Parabasalia

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Abstract

The Parabasalia are a clade of single-celled, anaerobic flagellates that are mainly obligate symbionts or parasites of insects and vertebrates. The group includes the common and widespread human sexually transmitted species *Trichomonas vaginalis*. Many species are found exclusively in the guts of termites and the wood-feeding roach *Cryptocercus*, where they contribute to wood digestion as part of a complex microbial community that sustains the insects. These insect symbionts often harbor an extensive and diverse assortment of ecto- and endosymbionts. The Parabasalia are characterized by a parabasal body (Golgi complex supported by a parabasal fiber), which is associated with the flagellar apparatus. Their mitochondria have evolved into hydrogenosomes, double-membrane-bounded organelles that derive energy from the breakdown of pyruvate to acetate, CO₂, and H₂. They vary in size from the minute *Tricercomitus*, which is only a few microns long, to the half-a-millimeter-long *Mastotermes* gut symbiont *Mixotricha paradoxa*. Historically, the Parabasalia have been treated as two groups: the smaller, simpler “trichomonads” which bear up to six flagella and the typically much larger, multiflagellate “hypermastigotes.” Ultrastructural and molecular evidence have shown that together these groups form a monophyletic Parabasalia, and though neither “trichomonads” nor “hypermastigotes” are monophyletic, they continue to be useful as descriptive terms.

Keywords

Anaerobic protists • Bacterial symbionts • Hydrogenosomes • Hypermastigotes • Karyomastigont • Parabasal body • Parasitic protozoa • Termite gut flagellates • *Trichomonas* • *Trichonympha*

Summary Classification

- Parabasalia
  - *Trichomonadida*
    - *Trichomonadidae*
    - Honigbergiellida
    - Honigbergiellidae
    - Hexamastigidae
    - Tricercomitidae
    - Tritrichomonadida
    - Tritrichomonadidae
    - Dientamoebidae
    - Monocercomonadidae
    - Simplicimonadidae
    - Hypotrichomonadida
    - Hypotrichomonadidae
    - Cristamonadida
Introduction

General Characteristics

Parabasalia is a clade of anaerobic protists, almost all of which are flagellates. Parabasalians are characterized by the presence of a parabasal body (a densely packed Golgi complex that is associated with striated fibers connected to the basal bodies), hydrogenosomes (anaerobic derivatives of mitochondria), closed pleuromitosis with an extranuclear mitotic spindle, and a particular arrangement of microtubular and non-microtubular elements of the mastigont, notably the axostyle and pelta (see below). Although belonging to the Excavata, parabasalians have lost the typical excavate features, particularly the ventral feeding groove and cytostome. Many species possess a characteristic undulating membrane formed by a recurrent flagellum and cytoplasmic projection. Most parabasalians are uninucleate, but multinucleate cells have evolved in some lineages. Although the parabasalians are not particularly species rich, including approximately 450 described species in 100 genera, they display immense variability in cell complexity and in the number of flagella, which ranges from zero to several thousands. Accordingly, Parabasalia has been historically divided into two assemblages, the trichomonads with up to six flagella per mastigont and usually simpler cells and the hypermastigotes, which can possess thousands of flagella and extraordinarily complex cells. However, it has been shown that an increase in the number of flagella has evolved several times independently from the trichomonad-like cells. The current taxonomy of the Parabasalia consists of six classes that better reflect the evolution of this group. Nonetheless, “trichomonad” and “hypermastigote” are still routinely used as terms of convenience to differentiate simpler cell types from multiflagellate forms.

Occurrence

Almost all parabasalians are symbionts of the digestive tracts of animals, both invertebrates and vertebrates, including humans. Much of the known diversity of
Parabasalia occurs in the guts of termites and their sister lineage, the wood-feeding roach *Cryptocercus*. Parabasalians belonging to the orders Trichonymphida, Spirotrichonymphida, and Cristamonadida are found nowhere else. These parabasalians form obligate, vertically inherited symbioses with the insect families Archotermopsidae, Hodontermitidae, Kalotermitidae, Mastotermitidae, Rhinotermitidae, Serritermitidae, Stolotermitidae, and Styloptermitidae of the infraorder Isoptera, collectively referred to as the lower termites, and *Cryptocercus*, the sole extant genus of infraorder Cryptocercoidea (Krishna et al. 2013). By contrast, termites from the most speciose family, Termitidae, only sporadically harbor small parabasalians such as *Trichomonas* and *Monocercomonas* and never hypermastigotes (Kirby 1937). While the lower termites are found on all continents except Antarctica, *Cryptocercus* has only been reported from the northern hemisphere, in certain mountainous regions of China, Korea, Russia, and the USA.

Besides termites, a number of trichomonad species have been described from other insects, such as cockroaches, crane flies, beetles, and true bugs, and some other invertebrates such as horse leeches and snails (Alexeieff 1911; Mackinnon 1913; Grassé 1926; Bishop 1932; Kozloff 1945; Brugerolle et al. 2003; Zhang 2003; Smejkalová et al. 2014), though nothing is known about their importance for their hosts. Trichomonads of vertebrates are mostly harmless intestinal commensals and can be found in diverse birds, fish, amphibians, reptiles, and mammals (e.g., Alexeieff 1910; Honigberg 1978; Cepicka et al. 2005, 2006; Smejkalová et al. 2012). The best-known parabasalians are the few human and livestock parasites that have escaped the lower intestinal tract and live in the genitourinary, upper digestive, or respiratory tracts: *Trichomonas vaginalis*, *Trichomonas gallinae*, *Trichomonas foetus*, and *Histomonas meleagridis*.

Although most parabasalian species are host-associated, a few free-living ones have been described as well, for example, *Monotrichomonas carabina*, *Ditrichomonas honigbergii*, *Pseudotrichomonas keilini*, and *Lacusteria cypriaca* (Bishop 1935, 1939; Farmer 1993; Bernard et al. 2000; Yubuki et al. 2010). They inhabit freshwater, brackish, and marine anoxic/microoxic sediments and have been found worldwide.

**Literature and History of Knowledge**

The first parabasalians to be described were trichomonads associated with humans and animals. The first was *Trichomonas vaginalis* from a human vaginal tract (Donné 1836), followed by three more species of *Trichomonas*, each now known by different names: *Tettratrichomonas limacis* from slugs (Dujardin 1841), *Tri-trichomonas suis* from pig intestines (Gruby and Delafond 1843), and *Trichomitus batrachorum* from frogs (Perty 1852). Another early description was of *Pentatri-chomonas hominis* from human intestines, originally named *Cercomonas hominis* (Davaine 1854, 1860). The first multi-flagellated species (hypermastigotes) were described not long after: *Lophomonas blattarum* (Stein 1860), from the hindgut of a common cockroach, and *Trichonympha agilis* (Leidy 1877) from the hindgut of a
termite. Leidy wrote that the multflagellate swimming cell he called *Trichonympha* reminded him of “nymphs in a recent spectacular drama, in which they appeared with their nakedness barely concealed by long cords suspended from the shoulders” (Leidy 1877), hence the origin of the -nympha suffix that proliferated through the nomenclature of hypermastigotes. Toward the end of the nineteenth century, more researchers began to study the protist hindgut community of termites, using only light microscopy and relatively unsophisticated staining techniques. The parabasalians, like the ciliates, proved accessible to iron hematoxylin and then protargol staining, however, revealing a wealth of taxonomically useful morphological characters. Many new genera of parabasalians from various hosts, both trichomonads and hypermastigotes, were described, and a classification system with elements recognizable in our current classification was in place by the early twentieth century (Grassi and Foà 1911).

The order Trichomonadida (corresponding roughly to the “trichomonad” assemblage) was created by Kirby (1947). His system of families and genera was revised by Honigberg (1963). Honigberg’s view on the evolution of the trichomonads was largely supported by electron microscopy (Brugerolle 1976), and his version of the trichomonad system survived to the beginning of the twenty-first century with some minor modifications. Then, it was gradually replaced by the contemporary system present in this chapter (see below), which is based both on morphology, including ultrastructure, and results of molecular phylogenetic studies.

The concept of hypermastigotes is even older than that of trichomonads. An affinity between the multflagellate *Lophomonas* and *Trichonympha* was first proposed after the discovery of *Joenia*, another termite hindgut protozoan that appeared morphologically intermediate between *Lophomonas* and *Trichonympha* (Grassi 1885). This led to the creation of the order Hypermastigida for multflagellate forms (Grassi and Foà 1911). Although much of the diversity of both trichomonads and hypermastigotes was described early in the twentieth century, it was still many years before their relatedness was understood. Hypermastigotes were initially thought to be ciliates, or intermediate between ciliates and gregarines (Leidy 1881; Kent 1882). Similarities between hypermastigotes and flagellates were soon recognized, however, and support for this view grew (Stein 1878; Kofoid and Swezy 1919; Cleveland 1923; Kirby 1947; Grassé 1952). With the advent of electron microscopy, ultrastructural studies began to reveal strong evidence that trichomonads and hypermastigotes were specifically related (Hollande and Valentin 1969a; Hollande and Carruette-Valentin 1971; Hollande and Carruette-Valentin 1972; Tamm and Tamm 1973). The superorder Parabasalia was proposed in 1973 to formally unite these two groups (Honigberg 1973). Molecular phylogenetic studies have confirmed the monophyly of Parabasalia but have also shown that neither trichomonads nor hypermastigotes are monophyletic, and at times their results have conflicted directly with morphology-based scenarios of parabasalian evolution (see Evolutionary History section).

