2 An Overview of Parasite Diversity

All living species are involved in parasitism, either as parasites or as hosts.
De Meeûs et al. (1998)

In the midst of your summer vacation reveries you suddenly come down with a particularly nasty, persistent and stubborn case of watery diarrhea, one accompanied by a feeling of bloating, loss of appetite and weight, nausea, vomiting and fever. A visit to the doctor culminates in the discovery in your stools of many tiny, fairly non-descript spherical structures that prove to be the oocysts of a parasite you've likely never heard of called ***Cyclospora cayetanensis***—you have been diagnosed with a case of cyclosporiasis. In what is becoming a more regular summer ritual even in developed countries, you have probably acquired this parasite by ingestion of imported vegetables or fruit (the hairy surfaces of raspberries and bagged salad greens are frequent culprits) contaminated with human fecal material containing ***Cyclospora*** oocysts. Although you may not appreciate it much at the time, you have just had an up-close and personal encounter with one of the most diverse groups of parasites on the planet, for ***C. cayetanensis*** is a member of a huge, diverse phylum of parasites called the Apicomplexa, which also includes the more familiar parasites that cause malaria and toxoplasmosis.

In Chapter 1 we described the basic features of parasitism and explained how parasitism differs from other kinds of biological associations. The goal of this chapter is to present a big picture of the diversity of the world's parasite species. The apicomplexans mentioned above are but a part of this impressive diversity. In this overview, we begin to develop an appreciation for the relationship of these many species to one another and other organisms.

Such an overview is helpful in many ways. It can help us understand how different groups of parasites have diversified and adapted to their hosts. From this we gain a better appreciation for predicting which parasites might colonize new host species, have the potential to cause an emerging disease, become invasive in new locations, or that may fare better or worse under changing climatic regimes. This knowledge facilitates efforts to control parasitic diseases by enabling us to determine the full spectrum of parasite species that might be involved and that are in need of control. The study of parasite diversity also allows us to develop more natural schemes to facilitate the identification and classification of parasites. It also enables us to assess the rate at which parasite species are becoming extinct and thus fits in with ongoing efforts to improve our understanding of the overall diversity of life

In This Chapter

Species names highlighted in red are included in the Rogues' Gallery, starting on page 505.

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forms in the world. Without a thorough inventory of parasite diversity, we may never know that certain species even existed before they are gone. An understanding of parasite diversity allows us to fully appreciate the place of parasites in natural environments, including their distinctive roles in ecosystems. It also puts us in a better position to exploit the unique biochemical capabilities of parasites for medicinal or other purposes. Finally, investigation of parasite diversity aids us in understanding the ecological and evolutionary processes that dictate how and why parasites have evolved and diversified as they have.

Need a quick tutorial on how to interpret evolutionary trees? Please see the tutorial provided with on-line materials accompanying the book. Visit https://www.routledge.com/Parasitology-A-Conceptual-Approach/Loker-Hofkin/p/book/9780367228880

In this chapter we first provide an overview of the immense diversity of parasites using evolutionary trees as a framework to portray this diversity. Thereafter, examples are provided that show how the evolutionary relationships of some enigmatic parasite groups have been revealed. Also discussed are examples of how humans have acquired some of our parasites and how we can retrace their evolutionary histories. The search for new parasite diversity is ongoing, and some examples of how this search is undertaken and the diversity cataloged are described in **Boxes 2.1** and **2.3**. We also discuss some examples of improved classification schemes that are based on a thorough knowledge of parasite diversity and evolutionary relationships. Examination of parasite diversity makes clear that parasites have frequently arisen from free-living ancestors. We also consider whether parasite lineages ever give rise to free-living organisms.

BOX 2.1
Elasmobranchs and Their Tapeworms—An Example of the Study of Parasite Biodiversity

Living within a peculiar part of the digestive system of elasmobranchs (sharks, skates, rays and sawfish) known as the spiral intestine is a large diversity of distinctive parasites representing nine major lineages of tapeworms, or cestodes. They provide a model for how the world's diversity of parasites can be characterized, including the following specific example. Specimens of the dwarf sawfish *Pristis clavata* (**Figure 1A**) were collected in an estuarine stream in northern Australia. Details regarding the origin of the sawfish, date and manner of capture and photographs were taken. Tissue samples were collected and saved to enable eventual extraction of the sawfish's DNA so that representative gene sequences could be obtained and compared with other elasmobranch species and portrayed in evolutionary trees depicting relationships among the elasmobranchs. The spiral intestine was then removed from each sawfish, and the tapeworms within, typically numerous and tiny, just a few millimeters in length, were removed and separated. Importantly, the tapeworms and any other parasites recovered from the sawfish host were given identifying labels such that host and parasite will be forever linked in museum records, leading to the eventual formation of large databases linking parasite specimens to their exact host of origin.

Some specimens of the tapeworms were fixed, stained and mounted on permanent slides or prepared for sectioning, to facilitate subsequent description of the morphological features. Parts of the same and other specimens were preserved in ethanol for eventual analysis of sequences of the 28S rDNA gene. The sequence obtained for this gene serves as an objective criterion to enable differentiation among species of tapeworms. It thus serves as a **barcode**, conceptually similar to a supermarket using distinctive barcodes to identify its products. This sequence along with sequences of other genes can also be used to generate phylogenetic trees depicting relationships among the tapeworms recovered from this and other elasmobranchs. Resulting from the scrutiny of the morphological features and sequence data in comparison to what is already known regarding tapeworms recovered from other elasmobranchs, an expert's opinion can be made to determine that the tapeworms recovered represent two new species of a new cestode genus, *Matticestus anneae* and *Matticestus kathleenae*. This was followed by a publication (Caira et al., 2018) describing the new genus and species, one of which is illustrated in **Figures 1B and C**. Specimens, including the actual specimens used to describe the species, were deposited in museum collections so they are part of the permanent record, aiding further future studies of this and similar tapeworms.

Imagine this process being repeated for different species of elasmobranchs from around the world. A vast record of host–parasite combinations is being developed, providing an increasingly complete compendium of the world's species of elasmobranch tapeworms (now numbering nearly 1,000 species), resulting in the publication of a monograph that summarizes much

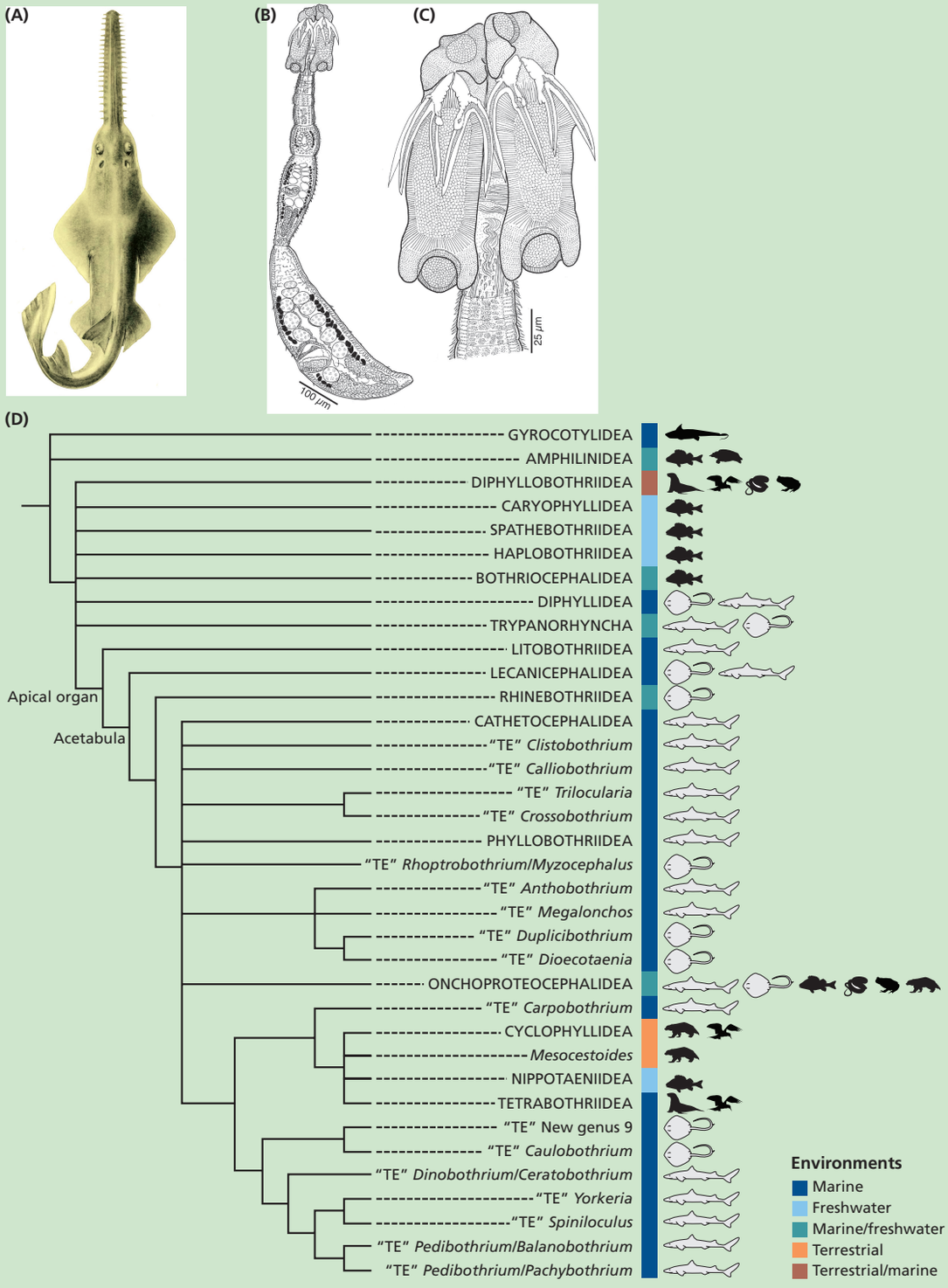

Figure 1 Elasmobranchs harbor a diverse array of cestode species. (A) A dwarf sawfish *Pristis clavata*, host to one of many new species of cestodes described from elasmobranchs. (B) A line drawing of the whole body and the remarkably intricate anterior holdfast or scolex (C) of the adult of *Matticestus anneae*, representing a new genus and species of tapeworm. (D) A summary of cestode relationships, shown relative to vertebrate hosts parasitized. Color-coded bars indicate the type of environment, icons indicate the groups of vertebrates parasitized, with solid icons indicating non-elasmobranchs. Note the prominent role played by elasmobranchs in the diversification of tapeworms. (A, public domain; B, C images from Figure 1 of Caira JN, Jensen K & Fyler CA (2018) *J Parasitol* 104:133–144. With permission from The Allen Press; D, From Caira JN & Jensen K (2014) A digest of elasmobranch tapeworms. *J Parasitol* 100:373–391 reproduced by permission of The Allen Press.)

of this information (Caira and Jensen, 2017). This systematic approach has also provided new information on the distribution and diversity of the world's species of elasmobranchs (Naylor et al., 2012) and has permitted the synthesis of a new overview of broad-scale patterns in tapeworm evolution and how they relate to the evolution of their hosts (**Figure 1D**). Lastly, although the life cycles of many of the elasmobranch tapeworms are unknown, the sequence and morphological database generated for adult tapeworms will provide unambiguous reference points for investigators who will someday find the larval stage of tapeworms in various hosts like fish or molluscs. By comparing the sequences from the larval stages with known sequences from the adult tapeworms, they can infer how the unknown life cycles of the tapeworms might fit together. Sadly, in a world where the abundance and diversity of elasmobranchs is being steadily eroded by profligate fishing practices, the record being established for their tapeworm diversity may increasingly come to represent a testament of lost parasite diversity.

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Although the primary emphasis of this chapter is to examine parasite diversity on a large scale, we must also remain mindful that parasite diversity can be gauged in other ways as well. Consequently, we also consider the genetic diversity inherent *within* parasite species and explain why this is relevant. One powerful means to consider the genetic resources available to any particular parasite is to sequence its genome. We will touch on characteristics of parasite genomes throughout the book, but this chapter will also delve into a goldmine of genetic diversity coming to light as a greater and greater diversity of parasite genomes is revealed.

2.1 THE DIVERSITY OF PARASITE SPECIES

What constitutes a parasite species requires some explanation

Let us begin with the definition of a parasite and a species. Recall the discussion of what constitutes a parasite in Chapter 1. For some, the term parasite applies only to eukaryotes with parasitic lifestyles. For others, there need be no kind of taxonomical constraint placed on an organism (such as a bacterium) or genetic entity (such as a virus) for it to be considered a parasite. Clearly these differing points of view will drastically influence one's estimate of how many different kinds of parasites exist.

Many different definitions of species have been put forth over the years and all have shortcomings. We use the following widely adopted version, frequently referred to as the biological species concept. A species is a group of individuals with similar properties that are able to interbreed with one another and produce fertile offspring and that don't regularly interbreed with other species. The members of a particular parasite species may be dioecious (or gonochoristic), meaning the species is comprised of separate male and female individuals, or they may be monoecious (or hermaphrodites), having functional reproductive organs of both sexes and capable of either cross-fertilization with another individual or, possibly, self-fertilization.

Although this definition readily fits many parasite species, it is less applicable for some organisms such as prokaryotes (eubacteria and archaea) that often have high levels of genetic variability. This variability arises in part from their enormous ability to exchange genetic information by **horizontal gene transfer** (HGT) with other organisms. HGT is a process in which one organism acquires genetic information from another organism

without being the offspring of that organism. The biological species concept also is problematic when applied to some eukaryotic parasites that may only rarely undergo sexual reproduction or that do so in unconventional or cryptic ways. As an example, for a species such as the unicellular ***Giardia lamblia***, even though population studies suggest recombination events consistent with sexual reproduction occur, no overt mating events have been observed. Meiosis-related genes occur in ***Giardia***, and there is evidence of genetic exchange between the two nuclei found in a single organism. Sex may be facultative in ***Giardia*** or may require different mating types we have yet to observe interacting with one another. Reproduction involving an infrequent, nonconventional, or cryptic exchange of genetic information applies to other important groups of parasites such as protists in the genera ***Entamoeba***, ***Trichomonas***, ***Leishmania*** and ***Trypanosoma***.

One approach to document that trypanosomes do in fact engage in sex is shown in **Figure 2.1**. Experimental crosses were made between two parental stocks of ***Trypanosoma brucei***, one transfected with a gene encoding a red fluorescent pigment and one transfected with a gene encoding a green pigment. The two stocks were mixed in a single tsetse fly (***Glossina***) vector by allowing it to feed on blood containing both types of parasites. Trypanosomes were removed from the fly and expanded in numbers in mice. Some trypanosomes retrieved from the mice exhibited either parental genotype (red or green) while others displayed a yellow phenotype (a color resulting from the presence of both red and green pigments), indicative that genetic recombination had occurred between red and green parental trypanosomes. Additionally, some trypanosomes from the fly's salivary glands have been shown to express genes associated with meiosis or to be haploid, both indicative of the operation of meiosis as an integral part of the sexual reproduction process. These results indicate that a named species like ***T. brucei*** is not just a collection of isolated clones but that these clones are united by sexual recombination events, even if such events do not occur as predictably or obviously as they do in other organisms. By contrast, some trypanosomes like ***T. vivax*** and ***T. brucei gambiense*** seem to have a more

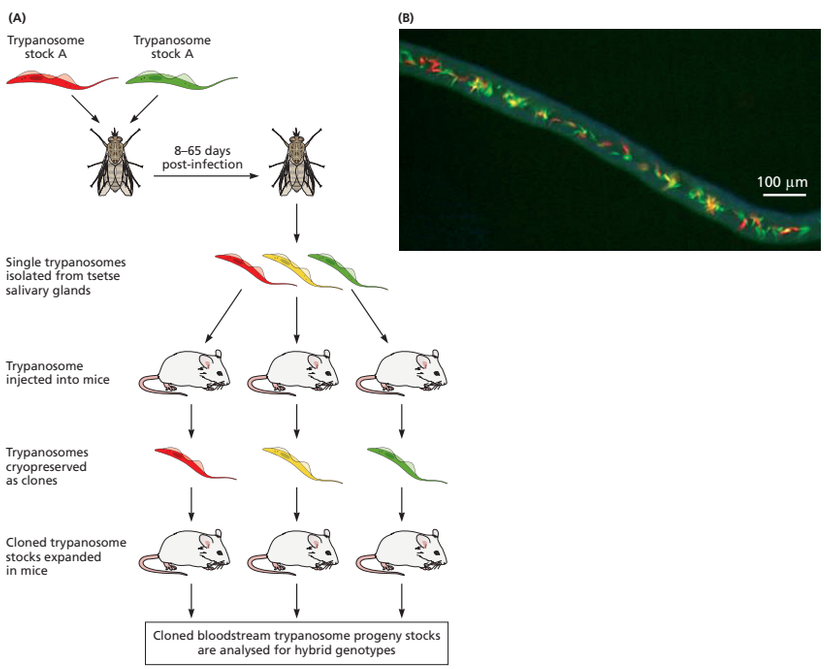

Figure 2.1 Sex is harder to document in some species than in others. (A) Experimental crosses made between two parental stocks (red and green) of *Trypanosoma brucei* in tsetse flies (*Glossina*) lead to retrieval of some progeny (yellow), indicative that sexual reproduction has occurred. (B) A salivary gland from *Glossina* showing trypanosomes of both parental types and of a yellow type, the latter indicative of genetic recombination. (A, From Hide G (2008) *J Parasitol* 24:425–428 reproduced by permission of Elsevier; B, From Gibson W et al. (2008) *Parasit Vectors*. doi:10.1186/1756-3305-1-4, published under Creative Commons Attribution License.)

Only slowly sharing its sexual secrets: *Trypanosoma cruzi*, the organism responsible for causing Chagas disease, has long been an enigma regarding its tendency to engage in sexual reproduction. What does recent evidence suggest? Read more here: https://doi.org/10.1038/s41467-019-11771-z.

purely clonal reproductive strategy for which sex has not been clearly demonstrated.

Because of such difficulties, some have advocated as an alternative to the biological species concept using an evolutionary species concept for parasites. In this view, a species is considered to be a group of organisms having a single lineage with a shared evolutionary trajectory. Ambiguities arise here as well because how different do lineages have to be if we are to consider them as one or more species? Also, analysis of different genes might return different answers with respect to the evolutionary relationships among the organisms being considered.

Even though different genes may have different histories and rates of divergence, sequence data acquired from both newly-collected parasite specimens or existing specimens in private or public collections are now used routinely to document a specimen's distinctiveness, possibly as a new species, and to reveal their relationships (see **Box 2.1**). There has been a strong trend to document for many eukaryotic organisms the sequence of a particular gene that can serve as a convenient species-specific marker, or **barcode**. The sequence chosen for barcoding is ideally one that is variable between species and relatively invariant within a species.

Often, but not always, the mitochondrial cytochrome *c* oxidase 1 gene (*CO1*) is used as such a marker. This is possible because the *CO1* gene is widely represented in eukaryotic genomes and, at least for animals, a 648-base-pair stretch of the gene is sufficiently variable among many species to provide a distinctive reference point. In general, mitochondrial genes are prone to higher rates of mutation than genes contained in the nucleus. The higher mutation rate may result from their proximity to reactive oxygen species that are produced in the mitochondria during respiration and that are capable of causing damage. The variability of *CO1* contrasts with the SSU rRNA gene discussed below that has worked effectively for building a tree of life incorporating very disparate organisms but that is too invariant to serve as a species marker. The trend is for more and more genes to be used to help delineate species, increasingly with the standard becoming the entire genome sequence for new specimens.

With respect to the use of gene sequences like *CO1* or others to aid identification, the actual specimen associated with a particular barcode sequence(s) must, as noted in **Box 2.1**, still be identified by traditional means, often with the involvement of an expert for that group. Ideally, that particular specimen including the host from which it came, its geographic locality, date of collection, sequence, and identification are then permanently linked and made publicly available in sequence databases. Specimens collected later can then be readily identified if they match the barcode sequence of the known species. Even if a database match is not available, the barcode sequence for an unknown specimen is a powerful tool to assist later with sound identification.

Molecular markers are also very useful tools for parasitologists interested in revealing the diversity of otherwise cryptic parasite species present in a host or in elaborating the unknown life cycle of a new parasite. For example, suppose that larval stages of an unknown parasite collected from a snail are barcoded and added to the sequence database. Years later, possibly in a very different location, another researcher might collect an adult parasite from the intestine of a bird that is identical in barcode sequence to the larvae originally acquired from the snail. In this way, an important connection can be made to illuminate for the first time the life cycle of the parasite

in question. Because minor variations within barcode sequences also occur among individuals within a species, barcoding also provides a means to monitor intraspecific diversity within a parasite species, even those from different locations.

We conclude this section by noting that some parasites defy easy application of any species concept. The important thing is to try to appreciate and not lose sight of the distinctive biology of the particular parasite being investigated.

Given these considerations, how many species of parasites inhabit the Earth?

A major shortcoming in our understanding of life on Earth is our inability to say with confidence how many species exist on our planet. Attempts to identify and enumerate species began with Linnaeus and his publication of the *Systema Naturae* in 1735. Since then, biologists have come up with widely varying estimates; in recent years these estimates ranged from 2 million to 1 trillion species. There may never be a definitive answer to the question. The calculation is complicated by a lack of thorough sampling of many habitats, by the presence of cryptic species (a topic we discuss later in this chapter), and by the potential loss of species to extinction faster than we can discover, describe and name them. Two recent estimates place the number of all species at 5 ± 3 million species and 8.7 ± 1.3 million species. The number of species already described is reckoned at about 1.9 million species, reduced to 1.5 million species if synonymies (one species given two or more names) are removed. The estimate of 8.7 million species takes the interesting approach that the higher levels of our classification schemes (phylum, class, order and family, for example) are relatively well known and follow a predictable pattern, such that we can use these values to extrapolate back to the number of species.

Although it is reassuring that these estimates seem to be converging on a more defined range, great uncertainties remain, including how many of the species are actually parasites. Some groups of parasites are much more tractable than others. For instance, credible estimates of the number of helminths (tapeworms, trematodes, nematodes and acanthocephalans) infecting vertebrates fall into the range of 75,000–400,000 species. The numbers of helminth species infecting invertebrates or plants are not as easy to estimate. Additional uncertainties arise in quantifying the number of parasitic protists, algae, plants and fungi. We are particularly ignorant regarding the global abundance of protists, fungi, arthropods (especially insects and mites) and nematodes. For each of these groups, many of the species to be discovered will prove to be parasites (**Box 2.2**).

The estimates given above mostly apply to eukaryotes. If we do not restrict the definition of a parasite to just the eukaryotic realm, parasites also include some of the prokaryotes, which have been systematically underrepresented in calculations of global species diversity. They are small and easily overlooked, and many cannot be easily isolated and cultured. Currently there are only about 9,500 named species of prokaryotes (including both bacteria and archaea). **Metagenomics**, the characterization of genetic material recovered directly from a particular environment (soil, water and air) without the need to culture the organisms present, has revolutionized our understanding of biodiversity for prokaryotes, and for eukaryotes too as we will soon see. The genetic material being targeted is what is referred to as **environmental DNA**, or **eDNA**, that may be derived

BOX 2.2

Some Different Viewpoints on the Number of Parasite Species Yet to be Discovered and How Common Parasite Species are Relative to Host Species

One oft-stated argument about parasite species diversity is as follows: if all host species have at least one parasite species, and maybe even more than one, then the number of parasite species on earth may be expected to equal or exceed the number of host or free-living species. One would indeed be hard-pressed to find a parasite-free host individual, let alone a parasite-free host species, so at face value the argument seems compelling. However, there is an assumption that all parasite species are able to infect just one host species, that is they are very host specific. Although some parasite species are host specific, many and probably most are not and have potentially very broad host ranges.

One study has noted that the parasites of animals comprise only about 5% of all named species and indicated the rate of discovery of new parasite species, especially when taking into account the number of parasitologists working with various parasite groups, is not rising but if anything, tending to level off or to decline (**Figure 1A**). In other words, it seems quite unlikely that the number of parasite species will ever rise to the level of the number of host species.

Figure 1B shows an effort to estimate the number of acanthocephalan species in fish using a random resampling of subsets of host species from an available data set enumerating numbers of acanthocephalan species in examined host species. Note that as sampling increases the relationship becomes non-linear. As compared to another method based on a linear relationship between parasite and host species richness, the resampling method gave more conservative estimates of the numbers of helminth species in vertebrates (~179,000) compared with the latter linear method (~370,000). The resampling method may provide better overall estimates when the number of host species sampled is lower.

We must also consider that modern methods provide powerful new alternative ways to look for and discover parasite diversity. For example, although we have come to appreciate that tropical rain forests are sources of amazing amounts of biological diversity, soil samples from such forests are not the first place you might think a parasitologist would choose to search for hidden parasite diversity. But consider the following example of the use of **metagenomics.** Using the heterogeneous eDNA extracted from a soil sample, the polymerase chain reaction is then used to specifically amplify one particular gene from many or most of the heterogeneous organisms represented in the sample. This is the gene that encodes the RNA that comprises the small subunit of the ribosome (the SSU rRNA gene) which in prokaryotes is the 16S rRNA gene and in eukaryotes the 18S rRNA gene. The SSU rRNA gene is chosen because it has proven to be a good marker to reveal genetic differences. The results clearly show that many different kinds of organisms are represented.

From 279 soil samples recovered from three rainforest locations, 26,860 sequences sufficiently different from one another to be recognized (called OTUs) were obtained just for the unicellular eukaryotes (conveniently called "protists" here). Each OTU can be considered indicative of a different species of protist. What came as a complete surprise is that 50.6% of these OTUs were classified as apicomplexans (**Figure 1C**). The Phylum Apicomplexa is one of the largest, if not *the* largest, lineage of parasites on the planet and is discussed further in this chapter. Of the apicomplexan sequences, 80.2% were from gregarines (**Figure 1D**), apicomplexans that predominantly infect arthropods and other invertebrates. The remaining non-gregarine apicomplexan sequences mostly grouped with *Plasmodium* species and relatives which cycle through arthropods and vertebrates including mammals and birds (Mahé et al., 2017). The soil samples likely contained the gametocysts or other life cycle stages of gregarines, or perhaps the partially decomposed corpses of hosts also containing parasite tissues and associated DNA.

Currently there are approximately 1,650 named species of gregarines. This single tropical forest soil study provides indirect evidence for the existence of possibly thousands more species of gregarines. This and other studies suggest we are not there yet with respect to arriving at more comprehensive and accurate estimates of the number of eukaryotic parasite species.

