported numbers of cases are considered to be unreliable, but it has been estimated that infection with

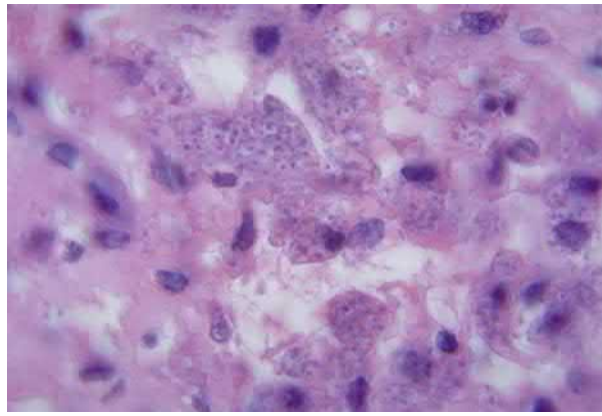

FIG. 4.4 Tissue biopsy of the deep layers of a skin lesion infected by Leishmania donovani. Individual macrophages are bloated by dozens of leishmania organisms in the amastigote stage. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

FIG. 4.5 The sandfly (Phlebotomus) is the insect vector for leishmaniasis and bartonellosis. If this sandfly looks more like a mosquito than a housefly, do not be surprised. Mosquitoes and sandflies are members of Class Diptera (flies). *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, and originally donated from the World Health Organization.)*

Trypanosoma brucei accounts for about 50,000 deaths each year. The intermediate host and vector for the disease is the tse-tse fly (Genus Glossina), and the reservoir for the tse-tse fly is infected humans or animals. As Trypanosoma brucei does not have an amastigote stage (capable of intracellular growth), it is found exclusively in extracellular fluids; principally, lymph, blood, and cerebrospinal fluid. Symptoms begin with swollen lymph nodes in the back of the neck, headache, and other clinical features of generalized infection. When the organism crosses the blood-brain barrier, the neurologic phase begins. Symptoms may include irritability, fatigue, insomnia, and irregular somnolence. The organism produces a chemical, tryptophol, known to induce sleep. Untreated cases are often fatal.

Trypanosoma cruzi is the cause of Chagas disease, also known as American trypanosomiasis. Chagas disease occurs almost exclusively in Central and South America. It affects about 8 million people [29]. Its intermediate host and vector is any of several species of the Triatominae subclass of Class Reduviiae insects. These blood-sucking triatomes are sometimes called kissing bugs, as they often bite the face, near the mouth. The triatome bug has a choice of about 100 different animals that serve as reservoirs for Trypanosoma cruzi. Infections have been known to occur following transfusion with contaminated blood.

Trypansoma cruzi has an intracellular amastigote stage. It is the amastigote that preferentially infects neurons of the peripheral nervous system. Infection of neurons in the wall of the heart can lead to conduction abnormalities, myocarditis, cardiomyopathy, and ventricular aneurysms. Infection of the neurons in the colon can lead to aganglionic megacolon.

Infectious Genera

Leishmania

- **Lineage**. Euglenozoa: Kinetoplastida: Trypanosomatida: Leishmania
- **Infection**. Leishmania aethiopica species complex: Leishmania aethiopica (cutaneous leishmaniasis)
- **Infection**. Leishmania amazonensis (cutaneous leishmaniasis)
- **Infection**. Leishmania chagasi (visceral leishmaniasis)
- **Infection**. Leishmania donovani (visceral leishmaniasis)
- **Infection**. Leishmania infantum (visceral leishmaniasis)
- **Infection**. Leishmania major (cutaneous leishmaniasis)
- **Infection**. Leishmania mexicana (cutaneous leishmaniasis)
- **Infection**. Leishmania peruviana (leishmaniasis)
- **Infection**. Leishmania tropica (cutaneous leishmaniasis)
- **Infection**. Leishmania venezualensis (cutaneous leishmaniasis)
- **Infection**. Leishmania viannia var brasiliensis (leishmaniasis)
- **Infection**. Leishmania viannia var guyayensis (leishmaniasis)
- **Infection**. Leishmania viannia var panamensis (leishmaniasis)

Trypanosoma

- **Lineage**. Euglenozoa: Kinetoplastida: Trypanosomatida: Trypanosoma
- **Infection**. Trypanosoma cruzi (American trypanosomiasis, Chagas disease)
- **Infection**. Trypanosoma brucei gambiense (African Trypanosomiasis, sleeping sickness)
- **Infection**. Trypanosoma brucei rhodesiense (African Trypanosomiasis, sleeping sickness)

Section 4.4 Percolozoa

The cell is basically an historical document.

Carl R. Woese [30]

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
          Heterolobosea
            Schizopyrenida
              Vahlkampfiidae
                Naegleria (genus)
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
```

Below Class Bikonta (two flagella) is Class Excavata (from Latin excavare, to make hollow, or, in this case, grooved). All subclasses of Class Excavata descend from an organism with a ventral feeding groove. Under Class Excavata is Class Discoba.

Class Discoba is a newly invented class, and its taxonomic origin is instructive. Recently acquired molecular data suggests that three subclasses of Class Excavata share a common direct ancestor: Class Percoloza, Class Euglenozoa, and Class Jakobid (which happens to contain no infectious organisms). The common ancestor of these three classes is apparently not shared with another subclass of Excavata: Class Metamonada. To preserve monophyly within Class Excavata, a newly named class needed to be inserted under Class Excavata. This class would contain Class Percolozoa, Class Euglenozoa, and Class Jakobid and would exclude class Metamonada. The newly named class is Discoba [31].

Under Class Discoba is Class Percolozoa, single-celled organisms containing mitochondria with discoid cristae, and the ability to shift between three

morphologic forms: amoeboid, flagellate, and cyst. The amoeboid form consists of a nonflagellated (i.e., without undulipodia) feeding cell. Like all amoeboid forms, it moves slowly, by extending a section of its cytoplasm (the pseudopod). Under adverse conditions, the amoeboid form can develop undulipodia, which presumably enhance its ability to move to a more hospitable location. As you would expect from organisms in a subclass of Bikonta, two undulipodia are observed in the flagellate form. Under conditions that are severely unsuitable for growth, the organism converts to a cyst form.

```
Percolozoa
  Heterolobosea
    Schizopyrenida
      Vahlkampfiidae
        Naegleria (genus)
```

There is only one known pathogenic genus in Class Percolozoa: Naegleria. Naegleria fowleri is the only species of Naegleria that is known to be infectious to humans. In older microbiology textbooks, Naegleria is grouped with the Acanthamoeba, under Class Amoebozoa. Naegleria rightly belongs to Class Percolozoa. The naeglerian life cycle includes three stages: cyst, trophozoite (amoeboid), and flagellate forms; whereas pathogenic members of Class Amoebozoa have only two stages: cyst and trophozoite. This distinction has taxonomic and diagnostic relevance. Though naeglerian meningoencephalitis can be confused histologically with amoebic meningoencephalitis, flagellate forms in cerebrospinal fluid would indicate a naeglerian infection (not an amoebic infection).

Naegleria fowleri is found in fresh water and in poorly chlorinated swimming pools, where it is free-living. Infections result from exposure to free-living organisms in their natural habitat, and not from exposure to infected individuals. The organism travels from the nose to the brain, where it causes a meningoencephalitis. Because these organisms are widely found in water, it is presumed that millions of people are exposed to the organism, but only rare individuals develop meningoencephalitis. It is not known why most people are unaffected by the organism, while others develop a rapidly progressive meningoencephalitis, with a very high mortality rate (about 95%). Naegleria meningoencephalitis accounts for several deaths in the United States each year (Fig. 4.6).

Infectious Genera

Naegleria

- **Lineage**. Percolozoa: Heterolobosea: Schizopyrenida: Vahlkampfiidae: Naegleria
- **Infection**. Naegleria fowleri (meningoencephalitis)

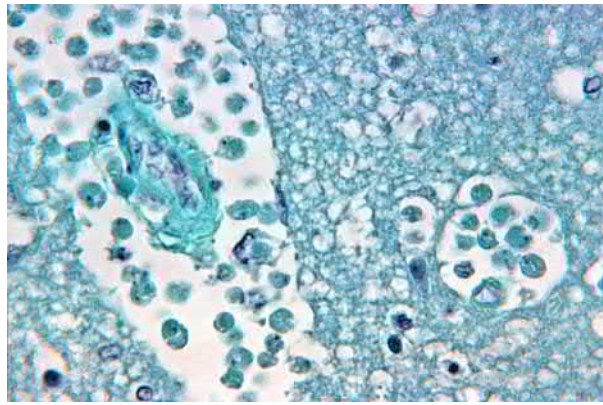

FIG. 4.6 Tissue section of brain infected with Naegleria fowleri. Extracellular naegleria organisms are seen in a large space surrounding a central vessel (left half of image) and in another, smaller perivascular space in the right half of the image. A few organisms infiltrate the brain parenchyma. The naegleria organisms are about the size of human macrophages, but with nuclei that are somewhat smaller and paler than human nuclei, and with foamy cytoplasm. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Section 4.5 Apicomplexa

The world appears to me to be put together in such a painful way that I prefer to believe that it was not created … intentionally.

Stanislaw Lem

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Ciliophora (ciliates)
        Apicomplexa
          Aconoidasida
            Haemosporida
              Plasmodiidae
                Plasmodium (genus)
            Piroplasmida
              Babesiidae
                Babesia (genus)
```

```
          Conoidasida
            Coccidia
              Eucoccidiorida
                Eimeriorina
                  Cryptosporidiidae
                    Cryptosporidium (genus)
                  Eimeriidae
                    Cyclospora (genus)
                    Isospora (genus)
                Sarcocystidae
                  Sarcocystis (genus)
                  Toxoplasma (genus)
    Heterokonta
Unikonta (1-flagellum)
  Amoebozoa
  Opisthokonta
    Choanozoa
    Animalia
    Fungi
```

Apicomplexa and Ciliophora are the two subclasses of Class Alveolata that contain organisms that produce human disease. Members of Class Alveolata are single-celled organisms with distinctive sacs underlying the plasma membrane (alveoli). They all have characteristic mitochondria, containing tubular cristae.

Members of Class Apicomplexa are all spore-forming parasites that live in animals. They all have a characteristic structure on the apex of their cells that helps them invade into animal cells; hence, the name Apicomplexa, referring to the apical complex. The lives of the apicomplexans are complex and individualized, but all members share several basic properties: the ability of apicomplexan cells to leave host cells, to travel to other host cells, to invade host cells, to feed and grow within host cells, and to divide into many small cells through a process called schizogony. Schizogony accounts for most of the histopathologic findings in apicomplexan infections, and inspires a collection of arcane terms that apply specifically to apicomplexans. When you understand schizogony, you understand apicomplexan pathology.

When human cells divide, they undergo a long process wherein the cell produces all of the material required for two full-sized cells, before it splits: two sets DNA, double the volume of cytoplasm, double the nuclear proteins, double the number of mitochondria, and a plasma membrane sufficient to cover two full-sized cells. In humans, cell division requires much energy and time. The apicomplexans have discovered a shortcut that greatly reduces the time and energy required for cell division. The shortcut is called schizogony, and consists of rapid nuclear replication without synchronous cytoplasmic

synthesis. Basically, the apicomplexan cell can divide into a large number of much smaller cells, and these smaller cells serve a specific purpose in the apicomplexan cell cycle. The two apicomplexan cell types that are capable of schizogony are the trophozoite and the oocyst.

The full-sized, vegetative apicomplexan cell is the trophozoite. It lives inside a host cell (such as an erythrocyte, a hepatocyte, or an intestinal epithelial cell), feeding off the host. After a time, the trophozoite undergoes schizogony, producing a collection of small, infective cells called merozoites, which travel (extracellularly) to other host cells, where they invade, feed, multiply, and grow into trophozoites within specific target cells. The trophozoite → merozoite cycle continues for a time, producing large numbers of infectious cells within the host organism. Eventually, the merozoites enter a new stage of life cycle, as male or female gametes. Depending on the species of Class Apicomplexa, fertilization may take place in the original host or in another organism (the female Anopheles mosquito in the case of malaria). The fertilized egg develops into an oocyst (that can survive outside the host). Under favorable conditions, the oocyst will undergo schizogony to produce small, sporozoites that infect cells within a new host. Like the trophozoite, the sporozoite produces merozoites. Members of Class Apicomplex have life cycles that vary somewhat from this description, and interested readers are encouraged to study the life cycles of the individual species. Nonetheless, knowledge of the most generic apicomplexan life cycle will help students understand the basic biology of the many important diseases produced by the many different apicomplexan species that infect animals [Glossary Mosquito].

Seven genera of Class Apicoomplexa cause diseases in humans: Plasmodium, Babesia, Cryptosporidium, Cyclospora, Cystoisospora, Sarcocystis, and Toxoplasma. Plasmodium and Babesia are subclasses of Class Aconoidasida (apicomplexans that lack a cone at the tip). The other apicomplexans are subclasses of Class Conoidasida (apicomplexans that have a cone at the tip).

```
Apicomplexa
  Aconoidasida
    Haemosporida
      Plasmodiidae
        Plasmodium (genus)
    Piroplasmida
      Babesiidae
        Babesia (genus)
```

Genus Plasmodium is responsible for human and animal malaria. About 300–500 million people are infected with malaria worldwide. About 2 million people die each year from malaria [32, 33]. Malaria accounts for more human deaths than any other vector-borne illness. There are several hundred species of Plasmodium that infect animals, but only a half dozen species are known

to infect humans [33]. Newly discovered species, causing human disease, may arise from animal reservoirs. For example, Plasmodium knowlesi causes malaria in macaque monkeys. It has emerged as a human pathogen in Southeast Asia, where it currently accounts for about two-thirds of malarial cases (Fig. 4.7).

In humans, malaria is contracted from sporozoites injected by female Anopheles mosquitoes. The sporozoites eventually develop into merozoites. In some species (including Plasmodium ovale, Plasmodium vivax, but not in Plasmodium malariae) a dormant variant of merozoite is produced that can produce a malarial relapse after decades of remission [34] (Fig. 4.8).

The Genus Plasmodium has some very distinctive features, not the least of which is its low G+C ratio. Plasmodium falciparum has a G+C ratio of about 20%, much lower than the ratios seen in the so-called low G+C bacteria (Class Bacilli and Class Clostridia) that hover just under 50%.

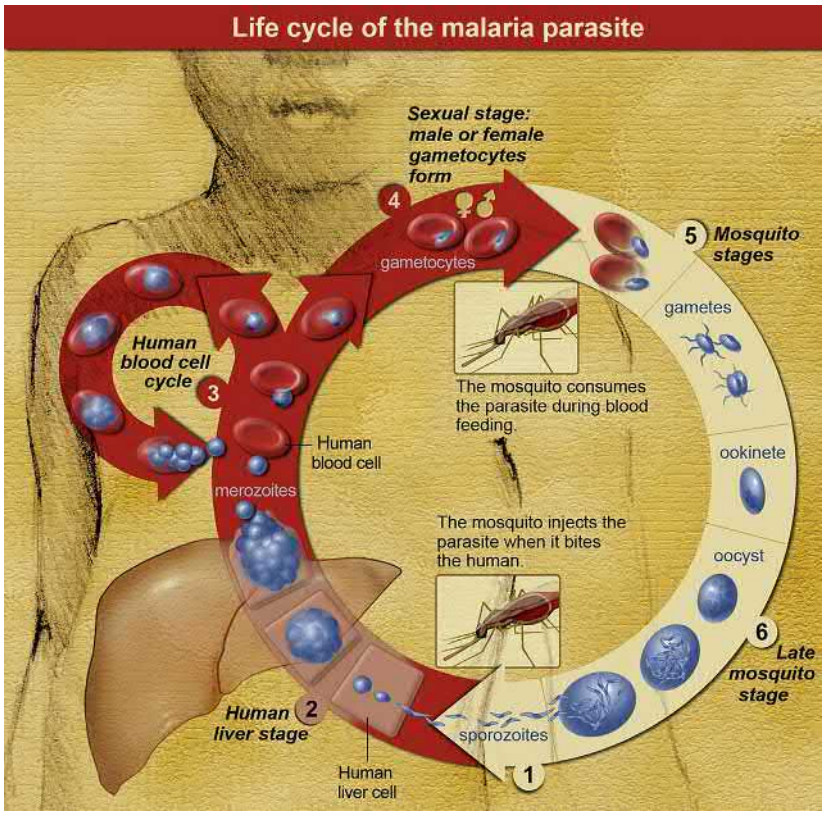

FIG. 4.7 The complex life cycle of the malaria parasite. *(Source, a public domain image provided by the US National Institutes of Health.)*

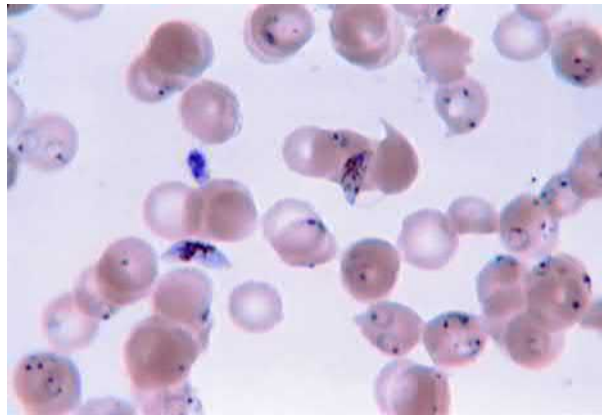

FIG. 4.8 Giemsa-stained blood smear showing Plasmodium organisms at different stages of maturation. The large, blue, banana-shaped forms are gametocytes. Inside red blood cells are rings with a blue setting, the so-called ring form of trophozoites. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Human babesiosis is uncommon, but this organism infects many mammals other than humans, and is the second most common parasitic blood disease of nonhuman mammals (after trypanosomal infections) (Fig. 4.9).

Two species infect humans: Babesia divergens, the most common cause of babesiosis in Europe, and Babesia microti, the most common type of babesiosis in North America [35]. The tick, Genus Ixodidae, serves an almost identical role for Genus Babesia as the Anopheles mosquito serves for Genus Plasmodium:

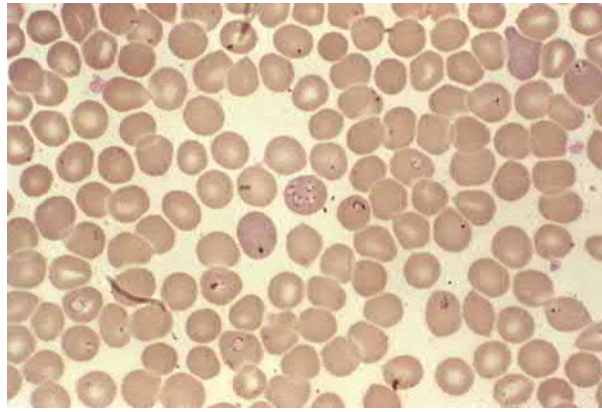

FIG. 4.9 Giemsa-stained blood smear showing Babesia organisms. Notice ring forms inside red blood cells that have similar morphology to the ring forms observed in Plasmodium infections. The ring forms of babesiosis, unlike those of malaria, are commonly found outside of erythrocytes. Clumps of ring forms are common in babesiosis; less so in malaria. Also, unlike the ring forms of malaria, the ring forms of babesiosis do not produce pigment. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

sporozoites develop in the salivary gland of the tick and are introduced into the human host via a bite. In babesiosis, unlike malaria, infection occurs exclusively in red cells and is not found in liver cells. Aside from humans, animal reservoirs of Babesia microti and Babesia divergens include mice and deer. Like malaria, babesiosis can be transmitted via transfusions with contaminated blood products.

```
Apicomplexa
  Conoidasida
    Coccidia
      Eucoccidiorida
        Eimeriorina
          Cryptosporidiidae
            Cryptosporidium (genus)
          Eimeriidae
            Cyclospora (genus)
            Isospora (genus)
        Sarcocystidae
          Sarcocystis (genus)
          Toxoplasma (genus)
```

Six species of Class Coccidia produce disease in humans: Cryptosporidium parvum, Cyclospora cayetanensis, Cystoisospora belli (Isospora belli), Sarcocystis suihominis, Sarcocystis bovihominis, and Toxoplasma gondii.

As a subclass of Class Apicomplexa, the members of Class Coccidia are obligate intracellular parasites. Nonetheless, Class Coccidia confers two features that makes it possible for coccidan species to survive outside the host, for a period of time. First, there is the durable coccidian oocyst. Most coccidians attach to enterocytes (particularly, the epithelial cells that line the small intestine). From this location, oocysts are carried within feces and eventually contaminate the water where feces are deposited. One method of coccidian infection is through oocysts that contaminate food and water. Second, some coccidians encyst within the tissues of the host organism. This is accomplished without violating the intracellular lifestyle typical of the apicomplexans. The coccidian cyst is formed from the husk of a host cell that has been filled with multiplying, small forms of the organism. These so-called cysts, which are really modified host cells filled with infective organisms, are stable and durable. When the host tissues are eaten by another animal, the cysts release an infective form of the organism, and the coccidian life cycle repeats, beginning in the intestines.

The diseases produced by any of the organisms belonging to Class Coccidia are known collectively as coccidiosis. This term is applied most often to coccidian infections in animals.

Cryptosporidiosis is caused by Cryptosporidium parvum. It is typically transmitted by drinking water that has been contaminated with relatively stable oocysts carried by the feces of an infected animal. Infection is confined to the

mucosa of the small intestine, typically producing a watery diarrhea that is self-limited in immune-competent individuals. Cryptosporidiosis infection can produce a severe gastroenteritis in immunodeficient individuals. In some cases of Cryptosporidiosis occurring in immunodeficient persons, the organism is never completely cleared from the small intestine. Cryptosporidiosis is a common disease that occurs worldwide (Fig. 4.10).

Like all members of Class Apicomplexa, Genus Cryptosporidium is an obligate intracellular organism. Casual histologic examination of infected mucosa shows that the organisms perch atop the apical surface of the epithelial cells lining the small intestinal villi (i.e., extracellular growth). Careful examination demonstrates that the cryptosporidia attached to the mucosal surface are actually wrapped by host cell membranes (i.e., intracellular growth). Students should avoid confusing cryptosporidiosis with similar-sounding cryptococcosis (Class Basidiomycota).

One species of Genus Cyclospora produces disease in humans: Cyclospora Cayetanensis. As in Cryptosporidiosis, stable oocysts carried in water contaminated with infected animals, pass into the GI tract, and produce sporozoites that grow in the small intestine. The infection produces diarrhea. On histologic stains, Cyclospora Cayetanensis can be mistaken for Cryptosporidium parvum, but Cyclospora Cayetanensis is the larger of the two organisms (Fig. 4.11).

Cystoisospora (synonymous with Isospora) belli has a life cycle, mode of transmission, and clinical presentation (enteritis) similar to that of Cryptosporidium parvum and Cyclospora Cayetanensis. Isosporiasis is an uncommon disease, but when it occurs, it tends to arise in immunodeficient individuals. Diagnosis can usually be made after careful stool examination. The oocysts are ellipsoidal and large.

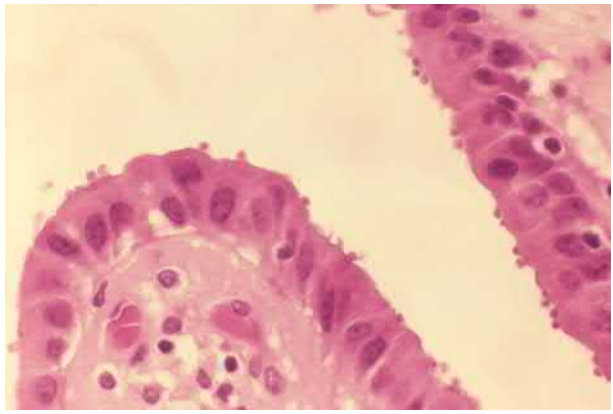

FIG. 4.10 Cryptosporidiosis involving gall bladder. The Cryptosporidium organisms are the small, round, eosinophilic (pink), bumps hugging the large epithelial cells forming the surface layer of the gall bladder. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, prepared by Dr. Edwin P. Ewing, Jr.)*

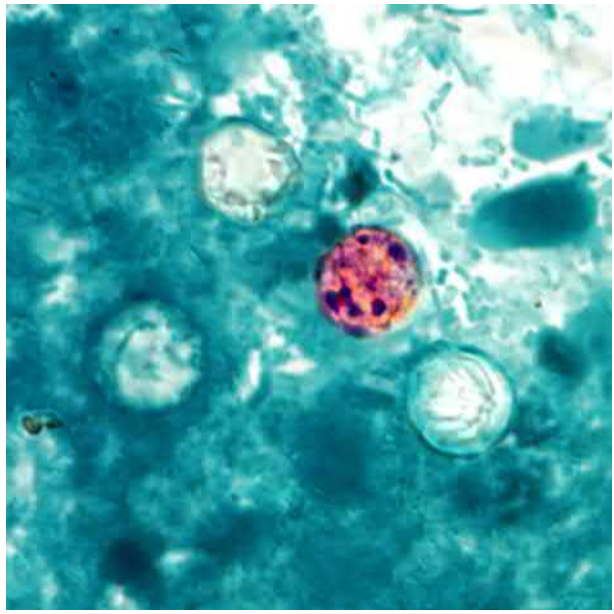

FIG. 4.11 Stool sample with modified acid fast stain demonstrating four oocysts of Cyclospora cayetanensis. The staining is variable, with one of the four organisms having a brown, mottled appearance. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention and prepared by Melanie Moser.)*

Cryptosporidium, Cyclospora, and Cystoisospora, the three enteric coccidians, are genera under Class Eimeriorina. Two of the coccidians are genera under Class Sarcocystidae: Sarcocystis and Toxoplasma. These genera produce a specialized structure, containing dormant, infectious organisms, filling the husk of a host cell. These structures have specialized names, but most health-care workers use the short but inaccurate term, "cysts."

Sarcocystis suihominis and Sarcocystis bovihominis can cause disease in humans. As with other coccidians, oocysts are released into the gut lumen and pass into the environment via feces. The intermediate host ingests contaminated water, and infection leads to the development of cysts in host cells, usually muscle; hence the name sarcocyst (from the Greek, "sarx," flesh). When the intermediate host is eaten by a primary host, the organisms within the sarcocyst reproduce, and their oocytes are eventually released into the gut, where they mix with fecal material and leave the host. The sarcocyst's primary host has a purely enteric infection. The intermediate host, in which ingested organisms invade muscle throughout the body, and develop sarcocysts within muscle cells, has an enteric and a systemic disease. As their names would suggest, infections occur in swine (hence, suihominis), or cows (hence, bovihominis). On rare occasion, humans become infected and may serve as either primary or secondary hosts. Though sarcosporidiosis is considered a rare disease in humans, the animal

reservoir for human infections is large. Various species of Genus Sarcocystis commonly infect a wide range of animals worldwide. In Southeast Asia, postmortem tongue biopsies revealed sarcocysts in 21% of the sampled population [36]. This indicates that many human infections are asymptomatic.

The sister genus to Sarcocystis is Toxoplasma. About one-third of the human population has been infected (i.e., about 2.3 billion people) by the only species that produces human toxoplasmosis: Toxoplasma gondii. This number is somewhat higher than the number of tuberculosis infections worldwide (about 2 billion). In the United States, the prevalence rate of Toxoplasma infection is about 11%.

Toxoplasma has a life cycle very similar to Sarcocystis. The most common primary host of Toxoplasma is the cat. Humans are the intermediate host. For most infected individuals, infection does not cause overt disease. Serious disease occurs most often in immune-compromised hosts, particularly individuals with AIDS or individuals who are pregnant. Transplacental infections may occur. Disease occurs in the locations where the cysts develop, often in the brain (causing encephalitis), eyes (causing chorioretinitis), or in tissues through which the organisms migrate (e.g., lymph nodes). Latent cysts can produced a reactivation of toxoplasmosis, and this occurs most often in individuals who were infected early in life and later became immune-compromised due to disease or immunosuppressive therapy (Fig. 4.12).

Before leaving the coccidioses, we must visit an unrelated fungal disease, with a similar sounding name: coccidioidomycosis. The story to this confusing terminology harkens back to a journal article, published in 1896, by two authors who had encountered a skin disease. Based on a misinterpretation of

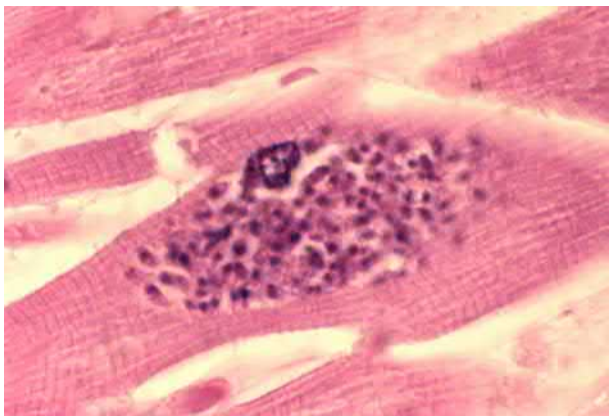

FIG. 4.12 Cardiac muscle cells infected by Toxoplasma gondii. The cardiac muscle cells are recognized by their numerous, evenly spaced cross-striations. In the central cell, there is a pseudocyst containing numerous small toxoplasma bradyzoites. *(Source, a public domain image provided by the US Center for Disease Control and Prevention, prepared by Dr. Edwin P. Ewing, Jr.)*

morphologic findings, the authors thought that they had encountered a novel coccidian organism, and named it Coccidioides immitis [37]. The misleading name has stuck for more than 120 years, up to the present. Through the years, the terms coccidiosis and coccidioidomycosis have been confused, and used interchangeably, to the detriment of clinical care.

As it happens, Coccidioides immitis is a fungus of class Ascomycota; not a coccidian. We will be discussing coccidioidomycosis in Section 6.4, "Ascomycota."

Infectious Genera

Plasmodium

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Aconoidasida: Haemosporida: Plasmodiidae: Plasmodium: Plasmodium
- **Infection**. Plasmodium falciparum (tertian malaria)
- **Infection**. Plasmodium vivax (tertian malaria)
- **Infection**. Plasmodium ovale (tertian malaria)
- **Infection**. Plasmodium malariae (quartan malaria)
- **Infection**. Plasmodium knowlesi (zoonotic malaria)

Babesia

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Aconoidasida: Piroplasmida: Babesiidae: Babesia
- **Infection**. Babesia divergens (babesiosis, piroplasmosis)
- **Infection**. Babesia microti (babesiosis, piroplasmosis)
- **Infection**. Babesia duncani (babesiosis, piroplasmosis)

Cryptosporidium

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Conoidasida: Coccidia: Eucoccidiorida: Eimeriorina: Cryptosporidiidae: Cryptosporidium
- **Infection**. Cryptosporidium parvum (cryptosporidiosis, beaver fever)

Cyclospora

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Conoidasida: Coccidia: Eucoccidiorida: Eimeriorina: Eimeriidae: Cyclospora
- **Infection**. Cyclospora cayetanensis (cyclosporiasis, gastroenteritis, some cases of traveler's diarrhea)

Cystoisospora

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Conoidasida: Coccidia: Eucoccidiorida: Eimeriorina: Sarcocystidae: Cystoisospora
- **Infection**. Cystoisospora belli, formerly and still used, Isospora belli (isosporiasis)

Sarcocystis

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Conoidasida: Coccidia: Eucoccidiorida: Eimeriorina: Sarcocystidae: Sarcocystis
- **Infection**. Sarcocystis suihominis, formerly Isospora hominis (sarcocystosis, sarcosporidiosis)
- **Infection**. Sarcocystis bovihominis, also known as Sarcocystis hominis, formerly Isospora hominis (sarcocystosis, sarcosporidiosis)

Toxoplasma

- **Lineage**. Eukaryota: Alveolata: Apicomplexa: Conoidasida: Coccidia: Eucoccidiorida: Eimeriorina: Sarcocystidae: Toxoplasma
- **Infection**. Toxoplasma: Toxoplasma gondii

Section 4.6 Ciliophora (ciliates)

There should be some things we don't name, just so we can sit around all day and wonder what they are.

George Carlin

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Ciliophora (ciliates)
          Litostomatea
            Vestibuliferida
              Balantiididae
                Balantidium (genus)
        Apicomplexa
    Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
```

Ciliophora and Apicomplexa are the two subclasses of Class Alveolata that contain human pathogens. Members of Class Alveolata are single-celled organisms

with distinctive sacs underlying the plasma membrane (alveoli). The mitochondria of alveolates have tubular cristae (internal partitions).

The ciliates (members of Class Ciliophora) are single-celled eukaryotes that are physically distinguished by the profusion of cilia visible on their surfaces (Fig. 4.13)

Each ciliate organism is endowed with two nuclei, with each nucleus having its own peculiar genomic architecture and cellular purpose. One nucleus is large and polyploid (the macronucleus), and serves the general physiologic needs of the cell (e.g., producing RNA and maintaining the cell's phenotype). The other nucleus (the micronucleus) is tiny and does not express its genes. It serves as the genetic repository preserving the germline of the organism. The micronucleus also produces the macronucleus, through a process that requires the amplification of its genome, followed by some severe editing. The macronucleus divides by a kinky process known as amitosis, but each amitotic division reduces its viability. After about 200 such divisions, the micronucleus must generate a new macronucleus. On such occasions, two ciliates may couple, and their micronuclei exchange DNA via a conjugal mechanism that involves the meiotic production and exchange of haploid chromosomes. If movie producers want to wow their audiences with a glimpse of otherworldly cellular behavior, they need go no further than the ciliates. There are an estimated 30,000 species of these organisms, and they can be found swimming in any pond or river [Glossary Polyploidy] (Fig. 4.14).

There is only one species of ciliates known to cause human disease: Balantidium coli (Fig. 4.15).

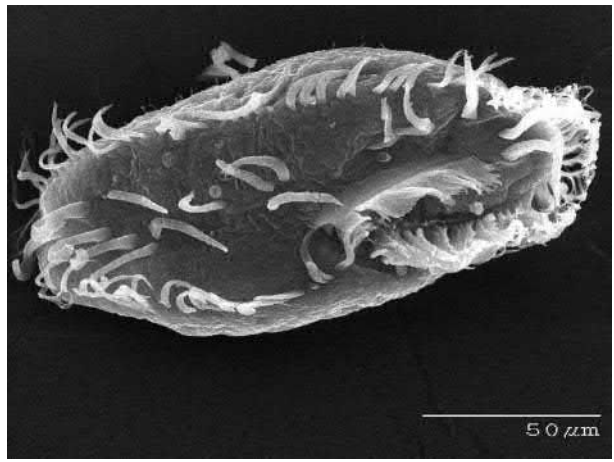

FIG. 4.13 Oxytricha trifallax, a typical ciliate, with ovoid shape and plentiful cilia. *(Source, Wikipedia, from a US government public domain work.)*

FIG. 4.14 Stentor roselii, a ciliate that lives in ponds and ditches. When attached to a firm surface, the cell stretches to form a trumpet shape that can reach up to 1.2 mm in length. Its cilia serve to brush passing microorganisms into its wide mouth. *(Source, Wikipedia, from a public domain image.)*

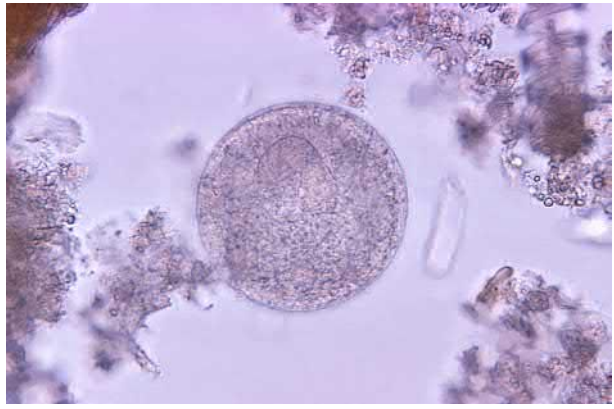

FIG. 4.15 Balantidium coli cyst (center) surrounded by fecal debris. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and prepared by Dr. L.L.A. Moore, Jr.)*

```
Ciliophora
  Litostomatea
    Vestibuliferida
      Balantiididae
        Balantidium (genus)
```

Balantidiasis is a zoonosis, found in a variety of animals, but the most important reservoir is the pig. In pigs, the organisms produce no apparent clinical disease. The organisms live in the pig's colon, and are passed into the feces. Human disease, which is rare, occurs when humans ingest food or water that has been contaminated by pig feces, or, rarely, from the feces of infected humans.

There are two forms of the organism: trophozoite and cyst. The trophozoites are the active, growing form of the organisms. Trophozoites transform into cysts under poor growth conditions. Encystation typically occurs in the distal colon, where the feces are relatively dry, and unfavorable for growth. The cysts are inactive, but can survive adverse conditions. Cysts are passed in the feces and are the passively infective form of the organism. Once in the intestine, cysts transform into trophozoites.

The trophozoites are capable of invading into the wall of the colon and occasionally produce ulcers. The ulcers of balantidiasis have a peculiar inverted-flask shape (i.e., a small opening on the lumenal surface overlying edges that extend laterally and ends sharply as a flat base, in the colon wall). Most cases of human balantidiasis are mild (some diarrhea or constipation) or asymptomatic. Cases of balantidiasis occur worldwide, but they are rare in the United States. Balantidiasis is a common infection in the Philippines.

Infectious Genera

- **Lineage**. Eukaryota: Alveolata: Ciliophora: Intramacronucleata: Litostomatea: Trichostomatia: Vestibuliferida: Balantidiidae: Balantidium
- **Infection**. Balantidium coli (balantidiasis)

Section 4.7 Heterokonta

Biology is the science. Evolution is the concept that makes biology unique.

Jared Diamond

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta (stramenopiles)
        Blastocystae
          Blastocystida
            Blastocystidae
              Blastocystis (genus)
  Unikonta (1-flagellum)
```

```
Amoebozoa
Opisthokonta
  Choanozoa
  Animalia
  Fungi
```

Heterokonta (also known as the stramenopiles) are bikonts (two flagella). The name "heterokont" derives from a characteristic morphologic feature; the two flagella are nonidentical. The class contains two strikingly different types of organisms; a colored group containing various types of algae and diatoms, and a colorless group containing organisms that have morphologic features similar to fungi.

Class Heteroka has undergone extensive taxonomic revision in the recent past. The golden and brown algae (currently in Class Heterokonta) were previously classified under Class Plantae. Adding to the confusion, green and red algae are currently placed in Class Archaeplastida. Various species of the colorless group (currently in Class Heterokonta) had been incorrectly placed in Class Fungi. Oomycota, a "colorless" class of heterokonts, contains the organisms that produce late blight of potato (Phytophthora infestans), and sudden oak death (Phytophthora ramorum). Oomycota, despite its suffix (mycota, an alternative name for fungus), is not a member of Class Fungi. Aside from its phylogenetic placement among the heterokonts (and not the opisthokonts to which the fungi belong), the oomycota cell wall is composed of cellulose, while the cell wall of fungi is made from chitin.

Given the taxonomic turmoil within Class Heterokonta, students should be grateful that there is only one heterokont genus that is infectious to humans: Blastocystis.

```
Heterokonta
  Blastocystae
    Blastocystida
      Blastocystidae
        Blastocystis (genus)
```

Blastocystis infects a variety of animals. The general rule for naming species of Blastocystis is to apply the genus to the species in which the organisms are recovered. Hence, every species of Blastocystis recovered from human feces was named Blastocystis hominis. Species of the organism recovered from rat feces were all named Blastocystis ratti. Obviously, this taxonomic disaster cannot persist. Efforts to identify individual species by species-specific genotyping (rather than bundling different species under the host species name) have begun [38].

Infection seems to be through acquisition of the cyst form of the organism, through a fecal-oral route. Human-human, animal-human, and human-animal transmission all may occur, but the relative frequencies of these

different transmissions are unknown. Incidence is highest where humans are exposed to animal feces, implying that animal-human transmission is common. In the United States, the prevalence rate of infection of Blastocystis hominis is 23%, with the highest rates found in the western states [39].

Four different morphologic forms of Blastocystis have been observed: cyst, vacuolar, ameboid, and granular. After the cyst is ingested, the other forms emerge in the intestine. Replication of the organism occurs in the vacuolar form. The length of infection varies from weeks to years. Many Blastocystis infections do not manifest clinically, and an asymptomatic carrier state is common. Disease, when it occurs, can closely mimic irritable bowel syndrome. When species of Blastocystis hominis are identified by type-specific laboratory testing, we will be in a better position to determine whether specific variants of the organism are correlated with clinical expression (e.g., asymptomatic disease, long-term carrier state, irritable bowel-like symptoms, and inflammatory bowel disease symptoms).

As a final point, it is important to avoid confusing Blastocystis with three similar-appearing but unrelated terms: blastomycosis, blastocyst, and blastomycetica. Blastomycoses is a fungal disease (Ascomycota). Blastocyst is the fluid-filled embryonic body characteristic of animals. Blastomycetica appears in the name of a fungal infection "erosio interdigitalis blastomycetica." This infection, despite what its name would imply, is a candidal infection of the web space between the third and fourth fingers of either hand. The infection was given its deceptive name in 1917, 5 years before the genus Candida was recognized [40]. As is often the case, the mistake stuck. All four terms come from the root word blastos (Greek for bud or embryo). Cystos (as in Blastocystis and blastocyst) is the Greek root meaning sac.

Infectious Genera

- **Lineage**. Eukaryota: Stramenopiles (same as Heterokonta): Blastocystae: Blastocystida: Blastocystidae: Blastocystis
- **Infection**. Blastocystis hominis (blastocystosis)

Section 4.8 Amoebozoa

Because all of biology is connected, one can often make a breakthrough with an organism that exaggerates a particular phenomenon, and later explore the generality.

Thomas R. Cech

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
```

```
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
Unikonta (1-flagellum)
  Amoebozoa
    Lobosea
      Discosea
        Thecamoebidae
          Sappinia (genus)
      Centramoebida
        Acanthamoebidae
          Acanthamoeba (genus)
          Balamuthia (genus)
    Conosa
      Archamoebae
        Pelobiontida
          Entamoeba (genus)
  Opisthokonta
    Choanozoa
    Animalia
    Fungi
```

Class Eukaryota is broadly divided into two large domains, the Bikonta (two flagella) and the Unikonta (one flagellum). The Amoebozoa belong to the Class Unikonta. Most extant Amoebozoan species have no undulipodia, but a few species of Amoebozoans retain their ancestral undulipodium. Because the Amoebozoans are Unikonts, they are related to Class Opisthokonta, which includes Class Choanozoa, Class Animalia, and Class Fungi

Amoebozoa move by flowing their cytoplasm from one area of the cell to another. Movement begins when a part of the cell wall, the lobopodium, is protruded. As cytoplasm flows into the lobopodium, the rest of the cell cannot help but follow. The amoebozoan genera vary greatly in size. Species producing disease in humans are about the size of human cells (10–40 μm), while at least one nonpathogenic species, Amoebozoa proteus, attains a size of 800 μm (on the verge of visibility with the unaided eye). The members of Class Amoebozoa engulf and eat smaller organisms. Most members of Class Amoebozoa live in the soil or aquatic environments, where they are beneficial bacterial predators (Entamoeba is an exception, vida infra) [3]. The pathogenic members of Class Amoebozoa occur in tissues as trophozoites (the amoeboid feeding cells) or as cysts (round, infective cells resistant to desiccation).

We include Class Amoebozoa among the single-cell eukaryotes, but it should be remembered that members of Class Amoebozoa are capable of communal behavior. Amoebae have been observed aggregating as small slug-like colonies that behave somewhat like multicellular organisms, that sense environmental stimuli, and that migrate in response to sensory input [3].

Determining the subclasses of Class Amoebozoa has proven to be very difficult [41]. It is likely that the class schema shown in this chapter will be changed in the next few years, when newly acquired genomic information is studied along with previously documented morphologic and physiologic data. In this book, Class Amoebozoa is divided into two major subclasses: Class Lobosea and Class Conosa. All of the pathogenic genera within Class Lobosea are capable of producing meningoencephalitis. Class Conosa includes one pathogenic genus: Entamoeba histolytica, which typically produces a dysentery-like condition. Aside from this division that neatly distinguishes the primary infections of the central nervous system and the primary infections of the intestines, the taxonomy of the infectious Amoebozoans is best studied genus-by-genus.

```
Amoebozoa
  Lobosea
    Discosea
      Thecamoebidae
        Sappinia (genus)
    Centramoebida
      Acanthamoebidae
        Acanthamoeba (genus)
        Balamuthia (genus)
```

Sappinia is a genus of free-living organisms, within Class Lobosea. There are two species capable of producing meningoencephalitis: Sappinia diploidea and its nearly identical species, Sappinia pedata [42]. Sappinia species can be found in trophozoite form (i.e., feeding amoeboid forms) and cyst form. The trophozoites are recognized by their two closely apposed nuclei, with a central flattening. Sappinia encephalitis may occur in immune-competent individuals. Only a few cases have been reported [43].

Genus Acanthamoeba and Genus Balamuthia are members of Class Acanthamoebidae. There are a variety of Acanthamoeba species that cause a similar set of human diseases. These include: Acanthamoeba rhysoides, Acanthamoeba polyphaga, Acanthamoeba palestinensis, Acanthamoeba hatchetti, Acanthamoeba culbertsoni, Acanthamoeba castellani, and Acanthamoeba astronyxis. These similar species are herein aggregated under the name Acanthamoeba species.

The Acanthamoeba are ubiquitous and found in the domestic water supply. Acanthamoeba species cause three distinctive clinical diseases: granulomatous amoebic encephalitis, amoebic keratitis, and cutaneous acanthamoebiasis

[43, 44]. Though many people are exposed to Acanthamoeba species, few people develop disease. Granulomatous encaphalitis and cutaneous acanthamoebiasis occur most often in people with immune deficiencies. Trophozoites and cyst forms can be observed in infected tissues. The trophozoite form may be confused histologically with macrophages. Scanning electron micrographic evaluation of the trophozoite would demonstrate the numerous surface spikes called acanthopodia, which distinguish members of the Acanthamoeba species from other amoebae [44]. Trophozoites have a single nucleus with a single large nucleolus. The cyst form can be visualized with a variety of histologic stains [43]. Acanthamoebic granulomatous encephalitis is a very rare disease, with a few hundred cases reported, worldwide. Only a few cases of cutaneous acanthamoebiasis have been reported

Acanthamoebic keratitis can occur in immune-competent individuals. It occurs most often in contact lens users, presumably from washing the lens in water containing the Acanthamoeba species.

The other pathogenic genus within Class Acanthamoebidae is Balamuthia. The only known pathogenic species of this genus is Balamuthia mandrillaris, which causes granulomatous encephalitis, much like that caused by Acanthamoeba species. The organism is found in soil, and possibly water. Like the other pathogenic members of Class Amoebozoa, it occurs in two forms: trophozoite and cyst. In tissue sections, Balamuthia mandrillaris is indistinguishable from Acanthamoeba species by standard microscopic examination. Cases of Balamuthia encephalitis are extremely rare, with only a few cases reported in the United States each year, primarily in immune-compromised patients (Fig. 4.16).

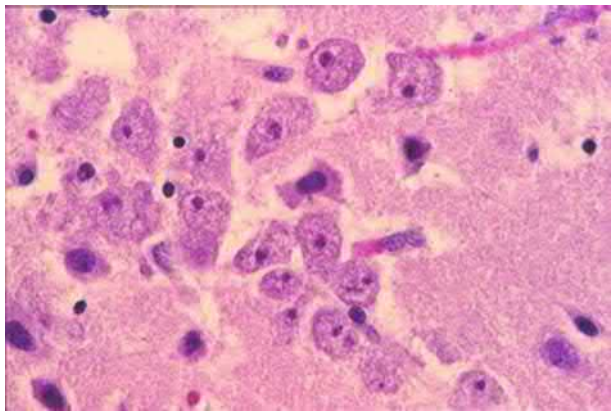

FIG. 4.16 Balamuthia infecting brain tissue. About 14 Balamuthia mandrillis trophozoites are scattered in the image. Morphologically, the Balamuthia organisms resemble foamy macrophages. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

```
Amoebozoa
  Conosa
    Archamoebae
      Pelobiontida
        Entamoeba (genus)
```

Genus Entamoeba contains the most commonly occurring pathogenic amoebic species, Entamoeba histolytica [3]. Infection occurs through fecal-oral transmission of the cyst form. It is estimated that about 50 million people are infected by Entamoeba histolytica, with about 70,000 deaths per year, worldwide. All of the other amoebozoan organisms discussed in this chapter, combined, account for just a few dozen deaths. Most Entamoeba histolytica infections do not result in clinical disease. The organism can live in the intestine for years as a commensal. Unlike the pathogenic amoebas discussed so far, Entamoeba histolytica is not found widely distributed in soil samples. Entamoeba histolytica has adapted itself to life inside a narrow range of preferred hosts: primates. As such, its life is restricted to the GI tract of humans and other primates, and to their feces. In the GI tract, the organism can be found in its amoeboid feeding form (i.e., Trophozoite). In stools, the trophozoites encyst; cysts can survive for months (Fig. 4.17).

Entamoeba gingivalis is a species of Entamoeba that is routinely found in a high percentage of specimens obtained from human saliva or from gingival scrapings. Its pathogenicity in oral diseases is controversial. Surprisingly, a recent case report has demonstrated Entamoeba gingivalis in a pulmonary abscess [45].

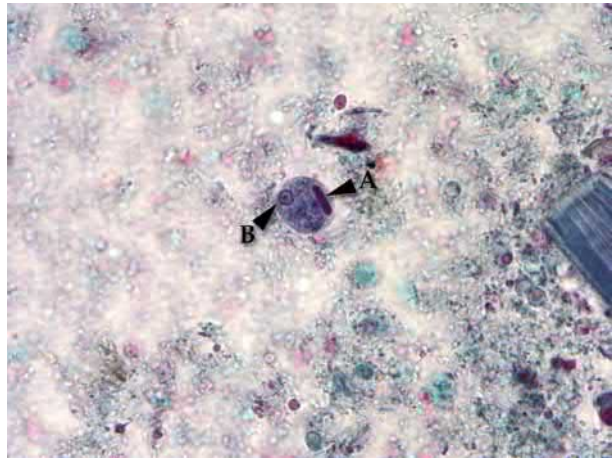

FIG. 4.17 Cyst of Entamoeba histolytica (center) surrounded by fecal matter. A rod-shaped chromatoid body is marked by arrow A. The cyst nucleus is marked by arrow B. Morphologically Entamoeba histolytica is virtually indistinguishable from Entamoeba dispar, a common, nonpathogenic inhabitant of human intestines. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention and prepared by Melanie Moser.)*

Entamoeba comes with four taxonomic traps that have been used to mortify generations of students.

–1. First and foremost, do not confuse Entamoeba coli (abbreviation E. coli) with Escherichia coli (likewise abbreviated as E. coli). Both live in the colon, and both can be reported in stool specimens.
–2. Of the Entamoeba genera that can be found on examination of human stools, only one is frequently pathogenic, Entamoeba histolytica. Entamoeba dispar, Entamoeba hartmanni, Entamoeba coli, Entamoeba moshkovskii, Endolimax nana, and Iodamoeba butschlii, occasionally found in stool specimens, are generally nonpathogenic.
–3. Do not confuse Entamoeba (Class Amoebozoa) with Dientamoeba (Class Metamonada).
–4. Terminology for amoebic infections is somewhat confusing. It is commonly agreed that the term "amoebiasis," with no qualifiers in the name, refers exclusively to the intestinal infection by Entamoeba histolytica, and is also known by the name "amoebic dysentery." Encephalitides caused by members of Class Amoebozoa (Acanthamoeba and Balamuthia) are named granulomatous amoebic encephalitis. Encephalitis caused by Naegleria fowleri (not an amoeba) is called primary amoebic meningoencephalitis, an accepted misnomer. Naegleria is a member of class Percolozoa. A better name for the Naeglerian disease would be primary percolozoan meningoencephalitis. Acanthamoeba castellanii causes amoebic keratitis, and is an occasional cause of granulomatous amoebic encephalitis. Sappinia diploidea causes Sappinia amoebic encephalitis.

Infectious Genera

Sappinia

- **Lineage**. Eukaryota: Amoebozoa: Discosea: Longamoebia: Thecamoebida: Sappinia
- **Infection**. Sappinia diploidea (Sappinia amoebic encephalitis)
- **Infection**. Sappinia pedata (Sappinia amoebic encephalitis)

Acanthamoeba

- **Lineage**. Eukaryota: Amoebozoa: Discosea: Longamoebia: Centramoebida: Acanthamoebidae: Acanthamoeba
- **Infection**. Acanthamoeba species (granulomatous amoebic encephalitis, amoebic keratitis, and cutaneous acanthamoebiasis)

Balamuthia

- **Lineage**. Eukaryota: Amoebozoa: Discosea: Longamoebia: Centramoebida: Balamuthiidae: Balamuthia
- **Infection**. Balamuthia mandrillaris (granulomatous amoebic encephalitis)

Entamoeba

- **Lineage**. Amoebozoa: Conosa: Archamoebae: Pelobiontida: Entamoeba
- **Infection**. Entamoeba histolytica (amoebic dysentery, amoebic colitis, amoebic liver abscess, amoebic brain abscess, entamoebiasis, amoebiasis)
- **Infection**. Entamoeba gingivalis (periodontal disease)

Section 4.9 Choanozoa

Half of being smart is knowing what you are dumb about.

Solomon Short

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
        Ichthyosporea
          Dermocystida
            Rhinosporidium (genus)
      Animalia
      Fungi
```

Class Choanozoa is a subclass of Class Opisthokonta, unicellular eukaryotes with a single, posterior flagellum. The precise hierarchical position of Class Choanaozoa is subject to change. In the schema below, Class Choanozoa has the same rank as Class Animalia, making it the sister class of all metazoans. The name "choanozoa" has a Greek root meaning "funnel" and refers to the cellular collar that is characteristic of the species in the class. Class Choanozoa contains one genus, with one species that is pathogenic to humans: Rhinosporidium seeberi.

```
Choanozoa
  Ichthyosporea (same as Mesomycetozoea)
    Dermocystida
      Rhinosporidium (genus)
```

Rhinosporidiosis presents clinically as a mass growing on the nasal respiratory lining, often causing nasal obstruction. These growths may occur on other mucosal surfaces, including the ocular conjunctivae, and can occur in animals other than humans. The diagnosis is usually established by histologic examination and by clinical presentation. Tissue sections are characterized by inflammatory tissue embedding large round organisms (the trophocytes).

Members of Class Choanozoa are aquatic, and cases of human rhinosporidiosis can usually be associated with exposure to water in ponds, lakes, or rivers. However, the organisms Rhinosporidium seeberi has never actually been isolated from these sources, nor has the organism been successfully cultured [46]. Because the organism has never been isolated from any presumed aquatic reservoir, and has never been cultured, its taxonomic assignment has not been easy. In the past, the organism was presumed to be fungal, as it looks somewhat like a large yeast on histologic cross section. Molecular analysis places the Rhinosporidium genus as a subclass within Class Choanozoa [46].

The geographic location with the highest incidence of rhinosporidiosis is Southern India and Sri Lanka. In general, the disease can be found in any tropical region, and cases have been reported in the Southeastern United States [46].

Rhinosporidiosis should not be confused with rhinoscleroma, a granulomatous lesion involving the nasal mucosa, caused by the bacteria Klebsiella rhinoscleromatis (Gamma Proteobacteria) (Fig. 4.18).

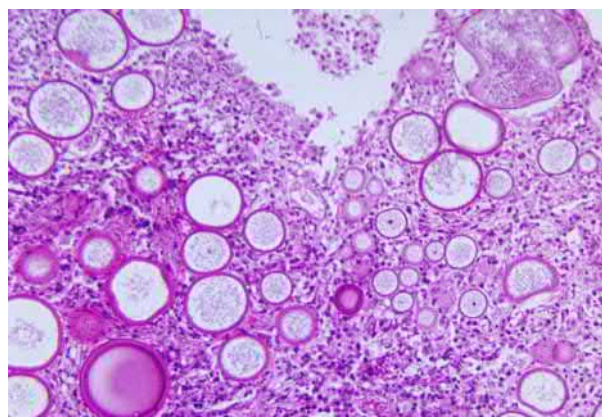

FIG. 4.18 An inflammatory nasal polyp produced by Rhinosporidium seeberi infection (rhinosporidiosis). Many round trophocytes are seen in an acute and chronic inflammatory background. The reactive inflammatory cells are dwarfed by the large trophocytes. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and prepared by Dr. Martin Hicklin.)*

Infectious Genera

Rhinosporidium

- **Lineage**. Eukaryota: Opisthokonta: Incertae sedis (problematic class): Ichthyosporea: Dermocystida: Rhinosporidium
- **Infection**. Rhinosporidium seeberi (rhinosporidiosis)

Section 4.10 Archaeplastida

If you want to make an apple pie from scratch, you must first create the universe.

