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ORIGINAL ARTICLE

Updated classification of the phylum Parabasalia

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Abstract

The phylum Parabasalia includes very diverse single-cell organisms that nevertheless share a distinctive set of morphological traits. Most are harmless or beneficial gut symbionts of animals, but some have turned into parasites in other body compartments, the most notorious example being *Trichomonas vaginalis* in humans. Parabasalians have garnered attention for their nutritional symbioses with termites, their modified anaerobic mitochondria (hydrogenosomes), their character evolution, and the wholly unique features of some species. The molecular revolution confirmed the monophyly of Parabasalia, but considerably changed our view of their internal relationships, prompting a comprehensive reclassification 14 years ago. This classification has remained authoritative for many subgroups despite a greatly expanded pool of available data, but the large number of species and sequences that have since come out allow for taxonomic refinements in certain lineages, which we undertake here. We aimed to introduce as little disruption as possible but at the same time ensure that most taxa are truly monophyletic, and that the larger clades are subdivided into meaningful units. In doing so, we also highlighted correlations between the phylogeny of parabasalians and that of their hosts.

KEYWORDS

Cristamonadea, *Cthulhu*, Cthulhuidae, Lophomonadea, *Nyarlathotep*, parabasalians, Spirotrichonymphea, taxonomy, Trichomonadea, Trichonymphea

INTRODUCTION

THE phylum Parabasalia includes morphologically diverse flagellate, amoeboid, and amoeboflagellate organisms mostly living inside animals (Brugerolle & Lee, 2000; Čepička et al., 2010; Čepička et al., 2017), with an especially successful radiation in the wood-eating non-Termitidae (often referred to as "lower") termites and the cockroach *Cryptocercus*. Parabasalians have been known and studied for two centuries (Čepička et al., 2017; Donné, 1836) due to their ecological, evolutionary, and medical importance. All anaerobes or microaerophiles, parabasalians were among the groups proposed to be ancestrally amitochondriate eukaryotes (or "Archezoa," Cavalier-Smith, 1987), until the discovery that one of their organelles, the hydrogenosome (Müller, 1993), is a modified mitochondrion (Bui et al., 1996; Germot et al., 1996; Roger et al., 1996). Hydrogenosome metabolism is itself an object of interest for its potential in bioproduction of hydrogen gas and for its syntrophic interactions with intracellular methanogens (Brune, 2014; Ohkuma, 2008). The ecological relevance of parabasalians is also tied to their role in termite biology: termites are so abundant, and their microbial symbiotic consortia so integral to their metabolism, that they are responsible for a significant amount of greenhouse methane in the atmosphere (Zanne et al., 2022). On the other end of the symbiotic spectrum, the parasitic

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Trichomonas vaginalis is notorious as the most common sexually transmitted disease in humans (Alsaad, 2022); some of its relatives are pathogenic in other mammals and birds (Amin et al., 2014; Petrželková et al., 2019). Individual parabasalian species have also drawn attention for puzzling and largely unexplored aspects of their biology. For example, they evolved rotating structures, something long touted as a rarity in biological evolution only ever achieved in bacterial flagella (Dawkins, 1996; Gould, 1983). One species of *Pseudotrichonympha* possesses a constantly rolling organelle of unknown function and ultrastructure (del Campo et al., 2017), and a few cristamonads rotate a whole portion of their cell relative to the rest (incidentally, a discovery that helped proving the fluid mosaic model), somehow without ripping apart the plasma membrane at the shear zone (Hehenberger et al., 2023; Tamm & Tamm, 1974).

The monophyly of Parabasalia is unquestioned and supported by a distinctive set of traits including hydrogenosomes, closed pleuromitosis, and the "parabasal body," an easily stained Golgi body supported by a unique cytoskeletal element, the parabasal fiber. Most parabasalians also share additional features, like the microtubular axostyle-pelta complex and the pattern of basal bodies and basal body-associated fibers. However, the internal classification of the phylum changed considerably with the rise of molecular phylogeny, and in interesting ways (Čepička et al., 2010; Céza et al., 2022; Gile & Slamovits, 2012; Keeling et al., 1998; Noda et al., 2012; Ohkuma et al., 2000). Classically, parabasalians were split according to two main morphotypes: "simple" organisms with few flagella closely associated to the nucleus, the "trichomonads," vs. large, complex organisms with dozens to thousands of flagella, the "hypermastigids" (Brugerolle & Lee, 2000). The idea that "hypermastigids" belong to a single radiation has been disproved, and trait evolution in Parabasalia is now understood to be more convoluted and involve parallel increases and decreases in morphological complexity.

A sound classification that reflects phylogeny is essential to carry out accurate character evolution analyses and is an important tool to understand evolution more generally. Two parabasalian taxonomic systems have been especially influential in the last decades: the classification of Brugerolle and Lee (2000) and that of Čepička et al. (2010). Both were impressive achievements significantly advancing the knowledge of the time and providing insightful evaluation of available evidence. The former organized and illuminated the relationships between morphological structures (and valuably catalogued authorities for various taxa), while the latter reconciled as much as possible classical insights with the upheaval caused by molecular trees. Much of the structure of the Čepička et al. (2010) classification still stands, despite the substantial increase in available data, but the number of new sequences has now rendered some of the taxa nonmonophyletic, or, conversely, created the opportunity to clarify the relationships within certain speciose and diverse clades (cristamonads and spirotrichonymphids), for which data were particularly sparse in 2010. Moreover, two new major lineages of parabasalians, the molecularly divergent pimpavickids (Céza et al., 2022) and a previously unnamed but morphologically distinct group including the genus *Cthulhu* (James, Okamoto, et al., 2013), have also been discovered since then. Here, we report a new organism related to *Cthulhu*, update the current classification of phylum Parabasalia to reflect the changes over the past 14 years, and highlight the importance of host identity as a diagnostic trait in parabasalian taxonomy, a topic that has been somewhat neglected in the past.

MATERIALS AND METHODS

Termite collection and identification

New data were generated from symbionts of three termite species. *Glyptotermes* sp. (Kalotermitidae; the species morphologically closest to the collected specimen is *Glyptotermes adamsoni*) was collected in Bolivia (coordinates: 17°29′56.15″ S, 63°39′8.78″ W); *Zootermopsis angusticollis* (Archotermopsidae) was collected in British Columbia, Canada (coordinates: 49°15′34.99″ N, 123°15′12.45″ W); *Procryptotermes hesperus* (Kalotermitidae) was collected in the Bahamas (coordinates: 26°41′21.05″ N, 78°58′4.80″ W). Termites were identified morphologically by R. H. Scheffrahn, and preserved representative specimens for *Glyptotermes* sp. and *Procryptotermes hesperus* are deposited at the University of Florida termite collection (https://www.termitediversity.org; codes: BO313 and BA2643, respectively).

New parabasalian morphological observations and SSU rRNA gene sequences

Single cells of parabasalians morphologically reminiscent of the genus *Cthylla* (James, Okamoto, et al., 2013) observed in the dissected hindguts of *Glyptotermes* sp. were individually collected, imaged, and stored for molecular analyses as described in detail elsewhere (Boscaro et al., 2017; del Campo et al., 2017). DNA extractions on these isolated cells were performed using the Masterpure Complete DNA and RNA Purification Kit (Epicenter, Madison, USA). SSU rRNA genes were amplified by PCR using primers PFI (Keeling, 2002) and FAD4 (Medlin et al., 1988), with the following PCR thermal profile: initial denaturation at 95°C (3 min); 30 cycles at 95°C (30 s), 55°C (30 s), and 72°C (90 s); final elongation at 72°C (7 min). PCR products were cloned using the TOPO-TA kit (Invitrogen, Carlsbad, USA) and Sanger-sequenced with BigDye Terminator v 3.1 (Applied Biosystem, Carlsbad, USA) at an in-house facility.

DNA extractions, PCR amplifications of the SSU rRNA gene, and cloning were performed using the same protocols, but starting from whole-gut material, in *Zootermopsis angusticollis* and *Procryptotermes hesperus*. All sequences discussed in the paper have been deposited in GenBank (accession numbers: PP297451-4).

Phylogenetic inference

A comprehensive dataset of 600 available parabasalian SSU rRNA gene sequences was assembled from publicly available data. Manual cleaning and selection to remove redundant and/or low-quality sequences produced a "species" database of 280 sequences, with approximately one representative per named species as well as many environmental sequences. Then, 15 sequences were flagged as "fast-evolving" based on their very long branches compared to relatives in preliminary phylogenetic analyses. The dataset excluding these sequences was labeled "species_nofast." A smaller "genus" database of 82 sequences was also assembled, with approximately one representative sequence per described genus, plus environmental sequences not closely related to any genus. Alignments were performed with MAFFT v. 7.520 (Katoh & Standley, 2013) using the -linsi option and removing columns with missing data at the beginning and end of the matrix, but without further masking fast-evolving sites (final alignment lengths: 1916, 1867, and 1590 columns for the "species," "species_nofast," and "genus" alignments, respectively). Maximum Likelihood analyses were performed with IQ-TREE v. 1.6.12 (Nguyen et al., 2015) using the GTR+F+I+G4 model (as suggested by the BIC parameter) and 1000 nonparametric bootstrap pseudoreplicates. All trees are unrooted (see Discussion).

RESULTS AND DISCUSSION

Preliminary considerations on the new classification of Parabasalia, with notes on two elusive genera: *Hexamastix* and *Tricercomitus*

Our new classification (Figure 1) follows in the footsteps of its two most influential predecessors (Brugerolle & Lee, 2000; Čepička et al., 2010); to save space, we will refer to the Brugerolle and Lee classification as "BL00," and the Čepička et al. classification as "C10." Other essential resources consulted for this paper were the metadata collections by Yamin (1979) and Gile (2024). A detailed discussion of an alternative classification suggested by Cavalier-Smith (2013) is not provided because that system, which changed the rank of many taxa present in C10 and assumed relationships that were not supported by phylogenetic trees (e.g., the monophyly of all "trichomonads" and their position as sister group of cristamonads), was not widely adopted. The next sections will start with a brief overview of the previous state