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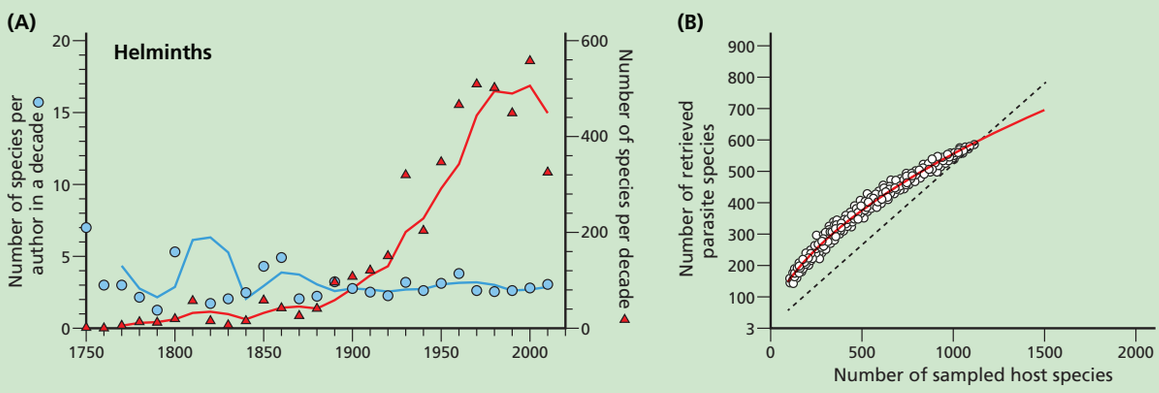
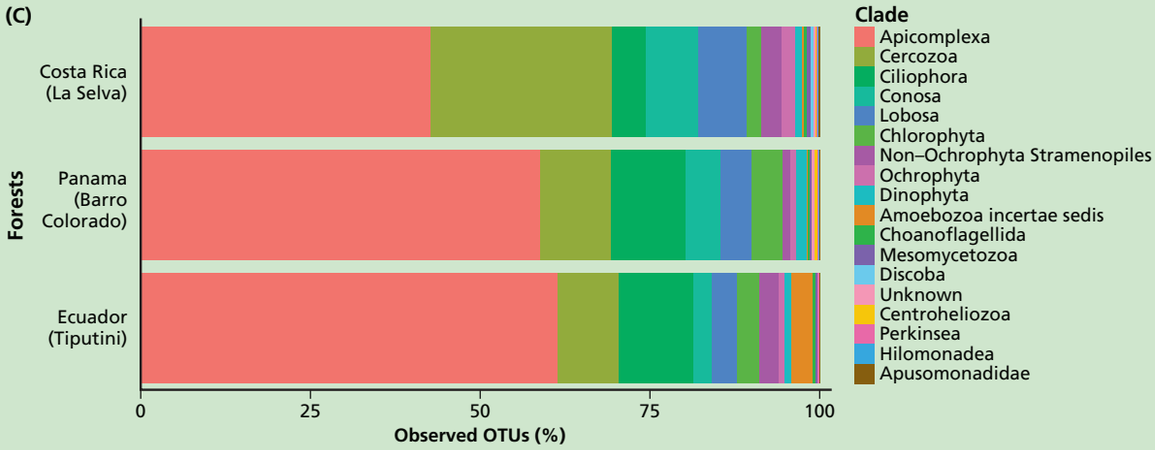




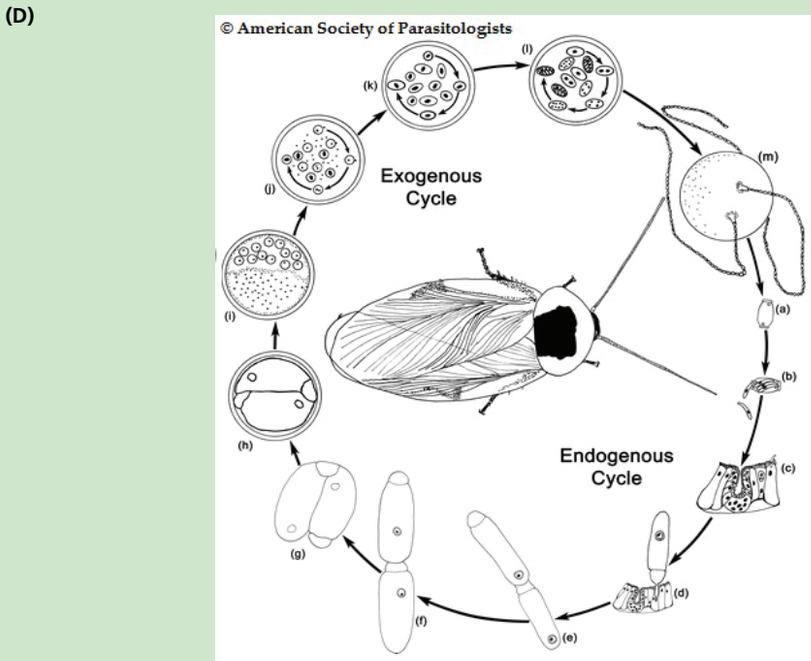


Figure 1 Various ways of assessing parasite diversity. (A) For helminths, the number of species described per decade (triangles and solid line) and numbers of species per decade divided by the number of authors per decade (circles and dotted line) is shown. Note the falling number of new species and the relatively flat line for species/author over the decades. (B) For acanthocephalan parasites of fish, the relationship between the number of sampled host species and the number of retrieved species for parasites is depicted. The circles indicate the values obtained from randomly sampling hosts from a host–parasite database. The red line indicates a curved regression line that best fits the data. The straight dotted line indicates the linear relationship predicted between the number of sampled host species and parasite species. Note how the two approaches yield different estimates of parasite species when extrapolated to a higher number of sampled host species. (C) For three different rainforests, the proportion of operational taxonomic units (OTUs) recovered from soil samples for each of the taxonomic groups listed on the right side of the figure is shown. Note for each habitat the predominance of samples that were Apicomplexa, especially gregarines. (D) The life cycle of a gregarine *Blabericola cubensis* parasitizing the Discoid Cockroach, *Blaberus discoidalis*. Cockroaches are infected by ingesting oocysts that release stages in the gut that penetrate gut epithelial cells and grow to eventually hang into the gut lumen. Eventually, reproductive stages are produced and paired in a gametocyst passed in the host's feces. Fertilization and formation of oocysts containing sporozoites occur in the external environment. (A, From Costello MJ (2016) *Integr Comp Biol* 56:588–599, with permission from Oxford University Press; B, From Strona G & Fattorini S (2014). *Int J Parasitol* 44:269–272, with permission from Elsevier; C, From Mahé F, deVargas C, Bass, D, Czech L et al. (2017) *Nat Ecol Evol*. https://www.nature.com/articles/s41559-017-009, with permission from Springer Nature; D, From Kolman JA, Clopton RE & Clopton DT (2015) *J Parasitol* 101:651–657, with permission of The Allen Press.)

How can a blood-feeding parasite be used to help monitor and sample vertebrate diversity in a national park? Read more about it at https://www.nature.com/articles/d41586-019-01987-w.

not only from unculturable microbes but also from decomposing organisms, shed skin or mucus, feces, hair, or other organismal products released into the environment. The study of eDNA has become a powerful new tool to study biodiversity.

Using this approach, the ongoing characterization of the rich assemblage of microbial species found in humans—the human microbiome—has revealed up to 1,600 species of gut-inhabiting bacteria per individual. If this line of thinking is extended to all animal species, we are presently just glimpsing the tip of the microbial iceberg.

Again bearing in mind the difficulties of applying the species concept to bacteria and archaea, estimates of the true diversity of bacteria species range as high as a trillion species. An unknown percentage of these many bacterial species will prove to be parasites. Add to these ranks the unnumbered hordes of distinct suborganismal entities, such as viruses and viroids, that can be considered parasites, and it becomes clear that we are still a long way from arriving at a firm estimate of the world's diversity of parasites. By extension from their present-day ubiquity, it seems probable that every species to have ever lived has been infected by some kind of parasite. For the moment, we can state with some justification that parasitism is indeed one of the most common lifestyles on Earth, but we are unable to answer with a very convincing number for how many parasite species exist.

Taxonomy, systematics, phylogenetics and evolutionary trees as essential approaches to understanding parasite diversity

Given that there are so many species of parasites, how can we possibly begin to organize them into some kind of comprehensible framework? See **Box 2.1** for one example of how this can be done. **Taxonomy** refers to the science of identification, description and naming of organisms. Since Linnaeus' time, biologists have labored to develop taxonomic schemes to organize biodiversity. The basic unit of such systems is the species, complete with its Latin binomial name (genus and species, such as *Homo sapiens*). Traditionally such taxonomic schemes were elaborated using morphological characteristics that enabled perceptive scientists to group them into ever more inclusive categories (species, genus, family, order, class, phylum and kingdom).

With the publication of Darwin's *Origin of Species* came the compelling metaphor of the **tree of life** (**Figure 2.2A**). It indicates the origin of all life from common ancestry followed by diversification of lineages of life as represented by branches and twigs on this growing tree. This view emphasizes the vertical acquisition of genetic information from one's ancestors and assumes there is a core complement of genes that is retained and reflects ancestry, with the gene encoding the RNA found in the small ribosomal subunit (SSU rRNA gene) being one. Some buds on the tree, representing successful lineages of organisms, gave rise to new branches that further diversified, whereas others withered and died out. Such a tree depicts the patterns of historical relatedness among all organisms. Given that such relationships exist, it would be optimal to have our modern taxonomic schemes follow the tree's branching patterns such that taxonomy mirrors the actual sequence of evolutionary events leading to the diversity we observe. For many years, we had no way to verify if our taxonomic schemes actually reflected evolutionary relationships. For some groups, the availability of fossils exemplifying transitional forms helped to verify taxonomy, but for parasites, which are mostly soft-bodied and have left a poor fossil record (but see **Box 7.4**), this is usually not an option.

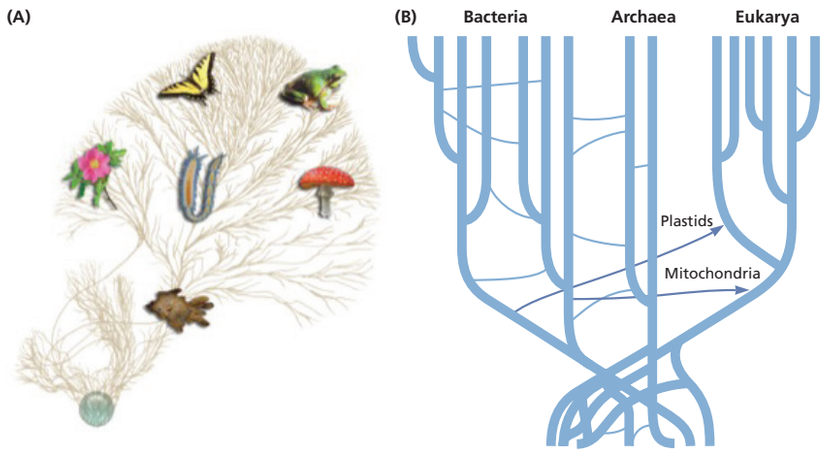


Figure 2.2 Darwin's concept of the tree of life and an updated alternative. (A) Darwin's concept of the tree of life, with life arising from common ancestry and an emphasis on the vertical acquisition of genetic information. (B) This tree takes into account horizontal gene transfer (HGT). According to this view, genetic information can be acquired from organisms other than one's ancestors. (A, From http://tolweb.org/tree/; B, Modified from Smets BF & Barkay T (2005) *Nat Rev Microbiol* 3:675–678, with permission from Springer Nature.)

Figure 2.2B modifies the concept of the Darwinian tree of life by taking into account the phenomenon of horizontal (or lateral) gene transfer (HGT), the movement of genetic information laterally between organisms that occurs without the involvement of sexual reproduction. According to this view, the tree of life is really a web of life. It has been further argued that instead of the diversity of all life being derived from common ancestry, that multiple genetic and environmental circumstances acting over a long time have created the diversity of life. Also quite ambiguous with respect to the tree of life are the viruses (**Figure 2.3**), which probably infect the cells of all types of organisms and are very adept at moving genetic information from one host cell to another. Standard versions of the tree of life do not include viruses, yet viruses have had a profound impact on all phases of organismal life. In addition to their parasitic role and their involvement in causing disease, viruses regularly provide their host organisms with novel genetic material. A thorough discussion of viruses is beyond the bounds of this book, and the reader is referred to virology textbooks for more discussion of these inherently parasitic, important and fascinating entities.

New realms of entities with fundamentally parasitic means of existence are now being revealed by metagenomics-driven studies: Check out "giant viruses" https://www.nature.com/articles/s41586-020-1957-x.pdf) and "huge phages" https://www.nature.com/articles/s41586-020-2007-4.pdf).

To help us better characterize and understand biodiversity, enter the discipline of **phylogenetics** referring to the study of the evolutionary relationships among organisms based on molecular sequence data or morphological traits. These relationships are conveniently depicted with the use of evolutionary trees. Phylogenetics is often involved in making and evaluating hypotheses about historical patterns of descent and can be thought of as part of a broader subject called **systematics**, which refers to the study of the diversification of life on Earth, including the relationships among organisms over time. Evolutionary trees are constructed using algorithms that assess the degrees of similarity in DNA or RNA nucleotide sequences or in

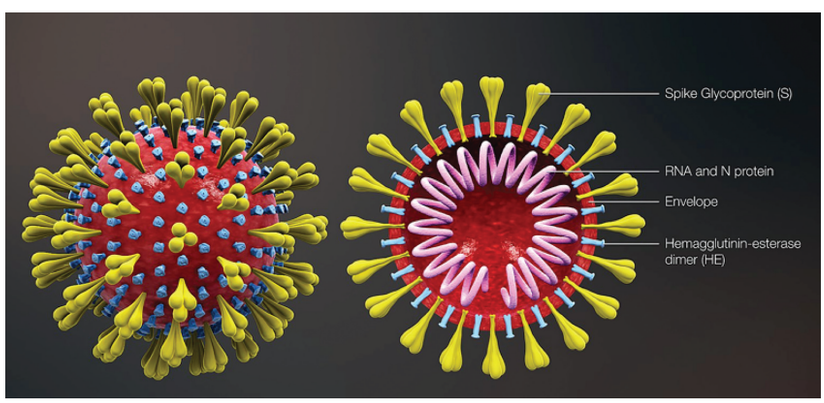


Figure 2.3 A schematic view of a coronavirus, like SARS-CoV-2, responsible for the pandemic beginning in late 2019. Even though they are not organisms in their own right and require a host cell to survive, viruses definitely fulfill other aspects of being parasitic: they cause harm to the host while they benefit, and they must regularly be transmitted to new hosts. Viruses also often infect eukaryotic parasites such as *Leishmania*, and as we will discuss, may play a major role in influencing the level of pathogenicity the eukaryotic parasite inflicts on its host. (Published under Creative Commons Attribution-Share Alike 4.0 International license.)

An inordinate fondness for … viruses? No, the most diverse biological entities on earth are not beetles nor bacteria, but viruses. Learn more about the ongoing quest to reveal the "virosphere" at: http://www.globalviromeproject.org.

protein amino acid sequences in the organisms being compared. Many trees are also constructed based on morphological characters or a combination of morphology and sequence data. The optimal ways to construct trees remain a topic of vigorous and ongoing debate, with entire professional societies and journals devoted to the topic.

Modern biologists portray relationships among organisms using such trees, and for good reason; trees provide a compelling way to order our thinking about the biodiversity that follows our best efforts to reconstruct the evolutionary trajectories taken. Evolutionary trees based on molecular sequence data have revolutionized our view of relationships among organisms. However, until we invent a machine that enables us to go back in time and directly witness how groups of organisms actually diversified, such trees should be considered hypotheses of relationships. For each of the trees shown in this chapter, many alternative versions could have been provided and each of those represented will become outmoded in time. Each is viewed as a relatively conservative statement of the current understanding of the relationships for the group in question. Someday the relationships among all organisms will be based on comparisons of complete genome sequences, a trend that is already well under way.

Enormous progress has been made in revealing the overall diversity of life

There is a steady, ongoing effort to reveal the overall topology of the tree of life. Such efforts began by identifying a target gene that is universally present in all organisms, a part of the so-called core genome, and that is relatively conserved such that sequence comparisons incorporating organisms as disparate as bacteria and tapeworms can be accommodated in the same analysis. Such a gene is the one already discussed above, the gene encoding the SSU rRNA. Using this approach, the pioneering efforts of Carl Woese and colleagues in the 1970s and 1980s revealed three major domains of life: the Bacteria, Archaea and Eukarya (**Figure 2.4A**). Efforts have since expanded beyond the single-gene approach to include phylogenetic trees

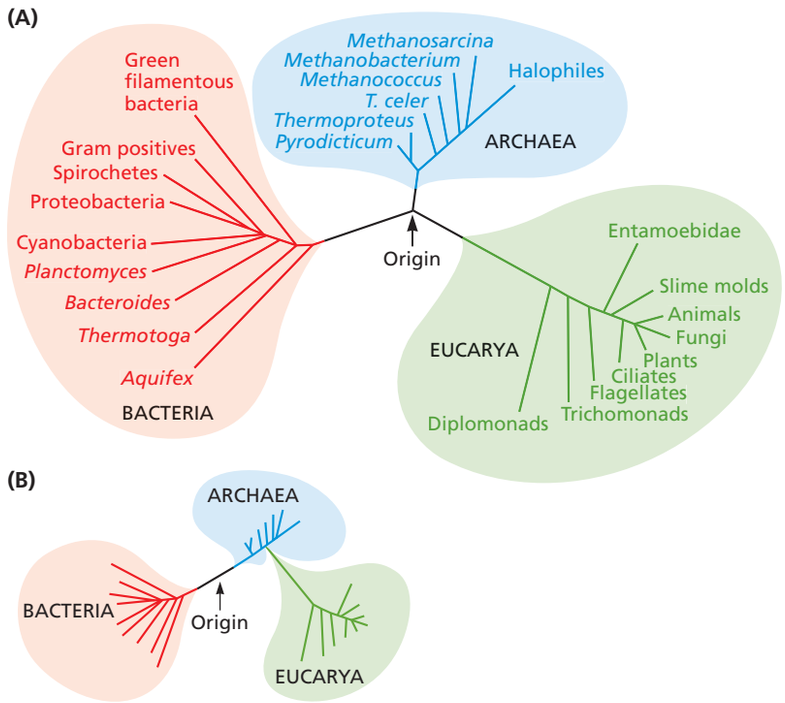


Figure 2.4 The tree of life, based on comparisons of the small ribosomal subunit gene. (A) Notice the origin (root) of the tree and that three major domains of life are demarcated: Bacteria, Archaea and Eucarya. (B) With the ongoing discovery of a greater variety of Archaea, including a group called the Asgard Archaea with eukaryotic-like features, it has been argued that the origins of Eucarya lie within the Archaea, suggesting there are really two major domains, Bacteria and Archaea/Eucarya. (B, Modified from Williams TA et al. (2013) *Nature* 504:231–236, with permission from Springer Nature.)

representing the entire genome sequences of selected organisms. Among the Archaea new representatives have recently been found called the Asgard archaea that have many properties in common with Eukarya, prompting the idea that Eukarya originated from within the Archaea, and thus we should instead consider that life has two major domains, not three (**Figure 2.4B**).

As presently known, the adoption of parasitism has not been a conspicuous feature in the Archaea. One species of Archaea, *Nanoarchaeum equitans*, has been discovered that parasitizes another member of the Archaea (**Figure 2.5**). Both host and parasite live in scalding hot, sulfur-rich water. We have come to think of Archaea as being extremophiles, inhabiting hot, salty or acidic environments, but it is becoming clear that Archaea are ubiquitous in nature and often occur in complex microbial consortia, including as part of the microbiome of humans (they are especially diverse in the nose), other animals and plants. Because it is difficult to grow Archaea in pure culture, progress has been somewhat slow in unraveling key aspects of their biology. We have archaea like *Methanobrevibacter* living in our mouths, populations of which seem to be enriched in patients suffering from periodontal disease. A member of this genus, *M. brevis*, has been recovered from brain abscesses. Most human-associated archaea are found in the gut and many like *Methanobrevibacter smithii* play a role in the production of methane. The archaeal methanogens of ruminants and termites add significant amounts of methane, a greenhouse gas, to the atmosphere! Human gut methanogens may indirectly favor the growth of pathogenic bacteria or cause constipation, but thus far, archaea have been described as being essentially **salutogenic**, that is playing a role in promoting human health and well-being. For the two remaining domains, the Bacteria and Eukarya, as we discuss in the sections that follow, adoption of parasitism has figured prominently.

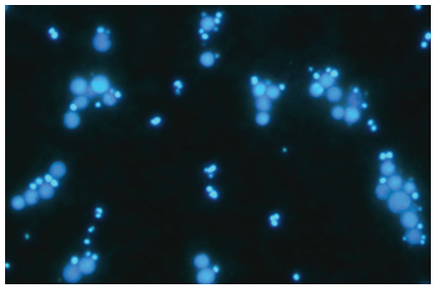


Figure 2.5 Parasitism is apparently rare in the Archaea. The only known parasitic archaea, *Nanoarchaeum equitans*, appears as the tiny bright spheres attached to the surfaces of bigger, fuzzier archaea host cells, *Ignicoccus* sp., which are less intensely stained. The tiny *N. equitans*, only 400 nm in diameter, has one of the smallest genomes known for any organism, consisting of only 490,000 base pairs. Associations like this one where Archaea attach to the surface of other microbes may prove common as we learn more about them. (Courtesy of Harald Huber, University of Regensburg.)

Many bacteria are parasites

Bacteria are normally covered in microbiology courses, and for that reason, their biology is not emphasized in this book. As discussed in Chapter 1, microbiologists typically refer to bacteria that cause disease as pathogens. Nevertheless, parasitism is a phenomenon that cuts across the boundaries of traditional disciplines, and it would be an oversight in this synopsis of parasite diversity to ignore bacteria completely. Consequently, we discuss them briefly here.

About 30 major groups of bacteria called phyla (singular, phylum) are recognized, for which at least some members of each can be cultured in the lab. There are at least 20 more "candidate phyla" identified, members of which are recognized for their genetic distinctiveness but thus far are unculturable. The number of bacterial phyla may someday climb to as high as 1,000. Of 16 relatively well-known bacterial lineages, 8 are particularly prominent with respect to containing human pathogens. Among them are bacteria causing many prominent human diseases, including tuberculosis, cholera, plague, syphilis, anthrax, Lyme disease and leprosy. Some prominent bacterial lineages such as *Chlamydia* and *Rickettsia* consist exclusively of intracellular parasites. Other groups, such as the Spirochaetes, not only have prominent parasitic representatives but also include many free-living species. Several bacterial lineages are predominantly free-living but contain a few parasitic representatives. It is clear that bacteria have readily adopted parasitism on several occasions. It is also clear that HGT has played an important role in the history of parasitism in bacteria. The first evidence for this process was the documentation of the transfer of drug resistance

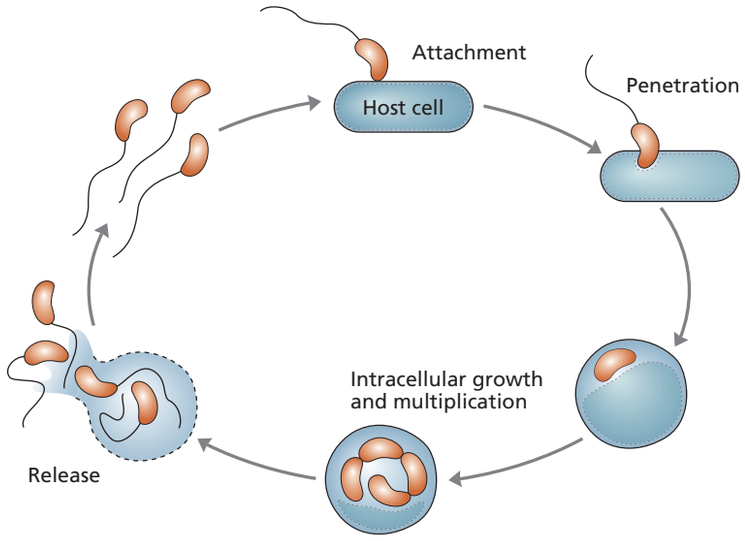


Figure 2.6 The parasitoid bacterium *Bdellovibrio*. *Bdellovibrio* attaches to, penetrates, and multiplies within its host cell (also a bacterium), eventually destroying it. It is then released and starts the cycle again.

or virulence genes on plasmids from one bacterium to another unrelated bacterial species.

Among the bacteria are several intriguing and lesser known parasites. *Bdellovibrio* is a parasitoid that bores into and parasitizes the bodies of other bacteria (**Figure 2.6**). It has been considered a possible means to control antibiotic-resistant bacterial pathogens. Some bacteria colonize unicellular eukaryotes like the ciliate *Paramecium* and can interfere with host reproduction (*Holospora*) or confer on their host the ability to kill other *Paramecium* whilst simultaneously rendering their host immune from similar attacks (*Caedibacter*). Unicellular eukaryotes like freshwater amebas serve as reservoir hosts for over 20 species of bacteria that are human pathogens, including *Legionella pneumophila* that causes Legionnaires' disease. Plants too suffer from bacterial infections as do invertebrates such as corals that are weakened by stressors like climate change and are thus more vulnerable to infections.

Eukaryotes are a very diverse group that includes many different kinds of parasites

The Eukarya, or eukaryotes, are characterized by the possession of membrane-bound structures within their cells, the most important and distinctive of these being the nucleus. As pointed out in **Figure 2.4**, the origin of eukaryotes is hotly debated, although it is generally considered there was a single common origin for eukaryotes, a group that today embraces most of all formally described and named species. Many eukaryotes are unicellular, microscopic and motile. They have traditionally been referred to as protists or protozoa, terms that today carry no formal taxonomic meaning but are still commonly used for convenience. Eukaryotes are also distinguished from the other two domains of life because they include multicellular organisms, such as animals, plants, many algae and most fungi.

Efforts to unravel the relationships among the many recognized lineages of eukaryotes are ongoing and **Figure 2.7** represents one such consensus effort, one built on the collective contributions of experts on the various groups of eukaryotes. Included in the figure are the main eukaryotic lineages and some of the important eukaryotic parasites they contain. Eukaryotes are believed to have begun to diverge over 1 billion years ago, which understandably makes it hard to retrace fully their history. One pattern

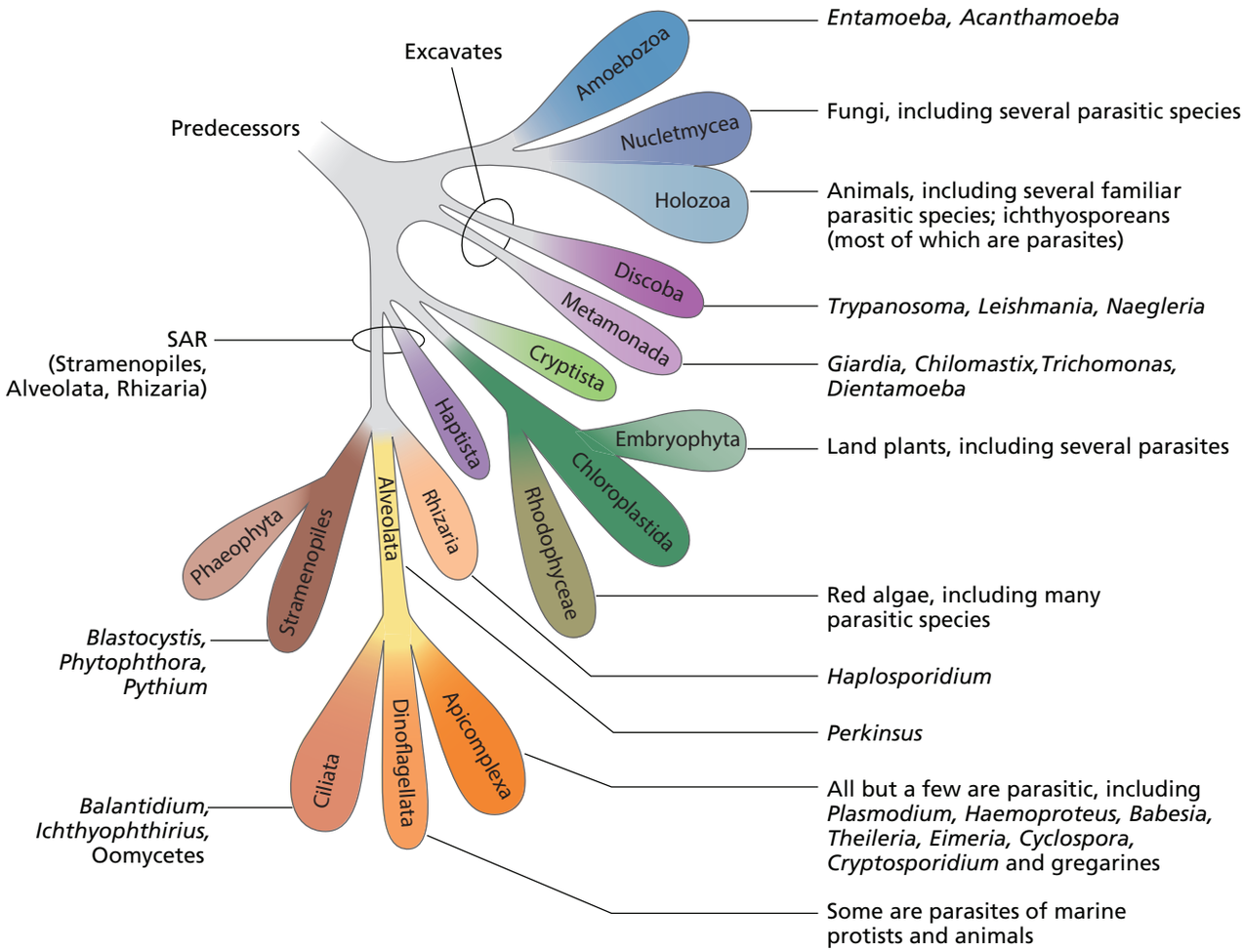


Figure 2.7 One portrayal of the diversity and relationships among the major groups of eukaryotes. Note that this is but one of several hypotheses offered for eukaryotic relationships. The position of some prominent groups of parasitic eukaryotes is shown in this portrayal. (Modified from Adl SM, Bass D, Lane CE et al. (2019) *J Eukarot Microbiol* 66:4–119, published under Creative Commons CC BY license.)

again emerging from a perusal of the overall eukaryotic tree is that parasitism has arisen independently in several distinct lineages.

