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ORIGINAL ARTICLE

New Parabasalia symbionts *Snyderella* spp. and *Daimonympha* gen. nov. from South American *Rugitermes* termites and the parallel evolution of a cell with a rotating "head"

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Abstract

Most Parabasalia are symbionts in the hindgut of "lower" (non-Termitidae) termites, where they widely vary in morphology and degree of morphological complexity. Large and complex cells in the class Cristamonadea evolved by replicating a fundamental unit, the karyomastigont, in various ways. We describe here four new species of Calonymphidae (Cristamonadea) from *Rugitermes* hosts, assigned to the genus *Snyderella* based on diagnostic features (including the karyomastigont pattern) and molecular phylogeny. We also report a new genus of Calonymphidae, *Daimonympha*, from *Rugitermes laticollis*. *Daimonympha*'s morphology does not match that of any known Parabasalia, and its SSU rRNA gene sequence corroborates this distinction. *Daimonympha* does however share a puzzling feature with a few previously described, but distantly related, Cristamonadea: a rapid, smooth, and continuous rotation of the anterior end of the cell, including the many karyomastigont nuclei. The function of this rotatory movement, the cellular mechanisms enabling it, and the way the cell deals with the consequent cell membrane shear, are all unknown. "Rotating wheel" structures are famously rare in biology, with prokaryotic flagella being the main exception; these mysterious spinning cells found only among Parabasalia are another, far less understood, example.

KEYWORDS
calonymphids, *Daimonympha friedkini*, Kalotermitidae, parabasalids, rubberneckia, *Snyderella caral*, *Snyderella chachapoya*, *Snyderella nazca*, *Snyderella valdivia*, SSU rRNA gene trees

INTRODUCTION

MANY protists from the phylum Parabasalia live in the hindguts of termites and have evolved great size and structural complexity (Brugerolle & Lee, 2000; Cepicka et al., 2010). The most complex, with hundreds to tens of thousands of flagella and various accompanying cytoskeletal features, are known as hypermastigotes, and were initially thought to form a discrete taxonomic clade (Brugerolle, 1986). However, molecular analyses have shown that hypermastigotes are not closely related to one another, and that large cell size and structural complexity arose many times independently (Cepicka et al., 2010; Céza et al., 2022; Gile & Slamovits, 2012; Noda et al., 2012; Ohkuma et al., 2005). Structurally, each hypermastigote group has also evolved complexity in slightly different ways, often involving replication of cellular elements. For example the Trichonymphea, which are the best studied, have replicated the basal bodies and flagella thousands of times, but retain a single nucleus (Brugerolle & Lee, 2000). By contrast, many members of Cristamonadea, which are especially common in termites of the family Kalotermitidae (Yamin, 1979), have replicated a more complex karyomastigont unit consisting of (usually) four flagella, the nucleus, and various cytoskeletal elements hundreds of times, resulting in multinucleate cells (Brugerolle & Patterson, 2001; Cepicka et al., 2010; Gile et al., 2011; Singh et al., 2021). Within Cristamonadea there is considerable variation in which elements have been replicated and how many times (Dolan et al., 2000; Gile et al., 2015). Some karyomastigont systems have been replicated in their entirety, whereas others have been replicated without the nucleus, or have subsequently lost the association between flagella and nuclei, producing akaryomastigonts; a mix of karyomastigonts and akaryomastigonts can also occur in the same cell. According to phylogeny it appears that large size and morphological complexity have evolved multiple times even within Cristamonadea (Cepicka et al., 2010; Gile et al., 2015; Noda et al., 2009).

Despite their diversity and their interesting character evolution, the Cristamonadea are chronically understudied compared to the Trichonymphea. Most taxa have only been described through light microscopy, there is little molecular data, and the phylogenetic relationships within the group are often untested. Even for some of the most diverse genera with molecular data, whether they are monophyletic is uncertain and the branching order of the main subgroups is not well supported (Cepicka et al., 2010; Ohkuma et al., 2005; Singh et al., 2021).