No treatise of the Parabasalia exists, and even the economically important species have not been reviewed in depth for nearly 20 years. The most recent books reviewing these species are *Trichomonads Parasitic in Humans* (Honigberg 1990)
and *Parasitic Protozoa* (Kreier 1991). An excellent genus-level descriptive key of the group by Brugerolle and Lee (2000) can be found in *An Illustrated Guide to the Protozoa* (Lee et al. 2000). Several chapters describing the biology and evolution of termite hindgut parabasalians can be found in *Intestinal Microorganisms of Termites and Other Invertebrates* (König and Varma 2006) and *Biology of Termites: A Modern Synthesis* (Bignell et al. 2011). The American Museum of Natural History houses the extensive microscope slide collections of Harold Kirby and Lemuel Roscoe Cleveland, two of the most prolific investigators of termite and *Cryptocercus* hindgut parabasalians. A set of 35 mm films made by Cleveland is also housed there.

**Practical Importance**

Parabasalians have evolved as symbionts of the animal digestive tract. Intestinal parabasalids generally cause little or no harm to their hosts (BonDurant and Honigberg 1994), with some pertinent possible exceptions (e.g., see below). A few species have moved to other areas of the body, where they are parasites. Humans are infected by several species, for example, *Trichomonas vaginalis* in the urogenital tract, *Trichomonas tenax* in the oral cavity, and *Pentarichomonas hominis* and *Dientamoeba fragilis* in the large intestine (Honigberg 1978; McDougald and Reid 1978). *Trichomonas vaginalis* is the most important by far, infecting 180 million people worldwide annually. It is the most common of the sexually transmitted urogenital infections in humans. The pathogenicity of *Dientamoeba fragilis* for humans is not well understood, but it seems that certain bowel disorders can be attributed to this species (Barratt et al. 2011). A single report of *Dientamoeba* being pathogenic for gorillas was published (Lankester et al. 2010). *Pentarichomonas hominis* is considered nonpathogenic (Honigberg and Burgess 1994). Several parabasal species have been found in the respiratory tract of humans, for example, *Trichomonas tenax*, *Trichomonas vaginalis*, *Tritrichomonas foetus*, *Pentarichomonas hominis*, *Tetratrichomonas gallinarum*, and *Tetratrichomonas empyemagena* (Jongwutiwes et al. 2000; Čepička et al. 2005; Kutisova et al. 2005; Duboucher et al. 2006, 2007; Carter and Whithaus 2008; Leterrier et al. 2012); their pathogenic potential is usually unclear. Although *Lophomonas blattarum*, a hypermastigote from cockroaches, has been repeatedly reported from the respiratory tract of humans, it was possibly confused with epithelial cells (see Li and Gao 2016).

*Histomonas meleagridis* causes histomoniasis or “blackhead” disease that affects the ceca and liver of turkeys, chicken, quail, and peafowl. It has been effectively treated with dimetridazole and nifursol in the feed, but these drugs are now banned in the European Union. Symptoms in turkeys are listlessness, anorexia, droopy wings, and yellow, sulfur-colored feces. *Histomonas* interacts with cecal worms, earthworms, coccidia, and other intestinal microbiota (AbdulRahman and Hafez 2009). It can be transmitted between birds by the nematode *Heterakis gallinarum*, and earthworms may serve as paratenic hosts (McDougald and Reid 1978).

Another parabasalian affecting birds is *Trichomonas gallinae*, which lives in the upper digestive tract of birds where it can cause disease. It infects turkeys, raptors,
and gulls, but its primary host is the domestic pigeon. In pigeons it is transferred in the “milk” from the crop of an affected bird to the offspring. Virulent strains cause ulcers of the mouth, pharynx, esophagus, and crop, from which the organism enters the blood stream and passes to the liver. From this site it can kill a bird in two weeks (BonDurant and Honigberg 1994). A large outbreak of T. gallinae recently led to population declines in two finch species in the UK (Lawson et al. 2011).

Cattle are the primary hosts of Tritrichomonas foetus, which is transmitted exclusively as a venereal infection. In infected females, fertilization of the ovum occurs but the embryo may die and be expelled or absorbed. Besides cattle, T. foetus is found also in the large intestine and nasal cavity of pigs, where it is considered a harmless commensal, and in the intestine of cats, where it causes diarrhea (Yao and Köster 2015). For a detailed account of trichomoniasis, see BonDurant and Honigberg (1994).

Aside from causing disease in humans and animals, the main impact of parabasalians on society is their role in the destruction of buildings by wood-eating termites. While Cryptocercus is found only within decaying tree trunks (Nalepa 1984), at least 370 of the 3000 living species of termites are considered pests. The most damaging of the termite pests are Cryptotermes brevis, Cryptotermes domesticus, Cryptotermes dudleyi, Coptotermes formosanus, Coptotermes gestroi, Reticulitermes flavipes, and Reticulitermes lucifugus; these all harbor symbiotic hindgut parabasalians (Krishna et al. 2013).

**Habitats and Ecology**

Parabasalians are all anaerobes, and most are intestinal symbionts or parasites. The majority of described species are obligate symbionts of wood-eating insects (the so-called “lower” termites and Cryptocercus wood-feeding roaches), and these associations in particular have received sustained attention in terms of evolutionary history and functional ecology. Each termite or wood roach usually harbors several species of parabasalians. These species help their host to digest cellulose, in cooperation with the other microorganisms present in the intestine. Their evolution as gut symbionts has led to many morphological adaptations including cell enlargement and multiplication of flagella in some lineages (see below). It has been shown that the symbiosis between termites and their parabasalians is obligate and that the insect host will starve to death despite continued feeding if the symbionts are removed (Cleveland 1925). The termite parabasalians are considered highly host specific and coevolving with their hosts, with few host switches (Kirby 1947; Kitade 2004; Noda et al. 2007; Tai et al. 2015).

Many parabasalian species found in vertebrates are understudied and have not been reported since the original description. The species living in the intestine are usually commensals, though a possible pathogenicity for the host is a consideration in some cases. The host specificity differs from species to species (e.g., Cepicka et al. 2006). Some species seem to be restricted to a few closely related hosts or a single host lineage such Tetratrichomonas limacis from gastropods or several trichomonad
Fig. 1 (continued)
species from guinea pigs (Nie 1950; Cepicka et al. 2006). Others can infect many species representing one or even more vertebrate classes, e.g., *Trichomitus batrachorum* from a wide diversity of amphibians and reptiles, *Pentatrichomonas hominis* from many mammalian orders, *Tetratrichomonas gallinarum* from birds and primates, and *Tetratrichomonas* sp. “lineage 10” from tortoises, cattle, and primates (Honigberg 1953; Honigberg and Burgess 1994; Cepicka et al. 2005; Smejkalová et al. 2012).

Several species of parabasalians, notably *Trichomonas* spp. and *Tritrichomonas foetus*, have colonized other internal organs, such as the oral cavity (e.g., *Trichomonas tenax* from humans and *Trichomonas gallinae* from birds) and genitourinary tract (*Trichomonas vaginalis* from humans and *Tritrichomonas foetus* from cattle). These species are often pathogenic for their hosts, causing various diseases. The trichomonads from extra-intestinal locations were generally believed to be highly host specific, with the exception of *Tritrichomonas foetus* that infects the intestine and nasal cavity of pigs as well as the genitourinary tract of cattle. Nonetheless, recently published studies have shown that the true host range may be wider in several cases (Šlapeta et al. 2012; Morin-Adeline et al. 2015).

**Characterization and Recognition**

**Light Microscopy**

Parabasalia is a morphologically diverse lineage and can be divided into two assemblages according to the cell complexity: trichomonads (relatively simple cells with up to six flagella per mastigont) and hypermastigotes (complex, often very large cells, with many flagella per mastigont). These two groups more or less correspond with the traditional orders Trichomonadida and Hypermastigida, but it has been shown that neither is monophyletic (see below). The trichomonads represent morphologically plesiomorphic forms of the Parabasalia, whereas the

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**Fig. 1** Light-microscopic morphology of Hypotrichomonadida (a–c), Tritrichomonadida (d–i), Trichomonadida (j–m), and Honigbergiellida (n–q). Protargol-stained cells, bright field. (a) *Trichomitus batrachorum* from *Bufo bufo*. (b) Parabasal body of *Trichomitus batrachorum* from *Testudo radiata*. (c) Hypotrichomonas acosta from *Leptopelis* sp. (d) Monocercomonas cobrurorum from *Tropidophis melanurus*. (e) Simplicimonas similis from *Melanphaeus faber*. (f) Tritrichomonas augusta from *Lacerta vivipara*. (g) Parahistomonas wenrichi from *Meleagris gallopavo*. (h) Histomonas meleagridis from *Meleagris gallopavo*. (i) Dientamoeba fragilis from *Homo sapiens*. (j) Tetratrichomonas sp. from *Macaca silenus*. (k) *Trichomonas tenax* from *Homo sapiens*. (l) *Trichomitus termopsidis* from *Zootermopsis angusticollis*. (m) Free-living *Pseudotrichomonas keilini*. (n) Hexamastix coercens from *Acomys* sp. (o) Tetratrichomastix sp., origin uncertain. (p) *Honigbergiella ruminantium* from *Bos taurus*. (q) Free-living *Monotrichomonas* sp. **Scale bar in Q** = 10 μm; it applies for the whole plate. **Labels:** arrows anterior flagella, arrowhead recurrent flagellum, Ax axostyle, C costa, P pelta, PB parabasal body, UM undulating membrane.
Morphology of Trichomonad Cells

Trichomonad cells are usually spindle-shaped or pyriform (Figs. 1, 2). They do not possess any cytostome. Instead, phagocytosis generally occurs anywhere on the cell surface. Certain taxa tend to be amoeboid, for example, *Histomonas meleagridis* and *Parahistomonas wenrichi* (Fig. 1g, h). *Trichomonas vaginalis* also becomes amoeboid when attached to the vaginal epithelium, though it retains its flagella, as does the cristamonad *Gigantomonas herculea*, which forms gigantic plasmodia as part of its life cycle. *Dientamoeba fragilis* has completely lost its flagella and is the only true amoeba within Parabasalia (Fig. 1i). Cells of trichomonads from vertebrates measure about ten micrometers; trichomonads from termites may have much larger cells with diameters of tens or even hundreds of micrometers, for example, cells of *Mixotricha paradoxa* measure up to half a millimeter in length (Cleveland and Grimstone 1964; Brugerolle 2004).