Carl Sagan

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
      Viridiplantae
        Chlorophyta
          Trebouxiophyceae
      Chlorellales
        Chlorellaceae
          Prototheca (genus)
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
```

There seems to be only one organism, in the entire kingdom of plants, that is capable of causing an infectious disease in humans. This organism is the algae, Prototheca wickerhamii. The emergence of plants, and the phylogenetic history of Genus Prototheca, makes a fascinating story.

Archaeplastida is the superclass of the kingdom of plants. As the story goes, oxygenic photosynthesis was discovered by cyanobacteria, about 2.5 billion

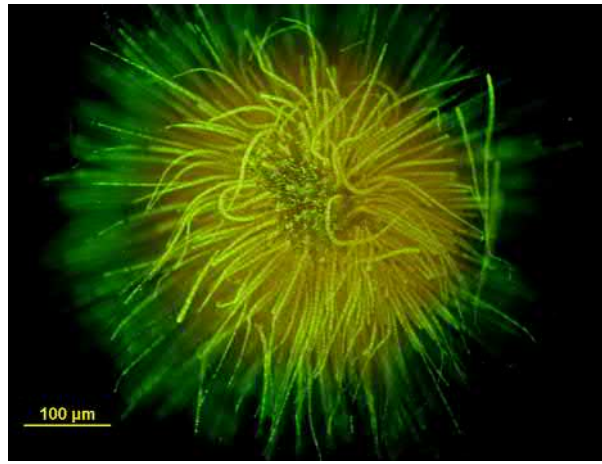

FIG. 4.19 Gloeotrichia echinulata, a member of Class Cyanobacteria, the first and only class of terrestrial organisms to evolve oxygenic photosynthesis. *(Source, Wikipedia, from a public domain image commissioned by the U.S. Geological Survey, and photographed by Barry Rosen.)*

years ago. Subsequently, the cyanobacteria refined the process, and no organism other than cyanobacteria can be credited with oxygenic photosynthesis (though other organisms use photosynthetic processes that do not produce oxygen). All oxygenic photosynthesis in eukaryotes is accomplished with the use of chloroplasts, a symbiotic organelle formed from captured cyanobacteria. An ancient member of Class Archaeplastida found that by engulfing cyanobacteria, it could photosynthesize. This indentured relationship between Arachaeplastida and cyanobacteria became permanent, and every descendant of Class Arhaeplastida, including all the green plants, have benefited from their ancestor's theft of a cyanobacteria. It is generally believed that the acquisition of cyanobacteria as a self-replicating synthesizing organelle occurred only once in earth's history. Chloroplast-containing organisms other than those within Class Archaeplastida, seized chloroplasts (not cyanobacteria) from green algae or other members of Class Archaeplastida [Glossary Cyanobacteria] (Fig. 4.19).

How do we know that chloroplast-containing organisms outside Class Archaeplastida acquired chloroplasts from other eukaryotic organisms, and not from cyanobacteria? By counting membranes around the chloroplast. Chloroplasts in the Archaeplastida are lined by two layers, corresponding to the inner and outer membranes of the original, indentured cyanobacteria. The chloroplasts of non-Archaeplastida eukaryotes have three or four membrane layers, suggesting that a member of Archaeplastida was engulfed, and the membranes of the chloroplast and the Arhaeplastida were entrapped permanently in the host cell [Glossary Kleptoplast].

As aforementioned, the creation of a chloroplast, from captured cyanobacteria, occurred once only, with all extant chloroplasts deriving from an early

union between a member of Class Archaeplastida and a cyanobacterium. This assertion may be false in one specific instance. Paulinella chromatophora, a member of the eukaryotic class Rhizaria, seems to have captured its own cyanobacteria and created its own permanent chloroplast-like organelle [47]. This conclusion is based, in part, on the dissimilarities between the photosynthesizing organelles of Paulinella chromatophora, and those of all other chloroplast-containing eukaryotes.

Chloroplasts can be acquired as a temporary symbiont through a process called kleptoplasty. The kleptoplastic cell captures a chloroplast from algae and uses the captured chloroplast for a short period (a few days to a few months) until the chloroplast degenerates. Such organisms refresh their depleted chloroplast population by more of the same kleptoplastic behavior. Permanent chloroplasts are never found in Class Animalia. Thanks to kleptoplasty, one member of Class Animalia, the sacoglassan sea slug, has achieved a photosynthetic life style.

In the past, the algae were all classified as types of plants, primarily because they contained chloroplasts and looked more like plants than any other type of organism. Today, different types of algae are assigned to plant and nonplant eukaryotic classes. Three classes of algae are now assigned to Class Archaeplastida: Class Rhodophyta (red algae), Class Chlorophyta (green algae), and Class Glaucophyta [3]. The golden and brown algae are currently assigned to Class Heterokonta, as are the diatomaceous algae. Though every member of Class Archaeplastida descended from a cell that contained a chloroplast, some of the descendants have lost the ability to photosynthesize.

```
Archaeplastida
  Viridiplantae
    Chlorophyta
      Trebouxiophyceae
        Chlorellales
          Chlorellaceae
            Prototheca (genus)
```

Genus Prototheca is a chloroplast-free member of Class Chlorophyta, a subclass of Class Archaeplastida.

Prototheca is a unicellular algae ubiquitous in sewage and soil. Presumably, all humans are exposed to Prototheca sometime in their lives, but the number of human clinical infections is exceedingly rare. Protothecosis can occur in any of the three clinical forms: cutaneous nodules, olecrenon (elbow) bursitis, and disseminated [48]. The two localized forms of protothecosis occur in either immune-competent or immune-compromised individuals, while the disseminated form seems to occur exclusively in immune-compromised individuals. Only about 100 cases were reported by 2004 [49] (Fig. 4.20).

Cutaneous protothecosis is characterized by nodules. Histologic examination usually shows chronic inflammation (lymphocytes and histiocytes dominating), and structures having the appearance of florets (i.e., resembling flowers).

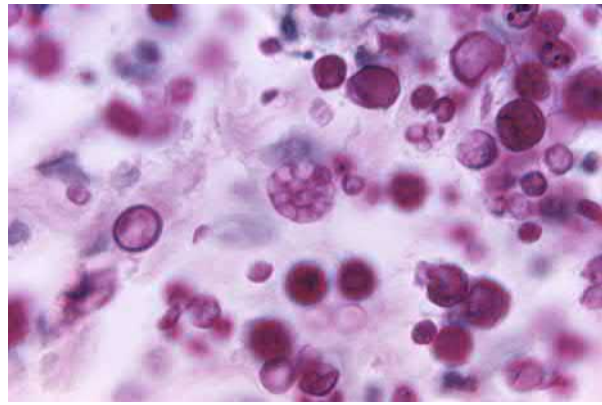

FIG. 4.20 Protothecosis (PAS stained tissue section). Numerous large round PAS-positive organisms are seen. Several of the organisms have begun the process of reproduction, with internal septations delineating numerous contained autospores. Eventually, the theca (wall) ruptures to release its autospores. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention and prepared by Dr. Kaplan.)*

These structures are produced by the organism's thick wall, known as the theca (the "theca" of "prototheca"), within which are several autospores (also called endospores) that formed by cleavage of the large organism [48]. Budding is not present.

Most human cases of protothecosis are caused by Prototheca wickerhamii. Prototheca zopfii, a species that causes disease in dogs, has been suspected to cause some cases of human disease.

Infectious Genera

Prototheca

- **Lineage**. Eukaryota: Viridiplantae: Chlorophyta: Trebouxiophyceae: Chlorellales: Chlorellaceae: Prototheca
- **Infection**. Prototheca wickerhamii (protothecosis)

Glossary

Cyanobacteria The most influential organisms on earth, cyanobacteria were the first and only organism to master the biochemical intricacies of photosynthesis (more than 3 billion years ago). Photosynthesis involves a photochemical reaction that uses carbon dioxide and water and releases oxygen. All photosynthesizing life forms are either cyanobacteria or eukaryotic cells (e.g., algae, plants) that have acquired chloroplasts (an organelle created in the distant past by endosymbiosis between a eukaryote and cyanobacteria). Before the emergence of oxygen-producing cyanobacteria, the earth's atmosphere had very little oxygen.

Histone The major protein in chromatin (i.e., the material composing chromosomes). A segment of DNA, wound around a histone protein core, is called a nucleosome, the basic

unit of DNA packaging in eukaryotes. When histones are modified by deacetylation (via deacetylases), the nucleosome tightens, reducing normal transcription by blocking transcription factors from attaching to target sites.

Intermediate host Same as secondary host. A eukaryotic organism that contains a parasitic eukaryotic organism for a period of time during which the parasite matures in its life cycle, but in which maturation does not continue to the adult or sexual phase. Maturation to the adult or sexual phase only occurs in the primary or definitive host. A parasitic eukaryotic organism may have more than one intermediate host. The survival advantages offered to the parasite by the intermediate host stage may include the following: provide conditions in which the particular stages of the parasite can develop that are not available within the primary host; disseminate the parasite (e.g., via water or air) to distant sites; protect the immature forms from being eaten by the adult forms; protect the parasite from harsh conditions that prevail in the primary host; and protect the parasite from external environmental conditions that prevail when the parasite leaves the primary host.

Kleptoplast Secondary chloroplast, wherein a host cell captures a chloroplast from another organism and uses the captured chloroplast as a temporary source of energy, until the chloroplast eventually ceases to function. Kleptoplasts typically capture chloroplasts throughout their lives, continuously replenishing exhausted organelles [3]. Most kleptoplasts are members of Class Protostista. The only animal known to practice kleptoplasty is a type of sea slug (Class Mollusca) that ingests chloroplast-rich marine organisms.

Mosquito Mosquitoes are members of Class Culicidae. Four genera of mosquitoes are vectors for human diseases: Aedes, Anopheles, Armigeres, and Culex. Among these genera, there are hundreds of individual species. Associating specific species of mosquito with specific diseases is a field of medicine unto itself. Mosquitoes are vectors for biologically diverse organisms (animals, single-cell eukaryotes, and viruses). As yet, mosquitoes are not known to be vectors for bacterial diseases; but this biological oversight may soon be corrected. It has recently been shown that mosquitoes carry pathogenic bacteria, including antibiotic-resistant species [50]. Mosquitoes are reviled throughout the world, and have been likened to flying, infected hypodermic needles. The mosquito seems to serve no useful ecologic purpose (other than as a food source for bats, and other insectivores). It has been speculated that if every genera of mosquito were eliminated as terrestrial species, there will be no significant negative ecologic repercussions [51]. The mosquito-borne viral diseases are discussed in Section 7.2, "Viral phylogeny." A few of the nonviral mosquito-transmitted diseases are listed below.

- Malaria (Class Apicomplexa) is transmitted by species of Genus Anopheles.
- Brugia malayi is transmitted by Aedes polynesiensis and other species.
- Wuchereria bancrofti transmitted by Armigeres subalbatus
- Dermatobia hominis (human botfly) in which mosquitoes carry the fly larvae and the larvae follow the mosquito entry point into the host organism.

Polyploidy A condition in which a cell or organism contains more than the usual complement of chromosomes type of the species, by some multiple of two. Polyploidization often results from the so-called endoduplication, in which the customary doubling of chromosome number during mitosis is retained in a single cell, rather than divided among two cells.

On occasion, such duplication events stabilize to produce an organism with double the typical complement of chromosomes for the species. These events can occur more than one time, producing species having very large numbers of chromosomes. Polyploidization

is particularly common in plants. For example, the black mulberry (Monus nigra) has 308 chromosomes.

Polyploidization confers an evolutionary advantage by providing "spare" chromosomes suitable for rapid evolution, and some of the most impressive evolutionary radiations (i.e., appearances of many species arising in a relatively short period of time) have occurred after polyploidization events [52–54].

Primary host Also called final host or definitive host, the primary host is infected with the mature or reproductive stage of the parasite. In most cases, the mature stage of the parasite is the stage that produces eggs, larvae, or cysts. See Intermediate host.

Sandfly Sandflies are small dipterans (flies), of several different genera, that live in sandy areas. The sandfly of genus Phlebotomus transmits Leishmania species (leishmaniasis) and the phleboviruses that cause Pappataci fever. The sandfly of genus Lutzomyia transmits Bartonella bacilliformis (bartonellosis).

Secondary host Synonymous with intermediate host.

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Chapter 5

Animals

Section 5.1 Overview of Class Animalia

Because all of biology is connected, one can often make a breakthrough with an organism that exaggerates a particular phenomenon, and later explore the generality.

Thomas R. Cech

Here is the abbreviated hierarchy leading from Class Eukaryota to Class Animalia.

```
Eukaryota
    Bikonta (2 undulipodia)
    Unikonta (1 undulipodium)
      Amoebozoa
      Opisthokonta
        Animalia (equivalent to Class Metazoa)
        Choanozoa
        Fungi
```

The highest class, Class Eukaryota, contains organisms with nucleated cells, and all the descendant subclasses will contain organisms that have nucleated cells. Furthermore, all classes that are not subclasses of Class Eukaryota will not contain organisms that have nucleated cells. For example, Class Bacteria, which is not a subclass of Class Eukaryota, contains no organisms that have nuclei.

Class Unikonta, one of the two major subclasses of Class Eukaryota, is characterized by organisms that have a single undulipodium. Class Opisthokonta is one of the subclasses of Class Unikonta. Class Animalia is a subclass of Class Opisthokonta. Animals have their own defining property (i.e., development from a blastula), along with inherited features descending from their ancestral superclasses.

Here is the schema for Class Eukaryota, including the subclasses of Class Animalia that we will be discussed later in this chapter.

```
Eukaryota
  Bikonta (2 undulipodia)
    Excavata
      Metamonada
```

Taxonomic Guide to Infectious Diseases. https://doi.org/10.1016/B978-0-12-817576-7.00005-5

```
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1 undulipodium)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
        Eumetazoa
          Bilateria
            Deuterostomia
              Chordata
                Craniata
            Protostomia
              Ecdysozoa
                Nematoda
                Arthropoda
                  Chelicerata
                  Hexapoda
                  Crustacea
              Platyzoa
                Platyhelminthes
                Acanthocephala
      Fungi
```

Section 5.2 Opisthokonts to Class ParaHoxozoa

An animal is a chemical reaction so unstable that it is instantly reversed at death.

Steve Jones, from his book "In the Blood"

The stretch of evolution extending from the opisthokonts to the metazoans (animals) account for two of the three major classes of multicellular organisms (Class Fungi and Class Metazoa), the third being Class Archaeplastida (plants), which split from the lineage leading to humans back at the bikont division of eukaryotes. In addition, all of the evolutionary innovations that define the animal kingdom were achieved prior to the Cambrian explosion, in a somewhat vague time frame that may have extended from about 1.5 billion years ago to about 600 million years ago.

Members of Class Opisthokonta, like all unikonts, has a single undulipodium (commonly but erroneously referred to as a flagellum). The undulipodium of the opisthokonts protrudes from the posterior pole, and serves to propel the organisms through water. The undulipodia (one or two in number) of other eukaryotic classes are most often anterior. Although the posterior undulipodium characterizes Class Opisthokonta, the cells of many of the descendant opisthokonts that have evolved into nonaquatic organisms (i.e., fungi and animals) lack the undulipodium. However, among the fungi, the aquatic chytrids produce gametes (i.e., fungal spores) that have an undulipodium [1]. Likewise, among the animals, the undulipodium is retained in spermatocytes. Chytrid spores and animal sperm use the undulipodium to propel themselves through fluid. Because each spermatocyte has an undulipodium, we can infer that the genes for building an undulipodium are retained in animals.

In addition to their characteristic posterior undulipodium, members of Class Opisthokonta have the ability to synthesize chitin, a long-chain polysaccharide found throughout Class Fungi and in many but not all members of Class Metazoa (e.g., chitin is not synthesized in mammals). All extant organisms that produce chitin are opisthokonts. Chitin is the opisthokont equivalent of cellulose, another long-chain polysaccharide, which is found in members of Class Plantae. Chytrids, unlike all other known opisthokonts, can synthesize both cellulose and chitin.

Animals are thought to have evolved from gallertoids; simple, spherical organisms floating in the sea. These living spheres were lined by a single layer of cells enclosing a soft center in which fibrous cells floated in an extracellular matrix. The earliest fossil remnants (seabed burrows and tracks) of these early animals date back to about 1 billion years ago. The broad classes of animals that we recognize today were all living during the Cambrian period, about 540 million years ago.

What is the single property that distinguishes animals from nonanimals? The answer is “the blastula”; an early embryo having a hollowed-out cavity. The reason that the blastula serves as the defining feature for every animal organism will take a bit of explanation. There are three classes within Class Eukaryota that contain multicellular organisms: Class Plantae, Class Fungi, and Class Metazoa. These three classes account for virtually every organism that we can see with the naked eye. Consequently, prior to the invention of the microscope (about 1590 AD) and the advent of scientific observations of the microscopic world (about 1676 AD), these three classes accounted for the totality of the observed living world. Fungi, plants, and animals can be distinguished by the method by which their included organisms develop. Fungi develop from spores. Plants, like animals, develop as embryos, but they do not have a blastula phase. Animals, unlike plants and fungi, develop from an embryonic blastula. Both fungi and plants have rigid cell walls, hardened by chitin in the case of fungi, and cellulose in the case of plants. Plants can form an embryo, but the way in which their cells are held together makes it impossible for them to form a blastula (Fig. 5.1).

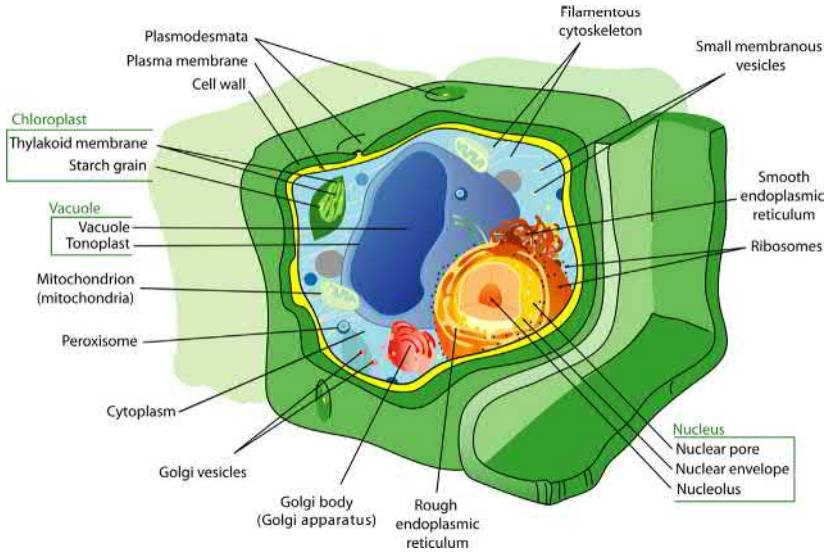

FIG. 5.1 Plant cells attain their shapes by their encasement in a rigid cellulose frame (green walls in the diagram). *(Source, Wikimedia, entered into the public domain by its author, LadyofHats.)*

Unlike plant cells (wrapped in cellulose) and fungal cells (wrapped in chitin), animal cells have no structural molecule in cell walls to constrain them into any particular shape. With the exception of cells that produces intracellular bands of protein that force a spindle or elongated shape (such as actin, myosin, or desmin), most animal cells are relatively floppy bags of protoplasm, somewhat like water-filled balloons (Fig. 5.2).

If you press two water-filled balloons, of the same size, together, you will find that their surface of union is a flat circle. If you crowd together, onto a flat surface, a monolayer of floppy balloons, you will get a matrix of polygonal forms, wherein each straight edge of the polygon is bounded by an edge of an adjacent balloon. It is easy to see how a collection of spheres can, when pushed together, yield a cuboidal or polygonal matrix (Fig. 5.3).

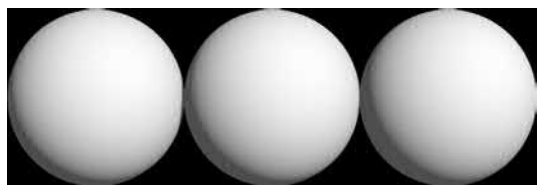

FIG. 5.2 Animal cells are essentially soft bags filled with water. When they are suspended in fluid, they assume their natural spherical shape. The illustration depicts how three epithelial cells might appear, if they simply touched one another. *(Source, Jules J. Berman, image created with Pov-ray rendering software.)*

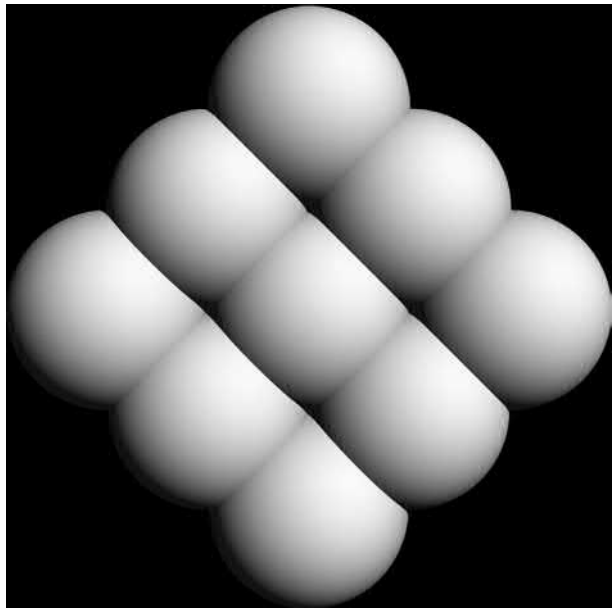

FIG. 5.3 Round animal cells, when pushed together, form straight edges at the surfaces of intersection. Touching spheres represent epithelial cells that are pushed together in a growing tissue. Notice that where spheres touch, flat surfaces are produced. The effect of many spheres pushing together is a polyhedral network, simulating an epithelium. *(Source, Jules J. Berman, image created with Pov-ray rendering software.)*

If polygonal epithelial cells were simply squeezed spheres, what would stop fluids from slipping between the spaces between the spheres? Why would glandular contents be confined within the lumen of an epithelium-lined gland? What would keep tissues from falling apart, when the squeezing stopped? The integrity of the polygonality of animal tissues is accomplished with two specialized structures that reside on the surface of animal cells: desmosomes and gap junctions. The first evolutionary achievement that can be credited to the desmosome is the development of the blastula, a stage in embryogenesis that is unique to metazoans. In the early morula stage of embryogenesis, the embryo consists of a small ball of cells. The cells of the early embryo, held together by desmosomes and tight junctions, soon thereafter begin to secrete fluid. Because the junctions holding cells together are watertight, fluids secreted by the cells accumulate in a central cavity (the blastocyst). An embryo, with a fluid-filled center, is known as a blastula. In summary, Class Metazoa owes its existence to soft cells (i.e., cells lacking a rigid cellulosic or chitinous wall) and specialized junctions (Fig. 5.4).

Class Metazoa has two subclasses: Class Parazoa and Class Eumetazoa. Class Parazoa contains two relatively small subclasses: Class Porifera (the sponges) and Class Placozoa. Sponges and placozoans are basal animals, consisting of a layer of jelly-like mesoderm sandwiched between simple epithelium. The parazoans

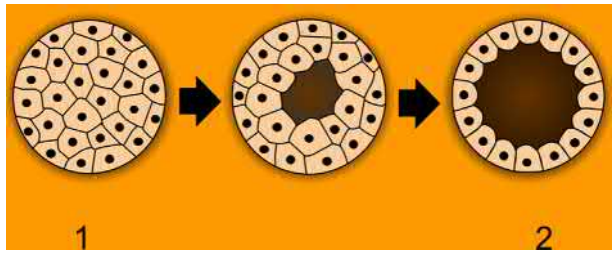

FIG. 5.4 Graphic of blastulation. The early, solid, embryo secretes fluid into a central viscus, the developing blastocyst. Blastulation is accomplished with specialized membrane channels that transport ions and water, and with desmosomes that provide a watertight boundary between adjacent cells. Blastocysts are characteristic of Class Metazoa. *(Source, Wikipedia, created and released into the public domain by Pidalka44.)*

evolved to extract food from the seabed floor. To do so, they flattened out, and stayed flat for a very long time. The placozoans were discovered in 1833, hugged against the wall of a seawater aquarium. These organisms are just under a millimeter in length and are composed of about 1000 epithelial cells. With the exception of being flat, rather than round, they resemble the gallertoids, with an outer lining of cuboidal cells, and an inner gelatinous matrix holding a suspension of fibrous cells. Class Placozoa contains a single species, Trichoplax adhaerens. Like all metazoans, the parazoans develop from blastulas [2]. Neither sponges nor placozoans have stomachs, or any other specialized organs. Furthermore, members of Class Parazoa lack anything that we might recognize as neurons [3].

Among the subclasses of Class Porifera is Class Demospongiae. Species of Class Demospongiae are the only living organisms known to methylate sterols at the 26-position, a fact used to identify the existence of demosponges at a date preceding the age of their first observed fossils [4, 5]. Some sponges may live for thousands of years, suggesting that evolution of sponges predates the evolved trait known as aging that arose later in the eumetazoan lineage (Fig. 5.5).

After the evolutionary development of the blastula, which characterizes Class Metazoa, there arrived another epithelial-lined structure, the stomach, which characterizes the eumetazoans [6]. Class Eumetazoa is a subclass of Class Metazoa and contains all animals other than sponges and placozoans, which lack stomachs (gastrulas). The stomach, it seems, is the first evolved somatic organ, and is the basis for the flippant generalization that a eumetazoan is little more than a traveling stomach. After the appearance of a stomach, a plethora of copy-cat organs, cavities, ducts, and epithelial-line surfaces evolved. During gastrulation, the tissues organize into embryonic germ layers, and we see the first appearance of neurons.

Class Eumetazoa has two subclasses: Class Ctenophora (comb jellies) and Class Parahoxozoa (whose descendants include humans). Members of Class ParaHoxozoa have a set of genes that is common to all its members. Among these genes are the Hox/ParaHox transcription factor genes. Class Parahoxozoa

FIG. 5.5 Acarnus erithacus, the red volcano sponge, an example of one of the 8800 known species of Class Demospongiae. *(Source, a public domain work of the US National Oceanic and Atmospheric Administration, photographed by SIMoN/MBNMS.)*

has two subclasses: Class Bilateria and Class Cnidaria. The cnidarians were formerly grouped with the ctenophorans (comb jellies) but have now been assigned their own class. The cnidarians contain over 21,000 extant species of aquatic animals, including sea anemones and corals, jellyfish, and hydra-like animals.

The cnidarians are now known to include the obligate parasitic myxozoans, a modified and simplified jellyfish that infects fish [7]. The myxozoans contains species having extremely small genomes, as small as 22.5 megabases in the case of Kudoa iwatai. **It is quite possible that the myxozoans are the earliest animal parasites on earth**. Like the long-lived sponges, the cnidarians include species that can regenerate themselves. The adult medusan form of the organism can revert back to the polyp, and fragments of the polyp can regenerate to create new organisms, with no apparent limit to the cycles of reversion and regeneration (Fig. 5.6).

Because the cnidarians and their preceding ancestors seem to have the ability to regenerate as needed, they are effectively ageless and immortal. The bilaterians, with possibly one exception, seem doomed to grow old and to die. Hence, if we wanted to determine the phylogenetic point marking where aging had evolved as a new phylogenetic trait, we should probably choose a spot somewhere near the cnidarian/bilaterian split.

Section 5.3 Bilaterians to Protostomes

The evolutionary relationships between the earliest branches of the animal kingdom—bilaterians, cnidarians, ctenophores, sponges, and placozoans—are contentious.

Maximilian J Telford [8]

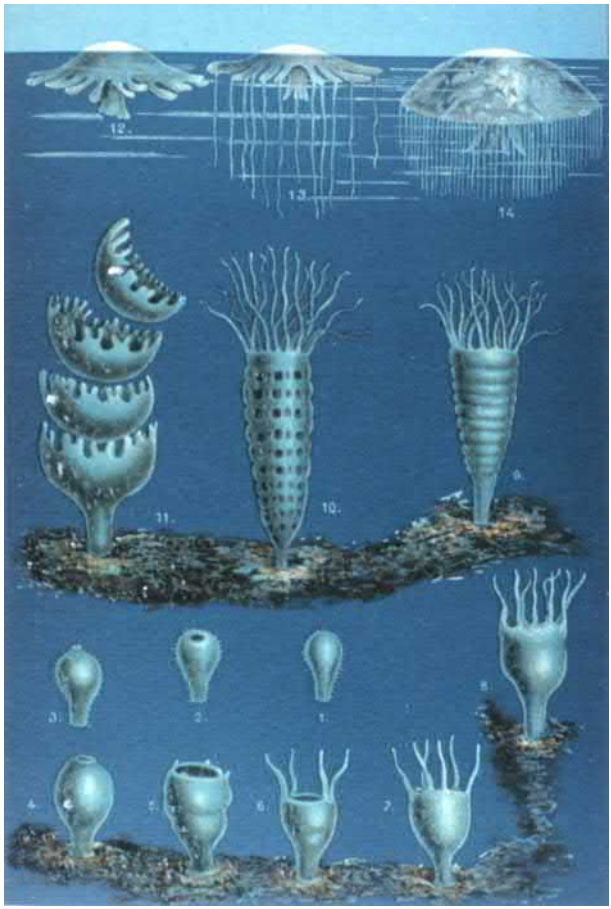

FIG. 5.6 The life cycle observed in some cnidarian organisms. Starting at the bottom of the figure, we see polyps transforming into the adult medusa (upper right). *(Source, Wikipedia, from a figure in Schleiden M. J. "Die Entwicklung der Meduse," in: "Das Meer," 1869.)*

Bilateria, also known as triploblasts, have three embryonic layers (endoderm, mesoderm, and ectoderm). Animals of Class Bilateria display bilateral symmetry (i.e., their bodies can be divided into two symmetrical halves by a plane that runs along a central axis). Hence, the bilaterians have laterality, with an anterior head, a posterior tail, a dorsal back, and a ventral belly. Starfish are descendant members of Class Bilateria, with axial symmetry and five arms. But wait. How is it possible for a five-armed organism to have bilateral symmetry? Shouldn't a symmetrical starfish have four arms, or maybe six arms? (Fig. 5.7)

We must remember that phylogeny has everything to do with sequential embryologic processes, and very little to do with what we observe in adult organisms. Adult organisms often display secondary changes due to developmental events that

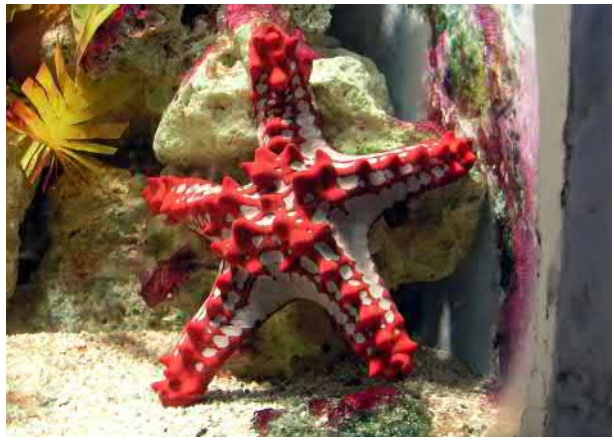

FIG. 5.7 A red-knobbed starfish with five arms, openly violating axial symmetry. *(Source, Wikipedia, released into the public domain by its author, Arpingstone.)*

occur after a fundamental embryologic process has occurred. Yes, the adult starfish violates axial symmetry, but the embryonic starfish has lovely symmetry, and it is the embryo that expresses the phylogenetic innovations of its ancestors (Fig. 5.8).

Likewise, humans are descendants of Class Bilateria. When we look at adult humans, we see some indication that we arose from a bilateral embryo (e.g., two kidneys, two lungs, two lobes to our brains), but we also see asymmetrical organs (one spleen, one liver, and a wandering alimentary tract that crisscrosses the body's central axis). If we confine our observations to the early human embryo, we see bilateral symmetry of every anlage (i.e., every predecessor to an adult organ). We can infer that all the twists, turns, and organ fusions came later.

Class Bilateria has two subclasses: Class Nephrozoa and Class Xenacoelomorpha. Class Xenacoelomorpha contains very simple, small animals, all of which lack a true gut (i.e., incomplete stomatogastric system). The coelom, the excretory organs, and nerve cords developed first in the Nephrozoa. The coelom is the body cavity that holds the digestive tract and the organs deriving from endoderm and mesoderm. The coelom is lined by either a specialized mesodermal cell (the mesothelial cell in humans) or by apparently undifferentiated mesodermal cells (as observed in molluscs). Nerve cords are the evolutionary precursors of the central nervous system and the spinal cord, as found in vertebrates. A primitive nerve cord would be a tract of nervous tissue, mostly bundled axons, that touch upon or emanate from ganglia (clusters of neuronal bodies) located at either extremity of the organism.

There is some evidence to suggest that smooth and striated muscle cells originated in Nephrozoa, as these cell types were present in an ancestral class of both protostomes and deuterostomes [9]. Nephrozoa, being the direct parent class of both protostomes and deuterostomes, is a candidate for the first class to have both smooth and striated muscles. The protostome/deuterostome split from Class

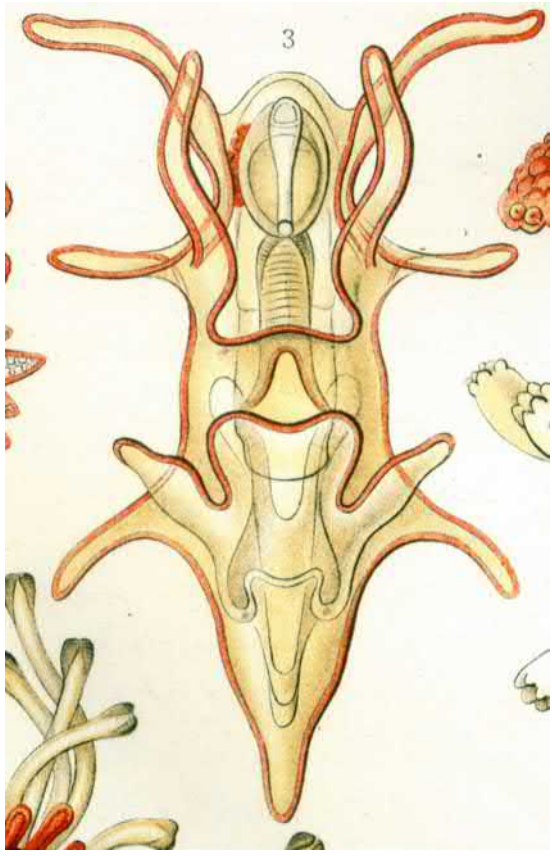

FIG. 5.8 A starfish larva illustrating a symmetric, bilateral body plan. *(Source, Wikipedia, from a drawing by Ernst Haeckel published in "Kunstformen der Natur," 1904.)*

Nephrozoa is thought to have occurred about 558 million years ago, just before the Cambrian explosion. The Cambrian explosion marks the period when predatory/prey activities moved into full swing; when animals needed muscles for chasing, seizing, and eating their prey. Hence, it makes sense that muscle cells would be largely unnecessary much earlier than the appearance of Class Nephrozoa. Lastly, it would not seem that muscle cells have been reported in animals other than those that have descended from Class Nephrozoa.

Class Nephrozoa has two subclasses: Class Deuterostomia and Class Protostomia. In most protostomes, the mouth forms first and the anus follows. In deuterostomes, the anus comes first, then the mouth. Without exception, all of the animals parasitic in humans are descendant members of Class Protostomia.

Why is Class Deuterostomia bereft of infectious organisms?

In deuterostomes, a dent forms at an early stage of development, which eventually forms the anus, from which endoderm tunnels to produce a mouth,

at the opposite end of the embryo. Class Deuterostomia is also known as Class Enterocoelomate, the reason being that every deuterostome develops its central coelom (body cavity) through a process known as enterocoely. In enterocoely, the coelom is formed by pinched-off pouches of endoderm. In classes other than the deuterostomes, the central coelom is formed by cavitations occurring within the mesoderm, a process called schizocoely.

One additional early developmental feature contributes to the dichotomy between protostomes and deuterostomes. All protostome blastulas derive from an eight-cell morula wherein the cells are spirally cleaved from one another and in which each of the eight cells are committed to a specific differentiation lineage. All deuterostome blastulas derive from an eight-cell morula wherein the cells are radially cleaved, and commitment of early embryonic cells to any particular differentiated lineage is delayed until the blastocyst is fully formed and the first two layers of the embryo appear.

Class Deuterostomia has four subclasses: Class Echinodermata (includes starfish), Class Chordata, Class Hemichordata (includes acorn worms, most living in the ocean beds), and Class Chaetognatha (planktonic arrow worms).

The subclass of the deuterostomes that is best known to most of us is Class Chordata. Class Chordata contains the deuterostomes that have evolved a notochord, a flexible rod of cells in a cartilaginous matrix that runs the length of the back, and which serves as the primary support of the endoskeleton. Class Chordata contains three extant subclasses: Class Craniata (whose descendants include fish, birds, amphibia, reptiles, and mammals), Class Tunicata (includes sea squirts), and Class Cephalochordata (includes lancelets, also known as amphioxus)

It is one of the great mysteries of medical biology that no member of Class Deuterostomia is infectious to humans. The deuterostomes include all the vertebrates (e.g., mammals, birds, fish, amphibians, reptiles). The descendants of Class Deuterostomia may be eager to attack and eat humans, but none of these species infect or parasitize humans. We can only guess as to why this is so. As a general rule, the descendants of Class Deuterostomia that live on land are large, and we can easily imagine that a large animal will have a tough time infecting animals of similar size. In turn, the large sizes of deuterostomes may be accounted for by the presence of lungs, in many deuterostome species, suspended in the thoracic coelom. Thoracic lungs function as a bellows, to efficiently blow oxygen and other gases into and out of the body. With an effective way to oxygenate tissues, deuterostomes can attain massive sizes. Protostomes lack anything that is closely equivalent to deuterostome lungs. Most protostomes rely on long tracheal tubes to conduct gases through the body. Not surprisingly, most protostomes are small, and many are flat (e.g., Platyhelminths), or long. In the rare instance we find a deuterostome that is small and shares its habitus with humans, something akin to infection may develop. Just such a situation pertains to Trichomycteridae plectrochilus, the toothpick catfish.

The toothpick catfish, also known as candiru, is a small fish, shaped like an eel, about an inch in length, that lives in the Amazon and Oronoco Rivers.

Its phylogenetic lineage as a deuterostome is shown below.

```
Deuterostomia
  Chordata
    Craniata
      Actinopterygii
        Siluriformes
          Loricarioidea
            Trichomycteridae (genus)
```

Trichomycteridae plectrochilus normally infects riverine fish, inserting itself between the gills, attaching to tissue with the aid of barbs, and feeding off of the host's blood (hematophagy). There are a few case reports, and many anecdotal accounts, of candiru entering the urethra, the vagina, or the anus of swimming or wading humans. Removal of the fish may require surgical excision.

The toothpick catfish does not reproduce within infected humans, and humans do not seem to constitute a usual habitus for its life cycle. It is best to think of the toothpick catfish as just another deuterostome that will feast on humans, when the opportunity arises; not as a human infection.

The protostome and deuterostome classes split about 558 million years ago. The protostomes, in terms of speciation, are much more successful than the deuterostomes. There are about a million extant protostome species, compared to a mere 60,000 species of deuterostomes. Every species of animal that infects humans (i.e., every parasitic animal discussed in the remainder of this chapter) is a descendant member of Class Protostomia. Class Protostomia includes the arthropods (ancestors of insects, chelicerata, crustaceans), as well as mollusks (including octopi and squid), annelids, nematodes, and platyhelminthes. In the next sections, we will be looking at the major classes of protostomes containing human parasites.

Section 5.4 Platyhelminthes (flatworms)

The proof of evolution lies in those adaptations that arise from improbable foundations.

Stephen Jay Gould

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
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  Chromalveolata
    Alveolata
      Apicomplexa
      Ciliophora (ciliates)
    Heterokonta
Unikonta (1-flagellum)
  Amoebozoa
  Opisthokonta
    Choanozoa
    Animalia
      Eumetazoa
        Bilateria
          Deuterostomia
            Chordata
              Craniata
          Protostomia
            Ecdysozoa
              Nematoda
              Arthropoda
                Chelicerata
                Hexapoda
                Crustacea
            Platyzoa
              Platyhelminthes
                Cestoda (tapeworms)
                  Cyclophyllidea
                    Taeniidae
                      Echinococcus (genus)
                      Taenia (genus)
                    Dipylidiidae
                      Dipylidium (genus)
                    Hymenolepididae
                      Hymenolepis (genus)
                  Pseudophyllidea
                    Diphyllobothriidae
                      Diphyllobothrium (genus)
                      Spirometra (genus)
                Trematoda (flukes)
                  Digenea
                    Echinostomida
                      Fasciolidae
                        Fasciola (genus)
                        Fasciolopsis (genus)
                    Opisthorchiida
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                    Heterophyidae
                      Metagonimus (genus)
                    Opisthorchiidae
                      Clonorchis (genus)
                      Opisthorchis (genus)
                  Plagiorchiida
                    Dicrocoeliidae
                      Dicrocoelium (genus)
                    Paragonimidae
                      Paragonimus (genus)
                  Strigeatida
                    Schistosomatidae
                      Schistosoma (genus)
                      Trichobilharzia (genus)
                      Schistosomatidae species (genus)
          Acanthocephala
Fungi
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Class Platyhelminthes contains the flatworms (from Greek "platys," flat, and "helmis," worm). Perhaps the most striking property of Class Platyhelminthes is its remarkable developmental plasticity. Class Turbellaria, a subclass of platyhelminthes that contains no human pathogens, is adept at growing whole organisms from excised parts. A tiny fragment of planaria (Schmidtea mediterranea), several hundredths the size of the original organism, can regenerate into a full-sized planaria. The pathogenic species of Class Platyhelminthes, though lacking the full regenerative abilities of the planaria, have a great capacity to modify their life cycles to enhance their parasitism.

The flatworms that cause human disease fall into two subclasses: Class Cestoda (the tapeworms) and Class Trematoda (the flukes).

```
Platyhelminthes
  Cestoda (tapeworms)
    Cyclophyllidea
      Taeniidae
        Echinococcus (genus)
        Taenia (genus)
      Dipylidiidae
        Dipylidium (genus)
      Hymenolepididae
        Hymenolepis (genus)
    Pseudophyllidea
      Diphyllobothriidae
        Diphyllobothrium (genus)
          Spirometra (genus)
```

The generalized life cycle of cestodes applies to all members of the class and accounts for the pathologic manifestations of these infections.

Adult tapeworms live in the intestinal tract of an animal (the primary host), absorbing nutrients from the partially digested food and other products in the bowel lumen. The adult tapeworm is composed of a scolex (the "head") which attaches to the intestinal wall, followed by a neck, and then followed by the proglottids that line up one after the other forming a "tape." The proglottids drop off from the end of the tape, and pass out of the organism in feces.

By the time the proglottid has dropped into the environment, it is gravid with infective eggs. The gravid proglottids are ingested by animals that eat grasses or food that had been contaminated by the proglottids. Animals infected by proglottids are the intermediate hosts. Eggs hatch out juvenile forms that migrate through the secondary host, eventually pausing to encyst in muscles and other organs. The cysts cause illness, in the secondary host, commensurate with their number, size, and anatomic locations.

Tapeworm infections for which humans are the primary host, are caused, in almost all cases, by eating undercooked tissues from an infected animal that is a secondary host. The disease that results consists of adult tapeworm, wherein the tapeworm, attached to the gut wall, lives off of nutrients in the intestinal lumen.

Tapeworm infections for which humans are a secondary host are caused by eating food that is contaminated by proglottids and eggs dropped by a primary host. The disease that results consists of larval cysts growing in human tissues.

The six genera of cestodes that infect humans are: Hymenolepis, Echinococcus, Taenia, Dipylidium, Diphyllobothrium, and Spirometra.

Echinococcus species use humans as secondary hosts. This means that humans become infected when they ingest the eggs or the proglottids that were passed into the ground in the feces of primary hosts (usually dogs and other carnivores). The subsequent disease, echinococcosis, also known as hydatid disease (from Greek, "hydatid," meaning watery cyst), results from the growth of larval cysts (cysticercoids). Because humans are seldom eaten by potential primary hosts, the human is a dead-end host for the organism. The severity of the disease is determined by the size and locations of the cysts. The most deadly forms of the disease produce large cysts in the central nervous system (Fig. 5.9).

Several different species of Genus Echinococcus cause human disease: Echinococcus granulosus, Echinococcus multilocularis, Echinococcus vogeli, and Echinococcus oligarthus.

Taenia solium (the pork tapeworm), and Taenia saginata (the beef tapeworm) use humans as the primary host for adult tapeworms. The resulting disease is often referred to as taeniasis. Humans become infected when they eat undercooked meat from infected animals (pork for Taenia solium, and beef for Taenia saginata) (Fig. 5.10).

Taenia solium can also use humans as a secondary host. When humans ingest eggs or proglottids (from soil contaminated with the feces of infected pigs), the hatched

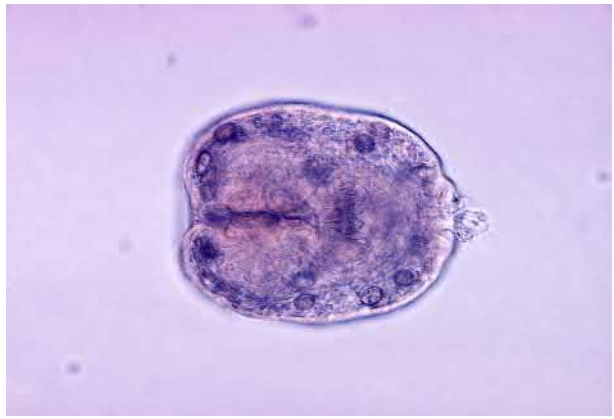

FIG. 5.9 Scolex (anterior segment of larval stage of Echinococcus granulosus, having protruding hooks and ventral suckers) extracted from a cyst (i.e., hydatid cyst). *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, prepared by Dr. L.L.A. Moore, Jr.)*

larvae may produce cysts (cysticercoids) throughout the body. This disease is known as cysticercosis. When cysts occur in the brain, it is referred to as neurocysticercosis.

Thus, Taenia solium can cause two separate diseases in the human population: taeniasis when the human is a primary host; and cysticercosis when the human is the secondary host.

Dipylidium caninum uses humans as a primary host. Humans become infected when they accidentally ingest fleas or lice (the secondary hosts) whose tissues are infected with cysticercoids. The fleas and lice are carried by dogs. After ingestion by humans (usually children), the cysts develop into adult tapeworms in the intestine. One of the names given to this adult tapeworm infestation is "double-pore tapeworm disease." This name derives from the characteristic anatomic feature of Class Dipylidiidae wherein genital pores appear on both sides of proglottids.

Hymenolepis nana, the last-to-be-described human tapeworm of Class Cyclophyllidea, has a complex life cycle that can only be understood in terms of its ability to combine the primary and secondary phases of its life cycle within a single host organism.

Hymenolepis nana, the dwarf tapeworm (the Greek, "nana" means dwarf), occurs worldwide and it is the most common cestode infection in humans. For Hymenolepis nana, humans serve as primary and secondary hosts, and both host roles occur concurrently in the same host. Here is how it works. Humans can become primary hosts when they eat animals (in this case, larval fleas and beetles) that contain infectious larval cysts (so-called cysticercoids). The larval cysts develop into adult tapeworms in the small intestine lumen, where gravid proglottids develop. Humans become secondary hosts of Hymenolepis nana when they ingest gravid proglottids or eggs dropped by a primary host. The hatched larvae penetrate into the mucosa of the intestinal villi, where they remain, to form larval cysts within the lymphatic channels of the intestinal submucosa.

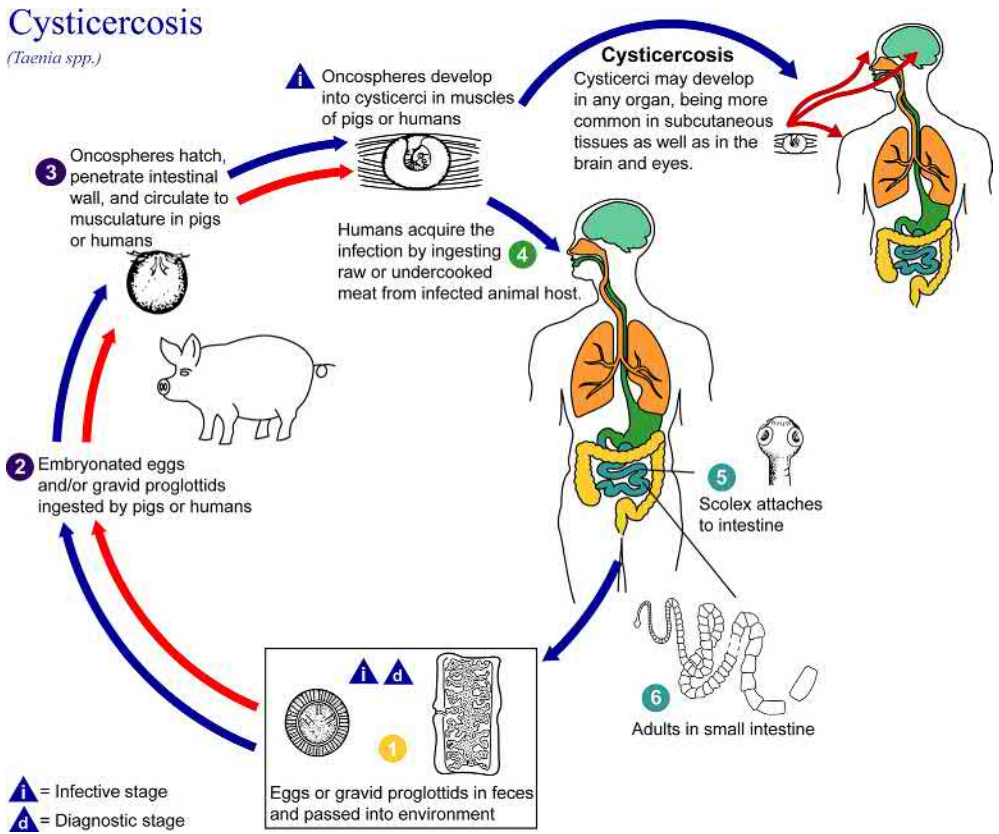

FIG. 5.10 Life cycle of Taenia species organisms that can produce either of two different diseases in humans (tapeworm disease or cysticercosis) depending on the phase of the Taenia life cycle in which the organism is ingested. *(Source, Wikipedia, from a public domain graphic produced by the US Centers for Disease Control and Prevention.)*

In the case of Hymenolepis nana, the infectious cysts (of the secondary host) nestled in the intestinal submucosa can mature in-place, to produce adult organisms in the small intestine. Even stranger, eggs deposited in the small intestine, by the adult proglottids within the primary host, can encyst in-place, in the submucosa. This means that a secondary host can assume the role of a primary host, and a primary host can assume the role of a secondary host.

Because a single human can serve as both the primary and the secondary host of Hymenolepis nana, the net result is that humans infected with Hymenolepis nana may have chronic infections, characterized by a huge worm burden in the small intestine, composed of adult tapeworms and larval cysts, resulting in the fecal passage of enormous number of eggs, over a long period of time. Other tapeworms, including other species within Genus Hymenolepis, lack this life-cycle flexibility. It is not surprising that Hymenolepis nana is the most common tapeworm infection in humans, worldwide (while Ascaris lumbricoides is the most common helminth infections, overall).

Rare cases of human hymenolepiasis are caused by the rodent tapeworms Hymenolepis microstoma and Hymenolepis diminuta (Fig. 5.11).

Diphyllobothrium latum uses humans as a primary host. Humans become infested when they eat undercooked meat from an infected fish (the secondary host). Diphyllobothrium latum has an unusual way of passing larvae through several secondary hosts before infecting the final secondary host consumed by humans. Copepods become secondary hosts after ingesting eggs dropped into water along with the feces of an infected primary host (various mammals including humans). When copepods are eaten by fish, infectious larvae persist in the larval stage (the so-called plerocercoid larvae), using the fish as another intermediate host. Humans break the chain when they ingest plerocercoid larvae that hatch and become adult tapeworms in the human small intestine.

FIG. 5.11 Egg of Hymenolepis diminuta. Eggs are slightly oval and up to 80 μm in length. Six hooks are present (of which four are visible in this image) within. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, prepared by Dr. Mae Melvin.)*

Spirometra species like Diphyllobothrium latum belong to Class Pseudophyllidea, and their larvae can develop in stages, within two secondary hosts. In this case, humans are used as the second intermediate host, and are infected by the plerocercoid larvae (the same role held by fish in the life cycle of Diphyllobothrium latum, via supra). Humans are a dead-end host. The plerocercoid larvae migrate to subcutaneous tissues and various organs, producing an inflammatory response. The resulting disease is called sparganosis. Clinical symptoms vary with the location of the larvae. Only a few hundred cases of sparganosis have been reported. The primary hosts vary and include dogs and birds. The first intermediate host is copepods. The natural second intermediate hosts are usually birds, reptiles, or amphibians. Humans usually contract the disease by consuming the raw flesh of one of the natural intermediate hosts or, more likely, drinking copepods, an animal small enough to go unnoticed in a glass of water.