PHYLUM **Parabasalia**
- CLASS **Hypotrichomonadea**
    - ORDER **Hypotrichomonadida**
        - FAMILY **Hypotrichomonadidae**
- CLASS **Pimpavickea**
    - ORDER **Pimpavickida**
        - FAMILY **Pimpavickidae**
- CLASS **Trichomonadea**
    - ORDER **Honigbergiellida**
        - FAMILY **Hexamastigidae**
        - FAMILY **Honigbergiellidae**
        - FAMILY **Cthulhuidae**
        - FAMILY **Tetratrichomastigidae**
    - ORDER **Trichomonadida**
        - FAMILY **Lacusteriidae**
        - FAMILY **Trichomonadidae**
- Clade "**Tla**"
    - CLASS **Lophomonadea**
        - ORDER **Lophomonadida**
            - FAMILY **Lophomonadidae**
    - CLASS **Trichonymphea**
        - ORDER **Trichonymphida**
            - FAMILY **Barbulanymphidae**
            - FAMILY **Hoplonymphidae**
            - FAMILY **Retractinymphidae**
            - FAMILY **Spirotrichosomidae**
            - FAMILY **Staurojoeninidae**
            - FAMILY **Teranymphidae**
            - FAMILY **Trichonymphidae**
- Clade "**Cadamassta**"
    - CLASS **Cristamonadea**
        - ORDER **Calonymphida**
            - FAMILY **Calonymphidae**
            - FAMILY **Deltotrichonymphidae**
            - FAMILY **Mixotrichidae**
        - ORDER **Devescovinida**
            - FAMILY **Devescovinidae**
        - ORDER **Gigantomonadida**
            - FAMILY **Gigantomonadidae**
    - CLASS **Dientamoebea**
        - ORDER **Dientamoebida**
            - FAMILY **Dientamoebidae**
    - CLASS **Monocercomonadea**
        - ORDER **Monocercomonadida**
            - FAMILY **Monocercomonadidae**
    - CLASS **Simplicimonadea**
        - ORDER **Simplicimonadida**
            - FAMILY **Simplicimonadidae**
    - CLASS **Tritrichomonadea**
        - ORDER **Tritrichomonadida**
            - FAMILY **Tritrichomonadidae**
    - CLASS **Spirotrichonymphea**
        - ORDER **Cononymphida**
            - FAMILY **Cononymphidae**
        - ORDER **Holomastigotoidida**
            - FAMILY **Holomastigotoididae**
        - ORDER **Spirotrichonymphida**
            - FAMILY **Brugerollinidae**
            - FAMILY **Fraterculidae**
            - FAMILY **Holomastigotidae**
            - FAMILY **Spirotrichonymphidae**

FIGURE 1 Updated classification of the phylum Parabasalia, with 11 classes, 16 orders, and 31 families. See text for lists of genera and diagnostic traits.

of affairs for each group, followed by the adjustments suggested by our analyses (Figure 2 and Figure S1).

Two genera of uncertain status and with conflicting molecular barcodes are, however, more easily discussed first and together: the "trichomonads" *Hexamastix* and *Tricercomitus*. *Hexamastix* has up to five forward-pointing flagella and a nonadherent or partially adherent recurrent flagellum, and a well-developed and protruding axostyle. It was assigned to the "morphologically simplest" family of "trichomonads," Monocercomonadidae, in BL00. As part of an extensive rearrangement of former "trichomonads," C10 established the family Hexamastigidae to highlight the molecular divergence of the three *Hexamastix* sequences available (attributed to *Hexamastix mitis*, *Hexamastix coercens*, and *Hexamastix kirbyi*), which fell within Order Honigbergiellida in Class Trichomonadea. The authors also argued that *Tetratrichomastix* could be a synonym of *Hexamastix*, from which it differs only in the number of anterior flagella (see also Brugerolle et al., 2003), and placed both in the same family. The same experts have more recently recharacterized *Tetratrichomastix* (but from free-living strains rather than in the insect type host, *Tipula*) and found that it in fact branches in a different position within Honigbergiellida (Céza et al., 2022). There are two remaining issues. First, *Hexamastix* has been reported from a very broad host range including vertebrates and insects (Honigberg & Christian, 1954; Kirby, 1930; Nie, 1950). While this is not necessarily unique among Parabasalia (see below), it should suggest caution, since the three barcoded *Hexamastix* species considered by C10 come from reptiles and rodents, while the type species, *Hexamastix batrachorum*, lives in an amphibian (Honigberg & Christian, 1954). The second issue is that other *Hexamastix* species were described from the termite *Zootermopsis* (Archotermopsidae) (Kirby, 1930), and short-read sequences from symbionts of *Zootermopsis laticeps* and *Zootermopsis nevadensis* obtained by Taerum et al. (2018) fall within Honigbergiellida, but not sister to other *Hexamastix*. We report here an almost identical (99.2%), longer sequence from *Zootermopsis angusticollis*, confirming that the small "trichomonads" of *Zootermopsis* do not form a monophyletic group with the sequenced *Hexamastix* from vertebrates (Figure 2). In our classification scheme we choose for practical reasons to follow C10 and keep the family Hexamastigidae (albeit without *Tetratrichomastix*) for *H. mitis*, *H. coercens*, and *H. kirbyi*, whose hosts are phylogenetically closer to the *Hexamastix* type host, but with the caveat that the genus is likely polyphyletic. Family Hexamastigidae as currently defined should only be kept if, once barcoded, *H. batrachorum* happens to branch with *H. mitis*, *H. coercens*, and *H. kirbyi*, and should otherwise be dismissed to generate less confusion once the genus is split. The organism currently called *Hexamastix* in termites will probably require reclassification when more data become available.

Tricercomitus has a similarly confusing history. A tiny (5–10 μm) flagellate with three forward-pointing flagella and a very long recurrent flagellum that adheres to the cell body and trails posteriorly, it was also assigned to Monocercomonadidae in BL00 and elevated to family status (Tricercomitidae) within Honigbergiellida in C10. The reference barcode was one tentatively assigned to *Tricercomitus* by previous authors (Ohkuma et al., 2000), who retrieved two similar environmental sequences from the hindguts of *Cryptotermes* and *Glyptotermes* (Kalotermitidae). While species of *Tricercomitus* have been described from kalotermitids (de Mello, 1952; Kirby, 1930), the type species *Tricercomitus termopsidis* was found in *Zootermopsis angusticollis* (Archotermopsidae) (Kirby, 1930). Taerum et al. (2018) assigned a clade of short reads from multiple *Zootermopsis* hosts to *Tricercomitus*, and we replicate here their findings with a longer but very similar (99.2%) sequence from *Z. angusticollis*. This clade of *Zootermopsis* symbionts falls in a completely different part of the parabasalian tree than the sequences from kalotermitids (Figure 2), specifically as close relatives of *Simplicimonas*. *Tricercomitus* and *Simplicimonas* are superficially similar, although the recurrent flagellum of *Simplicimonas* is only partially adhering to the cell body and is somewhat shorter, while its axostyle is stouter than that of *Tricercomitus*. However, these broad morphological traits are wholly insufficient to determine higher-level relationships among small "trichomonads" (Čepička et al., 2010; and see below). It is likely that what was considered a single parabasalian genus hosted by different termites might be a polyphyletic group of morphologically similar organisms. We agree with the interpretation of Taerum et al. (2018) that the sequences from *Zootermopsis* represent the "true" *Tricercomitus*, since they come from the type host. However, this parabasalian clearly requires more investigation, so we do not establish any above-genus taxon to accommodate *Tricercomitus*, and leave it *incertae sedis* in the phylum. The C10 family Tricercomitidae based on the sequences from kalotermitids is not included in our classification.

Morphology standing the test of time: Trichonymphea

Trichonymphids are large (60–400 μm) "hypermastigids" with a single nucleus, hundreds or thousands of flagella, inconspicuous and diffused axostylar fibers, and differentiated rostral and postrostral regions. The rostrum always bears flagella and is bilaterally symmetrical. At division, each daughter cell inherits one hemirostrum and its flagella, which are retained, then reconstitutes the symmetry. The trichonymphid clade (albeit assigned to various ranks) has been recognized for a long time (Cavalier-Smith, 2003, 2013; Grimstone & Gibbons, 1966; Hollande & Carruette-Valentin, 1971)

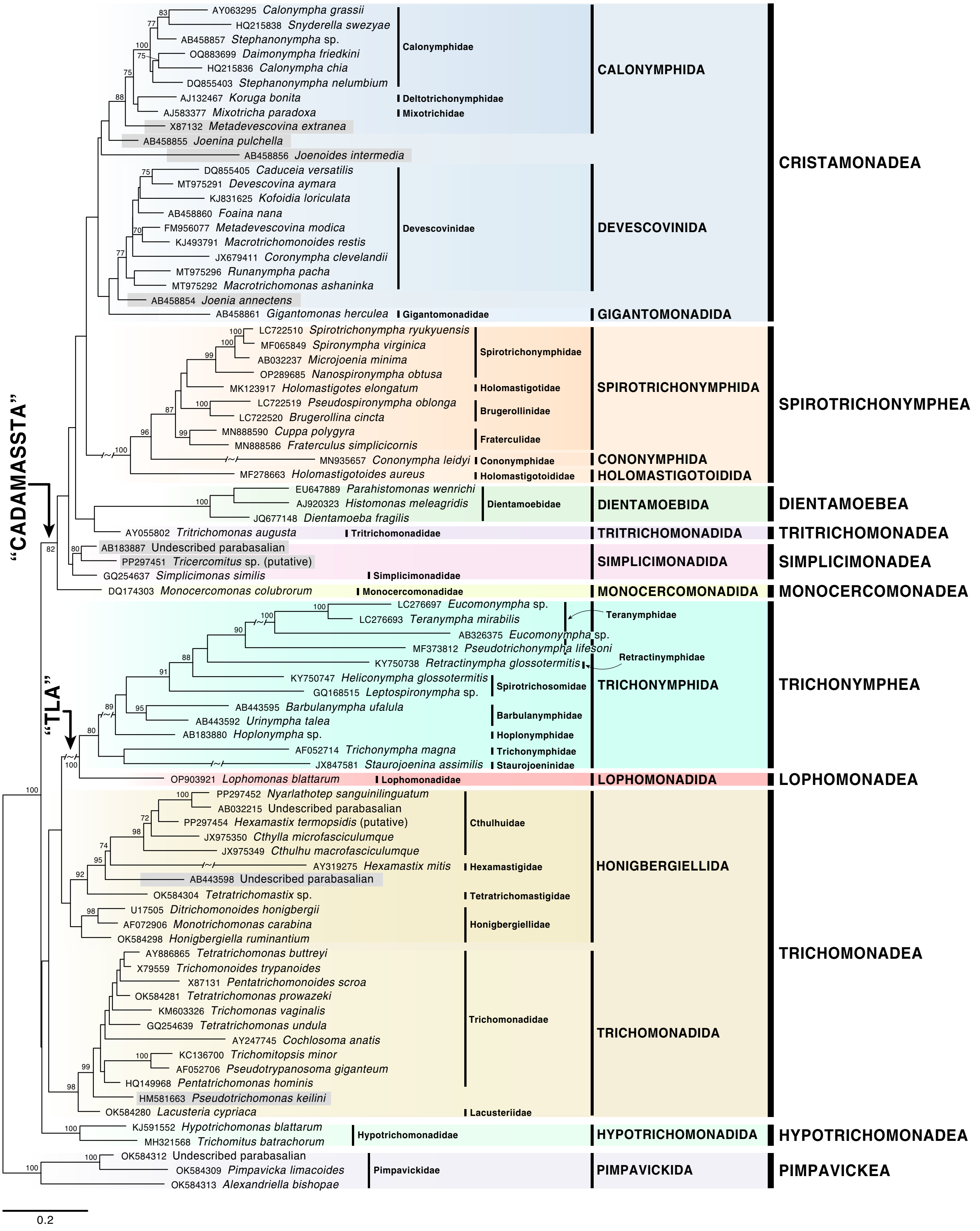

FIGURE 2 Unrooted Maximum Likelihood phylogeny of phylum Parabasalia, based on the "genus" dataset. Nonparametric bootstrap values are reported next to nodes; values below 70% are omitted. The updated classification is superimposed on the tree. Taxa *incertae sedis* within an order (i.e., not assigned to a family) or a class (i.e., not assigned to an order) are shown in gray boxes. A few branches were shortened (as indicated by a break in the branch) for space reasons.

and is consistently recovered in molecular phylogenies (Carpenter et al., 2009; Carpenter & Keeling, 2007; Čepička et al., 2010; Céza et al., 2022; Noda et al., 2012). Trichonymphid families are also long-standing (except for the recently established Retractinymphidae, Radek et al., 2023), with minimal changes occurring from the BL00 to the C10 classifications, and are morphologically well-defined, distinguished by characters like the presence of a rostral tube, the number of rostral flagellar areas, the presence and arrangement of flagella in the postrostral region, and the shape and size of parabasal bodies.