Stramenopiles

Stramenopiles are part of the SAR (Stramenopila-Alveolata-Rhizaria) lineage shown in **Figure 2.7**. Stramenopiles are also called heterokonts because in many, their motile stages produce flagella of two different shapes. Members of this group, dominated by diatoms, golden algae and brown algae, are largely free-living, but some parasitic lineages occur, most prominent among them the water molds or oomycetes (see also **Figure 2.33**), including the notorious *Phytophthora infestans*, cause of the blight responsible for the infamous Irish potato famine. Members of the oomycete genus *Pythium* frequently cause serious plant diseases and at least one species, *P. insidiosum*, can infect dogs, domestic animals and people.

Additionally, one noteworthy stramenopile (one lacking flagella) is of the genus *Blastocystis,* for which several subtypes are known, some of which have been recovered from humans and are then referred to as *Blastocystis hominis* (**Figure 2.8A**). These organisms have the distinction of colonizing over 1 billion people, making them the most prevalent eukaryotic microbe infecting the human intestine. They are probably transferred between

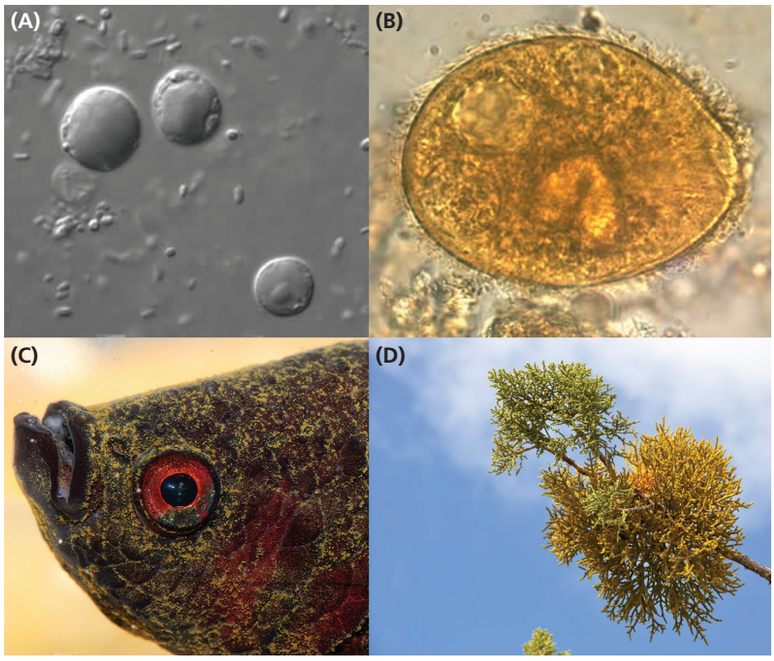


Figure 2.8 Examples of parasites representing major eukaryotic lineages. (A) Pre-cyst like forms of *Blastocystis hominis*, a stramenopile that is the microscopic eukaryote mostly commonly found in the human intestine. (B) A feeding trophozoite of *Balantidium coli*, the only ciliate parasite routinely infecting humans. Note the cilia at the periphery of the cell and the large, folded macronucleus. (C) An example of an infestation by a parasitic dinoflagellate *Oodinium* on the surface of fish causing the characteristic gold dust appearance. (D) Note the yellowish color of this mistletoe of juniper *Phoradendron juniperinum*, a common example of a plant that is a hemiparasite. (A, B, USA Govt Info Public Domain; C, CC The Creative Commons Public Domain Dedication; D, © Eric Loker.)

humans and domestic animals and have been implicated in causing diarrhea and nausea. *Blastocystis* has also been associated with cases of irritable bowel syndrome and may pose problems for immunocompromised people. Their role in causing pathology remains controversial and they are often considered components of a healthy gut microbiome. As with many eukaryotes inhabiting anaerobic or microaerophilic portions of the gut, *Blastocystis* does not possess conventional mitochondria capable of aerobic respiration. It has a mitochondrion-like organelle that does generate adenosine triphosphate (ATP) but seems intermediate in function between a mitochondrion and a hydrogenosome. The latter is an organelle that generates ATP from pyruvate, while giving off hydrogen (H_2) as a by-product. *Blastocystis* has a hydrogenase enzyme but does not produce H_2.

Alveolata

The Alveolata, also part of the SAR lineage, is a relatively well-defined clade of eukaryotes of immense importance because it contains many prominent groups of parasites. The name indicates their possession of alveoli, which are flattened vesicles lying beneath the plasma membrane. Included among the alveolates is the Apicomplexa, a huge, nearly exclusively parasitic lineage of great medical significance. We discuss them later in Section 2.1. Also included among the alveolates are the ciliates and dinoflagellates, some of which are prominent parasites. Ciliates typically have two nuclei and many short cilia arranged in rows. The one ciliate known to be parasitic in humans is ***Balantidium coli***, which causes balantidiasis, a zoonosis transmitted by the fecal–oral route, with pigs serving as reservoirs (see **Figure 2.8B**). Another familiar example of a parasitic ciliate, one frequently posing a problem for aquarists, is *Ichthyophthirius multifiliis*, better known as "ich," an ectoparasite that causes infestations of white spots to appear on the skin of freshwater fishes (see also **Figure 3.12**).

Although dinoflagellates are mostly free-living, mostly marine planktonic photosynthetic organisms, the group shows a predilection to form symbiotic associations. Zooxanthellae are mutualistic dinoflagellates that play an important role in formation of coral reefs, and some are parasites of

marine invertebrates like crabs. *Oodinium* are parasites of fish causing "velvet disease" or "gold dust disease" (see **Figure 2.8C**) owing to the golden color of the cysts produced on the infested skin of a fish. Related to dinoflagellates in a separate alveolate family are parasites of the genera *Parvilucifer and Perkinsus*. The former are parasites of dinoflagellates and have been considered potential control agents of harmful algal blooms. *Perkinsus marinus* is a parasite of oysters responsible for a disease called dermo that can cause mass die-offs of valuable oysters.

As if our frogs don't have enough disease problems already…: To go along with chytrid fungus and virus infections, a newly identified relative of *Perkinsus* termed "the amphibian Perkinsea" has been implicated in massive die-offs of frog tadpoles. Read more about it here: https://journals.sagepub.com/doi/full/10.1177/0300985818798132

Rhizaria

The third prominent group within the SAR lineage is the Rhizaria, mostly unicellular eukaryotes that produce pseudopods. Most, such as the foraminiferans and radiolarians, are free-living. Among the Rhizaria are the Phytomyxea (also called Plasmodiophorids), which are parasites developing within the cells of plants, often causing the infected tissue to form a scab or gall. Another group of rhizarian parasites is the spore-forming Ascetosporea, which are usually parasites of marine invertebrates. One example is *Haplosporidium nelsoni*, which causes a disease known as MSX (short for multinucleated sphere unknown) that has caused crashes in commercially valuable oyster populations.

Archaeplastida (Embryophyta, Chloroplastida and Rhodophyceae)

These organisms are characterized by the possession of a chloroplast and most possess a cell wall. Nested within the Chloroplastida are the Embryophyta which embraces most familiar land plants, several species of which are parasitic (see **Figure 2.8D**). The remaining Choroplastida are mostly green algae and are not parasitic. Some red algae (Rhodophyceae) are also parasitic, often on other red algae.

Metamonada

Members of this group of unicellular eukaryotes often live in environments where oxygen is limited and have mitochondria that are much reduced or instead have a hydrogenosome. Flagella are prominent. Several are noteworthy parasites. Consider ***Giardia lamblia***, also referred to as ***G. intestinalis*** or ***G. duodenalis*** (**Figure 2.9A**), the causative agent of giardiasis, first seen by van Leeuwenhoek using his microscope to examine his stools in 1681. We have since learned that ***Giardia*** does not possess typical mitochondria, and it was originally thought that this organism might have diverged from eukaryotic stock before the ancestral eukaryote had acquired the mitochondrion by primary endosymbiosis. **Primary endosymbiosis** in this context refers to the acquisition of a bacterium (probably an alpha-proteobacterium) by an ancestral proto-eukaryote, with the metabolically versatile bacterium thereafter serving as the mitochondrion. Although ***Giardia*** is still considered to be an early diverging eukaryote, we know today that it possesses a reduced version of the mitochondrion called a mitosome. **Mitosomes** are double-membrane structures like mitochondria and are almost certainly derived from them, but they lack mitochondrial DNA. They are incapable of aerobic respiration, in keeping with the limited oxygen environment of the small and large intestine in which ***Giardia*** lives, but they can still produce ATP. A related group of metamonads, members of the genus ***Trichomonas***, also have modified mitochondria that in their case are considered to be true hydrogenosomes (they do not require oxygen but can still produce ATP, with H_2 as a by-product), as described above. ***Trichomonas tenax*** inhabiting the mouth is associated with and can worsen periodontal disease. ***Trichomonas vaginalis*** is responsible for the common, sexually

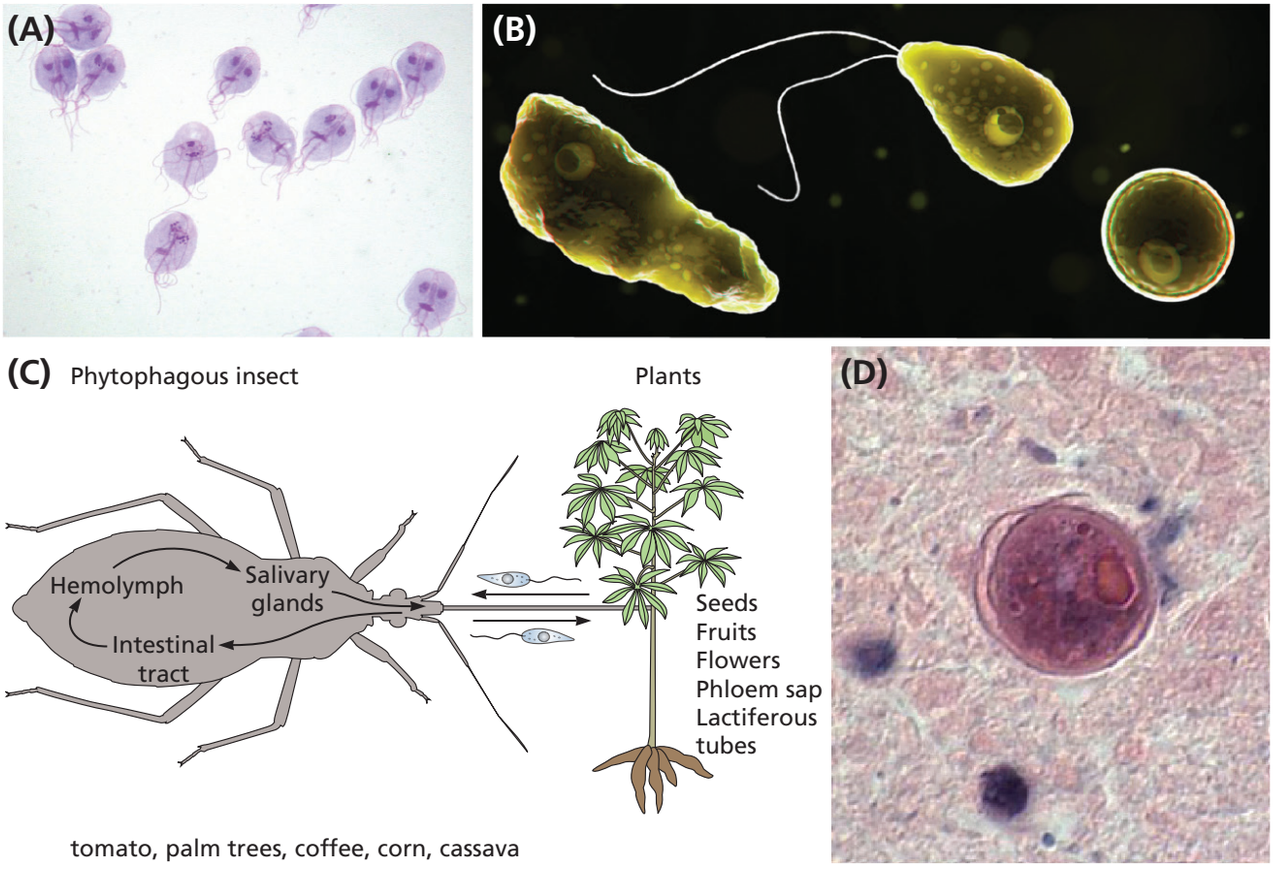


Figure 2.9 Examples of parasites representing major eukaryotic lineages. (A) Multiple trophozoites of the metamonad *Giardia lamblia*, each having two eye-like nuclei, and each propelled by four pairs of flagella. (B) The three characteristic life cycle stages of *Naegleria fowleri*, the trophozoites (on left) of which can invade the brain and cause primary amebic meningoencephalitis, or PAM. (C) The life cycle of a plant-infecting kinetoplastid, *Phytomonas* sp. Note this parasite, which has a prominent flagellum to propel it, is transmitted from plant to plant by phytophagous insects. *Phytomonas* undergoes multiplication in both plant and insect hosts. (D) A cyst stage of the amoebozoan *Balamuthia mandrillaris* in the brain, normally a soil-dwelling ameba only discovered in 1986 to be capable of initiating infections. When this parasite gains access to the body, like *N. fowleri*, it can also colonize the brain causing granulomatous amebic encephalitis (GAE). It can cause disseminated infections and can be transferred to new hosts during the transplantation of infected organs. (A, Published under CC BY 4.0; C, From Lopes AH, Souto-Padrón T, Dias FA, Gomes MT et al. (2010) *Open Parasitol J* 4:30–59, figure published under the Creative Commons Attribution 4.0 License; D, USA Govt Info Public Domain.)

transmitted infection known as trichomoniasis or "trich," and is the protist most commonly associated with pathogenicity in industrialized countries. A related species, ***Pentatrichomonas hominis*** lives in the large intestine and caecum and is occasionally implicated in causing diarrhea.

Two other metamonads commonly inhabit the human gut, *Dientamoeba fragilis* and *Chilomastix mesnili*. The former species lacks flagella and has an ameboid body. Its role in causing gastrointestinal disease is still debated. As resistant cyst stages are rare in this species, it may depend on colonizing the eggs of the human pinworm *Enterobius vermicularis* to be transmitted from one host to another. *C. mesnili* is found in the cecum and colon of humans, other primates and pigs, and is a commensal.

Discoba

Discoba is an example of a group of organisms strongly supported and united by similar sequences in a number of genes but for which it is hard to define a unifying morphological trait. Nonetheless, included are some

important parasite groups. One representative of note is *Naegleria fowleri* (**Figure 2.9B**) which typically dwells in warm, aerobic aquatic habitats, including some swimming pools. It can assume an amoeboid morphology, may transiently grow two flagella for swimming and dispersal and then disassemble them, or it can round up and encyst. It is worth mentioning here because occasionally people swimming in a habitat occupied by *N. fowleri* snuff this organism deep into their nasal chambers. If so, it can move through the small passages in the cribiform plate that separates the nasal chamber from the brain. It then begins to phagocytose cells of the brain, an ailment called primary amebic meningoencephalitis, or PAM. *N. fowleri* is frequently characterized as an **opportunistic parasite** though human infections for the otherwise free-living organism are a dead end. Infections, though rare, are almost always fatal for the affected person.

Among the most prominent of all parasites, also falling into the Discoba lineage, are the kinetoplastids, represented by ***Trypanosoma*** (see **Figure 2.9C**) and ***Leishmania***. Representatives of the former genus cause sleeping sickness and Chagas disease whereas ***Leishmania*** species cause a variety of forms of leishmaniasis. These parasites are discussed extensively throughout the book. Others, like *Phytomonas*, are important parasites of plants like coffee and palm trees. Kinetoplastids are unusual in possessing a single mitochondrion that contains a **kinetoplast**. The kinetoplast contains a network of concatenated circular DNA molecules (assembled like the chain mail in armor), some of which are maxicircles that encode in a peculiar, encrypted fashion the usual mitochondrial gene products. Many minicircles are also present and encode guide RNAs, which are used to decode the encrypted maxicircles. Guide RNAs either insert or delete uridine residues in maxicircle transcripts to accomplish this. It is not clear why kinetoplastids use this unusual RNA editing process. It may have been derived from genes transferred horizontally from viruses. Whatever the origin or purpose, it is clear that disabling RNA editing is lethal for kinetoplastids. Kinetoplastids are also unusual for sequestering the enzymes of glycolysis within distinct, membrane-bound **glycosomes**.

Amoebozoa

Amoebozoans are unicellular eukaryotes that move by the formation of blunt pseudopods. Within this group are prominent parasites such as ***Entamoeba histolytica***, which causes amebic dysentery, and others such as ***Endolimax***, *Acanthamoeba* and *Balamuthia* (see **Figure 2.9D**). ***E. histolytica*** is yet another example of an anaerobic or microaerophilic parasitic eukaryote with mitosomes. The mitosomes of ***Entamoeba*** appear to be among the most reduced of all endosymbiont-derived organelles; their function is still uncertain because they do not appear to participate in energy metabolism. Their abundance within ***E. histolytica*** trophozoites suggests that they have an essential yet still enigmatic role to play.

Nucletmycea

This is one of two major lineages of eukaryotes collectively called opisthokonts which ancestrally possessed a posteriorly directed flagella, though many representatives have lost this trait. The Nucletmycea is dominated by the fungi, with many parasitic representatives including the microsporidians, and is discussed in more detail later in the chapter.

Holozoa

This is the second major lineage of opisthokonts and includes major radiation of eukaryotes most familiar to us, the Metazoa, otherwise known

as animals. We will discuss the many parasitic representatives of animals extensively in the pages to follow. The study of opisthokonts points out that we still have much to learn about the variety of parasitic organisms in nature, as exemplified by the Mesomycetozoea (or Ichthyosporea), a lineage of mostly parasitic organisms that did not come to light until 1996.

The Apicomplexa is a huge, important, nearly exclusively parasitic group of organisms

The largest and arguably most important of all groups of parasites is the phylum Apicomplexa, part of the alveolate lineage shown in **Figure 2.7**. Some of the most deadly pathogens of humans (such as the malaria parasites), and of domestic animals (such as the coccidians) are apicomplexans. With a few exceptions such as the newly characterized symbionts of corals informally called corallicolids, apicomplexans are otherwise exclusively intracellular parasites. They make use of an **apical complex** (**Figure 2.10A**), for which they are named, to recognize, attach to and penetrate host cells. The apical complex is composed of a **conoid**, a set of microtubules arranged in a spiral configuration; **rhoptries**, which are secretory in function; and one or more **polar rings**. The complex may also contain more slender convoluted secretory structures called **micronemes** that connect to the rhoptries.

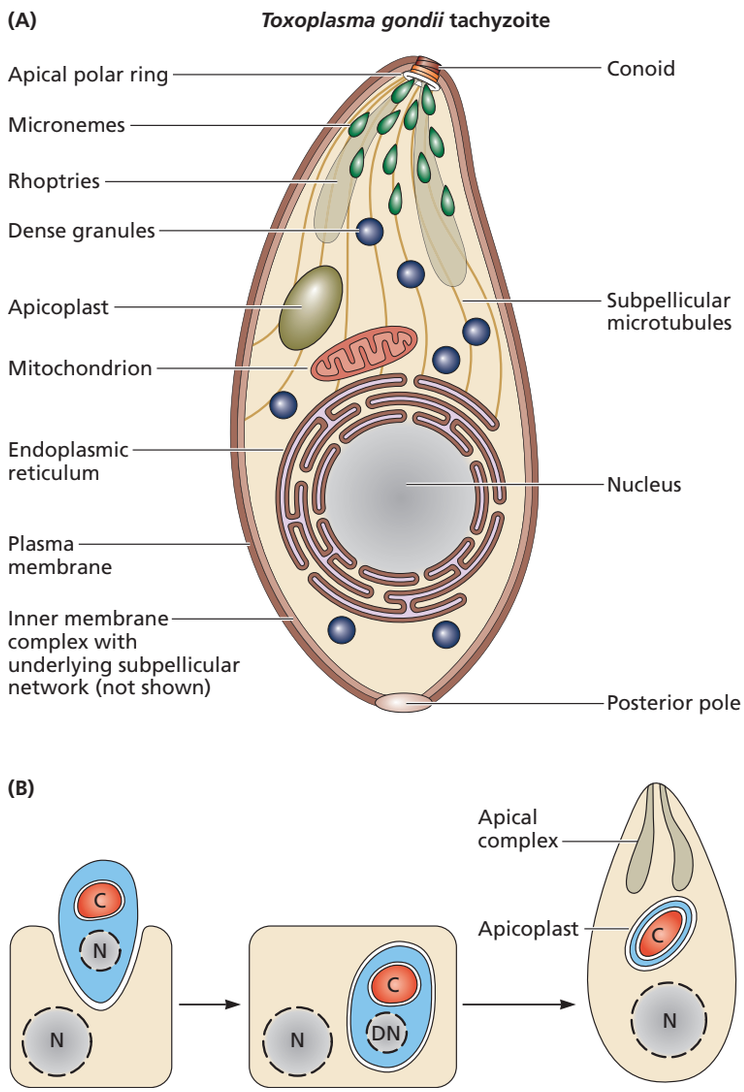


Figure 2.10 Characteristic features of apicomplexans and the origin of the apicoplast. (A) A typical apicomplexan, the tachyzoite of *Toxoplasma gondii*, shows details of the apical complex, a structure well named for indeed it is complex. Note also the presence of an apicoplast. (B) A possible sequence of events showing the origin of the apicoplast. Left, a nucleated algal cell is present (blue) containing a chloroplast (C) that is a modified cyanobacterium that was itself taken up by the ancestor of the host algal cell by **primary endosymbiosis**. The algal cell is then taken up by an ancestral protist (tan), an act called **secondary endosymbiosis**. In the middle, the nucleus of the engulfed algal cell is diminished in size (DN), and genes are transferred from both the nucleus and chloroplast of the alga to the protist nucleus. Note the persistence of the chloroplast. On the right, note that the algal cell has become much smaller, lost its nucleus and its chloroplast persists. The chloroplast is surrounded by four membranes and is called an apicoplast. The host cell is now an apicomplexan. C, chloroplast; N, nucleus; DN, diminished algal nucleus. (A, Modified from Baum et al. (2006) *Nat Rev Microbiol* 4:621–628 reproduced by permission of Springer Nature; B, Modified from Sheiner L & Striepen B (2013) *Biochim Biophys Acta* 1833(2):352–359, with permission from Elsevier.)

Most apicomplexans are also notable for possessing a circular plastid genome contained within a structure called the **apicoplast**. It is likely the apicoplast was acquired through a process called **secondary endosymbiosis** as illustrated in **Figure 2.10B**. The apicoplast is bound by four membranes within the apicomplexan cell. The membranes are derived from the inner chloroplast membrane of cyanobacterial origin, the outer chloroplast membrane also of cyanobacterial origin, the periplastid membrane of algal plasma membrane origin and the outer membrane from the apicomplexan endomembrane system.

Although genes involved in photosynthesis have been deleted from the original plastid chromosome and apicomplexans are not capable of photosynthesis, the apicoplast does retain functional genes. If the apicoplast is disabled, the apicomplexan may be killed or be unable to penetrate new host cells. The apicoplast is an appealing target for the development of new drugs that would selectively target apicomplexans and leave the host (lacking plastid genomes) unaffected (see Chapter 9). The newly-discovered corallicolids, which are symbionts in the gastric cells of corals, are unique among known apicomplexans in retaining a few genes involved in chlorophyll synthesis in their apicoplast. They represent an interesting intermediate step along the path beginning with a photosynthetic ancestor to the parasitic lifestyle of present-day apicomplexans.

It has been suggested that all free-living animal species harbour at least one apicomplexan parasite (recall the discussion of gregarine apicomplexans in **Box 2.2**). A remarkable and largely unappreciated degree of biodiversity exists even within a single apicomplexan genus. For example, there are already about 2,000 described species of *Eimeria*, with many more species awaiting description. As many as 10,000 species of *Haemoproteus* and *Plasmodium* may eventually be enumerated.

Traditional taxonomic schemes have recognized four groups of apicomplexans: the coccidians, gregarines now including *Cryptosporidium*, haemosporidians and the piroplasmids. Some gregarines including *Cryptosporidium* lack the apicoplast. Most of the apicomplexans have a conoid in their apical complex (see **Figure 2.10A**), but the haemosporidians (*Plasmodium* sp.) and the piroplasms (such as *Theileria* and *Babesia*) generally do not. The phylogenetic relationships among these groups are actively being pursued, and **Figure 2.11** presents one recent hypothesis of relationships. Note that other relatively closely related alveolate groups including chromerids, *Perkinsus* and dinoflagellates are used as outgroups to root the tree (see the website associated with this book). An outgroup represents an organism that is believed to be a close relative of the group being analyzed (in this case the Apicomplexa) but does not fall within that group. Several apicomplexan genera of medical significance (for example, *Cryptosporidium*, *Toxoplasma* and *Plasmodium*) will be mentioned often throughout this book.

Many well-known parasites belong to familiar groups of multicellular organisms

Also particularly worthy of discussion in this overview of parasite diversity are several groups of parasites that are multicellular (see **Figure 2.7**), found among the red algae, plants, fungi and animals.

The Parasitic Rhodophytes (Red Algae)

Red algae are photosynthetic, and most of the 7,000 species are multicellular and marine. They contain chloroplasts and distinctive red pigments.

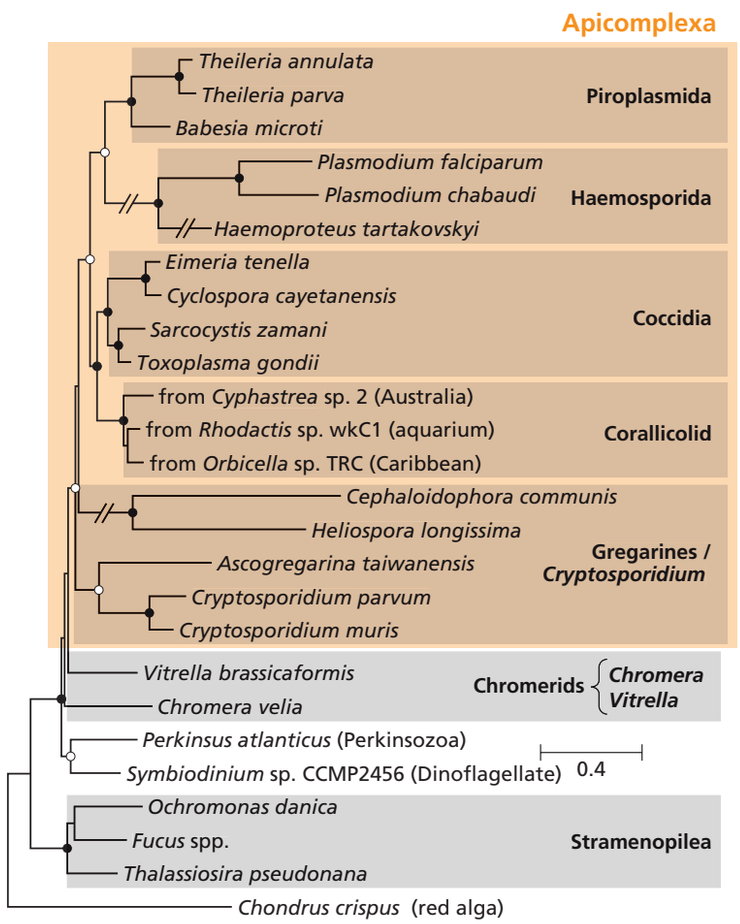


Figure 2.11 A phylogenetic tree of the Apicomplexa based on combined sequences of 18S, 5.8S, and 28S genes. Included on this tree are several important parasites representing the four major lineages within the Apicomplexa usually supported, including Piroplasmida (*Babesia* and *Theileria*), Haemosporida (*Plasmodium* and *Haemoproteus*), Coccidia (*Eimeria*, *Cyclospora*, *Sarcocystis* and *Toxoplasma*) and Gregarines including *Cryptosporidium.* Note that on this tree intercalated among the parasitic groups are the corallicolids, symbionts of corals. Similar analyses using other apicomplexan genes such as those found in the apicoplast place the corallicolids at the base of the apicomplexan tree. Outgroups for comparison include free-living phototrophs like *Vitrella*, and *Perkinsus* and dinoflagellates, all relatively closely related alveolates. The open and filled circles, respectively, show nodes that are strongly or very strongly supported by both the maximum likelihood and Bayesian methods of phylogenetic analysis used. (Redrawn from Kwong WK, Del Campo J, Mathur V, Vermeij MJA & Keeling PJ (2019) *Nature* 568:103–107, with permission from Springer Nature.)