```
Platyhelminthes
  Trematoda (flukes)
    Digenea
      Echinostomida
        Fasciolidae
          Fasciola (genus)
          Fasciolopsis (genus)
      Opisthorchiida
        Heterophyidae
          Metagonimus (genus)
        Opisthorchiidae
          Clonorchis (genus)
          Opisthorchis (genus)
      Plagiorchiida
        Dicrocoeliidae
          Dicrocoelium (genus)
        Paragonimidae
          Paragonimus (genus)
      Strigeatida
        Schistosomatidae
          Schistosoma (genus)
          Trichobilharzia (genus)
          Schistosomatidae species (genus)
```

Trematodes (flukes) are shaped like flattened worms. All of the flukes that infect humans are members of Class Digenea. Members of Class Digenea, like all trematodes, have a sucker at one end (the mouth) and a second, ventral, sucker on its underside. A distinctive anatomic feature of species in Class Digenea is its outer coat, wherein the junctions between cells disappear, forming a cytoplasmic syncytium. The flukes in Class Digenea have a life cycle that requires a minimum of two hosts (from Latin "di," meaning two and "ginus," meaning type). In several

cases, intermediate stages reproduce asexually. The complex life cycles of flukes, and their ability for larval stages to reproduce themselves, greatly expanding the number of larval organisms, are further indications of the remarkable generative abilities of Class Platyhelminthes (vida supra the planarian platyhelminth, Schmidtea mediterranea).

Here is the general life cycle of a Trematode:

–1. Eggs are released into the environment (usually water) by adult trematodes within the primary host. For example, Schistosoma haematobium females release their eggs into the bladder lumen, where they are washed out with the urine stream.

–2. The eggs hatch into swimming larvae known as miracidia that penetrate their first intermediate host (a snail or other mollusc).

–3. The miracidia, living in the first intermediate host, develop into a sac-like structure called a sporocyst.

–4. The sporocyst, unlike cystic stages in tapeworms, is capable of self-reproduction (in some species), producing a child sporocyst. The sporocyst also produces the next stage of larval development, the redia, a larval form that has an oral sucker.

–5. A redia, like the sporocyst, is capable of self-reproduction, and can produce more rediae. In addition, the redia is capable of producing a cercaria.

–6. A cercaria is an organism that develops from germinal cells of a sporocyst or a redia. This means that the cercaria is not just a phase of larval development produced by morphologic transformation of one larval form into another; it is a new organism that arises from a particular type of cell within a larval organism. The cercaria is motile.

–7. The motile cercaria may infect a new host (the primary host), where it becomes an adult fluke. Or the cercaria may transform into one of two dormant forms that persist in the environment, often attached to edible vegetation, or in another intermediate host. These two forms are: mesocercaria and metacercaria. The mesocercaria is a larval form of the organisms, while the metacercaria is an encysted form.

It is obvious that the flukes are a form of life with an incredibly complex and flexible life cycle. Trematode infections in humans can be simplified by remembering the following rule: all pathogenic classes of trematodes, with one exception, produce human infections wherein the human is the primary host (i.e., humans host the egg-laying adult fluke); and in which humans become infected by eating infectious metacercariae that have settled on vegetation, or that have infected a second intermediate animal host. The exception to this rule is Class Schistosomatidae, which will be discussed at the end of the chapter.

Members of Class Fasciolidae produce adult flukes that live in the liver, gall bladder, and intestines of the primary host (e.g., humans). All pathogenic genera of Class Fasciolidae use freshwater snails as one of the intermediate hosts (though some species have expanded their host range to include other

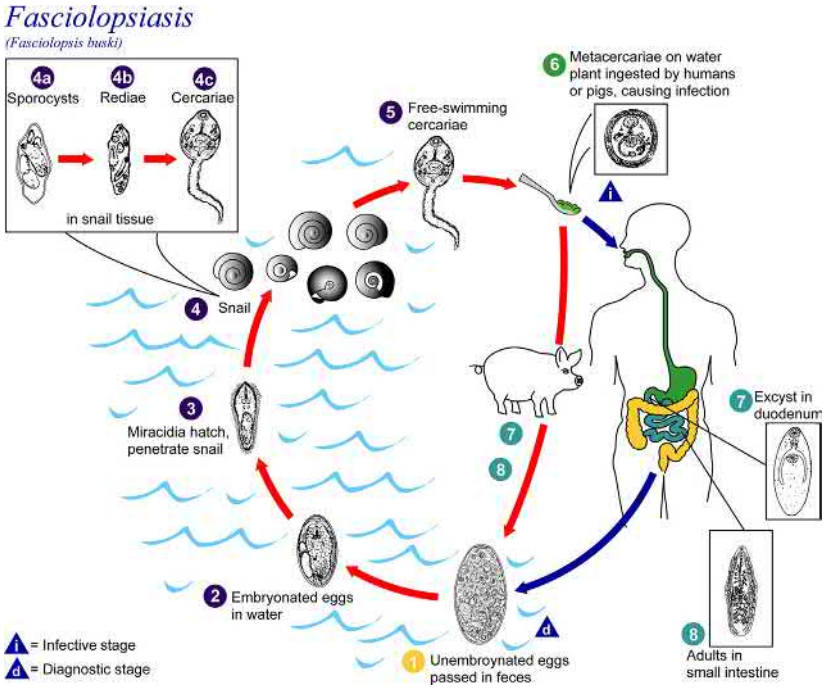

FIG. 5.12 Life cycle of Fasciolopsis buski. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, and prepared by Dr. Alexander J. Da Silver and Melanie Moser.)*

molluscans). Two polysyllabic diseases are caused by members of Class Fasciolidae: fascioliasis and fasciolopsiasis (Fig. 5.12).

Most cases of fascioliasis are caused by Fasciola gigantica (liver fluke disease). This disease, common in Southeast Asia, occurs when a human ingests metacercaria that contaminate vegetables. The metacercaria develop in the small intestine, and eventually migrate to the biliary ducts of the liver. The adult flukes lay eggs that are passed in feces. A variety of animals serve as hosts for Fasciola gigantica including cattle (Fig. 5.13).

Fasciola hepatica, the sheep liver fluke, infects ruminants (particularly sheep) but may also infect humans. Human infection occurs when metacercaria on vegetation (particularly watercress) are ingested.

Fasciolopsiasis is caused by Fasciolopsis buski, a large (us to 7.5 cm length) fluke that lives in the intestines of the primary host (pigs and humans). The number of humans infected is about 10 million. Surprisingly, most infections are asymptomatic. Humans become infected by ingesting metacercaria on vegetation. The disease occurs almost exclusively in Southeast Asia and India.

Metagonimiasis is caused by any of the several species of small flukes that live in the small intestines of the primary host. The metacercaria cysts attach to the underside of fish scales. Humans become infected when they eat

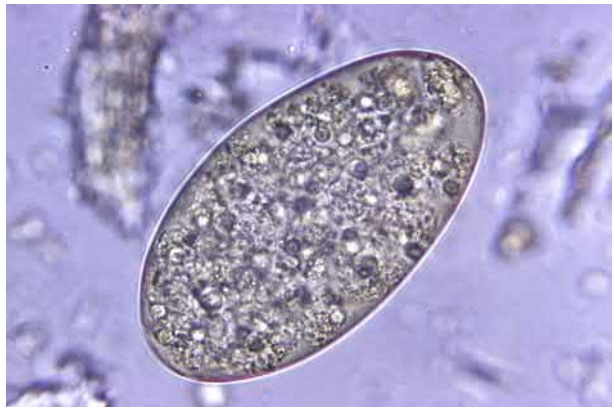

FIG. 5.13 Fasciolopsis buski egg in fecal specimen. Fasciolopsis buski eggs in feces are unembryonated, and are morphologically indistinguishable from the eggs of Fasciolopsis hepatica.*(Source, a public domain image provided by the US Centers for Disease Control and Prevention, prepared by Dr. Mae Melvin.)*

undercooked fish that contain the metacercaria. The disease occurs wherever infected species of fish are eaten raw or uncooked. Most cases occur in Asia; cases are particularly common in Korea.

Class Opisthorchiidae contains two infectious genera: Clonorchis and Opisthorchis. Infectious genera within Class Opisthorchiidae produce the condition known as opisthorchiasis, sometimes called clonorchiasis, in which adult flukes live in the bile ducts of the liver. Humans become infected after eating undercooked fish containing metacercaria.

Clonorchis sinensis, the Chinese liver fluke (also known as the Oriental liver fluke) infects about 30 million people. As the name suggests, most cases occur in Asia. Humans become infected when they eat uncooked or raw fish infected with the metacercaria. The metacercaria develop in the small intestine, and eventually migrate to the bile ducts. The adult flukes live in the bile ducts. These flukes feed on bile produced by the liver, excreted through the bile ducts and stored in the gall bladder. Hence, bile does not reach the small intestine; and the normal digestion of food, in the human host, is interrupted. Flukes in the liver produce a chronic inflammatory response, and untreated infections may eventually lead to the development of bile duct cancer (cholangiocarcinoma).

Opisthorchis felineus, the cat liver fluke, infects humans who eat undercooked fish containing the metacercaria. The adult fluke lives in the bile ducts of the liver. Untreated infections may lead to cirrhosis (diffuse fibrosis) of the liver and to an increased risk of liver cancer. Human infections of Opisthorchis felineus occur most often in Siberia.

Clonorchis viverrini, the Southeast Asian liver fluke, is likewise transmitted when humans ingest metacercaria in undercooked fish, and adult flukes live in the bile ducts.

Class Plagiorchiida contains two infectious genera: Dicrocoelium and Paragonimus.

Two species of Genus Dicrocoelium (Dicrocoelium dendriticum and Dicrocoelium hospes) are rare causes of liver fluke disease in humans. The fluke is small, narrow, and long; hence, the alternate name, lancet liver fluke. The adult flukes live in the distal branches of the biliary tree, where they tend to produce mild disease (compared with the biliary infections produced by members of Class Opisthorchiidae, vida supra). The distinguishing feature of Dicrocoelium infections is the second intermediate host: the ant, Formica fusca. Humans become infected when they ingest ants infected by metacercariae; hence, the rarity of human disease.

Paragonimus westermani, along with dozens of less common species within Genus Paragonimus, causes the condition known as paragonimiasis. Paragonimus species are lung flukes. Adult flukes live in the respiratory tree of various infected animals (including rodents, pigs, and humans). Humans become infected when they eat undercooked crabs or crayfish that are infected with metacercariae. Adult flukes and their eggs produce pulmonary inflammation. Paragonimiasis is a common cause of hemoptysis (coughing up blood). Paragonimus eggs can be found in the sputum of infected individuals. About 22 million people are infected worldwide, with most cases occurring in Southeast Asia, Africa, and South America. Cases also occur in the United States.

Trematode infections by pathogenic members of Class Schistosomatidae are fundamentally different, in their method of transmission, from the previously discussed fluke infections. All the other subclasses of trematodes infect humans when the metacercaria (cysts) are eaten. The schistosomes infect humans when cercaria (the phase of larval development that precedes metacercaria), swimming in water, actively penetrate the skin of humans (Fig. 5.14).

Schistosomiasis is the disease caused by any of the five pathogenic species of Genus Schistosoma: Schistosoma haematobium (urinary schistosomiasis), Schistosoma intercalatum (intestinal schistosomiasis), Schistosoma japonicum (schistosomiasis), Schistosoma mansoni (intestinal schistosomiasis), and Schistosoma mekongi (Asian intestinal schistosomiasis). About 200 million people are infected by schistosomes. Infections are common in developing countries, with about half of the infections occurring in Africa [Glossary Exotic diseases].

Trichobilharzia regenti is another member of Class Schistosomatidae. Humans are an accidental dead-end host for this species. As in schistosomiasis, the cercaria penetrates the skin of humans, but the cercaria cannot develop further. The disease is a localized infection known as swimmer's itch or cercarial dermatitis. Other genera of Class Schistosomatidae are known to produce skin rashes in humans.

Infectious Genera

Hymenolepis

– **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Cyclophyllidea: Hymenolepididae: Hymenolepis

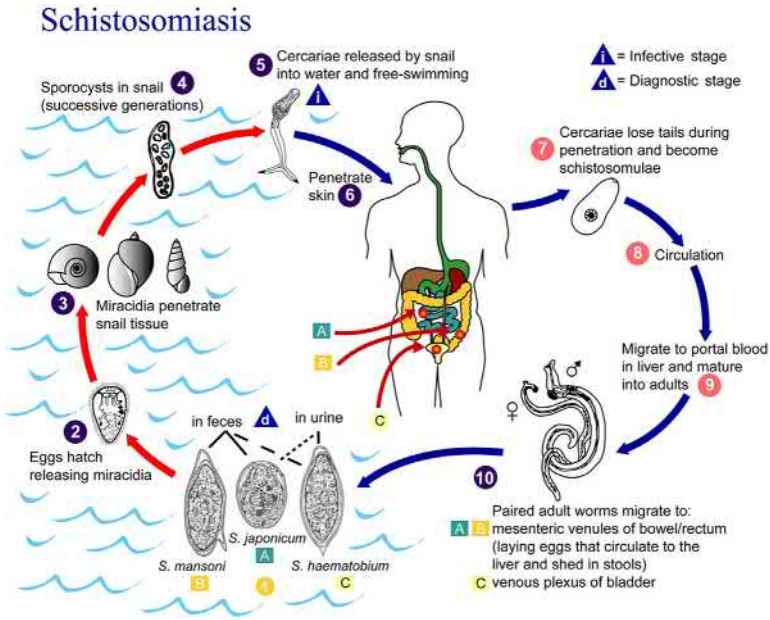

FIG. 5.14 Life cycle of schistosomes.*(Source, Wikipedia, from a public domain graphic produced by the US Centers for Disease Control and Prevention.)*

- **Infection**. Hymenolepis diminuta (rat tapeworm, rarely causing hymenolepiasis in humans)
- **Infection**. Hymenolepis nana (hymenolepiasis)

Echinococcus

- **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Cyclophyllidea: Taeniidae: Echinococcus
- **Infection**. Echinococcus granulosus (hydatid disease, echinococcal disease, cystic echinococcosis, echinococcosis)
- **Infection**. Echinococcus multilocularis (hydatid disease, echinococcal disease, alveolar echinococcosis, echinococcosis)
- **Infection**. Echinococcus vogeli (hydatid disease, echinococcal disease, polycystic echinococcosis, echinococcosis)
- **Infection**. Echinococcus oligarthus (hydatid disease, echinococcal disease, polycystic echinococcosis, echinococcosis)

Taenia

- **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Cyclophyllidea: Taeniidae: Taenia
- **Infection**. Taenia saginata (beef tapeworm disease)
- **Infection**. Taenia solium (pork tapeworm disease, neurocysticercosis, cysticercosis)

Dipylidium

- **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Cyclophyllidea: Dipylidiidae: Dipylidium
- **Infection**. Dipylidium caninum (cucumber tapeworm disease, double-pore tapeworm disease)

Diphyllobothrium

- **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Pseudophyllidea: Diphyllobothriidae: Diphyllobothrium
- **Infection**. Diphyllobothrium latum (diphyllobothriasis or broad tapeworm disease or fish tapeworm disease)

Spirometra

- **Lineage**. Platyhelminthes: Cestoda (tapeworms): Eucestoda: Pseudophyllidea: Diphyllobothriidae: Spirometra
- **Infection**. Spirometra species (sparganosis)

Fasciola

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Plagiorchiida: Echinostomata: Echinostomatoidea: Fasciolidae: Fasciola
- **Infection**. Fasciola gigantica (fascioliasis, liver fluke disease)
- **Infection**. Fasciola hepatica (sheep liver fluke)

Fasciolopsis

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Plagiorchiida: Echinostomata: Echinostomatoidea: Fasciolidae: Fasciolopsis
- **Infection**. Fasciolopsis buski (fasciolopsiasis)
- **Infection**. Fasciolopsis magna (fasciolopsiasis)

Metagonimus

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Opisthorchiida: Opisthorchiata: Heterophyidae: Metagonimus
- **Infection**. Metagonimus yokagawai (metagonimiasis)
- **Infection**. Metagonimus takashii (metagonimiasis)
- **Infection**. Metagonimus miyatai (metagonimiasis)

Clonorchis

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Opisthorchiida: Opisthorchiata: Opisthorchiidae: Clonorchis
- **Infection**. Clonorchis sinensis, alternately Opisthorchis sinensis (Chinese liver fluke, Oriental liver fluke)

Opisthorchis

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Opisthorchiida: Opisthorchiata: Opisthorchiidae: Opisthorchis

- **Infection**. Opisthorchis felineus (Cat liver fluke)
- **Infection**. Opisthorchis viverrini (Clonorchis viverrini)

Dicrocoelium

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Plagiorchiida: Xiphidiata: Gorgoderoidea: Dicrocoeliidae: Dicrocoelium
- **Infection**. Dicrocoelium dendriticum (lancet liver fluke)
- **Infection**. Dicrocoeliidae: Dicrocoelium: Dicrocoelium hospes (lancet liver fluke)

Paragonimus

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Plagiorchiida: Troglotremata: Troglotrematidae: Paragonimus
- **Infection**. Paragonimus westermani (Paragonimiasis, lung fluke)

Schistosoma

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Strigeidida: Schistosomatidae: Schistosoma
- **Infection**. Schistosoma haematobium (urinary schistosomiasis)
- **Infection**. Schistosoma intercalatum (intestinal schistosomiasis)
- **Infection**. Schistosoma japonicum (schistosomiasis)
- **Infection**. Schistosoma mansoni (intestinal schistosomiasis)
- **Infection**. Schistosoma mekongi (Asian intestinal schistosomiasis)

Trichobilharzia

- **Lineage**. Platyhelminthes: Trematoda (flukes): Digenea: Strigeidida: Schistosomatidae: Trichobilharzia
- **Infection**. Trichobilharzia regenti (swimmer's itch)

Section 5.5 Nematoda

Life is hard. Then you die. Then they throw dirt in your face. Then the worms eat you. Be grateful it happens in that order.

David Gerrold

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
```

- Alveolata
  - Apicomplexa
  - Ciliophora (ciliates)
- Heterokonta

Unikonta (1-flagellum)

- Amoebozoa
- Opisthokonta
  - Choanozoa
  - Animalia
    - Eumetazoa
      - Bilateria
        - Deuterostomia
          - Chordata
            - Craniata
        - Protostomia
          - Ecdysozoa
            - Nematoda, roundworms
              - Secernentea
                - Ascaridida
                  - Anisakidae
                    - Anisakis (genus)
                    - Pseudoterranova (genus)
                    - Contracaecum (genus)
                  - Ascarididae
                    - Ascaris (genus)
                    - Baylisascaris (genus)
                  - Toxocaridae
                    - Toxocara (genus)
                  - Dioctophymatidae
                    - Dioctophyme (genus)
                - Spirurida
                  - Spirurina
                    - Filarioidea
                      - Onchocercidae
                        - Brugia (genus)
                        - Loa (genus)
                        - Onchocerca (genus)
                        - Mansonella (genus)
                        - Wuchereria (genus)
                  - Camallanida
                    - Dracunculoidea
                      - Dracunculidae
                        - Dracunculus (genus)
                - Oxyurida
                  - Oxyuridae

```
                        Enterobius (genus)
                    Rhabditida
                      Strongyloididae
                        Strongyloides (genus)
                      Ancylostomatidae
                        Ancylostoma (genus)
                        Necator (genus)
                      Metastrongylidae
                        Angiostrongylus (genus)
                        Trichostrongylidae
                          Trichostrongylus (genus)
                  Enoplea
                    Dorylaimida
                      Trichocephalida
                        Trichinellidae
                          Trichinella (genus)
                          Capillaria (genus)
                        Trichuridae
                          Trichuris (genus)
              Arthropoda
                Chelicerata
                Hexapoda
                Crustacea
            Platyzoa
              Platyhelminthes
              Acanthocephala
Fungi
```

Three large subclasses of animals comprise Class Protostomia: Class Platyzoa, Class Ecdysozoa, and Class Lophotrochozoa. Only the first two classes contain organisms that infect humans. Class Platyzoa contains Class Platyhelminthes and Class Acanthocephala. Class Ecdysozoa accounts for Class Arthropoda, and Class Nematoda.

Nematodes, also known as roundworms, lack a circulatory system, and a respiratory system. They are all pseudocoelomates, meaning that their body cavities are not fully lined by mesoderm. Compare this with the flatworms (Class Platyhelminthes) that are acoelomate (i.e., without body cavities). The Nematodes have a tube-shaped digestive tract, open at both ends (mouth and anus). The presence of a complete digestive tract is another property that distinguishes the roundworms from the flatworms; the digestive tract of flatworm has a single opening for the ingestion of food and the excretion of waste.

Nematodes are covered by a cuticle composed largely of extracellular collagen and other proteins (e.g., cuticulins) excreted by epidermal cells. The cuticle is shed repeatedly at various life stages of the nematode.

Two major subclasses of Class Nematoda contain the organisms that infect humans: Secernentea and Enoplea. These two classes have various anatomic features that distinguish one from the other. Their most relevant distinction, for health-care workers, is that members of Class Secernentea are terrestrial dwellers, while members of Class Enoplea are marine inhabitants.

Many pathogenic members of Class Secernentea are best described within their subclasses

```
Nematoda
  Secernentea
    Ascaridida
      Anisakidae
        Anisakis (genus)
        Contracaecum (genus)
        Pseudoterranova (genus)
```

Anisakiasis is the disease produced by members of Class Anisakidae, which includes the following genera: Anisakis, Contracaecum, and Pseudoterranova. Humans are infected by eating undercooked shellfish and fish infected with larvae. Most cases occur in countries where raw fish is regularly consumed. Only a handful of cases are reported in the United States, annually. Humans are a dead-end host for the worms. After ingestion, the larvae travel to the small intestine, and live for a time, eventually dying without reproducing. In rare cases, they may cause abdominal obstruction, but their most clinically significant effect is through an acute allergic reaction. An acute enteritis often occurs, and, in some cases, a generalized anaphylactic reaction. Some fishermen are so highly sensitized that they develop hyper-immune reactions simply from handling fish or crustaceans that are infected with the larvae.

```
Nematoda
  Secernentea
    Ascaridida
      Ascarididae
        Ascaris (genus)
        Baylisascaris (genus)
```

Ascaris lumbricoides, the cause of ascariasis, infects about 1.5 billion people worldwide, making it the most common helminth (worm) infection of humans [10]. Most cases occur in tropical regions, particularly in Africa and Asia. Humans are the exclusive primary host for the organism.

Infection occurs when humans ingest embryonated eggs contaminating water or food.

The ingested embryonated eggs produce larvae in the small intestine. The larvae invade through the intestinal wall and into the portal system, where they are transported to the alveoli of lungs. While in the lungs, they can produce a pneumonitis. From the lungs, they move upwards, through the bronchial

system, to the pharynx, and drop back into the intestinal system via the esophagus. Among the largest of the nematodes, A. lumbricoides reach adulthood in the intestine, where they can grow to nearly 40 cm in length and have a lifespan of up to 2 years. Ascaris lumbricoides is not the only helminth that migrates through the lungs. In Class Rhabditida, Ancylostoma duodenale, Necator americanus, and Strongyloides stercoralis invade the pulmonary system.

Eggs produced by the adult worms leave the intestine via the feces and contaminate soil and water where sanitation is deficient. Adult worms may produce a wide range of symptoms due to obstruction of the intestines and ducts that connect with the intestine. Like members of Class Anisakidae, Ascaris lumbricoides may provoke an allergic reaction. An allergy to Ascaris lumbricoides may precipitate allergic reactions to shrimp and dust mites, as these unrelated species have some antigens in common (Fig. 5.15).

FIG. 5.15 CDC laboratory technician Henry Bishop poses with Ascaris lumbricoides worms passed by a single child living in Kenya, Africa. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Readers should be careful not to confuse ascariasis with the similar-sounding anisakiasis (vida supra).

Baylisascaris procyonis causes baylisascariasis in humans. Raccoons are the primary host. The adult nematode lives in the raccoon intestine and eggs are dropped with raccoon feces. The eggs of Baylisascaris procyonis can survive for years, and they are extremely resistant to disinfectants and heat. In rare circumstances, humans may become infected, as the secondary host, by ingesting eggs. Eggs develop into larvae in the human intestine, and the larvae migrate out of the intestines and through various organs, where they eventually encyst. Involvement of the central nervous system by encysted larvae is an extremely serious condition.

```
Nematoda
  Secernentea
    Ascaridida
      Toxocaridae
        Toxocara (genus)
```

Humans are dead-end, accidental hosts for Toxocara canis (the dog toxocara) and Toxocara cati (the cat toxocara). Infected animals pass eggs in their feces. Larval development occurs within the eggs, and if matured eggs are ingested by humans, the larvae can hatch in the small intestine. The larvae migrate through various tissues: eyes, lung, liver, and brain being common destinations.

The disease caused by toxocara organisms is toxocariasis. During the migratory stage, the disease is often referred to as visceral larva migrans. Eye involvement is referred to as ocular larval migrans. Readers should not be lulled into a false sense of terminologic security. When toxocara migrate through the skin, the condition is NOT called cutaneous larva migrans: this term is reserved for cutaneous manifestations of Ancylostoma braziliense. An immune response to the migrating toxocara larvae may produce eosinophilia (i.e., an increase in eosinophils in the peripheral blood), and the term eosinophilic pseudoleukemia has been used to describe this condition.

After a period of migration, the worms, which cannot mature further in the human body, encyst to produce small, localized, permanent nodules in tissues.

Readers should not confuse "toxocara" with the similar-sounding "toxoplasma" (Class Apicomplexa), a problem aggravated when insouciant clinicians use the abbreviated and ambiguous form "toxo," which can refer to either organism.

```
Nematoda
  Secernentea
    Ascaridida
      Dioctophymatidae
        Dioctophyme (genus)
```

Dioctophyme renale, also known as the giant kidney worm, is a rare cause of human disease. Humans, one of many animals serving as the primary host, are infected by ingesting an undercooked second intermediate host (usually fish or frogs) that had, in turn, ingested the first intermediate host (a freshwater earthworm). Ingested larvae penetrate the human intestines and migrate to the liver.

From the liver, they migrate to a kidney (unilateral, usually the right kidney), where they become adults. Eggs laid by the adult worm are excreted in the urine. The infestation of large adult worms typically leads to the destruction of the kidney, if left untreated. Human disease is rare, and can occur anywhere in the world.

```
Nematoda
  Secernentea
    Spirurida
      Spirurina
        Filarioidea
          Onchocercidae
            Brugia (genus)
            Loa (genus)
            Onchocerca (genus)
            Mansonella (genus)
            Wuchereria (genus)
```

Filarial nematodes are string-like worms that are sufficiently small to fit into lymphatic vessels. About 150 million people are infected by the filarial nematodes (genera Brugia, Loa, Onchocerca, Mansonella, and Wuchereria) [11]. Wuchereria bancrofti and Brugia malayi, together, infect about 120 million individuals [11]. Most cases occur in Africa and Asia.

The life cycle for these organisms involves a human primary host, in which the adult filarial worms produce juveniles (microfilaria) that migrate through lymphatics and blood vessels, where the microfilaria are sucked out by a secondary host (i.e., a blood-feeding mosquito or fly). In the secondary host, they develop into larvae. The secondary host serves double duty as a vector by injecting larvae into the primary host when the insect has a blood meal. The larvae grow into adult threadworms within the primary host, a process that takes place over a year or more, and the cycle continues. The symptoms and severity of the disease are largely determined by the anatomic destinations of the migrating worms, and on the total load of worms, as multiple infections in the same person lead to a continuously increasing filarial burden.

Wuchereria bancrofti, Brugia malayi, and Brugia timori tend to cause lymph system obstruction, which can lead to elephantiasis. Elephantiasis is a condition wherein one or more extremities, usually the legs, are chronically swollen.

Other filarial worms preferentially inhabit the fatty tissue within the subcutis of skin: loa loa (the African eye worm), Mansonella streptocerca, and Onchocerca volvulus. The subcutaneous tissue is also the cause of infection by a nematode in Class Camallanida: Dracunculus medinensis (vida infra). Mansonella perstans and Mansonella ozzardi live in the abdominal peritoneum.

Onchocerca volvulus is the cause of river blindness, the second leading cause of infection-produced blindness (behind trachoma, caused by Chalmydia trachomatis, Class Chlamydiae). The ocular pathogenicity of Onchocerca volvulus is caused by an endosymbiont, Wolbachia pipientis (See discussion in Class Alpha Proteobacteria).

In addition to the filarial infections for which humans are the natural, primary host, there are reported cases of the so-called zoonotic filariasis, in which humans are a dead-end host. The zoonotic infections, as in the filarial infections for which humans are the natural hosts, are all transmitted by blood-feeding insects. The filaria live for a time in human tissues, where they eventually die, producing a localized inflammatory reaction. Though various species of filaria are found in a wide assortment of animal hosts, including birds and reptiles, only mammalian hosts have been associated with zoonotic filariasis in humans [12].

```
Nematoda
  Secernentea
    Spirurida
      Camallanida
        Dracunculoidea
          Dracunculidae
            Dracunculus (genus)
```

Dracunculus medinensis is the single pathogenic species in Class Dracunculidae. Dracunculiasis, also known as guinea worm, has a dramatic clinical presentation and treatment. The adult female worm bursts forth from the skin, usually just above or below the knee. The astute physician coaxes the living worm, onto a stick, which he or she then winds slowly, thus delivering the intact worm and relieving the patient of his parasitic burden. The Rod of Asclepius, historically symbolizing the practice of medicine, is inspired by the ancient ritual whereby the Dracunculus worm is extracted (Fig. 5.16).

Human become infected when they drink water that has been contaminated with copepods (tiny organisms in Class Crustacea) containing a juvenile form of the organism. Readers will remember that copepods are a favored secondary host for cestodes (Class Platyhelminthes). After ingestion by humans, the copepods die, and larvae are released to penetrate the stomach wall. The male worms die, but the females migrate to subcutaneous tissue and continue to grow into adulthood (about 1 year later). The infectious cycle is perpetuated when the matured female adult (approaching a meter in length) pokes through the skin and releases its larvae, predestined for ingestion by secondary hosts (i.e., copepods).

As with several of the previously described nematodes (i.e., Ascaris lumbricoides, Enterobius vermicularis, and the common filarial species) humans are the exclusive primary host of Dracunculus medinensis. Public health measures have drastically reduced the occurrences of dracunculiasis in Africa and Pakistan.

```
Nematoda
  Secernentea
    Oxyurida
      Oxyuridae
        Enterobius (genus)
```

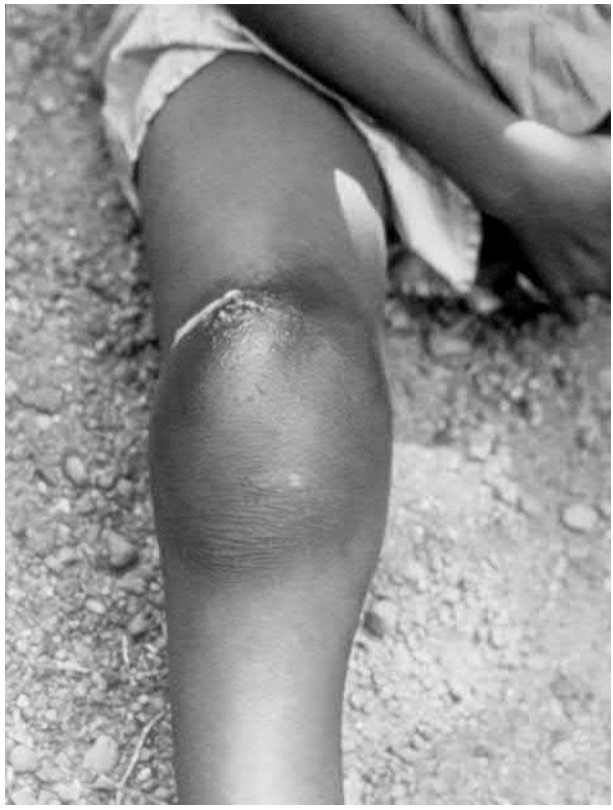

FIG. 5.16 Female Dracunculus medinensis worm, sometimes called the guinea worm, beginning to emerge from an infected knee. The worm may attain a length of 2–3 ft. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Enterobius vermicularis, known as the pinworm in the United States, causes enterobiasis, sometimes called oxyuriasis.

Humans are the primary and exclusive host of the pinworm. Eggs are ingested, and the larvae emerge in the duodenum. The larvae migrate in the direction of peristalsis, as they mature into adults. The adults mate in the ileum and settle in the distal ileum, the proximal colon, or the appendix, where the females become bloated with eggs. Afterwards, the adult females (now about 1 cm in length) migrate to the anal skin where they lay their eggs. The eggs infect other humans or re-infect the original host, through fecal-oral contamination or through dispersal in the air (e.g., when bed linens are shaken).

The most common symptom of pinworm is anal itching. Cases have been reported of appendicitis caused by gravid pinworms obstructing the lumen of the appendix.

```
Nematoda
  Secernentea
    Rhabditida
      Strongyloididae
        Strongyloides (genus)
      Ancylostomatidae
        Ancylostoma (genus)
        Necator (genus)
      Metastrongylidae
        Angiostrongylus (genus)
        Trichostrongylidae
          Trichostrongylus (genus)
```

Class Rhabditida is characterized by juvenile forms that are adept at free-living (usually in soil) and capable of a range of behavior usually associated with adult forms; particularly the ability to penetrate skin, to migrate through tissues, and to survive for extended periods.

Strongyloides stercoralis is a nematode that primarily infects humans; naturally occurring infection by Strongyloides stercoralis in animals other than humans is not known at this time. Humans become infected when larvae, passed from human feces, and free-living in contaminated soil, penetrate exposed skin and enter the circulation system. No secondary host is involved in the life cycle of Strongyloides stercoralis. Once in the bloodstream, Strongyloides stercoralis larvae move to the lungs, where they invade the bronchial tree, advance to the pharynx, and drop into the esophagus. When they reach the small intestine, they are adults, and female worms begin to lay eggs, thus renewing the infectious life cycle. Infections in humans are uncommon and occur in countries with poor sanitation.

Strongyloides has one important biological trick that contributes to its clinical presentation. Larvae that develop from eggs laid in the small intestine are capable of autoinfecting the host, by invading though the intestinal mucosa, or by invading through the anal skin (when they emerge from the large intestine). The larvae pass to the lung, renewing the infection cycle within their original human host. This step short-circuits the step wherein larvae dwell as free-living organisms in soil.

The consequences of autoinfection are several: infection can continue for a long time, sometimes extending throughout the lifespan of the host; the infectious load (i.e., Number of organisms in the body) can be immense.

The term applied to a high infectious load of Strongyloides stercoralis is "hyperinfection syndrome" [13]. Immune-compromised patients are at highest risk for hyperinfection syndrome. When hyperinfection occurs, the disease can be fatal.

In addition to Strongyloides stercoralis, other species that have been reported to infect humans are: Strongyloides fuelleborni and Strongyloides kellyi.

Class Anyclostomadea contains the two species responsible for nearly all cases of hookworm disease in humans: Ancylostoma duodenale and Necator americanus. The two species have similar clinical presentations, but different geographic

distributions. Ancylostoma duodenale is common in the Middle East, North Africa, and India. Necator Americanus is common in North and South America, as well as parts of Africa and Asia. Hookworms infect about 1 billion people.

Hookworm eggs and larvae live in soil. Like the previously described member of Class Rhabditida, Strongyloides stercoralis, larvae of Ancylostoma duodenale and Necator Americanus penetrate the skin of human hosts. Ancylostoma duodenale may also infected by oral ingestion. The larvae invade tissues and travel to the lung, where they travel up the bronchial tree, eventually dropping into the esophagus, passing down the alimentary tract to the small intestine, where they mature into adults. Eggs laid by the female adult worms pass into the environment, with feces. Humans are the only natural host for the hookworms. There is no secondary host (Fig. 5.17).

The distinctive biological feature of the hookworms, distinguishing them from other nematodes, is hemophagia (i.e., blood eating). The hookworms suck blood from the host, producing anemia and malnutrition. Children are particularly vulnerable to the effects of hookworm infection, which may causes delays in mental and physical development.

Ancylostoma braziliense is a hookworm of dogs and cats. It occasionally penetrates the skin of humans, a dead-end host, and causes localized inflammation of the skin or limited subcutaneous migration (cutaneous larva migrans or creeping eruption).

Class Metastrongylidae contains two genera that contain infectious organisms: Angiostrongylus and Trichostrongylus.

Angiostrongylus cantonensis is a parasitic nematode (roundworm) that causes angiostrongyliasis, the most common cause of eosinophilic meningitis in Southeast Asia. Angiostrongylus cantonensis lives in the pulmonary arteries of rats; hence its common name, rat lungworm. Snails are the most common intermediate hosts, where larvae develop until they are infective. Humans are

FIG. 5.17 Hookworm filariform larva surrounded by clumps of fecal debris. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, prepared by Dr. Mae Melvin.)*

incidental hosts of this roundworm, and may become infected through ingestion of raw or undercooked snails or from water or vegetables contaminated by snails or slugs or deposited larvae. Ingested larvae travel through the blood to the brain, where the larvae may die or may progress to juvenile adults before eventually dying. The dead and dying organisms produce an allergic inflammatory reaction in the brain, with an increase in eosinophils in cerebral spinal fluid. Other species of Genus Angiostrongylus that may infect humans include Angiostrongylus costaricensis and Angiostrongylus mackerrasae.

Genus Trichostrongylus contains a variety of species that infect a wide range of animals; 10 species are known to infect humans. Humans are infected when larvae are ingested by ingesting contaminated water or vegetables. The larvae mature in the gut, and adult organisms live in the small intestine. Infections are often asymptomatic. Symptoms, when they occur, are typically those of enteritis. Eosinophilia (increased eosinophils in the blood) often occurs, and severe symptoms arise in some individuals.

```
Nematoda
  Enoplea
    Dorylaimida
      Trichocephalida
        Trichinellidae
          Trichinella (genus)
          Capillaria (genus)
        Trichuridae
          Trichuris (genus)
```

The nematodes that cause infection in humans belong to the Class Secernentea or Class Enoplea. The human pathogens in Class Secernentea have been described (via supra). Class Enoplea has three genera containing species that are infectious to humans: Trichinella, Capillaria, and Trichuris.

Trichinella spiralis is the cause of trichinosis, also known as trichinellosis. Humans are simultaneously the primary and the secondary host for this organism as the adult worms live in the intestines, producing eggs that hatch into larvae within the body of the female adult. The female adult worms live for about 6 weeks. The larvae leave the adult and penetrate the intestinal mucosa and migrate to muscles, where they encyst, waiting to be eaten by another potential host. Encysted larvae live within individual muscle cells. A full developed larva is about 80 μm in length, so the larva curls tightly within the muscle cell, allowing it to fit in a tight space (Fig. 5.18).

Humans typically become infected after eating the undercooked infected meat. More than 150 different animals have been reported as sylvatic hosts. Most infections in the United States come from eating pork. In the early decades of the 20th century, hogs were fed on pig meat, thus magnifying the infectious burden in the hog population. Methods for cooking pork were lax, and trichinosis in humans was common. With improved, regulated diets for hogs, and with proper methods of cooking pork, the incidence of trichinosis in the

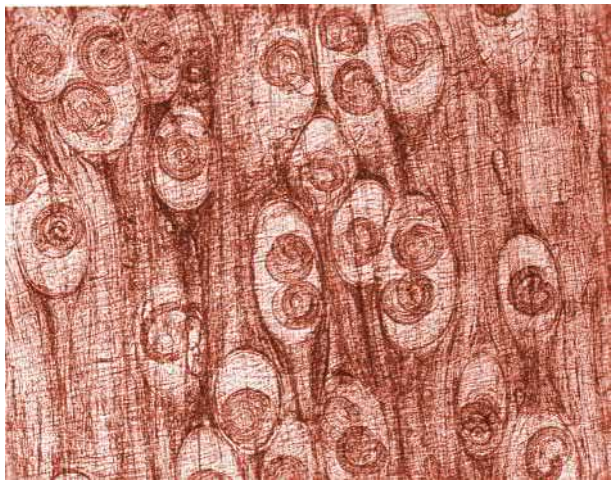

FIG. 5.18 Heavy infection of Trichinella spiralis encysted larvae in muscle. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

United States has dropped. Clinically, trichinosis produces enteric symptoms when the adult worms are reproducing in the intestines; muscle aches when the larvae are invading muscle cells.

Hepatic capillariasis, caused by Capillaria hepatica, is a rare human infection, with only a few dozen cases reported, most them occurring in children. Eggs in soil are ingested, hatched larvae penetrate the intestinal mucosa, and the larvae are carried through the portal system to the capillaries of the liver, where they mature to adults. The adults lay their eggs in the liver. This leads to liver fibrosis. If the parasite burden is high, cirrhosis may eventually develop. The parasite infects a wide range of animals, but rats are the most likely source of human infections. When an infected rat dies, its body decomposes, and eggs within the liver are released into the soil, where the life cycle resumes.

Trichuris trichiura is called the human whipworm, named for its tightly wound, thick segment, from which a straight, thinner segment extends (i.e., handle and whip). The disease caused by Trichuris trichiura is trichuriasis. Humans are the natural primary host for the organism. No secondary host is involved. Ingested eggs hatch in the small intestine, and larvae mature in the large intestine. The matured worms, which can live up to 5 years, anchor in the colonic mucosa and release eggs that pass out of the colon with feces. Eggs in the soil contaminate food, and the life cycle resumes.

The degree of morbidity is determined by the number of parasitic worms. Heavy infections can produce bloody diarrhea, anemia, and even rectal prolapse (from the aggregate weight of worms the rectum). Trichuriasis often accompanies other parasitic infections in the same individual.

Infectious Genera

Anisakis

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Ascaridomorpha: Ascaridoidea: Anisakidae: Anisakis
- **Infection**. Anisakis simplex complex (anisakiasis)

Baylisascaris

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Ascaridomorpha: Ascaridoidea: Ascarididae: Baylisascaris
- **Infection**. Baylisascaris procyonis (baylisascariasis, larva migrans with brain involvement)

Pseudoterranova

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Ascaridomorpha: Ascaridoidea: Anisakidae: Pseudoterranova
- **Infection**. Pseudoterranova decipiens (anisakiasis)

Ascaris

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Ascaridomorpha: Ascaridoidea: Ascarididae: Ascaris
- **Infection**. Ascaris lumbricoides (ascariasis, ascaris pneumonitis)

Toxocara

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Ascaridomorpha: Ascaridoidea: Toxocaridae: Toxocara
- **Infection**. Toxocara canis, the dog roundworm (toxocariasis, visceral larva migrans, ocularis larva migrans)
- **Infection**. Toxocara cati, or Tococara mystax, or the feline roundworm (toxocariasis, visceral larva migrans, ocularis larva migrans)

Enterobius

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Oxyuridomorpha: Oxyuroidea: Oxyuridae: Enterobius
- **Infection**. Enterobius vermicularis, also called pinworm in the United States and as threadworm in the United Kingdom, or sometimes as seatworm (enterobiasis or oxyuriasis)

Strongyloides

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Tylenchina: Panagrolaimomorpha: Strongyloidoidea: Strongyloididae: Strongyloides
- **Infection**. Strongyloides stercoralis, known as threadworm in US and pinworm in United Kingdom (strongyloidiasis)

Ancylostoma

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Rhabditina: Rhabditomorpha: Strongyloidea: Ancylostomatidae: Ancylostomatinae: Ancylostoma
- **Infection**. Ancylostoma duodenale (hookworm, along with Necator americanus)

Necator

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Rhabditina: Rhabditomorpha: Strongyloidea: Ancylostomatidae: Bunostominae: Necator
- **Infection**. Necator americanus (hookworm, along with Ancylostoma duodenale)

Angiostrongylus

- **Lineage**. Nematoda: Chromadorea: Strongylida: Metastrongyloidea: Angiostrongylidae: Angiostrongylus
- **Infection**. Angiostrongylus cantonensis (angiostrongyliasis)
- **Infection**. Angiostrongylus costaricensis (abdominal angiostrongyliasis, intestinal angiostrongyliasis)
- **Infection**. Angiostrongylus mackerrasae (eosinophilic meningitis)

Trichostrongylus

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Rhabditina: Rhabditomorpha: Strongyloidea: Trichostrongylidae: Trichostrongylus
- **Infection**. Trichostrongylus orientalis (trichostrongyliasis, trichostrongylosis)

Dracunculus

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Dracunculoidea: Dracunculidae: Dracunculus
- **Infection**. Dracunculus medinensis (dracunculiasis, guinea worm disease)

Brugia

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Spiruromorpha: Filarioidea: Onchocercidae: Brugia
- **Infection**. Brugia malayi (lymphatic filariasis, elephantiasis)
- **Infection**. Brugia pahangi (animal filariasis, rarely infecting humans) [14]
- **Infection**. Brugia timori (lymphatic filariasis)

Loa

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Spiruromorpha: Filarioidea: Onchocercidae: Loa
- **Infection**. Loa loa (loa loa filariasis, loiasis, loaiasis, Calabar swellings, Fugitive swelling, Tropical swelling, African eye worm)

Mansonella

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Spiruromorpha: Filarioidea: Onchocercidae: Mansonella
- **Infection**. Mansonella ozzardi (serous cavity filariasis, seldom causes clinical disease, but generalized symptoms including fever, lymphadenopathy, joint pain, headache and pruritis are occasionally encountered)
- **Infection**. Mansonella perstans, formerly Dipetalonema perstans (serous cavity filariasis, bung-eye disease)
- **Infection**. Mansonella streptocerca, formerly Dipetalonema streptocerca (streptocerciasis)

Onchocerca

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Spiruromorpha: Filarioidea: Onchocercidae: Onchocerca
- **Infection**. Onchocerca volvulus (onchocerciasis, river blindness)

Wuchereria

- **Lineage**. Nematoda: Chromadorea: Rhabditida: Spirurina: Spiruromorpha: Filarioidea: Onchocercidae: Wuchereria
- **Infection**. Wuchereria bancrofti (filariasis)

Trichinella

- **Lineage**. Nematoda: Enoplea: Dorylaimia: Trichinellida: Trichinellidae: Trichinella
- **Infection**. Trichinella nativa (trichinellosis)
- **Infection**. Trichinella nelsoni (trichinellosis) [15]
- **Infection**. Trichinella pseudospiralis (trichinellosis) [16]
- **Infection**. Trichinella spiralis (trichinellosis, trichinosis)

Capillaria, synonymous with Calodium

- **Lineage**. Nematoda: Enoplea: Dorylaimia: Trichinellida: Capillariidae: Capillaria
- **Infection**. Capillaria philippinensis (intestinal capillariasis)
- **Infection**. Capillaria hepatica (hepatic capillariasis)

Trichuris

- **Lineage**. Nematoda: Enoplea: Dorylaimia: Trichinellida: Trichuridae: Trichuris
- **Infection**. Trichuris trichiura, human whipworm (trichuriasis)

Dioctophyme

- **Lineage**. Nematoda: Enoplea: Dorylaimia: Dioctophymatida: Dioctophymatoidea: Dioctophymatidae: Dioctophyme
- **Infection**. Dioctophyme renale (giant kidney worm infection)

Section 5.6 Acanthocephala

Nothing in biology makes sense except in the light of evolution.

Theodosius Dobzhansky [17]

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
        Eumetazoa
          Bilateria
            Deuterostomia
              Chordata
                Craniata
            Protostomia
              Ecdysozoa
                Nematoda
                Arthropoda
                  Chelicerata
                  Hexapoda
                  Crustacea
              Platyzoa
                Platyhelminthes
                Acanthocephala
                  Archiacanthocephala
                    Moniliformida
                      Moniliformidae
                        Moniliformis (genus)
      Fungi
```

Acanthocephalans, also known as thorny-headed worms or spiny-headed worms, live purely parasitic lives. As is so often the case among dedicated parasites, these organisms have unburdened themselves of some anatomic features that may have been useful to their ancestors, but which serve no purpose when host resources are readily available. For taxonomists, this is a vexing problem; without inherited anatomic features, it is difficult to establish an organism's lineage, with any certainty.

Taxonomists have placed the Acanthocephalans as a subclass of Class Platyzoa, along with the Platyhelminthes, and Class Rotifera, a class that does not contain human pathogens. Based on gene comparisons, it seems likely that Class Acanthocephala will soon be moved a notch over, to become a subclass of Class Rotifera.

All adult acanthocephalans have an extensible proboscis that is armed with hooks for attachment to gut wall. There is variation in the size and morphologic features of the acanthocephalans. In the case of the Moniliformis moniliformis, a known human pathogen, worms, found in stool are just over a centimeter in length and about 4 mm wide. The proboscis is armed with 14 rows, each row with 6–8 hooks.

There are well over 1000 species within Class Acanthocephala, infesting an enormous variety of animals, including dogs, squirrels, rats, birds, fish, and insects, including cockroaches. Like many dedicated parasitic animals, members of Class Acanthocephala display a bewildering array of life cycles, with one or more intermediate hosts. The complete life cycles of many of the known genera have not been fully established.

Parasitologists have taken a particular interest in Class Acanthocephala because these parasites have evolved a gruesome strategy for host parasitism known as "brain-jacking." All studied acanthocephalan species are able to alter the behavior of their intermediate hosts so as to increase the likelihood that the acanthocephalan will be delivered to its final host; typically in the form of a snack whose main ingredient is the intermediate host. In many cases, the parasite provokes the intermediate hosts to move to a vulnerable location (e.g., the surface of a pond, or a well-lit and exposed spot of soil), where the final host can eat them. The mechanism underlying brain-jacking is not fully understood, but it seems to be mediated, in at least some cases, by the parasite-induced release of host serotonin.

```
Acanthocephala
  Archiacanthocephala
    Moniliformida
      Moniliformidae
        Moniliformis (genus)
```

Though all genera of Class Acanthocephala are parasitic in animals, none are particularly well adapted to life within humans. Human infection is extremely rare; when it occurs, it is secondary to the unintentional ingestion of whole or part of an uncooked, natural host. A single genus of Class Acanthocephala is known to have produced disease in humans: Moniliformis. Another genus, Apororhynchus is occasionally listed as a human parasite, but review of the literature yields no

specific report documenting human pathogenicity. Though the literature describing Acanthocephaliasis is scant, it seems that human infections are characterized by diarrhea, secondary to worm attachments to the walls of the small intestine. Reports of human infections usually come from Middle Eastern countries [18].

Infectious Genera

Moniliformis

- **Lineage**. Protostomia: Lophotrochozoa: Acanthocephala: Archiacanthocephala: Moniliformida: Moniliformidae: Moniliformis
- **Infection**. Moniliformis dubius (Acanthocephaliasis)
- **Infection**. Moniliformis moniliformis (Acanthocephaliasis)

Section 5.7 Chelicerata

The truly privileged theories are not the ones referring to any particular scale of size or complexity, nor the ones situated at any particular level of the predictive hierarchy, but the ones that contain the deepest explanations.

David Deutsch

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
        Eumetazoa
          Bilateria
            Deuterostomia
              Chordata
                Craniata
            Protostomia
              Ecdysozoa
```

```
                Nematoda
                Arthropoda
                  Chelicerata
                    Arachnida
                      Acari
                        Sarcoptiformes
                          Sarcoptidae
                            Sarcoptes (genus)
                        Trombidiformes
                          Demodicidae
                            Demodex (genus)
                          Cheyletidae
                            Cheyletiella (genus)
                  Hexapoda
                  Crustacea
              Platyzoa
                Platyhelminthes
                Acanthocephala
Fungi
```

The subclasses of Class Arthropoda that harbor human infectious organisms are Class Chelicerata, Class Hexapoda, and Class Crustacea. Class Chelicerata contains a variety of organisms that would appear, at first glance, to be unrelated: spiders, mites, ticks, scorpions, and horseshoe crabs. The common names do not help much. Horseshoe crabs are not true crabs; true crabs belong to Class Crustacea.

All the members of Class Chelicerata have chelicerae, embryonic appendages that form prior to, and in the vicinity of, the mouth. In most species within Class Chelicerata, the chelicerae are feeding pincers. In spiders, the chelicerae are fangs. Another feature of the chelicerates, which helps to distinguish them from insects, is the absence of antennae.

The enormous difference in size between a horseshoe crab (60 cm or less) and a Demodex mite (less than 1 mm) is yet another reminder that biological classes are not determined by similarities (such as size), but by phylogenetic relationships (such as chelicerae). Aside from their class-specific chelicerae, chelicerates inherit the body plan of their superclass, Class Arthropoda. This means that they have a heart that pumps blood though a major body cavity called a haemocele.

Most members of Class Chelicerata are noninfectious in humans. Only three genera of Class Chelicerata live in, or on, humans, and both genera belong to the subclass of arachnids named Class Acari, which includes mites and ticks. Readers should not confuse mites and ticks with insects. Insects are members of Class Hexapoda. In addition, Class Acari (in Class Chelicerata) should not be confused with the similar-sounding Class Ascaris (in Class Nematoda).

Ticks are vectors for a variety of infectious pathogens. Tick species Ixodes scapularis transmits Babesia microti (babesiosis), Borrelia burgdorferi (Lyme disease), and Anaplasma phagocytophilum (human granulocytic

anaplasmosis) [19]. Tick-borne viruses include: Crimean-Congo hemorrhagic fever, Tick-borne encephalitis, Powassan encephalitis, Deer tick virus encephalitis, Omsk hemorrhagic fever, Kyasanur forest disease (Alkhurma virus), Langat virus, and Colorado tick fever [Glossary Hemorrhagic fevers].

In this book ticks are not considered infectious organisms. Basically, ticks leave the host after collecting their blood meal. Because ticks are temporary guests and do not actually live in humans, they will not be discussed further in this chapter.

```
Chelicerata
  Arachnida
    Acari (includes mites and ticks)
      Sarcoptiformes
        Sarcoptidae
          Sarcoptes (genus)
      Trombidiformes
        Demodicidae
          Demodex (genus)
        Cheyletidae
          Cheyletiella (genus)
```

There are three infectious genera of mites: Demodex, Cheyletiella, and Sarcoptes. Genera Demodex and Cheyletiella belong to the Acari subclass Class Trombidiformes. Genus Sarcoptes belongs to the Acari subclass, Class Sarcoptiformes. Both these classes contain extremely small mites that can live on humans without being identified with the naked eye.

Genus Sarcoptes contains one infectious organism for humans; Sarcoptes scabiei, the cause of scabies in humans and sarcoptic mange in animals. This small mite burrows into the superficial skin. Burrows are visible to astute searchers. Once in the skin, the mites produce an allergic reaction, producing itching. The name scabies comes from the Latin scabere, to scratch. Scabies is an exceedingly common, global disease, with about 300 million new cases occurring annually. The organism infects humans through contact with other infected humans and animals. Spread of the scabietic rash is encouraged by scratching, which results in self-inoculation from one area of skin to another. The organism prefers intertriginous sites (skin creases such as groin, under breast, between fingers).

Scabies infestation can be particularly severe for immune-compromised patients, particularly those with AIDS. In this circumstance, the infection can become generalized, extending over the entire surface of the body, excluding only the face.

Demodex is a tiny mite that lives in facial skin. Demodex folliculorum favors the hair follicles. Demodex brevis favors the sebaceous glands. Both mites can be found in the majority of humans, and infections seldom produce clinical disease. It is common for pathologists to encounter these mites, on cross sections of hair follicles or sebaceous glands, when studying histologic samples of facial skin; a finding of no consequence. In the rare instances when Demodex infections are

heavy, a condition characterized by itching and inflammation may occur. This condition is known as demodicosis. When demodicosis occurs on the eyelashes, the condition is demodectic blepharitis. Demodex canis, an infection of dogs that can produce demodectic mange, may rarely cross-infect humans.

Cheyletiella mites infect the skin of dogs, cats, and rabbits. Occasionally, Cheyletiella yasguri or Cheyletiella blakei infect humans, producing a self-limited dermatitis, cheyletiellosis, one of several different diseases characterized by the occurrence of red, itchy bumps.

Infectious Genera

Sarcoptes

- **Lineage**. Arthropoda: Chelicerata: Arachnida: Acari: Sarcoptiformes: Sarcoptidae: Sarcoptes
- **Infection**. Sarcoptes scabiei (scabies)

Demodex

- **Lineage**. Arthropoda: Chelicerata: Arachnida: Acari: Trombidiformes: Demodicidae: Demodex
- **Infection**. Demodex folliculorum (demodicosis)
- **Infection**. Demodex brevis (demodicosis)
- **Infection**. Demodex canis (dog mite, rarely infecting humans; demodectic mange in dogs)

Cheyletiella

- **Lineage**. Arthropoda: Chelicerata: Arachnida: Acari: Trombidiformes: Cheyletidae: Cheyletiella
- **Infection**. Cheyletiella yasguri (cheyletiellosis, cheyletiella dermatitis, walking dandruff)
- **Infection**. Cheyletiella blakei (cheyletiellosis, cheyletiella dermatitis, walking dandruff)

Section 5.8 Hexapoda

It has become evident that the primary lesson of the study of evolution is that all evolution is coevolution: every organism is evolving in tandem with the organisms around it.

Kevin Kelly

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
```

- Euglenozoa
- Percolozoa
- Archaeplastida
- Chromalveolata
  - Alveolata
    - Apcomplexa
    - Ciliophora (ciliates)
  - Heterokonta
- Unikonta (1-flagellum)
  - Amoebozoa
  - Opisthokonta
    - Choanozoa
    - Animalia
      - Eumetazoa
        - Bilateria
          - Deuterostomia
            - Chordata
              - Craniata
          - Protostomia
            - Ecdysozoa
              - Nematoda
              - Arthropoda
                - Chelicerata
                - Hexapoda
                  - Insecta
                    - Hemiptera
                      - Cimicoidea
                        - Cimicidae
                          - Cimicinae
                            - Cimex (genus)
                    - Phthiraptera
                      - Anoplura
                        - Pediculidae
                          - Pediculus (genus)
                        - Pthiridae
                          - Pthirus (genus)
                    - Diptera
                      - Calliphoridae
                        - Cochliomyia (genus)
                      - Oestroidea
                        - Calliphoridae (genus)
                        - Sarcophagidae (genus)
                        - Dermatobia (genus)
                    - Siphonaptera

```
                    Hectopsyllidae
                      Tunga (genus)
                Crustacea
            Platyzoa
              Platyhelminthes
              Acanthocephala
    Fungi
```

Class Arthropoda accounts for 80% of the known species of animals and has three subclasses containing organisms that are pathogenic to humans: Class Chelicerata, Class Hexapoda, and Class Crustacea.

Class Hexapoda is the largest class of animals, in terms of the number of different class species. Molecular evidence would suggest that Class Hexapoda first appeared about 425 million years ago, coinciding with the earliest fossils of large, vascular land plants [20]. Presumably, the species diversity of Class Hexapoda is intimately related with the diversity of land plants, particularly plants in Class Angiospermae, the flowering plants. Insects pollinate the plants, the plants serve as primary food sources for the insects; plant and insect co-evolve to serve one another's survival interests. As the name would suggest, members of Class Hexapoda have six ("hex") legs ("poda").

Class Hexapoda has two major subclasses: Entognatha and Ectognatha; hexapods with enclosed mouthparts and hexapods with exposed mouthparts, respectively. Class Insecta belongs to the Class Ectognatha (exposed mouthparts). The insect body is built from fused segments, and the head has a mandible and a maxilla. All of the infectious members of Class Hexapoda belong to Class Insecta. Members of Class Entognatha contain some of the most common organisms on earth (e.g., Collembola, also known as springtails), but none of the entognathans infect humans [21].

Despite its precise taxonomic definition, members of other animal classes are often mistaken for members of Class Hexapoda. This is particularly true for Class Chelicerata, which contains spiders, mites, ticks, and scorpions, none of which are hexapods.

```
Hexapoda
  Insecta
    Hemiptera
      Cimicoidea
        Cimicidae
          Cimicinae
            Cimex (genus)
```

Class Hemiptera, a subclass of Class Hexapoda, are the so-called "true bugs." They are distinguished from other insects by their mouth parts, which are shaped as a proboscis and covered by a labial sheath. The mouth parts of Class Hemiptera are designed for sucking. Class Hemiptera includes cicadas

and aphids. The triatome species that are vectors for Trypanosoma cruzi (Euglenozoa) are members of Class Hemiptera.

It is worth reminding readers that the word "bug" has no taxonomic meaning, as it cannot be applied to any particular class of organisms. It is meaningless to use the word "bug" to refer to any small crawling hairless organism, as the organism may be an insect (Class Insecta), a hexapod that is not an insect (Class Entognatha), a mite (Class Chelicerata), or a pill bug (Class Crustacea).

Cimicidae are true bugs (i.e., Class Hemiptera). Bedbugs (Cimex lenticularis) suck blood and produce an inflammatory response, but they are not a true infection (they don't live in the skin), and they are not known to be carriers of other infectious agents. Bedbugs are included in this book because there is currently a bedbug epidemic in US cities; bedbug enthusiasts would be offended if this species were omitted.