Our phylogenetic analysis confirms, with high support, the monophyly of Class Trichonymphea (Figure 2). Trichonymphea families are also recovered as monophyletic, with two exceptions. Firstly, the three molecularly barcoded genera in family Hoplonymphidae do not form a clade (see also Carpenter et al., 2011). This fits with the fact that the type genus, *Hoplonympha*, is morphologically different from the other genera of Hoplonymphidae, having a slender cell body characterized by vertical furrows and ridges that are absent from *Barbulanympha*, *Urinympha*, and the yet unsequenced *Rhynchonympha* (Brugerolle & Lee, 2000; Mee et al., 2019). Moreover, *Hoplonympha* has only been observed in termites, while the other genera are symbionts of *Cryptocercus*. We hence transfer the hoplonymphids of *Cryptocercus* to their own family, Barbulanymphidae fam. nov. A somewhat similar situation involves the morphologically distinctive family Spirotrichosomidae, in which the postrostral flagella are arranged in two main left-hand spiraling bands (and thinner "secondary" flagellar rows that are usually lost during cell division). No sequence was available at the time of C10, but the two genera currently barcoded (*Leptospironympha* and *Heliconympha*) are not sister lineages. Indeed, both Carpenter et al. (2010) and Radek et al. (2018) have argued for a possible split between two spirotrichosomid groups. However, five other described genera in the family, including the type genus, *Spirotrichosoma*, have not been molecularly characterized yet, and the support for the nonmonophyly of the family is not strong in some of our analyses. Accordingly, we feel this group is likely to require revision but decided it is not yet advisable to split it, at the very least until a sequence from *Spirotrichosoma* become available.

Since few nodes above the family level are supported or consistent among trees, we maintain a single order (Trichonymphida) within Class Trichonymphea. We note, however, that the three families considered more closely related by some authors (Trichonymphidae, Teranymphidae, and Spirotrichosomidae, all with a rostral tube and postrostral flagella; Brugerolle & Lee, 2000) never form a monophyletic group in molecular phylogenies, suggesting some degree of parallel evolution.

Most trichonymphid genera, including species-rich ones like *Trichonympha* and *Pseudotrichonympha*, are also monophyletic, although future revisions might be required for *Eucomonympha*, which does not form a clade within Family Teranymphidae (Carpenter & Keeling, 2007). *Trichonympha* is a morphologically and phylogenetically coherent taxon, but it is molecularly diverse (Boscaro et al., 2017; Carpenter et al., 2009), divided in three main lineages reflecting different hosts and bacterial symbiont communities (Ikeda-Ohtsubo & Brune, 2009; Ohkuma et al., 2009). The SSU rRNA gene of *Trichonympha* species found in *Cryptocercus* (Carpenter et al., 2009) are extremely fast-evolving (Figure S1).

Class Trichonymphea, as well as many of its genera, has a relatively broad range of hosts, being found in many termite families as well as *Cryptocercus*. In *Cryptocercus*, trichonymphids are by far the most commonly reported parabasalians, the only others being *Prolophomonas* and sequences of honigbergiellids with no associated morphological description (see below).

Morphology failing: Cristamonadea and Lophomonadea

The C10 classification elevated a clade previously suggested by Brugerolle and Patterson (2001) to the status of class: Cristamonadea. Cristamonads are probably the most morphologically diverse parabasalian lineage, as they encompass several different morphogroups: the tetrakont devescovinids, with a thick recurrent flagellum subtended by a unique structure, the cresta; the calonymphids, which have replicated their ancestral single nucleus and associated flagella (the "karyomastigont" unit) in various ways; and a variety of "hypermastigids" (*Lophomonas*, *Kofoidia*, *Rhizonympha*, deltotrichonymphids, and joeniids) that were formerly grouped together (Order Lophomonadida in BL00) due largely to differences between them and other "hypermastigids" (trichonymphids and spirotrichonymphids), such as the loss of flagella during division, rather than genuine shared features. By the time of C10, the monophyly of cristamonads had received molecular support; however, the cristamonad subgroups were phylogenetically entangled, with virtually none of the classically identified lineages appearing monophyletic. For this reason, the C10 authors cautiously decided to establish a single order (Cristamonadida) and a single family (Lophomonadidae) within the class.

Many more cristamonad sequences, including some of key taxonomic relevance, have since become available (Gile et al., 2011; Gile & Slamovits, 2012; Hehenberger et al., 2023; Singh et al., 2021; Tai, Gile, et al., 2015), consolidating the monophyly of Class Cristamonadea (with one important exception, see below) and allowing to make some sense of the phylogenetic structure within the class (Figure 2). First, we reinstate an emended version of the old Family Calonymphidae for all multinucleated parabasalians with replicated karyomastigonts—save for *Coronympha* (and its junior synonym *Metacoronympha*; Harper et al., 2009), which is closer to devescovinids. The convergent evolution is in this case evident: the karyomastigonts of *Coronympha* bear four flagella, one of which is thicker, posteriorly-directed, and subtended by a cresta, clearly reminiscent of a devescovinid with radially-replicated karyomastigonts. This has been noted for a long time (Brugerolle & Lee, 2000; Dolan & Kirby, 2002), but was interpreted as *Coronympha* being a "less-derived" member of Calonymphidae with telltale remnants of its devescovinid ancestry. Our classification separates a "true" Calonymphidae clade from the morphologically convergent, cresta-bearing *Coronympha*. Branching close to Calonymphidae are *Deltotrichonympha* and *Koruga*, large "hypermastigids" that superficially resemble trichonymphids but possess a central bundle of axostylar fibers and flagella that regress during cell division. For them, we reinstate the family Deltotrichonymphidae from BL00. In the same area of the tree also branches *Mixotricha paradoxa*, an enormous organism covered not by flagella but spirochetes whose movement propels the host in an astounding example of symbiotic locomotion (Radek & Nitsch, 2007; Tamm, 1982). *Mixotricha* used to be considered a devescovinid due to some similarity in the arrangement of its only four flagella. We now assign it to its own family, Mixotrichidae fam. nov. Calonymphidae, Deltotrichonymphidae, and Mixotrichidae form a supported, monophyletic clade of cristamonads without a cresta: Order Calonymphida ordo nov.

Another main branch of Cristamonadea includes mostly cristate organisms: classic devescovinids and the "derived" devescovinid *Coronympha*. We hence reinstate Family Devescovinidae as the only family in Order Devescovinida, ordo nov., with a modified diagnosis. This clade still includes a few odd ducks, like the joeniid-looking *Runanympha* (Singh et al., 2021) and the peculiar *Kofoidia* (Tai, Gile, et al., 2015), with multiple apical bundles of flagella. However, since no ultrastructural analysis of either is available, it is at present difficult to assess how morphologically out of place these genera really are in the molecularly well-supported Devescovinidae.

The third and last Cristamonadea branch we elevate to order (Gigantomonadida ordo nov.) and family (Gigantomonadidae fam. nov.) status contains a single genus, *Gigantomonas*, which used to be considered a devescovinid but possesses uncommon traits among parabasalians, such as an amoeboid life stage with multiple nuclei and no flagella (Noda et al., 2009).

This leaves out the joeniids, "hypermastigids" with numerous apical flagella but a "trichomonad"-like (i.e., single and central, rather than diffused) pelta-axostyle complex. There are currently molecular data from only three joeniid genera: *Joenia*, *Joenoides*, and *Joenina* (Noda et al., 2009). They do not form a monophyletic group, but are instead scattered in unsupported positions outside the cristamonad families discussed above, with inconsistences among trees (*Joenoides* usually branches as the sister group of all other cristamonads, but this might be due to long-branch attraction). We think it more prudent to leave all joeniid genera as *incertae sedis* within Class Cristamonadea. More sequences are needed to determine if joeniids might end up clustering together, or if they instead are a polyphyletic assemblage. The position within Devescovinidae of the recently described "joeniid" *Runanympha* (Singh et al., 2021), and the large variability of parabasal body shapes in joeniids (which might form the basis for future taxonomic revisions) would suggest the latter hypothesis.

Cristamonadea is the most diverse, genus-, and species-rich class of Parabasalia, so it is not surprising that, below the largely reliable phylogenetic structure outlined above, there remain many issues to address at the genus level. Within Calonymphidae, the traditional genera *Calonympha*, *Stephanonympha*, and *Snyderella*, despite being defined by clear diagnostic features, are entangled to different degrees in molecular trees (Gile et al., 2011; Hehenberger et al., 2023; Singh et al., 2021). Difficulties in resolving the devescovinids *Macrotrichomonas* and *Metadevescovina* (among others) are also detailed elsewhere (Gile et al., 2015; Noda et al., 2009; Singh et al., 2021); in most analyses the species *Metadevescovina extranea* branches far from its congeners, within Order Calonymphida. A revision of the genus might formally assign this species to a fourth family of Calonymphida.

With one possible exception (de Mello & Uttangi, 1950), all Cristamonadea are found in termite hindguts, and there are strong correlations between the subgroups established here and the host termite families (Figure S1). Within Order Calonymphida, Calonymphidae are symbionts of Kalotermitidae, and the other lineages (including *Metadevescovina extranea*) are found in *Mastotermes* (Mastotermitidae). Incidentally, this is another difference between *M. extranea* and its congeners which, like most representatives of Order Devescovinida, are symbionts of Kalotermitidae. Two traditional devescovinid genera are the only exceptions to this pattern; they also happen to be morphologically divergent, and are not currently represented by molecular data, so their affiliation to Devescovinida in our classification is putative: the needle-like *Polymastigoides* and the polymorphic *Kirbynia*, both described from Hodotermitidae. Other Cristamonadea (*Gigantomonas* and the joeniids) are found in a variety of termite families, reflecting their undetermined relationship to each other and the two larger orders.