Although red algae hardly spring to mind as iconic parasites, at present about 8% of the 66 described genera of red algae (in the Florideophyte lineage) include parasites. Altogether, there are about 116 known species of parasitic red algae. Parasitism is believed to have arisen independently over 100 times in this group. Red algae are distinctive in their mode of parasitism. They are able to form fusions among adjacent cells, the fusions being called secondary pit connections, that provide an avenue through which organelles such as the nucleus, mitochondria and plastids from a parasitic cell can gain access to the cytoplasm of a host cell. Once the parasite organelles are present, they proceed to divide and spread via holes in the septa separating adjacent host cells called pit connections (**Figure 2.12A**). The mitochondria the parasite brings with it are functional and required for its success, and although plastids are also transferred they are incapable of photosynthesis, so the parasite is dependent on nutrients provided by the host cells. Parasitic red algae often appear as small colorless eruptions from the host's surface. Another peculiar feature of parasitism in red algae is that most parasites share a recent common ancestry with an extant, free-living red alga species that they specifically parasitize. Such parasites are called **adelphoparasites** (adelphose being a Greek term for kin). Other red algal parasites are called **alloparasites** and typically have broader host ranges (but the hosts are always other red algae) that include more distantly related host species in other genera or families (**Figure 2.12B**). One idea awaiting further study is that adelphoparasites represent an early phase in parasitism followed by adoption of more distantly related hosts as characteristic

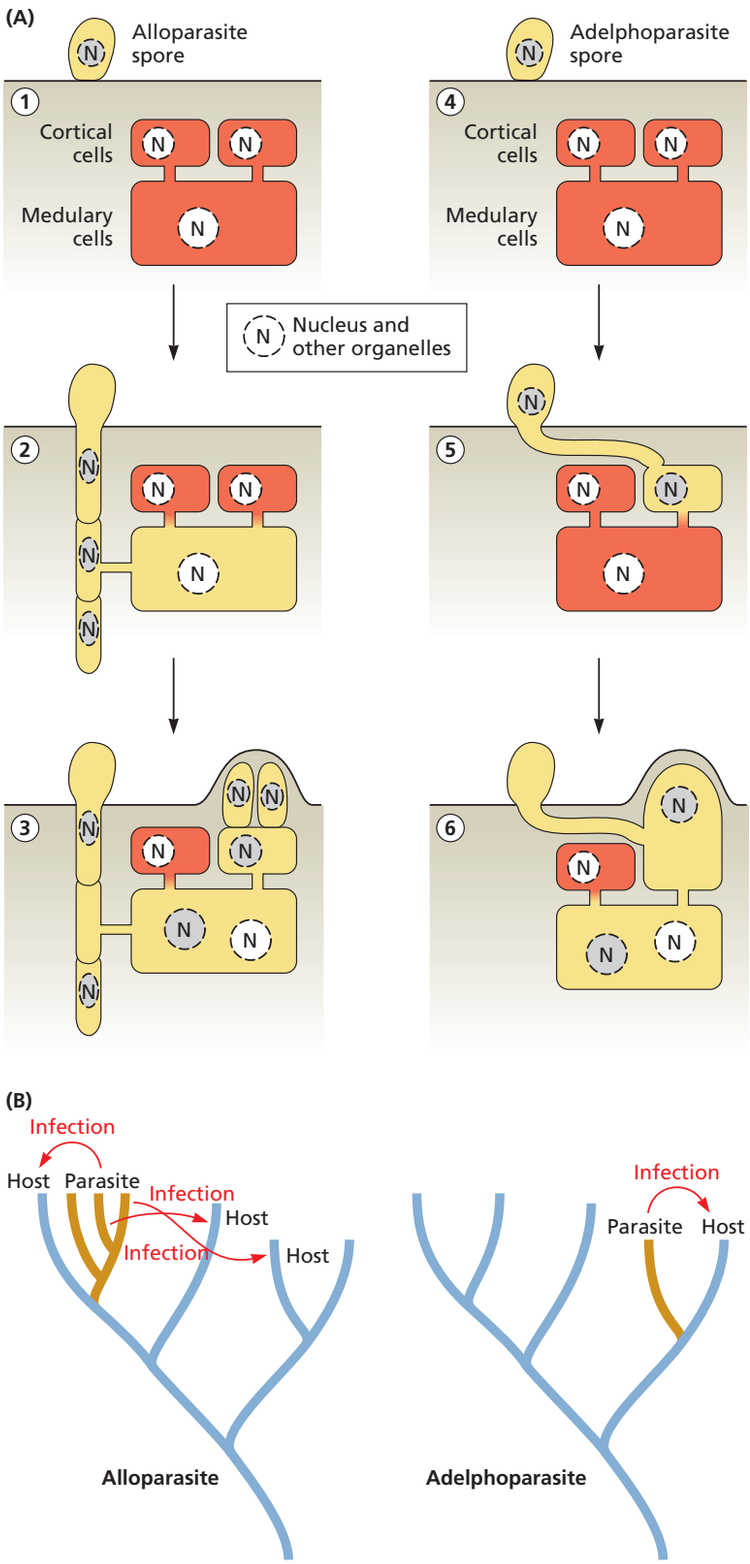


Figure 2.12 The peculiar nature of parasitism in red algae. (A) Host red algal cells are shown in red with their nuclei (N) in white. Parasite cells are shown in yellow with their nuclei (N) in gray. Germination and development for an alloparasite (*Choreocolax polysiphoniae*) are shown in panels 1–3 and for an adelphoparasite (*Gracilariophyla oryzoides*) in panels 4–6. Note how the nucleus and other organelles of the parasite spread through host cells via connections, basically taking over the host cells in the process. (B) Note the close relationship between the adelphoparasite (brown) and the host it infects (red arrow). In alloparasites, infection (red arrows) of more distant relatives can occur. (Modified from Blouin NA & Lane CE (2012) *BioEssays* 34:226–235. With permission from John Wiley and Sons.)

of alloparasites. Molecular studies have shown that many red algal parasites exist in a continuum between these two poles, suggesting some revision in emphasis on the use of these two terms may be needed. As discussed further in Chapter 7, red algae provide a compelling model to examine the origins of parasitism from free-living ancestors.

A parasitic plant that kills an animal parasite on their shared host plant: Carnivorous plants have long been known, but here is a first. Read more at https://www.cell.com/current-biology/pdf/S0960-9822(18)30815-7.pdf.

Parasitic Flowering Plants

Flowering plants, or angiosperms, are multicellular, photosynthetic, autotrophic eukaryotes that are a prominent lineage within the Embryophyta (or land plants) in **Figure 2.7**. They produce cell walls containing cellulose. Among the 370,000 species of flowering plants, about 4,530 species (1.2%) are parasitic. Parasitism is estimated to have arisen 12–13 separate times during the evolution of angiosperms (**Figure 2.13**), with parasitic representatives known in about 30 different families.

Parasitic angiosperms invade the roots or stems of other land plants with their specialized invasive roots called **haustoria**, apparently having no capacity to become invasive within the bodies of other major groups of multicellular organisms (but see margin callout). Some plants do however parasitize mycorrhizal fungi (see **Figure 1.17**). The formation of haustoria is an essential feature of plant parasitism, and it is through these structures that parasitic plants absorb their nutrition from their hosts (**Figure 2.14**). We are learning the haustorium allows two-way exchange of a surprising list of items between parasite and host, including viruses, retrotransposons, mRNA molecules and microRNAs that may regulate host gene expression, as well as water, basic nutrients and proteins.

Plant parasites are either **facultative parasites** and are able to live autotrophically without the need to parasitize a host, or they are **obligatory parasites** and require a suitable host to complete their life cycles. Parasitic plants are also classified according to their ability to engage in photosynthesis: some are **hemiparasites** and are still capable of photosynthesis, whereas others are **holoparasites**, which are incapable of photosynthesis and must obtain all their energy through their haustoria.

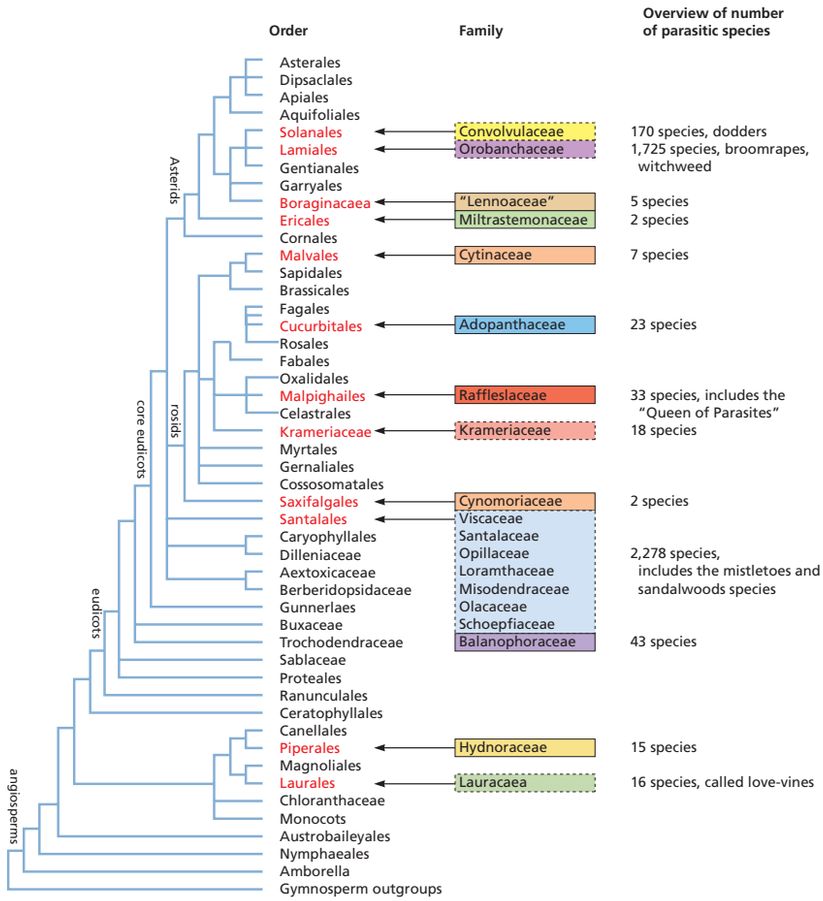
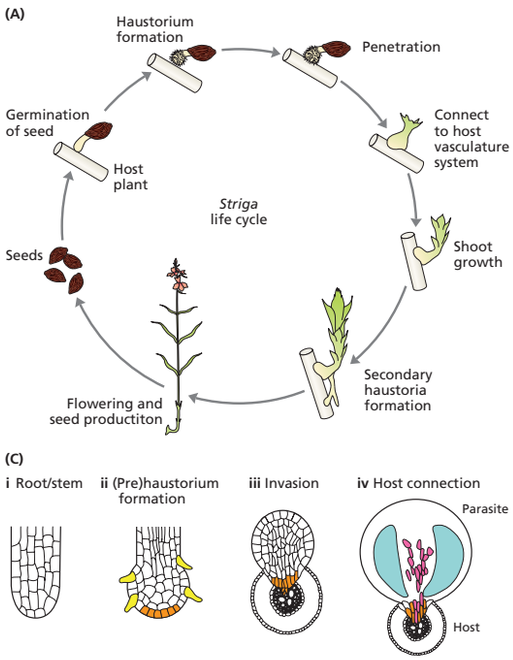


Figure 2.13 An overview of parasitism in flowering plants. This tree provides an overview of the diversity of flowering plants and indicates the lineages in which parasitism has occurred (in red) and the approximate number of parasitic species for each. Those groups enclosed by a dashed box are hemiparasites and those enclosed by a box with solid lines holoparasites. Convolvulaceae and Orobanchaceae include both. See http://www.parasiticplants.siu.edu/ for an excellent overview of parasitic plants.



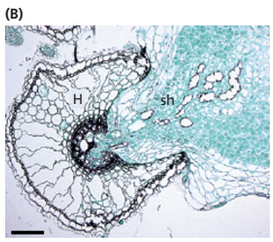


Figure 2.14 Life cycle and host colonization for a typical parasitic plant. (A) The life cycle of witchweed (*Striga* spp). (B) Cross-section of *Striga hermonthica* (Sh) parasitizing a rice root (H). (C) For *Striga* sp., starting with the terminal end of its primary root, shown are four steps leading to colonization and invasion of the vasculature of a host plant. Note the invasive cells shown in orange, some of which are modified to become xylem cells, thereby favoring nutrient transport to the parasite. (A, Modified from IITA Striga Manual (1997); B, From Yoshida S & Shirasu K (2012) *Curr Opin Plant Biol* 15:708–713. With permission from Elsevier.)

Some parasitic plants, especially facultative parasites, can infect hundreds of different host plant species, whereas others, especially obligate holoparasites, have very specific host requirements. For example, some mistletoes can parasitize only other species of mistletoes, a phenomenon termed **obligate epiparasitism**. Although reminiscent of parasitic red algae described above, obligate epiparasites are not necessarily close relatives of their hosts. Mistletoes that are parasites of other mistletoes, which are themselves parasites of a plant host, also exemplify the phenomenon of **hyperparasitism**. Some parasitic plants, such as witchweeds (*Striga*), dodder (*Cuscuta*) (**Figure 2.15A**) and broomrapes (*Orobanche*), have a major impact on crop production. Losses in cereal and legume crops due to *Striga* are estimated to cost Asia and Africa $10 billion annually. One remarkable parasitic plant, the "queen of parasites," produces the world's biggest flower (**Figure 2.15B**). Members of the genus *Rafflesia* have been shown to express hundreds of genes likely acquired from their host plants, thus providing an example of host-to-parasite HGT.

Figure 2.15 Some remarkable parasitic plants. (A) A species of dodder (*Cuscuta*) enveloping an acacia tree. Some species of *Cuscuta* have small amounts of chlorophyll and can engage in photosynthesis and are classified as hemiparasites, whereas others are totally dependent on their host for nutrition and are holoparasites. (B) A flower of the parasitic plant *Rafflesia arnoldsi*, the world's largest at 100 cm in diameter. The flower smells like rotting flesh and thereby attracts flies that serve as pollinators. This remarkable plant, sometimes called the "queen of parasites," lacks leaves, stems, and roots. It produces an invasive haustorium that colonizes vines of the genus *Tetrastigma*. (A, Courtesy of Khalid Mahmood, published under CC BY-SA 3.0; B, Courtesy of Ma Suska, published under CC BY-SA 2.0.)

Parasitic Fungi

The kingdom Fungi includes organisms ranging from tiny single-celled microsporidians and chytrids to complex, multicellular mushrooms. Fungal parasites are usually discussed in mycology courses and most will not be emphasized in this book. **Mycology** is the branch of biology that deals with the study of fungi. However, parasitism is such a pervasive feature of fungal biology, and fungi are so frequently implicated as the cause of emerging diseases or extirpations of endangered species, it would be remiss not to include them in this overview of parasite diversity. Examples of the effects of parasitic fungi appear throughout the book.

Fungi are heterotrophic with an **osmotrophic** (absorptive) rather than **phagotrophic** (ingestive) mode of acquisition of nutrients. They usually possess a thallus (body) composed of branching filaments called **hyphae** that together can form a densely branched network called a **mycelium**. The hyphae grow by apical extension and have polymers of *N*-acetyl glucosamine (**chitin**) in their cell walls. It is estimated that two-thirds of the known ~100,000 species of fungi enter into some form of intimate association with another living organism, and many different kinds of fungi parasitize a huge variety of organisms including other fungi, single-celled eukaryotes like amebas, plants and many different kinds of animals, including humans. In fact, it has been suggested that the ancestors of all fungi were parasites of algae. Common estimates of the number of fungal species in the world range from 1.5 to 5 million species.

Although the phylogenetic relationships among fungi are far from settled, there has been considerable progress in resolving relationships and identifying component groups. **Figure 2.16**, representing one hypothesis of relationships, provides an overview of some of the major parasitic groups.

The chytrids (see Chytridiomycetes on tree in **Figure 2.16A**) used to be regularly excluded from the fungi but are now recognized as being among the earliest diverging members of this kingdom. Chytrids are mostly aquatic forms but there are representatives that parasitize animals or plants. One chytrid *Batrachochytrium dendrobatidis* can infect over 500 species of amphibians on all continents where amphibians are found. It has contributed to nearly half of all amphibian species being in decline and has already caused the extinction of some frog species (see Chapter 8 and **Box 8.1**). A related species, *B. salamandrivorans*, has recently emerged and is particularly lethal for salamanders. The Microsporidia, long considered to be protists because of their unicellular spores, are now recognized as fungi. Microsporidians are intracellular parasites with highly reduced genomes and much-reduced mitochondria called mitosomes. Most are parasites of animals but a few species are hyperparasites of gregarines. These host-specific parasites colonize all groups of animals, especially insects, and are probably grossly underrepresented by the approximately 1,500 species thus far described; some have suggested as many as 1 million species exist. Among them are species of *Nosema* implicated in declines of bee populations.

The Ascomycota (**Figure 2.16B**) is a vast group of fungi that includes several important plant parasites. These include the causative agents of Dutch elm disease (*Ophiostoma ulmi*), chestnut blight (*Cryphonectria parasitica*) (**Figure 2.17A**), and the recently problematic ash dieback (*Chalara fraxinea*). Chestnut blight devastated the American chestnut tree (*Castanea dentata*) across the North American continent in the early 1900s. *Pneumocystis jirovecii* (Pneumocystidiomycetes) is a frequent cause of pneumonia in humans (**Figure 2.17B**). This organism used to be called *P. carinii*, the latter a name now reserved for a species found in animals. Fungal infections

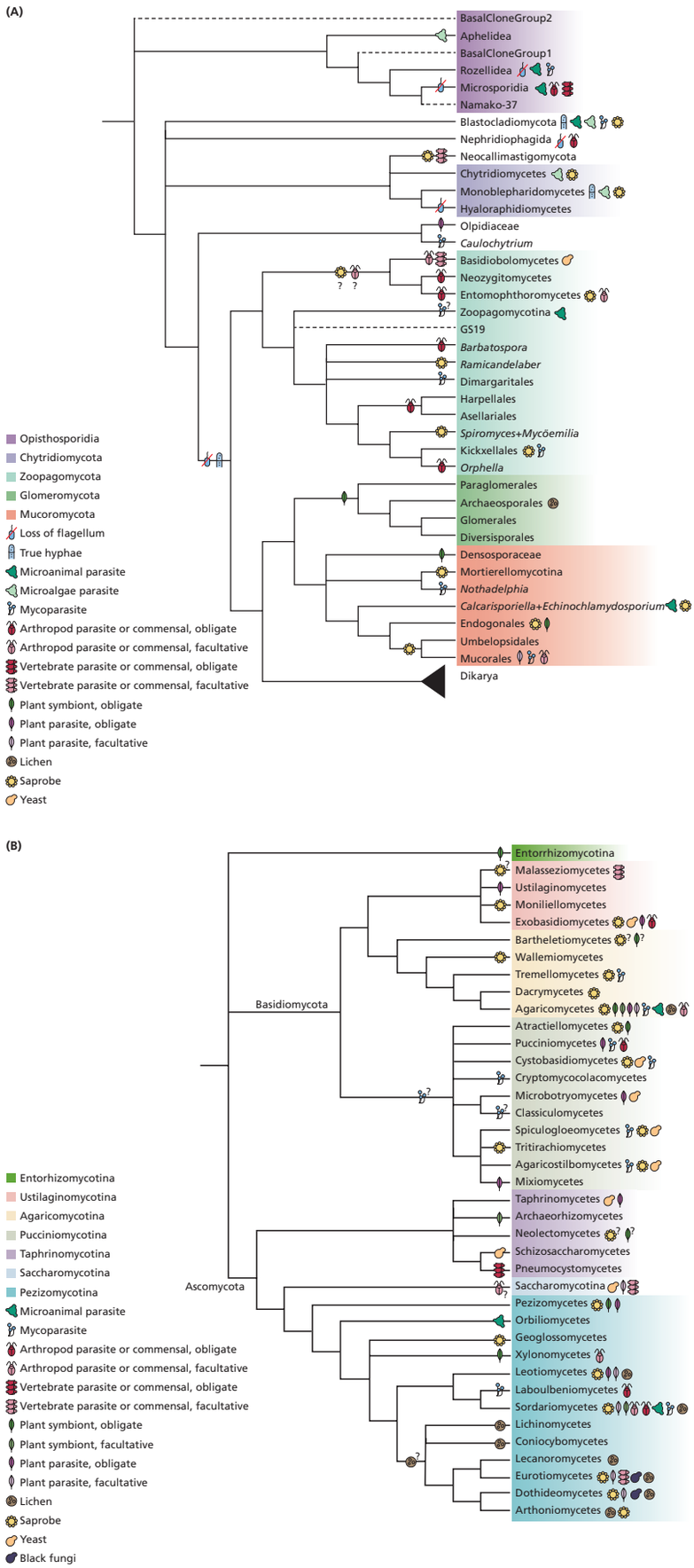


Figure 2.16 Parasitism is ubiquitous among the fungi. These two phylogenetic trees provide an overview of fungal diversity, with tree A showing the non-Dikaryon fungi, and tree B showing the Dikaryon (possessing 2 nuclei per cell in some life cycle stages) fungi, namely the Basidiomycetes and Ascomycetes. Note that of the many lineages of fungi, most include species that are involved in symbiotic associations, and most (over 60%) include parasitic representatives of one type or another, indicative of many separate origins of distinctive types of parasitism within the fungi. (Modified from Naranjo-Ortiz MA & Gabaldon, T (2019) Fungal evolution: diversity, taxonomy and phylogeny of the fungi. *Biol Rev* 94:1443–1476. doi:10.1111/brv.12550. With permission from John Wiley and Sons.)

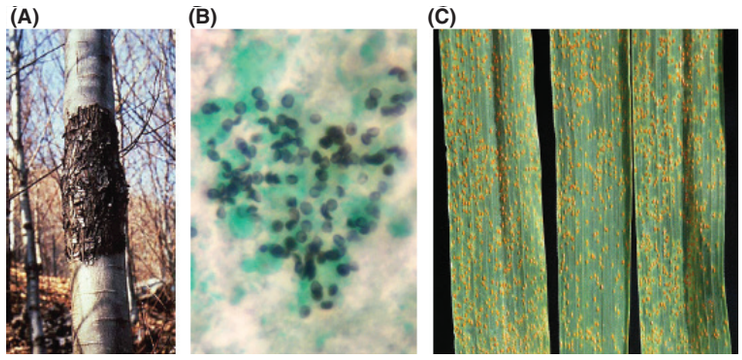


Figure 2.17 Examples of the impact and diversity of fungal parasites. (A) A lesion (canker) on a chestnut tree (*Castanea*) afflicted by chestnut blight (*Cryphonectria parasitica*). (B) Cysts of *Pneumocystis jirovecii*, an ascomycete fungus, from the lungs of a patient with pneumonia. (C) The wheat leaf rust (*Puccinia triticina*), a basidiomycete fungus that infects wheat and other related food grain plants. (A, Courtesy of Daniel Rigling; B, Courtesy of Pulmonary Pathology, published under CC BY-SA 2.0; C, Courtesy of James Kolmer, US Department of Agriculture.)

such as pneumonia caused by *P. jirovecii* are often **opportunistic** because they do not cause disease in healthy hosts but can become problematic in unhealthy individuals. This parasite can cause fatal infections in immunocompromised individuals. The parasite is often found in those who have a concurrent infection with HIV or are taking immunosuppressive drugs following organ transplants. Another ascomycete that has achieved unwanted prominence is *Pseudogymnoascus destructans* (Dothideomycetes), the fungus responsible for white-nose syndrome currently decimating some species of North American bats (see **Box 8.1**).

The Basidiomycetes includes the familiar mushrooms, stinkhorns and puffballs, as well as several substantial parasites of plants such as the rusts (**Figure 2.17C**), smuts and wood-rotting fungi. *Puccinia triticina* and its relatives are of great concern because of their potential for devastating wheat and other food crops around the world.

A fungus associated with pancreatic cancer: *Malassezia*, an exobasidiomycete commonly found on the skin and scalp where it can cause irritation, can also colonize the pancreas from the gut and seems to be associated with pancreatic cancer. Read more about it here: https://www.nature.com/articles/s41586-019-1608-2.pdf

Parasitic Animals

Members of the kingdom Animalia (animals, or metazoans) are unique among the world's organisms for the development in most of integrated nervous and muscular systems that give them unprecedented mobility and responsiveness to environmental circumstances. Animals are multicellular heterotrophs that usually acquire their energy from ingestion of organic compounds (phagotrophy), although several parasitic groups acquire nutrients by absorption across their body walls. Many of the world's most familiar and medically significant parasites are found among animals. Parasitism has arisen independently on several occasions with estimates ranging from 60 to over 223 occasions, in both major and minor lineages, but especially in the Arthropoda, the largest phylum by far in the animal kingdom. Some lineages of animals are exclusively parasitic, some have a mixture of free-living and parasitic species, and some as best we know are without parasitic representatives (**Figure 2.18**).

Establishing the relative phylogenetic positions of the 35–40 phyla of animals in the overall tree of animal evolution is a subject of ongoing study. As is true with most groups of organisms, nucleotide sequence data (increasingly based on whole-genome sequences) have dramatically improved our understanding of the relationships among animals. This is particularly true for parasites because often their morphological features have been greatly modified, rendering their origins and relationships obscure. A conservative view of animal relationships based on genome-scale datasets provides many of the salient features.