Trichomonads are predominantly uninucleate. Many cells of *Dientamoeba fragilis* are binucleate, because they are arrested in the telophase stage of the cell cycle (Camp et al. 1974) (see Fig. 1i). The nucleus of a trichomonad is typically in close association with the flagellar basal bodies (which in simple forms are grouped together into a single “mastigont”) and associated cytoskeletal fibers; in other words most trichomonads have a “karyomastigont.” The number of flagella in a mastigont varies in trichomonads from zero in the amoeboid *Dientamoeba fragilis* (Fig. 1i) to six in genera *Hexamastix*, *Pentatrichomonas*, *Pentatrichomonoides*, *Cochlosoma*, and *Cithylla* (Fig. 1m). The ancestral number is four (e.g., *Trichomitus*, *Trichomonas*, *Parahistomonas*, *Monocercomonas*, *Simplicimonas*, *Honigbergiella*, *Devescovina*; Figs. 1a–g, m, p and 2d, g), but five flagella are common (e.g., *Trichomonas*, *Tetrastrichomonas*, *Pseudotrypanosoma*, *Trichomitopsis*, *Tetra-trichomastix*; Fig. 1j–l, n), and trichomonads with three flagella (*Ditrichomonas*), two flagella (*Monotrichomonas*, Fig. 1q), or a single flagellum (*Histomonas*, Fig. 1h) are known as well.

Two independent lineages of Cristamonadida, the first one being represented by the genera *Calonympha*, *Stephanonympha*, and *Snyderella* and the second one by the genus *Coronympha*, have multiplied their nuclei and possess eight (Coronympha young forms) to hundreds or even hundreds (e.g., *Snyderella*) of nuclei per cell (see Figs. 2h, j and 6c) (Harper et al. 2009; Gile et al. 2011). Such “polymonad” trichomonads are collectively called the calonymphs (Cepicka et al. 2010). As in simpler trichomonads, the nuclei of calonymphs are each associated with flagellar...
Fig. 2  Light-microscopic morphology of Cristamonadida. (a) Protargol-stained *Foaina dogieli* from *Kalotermes flavicollis*. (b) Protargol-stained *Foaina* sp. from *Neotermes cubanus*. The dots on the surface are the sites of attachment of epibiotic bacteria. (c) Protargol-stained *Foaina* sp. from *Neotermes cubanus* showing epibiotic bacteria, observed under DIC. The partial cell in the picture belongs to a polymastigid oxymonad. (d) Living *Caduceia versatilis* from *Cryptotermes cavifrons* observed under phase contrast. (e) Protargol-stained *Macrotrichomonas* sp. from *Neotermes cubanus*. (f) Protargol-stained *Macrotrichomonoides* sp. from *Neotermes cubanus*, detail of the parabasal body coiling around the axostyle. (g) Living *Macrotrichomonoides restis* from *Neotermes jouteli* observed under DIC. (h) Living *Snyderella* sp. from *Cryptotermes cavifrons* observed under phase contrast. (i) Protargol-stained *Joenia annectens* from *Kalotermes flavicollis*. (j) Top view of
basal bodies in a mastigont, forming an organelle system called the karyomastigont (as above). In the genera *Calonympha* and *Prosnyderella*, there is a proliferation of mastigonts that lack an associated nucleus, the akaryomastigonts. Only in the genus *Snyderella* are the nuclei disassociated from the mastigonts and suspended in the cytoplasm. In this case, all flagella are found in akaryomastigonts across the surface of the cell. Each karyo- or akaryomastigont has four flagella. Although the calonymphs are multiflagellate, their mastigont organization is trichomonad-like and clearly derives from a multiplication of nucleomotor systems, rather than the proliferation of individual flagella that has occurred multiple times in hypermastigotes. Accordingly, two to four flagella are present in individual karyo- or akaryomastigonts.

Flagella of trichomonads insert apically or subapically. One flagellum is usually recurrent and runs posteriorly along the cell body, while the other flagella are directed anterolaterally (Figs. 1 and 2a, e, g). The posterior flagellum is acronematic, while the anterior flagella usually end with structures called “knobs” when stained (Fig. 1a, c, e, j, n–q), though the knobs may be artifacts due to the cell shrinkage during the fixation (Céza et al. 2015). The recurrent flagellum of some trichomonads is associated with the cell body, forming an undulating membrane. The undulating membrane may reach the posterior end of the cell (e.g., *Tetratrichomonas*, *Pentatrichomonas*, *Tririchomonas*, *Trichomitus*; Fig. 1a, c, f, j, l), or it can be shorter (e.g., *Trichomonas*, *Ditrichomonas*, *Monotrichomonas*; Fig. 1k, q). In most cases, the recurrent flagellum extends beyond the undulating membrane (e.g., *Tetratrichomonas*, *Tririchomonas*, *Trichomitus*), but in *Trichomonas* and *Pseudotrichomonas*, the recurrent flagellum is associated with the cell body along its whole length, and no free portion is developed (Fig. 1k, m). The undulating membrane is usually underlain by a fiber of varying thickness called a costa (Fig. 1a, f, j–l). Some genera, e.g., *Hypotrichomonas*, *Pseudotrichomonas*, *Ditrichomonas*, and *Monotrichomonas*, possess an undulating membrane but no costa (Fig. 1c, m, q). The undulating membrane of some members of Cristamonadida is instead underlain by a fibrous cresta (Fig. 2a, e) that is not homologous to the costa (Kirby 1942; Holland and Valentin 1969b; Brugerolle 1976; Brugerolle and Lee 2000). The presence/absence of costa and cresta was historically suggested to be an important taxonomic feature (Kirby 1947; Honigberg 1963).

The karyomastigont of trichomonads is associated with characteristic cytoskeletal elements. Four of these are visible under the light microscope: costa/cresta, pelta, axostyle, and, with appropriate staining, parabasal fibers (Fig. 1). The axostyle is a hyaline rod and is differentiated into the proximal, spatulate capitulum, which laterally covers the nucleus, and a distal trunk, which usually protrudes from the

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**Fig. 2** (continued) cell apex of *Coronympha* (*Metacoronympha*) sp. from *Incisitermes snyderi* showing nuclei stained with DAPI. Scale bars = 10 μm for a–c, e, f, h, and j and 20 μm for d, g, and i. **Labels:** arrows anterior flagella, arrowhead recurrent flagellum, Ax axostyle, Cr cresta, double arrowhead epibiotic bacteria, PB parabasal body, UM undulating membrane
posterior end of the cell. Trichomonad taxa differ in the shape of the capitulum, thickness of the trunk, and shape of its ending. In general, two types of axostyles are recognized (Cepicka et al. 2010): *Trichomonas* type, which tapers gradually (e.g., *Trichomonas*, *Trichomatius*, *Monocercomonas*, and many others; Fig. 1a, c, d, j, k, m–o), and *Tritrichomonas* type, which tapers abruptly (e.g., *Tritrichomonas*, *Simplicimonas*, and many cristamonad genera; Fig. 1e, f, l). The pelta is a crescent-shaped structure that curves over the anterior side of the nucleus. In the bird parasite genus *Cochlosoma*, the pelta has been modified to support an adhesive disc superficially resembling that of the diplomonad *Giardia* (Pecka et al. 1996). Each mastigont of the calonymphs includes an individual axostyle and pelta. Trunks of the axostyles are either separated from each other (e.g., in *Coronympha*) or they form a bundle along the cell’s axis (e.g., *Calonympha* and *Stephanonympha*) (Kirby 1929; Rösel et al. 1996). Both axostyle and pelta are absent in the amoeboid *Dientamoeba fragilis* (Camp et al. 1974).

Usually one or two parabasal fibers run from the basal bodies into the cell. These are associated with the Golgi apparatus and together with the Golgi form the so-called parabasal body, which is the apomorphy for which Parabasalia is named. The parabasal body may be V-shaped (e.g., *Trichomonas*, *Trichomatius*, *Hypo-trichomonas*; Fig. 1b), sausage-shaped (e.g., *Tritrichomonas*, *Monocercomonas*; Fig. 1f), discoid (e.g., *Tetrarichomonas* and *Pseudotrichomonas*; Fig. 1j, m), drop-shaped (e.g., *Pentarichomonas*, *Simplicimonas*, *Hexamastix*; Fig. 1e, n), elongate (e.g., *Trichomitopsis*; Fig. 1l), or branched (*Pseudotrypanosoma*).

The parabasal body shape is particularly striking in the devescovinids, a grouping of large trichomonads from termite hindguts. In genera such as *Devescovina*, *Metadevescovina*, *Caduceia*, and *Macrotrichomonas*, the parabasal body winds around the axostyle, with the number of turns being used as a species-level taxonomic feature (Fig. 2f). The coiling of the parabasal body reminded Kirby of the snakes winding around Hermes’ staff, the caduceus, and prompted him to name a new genus *Caduceia* (Kirby 1942). Apart from being larger than other trichomonads, the devescovinids have a similar overall morphology, with three anterior and one recurrent flagellum. The latter sometimes adheres to the cell, forming an undulating membrane. The recurrent flagellum is typically thickened to form a cord or a ribbon-like band (Foà 1905; Janicki 1915; Brugerolle and Lee 2000).