The one exception to the monophyly of Cristamonadea (as defined in C10) is the genus *Lophomonas*. No *Lophomonas* sequence existed at the time of the C10 classification, but barcodes are now available for two species, including the type *Lophomonas blattarum* (Gile & Slamovits, 2012; Nguyen et al., 2023). They branch with strong support somewhere else entirely in the tree of Parabasalia: as sister group of Class Trichonymphea (Figure 2). No observed morphological trait suggests or supports this relationship, but there is a key difference between *Lophomonas* and "true" cristamonads: in *Lophomonas* the privileged basal bodies are arranged in parallel, rather than having basal body R4 lie perpendicularly to the other three (Gile & Slamovits, 2012). Based on this character and the strongly supported tree topology, Cavalier-Smith (2013) established the Order Lophomonadida exclusively for the genus *Lophomonas* and its closest relatives within Class Trichonymphea; Čepička et al., 2017 confirmed this definition of the genus, but left it outside any class. We formalize here the Class Lophomonadea classis nov. with the single order Lophomonadida, rather than subsuming it in the well-established and morphologically coherent Trichonymphea.

Lophomonas was always an odd fit for Cristamonadea as the only genus found exclusively in omnivorous cockroaches (e.g. *Blatta*, *Periplaneta*) rather than termites. Similar genera, even considered synonyms of *Lophomonas* by some authors (Brugerolle & Lee, 2000), were reported from *Cryptocercus* (*Prolophomonas*; Cleveland et al., 1934) and Kalotermitidae (*Eulophomonas*, the description of which is very incomplete and probably impossible to replicate; Grassi & Foà, 1911), but these observations were never confirmed after the original publications. Since there is additional confusion on the identification of the "lophomonad" morphotype, as shown by mistaken reports from human hosts (Mewara et al., 2024), more data on *Lophomonas*, *Prolophomonas*, and *Eulophomonas* are certainly still needed.

Finally, the thoroughly unique genus *Rhizonympha* remains *incertae sedis* in the phylum Parabasalia in our classification. *Rhizonympha* are large plasmodial cells living attached to the gut wall of *Anacanthotermes* (Hodotermitidae) and bearing hundreds of karyomastigonts each complete with nucleus, axostyle, parabasal body, and a tuft of flagella. It used to be classified with the other hypermastigids that shed flagella during cell division, but without a molecular barcode it would now be difficult to argue for a closer relationship with *Lophomonas*, the Cristamonadea, or neither.

The successful radiation of "simple" parabasalians: Trichomonadea

"Trichomonads" (i.e., parabasalians with 4–6 or fewer flagella in close association with nucleus and axostyle-pelta complex) constituted one of the two main subdivisions of the phylum in the BL00 classification, but were shown to be an artificial assemblage and were distributed among multiple classes in C10 based on phylogeny and combinations of morphological characters, including the shape of the axostyle (either of the "*Trichomonas* type," tapering gradually, or of the "*Tritrichomonas* type," cylindrical and tapering abruptly at the end) and the presence of certain ultrastructural features. The small-sized "trichomonads" were mostly (but by no means all, see below) grouped in a monophyletic, SSU rRNA gene-supported class: Trichomonadea. Within Trichomonadea, two orders were established: Trichomonadida, including all parabasalians with a lamellar undulating membrane (in which the membrane is formed by a projection of the cell body extending to the flagellum) underlined by a structure called costa; and Honigbergiellida, a more diverse group of organisms without, or with an inconspicuous, undulating membrane and lacking a costa.

The taxonomic structure suggested by C10 for Class Trichomonadea is still largely consistent with the updated trees, but some adjustments need to be made (Figure 2). The monophyly of Family Trichomonadidae in Order Trichomonadida, and hence of its diagnostic character, a costa with a "B-type" ultrastructure, is confirmed and corroborated here (costa of the "A-type," which are more similar in composition to the parabasal fiber, evolved independently in unrelated "trichomonads," see below). The sister lineage of Trichomonadidae is *Pseudotrichomonas*, an organism with an undulating membrane but no costa, formally placed in Honigbergiellida when there was no available sequence. The sister lineage of Trichomonadidae + *Pseudotrichomonas* is *Lacusteria*, which has an inconspicuous undulating membrane. Yubuki et al. (2010), who first obtained sequences from these genera, argued for their inclusion in Trichomonadidae on phylogenetic grounds. More recently, Céza et al. (2022) separated *Lacusteria* into its own family, Lacusteriidae, and left the affiliation of *Pseudotrichomonas* undetermined. Out of caution, we follow Céza et al. (2022) and leave *Pseudotrichomonas incertae sedis* in Order Trichomonadida, due to the low statistical support for its status as sister group, rather than part of, Family Trichomonadidae. Some previous trees (Malik et al., 2011; Yubuki et al., 2010) recovered the genus clustering within the family, and it cannot be excluded that the absence of a costa might be, in this case, a derived character. However, should our topology be confirmed in the future, another family could be established within the order to accommodate *Pseudotrichomonas*.

Order Honigbergiellida has, since the last classification, been enriched by several taxa and requires more restructuring (Figure 2). We maintain the family Honigbergiellidae (without *Pseudotrichomonas*), even though it appears nonmonophyletic in some of our trees, admittedly with very low bootstrap support. We also maintain the family Hexamastigidae, but with the caveats previously mentioned when discussing *Hexamastix*, and by removing and assigning to its own family (Tetratrichomastigidae fam. nov.) the recently recharacterized genus *Tetratrichomastix*

(Céza et al., 2022). The main change we introduce is the formal establishment of a taxon for a group of morphologically distinctive organisms (informally called "cthulhumonads"), observed for the first time eleven years ago (James, Okamoto, et al., 2013): *Cthulhu*, *Cthylla*, and a third genus described here (see below). We assign them to Family Cthulhuidae fam. nov. within Order Honigbergiellida: they are characterized by small cell size, a single nucleus, a prominent axostyle, and a subapical bundle of 5–20 flagella (hence, they are technically not necessarily "trichomonads") that beat together in a distinctive power-stroke / recovery-stroke pattern. There is a fifth branch within Order Honigbergiellida that only contains environmental sequences from *Cryptocercus* (Ohkuma et al., 2009) and an unnamed free-living strain (Céza et al., 2022). There is no report of "trichomonads" in *Cryptocercus* in the older literature (Gile, 2024; Yamin, 1979), so the environmental sequences probably belong to new genera. A fifth family within the order should be established when an organism within this clade receives a formal name.

It is worth mentioning that support for the monophyly of Class Trichomonadea (actually non-monophyletic in our "genus" dataset, but monophyletic in our "species" and "species_nofast" datasets, Figure S1) and Order Honigbergiellida is low. We do not think this is a sufficient reason to further modify the classification, but more work on this part of the parabasalian phylogeny is warranted, especially considering that within the class, only families Trichomonadidae and Cthulhuidae present unambiguous and reliable diagnostic features (unique type of costa and arrangement and movement of flagella, respectively). Furthermore, some Trichomonadidae genera might require reassessment, in particular the species-rich *Tetratrichomonas* (dos Santos et al., 2017; Petrželková et al., 2019).

Trichomonadea have broad host ranges. Within Order Honigbergiellida, Cthulhuidae are found in the hindgut of various termite families, Hexamastigidae in tetrapod vertebrates, and several unassigned sequences come from *Cryptocercus*; Honigbergiellida also includes free-living organisms. In particular, all genera in Honigbergiellidae and Tetratrichomastigidae have free-living representatives, although some species have been occasionally observed as endobionts (e.g., *Honigbergiella* strains in cows, and *Tetratrichomastix* strains in non-termite insects). Free-living are also *Lacusteria* and *Pseudotrichomonas* in the other order, Trichomonadida (Céza et al., 2022). The phylogenetic position of the free-living taxa in Trichomonadea would suggest that the common ancestor of the class might have been free-living, but the strength of this conclusion depends on the position of the root of Parabasalia (which has not yet been determined, see below). Some Trichomonadidae genera live in termites; these are commonly reported from multiple termite families in literature, but not enough molecular data is available yet to confirm this distribution (Berchtold & König, 1995; Keeling et al., 1998; Taerum et al., 2018; Tai et al., 2013). It is instead clear that Trichomonadidae occurring in tetrapod vertebrates have low host specificity, with closely related strains of *Pentatrichomonas* or *Trichomonas*, for example, found in mammals and birds (Malik et al., 2011).

Description of *Nyarlathotep sanguinilinguatum* gen. nov., sp. nov.

The new "cthulhumonad" parabasalian observed in *Glyptotermes* sp. (Figure 3) shares many characteristics with *Cthylla*, described in *Reticulitermes virginicus* (James, Okamoto, et al., 2013). It is a small (4.5–6 µm wide × 7–9 µm long, excluding the axostyle), round or slightly elongated cell with a single nucleus, a thin but very long axostyle protruding from the posterior end for a length higher than the length of the cell body, and a bundle of several (impossible to count in vivo) long (26–29 µm) flagella protruding from a subapical anterior nook just beneath, and running perpendicular to, the apical papilla. Contrary to *Cthulhu* and *Cthylla* (James, Okamoto, et al., 2013), the flagellar bundle maintains its integrity during both power and recovery strokes, resulting in a twitchy back-and-forth movement (Videos S1 and S2). Occasionally, one flagellum temporarily adheres to the axostyle and resembles a recurrent flagellum; otherwise, the bundle does not splay and behaves like a single structure. In phylogenetic analyses (Figure 2 and Figure S1), the new parabasalian clusters with Cthulhuidae environmental sequences from other Kalotermitidae, including a newly obtained one from *Procryptotermes hesperus*. The *P. hesperus* symbiont will probably be assigned to the same genus as the *Glyptotermes* symbiont, but in a

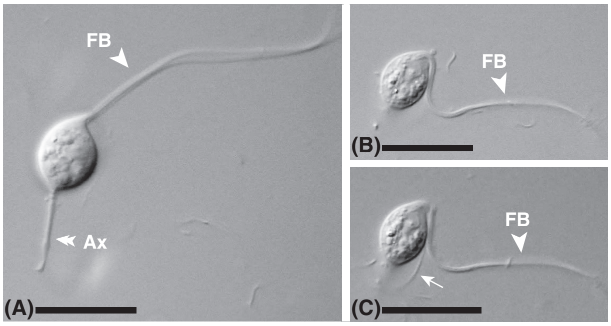

FIGURE 3 In vivo morphology of *Nyarlathotep sanguinilinguatum* gen. nov., sp. nov. A, representative cell with extended flagellar bundle; B and C, same cell at different timepoints during a recovery stroke. Ax. and double arrowhead, axostyle; FB and single arrowhead, flagellar bundle; the arrow points at a stray (recurrent?) flagellum possibly separating from the bundle after the power stroke, seemingly sticking to the axostyle. All bars are 10 µm.

different species, once morphological data are available. In keeping with the Lovecraftian theme of previous "cthulhumonad" genus names, we name the new taxon after the Cthulhu Mythos god Nyarlathotep, with specific reference to its Bloody Tongue aspect, which has a single long, apical tentacle in lieu of a face: *Nyarlathotep sanguinilinguatum* gen. nov. sp. nov.