Among those animals with bilateral symmetry, in addition to a number of smaller phyla whose affinities are debated, three major lineages of animals are recognized, as indicated by the colors in **Figure 2.18**: the Deuterostomia, Lophotrochozoa and the Ecdysozoa. Molecular studies have been particularly important in defining this last major lineage, one united

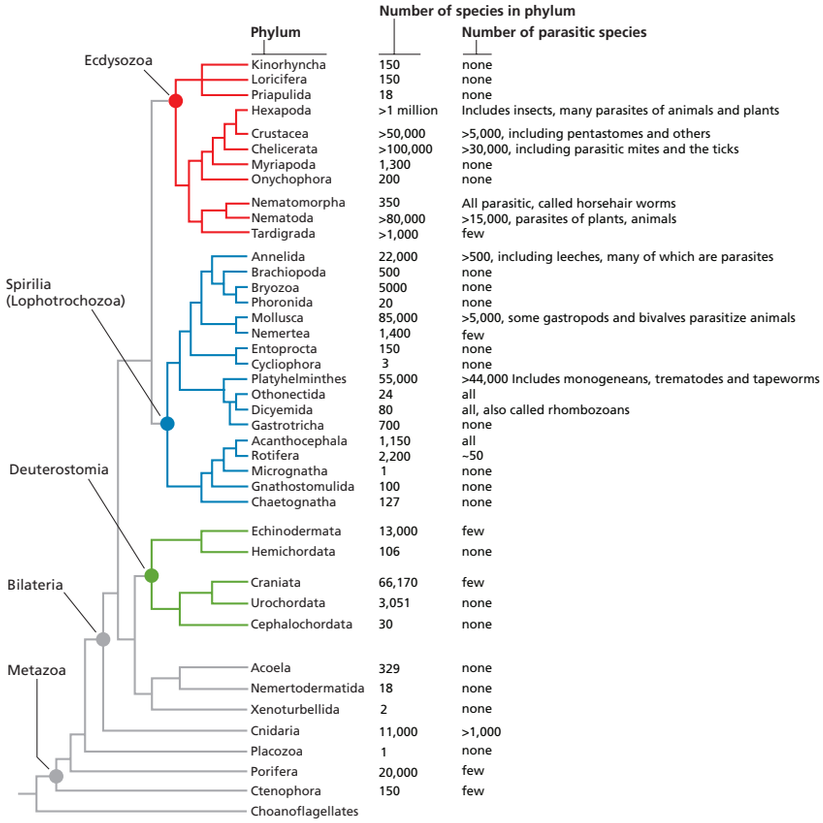


Figure 2.18 An overview of animal (metazoan) phylogeny with an emphasis on parasitic animals. This composite view of animal relationships is based largely on genome-scale data, not a single reference gene like SSU rDNA. The dots on the tree indicate branching points delineating major groups. To the right of the tree is indicated the estimated number of species in each phylum, followed by the estimated number of parasitic species in that phylum. The tree is meant to convey hypothesized patterns of branching order, some of which are still not finally resolved. Branch lengths are not meant to convey elapsed time. (Based on Laumer CE, Fernández R, Lemer S, Combosch D et al. (2019) *Proc R Soc B* 286:20190831. doi:10.1098/rspb.2019.0831 and Lu TM, Kanda M, Satoh N & Furuya H (2017) *Zool Lett* 3:6. doi:10.1186/s40851-017-0068-5, published under Creative Commons Attribution 4.0 International License.)

by the common property of molting (**ecdysis**) of an external cuticle. The Guinea worm, ***Dracunculus medinensis*** (**Figure 2.19A**), a member of the phylum Nematoda that includes many parasitic representatives, is an example of an animal that undergoes molting (Ecdysozoa). See Chapters 8 and 9 for additional discussion of this species, now on the verge of elimination in its human hosts owing to control measures to break its life cycle by filtering drinking water and preventing ingestion of infected copepods. Also included among the molting phyla is a huge group of organisms, the Arthropoda, that

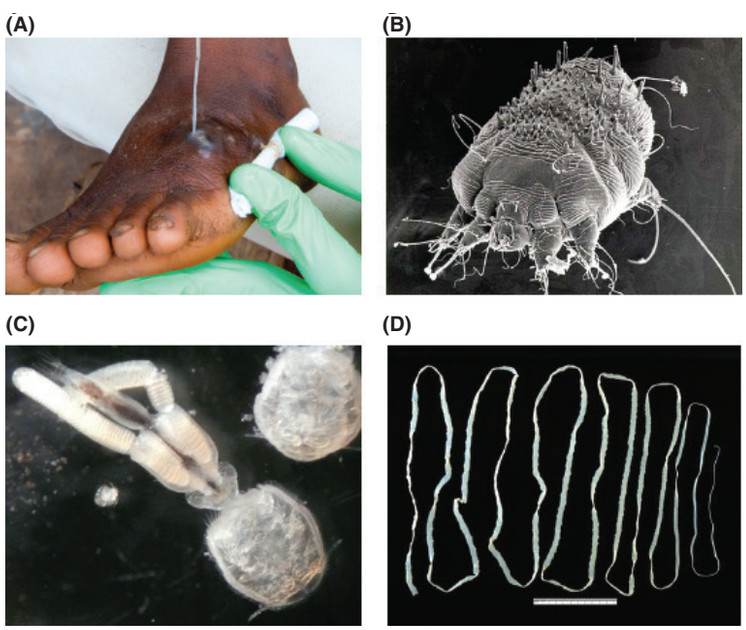


Figure 2.19 Examples of some prominent groups of animal parasites. (A) The Guinea worm *Dracunculus medinensis*, a nematode, extruding from a victim's foot. (B) *Sarcoptes scabiei*, a parasitic mite that burrows through the skin of its human host. (C) A parasitic copepod, like the mite depicted in (B), also an arthropod. This female copepod burrows into the gills and mouth of its host, a shark, and produces two large ovisacs. (D) *Taenia saginata*, the beef tapeworm. (A, © The Carter Center, L. Gubb; B, Courtesy of Louis De Vois; C, With permission from Kelly Weinersmith; D, Courtesy of the CDC.)

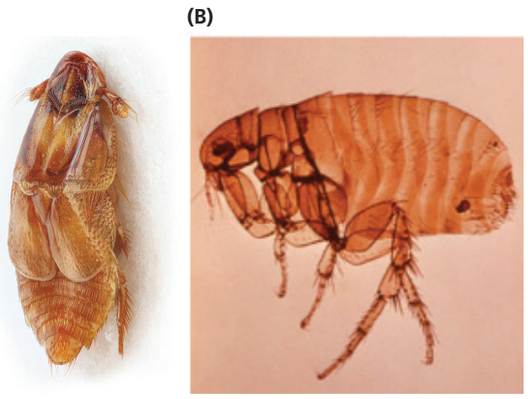


Figure 2.20 Examples of ectoparasites among a huge lineage containing many parasites, the Insecta. (A) The beaver beetle *Platypsyllus castoris*, about 2.5 mm in length, is the only representative of its genus. It is a peculiar dorsoventrally flattened, eyeless, and wingless beetle that feeds on skin and its secretions (and possibly blood) of the American beaver, *Castor canadensis* and the European beaver, *Castor fiber*. (B) The flea *Xenopsylla cheopis*, a laterally flattened blood-feeding ectoparasitic insect that also serves as a vector for *Yersinia pestis*, the bacterium that causes plague. (A, Courtesy of Stanislav Snall, published under CC BY 3.0; B, Courtesy of CDC.)

comprise over 1.1 million species, most of which are insects. The Arthropoda includes several other groups with many parasite species such as ***Sarcoptes scabiei***, a mite that causes intense itching when it tunnels through human skin (**Figure 2.19B**). More specifically, it is a chelicerate arthropod meaning it has pincerlike chelicerae instead of jaws. Many species of mites are parasitic. Another prominent group of arthropods is the crustaceans, many of which are parasitic, often becoming highly modified in the process of becoming parasitic (**Figure 2.19C**). Prominent among the Lophotrochozoa are members of the phylum Platyhelminthes, most of which are parasitic. One spectacular example is ***Taenia saginata***, a tapeworm that routinely grows to 4–12 m in length, living in the small intestine of its human host (**Figure 2.19D**).

Figure 2.18 reveals that much of the uncertainty with respect to quantifying the number of eukaryotic species in general, and of animal parasites in particular, lies within the vast phylum Arthropoda. The phylum is dominated by insects of which there are an estimated 900 thousand named species. However, there are many more insect species to be described and we do not know if this number will prove to be in the millions or thousands of new species. The variety of parasitic lifestyles among the insects alone is immense (**Figure 2.20**), including organisms as diverse as gall-making parasites of plants, hundreds of thousands of wasp species that are parasitoids undergoing their larval development in other insects and invertebrates, ectoparasites such as fleas and lice, and additional blood-feeding insects such as mosquitoes, black flies and kissing bugs that also are frequently implicated in the transmission of viruses or other disease-causing organisms.

Below in Section 2.2, we discuss some specific examples of how parasites have provided remarkable insights into animal relationships.

2.2 INSIGHTS INTO PARASITISM FROM THE STUDY OF DIVERSITY

The quest to reveal the full measure of parasite diversity and to understand the relationships of parasites to one another and to other organisms has led to many novel insights about parasitism. In this section, we provide some examples. Box 2.3 also provides a perspective on ongoing efforts to reveal the diversity inherent in an engrossing and manipulative group of parasites, the Nematomorpha.

The phylogenetic affinities of enigmatic parasites can be revealed

One baffling group of parasites is the tongue worms (because they have a vague resemblance to a tongue), also known as **pentastomes** (**Figure 2.21A**).

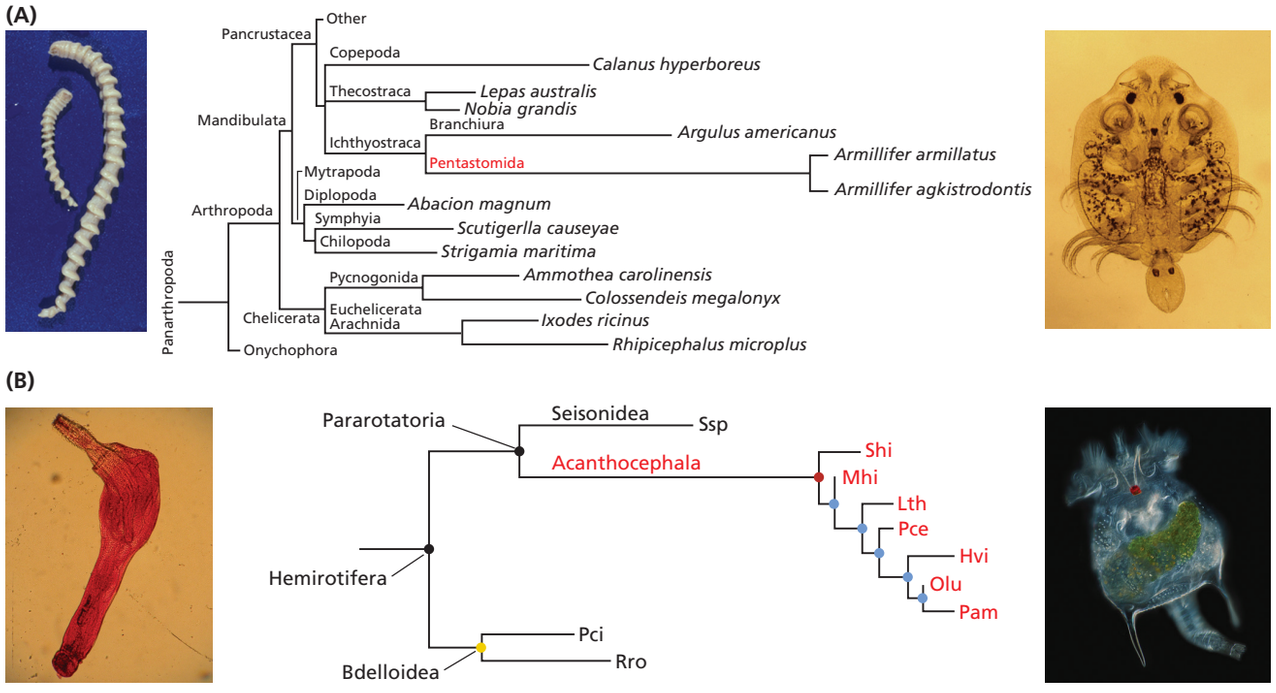


Figure 2.21 Two examples of perplexing parasites with surprising relatives. (A) left, a pentastome; center, a tree portraying results indicating the similarity in mitochondrial gene sequences between the pentastome *Armillifer* and the branchiuran crustaceans *Argulus*, the fish louse shown at right. (B) left, an acanthocephalan or thorny-headed worm, an exclusively parasitic group; center, acanthocephalans hypothesized in the analysis of mitochondrial gene order to be related to rotifers, predominantly a free-living group; right, a rotifer. Note that names in black on this tree are all of rotifers whereas the acanthocephalans are shown in red, with the three-letter codes on the right being abbreviations of names of different species. (A(i), From Tappe D & Büttner DW (2009). *PLoS Negl Trop Dis* 3:e320, open-access under the terms of the Creative Commons Attribution License; A(ii), Tree from Li J et al. (2016) *Korean J Parasitol* 54:813–817, with permission from the *Korean Journal of Parasitology*; A(iii), *Argulus* courtesy of H. Yokoyama, University of Tokyo; B(ii) Tree from Sielaff M et al. (2016) *Mol Phylogenet Evol* 96:79–92, permission from Elsevier; B(iii), rotifer with permission from Frank Fox and the website www.mikro-foto.de.)

The latter name stems from the appearance in some of five mouth-like openings on one end of the elongated, segmented body. In fact, there is one mouth surrounded by two pairs of hooks used as attachment structures. They are typically found as adults in the respiratory tracts of vertebrates, especially in predatory reptiles like snakes, but also from amphibians, crocodilians, turtles, birds and mammals. Their larvae are found in many of these same groups and in marine and freshwater fishes, and insects. They are predominantly a terrestrial group. About 130 species of these strange parasites are known -strange because they seem so unrelated to anything else. What are they?

Over the years, scientists have noted features of pentastomes reminiscent of those found in a number of animal groups—tardigrades, mites, onychophorans, annelids and myriapods—but given their lack of obvious similarity with other animals, pentastomes were frequently accorded the status of a separate phylum, the Pentastomida. Then it was noted that details of the structure of the spermatozoa of pentastomes shared surprising similarities with those of another parasite, but one totally dissimilar in appearance and habitat, the fish louse *Argulus* (**Figure 2.21A**). *Argulus* is a crustacean and is typically found living in the gill chambers or on the external surfaces of fish.

Following this lead, first the SSU rDNA sequences of pentastomes and *Argulus* were examined and were similar, supporting the relationships suggested by sperm morphology. This result has since been confirmed by more extensive comparisons of sequences such as whole mitochondrial genomes.

Pentastomids are now generally considered to be nearly unrecognizably modified members of the Crustacea, having as relatives not only fish lice, but crabs and copepods as well, organisms we far more intuitively unite as crustaceans.

This example teaches us that painstaking anatomical work can reveal relationships otherwise not at all foreseen based on general appearance. Also, sequence data can provide an objective independent assessment of relationships. Pentastomes, like many parasites, are under selective pressure to accommodate radically different kinds of habitats such that the normal anatomical features of the group to which they belong become obscured. Keep in mind that although the pentastome–*Argulus* relationship now seems to be reasonably well supported, it remains a hypothesis awaiting further testing. After all, exactly how was a presumptive ancestral lineage of fish ectoparasites transformed into the enigmatic endoparasitic pentastomes of reptiles, crocodilians and mammals that we see today? Further layers of intrigue are added by the discovery of what look to be minute pentastomids in the ancient *marine* fossil record (see **Box 7.4**).

The second example (see **Figure 2.21B**) reveals a surprising relationship between an exclusively parasitic group, the **acanthocephalans** or thorny-headed worms, and the mostly free-living phylum Rotifera (the rotifers or wheel animals). Acanthocephalan adults use their spiny proboscis to embed in the intestinal wall of a vertebrate host, with their body, which lacks a gut, hanging into the intestinal lumen. Their larval stages are usually encysted in arthropods. Thorny-headed worms used to be placed in their own phylum, the Acanthocephala, but now they are often considered to be highly modified rotifers. Again, it seems like a long evolutionary journey from a free-living rotifer living in an aquatic environment to an endoparasite of vertebrates that lacks any semblance of its own gut.

How might free-living rotifers step-by-step evolve into acanthocephalans with complex life cycles with adults living as intestinal parasites in vertebrates? See https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5266366/ for one scenario.

A third major group of parasites slow to be resolved with more certainty with respect to their position in animal phylogeny is the **Myxozoa**. Myxozoans are an exclusively parasitic group of animals inhabiting either annelid worms or bryozoans as definitive hosts, and vertebrates, especially fish, as intermediate hosts. Some like ***Myxobolus cerebralis*** cause prominent fish diseases (**Figure 2.22**). Although once thought to be unicellular protists or a separate group of early diverging bilaterians, molecular study has clarified their status as unusual cnidarians, the phylum containing jellyfish and corals. This result nicely helps to explain the provocative similarity between cnidarian nematocysts and the polar capsules of myxozoan spores that had long puzzled zoologists (**Figure 2.22**). Myxozoans remain intriguing because at least one species, *Henneguya salminicola*, lacks a mitochondrial genome and functional aerobic metabolism, the first animal unequivocally shown to have this distinction. To go along with their anatomical simplification, myxozoans have among the smallest of genomes known from animals. As another striking and possibly related experiment in parasitism, the

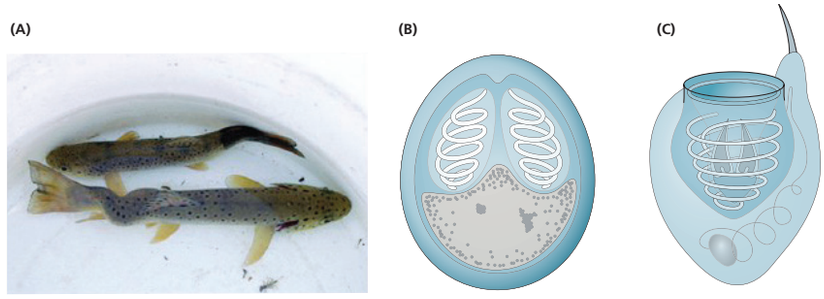


Figure 2.22 Myxozoans, exemplified by *Myxobolus cerebralis,* the organism that causes whirling disease in salmonid fishes. (A) Trout suffering from whirling disease infection. Note the misshapen spine and blackened tail. (B) Example of a myxospore showing coiled polar filaments within. These are extruded and provide attachment to the host cell to be infected. (C) An example of a cnidarian nematocyst showing the coiled tube within, which is believed to be homologous to the polar filaments of the myxospore. (B, From Eiras JC, Malta JCO, Varella AMB & Pavanelli GC (2005) *Mem Inst Oswaldo Cruz* 100:245–247, permission granted under Creative Commons attribution-type BY; C, Courtesy of Ivy Livingstone.)

oocytes of sturgeons are often infected with a bizarre nematocyst-bearing cnidarian called *Polypodium* that even produces jellyfish-like forms in the freshwater stage of its life cycle.

Studies of parasite diversity reveal how particular parasites came to infect humans

An advantage of having a solid understanding of the phylogenetic relationships for both host and associated parasite lineages is that we gain insights into when and how hosts acquired their parasites. This is certainly also true for human parasites, as indicated in the following two examples. The first pertains to the sucking lice (Anoplura) that we harbor. All sucking lice are blood-feeding ectoparasites of mammals. Humans are unusual as compared to our nearest relatives for harboring lice representing two different species, each of a different genus, ***Pediculus humanus*** and ***Pthirus pubis***. The former species is of particular note for serving as a vector of the bacterium causing epidemic typhus (*Rickettsia prowazekii*) and other pathogens. In contrast, chimpanzees and gorillas each harbor one sucking louse species (*Pediculus schaeffi* and *Pthirus gorillae*, respectively). A number of phylogenetic studies have ascertained both the pattern of relationships among primates, including apes, and among their sucking lice (**Figure 2.23A**). Such studies have estimated the time of divergence based on the amount of sequence change occurring for both lice and primates, an application of the **molecular clock hypothesis**.

The essential idea behind the molecular clock hypothesis is that DNA sequences change by mutation at a constant rate, such that the degree of divergence in a particular gene sequence between two related species could be used to date the time when they diverged. Although the clock hypothesis is somewhat controversial and the rate of nucleotide substitution change varies among different groups of organisms, it is still widely used. Using the molecular clock hypothesis, the divergence time between *Pediculus* and *Pthirus* was estimated to be 13 million years ago.

Figure 2.23B shows one hypothesis that explains how humans acquired their two louse species. Thick tan lines show the phylogeny for humans,

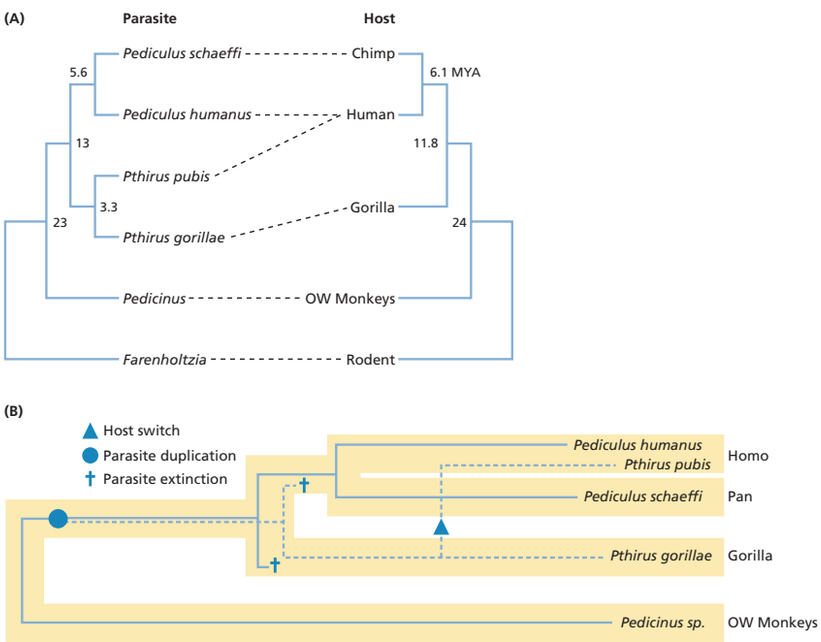


Figure 2.23 The origins of humans and sucking lice. (A) Phylogenetic trees for both primates (emphasizing humans and our closest relatives) and their anopluran sucking lice. (B) This tree provides a hypothesis for how humans acquired their two louse species. (A, B Image from Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice. BMC Biol 5:1–11. Adapted from Reed et al. (2007) *BMC Biol* 5:1–11 used under the terms of the Creative Commons Attribution License 2.0.)

chimpanzees, gorillas and Old World monkeys. The relationships among lice are shown by the thin blue lines (solid and dashed). This scenario is of a parasite duplication that occurred about 13 million years ago (estimated from the degree of sequence divergence) leading to *Pediculus* (solid lines) and *Pthirus* (dashed lines). An extinction (represented as a cross) occurred in each louse lineage. Both humans and chimpanzees acquired *Pediculus* from a common ancestor 5–6 million years ago: the estimated time of divergence between chimpanzees and humans and between our respective *Pediculus* species match. In contrast, the estimated divergence time between our *P. pubis* and the gorilla's *P. gorillae* occurred only 3–4 million years ago, a much shorter time than the time of the last common ancestor between gorillas and our human lineage (7 million years ago). It thus seems likely that we acquired *Pthirus* lice by a host switch from gorillas (represented by the vertical arrow within the *Pthirus* lineage). Such a switch may have been favored by humans sharing habitats with gorillas or when humans preyed on gorillas allowing lice the opportunity to move onto a new host. According to this parsimonious scenario for humans, which is by no means the only possible interpretation, *P. humanus* is an example of an **heirloom parasite** (one acquired from our ancestors), whereas *P. pubis* is a **souvenir parasite**, acquired via a host shift along the way. Recent studies suggest the divergence times between humans and our closest relatives may be longer than suggested here, so stay tuned for alternative ideas about our relationships with our lice.

The second example involves one of our most deadly parasites, ***Plasmodium falciparum***. How did we acquire it? ***P. falciparum***, an apicomplexan, is the most lethal of the four species commonly implicated in human malaria. The considerable pathogenicity of ***P. falciparum*** has left its mark on human evolution (see discussion of sickle cell anemia in Chapter 7). One possibility for its acquisition is that, just as with ***Pediculus*** lice, the last common ancestor of humans and chimpanzees harbored a malaria parasite that subsequently diversified into ***P. falciparum*** in humans and *Plasmodium reichenowi* in chimpanzees. However, it was shown that the amount of genetic diversity in ***P. falciparum*** was far less than in *P. reichenowi* from chimpanzees, suggesting that humans may have acquired a parasite similar to the ***P. falciparum*** parasite from chimpanzees long after their split from a common ancestor. Although attractive, this idea has since been superseded by data suggesting that gorillas from western Africa are the source of ***P. falciparum***. The tree in **Figure 2.24** shows the diversity inherent in ***Plasmodium*** parasites of African apes. This diversity was revealed

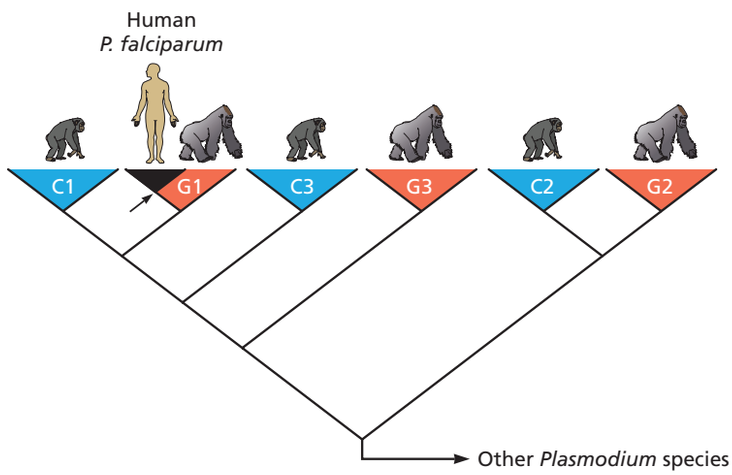


Figure 2.24 The origins of one of the most dangerous species of human parasite, *Plasmodium falciparum*. Isolates obtained from chimpanzees are shown in blue and those from gorillas from western Africa in red. All isolates of the human parasite, *Plasmodium falciparum*, are shown in black. (From Holmes EC (2010) *Nature* 467:404–405. https://www.nature.com/articles/467404a. With permission from Springer Nature.)

by the extensive sampling of ***Plasmodium*** DNA acquired and subsequently amplified from gorilla, chimpanzee and bonobo fecal samples (malaria parasites normally inhabit the blood, but some of their genetic material ends up in the feces of infected animals). All isolates of the human parasite ***P. falciparum*** exhibit only modest diversity in comparison and fall within one lineage (G1), indicating their closest relatives are from gorillas. A comparison of genomes of ***Plasmodium*** isolates from great apes suggests that ***P. falciparum*** is most closely related to the gorilla species *P. praefalciparum* and they shared a common ancestor 40,000–60000 years ago.

As we will detail below, based on insights from genome sequences obtained from several more ***Plasmodium*** isolates from great apes, we have gained further insight into *how* the colonization of humans might have occurred.

Studies of diversity can help reconstruct the historical biogeography of parasites

Another reason to pursue studies of parasites based on an understanding of their overall diversity and relationships is to gain insight into the historical biogeography of the parasite in question. **Historical biogeography** refers to the study of how historical aspects of geology, ecology or climate may have influenced the past and present distributions of species. How did the parasite come to be where it is today? What can historical biogeography tell us about where it might someday go? One example is provided by the human parasite ***Schistosoma mansoni***, a causative agent of intestinal schistosomiasis. This species today infects about 90 million people, mostly in Africa but also in South America. In its adult stage, this parasite inhabits the veins around the intestine of humans, whereas its larval stages undergo obligatory development in freshwater snails of the genus *Biomphalaria*. The geographic range of ***S. mansoni*** is dictated by the presence of compatible *Biomphalaria* snails. Phylogenetic studies of ***Schistosoma*** suggest this group has diversified in both Asia and Africa (**Figure 2.25**).