**Morphology of Hypermastigote Cells**

Hypermastigotes measure from several to several hundred micrometers in length and bear more flagella than trichomonads, ranging from several tens to several thousands. Almost all hypermastigote cells possess a single nucleus. Most structures typical for trichomonads, i.e., pelta, axostyle, and parabasal body, are also present in hypermastigotes but usually have been expanded or transformed. Three broad morphological categories of hypermastigotes can be recognized. Trichonymphida (e.g., *Trichonympha*, *Staurojoenina*; Fig. 3a–h) have many flagella arranged along and around a bilaterally symmetrical rostrum. Spirotrichonymphida (e.g.,
Spirotrichonympha, Holomastigotes; Fig. 4) lack a true rostrum, and the complex cytoskeletal structure supporting the flagella is arranged in two counterclockwise spirals (Brugerolle and Lee 2000) (see below for more details). The third group, collectively and informally called the lophomonads, were placed together in early taxonomies because of their shared anterior tuft of many flagella but are now known to be polyphyletic. All lophomonads, with the exception of Lophomonas itself, belong to the Cristamonadida, though they do not branch together within that order. Another shared feature of lophomonads is the resorption of parabasal bodies, axostyle, flagella, and basal bodies during cell division (Brugerolle and Patterson 2001). Only the four privileged basal bodies (homologous to the ancestral four basal bodies, see below) are not resorbed. These are duplicated and then separated, and the
additional flagella and associated structures are rebuilt in each daughter cell (Hollande and Carruette-Valentin 1971).

Projoenia sawayai (Cristamonadida) displays the most plesiomorphic morphology among cristamonad hypermastigotes in general (Lavette 1970). Its cells are 45–100 μm long and strongly resemble cells of devescovinids by possessing a recurrent flagellum proximally supported by a cresta, a stout axostyle, and a single, spiral parabasal body, yet its mastigont is an apical flagellar area with as many as 500 flagella. The morphology of other cristamonad hypermastigotes (informally

Fig. 4 Light-microscopic morphology of Spirotrichonymphida. (a) Protargol-stained juvenile Microjoenia sp. from Reticulitermes lucifugus. (b) Protargol-stained adult Microjoenia sp. from Reticulitermes lucifugus. (c) Protargol-stained Spironympha sp. from Reticulitermes flaviceps. (d) Living Spirotrichonympha flagellata from Reticulitermes hesperus observed under DIC. (e) Protargol-stained juvenile Holomastigotes elongatum from Reticulitermes lucifugus. (f) Protargol-stained adult Holomastigotes elongatum from Reticulitermes lucifugus. (g) Living Holomastigotoides sp. from Coptotermes testaceus observed under DIC. Scale bars = 10 μm for a–c, e, and f; 20 μm for d; and 50 μm for g. Labels: Ax axostyle/axostylar filaments, PB parabasal body
referred to as “joeniiids,” e.g., *Joenia*, *Joenoides*, *Placojoenia*, *Joenina*, and *Joenopsis*) is similar to that of *Projoenia*, though the cells are more complex, bear more flagella, and lack the cresta (Brugerolle and Lee 2000) (Fig. 2i). Joeniid genera differ mainly in the shape of the flagellar area. *Rhizonympha jahieri* is a peculiar lophomonad whose cells are plasmodia with several hundred karyomastigonts, each containing multiple flagella (Grassé and Hollande 1951). *Kofoidia loriculata* is another unusual lophomonad with several bundles of flagella on the apex that are arranged in an open circle (Light 1927). Genera *Deltotrichonympha* and *Koruga* (which is likely a synonym of *Deltotrichonympha*) have rows of flagella extending down the cell body in addition to the apical flagellar area.

*Lophomonas* cells are 20–60 μm long and contain a single karyomastigont (Fig. 3i, j). The many flagella are arranged in an ear-shaped row partially encircling the nucleus. The axostyle is thin and can protrude through the cell body (Kudo 1926a, b; Hollande and Carruette-Valentin 1972).

In the monophyletic hypermastigote order Trichonymphida, cells are divided into a rostrum and postrostral area (Fig. 3a–h). The postrostral area contains the nucleus, which usually lies close to the boundary of the two areas. The rostrum is bilaterally or tetraradially symmetrical and bears two (most trichonymphids) or four (Staurojoeninidae; Fig. 3e) flagellar areas, each composed of longitudinal rows of flagella. The areas separate from each other during cell division and are distributed into the daughter cells (Hollande and Carruette-Valentin 1971). Some trichonymphids bear flagella also on the postrostral area: in Spirotrichosomidae (e.g., *Spirotrichosoma*, *Leptospironympha*, *Apospironympha*; Fig. 3c, d), the postrostral basal bodies are arranged in spiral rows, which makes them superficially similar to Spirotrichonymphida (see below), while in Trichonymphidae (Fig. 3g, h), the rows are longitudinal. In Teranymphidae, the postrostral flagella are either arranged in longitudinal rows as well (*Eucomonympha* and *Pseudotrichonympha*; Fig. 3a, b) or form multiple circular transverse rows (*Teranympha*). In Hoplonymphidae (e.g., *Hoplonympha*, *Barbulanympha*) and Staurojoeninidae (e.g., *Staurojoenia*), the postrostral area is devoid of flagella (Fig. 3e, f). The parabasal complex of trichonymphids is divided into numerous branches around the nucleus (*Trichonympha*) or consists of numerous bodies within the cell (Brugerolle and Lee 2000).

The flagella of Spirotrichonymphida are arranged in two or more counterclockwise spiral rows that are distributed into daughter cells during the division (Fig. 4). The number of flagellar lines can vary among cells of a single species (Brugerolle 2006a). Some genera (*Spirotrichonympha*, *Spironympha*, *Spirotrichonymphella*) possess an apical structure (“pseudorostrum”) that resembles the rostrum of Trichonymphida, while the others (e.g., *Holomastigotes*, *Holomastigotoides*) do not. The axostyle is either absent (*Spirotrichonymphella*), split into individual filaments (*Spirotrichonympha* and *Holomastigotoides*), or resembles the axostyle of trichomonads (*Microjoenia*, *Micromastigotes*). Parabasal fibers and multiple, small parabasal bodies are associated with the flagellar rows or are scattered in the cytoplasm (Brugerolle and Lee 2000; Brugerolle 2001).
*Cthulhu macrofasciculumque* is the only hypermastigote member of Honigbergiellida (James et al. 2013). Its cells are only about 20 μm long and bear about 20 flagella. Otherwise, their morphology is largely trichomonad-like.

**Structure of the Cytoskeleton**

Although Parabasalia belongs to the supergroup Excavata, they do not possess the ventral feeding groove supported by cytoskeleton, which is typical for plesiomorphic excavates such as *Carpediemonas*, *Trimastix*, and *Jakoba*. Instead, they have developed a characteristic system of microtubular and non-microtubular cytoskeletal elements, for which homology with elements of the flagellar apparatus of typical excavates usually cannot be determined (Simpson 2003). The mastigont system that is most similar to the hypothetical last common ancestor of Parabasalia is found in some trichomonads (Fig. 5b, c) and comprises four basal bodies, three of which (here referred to as B2, B3, and B4) are parallel, are directed anteriorly, and bear the three anterior flagella. The remaining basal body (B1) lies in proximity to B2–B4 but is perpendicular to them and bears the recurrent flagellum. Note that in much of the Parabasalia literature, the basal body of the recurrent flagellum is instead designated R, and the anterior flagellar basal bodies are B1–B3. The B1–B4 scheme adopted here allows microtubular roots to be designated and compared across eukaryotic lineages (Moestrup 2000; Yubuki and Leander 2013; Yubuki et al. 2016). In genera with four anterior flagella, such as *Trichomonas*, *Tetratrichomonas*, and *Pseudotrypanosoma* (Trichomonadida), the basal body bearing the additional flagellum (B5) lies in the same plane as B2–B4 and is parallel to them, making a four-sided bundle (Fig. 5a). A fifth anterior flagellum has been added into the mastigont of Parabasalia in two different ways. In *Pentatrichomonoides* (Trichomonadida) and *Hexamastix* (Honigbergiellida), the basal body of the fifth anterior flagellum (B6) is adjacent and parallel to B2–B5 (Hampl et al. 2007; Fig. 5e, f). In *Pentatrichomonas* and *Cochlosoma* (Trichomonadida), on the other hand, B6 is separate and not parallel to the others (Honigberg et al. 1968; Pecka et al. 1996). In species with fewer than four flagella, the four basal bodies remain but one or more of them are barren. For example, *Ditrichomonas*, which has two anterior flagella, has one barren basal body, while *Histomonas*, with only one flagellum, has three barren basal bodies (Schuster 1968; Farmer 1993).