Standalone clades with uncertain relationships: Hypotrichomonadea, Tritrichomonadea, Dientamoebea, Simplicimonadea, Monocercomonadea, and Pimpavickea

As mentioned above, many "simple trichomonads" are not phylogenetically affiliated to the large Trichomonadea clade, but are instead scattered in various positions in the parabasalian tree; morphological traits are generally ineffective in guiding us through their relationships. For example, the authors of C10 observed that *Hypotrichomonas* and *Trichomitus*, two genera not previously considered particularly close, consistently branched together in molecular trees, either inside or outside Trichomonadea depending on the marker used. The C10 scheme placed the two genera in their own class, Hypotrichomonadea. With many more sequences available, that insight is now thoroughly corroborated: in our analyses, Hypotrichomonadea never clustered within another class (Figure 2 and Figure S1). *Hypotrichomonas* and *Trichomitus* have little to unite them to the exclusion of other tetrakont "trichomonads," and they have broad host ranges. Both are found in the intestine of squamates, chelonians, amphibians, and mammals (Agripo et al., 2020; Céza et al., 2015; Smejkalová et al., 2012). *Hypotrichomonas* has also been reported from omnivorous cockroaches and beetles (Céza et al., 2015). A potential third genus in the class, represented by sequence GQ254642, was collected from a marsupial (Čepička et al., 2010).

Four lineages formed Class Tritrichomonadea in C10: *Tritrichomonas* (Tritrichomonadidae), *Simplicimonas* (Simplicimonadidae), *Monocercomonas* (Monocercomonadidae), and a few genera with extremely reduced, or completely absent, flagellar apparatus and pelta-axostyle complex (Dientamoebidae). However, no tree ever recovered these four families as one monophyletic group, but rather as a paraphyletic assemblage in uncertain and unsupported relationships with Classes Cristamonadea and Spirotrichonymphea. Additionally, there is no reliable morphological trait uniting them. Čepička et al. (2010) noted that three of these families were characterized by two distinctive ultrastructural features: an "infrakinetosomal body" supporting the recurrent flagellum and a "comb-like structure" connecting the parabasal fiber with basal body-associated structures; however, the same authors noted that both are probably ancestral for cristamonads, too, and the distantly related Hypotrichomonadea also have an analogue (or possibly, homologue) of the comb-like structure. For these reasons, and considering that our updated trees still do not support the monophyletic status of Class Tritrichomonadea as previously defined, we suggest a simple fix in our classification system: recognize the four lineages, but separate them in as many classes, in unknown relationship with each other: Tritrichomonadea (redefined), Dientamoebea classis nov., Simplicimonadea classis nov., and Monocercomonadea classis nov.

These classes largely correspond to the C10 families. The clustering of all morphologically reduced genera (*Dientamoeba*, *Histomonas*, and *Parahistomonas*) supports the idea that extensive losses of parabasalian key features, such as recognizable basal bodies-associated structures and the pelta-axostyle complex, only occurred once. One difference between C10 and our classification is that, for reasons discussed above, we putatively interpret a sequence now falling close to *Simplicimonas* as the "real" *Tricercomitus* (assigned by other authors to Class Trichomonadea instead).

The four classes formerly grouped in C10 are most commonly symbionts of vertebrates. *Monocercomonas* (once a large genus, later proven to be polyphyletic and redefined in its current form by Čepička et al., 2010 and Hampl et al., 2007) has only been molecularly detected in squamate hosts (Tuska-Szalay et al., 2022); *Tritrichomonas* has instead been found in various kinds of tetrapods (Tachezy et al., 2002); Dientamoebea genera are symbionts of birds or humans, and one not-yet-barcoded genus, *Protrichomonas*, was also reported from fish (Brugerolle & Lee, 2000; Mantini et al., 2009; Stark et al., 2016). Simplicimonadea as defined here includes two subgroups: *Simplicimonas*, confirmed in squamates and bugs (Čepička et al., 2010; Smejkalová et al., 2014), and a clade of termite (Kalotermitidae and Archotermopsidae) symbionts including the putative *Tricercomitus* (Taerum et al., 2018).

Finally, the most recently discovered group of parabasalians, the free-living pimpavickids (Céza et al., 2022), are assigned to their own class, Pimpavickea classis nov., in addition to the previously established Order Pimpavickida, formerly not belonging to any class.

Based on node support and consistency across many papers, all the groups discussed are monophyletic; it seems however clear at this point that SSU rRNA gene sequences are not able to definitively confirm their relationships with each other and other classes. Multigene phylogenies with representatives from each lineage will hopefully resolve the more ancient nodes in the future.

A familiar branch with many new leaves: Spirotrichonymphea

Spirotrichonymphids are small-, medium-, or large-size "hypermastigids" with a single nucleus and many flagella organized in right-hand spiraling bands; their axostyles vary in shape from stout structures similar to those of "trichomonads" to diffused fibers, as in trichonymphids. Like trichonymphids, to which they superficially resemble and share convergent structures ("pseudorostra" and "columellae" are analogous to trichonymphids' rostra and rostral tubes, respectively), spirotrichonymphids have been recognized as a coherent clade of parabasalians for a long time: an order in BL00 and a class (Spirotrichonymphea) in C10. Like cristamonads, they were not further subdivided in C10 because many genera had not been sequenced at the time, and some of the classically suggested relationships were not confirmed or were poorly supported in molecular trees.

Molecular data from most old as well as several newly described genera are now available (Gile et al., 2018, 2021; Noda et al., 2023; Taerum et al., 2019, 2020), and there are enough stably supported nodes in the Spirotrichonymphea tree that a phylogeny-based classification can now be established (Figure 2 and Figure S1). As a convenient starting point, it is useful to recognize that families already identified by BL00 are indeed monophyletic, thanks in part to efforts to better define many genera in recent years (Jasso-Selles et al., 2017; Noda et al., 2023; Taerum et al., 2020). We hence re-establish Family Spirotrichonymphidae, now also including the former joeniid *Microjoenia* (see Gile et al., 2021) and the recently described *Nanospironympha* (Noda et al., 2023), as well as Family Holomastigotidae, including *Holomastigotes* and a few other genera still lacking molecular characterization (some of which might be synonyms). Spirotrichonymphidae and Holomastigotidae belong to a larger, strongly supported clade, for which we use the existing name Order Spirotrichonymphida, that also contains other branches composed of recently discovered taxa. We establish Family Brugerollinidae fam. nov. for *Brugerollina* and *Pseudospironympha* (Noda et al., 2023); and Family Fraterculidae fam. nov. for *Fraterculus* and *Cuppa*, which are morphologically different but are the only sequenced Spirotrichonymphea to be found in Kalotermitidae (they co-occur in *Paraneotermes simplicicornis*; Taerum et al., 2020).

Only two genera of spirotrichonymphids remain outside of Order Spirotrichonymphida, but they are both molecularly diverse and divergent, in unsupported relationships with each other and Spirotrichonymphida. We hence elect to create one order for each of them. *Holomastigotoides* is reassigned to the classical Family Holomastigotoididae (together with the yet unsequenced *Rostronympha*) in Order Holomastigotoidida ordo nov; *Cononympha* is assigned to Family Cononymphidae fam. nov. and Order Cononymphida ordo nov.

Spirotrichonymphea live exclusively in the hindgut of termites. The majority of sequenced strains were found in Rhinotermitidae, which might have been the ancestral hosts, and (to a lower extent) Hodotermopsidae (Figure S1). *Anacanthotermes* (Hodotermitidae) contains many of the described Spirotrichonymphea diversity still lacking molecular representation.

Parabasalian root, above-class relationships, and *incertae sedis* genera

Many previous studies lamented that it is difficult to ascertain the position of the parabasalian root using molecular markers (Čepička et al., 2010; Céza et al., 2022; Hampl et al., 2004; Malik et al., 2011; Noda et al., 2012). We confirm this assessment. Combinations of three different datasets and two outgroups—representatives of *Anaeramoeba* and oxymonads, respectively—produced five different positions for the root, none of which were well-supported. The pimpavickid *Alexandriella*; the clade Tritrichomonadea + Dientamoebea; the honigbergiellid clade *Monotrichomonas* + *Ditrichomonoides*; the whole genus *Trichonympha*; or just the *Trichonympha* species harbored by *Cryptocercus* appeared as sister group of all other parabasalians in different trees. We think it is unlikely, although not impossible, for the true root to fall within any of the classes discussed here, but not much more can be said on this topic until a species-rich multigene phylogeny will become available.

Similarly, few above-class nodes are supported in our, as well as previous, trees. The two exceptions are however consistent: a sister-group relationship between Trichonymphea and Lophomonadea, and a clade including (in unresolved branching order) Spirotrichonymphea, Cristamonadea, and the four former "tritrichomonad" classes (in our classification: Tritrichomonadea, Dientamoebea, Simplicimonadea, Monocercomonadea). We informally name these above-class clades "Tla" and "Cadamassta" (from the initial letters of each class), respectively, without assigning them to a Linnean rank. While in all likelihood monophyletic, "Tla" and "Cadamassta" cannot be assigned any obvious shared trait, which is maybe not surprising, considering how ancient they are compared to other groups discussed.

Three genera remain *incertae sedis* in phylum Parabasalia in our classification because they are not molecularly barcoded and cannot be unambiguously assigned to any class based on morphology. *Rhizonympha* has already been discussed in the Cristamonadea section. *Chilomitus* is a "trichomonad" symbiont of rodents with a few unique characteristics that set it apart in the BL00 classification; it could be related to any of the current classes of "trichomonads," or none of them. Finally, *Trichocovina* shares some traits with Devescovinidae (e.g., the shape of the parabasal body) but has a costa and an undulating membrane, like the members of Trichomonadidae and a few other clades (Maaß & Radek, 2006). Finally, as discussed above, *Tricercomitus* is also not formally assigned to a higher-level taxon, in this case because of two conflicting possible phylogenetic positions (either in Simplicimonadea or Trichomonadea), neither of which is yet corroborated by morphological data.

Final considerations and notes on host specificity of parabasalians

A synopsis of our current views on the evolution and classification of Parabasalia is shown in Figure 4. The considerations made by Čepička et al. (2010) about the distribution and evolution of morphological traits in the phylum are still largely applicable to the revised trees, so on that topic we refer to their paper, and we focus here instead on patterns concerning hosts and host ranges.

All "hypermastigid" parabasalians (including Cthulhuidae) as well as the larger and more complex among "trichomonads" (i.e., Cristamonadea) are almost exclusively found in termites and cockroaches (Blattodea). Since it is now clear that complex parabasalians do not belong to a single radiation, we must assume that there is something about the blattodean hindgut that favors the multiplication of flagella and, to a smaller extent, the evolution of large cell sizes. The latter is likely correlated to the consumption of wood particles by phagocytosis. Another trend discernible among parabasalians, especially "trichomonads," is that the vast majority live in the host digestive system and are either beneficial or harmless, while the relatively few exceptions that moved to other compartments, like the urogenital system, become parasitic.