```
Hexapoda
  Phthiraptera
    Anoplura
      Pediculidae
        Pediculus (genus)
      Pthiridae
        Pthirus (genus)
```

Three species of lice (Class Pediculus and Class Phthirus) have adapted to infect humans: Pediculus humanus capitus, the head louse, Pediculus humanus corporis, the body louse, and Phthirus pubis (crab louse or pubic louse). These lice are all hematophagus (blood sucking), and can produce an inflammatory skin reaction at the site of infection. The eggs of lice are called nits. The nits of head lice and pubic lice are deposited on hairs. The nits of body lice are deposited on skin and clothing (Fig. 5.19).

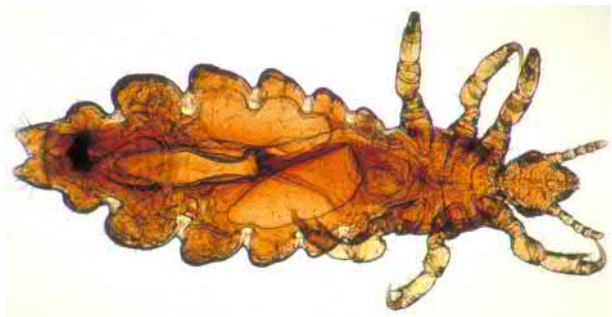

FIG. 5.19 Female Pediculus humanus corporis louse. Aside from causing pediculosis, this insect is a vector for Rickettsia prowazekii, Borrelia recurrentis, and Bartonella quintana. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, and obtained from Dr. Dennis D. Juranek.)*

```
Hexapoda
  Insecta
    Diptera
      Calliphoridae
        Cochliomyia (genus)
      Oestroidea
        Calliphoridae (genus)
        Sarcophagidae (genus)
        Dermatobia (genus)
```

Myiasis is an infection of the larvae (maggots) of flies (Class Diptera). The screwworm (Cochliomyia hominivorax), despite its vivid name, is a type of fly. Because insect larvae are small and squishy, they are often mistaken for worms. The disease caused by Cochliomyia hominivorax is manifested by worm-like larvae growing in skin. Whereas myiasis caused by most other species of fly is an infection of dead animals, or necrotic parts of living animals (e.g., blowfly, flesh fly), the screwworm lays its larvae in living flesh (human or animal). Fortunately, the screwworm has been eradicated in the United States. Human and animal screw-worm infections occur in Central and South America (Fig. 5.20).

Another fly that can infect living human skin is the human botfly (Dermatobia hominis). The botfly larvae attach to the skin, sometimes using a passive vector, such as a mosquito, to arrive at a convenient entry point, at which they burrow downwards. The botfly is found in Central and South America.

When the larvae of flies are accidentally ingested, they can be found anywhere in the intestinal tract. If the larvae are actively growing, then the condition is referred to as intestinal myiasis. If the larvae are simply passing through, without growth, then the condition is called intestinal pseudomyiasis. Both conditions are rare [22].

```
Hexapoda
  Insecta
    Siphonaptera
      Hectopsyllidae
        Tunga (genus)
```

Fleas (Class Siphonaptera) are another family of insects that draws blood. Fleas carry a variety of infectious organisms. Fleas are vectors for bacteria: Yersinia pestis (plague), Rickettsia typhi (endemic typhus), Rickettsia felis (endemic typhus), Bartonella henselae (bacillary angiomatosis, infectious peliosis hepatis, cat-scratch disease, bartonellosis). Fleas are an intermediate host for hymenolepis diminuta, a rare cause of human tapeworm disease (hymenolepiasis). Despite early speculation, fleas are not carriers of the HIV (AIDS) virus.

With one exception, fleas do not live on or in humans, and are not the cause of infectious diseases in humans. The one exception is tungiasis, a skin disease

FIG. 5.20 Cochliomyia hominivorax larva (screwworm larva). The mandibles near the mouth of the larva grasp living flesh. Hundreds of larvae may live within a single wound. *(Source, Wikipedia, from a public domain image provided by the US Centers for Disease Control and Prevention.)*

caused by Tunga penetrans (alternately known as chigoe, jigger, and nigua), a very small flea. Female fleas burrow into the skin, producing intense localized inflammation and pain. The skin lesion is characterized by a black dot surrounded by edematous and erythematous (red) skin. The disease occurs in Africa, South America and the Caribbean, where the infection rate can be very high (about 50% of the population in endemic areas such as Nigeria, Brazil, and Trinidad).

Infectious Genera

Cimex

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Paraneoptera: Hemiptera: Prosorrhyncha: Heteroptera: Euheteroptera: Neoheteroptera: Panheteroptera: Cimicomorpha: Cimicoidea: Cimicidae: Cimex
- **Infection**. Cimex lectularius, bedbug (bedbug bites)

Siphonaptera (fleas)

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Siphonaptera
- **Infection**. Siphonaptera species (flea bites)

Pediculus

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Paraneoptera: Psocodea: Phthiraptera: Anoplura (sucking lice): Pediculidae: Pediculus
- **Infection**. Pediculus humanus (capitus), head louse (pediculosis)
- **Infection**. Pediculus humanus (corporis), body louse (pediculosis)

Phthirus

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Paraneoptera: Psocodea: Phthiraptera: Anoplura (sucking lice): Pthiridae: Pthirus
- **Infection**. Phthirus pubis (crab louse or pubic louse)

Cochliomyia

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Diptera: Brachycera: Muscomorpha: Eremoneura: Cyclorrhapha: Schizophora: Calyptratae: Oestroidea: Calliphoridae (blow flies): Chrysomyinae: Chrysomyini: Cochliomyia
- **Infection**. Cochliomyia hominivorax (screw-worm myiasis)

Calliphoridae (blow flies)

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Diptera: Brachycera: Muscomorpha: Eremoneura: Cyclorrhapha: Schizophora: Calyptratae: Oestroidea: Calliphoridae
- **Infection**. Calliphoridae species (blowfly myiasis)

Sarcophagidae (flesh flies)

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Diptera: Brachycera: Muscomorpha: Eremoneura: Cyclorrhapha: Schizophora: Calyptratae: Oestroidea: Sarcophagidae
- **Infection**. Sarcophagidae species (flesh fly myiasis)

Dermatobia

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Diptera: Brachycera: Muscomorpha: Eremoneura: Cyclorrhapha: Schizophora: Calyptratae: Oestroidea: Oestridae: Cuterebrinae: Dermatobia
- **Infection**. Dermatobia hominis (human botfly myiasis)

Tunga

- **Lineage**. Insecta: Dicondylia: Pterygota: Neoptera: Holometabola: Siphonaptera: Pulicomorpha: Pulicoidea: Hectopsyllidae: Tunga
- **Infection**. Tunga penetrans (tungiasis, nigua)

Section 5.9 Crustacea

Probably a crab would be filled with a sense of personal outrage if it could hear us class it without ado or apology as a crustacean, and thus dispose of it. "I am no such thing," it would say; "I am MYSELF, MYSELF alone."

William James

```
Eukaryota
  Bikonta (2-flagella)
    Excavata
      Metamonada
      Discoba
        Euglenozoa
        Percolozoa
    Archaeplastida
    Chromalveolata
      Alveolata
        Apicomplexa
        Ciliophora (ciliates)
      Heterokonta
  Unikonta (1-flagellum)
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
        Eumetazoa
          Bilateria
            Deuterostomia
              Chordata
                Craniata
            Protostomia
              Ecdysozoa
                Nematoda
                Arthropoda
                  Chelicerata
                  Hexapoda
                  Crustacea
                    Maxillopoda
                      Pentastomida
                        Linguatulidae
                          Linguatula (genus)
                        Porocephalidae
                          Armillifer (genus)
                          Porocephalus (genus)
```

```
            Platyzoa
              Platyhelminthes
              Acanthocephala
    Fungi
```

Class Arthropoda has three subclasses that contain organisms that are pathogenic to humans: Class Chelicerata, Class Hexapoda, and Class Crustacea. Class Crustacea includes many of the menu items that are commonly called shellfish: lobsters, crabs, shrimp. It also contains smaller organisms, such as copepods that are commonly found in plankton; and barnacles, which are found encrusted over ocean surfaces. Most crustaceans live in water, but some live on land. An example of the latter are organisms of Genus Armadillidium, variously known as woodlice, pill bugs, potato bug, and other misleading names that would wrongly suggest a non-Crustacean identity. Crustaceans are characterized by a body plan composed of fused segments covered by a single carapace and protected by a hard exoskeleton. Moulting occurs in all growing crustaceans.

Perhaps the most distinctive feature of Crustaceans is the multiple larval stages that precede the emergence of the adult form. These larval forms vastly amplify the complexity of carcinology (the study of crustaceans). In past years, many larval forms of crustaceans have been incorrectly identified as separate animal species.

```
Crustacea
  Maxillopoda
    Pentastomida
      Linguatulidae
        Linguatula (genus)
      Porocephalidae
        Armillifer (genus)
        Porocephalus (genus)
```

Only one subclass of Class Crustacea contains organisms that are infectious to humans: Class Pentastomida, the so-called tongue worms. This class contains three genera that cause different forms of the same disease: pentastomiasis [23].

The pentastome adult has the appearance of a worm. Unlike worms of Classes Platyhelminthes or Nematoda, tongue worms display the typical crustacean body type: fused segments all covered by a hard, chitinous carapace. Tongue worms have five anterior appendages, leading to the class name "Pentastomida."

The primary hosts of tongue worms (i.e., the host that carries the adult form of the organism) are vertebrate animals. Typically, the adult tongue worm parasitizes the respiratory tract of these animals. Humans are the intermediate host, carrying only the larval forms. Transmitted by food, pentastomid eggs hatch in the intestines, and the larvae invade tissues to encyst anywhere in the body. The nasopharynx is the most likely place to find the cysts.

Typically, the cysts are walled off by an inflammatory reaction, and eventually become calcified. With the exception of the obstructive effect produced by the

presence of a cyst, infections are often asymptomatic. In areas where pentastomiasis is common, the cysts are often discovered as incidental findings, at autopsy.

Pentastomiasis occurs most often in Africa, Malaysia, and the Middle East. Less often, the disease may occur in China or South America. In the Middle East, pentastomiasis is known as Halzoun.

Three genera of Class Pentastomida are involved: Linguatula, Armillifer, and Porocephalus. One of the more confusing terms associated pentastomiasis is "porocephaliasis," named for a pentastome genus, Porocephalus. The genus "Porocephalus" and the infection "porocephaliasis" should not be confused with "porocephaly," a rare developmental disorder in which cysts or cavities are found in the brains of infants.

Infectious Genera

Linguatula

- **Lineage**. Eumetazoa: Bilateria: Protostomia: Ecdysozoa: Panarthropoda: Arthropoda: Mandibulata: Pancrustacea: Crustacea: Oligostraca: Ichthyostraca: Pentastomida: Porocephalida: Linguatulidae: Linguatula
- **Infection**. Linguatula serrata (pentastomiasis, marrara, halzoun Syndrome, tongue worm disease, linguatulosis)

Armillifer

- **Lineage**. Eumetazoa: Bilateria: Protostomia: Ecdysozoa: Panarthropoda: Arthropoda: Mandibulata: Pancrustacea: Crustacea: Oligostraca: Ichthyostraca: Pentastomida: Porocephalida: Armilliferidae: Armillifer
- **Infection**. Armillifer armillatus (pentastomiasis, porocephaliasis)
- **Infection**. Armillifer grandis (pentastomiasis, porocephaliasis)
- **Infection**. Armillifer moniliformis (pentastomiasis, porocephaliasis)

Porocephalus

- **Lineage**. Eumetazoa: Bilateria: Protostomia: Ecdysozoa: Panarthropoda: Arthropoda: Mandibulata: Pancrustacea: Crustacea: Oligostraca: Ichthyostraca: Pentastomida: Porocephalida: Porocephalidae: Porocephalus
- **Infection**. Porocephalus crotali (pentastomiasis, porocephaliasis)

Glossary

Exotic diseases The word "exotic" brings to mind all things strange and exciting. For clinical microbiologists, "exotic" refers to infectious diseases that occur commonly in a specific locality; and which suddenly emerge as a newly encountered disease in a distant geographic location. In the United States, numerous exotic diseases have been introduced in the past few decades due, primarily, to two influences: global warming and global travel. These exotic infections include Zika, Ebola, Dengue, Chikungunya, SARS, West Nile fever, Yellow fever, Mayaro fever, Monkeypox, HIV/AIDS, Lassa fever, Malaria, Leishmaniasis, Chagas disease, Cyclospora, and Cholera [24]. We can expect to encounter additional exotic diseases as global warming expands the geographic range of tropical disease vectors.

Today, the preferred transportation venue for exotic infections is the jet airliner. An outbreak that may have taken years to cross the oceans, in the recent past, may now spread around the globe in a matter of days. Can it be a coincidence "airline" is synonymous with "carrier"?

Aside from causing disease in humans, exotic infections may ravage animals and plants. The much-dreaded New World screwworm (Cochliomyia hominivorax), whose maggots eat the living flesh of the animals they infect, has, after a long absence, returned to the United States Likewise, the medfly, or Mediterranean fruit fly (Ceratitis capitata) is a recurring threat to US crops.

Hemorrhagic fevers Hemorrhagic fevers are produced by an agent (almost always a virus) that elicits vasoactive mediators (e.g., kinins, histamine); thus increasing endothelial permeability (i.e., producing leaky vessels) and leading to hypovolemic shock (i.e., shock due to lack of blood in vessels). Typically, the vasoactive mediators are produced as a viral cytopathic effect of infected reticuloendothelial cells (e.g., macrophages). The pathogenesis varies somewhat from virus to virus and patient to patient. The presence or absence of liver involvement, CNS involvement, and DIC (disseminated intravascular coagulation) will alter the clinical course of disease. The hemorrhagic fever viruses come from Group IV or Group V, the single-stranded RNA viruses. They all have a nonhuman reservoir that is restricted to a specific geographic location, but can be spread by between infected humans. The exception is Dengue hemorrhagic fever, which seems to never have a direct human-human spread (i.e., always infects via a viral vector). Vaccines have been developed for yellow fever and for Argentine hemorrhagic fever (Junin virus). Once clinical symptoms emerge, the primary goal of therapy for the viral hemorrhagic fevers is to provide supportive care, and to take precautions against the spread of infection. Rickettsia bacteria can produce nonviral hemorrhagic fevers. Scrub typhus, caused by Orientia tsutsugamushi, and transmitted by trombiculid mites, is another example of a nonviral fever. Here is a list of hemorrhagic fever viruses.

```
Arenaviridae (Group V)
  Lassa fever
  Argentine hemorrhagic fevers (Junin virus)
  Bolivian hemorrhagic fevers (Machupo virus)
  Brazilian hemorrhagic fevers (Sabia virus)
  Venezuelan hemorrhagic fevers (Guanarito virus)
Bunyaviridae (Group V)
  Hantaviruses that cause hemorrhagic fever with renal syndrome
(HFRS)
  Nairovirus that causes Crimean-Congo hemorrhagic fever (CCHF)
  Phlebovirus that causes Rift Valley fever (RVF)
Filoviridae (Group V)
  Ebola hemorrhagic fever
  Marburg hemorrhagic fever
Flaviviridae (Group IV)
  Dengue fever (severe form)
  Yellow fever
  Tick-borne encephalitis group that cause Omsk hemorrhagic
  fever and Kyasanur Forest disease
Arenaviridae (Group V)
  Lujo virus
```

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Chapter 6

Fungi

Section 6.1 Overview of Class Fungi

Definition of a lexicographer: "harmless drudge."

Samuel Johnson

```
Eukaryota
  Bikonta, 2-flagella
  Unikonta, 1-flagellum
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
        Zygomycota
        Dikarya
          Basidiomycota
          Ascomycota
        Microsporidia
```

In the past decade, Class Fungi has become the most intellectually frustrating branch of clinical microbiology. There are many reasons why mycology (the study of fungi) has become so very difficult, but two reasons seem to dominate.

–1. Number of offending organisms. Approximately 54 fungi account for the vast majority of fungal infections, but the actual number of fungi that are pathogenic to humans is much higher. To provide some idea of the ubiquitous nature of fungi, it is estimated that, on average, humans inhale about 40 conidia (asexual fungal propagative spores) each hour. Most of these organisms are nonpathogenic under normal circumstances. However, in the case of immune-compromised patients, or in the case of patients who provide a specific opportunity for ambient fungi to attach and grow within a body (e.g., an indwelling vascular line), an otherwise harmless fungus may produce a life-threatening illness. As the number of immune-compromised patients increases, due to transplants, AIDS, cancer treatment, long-term steroid use; and with the proliferation of medical devices that provide potential entry points for fungi, the number of newly recognized fungal pathogens will increase. It is estimated that there are about 20 new fungal diseases reported each year [1]. If the

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number of diseases caused by other types of organisms (i.e., bacteria, protists, animals, viruses, and prions) remains steady, then it will not be long before the number of different fungal diseases exceeds the number of different diseases produced by all other organisms, combined.

–2. Increased sensitivity of diagnostic tests. It is now possible to identify heretofore undiagnosed cases of pathogenic species [2]. In the past, when clinical mycology laboratories had fewer sophisticated tests than available today, it was easier to lump diseases under a commonly encountered species or a genus. For example, Aspergillus fumigatus is a common cause of severe pulmonary infections in immune-compromised patients. With advanced typing techniques, an additional 34 species of Aspergillus have been isolated from clinical specimens [1].

It is ironic, but for much of human history, the fungi were misclassified as plants. Class Fungi is now recognized as a subclass of Class Opisthokonta. As a subclass of Class Opisthokonta, the fungi are much more closely related to humans and other animals than to the plants that they superficially resemble.

The most salient clue to the phylogenetic origin of fungi was the ancestral posterior single undulipodium, common to all opisthokonts. As previously discussed, the only fungal class with a posterior undulipodium is Class Chytrid, considered to be the most primitive fungal class [3]. The chytrids are aquatic, and use their posterior undulipodium to propel themselves through water. All the other fungi have adapted to life in soil, where the posterior undulipodium serves no essential purpose. It is presumed that the soil-based fungi lost their single posterior undulipodia through evolution. Nonetheless, phylogenetic lineage is determined by ancestry, and the undulipodium of chytrids demonstrates that fungi had a posterior undulipodium in their past. None of the chytrids infect humans, but several species are currently ravaging the amphibian population. The chytrid Batrachochytrium dendrobatidis is capable of infecting thousands of different amphibian species and threatens many of these species with extinction.

Aside from the ancestral single posterior undulipodium, which establishes a close relationship between fungi and animals, there is also the presence of chitin. Chitin is a long-chain polymer built from units of N-acetylglucosamine, and found in the cell walls of every fungus. It is analogous to cellulose, which is built from units of glucose. Importantly, chitin is never found in plants, and cellulose is never found in fungi. Aside from its presence in fungi, chitin is found in some member of Class Protoctista and in some members of Class Animalia (particularly arthropods). Chitin is the primary constituent of the exoskeleton of insects. The important structural role of chitin in fungi and animals should have been a clue to the close relationship between these two classes. It happens that chitin was not discovered

until 1930 (by Albert Hoffmann); well before that time, Class Fungi had been incorrectly assigned to the plant kingdom.

Lastly, fungi and animals are heterotrophic, acquiring energy by metabolizing organic compounds obtained from the environment. Plants, unlike animals and fungi, are phototropic autotrophs, producing organic compounds from light, water, and carbon dioxide. Fungi, unlike plants, lack chloroplasts, the organelle wherein oxygenic photosynthesis occurs [Glossary Chloroplast evolution, Heterotrophic].

Interactions between fungi and humans vary, following one or more of the following scenarios, listed in order of increasing clinical consequence:

–1. The fungus grows in the external environment, usually in soil or on plants, never interacting in any way with humans.

–2. Spores and asexual reproductive forms are emitted into the air. In warm and tropical locations, fungal elements are the predominant particulate matter found in air samples. Humans are exposed constantly to a wide variety of fungi just by breathing (airborne conidia), by ingestion (fungi growing on the plants we eat), and by direct skin contact with fungal colonies in soil and airborne organisms; without consequence.

–3. After exposure, fungi dwell in or on the human body for a limited time, eventually sloughing off, or dying; without consequence.

–4. After exposure, fungi may transiently colonize a mucosal surface, such as the oral cavity, the nose, the gastrointestinal tract, the respiratory tract, or the skin. Once on a mucosal surface, an acute allergic response may occur (e.g., sneezing). After a time, the colony fails to thrive due to an inhospitable environment (e.g., insufficient food, poor ionic milieu, effective host immune response).

–5. After exposure, fungi permanently colonize the mucosal surface, with no immediate clinical effect. Candida species commonly colonize the mouth and the vagina. Aspergillus species may colonize the respiratory surfaces (e.g., bronchi). In many cases, we simply carry fungal colonies as commensals (organisms that live within us, without causing disease).

–6. Colonies persist, but the host reacts with an acute or chronic immune response. Chronic allergic aspergillosis of the bronchi is a good example. The patient may have a chronic cough. Microscopic examination of bronchial mucosa may reveal some inflammation, the presence of eosinophils, and the occasional hypha. Sometimes the host response is granulomatous, producing small nodules lining the bronchi, containing histiocytes and lymphocytes. A truce between the fungal colony and the host response is sometimes attained, in which the fungus colonies never leave, the inflammation never regresses, but the fungus does not invade into the underlying mucosa.

–7. Fungi invade through the mucosa into the submucosa and underlying tissue. These locally invasive infections often manifest as a fungal ball, consisting of varying amounts of inflammatory tissue, necrosis, and fungal elements.

–8. Fungal elements invade into lymphatics, traveling with the lymph fluid, and producing regional invasive fungal disease along the route of lymphatic drainage. The prototypical example of this process is found in infections with Sporothrix schenckii, which typically gains entrance to the skin, from the soil, through abrasions. Infection yields multiple skin papules, emanating from the point of primary infection (usually the hand or the foot), and following line of lymphatic drainage.

–9. Fungal elements invade into blood vessels.

–10. Fungal elements become a blood constituent (i.e., fungemia) and disseminate throughout the body.

–11. Fungal elements spread throughout the body to produce invasive fungal infections in multiple organs.

The most perplexing aspect of fungal infections is that a single fungus may manifest itself by any and all of these biologic options (e.g., Aspergillus and Malassezia species, vida infra). In general, the more immune-competent the individual, the less likely that a fungal infection will become clinically significant or life threatening.

Readers should be aware that pathologists have developed a wide variety of techniques to identify fungi based on their morphologic features in tissue biopsies (e.g., the presence or absence of pigment, the presence or absence of hyphal septation, the presence or absence of hyphal branching, the angulation of branches, hyphal thickness, the presence or absence of yeast forms, whether yeast forms grow by budding, the morphologic appearance of buds, etc.). Pathologists also use anatomic information (e.g., whether the infection is superficial or deep, local or systemic, the anatomic site of the lesion), and clinical history (whether the infection occurs in an immunodeficient patient, a diabetic, a child, a gardener, etc.).

Clinical mycologists find it useful to determine whether a fungus is thermally dimorphic. Thermally dimorphic fungi grow in the laboratory as yeasts (round organisms) or as hyphae, depending on temperature. For example, Penicillium marneffei grows as hyphae at room temperature and as yeasts at body temperature. Penicillium marneffei is seldom pathogenic, except in immune-compromised individuals, but most of the highly pathogenic fungi capable of causing disease in healthy individuals are thermally dimorphic. These organisms are: Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces dermatitidis, and Histoplasma capsulatum. Candia albicans and Sporothrix schenckii are also thermally dimorphic and infect immune-competent individuals, but typically cause mild, localized disease. All of these dimorphic fungi grow in human tissue as yeast forms, and all happen to be members of Class Ascomycota. Here's the problem; thermal dimorphism

is a property that is sometimes clinically useful, but sometimes not, as it includes both harmful and harmless fungi. Thermal dimorphism is applicable only to fungi that can be cultured (e.g., Lacazia loboi, vida infra, cannot be cultured and dimorphism cannot be determined). Thermal dimorphism is sometimes taxonomically specific and sometimes not; most of the pathogenic dimorphic fungi belong to Class Ascomycota, but some do not (e.g., Ustilago maydis, the dimorphic fungus that produces smut in corn, is a member of Class Basidiomycota). Thermal dimorphism has value to clinical mycologists, who are well versed in the limitations of its clinical utility. For the rest of us, nontaxonomic approaches to medical mycology are devices best left to the experts.

With the aforementioned caveats, there are a few general properties of the fungi that most students will find useful.

–1. Fungi propagate by ejecting reproductive elements into the air. Humans become infected when they inhale, ingest, or come into surface contact with fungi that contaminate air, soil, and water. Animal vectors are not required, and students need not memorize long lists of fungal vectors. Animals can, however, serve as reservoirs for fungi (e.g., Microsporidia). If a fungus is growing in your environment, the overwhelming likelihood is that you are constantly exposed to numerous potentially infective fungal elements.

–2. Most pathogenic fungi are globally ubiquitous, or they reside in the tropics. Thus, we seldom need to memorize their geographic distribution. The few exceptions (e.g., Histoplasma, Coccidioides, and Paracoccidioides) are mnemonically tolerable.

–3. Very few fungal diseases are contagious from person to person, though there are exceptions (e.g., tinea infections).

–4. Fungal colonization that does not result in disease is quite common. Pneumocystis, Aspergillus, and Cryptococcus are just a few examples of potentially life-threatening infections that are found to inhabit the lungs of a significant percentage of healthy persons. When colonized individuals become immunosuppressed, endogenized fungi may emerge as serious pathogens.

–5. Though there are over a million fungal species, and hundreds of potential fungal pathogens, the vast majority of human fungal diseases can be accounted for by a few dozen genera, falling into four classes: Class Zygomycota, Class Basidiomycota, Class Ascomycota, and Class Microsporidia. By far, Class Ascomycota contains the majority of the pathogenic fungal organisms, with 20 infectious genera. Readers are highly encouraged to memorize the fungi that fall into the classes containing the fewest infectious organisms (i.e., Zygomycota, Basidiomycota, and Microsporidia); all the other fungal pathogens belong to Class Ascomycota.

–6. Most fungal diseases do not occur in immune-competent individuals. Of the hundreds of fungal infections that can occur in humans, only a

dozen or so produce disease in healthy persons. With few exceptions (e.g., Cryptococcus gattii, Class Basidiomycota), the clinically serious systemic mycoses that regularly occur in otherwise healthy individuals belong to Class Ascomycota.

Section 6.2 Zoopagomycota and Mucoromycota (formerly Zygomycota)

You can't teach an old dogma new tricks.

Dorothy Parker

```
Eukaryota
  Bikonta, 2-flagella
  Unikonta, 1-flagellum
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
        Zygomycota
          Mucoromycota
            Mucormycotina
              Mucorales
                Mucoraceae
                  Rhizopus (genus)
                  Mucor (genus)
                  Absidia (genus)
                Syncephalastraceae
                  Syncephalastrum (genus)
          Zoopagomycota
            Entomophthoramycotina
              Entomophthorales
                Basidiobolaceae
                  Basidiobolus (genus)
                Ancylistaceae
                  Conidiobolus (genus)
        Dikarya
          Basidiomycota
            Agaricomycotina
          Ascomycota
            Pezizomycotina
            Saccharomycotina
            Taphrinomycotina
        Microsporidia
```

Currently, there are eight generally recognized major classes of fungi [4]. Of these eight classes, there are four classes currently known to contain pathogenic organisms: Class Zygomycota, Class Basidiomycota, Class Ascomycota, and

Class Microsporidia. Members of Class Zygomycota have recently been divided among two new classes: Class Mucoromycota and Class Zoopagomycota. In this discussion, we will retain Class Zygomycota as the parent class of Mucoromycota and Zoopagomycota, though the newest classifications dispense with Class Zygomycota entirely.

Like all the major divisions of fungi, Class Zygomycota is characterized by its sexually reproductive form. In the zygomycotes, the sexual form is the zygospore. Like so much of clinical mycology, the defining morphologic features of the zygomycotes are never observed in clinical specimens. In tissues, these organisms are present as hyphal forms, without a yeast phase. In the laboratory culture dish, they are present as hyphal colonies with asexual reproductive forms (sporangia containing spores, and free spores). The identification of species is typically made by expert evaluation of the available structures: hyphae and sporangia. In many instances, members of Class Zygomycota can be distinguished from members of the other major classes of fungi (i.e., ascomycotes and basidiomycotes) by three features: (1) nonseptate hyphae, (2) wide hyphae with thick walls, and (3) absence of yeast phase. Nonseptation of hyphae refers to a type of hyphal growth wherein walls (i.e., septations), do not separate individual hyphal cells, and in which multiple nuclei float in the filamentous hyphae (i.e., coenocytic growth).

Most of the pathogenic zygomycotes are noncommensal opportunists. They grow in soil, water, or air, on plants or on dung. Humans are constantly being exposed to their infective spores, by inhalation or by ingestion. Virtually all infections occur in patients who provide these fungi with a physiologic opportunity for growth (e.g., malnutrition, diabetes, advanced cancer, immunodeficiency, or an infection portal such as an indwelling catheter or an intravenous line).

```
Zygomycota
  Mucoromycota
    Mucormycotina
      Mucorales
        Mucoraceae
          Rhizopus (genus)
          Mucor (genus)
          Absidia (genus)
        Syncephalastraceae
          Syncephalastrum (genus)
  Zoopagomycota
    Entomophthoramycotina
      Entomophthorales
        Basidiobolaceae
          Basidiobolus (genus)
        Ancylistaceae
          Conidiobolus (genus)
```

The pathogenic members of Class Zygomycota belong to one of the two subclasses: Class Mucorales or Class Entomophthoramycotina. Infection with any zygomycote is known as zygomycosis. When the infectious agent is known to be a member of Class Mucorales, the disease is more specifically

known as mucormycosis. Class Mucorales account for the bulk of infections caused by zygomycotes. Regardless of the mucorales species, the clinical infections are similar. Common primary sites of infection are lungs, gastrointestinal tract, kidneys, and skin. Sinus infections, spreading to the nasopharynx, eyes, and brain, seem to have a particular affinity for diabetic individuals. Primary infections tend to be invasive, and may lead to disseminated disease. Rare infectious genera in Class Mucorales, aside from those listed here, have recently been isolated: Cokeromyces, Saksenaea, Apophysomyces, and Chlamydoabsidia [1] (Fig. 6.1).

Infections caused by species of Class Zoopagomycota produce somewhat different clinical picture than that of Class Mucoromycota. The zoopagomycoses are most often primary skin infections, and can occur in immunecompetent hosts. Infections caused by Genus Basidiobolus often arise on the trunk and thighs. Infections from members of Genus Conidiobolus typically arise on the nose and face [5]

Infectious Genera

Rhizopus

- **Lineage**. Fungi: Incertae sedis (problematic class): Mucoromycota: Mucoromycotina: Mucoromycetes: Mucorales: Mucorineae: Rhizopodaceae: Rhizopus
- **Infection**. Rhizopus oryzae (mucormycosis, zygomycosis)
- **Infection**. Rhizomucor pusillus

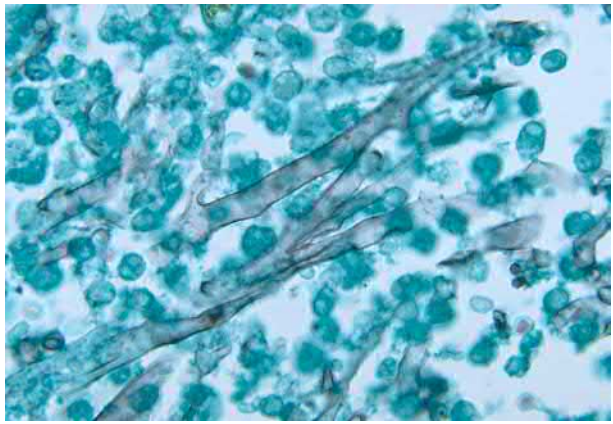

FIG. 6.1 Mucor pusillus infection of heart valve. Notice wide fungal hyphae, with few or no septations along their lengths. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and produced by Dr. Libero Ajello.)*

Mucor

- **Lineage**. Fungi: Incertae sedis (problematic class): Mucoromycota: Mucoromycotina: Mucoromycetes: Mucorales: Mucorineae: Mucoraceae: Mucor
- **Infection**. Mucor indicus (mucormycosis, zygomycosis, phycomycosis)
- **Infection**. Mucor racemosus (mucormycosis, allergic skin reaction)

Rhizomucor

- **Lineage**. Fungi: Fungi incertae sedis: Mucoromycota: Mucoromycotina: Mucoromycetes: Mucorales: Lichtheimiaceae: Rhizomucor
- **Infection**. Rhizomucor pusillus, formerly mucor pusillus (rare cases of zygomycosis, typically as nosocomial infections in immune-compromised individuals)

Absidia

- **Lineage**. Fungi: Incertae sedis (problematic class): Mucoromycota: Mucoromycotina: Mucoromycetes: Mucorales: Cunninghamellaceae: Absidia
- **Infection**. Absidia corymbifera (mucormycosis, zygomycosis)

Syncephalastrum

- **Lineage**. Fungi: Incertae sedis (problematic class): Mucoromycota: Mucoromycotina: Mucoromycetes: Mucorales: Syncephalastraceae: Syncephalastrum
- **Infection**. Syncephalastrum racemosum (mucormycosis, zygomycosis, nail infection)

Basidiobolus

- **Lineage**. Fungi: Incertae sedis (problematic class): Zoopagomycota: Entomophthoromycotina: Basidiobolomycetes: Basidiobolales: Basidiobolaceae: Basidiobolus
- **Infection**. Basidiobolus ranarum (basidiobolomycosis, zygomycosis, entomophthoramycosis)

Conidiobolus

- **Lineage**. Fungi: Incertae sedis (problematic class): Zoopagomycota: Entomophthoromycotina: Entomophthoromycetes: Entomophthorales: Ancylistaceae: Conidiobolus
- **Infection**. Conidiobolus coronatus (conidiobolomycosis, zygomycosis, entomophthoramycosis)
- **Infection**. Conidiobolus incongruus (conidiobolomycosis, zygomycosis, entomophthoramycosis)

Section 6.3 Basidiomycota

In the particular is contained the universal.

James Joyce

```
Eukaryota
  Bikonta, 2-flagella
  Unikonta, 1-flagellum
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
        Microsporidia
        Zygomycota
          Entomophthoromycotina
          Mucoromycotina
        Dikarya
          Ascomycota
          Basidiomycota
            Agaricomycotina
              Tremellomycetes
                Trichosporonales
                  Trichosporonaceae
                    Trichosporon (genus)
                Tremellales
                  Tremellaceae
                    Cryptococcus (genus)
            Ustilaginomycotina
              Exobasidiomycetes
                Malasseziales:
                  Malasseziaceae
                    Malassezia (genus)
```

Pathogenic subclasses of organisms belonging to Class Fungi can be divided into those subclasses that form dikaryons (i.e., Class Basidiomycota and Class Ascomycota), and those subclasses that do not form dikaryons (Class Zygomycota and Class Microsporidia). Dikaryons occur exclusively in the ascomycotes and basidiomycotes (i.e., in no other earthly organisms).

A dikaryon is a cell with a double nucleus, composed of two haploid nuclei that came to occupy the same cell, through conjugation, and without fusion of the two nuclei (i.e., two cells fused, but the nuclei within the cells do not fuse). A dikaryon is a dividing cell, wherein both nuclei divide synchronously, and both nuclei are metabolically active. The somatic cells of hyphae are haploid (from the Greek, "haplous," meaning single), having a complete set of unpaired chromosomes. Likewise, the gametes of fungi are haploid. Dikaryons can be formed by the fusion of haploid cells from two physically adjacent compatible

mycelia (i.e., hyphae), from the sexual fusion of two gametes, or from the fusion of a gamete with a haploid somatic cell. The dikaryotic state may be very short, or relatively long, but it eventually leads to a fused, diploid state. Diploid cells can yield, through meiosis, two haploid spore cells.

The two subclasses of Class Dikarya (Class Basidiomycota and Class Ascomycota) each have their own characteristic sexual and asexual bodies that produce cells that leave the organism and enter the environment, often as airborne spores. In Class Basidiomycota organisms reproduce sexually using a club-shaped structure, called a basidium, that produces basidiospores. They can also reproduce asexually by producing hardened spores (conidia) from specialized cell structures (conidiophores), extending from hyphae. The food we eat, the water we drink, and the air we breathe carry the sexual spores (basidiospores) of the basidiomycotes, and the asexual spores (conidia) of ascomycotes and basidiomycotes (Fig. 6.2).

```
Agaricomycotina
  Tremellomycetes
    Trichosporonales
      Trichosporonaceae
        Trichosporon (genus)
    Tremellales
      Tremellaceae
        Cryptococcus (genus)
  Ustilaginomycotina
    Exobasidiomycetes
      Malasseziales:
        Malasseziaceae
          Malassezia (genus)
```

Three genera account for the human infections caused by members of Class Basidiomycota: Genus Trichosporon, Genus Cryptococcus, and Genus Malassezia.

At least two species of Genus Trichosporon (Trichosporon inkin and Trichosporon ovoides) may cause white piedra, a relatively rare infection of hair shafts, in which small white, somewhat gelatinous nodules are found growing on hairs. In the United States, most cases occur in humid, warm climates. Trichosporon species have been suspected of causing a variety of superficial infections of skin and nails [6].

Cryptococcus species grow in tissues exclusively as yeasts (i.e., hyphal mycelia are never observed). Cryptococcus neoformans may disseminate to many different organisms, but is a prominent cause of cryptococcal meningitis or meningoencephalitis in immunodeficient patients. Occasional cases of cryptococcal meningitis are caused by Cryptococcus laurentii and Cryptococcus albidus (Fig. 6.3).

Cryptococcus gattii produces highly virulent infections that involve the lungs, the meninges, or the brain. Until recently, it was confined to tropical

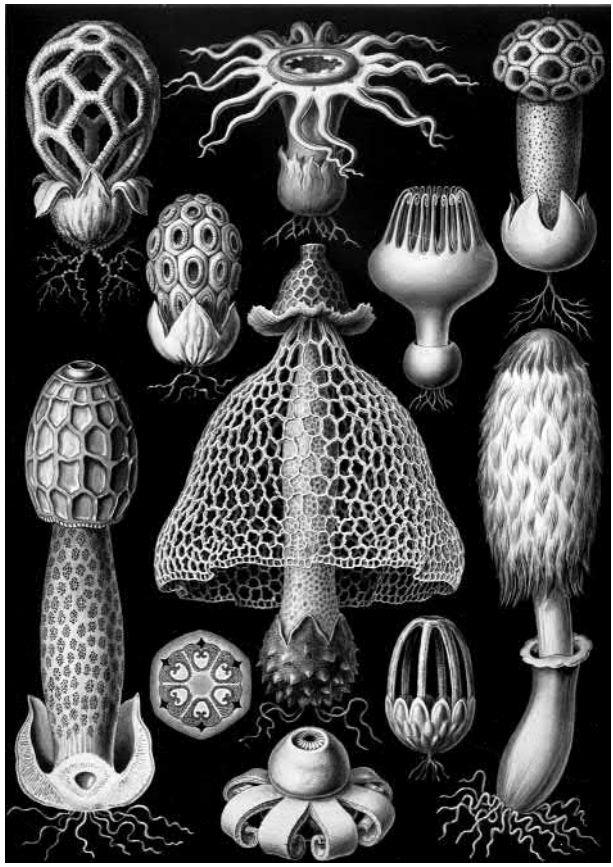

FIG. 6.2 Drawings of various sporocarps (more specifically, basidiocarp) of basidiomycotic fungi. The sporocarp, also known as the fruiting body, is a multicellular structure on which sexual spore production proceeds. A deep understanding of the morphology of basidiomycotic reproduction is not particularly useful for pathologists, insofar as the tissue growth of virtually all infectious fungi is vegetative (i.e., restricted to dividing yeast or mycelia-forming hyphae, and without the production of conidia, basidiospores, or sporocarps, that might otherwise assist in the identification of the organism). *(Source, Wikipedia, from a drawing by Ernst Haeckel published in "Kunstformen der Natur", 1904 [Glossary Ascocarp, Basidiocarp, Mushroom, Sporocarp].)*

or subtropical climates, and was particularly frequent in Papua New Guinea and Northern Australia. In recent years, a few hundred cases have occurred in the Northwest United States and in Vancouver. Unlike Cryptococcus neoformans, which causes disease almost exclusively in immune-compromised individuals, Cryptococcus gattii occurs in immune-competent individuals [Glossary Virulence factor].

Malassezia species produce a type of ringworm called tinea versicolor (alternately known as pityriasis versicolor), and a folliculitis called pityrosporum

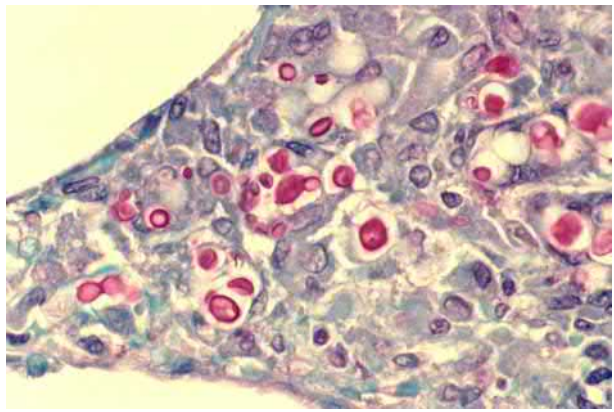

FIG. 6.3 Cryptococcosis, lung. Large, round organisms of Cryptococcus neoformans, with deeply stained cell walls indicate thick polysaccharide capsules (mucicarmine stain). The synthesis of protective capsules is considered the chief mechanism whereby cryptococcal organisms manage to resist host defenses and attain dormancy within tissues *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and prepared by Dr. Dr. Edwin P. Ewing, Jr.)*

folliculitis (Malassezia was formerly known as Pityrosporum). Malassezia species are part of normal skin flora. Active infections, when they occur, arise from endogenous skin organisms, not through contagion with infected humans. Tinea versicolor occurs in up to 8% of the general population, most frequently in adolescents. It consists of a round itchy macular rash with a sharply demarcated circumference; hence, falling into the broad clinical category of the so-called ringworm fungal infections. In biopsies of infected skin, yeast forms admixed with hyphae are seen, producing a histologic appearance likened to spaghetti and meatballs. Most cases of tinea versicolor are caused by Malassezia globosa. In pityrosporum folliculitis, organisms descend into hair follicles, producing inflammation, with papule formation.

The clinical term "ringworm" is irreconcilable with taxonomic nomenclature, and a few words of explanation are required. Ringworm is synonymous with dermatophytosis, skin infections caused by one of the several fungi that live in the top, keratin layer of the epidermis, producing round macules or plaques. Within Class Ascomycota, there is a subclass called Arthrodermataceae that contains the traditional dermatophytic genera: Epidermophyton, Microsporum, and Trichophyton. Nonetheless, additional fungal genera, outside Class Arthrodermataceae may produce ringworm infections: Hortaea (an ascomycote) and Malassezia (a basidiomycote).

Malassezia species are ubiquitous and grow, in yeast form, as commensals on normal keratinized skin. They require fatty acids, and are thus found in highest concentrations in sebum-rich areas, including the face. They have been found in a high percentage of cases of several common and mild skin disorders,

including dandruff, seborrheic dermatitis, and even hyperhidrosis. The pathogenic role of Malassezia species in these diseases is obscure.

As with most fungal infections, otherwise mild conditions can progress into life-threatening diseases in individuals who are malnourished or immunodeficient. Malassezia species have been involved in serious fungal infections arising in low-birth weight infants. Additional cases have occurred in adults who receive intravenous parental nutrition, presumably through the introduction of fungi via intra-catheter growth on lipid-rich alimentation fluids [7].

Infectious Genera

Trichosporon

- **Lineage**. Fungi: Dikarya: Basidiomycota: Agaricomycotina: Tremellomycetes: Trichosporonales: Trichosporonaceae: Trichosporon
- **Infection**. Trichosporon sp. (white piedra)

Cryptococcus

- **Lineage**. Fungi: Dikarya: Basidiomycota: Agaricomycotina: Tremellomycetes: Trichosporonales: Trichosporonaceae: Cryptococcus
- **Infection**. Cryptococcus neoformans (cryptococcal meningitis)
- **Infection**. Cryptococcus gattii (pulmonary cryptococcosis, basal meningitis, and cerebral cryptococcomas)

Malassezia, formerly Pityrosporum

- **Lineage**. Fungi: Dikarya: Basidiomycota: Ustilaginomycotina: Malasseziomycetes: Malasseziales: Malasseziaceae: Malassezia
- **Infection**. Malassezia globosa (tinea versicolor, pityrosporum folliculitis)
- **Infection**. Malassezia ovale, formerly Pityrosporum ovale (tinea versicolor, pityrosporum folliculitis)
- **Infection**. Malassezia furfur (fungemia in low birth weight neonates)
- **Infection**. Malassezia pachydermatis (fungemia in low birth weight neonates)

Section 6.4 Ascomycota

One does not discover new lands without consenting to lose sight of the shore for a very long time.

Andre Gide

```
Eukaryota
  Bikonta, 2-flagella
  Unikonta, 1-flagellum
    Amoebozoa
    Opisthokonta
```

```
Choanozoa
Animalia
Fungi
  Zygomycota
    Entomophthoromycotina
    Mucoromycotina
  Dikarya
    Basidiomycota
    Ascomycota
      Saccharomycotina
        Saccharomycetes
          Saccharomycetales
            Saccharomycetaceae
              Candida (genus)
      Taphrinomycotina
        Pneumocystidomycetes
          Pneumocystidales
            Pneumocystidaceae
              Pneumocystis (genus)
      Pezizomycotina
        Dothideomycetes
          Dothideales
            Dothioraceae
              Hortaea (genus)
          Capnodiales
            Piedraiaceae
              Piedraia (genus)
          Pleosporomycetidae
            Pleosporales
              Testudinaceae
                Neotestudina (genus)
        Sordariomycetes
          Microascales
            Microascaceae
              Scedosporium (genus)
          Hypocreales
            Nectriaceae
              Fusarium (genus)
          Sordariales
            Incertae sedis (problematic class)
              Madurella (genus)
        Eurotiomycetes
          Eurotiales
            Trichocomaceae
              Aspergillus (genus)
                Penicillium (genus)
          Herpotrichiellaceae
            Fonsecaea (genus)
              Cladophialophora (genus)
              Phialophora (genus)
          Onygenales
            Ajellomycetaceae
              Emmonsia (genus)
              Histoplasma (genus)
```

```
                Blastomyces [8] (genus)
                Paracoccidioides (genus)
              Arthrodermataceae (the dermatophytes)
                Epidermophyton (genus)
                Microsporum (genus)
                Trichophyton (genus)
              Onygenaceae
                Coccidioides (genus)
              Incertae sedis (problematic class)
                Lacazia (genus)
          Ophiostomatales
            Ophiostomataceae
              Sporothrix (genus)
    Microsporidia
```

There are four classes of fungi that contain pathogenic organisms: Class Zygomycota, Class Basidiomycota, Class Ascomycota, and Class Microsporidia. Class Ascomycota contains the greatest number of fungal organisms infectious in humans, and contains most of the fungi that regularly cause clinically life-threatening disease in otherwise healthy individuals. Class Ascomycota, along with Class Basidiomycota, comprise the dikaryotic fungi

All members of Class Ascomycota that reproduce sexually produce an ascus (from the Greek "askos," meaning sac) containing spores. Unfortunately for taxonomists, many members of Class Ascomycota simply do not reproduce sexually; hence, they do not produce the ascus that characterizes their taxonomic class. Taxonomists invented a temporary class of organisms known as the deuteromycotes (or imperfect fungi) to hold these apparently asexual species. Thanks to molecular analyses, many of these ascus-impaired species have been sorted into proper subclasses within Class Ascomycota. Currently, three major classes account for all of the pathogenic members of Class Ascomycota: Saccharomycotina, Taphrinomycotina, and Pezizomycotina. Class Saccharomycotina are yeasts; round, unicellular fungi that reproduce by budding. This class contains a single genus that is pathogenic in humans: Candida. Class Taphrinomycotina contains a single species that is pathogenic in humans: Pneumocystis jirovecii. All of the remaining Ascomycotes, and there are many, belong to Class Pezizomycotina (Fig. 6.4).

```
Ascomycota
  Saccharomycotina
    Saccharomycetes
      Saccharomycetales
        Saccharomycetaceae
          Candida (genus)
```

Candida, the sole pathogenic genus in Class Saccharomycotina, is a normal inhabitant of humans, and various species are found on the skin, respiratory tract, gut, and female genital tract of healthy individuals. An ecological balance exists between Candida species and various bacterial commensals. When this balance is disrupted by the use of antibiotics, overgrowth of Candida species may occur.

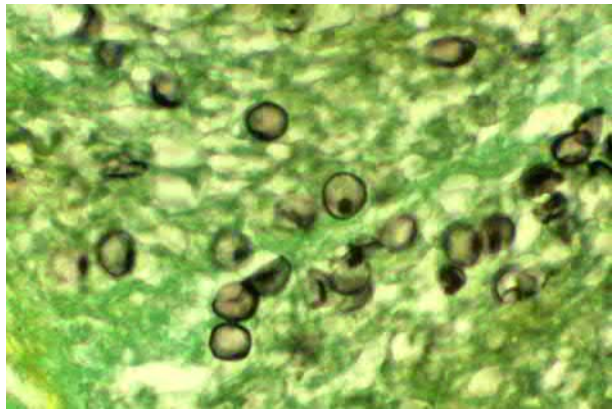

FIG. 6.4 Tissue section of showing the so-called cyst forms of Pneumocystis jirovecii organisms. With silver stain, the cyst walls are a solid black. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and prepared by Dr. Edwin P. Ewing, Jr.)*

In addition, as with virtually all of the pathogenic fungi, overt diseases may occur in immunodeficient individuals. Patients undergoing intense chemotherapy are at particular risk for life-threatening candidal infections.

The least worrisome of the candidiases are superficial infections confined to mucosal surfaces. These are common in the mouth and GI tract and are characterized by thick colonies of yeast that form a white surface crust. The so-called invasive candidiasis involves the penetration of organisms through the mucosa into deeper tissue layers, and the transition to invasiveness is often accompanied by a change in morphology from the yeast form to pseudohyphae and hyphae. The most serious stage of candidiasis involves growth in blood (candidemia) and dissemination to distant organs. Candida albicans is the most common pathogenic species, but there are many more known pathogenic types, including Candida auris, Candida dubliniensis, Candida glabrata, Candia parapsilosis, Candida rugosa, and Candida tropicalis [Glossary Pseudohyphae].

```
Ascomycota
  Taphrinomycotina
    Pneumocystidomycetes
      Pneumocystidales
        Pneumocystidaceae
          Pneumocystis (genus)
```

Pneumocystis, the sole pathogenic genus in Class Taphrinomycotina, was, until recently, presumed to be a single-celled eukaryote. Early papers invented a detailed life cycle for Pneumocystis, complete with morphologically distinct developmental stages that included cyst, trophozoite, sporozoite, and intracystic bodies [9]. To be fair to the early taxonomists, the classification of Pneumocystis was particularly difficult because the organism could not be grown in culture. Owing to molecular analyses, we now know that Pneumocystis is a fungus

that grows as a yeast [10]. The so-called trophozoite stage of Pneumocystis is equivalent to the vegetative stage of well-studied Schizosaccharomyces pombe, a nonpathogenic member of Class Taphrinomycotina. The yeasts form an enclosed cyst, which eventually ruptures, releasing spores. These different forms of Pneumocystis comprise the various morphologic forms of the fungus that are seen in histologic sections of infected lungs. Pneumocystis jirovecii (formerly Pneumocystis carinii) produces pneumonia in immunodeficient individuals. AIDS patients are particularly vulnerable to Pneumocystis infections.

```
Ascomycota
  Pezizomycotina
    Dothideomycetes
      Dothideales
        Dothioraceae
          Hortaea (genus)
      Capnodiales
        Piedraiaceae
          Piedraia (genus)
      Pleosporomycetidae
        Pleosporales
          Testudinaceae
            Neotestudina (genus)
```

Hortaea werneckii causes Tinea nigra, from the Latin "tinea," meaning worm and "niger," meaning black. Tinea, also known as ringworm, is a localized infection of the keratin layer of the skin. The tinea lesions produced by Hortaea werneckii are black because the organism produces melanin. Melanin production is a feature that can help clinical mycologists identify a fungal species. Species that produce melanin are called dematiaceous fungi [Glossary Dematiaceous fungi].

Piedraia hortae causes black piedra, an infection of hair characterized by small black fungal nodules growing on hair shafts.

Note that Piedraia hortae is not a member of genus Hortaea; being as it is a species of Genus Piedraia. Likewise, Hortaea werneckii does not share its genus with Piedraia hortae. The mycologists responsible for this nomenclature could not have been more confusing, if they tried.

Neotestudina is one of various genera that has been cultured from mycetoma, an uncommon and enigmatic skin infection. Mycetoma, also known as Madura foot and maduromycosis, occurs most often in India, Africa, and South America. It presents as a slowly growing, fungating mass arising in the subcutaneous tissues, usually of the foot. As the mass grows, draining sinuses discharge fluid and hard grains (white, white-yellow or black grains). These masses often become superinfected, making it very difficult to determine the primary pathogen that causes the disease. More than 30 different species of bacteria and fungi have been grown from these lesions. It has been claimed that black grain mycetomas is caused by Leptosphaeria senegalensis, Madurella grisea, Madurella mycetomatis, or Pyrenochaeta romeroi. White grain mycetomas are reputedly

caused by Acremonium species, Aspergillus nidulans, Neotestudina rosatii, or Pseudallescheria boydii. White-yellow grain mycetomas are said to be caused by Actinomadura madurae, Nocardia asteroides, and Nocardia brasiliensis. Brown-red grain mycetomas are said to be caused by Actinomadura pelletieri or Streptomcyes somaliensis. Taken at face value, these claims would indicate that many different organisms, both bacterial and fungal, can produce a disease of remarkably specific, even unique clinical features. Suffice it to say that clinical science has much to learn about mycetoma.

```
Ascomycotga
  Pezizomycotina
    Sordariomycetes
      Microascales
        Microascaceae
          Scedosporium (genus)
      Hypocreales
        Nectriaceae
          Fusarium (genus)
      Sordariales
        Incertae sedis (problematic class)
          Madurella (genus)
```

Scedosporium prolificans accounts for a sizable portion of the instances of an uncommon lesion: disseminated phaeohyphomycosis. The prefix derives from the Greek "phaeo," meaning dusky. The suffix "hyphomycosis" indicates that the fungal organism produces hyphae. Phaeohyphomycosis presents as one or more abscesses that are brown on gross examination. The fungi that cause phaeohyphomycosis (e.g., Scedosporium prolificans) are dematiaceous (melanin-producing). Other genera of dematiaceous (brown-pigmented) fungi that may cause phaeohyphomycotic lesions, particularly in immune-compromised patients include Alternaria, Curvularia, Phialophora, Cladiophora, and any fungus of Class Herpotrichiellaceae known to produce chromoblastomycosis (vida infra).

Species of Fusarium can cause corneal keratitis and onychomycosis (fungal nail infection) in otherwise healthy individuals. In immune-compromised patients with very low white blood cell counts, various Fusarium species can produce life-threatening disseminated infections. These species include Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, and Fusarium verticillioides. Aside from their role in human infection, Genus Fusarium has been studied for its ability to produce various powerful mycotoxins. Poisoning outbreaks from Fusarium-contaminated food have been reported [11].

Madurella species were included in the discussion of Genus Neotestudina (vida supra). They are among the many putative causes of maduromycosis.