Surprisingly, *Biomphalaria* snails originated and first diversified in South America, raising the question, why is ***S. mansoni*** so common in Africa today? Phylogenetic studies suggest that a South American *Biomphalaria* snail colonized Africa sometime in the last 2 million years (see **Figure 2.25**),

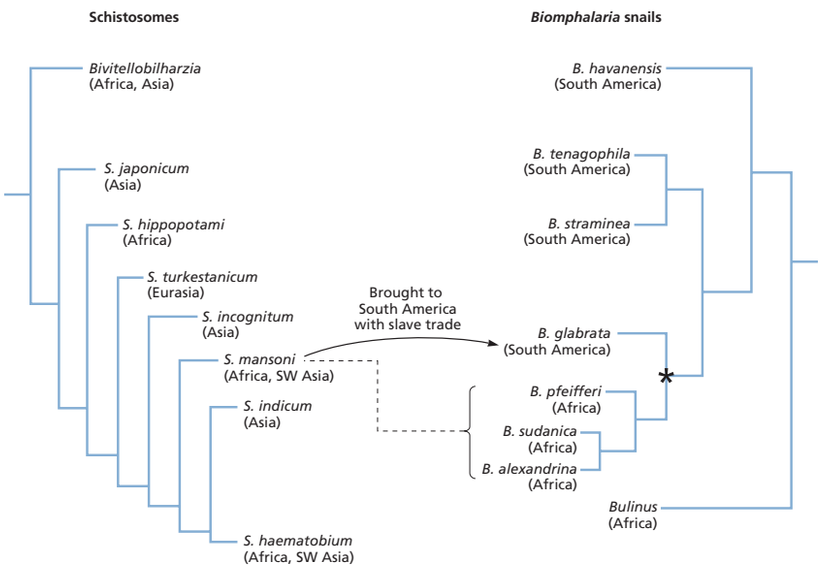


Figure 2.25 Phylogenetic studies help us reveal the historical biogeography of *Schistosoma mansoni*. Shown on the left is a phylogeny for *Schistosoma,* primarily an African and Asian genus. The distribution of *S. mansoni* is mostly African. Shown on the right is a phylogeny for *Biomphalaria* snails. Note that *Biomphalaria* originated in South America but that Africa was later colonized (asterisk). The dashed line indicates the species of *Biomphalaria* with which *S. mansoni* evolved. Note that *S. mansoni* was later brought to South America (arrow) and was able to colonize *B. glabrata* there. Although *S. haematobium* was also brought to South America, it was unsuccessful because its snail host *Bulinus* is confined to Africa, Europe and Asia.

long after the continents had drifted far apart. The long-distance colonization of Africa was facilitated by the fact that *Biomphalaria* snails are hermaphrodites (they can self-fertilize so only one snail is needed to start a population) and because they are often caught in the feathers or on the feet of birds thereby aiding their dispersal to new areas. Once in Africa, *Biomphalaria* diversified and provided a new option for resident ***Schistosoma*** species, one of which must have switched into this newly available snail and evolved into a separate species that we know today as ***S. mansoni***.

So how did this parasite then come to colonize South America? Several lines of evidence indicate the colonization of South America by ***S. mansoni*** was fairly recent and was likely a consequence of the trade that brought infected Africans to the New World 400–500 years ago. Interestingly, because a species of *Biomphalaria* (*B. glabrata*) similar to the one speculated to have given rise to the African species was still present in South America, ***S. mansoni*** fortuitously found the necessary snail hosts needed to support its life cycle and to enable its persistence (see **Figure 2.25**). A related species, ***Schistosoma haematobium***, that causes urinary schistosomiasis in Africa was also almost certainly brought to the New World in slaves but did not gain a foothold there. Because its snail hosts (in the genus *Bulinus*) originated in Africa and never colonized South America, the necessary snail hosts for ***S. haematobium*** were unavailable to support transmission in South America at the time of the slaves' arrival. This example indicates that ***S. mansoni*** is a relatively newly acquired parasite of the human lineage. It reveals the dependence of parasites on key hosts such as snails and explains why this parasite so readily spread to the New World, whereas close relatives did not.

BOX 2.3

Finding New Parasite Diversity: The Nematomorpha as a Model

One of the most exciting aspects of science is to discover something never before seen, and the ongoing efforts by parasitologists to find new species of parasites unknown to science is an example of this pursuit. How are such discoveries made, how is the new species characterized, and what is the benefit of this information? One good model that demonstrates how these questions are answered is that of a relatively small, exclusively parasitic phylum of worms called the Nematomorpha, or nematomorphs. These also are known as horsehair worms, hairworms or gordiacea. The long, sinuous nematomorph adults are free-living, often being found in knotlike aggregations of copulating groups along the margins of streams or ponds (see **Figure 1A**). The name gordiaceans comes from the knot of rope from the city of Gordius that was famously cut by Alexander the Great. Fossil nematomorphs are known from the early Cretaceous (100–110 million years ago).

Nematomorph larvae are often ingested by invertebrates like aquatic insect larvae, such as midges and mayflies, where they undergo initial essential developmental steps. Eventually these early nematomorph larval stages are ingested by terrestrial arthropods such as crickets, where they grow and proceed through a single molt, just before completing their larval development. The mature adults then make a dramatic exit from their host's body (**Figure 1B**). Being parasitic as larvae but free-living as adults, they are examples of protelean parasites. Nematomorphs can have profound effects on their hosts, such as castration. Although these effects may be temporary, they nonetheless can provoke behavioral changes believed to promote nematomorph transmission (see Chapter 3). The extent to which developing nematomorphs fill the body cavities of their hosts without actually killing them (**Figure 1C**) is a testament to the ability of parasites to manipulate their host's anatomy and physiology to their benefit.

Currently, there are about 350 species of nematomorphs known to science, and experts estimate there are as many as 2,000 species in the world. Using a detailed understanding of the known nematomorphs as a starting point, nematomorph specialists have embarked on a series of collections of new specimens that are often found as adult worms in aquatic habitats. Some of the new specimens are prepared for scanning electron microscopy so external features can be accurately described (**Figure 1D**). Some are extracted to acquire DNA, so that diagnostic sequences like the *CO1* barcode region or other signature genes can be obtained and checked against other known nematomorph sequences. If distinctive, an evolutionary tree placing the new worm into a broader context is constructed. Typically an exhaustive description of a new species will be made and submitted

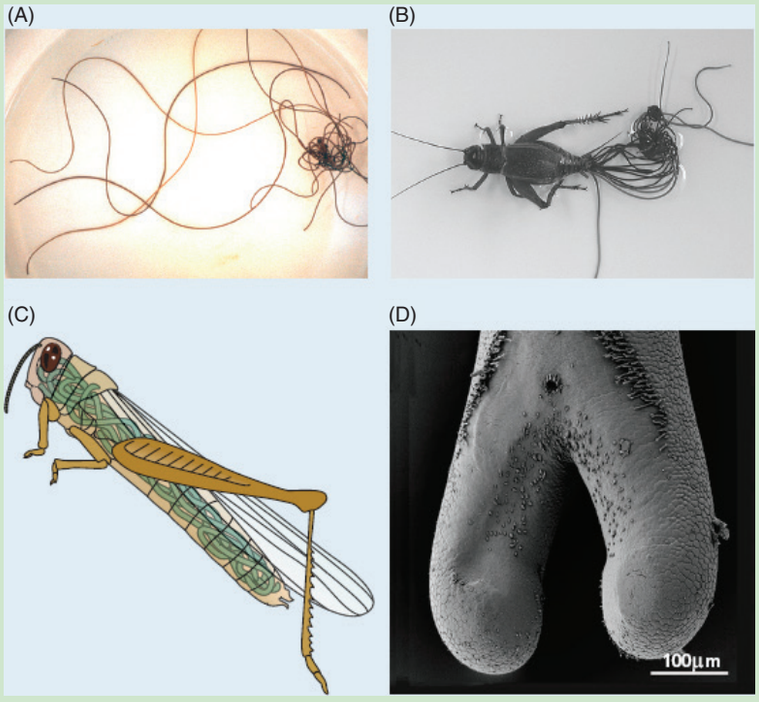


Figure 1 Discovering new parasite diversity as exemplified by nematomorphs. (A) A tangle of nematomorph adults, each about 25 inches in length. (B) Adults of *Paragordius varius* exiting from a cricket host, 28 days after initial infection. (C) Note the extent to which the body of the insect host is occupied by the developing nematomorph worm within. (D) Scanning electron micrograph showing the forked posterior end of a male nematomorph (*Gordionus*), with a cloacal opening. (A, B Courtesy of Ben Hanelt, University of New Mexico; C, Courtesy of Rebecca Strich; D, From Begay A, Schmidt-Rhaesa A, Bolek M & Hanelt B (2012) *Zootaxa* 3406:30–38. https://www.biotaxa.org/Zootaxa/article/view/zootaxa.3406.1.2. With permission from © Magnolia Press.)

to a peer-reviewed scientific journal, and then voucher specimens will be deposited in an appropriate museum. By having a more complete picture of the diversity of nematomorphs, we will be able to clarify how parasitism evolved in this group, how frequently horsehair worms have shifted into new lineages of hosts and how nematomorphs have spread around the world. We might eventually understand why they are particularly common in arid climates and how their diversity might serve as a way to understand the impact of climate change.

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Complexes of cryptic parasite species are coming to light

One problem in quantifying parasite diversity that has come to light with the advent of molecular systematics methods is the surprising extent to which cryptic species have been reported. **Cryptic species** are closely related organisms that are morphologically similar and, on these grounds, are described as a single species. Yet within the group are clusters of individuals that are sufficiently distinct genetically from other clusters to be accorded the status of distinct species. Reports of cryptic species have grown dramatically in the last couple of decades, with an estimated 60% of all new species descriptions arising from complexes of cryptic species.

One striking example of cryptic diversity is provided by digenetic trematodes (also known as digeneans) that infect fish in the St. Lawrence River. The fish are infected with a life cycle stage (metacercariae) of a particular group of digeneans, the diplostomoids. These digeneans cannot be easily identified to species based on their morphology. Among over a thousand metacercariae examined, 47 diplostomoid species were detected based on distinctive *CO1* barcode sequences, representing a large increase in known diversity for this group of digeneans.

Nematodes of the genus ***Trichinella***, responsible for causing trichinellosis (**Figure 2.26**), also provide a good example of how improved breadth and

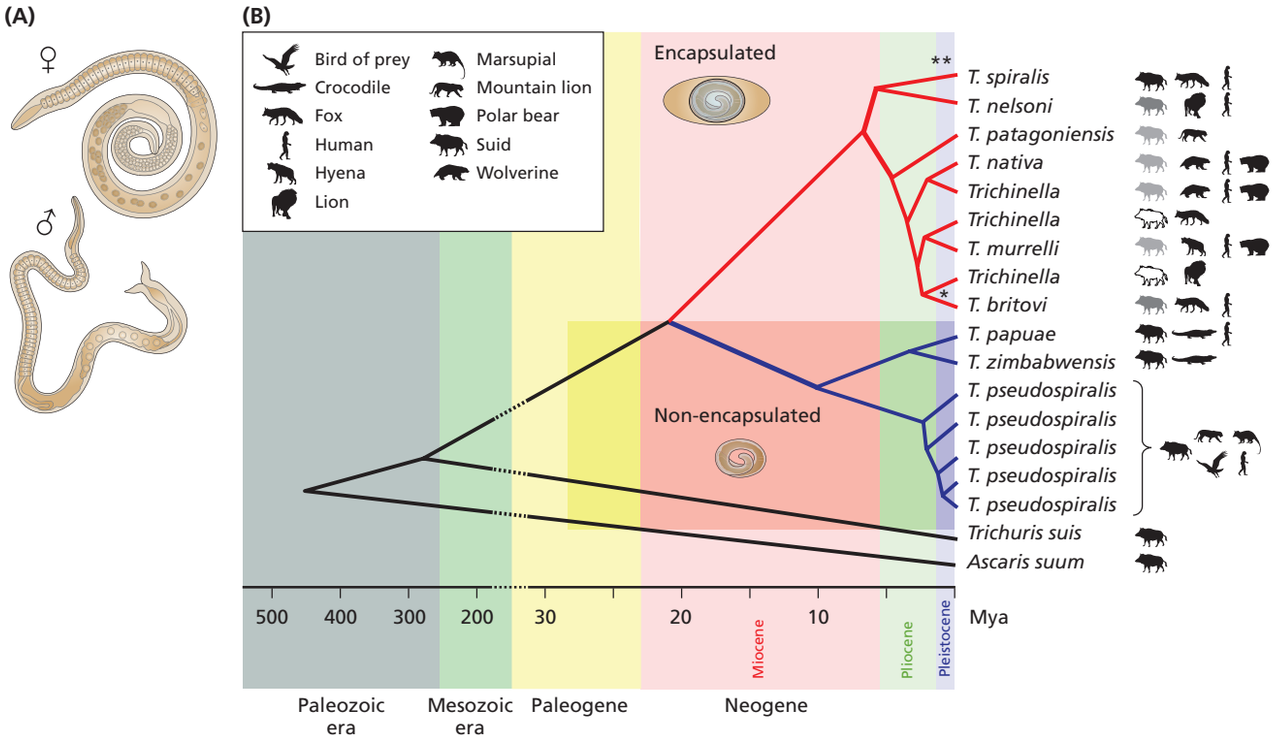

Figure 2.26 The hidden diversity of *Trichinella* species. (A) Adult worms of *Trichinella spiralis*, the nematode responsible for causing trichinellosis. (B) A phylogenetic hypothesis for *Trichinella*, showing all 12 known taxa, with *Trichuris* and *Ascaris* species as outgroups. The tree is based on comparisons of thousands of shared gene sequences. Note two major lineages, one producing cysts with a heavy encapsulated wall within a host muscle cell, and a non-encapsulated lineage with a thin capsule wall. The encapsulated species infect only mammals where non-encapsulated species infect both mammals and possibly birds or crocodiles. Estimated rates of nucleotide divergence have been used to put a timeline on when the various branching events have occurred. The extent to which an indicated lineage uses suid (pig) hosts ranges from black (high) to gray (medium) to light gray (low) to white (not assessed). (A, Courtesy of Ivy Livingstone; B, Modified from Korhonen PK, Pozio E, LaRosa G, Chang BCH et al. (2016) *Nat Commun* 7. doi:10.1038/ncomms10513. Published under Creative Commons Attribution 4.0 International License.)

depth of collections coupled to the application of biochemical and molecular techniques have resulted in a dramatic increase in our understanding of the diversity inherent in the genus. ***Trichinella spiralis*** females living in the small intestine give birth to larvae that seek out, penetrate and encyst within muscle cells, demonstrating, as we noted in Chapter 1, that even helminths can become intracellular parasites. Under the parasite's influence, the host muscle cell loses its contractility and becomes a highly modified nurse cell that supports the survival of the nematode larva within. From the originally recognized single species, ***T. spiralis***, there are now nine named species, with at least three additional sequence-distinctive unnamed ***Trichinella*** forms awaiting further study.

Revealing such diversity is important because the individual species involved in cryptic species complexes may have very different modes of transmission or patterns of host use. For example, the different cryptic diplostomoid species inhabit different organs in their fish hosts and reveal patterns of host specificity not previously known. A full accounting of such diversity could give important clues for tracing the origins of outbreaks or explaining shifts to new host species. Some species of ***Trichinella*** are adapted to Arctic climates, whereas others thrive in the tropics, and the recognized species differ in host preference with some more likely to infect domestic swine, for example, than others.

Similar considerations apply to arthropod vectors that transmit disease, which often exist in cryptic species complexes, the species of which vary in

their vectorial capacity. The ***Anopheles culicifacies*** complex involved in malaria transmission in India and the ***Simulium damnosum*** complex involved in the transmission of onchocerciasis in Africa are two prominent examples.

It has been argued that cryptic species may be particularly common among parasites because they are often small with few distinguishing morphological features and have short generation times, so they evolve more quickly than their hosts. However, one recent study found that among major groups of animals, there is no greater propensity to exhibit cryptic species among parasitic representatives than free-living counterparts. The study of cryptic species among parasites will nonetheless continue to reveal surprises.

Studies of parasite diversity help provide a better foundation for taxonomy

One of the goals of the study of parasite diversity is to reveal the evolutionary relationships among parasite groups, such that existing taxonomic schemes for classification can be brought into agreement with these relationships. Examples such as the acanthocephalan–rotifer and pentastomid–crustacean connections have already been discussed.

One example of the reconciliation of phylogenetic relationships with taxonomy is provided by the schistosomes infecting mammals. One genus *Orientobilharzia*, consisting of a few species infecting Eurasian ruminants, was established that differs from ***Schistosoma*** (found in many mammals from Asia and Africa) primarily on the basis of the number of testes present in male worms, 37–80 and <10, respectively (**Figure 2.27A**). This is a clear morphological difference that makes the separation of the two genera straightforward. However, several molecular phylogenetic studies consistently identified *Orientobilharzia* as nested within a larger clade that otherwise contained only ***Schistosoma*** species. At the same time, worms in other described schistosome genera fall into distinct clades (**Figure 2.27B**). Given that other morphological characters unite *Orientobilharzia* and *Schistosoma*, it would seem in this case that the number of testes simply is not a characteristic that reflects major evolutionary changes. As a consequence of

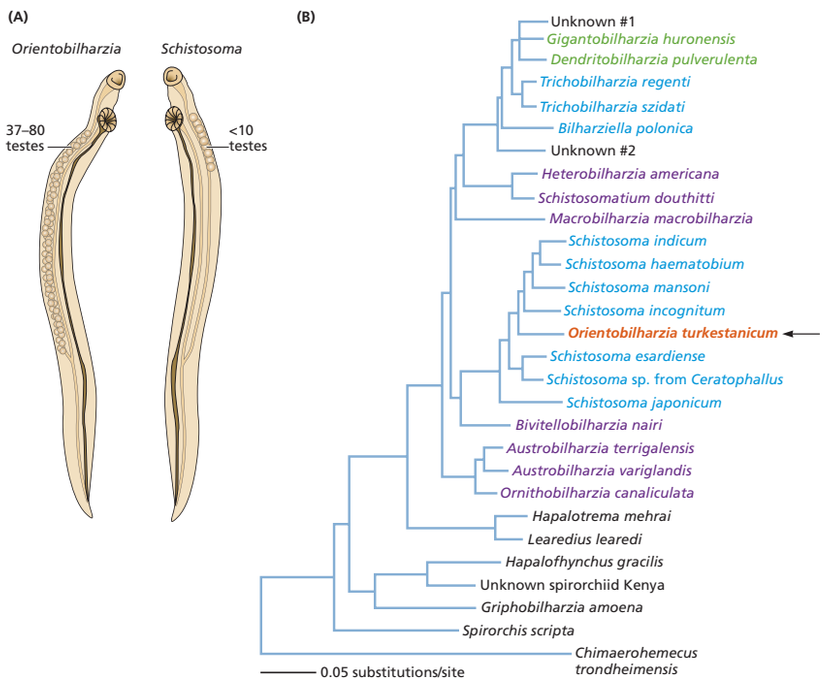

Figure 2.27 Improving taxonomy through a better fundamental understanding of parasite diversity. (A) Male schistosomes of the genus *Orientobilharzia* have 37–80 testes, whereas males of *Schistosoma* have fewer than 10. (B) On this tree, *Orientobilharzia turkestanicum* (black arrow) nests within a group containing several species of *Schistosoma*. (B, Image from Loker ES & Brant SV (2006) *Trends Parasitol* 22:521–528 reproduced with permission from Elsevier.)

such molecular studies, the generic name *Orientobilharzia* has since been relegated to the status of a junior synonym of ***Schistosoma***, and *Orientobilharzia turkestanicum,* for example, is now most properly known as *Schistosoma turkestanicum*.

It should by no means be concluded that morphological characters are inevitably misleading in attempting to define parasite diversity. Our taxonomic hierarchies have for decades been constructed using morphological criteria and many have proven to be robust. Morphological characters often provide convenient ways to identify species and can be used in conjunction with results from molecular studies to identify traits called **synapomorphies**, which are shared exclusively by members of a monophyletic group and the group's immediate common ancestor. A **monophyletic group** is one that includes only taxa derived from a most recent hypothetical common ancestor of that group. One good example of the complementary use of molecules and morphology is provided by monogeneans of the genus *Cichlidogyrus* from African cichlid fish. These flukes are ectoparasitic flatworms living on the gills of the fish. Trees based on sequence data corroborate that characteristics of the haptor (the posterior attachment organ of monogeneans) provide reliable information about main lineages of monogeneans whereas morphological differences in reproductive organs are better for delineating among closely related species. The combined analysis of sequence data and morphological traits also helps to reveal **homoplasy**, referring to similarities resulting from convergent evolution rather than common ancestry. Homoplasy is likely to be a common outcome in parasite evolution because parasites often experience similar hosts and microhabitats within those hosts that favor the evolution of similar traits. Myxozoans seem to exemplify this issue. Our best understanding of parasite diversity will continue to derive from information from as many sources as possible, including both morphology and molecules.

Do parasites give rise to free-living organisms?

The survey of parasite diversity above emphasizes the concept that parasitism has been derived multiple times from free-living progenitors. But what about the opposite possibility? Traditionally, it has been thought that the move to parasitism is a one-way trip because organisms lose genes and structures required to return to a free-living state and they acquire adaptations that specialize them to a life of parasitism. This is in essence what is called **Dollo's law**. Indeed, the discussion of genomes below highlights that genomic reduction is a frequent but by no means inevitable consequence of parasitism.

Nonetheless, examples have come to light, aided by molecular phylogenetics analyses, in which apparent reversions to free-living existence within groups that are parasitic have occurred. Such reversions are particularly noteworthy in mites. Consider the tiny (0.2–0.3 mm) free-living house dust mites (Family Pyroglyphidae) like *Dermatophagoides farina,* which are common inhabitants of nests and houses. They are also one of the most common causes of allergies in people. A strong case has been made that they evolved from mites parasitic on warm-blooded hosts. An ability to survive in low humidity and the presence of enzymes used to digest keratinous material, useful while inhabiting their hosts (and their nests), may have enabled the house dust mites to adapt to life without hosts. Furthermore, house dust mites have diversified into new species in their post-parasitic existence, indicating that parasitism has not inevitably foreclosed subsequent diversification.

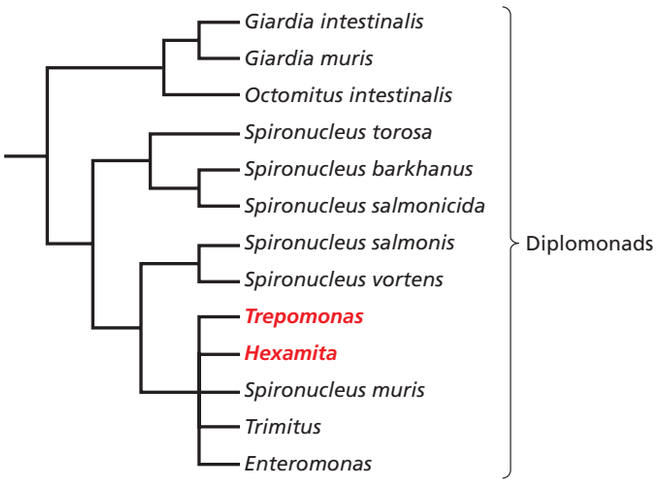

Figure 2.28 A phylogenetic tree for diplomonads, showing the free-living genus *Trepomonas* nested among lineages of parasitic organisms including *Giardia* or *Spironucleus*. Both a ribosomal RNA gene and multigene analysis were used to establish this tree. Predominantly or totally free-living taxa are shown in red, parasitic taxa in black. (From Xu F et al. (2016) *BMC Biol* 14:62. doi:10.1186/s12915-016-0284-z. Published under the Creative Commons Attribution 4.0 International License.)

Another fascinating example is provided by a particular isolate of *Trepomonas* (**Figure 2.28**), a free-living protist recovered from marine sediments, that nests within a lineage dominated mostly by parasites like ***Giardia*** or *Spironucleus*, the latter being parasites of fish, birds and rodents. *Trepomonas* provides an explanation for how loss of genetic material during genome reduction associated with parasitism can be countered: acquisition of new genes by HGT from bacteria. Genes acquired include those required to digest bacterial cell walls and to provide nucleotides and sterols, the latter two capacities often lost in parasites. These are all genes the parasitic ancestors of *Trepomonas* did not possess. It is not yet known if free-living representatives of *Hexamita* (see figure) has undergone similar episodes of HGT. Although these examples indicate it is possible for parasites to adopt a free-living lifestyle, such examples seem to be rare by comparison to the many noted adoptions of parasitic lifestyles by free-living organisms (see also a discussion of the origins of parasitism in Chapter 7).

2.3 GENETIC DIVERSITY ALSO EXISTS WITHIN PARASITE SPECIES

The discussion above focuses on named parasite species as one metric to define, measure and study parasite diversity. There are at least two other forms of parasite diversity to consider as well, both of which contribute enormously to the properties we associate with parasites and that prove to be relevant with respect to understanding the nature of parasites, including their transmission, virulence, treatment and control. The first is the genetic diversity inherent within each species, the so-called intraspecific variation, which is addressed in the next section. The second is the diversity of genes that constitute the genetic repertoire, or genome, of each parasite species: a topic we will cover in Section 2.4.

Diversity within parasite species is extensive and important

No species consists of individuals that are completely uniform genetically. Even in species that routinely engage in self-fertilization or asexual reproduction, genetic variants nonetheless occur thanks to the ongoing process of mutation or other genetic modifications that might arise. It is this intraspecific genetic variation that forms the substrate upon which natural selection acts, favoring some variants over others. For example, individual parasites with a variant gene that confers resistance to a particular drug

might be favored during a control program testing the use of that drug. In Chapter 7, we revisit the importance of genetic variation and population structure within parasite species when we discuss the evolution of parasites. For now, the mission is to show that diversity exists within parasite species and that this variation is consequential and needs to be considered.

First we introduce several terms that are applied to variant forms within a species. The term **isolate** describes a sample of a parasite species derived from a particular host at a particular time. **Strain** refers to an intraspecific group of parasites that differs from other such groups in one or more traits, including traits that might be relevant to control or treatment. **Subspecies** is used to identify a distinct group of organisms within a species that may occupy a particular region and that can interbreed with other subspecies. In this case, however, the organisms typically do not interbreed because of their isolation or some other reason. Subspecies are given a formal name, such as *Trypanosoma brucei rhodesiense*, with *rhodesiense* being the subspecies name. Also, although studies to discriminate among species use less variable genetic markers such as SSU rDNA or cytochrome oxidase, studies of intraspecific diversity rely on more variable markers, such as **microsatellite markers** or single nucleotide polymorphisms (**SNPs**, pronounced "snips)." See **Figure 7.2** for further details. Both microsatellite markers and SNPs can be used to differentiate one individual parasite from another or to identify differences among populations of the same parasite species across small geographic scales.

One important example of how variation within a parasite species matters, is illustrated by *T. brucei*, which is commonly recognized to consist of three subspecies, *T. brucei brucei*, *T. brucei rhodesiense*, and *T. brucei gambiense*. These subspecies can be thought of as intraspecific variants with distinctive host ranges, diseases caused and geographic distributions (**Table 2.1**).

One of the mysteries presented by this complex of subspecies is why epidemics of the subspecies that afflict humans only appear at certain times and often in the same defined locations. There is some evidence that the acquisition of a particular gene, the *SRA* gene (serum resistance-associated gene), by a relatively rare recombination event (recall **Figure 2.1**) transforms the nonhuman parasite *T. b. brucei* into an organism able to survive in and infect people. The presence of the protein encoded by this gene confers resistance to lysis of trypanosomes when placed in human serum (see also **Figure 7.11**). The *SRA* gene happens to be present in all isolates of human-infective *T. brucei rhodesiense* and thus seems to serve as a marker of the presence of a trypanosome capable of infecting people

Table 2.1 Characteristics of the subspecies of *Trypanosoma brucei*.

Species/ Subspecies	Host Range	Disease	Disease Profile in Humans	Distribution
Trypanosoma brucei brucei	Wild and domestic animals	Nagana	—	Tropical Africa
Trypanosoma brucei rhodesiense	Humans, wild and domestic animals	Rhodesian sleeping sickness	Acute	Eastern and Southern Africa
Trypanosoma brucei gambiense	Humans, primarily. Wild and domestic animals	Gambian sleeping sickness	Chronic	Western and Central Africa

Table from Hide G & Tait A (2009) Molecular epidemiology of African sleeping sickness. Parasitology 136:1491–1500. With permission from Cambridge University Press.

and potentially able to cause an epidemic. Lest we become too confident in thinking we now hold the secret for how, where and why all human sleeping sickness epidemics might occur, *SRA* does not serve as a marker to identify the human-infective ***T. brucei gambiense*** isolates more commonly found in West and Central Africa. Nonetheless, this example serves to inform us that differences in genetic composition among representatives of what is still defined as a single species, ***T. brucei***, can have major implications for human and animal health.

An appreciation for the extent of variation within a parasite species gives us a valuable way to both interpret the evolutionary history of the parasite and to understand how this variation might influence eventual control efforts. For example, different isolates of the nematode *Trichostrongylus colubriformis* have different forms of an immunodominant carbohydrate surface antigen that is a promising potential vaccine target (**Figure 2.29**). This forces us to take into account the intraspecific variation inherent in the target species with respect to developing a vaccine that can be broadly effective.