Many if not most parabasalians live in close symbiosis with prokaryotic endo- and/or ectosymbionts (Carpenter et al., 2011; Hongoh et al., 2008; Husnik et al., 2021; Ohkuma, 2008; Tai et al., 2016), and studies have investigated the coevolution between these protists and their microbiomes (Ikeda-Ohtsubo & Brune, 2009; Noda et al., 2007, 2018). Recently, some attention has been given also to the phylogenetic correlations between parabasalians and their animal hosts (De Martini et al., 2021; Michaud et al., 2019; Noda et al., 2007; Ohkuma et al., 2009; Song et al., 2021; Tai, James, et al., 2015), but almost exclusively in wood-eating insects. While the conclusions often show a high degree of coevolution and cospeciation, the genus- and species-level taxonomy is still permeated by the assumption, influenced by the old literature, that a single parabasalian species can be found in many unrelated hosts. Molecular data have shown that this is rarely the case (James, Tai, et al., 2013). Many of the discrepancies between morphology and molecular phylogeny discussed in this paper (e.g. *Lophomonas*, *Metadevescovina*, *Hoplonympha*, *Hexamastix* …) make more sense when taking into consideration differences among the hosts. It is probably safe to assume, at this point, that parabasalians in Blattodea are quite host-specific, and that more low-rank rearrangements will be needed in the future to reflect this. At the same time, this seems to be less true for parabasalians in vertebrates, where the extent of horizontal symbiotic transfers is apparently a more significant factor.

TAXONOMIC SUMMARY

Please note that a small number of genera listed below might be synonyms, but were included if they appeared again in the literature after being synonymized.

Taxonomic assignment. Parabasalia (Honigberg, 1973).

Class Hypotrichomonadea Čepička, Hampl, & Kulda, 2010

Single nucleus/karyomastigont. Three forward-pointing flagella; one recurrent flagellum forming a lamellar undulating membrane supported by an A-type costa (in *Trichomitus*) or without a costa (in *Hypotrichomonas*). Comb-like structure, but no infrakinetosomal body. Protruding axostyle of the *Trichomonas* type. Biramous parabasal body. In vertebrates and nonxylophagous insects. Single order.

Order Hypotrichomonadida Čepička, Hampl, & Kulda, 2010

Diagnosis as for Class Hypotrichomonadea. Single family.

Family Hypotrichomonadidae Honigberg, 1963 emend. Čepička, Hampl, & Kulda, 2010

Diagnosis as for Order Hypotrichomonadida. Two genera: *Hypotrichomonas* Lee, 1960 (type), *Trichomitus* Swezy, 1915.

Class Pimpavickea classis nov. Boscaro & Keeling, 2024

Single nucleus, relatively deep in cytoplasm. Prominent anterior projection, motile in at least one genus (*Pimpavicka*). Two or three forward-pointing flagella; one recurrent flagellum either nonadhering or forming an inconspicuous undulating membrane without a costa. Protruding axostyle of the *Trichomonas* type. Free-living. Single order.

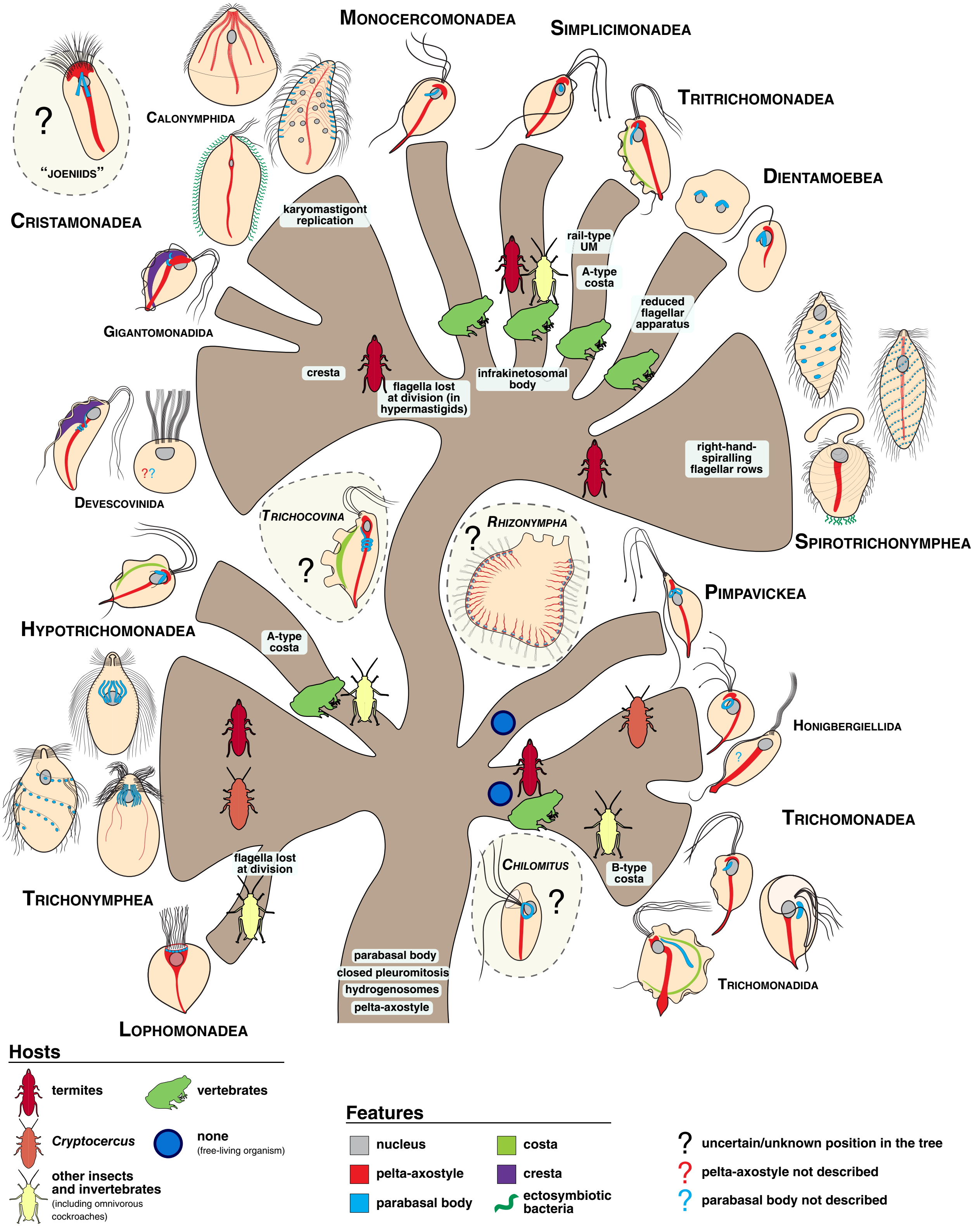

FIGURE 4 Simplified pictorial summary of the phylogenetic relationships within phylum Parabasalia according to the revised classification system. The root is arbitrarily placed to create a polytomy between five major clades: "Cadamassta," "Tla," Hypotrichomonadea, Pimpavickea, and Trichomonadea. Broad host categories confirmed by molecular studies are depicted, as well as selected parabasalian features. The drawings represent the following genera (from the bottom left, going clockwise around the tree): *Lophomonas*, *Idionympha*, *Leptospironympha*, *Trichonympha*, *Trichomitus*, *Trichocovina*, *Kofoidia*, *Macrotrichomonas*, *Gigantomonas*, *Joenoides*, *Mixotricha*, *Deltotrichonympha*, *Snyderella*, *Monocercomonas*, *Simplicimonas*, *Tritrichomonas*, *Dientamoeba*, *Histomonas*, *Holomastigotes*, *Spirotrichonympha*, *Rostronympha*, *Rhizonympha*, *Pimpavicka*, *Honigbergiella*, *Cthulhu*, *Pseudotrichomonas*, *Cochlosoma*, *Trichomitopsis*, and *Chilomitus*. UM, undulating membrane.

Order Pimpavickida Céza & Čepička, 2022

Diagnosis as for Class Pimpavickea. Single family.

Family Pimpavickidae Céza & Čepička, 2022

Diagnosis as for Order Pimpavickida. Two genera: *Pimpavicka* Céza & Čepička, 2022 (type), *Alexandriella* Céza & Čepička, 2022.

Class Trichomonadea Kirby, 1947 sensu Čepička, Hampl, & Kulda, 2010

Single nucleus/karyomastigont. Usually four to six flagella, but fewer in Family Honigbergiellidae and up to 20 or more in Family Cthulhuidae; undulating membrane may or may not be present; when present, it is not supported by a costa, except in Family Trichomonadidae, where it is always supported by a B-type costa. No comb-like structure or infrakinetosomal body. Protruding axostyle of the *Trichomonas* (most common) or *Tritrichomonas* type, absent in a few genera in Family Trichomonadidae. Variously shaped parabasal body. Free-living or symbionts in vertebrates and invertebrates, including insects (xylophagous: *Cryptocercus* and many families of termites; and nonxylophagous). Two orders.

Order Honigbergiellida Čepička, Hampl, & Kulda, 2010 emended Boscaro & Keeling, 2024

Usually two to six flagella, but up to 20 or more in Family Cthulhuidae; recurrent flagellum common, developing into a lamellar undulating membrane (not supported by a costa) in some genera. Axostyle of the *Trichomonas* or *Tritrichomonas* type. Variously shaped parabasal body. Free-living or symbionts in vertebrates and xylophagous insects (*Cryptocercus* and various families of termites). Four families.

Family Hexamastigidae Čepička, Hampl, & Kulda, 2010 emended Boscaro & Keeling, 2024

Five (fewer in immature individuals) forward-pointing flagella; one nonadherent or partially adherent recurrent flagellum. Axostyle of the *Trichomonas* type. Variously shaped parabasal body. In vertebrates, but also reported from various invertebrates, including xylophagous insects. Single genus: *Hexamastix* Alexeieff, 1912 (type).

Family Honigbergiellidae Čepička, Hampl, & Kulda, 2010

One to three forward-pointing flagella; one recurrent flagellum either nonadherent or forming a short undulating membrane at the proximal end, not supported by a costa. Axostyle of the *Trichomonas* type. Disc-shaped parabasal body. Free-living or symbionts in vertebrates. Four genera: *Honigbergiella* Hampl, Čepička & Kulda, 2007 (type), *Ditrichomonoides* Farmer & Čepička, 2022, *Honigbergiellopsis* Céza & Čepička, 2022, *Monotrichomonas* Bernard, Simpson & Patterson, 2000.

Family Cthulhuidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:66F32D6C-E51E-4074-8210-49E70EC37392

Five to twenty or more forward-pointing flagella originating from a subapical nook, and forming a tight bundle; weakly-adhering recurrent flagellum sometimes observed. Axostyle of the *Tritrichomonas* type. In various families of termites. Three genera: *Cthulhu* James & Keeling, 2012 (type), *Cthylla* James & Keeling, 2012, *Nyarlathotep* Boscaro & Keeling, 2024.

Nyarlathotep gen. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act: 8F4FF2F6-9A86-411F-8ED5-4F614AB44B22

Etymology: Named after one of the dark gods of the Lovecraftian Mythos, Nyarlathotep, the Crawling Chaos (gender: neuter).

Type species: *Nyarlathotep sanguinilinguatum*.

Type host: *Glyptotermes* sp. (Isoptera, Kalotermitidae).

Description: Parabasalian with one nucleus, several subapical flagella tightly bundled together, and a stout, protruding, *Tritrichomonas* type axostyle. The flagellar bundle does not splay during the recovery stroke.