The basal bodies of the anterior flagella, B2–B4, bear striated rootlets running posteriorly into the cytoplasm. The rootlets of B3 and B4 are short and single, while B2 bears multiple, long fibers, called sigmoid fibers or preaxostylar filaments, that are curved toward the dorsal side of the cell and run toward the pelta-axostyle junction (Fig. 5a–c, f). In addition to striated rootlets, B3 and B4 each bear a short, hooked lamina along their length (Brugerolle 1991). Another typical cytoskeletal structure that originates from the area of the basal bodies (specifically, between B1 and B4) is the striated marginal lamella. It underlies the proximal part of the recurrent flagellum and participates in the undulating membrane structure (Fig. 5a, b, g).
**Fig. 5** Ultrastructure of trichomonads. (a) Organization of the cytoskeleton of *Tetratrichomonas* sp. (Trichomonadida). (b) Organization of the cytoskeleton of *Tritrichomonas muris*.
The recurrent flagellum of some species is attached to the ventral cell surface, and an undulating membrane develops (Fig. 5a, b, g). The side of the cell where the recurrent flagellum runs is referred to as “dorsal” in the older literature, but here we consider it “ventral,” because the ventral feeding groove had been located here before it was lost. There are two basic types of undulating membrane in trichomonads. The first one, called a lamelliform undulating membrane, is found in Trichomonadida, Honigbergiellida, and Hypotrichomonadida, and a very simple version is also seen in Simplicimonas (Trichomonadida) (Fig. 5a, g). It is formed by a thin cytoplasmic projection that is laterally supported by the recurrent flagellum. The recurrent flagellum usually has a typical structure with no modifications, but in some genera from termites, such as Trichomitopsis, Pseudotrypanosoma, Trichomonoides, and Pentatrichomonoides, it is enlarged and contains paraxonemal fibers (Hollande and Valentin 1968; Brugerolle et al. 1994; Brugerolle 1999). The distal part of the cytoplasmic projection of the cell body contains the marginal lamella. The second type of undulating membrane is found in the genus Trichomonas (Trichomonadida) and is called a “rail”-type undulating membrane (Brugerolle 1976) (Fig. 5b). Here, the recurrent flagellum is applied directly to the distal part of the cytoplasmic projection, which is much thicker than in the lamelliform undulating membrane. Both the cytoplasmic projection and recurrent flagellum contain electron-dense material derived from the marginal lamella. Trichomonas species differ considerably in the fine structure of the rail-type undulating membrane (Joyon et al. 1969) (see Fig. 5b–d in Brugerolle & Lee 2000). In some members of Cristamonadida, the recurrent flagellum adheres to the cell body as well, and a homolog of the rail-type undulating membrane is developed. In this case, however, there is no cytoplasmic projection, though a sharp transition can be seen where one side of the undulating membrane meets the cell membrane, and the enlarged recurrent flagellum applies directly to the cell surface and is subtended by an electron-dense fiber, the cresta (Fig. 6b). The cresta is thus not homologous to the costa that underlies the undulating membrane of many trichomonads, but instead

Fig. 5 (continued) (Trichomonadida). (c–k) Transmission electron micrographs. (c) Mastigont of Monocercomonas colubrorum (Trichomonadida). (d) Apical portion of Honigbergella ruminantium (Honigbergiellida). (e, f) Mastigont of Hexamastix kirbyi (Honigbergiellida). (g) Simple lamelliform undulating membrane of Simplicimonas similis (Trichomonadida). (h, i) Cyst of Honigbergella ruminantium (Honigbergiellida). (j, k) Trunk of the axostyle of Simplicimonas moskowitzi (Trichomonadida). Scale bars = 200 nm for c, f, g, and i and 500 nm for d, e, h, j, and k. Labels: 1, 2, 3, 4, 5, 6 basal bodies 1–6, A axostyle, CA costa (A-type), CB costa (B-type), CS comb-like structure, CW cyst wall, F1 recurrent flagellum (flagellum 1), G glycocalyx, GB Golgi body (parabasal body), H hydrogenosome, IF internalized flagellum, IKB infrakinetosomal body, ML marginal lamella, N nucleus, P pelta, PER periaxostylar ring, PF parabasal fiber, SF sigmoid fibers, SKB suprakinetosomal body, UML undulating membrane (lamelliform), UMR undulating membrane (rail type). (a) After Brugerolle (1976), (b) after Brugerolle (1991), and (e–i) after Hampl et al. (2007), with permission from Elsevier, modified, and (j and k) after Cepicka et al. (2010), with permission from Elsevier, modified.
**Fig. 6** Ultrastructure of Cristamonadida. (a, b) Electron micrographs of *Caduceia versatilis*. (c) Electron micrograph of *Calonympha grassii*. Scale bars = 1000 nm for a and b and approximately 3000 nm for c. **Labels:** A axostyle, B unidentified bacteria in the nucleus of *Caduceia*, BC “bacterial cup,” a group of undescribed bacteria surrounding the axostyle directly posterior to the parabasal body, C cresta, F symbiotic fusiform bacteria of *Caduceia*, F1 recurrent flagellum (flagellum 1), GB Golgi body (parabasal body), M mastigont of *Calonympha* with four basal bodies, P Carter-pelta-axostyle complex, PF parabasal fiber, N nucleus, *T. candidatus* Tammella caduceiae ectosymbiotic bacteria.
may be homologous to the proximal part of the rail-type undulating membrane of *Tritrichomonas* (Hollande and Valentin 1969b; Gile et al. 2015).

The undulating membrane of *Trichomitus* (Hypotrichomonadida), *Tritrichomonas* (Trichomonadida), and most members of Trichomonadida is underlain along its length by a thick striated fiber, the costa (Fig. 5a, b). Although costae are relatively uniform when observed under the light microscope, their structure and the exact site of their origin in the area of the basal bodies differ among lineages. Generally, two types are distinguished: A-type and B-type (historically, they had been also referred to as C1- and C-type, respectively). The A-type costa is found in *Tritrichomonas* and *Trichomitus*. Its striations have a periodicity of about 40 nm and consist of repetitions of four transverse bands. In contrast, the B-type costa of Trichomonadidae, although with similar periodicity of 40 nm, consists of the repetition of a single basic line of dense filaments in cross section (Honigberg et al. 1972). The costa of most trichomonads is a rigid structure; in the closely related genera *Trichomitopsis* and *Pseudotrypanosoma*, it is contractile, and its movement contributes to the movement of the entire cell (Mattern and Honigberg 1971; Amos et al. 1979).

The parabasal apparatus is a defining feature of parabasalians. In trichomonads it includes two striated filaments (PF1 and PF2) with periodicity (ca. 40 nm) and structure very similar to that of the A-type costa (Mattern et al. 1967; Honigberg et al. 1971; Honigberg et al. 1972; Brugerolle 1976). They emerge from the basal bodies, run posteriorly into the cytoplasm, and are associated with the cisternae of stacked Golgi apparatus, which is extensively developed (Fig. 5d, f).

The axostyle and pelta are conspicuous structures of trichomonad cells under the light microscope (see above). Transmission electron microscopy revealed that each consists of a single, broad sheet of cross-linked microtubules (Brugerolle 1986) (Fig. 5c, f). Although pelta and axostyle are two separate structures, they meet in the area called the pelta-axostylar junction, where their microtubules overlap for some length. The inner side of the pelta-axostylar junction is associated with the sigmoid fibers that descend from B2. The pelta curves to the right and encircles the area where the basal bodies are located and supports the periflagellar canal, an external pit or chamber that houses the proximal portion of the flagella (Honigberg and Brugerolle 1990). The axostyle is divided into an anterior, spoon-shaped capitulum that curves over the nucleus (Fig. 5a–c) and a posterior, tubular trunk that extends axially to the posterior taillike tip of the cell (Fig. 5j, k). The axostylar trunk is formed from a sheet of microtubules, either with edges joined to form a hollow tube, as in *Simplicimonas* (Cepicka et al. 2010), or rolled into a spiral, as in *Tritrichomonas* (Brugerolle and Lee 2000). In *Pentatrichomonoides* (Trichomonadida), the trunk has been modified into a microtubular corset that underlies most of the cell surface (Brugerolle et al. 1994).

There are several non-microtubular structures in the mastigont of trichomonads that are lineage specific. Some of these are various kinds of striated fibers. Although their structure has been well documented by TEM studies, their compositions and functions are unknown. For example, the so-called infrakinetosomal body is typical for some Tritrichomonadida and Cristamonadida, and the comb-like structure has
been documented from the mastigont of Tritrichomonadida, Cristamonadida, and Hypotrichomonadida (Fig. 5b, c; see Cepicka et al. 2010).

Hypermastigotes have many unique ultrastructural features, though certain aspects of their cytoskeletons are directly comparable to those of simpler trichomonads. In particular, the “privileged” basal bodies, those that are homologous to the ancestral B1–B4, can be found among the many additional flagella (Hollande and Carruette-Valentin 1971). These are most clearly apparent in the lophomonads, where they are at the heart of the apical flagellar bundle and polarize the parabasal bodies and pelta-axostyle complex just as in trichomonads (Hollande and Carruette-Valentin 1972; Brugerolle 1991). Upon cell division, all flagella are resorbed and only the privileged basal bodies remain. The privileged basal bodies are arranged in the three anterior, one recurrent arrangement typical of trichomonads, with a hooked lamina on B2 and B4 (Honigberg and Brugerolle 1990). One exception to this is found in *Lophomonas*, where the direction of B1 has changed such that all four privileged basal bodies are parallel (Hollande and Carruette-Valentin 1972; Brugerolle 1991). This significant ultrastructural difference is consistent with the distant relationship between *Lophomonas* and the cristamonad hypermastigotes according to molecular phylogenies (Gile and Slamovits 2012). Furthermore, the basal bodies of the proliferated flagella in *Joenia* and *Deltotrichonympha* (Cristamonadida) each bear a hooked lamina, suggesting they arose by multiplication of B2 or B4, while the proliferated flagella in *Lophomonas* have unadorned basal bodies, suggesting that they derive from B1 (Brugerolle 1991).

In *Trichonymphida* (Fig. 7), the proliferated flagella are organized into two regions, with the parabasal fibers forming a base plate for each. These plates meet along their longitudinal edges to form the distinctive rostral tube characteristic of *Trichonympha*, *Pseudotrichonympha*, and *Teranympha*, or they are separated by lobes of ectoplasm, as in, e.g., *Hoplonympha* and *Barbulanympha*. In *Staurojoenina*, there are four such rostral plates separated by four ectoplasmic lobes. At the apex of each rostral plate can be found one (*Staurojoenina*), two (*Trichonympha*), or three (*Urinympa*) privileged basal bodies (Hollande and Carruette-Valentin 1971; Brugerolle and Lee 2000).