Studies of intraspecific variation, as exemplified by investigations of tapeworms in the genus ***Echinococcus***, can provide new insights into the evolutionary history of parasites. Both distinctive North American and Asian variants of ***E. multilocularis*** have been discovered on St. Lawrence Island, which lies between Alaska and Siberia in the Bering Sea. This discovery provides supportive evidence that the island was part of the Beringian land bridge that once connected the two continents and enabled variants from both continents to co-occur there.

We also gain deeper insights into the nature and consequences of parasitism (including important topics such as virulence) when we have an appreciation for the variation occurring within parasite species. Strains of ***Toxoplasma gondii*** found in people in the Northern Hemisphere are much more likely to be clonally propagated by carnivory of domestic herbivores, whereas other strains found in South America show much more evidence of this parasite undergoing regular sexual recombination and in inhabiting wild animals. This latter point is of considerable relevance to conservation biologists interested in protecting endangered species (see also **Figure 8.25**).

The symbionts harbored by parasites may also be variable among individuals. The wasp *Cotesia sesamiae* is considered to be a generalist parasitoid because it has a broad spectrum of infectivity for different insect species. However, the host species colonized depends on the particular variant of immunosuppressive polydnavirus carried by the wasp (see also Chapter 4). Different *C. sesamiae* wasps with different host preferences harbor distinctive polydnaviruses. The particular form of polydnavirus present is also influenced by symbiotic bacteria (*Wolbachia*) harbored by the wasps. Thus the variability in insect host species infectivity shown by the wasp is influenced by the version of the polydnavirus present, which is in turn influenced by the presence or absence of *Wolbachia*.

As a final comment on the diversity inherent within a parasite species, recent studies have brought to light the extent to which the parasites we

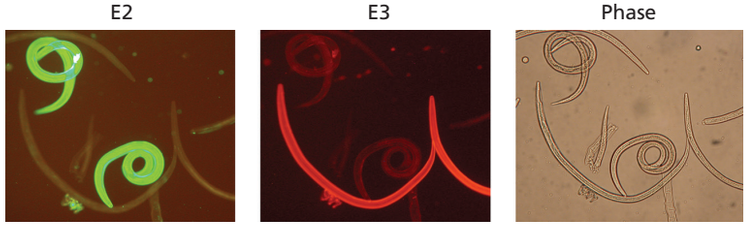

Figure 2.29 A demonstration of intraspecific variability on the surface of larvae of the nematode *Trichostrongylus colubriformis*. A sample of L3 larvae (all worms present are revealed by the phase contrast image at right) was stained with two different antibodies (E1 and E2), each labeled with a different fluorochrome (green or red), and each recognizing a different carbohydrate antigen. Note how different subsets of worms were stained by each antibody. (From Maass DR, Harrison GB, Warwick N et al. (2009) *PLoS Pathog* 5:e1000597, published under the Creative Commons Attribution license.)

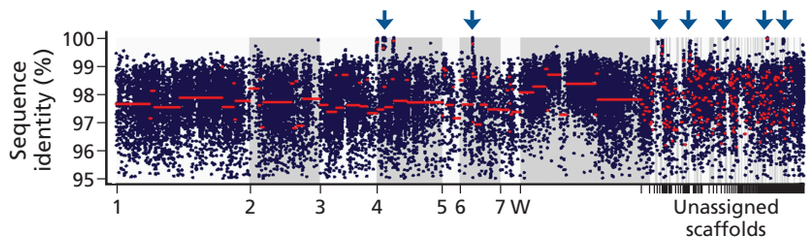

Figure 2.30 Interspecific hybridization between *Schistosoma bovis* and *S. haematobium* as shown by a whole-genome scan. Shown on the horizontal axis are alignable and identifiable positions for both species along the seven autosomal chromosomes, and sex chromosome (W), along with some scaffold regions that could not be assigned to particular chromosomes. The vertical axis indicates for the various regions the percentage of sequence identity (the purple dots) between the two species. Note that generally the two species are very similar (overall 97% sequence identity), but that in a few locations (arrows), they are >99% identical. These near-identical blocks encompass long stretches of DNA in some cases several hundred kilobases in length and are believed to represent areas where portions of the *S. bovis* genome have been acquired by *S. haematobium* by interspecific hybridization. (From Oey H et al. (2019) *PLoS Pathog* 15:e100751. Published under Creative Commons Attribution license.)

might normally recognize as a single species actually carry genetic information from two species; that is hybridization has occurred somewhere in its ancestry. **Hybridization** occurs when two species, generally considered to be related but distinct, nonetheless engage in genetic exchange and viable mixed progeny result. The resultant hybrids often show distinctive and troublesome traits, including greater fecundity and potential to cause pathogenicity, or have the ability to infect broader spectra of hosts than either parental species. The recent emergence of urogenital schistosomiasis in streams in Corsica, facilitated by the presence there of *Bulinus* snail hosts, came as a surprise, particularly so when it was discovered the introduced schistosome was a hybrid between ***S. haematobium***, the usual causative agent of urogenital schistosomiasis, and the closely-related ruminant schistosome, *Schistosoma bovis* (see **Figure 2.30** for evidence from genome studies of hybridization between these two species). The hybrid Corsican schistosome is better able to infect the snail host and causes greater pathology in experimental rodent hosts. Hybridization among schistosomes has been found to be common in West Africa, complicating not only species identification and attempts to sort out patterns of transmission but also raising the unsettling prospects that hybrids may possess traits that make ongoing control efforts more difficult.

Hybridization has been observed in other prominent parasites including ***Plasmodium***, ***Leishmania*** and ***Trypanosoma cruzi***. Our improved ability to discriminate genetic differences among parasites has highlighted a phenomenon that has turned out to be far more common than expected, and that may explain the sudden emergence of parasite outbreaks such as have occurred in Corsica. The ultimate repositories of genetic information and diversity lie within the genomes of organisms, the topic we consider next.

2.4 THE GENOMES OF PARASITES—A LARGELY UNTAPPED GOLDMINE OF DIVERSITY

BOX 2.4

Some Genome Basics

One of the most noteworthy features of the age in which we live, one to be long remembered, is that this is the time when genome sequences first became available. A **genome** is a complete set of genetic information, encoded in DNA (by RNA in RNA viruses), inherited from an organism's predecessors. The sequence of nucleotides (A, T, C and G) that comprise the entire genome is known as the **genomic sequence**. The study of genomes and their properties is referred to as **genomics**. The first genomic sequence of any kind was obtained for an RNA virus (Bacteriophage MS2) in 1976. The first complete genome sequence for any organism was for the bacterial pathogen *Haemophilus influenzae*, published in 1995. The nematode *Caenorhabditis elegans* was the first animal genome sequence obtained, in 1998. The sequencing of the draft human genome was reported in 2001, marking a momentous achievement in our basic understanding of life. Since that time, with ever-improving sequencing technology making the process faster and cheaper, a torrent of genome sequences have become available. Genomic sequences for organisms range in size from about 112,000 base pairs (or 11.2 kbp) for the intracellular bacterial symbiont of

insects, *Nasuia deltocephalinicola*, to 6.37 billion base pairs (6.37 Gbp) for the diploid female human genome, to the largest known genome, for a free-living single-celled freshwater ameba, *Polychaos dubium*, of 670 Gbp.

A genome typically includes genes arrayed on circular chromosomes in prokaryotes and linear chromosomes in eukaryotes. Additional important parts of the overall eukaryotic genome include the separate circular genome found within each mitochondrion, and for photosynthetic eukaryotes, the circular chromosome found within chloroplasts. The estimated 19,000–20,000 protein-encoding genes in humans comprise but 1.5% of the genome. Also present are non-coding DNA sequences like promoters and enhancers that play a role in regulating the transcriptional activity of genes, and the introns found in eukaryotic genes that separate the coding exon regions. Also present are non-protein-coding regions for making RNA like those for ribosomal structure, transfer RNAs or that produce a variety of small RNAs for various purposes, including microRNAs. Micro RNAs are small stretches of RNA of about 22 base pairs in length that bind to messenger RNAs and alter their translation into proteins (an example of post-transcriptional gene regulation). Also present are transposable or mobile genetic elements like transposons and retrotransposons, highly repetitive sequences, and sequences for which no function has yet been ascribed. It is not uncommon to find large proportions of the genome to be comprised of transposable elements (50% of the human genome for instance) that can propagate themselves along with the genome. Although most are silent and do not move, some can increase dramatically in representation, even copying themselves and moving into new locations in the genome. Although long considered "parasitic DNA," some transposons have been shown to play important roles in gene regulation and provide a source of genetic novelty by shuffling genetic material in new ways.

Significantly, about 4 million "switch" regions have been identified in the human genome. These play an important role in controlling gene expression or regulation in ways to enable humans to develop successfully and to allow differentiation of many different specialized cell types.

Lastly, it should be noted that the transcriptional activity of parts of the genome can be modified **epigenetically**, meaning that although the actual sequence of bases is not modified, the likelihood that particular genes are expressed can be altered by changing the physical structure of DNA or its histone protein packing material. At the DNA level, this usually occurs by the addition of a methyl group ($-CH_3$) to particular bases, which tends to silence the associated gene, or by modification of the histone proteins, the components of nucleosomes around which DNA strands are wrapped. Some epigenetic modifications enable the DNA to be more tightly wrapped, and in such regions termed **heterochromatin**, is less likely to be transcribed (it is "silenced"). In contrast, in changes that relax histone winding of DNA, leading to what is termed **euchromatin**, the associated genes are more likely to be expressed. Histones are modified by the addition of methyl groups or acetyl groups ($-COCH_3$). Epigenetic modifications are significant because they provide a means whereby the activity of genes in the genome can be influenced by exposure to environmental conditions, without changing the actual genomic sequence. Furthermore, some epigenetic changes can be preserved from one generation to the next.

Parasites have been an important part of the genome sequencing effort

Owing to their considerable impact on public health, there has been a strong push to sequence the genomes of pathogenic viruses and bacteria, as well as parasitic eukaryotes. Major rationales for having detailed knowledge of parasite genomes are to understand how they cause pathogenicity, to identify new potential targets that can be treated by drugs or that might form the basis of development of a new vaccine and to gain a greater appreciation for how parasites orchestrate their complex life cycles. The availability of genome sequences has provided an exciting new impetus to learn in greater depth all aspects of the secrets of parasitism. Among the first eukaryotic genomes to be sequenced was for the microsporidian parasite of mammals ***Encephalitozoon cuniculi*** in 2001, in part because its genome is very small for a eukaryote (2.9 Mbp). The genomes of the major human malaria parasite ***Plasmodium falciparum*** and its most important mosquito vector ***Anopheles gambiae*** were both published in 2002.

As of this writing, 590 genomes representing many familiar parasitic protists and fungi, and animal vectors of disease (like mosquitoes and snails) can be viewed and interrogated at the VEupathDB site (https://veupathdb.org/veupathdb/app). Additionally, 202 genomes representing over 163 mostly parasitic species with a few free-living species of parasitic flatworms and nematodes are available at the Wormbase site (https://parasite.wormbase.org). The basic properties of some helminth genomes are highlighted in **Table 2.2**.

Table 2.2 Some basic characteristics of prominent helminth genome sequences.

Species	Clade	Genome Size (Mb)	Number of Protein-Coding Genes	Source	Type of Parasitism
Nematodes					
Ascaris suum	Ascaridida III	273	18,542	WormBase	V
Brugia malayi	Spirurida III	96	11,515	WormBase	I/V
Bursaphelenchus xylophilus	Tylenchida IV	75	18,074	GeneDB	P
Caenorhabditis briggsae	Rhabditia V	108	19,507	WormBase	NA
Caenorhabditis elegans	Rhabditia V	100	19,099	WormBase	NA
Dirofilaria immitis	Spirurida III	84	10,179	WormBase	I/V
Globodera pallida	Tylenchida IV	125	16,419	WormBase	P/F
Haemonchus contortus	Rhabditia V	320	23,610	WormBase	V/F
Haemonchus contortus	Rhabditia V	370	21,799	Sanger FTP	V/F
Heterorhabditis bacteriophora	Rhabditia V	77	21,250	WormBase	I/F
Loa loa	Spirurida III	91	14,907	WormBase	I/V
Meloidogyne hapla	Tylenchida IV	54	14,420	WormBase	P/F
Meloidogyne incognita	Tylenchida IV	86	19,212	www6.inra.fr/meloidogyne_incognita	P/F
Necator americanus	Rhabditia V	244	19,151	GenBank	Bacterivore/V
Onchocerca volvulus	Spirurida III	NA	—	WormBase	I/V
Panagrellus redivivus	Rhabditia V	64	24,249	WormBase	NA
Pristionchus pacifus	Rhabditia V	169	29,201	WormBase	I/F
Romanomermis culicivorax	Dorylaimia II	323	48,171	nematodes.org/genomes/romanomermis_culicivorax/	I/F
Strongyloides ratti	Rhabditia V	—	—	WormBase	V/F
Trichinella spiralis	Dorylaimia II	64	15,808	WormBase	V/V
Trichuris muris	Dorylaimia II	85	11,004	GeneDB	V
Trichuris trichiura	Dorylaimia II	73	9,650	Sanger FTP	V
Flatworms					
Clonorchis sinensis	Trematoda	547	13,634	fluke.sysu.edu.cn	I/V
Clonorchis sinensis	Trematoda	516	16,258	NA	I/V
Echinococcus granulosus	Cestoda	152	11,325	GenBank	V/V
Echinococcus granulosus	Cestoda	115	10,231	GeneDB	V/V
Echinococcus multilocularis	Cestoda	115	10,345	GeneDB	V/V
Hymenolepsis microstoma	Cestoda	141	10,241	GeneDB	V/I
Schistosoma haematobium	Trematoda	385	13,073	schistodb.net	I/V
Schistosoma japonicum	Trematoda	397	13,469	GeneDB	I/V
Schistosoma mansoni	Trematoda	363	11,809	NA	I/V
Schistosoma mansoni	Trematoda	364	10,852	GeneDB	I/V
Schimidtea mediterranea	Turbellaria	902	29,850	McDonnell Gen Inst.	F
Taenia solium	Cestoda	122	12,490	GeneDB	V/V

The statistics are extracted from the genome papers, and may not correspond with the data utlilized, or statistics reported by other sources.
Systematic classification according to Blaxter et al. (1998) reported.
Type of parasitism: I, invertebrate host; V, vertebrate host; P, plant parasitic; F, free-living.

The evolution of compact genomes of reduced size is characteristic of some parasites

Given that parasites inhabit the bodies and even in some cases the individual cells of their hosts, it makes sense to think they would borrow heavily from their hosts and rely on them for provision of energy, and biochemical building blocks needed for their growth and reproduction. Also, many parasites are anatomically simplified, lacking some sensory structures like eyes, elaborate organs of locomotion, or even a gut in some cases.

It is thus reasonable to consider that parasite genomes might exhibit loss of many genes involved in the synthesis of compounds they can acquire directly from the host milieu or that are responsible for elaborating body structures that are not needed. Some genomes, like that of the microsporidian ***E. cuniculi***, an intracellular parasite of mammals, fulfill this expectation. The ***E. cuniculi*** genome is very small (2.9 Mbp) as eukaryotic genomes go, has relatively few protein-encoding genes (about 2,000) and it lacks genes for some biosynthetic pathways, such as those of the centrally important tricarboxylic acid cycle. It is an "energy parasite," reliant on its host cells for energy-rich compounds like ATP.

Signatures of genome reduction are also seen in parasitic flatworms like flukes and tapeworms (**Table 2.2**) which have lost the *de novo* capacity to make fatty acids, sterols, cholesterol, amino acids and purines, and auxiliary factors like co-factors and vitamins. Homeobox genes that are involved in laying down the patterning in body plans are extensively reduced in parasitic flatworms, from an expected number of about 96 as in other invertebrates to about 72 in flukes and 62 in tapeworms. Among the losses are genes involved in neural development (possibly including genes involved in sensory development) and in gut development (tapeworms lack a gut). Reduced genome size compared to free-living relatives has also been noted in apicomplexans and some parasitic amebas, fungi, nematodes and arthropods.

The ***E. cuniculi*** genome is also "compact" in the sense that intergenic regions are small, and some genes are truncated, such as those encoding enzymes involved in attaching particular amino acids to their appropriate transfer RNA. This leads to error-prone translation. Whether such imprecision is ultimately detrimental or advantageous to the parasite is not yet clear. Other forms of genome compaction include the presence of fewer introns and fewer mobile genetic elements and repetitive sequences. As already noted, several groups of parasites including ***Entamoeba***, ***Giardia***, ***Trichomonas***, some apicomplexans and microsporidians either lack mitochondria or retain highly reduced mitochondrial-derived mitosomes. Parasitic plants, like dodder *Cuscuta campestris* (**Figure 2.15**), often lack genes for metabolic pathways associated with high photosynthetic activity or nutrient uptake from the soil, instead relying on their host plant for the provision of such functions.

But parasite genomes also show several novel capabilities and gene family expansions

Lest we draw an erroneous conclusion that parasites might always be expected to have genomes of reduced size compared to free-living relatives, consider that the parasitic lifestyle offers many challenges to overcome, requiring distinctive genetic innovations. These include the need to locate a host, to penetrate and navigate through the host's body and to overcome host defenses and acquire nutrition. Many parasites have complex life cycles involving in some cases residence in multiple, often quite different host species living in distinct environments. Consequently, parasitism is not necessarily a one-way trip to genome reduction and reduced synthetic

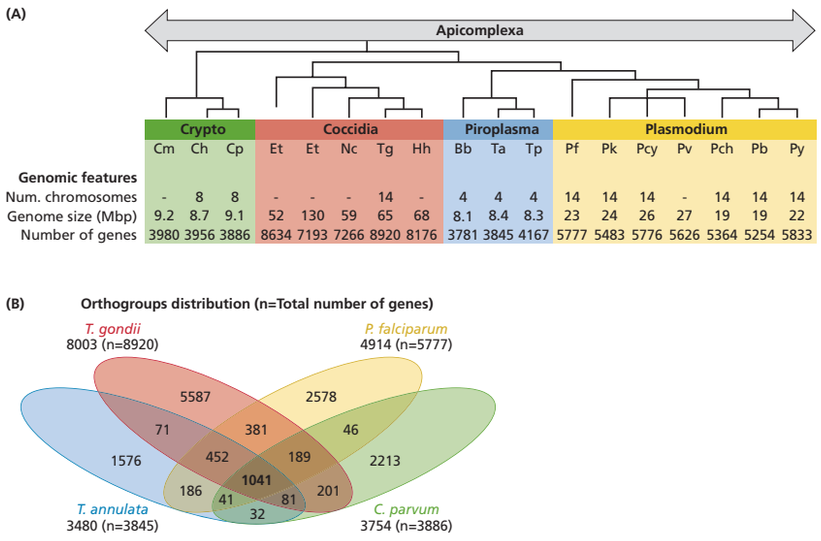

Figure 2.31 A comparative overview of some apicomplexan genomes. (A) A tree showing basic relationships among 18 different species (see code for names below), along with the number of chromosomes, genome size and the number of genes for each. Note the variation in genome size even within a parasite genus. (B) A Venn diagram showing for four representative groups the distribution of orthologous genes among them. Note that there are some genes in common to some or all groups and others are distinct for each group. The apicomplexan species listed are Cm, *Cryptosporidium muris*; Ch, *Cryptosporidium hominis*; Cp, *Cryptosporidium parvum*; Et, *Eimeria tenella*; Sn, *Sarcocystis neurona*; Nc, *Neospora caninum*; Tg, *Toxoplasma gondii*; Hh, *Hammondia hammondi*; Bb, *Babesia bovis*; Ta, *Theileria annulata*; Tp, *Theileria parva*; Pf, *Plasmodium falciparum*; Pk, *Plasmodium knowlesi*; Pcy, *Plasmodium cynomolgi*; Pv, *Plasmodium vivax*; Pch, *Plasmodium chabaudi*; Pb, *Plasmodium berghei*; Py, *Plasmodium yoelii*. (Modified from Lakshmipuram SS & Parkinson J (2017) *Crit Rev Biochem Mol Biol* 52:254–273. doi.10.1080/10409238.2017.1290043 with permission from Taylor & Francis and from Lakshmipuram Seshadri Swapna and John Parkinson.)

or biochemical versatility but can lead to impressive expansions of particular gene families. For instance, it is intriguing that not all microsporidian genomes have gone the reductive route of *E. cuniculi*. Some like *Ordospora colligata*, a parasite of water fleas (*Daphnia* sp.), has a genome of 24 Mb, almost ten times the size of the *E. cuniculi* genome. Another example of the extent to which genome properties vary is provided by an overview of some prominent apicomplexan genomes in **Figure 2.31**. Central to this discussion are **orthologous genes**, or orthologs, from different species that originate from a common ancestral gene found in the ancestor of those species.

Recent reviews have concluded that prominent parasite groups like trypanosomes, apicomplexans and helminths have acquired new genes through horizontal transfer and gene duplication. These processes seem to be favored and accelerated by the need to interact with a host that can fight back via its immune system and other defenses. The net result is that genome reduction is by no means the dominant theme. For instance, various protist parasites have the capacity to generate large numbers of surface molecules, which can be varied at different timepoints, stymieing any host immune response to specific parasite molecules. The phenomenon, known as antigenic variation, requires a large number of genes, each encoding a different membrane-associated molecule. ***Trypanosoma***, ***Trichomonas vaginalis***, ***Plasmodium falciparum and Giardia lamblia*** and others avail themselves of this immune evasion strategy, a topic that will be explored more fully in Chapter 4.

Helminths seem not to have elaborate mechanisms for antigenic variation but do have complex and resilient surface coats adorned with a variety of surface proteins encoded by expanded gene families. Surface glycoproteins may be bound by components of the host innate immune system like lectins, sending inhibitory signals in the process. Glycosyltransferase enzymes, expanded in both nematodes and tapeworms, can modify parasite surface glycoproteins, potentially altering immune recognition. Some surface molecules like tetraspanins exist in expanded gene families and are also of interest because they can be included in small extracellular vesicles produced by parasites that may be delivered to, enter and affect host cells.

The process of invading the host's body or cells also requires distinct genetic attributes (**Figure 2.32**). In apicomplexans, the process of invasion of a host cell involves hundreds of specialized proteins that are sequestered in the various components of the apical complex region. A common feature of parasite genomes is to have greatly expanded families of both proteases

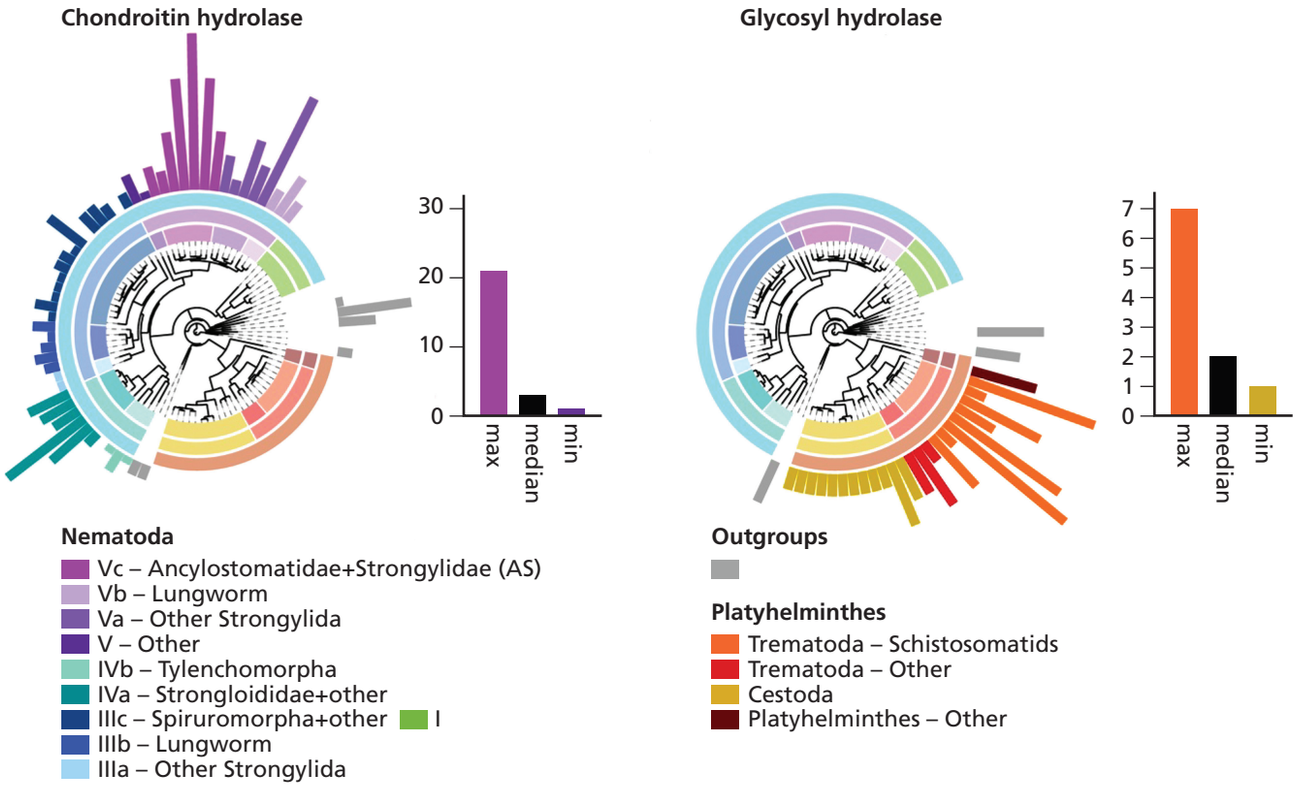

Figure 2.32 Two examples of expanded gene families of relevance to parasitic helminths, chondroitin hydrolase and glycosyl hydrolase. Chondroitin hydrolase and glycosyl hydrolase are involved in the breakdown of extracellular matrix components that provide structural integrity to tissues, so probably facilitate parasite passage through host tissues. The central part of each figure shows in a circular form a phylogenetic tree for the various parasites studied (see color key). Note for each tree that certain parasites have expanded representations of the gene families indicated, as shown by the height of the bars emanating from each circular image. For example, tissue-penetrating nematodes like hookworms (Ancylostomatidae and Strongylidae) have expanded families of chondroitin hydrolases, and schistosomes have expanded families of glycosyl hydrolases, the latter possibly to facilitate passage of their eggs through host tissues. (Modified from International Helminth Genomes Consortium & Day TA (2018) *Nat Genet* 51:163–174. doi:10.1038/s41588-018-0262-1. Published under Creative Commons Attribution 4.0 International License.)

and protease inhibitors. Proteases, also referred to as peptidases or proteinases, are enzymes that attack and cleave the peptide bonds between amino acids in proteins in various ways. At least seven major groups of proteases are known including serine proteases, metalloproteases and cysteine proteases, characterized by the particular chemical groups and peptide bonds they attack. They are commonly deployed by parasites to facilitate passage through host tissues, to digest ingested host proteins including those found in blood, and to prevent coagulation of blood around them. Cathepsin B C1-cysteine proteases are highly expressed in blood-feeding nematodes and in flukes like ***Schistosoma*** and ***Fasciola***. No single family of proteases is emphasized in all parasites, although an M8 metalloprotease is repeatedly found as a major surface protease. Different parasites, even those closely related, show expansions of different protease families. Switching to new hosts and exposure to novel substrates followed by gene duplication have been considered drivers of the expansion of protease families. Hosts often produce proteases to attack parasite surfaces so it is not surprising that parasites produce expanded families of protease inhibitors to prevent their bodies from being digested, or to modulate host immune responses. For example, trypsin inhibitors are abundant in nematodes and flukes

Not surprisingly given our persistent attempts to eliminate parasites by drug treatments, helminths often have expanded families of glycoprotein

transporters relative to vertebrates. These molecules can pump toxins (including drugs) from the parasite's body. Intriguingly, though we tend to think of parasites in light of them being a challenge to our immune systems, they too have to contend with an invasion of their bodies by other infectious agents and show expression of some gene families known from other invertebrates to be involved with innate immune responses.