```
Ascomycota
  Pezizomycotina
    Eurotiomycetes
      Eurotiales
```

```
  Trichocomaceae
    Aspergillus (genus)
    Penicillium (genus)
Herpotrichiellaceae
  Fonsecaea (genus)
  Cladophialophora (genus)
  Phialophora (genus)
Onygenales
  Ajellomycetaceae
    Emmonsia (genus)
    Histoplasma (genus)
    Blastomyces [8] (genus)
    Paracoccidioides (genus)
  Arthrodermataceae (the dermatophytes)
    Epidermophyton (genus)
    Microsporum (genus)
    Trichophyton (genus)
  Onygenaceae
    Coccidioides (genus)
  Incertae sedis (problematic class)
    Lacazia (genus)
```

Genus Aspergillus contains the ubiquitous species that cause aspergillosis. The most common species associated with aspergillosis is Aspergillus fumigatus. Aspergillosis can manifest clinically in many different forms. Infections begin in the lung, where they may produce colonization of the airways, without disease. Alternately, they may provoke an acute or chronic allergic reaction in the lungs. The organism can grow in respiratory mucosa or may invade into the lung tissue. Infection may produce large fungal masses or may invade diffusely through the lung, like a pneumonia. Or the infection may produce a fungemia, and disseminate throughout the body. Spores of Aspergillus species are found in the air, and everyone is exposed to these fungi. Disease most often occurs in immune-compromised individuals. Primary cutaneous aspergillosis is a rare form of aspergillosis that occurs in the skin of immune-compromised patients near the site of indwelling intravenous lines.

Genus Penicillium contains one species that is known to produce human disease: Penicillium marneffei. Infections occur primarily in Southeast Asia, and most reported cases have occurred in AIDS patients. The disease, known as penicilliosis, can produce systemic infection. This species, like many other highly pathogenic members of Class Ascomycota, is a dimorphic fungus [Glossary Dimorphic fungi].

Chromoblastomycosis can be caused by a variety of organisms that belong to Class Herpotrichiellaceae: Fonsecaea pedrosoi, Phialophora verrucosa, Cladophialophora carrionii, and Fonsecaea compacta. Chromoblastomycosis begins as a skin papule at the site of entry, and over the years may slowly spread.

Cladophialophora bantiana can produce phaeohyphomycotic (i.e., brown-colored) brain abscesses and subcutaneous lesions in both normal and immunosuppressed patients.

Class Onygenales contains many of the fungi that characteristically cause disease in immune-competent individuals, and it is the only class of fungi containing organisms that infect, disseminate, and sometimes kill otherwise healthy persons, with one important qualification. Good health is hard to establish with certainty, and the biological relationships between human host and fungal infection can be very complex. Although most pathogenic fungi produce clinical disease in immunodeficient individuals, you will occasionally encounter a supposedly normal person who develops a fulminant infection from a supposedly nonpathogenic or opportunistic fungus. Nonetheless, there are a few fungi that produce disease in seemingly healthy patients, and most of these belong to Class Onygenales: Paracoccidioides brasiliensis, Coccidioides immitis, Blastomyces dermatitidis, and Histoplasma capsulatum. Each is characterized by the growth of yeast (round cells) in diseased tissues, and each is dimorphic in culture medium (Fig. 6.5).

Most pathogenic fungi have no specific geographic locality. Histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis are exceptions. Histoplasmosis is found most frequently in the Central and Eastern US around the Ohio and Mississippi River Valleys. Coccidioidomycosis, also known as San Joaquin fever or valley fever, is found mostly in the Southwest US and Mexico, where the fungus lives in the soil. Paracoccidioidomycosis is found almost exclusively in Central and South America, particularly Brazil, Colombia, and Venezuela (Fig. 6.6).

Lacazia loboi, formerly loboa loboi, is another species in Class Onygenales that causes disease in otherwise healthy individuals. The disease, endemic to the Amazon, is known by a number of names: Lobo disease, lacaziosis, keloidal blastomycosis, Amazonian blastomycosis, miraip,

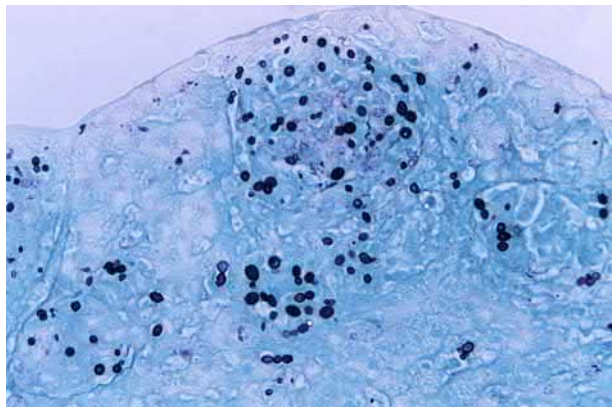

FIG. 6.5 Histoplasmosis. Numerous Histoplasma capsulatum organisms are found growing in tissue (observed as black yeast forms on silver stain). H. capsulatum grows in soil and is found in high concentration in bat or bird guano. Spores from dried materials in the ground becomes airborne and is inhaled. The condition is not transmitted from person to person. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention, and prepared by Dr. Libero Ajello.)*

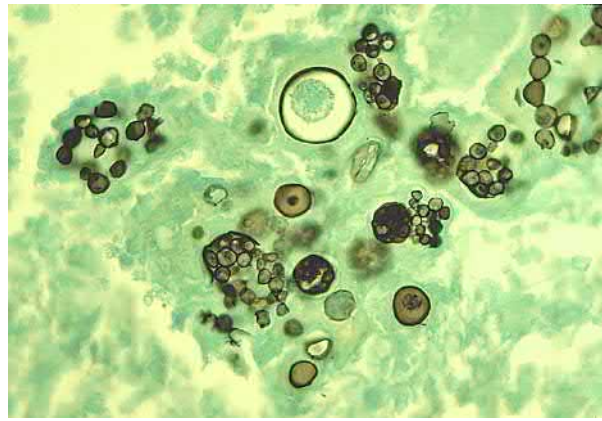

FIG. 6.6 Coccidioidomycosis of lung (methenamine silver stain). Spherules of Coccidioides immitis are seen, some containing numerous endospores. Several of the spherules have ruptured, releasing their contents. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, prepared by Dr. Edwin P. Ewing, Jr.)*

piraip, and lobomycosis. Clinically, Lobo's disease is a granulomatous infection of the skin. The disease can be mistaken for Paracoccidioides brasiliensis and with Blastomyces dermatididis due to the similar morphology of the yeast in tissues. Lacazia loboi has not been successfully cultured. Like most fungal infections, transmission comes from the environment, not through human contagion.

Genus Emmonsia contains two pathogenic organism: Emmonsia parva, alternately known as Chrysosporium parvum var. parvum, and Emmonsia crescens, alternately known as Chrysosporium parvum var. crescens. These organisms are the causative agents of a adiaspiromycosis, a disease with a unique pathogenic mechanism. Adiaspiromycosis causes pulmonary disease in various animal species, particularly rodents. It is a rare cause of disease in humans. Though it is referred to as an infection, it is actually a foreign body reaction, resulting from the inhalation and sequestration of conidial spores in the small branches of the respiratory tree. The spores are large, about 300 μm in diameter. Histologic cross sections of infected lungs show a walled spore, surrounded by acute and chronic inflammation and foreign body reaction granulomas. There is no growth of the organism. In the literature, the term "disseminated adiaspiromycosis" is sometimes encountered [12], referring to lesions that involve most of the lung parenchyma. In this case, dissemination is not an indication that a lesion has spread from one part of the lung to another, but that the load of inhaled spores involves the respiratory tree bilaterally. For the logophiles, the term "adiaspiromycosis" when disassembled into its Greek roots (a = the negation "not," diaspira = diaspora or dissemination, mycosis = fungal condition) tells us that the fungus does not spread (Fig. 6.7).

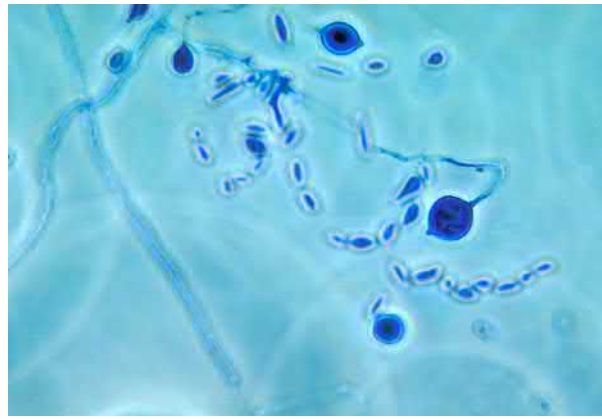

FIG. 6.7 Adiasporomycosis. Small conidiospores amidst large adiaspores. The conidiospores are the airborne, infective forms of the organism. Once in the lungs of an animal, the conidiospores develop as large adiaspores. In the host lungs, the adiaspores are end-stage organisms that do not replicate. The lung disease produced by adiaspores (adiaspiromycosis or haplomycosis) is due to a foreign body reaction, and subsequent granuloma formation, or to obstruction of small airways, and subsequent reduction in respiration. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, and prepared by Dr. Libero Ajello.)*

Recently, new species, tentatively classified as Emmonsia, have been identified that do not form adiaspores (i.e., whose conidia do not enlarge and block airways). These species of Emmonsia grow as yeast, and produce serious, disseminated disease in susceptible (e.g., immunosuppressed) populations [13].

On tissue examination, the pure yeast-form Emmonsia infections can be easily misdiagnosed as histoplasmosis or blastomycosis. About a half dozen different yeast-form Emmonsia species have been identified in human disease. Most are referred to as Emmonsia species, not further designated. One species with an assigned name is Emmonsia pasteuriana.

Class Onygenales also contains Class Arthrodermataceae, the dermatophytes: Genus Epidermophyton, Genus Microsporum, and Genus Trichophyton. The dermatophytes, unlike all other fungal species that are pathogenic in humans, are obligate parasites [1]. They live in the keratinized layers of epidermis, causing infections of skin, nails, and hair. Species within these three genera account for most of the tinea, also known as ringworm, infections of humans. Exceptions are Hortaea werneckii (an ascomycote in Class Dothideomycetes), the cause of tinea nigra; and Malassezia furfur (a basidiomycota), the cause of Tinea versicolor. All tinea infections are colonizations of the superficial layers of the epidermis. Like other infections caused by members of Class Onygenales, tinea infections can occur in immune-competent hosts. Like most fungal infections, the clinical features of the disease tend to worsen in immune-compromised patients. In immune-compromised patients, a case of superficial tinea may progress into a locally invasive process (tinea profunda) or recurrent infections [14].

As previously described, fungal diseases are not generally spread from person to person. They are spread by fungi that grow in the environment and release infective spores into air, soil, or water. In the case of the dermatophytes, direct transmission from person to person or animal to person (e.g., tinea due to Microsporum canis infections on cats and dogs) is possible.

It is important not to confuse fungi of Genus Microsporum with the fungi of Genus Microsporidium (Class Microsporidia).

```
Ascomycota
Pezizomycotina
 Ophiostomatales
 Ophiostomataceae
  Sporothrix (genus)
```

Sporotrichosis is caused by Sporothrix schenckii, a fungus that grows in soil, particularly peat moss. Gardeners who handle soil and peat are infected through abrasions on their hands. The lesion begins as a localized mass. As time passes, the satellite lesions advance up the arm, via lymphatic spread. Sporotrichosis is endemic to Peru.

Infectious Genera

Emmonsia

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Ajellomycetaceae: Emmonsia
- **Infection**. Emmonsia parva, also known as Chrysosporium parva (adiaspiromycosis or haplomycosis)
- **Infection**. Emmonsia crescens, also known as Chrysosporium crescens (adiaspiromycosis or haplomycosis)
- **Infection**. Emmonsia pasteuriana (disseminated pulmonary infection)
- **Infection**. Emmonsia species other than E. parva and E. crescens (disseminated pulmonary infection)

Fusarium

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: sordariomyceta: Sordariomycetes: Hypocreomycetidae: Hypocreales: Nectriaceae: Fusarium
- **Infection**. Fusarium species (corneal keratitis)

Histoplasma

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Ajellomycetaceae: Histoplasma
- **Infection**. Histoplasma capsulatum, var capsulatum (histoplasmosis)
- **Infection**. Histoplasma capsulatum, var duboisii (histoplasmosis)
- **Infection**. Histoplasma capsulatum, var farcinimosum (histoplasmosis)

Fonsecaea

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Chaetothyriomycetidae: Chaetothyriales: Herpotrichiellaceae: Fonsecaea
- **Infection**. Fonsecaea compacta (chromoblastomycosis, also known as chromomycosis, and cladosporiosis)

Madurella

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: sordariomyceta: Sordariomycetes: Sordariomycetidae: Sordariales: Incertae sedis (problematic class): Madurella
- **Infection**. Madurella grisea (one of several putative causes of black grain mycetomas)
- **Infection**. Madurella mycetomatis (one of several putative causes of black grain mycetomas)

Piedraia (genus)

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: dothideomyceta: Dothideomycetes: Dothideomycetidae: Capnodiales: Piedraiaceae: Piedraia
- **Infection**. Piedraia hortae (black piedra)

Neotestudina

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: dothideomyceta: Dothideomycetes: Pleosporomycetidae: Pleosporales: Testudinaceae: Neotestudina
- **Infection**. Neotestudina rosatii (white grain mycetoma)

Hortaea

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: dothideomyceta: Dothideomycetes: Dothideomycetidae: Capnodiales: Teratosphaeriaceae: Hortaea
- **Infection**. Hortaea werneckii (tinea nigra)

Aspergillus

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Eurotiales: Aspergillaceae: Aspergillus
- **Infection**. Aspergillus fumigatus (aspergillosis)

Penicillium

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Eurotiales: Aspergillaceae: Penicillium
- **Infection**. Penicillium marneffei (penicilliosis)

Epidermophyton

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Arthrodermataceae: Epidermophyton
- **Infection**. Epidermophyton floccosum (athlete's foot, tinea pedis, tinea cruris, tinea corporis, onychomycosis)

Microsporum

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Arthrodermataceae: Microsporum
- **Infection**. Microsporum canis (ringworm, dermatophytosis, tinea)

Trichophyton

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Arthrodermataceae: Trichophyton
- **Infection**. Trichophyton rubrum (ringworm, dermatophytosis, tinea)
- **Infection**. Trichophyton tonsurans (ringworm, dermatophytosis, tinea, 90% of cases of tinea capitis in North America, tinea favosa)

Blastomyces

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Ajellomycetaceae: Blastomyces
- **Infection**. Blastomyces dermatitidis (blastomycosis)

Coccidioides

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Incertae sedis (problematic class): Coccidioides
- **Infection**. Coccidioides immitis (coccidioidomycosis, also known as San Joaquin valley fever or valley fever)
- **Infection**. Coccidioides posadasii (another cause of coccidioidomycosis)

Paracoccidioides

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Incertae sedis (problematic class): Paracoccidioides
- **Infection**. Paracoccidioides brasiliensis (paracoccidioidomycosis, South American Blastomycosis, or Brazilian Blastomycosis)

Lacazia

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Eurotiomycetidae: Onygenales: Incertae sedis (problematic class): Lacazia
- **Infection**. Lacazia loboi (Jorge Lobo disease, Lobo disease, lacaziosis, keloidal blastomycosis, Amazonian blastomycosis, blastomycoid granuloma, miraip, piraip, lobomycosis)

Scedosporium

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: sordariomyceta: Sordariomycetes: Hypocreomycetidae: Microascales: Microascaceae: Scedosporium
- **Infection**. Scedosporium apiospermum, Pseudallescheria boydii (lung disease, disseminated infection, mycetoma, in immune-compromised individuals)
- **Infection**. Scedosporium proliferans (inflatum)

Sporothrix

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: sordariomyceta: Sordariomycetes: Sordariomycetidae: Ophiostomatales: Ophiostomataceae: Sporothrix
- **Infection**. Sporothrix schenckii (sporotrichosis, rose-handler's disease)

Candida

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Saccharomycotina: Saccharomycetes: Saccharomycetales: Debaryomycetaceae: Candida/Lodderomyces clade: Candida
- **Infection**. Candida albicans (candidiasis, candidemia, thrush)
- **Infection**. Candida glabrata (urinary tract infection and sepsis in immune-compromised individuals)
- **Infection**. Candida parapsilosis (wound infection and sepsis in immune-compromised individuals)
- **Infection**. Candida dubliniensis (infections in immune-compromised individuals)

Candida tropicalis (frequent cause of sepsis and disseminated candidiasis in immune-compromised individuals)

Pneumocystis

- **Lineage**. Fungi: Dikarya: Ascomycota: Taphrinomycotina: Pneumocystidomycetes: Pneumocystidales: Pneumocystidaceae: Pneumocystis
- **Infection**. Pneumocystis jirovecii, formerly Pneumocystis carinii (Pneumocystis Pneumonia)

Cladophialophora

- **Lineage**. Fungi: Dikarya: Ascomycota: saccharomyceta: Pezizomycotina: leotiomyceta: Eurotiomycetes: Chaetothyriomycetidae: Chaetothyriales: Herpotrichiellaceae: Cladophialophora
- **Infection**. Cladophialophora bantiana, formerly Xylohypha bantiana, formerly Cladosporium bantianum (cerebral phaeohyphomycosis)

Section 6.5 Microsporidia

The most savage controversies are those about matters as to which there is no good evidence either way.

Bertrand Russell

```
Eukaryota
  Bikonta, 2-flagella
  Unikonta, 1-flagellum
    Amoebozoa
    Opisthokonta
      Choanozoa
      Animalia
      Fungi
        Zygomycota
        Dikarya
          Basidiomycota
          Ascomycota
        Microsporidia
          Encephalitozoonidea
            Encephalitozoon (genus)
          Enterocytogoonidea
            Enterocytozoon (genus)
          Microsporidea
            Microsporidium (genus)
          Nosematidea
            Brachiola (genus)
            Nosema (genus)
            Vittaforma (genus)
          Pleistophoridea
            Pleistophora (genus)
            Trachipleistophora (genus)
```

In decades past, members of Class Microsporidia were included with the archaezoans, a proposed class of eukaryotes so ancient in origin that they evolved without the benefit of mitochondria [15]. Class Archaezoa has been abandoned, insofar as current thought holds that all eukaryotes derived from a root organism that contained mitochondria. Furthermore, using modern molecular techniques, the members of Class Microsporidia have been shown to contain ribosomal RNA sequences typical of fungi [15, 16]. Apart from their phylogenetic relationship

to fungi, these organisms have striking "nonfungal" features, being instead obligate intracellular parasites that have adapted themselves to parasitic lives in a wide range of eukaryotic organisms. Unlike virtually all other members of Class Fungi, the members of Class Microsporidia lack mitochondria [17], lack a hyphal form, and do not produce multicellular tissue structures. As with other so-called amitochondriate eukaryotic classes (e.g., Class Metamonada [18], and Class Amoebozoa 22 [19]), the members of Class Microsoridia have retained remnant forms of mitochondria (i.e., mitosomes, hydrogenosomes) [17]. In addition, the microsporidia synthesize chitin, a structural feature found in fungi. With the addition of additional molecular studies over the past few decades, there is little doubt that microsporidians are true fungi [15].

All members of Class Microsporidia form spores, thick-walled cells that can survive in the environment. Infected animals pass spores in their urine and feces, and the spores infect humans by contact or inhalation. The spores pass to the intestines, where they extrude a polar tube into the intestinal lining cells. Through the polar tube, the cytoplasm of the spore (sporoplasm) enters the host cell and organizes into a cell capable of division. Eventually, more spores are formed. When the host cells lyse (break open and die), spores are released into the intestine, and are passed with feces into the environment.

A wide variety of animals are reservoirs for the various species of Microsporidia: mammals, birds, and insects. The spores are passed in the stools, and infect humans through direct contact, water contamination, or through respiration of airborne spores. Preliminary evidence suggests that microsporidial infections are common [20]. Most, but not all, cases of symptomatic microsporidiosis occurs in immune-compromised individuals, particularly in patients who have AIDS.

Though microsporidiosis is considered a rapidly emerging disease, we lack important and fundamental epidemiologic information. How prevalent is the organism in the immune-competent population? How prevalent is the organism in the population of immunodeficient but asymptomatic patients? How often is a microsporidium the causative agent of diarrheal diseases among different age groups? In which geographic regions does microsporidiosis occur? What are the most important animal reservoirs for human microsporidiosis?

```
Microsporidia
  Encephalitozoonidea
    Encephalitozoon (genus)
  Enterocytogoonidea
    Enterocytozoon (genus)
  Microsporidea
    Microsporidium (genus)
  Nosematidea
    Brachiola (genus)
```

```
    Nosema (genus)
    Vittaforma (genus)
  Pleistophoridea
    Pleistophora (genus)
    Trachipleistophora (genus)
```

At least 14 species of Microsporidia, in 8 genera, can cause microsporidiosis. Most produce symptoms of diarrhea that can be chronic, and wasting, in susceptible individuals. Some microsporidian species produce a variety of additional conditions, associated with ocular infections, muscle infections, and even systemic disease (Fig. 6.8).

Readers should remember not to confuse Microsporidia with Microsporum, a fungal genus causing dermatophytosis. It is also important not to confuse microsporidiosis with cryptosporidiosis, an apicomplexan disease (Apicomplexa), that also produces diarrhea in immune-compromised patients.

Infectious Genera

Brachiola

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Apansporoblastina: Nosematidae: Brachiola
- **Infection**. Brachiola algerae (microsporidiosis, Keratoconjunctivitis, skin and deep muscle infection)
- **Infection**. Brachiola connori (microsporidiosis, ocular infection)
- **Infection**. Brachiola vesicularum (microsporidiosis)

FIG. 6.8 Electron micrograph of Encephalitozoon intestinalis organisms, within an intracellular vacuole. Red arrows point to immature forms, and black arrows point to mature spores. *(Source, a public domain image provided by the US Centers for Disease Control and Prevention.)*

Encephalitozoon

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Apansporoblastina: Unikaryonidae: Encephalitozoon
- **Infection**. Encephalitozoon cuniculi (microsporidiosis, keratoconjunctivitis, infection of respiratory and genitourinary tract, disseminated infection)
- **Infection**. Encephalitozoon hellem (microsporidiosis, keratoconjunctivitis, infection of respiratory and genitourinary tract, disseminated infection)
- **Infection**. Encephalitozoon intestinalis, formerly Septata intestinalis (microsporidiosis, infection of the GI tract causing diarrhea, and dissemination to ocular, genitourinary and respiratory tracts)

Enterocytozoon

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Apansporoblastina: Enterocytozoonidae: Enterocytozoon
- **Infection**. Enterocytozoon bieneusi (microsporidiosis, diarrhea, acalculous cholecystitis)

Microsporidium

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Incertae sedis (problematic class): Microsporidium
- **Infection**. Microsporidium ceylonensis (microsporidiosis, infection of the cornea)
- **Infection**. Microsporidium africanum (microsporidiosis, infection of the cornea)

Nosema

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Apansporoblastina: Nosematidae: Nosema
- **Infection**. Nosema ocularum (microsporidiosis, ocular infection)

Vittaforma

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Apansporoblastina: Nosematidae: Vittaforma
- **Infection**. Vittaforma corneae, same as Nosema corneum (microsporidiosis, urinary tract infection, ocular infection)

Pleistophora

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Pansporoblastina: Pleistophoridae: Pleistophora
- **Infection**. Pleistophora sp. (microsporidiosis, muscular infection)

Trachipleistophora

- **Lineage**. Fungi: Incertae sedis (problematic class): Microsporidia: Pansporoblastina: Pleistophoridae: Trachipleistophora

- **Infection**. Trachipleistophora hominis (microsporidiosis, muscular infection, keratitis)
- **Infection**. Trachipleistophora anthropophthera (microsporidiosis, disseminated infection)

Glossary

Ascocarp The sporocarp of a fungus of Class Ascomycosis.

Basidiocarp The sporocarp of a fungus of Class Basidiomycota.

Chloroplast evolution Chloroplasts are the organelles (little membrane-wrapped replicating structures within cells) that produce glucose and oxygen, via a process that can be loosely described as:

```
carbon dioxide + water + light energy -> carbohydrate + oxygen.
```

With very few exceptions, chloroplasts are found in all members of Class Plantae. Aside from photosynthesis occurring in plants, we can also observe photosynthesis in cyanobacteria. Photosynthesis produced by cyanobacteria is thought to account for the conversion of our atmosphere from an anoxic environment to an oxygen-rich environment. Did photosynthesis, a complex chemical pathway, evolve twice in terrestrial history; once in cyanobacteria and once again in primitive plants?

Present thinking on the subject holds that the evolution of photosynthesis occurred only once, in the distant past, and all photosynthesis ever since, in cyanobacteria and in plants, arose from this one event. It is presumed that plants acquired photosynthesis when they engulfed photosynthesizing cyanobacteria that evolved into self-replicating chloroplasts. This conclusion is based on the observation that chloroplasts, unlike all other plant organelles, are wrapped by two membranous layers. One layer is believed to have been contributed by the captured cyanobacteria, and one layer presumably came from the capturing plant cell as it wrapped the cyanobacteria in its own cell membrane.

Dematiaceous fungi Fungi that are pale brown, dark brown, or black due to melanin pigment. The term chromoblastomycosis refers to any chronic skin infection caused by a dematiaceous fungus. The term phaeohyphomycosis is more general, and refers to any fungal infection produced by a dematiaceous fungus, anywhere in the body. Examples of dematiaceous species are: Alternaria species, Exophiala species, and Rhinocladiella mackenziei. Most infections associated with dematiaceous fungi occur in immune-compromised individuals. In addition to causing human infection, dematiaceous fungi may sometimes serve as human allergens (e.g., allergic sinusitis).

Dimorphic fungi Fungi that can grow as a yeast form or as a mycelia (hyphal growth) depending on environmental conditions (such as temperature). Many of the dimorphic fungi grow as mycelia between 25 and 30°C; growing as yeast at body temperature (35–37°C). Coccidioides immitis is an example of a dimorphic fungus whose morphology is not strictly determined by temperature. C. immitis grows as a mycelium in soil and as a yeast (morphologically, as thick large spherules) in living tissue.

The dimorphic fungi that are human pathogens are as follows:

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Blastomyces dermatididis
Histoplasma capsulatum
Coccidioides immitis
Paracoccidioides brasiliensis
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Sporothrix schenckii
Candida albicans
Penicillium marneffei

The first four of the listed dimorphic fungi are considered potent pathogens in healthy humans (i.e., they do not require an immune-compromised human host). Sporothrix schenckii does not require its host to be immunocompromised, but it tends to produce lesions that are confined to skin (i.e., that do not widely disseminate). Penicillium marneffei tend to produce disease in immune-compromised hosts.

Candida albicans tends to produce systemic disease in individuals who are immune-compromised, but can produce local infections in otherwise healthy persons. Interestingly, Candida albicans is capable of exhibiting dimorphism in tissue and culture. In tissue, most infections are produced by yeast forms of C. albicans, but when fungus invades deeply into tissue, one tends to see both yeast forms and hyphal forms. The hyphal form, often seen as pseudohyphae, seems to invade through tissues more readily than the yeast form.

All of the known pathogenic dimorphic fungi are descendant members of Class Pezizomycotina, which is a descendant subclass of Class Ascomycota.

The term "dimorphic fungi" (i.e., yeast vs hyphae) should not be confused with another paired morphologic aspect of fungal growth: anamorphic versus teleomorphic growth. Anamorphic growth refers to the asexual growth phase of fungi. Teleomorphic growth refers to the morphologically distinctive sexual growth phase of fungi. Disease-causing fungi growing in human tissues are always anamorphic.

Heterotrophic An organism is heterotrophic if it must acquire organic compounds from the environment as its energy source. All animals and all fungi (both of Class Opisthokonta) are heterotrophs. In contrast, members of Class Plantae, and chloroplast-containing members of Class Protoctista, are phototropic autotrophs, producing organic compounds from light, water, and carbon dioxide.

Mushroom A mushroom is a fleshy fungal sporocarp that we can observe with the naked eye as an outgrowth, usually from the ground or from a tree trunk. Most, but not all, mushrooms are ascocarps (i.e., the sporocarp of a fungus of Class Ascomycota).

Pseudohyphae A nonspecific term referring to any of several instances wherein a fungal structures grow or coalesce as linear strings of thin or rod-shaped cells that look like hyphae. In this book, the term "pseudohyphae" appears only as it applies to the growth of Candida yeast organisms. When the replicating yeast cells stretch and stay attached to one another, the lines of cells are called pseudohyphae.

Sporocarp Refers to the fungal structure in which spores are produced. A mushroom is the most widely recognizable type of sporocarp.

Virulence factor These are molecules that enhance the ability of an infectious organism to survive in its host. Many of the best-studied virulence factors are produced by bacteria, and because virulence factors always work to the detriment of the host, the terms "virulence factor" and "bacterial toxin" are mistakenly used interchangeably. You will find it useful to separate these two concepts because virulence factors and bacterial toxins have very different functions. Toxins work by damaging or killing host cells. Virulence factors work by making it easier for organisms to invade, grow, and persist within the host. Virulence is attained by helping the infectious organism obtain nutrition from host cells, by evading or suppressing the host immune response, by enhancing the ability of the organism to adhere to host cells, or by enabling the organisms to enter host cells or to invade through host tissues.

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Chapter 7

Viruses

Section 7.1 Viruses and the meaning of life

The question whether or not "viruses are alive" has caused considerable debate over many years. Yet, the question is effectively without substance because the answer depends entirely on the definition of life or the state of "being alive" that is bound to be arbitrary.

Eugene V. Koonin and Petro Starokadomskyy [1]

The first edition of this book, published 7 years ago, includes the following ill-conceived statement: "Viruses lack key features that distinguish life from nonlife. They depend entirely on host cells for replication; they do not partake in metabolism, and do not yield energy; they cannot adjust to changes in their environment (i.e., no homeostasis), nor can they respond to stimuli. Most scientists consider viruses to be mobile genetic elements that travel between cells (much as transposons are mobile genetic elements that travel within a cell)" [2].

As nonliving organisms, dependent entirely on host cells for their continued existence, viruses have done extremely well for themselves [3]. Every class of living organism hosts viruses. Viruses are literally everywhere in our environment, and are the most abundant life form in the oceans, in terms of numbers of organisms [4]. The number of different species of viruses seems to far exceed the species of eukaryotes, bacteria, and archaea [5, 6]. A single class of newly characterized aquatic viruses, the Megaviridae, appears to have more species and more phylogenetic diversity than all of the bacterial and archaean species found in ocean water [7].

Viruses have been on earth for a very long time [8, 9]. The evidence suggesting that viruses preceded the appearance of other classes of living organisms is based in part on the observation that some archaeans, bacteria, and eukaryotes have homologous genes that code for capsid proteins, such as would be derived from a class of viruses. The simplest explanation for homology for a viral-type gene, in all extant cellular forms of life, is that an ancient gene came from viral species that lived at a time that preceded the emergence of newer forms of life [10]. Over the aeons, retroviruses have had a chance to insert lots of DNA fragments into eukaryotic species. At least 8% of the human genome is composed of fragments of RNA viruses [11–13] [Glossary Capsid].

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Over the past 7 years, the evidence and the arguments, supporting viruses as living organisms, has won a great deal of support. Consider the following points:

Viruses replicate and create virocells

Viruses employ the replicative machinery that they find in host cells, manufacturing viral assembly plants in the process. After infection, host cells forego many of their normal functions, and the newly synthesized viral products produce disturbances in cellular physiology (so-called cytopathic effects). The host cells become something more akin to a viral factory than to eukaryotic organisms, and these new living entities are referred to as virocells, indicating that the virus has created its own form of cellular life [Glossary Virion].

Viruses exhibit chemical, structural, and physiological diversity

Viruses display great diversity in their range of hosts, habitat, size, genes, mechanisms of infection, and capsids. Unlike eukaryotes, viruses have a range of genomic types, which include: double-stranded DNA; single-stranded DNA; double-stranded RNA; positive sense single-stranded RNA; negative sense single-stranded RNA, single-stranded RNA with a reverse transcriptase, and double-stranded DNA with a reverse transcriptase.

Viruses of each type breed true, meaning that a virus of any type will replicate to produce viruses of the same type. Thus, the types of viruses, determined by their genomic structure and replicative modes, can be organized as biological classes.

Viruses do not lead a totally intracellular life

Viruses are accused of having no life outside of their host cells. It has long been assumed that extracellular viral existence is relegated to long-term storage: a lifeless viral genome double-wrapped in a protein capsid, and an envelope that is mostly purloined from the cell membranes of a former host.

It has been recently demonstrated that some viruses enjoy life outside of their hosts. The Acidianus Tailed Virus can be cold-stored at room temperature for long periods, without changing their morphology. When the temperature rises they undergo a structural transformation, forming bipolar tails [14]. This tells us that viruses can react to external stimuli and perform biological activities, much as free-living organisms do.

Viruses evolve and speciate

It has long been accepted that there are different distinctive species of viruses. The definition of a viral species, as suggested in 1985, is essentially the same definition as any other species of living organism: an evolving gene pool [15]. Despite the high mutation rate of viral genomes, there is sufficient stability in their genomes, particularly in genes that code for constitutive proteins, to establish a phylogenetic lineage for classes of viruses, by examining relatively conserved genomic sequences [16].

Presumably, much of the stability of viral species is imposed by host organisms. How so? All viruses must replicate within a host, and the process by

which a eukaryotic cell is infected and transformed into a virocell is very complex. Viruses are forced to adapt some level of host preference, and must fine-tune all their functions to the available conditions within the host [17]. Thus, for viruses to successfully replicate, they must evolve into relatively stable species. And this is what we observe when we study viruses [Glossary Evolvability].

Virus speciation yields phylogenetic lineages (i.e., descendant families)

In 2017, the ICTV (International Committee on Taxonomy of Viruses) recognized 9 orders, 131 families, 46 subfamilies, 803 genera, and 4853 different viral species [18]. The number of known viral species (4853) is thought to represent a tiny fraction of the total number. In the next section, "Viral Phylogeny" we will discuss the various concepts and methods that have led to a rudimentary phylogeny of the viruses.

Viruses participate in the evolution of eukaryotic organisms

Some of the greatest evolutionary leaps among eukaryotic organisms can be attributed to genes taken from noneukaryotes (e.g., mitochondria and chloroplasts). The viruses have also contributed to eukaryotic evolution, and it's fair to say that if it were not for viral genes, you would not be reading this book (it would be left to some octopus or insect to finish out the chapter).

Specifically, at least four major advancements can be attributed to retroviral genes inserted into ancient eukaryotic genomes:

–1. The acquisition of adaptive immunity in Class Gnathostomata (the jawed vertebrates), and every descendant class [19] [Glossary Adaptive immunity].
–2. The acquisition of the placenta in Class Theria (i.e., the placental mammals plus the marsupials, which have a rudimentary placenta).
–3. The maintenance of pluripotent stem cells, important in embryogenesis, is achieved, in part, with transcripts of stem cell-specific long terminal repeat retrotransposons, themselves derived from retroviral sequences [20, 21] [Glossary Transposon].
–4. In Class Mus (containing the common house mouse), an MuERV-L retrovirus capsid gene domesticated to code an antiviral factor [22, 23].

We should note that the adaptation of retroviruses does not always have purely beneficial consequences for the individual members of a species. Oncogenes, many of which have a retroviral origin, are genes that play a key role in driving cells toward a cancer phenotype. Oncogenes are highly conserved genes, indicating a beneficial role for the species (otherwise, they would not be conserved). Nonetheless, oncogenes clearly have a deleterious effect on some individuals within the species (i.e., by causing cancer). One particularly striking example occurs in a polycythemia strain of mice carrying the Friend murine leukemia virus. All the mice of this particular strain have polycythemia, a condition characterized by a sustained increase in the number of circulating red blood cells. It seems that the resident virus codes for a nonfunctional Env (viral envelope) gene. The protein coded by the degenerate

Env gene serves as a mimic for the normal erythropoietin gene of the mouse, causing an increase in red cell production (i.e., polycythemia) [24, 25].

Participating in eukaryotic evolution, and accounting in no small way for the generation of new species and new strains of species, as viruses certainly do, are itself a biological function, and another indicator of viral life.

Life is what we choose to make of it.

Are viruses living organisms, or are they simply sequences of nucleic acid that have the wherewithal to move from cell to cell. Much of the debate focuses around the definition of life [1, 26]. How we define life is somewhat arbitrary, and we can certainly produce a definition of life that includes the viruses, if that is what we choose. There's no urgency to the matter. Once our robots become self-replicating, we'll need to address the definition of life anew. When that time comes, perhaps robots will take the lead and create a definition for life that includes organic viruses and software viruses.

Who is the better class of organism: virus or human?

For a moment, let's put our tongues in our cheeks and pretend that we are viruses [8]. As viruses, what would we think of humans and other eukaryotic organisms? Would we be willing to accept humans as fellow living organisms, or would we point to the following list of disqualifiers to conclude that humans just don't make the grade?

–1. Humans cannot replicate (they merely procreate).

In an effort to strengthen their species' gene pool, humans undergo a strange mating process in which the chromosomes of both parents are hopelessly jumbled together to produce an offspring that is unique, and unlike either the father or the mother. In doing so, humans miss out on the replicative process, performed with the greatest enthusiasm by every virus.

Self-replication is one of the fundamental features of life. Because humans cannot replicate themselves, they barely qualify as living organisms. At least, this is what the viruses think.

–2. Humans do not react in a manner that preserves the survival of their own species.

Humans have created a variety of weapons that are fully capable of wiping out all of human life. Viruses generally respect one another's right to co-exist.

–3. We can thank viruses for the existence of humans.

Viruses are constantly donating DNA to humans, and humans have used this DNA to evolve [12]. In point of fact, if there were no viruses, there would be no DNA replication, no adaptive immune system, no placentas, and no humans [27].

We also see that viral species acquire genes from their hosts, and that viruses retain these genes as they evolve. Presumably, the acquisition of host genes confers some survival advantage upon the virus. Nonetheless, viruses are a self-sufficient class of organism, and no specific instance comes to mind wherein a viral species depended on a human gene for its survival.

–4. Humans may have descended from viruses.

Nobody really knows much about the earliest forms of life, and there is plenty of room for conjecture. It's quite feasible that the earliest genetic material consisted of sequences of RNA, and that these RNA molecules moved between the earliest forms of cells. If this were the case, then the earliest genomes were essentially RNA viruses, and this would place humans as direct, but distant descendants of viruses.

There is a current theory, among many competing theories, that the first eukaryotic nucleus was a giant virus that was not totally successful in transforming its proto-eukaryotic host into a virocell [8, 28, 29]. A hybrid giant virus/virocell/proto-eukaryote may have stabilized and replicated to form an early, nucleated cell; the first eukaryote.

–5. Humans serve viruses; not vice versa.

We are taught to think of viruses as fragments of nucleic acids wrapped by a capsid (i.e., the virion). To a virus, extracellular existence must be akin to a state of suspended animation. Virions come back to life when they invade a eukaryotic cell and create a virocell; a living organism consisting of the hijacked eukaryotic cell whose nuclear machinery is redirected to synthesize viral progeny. If every eukaryotic cell is conceptualized as a potential virocell, then every eukaryotic species is a potential slave owned by the viral kingdom. The viruses probably think they're doing us a favor. Frankly, most of the cells in a metazoan body lead a vegetative existence, doomed to a fully differentiated, postmitotic, and short existence. Viruses re-animate postmitotic cells, and create a thriving center for viral life from a lackluster population of eukaryotic cells, as virocells.

Section 7.2 Viral phylogeny

How is it that you keep mutating and can still be the same virus?

Chuck Palahniuk, in his novel, *Invisible Monsters*

If we accept that viruses are living organisms, on equal footing with bacteria, achaeans, and eukaryotes, then we must accept the challenge of creating a classification of viruses based on phylogeny (descent from evolving ancestral species), and abandon viral groupings based solely on phenetics (i.e., based on physical similarities). **There is a problem with the notion of a purely phylogenetic classification of viruses; it may be impossible** [Glossary Phenetics].

Let's take a look at a few of the impediments to establishing a viral phylogeny that is comprehensive, testable, and credible.

–1. Large number of known and unknown viral species

Simply put, the greater the number of species, the more work is required to prepare a taxonomy. Every new species requires a certain irreducible amount of study, and if new species are being discovered at a rate that exceeds our ability to describe and classify known species, then the list of unassigned species will become infinitely long over time.

–2. Lack of any accepted concept of a "root" virus

The classification of cellular organisms is built on the premise that each of the major classes (i.e., bacteria, archaeans, and eukaryotes) has a root or founder class, with a hypothesized set of class-defined features, from which all subclasses descended. In the case of viruses, we really have no way of describing the ancestor of all extant viruses, and we do not have a strong reason to assume that all the viruses we see today came from any single class of viruses.

Furthermore, we define viruses as being obligatory parasites, requiring one of the major classes of cellular organisms for a host. If this were the case, then viruses, as we have come to define them, could not have existed prior to the existence of host organisms to parasitize. Hence, if viruses existed prior to the emergence of cellular life, then the root of the viruses was not a virus, insofar as they could not have parasitized cellular hosts. If the root of the viruses was not a virus, then it may have been almost anything, and we could not rule out the existence of multiple root organisms, accounting for the widely varying versions of viral genomes that we observe today.

Games of phylogenetic logic are harmless fun, but they illustrate how it is impossible to create a top-down classification of viruses, if we know nothing about the biological features that would define the top class.

–3. High rate of mutation in viruses

For the most part, viruses do not repair their genomes. A notable exception is the megavirus Cafeteria roenbergensis [30]. Presumably, we will find that other megaviruses have DNA repair pathways, but the small, simple viruses have rates of mutation in DNA and RNA, with no mechanism to repair the damage. This means that genome-damaged viruses have two choices: to die or to live with, and replicate, their mutations. Consequently, viral genomes tend to degenerate quickly, producing lots of variants. Species mutability is particularly prevalent among the RNA viruses (e.g., influenza virus, Newcastle disease virus, and foot and mouth disease virus).

Mutational variations of a virus seldom produce a new species. Instead, variations produce diversity in the viral gene pool of the species. If new mutations do not produce an alteration in the specificity of host organisms, or in the construction of the virocell, then the variant viral replicants resulting from mutations will usually preserve their membership in the same viral species [Glossary Virocell].

Still, all those viral genomic variants complicate the job of the viral taxonomist. Basically, the high rate of mutation in viruses yields lots of genomic variation among viral populations, making it easy for bioinformaticians to detect species diversity where none exists. We can easily imagine a situation wherein new species are discovered, and old species are declared extinct, because we simply do not have the time and manpower to carefully examine every genomic variant for the structural and physiologic features that determine its correct taxonomic classification. Bioinformaticians off-handedly refer to the variant genomes, resulting from mutations and replication errors, as quasispecies [31]. For the traditional taxonomist who is trying to create a simple phylogenetic classification of viruses, the vague concept of "quasispecies" must be particularly exasperating.

–4. Multiparental lineage of viruses

Viral reassortment is a process wherein whole segments (the equivalent of viral chromosomes) are exchanged between two viruses infecting the same cell. Viral reassortment has been observed in four classes of segmental RNA viruses: Bunyaviridae, Orthomyxoviridae, Arenaviridae, and Reoviridae. Following reassortment, a new species of virus may appear, and this new species will contain segments of two parental species. This poses a serious problem for traditional taxonomists, who labor under the assumption that each new species has one and only one parental species [32]. It is the uniparental ancestry of biological classifications that accounts for their simplicity, and for the concept of lineage, wherein the ancestry of any species can be computed from an uninterrupted line of classes stretching from the species level to the root level. When a species has more than one parent, then its lineage is replaced by an inverted tree. The tree branches outwards with each class reaching to more than one parent class, iteratively, producing a highly complex ancestry wherein the individual classes have mixed heritage.

Bioinformaticians have no problem creating multiparental classifications, and have used them to organize and model for biological processes (e.g., pathways), and molecular components (e.g., genes and proteins) [33]. They call such constructs ontologies, and have an assortment of computational tools to construct, deconstruct, and analyze complex representations of class relationships.

The pros and cons of single-parent class relationships versus multiparent class relationships have been argued at great length in the bioinformatics literature and cannot be fully explored here [34, 35]. Suffice it to say that no matter how many species we must accommodate, a simple classification will always provide an ordered set of class relationships that can be fully absorbed by the human mind. Ontologies are highly complex, often uncomputable, and chaotic (e.g., providing different analytic solutions with repeated analyses of the same data) [34]. It remains to be seen whether the ontologic model can be usefully applied to viral classifications.

Hope for a viral classification, based on phylogeny

Despite these four listed impediments to viral classification, there is reason to hope that there will one day be a complete set of viral classes and a simple set of lineages that connect each viral class to a defined "root" organism. A sense of hopefulness is based, in no small part, on observations of the physical world. Despite our sense that anything is possible in the vastness of space, we see an awful lot of sameness throughout the universe. Wherever we aim our telescopes, we see galaxies, most of which are flat and spiral, often having about the same size, and composed of the same objects: stars, planets, gas, dust, and black holes. A small set of physical laws impose stability everywhere at once, and the result is the somewhat repetitious universe in which we live. Likewise, despite the large number of species living on our planet, they are all variations of a few common themes that can be encapsulated under a simple classification.

About a half billion years ago, the early metazoan classes (i.e., animals) evolved at a rapid rate, producing dozens of body plans that we can examine in ancient shale deposits. This period, which lasted about 40 million years or so, is known as the Cambrian Explosion. The same body plans that evolved during the Cambrian explosion account for nearly all of the classes of animals that live today. This is to say that since the Cambrian, no new body plans have gained entry into the metazoan world. Much has been written about the Cambrian explosion, much of it focused on why metazoan body plans are so few, and why the world lacks newly evolved entries [36]. We have observed as a general rule of biology, that no matter how easy it may be for a class of organisms to speciate, there always seems to be a limited number of general classes into which all the species can be assigned. It is as though the evolutionary process itself confines classes to a smaller and smaller repertoire of available designs.

When we think that we have encountered a class of life that is too complex for simple classification, we are usually proven wrong. For example, in the first two-thirds of the 20th century, the classification of bacteria was considered a hopeless task, insofar as bacteria of different classes were known to exchange DNA among themselves, in a general process known as horizontal gene transfer. If bacteria were constantly exchanging genetic material, then it seemed that every bacteria was an amalgam of other bacteria and could not be sensibly classified. Be that as it may, bacteria were found to have a convincing phylogenetic order, based on the ancestral lineage of highly conserved species of rRNA that distinguished the bacteria from archaeal bacteria and further distinguished the classes and subclasses of bacteria [37, 38].

In the case of viruses that mutate at a high rate, that exchange large pieces of their DNA, that extract DNA from their host organisms, and that produce an uncountably large number of diverse species, one might think that a classification would be an impossible task. Not so. Instead, we are finding fundamental molecular motifs that can be used to classify viruses into biological groups that share phylogenetic origins [39, 16]. For example, despite the sequence variations that

occur in rapidly mutating viruses, scientists are finding that the three-dimensional folds of protein molecules are conserved, and that viruses can be grouped into the so-called fold families, which can in turn be grouped into fold super families, that preserve phylogenetic relationships among viral lineages [39].

Among the retroviruses, it has been shown that viral ancestral lineages can be determined by looking at inherited variations in the so-called "global" genomic properties (e.g., translational strategies, motifs in Gag and Pol genes and their associated enzymes) [16].

We shouldn't be surprised that viruses, like every other class of organism, falls into a rather limited set of phylogenetic classes. Because all viruses are parasitic, we can see why all viral species are constrained to evolve in a manner that maintains their host compatibility [17].

A demonstration of host-specific constraints on viruses is found by examining the specificity of viruses for the highest classes of organisms. Virus infections are found in Class Archaea, Class Bacteria, and Class Eukaryota, but there is no instance in which any single class of viruses is capable of infecting more than one of these classes of cellular organisms. Furthermore, within a class of cellular organisms, there are only rare instances of classes of virus that can infect distantly related subclasses. For example, there are virtually no viruses that can infect both Class Animalia and Class Plantae (rare exceptions are claimed [40]). Furthermore, as the host evolves, so must the virus. Hence, we might expect to find ancestral lineages of viruses that shadow the lineage of their host organisms.

The relatively recent discovery of NCLDVs (nucleocytoplasmic large DNA viruses, popularly known as giant viruses) has greatly expanded our notion of viral existence [45, 46]. The life of an NCLDV is not much different from that of obligate intracellular bacteria (e.g., Rickettsia). The NCLDVs, with their large genomes and complex sets of genes, have provided taxonomists with an opportunity to establish ancestral lineages among some of these viruses [45, 46].

At this time, the classification of viruses is somewhat crude. Anything you choose as a classifying principle fails to biologically unify the subclasses. For example, if you classify viruses by their genomic molecules (i.e., DNA or RNA, single strandedness or double strandedness), you will find that subclasses with the same genomic type will have dissimilar structures: envelope, size, shape, proteins, and capsid. When we list viruses based on method of contagion, by persistence within host (i.e., acute, chronic, latent, or persistent), toxicity (lytic, immunogenic), or by target cell specificity, no consistent taxonomic correlation is found.

Though we cannot as yet classify viruses strictly by their evolutionary lineage, we can usefully group viruses based on the physical characteristics of their genomes. The Baltimore Classification divides viruses into seven groups based on whether their genome is DNA, RNA, single stranded, or double stranded, the sense of the single strand, and the presence or absence of a reverse transcriptase. Here are the classes of the pathogenic viruses. This classification,

though nonphylogenetic in concept, has the great advantage of being comprehensive: every known virus can be assigned to a group within the Baltimore Classification.

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Group I, double-stranded DNA
Group II, single-stranded DNA
Group III, double-stranded RNA
Group IV, positive sense single-stranded RNA
Group V, negative sense single-stranded RNA ssRNA
Group VI, single-stranded RNA with a reverse transcriptase
Group VII, double-stranded DNA with a reverse transcriptase
```

It is worth repeating that when we use the Baltimore Classification (or any alternate viral classification, for that matter) we must grudgingly accept the fact that biologically relevant features of grouped viruses will cross taxonomic boundaries. Consider the arboviruses. Arbovirus is a shortened name for Arthropod borne virus. The arboviruses fall into several different groups of viruses. The principle vectors of the arboviruses are mosquitoes, ticks. Mosquito-borne arboviruses are members of Class Bunyaviridae (Group V), Flaviviridae (Group IV), or Togaviridae (Group IV). Tick-borne arboviruses are members of Class Bunyarviridae (Group V), Flaviviridae (Group IV), or Reoviridae (Group III). Over 500 arboviruses, infecting a variety of animals, have been described [47]. The arboviruses, organized by their transmission vectors, as shown below, cross multiple viral groups.

```
Mosquito-borne viruses.
  Bunyaviridae (Group V)
    La Crosse encephalitis virus
    California encephalitis virus
    Rift Valley fever virus
  Flaviviridae (Group IV)
    Japanese encephalitis virus
    Australian encephalitis virus
    St. Louis encephalitis virus
    West Nile fever virus
    Dengue fever virus
    Yellow fever virus
    Zika fever virus
  Togaviridae (Group IV)
    Eastern equine encephalomyelitis virus
    Western equine encephalomyelitis virus
    Venezuelan equine encephalomyelitis virus
    Chikungunya virus
    O'Nyong-nyong fever virus
    Ross River fever virus
    Barmah Forest virus
```

```
Tick-borne viruses.
  Bunyaviridae (Group V)
    Crimean-Congo hemorrhagic fever virus
  Flaviviridae (Group IV)
    Tick-borne encephalitis virus
    Powassan encephalitis virus
    Deer tick encephalitis virus
    Omsk hemorrhagic fever virus
    Kyasanur forest disease virus (Alkhurma virus)
    Langat virus
  Reoviridae (Group III)
    Colorado tick fever virus
```

The term "arbovirus" excludes viruses transmitted by nonarthropod vectors, such as rodents and bats[48] [Glossary Bat].

```
Rodent-borne viruses (roboviruses)
  Arenaviridae (Group V)
    Lassa fever
    Venezuelan hemorrhagic fever (Guanarito virus)
    Argentine hemorrhagic fever (Junin virus)
    Bolivian hemorrhagic fever (Machupo virus)
    Lujo virus
  Bunyaviridae (Group V)
    Puumala virus
    Andes virus
    Sin Nombre virus
    Hantavirus
Bat-borne viruses [48] [Glossary Bat].
  Filoviridae (Group V)
    Ebola hemorrhagic fever
    Marburg hemorrhagic fever
  Rhabdoviridae (Group V)
    Australian bat lyssavirus
    Rabies virus
    Mokola virus
    Duvenhage virus
    Lagos bat virus
    Duvenhage virus
```

It would seem that we do not know enough about the origin and phylogeny of the different classes of viruses to create a true classification, wherein viruses of a class share a common set of inherited relationships. There is, however, hope for a future in which viruses can be organized by phylogenetic principles. Highly innovative work in the field of viral phylogeny is proceeding, from a

variety of different approaches, including: inferring retroviral phylogeny by sequence divergences of nucleic acids and proteins in related viral species [16]; tracing the acquisition of genes in DNA viruses [41]; and dating viruses by the appearance of viral-specific antibodies in ancient host cells [12]. Because viruses evolve very rapidly, it is possible to trace the evolution of some viruses, with precision, over intervals as short as centuries or even decades [39, 42–44]. It should be noted that before the advent of ribosomal sequence analysis, and as recently as the early 1970s, bacterial phylogeny was considered a hopeless field [37]. Bacteria were grouped by morphology, nutritional requirements, and enzymatic reactions (e.g., hemolysis, coagulase, etc.) without much attention to phylogenetic relationships. The field of viral phylogeny is quickly catching up with the phylogeny of living organisms.

Section 7.3 Group I viruses: Double-stranded DNA

What trap is this? Where were its teeth concealed?

Philip Larkin, from his poem "Myxomatosis"

```
Group I, dsDNA
  Herpesvirales
    Herpesviridae
      Epstein-Barr virus
      Herpes simplex virus type
      Herpes simplex virus type
      Herpes virus varicella
      Herpesvirus simiae
      Human herpesvirus type
      Human herpesvirus type
      Human herpesvirus type
      Cytomegalovirus
  Unassigned
    Nonenveloped
      Adenoviridae
        Human adeonviruses A through G
      Papillomaviridae
        Human papillomavirus
      Polyomaviridae
        BK polyomavirus
        JC polyomavirus
        Simian virus
  Nucleocytoplasmic large DNA viruses (NCLDV viruses)
    Poxviridae
      Orthopoxvirus
```

```
          Buffalopox virus
          Cowpox virus
          Monkeypox virus
          Vaccinia virus
          Variola major virus
          Variola minor virus
        Parapoxvirus
          Orf
          Milker
        Molluscipoxvirus
          Molluscum contagiosum virus
        Yatapoxvirus
          Tanapoxvirus
          Yaba monkey tumor virus
      Mimiviridae
        Mimivirus
          Acanthamoeba polyphaga mimivirus
Group II, ssDNA
Group III, dsRNA
Group IV (+)ssRNA
Group V (-)ssRNA
Group VI, ssRNA-RT
Group VII, dsDNA-RT
```

The Group I viruses all have a double-stranded DNA genome. Aside from this property, these viruses vary greatly. Some species have envelopes; others do not. Some species have circular genomes; others have linear genomes. The size of the viral genome can vary as much as 50-fold among different species of the group. The host range covers the range of living organisms. Bacteria, archaeans, eukaryotes are infected by one or the other Group I viruses. The group has been subclassed based on shared morphologic properties, six of these subclasses contain human pathogens: Adenoviridae, Herpesviridae, Poxviridae, Papillomaviridae, Polyomaviridae, and Mimiviridae.

Most of the DNA transforming viruses (i.e., DNA viruses that cause cancer) belong to Group I: Polyomaviruses, Adenoviruses, Papillomaviruses, and Herpesviruses (including Epstein Barr virus). One exception is Hepatitis B virus, which belongs to Group VIII. Unlike the retroviruses (Group VI), which contain genes that are homologous with cancer-causing oncogenes, the DNA transforming viruses do not contain oncogenes. The Group I DNA transforming viruses seem to cause cancer through a mechanism related to their ability to induce replication in their host cells [Glossary Hepatitis viruses].

```
Group I, dsDNA
  Herpesvirales
    Herpesviridae
```

```
Epstein-Barr virus
Herpes simplex virus type
Herpes simplex virus type
Herpes virus varicella
Herpesvirus simiae
Human herpesvirus type
Human herpesvirus type
Human herpesvirus type
Cytomegalovirus
```

Members of Class Herpesviridae are commonly known as herpesviruses. These viruses produce acute disease characterized by lytic (i.e., cytopathic) effects in infected cells; and latent disease, characterized by recurrences of disease, sometimes spanning the life of the host. After cells are infected by virus particles, the viral genome migrates to the host nucleus, where replication and transcription of the viral genes occurs. After a latent phase, viruses may precipitate a lytic phase, manifesting as clinical disease. The recurring disease may be clinically distinct from the initial infection (e.g., chicken pox, the initial varicella virus infection, is followed decades later by shingles). Some of the herpesviruses are DNA transforming viruses.

The human herpesviruses are: Epstein-Barr virus, Herpes simplex viruses, Varicella virus, and Human herpesviruses 6, 7, and 8, and Cytomegalovirus.

Epstein-Barr virus infects almost all adults. Its persistence makes it one of the most prevalent human pathogens. It manifests acutely as mononucleosis, a pharyngitis accompanied by lymphocytosis (increases lymphocytes in the peripheral blood) with morphologic alterations in infected lymphocytes. Splenomegaly and hepatomegaly may occur. The generalized symptoms of the disease, particularly fatigue, may extend for months or longer, and some cases of mononucleosis recur. Epstein-Barr virus is a DNA transforming virus and accounts for several cancers, including Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and central nervous system lymphoma. A role for the virus in several autoimmune diseases has been suggested.

Herpes simplex type 1 causes cold sores, and Herpes simplex types 2 causes genital herpes. Both diseases may recur after initial infection.

As mentioned, Herpes virus varicella-zoster causes chickenpox on first infection and herpes zoster, also known as shingles, on reactivation.

Herpesvirus simiae, also known as B virus, infects macaque monkeys, without causing severe disease. In rare circumstances, humans may become infected with this virus, from the monkey reservoir. Human infection typically results in a severe encephalopathy.

Human herpesvirus type 6 (HHV6) and type 7 (HHV7) produce exanthem subitum, also known as roseola infantum and as sixth disease. Readers should not confuse sixth disease with fifth disease. Fifth disease, also known as erythema infectiosum and slapped face disease, is caused by

Parvovirus B19. These diseases take their names from an historical diagnostic dilemma faced by pediatricians, who regularly encountered six clinical syndromes of childhood rashes. Four of the childhood rashes had known etiologies. The fifth and sixth rashes, both caused by organisms that were not yet identified, were referred to as "fifth disease" and "sixth disease." Subsequently, the viral causes of these two diseases were discovered, but the numeric names held.

Human herpesvirus type 8 (HHV8) is a DNA transforming virus that can cause Kaposi sarcoma, primary effusion lymphoma, and some forms of Castleman disease. Kaposi sarcoma is a cancer characterized by focal proliferations of small blood vessels, occurring most often in the skin. Immunosuppressed patients (e.g., transplant recipients), who are carriers of the latent HHV8 virus, may develop Kaposi sarcoma within a few months of immunosuppression. Interestingly, if immunosuppression is halted, the Kaposi sarcoma may regress [49]. It is presumed that sustained viral replication is necessary for early tumor growth.