In *Spirotrichonymphida*, the proliferated flagella are organized into two to six helical rows in which the basal bodies are connected by short connecting fibers and longer fibrous bands (Lingle and Salisbury 1995). Depending on the genera, these bands might reach the cell’s posterior (e.g., *Spirotrichonympha*) or traverse most of the cell (e.g., *Holomastigotoides*) or remain confined to the cell’s apex (*Microjoenia*) (Brugerolle 2001, 2005, 2006b; Brugerolle and Bordereau 2004). Parabasal bodies may be interspersed regularly or irregularly between the rows (Brugerolle and Lee 2000). Each flagellar line has a set of one to three basal bodies at its apex, one of which bears the hooked lamina characteristic of B2 and B4 of trichomonads, while homologs of the recurrent basal body (B1) have not been identified (Brugerolle 2001).
Parabasalians are characterized by a distinctive double-membrane-bounded organelle called the hydrogenosome. The hydrogenosome’s basic biochemistry was first defined by Lindmark and Müller in *Tritrichomonas foetus* (1973). These organelles...
function in anaerobic ATP generation via the partial oxidation of pyruvate to acetate, carbon dioxide, and molecular hydrogen (Tachezy 2008). They are homologous to mitochondria (although the precise evolutionary history vis-à-vis obligately aerobic mitochondria has been extensively debated; Martin and Müller 2007), a relationship that was demonstrated through molecular/biochemical evidence such as the presence within hydrogenosomes of mitochondrial-type chaperones (Germot et al. 1996) and of the NADH dehydrogenase module of the mitochondrial respiratory chain (Hrdý et al. 2004). Like most hydrogenosomes, those from parabasalians lack a genome (Turner and Müller 1983; Clemens and Johnson 2000; Van Der Giezen et al. 2005). The anti-trichomoniasis drug metronidazole (Flagyl) receives an electron in the hydrogenosome, making it cytotoxic (Benchimol 2009).

Using TEM, the hydrogenosome is seen as an organelle delimited by two closely appressed membranes, with a homogenous, granular matrix (Figs. 5d, g, 7c). It lacks cristae. In *Trichomonas vaginalis* the hydrogenosomes occupy about 6% of the cell volume (Nielsen and Diemer 1976). Hydrogenosomes proliferate in the cell by a fission process similar to that described for peroxisomes and mitochondria (Wexler-Cohen et al. 2014).

**Mitosis and Reproduction**

The mitotic process used by parabasalians in cell division is a form of “cryptopleur-omitosis.” In this case the nuclear envelope remains intact, while the chromosomes’ kinetochores are embedded in the envelope. The mitotic spindle, also called a parasdesmose, remains outside the nucleus and consists of pole-to-pole microtubules and pole-to-kinetochore microtubules. The spindle pole bodies, called atractophores, are associated with the mastigont structures, such that the spindle segregates the two groups of kinetosomes as it separates the two sets of chromosomes in the nucleus. This is best seen in Fig. 3.7 in Honigberg and Brugerolle (1990). The atractophore is a somewhat amorphous granular material in the trichomonads but has a distinctive bell-clapper appearance in the trichonymphid and spirotrichonymphid hypermastigotes (Hollande and Carruette-Valentin 1972; Ritter et al. 1978). Kubai (1973) gives a very detailed ultrastructural study of the kinetochores and their movement within the nuclear envelope prior to attachment to the spindle tubules in *Trichonympha*.

Mitosis of the multiple nuclei in calonymphs occurs simultaneously, whether in the nonmastigont-associated nuclei of *Snyderella* or in the karyomastigonts of all other genera, but the nuclei can then be segregated asymmetrically, so that the two offspring cells do not have the same number of nuclei. For example, a cell with 100 nuclei can divide into two cells of 70 and 30 nuclei (Dolan et al. 2000a, b). It is often difficult to resolve the chromosomes in these mitoses. Among all the trichomonad species described, only a few karyotypes have been reported (Zubáčková et al. 2008).

Most parabasalian species are assumed to reproduce only asexually. The sexual cycles of many *Cryptocercus* parabasalians have been studied extensively by
Cleveland (1947) and involve whole cell fusion of haploid gametes. In *Trichonympha* the male gamete enters the female gamete from the posterior end and is fully absorbed. The male’s organelles disintegrate, and the two nuclei fuse. The cell then undergoes meiosis. This sex cycle is triggered by ecdysone and the molting of the insect and does not occur otherwise. For a critical view of Cleveland’s work, see Raikov (1995). Though not observed by other workers or in parabasalians outside the *Cryptocercus* hindgut, the presence of a sexual cycle in parabasalians is supported by genes for meiotic machinery in the genome of *Trichomonas vaginalis* (Malik et al. 2008).

**Cysts**

While many intestinal symbiotic protists are propagated between hosts by a cyst stage, few of the parabasalian gut flagellates do this. Certain hypermastigotes are the main exceptions to this rule, for example, *Staurojoenina* from *Neotermes* and *Macrospironympha* from the wood roach *Cryptocercus* (Cleveland et al. 1934; Dolan et al. 2004). *Trichonympha* from *Cryptocercus* encyst when their host molts. While encysted, the cells divide such that two daughter cells are released upon excystment (Cleveland et al. 1934). The cockroach symbiont *Lophomonas* also forms cysts in which one or more nuclear divisions take place (Kudo 1926a, b). Among non-termite gut parabasalians, true cysts have been observed from *Honigbergiella ruminantium* (Fig. 5h, i), *Trichomitus batrachorum*, *Trichomitus sanguisugae*, *Monocercomonas tipulae*, *Ditrichomonas honigbergii*, and possibly *Dientamoeba fragilis* (Brugerolle 1973; Farmer 1993; Hampl et al. 2007; Clark et al. 2014).

In other parabasalians, the rounded, resistant, resting form is called a pseudocyst because it lacks a cell wall (Pereira-Neves et al. 2003). Pseudocysts are particularly well characterized in *Trichomonas vaginalis* and *Tritrichomonas foetus* (Pereira-Neves et al. 2003; Pereira-Neves and Benchimol 2009). *Barbulanympha* forms pseudocysts upon molting of its host, *Cryptocercus* (Cleveland et al. 1934).

**Prokaryotic Symbionts**

Many of the parabasalian symbionts in the guts of termites and *Cryptocercus* are themselves host to a wide diversity of ecto- and endosymbiotic bacteria (Fig. 6a, b). A single host can harbor multiple types of bacterial symbionts that occupy distinct regions of the host cell (Sato et al. 2009; Strassert et al. 2010). While this has been known since early descriptions of the flagellates and was often incorporated into their name, e.g., *Devescovina striata* (Dolan 2001), only the development of molecular phylogenetic techniques has allowed researchers to place them into new and existing bacterial phyla and study their metabolic interactions. Many of these bacterial symbionts are from the *Bacteroidales*, the *Elusimicrobia* (formerly Termite Group 1), and the *Spirochaetales*. They have been found to fix nitrogen, produce acetate, and serve as motility symbionts (Tamm 1982; Ohkuma et al. 2015). Just as
many of the termite gut symbiotic parabasalians remain to be described, so are there many cases of bacterial symbioses of flagellates that need investigation. There is considerable evidence that these bacterial symbionts are specific to their host flagellates and that the two groups of organisms have coevolved within the termite’s gut (Noda et al. 2007; Desai et al. 2010; Strassert et al. 2010).

Many of the cases of ectosymbiotic bacteria are from the order Bacteroidales. These include the ectosymbionts of the cristamonads Joenia annectens and Devescovina spp. and the Cryptocercus trichonymphids Barbulanympha and Urinympha, which are all likely nitrogen fixers (Noda et al. 2006; Strassert et al. 2010; Desai and Brune 2012; Tai et al. 2016). The order Bacteroidales is well represented on the termite’s gut wall (Nakajima et al. 2006). It has been hypothesized that these ectosymbionts may consume small amounts of oxygen, in effect protecting the anaerobic host (Noda et al. 2006). A nitrogen-fixing Bacteroidales endosymbiont has been found in Pseudotrichonympha grassii from Coptotermes formosanus (Hongoh et al. 2008).

Spirochetes can be either ecto- or endosymbionts of parabasalians in the termite hindgut, or they may be free in the gut fluid (Ohkuma 2008). The cristamonad Mixotricha paradoxa from Mastotermes darwiniensis is an example of motility symbiosis with hundreds of spirochetes arrayed across the parabasalian cell surface, propelling the huge 500 micron-long flagellate through the gut (Cleveland and Grimstone 1964). Molecular phylogenetic work has found three species of Treponema spirochetes occupying distinct regions of the M. paradoxa surface, each associated with a Bacteroides-related rod-shaped bacterium (Wenzel et al. 2003; König et al. 2005). Three distinct spirochetes were also described from Spirotrichonympha leidyi in Coptotermes formosanus (Inoue et al. 2008). Acetogenesis and nitrogen fixation were confirmed from the complete genome of an unusual spirochete endosymbiont of Eucomonympha from the termite Hodotermopsis sjoestedti. In this case the spirochete is a short rod, devoid of its normal periplasmic flagella (Ohkuma et al. 2015).

Recent studies have found distinct termite gut lineages of several bacterial phyla associated with parabasalian flagellates, including the Synergistes, Verrucomicrobia, and Elusimicrobia. The motility symbionts on Caduceia versatilis, first reported by Tamm (1982), have been identified as affiliated with the Synergistes and named “Candidatus Tammella caduceiae” (Hongoh et al. 2007). The endonuclear organisms seen in Trichonympha agilis have been found to be Verrucomicrobia and named “Candidatus Nucleococcus spp.” (Sato et al. 2014). The symbionts originally affiliated with Termite Group 1 have been named the Elusimicrobia and include the group Endomicrobia, which have been found widely in Trichonympha both from termites and Cryptocercus (Geissinger et al. 2009; Ikeda-Ohtsubo and Brune 2009).