Nyarlathotep sanguinilinguatum sp. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:E86F122A-FED7-438C-BAF5-54568E398436

Etymology: From *sanguis* (Latin, blood) and *lingua* (Latin, tongue), *sanguinilinguatum* (bloody-tongued). Referring to the single tentacle that characterizes the God of the Bloody Tongue, one of the many avatars of Nyarlathotep.

Type host: *Glyptotermes* sp. (Isoptera, Kalotermitidae).

Type locality: Bolivia (coordinates: 17°29′56.15″ S, 63°39′8.78″ W).

Host collection: specimen BO313 from the University of Florida termite collection.

Description: Morphological characteristics as per the genus. Cell body rounded or slightly elongated, 7–9 μm long. Flagella three times as long as the cell. Axostyle protruding for approximately the body length.

Holotype: Specimen in Figure 3A of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: PP297452.

Family Tetratrichomastigidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:1E79DC5E-95E3-4F5F-BB3E-E11C9D4DC711

Four (fewer in immature individuals) forward-pointing flagella; one nonadherent recurrent flagellum. Axostyle of the *Trichomonas* type. Free-living or symbionts in nonxylophagous insects (genus *Tipula*). Single genus: *Tetratrichomastix* Mackinnon, 1913 (type).

Order Trichomonadida Kirby, 1947 sensu Čepička, Hampl, & Kulda, 2010

Two to five forward-pointing flagella; recurrent flagellum always forming a lamellar undulating membrane, with or without a costa. Axostyle of the *Trichomonas* (most common) or *Tritrichomonas* type. Variously shaped parabasal body. Free-living, or symbionts in vertebrates and invertebrates (including various families of termites, but not *Cryptocercus*). Three families.

Family Lacusteriidae Céza & Čepička, 2022

Two or three forward-pointing flagella; recurrent flagellum forming a weakly developed undulating membrane not supported by a costa; distal end of the recurrent flagellum free. Axostyle of the *Trichomonas* type. Small disc- or drop-like parabasal body. Free-living. Single genus: *Lacusteria* Yubuki, Céza, Čepička, Yabuki, Inagaki, Inouye & Leander, 2010 (type).

Family Trichomonadidae Chalmers & Pekkola, 1918 sensu Hampl, Vrlík, Čepička, Pecka, Kulda & Tachezy, 2006

Four or five forward-pointing flagella; recurrent flagellum always forming a lamellar undulating membrane supported by a B-type costa. Axostyle of the *Trichomonas* (most common) or *Tritrichomonas* (in *Trichomitopsis* and *Pseudotrypanosoma*) type; reduced or nonprotruding in *Pentatrichomonoides*, *Cyathosoma*, and *Ptychostoma*. Variously shaped parabasal body. In vertebrates and invertebrates. Ten genera: *Trichomonas* Donné, 1936 (type), *Cochlosoma* Kotlán, 1923, *Cyathosoma* Tyzzer, 1930, *Pentatrichomonas* Mesnil, 1914, *Pentatrichomonoides* Kirby, 1931, *Pseudotrypanosoma* Grassi, 1917, *Ptychostoma* Tyzzer, 1930, *Tetratrichomonas* Parisi, 1910, *Trichomitopsis* Kofoid & Swezy, 1919, *Trichomonoides* Brugerolle & Bordereau, 2004.

***Incertae sedis* in Order Trichomonadida**: *Pseudotrichomonas* Bishop, 1939.

Class Lophomonadea classis nov. Boscaro & Keeling, 2024

Single nucleus. Many flagella in one apical tuft, regressing during cell division; four parallel privileged basal bodies on one side of the flagellar area. Thin, protruding axostyle of the *Trichomonas* type, anteriorly opening in a calyx-like structure that envelopes nucleus, basal bodies, and parts of the many parabasal bodies, which are arranged into a ring. In omnivorous cockroaches, but reported also from *Cryptocercus* (*Prolophomonas*) and termites (*Eulophomonas*). Single order.

Order Lophomonadida Light 1927 emended Cavalier-Smith, 2013

Diagnosis as for Class Lophomonadea. Single family.

Family Lophomonadidae Kent 1880 emended Cavalier-Smith, 2013

Diagnosis as for Order Lophomonadida. Three genera: *Lophomonas* Grassi, 1885 (type), *Eulophomonas* Grassi & Foà, 1911, *Prolophomonas* Cleveland, Hall, Sanders & Collier, 1934.

Class Trichonymphea Poche, 1913

Large organisms with a single nucleus not always closely associated to basal bodies. Distinct rostral region, always flagellated and with a bilateral symmetry (defined by two hemirostra, with separate flagellar areas, each inherited by one daughter cell during division). The postrostral region may or may not be flagellated. Many flagella, not regressing during cell division (except for secondary kineties in Family Spirotrichosomidae). No single central axostyle. Parabasal bodies may or may not be conspicuous. In *Cryptocercus* and several families of termites. Single order.

Order Trichonymphida Poche, 1913

Diagnosis as for Class Trichonymphea. Seven families.

Family Barbulanymphidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:8854FEBD-5020-47BA-A5CA-93F3767DD32A

No rostral tube. No postrostral flagella. One triangular flagellar area in each hemirostrum, separated by ectoplasmic lobes. Round or elongated cell body. Short parabasal bodies arranged around the nucleus. In *Cryptocercus*. Three genera: *Barbulanympha* Cleveland, Hall, Sanders & Collier, 1934 (type), *Rhynchonympha* Cleveland, Hall, Sanders & Collier, 1934, *Urinympha* Cleveland, Hall, Sanders & Collier, 1934.

Family Hoplonymphidae Light, 1926 emended Boscaro & Keeling, 2024

No rostral tube. No postrostral flagella. One triangular flagellar area in each hemirostrum; conspicuous structures (rhizoplasts) connecting flagellar areas to the nucleus. Slender cell body with prominent ridges and grooves. In termites (Kalotermitidae and Hodotermopsidae). Single genus: *Hoplonympha* Light, 1926 (type).

Family Retractinymphidae Radek & Brune, 2023

Rostral tube with two rostral plates. Postrostral flagella as long or longer than rostral flagella, beating independently, and arranged in longitudinal rows. No axostylar fibers. Parabasal bodies close to the cell surface but not associated to flagellar rows. In termites (Serritermitidae). Single genus: *Retractinympha* Radek & Brune, 2023 (type).

Family Spirotrichosomidae Hollande & Carruette-Valentin, 1971

Rostral tube with two rostral plates. Two flagellar bands originating in the rostrum and continuing in the postrostral region in left-hand spirals around the cell body. Secondary flagellar rows, with flagella shed during cell division, may subtend the main bands. Parabasal bodies arranged below the flagellar bands. In *Cryptocercus* and termites. Seven genera: *Spirotrichosoma* Sutherland, 1933 (type), *Apospironympha* Cleveland & Day, 1958, *Bispironympha* Bobyleva, 1969, *Colospironympha* Cleveland & Day, 1958, *Heliconympha* Radek, Meuser, Strassert, Arslan, Teßmer, Šobotník, Sillam-Dussès, Nink & Brune, 2018, *Leptospironympha* Cleveland, Hall, Sanders & Collier, 1934, *Macrospironympha* Cleveland, Hall, Sanders & Collier, 1934.

Family Staurojoeninidae Grassi, 1917

No rostral tube. No postrostral flagella. Two flagellar areas, separated by ectoplasmic lobes, per hemirostrum (tetraradial symmetry superimposed on the bilateral symmetry). Parabasal bodies arranged in a fan that surrounds and extends beyond the nucleus. In termites (Kalotermitidae) and *Cryptocercus*. Two genera: *Staurojoenina* Kirby, 1926 (type), *Idionympha* Cleveland, Hall, Sanders & Collier, 1934.

Family Teranymphidae Koidzumi, 1921

Rostral tube with two rostral plates. One flagellar area per hemirostrum, not clearly separated by ectoplasmic lobes; thin lines of ectoplasm between flagellar rows. Postrostral flagella shorter than rostral flagella; flagellar rows of the two regions continuous or arranged in rings (*Teranympha*) in the postrostral region. Parabasal fibers extend along the postrostral flagellar rows; inconspicuous parabasal bodies sometimes scattered in the cytoplasm. In *Cryptocercus* and various families of termites. Three genera: *Teranympha* Koidzumi, 1917 (type), *Eucomonympha* Cleveland, Hall, Sanders & Collier, 1934, *Pseudotrichonympha* Grassi & Foà, 1911.

Family Trichonymphidae Kent, 1880

Rostral tube with two rostral plates. One flagellar area per hemirostrum, not clearly separated by ectoplasmic lobes; thin lines of ectoplasm between flagellar rows. Postrostral flagella longer than rostral flagella and arranged in independent rows. Many, large parabasal bodies arranged around the nucleus. In *Cryptocercus* and various families of termites. Single genus: *Trichonympha* Leidy, 1877 (type).

Class Cristamonadea Čepička, Hampl, & Kulda, 2010

One or many nuclei. Either four flagella, one of which is usually thickened, backward-pointing, and subtended by a cresta (exception: *Mixotricha*), or many flagella, independent from the nucleus/nuclei or associated to them in multiple karyomastigonts. Flagella other than the four privileged (ancestral) ones generally shed at each cell division. Axostyle usually central and stout, more often of the *Tritrichomonas* type; multinucleate taxa have multiple axostyles. Parabasal bodies in various shapes and number. In termites (various families, but most commonly Kalotermitidae). Three orders.

Order Calonymphida ordo nov. Boscaro & Keeling, 2024

One or many nuclei; four or many flagella, not always arranged in karyomastigonts. No cresta. One or many thin, long axostyles. Many small parabasal bodies, not necessarily associated to nuclei. In several termite families. Three families.

Family Calonymphidae Grassi, 1911 sensu Gile, 2011

Many nuclei, either closely associated with 2–4 flagella in a multiple-karyomastigont system, or independent from flagella; both patterns occur in some species. One thin, long axostyle branching from each cluster of flagella, regardless of the presence of a nucleus; axostyles coalescing posteriorly in an axostylar bundle. Parabasal bodies peripheral, close to nuclei in karyomastigonts or only to basal bodies when there are no karyomastigonts. In termites of the family Kalotermitidae. Nine genera: *Calonympha* Foà, 1905 (type), *Criconympha* Dolan & Kirby, 2002, *Daimonympha* Hehenberger, Boscaro & Keeling, 2023, *Diplonympha* Grassi, 1920, *Gyronympha* Dolan & Kirby, 2002, *Metastephanonympha* de Mello & de Brito, 1929, *Prosnyderella* Dolan & Kirby, 2002, *Snyderella* Kirby, 1929, *Stephanonympha* Janicki, 1911.