Cytomegalovirus infects about half of the world population, with most individuals suffering no ill effects. Once infected, the virus usually persists for the life of the individual. In a minority of cases, particularly among immunecompromised individuals (e.g., organ transplant recipients and AIDS patients) and newborns, the virus may produce severe neurologic disease. The disease is known as cytomegalic inclusion body disease, and, as the name suggests, cytoplasmic and nuclear inclusion bodies characterize actively infected cells. When the virus is transmitted transplacentally, by mothers infected during their pregnancy, the newborn may suffer developmental damage to the brain and other organs (Fig. 7.1).

```
Group I, dsDNA
  Unassigned
    Nonenveloped
      Adenoviridae
        Human adeonviruses A through G
      Papillomaviridae
        Human papillomavirus
      Polyomaviridae
        BK polyomavirus
        JC polyomavirus
        Simian virus
```

Class Adenoviridae contains the human adenoviruses of which there are 57 types, with different clinical syndromes associated with specific subtypes of the virus. Most adenoviral diseases present clinically as respiratory illness, conjunctivitis (i.e., viral conjunctivitis), or gastroenteritis. Infections may present clinically as tonsillitis (simulating strep throat), pharyngitis (croup), otitis media,

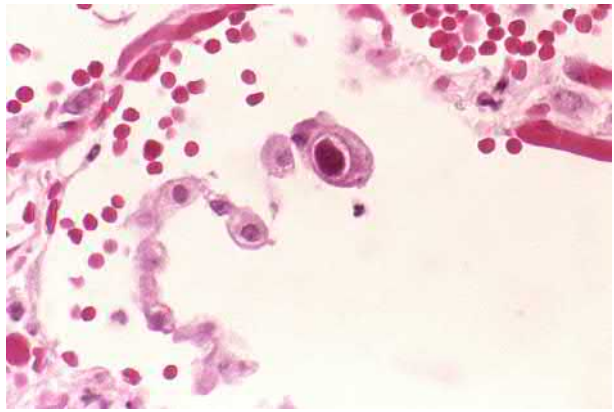

FIG. 7.1 Cytomegalovirus infection of the lung. Near the top, center of the image is an infected pneumocyte with a highly enlarged nucleus. The bulk of the nucleus is occupied by a dense inclusion, sometimes called Cowdry body, containing viral nucleocapsids. Surrounding the inclusion is a clear zone. Such nuclear inclusions, observed with all species of herpes viruses that infect humans, have long served as an important clue to the diagnosis and the pathogenesis of viral diseases. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention Public Health Image Library.)*

pneumonia, meningoencephalitis, and hemorrhagic cystitis. Adenoviruses are commonly spread by aerosolized droplets, and are particularly stable in the external environment.

Human papillomaviruses cause skin warts, laryngeal warts, and genital warts. Warts are benign tumors composed of proliferating squamous cells. In some cases, these human papillomavirus-induced warts progress to become invasive squamous cell carcinomas (Fig. 7.2).

Class Polyomaviridae contains several viruses that infect humans: BK polyomavirus, JC polyomavirus, and Simian virus 40.

The BK polyomavirus rarely causes disease in infected patients, and the majority of humans carry the latent virus. Latency can shift to lytic infection after immunosuppression, producing a clinical nephropathy.

The JC polyomavirus persistently infects the majority of humans, but it is not associated with disease in otherwise healthy individuals. Rarely, in immunecompromised patients, JC polyomavirus may produce progressive multifocal leukoencephalopathy. The virus targets myelin-producing oligodendrocytes in the brain to produce areas of demyelination and necrosis.

Simian virus 40 (SV40) infects monkeys and humans, but there is no evidence at this time confirming a role in human disease.

```
Group I, dsDNA
  Unassigned
    Nucleocytoplasmic large DNA viruses (NCLDV viruses)
```

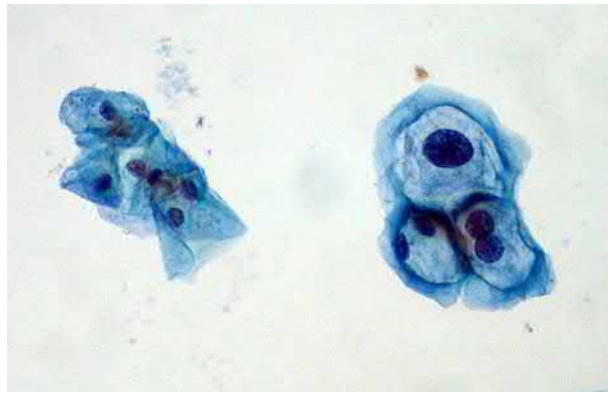

FIG. 7.2 Koilocytosis. The clump of flat epithelial cells on the left is normal squamous cells that line the uterine cervix. The clump of three cells on the right is squamous epithelial cells demonstrating koilocytosis, a cytopathic effect produced by human papillomavirus infection. Notice that the nuclei are enlarged, appearing approximately 2-3 times as large as normal nuclei (on left). Surrounding each nucleus (right clump) is an abnormal zone of pale cytoplasm, typical of koilocytosis. Beyond the pale zone is a thinner zone of normal-appearing cytoplasm extending out to the cell membrane. *(Source, Wikipedia, from a public domain image contributed by Euthman.)*

```
Poxviridae
  Orthopoxvirus
     Buffalopox virus
     Cowpox virus
     Monkeypox virus
     Vaccinia virus
     Variola major virus
     Variola minor virus
  Parapoxvirus
     Orf
     Milker
  Molluscipoxvirus
     Molluscum contagiosum virus
  Yatapoxvirus
     Tanapoxvirus
     Yaba monkey tumor virus
     Yaba
```

Members of Class Orthopoxvirus produce disease characterized by pustules of the skin, and lymphadenopathy.

The smallpox virus is remarkable for its extremely narrow host range: humans only. The virus infects the skin and the mucosa of the upper respiratory tract, where it produces a pustular, weeping, and rash. In the respiratory mucosa, the rash interferes with breathing. If the disease becomes hemorrhagic, the

prognosis worsens. Smallpox is reputed to have killed about 300 million people in the 20th century, prior to the widespread availability of an effective vaccine. Smallpox has been referred to as the greatest killer in human history. Its mortality rate was 30%–35%, which is significantly less than some of the hemorrhagic viruses (e.g., Ebola virus, Group V, has a mortality rate of nearly 90%). No doubt, death rates climbed because the disease was easily communicable via aerosols, fomites, bodily fluids, or direct contact with patients with rash.

Currently, smallpox has the distinction of being the only infection of humans which has been declared "eradicated." It should be noted that there are a few closed societies for which the status of smallpox in the population is unknown (e.g., North Korea). Aside from the remarkable success of vaccination, eradication was no doubt made possible because smallpox has no animal reservoir. At this time, vaccination is not routinely performed and is reserved primarily as an antiterror measure, for personnel entering a zone where there is a bioweapons threat.

Variola minor is a virus closely related to Variola major that produces a milder disease. These diseases are known by various names including alastrim, cottonpox, milkpox, whitepox, and Cuban itch. Infection with Variola minor is thought to produce cross-resistance to Variola Major (and vice versa).

Vaccinia virus is the laboratory-grown poxvirus, of obscure heritage, that does not precisely correspond to known viruses that reside outside the laboratory or clinic (i.e., not quite cowpox, not quite variola). Vaccinations with vaccinia virus have been known to produce, in rare cases, a variety of clinical disorders, ranging from vaccinia (localized pustular eruptions) to generalized vaccinia, to progressive vaccinia, to vaccinia gangrenosum, and to vaccinia necrosum. Other conditions associated with vaccinations include eczema vaccinatum and postvaccinial encephalitis).

Smallpox vaccination, aside from eradicating mankind's greatest killer, may have heretofore unrecognized public health value. The number of currently known pathogenic organisms, their variant subtypes, their ability to mutate, and the emergence of newly encountered pathogens make it impossible to develop vaccines for every organism that infects humans. Consequently, vaccine experts are searching for vaccines that confer immunity, partial or full, for several different pathogens or for several variants of a single pathogen [50]. An interesting development in this field is that the smallpox vaccine may confer limited protection against HIV (human immunodeficiency virus) infection. Both viruses enhance their infectivity by exploiting a receptor, CCR5, on the surface of white blood cells. This shared mode of infection may contribute to the cross-protection against HIV that seems to come from smallpox vaccine. It has been suggested that the emergence of HIV in the 1980s may have resulted, in part, from the cessation of smallpox vaccinations in the late 1970s [51].

Buffalopox, cowpox, and monkeypox produce diseases in animal reservoirs and rarely infect humans. Human infections occur from close contact with infected animals and manifest much like smallpox, but milder.

Members of Class Parapoxvirus infect vertebrates, particularly sheep, goats, cattle, and red squirrels. Orf virus causes "sore mouth" or "scabby mouth" disease of sheep and goats. Humans, though rarely infected, may develop painful hand sores. A similar condition can occur in humans who handle the udders of cows infected with Milker's nodule virus.

Class Molluscipoxvirus contains one species infectious in humans, Molluscum contagiosum virus. Molluscum contagiosum is an eruption of wart-like skin lesions that are easily diagnosed on histological examination by their distinctive cellular inclusions (so-called molluscum bodies). There are no known animal reservoirs. Infection is spread from human to human. Treatment is not always necessary, as individual lesions will regress within two months. However, autoinoculation of the virus may produce new skin lesions, thus prolonging the disease.

Members of Class Yatapoxvirus infect primates in equatorial Africa. Infections can spread to humans by insect vectors. Tana poxvirus produces a pock-forming skin infection, with fever and lymphadenopathy in infected humans (i.e., like a mild form of smallpox). The Yaba monkey tumor virus produces histiocytomas in monkeys. Histiocytomas are proliferative lesions of fibrous tissue that yield tumor-like nodules. These virally induced histiocytomas in monkeys grow rapidly following infection, and then regress over the ensuing month [52]. Yaba monkey tumor virus and Yaba-like disease virus, like all members of Class Yatapoxvirus, are considered potential human pathogens.

```
Group I, dsDNA
  Unassigned
    Nucleocytoplasmic large DNA viruses (NCLDV viruses)
      Mimiviridae
        Mimivirus
          Acanthamoeba polyphaga mimivirus
```

Class Mimiviridae, discovered in 1992, occupies a niche that seems to span the biological gulf separating living organisms from viruses. Members of Class Mimiviridae are complex, larger than some bacteria, with enormous genomes (by viral standards), exceeding a million base pairs and encoding upwards of 1000 proteins. The large size and complexity of Class Mimiviridae exemplifies the advantage of a double-stranded DNA genome. DNA is much more chemically stable than RNA, and can be faithfully replicated, even when its length exceeds a billion base pairs. A double-stranded DNA genome can be protected by DNA repair enzymes, and by external modifications to the DNA structure. Class Megaviridae is a newly reported (October 2011) class of viruses, related to Class Mimiviridae, but larger [45].

As previously noted, the life of a mimivirus is not very different from that of obligate intracellular bacteria (e.g., Rickettsia). The discovery of Class Mimiviridae inspires biologists to reconsider the "nonliving" status relegated to viruses and compels taxonomists to examine the placement of viruses within the phylogenetic development of prokaryotic and eukaryotic organisms.

Acanthamoeba polyphaga mimivirus is a possible human pathogen. Some patients with pneumonia have been shown to have antibodies against the virus [53].

Though Myxoma virus is not a human pathogen, it seems appropriate to include some mention of this member of Class Poxviridae, due to the role humans have played in its history. Myxoma virus produces a fatal disease, myxomatosis, in rabbits. The disease is characterized by the rapid appearance of skin tumors (myxomas), followed by severe conjunctivitis, systemic symptoms, and fulminant pneumonia. Death usually occurs in 2-14 days after infection. In 1952, a French virologist, hoping to reduce the rabbit population on his private estate, inoculated a few rabbits with Myxoma virus. The results were much more than he had bargained for. Within two years, 90% of the rabbit population of France had succumbed to myxomatosis.

European rabbits, introduced to Australia in the 19th century, became feral and multiplied. By 1950 the rabbit population of Australia was about 3 billion. Seizing upon the Myxoma virus as a solution to rabbit overpopulation, the Australians launched a Myxoma virus inoculation program. In less than 10 years, the Australian rabbit population was reduced by 95% [54]. Nearly 3 billion rabbits died, a number very close to the number of humans living on the planet in the mid-1950s. This plague on rabbits was unleashed by a committee of humans who decided that it was proper to use a lethal rabbit virus as a biological weapon. Without commenting on the moral implications of animal eradication efforts, it is worth noting that rabbits are not the only mammals that can be exterminated by a pathogenic virus. Humans should take heed.

Infectious Genera

Adenoviridae species

- **Lineage**. dsDNA viruses, no RNA stage: Adenoviridae: Mastadenovirus: unclassified Human adenoviruses: Human adenovirus sp.
- **Infection**. Human adenoviruses (produce infections of respiratory tract, pharyngitic and pneumonic, plus conjunctival, gastroenteritic, or bacteremic infections)

```
Human adenovirus A, types 12, 18, 31
Human adenovirus B, types 3, 7, 11, 14, 16, 21, 34-35, 50, 55
Human adenovirus C, types 1, 2, 5-6, 57
Human adenovirus D, types 8-10, 13, 15, 17, 19-20, 22, 23-30,
32-33, 36-39, 42-49, 51, 53-54, 56
Human adenovirus E, type 4
Human adenovirus F, types 40-41
Human adenovirus G, type 52
```

Herpesviridae

- **Lineage**. dsDNA viruses, no RNA stage: Herpesvirales: Herpesviridae: Gammaherpesvirinae: Lymphocryptovirus: Human gammaherpesvirus 4 (Epstein-Barr virus)

- **Infection**. Epstein-Barr virus (include infections such as mononucleosis, and neoplasms such as Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and central nervous system lymphoma, as well as various autoimmune diseases)
- **Infection**. Herpes simplex types 1 (cold sores)
- **Infection**. Herpes simplex types 2 (genital herpes)
- **Infection**. Herpes virus varicella-zoster (chickenpox on first infection, herpes zoster or shingles on re-activation)
- **Infection**. Herpesvirus simiae, also known as B virus (encephalopathy)
- **Infection**. Human herpesvirus type 6, HHV6 (exanthem subitum, roseola infantum, sixth disease)
- **Infection**. Human herpesvirus type 7, HHV7 (exanthem subitum, roseola infantum, sixth disease; suggested but disputed cause of pityriasis rosea)
- **Infection**. Human herpesvirus type 8, HHV8 (Kaposi sarcoma, primary effusion lymphoma, Castleman's disease)
- **Infection**. Cytomegalovirus, also known as Human herpesvirus 5 (cytomegalic inclusion body disease)

Orthopoxvirus

- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Orthopoxvirus: Variola virus
- **Infection**. Variola major (smallpox)
- **Infection**. Variola minor (alastrim, cottonpox, milkpox, whitepox, and Cuban itch)
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Orthopoxvirus: Vaccinia virus
- **Infection**. Vaccinia virus (vaccinia, generalized vaccinia, progressive vaccinia, vaccinia gangrenosum, vaccinia necrosum, eczema vaccinatum, postvaccinial encephalitis)
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Orthopoxvirus: Vaccinia virus: Buffalopox virus
- **Infection**. Buffalopoxvirus (Buffalopox)
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Orthopoxvirus: Monkeypox virus
- **Infection**. Monkeypox virus (Monkeypox)
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Orthopoxvirus: Cowpox virus
- **Infection**. Cowpox virus (Cowpox)

Molluscipoxvirus

- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Molluscipoxvirus: Molluscum contagiosum virus
- **Infection**. Molluscum contagiosum virus (Molluscum contagiosum)

Parapoxvirus

- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Parapoxvirus
- **Infection**. Milker's nodule virus (Milker's nodes)
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Parapoxvirus: Orf virus
- **Infection**. Orf virus (Orf, also known as ecthyma contagiosum)

Yatapoxvirus

- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Yatapoxvirus: Tanapox virus
- **Infection**. Tanapox virus(mild pock-forming skin infection) [52]
- **Lineage**. dsDNA viruses, no RNA stage: Poxviridae: Chordopoxvirinae: Yatapoxvirus: Yaba monkey tumor virus
- **Infection**. Yaba monkey tumor virus (regressing histiocytoma) [52]

Papillomaviridae

- **Lineage**. dsDNA viruses, no RNA stage: Papillomaviridae
- **Infection**. Human papillomavirus (warts, genital warts, laryngeal papillomas, squamous carcinoma)

Polyomaviridae

- **Lineage**. dsDNA viruses, no RNA stage: Polyomaviridae
- **Infection**. BK polyomavirus or BK virus (nephropathy in immune-compromised individuals)
- **Infection**. JC polyomavirus or JC virus (progressive mulifocal leukoencephalopathy in immune-compromised individuals)
- **Infection**. Simian virus 40 or SV40 virus (highly controversial potential cause of human cancer)

Mimiviridae:

- **Lineage**. dsDNA viruses, no RNA stage: Mimiviridae: Mimivirus
- **Infection**. Acanthamoeba polyphaga mimivirus (pneumonia)

Section 7.4 Group II viruses: Single-stranded (+) sense DNA

Everything should be made as simple as possible, but not simpler.

Albert Einstein

```
Group I, dsDNA
Group II, ssDNA
  Parvoviridae
    Bocavirus
```

```
    Human parvovirus B
Group III, dsRNA
Group IV (+)ssRNA
Group V (-)ssRNA
Group VI, ssRNA-RT
Group VII, dsDNA-RT
```

Group II, The single-stranded DNA viruses, contains only one family of viruses that are pathogenic in humans: Class Parvoviridae. Class Parvoviridae contains environmentally resistant viruses that infect a wide range of animals. Parvoviruses are the smallest viruses currently known.

```
Group II, ssDNA
  Parvoviridae
    Bocavirus
    Human parvovirus B
```

Parvovirus B19 is the agent that causes fifth disease, so named because the diseases was the fifth type of common childhood exanthem among six rashes listed in textbooks. Until the 1980s, the cause of this fifth listed exanthem was unknown; so it came to be known as "fifth disease." Other names for fifth disease are erythema infectiosum and slapped face disease. Human herpesviruses 6 and 7 cause the sixth childhood rash, "sixth disease." The rash of fifth disease results from an immune response of the host to the virus particles. Essentially, fifth disease is an allergic phenomenon, and not the direct, cytopathic effect of the virus.

Infection by parvovirus occurs from contact (usually via respiratory droplets) with actively infected hosts. The virus is known to infect humans and dogs. Serologic evidence indicates that at least half of the human population has been infected with parvovirus B19.

Members of Class Parvoviridae characteristically grow in rapidly dividing host cells, using host processes to support their own replication. The target cells for the parvovirus B19 are the dividing precursor erythroid cells. Another name for parvovirus B19 is erythrovirus B19, indicating the target cell for the virus. In the active stage of infection, huge amounts of virus are produced. Death or dysfunction of the target hematopoietic (blood precursor) cells can lead to a transient pancytopenia (i.e., anemia of all blood cell lineages). In rare cases, aplastic anemia may occur, in which most of the precursor erythroid cells are destroyed, leading to a massive decline in circulating mature forms. When aplastic anemia occurs, it usually occurs in individuals who have a concurrent condition that requires an excessive production of blood cells to maintain the normal blood profile of mature cells. These conditions include: autoimmune hemolytic anemia, sickle cell anemia, and inherited blood dyscrasias that increase the fragility of red blood cells or that decrease the life span of red blood cells. Basically, a coinfection with parvovirus B19 is the last straw for bone marrows that are barely keeping pace with the body's demand for erythrocytes.

The intense viremia that occurs in parvovirus B19 infection, and the small size of parvovirus particles, may predispose to cross-placental transmission occurring in some cases of infection in pregnant women. Though rare, parvovirus may cause miscarriage or hydrops fetalis (fluid accumulation in the fetus) with anemia.

Bocavirus has been associated with some cases of respiratory disease and diarrhea in young children. Though it is rarely detected in healthy persons, there is indication that it can occur in up to 9% of pediatric patients hospitalized with lower respiratory infections [55]. Bocavirus should not be confused with Bocas virus, a type of Coronavirus (Group IV).

SEN virus (SEN-V) is a newly discovered single-stranded nonenveloped DNA virus that has been found in the blood of donors and recipients of transfusion blood [56]. In addition, another Group II virus, TT virus, also known as Transfusion Transmitted virus or Torque teno virus, has been isolated from transfusion blood. TT virus is currently a suspected hepatitis virus. At this time, the pathogenicity of both SEN-V and TT viruses are in doubt; hence neither virus is included in the list of Group II virus pathogens.

Infectious Genera

Bocavirus

- **Lineage**. ssDNA viruses: Parvoviridae: Parvovirinae: Bocaparvovirus (Bocavirus)
- **Infection**. Bocavirus (respiratory disease and diarrhea in children)

Human parvovirus

- **Lineage**. ssDNA viruses: Parvoviridae: Parvovirinae: Erythroparvovirus: Primate erythroparvovirus 1: Human parvovirus B19
- **Infection**. Human parvovirus B19, alternately known as erythrovirus B19 (Fifth disease, erythema infectiosum, slapped face disease, transient hemolytic anemia, aplastic anemia)

Section 7.5 Group III viruses: Double-stranded RNA

What is essential is invisible to the eye.

Antoine de Saint-Exupery

```
Group I, dsDNA
Group II, ssDNA
Group III, dsRNA
  Reoviridae
    Rotavirus
    Coltivirus
    Orbivirus
Group IV (+)ssRNA
```

```
Group V (-)ssRNA
Group VI, ssRNA-RT
Group VII, dsDNA-RT
```

The Group III viruses have a double-stranded RNA genome. Replication of Group III viruses takes place exclusively in the cytoplasm, where the viral RNA codes for the proteins needed for viral replication. Viral proteins are synthesized using the host cell machinery (i.e., ribosomes).

Group III contains six major classes, of which only one contains organisms that are infectious to humans: Class Reoviridae. The name derives from "Respiratory enteric orphan" viruses. The term "orphan," when applied to a virus indicates that no known diseases are associated with the virus. This is no longer the case for the Reoviruses.

```
Group III, dsRNA
  Reoviridae
    Rotavirus
    Coltivirus
    Orbivirus
```

The most clinically significant species in Class Reoviridae is rotavirus. In 2004, rotavirus infections accounted for about a half million deaths in young children, from severe diarrhea [57]. Most of the deaths occurred in developing countries. The death rate is expected to decline due to the introduction of an apparently safe and effective vaccine [57]. Rotavirus, when observed with transmission electron microscopy, resembles a wagon wheel. It was formerly known as gastroenteritis virus type B. It is passed from human to human by fecal-oral route (Fig. 7.3).

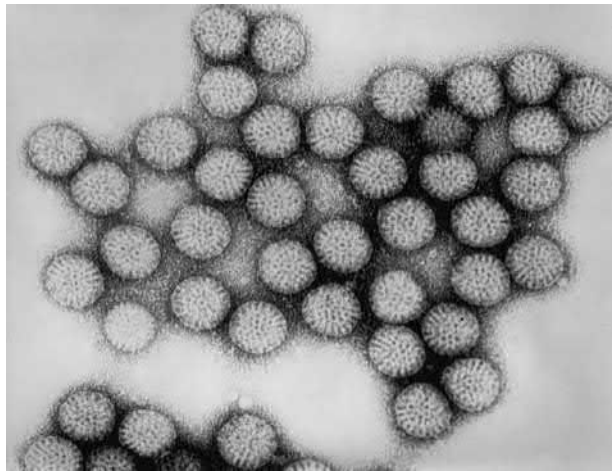

FIG. 7.3 Transmission electron micrograph of rotavirus particles.*(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention, prepared by Dr. Erskine Palmer. Rotavirus_TEM_B82-Rotavirus_TEM_B82-.)*

Aside from rotavirus, there are three genera that infect humans, of which two produce disease. The Orothoreoviruses infect vertebrates, including humans, but no disease has been linked to the infections. The two disease-producing infectious genera are Coltivirus and Orbivirus.

Colorado tick fever is endemic in the Rocky Mountains (in contradistinction to Rocky Mountain spotted fever, a rickettsial infection that has no restricted affinity for the Rocky Mountains). Coltivirus takes its name from the disease (i.e., COLorado TIck fever VIrus). As the name suggests, Colorado tick fever is carried by a tick (in this case, Dermacentor andersoni) and produces a fever. The fever is often accompanied by myalgia, headache, and photophobia. In a small percentage of children with Colorado tick fever, encephalitis may follow.

Orbiviruses have been implicated in several rather obscure fever-associated conditions: Kemerovo fever, found in Western Siberia and transmitted by ticks; Orungo fever, found in Central African and transmitted by mosquitoes; and Changuinola fever, found in Northern South America and transmitted by sand flies of Class Phlebotomus.

Human rotavirus

- **Lineage**. dsRNA viruses: Reoviridae: Sedoreovirinae: Rotavirus
- Human rotavirus (gastroenteritis, diarrhea)

Coltivirus

- **Lineage**. dsRNA viruses: Reoviridae: Spinareovirinae: Coltivirus
- Coltivirus (Colorado tick fever)

Orbivirus

- **Lineage**. dsRNA viruses: Reoviridae: Sedoreovirinae: Orbivirus
- Orbivirus (Colorado tick fever)

Section 7.6 Group IV viruses: Single-stranded (+) sense RNA

Simplicity is the ultimate sophistication.

Leonardo da Vinci

```
Group I, dsDNA
Group II, ssDNA
Group III, dsRNA
Group IV (+)ssRNA
  Nidovirales
    Coronaviridae
     SARS virus
     Torovirus species
```

Hepeviridae:
  Hepatitis E
Caliciviridae:
  Norovirus
  Sapporo virus
Togaviridae
  Alphavirus Viral diseases
    Chikungunya
    Eastern equine encephalomyelitis virus
    Getah virus
    Mayaro virus
    Mucambo virus
    O'nyong'nyong virus
    Ross river virus
    Barmah forest virus
    Sagiyama virus
    Semliki forest virus
    Sindbis virus
    Tonate virus
    Venezuelan equine encephalomyelitis virus
    Western equine encephalomyelitis virus
  Rubivirus
    Rubella virus
Flaviviridae
  Hepacivirus
    Hepatitis C
  Flavivirus
    Dengue virus types
    Hepatitis G virus
    Japanese B encephalitis virus
    Murray Valley encephalitis virus
    Rocio virus
    Spondweni virus
    St Louis encephalitis
    Wesselsbron
    West Nile virus
    Yellow fever virus
    Tick-borne virus group:
      Absettarov
      Hanzalova
      Hypr
      Kumlinge
      Kyasanur forest disease
      Louping ill

```
          Negishi
          Omsk
          Powassan
          Langat
          Russian spring summer encephalitis
      Hepatitis G virus group
        Hepatitis G virus
    Picornaviridae
      Enterovirus:
        Coxsackievirus
        Echovirus
        Poliovirus
        Enterovirus
        Rhinovirus A and B
      Hepatovirus:
        Hepatitis A
    Astroviridae
      Astrovirus species
Group V (-)ssRNA
Group VI, ssRNA-RT
Group VII, dsDNA-RT
```

The Group IV viruses have a positive sense RNA genome. Positive sense RNA can be translated directly into protein, without a DNA intermediate and without creating a complementary RNA strand. To replicate its genome, though, a complementary RNA strand is required. The positive RNA strand serves as a template for an RNA-dependent RNA polymerase, yielding a complementary RNA strand, to form a dimer with the template strand. The double-stranded RNA subsequently serves as the template for a new positive sense genome.

The positive-strand RNA genome is independently infectious, for most Group IV viruses. This means that in the absence of a capsid, envelope, or enclosed proteins, the RNA molecule, when inserted into a cell, is capable of using host cell machinery to construct additional viruses. Such viruses can be extremely small. In an experiment conducted in the late 1960s, Sol Spiegelman and his coworkers developed a method by which smaller and smaller viral RNA molecules could be isolated that were capable of replicating if provided with a specific RNA-dependent RNA polymerase and substrate nucleotides. A minimalist infectious viral genome was eventually selected which was only 220 nucleotides (bases) in length [58].

As discussed in the overview, we know very little about the phylogenetic relationship among viruses. Consequently, we subclassify the Group IV viruses based on structural similarities: symmetry of capsid, presence of absence of a viral envelope, and size. There are six subclasses of the Group IV single-stranded positive-sense RNA viruses: Picornaviridae, Togaviridae, Coronaviridae, Hepeviridae, Caliciviridae, Flaviviridae, and Astroviridae. As expected, within

each class, viruses share structural similarities; but there are no properties, other than the defining property of a (+)sense single-stranded RNA genome, that extends to all six classes of the Group IV viruses. For example, some classes have envelopes (i.e., Flaviviridae, Togaviridae, Coronaviridae), and others do not (i.e., Caliciviridae, Picornaviridae, Hepeviridae, and Astroviridae).

```
Group IV (+)ssRNA
  Nidovirales
    Coronaviridae
      SARS virus
      Torovirinae
        Human torovirus
```

Members of Class Nidovirales do not package polymerase in the viral particle. The genome is read directly using host enzymes. Class Nidovirales contains one subclass with viruses that infect humans: Class Coronaviridae. The coronaviruses are characterized by gylocoprotein spikes (peplomers) that protrude from the envelope that encloses the round nucleocapsid. The arrangement of peplomers resembles a corona (hence, coronavirus).

SARS (severe acute respiratory syndrome) virus produces a severe flu-like illness, and is spread by close human contact. The earliest outbreak of SARS occurred in Southeast Asia, in 2002. Soon thereafter, cases occurred in distant locations, including Toronto, Canada, and Bangalore, India. The worldwide response to SARS was possibly the most intensive public health effort ever launched to stem an epidemic of a new, and potentially fatal, viral disease. By 2004, China, the epicenter of the fledgling epidemic, was declared SARS free.

Toroviruses infect a variety of mammals. Human torovirus is a rare cause of enteritis.

```
Group IV (+)ssRNA
  Hepeviridae:
    Hepatitis E
```

Class Hepeviridae is a tentative class, with just one genus and one species, the Hepatitis E virus. It is possible that the Hepatitis E virus will be reassigned to an existing subclass of Group IV or will be assigned to a newly named subclass.

Hepevirus should not be confused with the orthographically similar "herpesvirus."

Also, readers should not confuse Class Hepeviridae (Hepatitis E virus) with Class Hepacivirus, a subclass of Class Flaviviridae that contains the Hepatitis C virus. Neither of these Group IV subclasses should be confused with Class Hepadnaviridae (Group VII).

```
Group IV (+)ssRNA
  Caliciviridae
    Norovirus
    Sapporo virus
```

Class Caliciviridae contains small nonenveloped viruses, 35–40 nm in diameter, just a tad larger than members of Class Picornaviridae. They take their name from the Latin root, calyx, meaning goblet, referring to the cup-shaped capsid depressions. Members of Class Caliciviridae cause acute gastroenteritis in humans.

```
Group IV (+)ssRNA
  Togaviridae
    Alphavirus Viral diseases
      Chikungunya
      Eastern equine encephalomyelitis virus
      Getah virus
      Mayaro virus
      Mucambo virus
      O'nyong'nyong virus
      Ross river virus
      Barmah forest virus
      Sagiyama virus
      Semliki forest virus
      Sindbis virus
      Tonate virus
      Venezuelan equine encephalomyelitis virus
      Western equine encephalomyelitis virus
    Rubivirus
      Rubella virus
```

Class Togaviridae is named for its distinctive coat (the "toga"). Togaviruses have a genome approximately 12 kbases in length, somewhat larger than the genome of Class Picornaviridae. Togaviruses live in the cytoplasm of their host cells, where viral replication and gene expression take place. Class Togaviridae contains two subclasses: Class Alphavirus and Class Rubivirus. All the members of Class Alphavirus are arboviruses (arthropod-borne viruses) spread by mosquitoes (primarily) or ticks. Class Rubivirus contains only one species that is infective in humans: Rubella virus, the cause of German measles. Rubella is spread directly from person to person, without an insect vector. Readers should not confuse Rubella virus with the measles virus, Rubeola. Rubeola virus is a paramyxovirus (Group V), unrelated to Rubella virus.

A few of the alphaviruses typify the group. Chikungunya is a disease that produces a clinical syndrome similar to that seen with Dengue virus (Class Flaviviridae), Ross river virus and Barmah forest virus; namely, an acute febrile phase followed by a prolonged arthralgic phase. Chikungunya fever is spread by the Aedes mosquito, and the reservoir is human (i.e., transmission is human to mosquito to human). In recent years, the incidence of Chikungunya has recently increased in Asia and Africa and is now an emerging disease in the Europe [47].

Ross river virus and Barmah forest virus produce clinically and geographically indistinguishable diseases, sometimes referred to collectively as epidemic

polyarthritis. The diseases are spread by various species of mosquito, and both are endemic to Australasia. They produce an acute influenza-like illness, followed by arthralgia. Joint pains may persist for many months. The reservoir for both viruses seems to be, primarily, marsupials.

```
Group IV (+)ssRNA
  Flaviviridae
    Hepacivirus
     Hepatitis C
  Flavivirus
     Dengue virus types
     Japanese B encephalitis virus
     Murray Valley encephalitis virus
     Rocio virus
     Spondweni virus
     St Louis encephalitis
     Wesselsbron
     West Nile virus
     Yellow fever virus
     Tick-borne virus group:
       Absettarov virus
       Hanzalova virus
       Hypr virus
       Kumlinge virus
       Kyasanur forest disease virus
       Louping ill
       Negishi virus
       Omsk virus
       Powassan
       Langat
       Russian spring summer encephalitis
  Hepatitis G virus group
     Hepatitis G virus
```

The members of Class Flaviviridae are enveloped, spherical, and have a diameter of about 50 nm. Most members of Class Flaviviridae are arthropod-borne, being transmitted by a tick (Class Chelicerata) or a mosquito (Class Hexapoda). The subclasses of Class Flaviviridae that contain infectious viruses in humans are: Hepacivirus, Flavivirus, Tick-borne virus group, and Hepatitis G virus group.

Hepatitis C virus is the only member of Class Hepacivirus known to cause human disease. Hepatitis C is a common cause of hepatitis and chronic liver disease. It is spread from person to person by sexual transmission, by contact with infected blood, or blood products, and can be spread by contaminated needles. It can be transmitted to the infants born to infected mothers. People who develop hepatitis from this virus often develop chronic infection of the

liver, which varies from person to person in intensity and in the likelihood of progressing to cirrhosis. Over 1% of the US population is infected with Hepatitis C.

Class Flavivirus (from the Latin "flavus," meaning yellow) is a subclass of Class Flaviviridae, both named for the yellow (jaundiced) skin resulting from infection with its most notorious species, Yellow fever virus. The flaviviruses include some of the most common and deadly viruses on earth, led by Yellow fever virus and Dengue fever virus. Among the flaviviruses are numerous encephalitis-producing viruses that have specific geographic distributions: Japanese encephalitis virus (mosquito-borne), Murray Valley encephalitis virus (mosquito-borne), St. Louis encephalitis virus (mosquito-borne), West Nile encephalitis virus (mosquito-borne), and a host of viruses collectively known as Tick-borne encephalitis viruses.

Yellow fever virus seems to have originated in Africa and spread to other continents in the mid-17th century. It is responsible for hundreds of thousands of deaths in North America alone. The disease is carried by primates, including humans, and transmitted from person to person by the bite of a mosquito (Aedes aegypti). It produces hepatitis and hemorrhaging (hence, it is included among the hepatitis viruses and the hemorrhagic fever viruses).

Yellow fever virus is associated with an impressive number of medical breakthroughs, being the first disease demonstrated to be transmitted by an arthropod, among the first diseases shown to be caused by a virus, and among the first infections controlled by a live vaccine. Effective methods of yellow fever prevention, through the eradication of the Aedes aegypti were developed in the 1890s, and an effective vaccine was developed in the 1930s. Today, there are about 200,000 cases of yellow fever worldwide, with about 30,000 deaths [59]. Most infections occur in Africa.

While the incidence of yellow fever has diminished over the past century, the incidence of dengue fever is increasing. Dengue, like yellow fever, is transmitted primarily by the Aedes aegypti mosquito. More than 50 million dengue virus infections occur each year worldwide. Most infections are asymptomatic or cause only mild disease, but a minority of infections are severe and may cause death. Typical cases exhibit fever and intense pain in muscles and joints (hence the alternate name of the disease, breakbone fever). Fevers can come and go. Capillary permeability is a common feature of the disease, and this may result in petecchiae, the egress of fluid from the vascular compartment, shock (so-called Dengue shock syndrome), and hemorrhage (hemorrhagic syndrome). Severe cases of dengue, if untreated, may have a fatality rate approaching 20%. Like yellow fever, Dengue is included in the group of hemorrhagic viruses.

The Hepatitis G virus group, in Class Flaviviridae, contains only one species, Hepatitis G virus, which had been traditionally included among the named hepatitis viruses. Hepatitis G is now considered to be an "orphan virus" (i.e., a virus that has no associated disease). The Hepatitis G virus is found in a small percentage of donated blood units.

```
Group IV (+)ssRNA
  Picornaviridae
    Enterovirus:
    Coxsackievirus
    Echovirus
    Poliovirus
    Enterovirus
    Rhinovirus A and B
  Hepatovirus:
    Hepatitis A
```

Members of Class Picornaviridae have a small RNA genome, as small as 7 kbases (i.e., 7000 nucleotides) in length. The picornaviruses include two subclasses: Enterovirus and Hepatovirus.

Members of Class Enterovirus are among the most prevalent human pathogens. In active infections, the virus can often be recovered from feces and respiratory secretions. Poliovirus, spread by contaminated fecal material, produces a paralytic syndrome characterized by inflammation and destruction of the anterior horn cells of the spinal cord. Many poliovirus infections do not result in disease, but disease-free infected individuals are carriers, transiently producing infective virus. Aside from poliovirus, many of the enteroviruses display neurotropism, producing aseptic meningitis and flacid paralysis [60].

There are a huge number of serotypes in Class Enterovirus, spread among the Coxsackievirus, Echovirus, and Enterovirus genera. They produce nonspecific flulike illnesses, and various strains produce distinctive syndromes such as hand-foot-mouth disease, herpangina, and hemorrhagic conjunctivitis, and Bornholm disease (epidemic pleurodynia). Enterovirus infections are common pediatric maladies. Infections in newborns can be severe, with hepatitis, encephalitis, and sepsis.

Class Enterovirus also includes Genus Rhinovirus, which contains more than 100 variant strains. The rhinoviruses account for most instances of the common cold.

Class Hepatovirus contains Hepatitis A, a cause of hepatitis. As you would expect from a member of Class Enterovirus, Hepatitis A is typically spread by the fecal-oral route. The resulting hepatitis is acute, and generally subsides without sequelae. Unlike Hepatitis B and C, Hepatitis A does not progress to chronic hepatitis and cirrhosis.

```
Group IV (+)ssRNA
  Astroviridae
    Astrovirus species
```

Members of Class Astroviridae, like those of Class Picornaviridae and Class Caliciviridae, lack an envelope. The class contains one species that is pathogenic in humans: Astrovirus. Astrovirus causes enteritis. Infections are especially common in children, accounting for more than 5% of enteritis cases in the pediatric age group.

Infectious Genera

Enterovirus

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Picornavirales: Picornaviridae: Enterovirus
- **Infection**. Coxsackievirus (flu-like illness, hand-food-mouth disease, herpangina, hemorrhagic conjunctivitis, aseptic meningitis; in newborns, myocarditis, meningoencephalitis, hepatitis)
- **Infection**. Echovirus, Enteric Cytopathic Human Orphan virus (flu-like illness, aseptic meningitis; in newborns, severe myocarditis hepatitis, and systemic infection)
- **Infection**. Poliovirus (polio)
- **Infection**. Enterovirus 68–109 (flu-like illnesses and other syndromes associated with Coxsackievirus and Echovirus)
- **Infection**. Rhinovirus (common cold)

Hepatovirus

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Picornavirales: Picornaviridae: Hepatovirus
- **Infection**. Hepatitis A (hepatitis A)

Alphavirus

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Togaviridae: Alphavirus
- **Infection**. Chikungunya virus, a member of Semliki Forest virus complex (Chikungunya fever, rash, arthritis)
- **Infection**. Eastern equine encephalomyelitis virus (Encephalitis)
- **Infection**. Getah virus (asymptomatic in humans)
- **Infection**. Mayaro virus, a member of Semliki Forest virus complex (rash, arthritis)
- **Infection**. Mucambo virus (encephalitis)
- **Infection**. O'nyong'nyong virus, a member of Semliki Forest virus complex (rash, arthritis)
- **Infection**. Ross river virus, a member of Semliki Forest virus complex (epidemic polyarthritis)
- **Infection**. Barmah forest virus (epidemic polyarthritis)
- **Infection**. Sagiyama virus, a subtype of Ross River virus (asymptomatic in humans)
- **Infection**. Semliki forest virus, a member of Semliki Forest virus complex (rash, arthritis)
- **Infection**. Sindbis virus (Sindbis fever, rash, arthritis)
- **Infection**. Tonate virus (encephalitis)
- **Infection**. Venezuelan equine encephalomyelitis virus (encephalitis, often causing, in humans, a flu-like illness with high fever and headache)
- **Infection**. Western equine encephalomyelitis virus (Western equine encephalomyelitis)

Rubivirus

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Togaviridae: Rubivirus
- **Infection**. Rubella virus (German measles)

Coronaviridae

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Nidovirales: Cornidovirineae: Coronaviridae
- **Infection**. SARS virus
- **Infection**. Torovirus species (gastroenteritis)

Hepeviridae

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Hepeviridae
- **Infection**. Hepatitis E (hepatitis)

Caliciviridae

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Caliciviridae
- **Infection**. Norovirus, formerly Norwalk virus (epidemic gastroenteritis)
- **Infection**. Sapporo virus (mild gastroenteritis in children)

Hepacivirus

- **Lineage**. ssRNA positive-strand viruses, no DNA stage: Flaviviridae: Hepacivirus
- **Infection**. Hepacivirus C (Hepatitis C)

Flavivirus

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Flaviviridae: Flavivirus
- **Infection**. Dengue virus types 1–4 (dengue fever; severe form is called dengue hemorrhagic fever)
- **Infection**. Japanese encephalitis virus (encephalitis, with less than 1% of human infections leading to disease)
- **Infection**. Murray Valley encephalitis virus (Murray Valley encephalitis, formerly known as Australian encephalitis)
- **Infection**. Rocio virus (meningoencephalitis)
- **Infection**. Spondweni virus (acute febrile illness)
- **Infection**. St Louis encephalitis (encephalitis)
- **Infection**. Wesselsbron virus (fever, flu-like illness, most infections are subclinical)
- **Infection**. West Nile virus (West Nile fever)
- **Infection**. Yellow fever virus (yellow fever)

Tick-borne encephalitis group of flaviviruses

- **Infection**. Absettarov virus (encephalitis)
- **Infection**. Hanzalova virus (encephalitis)
- **Infection**. Hypr virus (encephalitis)
- **Infection**. Kumlinge virus (fever and encephalitis)
- **Infection**. Kyasanur forest disease virus (hemorrhagic fever)
- **Infection**. Louping ill virus (tick-borne encephalitis)
- **Infection**. Negishi virus (encephalitis)
- **Infection**. Omsk virus (hemorrhagic fever)
- **Infection**. Powassan virus (tick-borne encephalitis)
- **Infection**. Langat virus (tick-borne encephalitis)
- **Infection**. Russian spring summer encephalitis virus (encephalitis)

Hepatitis G virus group

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Flaviviridae: Pegivirus: Pegivirus C: GB virus C
- **Infection**. Hepatitis G virus, alternately GB virus CF ("orphan virus" not associated with any human disease)

Astroviridae:

- **Lineage**. ssRNA viruses: ssRNA positive-strand viruses, no DNA stage: Astrovirid
- **Infection**. Astrovirus species (gastroenteritis)

Section 7.7 Group V viruses: Single-stranded (–) sense RNA

An inefficient virus kills its host. A clever virus stays with it.

James Lovelock

```
Group I, dsDNA
Group II, ssDNA
Group III, dsRNA
Group IV (+)ssRNA
Group V (-)ssRNA
  Mononegavirales (nonsegmented)
    Paramyxoviridae
      Henipavirus
        Hendra virus
        Nipah virus
      Rubulavirus
        Mumps virus
        Parainfluenza types
```

```
    Morbillivirus
      Measels virus
    Avulavirus
      Newcastle disease virus
    Metapneumovirus
    Pneumovirus
      Respiratory syncytial virus
    Parainfluenza Types
  Rhabdoviridae
    Lyssavirus
      Duvenhage
      Rabies virus
    Vesiculovirus
      Vesicular stomatitis
      Chandipura
  Filoviridae
    Marburg virus
      Marburg virus
    Ebolavirus
      Ebola Reston
      Ebola Siena
      Ebola Sudan
      Ebola Zaire
  Bornaviridae
    Bornavirus
Unassigned Classes
  Arenaviridae
    Mammarenavirus
    Lassa_virus complex:
      Lassa virus
      Lujo virus
      Lymphocytic choriomeningitis virus
      Mobala virus
      Mopeia virus
    Tacaribe-virus complex:
      Amapari virus
      Chapare virus
      Flexal virus
      Guanarito virus
      Junin virus
      Latino virus
      Machupo virus
      Oliveros virus
      Parana virus
```

```
          Pichinde virus
          Pirital virus
          Sabia virus
          Tacaribe virus
          Tamiami virus
          Whitewater Arroyo virus
      Bunyaviridae
        Akabane virus
        Bunyamwera virus
        California encephalitis virus
        Hantavirus
          Hantavirus Belgrade virus
          Hantavirus Hantaan virus
          Hantavirus Prospect Hill virus
          Hantavirus Puumala virus
          Hantavirus Seoul virus
          Hantavirus Sin Nombre virus
        Nairovirus
          Nairovirus Bhanja virus
          Nairovirus Crimean virus
          Nairovirus Hazara virus
          Oropouche virus
          Phlebovirus
            Rift Valley Fever
            Pappataci fever virus
        Orthomyxoviridae
          Influenza
          Thogotovirus
            Tick-borne orthomyxoviridae Dhori
            Tick-borne orthomyxoviridae Thogoto
    Unassigned genus
      Deltavirus
        Hepatitis delta virus
Group VI, ssRNA-RT
Group VII, dsDNA-RT
```

Single-stranded RNA viruses are grouped into viruses with a positive sense, negative sense, and ambisense.

Positive sense RNA viruses are those that have the same direction as human mRNA. Positive sense virus genes can be translated (to yield protein) with the same cellular machinery that translates mRNA. These are the Group IV viruses.

Negative sense RNA is complementary to mRNA. Negative sense RNA must be converted to positive sense RNA or to DNA before it becomes biologically available for translation or replication, respectively.

The Group V viruses are single-stranded negative sense RNA viruses that use an RNA-dependent RNA polymerase, packaged within the virus particle, to produce positive sense RNA, within the host cell. The Group VI viruses are single-stranded negative sense RNA viruses that use an RNA-dependent DNA polymerase (so-called reverse transcriptase), packaged within the virus particle, to produce a complementary strand of DNA. The synthesized strand of DNA is subsequently used as a template to yield a double-stranded DNA molecule that contains the genetic information from the viral genome.

The ambisense single-strand RNA viruses contain at least one positive sense RNA segment admixed with negative sense RNA. Genomically, an ambisense virus is a concatenation of a Group IV virus (positive sense single-stranded) and a negative sense virus. Nevertheless, transcription in ambisense viruses is coupled with translation of the viral genome to a complementary RNA strand, a process that is characteristic of the Group V viruses [61]. Consequently, the ambiviruses are currently included in the "unassigned" subclasses of the Group V viruses.

Among the Group V RNA viruses pathogenic to humans, there is one assigned class of viruses, Class Monongavirales, which includes the following subclasses: Bornaviridae, Filoviridae, Paramyxoviridae, and Rhabdoviridae. The remaining Class V viruses belong to unassigned subclasses (i.e., with no named taxonomic superclass) or unassigned genera (i.e., belonging to no assigned class). The unassigned viruses include the ambisense viruses. The unassigned classes are: Arenaviridae, Bunyaviridae, and Orthomyxoviridae. Deltavirus is an unassigned genus.

The Group V viruses are numerous, and it would be unproductive to describe each virus in detail. The following discussion will include viral disorders that typify their class or that highlight recent findings that might have been omitted from previously published virology texts. A listing of the Group V viruses, along with their associated diseases and clinical conditions, arranged by subclass, is provided at the end of this section.

```
Group V (-)ssRNA
  Mononegavirales (nonsegmented)
    Paramyxoviridae
      Henipavirus
        Hendra virus
        Nipah virus
      Rubulavirus
        Mumps virus
        Parainfluenza types
      Morbillivirus
        Measels virus
      Avulavirus
        Newcastle disease virus
      Metapneumovirus
```

```
    Pneumovirus
      Respiratory syncytial virus
    Parainfluenza Types
  Rhabdoviridae
    Lyssavirus
      Duvenhage
      Rabies virus
    Vesiculovirus
      Vesicular stomatitis
      Chandipura
    Filoviridae
      Marburg virus
        Marburg virus
      Ebolavirus
        Ebola Reston
        Ebola Siena
        Ebola Sudan
        Ebola Zaire
    Bornaviridae
      Bornavirus
```

Class Paramyxoviridae includes the viruses that cause measles (Rubeola or Morbilli virus) and mumps. Measles remains one of the most fatal virus disease, in terms of deaths worldwide. In 2001, measles virus accounted for about 745,000 deaths. Class Paramyxoviridae also includes several viruses that account for many respiratory diseases, particularly in children: the parainfluenza viruses, respiratory syncytial virus, and (somewhat less severe, clinically) human metapneumovirus.

Class Rhabdoviridae includes Rabies virus, a species of lyssavirus (from Lyssa, the Greek goddess of madness).

Class Filoviridae includes Ebola virus, Marburg virus. Members of Class Filoviridae infect primates (including humans) and produce potentially fatal viral hemorrhagic fevers.

Class Bornaviridae contains one species that infects humans, Borna virus. This virus, which infects a variety of animals, is neurotropic, producing nervous system inflammation (e.g., meningoencephalitis), neurologic impairment (e.g., ataxia), and behavior disorders (e.g., excitation or depression) in infected animals.

The role of Bornavirus in humans is undetermined at this time, but Bornavirus infections have been implicated as a cause of mental illnesses in humans, including bipolar disorder. It is the only member of Class Mononegavirales that replicates inside the host nucleus. Bornavirus has recently gained scientific attention as the only nonretrovirus that has been shown to integrate (permanently) into the mammalian genome [11]. Inherited

fragments of bornavirus have been found in various types of mammals, suggesting that bornavirus is not new [12].

```
Group V (-)ssRNA
  Arenaviridae
    Mammarenavirus
      Lassa_virus complex:
        Lassa virus
        Lujo virus
        Lymphocytic choriomeningitis virus
        Mobala virus
        Mopeia virus
      Tacaribe-virus complex:
        Amapari virus
        Chapare virus
        Flexal virus
        Guanarito virus
        Junin virus
        Latino virus
        Machupo virus
        Oliveros virus
        Parana virus
        Pichinde virus
        Pirital virus
        Sabia virus
        Tacaribe virus
        Tamiami virus
        Whitewater Arroyo virus
  Bunyaviridae
    Akabane virus
    Bunyamwera virus
    California encephalitis virus
    Hantavirus
      Hantavirus Belgrade virus
      Hantavirus Hantaan virus
      Hantavirus Prospect Hill virus
      Hantavirus Puumala virus
      Hantavirus Seoul virus
      Hantavirus Sin Nombre virus
    Nairovirus
      Nairovirus Bhanja virus
      Nairovirus Crimean virus
      Nairovirus Hazara virus
       Oropouche virus
```

```
    Phlebovirus
      Rift Valley Fever
      Pappataci fever virus
  Orthomyxoviridae
    Influenza
    Thogotovirus
      Tick-borne orthomyxoviridae Dhori
      Tick-borne orthomyxoviridae Thogoto
```

Along with the bunyaviruses, the viruses in Class Arenaviridae account for the majority of roboviruses (rodent-borne viruses). In general, each arenavirus infects a specific species of rodent. Rodents occasionally transmit the virus to humans, either through aerosolized excreta (e.g., urine, feces) or by direct contact of infectious material with cuts and abrasions in human skin. Most of the diseases caused by bunyaviruses are encephalitides, hemorrhagic fevers, or nonhemorrhagic fevers; the severity vary with the viral species and host resistance.

The arenaviruses have been separated by geographic locale into two groups: New World Viruses and Old world viruses. Old world arenaviruses and their approximate locales are: Ippy virus (Central African Republic), Lasssa virus (West Africa), Lymphocytic choriomeningitis virus (worldwide), Mobala virus (Central African Republic), and Mopeia virus (Mozambique).

The New World arenaviruses and their approximate locales are: Amapari virus (Brazil), Flexal virus (Brazil), Guanarito virus (Venezuela), Junin virus (Argentina), Latino virus (Bolivia), Machupo virus (Bolivia), Oliveros virus (Argentina), Parana virus (Paraguay), Pichinde virus (Columbia), Pirital virus (Venezuela), Sabia virus (Brazil), Tacaribe virus (Trinidad), Tamiami virus (Florida, USA), and Whitewater Arroyo virus (New Mexico, USA). Several of the arenaviruses isolated from humans have not, as yet, been associated with human disease; these species are omitted from the list of infectious diseases associated caused by arenaviruses (vida infra).

One member of Class Arenaviridae, Lassa virus, the cause of Lassa fever, should not be confused with Lyssa virus, a member of Class Rhabdoviridae and the cause of rabies.

Class Bunyaviridae along with Class Arenaviridae account for the majority of Roboviruses (rodent-borne viruses). In addition, like the virus syndromes produced by members of Class Arenaviridae, the members of Class Bunyaviridae tend to produce fever syndromes, hemorrhagic fever syndromes, or meningoencephalitides.

Class Orthomyxoviridae includes Influenza virus (types A, B, and C) and the thogotoviruses. Seasonal influenza kills between a quarter million and a half million people worldwide each year. In the United States seasonal

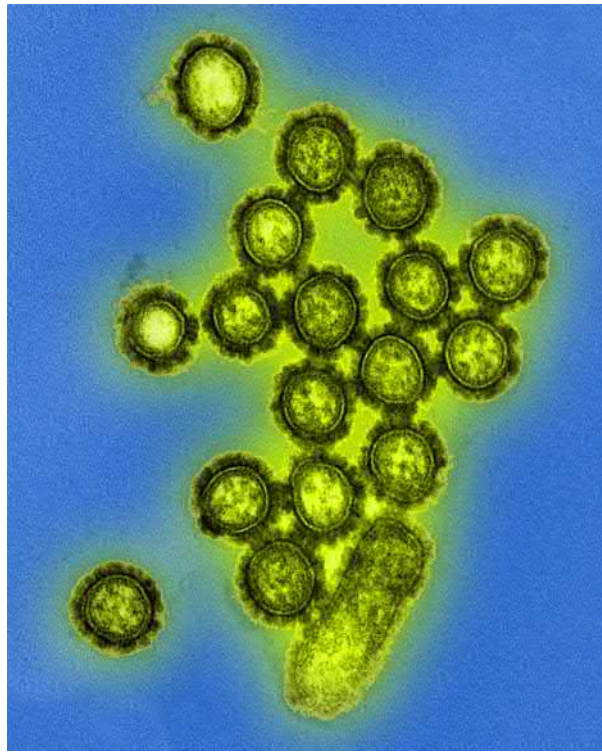

FIG. 7.4 H1N1 influenza (swine flu) viruses. *(Source, a public domain image provided by the U.S. National Institute of Allergy and Infectious Diseases.)*

influenza accounts for about 40,000 deaths annually. The 1917–18 influenza pandemic caused somewhere between 50 million and 100 million deaths (Fig. 7.4).

The thogotoviruses (Dhori virus and Thogoto virus) infect ticks, and ticks transmit the infection to humans. Thogotoviruses produce fever and encephalitis.

```
Group V (-)ssRNA
  Unassigned Classes
    Unassigned genus
      Deltavirus
        Hepatitis delta virus
```

Genus Deltavirus is a genus in Group V that has not been assigned a viral class. The genus contains one species, the Hepatitis D virus. The Hepatitis D virus cannot replicate on its own. Replication requires the presence of Hepatitis B virus in the same host cell. Coinfections of Hepatitis B and Hepatitis D viruses (sometimes referred to as Labrea fever) have a more severe clinical course than infections with

Hepatitis B alone (e.g., increased likelihood of developing liver failure, shortened time interval for initial infection to the development of cirrhosis, increased likelihood of developing liver cancer, and overall increased mortality rate).