**Taxonomy**

The taxonomic system of Parabasalia adopted here (outlined at the end of this section) consists of six classes, eight orders, and 17 families and corresponds as closely as possible to the current consensus of molecular phylogenetic analyses
while remaining consistent with morphological data. However, molecular phylogenies are not resolved in all areas of the tree, and some parabasalians have not yet been included. Names may yet change as new data become available, as they have many times in the last century. In order to allow readers to understand the meaning of taxon names at different time points in the literature on parabasalians, a brief historical account of parabasalian taxonomy follows.

Traditionally, the Parabasalia was divided into two orders, Trichomonadida and Hypermastigida, according to the number of flagella per mastigont and cell complexity (e.g., Levine et al. 1980). Trichomonadida included the simpler forms along with polymonad ones (those whose nuclei and flagella were multiplied together) (Honigberg 1963; Pecka et al. 1996). Hypermastigida was divided into three sub-orders according to the arrangement of flagella. Lophomonadina had flagella arranged in a tuft at the cell apex, Trichonymphina had many flagella arranged along a bilaterally symmetrical rostrum, and Spirotrichonymphina had flagella arranged in spiral rows (Grassi and Foà 1911; Hollande and Carruette-Valentin 1971). Although early phylogenetic trees already showed that this classification system did not correspond to the actual phylogeny of Parabasalia because of a paraphyletic Trichomonadida and extensively polyphyletic Hypermastigida (see above), this taxonomy remained unrevised until the twenty-first century. Brugerolle and Patterson (2001) were the first to formally recognize the polyphyletic nature of hypermastigid in a taxonomic system and united certain genera of trichomonads and the whole Lophomonadina within a new order, Cristamonadida (Brugerolle and Patterson 2001).

Cepicka et al. (2010) revised the higher taxonomy of Parabasalia in order to bring it in line with the contemporary results of molecular phylogenetic studies. They divided Parabasalia into six classes, Trichomonadea, Tritrichomonadea, Hypotrichomonadea, Cristamonadea, Trichonympha, and Spirotrichonympha. Most classes include a single order, but Trichomonadea was further divided into two orders, Trichomonadida and Honigbergiellida. The first three classes contain only trichomonads, whereas all members of Trichonympha and Spirotrichonympha were hypermastigotes; Cristamonadea and Honigbergiellida contain both trichomonads and hypermastigotes (though only a single hypermastigid species, \textit{Cthulhu macrofasciculumque}, belongs to the latter). The system of six classes was adopted in the recently revised classification of eukaryotes (Adl et al. 2012).

Establishing an internal taxonomy for Cristamonadea has proven problematic. Molecular phylogenies have been unable to resolve the internal relationships. Similarly, while morphological and ultrastructural features are able to circumscribe individual genera, few characters have been identified to group genera into families. Finally, some of the traditional families have proven non-monophyletic. For these reasons, all genera of Cristamonadea were lumped into a single family, Lophomonadidae (Cepicka et al. 2010). However, it was later shown that the genus \textit{Lophomonas} is not related to the rest of Cristamonadea, but instead forms a sister lineage to Trichonympha (Gile and Slamovits 2012), making Lophomonadidae and Cristamonadea sensu Cepicka et al. (2010) polyphyletic.
The problem of the polyphyly of Cristamonadea was addressed in a recent system by Cavalier-Smith (2013). He divided Parabasalia (which he treated as a superclass) into two classes, Trichomonadea and Trichonymphea. Trichomonadea was further divided into subclasses Eotrichomonadea (order Trichomonadida with suborders Trichomonadina and Honigbergiellina and order Tritrichomonadina) and Cristamonadea (orders Cristamonadida and Spirotrichonymphida). Trichonymphea was divided into orders Trichonymphida and Lophomonadida; the latter consisted of the genus *Lophomonas*. However, according to the current understanding of the evolution of the phylogeny of Parabasalia, several taxa of this system are paraphyletic or polyphyletic (Cavalier-Smith 2013).

Here, we mostly follow the system of Cepicka et al. (2010) and divide Parabasalia into six classes: Trichomonadea, Tritrichomonadea, Hypotrichomonadea, Cristamonadidea, Spirotrichonymphida, and Trichonymphea. We also recognize the order Lophomonadida sensu Cavalier-Smith (2013) (within Trichonymphea) as well as the family Lophomonadidae containing *Lophomonas* and Joeniidae as the only family of Cristamonadida comprising all genera contained in Lophomonadidae sensu Cepicka et al. 2010 except *Lophomonas* itself. The detailed taxonomy used here is as follows:

Class Trichomonadea
   Order Trichomonadida
      Family Trichomonadidae (*Cochlosoma, Lacusteria, Pentatrichomonas, Pentatrichomonoides, Pseudotrichomonas, Pseudotrypanosoma, Tetra-trichomonas, Trichomitopsis, Trichomonas, Trichomonoides*)
   Order Honigbergiellida
      Family Honigbergiellidae (*Ditrichomonas, Honigbergiella, Monotrichomonas*)
      Family Hexamastigidae (*Hexamastix, Tetratrichomastix*)
      Family Tricercomitidae (*Tricercomitus*)
      Unplaced genera *Cthulhu* and *Cthylla*

Class Tritrichomonadea
   Order Tritrichomonadidae
      Family Tritrichomonadidae (*Tritrichomonas*)
      Family Dientamoebidae (*Dientamoeba, Histomonas, Parahistomonas, Protrichomonas*)
      Family Monocercomonadidae (*Monocercomonas*)
      Family Simplicimonadidae (*Simplicimonas*)

Class Hypotrichomonadea
   Order Hypotrichomonadida
      Family Hypotrichomonadidae (*Hypotrichomonas, Trichomitius*)
Class Cristamonadea
  Order Cristamonadida
    Family Joeniidae (*Achemon*, *Astronympha*, *Bullanympha*, *Caduceia*,
    *Calonympha*, *Coronympha*, *Criconympha*, *Cyclojoenia*,
    *Deltotrichonympha*, *Devescova*, *Diplonympha*, *Evemonya*, *Foaina*, *Gigan-
    tomonas*, *Gyronympha*, *Hyperdevescova*, *Joenia*, *Joenia*, *Joenoides*,
    *Joenopsis*, *Kirbyina*, *Kofoidia*, *Koruga*, *Macrotrichomonas*, *Macro-
    trichomonoides*, *Metadevescova*, *Mixotricha*, *Pachyjoenia*, *Parajoenia*,
    *Parajoenopsis*, *Placojoenia*, *Polymastigotoides*, *Projoenia*, *Prosnyderella*,
    *Pseudodevescova*, *Rhizonympha*, *Snyderella*, *Stephanonympha*)

Class Spirotrichonymphea
  Order Spirotrichonymphida
    Family Holomastigotoididae (*Holomastigotes*, *Holomastigotoides*, *Micro-
    joenia*, *Micromastigotes*, *Rostronympha*, *Spiromastigotes*, *Spironympha*,
    *Spirotrichonympha*, *Spirotrichonymphella*, *Uteronympha*)

Class Trichonymphea
  Order Trichonymphida
    Family Trichonymphidae (*Trichonympha*)
    Family Hoplonymphidae (*Barbulanympha*, *Hoplonympha*, *Rhynchonympha*,
    *Urinympa*)
    Family Staurojoeninidae (*Idionympha*, *Staurojoenina*)
    Family Teranymphidae (*Eucomonympha*, *Pseudotrichonympha*, *Teranympa*)
    Family Spirotrichosomidae (*Apospironympha*, *Bispironympha*,
    *Colospironympha*, *Leptospironympha*, *Macrosplironympha*,
    *Spirotrichosoma*)

Order Lophomonadida
  Family Lophomonadidae (*Lophomonas*)

Parabasalian genera *Incertae sedis*
  *Trichocovina* (Trichomonadida or Tritrichomonadida or Cristamonadida)
  *Prolophomonas* (Lophomonadida or Cristamonadida)
  *Eulophomonas* (Lophomonadida or Cristamonadida)
  *Chilomitus* (formerly Monocercomonadidae)
**Maintenance and Cultivation**

Many trichomonad species from vertebrates (including most species from humans) and some species from non-termite insects can be maintained relatively easily in polyxenic cultures with bacteria, using various media such as Dobell and Laidlaw’s biphasic medium (Dobell and Laidlaw 1926) or liquid medium TYSGM-9 (Diamond 1982). Trichomonads from mammals and birds are maintained at 42 °C and are subcultured approximately every third day; those isolated from poikilotherms and insects are maintained at room temperature and are subcultured approximately once a week (Cepicka et al. 2006). Free-living trichomonads such as *Pseudotrichomonas keilini*, *Tetratrichomonas undula*, or *Ditrichomonas honigbergii* were also cultured in Dobell and Laidlaw’s biphasic medium or TYSGM-9 (Farmer 1993; Cepicka et al. 2006; Yubuki et al. 2010), though various media used for free-living protists such as Sonneborn’s *Paramecium* medium (ATCC medium 802), its 9:1 mixture with TYSGM-9, or 5% PYNFH medium (ATCC medium 1034) have been used as well (Yubuki et al. 2010).

Several species from vertebrates, such as *Trichomonas vaginalis*, *Pentatrichomonas hominis*, *Tririchomonas foetus*, *Trichomitus batrachorum*, *Hypotrichomonas acosta*, *Monocercomonas colubrorum*, and *Simplicimonas moskowitzi* can be cultured axenically in the TYM medium. The pH of the medium is usually adjusted to 7.2; for *Trichomonas vaginalis*, the optimum pH is 6.2. Most cultured species can be easily cryopreserved. Cells at early-stationary or late-log growth phase are supplemented by DMSO to a final concentration of 5%. The suspension is then cooled at 6 to 8 °C per minute to the point of release of the latent heat of fusion. Then, the suspension is subjected to rapid cooling to take the organisms past the latent heat of fusion zone within 1.5 minutes. Then, the suspension is cooled at the rate of 1 to 2 °C per minute to −60 °C, and then it is immersed in liquid nitrogen (Honigberg and Burgess 1994).