Genome studies have revealed horizontal gene transfer (HGT) to be a frequent feature of parasite genomes

It has been argued that eukaryotes are largely refractory to HGT because they often maintain separate somatic and germinal cells, with the latter more difficult for foreign DNA to invade. Yet a growing body of evidence, in many cases coming from the study of parasite genomes, suggests there are numerous examples of HGT involving eukaryotes. Bacteria and viruses are likely sources of genes for eukaryotic parasites because they are ubiquitous, metabolically very diverse, and often ingested by eukaryotes. Mobile genetic elements like transposons (what has been called the **mobilome**) are also another source of genetic information for eukaryotic species. The genomes of ***Trypanosoma***, ***Trichomonas***, ***Entamoeba*** and microsporidians show evidence of repeated and considerable HGT from prokaryotes and viruses, especially those sharing environments with them.

Although the percentage of a eukaryotic parasite's genes acquired by HGT is low, less than 2.5% for parasitic protists and lower for multicellular parasites, the genes acquired are often key to parasite success. For instance, microsporidians have lost their capacity to make ATP but have acquired from bacteria genes encoding nucleotide transport proteins that can be used to import purine nucleotides. The stramenopile *Blastocystis* has acquired 2.5% of its genes by HGT, enabling it to live in the gut, evade host defenses and influence the activity of bacteria in the gut environment. For ***T. brucei***, their ornithine decarboxylase genes (essential for cell growth) are most similar to those of vertebrates, implying they have been acquired from their hosts. There is even some indication of retrotransposon-mediated transfer of genetic information between birds and their filarial nematode parasites.

Another example of HGT involving two distantly related eukaryotic kingdoms is the transfer of multiple genes from fungi to oomycetes (**Figure 2.33**). Oomycetes (see stramenopiles) comprise a distinct lineage of fungus-like microorganisms that are often called water molds, even though most species infect terrestrial plants. Unlike fungi with cell walls made of chitin, the cell walls of oomycetes are made of cellulose. Like fungi, they produce filamentous structures for the absorption of nutrients. HGT is believed to have involved genes giving oomycetes the ability to take up soluble nutrients, a phenomenon called osmotrophy, and to resist host immune responses, considered essential for the ability of oomycetes to parasitize plants. One oomycete benefiting from HGT is *P. infestans*, the organism responsible for potato blight that caused the Great Potato Famine in Ireland from 1845 to 1852. A million Irish citizens died during the famine and another million emigrated from Ireland. Potato blight is still a formidable problem today.

The obligatory plant parasites *Orobanche minor* and *Aeginetia indica* have both acquired genes from host plants. Furthermore, some of the acquired genes occur in the same positions relative to one another and have the same gene structure as in their hosts, suggestive of the acquisition of large pieces of host DNA up to 100 kbp long by the parasites. It also appears these genes are undergoing higher than average rates of base changes

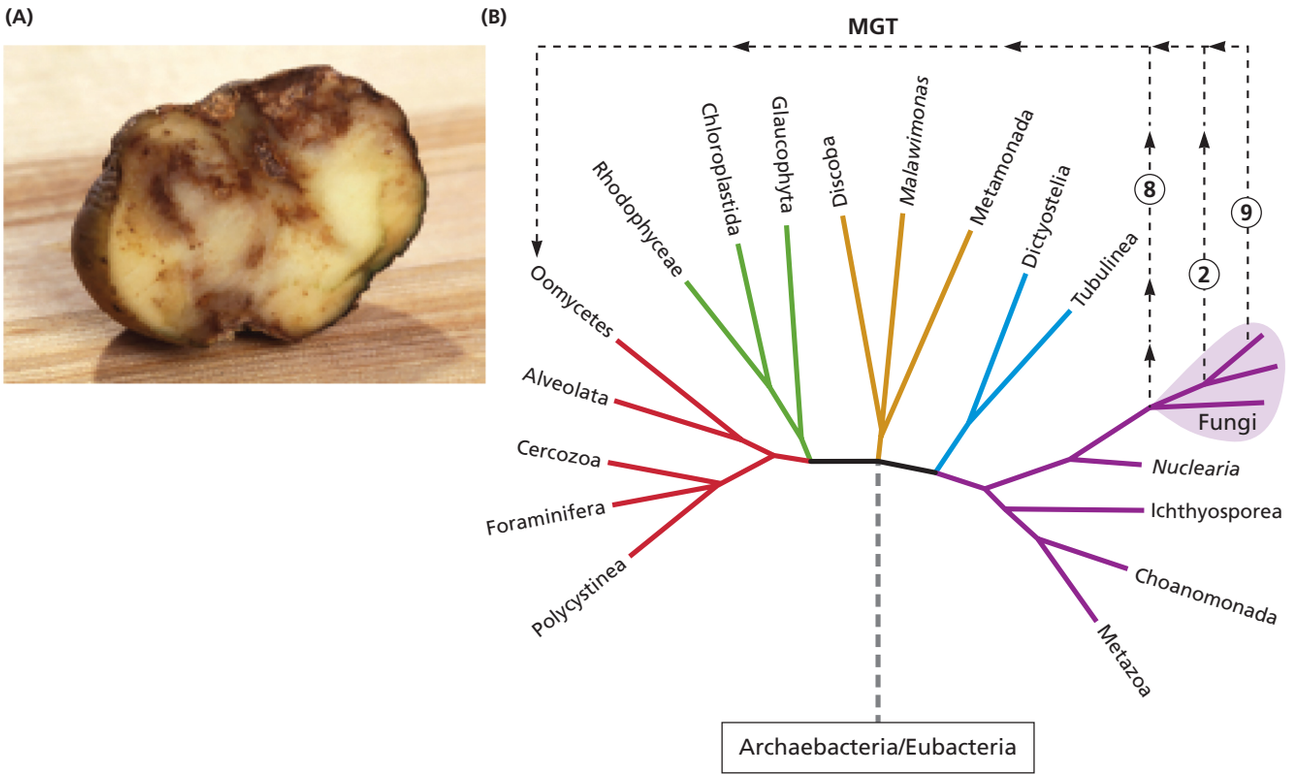

Figure 2.33 ***Phytophthora infestans,*** **an oomycete responsible for the infamous late potato blight, has engaged in horizontal gene transfer (HGT)**. (A) Note the shrunken appearance of the infected potato and its rotten interior. (B) Shown on this tree are eight, two and nine different instances in which genetic material was likely transferred by HGT (dashed lines) from fungi at different points in their diversification to members of the oomycete (including *Phytophthora)* lineage. (B, From Richards TA et al. (2011) *PNAS* 108:15258. With permission from PNAS.)

suggesting they are being rapidly re-purposed within the environment of the parasite genome. This is just one example of how dynamic parasite genomes can be, the topic we next discuss.

Why would a wheat farmer care about horizontal gene transfer? Read more at: https://science.sciencemag.org/content/368/6493/eaba5435

Parasite genomes are dynamic and changeable

Given that parasites must cope with hosts that can dramatically modify and improve their anti-parasite responses over time, it is hardly surprising that parasites too have mechanisms to respond to their hosts. Antigenic variation is one way, and this is often facilitated by tandem arrays of related but different genes encoding surface proteins, often located near telomeres (ends of chromosomes). Telomeric locations facilitate both a specific means to regulate the expression of individual genes in the arrays and favor the generation of the arrays in the first place, through recombination and gene duplication. In cases where such gene families are not located near telomeres, the sequences in which they are embedded may show telomere-like repeats thought to favor recombination and thus generation of diversity. The expression of different individual genes within such clusters provides an important way to counter the adaptive immune responses of their hosts.

As more genomes for a particular parasite species are acquired, we are learning that there is considerable variability in size and content among them. Genome-based studies of ***Leishmania*** have shown that even closely related strains exhibit differences in the numbers of their chromosomes, resulting in variations in the numbers of copies of particular genes. These include genes important in infectivity, including those encoding the metalloprotease

GP63, known to attack host immune and cell signaling pathways, and amastins, which help anchor the intracellular amastigote stages of this parasite to the wall of the parasitophorous vacuole in which they reside in host macrophages. Novel mechanisms whereby ***Leishmania*** can amplify telomeric and subtelomeric regions of particular chromosomes have also been proposed. Similar fluctuations in the number of copies of critical genes have been noted for *Blastocystis* and its genes acquired by HGT. So, providing an archetypical genome for any parasite species must be accompanied by a realistic assessment that other individuals of the same species may differ quite substantially in genome content. By sequencing more genomes, we surely will gain greater insights into the functional significance of these variations. In addition, we are rapidly learning that the activity of particular genes can be modified through other means, the topic of our next section.

The study of epigenetic modifications of parasite genomes is in its infancy but likely to be transformative

The ability of parasites to alter the likelihood of expression of particular genes by epigenetic changes of either DNA directly, or of the histones wrapping the DNA (see **Box 2.4**, **Figure 2.34**), is increasingly recognized as playing an important role in orchestrating the many changes occurring in gene expression throughout a complex life cycle. We are only just beginning to glimpse the important role of epigenetic "marks" in parasite genomes and how they might be exploited to limit virulence or as new targets for therapy. Among apicomplexans and other protist parasites, DNA methylation does not as yet appear to be a commonly used epigenetic tactic, but prominent roles for histone modifications have been found. Expression of specific members of the *var* multicopy gene family in ***P. falciparum*** represents a system that has been relatively well studied. As discussed in Chapters 4, 5, and 10, proteins encoded by *var* genes play an important role in immune evasion and in the pathogenicity of ***P. falciparum***. Inactive *var* genes are associated with methylated versions of histone H3 that favor chromatin condensation, transfer to the periphery of the nucleus, and transcriptional silencing. An actively transcribed *var* gene is associated with a distinctive acetylated version of histone H3, which promotes uncoiling of chromatin

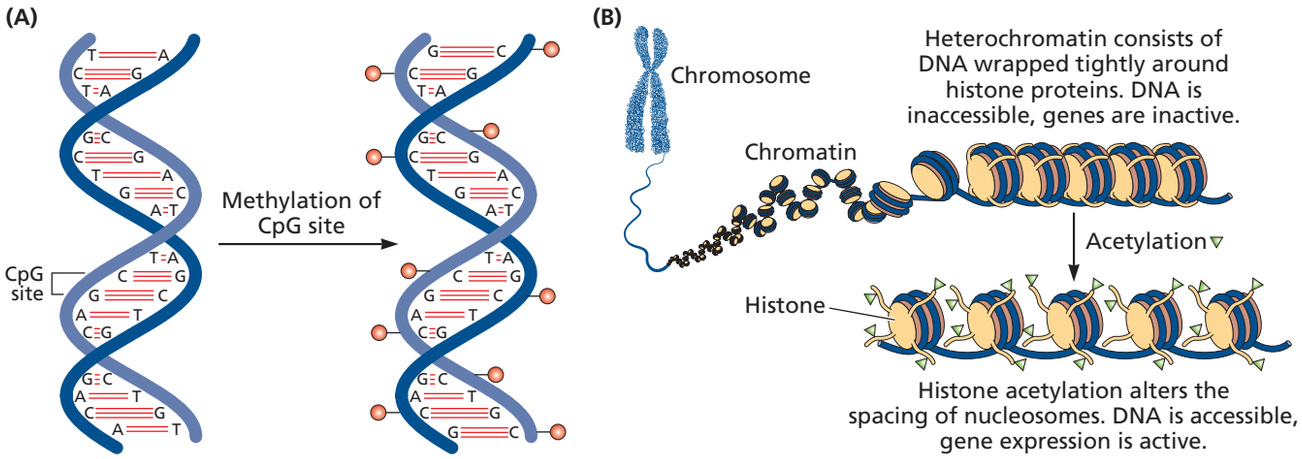

Figure 2.34 Some epigenomics basics. (A) DNA can be epigenetically modified directly by the addition of methyl groups at sites where a cytosine (C) is followed by a guanine (G), suppressing transcription of the affected gene. (B) Histone complexes used to package DNA can also be epigenetically modified by the addition of acetyl groups, resulting in less tight packaging with the associated genes more likely to be transcribed. (A, From Koch MW, Metz LM & Kovalchuk O (2013) *Nat Rev Neurol* 9:35–43, with permission from Springer Nature.)

and gene expression. Many mysteries remain, including how histone modifications required for transcriptional activation or inactivation of particular *var* genes are achieved, and how malaria parasites distinguish *var* from other genes.

Another important example of epigenetic modification controlling a key step in a parasite's life cycle is provided by ***Giardia***, for which late stages in cyst formation appear to be regulated by histone modifications. In ***Leishmania*** and other kinetoplastids, particular thymine residues in DNA in telomeric repeat regions are modified by the addition of glycosyl groups. These so-called "Base J" signals are often associated with the repression of transcription. ***Leishmania*** parasites also engage in histone modification to affect gene expression and as one might expect, patterns of modification are quite different in sandfly-inhabiting promastigote stages vs. amastigote stages found in mammalian macrophages.

One of the most remarkable features of epigenetic modification in ***Leishmania*** relates to the extent to which these parasites can epigenetically modify the expression of *host* genes. For instance, infections of ***L. donovani*** have been shown to result in methylation and inactivation of innate immune genes in host macrophages thereby favoring intramacrophage survival of the parasites. An exciting area of growth for parasitology research in the future is to more fully unravel the puzzling process of how the expression of host defense-related genes can be modified for the parasite's benefit by signals directly or indirectly affecting epigenetic changes in host genomes.

What parasite genomes have yet to tell us

Having a genome sequence is one thing; fully understanding how it works is quite another. For one thing, most parasite genomes contain many, and perhaps even a majority, of genes for which the function is unknown. Although tools like CRISPR (discussed at several points in the book) are actively being developed to dissect the functional roles and phenotypic impacts of individual genes, full functional characterization of parasite genes remains a daunting task. Connecting particular networks of genes to the emergent properties of host-parasite interactions like host specificity, manipulation of host behaviors, and orchestration of complex life cycles largely remains elusive, although progress is being made.

For instance, the power of sequencing techniques like RNA-Seq allows one to rapidly profile the mRNA molecules being made within a biological sample (referred to as the **transcriptome**), thereby gaining a picture of what genes are being expressed. Many studies have now examined the transcriptome in different parasite life cycle stages, different tissues or organs within a parasite like a helminth, or even from single parasite cells. All provide distinctive insights into how the genome is used. **Figure 2.35** is of a "heat map" that measures for a long list of parasite genes the extent to which each is expressed in different life cycle stages of ***S. mansoni***. Note the distinctive patterns of gene expression for the six different life cycle stages shown. Increasingly detailed transcriptional profiles of individual organs of ***S. mansoni*** are available highlighting, for example, the different suites of genes produced in testes of male, or ovaries of female worms, including from worms that are paired or not. As another example, studies of different life cycle stages of the gut-inhabiting apicomplexan ***Cryptosporidium*** (sporozoites, epicellular forms and oocysts) reveal that the epicellular/intracellular stages of the parasite produce many more transcripts than found in oocysts, many of which are related to active biosynthesis for these rapidly growing stages.

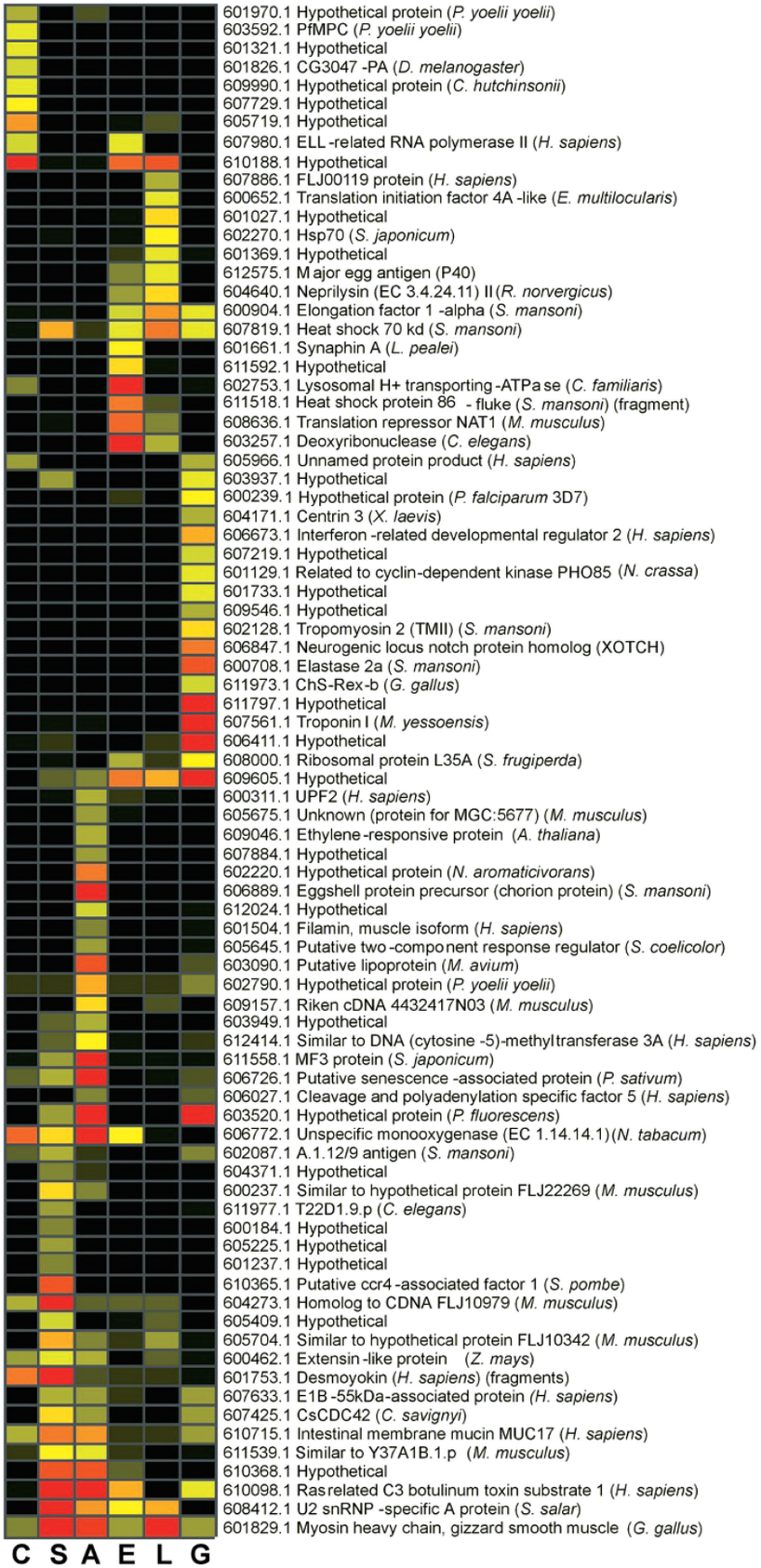

Figure 2.35 A heat map for different life cycle stages of *Schistosoma mansoni*. (C, cercaria; S, schistosomulae; A, adults; E, eggs; L, miracidia; G, germ balls from intra-molluscan stages). For each of the transcripts listed, shown is its relative frequency, with black representing no counts (cool) and red indicating the most (hot). Note how each different life cycle stage indicates different suites of transcripts with relatively high expression levels. (Modified from Verjovski-Almeida S et al. (2003) *Nat Genet* 35:148–157. With permission from Springer Nature.)

Single-cell transcriptomics offers the prospect of unparalleled precision and lack of bias in determining how a parasite uses its genome. Imagine too that for parasites like ***Cryptosporidium*** or ***T. gondii*** that live within host cells, the capture of a single infected host cell enables one to simultaneously and precisely profile both the parasite and host transcriptomes. By comparing samples taken from different points in the infection cycle or different host cell types, exquisitely detailed portraits of genome usage will emerge, bringing us closer to understanding the how and why of parasite genomes.

Also, by comparing multiple genomes from related parasites and searching for differences among them, we can begin to understand what makes each parasite species distinctive, allowing us to glimpse how knowledge of genomes leads to ever deeper insights into the specific mechanisms underpinning host-parasite interactions. For instance, let's consider once again the question of the origin of the human parasite ***P. falciparum*** from related parasites found in gorillas. Sequences obtained for several related ***Plasmodium*** species infecting our great ape cousins are remarkably similar, but some diverging genes have been identified. One such gene *rh5*, encodes a protein involved in attaching malaria parasites to a protein called basigin on the surface of human erythrocytes (**Figure 2.36**), an essential feature in infectivity. By comparing *rh5* sequences across ***Plasmodium*** species from different apes, it was possible to infer an ancestral state for this gene, one that encoded a version of the protein able to bind both human and gorilla erythrocytes. By systematically mutating bases at six key sites differentiating the ancestral and human forms of *rh5*, it was possible to identify a single amino acid mutation (H200Y) that was responsible for the production of a version of the RH5 protein able to bind human but not gorilla erythrocytes. Thus, scanning entire genomes, each of approximately 23Mbp, helped locate key genomic differences that pinpointed a particular gene, a single mutation of which can help account for the emergence of one of the deadliest infectious diseases of humanity, ***P. falciparum***.

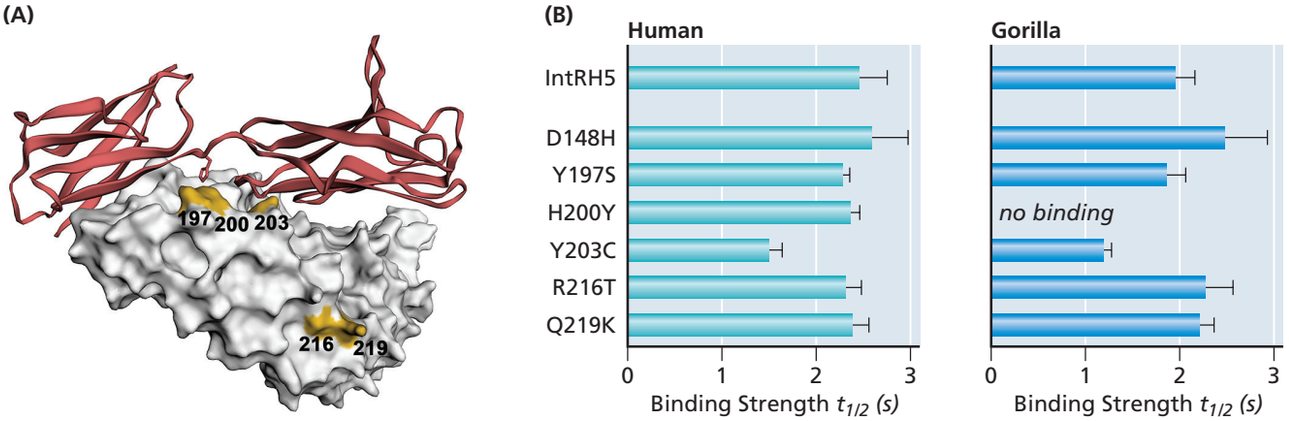

Figure 2.36 Interaction of proteins encoded by different mutated versions of the *rh5* gene of *Plasmodium falciparum* with human and gorilla erythrocytes. (A) A protein corresponding to a hypothetical ancestral form of the RH5 protein (the gray shape) showing positions of various mutations (in yellow) that differentiate it from the version of RH5 in human-infecting *P. falciparum*. The red ribbon indicates the basigin receptor protein on host erythrocytes. Note that one of these mutations at position 200 (H200Y) affects RH5 in a way (B) that it can still bind erythrocytes from humans, whereas binding to gorilla erythrocytes does not occur. This difference provides a way to account for the emergence of *P. falciparum* specifically in humans. (From Galaway F et al. (2019) *PLoS Biol* 17:e3000490. doi:10.1371/journal.pbio.3000490. Published under the Creative Commons Attribution license.)

SUMMARY

An enormous diversity of parasites inhabits our world, and there is an ongoing quest to reveal the full extent of this diversity. The unit of diversity studies is often the species and although a conventional species definition (populations of similar and interbreeding individuals) has been used for parasites, we have seen that this definition often does not describe many parasite groups in which sexual reproduction is cryptic, rare, or absent. It would be very satisfying to say with confidence how many parasite species are present on Earth, but the answer is still elusive as some geographic areas, habitat types, and groups of host species remain poorly sampled. We are getting closer to an answer for some parasites, such as the number of helminth species that infect vertebrates, but we are still in the dark for many other groups. Despite these uncertainties, a strong case can be made for the existence of more species of parasites than species of free-living organisms.

Phylogenetic trees provide a compelling way to order our thinking about parasite diversity. Although such trees are designed to trace the vertical patterns of inheritance from ancestors to progeny, we have also seen that HGT has undoubtedly been a factor in parasite evolution and may well prove in time to be even more important than we currently realize.

Among the unicellular eukaryotes are several prominent parasite groups that show peculiar modifications to their parasitic lifestyles, and none are more prominent than the apicomplexans, some of which cause important diseases such as malaria or toxoplasmosis. The apicomplexans harbor a huge, still largely unrevealed reservoir of diversity. Many multicellular eukaryote groups include parasites, such as the red algae, which engage in surprising and insidious strategies of infection, and plants, which have frequently adopted parasitism, even to the extent of losing their ability to engage in photosynthesis. The Fungi comprise a huge group of poorly characterized organisms, many of which are parasitic in plants or animals; fungi are also increasingly implicated in causing emerging diseases. Parasitic animals are relatively well known for their effect on the health of plants, animals and people and it is interesting to note that parasitism has arisen many different times among animals. We have also seen that sometimes parasites give rise to free-living organisms, though this seems to be comparatively uncommon.

Studies of parasite diversity have helped clarify many (but not yet all) enigmatic relationships among disparate organisms that have long mystified biologists. The origins and biogeographic distributions of human-infective parasites have been revealed by modern diversity studies, which have also helped to provide convenient benchmarks (such as DNA barcodes) to categorize species. Modern molecular studies have in many cases revealed that what we once thought was a single parasite species is in fact a complex of species and have enabled biologists to identify entire new lineages of parasites that were previously missed. Metagenomics approaches have also revealed how surprisingly common and diverse some parasite groups are. Modern studies of parasite diversity have also provided fresh insights into the considerable and biologically relevant diversity that is inherent within a single parasite species. We are learning that processes like hybridization further augment intraspecific diversity, in some cases blurring the boundaries between parasite species.

Lastly, we explore the topic of parasite genomes, the complete DNA sequences found in a particular species. We note that parasitic organisms have been a frequent target for genome sequencing studies, and parasite genomes have proven to be fascinating and novel in several ways. Although the reduction in size is a common feature of parasite genomes, many also show remarkable expansions of particular gene families involved in achieving infectivity

or evading host immunity. Parasite genomes also prove to be dynamic and changeable and frequently show evidence of acquisition of novel genetic capacities by HGT. The study of parasite genomes has identified new targets for drug or vaccine development, provides distinctive viewpoints on how parasites have evolved and has enabled a huge variety of follow-up studies documenting how individual life cycle stages or even individual parasite cells employ different suites of genes. But we have yet to achieve a full understanding of all that parasite genomes have to teach us, particularly regarding complex, emergent phenomena like host specificity or immune evasion.

REVIEW QUESTIONS

1. Why are studies of parasite diversity important and useful?

2. The biological species concept is difficult to apply to some parasite groups. Which ones and why?

3. How many species of parasites are there, and what groups or factors make the answer particularly hard to quantify?

4. How do the patterns of inheritance implied by Darwin's tree of life compare to those emerging from the concept of horizontal gene transfer, and how are parasites affected?

5. Many of the parasitic protists have unusual cell biology that features peculiar organelles. Provide some examples.

6. Why do you suppose there have been many origins of parasitic species from free-living ancestors, but relatively few examples of parasites evolving to become free-living?

7. What are metagenomics studies? Give some examples where they are relevant to the study of parasites in general.

8. What is a monophyletic group and how does the process of homoplasy obscure relationships?

9. Differentiate between an isolate, a strain, and a subspecies. Use the example of *Trypanosoma brucei* to show why intraspecific diversity matters.

10. Why is it that we are just now learning the extent to which hybridization has occurred among parasite species. Why is hybridization important?

11. Parasites have been a popular subject for genome studies. Why might this be? Why does it make sense that parasite genomes often show reduction and/or compaction in comparison to free-living relatives? Why though, do parasite genomes often show dramatic expansions of particular families of genes? What are these genes used for?

12. Can you provide an example of how comparative genomics studies help to provide distinctive insights into the origins of human parasitism?

13. Check out the websites listed that manage and curate genomic information for parasites and explore them for the amazing amount of content they contain.

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THE DIVERSITY OF PARASITE SPECIES

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