Infectious Genera

Paramyxoviridae

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Henipavirus: Hendra henipavirus (also known as equine morbillivirus)
- **Infection**. Hendra virus, a henipavirus transmitted by Pteropid fruit bats (pulmonary edema and hemorrhage, encephalitis)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Henipavirus: Nipah henipavirus
- **Infection**. Nipah, a henipavirus transmitted by Pteropid fruit bats (encephalitis, relapse encephalitis)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Morbillivirus: Measles morbillivirus
- **Infection**. Measles virus (measles, rubeola, morbilli)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Rubulavirus: Mumps rubulavirus
- **Infection**. Mumps virus (mumps)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Avulavirus: Avian avulavirus 1 (Newcastle disease virus)
- **Infection**. Newcastle disease virus, transmitted by infected birds (conjunctivitis and flu-like illness)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Paramyxoviridae: Respirovirus: Human respirovirus 1
- **Infection**. Human Parainfluenza virus 1, HPIV-1 (most common cause of croup; also other upper and lower respiratory tract illnesses typical)
- **Infection**. Human Parainfluenza virus 2, HPIV-2 (causes croup and other upper and lower respiratory tract illnesses)
- **Infection**. Human Parainfluenza virus 3, HPIV-3 (associated with bronchiolitis and pneumonia)
- **Infection**. Human Parainfluenza virus 4, HPIV-4 (mild respiratory infections, often clinically silent)

Pneumoviridae

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Pneumoviridae: unclassified Pneumoviridae
- **Infection**. Respiratory syncytial virus, RSV (pneumonia, pneumovirus pneumonia)

- **Lineage**. ssRNA viruses: ssRNA negative-strand viruses: Mononegavirales: Pneumoviridae: Metapneumovirus
- **Infection**. Human metapneumonovirus (mild respiratory illness in healthy individuals, occasionally severe respiratory illness in children, elderly, and immune-compromised individuals)

Rhabdoviridae: Lyssavirus

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Rhabdoviridae: Lyssavirus: Duvenhage lyssavirus
- **Infection**. Duvenhage (rabies-like encephalitis)

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Rhabdoviridae: Lyssavirus: Rabies lyssavirus
- **Infection**. Rabies virus (rabies, hydrophobia, lyssa)

Rhabdoviridae: Vesiculovirus

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Rhabdoviridae: Vesiculovirus: unclassified Vesiculovirus: Vesicular stomatitis virus
- **Infection**. Vesicular stomatitis virus (flu-like illness in humans)

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Rhabdoviridae: Vesiculovirus: Chandipura vesiculovirus: Chandipura virus
- **Infection**. Chandipura (encephalitis)

Filoviridae:

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Filoviridae: Ebolavirus: Zaire ebolavirus: Ebola virus
- **Infection**. Ebola virus (Ebola hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Filoviridae: Marburg virus
- **Infection**. Marburg virus (Marburg hemorrhagic fever)

Bornaviridae:

- **Lineage**. ssRNA negative-strand viruses: Mononegavirales: Bornaviridae: Orthobornavirus
- **Infection**. Bornavirus, alternately Borna disease virus (Borna disease in mammals, possible cause of mental illness, including bipolar disorder in humans)

Mammarenavirus

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Lassa mammarenavirus
- **Infection**. Lassa virus (Lassa fever, multisystem disease characterized by hyperpyrexia, coagulopathy, hemorrhaging, and necrosis of liver and spleen)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Lujo mammarenavirus
- **Infection**. Lujo virus (viral hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Lymphocytic choriomeningitis mammarenavirus
- **Infection**. Lymphocytic choriomeningitis virus (lymphocytic choriomeningitis)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Chapare mammarenavirus
- **Infection**. Chapare virus (hemorrhagic fever) [62]

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Flexal mammarenavirus
- **Infection**. Flexal virus (flu-like illness)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Guanarito mammarenavirus
- **Infection**. Guanarito virus (Venezuelan hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Argentinian mammarenavirus
- **Infection**. Junin virus (Argentine hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Machupo mammarenavirus
- **Infection**. Machupo virus (Bolivian hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Oliveros mammarenavirus
- **Infection**. Oliveros virus (severe hemorrhagic fever in South America) [63]

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Brazilian mammarenavirus (Sabio virus)
- **Infection**. Sabia virus (Brazilian hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Whitewater Arroyo mammarenavirus
- **Infection**. Whitewater Arroyo virus (hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Wenzhou mammarenavirus
- **Infection**. Wenzhou virus (transmitted by rodents and shrews, of undetermined human pathogenicity)

- **Lineage**. ssRNA negative-strand viruses: Arenaviridae: Mammarenavirus: Mopeia mammarenavirus
- **Infection**. Mopeia virus (transmitted by Mastomys natalensis rodents, of undetermined human pathogenicity)

Bunyaviridae:

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Peribunyaviridae: Orthobunyavirus: Bunyamwera orthobunyavirus: Bunyamwera virus
- **Infection**. Bunyamwera virus (headache systemic symptoms, and rash)

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Peribunyaviridae: Orthobunyavirus: California encephalitis orthobunyavirus: California encephalitis virus
- **Infection**. California encephalitis virus (encephalitis)

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Hantaviridae: Orthohantavirus
- **Infection**. Hantavirus Belgrade, also known as Hantavirus Dobrava-Belgrade (hemorrhagic fever with renal syndrome)
- **Infection**. Hantavirus Hantaan (Korean hemorrhagic fever, hemorrhagic fever with renal syndrome)
- **Infection**. Hantavirus Prospect Hill (not associated with human disease)
- **Infection**. Hantavirus Puumala (hemorrhagic fever with renal syndrome)
- **Infection**. Hantavirus Seoul (hemorrhagic fever with renal syndrome)
- **Infection**. Hantavirus Sin Nombre (formerly Muerto Canyon)

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Nairoviridae: Orthonairovirus
- **Infection**. Nairovirus Bhanja (rare, flu-like illness ranging from mild to severe disease)
- **Infection**. Nairovirus Crimean (Congo haemorrhagic fever, Crimean-Congo hemorrhagic fever)
- **Infection**. Nairovirus Hazara (hemorrhagic fever)

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Peribunyaviridae: Orthobunyavirus: Oropouche orthobunyavirus: Oropouche virus
- **Infection**. Oropouche virus (Oroopouche fever, characterized by fever with systemic symptoms)

Phlebovirus:

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Phenuiviridae: Phlebovirus: Rift Valley fever phlebovirus: Rift Valley fever virus
- **Infection**. RVF virus (Rift Valley fever)

- **Lineage**. ssRNA negative-strand viruses: Bunyavirales: Phenuiviridae: Phlebovirus: unclassified Phlebovirus: Sandfly fever Sicilian virus
- **Infection**. Pappataci fever virus (Pappataci fever, phlebotomus fever, sandfly fever, three-day fever)

Orthomyxoviridae:

- **Lineage**. ssRNA negative-strand viruses: Orthomyxoviridae: unclassified Orthomyxoviridae: unidentified influenza virus
- **Infection**. Infuenza types A, B, and C (viral influenza)

Thogotovirus

- **Lineage**. ssRNA negative-strand viruses: Orthomyxoviridae: Thogotovirus: Dhori thogotovirus
- **Infection**. Tick-borne orthomyxoviridae Dhori (fever, encephalitis)

- **Lineage**. ssRNA negative-strand viruses: Orthomyxoviridae: Thogotovirus: Thogoto thogotovirus
- **Infection**. Tick-borne orthomyxoviridae Thogoto (respiratory disease)

Deltavirus:

- **Lineage**. ssRNA negative-strand viruses: Deltavirus: Hepatitis delta virus
- **Infection**. Hepatitis delta virus (high mortality hepatitis when co-infected or superinfected with Hepatitis B virus)

Section 7.8 Group VI viruses: Single-stranded RNA reverse transcriptase viruses with a DNA intermediate in life cycle

The origin of retroviruses is lost in a prebiotic mist.

Patric Jern, Goran Sperber, Jonas Blomberg [16]

```
Group I, dsDNA
Group II, ssDNA
Group III, dsRNA
Group IV (+)ssRNA
Group V (-)ssRNA
Group VI, ssRNA-RT
  Retroviridae
    Deltaretrovirus
      Human T-cell lymphotropic virus
    Lentivirus
      Human immunodeficiency virus
Group VII, dsDNA-RT
```

Single-stranded RNA viruses can be positive sense or negative sense. Positive sense RNA, for example, eukaryotic mRNA and Group IV viruses, can be directly translated to produce protein. Negative sense RNA is complementary to mRNA. Negative sense RNA must be copy-converted to positive sense RNA or to DNA before becoming biologically available for translation or replication, respectively.

The Group VI viruses are single-stranded negative sense RNA viruses that use an RNA-dependent DNA polymerase (so-called reverse transcriptase), packaged within the virus particle to produce a complementary strand of DNA. The synthesized strand of DNA is subsequently used as a template to yield a double-stranded DNA molecule containing the genetic information from the viral genome. Group VI viruses can integrate this double-stranded DNA into the host genome. The Group VI viruses are referred to as retroviruses.

The Group V viruses, like the Group VI viruses, are single-stranded negative sense RNA viruses. These viruses do not employ reverse transcriptase. Instead,

they use an RNA-dependent RNA polymerase, packaged within the virus particle, to produce positive sense RNA within the host cell. The positive sense RNA is subsequently used to synthesize proteins.

Group VI viruses share their genetic legacy with the human genome. About 8% of human genes are retroviral. Human DNA of retroviral origin is referred to as endogenous retrovirus, or as a retroviral provirus. Retroviruses in the external environment, capable of infecting eukaryotic host cells, are referred to as exogenous retrovirus (i.e., a retrovirus that is outside the gene).

Despite the legacy of retroviruses within the genome of eukaryotic cells, there are only a few exogenous retroviruses that cause infectious disease in humans. The Group VI human pathogens are restricted to one class of retroviruses, Class Retroviridae, and to two genera within this class: Deltaretrovirus and Lentivirus.

```
Group VI, ssRNA-RT
  Retroviridae
    Deltaretrovirus
      Human T-cell lymphotropic virus
    Lentivirus
      Human immunodeficiency virus
```

Genus Deltaretrovirus contains four human t-cell lymphotropic viruses: HTLV-1, HTLV-2, HTLV-3, and HTLV-4. Of these four viruses that infect humans, only HTLV-1 virus has been associated with human disease. Infection with HTLV-1 greatly increases the risk of developing adult T-cell leukemia/lymphoma, with about 1 out of every 25 infected individuals eventually developing the disease). HTLV-1 has also been implicated as a cause of a human myelopathic condition, tropical spastic paraparesis. Although millions of individuals have been infected by HTLV-1, worldwide, fewer than 2% of infected individuals will develop an HTLV-1 associated myelopathic condition.

Readers should be careful not to confuse Class Deltaretrovirus (a class of Group VI retroviruses) with Class Deltavirus, a Group V single-strand RNA negative-strand virus containing Hepatitis delta virus.

Genus Lentivirus contains the HIV, which produces HIV infection and the syndrome of associated diseases known as AIDS.

Readers should not confuse HTLV-III, a virus discovered in 2005, and which is not known at this time to produce disease in infected humans, with an early name (long since abandoned) that was assigned to the HIV virus.

Infectious Genera

Deltaretrovirus

– **Lineage**. Ortervirales: Retroviridae: Orthoretrovirinae: Deltaretrovirus: unclassified Deltaretrovirus: Untyped Human T-lymphotropic virus: Human T-cell lymphotropic virus

– **Infection**. HTLV-1 (some cases of T-cell leukemia and T-cell lymphoma in adults, HTLV-1 associated myelopathy/tropical spastic paraparesis or HAM/TSP)

Lentivirus

– **Lineage**. Ortervirales: Retroviridae: Orthoretrovirinae: Lentivirus: Primate lentivirus group: unclassified Primate lentivirus group: Human immunodeficiency virus
– **Infection**. Human immunodeficiency virus (HIV infection and AIDS)

Section 7.9 Group VII viruses: Double-stranded DNA reverse transcriptase viruses

The human genome is a living document of ancient and now extinct viruses.

Michael Emerman and Harmit Malik [12]

```
Group I, dsDNA
Group II, ssDNA
Group III, dsRNA
Group IV (+)ssRNA
Group V (-)ssRNA
Group VI, ssRNA-RT
Group VII, dsDNA-RT
  Hepadnaviridae
    Orthohepadnavirus
      Hepatitis B
```

Group VII viruses are double-stranded DNA viruses that can integrate their DNA into the host genome, using a reverse transcriptase enzyme. The reverse transcriptase enzyme acts upon RNA, transcribed from viral DNA, as the template for genomic DNA. Hence, the Group VII viruses are an unusual type of retrovirus that do not belong to Class Retroviridae (Group VI), because the genome is double-stranded DNA. Likewise, the Group VII viruses are not classed within the double-stranded DNA viruses (Group I) because their replication requires the synthesis of an RNA intermediate.

```
Group VII, dsDNA-RT
  Hepadnaviridae
    Orthohepadnavirus
      Hepatitis B
```

Group VII has one class of viruses that is pathogenic in humans: Class Hepadnaviridae. Hepadnaviruses (short for HEPAtic DNA VIRUS) have a small, circular DNA genome. Class Hepadnaviridae contains one viral species that is pathogenic in humans: hepatitis B virus.

Hepatitis B infects more than 200 million people, worldwide, causing 2 million deaths each year. Deaths are due to acute or chronic hepatitis or due to ensuing conditions such as cirrhosis and hepatocellular carcinoma. Infection is spread from infected persons through contact with body fluids (e.g., sexual intercourse, through inoculation with contaminated needles or tattoo instruments, or through the use of contaminated blood transfusion products) [Glossary Blood contamination].

As a virus that can insert part of its genome into host DNA, you might expect that it would be oncogenic (able to produce cancers). You would be correct. Hepatocellular carcinoma occurs in about 10% of infected patients who develop chronic hepatitis. Hepatitis B is the only DNA transforming virus that is not a Group I virus. Sections of the viral genome, inserted into host DNA, persist in cells of the hepatocellular carcinomas that eventually develop.

In addition to causing hepatitis B, the hepatitis B virus is essential for the replication of hepatitis delta virus. Hepatitis delta virus (Hepatitis D virus) is an RNA virus of Group V. As previously noted, the hepatitis delta virus is a defective virus that cannot replicate without the help of the hepatitis B virus, which produces the protein coat for hepatitis delta virus. A coinfection with hepatitis B and hepatitis D produces a more aggressive disease than that produced with hepatitis B alone [64].

Infectious Genera

Hepadnaviridae

- **Lineage**. Retro-transcribing viruses: Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus
- **Infection**. Hepatitis B (acute hepatitis, chronic hepatitis, cirrhosis, hepatocellular carcinoma)

Glossary

Adaptive immunity Immunity in which the response adapts to the specific chemical properties of foreign antigens. Adaptive immunity is a system wherein somatic T cells and B cells are produced, each with a unique and characteristic immunoglobulin (in the case of B cells) or T-cell receptor (in the case of T cells). Through a complex presentation and selection system, a foreign antigen elicits the replication of a B cell that produces an antibody whose unique immunoglobulin attachment site matches the antigen. Antigen-antibody complexes may deactivate and clear circulating antibodies, or may lead to the destruction of the organism that carries the antigen (e.g., virus or bacteria).

The process of producing unique proteins requires that recombination and hypermutation take place within a specific gene region. Recombinations yield on the order of about a billion unique somatic genes, starting with one germinal genome. This process requires the participation of recombination-activating genes (RAGs). The acquisition of an immunologically active recombination-activating gene from a retrovirus is presumed to be the key evolutionary event that led to the development of the adaptive immune system. This event, which occurred in one of the early species of gnathostomes (jawed vertebrates),

established the adaptive immune system in all jawed vertebrates and their descendants. As one might expect, inherited mutations in RAG genes cause immune-deficiency syndromes [65, 66].

Bat A bat is not a flying mouse and is not a member of Class Rodentia, flying or otherwise. Bats are mammals of Class Chiroptera. With forelimbs that have evolved into wings, they are the only mammals capable of sustained, self-propelled flight. Their relevance in this book stems from their status as viral vectors. Currently, bat populations of many species are being decimated by Geomyces destructans, a fungus in Class Ascomycota, the cause of white-nose syndrome. Apparently, the fungal infection, which grows in cold conditions, awakens bats from their deep hibernation; starvation results.

Blood contamination When a blood donor is infected with a pathogenic organism, the disease can be passed to the recipient. Examples of organisms and diseases that can be spread through blood transfused blood or blood components include:

Human Immunodeficiency Virus
Human T-Lymphotropic Viruses type I and type II
Hepatitis A
Hepatitis B
Hepatitis C
Hepatitis E
Cytomegalovirus
Epstein-Barr Virus
Human Parvovirus B19
Human Herpesvirus 6
Human Herpesvirus 8
TT virus or Transfusion Transmitted Virus or Torque teno virus [67]
SEN Virus [56]
CJD and vCJD
Syphilis
Malaria [68]
Chagas Disease
African trypanosomiasis
Toxoplasmosis
Leishmaniasis
Babesiosis
Rocky Mountain Spotted Fever
Ehrlichiosis

Capsid The protein shell of a virus that encloses the genetic material of the virus when the virus is outside its host cell. The capsid aids the virus with its attachment to the target host cell, and with the penetration of the viral genome into the host cell.

Evolvability Evolution by natural selection is not a physiological process, like respiration or replication. The theory of evolution is little more than a restatement of a probabilistic truism, in biological terms. Namely, organisms that are most likely to survive will be the organisms most likely to reproduce. Biologists speak in terms of evolvability to indicate certain factors that may tip the evolutionary scales in an organism's favor.

For the most part, these features all involve mechanisms by which new or modified genetic material that may serve as the source of new genes, is obtained. These would include:

- Having mechanisms for horizontal gene transfer
- Having mechanisms for increasing the rate of mutation under environmentally stressful circumstances (e.g., radiation, heat, cold)
- Tendency toward endoduplication of genes
- Having large, diverse gene pool
- Presence of pseudogenes and junk DNA

Hepatitis viruses Several of the viruses that cause hepatitis are provided with names that are easy to remember but impossible to reconcile as a coherent biological class. These are Hepatitis A, B, C, D, E, F, and G. Pathogenic viruses that attack any particular organ need not all belong to the same biological class, and the named hepatitis viruses are no exception, belonging to Groups IV, V, and VII. In addition, not all pathogenic viruses that infect the liver belong to the named hepatitis viruses. Yellow fever virus, which has killed millions of people throughout history, is a Group IV hepatitis virus.

Here is a list of the named hepatitis viruses:

- Hepatitis A virus is a member of Class Picornaviridae (Group IV).
- Hepatitis B virus is a member of Class Hepadnaviridae (Group VII).
- Hepatitis C virus is a member of Class Flaviviridae (Group IV), the same class that contains yellow fever virus, which also produces hepatitis.
- Hepatitis D is a member of an unassigned class in Group V.
- Hepatitis E virus is a member of class Hepeviridae (Group IV).
- Hepatitis F virus is a hypothetical organism, supposedly responsible for some cases of hepatitis that cannot be diagnosed under any of the nonimaginary taxa.
- Hepatitis G virus is now thought to be the same virus as GB virus C, a virus not known to produce any human disease.

Phenetics An approach to classification wherein objects are grouped together based on a shared set of physical features. In this book, we make the argument that phenetics is an improper approach for biological classifications insofar as two distantly related species may share a physical similarity (e.g., ability to fly or aquatic life) without having any close ancestral relationship, without having a close genetic relationship, and without sharing many metabolic pathways. As the biologist George Gaylord Simpson put it, "Individuals do not belong in the same taxon because they are similar, but they are similar because they belong to the same taxon" [69].

Transposon Also called transposable element, and informally known as jumping gene. The name "transposable element" would seem to imply that a fragment of the genome (i.e., the transposable element) physically moves from one point in the genome to another. This is not the case. What actually happens (in the case of Class II transposons) is that a copy of the DNA sequence of the transposon is inserted elsewhere in the genome, resulting in the sequence now occupying two different locations in the genome. In the case of Class II transposons, the DNA sequence of the transposon is translated into RNA, then reverse-transcribed as DNA, and reinserted at another location; likewise resulting in two of the same sequence in two locations in the genome [70]. You can see how transposable elements might bloat the genome with repeated elements. Some transposons are the ancient remnants of retroviruses and other horizontally transferred genes that insinuated their way into the eukaryotic genome. Because transposon DNA is not necessary for cell survival, the sequences of transposons are not conserved, and mutations occurring over time yield

degenerate sequences that no longer function as retroviruses. A role for transposons in the altered expression of genes in cancer cells has been suggested [71].

Virion The infective form of a virus outside the host cell. Viruses can be thought of as having two alternating forms: the virion and the virocell.

Virocell The name given to a host cell that has been commandeered by a virus to devote its cellular machinery to the mass production of virus particles. When we think of viruses a living organisms, it is best to envisage the virocell, which consists of a viral factory built from the wreckage of the host cells; and not the virion, which is simply a vehicle for transporting a virus safely from host to host.

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Chapter 8

Changing how we think about infectious diseases

Section 8.1 Abandoning Koch's postulates

It isn't that they can't see the solution. It's that they can't see the problem.

G.K. Chesterton

Robert Koch's postulates, published in 1890, are a set of criteria that establish whether a particular organism is the cause of a particular disease. Today, Koch's postulates are taught in high school and college classrooms as a demonstration of the rigor and legitimacy of clinical microbiology. To review, the four postulates of Koch are as follows:

–1. The microorganism must be found in the diseased animal, and not found in healthy animals.

–2. The microorganism must be extracted and isolated from the diseased animal and subsequently grown in culture.

–3. The microorganism must cause disease when introduced to a healthy experimental animal.

–4. The microorganism must be extracted from the diseased experimental animal and demonstrated to be the same microorganism that was originally isolated from the first diseased animal.

Let's go over these four postulates once more, this time explaining how they ignore or contradict what we now know about infectious diseases.

–1. The microorganism must be found in the diseased animal, and not found in healthy animals.

As previously discussed, lots of pathogenic organisms are found in healthy animals, producing disease in only a tiny fraction of the individuals who are infected. For example, Bartonella species can live in blood without causing disease, producing an asymptomatic bacteremia in the wide assortment of animals that they may infect. Hence, we can no longer assume that blood samples from healthy animals are sterile. The mechanism of Bartonella transmission from animal to animal is not fully understood, but arthropod vectors (ticks, fleas, and

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lice) are suspected, as well as scratches and bites from infected animals (e.g., cats and rats) [1] [Glossary Vector].

There are now about eight species of Bartonella that are known or suspected to be human pathogens. Until just a few decades ago, only two such species were known. Today, the species of Bartonella, which are ubiquitous among mammals, are known or suspected to cause a variety of phenotypically dissimilar diseases [1]:

- Bartonella bacilliformis → Carrion disease
- Bartonella quintana → Bacillary angiomatosis, trench fever, endocarditis
- Bartonella henselae → Bacillary angiomatosis, cat-scratch disease, peliosis hepatis B. henselae
- Bartonella clarridgeiae → Cat-scratch disease [2]
- Bartonella elizabethae → Endocarditis
- Bartonella vinsonii var berkhoffii → endocarditis
- Bartonella vinsonii var arupensis → fever and a valvulopathy
- Bartonella grahamii → uveitis

The precise diagnosis of Bartonella species in human blood and lesions has provided us with the names of infectious organism associated with a number of diseases, but this new knowledge has not shed much light on why Bartonella can circulate in the blood without causing any reaction, for indefinite periods of time, or why any given Bartonella species may be associated with any of several diverse clinical manifestations. Furthermore, Koch's third postulate fails miserably for genus Bartonella; injecting any of these Bartonella species into experimental animals, will more than likely produce no symptoms.

–2. The microorganism must be extracted and isolated from the diseased animal (and grown in culture).

Many pathogens do not grow in nutrient medium culture. This applies generally to common Mollicute bacteria, including Erysipelothrix, Mycoplasma, and Ureoplasma. This would also apply to viruses, none of which grow in cell-free media. Paradoxically, some of the organisms known to produce bacteremias in human blood grow very poorly in blood cultures, and this would include the aforementioned Bartonella species and the HACEK organisms [1, 3]. The HACEK organisms are a group of proteobacteria, found in otherwise healthy individuals, that are known to cause some cases of endocarditis, especially in children, and which do not grow well in culture. The term HACEK is created from the initials of the organisms of the group: Haemophilus, particularly Haemophilus parainfluenzae; Aggregatibacter, including Aggregatibacter actinomycetemcomitans and Aggregatibacter aphrophilus; Cardiobacterium hominis; Eikenella corrodens; and Kingella, particularly Kingella kingae.

–3. The microorganism must cause disease when introduced to a healthy experimental animal.

Again, some of the worst microorganisms will not produce disease in healthy animals. To confuse matters further, we now have examples of nonliving agents that will produce transmissible disease in healthy animals (prions).

This third postulate of Koch presumes that each occurrence of an infectious disease has a particular organism that is "the cause" of the disease. We must return here to our often-repeated theme that diseases do not have "a cause," and infectious diseases are no exception to the rule that pathogenesis is a multistep process. We have already seen that myocardial infarction results from a multitude of conditions that occur through time. In some cases, the last event is infectious, wherein a focal bacterial endocarditis precipitates a thrombus that blocks a narrowed coronary artery. It would be folly to believe that the sequence of events that lead to a myocardial infarction can be precipitated simply by injecting an organism into an animal. Later in this chapter, we will see two examples of rare infections for which several conditions must prevail before a disease emerges [4, 5].

–4. The microorganism must be extracted from the infected experimental animal and demonstrated to be the same microorganism that was originally isolated from the original diseased animal.

Many infections, considered the underlying cause of a disease, are absent from the lesions that ultimately develop. For example, Group A streptococcus infection is considered to be the underlying cause of rheumatic fever. The infection is long gone prior to the appearance of the valvular and endocardial lesions of rheumatic fever. As another example, several species of human papillomavirus are considered to be the underlying cause of nearly all cases of squamous carcinoma of the uterine cervix. Morphologic cytopathic effects are visible in the earliest precancers that precede the development of invasive carcinoma. The cancers, which may occur years following the early papillomavirus infections, may lack recoverable virus.

Let's look at an example of an infectious disease that violates every one of Koch's postulates. Whipple disease, previously a disease of unknown etiology, is characterized by organ infiltration with foamy macrophages (i.e., specialized reticuloendothelial cells that "eat" bacteria and debris). The organ most often compromised in Whipple disease is the small intestine, where infiltration of infected macrophages in the lamina propria (i.e., a strip of loose connective tissue subjacent to the epithelial lining of the small intestine) causes malabsorption. Whipple disease is rare. It occurs most often in farmers and gardeners who work with soil.

Whipple disease was first described in 1907 [6], but its cause was unknown until 1992, when researchers isolated and amplified, from Whipple disease tissues, a 16s ribosomal RNA sequence that could only have a bacterial origin [7]. Based on molecular features of the ribosomal RNA molecule, the researchers

assigned it to Class Cellulomonadacea, and named the species Tropheryma whipplei, after the man who first described the disease, George Hoyt Whipple.

Particularly noteworthy, in the case of Whipple disease, is that Koch's postulates never came close to being satisfied. For the experimentalist, the most important of Koch's postulates require the extraction of the organism from a lesion (i.e., from diseased, infected tissue), the isolation and culture of the organism in the laboratory, and the consistent reproduction of the lesion in an animal injected with the organism. In the case of Whipple disease, none of these criteria were satisfied. The consistent identification in Whipple disease tissue of a particular molecule, characteristic of a particular species of bacteria, was deemed sufficient to establish the infectious origin of the disease.

In the general scheme of events, bacteria in the human body are eaten by macrophages, wherein they are degraded. In the case of T. whipplei, only a small population of susceptible individuals lack the ability to destroy T. whipplei organisms. In susceptible individuals, the organisms multiply within macrophages. When organisms are released from dying macrophages, additional macrophages arrive to feed, but this only result in the local accumulation of macrophages bloated by bacteria. Whipple disease is a good example of a disease caused by an organism but dependent on a genetic predisposition, expressed as a defect in innate immunity; specifically, a reduction of macrophages expressing CD11b (also known as macrophage-1 antigen) [8]. Whipple disease cannot be consistently reproduced in humans or any other animals, because it can only infect and grow in a small portion of the human population.

As we learn more and more about the complexity of disease causation, formerly useful paradigms, such as Koch's postulates, seem burdensome and useless. When we encounter rare diseases of infectious cause, we might expect to find that the pathogenesis of disease (i.e., the biological steps that lead to a clinical phenotype) may require several independent causal events to occur in sequence. In the case of Whipple disease, the infected individual must be exposed to a soil organism, limiting the disease to farmers and gardeners. The organism, residing in the soil, must be ingested, perhaps by the inhalation of dust. The organism must evade degradation by gut macrophages, limiting disease to individuals with a specific type of defect in cell-mediated immunity, and the individual must have disease that is sufficiently active to produce clinical symptoms. It is unlikely that we could reproduce a complex sequence of steps, leading to a disease, by simply inoculating an organism into an experimental animal [Glossary Underlying cause, Proximate cause, Root cause].

Side-stepping Koch's postulates has become de rigueur in the practice of modern medicine. For example, the United States has experienced a recent increase in cases of acute flaccid myelitis, a rare disease of children [9]. Diagnosis is based on a metagenomic analysis (i.e., culture-independent sequence searches conducted on an assemblage of microbial gene sequences in a biologic sample)

of DNA obtained from nasopharyngeal swabs. The organism that is present in most of the examined cases is enterovirus-D68, and this virus is the presumed causal organism of acute flaccid myelitis, until proven otherwise.

Genotyping species of organisms has become quite easy, but there are many millions of microorganism species, and it may never be feasible to complete a database of genome sequences of all living species. Though the number of individual species is too large to sequence, we can do a fair job at sequencing most of the different genera of living species. We now have a fairly accurate way of identifying the genus of any organism found within a tissue sample, by sequencing its ribosomal RNA and comparing the sequence against references sequences in public databases [10–13]. There are limitations to this technique, but when we combine our analysis of ribosomal RNA with our accumulated knowledge of clinical features of the infection, we can often arrive at candidate pathogen [11, 14, 15].

Modern medicine has changed the vocabulary of infection. Familiar terms such as primary pathogen, opportunistic infection, and immunocompetent patient need to be reexamined in light of what we have come to know. Even a fundamental concept, such as "the organism causing the disease" should probably be abandoned in light of the multistep pathogenesis of all diseases. Because a microorganism may contribute to the pathogenesis of a disease at a single moment of time, long before the disease becomes clinically manifest, we can expect to see cases in which screening tests for a putative causal organism will be negative in affected patients [16]. Koch, in his own time, understood the practical limitations of his postulates. Maybe it's time to reconsider Koch's postulates in light of the analytic methods now available that assign a taxonomic class to an infective organism, without isolating or characterizing the agent [17].

Section 8.2 Prion diseases: Fulfilling Koch's postulates, but without an organism

Life is a concept.

Patrick Forterre [18]

Everyone has heard the aphorism: "One bad apple spoils the bunch." This trite adage seems to be the principle underlying the prion diseases. A prion is a misfolded protein that can somehow serve as a template for proteins of the same type to misfold, producing collections of nonfunctioning protein globs that accumulate, causing cells to degenerate. The cells of the body that are most vulnerable to prion-produced disease are the neurons of the brain. The reason for the particular sensitivity of neurons to prion disease relates to the limited ability of neurons to replicate (i.e., to replace damaged neurons with new neurons), and to repair damage occurring in their uniquely long cytoplasmic extensions (e.g., axons).

The term prion was introduced in 1982, by Stanley Prusiner [19]. Prions are the only infectious agents that contain neither DNA nor RNA. Though few scientists would consider prions to be organisms, living or otherwise, they are included here to ensure that readers are aware of these biological agents. Prions are not confined to mammals, and not confined to the brain [20]. They have been observed in fungi, where their accumulation does not seem to produce any deleterious effect, and may even be advantageous to the organism [21].

One peculiar feature of the prion diseases is that they fulfill Koch's postulates. Let's review Koch's postulates, substituting the word "agent" for "microorganism."

1. The agent must be found in the diseased animal, and not found in healthy animals.
2. The agent must be extracted and isolated from the diseased animal (and subsequently grown in culture).
3. The agent must cause disease when introduced to a healthy experimental animal.
4. The agent must be extracted from the diseased experimental animal and demonstrated to be the same agent that was originally isolated from the first diseased animal.

Allowing a bit of leeway for the interpretation of these four requirements, in the particular case of prion molecules, we can contrive a fairly convincing argument that the prion agent satisfies all of Koch's postulates, and that prions are the infection causing Creutzfeld-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalitis in cows. Referring to the requirements, we have a large literature in which the transmissibility of prion extracts from human to nonhuman animal, and from nonhuman animal to nonhuman animal has been demonstrated [22, 23]. The prion agents have been extracted and studied. Prions can be examined in growing yeast cultures, and the propagation of prions can be observed in controlled, experimental settings [24–26]. In culture and in ex vivo systems, we can now study how various cellular processes may participate in the conversion of normal prion proteins into infectious prion agents [26, 27].

The key difference between prion infections and infections caused by living microorganisms is that prions propagate without replicating. It would seem that the mere presence of a prion molecule in company with normal prion proteins is sufficient to produce a characteristic misfolding of the normal form of the protein, which can propagate to other normal prion proteins in the cell, and from one cell to another.

Because the prion protein, in its proper molecular configuration, is a normal constituent of cells, conventional terminology seeks to avoid confusion by labeling the infectious prion protein as "PrP(Scr)" (i.e., prion protein as it might occur in scrapie) and the normal prion protein as either "PrP" or as "PrP(C)" (i.e., prion protein as it might occur in normal cells).

The normal prion protein (PrP(C)) serves many different purposes, including the regulation of myelin maintenance, and seems to have a role in various processes wherein cellular differentiation is controlled [28]. It is significant that PrP(C) knockout mice [i.e., mice with no PrP(C)] are essentially normal at birth, and survive over the following months with only marginal behavior deficits [29]. Animals that lack PrP(C) are completely resistant to all forms of prion-induced disease, simply because they lack any protein that can be converted to a pathogenic form. Hence, it is important to understand the role of PrP(C), if we plan to develop drugs that treat or prevent prion diseases based on targeting the PrP(C) molecule.

Unlike most of the infectious diseases we have discussed so far, the prion diseases occur in three pathogenetic forms [30]:

–1. Transmitted. Caused by an infectious prion (e.g., PrP(Scr)) that is introduced into a host.

In the prion disease known as Kuru, members of the Fore tribe in New Guinea practice a form of ritualistic cannibalism. Kuru was contracted when tribe members ate the brains of individuals harboring the Kuru infection. When the practice of ritualistic cannibalism was stopped, the incidence of Kuru fell.

In the case of the so-called variant Creutzfeldt-Jakob disease, the disease is contracted after individuals eat the meat of cows infected with bovine prions.

In the case of iatrogenic Creutzfeldt-Jakob disease, disease is contracted when prion-contaminated blood products (e.g., human growth hormone preparations) or tissues (e.g., dura mater grafts, pituitary extracts) are injected or transplanted into recipient patients.

–2. Genetic

Genetic prion disease results from inherited germ-line mutations of the PRNP gene (i.e., the gene that codes for PrP(C)). The genetic diseases include familial Creutzfeldt-Jakob disease, fatal familial insomnia, and Gerstmann-Straussler-Scheinker disease.

–3. Sporadic

Little is known about sporadic prion diseases, which includes sporadic Creutzfeldt-Jakob disease, sporadic fatal insomnia, and variable protease-sensitive prionopathy. The two mechanisms that seem to be most credible involve: (1) a somatic mutation of the PRNP gene within a single cell, which yields new molecules of PrP(Scr), which propagate through the nervous system to produce a prionopathy and (2) an acquired conformational change of PrP(C) into PrP(Scr), which then propagates to produce a prionopathy [26]. The clinical form of the prionopathy that would result from either of these hypothetical mechanisms would be somewhat dependent on how the disease

spreads through the brain [31]. This, in turn, may depend on where in the central nervous system (CNS) the process begins (i.e., which neurons are the first to be altered).

In all the three pathogenetic forms, the process seems to take decades to develop. In the case of kuru, infections may require as long as 50 years to become clinically evident [32]. All forms of prionopathy are progressive, with clinical signs dominated by decreasing cognitive ability and impaired motor coordination. Currently, all of the prion diseases are 100% fatal. All forms of prionopathy lead to the same morphologic changes in cells, this being a spongiform encephalopathy [33] (Fig. 8.1).

When we think of the prionopathies as a disease of a self-propagating misfolded protein that accumulates in neurons, and we stop thinking in terms of any particular protein as being the defining agent of disease, then we begin to see additional diseases that might fit the definition of a prionopathy. In particular, it has been suggested that the disease known as multiple system atrophy (MSA) with parkinsonism may qualify. MSA is a CNS disorder that develops very slowly, and produces a progressive loss of autonomic nervous system function. In MSA, there is always an accumulating ball of sticky, misfolded alpha-synuclein protein, observed as cytoplasmic inclusion bodies in glial cells. In 2015, it was shown that a neurodegenerative disease could be transmitted to transgenic mice expressing normal human alpha-synucleoprotein, after injection with a brain homogenate prepared from human MSA cases [34]. Hence, MSA can be considered a type of prionopathy produced by an altered protein other than PrP(Scr).

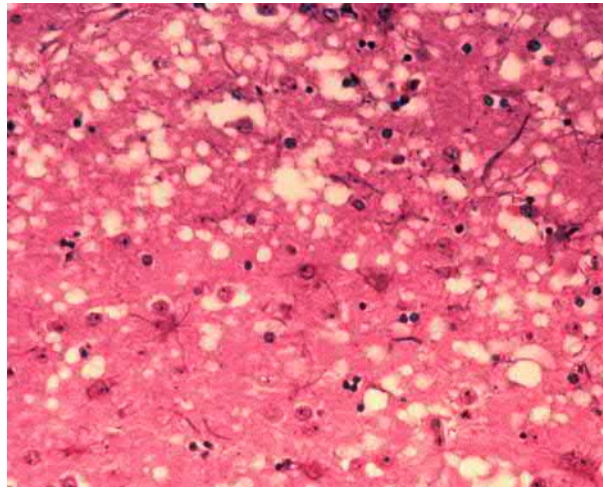

FIG. 8.1 Spongiform encephalopathy (brain tissue section, hematoxylin-eosin stain). Numerous vacuolated neurons are seen (i.e., spongiform change), along with small degenerate cells and scattered inflammatory cells. *(Source, a public domain image prepared at the U.S. Department of Agriculture, by Dr. Al Jenny.)*

If we accept that prion diseases can be produced by self-propagating proteins other than PrP(Scr), then what other diseases might we now investigate as potential prion diseases. Without diverting too far from the subject of this book, we can say that there seems to be a newly described class of diseases characterized by misfolded self-propagating and, under some circumstances, transmissible proteins, which may include the prionopathies, the tauopathies (including Alzheimer disease), and the amyloidoses [31, 35–37].

Section 8.3 Diagnostic challenges

> *In fact, diseases that exhibit simple Mendelian patterns of inheritance tend to be rare. Rather, complex diseases arise from numerous genetic and environmental factors working together.*
>
> Johanna Craig [38]

Diagnosis of infectious disease isn't easy; the chief problem being that patients with early signs of infection will often present with nonspecific symptoms that could accompany the common cold, or the so-called "intestinal" flu, or as any other common condition producing generalized malaise and mild fever. American health-care workers will not soon forget the first US case of Ebola. Thomas Duncan first arrived at the Emergency Room of the Texas Health Presbyterian Hospital, on the evening of September 25, 2014, complaining of fever, abdominal pain, dizziness, and nausea. After a few hours in the emergency room, Mr. Duncan was discharged with the diagnosis of sinusitis and a prescription of antibiotics. Mr. Duncan returned to the same emergency room 2 days later, much sicker. It turns out that Mr. Duncan had arrived in the United States just days previously, after having resided in Liberia, Africa. This time around, the correct diagnosis of Ebola virus infection was rendered. On October 8, Mr. Duncan died from Ebola virus hemorrhagic fever. Before his death, two health-care workers from the hospital were infected; they were the first to contract Ebola virus infection while on US soil. Both were diagnosed in early stages of the disease, and both survived.

Let's list some of the reasons why diagnosing an infectious disease can be very difficult, even for the most astute physicians.

– Huge variety of potential clinical presentations for a single organism.

As mentioned, the clinical presentation of an infectious disease is often nonspecific and may closely mimic other infectious and noninfectious conditions, particularly in the early stages of the disease. Many infections produce mild, self-limited disease, and are not worth investigating clinically.

Infectious diseases may closely mimic one another, leading the unwary physician to mistakenly apply a common diagnosis to an uncommon infection. For example, Neorickettsia sennetsu causes a rare disease that closely mimics infectious mononucleosis, a common disease caused by the Epstein Barr virus. As another example, patients with HIV/AIDS are susceptible to

two different skin conditions that closely resemble one another: bacillary angiomatosis and Kaposi sarcoma. Bacillary angiomatosis is an exaggerated overgrowth of small vessels of the skin, produced by infection with species of Bartonella (i.e., B. quintana and Bartonella henhenselae). Kaposi sarcoma is a cancer of vascular origin (i.e., a type of angioscarcoma) that is caused by Herpesvirus-8. Both bacillary angiomatosis and Kaposi sarcoma look very similar by gross examination and by microscopic examination with standard histological stains (i.e., Hematoxylin and eosin stains). Both bacillary angiomatosis and Kaposi sarcoma occur in AIDS patients, but their respective prognoses and treatments are very different. The correct diagnosis requires an astute pathologist who understands and anticipates the rare and the common causes of vascular proliferative lesions in immune-compromised patients [Glossary Hematoxylin and eosin].

– The list of nonpathogenic organisms is growing shorter.

In 1950, the United States navy conducted an ill-advised experiment on the unsuspecting citizens of San Francisco [39]. Large hoses sprayed out a fog of Serratia marcescens and Bacillus globigii to determine whether this kind of dispersal mechanism might be an effective way of exposing a large population to a biological warfare agent. S. marcescens and B. globigii were used because these organisms were considered to be completely harmless. From the viewpoint of the navy, this experiment was a success in that the bacteria were distributed widely over the Bay area, delivering a small dose of organisms to the target population.

The US navy declared a victory for itself in its undeclared war on San Francisco. The generals did not suspect that there would be any collateral damage. Unexpectedly, a small epidemic of S. marcescens infections was reported among the exposed population. In all, 11 individuals required hospitalization and one individual died. No cases of S. marcescens infections had been previously reported in the hospital where the death occurred, and no clusters of S. marcescens infections had occurred following the epidemic that coincided with the navy's experiment. It seems that S. marcescens, though harmless to most individuals, was pathogenic to a tiny subpopulation of the population. Presumably, genetic susceptibility accounted for the shift from harmless organism to deadly pathogen, in at least one hapless victim. At the time, nobody understood why this mini-epidemic had hit San Francisco. It was not until 1976, when the navy experiment was declassified, that the truth came to light.

What does it take to be a pathogen? Basically, any organism that can grow in human blood, in human tissues, on human endothelial or serosal surfaces, or in any internal sources of fluid (i.e., joint fluid, pleural fluid, and urine) can be a pathogen. Even organisms that don't grow well in human blood and tissues can be pathogenic in a select group of individuals.

The proteobacteria Eikenella corrodens is a normal inhabitant of the mouth that is harmless under most, but not all, conditions. When the organism is mechanically forced into the blood stream (e.g., by accidentally biting through the oral mucose while eating), it can produce a cellulitis or a bacteremia with endocarditis. It is included with the HACEK group of endocaditis-producing organisms. E. corrodens can also produce disease in diabetics and immunocompromised individuals, apparently without inadvertent biting. Genus Prevotella contains oral inhabitants that can produce plaque, halitosis, and periodontal disease. Prevotella dentalis, like E. corrodens, produces the so-called bite infections, wherein oral bacteria are inoculated, by a bite or abrasion, into adjacent tissues, producing abscesses, wound infections, or bacteremia. P. dentalis bacteremia can lead to disseminated infections.

We now encounter instances wherein once-obscure organisms have risen to the level of common pathogens. Blastocystis hominis was a eukaryotic organism seen as an incidental finding, of no known significance, on stool examinations. For a long time, the proper taxonomic classification of this organism was undetermined, and it has been variously referred to as a yeast, a fungus, an amoeba, a flagellated single-celled eukaryote, or a sporozoan (former name for apicomplexan), at various times [40]. Today, Blastocystis is considered a genus belonging to the heterokonts, and is the only heterokont known to produce a human infection. Infection follows ingestion of the cyst through the fecal-oral route. Most infections do not result in any clinical symptoms, but sometimes, a syndrome mimicking irritable bowel syndrome may occur. Among individuals who have microscopic school examinations, for any reason, up to 25% of specimens contain Blastocystis [41]. Because Blastocystis is found in the stools of healthy individuals, the finding of the organism in the stool of a symptomatic patient does not necessarily establish a causal relationship. Treatment with metronidazole, an antibiotic effective against eukaryotes and prokaryotes, has its advocates [42, 43], but can we do better? Blastocystis is the only heterokont known to cause disease in humans, and we know almost nothing about the heterokonts that would help us design a drug that would be effective against Blastocystis. **Our experience with Blastocystis reminds us that as we may discover pathogens belonging to classes of organisms that were previously thought to be devoid of infectious agents. In such cases, we will need to find new, class-specific antibiotics.** Let's look at another example wherein a taxonomic change requires us to develop a new approach to treatment.

– Growing number of rare infectious diseases

While the list of common infections is growing slowly, the list of rare infectious diseases is exploding. Improvements in the taxonomic designations of infectious organisms, the availability of highly advanced reference laboratories capable of accurately identifying infectious organisms, increases in the number of immune-compromised patients susceptible to infections by organisms that are

not otherwise pathogenic, and the ease with which infections can be transported from place to place throughout the modern world, have all contributed to the increase in newly encountered rare infectious diseases.

A source of new, rare infections is invasive instruments and catheters, particularly those that dwell inside the body for prolonged periods, such as bladder catheters, ventilator tubes and pulmonary assistive devices, shunts, venous and arterial lines, and indwelling drains and tubes. These devices provide a path of entry to a wide variety of organisms that would otherwise be halted by normal anatomic barriers. Of the different organisms that invade via indwelling devices, most are bacteria. Fungal disease has occurred in adults who receive intravenous parental nutrition; the fungi growing in the lipid-rich alimentation fluids [44]. The bacterial organisms that invade via indwelling devices include species of Pseudomonadales, Bacillales, Bacteroidetes, Fusobacteria, and Legionallales. Despite their taxonomic diversity, all these organisms seem to share an ability to secrete biofilms over surfaces, and to glide through the biofilms they create. Biofilms are invisible, slimy coatings, composed of polysaccharides and cellular debris that provide sanctuary from the antibacterial sprays and solutions used in hospitals. Bacterial species that can glide through a biofilm can track a catheter into the body. For example, Staphylococcus epidermidis is a commensal organism that lives on human skin. Some of the organisms now known to cause catheter-associated hospital infections were previously obscure [e.g., Leclercia adecarboxylata [45]. The list of such organisms is constantly growing.

Most of the newly recognized rare infections have a fungal origin. Approximately 54 fungi account for the vast majority of fungal infections, but the total number of fungi that are pathogenic to humans is much higher, and growing rapidly [46]. With advanced typing techniques, it is now possible to identify new species of fungi. For example, 34 new species of Aspergillus have been isolated from clinical specimens Aspergillus fumigatus, a common cause of severe pulmonary infections in immune-compromised patients [46]. We can now identify the specific fungal species responsible for cases that would formerly have been impossible to diagnose accurately [47].

– Properly classifying infectious diseases

For a long time, brain infections due to Naegleria fowleri were lumped with the amoebic encephalitides, and Naegleria was classified as an Acanthameoba, under Class Amoebozoa. We now know that N. is a member of Class Percolozoa, and the encephalitis caused by Naegleria fowleri is a percolozoan encephalitis. Why is this significant? Naegleria happens to be the only pathogenic species in Class Percolozoa, and we know almost nothing about the Percolozoan pathways that might render Naegleria sensitive to antibiotics. Currently, Naegleria encephalitis is treated as though it were one of the amoebic encephalitides, with

amphotericin B. With or without amphotericin B treatment, nearly all cases of percolozoan encephalitis are fatal [48]. Clearly, we need to learn a lot more about the biological pathways of members of Class Percolozoa, so that we can design a class-based strategy to prevent and treat Percolozoan encephalitis. Most importantly, we need to stop pretending that Naegleria is a genus in Class Amoebozoa, simply because it looks like an amoeba.

In the past, the rational basis for splitting a group of organisms into differently named species required, at the very least, heritable functional or morphologic differences among the members of the group. Gene sequencing has changed the rules for assigning new species. For example, various organisms with subtle differences from Bacteroides fragilis have been elevated to the level of species based on DNA homology studies. These include Bacteroides distasonis, Bacteroides ovatus, Bacteroides thetaiotaomicron, and Bacteroides vulgatus [3, 49].

There is a growing list of infections known to be resistant to most types of antibiotic treatments. For the most part, these are not rare diseases; they are common diseases that happen to be resistant to antibiotics. Examples are resistant strains of Staphylococcus aureus, Acinetobacter baumanii, and Klebsiella pneumoniae. New techniques for subtyping strains based on antibiotic resistance or susceptibilities are being developed and will probably replace older agar growth tests [50] [Glossary Serotype, Serovar].

Genus Plasmodium is responsible for human and animal malaria. About 300–500 million people are infected with malaria worldwide. About 2 million people die each year from malaria [51, 52]. There are several hundred species of Plasmodium that infect animals, but only a half dozen species are known to infect humans [52]. Newly emerging species, causing human disease, may arise from animal reservoirs. For example, Plasmodium knowlesi, a known cause of malaria in macaque monkeys, has emerged as a cause of human malaria in Southeast Asia, where it has grown in incidence to the point that it currently accounts for about two-thirds of malarial cases in this region. Malaria is commonly diagnosed with an antigen test developed against the common forms of human plasmodia (e.g., Plasmodium falciparum). Patients with P. knowlesi may have a negative reaction to the standard Plasmodium antigen test [53]. If this were to occur, a P. knowlesi infection may go undiagnosed, and the patient might not be provided with needed antimalarial medication. Careful examination of blood will usually indicate the presence of parasites in the cases of P. knowlesi malaria, and a specific diagnosis can be confirmed with advanced molecular tests. Here is an example of a previously rare infection, emerging as an endemic infection, whose diagnosis can be missed with testing methods designed for the common forms of disease.

Technical advances in the past decade have greatly improved our ability to diagnose species and subtypes (i.e., serotypes or serovars) [54, 55]. A striking case in point was reported in 2014, when DNA was extracted from the spinal fluid of a child with meningitis of undetermined cause [56]. After 2 days of

sequencing and computer searches through multiple DNA databases, a match was found with Leptospira. The patient was started on penicillin, and recovered soon thereafter. In this case, modern genetic analysis helped rescue a gravely ill child. Nevertheless, our ability to diagnose a specific species present within a sample specimen (e.g., tissue, blood, body fluid) will always be limited by our incomplete pathogen database. There are just too many potential pathogens to include every organism's genome, and the list of pathogens keeps growing longer. Fortunately, microbial taxonomy provides some relief to this seemingly intractable problem.

While the number of potential pathogenic organisms is large and unknowable, the number of genera that contain pathogens is quite small. A genomic database that provides the characteristic sequences for every genus of pathogenic organism would, in theory, allow us to determine whether a clinical sample contains organisms belonging to a genus that is known to contain known pathogens. Such information would be helpful in several ways:

- If clinical findings suggest a particular pathogen, then knowing that the clinical sample contains organisms belonging to the genus of the suspected pathogen would provide a level of corroboration for our clinical impression.
- If genetic analysis of the clinical sample indicates that the sample contains organisms belonging to a single genus known to contain pathogens, then we might consider initiating treatment with a drug that is known to be effective against organisms of the genus.
- If genetic analysis of the clinical sample indicates that genetic analyses of multiple clinical samples fail to match against any known genus of micro-organism, including viruses, then the clinician might begin to search for noninfectious causes of the patient's condition.

In point of fact, the genus of an organism can often be determined by examining the sequence of the rRNA gene, and such an approach has proven useful in the diagnosis of bacteria and fungi [2, 10–14]. Not only has this methodology proven useful in identifying the genus of organisms in a sample, but an extension of the method has also been developed to locate individual organisms within a mixed clinical sample of organisms. Roughly, here is how it works [57, 58]:

1. Genus-specific rRNA oligonucleotides are prepared as fluorescent probes.
2. The rRNA probes are incubated with a mixture of organisms.
3. The target organisms are detected (via fluorescence in situ hybridization).
4. The fluorescent organisms are extracted and examined by ancillary methods (e.g., scanning electron microscopy, culture) to determine the species name of the organism.

It should be noted that genetic analyses, powerful as they are, can often be circumvented by clinical acumen. In the aforementioned case of gene-based diagnosis of childhood meningitis caused by leptospirosis, we note that an astute clinician may have reached the same diagnosis sooner, and without state-of-the-art technology. Empirically, it is known that children with meningitis whose cerebrospinal fluid (CSF) specimen does not grow organisms will have leptospirosis in about a third of cases [59]. The diagnosis could be confirmed quickly using older technology that directly tested for the presence of the suspected organism (rather than laboriously searching through a large database) [59]. We should be prepared to accept that there will always be circumstances wherein a gene-based diagnosis must give way to common sense.

- Some diagnoses can only be established by highly specialized laboratories. Some of these laboratories were created to find the cause of epidemics, not of isolated illnesses. Under normal conditions, empiric treatments may be a practical alternative to precision diagnoses.
- Sometimes, it is faster and more effective to deduce a diagnosis than to resort to sequence analyses.
- Sometimes, precision diagnosis can be overly sensitive, detecting sequences of contaminants or detecting organisms that are present in such low numbers that they could not have caused the disease.
- Sometimes precise diagnoses are mistaken. Aside from sample contamination, which is a persistent problem whenever tiny quantities of diagnostic material are analyzed, we must be prepared to encounter instances when DNA sequences, thought to be characteristic of a species, are found in other organisms [60, 61].
- Sometimes precise diagnoses will be irreproducible by other laboratories examining samples of the same specimen. The difficulties in verifying and reproducing the results obtained through precision measurements are a matter of deep concern to those who regulate new diagnostic tests [62–77].

Section 8.4 Discovering new infections among the diseases of unknown origin

That which can be asserted without evidence, can be dismissed without evidence.

Christopher Hitchens

What shall we do with all the human diseases whose pathogenesis is unknown, despite our best efforts? We can start by grouping them to see if they share any common biological properties. Here is a partial list of diseases, some being common but most being rare, that are mysteries of modern medicine:

- Acrocyanosis

- Acute flaccid myelitis
- Alzheimer disease
- Aphthous ulcers
- Balanitis xerotica obliterans
- Behcet disease
- Benign fasciculation syndrome
- Brainerd diarrhea
- Cardiac syndrome X
- Chronic fatigue syndrome
- Chronic prostatitis/chronic pelvic pain syndrome
- Cluster headache
- Complex regional pain syndrome
- Copenhagen disease
- Cronkhite-Canada syndrome
- Cyclic vomiting syndrome
- Dancing mania
- Danubian endemic familial nephropathy
- Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome)
- Electromagnetic hypersensitivity
- Encephalitis lethargica
- Exploding head syndrome
- Fibromyalgia
- Fields' disease
- Functional colonic disease
- Giant cell (temporal) arteritis
- Gluten-sensitive idiopathic neuropathies
- Gorham vanishing bone disease
- Granuloma annulare
- Granulomatosis with polyangiitis (Wegener syndrome)
- Gulf War syndrome
- Hallermann-Streiff syndrome
- Heavy legs
- Henoch-Schonlein purpura
- Interstitial cystitis
- Irritable bowel syndrome
- Kashin-Beck disease
- Kawasaki disease
- Lichen sclerosus
- Lytico-Bodig disease
- Mesoamerican nephropathy
- Microscopic polyangiitis
- Morgellons disease
- Mortimer disease
- Myofascial pain syndrome

- New daily persistent headache
- Nodding disease
- Picardy sweat
- Pigmented villonodular synovitis
- Pityriasis rosea
- Polyarteritis nodosa
- Posterior cortical atrophy
- Prurigo nodularis
- SAPHO syndrome
- Sarcoidosis
- Sick building syndrome
- Sjogren's syndrome
- Spontaneous cerebrospinal fluid leak
- Stiff person syndrome
- Sudden unexpected death syndrome
- Sweating sickness
- Synovial osteochondromatosis
- Takayasu arteritis
- Torticollis
- Trichodynia
- Trigger finger
- Tropical sprue

We do not know the root causes, the steps of development, or the fundamental biological properties of these diseases. Based on our experiences with diseases of known origin, is there anything that we can say that may help us to understand and classify these strange diseases? Most certainly, yes. If the diseases occur primarily in children, and if the disease runs in families, then we can say that, in all likelihood, a strong genetic influence is at work. If the disease causes fever and signs of inflammation and responds to anti-inflammatory medication, then we can infer that the condition involves a primary disorder of the inflammatory system, or a secondary disorder (such as the response to an infection). If the disease arises as an epidemic, then an infection or a newly introduced environmental toxin is likely to blame. Most importantly, if all of the medical research at our disposal is brought to bear on a disease, and no root cause is found, then it's a very safe bet that multiple conditions and events converge to produce the disease, and that a single cause will never be assigned. Our attention should be focused on finding the factors that contribute to the development of the disease.

In point of fact, if we look at former diseases-in-waiting, we find that most are diseases whose causes are amalgamations of infectious, genetic, and environmental conditions. Let's consider Whipple disease, discussed previously in this chapter. The disease is only observed in tillers of the soil (i.e., an occupational disease), who ingest the T. whipplei organism (i.e., an

infectious disease), and who also happen to have a rare immune defect (i.e., a genetic disease). The multiplicity of events and conditions contributing to the development of Whipple disease confused early generations of medical researchers and earned it a prominent spot on the list of diseases-in-waiting. Using modern laboratory techniques and the knowledge that diseases are sequential, multievent processes, the mystery of Whipple disease was solved, 85 years after the first report of a human infection.

Celiac disease, a former disease-in-waiting, remains shrouded in biological ambiguity. Regardless, medical researchers believe that they know enough about this disease to drop it from the list. Celiac disease is triggered by the ingestion of gluten, a component of wheat (i.e., an environmental disease), and occurs in individuals who inherit Human Leucocytic Antigens DQ2 and DQ8 (i.e., a genetic disease) [78]. Gluten peptides are resistant to complete proteolytic digestion and enter the small intestinal lamina propria (i.e., the layer just beneath the epithelial cells lining the gut) where they elicit a strong, local immune reaction [78] (i.e., a disease of immunity). Recent literature suggests that the inflammatory response to gluten is primed by reovirus infection (i.e., a microbial infection) [79]. Despite the complex etiology of the disease, symptoms of the disease can be ameliorated by omitting gluten from the diet.