As obligate anaerobic symbionts, often with bacterial symbionts of their own, the parabasalians of the termite gut have proven difficult to culture. Only a few termite gut parabasalians, such as *Trichomitopsis termopsidis* (Cleveland) from *Zootermopsis angusticollis*, have been brought into axenic culture (Yamin 1978; Odelson and Breznak 1985). None has been cultured on a defined medium. In brief, a buffered salt solution (pH 6.9) is used: K₂HPO₄, 10.8 mM; KH₂PO₄, 6.9 mM; KCl, 21.5 mM; NaCl, 24.5 mM; MgSO₄, 5.2 mM; and CaCl₂, 0.53 mM. To this solution is added 0.1% (w/v) cellulose particles small enough for the cells to ingest. This salt solution supplemented with cellulose is boiled and then cooled, while bubbling with O₂-free N₂. The solution is poured into tubes and sealed under N₂ with rubber stoppers and autoclaved. N₂-flushed plastic syringes are used to add the following after autoclaving: NaHCO₃ to 10 mM and heat-inactivated fetal calf serum to 2.5% (v/v). After surface-sterilizing the termite with 70% ethanol, the hindgut is removed by forceps and broken open with a syringe plunger tip. The plunger is inserted in the syringe, which is flushed with N₂. The syringe is used to draw up medium from the tube and then to plunge the medium, with protist cells, back into the tube. Cultures are incubated at 27 °C with subcultures made every 2–4 weeks (Yamin 1978).
Evolutionary History

External Relationships

Parabasalia belongs to the Metamonada clade within the Excavata supergroup, a supergroup whose members are characterized in part by a feeding groove (Simpson 2003). Parabasalia is one of the three major subclades of metamonads, along with Preaxostyla (comprising Oxymonada and trimastigids) and Fornicata (comprising Diplomonadida, Retortamonadida, and Carpediemonas-like organisms) (Simpson 2003; Adl et al. 2012; Zhang et al. 2015). Though the Parabasalia and Oxymonada have lost the ancestral excavate-type feeding groove, molecular phylogenetic evidence links them to their more plesiomorphic relatives in Fornicata and the trimastigids. Multigene phylogenies suggest that Fornicata is the sister group of Parabasalia, while Preaxostyla is the deepest branch in the clade (Hampl et al. 2005, 2009; Katz and Grant 2015).

Metamonads are mostly anaerobic gut commensals. The most recent ancestor of Parabasalia was probably a gut commensal, and the free-living species are secondarily adapted to life outside an animal host. However, the free-living species have not yet been included in rooted, multigene analyses, so the possibility that they might form the deepest branches cannot be completely excluded (Hampl et al. 2007; Noda et al. 2012). The other two metamonad lineages have deep-branching, free-living representatives, e.g., Trimastix in Preaxostyla and Carpediemonas in Fornicata (Kolisko et al. 2008; Zhang et al. 2015), so it is likely that Metamonada as a whole is ancestrally free-living.

Parabasalia is likely a relatively young phylum. Parabasalia certainly predates the origin of termites, which has been dated back to the Jurassic/Cretaceous boundary, roughly 150 million years ago (Misof et al. 2014; Bourguignon et al. 2015). This provides a minimum age for the group. There are no sound estimates to provide a maximum age: molecular clock age estimates have not yet been applied to the Parabasalia specifically, and such age estimates for Excavata are highly method sensitive and range between 900 million and 1.8 billion years (Parfrey et al. 2011; Eme et al. 2014). Parabasalians attributable to the orders Trichonymphida, Cristamonadida, and Spirotrichonymphida have been described from 100-million-year-old early Cretaceous amber (Poinar 2009).

Internal Relationships

Inferences of character evolution in Parabasalia depend largely on the position of the root for the clade, an inference that eluded molecular phylogenetic analyses for years (Hampl et al. 2004). Outgroup rooting with SSU rDNA or protein sequences failed to find a supported position for the root but tended to place the root near or within the Trichonymphida (Keeling et al. 1998; Ohkuma et al. 2000, 2007a). This position is clearly artifactual, deriving from the attraction of the long stem branch of Parabasalia to the long branches of the Trichonymphida (Keeling and Palmer 2000; Hampl et al.
Also, the Trichonymphida rooting contradicts morphology-based scenarios in which the simpler, smaller cells are considered most similar to the ancestral form (Kirby 1947; Honigberg 1963; Brugerolle 1976). With the addition of elongation factor 1-alpha sequences to multi-protein analyses, a different root position was inferred, between the clade of Trichomonadida and Trichonymphida on one hand and Hypotrichomonadida, Spirotrichomonadida, Tritrichomonadida, and Cristamonadida on the other (see Fig. 8). Honigbergiellida, Lophomonadida, and free-living members of Trichomonadida were not included in the analysis (Noda et al. 2012). This root position results in simpler parabasalians forming the deeper branches and the complex hypermastigotes arising later, a more intuitively plausible scenario (Fig. 8).

The ancestral morphology of parabasalians under this rooting was therefore likely similar to *Trichomitus* and *Hypotrichomonas* (Hypotrichomonadida): small cells with four flagella, a costa, and a lamelliform undulating membrane (Cepicka et al. 2010). Variations on this body plan have taken different directions among the simpler parabasalians. The undulating membrane has been lost several times, for example, in *Honigbergiella*, *Simplicimonas*, *Monocercomonas*, and *Dientamoebidae*, and altered to a rail type in Tritrichomonadida (Brugerolle 1976; Cepicka et al. 2010). Flagellar number is particularly changeable among trichomonads and has increased to five or six in the Trichomonadida and Honigbergiellida and reduced to three or two in certain Honigbergiellida and to zero in *Dientamoeba*, with an anomalous increase to at least 20 flagella in *Cthulhu* (James et al. 2013).

It is in the hypermastigote taxa that the most impressive morphologies have evolved. Though traditionally united on the basis of many flagella but just one nucleus, molecular phylogenetic analyses have demonstrated the polyphyly of hypermastigotes. While trichonymphids and spirotrichonymphids are each monophyletic groups, their complex multi-flagellate morphologies evolved independently of each other. Lophomonads, on the other hand, are actually polyphyletic. *Lophomonas* forms the sister lineage to trichonymphids (Gile and Slamovits 2012); *Kofoidia* is closely related to the cristamonad genera *Devescovina* and *Metadevescovina*, which have trichomonad cell organization (Tai et al. 2014); and the rest, genera such as *Joenia*, *Joenia*, *Joenoides*, and *Deltotrichonympha*, branch separately near the base of the Cristamonadida (Ikeda-Ohtsubo et al. 2007; Noda et al. 2009).

The evolutionary tendency to multiply flagella is restricted to parabasalians that live in the termite or roach hindgut. It is difficult to count the number of distinct flagellar multiplication events in Parabasalia because relationships among cristamonads are not resolved, but within the boundaries of this uncertainty, there must have been at least five and possibly more than seven distinct instances (not including cases of nuclear multiplication). This number includes the recently described genus *Cthulhu*, which bears at least 20 flagella and branches with *Hexamastix* and *Cthylla* in the Honigbergiellida (James et al. 2013). The termite/roach hindgut environment also appears to favor evolutionary increases in cell size, as, for example, in the large (50–100 μm long) trichomonad *Trichomitopsis termopsidis* (Keeling 2002) and in cristamonads such as *Devescovina* and *Macrotrichomonas*.
**Fig. 8** Schematic phylogenetic tree of *Parabasalia* based on multiple molecular phylogenetic analyses (see text for details). The eight orders according to this scheme, based on Čepička et al. (2010) and Cavalier-Smith (2013), are indicated to the right. Circles at tips indicate habitat: genera with open circles are exclusive to termite and/or cockroach hindguts. Black circles indicate genera with free-living species. Colors indicate a broad host range of described species, yellow for vertebrates and green for vertebrates and invertebrates. Asterisks indicate genera in which one or more species has been found in humans. Bold type indicates “hypermastigote” genera, i.e., parabasalians with many flagella.
that have only four flagella despite reaching lengths of 80–90 μm (Brugerolle and Lee 2000; Gile et al. 2015). Meanwhile, parabasalians that are not restricted to the roach or termite hindgut tend to remain small (under 30 μm, usually under 20 μm) and retain six or fewer flagella (Brugerolle and Lee 2000).

Intergeneric relationships in the Parabasalia are resolved to differing degrees in different parts of the tree. The schematic representation of these relationships (Fig. 8) is a synthesis of results from multiple phylogenetic analyses: some using protein-coding sequences (Gerbod et al. 2004; Ohkuma et al. 2007; Cepicka et al. 2010; Noda et al. 2012), but most using SSU rDNA (Gerbod et al. 2002; Hampl et al. 2004; Hampl et al. 2006; Noël et al. 2007; Noda et al. 2009; Carpenter et al. 2010; Cepicka et al. 2010; Yubuki et al. 2010; Gile et al. 2011; Gile and Slamovits 2012; Tai et al. 2014; Gile et al. 2015). Most genera with at least some molecular data are included in the figure, but many important genera have yet to be included in molecular phylogenetic analyses and are not represented. Some evolutionary trends in Parabasalia are also indicated in Fig. 8. Multiplications of flagella (hypermastigote genera) are indicated by bold text. Termite and cockroach gut residents are indicated by open circles. Note that the orders Cristamonadida, Spirotrichonymphida, and Trichonymphida have radiated entirely within this habitat (Lophomonadida are from cockroaches but not termites). Many trichomonad genera have broad host ranges, with species found across vertebrates (yellow circles) or across vertebrates and invertebrates, in some cases including the termite/roach hindgut (green circles). The few free-living species belong to genera indicated by black filled circles.

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