Family Deltotrichonymphidae Brugerolle & Lee, 2000

Large amoeboflagellate organisms with one nucleus, deep in the cytoplasm. Many flagella separated in a rostral and a postrostral region (no flagella in the amoeboid posterior end of the cell, below a well-defined girdle), shed during cell division. Many axostylar fibers, some of which coalesce in a single axostyle posterior to the nucleus. Many parabasal bodies scattered in the cytoplasm. In the termite genus *Mastotermes* (Mastotermitidae). Two genera: *Deltotrichonympha* Sutherland, 1933 (type), *Koruga* Cleveland, 1966.

Family Mixotrichidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:C06586E4-B8EE-4AD8-827C-028D58A45357

Huge organisms with one nucleus, deep in the cytoplasm. Four subapically-inserted flagella. Single long, thin, nonprotruding axostyle enveloping the nucleus. Hundreds of inconspicuous parabasal bodies scattered in the cytoplasm. Most of the cell body covered in symbiotic spirochetes. In the termite genus *Mastotermes* (Mastotermitidae). Single genus: *Mixotricha* Sutherland, 1933 (type).

Order Devescovinida ordo nov. Boscaro & Keeling, 2024

Typically one nucleus/karyomastigont, but sometimes more. Four flagella per karyomastigont, one of which thickened, backward-pointing, and associated to a cresta. Axostyle stout, often protruding, more commonly of the *Tritrichomonas* type. Parabasal body of various shapes, but typically large, complex and coiled around the axostyle in taxa with a single karyomastigont. Whole karyomastigont, including axostyle, parabasal body, and cresta, replicated 8 or more times in *Coronympha*, in a ring or spiral arrangement; *Kirbynia* has multiple forms, one of which with the typical single karyomastigont, others with multiple karyomastigonts as well as flagella independent from the nuclei. Two genera do not fit this description and are included based on phylogeny: *Runanympha*, with a single nucleus, typical axostyle and coiled parabasal body, but an apical tuft with many flagella; and *Kofoidia*, with a single nucleus and multiple separate bundles of flagella (axostyle and parabasal body shape unknown). In termites of the family Kalotermitidae, but reported also in Hodotermitidae. Single family.

Family Devescovinidae Doflein, 1911 emended Boscaro & Keeling, 2024

Diagnosis as for Order Devescovinida. Eighteen genera: *Devescovina* Foà, 1905 (type), *Achemon* Grassé & Hollande, 1950, *Astronympha* Grassé, 1952, *Bullanympha* Kirby, 1939, *Caduceia* França, 1918, *Coronympha* Kirby, 1929, *Evemonia* Grassé & Hollande, 1950, *Foaina* Janicki, 1915, *Hyperdevescovina* Kirby, 1947, *Kirbynia* Grassé & Hollande, 1950, *Kofoidia* Light, 1927, *Macrotrichomonas* Grassi, 1920, *Macrotrichomonoides* Gile & Keeling, 2014, *Metadevescovina* Light, 1926, *Parajoenia* Janicki, 1911, *Polymastigoides* Grassé & Hollande, 1951, *Pseudodevescovina* Sutherland, 1933, *Runanympha* Singh & Keeling, 2021.

Order Gigantomonadida ordo nov. Boscaro & Keeling, 2024

Single nucleus/karyomastigont form with four flagella (including a thickened one associated with a large cresta), *Tritrichomonas* type axostyle, and noncoiled parabasal body. Amoeboid form with two or more nuclei, no flagellar apparatus and no axostyle. In termites of the family Hodotermitidae. Single family.

Family Gigantomonadidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:9A83903C-5B7B-45CD-8D45-A4C485E6FF5F

Diagnosis as for Order Gigantomonadida. Single genus: *Gigantomonas* Dogiel, 1916 (type).

***Incertae sedis* in Class Cristamonadea**: *Cyclojoenia* Nurse, 1945, *Joenia* Grassi, 1885, *Joenina* Grassi, 1920, *Joenoides* Grassé, 1952, *Joenopsis* Cutler, 1920, *Pachyjoenia* Brugerolle & Bordereau, 2005, *Parajoenopsis* Saleem, 1955, *Placojoenia* Radek & Hausmann, 1994, *Projoenia* Lavette, 1970.

Class Dientamoebea classis nov. Boscaro & Keeling, 2024

Amoeboid or amoeboflagellate organisms. One or two (in *Dientamoeba*) nuclei; nucleus associated with flagella, when present, in a karyomastigont. One to four forward-pointing flagella (no recurrent flagellum), sometimes with additional nonflagellated basal bodies, except in *Dientamoeba*, which has no flagellar apparatus. Reduced, nonprotruding axostyle of the *Trichomonas* type, completely absent in *Dientamoeba*. Rod or biramous parabasal body close to the nucleus (one parabasal body per nucleus in *Dientamoeba*). In vertebrates. Single order.

Order Dientamoebida ordo nov. Boscaro & Keeling, 2024

Diagnosis as for Class Dientamoebea. Single family.

Family Dientamoebidae Grassé, 1953

Diagnosis as for Order Dientamoebida. Four genera: *Dientamoeba* Jepps & Dobell, 1918 (type), *Histomonas* Tyzzer, 1920, *Parahistomonas* Honigberg & Kuldová, 1969, *Protrichomonas* Alexeieff, 1911.

Class Monocercomonadea classis nov. Boscaro & Keeling, 2024

Single nucleus/karyomastigont. Three forward-pointing flagella; one recurrent flagellum (without undulating membrane or costa). Comb-like structure and infrakinetosomal body present. Protruding axostyle of the *Trichomonas* type. Variously shaped parabasal body. In vertebrates, but reported also in invertebrates, including xylophagous insects. Single order.

Order Monocercomonadida ordo nov. Boscaro & Keeling, 2024

Diagnosis as for Class Monocercomonadea. Single family.

Family Monocercomonadidae Kirby, 1944

Diagnosis as for Order Monocercomonadida. Single genus: *Monocercomonas* Grassi, 1879 (type).

Class Simplicimonadea classis nov. Boscaro & Keeling, 2024

Single nucleus/karyomastigont. Three forward-pointing flagella; one recurrent flagellum (without undulating membrane or costa). Comb-like structure and infrakinetosomal body present. Protruding axostyle of the *Tritrichomonas* type (at least in *Simplicimonas*). Small disc-like parabasal body close to the nucleus. In vertebrates and invertebrates, including various families of termites. Single order.

Order Simplicimonadida ordo nov. Boscaro & Keeling, 2024

Diagnosis as for Class Simplicimonadea. Single family.

Family Simplicimonadidae Čepička, Hampl, & Kulda, 2010

Diagnosis as for Order Simplicimonadida. Single genus: *Simplicimonas* Čepička, Hampl, & Kulda, 2010 (type).

Class Tritrichomonadea Čepička, Hampl, & Kulda, 2010 emended Boscaro & Keeling, 2024

Single nucleus/karyomastigont. Three (rarely four) forward-pointing flagella; recurrent flagellum forming a rail-type undulating membrane (i.e., the membrane is formed by a lateral projection of the flagellum) supported by an A-type costa. Comb-like structure and infrakinetosomal body present. Protruding axostyle of the *Tritrichomonas* type. Elongated parabasal body. In vertebrates, but reported also in termites. Single order.

Order Tritrichomonadida Čepička, Hampl, & Kulda, 2010 emended Boscaro & Keeling, 2024

Diagnosis as for Class Tritrichomonadea. Single family.

Family Tritrichomonadidae Čepička, Hampl, & Kulda, 2010

Diagnosis as for Order Tritrichomonadida. Single genus: *Tritrichomonas* Kofoid, 1920 (type).

Class Spirotrichonymphea Grassé 1952 sensu Čepička, Hampl, & Kulda, 2010

Single nucleus not closely associated to basal bodies. Many flagella, not regressing during cell division and arranged in right-hand spirals around the cell body. Axostyle variable, either central (protruding or not protruding), composed of multiple axostylar fibers, or undetectable. Multiple small parabasal bodies, close to the cell surface (most common) or surrounding the nucleus in a ring. In termites (many families). Three orders.

Order Cononymphida ordo nov. Boscaro & Keeling, 2024

Cone-shaped cells with apical pseudorostrum and columella, and broad posterior end. Posterior flagella long and characteristically "sticky." Single central axostyle, usually not protruding. In termites of the genera *Coptotermes* and *Heterotermes* (Rhinotermitidae). Single family.

Family Cononymphidae fam. nov. Boscaro & Keeling, 2024

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Diagnosis as for Order Cononymphidae. Single genus: *Cononympha* Koidzumi, 1917 (type).

Order Holomastigotoidida ordo nov. Boscaro & Keeling, 2024

No columella. Flagellar bands do not reach the posterior end of the cell. Separate axostylar fibers running below the flagellar rows (*Holomastigotoides*) or a central, nonprotruding axostyle (*Rostronympha*). Parabasal bodies small, underlining flagellar rows. In termites (Rhinotermitidae and Hodotermitidae). Single family.

Family Holomastigotoididae Grassi & Foà, 1911

Diagnosis as for Order Holomastigotoidida. Two genera: *Holomastigotoides* Grassi & Foà, 1911 (type), *Rostronympha* Duboscq, Grassé & Rose, 1937.

Order Spirotrichonymphida Grassé, 1952

Apical pseudorostrum and columella probably ancestrally present, but may be inconspicuous or absent. Various axostyles and arrangement of parabasal bodies. In several families of termites. Four families.

Family Brugerollinidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:3252544B-8621-4E38-8182-694103624F04

No unifying morphological character. The most recent common ancestor of *Brugerollina* and *Pseudospironympha*, and all its descendants. In termites (Hodotermopsidae and Rhinotermitidae). Two genera: *Brugerollina* Taerum & Gile, 2023 (type), *Pseudospironympha* Noda & Kitade, 2023.

Family Fraterculidae fam. nov. Boscaro & Keeling, 2024

urn:lsid:zoobank.org:act:48493883-876F-4F31-BF6F-85741ABA3331

No unifying morphological character. The most recent common ancestor of *Fraterculus* and *Cuppa*, and all its descendants. In the termite genus *Paraneotermes* (Kalotermitidae). Two genera: *Fraterculus* Taerum & Gile, 2020 (type), *Cuppa* Taerum & Gile, 2020.

Family Holomastigotidae Janicki, 1915

No axostylar fiber. Parabasal bodies scattered between flagellar rows. In various families of termites. Four genera: *Holomastigotes* Grassi, 1892 (type), *Spiromastigotes* Duboscq & Grassé, 1943, *Spirotrichonymphella* Grassi, 1917, *Uteronympha* Brugerolle, 2006.

Family Spirotrichonymphidae Grassi, 1917

Central axostylar bundle, or single stout axostyle, often protruding. In several families of termites. Six genera: *Spirotrichonympha* Grassi & Foà, 1911 (type), *Microjoenia* Grassi, 1982, *Micromastigotes* Hollande & Carruette-Valentin, 1971, *Nanospironympha* Noda & Kitade, 2023, *Spironympha* Koidzumi, 1917, *Torquenympha* Brown, 1930.

***Incertae sedis* in Phylum Parabasalia**: *Chilomitus* da Fonseca, 1915, *Rhizonympha* Grassé & Hollande, 1951, *Tricercomitus* Kirby, 1930, *Trichocovina* Maaß & Radek, 2006.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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