Another disease that seems to follow a pathogenesis similar to that of celiac disease is reactive arthritis, formerly known as Reiter's syndrome. This inflammatory condition affects the joints, eyes, urethra, and skin. Symptoms from these different tissues may occur asynchronously, and the disease may occur as a succession of remissions and relapses. As in celiac disease, there seems to be a genetic component to the syndrome, with about 75% of affected individuals having the Human Leucocytic Antigens B27 marker. Human Leucocytic Antigen B27 is a major histocompatibility locus that has been associated with several other inflammatory conditions, including ankylosing spondylitis and acute anterior uveitis [80, 81]. As in celiac disease, Reiter's syndrome seems to have an infectious component, with most new cases following sexually transmitted infection by Chlamydia trachomatis or Ureaplasma urealyticum. Disease can also arise after gastrointestinal infection with shigella, salmonella, yersinia, or campylobacter bacteria.

Nodding disease, which may soon be removed from the list of diseases-in-waiting, is a frightening condition, first documented in the 1960s, that occurs almost exclusively in young children and adolescents living in regions of South Sudan, Tanzania, and Uganda. The disease stunts normal growth of the brain, and produces seizures. During the seizures, the neck muscles do not support the weight of the head, resulting in a characteristic nod, emphasized in the name of the disease. It was noticed that nodding disease occurs in areas where river blindness is endemic. River blindness is the second most common cause of infectious blindness worldwide and occurs in individuals infected by the filarial nematode *Onchocerca* volvulus [82]. The nematode migrates to the eyes,

where a peculiar secondary infection takes control of the pathogenic process. Wolbachia pipientis happens to be an endosymbiont that infects most members of the filarial Class Onchocercidae [83]. It is the Wolbachia pipientis living within O. volvulus that causes the local inflammatory reaction that leads to blindness.

A recent paper found that patients with nodding disease have antibodies to Onchocerca volvulus proteins that cross-react with leiomodin-1, a protein expressed in areas of the brain affected by the disease [84]. If this early research is confirmed, then nodding disease will be seen as an infectious disease that elicits an antibody response, which subsequently elicits a neurologic disorder. If so, nodding disease should be preventable by the avoidance or early treatment of O. volvulus infections. Hopefully, another disease with a complex etiology will be removed from the list of diseases-in-waiting.

Keshan disease was a disease of unknown etiology that has been tentatively removed from the list of diseases-in-waiting. Endemic to the Keshan region of Northeastern China, this disease, first reported in 1935, produces a cardiomyopathy, mostly in boys under 15 years old and women of childbearing age. This disease is often fatal, and thousands of deaths were reported in its peak incidence years (1960–70). Knowing that the region with the highest incidence of disease corresponded to a region with selenium deficiency, Chinese authorities treated spring crops with sodium selenite. It worked. The incidence of Keshan disease dropped in the areas where the crops were sprayed [85]. In 1981, a report came out indicating that Keshan disease patients had high levels of antibodies against Coxsackie virus, a virus known to produce cardiomyopathy [85, 86]. Current thinking would suggest that Keshan disease occurs in individuals whose hearts are weakened by selenium-deficient individuals, and who subsequently become infected with a virulent and cardiotoxic strain of Coxsackie virus [4].

Last but not least, we should consider Alzheimer disease, a common condition whose cause is unknown, and for which we have no prevention, no cure, and no way to impede the relentless progress of the disease [87]. We have already discussed Alzheimer disease in Section 8.2, “Prion Diseases: Fulfilling Koch's Postulates, but Without an Organism.” In our earlier discussion, we observed that misfolded amyloid, as extracellular amyloid plaques, and tau proteins, as intracellular neurofibrillary tangles, accumulate in the areas of the brain most affected by the neurodegeneration of Alzheimer disease. Although the origin of aggregates of misfolded protein in Alzheimer disease is unknown, we see such changes, to some degree, in the brains of virtually every individual who dies at an advanced age. We tend to credit such changes to long-term effects that accumulate in aging (i.e., nonrenewing or postmitotic) cells. Like many of the diseases of unknown origin, there seems to be a genetic component of indeterminate significance. We observe that individuals who have inherited two copies of the ApoE4

gene, sometimes called the Alzheimer gene, have a much increased risk of developing the disease.

Having found both nongenetic (aging) and genetic (ApoE4) risk factors for Alzheimer disease, might we not expect an infectious etiology is also involved? In fact, a specific organisms, Porphyromonas gingivalis (Class Bacteroidetes), an oral bacteria that can produce gingivitis, is currently being investigated as a potential cause of Alzheimer disease. The evidence so far would seem to indicate that individuals with chronic gingivitis have higher than normal incidence of the disease, the organism can infect brains, and that toxins produced by the organism and found in the brain are neurotoxic [88, 89]. We need not speculate on the potential significance of finding that a bacterium is an underlying cause of Alzheimer disease. Clinical trials, as they say, are underway. The point emphasized here is that diseases of unknown origin are often caused by a combination of genetic, environmental, and infectious conditions that work in steps, over time. Sometimes the best approach to prevent these types of diseases relies on a multifront effort. Such an approach has been attempted, by enhancing the diet, physical fitness, and metal activity of at-risk individual; with positive results [90].

Summarizing, the diseases-in-waiting seem to fit a common pattern as follows:

- Complex chain of causal events
- Tend to occur in rather specific populations, often based on age, geographic location, and occupation
- Unraveling their pathogenesis requires advanced diagnostic methodologies
- Hard to absolutely prove (there could be more to the story or the story could be wrong)
- Provide multiple interventional opportunities to stop or slow the development of disease (i.e., treatments can be aimed at any of the multiple steps in pathogenesis)
- Most importantly, **an infectious agent is often involved somewhere in the process**

We should think of pathogenesis as a sequence of biological events that occur over time, and that eventually produce a clinical phenotype that we recognize clinically as a disease. When infectious agents are involved in one step of a sequence of steps that eventually lead to disease, we can no longer equate the infectious agent with the "cause" of the disease. It may be difficult to understand how we might diagnose, prevent, or treat a disease that has no single, assignable cause. Nonetheless, the diseases of unknown origin seem to be teaching us that diseases often have complex pathogeneses. Identifying a disease-related organism is often just the beginning of a long discovery processes that will lead to a full understanding of these challenging diseases.

Section 8.5 Unstable taxonomies

I learned very early the difference between knowing the name of something and knowing something.

Richard Feynman

A taxonomy is a theoretical construct. As such, it needs to be constantly tested to determine whether the defined properties of each class actually extend to all of the members of the class, and whether the hierarchy of the classes is true. As new information is acquired, taxonomies will need to change.

Some of the recent changes in the taxonomy of living organisms have involved the highest classes in the hierarchy. The first division of the Eukaryotes was assigned to Class Bikonta and Class Unikonta (analogous to dicot and monocot division in Class Angiospermae) [91]. Class Protoctista has been dropped from the formal taxonomy. Class Microsporidia was moved from Class Protoctista to Class Fungi. The parasitic myxozoans have been added to Class Cnidaria (which includes sea anemones, corals, jellyfish, and hydra-like animals). Class Chlorophyta was moved from Class Protoctista to Class Archaeplastida.

Class Fungi has recently undergone profound changes, with the exclusion of myxomycetes (slime molds) and oomycetes (water molds), and the acquisition of Class Microsporidia. The instability of fungal taxonomy negatively impacts the practice of clinical mycology. When the name of a fungus changes, so must the name of the associated disease. Consider "Allescheria boydii," people infected with this organism were said to suffer from the disease known as allescheriasis. When the organism's name was changed to Petriellidium boydii, the disease name was changed to petriellidosis. When the fungal name was changed, once more, to Pseudallescheria boydii, the disease name was changed to pseudallescheriasis [46]. All three names appear in the literature (past and present).

The fungi are known for wide variations in the morphologic appearance of a single species Pathogenic fungi grow within human tissues without reproduction (i.e., in an asexual morphologic phase). For the most part, pathologists see hyphae or yeast forms, and these forms and most of the many species of infectious fungi can look more or less equivalent when observed in tissue. As a partial remedy for this situation, pathologists have discovered a wide range of subtle clues that may help them distinguish one fungus from another: width of hyphae, angle of hyphal branching, septation of hyphae, the presence or absence of pigment, the size of yeasts and the morphology of budding, the type of host response to the fungal organisms (e.g., granuloma, acute inflammation), the anatomic site of infection, and so on. Clever as they may be, the definitive diagnosis for fungal species is determined in the clinical mycology lab, whenever feasible. Fungal specimens successfully grown in culture often have a different morphology from

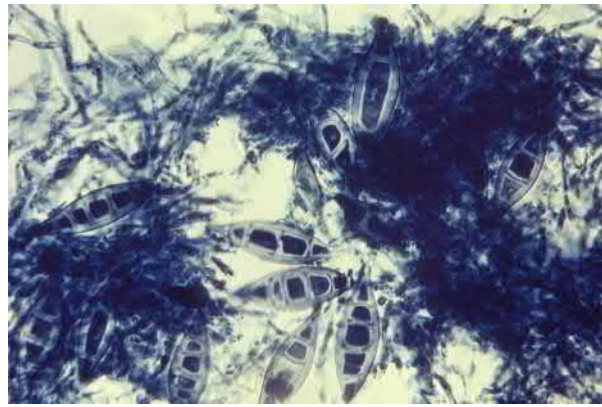

FIG. 8.2 Culture specimen of Microsporum canis, exhibiting mycelia and macrocondia. A dense mycelia composed of tangled hyphae forms the background from which about 10 pod-shaped septate structures are observed. These structures are macroconidia (i.e., large, multiseptate conidia), which grow asexually from conidiophores growing from mycelial hyphae. Conidia are the propagules of fungi, which are swept into the air and water. It is estimated that humans inhale about 40 conidia, of diverse provenance, every hour. Most human encounters with conidia are inconsequential, but infections or allergic reactions may occur in susceptible individuals. It is notable that conidia form in cultured fungi, but are virtually never observed in infected human tissues. *(Source, a public domain image provided by the U.S. Centers for Disease Control and Prevention and prepared by Dr. Lucille K. Georg.)*

that of the same fungus growing in human tissue. This situation is very different from that of bacterial infections, which have about the same morphology in tissues as they have in the culture dish (Fig. 8.2).

Fungal organisms have two options for reproduction: sexual and asexual. Both these forms of reproduction have their own morphologic appearances, in the same species of organism. Factors that determine the mode of reproduction for a cultured fungus are a mystery. It is possible for one mycologist to observe a fungus that reproduces exclusively asexually, while another mycologist, observing the same species in a culture dish or growing in the wild, may observe sexual reproduction (e.g., fruiting bodies). Depending on the phase of reproduction observed, and ignorant of the existence of an alternate morphologic form, taxonomists have assigned different names (sexual and asexual) to the same organism. Rather than harmonizing a dichotomous nomenclature under one preferred name, the International Code of Botanical Nomenclature (ICBN) had ruled, in the past, that it is acceptable to assign two different binomials to an organism: a sexual (also called teleomorphic, perfect, or meiotic form) and an asexual (also called anamorphic, imperfect, or mitotic form). For instance, two binomials legitimately apply to the same organism: *Filobasidiella neoformans* (the teleomorphic form) and *Cryptococcus neoformans* (the anamorphic form). Clinical mycologists typically use the asexual name, because it is the asexual form that grows in human tissues.

The unbridled craziness of one species having several different names could not continue forever. The 18th International Botanical Congress assembled in 2011, in Melbourne, Australia decided that a fungus was not a plant, and renamed themselves "The International Code of Nomenclature for Algae, Fungi, and Plants." More importantly, the Congress abolished longstanding provisions permitting separate names for the different morphologic forms of a pleomorphic fungal species (i.e., names for anamorphs and teleomorphs) [92]. The "One fungus = One name" rule, as it has come to be known, faces many practical obstacles to its quick implementation. For example, many fungi have never exhibit sexual reproduction in culture. Many other fungi cannot be cultured. A special pseudoclass of fungi, deuteromyctetes (spelled with a lowercase "d," signifying its questionable validity as a true biologic class) has been created to hold these indeterminate organisms until definitive classes can be assigned. Currently, there are several thousand such fungi, sitting in a taxonomic limbo, until they can be placed into a definitive taxonomic class [46]. Still, the "one fungus = one name" rule is a step in the right direction, and we can expect that nonmorphologic (e.g., genetic) identifiers of fungal species will eventually lead to a coherent and logical fungal taxonomy.

Here is a small sampling of recent name changes in species or genus of infectious organisms:

- Aggregatibacter actinomycetemcomitans, formerly Actinobacillus actinomycetemcomitans
- Anaplasma phagocytophilum, formerly assigned two different species names: Ehrlichia phagocytophilium and Ehrlichia equi [93].
- Aonchotheca philippinensis, formerly Capillaria philippinensis
- Arcanobacterium haemolyticum, formerly Corynebacterium haemolyticum
- Arcanobacterium pyogenes, formerly Actinomyces pyogenes
- Bartonella quintana, formerly Rochalimaea quintana
- Brachyspira pilosicoli, formerly Serpulina pilosicoli
- Burkholderia mallei, formerly Pseudomonas mallei
- Cladophialophora bantiana, formerly Xylohypha bantiana
- Cystoisospora belli, formerly Isospora belli
- Elizabethkingia meningoseptica, formerly Chryseobacterium meningosepticum
- Encephalitozoon intestinalis, formerly Septata intestinalis
- Fluoribacter bozemanae, formerly Legionella bozemanae
- Gardnerella vaginalis, formerly Corynebacterium vaginalis, formerly Haemophilus vaginalis
- Helicobacter pylori, formerly Campylobacter pylori
- Klebsiella granulomatis, formerly Calymmatobacterium granulomatis, formerly Donovania granulomatis
- Malassezia furfur, formerly Pityrosporum ovale

- Micromonas micros, formerly Peptostreptococcus micros formerly Parvimonas micros
- Mycolcadus corymbifera, formerly Absidia corymbifera
- Neorickettsia sennetsu, formerly Ehrlichia sennetsu
- Norovirus, formerly Norwalk virus (epidemic gastroenteritis)
- Pneumocystis jirovecii, formerly Pneumocystis carinii
- Rhodococcus equi, formerly Corynebacterium equi, formerly Bacillus hoagii, formerly Corynebacterium purulentus, formerly Mycobacterium equi, formerly Mycobacterium restrictum, formerly Nocardia restricta, and formerly Proactinomyces restrictus.
- Rotavirus formerly known as gastroenteritis virus type B.
- Sarcocystis suihominis, formerly Isospora hominis
- Stenotrophomonas maltophilia, formerly Pseudomonas maltophilia
- Volutella cinerescens, formerly Psilonia cinerescens

Regional differences in nomenclature account for considerable confusion. For example, Enterobius vermicularis is called pinworm in the United States and threadworm in the United Kingdom; while strongyloides stercoralis is just the opposite (threadworm in the United States and pinworm in the United Kingdom). The only way to escape this trans-Atlantic confusion is to translate the common name of the organism back to its standard Latin binomial.

Taxonomic instability negatively impacts clinical practice. Changes in the standard names of a fungus, appearing in the ICBN, should trigger concurrent changes in the standard nomenclatures of medicine, such as the World Health Organization's International Classification of Disease, and the National Library of Medicine's Medical Subject Headings, and a variety of specialized disease nomenclatures. Some of these nomenclatures update infrequently. When disease nomenclatures lag behind official fungal taxonomy, errors in coding and reporting infectious fungal diseases will ensue [Glossary Dictionary, Nomenclature].

Section 8.6 Taxonomic stupidity

I personally think we developed language because of our deep need to complain.

Lily Tomlin

Nearly every medical science is a child compared with medical microbiology. Think a moment about the youthfulness of bioinformatics, genetics, and molecular biology. Medical subjects with ancient roots, such as physiology, embryology, histology, and cell biology, were relatively obscure until the 20th century. In the case of infectious illness, scientists were providing names to living organisms and to the biological processes of living organisms, for hundreds of years, often predating the invention of the microscope, and often invoking ancient and magical notions to explain invisible, malicious

entities. Consequently, biologists have expended a great deal of effort toward the expulsion of nonscientific terms from the vocabulary of modern microbiology.

Class Protoctista

A good classification is never complete until every member of the classification has a place in a class, every class has at least one member, and every member within a class has a defined relationship with every other member within the same class. Consequently, we avoid undefined classes with names such as "miscellaneous" or place-holding classes, named "not otherwise specified," filled with objects waiting for their proper class assignments. Adhering to this last provision may be very difficult. When a classification is being constructed, it is common to have some objects whose properties are a mystery or objects that simply cannot be easily related to other objects. Taxonomists often have no choice but to put all their leftover objects into a miscellaneous class, until such time as additional information is obtained. The Latin term incertae sedis, meaning "of uncertain placement," and indicating a problematic class, puts a veneer of classical authority on the practice [Glossary Problematica, Unclassifiable objects].

In the classification of living organisms, 19th century taxonomists did not know quite what to do with the many different one-celled eukaryotes they were collecting. As a stop-gap measure, they invented the Kingdom Protozoa, the class of all one-celled animals, the parent class of all multicellular organisms. Kingdom protozoa was a pseudoclass, consisting of all manner of organisms that were not closely related to one another, and which contained organisms that should have been assigned to separately named classes of single-celled eukaryotic organisms [94]. Many decades passed before taxonomists caught up with the blunder, and reassigned individuals in class protozoa to proper classes of their own. Nonetheless, biologists are unwilling to abandon long-cherished terminology. Taxonomists who should know better have pleaded to retain obsolete and misleading plesionyms, such as "protist," "protoctista," and "protozoa" on the grounds that doing so will enhance communication and facilitate teaching [95]. They make a fair point, but surely the chief objective of science is to correct misconceptions, not perpetuate them. We have seen some evidence of progress. For example, the Society of Protozoologists, founded in 1947, has seen fit to update their name to Society of Protistologists, in 2005. It would be asking too much for them to change their name, once more, to the awkward but accurate "Society of single-celled Eukaryotic Organisms." In this book, the term "protozoa" is avoided altogether.

Informal classes of organisms that violate the rules of classification

Class Anamniota sometimes appears in the literature, indicating the sister class to Class Amniota. In actuality, there is no legitimate Class Anamniota; there

are simply lots of anamniotes belonging to lots of distantly related classes. The source of the confusion came with the three subclasses of Class Tetrapoda: Class Amphibia, Class Sauropsida, and Class Synapsida. The Sauropsids and the Synapsids are the first classes of animals to lay their eggs on land or to retain eggs in gestating mother. To do so, the embryo acquired a set of protective extra-embryonic membranes (i.e., allantois, amnion, and chorion). If the Sauropsids (including future birds and reptiles), and synapsids (including future mammals) belong to Class Amniota, it would seem only fitting to presume that Class Amphibia, whose eggs lack an amnion, would be a subclass of Class Anamniota. This would be a mistake, insofar as none of the classes of animals other than the Sauropsids and Synapsids, and their descendants, have amnions; hence, the anamniotes do not constitute a sensible class of animals [Glossary Negative classifier].

We commonly encounter groupings of animals that have no logical place within a formal classification. While we are on the subject of amphibian classification, we may as well examine the term "herpetology," it being the branch of zoology concerned with the study of amphibians and reptiles. Amphibians and reptiles belong to sister subclasses of Class Tetrapoda (i.e., Class Amphibia and Class Synapsida). The present-day descendants of these respective classes are only distantly related to one another. For example, Class Reptilia is a descendant of Class Sauropsida, but the pairing of present-day amphibians with present-day reptiles creates a grouping with a tenuous phylogenetic relationship. Furthermore, among the members of Class Reptilia are birds, which are not studied by herpetologists. In summary, the field of herpetology covers animals of two distantly related classes, but excludes certain subclasses of animals within its member classes (i.e., excludes birds). Herpetologists should choose one or two classes of animals to study, and not confuse the rest of us with a name that violates the laws of biological classification.

Gender assignment for asexuals

Biologists love assigning gender to any and every biological entity, whatever the consequences. When a mitotic cell divides asexually, the progeny are referred to as daughter cells. Whether the organism is a male or a female, it makes no difference. The product of cell division is a daughter cell. Similarly, Echinococcal cysts may harbor cysts within themselves, and these are referred to as daughter cysts.

Even abstractions are provided with a gender. When a class produces two subclasses, the two sexless subclasses become the sister classes of one another.

The problem is not confined to biology, and is not exclusively a foible of the English language. In French and Spanish, the uterus is assigned the male gender (preceded by "le" and "el," respectively). In French and Spanish, the prostate is feminine (preceded by "la" in either language). Why do we do this to ourselves?

Class-specific terms applied to the wrong classes

As previously mentioned, Class Fungi were originally assigned as a subclass of Class Archaeplastida (plants), based on a few shared similarities (e.g., both live in soil and both grow as immobile, sessile multicolored structures). The misclassification of fungi as a class of plants has been hard to expunge. Universities, steeped in tradition, continue to assign their mycologists to the Department of Botany. Until earlier in this decade, the taxonomy of fungi was determined by the International Committee for BOTANICAL Nomenclature (ICBN). To this day, we find published taxonomies that identify fungi as types of flowers [96].

The language of biological science is peppered with taxonomically false terms. For example, the suffix "phyte" or "phyta" comes from the Greek "phyton" meaning plant. It is attached to all manner of nonplant organisms. As a case in point, heterokontophyta, the former name for the heterokonts, and still in common usage, is a single-celled eukaryote that is certainly not a plant.

Much of our terminological mischief harkens back to a time when it was common to divide all objects on earth into three classes: animal, vegetable, and mineral. Consequently, all of the nonmetazoan eukaryotes were thought of as vegetables or plants, and given names taken from the field of botany. So too the bacteria. Hence, the gut bacteria were called the human microflora. Mycelial growth was described as vegetative. The sporocarp of fungi continues to be called a "fruiting body." The nearly meaningless term "fruiting body" is haphazardly applied to bacteria and slime molds, as well.

Just as we falsely apply strictly botanical nomenclature to nonbotanical terms, we also apply metazoan terms to nonmetazoan organisms. For example, the term "zoo" derives from the Greek "zoion," meaning an animal. We find "zoo" or "zo" appearing as a prefix, infix, or suffix for organisms that are single-celled eukaryotes, fungi, and almost anything but animals.

Here is a list of confusing "zoo" terms, selected from this book:

- Amoebozoa (a one-celled eukaryote and not an animal)
- Apoikozoa (synonymous with Choanozoa, a sister class to animals, and not an animal)
- Bradyzoites (an encysted form of sporozoan, such as Toxoplasma gondii, and not belonging to any member of Class Animalia)
- Choanozoa (same as aforementioned Apoikozoa),
- Encephalitozoon (a member of Class Microsporidia, a descendant of Class Fungi, and not an animal)
- Enterocytozoon (another member of Class Microsporidia, a descendant of Class Fungi, and not an animal)
- Euglenozoa (a class of single-cell eukaryotes, and not an animal)
- Filozoa (an ancestral class for the metazoans, and hence not an animal)
- Holozoa (another ancestral class for the metazoans and hence, not an animal)
- Mesomycetozoea (a nonmetazoan opisthokont; hence, not an animal)
- Percolozoa (a descendant of Class Excavata, and not an animal)

- Protozoa (single-celled eukaryotes and hence, not animals)
- Sporozoan (synonymous with apicomplexan, single-celled eukaryotes that are not animals)
- Sporozoites (an infective, motile form of some sporozoans, and hence not an animal)
- Sulcozoa (a class of single-celled eukaryotes, and hence not an animal)
- Trichozoa (a subclass of Class Excavata, and hence not an animal)
- Trophozoite (a growth stage of some sporozoans, and hence not an animal)
- Zoopagomycota (a type of fungus, and hence not an animal)
- Zoospores (a taxonomically nonspecific term referring to a swimming spore, and not an animal)

Too many spores

The term spore (from the Greek "spora" meaning sowing) is used widely and indiscriminately in the biological literature to indicate an agent of microbial reproduction, often when an exact and unambiguous term is available. Hence, bacterial endospores, fungal conidia, and eukaryotic cysts are commonly lumped as "spores." Within this very book, we have witnessed "spore" appearing as a prolific suffix, prefix, or infix of unrelated biological terms, including Autospores, Chrysosporium, Cladosporiosis, Cladosporium, Cryptosporidiidae, Cryptosporidiosis, Cryptosporidium, Cyclospora, Cystoisospora, Endospores, Haemosporida, Ichthyosporea, Isospora, Micropolyspora, Microsporidea, Microsporidia, Microsporidiosis, Microsporidium, Microsporum, Oxysporum, Pityrosporum, Pleosporales, Pleosporomycetidae, Rhinosporidiosis, Rhinosporidium, Sarcosporidiosis, Scedosporium, Sporocyst, Sporothrix, Sporotrichosis, Sporozoan, Sporozoite, Streptosporangineae, Thermomonosporaceae, Tichosporonales, Zoospores, and Zygospore. It's time to call for a moratorium on spores, which we shall not label as a "sporatorium."

In addition, "spora" is the root for "sporadic" which extends the Greek meaning of "sowing" to conjure the notion of an aimless toss of seed, without any specific pattern. Hence, a sporadic disease has no known cause, and no discernible pattern of occurrence (e.g., neither genetic nor environmental, and without any particular geographic origin). The term "sporadic" is fraught with scientific ambiguity and should probably be abandoned altogether. To label a disease "sporadic" legitimizes and perpetuates the dubious notion that diseases can occur without cause (e.g., entirely by "chance"). Many of the diseases that were considered to be sporadic, decades ago, are now known to have specific causes. Would it not be more accurate to use the phrase "undetermined pathogenesis" in place of "sporadic," for occurrences of a disease whose cause is currently unknown?

Squiggly things

Parasitic worms are called helminths. Class Platyhelminthes and Class Nematoda account for all the so-called helminthic diseases of humans. Readers should be warned that the term "worm" has no taxonomic meaning; soft, squiggly organisms colloquially known as "worms" are scattered throughout animal taxonomy, with no close relationship with one another. A small squirming organism referred to as a "worm" may be an insect larva (i.e., not a helminth), or it may be one of several unrelated classes of organisms. Class Acanthocephala includes the thorny-headed worms. Class Annelida (earthworms) descends from Class Lophotrochozoa, which includes molluscs. Acorn worms (Class Enteropneusta) are hemichordates whose closest phylogenetic relative are the echinoderms, which include sand dollars and starfish. Class Chaetognatha contains the predatory marine arrow worms. Class Lophotrochozoa contains the Nemertea, or ribbon worms. Class Nematoda (roundworms) and Class Annelida (ringed worms, including earthworms) are more closely related to spiders and clams, respectively, than either one is related to Class Platyhelminthes (flatworms).

Many so-called worms are actually the larval forms of animals whose adult stage bears no resemblance to worms. An example is Linguatula serrata (Class Crustacea), the agent causing tongue worm disease. The tongue worm is the larval stage of a crustacean. Likewise, the screwworm (Cochliomyia hominivorax) is actually a type of fly. It is called a screwworm because the disease is manifested by worm-like larvae growing on skin. Lastly, the ineptly named "ringworm" infections are not caused by worms or by any animals; they are fungal infections of the skin. The word "worm" may even refer to a marsupial joey (Class Mammalia), which is typically a smooth hairless slug-shaped organism the size of a jellybean.

The same form of objection can be applied to the word "fly." The term "fly" should refer exclusively to member of Class Diptera (flies). As it happens, the term "fly" has been assigned, at one time or another, to just about any small flying insect, few of which are dipterans: butterflies (Class Lepidoptera), dragonflies (Class Palaeoptera), and mayflies (Class Palaeoptera). When a word is applied to morphologically similar, but phylogenetically unrelated organisms, what useful meaning could it convey?

Unnecessary ranks

In hierarchical biological nomenclatures, classes are given ranks. In early versions of the classification of living organisms, it was sufficient to divide the classification into a neat handful of divisions: Kingdom, Phylum, Class, Order, Family, Genus, and Species. Today, the list of divisions has nearly quadrupled. For example, Phylum has been split into the following divisions: Superphylum, Phylum, Subphylum, Infraphylum, and Microphylum. The other divisions are likewise split. The process whereby classes are split is somewhat arbitrary and subject to dispute among taxonomists [97].

Taxonomists referring to a class, without specifying its rank, will sometimes use the word "taxon."

Most importantly, the complex taxonomic ranking system for living organisms does not carry over to the ranking systems that might be used for other scientific domains (e.g., classification of diseases, classification of genes, etc.) and creates an impediment for anyone wanting to bridge classifications held within diverse, but related, fields.

In this book, taxonomic complexity is drastically simplified by dropping named ranks and simply referring to every class as "Class." When every class of organism were associated with the name of its one parent class, then it becomes possible to computationally trace the complete ancestral lineage for every class or species [98]. Likewise, knowing the direct parent class for every class and species permits us to find all the child classes (i.e., direct subclasses) for every class and, consequently, all the sibling classes for every child class.

Computer savvy biologists and bioinformaticians rely upon large taxonomic listings to trace the ancestry and to determine phylogenetic relationships among specific organisms and their classes. For example, a listing of species and classes of organisms that is used by bioinformaticians throughout the world is available at no cost from the European Bioinformatics Institute.

The taxonomy.dat, which is currently over 468 megabytes in length, can be downloaded via anonymous ftp at:

ftp://ftp.ebi.ac.uk/pub/databases/taxonomy/

Additional information on the taxonomy.dat file is found at:

http://www.ebi.ac.uk/msd-srv/docs/dbdoc/ref_taxonomy.html

A sample entry (one of hundreds of thousands in the file) is shown:

```
ID                    : 24
PARENT ID             : 22
RANK                  : species
GC ID                 : 11
SCIENTIFIC NAME       : Shewanella putrefaciens
SYNONYM               : Alteromonas putrefaciens
SYNONYM               : Pseudomonas putrefaciens
MISSPELLING           : Shewanella putrifaciens
MISSPELLING           : Alteromonas putrifaciens
```

The sample entry (above) provides an ID number for the entry organism, and for it's parent class. Since every organism and class has a parent, we can trace the taxonomic lineage of any organism or class by iterating through the parental

links [98]. For example, the following lineage, for Homo sapiens, was computed on the fly from the taxonomy.dat file:

```
Eukaryota: Opisthokonta: Metazoa: Eumetazoa: Bilateria:
Deuterostomia: Chordata: Craniata: Vertebrata: Gnathostomata:
Teleostomi: Euteleostomi: Sarcopterygii: Dipnotetrapodomorpha:
Tetrapoda: Amniota: Mammalia: Theria: Eutheria: Boreoeutheria:
Euarchontoglires: Primates: Haplorrhini: Simiiformes: Catarrhini:
Hominoidea: Hominidae: Homininae: Homo: Homo sapiens.
```

Likewise, we can instantly compute the subclasses of any class we choose. For example, let's look at three classes and their computed subclasses:

```
Eumetazoa: Subclasses are Cnidaria, Ctenophora, and Bilateria
Fungi: Subclasses are Chytridiomycota, Microsporidia,
environmental samples, unclassified Fungi, Fungi incertae
sedis, Blastocladiomycota, Dikarya, Mixed fungal DNA libraries,
Cryptomycota, Mucoromycota, and Zoopagomycota

Apicomplexa: Subclasses are unclassified Apicomplexa, environmental
samples, Aconoidasida, Apicomplexa incertae sedis, and Conoidasida
```

In summary, modern taxonomists do not need to rely on class rankings. We only need to know the parent class for each class contained in a taxonomy. Every taxonomist and every bioinformaticians should have access to simple software that can compute the ancestral lineage, and the sister classes, for any given class of living organism. Very soon, scientists will have genomic sequences for representative species covering all the known classes of living organisms.

Section 8.7 Recurring sources of error

Many errors, of a truth, consist merely in the application the wrong names of things.

Baruch Spinoza (1632–77)

Alpha Proteobacteria—Readers should be careful not to confuse Bartonella with the similar-sounding Bordetella (Beta Proteobacteria).

Alpha Proteobacteria—Readers should be aware that brucellosis has been known by a great number of different names, including Mediterranean fever. Mediterranean fever, an arcane synonym for brucellosis, should not be confused with familial Mediterranean fever (a gene disorder characterized by fever and abdominal pain) or with Mediterranean anemia (a synonym for thalassemia).

Alpha Proteobacteria—Readers should be aware that Neorickettsia, despite its name, is not a type of Rickettsia (i.e., not a member of Class Rickettsiaceae).

Neorickettsia is a member of Class Anaplasmataceae; hence, the disease it causes is an ehrlichiosis.

Alpha Proteobacteria—Readers should not be confused by the term "scrub typhus" for infection by Orientia tsutsugamushi (alternately named Rickettsia tsutsutgamushi). This disease is grouped as a "spotted fever," not a form of typhus.

Gamma Proteobacteria—Organisms of Genus Cardiobacterium should not be confused with the similar-sounding Genus Corynebacterium (Class Actinobacteria).

Gamma Proteobacteria—The term "dysentery" (from the Latin "dys" and Greek "dus," meaning bad, and the Greek "enterikos" meaning intestine) is often used to connote a specific disease, but dysentery is nonspecific term that can be applied to any enteric disorder associated with severe or bloody diarrhea. Because the group of diseases known as "dysentery" is the most frequent cause of childhood morbidity and mortality, it is important to use the term correctly. In developed countries, the term "dysentery" most often refers to salmonellosis, while in less developed countries, "dysentery" usually refers to shigellosis (also called bacillary dysentery, another misnomer) [99]. Other bacterial causes for dysentery are: Vibrio cholerae, Escherichia coli, Clostridium difficile, Salmonella, Campylobacter jejuni, and Yersinia enterocolitica. Viruses that cause dysentery include Rotavirus and Norwalk virus. The term "amoebic dysentery" is usually reserved for gastroenteritis caused by Entamoeba histolytica.

Gamma Proteobacteria—Granuloma venereum, caused by Klebsiella granulomatis, can be mistaken clinically with two other diseases that are characterized by genital ulcers: syphilis (Treponema pallidum Class Spirochaetae) and chancroid (Haemophilus ducreyi, Class Gamma Proteobacteria). Adding to the confusion, the syphilitic genital ulcer known as a chancre must be distinguished from chancroid. One last caveat. Granuloma venereum, caused by Klebsiella granulomatis, must not be confused with lymphogranuloma venereum, caused by Chlamydia trachomatis (Class Chlamydiae).

Gamma Proteobacteria—Readers should not confuse rhinscleroma, caused by Klebsiella rhinoscleromatis (Class Gamma Proteobacteria), with rhinosporidiosis, caused by Rhinosporidium seeberi (Choanozoa).

Gamma Proteobacteria—Salmonella paratyphi and Salmonella typhimurium cause typhoid fever and paratyphoid fever, respectively. Neither of these diseases should be confused with typhus fevers, caused by Rickettsia typhi and Rickettsia prowazekii. Both diseases (typhoid and typhus) take their root from a Greek word meaning stupor, referring to the neurologic manifestations of the diseases.

Gamma Proteobacteria—Species of Genus Shigella cause Shigellosis, also known as bacillary dysentery. Despite its name, readers should not assume that the cause of bacillary dysentery is a member of Class Bacilli. The exclusive cause of bacillary dysentery are Shigella belonging to the Gamma Proteobacteria.

Gamma Proteobacteria—Shigella boydii, one of the causes of shigellosis, should not be confused with Pseudallescheria boydii, a fungus in Class Ascomycota, one of many fungal organisms associated with the skin infection maduromycosis.

Gamma Proteobacteria—Readers should not confuse Plesiomonas shigelloides, containing the species name "shigelloides," with the genus name "Shigella" (vida supra).

Gamma Proteobacteria—Haemophilus influenzae causes pneumonitis, meningitis, and bacteremia, in infants and young children. Its species name, infuenzae, was assigned when the bacteria was mistakenly thought to be the cause of influenza. Influenza, also known as the flu, is caused exclusively by the influenza virus, a Group V orthomyxovirus.

Gamma Proteobacteria—Haemophilus parainfluenzae causes some cases of endocarditis. Despite its name, Haemophilus parainfluenzae is not the cause of the disease known as parainfluenza. Parainfluenza is a type of croup (laryngotracheobronchitis), and about 75% of the cases of croup are caused by the parainfluenza virus, a Group V virus.

Gamma Proteobacteria—Despite its name, Haemophilus parainfluenza is not the cause of the disease known as parainfluenza. Parainfluenza is a type of croup (laryngotracheobronchitis), and about 75% of the cases of croup are caused by the parainfluenza virus, a Group V virus.

Gamma Proteobacteria—Haemophilus ducreyi is the cause of chancroid, a sexually transmitted disease. It must not be confused with Klebsiella granulomatus, in Class Enterobacteriaceae, the cause of granuloma inguinale.

Gamma Proteobacteria—Readers should not confuse Genus Acinetobacter (Gamma Proteobacteria) with Class Actinobacteria.

Spirochaetae—Rat-bite fever is caused by either Spirillum minus or by Streptobacillus moniliformus (Class Fusobacteria) [100]. Regardless of the causative organism, or the phylogenetic classes to which the organisms are assigned, the clinical symptoms are similar, as is the treatment.

Mollicutes—Erysipelothrix contains one infectious species; Erysipelothrix rhusiopathiae, the cause of erysipeloid, a type of cellulitis (subcutaneous infection). Students should not confuse erysipeloid with the similar-sounding disease, erysipelas. Both erysipeloid and erysipelas produce cellulitis. Erysipelas is more common and, potentially, a more serious disease than eysipeloid. Erysipelas is caused by members of Genus Streptococcus (Class Bacilli). Two additional similar-sounding skin conditions are erythrasma, characterized by brown scaly skin patches; caused by Corynebacterium minutissimum (Class Actinobacteria), and erythema infectiosum, caused by Parvovirus B19. All four skin conditions are associated with reddened skin, and all three diseases take their root from the Greek "erusi," meaning red.

Class Bacilli plus Class Clostridia—The term "bacillary" is misleading. You might think that the adjective "bacillary" would be restricted to members of Class Bacilli, its subclasses, and Genus Bacillus. It seldom does. The word "bacillus" has its root in Latin, from "baculum" a rod or staff, so the name has been applied to the second term in the binomial name of bacteria that do not belong to Class Bacilli.

An example of a species with "bacilla" in its name that is not a member of Class Bacilli is Bartonella bacilliformis (the cause of verruga peruana). Examples of genera with "bacillus" in their name that are not members of Class Bacilli are: Actinobacillus and Streptobacillus. Genus Streptobacillus (Class Fusobacteria) is a terminological catastrophe, as it is not a sister genus to Streptococcus, and it is not a member of Class Bacilli.

The subclasses of Class Bacilli were assigned based on phylogeny, not on morphology. Therefore, there are members of Class Bacilli that are not rod-shaped (e.g., Genus Staphylococcus and Streptococcus).

Diseases containing the term "bacillary" are not caused by members of Class Bacilli. These include bacillary angiomatosis (caused by Bartonella quintana and Bartonella henselae) and bacillary dysentery (caused by four different Shigella species and by Yersinia enterocolitica).

Class Bacilli plus Class Clostridia—Listeria monoctyogenes is the organism that causes listeriosis. Listeriosis should not be confused with the similar-sounding disease, leptospirosis (Spirochaetae) Spirochaetae.

Chlamydiae—Readers should not confuse trachoma with inclusion conjunctivitis, as each disease is caused by distinct variants of the same species (Chlamydia trachomatis). Trachoma is contracted by exposure to eye secretions from people with trachoma. Inclusion conjunctivitis is caused by ocular exposure to secretions from the sexually transmitted infection.

Chlamydiae—Chlamydia trachomatis may also cause lymphogranuloma venereum, a disease that usually presents as swollen lymph nodes in the groin. The lymph nodes often have draining abscesses. The disease is rare, with only a few hundred cases occurring in the United States each year. Lymphogranuloma venereum must not be confused with granuloma inguinale, also known as granuloma venereum, caused by the bacterium Klebsiella granulomatis.

Actinobacteria—Members of Class Actinobacteria tend to be filamentous, and this morphologic feature led to great confusion. In the past, these filamentous bacteria were mistaken for fungal hyphae, and many of the diseases caused by members of Class Actinobacteria are given fungal names (e.g., actinomycosis, mycetoma, and maduromycosis).

Actinobacteria—Readers should be aware of the highly confusing term, "diphtheroid," from the Greek diphththera, meaning leather membrane, and commonly applied to all the nonpathogenic species within Genus Corynebacterium. As nonpathogens, the diphtheroids do not cause diphtheria. Diphtheria is caused Corynebacterium diphtheriae (i.e., a nondiphtheroid).

Actinobacteria—Readers should not confuse the bacterial genus Tropheryma with the similar-sounding term Taphrinomycotina, the fungal genus that includes Pneumocystis.

Actinobacteria—Actinomycosis (Greek "actino," ray and "myco," fungus), despite its misleading name, is a bacterial infection, not a fungal (i.e., mycotic) infection. Actinomycosis, also referred to as actinomycotic abscess, can be caused by any of several actinobacterial species.

Metamonada—It is important not to confuse trichomonas, caused by the metamonad Trichomonas vaginalis, with the similar-sounding name trachoma. Trachoma is a bacterial infection of the eyes caused the Chlamydia trachomatis.

Apicomplexa—The disease produced by any of the organisms belonging to Class Coccidia is known collectively as coccidiosis. This term is applied most often to coccidian infections in animals, excluding humans. Coccidiosis in humans must not be confused with the similar-sounding coccidioidomycosis (Ascomycota). Needless to say, Coccidia, the Apicomplexan subclass, should never be confused with conidia, the asexual fungal spore, with which it rhymes.

Heterokonta—Formerly known as Heterokontophyta. The roots "phyta," "phytes," "phyte," and "phytic" all derive from the Greek "phyton" meaning plant. Despite its deceptive former name, the heterokonts are not plants.

Heterokonta—Oomycota, a "colorless" class of heterokonts, contains the organisms that produce late blight of potato (Phytophthora infestans), and sudden oak death (Phytophthora ramorum). Oomycota, despite its suffix (mycota, an alternative name for fungus), is not a member of Class Fungi.

Heterokonta—It is important to avoid confusing Blastocystis with three similar-appearing but unrelated terms: blastomycosis, blastocyst, and blastomycetica. Blastomycoses is a fungal disease (Ascomycota). Blastocyst is the fluid-filled embryonic body characteristic of animals. Blastomycetica appears in the name of a fungal infection "erosio interdigitalis blastomycetica." This infection, despite what its name would imply, is a candidal infection of the web space between the third and fourth fingers of either hand. The infection was given its deceptive name in 1917, 5 years before the genus Candida was recognized [101]. As is often the case, the mistake stuck. All four terms come from the root word blastos (Greek for bud or embryo). Cystos (as in Blastocystis and blastocyst) is the Greek root meaning sac.

Amoebozoa—Do not confuse Entamoeba coli (abbreviation E. coli) with Escherichia coli (likewise abbreviated as E. coli). Both live in the colon, and both can be reported in stool specimens. Do not confuse Entamoeba (Class Amoebozoa) with Dientamoeba (Class Metamonada).

Amoebozoa—It is commonly agreed that the term "amoebiasis," with no qualifiers in the name, refers exclusively to the intestinal infection by Entamoeba histolytica. Encephalitides caused by members of Class Amoebozoa (Acanthamoeba and Balamuthia) are named granulomatous amoebic encephalitis. Encephalitis caused by Naegleria fowleri (not an amoeba) is called primary amoebic meningoencephalitis, an accepted misnomer. Naegleria is a member of class Percolozoa. A better name for the Naeglerian disease would be primary percolozoan meningoencephalitis.

Archaeplastida—"Kleptoplast" should not be confused with the similar-sounding word, "kinetoplast." A kleptoplast is a chloroplast that has been stolen by another organism. A kinetoplast, uniquely found in members of Class Kinetoplastida, is a clump of DNA composed of multiple copies of the mitochondrial genome, tucked inside a mitochondrion.

Nematoda—Astute readers will notice that the prefix "trich" appears often in connection with Class Nematoda: Trichostrongylus, Trichocephalida, Trichinellidae, Trichinella, and Trichuris. A wide assortment of organisms, diseases, and medical terms contain the root "trich" (pronounced trick) and produce similar-sounding terms (i.e., homonyms). If you want to avoid confusing one disease with another, it is best to "come to terms" with this "trichy" nomenclature. The suffix "trich" comes from the Greek "thrix," meaning hair. Various unrelated organisms with a hair-like appendage are provided with the "trich" suffix. Likewise, medical conditions of the hair are provided the same suffix: trichosis is any pathologic condition of hair; trichilemmoma is a tumor of hair, trichobezoar is the medical term for hairball, and trichotillomania is compulsive hair pulling. Words that sound somewhat like "trich" include trachoma (caused by the bacteria, Chlamydia trachomatis) and trachea (the windpipe). In addition to Trichomonas, there are several unrelated "trich" organisms that cause disease in humans: Trichinella, Trichomonas, Trichomonad, and Trichophyton. Other "trich" diseases: Trichostrongylosis trichinosis, trichuriasis, trichomoniasis, trichiasis (everted eyelashes that touch the cornea or conjunctiva, often a post-infectious condition).

Nematoda—When toxocara migrate through viscera, the condition is called visceral larva migrans. When toxocara migrate through an eye, the condition is called ocular larva migrans. When toxocara migrate through the skin, the condition is NOT called cutaneous larva migrans: this term is reserved for cutaneous manifestations of Ancylostoma braziliense.

Nematoda—Readers should not confuse "toxocara" with the similar-sounding "toxoplasma" (Class Apicomplexa), a problem aggravated when clinicians use the abbreviated form "toxo," when referring to "toxoplasmosis."

Chelicerata—Most members of Class Chelicerata are noninfectious in humans. Only two genera of Class Chelicerata live in, or on, humans, and both genera belong to the subclass of arachnids named Class Acari, which includes mites and ticks. Readers should not confuse mites and ticks with insects. Insects are members of Class Hexapoda. In addition, Class Acari (in Class Chelicerata) should not be confused with the similar-sounding Class Ascaris (in Class Nematoda).

Hexapoda—Class Hemiptera are the so-called "true bugs." They are distinguished from other insects by the shape of their mouth parts, which are shaped as a proboscis and covered by a labial sheath. The mouth parts of Class Hemiptera are designed for sucking. Class Hemiptera includes cicadas and aphids. The triatome species that are vectors for Trypanosoma cruzi (Euglenozoa) are members of Class Hemiptera.

Crustacea—One of the more confusing terms associated with pentastomiasis is "porocephaliasis," named for a pentastome genus, Porocephalus. The genus "Porocephalus" and the infection "porocephaliasis" should not be confused with "porocephaly," a rare developmental disorder in which cysts or cavities are found in the brains of infants.

Ascomycota—Readers should be alerted that the term "Candida" is a source of some taxonomic confusion: candida, in Latin, means white. Many organisms are white, and have taken "candida" as part of a binomial name. Though there is only one Genus Candida (the fungus), there are many species named candida, particularly in Kingdom Plantae. For example, there are three M. candida species: Mammilloydia candida, a cactus; Miltonia candida, an orchid; and Masdevallia candida, another orchid.

Ascomycota—Readers should remember not to confuse Microsporum with Microspora, a genus in Class Microsporaceae, a Chlorophyte. It is also important not to confuse fungi of Genus Microsporum with the fungi of Genus Microsporidium (Class Microsporidia).

Ascomycota—Readers should not assume that Lobo disease is caused by a member of Class Lobosea, a subclass of amoebozoans that includes Genus Acanthaemoeba. Lobo disease is caused by Lacazia loboi, an ascomycote fungus.

Ascomycota—Note that Piedraia hortae is not a member of genus Hortaea; being as a species of Genus Piedraia. Likewise, Hortaea werneckii does not share its genus with Piedraia hortae. The mycologists responsible for this nomenclature could not have been more confusing, if they tried.

Basidiomycota—Fungi of Genus Trichosporon, basidiomycotes causing white piedra, should not be confused with organisms of Genus Trichsporum, a name that includes both fungi in Class Ascomycota and plants in Class Gesneriaceae [102].

Microsporidia—It is important not to confuse microsporidiosis with cryptosporidiosis, an apicomplexan disease (Apicomplexa), that also produces diarrhea in immune-compromised patients.

Group I Viruses—Class Herpesviridae (Group I virus) should not be confused with Class Hepeviridae (Group IV virus).

Group II Viruses—Bocavirus, a Group II parvovirus, should not be confused with Bocas virus, a type of Coronavirus (Group IV).

Group IV Viruses—Hepevirus should not be confused with the orthographically similar "herpesvirus." Also, readers should not confuse Class Hepeviridae (Hepatitis E virus) with Class Hepacivirus, a subclass of Class Flaviviridae that contains the Hepatitis C virus. Neither of these Group IV subclasses should be confused with Class Hepadnaviridae (Group VII).

Group IV Viruses—Readers should not confuse Rubella virus with the measles virus, Rubeola. Rubeola virus is a paramyxovirus (Group V), unrelated to Rubella virus.

Group V Viruses—One member of Class Arenaviridae, Lassa virus, the cause of Lassa fever, should not be confused with Lyssa virus, a member of Class Rhabdoviridae and the cause of rabies.

Group V and VI viruses—Readers should be careful not to confuse Class Deltaretrovirus (a class of Group VI retroviruses) with Class Deltavirus, a Group V single-strand RNA negative-strand virus containing Hepatitis delta virus.

Group VI Viruses—Readers should not confuse HTLV-III, a virus discovered in 2005, and which is not known at this time to produce disease in infected humans, with an early name (long since abandoned) that was assigned to the HIV virus.

Glossary

Dictionary A terminology or word list accompanied by a definition for each item.

Hematoxylin and eosin Abbreviation: H&E. The most common stain used in pathology laboratories. With H&E staining, the nuclei of cells are blue, and the cytoplasm is pink. Without staining, cells are colorless (except for those cells that are pigmented, such as melanocytes), and the anatomic parts of the cell (nucleus, cell membrane, nucleolus, brush borders, etc.) cannot be easily distinguished. The development of histologic staining techniques in the 19th century was among the most important advances in medical science.

Negative classifier A negative classifier is a feature whose absence is used to exclude an organism from a taxonomic class; it is the riskiest way to assign classes. A species may lack a particular feature because none of its ancestors ever had the feature, as might be the case in a valid lineage of organisms. An example is the Collembola, popularly known as springtails, a ubiquitous member of Class Hexapoda, and easily found under just about any rock. These organisms look like fleas (same size, same shape) and were formerly included among the true fleas (Class Siphonaptera). Like fleas, springtails are wingless, and it was assumed that springtails, like fleas, lost their wings somewhere in evolution's murky past. However, true fleas lost their wings when they became parasitic. Springtails never had wings, an important taxonomic distinction separating springtails from fleas. Today, springtails (Collembola) are not classed with fleas or with any member of Class Insecta. They belong to Class Entognatha, a separate subclass of Class Hexapoda. Alternately, taxonomists may be deceived by a feature whose absence is falsely conceived to be a fundamental property of a class of organisms. For example, Class Fungi was believed to have a characteristic absence of a flagellum. Based on the absence of a flagellum, the fungi were excluded from Class Opisthokonta and were put in Class Plantae, which they superficially resembled. However, the chytrids, recently shown to be a primitive member of Class Fungi, have a flagellum. This places fungi among the true descendants of Class Opisthokonta.

Nomenclature A nomenclature is a listing of terms that cover all of the concepts in a knowledge domain. A nomenclature is different from a dictionary for three reasons: (1) the nomenclature terms are not annotated with definitions, (2) nomenclature terms may be multiword, and (3) the terms in the nomenclature are limited to the scope of the selected knowledge domain. In addition, most nomenclatures group synonyms under a group code. For example, a food nomenclature might collect submarine, hoagie, po' boy, grinder, hero, and torpedo under an alphanumeric code such as "F63958." Nomenclatures simplify textual documents by uniting synonymous terms under a common code. Documents that have been coded with the same nomenclature can be integrated with other documents that have been similarly coded, and queries conducted over such documents will yield the same results, regardless of the term is entered (i.e., a search for either hoagie or po' boy will retrieve the same information, if both terms have been annotated with the synonym code, "F63948"). Optimally,

the canonical concepts listed in the nomenclature are organized into a hierarchical classification [103, 104].

Problematica The term "problematica" is used by taxonomists to indicate a class of organism that defies robust classification [105]. The very existence of this term tells us that taxonomy is a delicate and tentative science. We must always be prepared to examine and test our current classification, and to make corrections wherever warranted.

Proximate cause Synonymous with the immediate cause. The proximate cause of a biological event is the closest action that can be held responsible for the cause of the event. For example, the rupture of a blood vessel in the lung may be the proximate cause of death, while an invasive lung cancer may have been the underlying cause of death. The erosion of a vessel by tumor cells was one of the sequence of events leading from the underlying cause of death to the proximate cause of death. The underlying cause of death satisfies the "but-for" condition. But for the lung cancer, the vessel would not have eroded, and blood would not have flooded the lung tissue. The proximate cause of death need not be a necessary condition resulting from the underlying cause of death. Had the vessel not ruptured, the individual may have died from an alternate proximate cause (e.g., metastasis, pneumonia).

Root cause The earliest event or condition that is known to set in motion a chain of additional events that can result in some specified result. The term "root cause" is preferable to another term "underlying cause" that is often applied to the same concept. Any of the events that precede a result could be construed as underlying causes. The term "root cause" conveys the idea of a first or earliest event in a multievent process.

Of course, we can never be certain what the earliest event is in any process. For example, infections from Naegleria fowleri result from exposure to free-living organisms in their natural habitat (pond and river water). The organism travels from the nose to the brain, where it causes a meningoencephalitis. It seems self-evident that Naegleria fowleri is the root cause of Naeglerian meningoencephalitis. Maybe not.

Because these organisms are widely found in water, it is presumed that millions of people are exposed to the organism, but only very few individuals develop meningoencephalitis. It is not known why most people are unaffected by the organism, while others develop a rapidly progressive meningoencephalitis. Perhaps the root cause of disease is a very specific deficiency in the host defense system, rendering a few individuals susceptible to infection with a ubiquitous organism. If so, the root cause shifts from the organism to the host.

Even in the simplest of cases, it is difficult to assign a root cause with any certainty. We never know if we've looked backwards far enough. Still, we do the best that we can, and we apply the term "root cause" with the understanding that we may need to modify our thinking, if evidence of an earlier event comes to light.

Serotype Subtypes of a species of bacteria or virus that differ in their surface antigens.

Serovar Same as serotype.

Unclassifiable objects Classifications create an hierarchical collection of classes, and their taxonomies assign each and every named object to its correct class. This means that a classification is not permitted to contain unclassified objects; a condition that puts fussy taxonomists in an untenable position. Suppose you have an object, and you simply do not know enough about the object to confidently assign it to a class. Or, suppose you have an object that seems to fit more than one class, and you can't decide which class is the correct class. What do you do? Historically, scientists have resorted to creating a "miscellaneous," "problematica," or "incertae sedis" (uncertain placement) class into

which otherwise unclassifiable objects are given a temporary home, until more suitable accommodations can be provided [105].

Historically, the promiscuous application of "miscellaneous" classes has proven to be a huge impediment to the advancement of the biological sciences. In the case of the classification of living organisms, the class of protozoans stands as a case in point. Ernst Haeckel, a leading biological taxonomist in his time, created the Kingdom Protista (i.e., protozoans), in 1866, to accommodate a wide variety of simple organisms with superficial commonalities. Haeckel himself understood that the protists were a blended class that included unrelated organisms, but he believed that further study would resolve the confusion. In a sense, he was right, but the process took much longer than he had anticipated; occupying generations of taxonomists over the following 150 years. Today, the former members of Kingdom Protista have been reassigned to positions among the animals, plants, and fungi. In the meantime, therapeutic opportunities for eradicating the so-called protozoal infections, using class-targeted agents, have no doubt been missed [3]. For practical reasons, textbooks still use the term "protozoan," but strictly speaking, Kingdom Protista no longer exists [95].

You may think that the creation of a class of living organisms, with no established scientific relation to the real world, was a rare and ancient event in the annals of biology, having little or no chance of being repeated. Not so. A special pseudoclass of fungi, deuteromycetes (spelled with a lowercase "d," signifying its questionable validity as a true biologic class) has been created to hold fungi of indeterminate speciation. Currently, there are several thousand such fungi, sitting in a taxonomic limbo, waiting to be placed into a definitive taxonomic class [3, 46].

Underlying cause The event that initiated the sequence of events leading to some clinical outcome (e.g., disease). Death certificates require physicians to list the underlying cause of death. The World Health Organization, aware of the difficulties in choosing an underlying cause of death, and assigning a sequential list of the ensuing clinical consequences leading to the proximate cause of death, has issued reporting guidelines [106]. Instructions notwithstanding, death certificate data are notoriously inconsistent, giving rise to divergent methods of reporting the diseases that cause death [107–109].

Reluctantly, we must acknowledge that, in any biological system, we can seldom designate the underlying causes with any certitude. One event may lead to many other events, and events which we believe to be initiating may have their own predicative causes.

Vector An organism that moves a disease-causing organism from one host to another. Diseases spread by vectors include malaria, plague, leishmaniasis, African trypanosomiasis, relapsing fever, yellow fever, dengue fever and dengue hemorrhagic fever, hantavirus disease, West Nile encephalitis, Japanese encephalitis, Rift Valley fever, Venezuelan equine encephalitis, and chikungunya. All arboviruses have arthropod vectors, and there are about 100 known arboviruses that cause human disease [52]. One vector can carry more than one type of infectious organism. For example, a single species of Anopheles mosquito can transmit Dirofilaria immitis, O'nyong'nyong fever virus, Wuchereria bancrofti, and Brugia malayi. Obversely, one disease organism can be spread by more than one vector. For example, orbiviruses are spread by mosquitoes, midges, gnats, sandflies, and ticks.

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