Here we use light microscopy and small subunit ribosomal RNA (SSU rRNA) gene phylogeny to investigate several large Cristamonadea from the family Calonymphidae (sensu Gile et al., 2011) from *Rugitermes*, a genus of Kalotermitidae from South America whose symbionts have seldom been characterized, and which is therefore a good candidate to host unexplored diversity of Parabasalia. We describe four new species in the genus *Snyderella*, which have the expected pattern of numerous apical akaryomastigonts, and which form a weakly supported group with other described *Snyderella* isolates within a strongly supported Calonymphidae clade in the SSU rRNA tree. We also describe another multinucleate Cristamonadea symbiont whose overall morphological features and behavior do not match any previously described genus, and which does not branch close to any other named species in the molecular tree of Calonymphidae. This large cell has several whorls of karyomastigonts clustered around a physically rotating cellular apex. Apical rotation is found in another Cristamonadea, *Caduceia versatilis* (informally, "rubberneckia"; Tamm, 2008; Tamm & Tamm, 1974), but the two taxa are only distantly related and share no other morphological similarity (e.g. *Caduceia* is mononucleate and branches within the Devescovinidae; Noël et al., 2007), suggesting this complex behavioral trait has evolved multiple times in parallel in Parabasalia.

MATERIALS AND METHODS

Termite and parabasalian collection and identification

The collection, identification, and molecular barcoding (using the mitochondrial LSU rRNA gene) of the termite hosts in this study are described in Boscaro et al. (2017; for *Rugitermes laticollis* specimen EC1465 and *Rugitermes bicolor* specimen PU946), and Scheffrahn & Carrijo, 2020 and Singh et al., 2021 (for *Rugitermes aridus* specimen PU991). All specimens are deposited at the University of Florida Termite Collection (https://www.termitediversity.org).

The protist symbionts in the termite hindguts were collected and observed as described in del Campo et al. (2017). Briefly, the dissected hindgut's content was suspended in Trager's medium U (Trager, 1934), the organisms were observed under an Axioplan 2 compound microscope (Zeiss), and photographed and filmed with a 3CCD HD video camera XL H1S. Nuclei were visualized on paraformaldehyde-fixed cells treated with Hoechst stain (Sigma) for 10 min. Individual cells were manually picked for molecular analysis using custom-made glass pipettes and an Axiovert 200 (Zeiss) inverted microscope.

SSU rRNA gene sequencing and phylogenetic inference

DNA extractions were performed on single cells using the Masterpure Complete DNA and RNA Purification

Kit (Epicentre). SSU rRNA genes were amplified using eukaryotic 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). Purified PCR products were cloned with the TOPO-TA cloning kit (Invitrogen) and Sanger-sequenced with BigDye Terminator v 3.1 (Applied Biosystem) at an in-house facility. All clone sequences were submitted to GenBank (accession numbers: OQ883698–OQ883718).

Representative sequences were aligned together with 74 orthologs from available Cristamonadea (or unassigned sequences falling within Cristamonadea) and 7 Tritrichomonadea (as the outgroup). The dataset was aligned with MAFFT v. 7.520 using the L-INS-i method (Katoh & Standley, 2013). Columns with missing data at the beginning and end of the alignment were removed, but no internal column was trimmed (attempts to do so did not change the tree topology, nor significantly altered statistical support; data not shown). The final alignment had 1496 columns. Maximum likelihood (ML) 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 100 nonparametric bootstrap pseudoreplicates. Bayesian analyses were performed with MrBayes v. 3.2.7a (Ronquist et al., 2012) using the GTR+I+G4 model; three independent runs with one cold and three heated chains each were run for 1,000,000 generations, sampling every 100 generations and discarding as burn-in the first 25% sampled datapoints.

RESULTS AND DISCUSSION

Host collection, identification, and barcoding

Three species of *Rugitermes* were collected in Ecuador and Peru. The first, collected in Quito, Ecuador was previously identified morphologically and by DNA barcoding using the mitochondrial LSU rRNA gene as *Rugitermes laticollis* (Boscaro et al., 2017; Scheffrahn et al., 2015). This species had until recently no historical record of symbionts (Yamin, 1979), the exceptions now being *Trichonympha hueyi* (Boscaro et al., 2017) and *Devescovina sapara* (Singh et al., 2021). The second host species, collected in Tingo Maria, Peru was also identified previously by morphology and barcoding as *Rugitermes bicolor* (Boscaro et al., 2017), which also has no historical record of symbionts (Yamin, 1979), except for *Trichonympha webbyae* (Boscaro et al., 2017). Lastly, a third species of *Rugitermes* was collected in Huánuco, Peru, and has recently been described as *Rugitermes aridus* (Scheffrahn & Carrijo, 2020). The only known protist symbiont of this species, previously labeled *Rugitermes* sp., was *Devescovina aymara* (Singh et al., 2021).

Morphology of the new *Snyderella* species

There are three common genera within the family Calonymphidae that share a similar overall appearance: *Snyderella*, *Stephanonympha*, and *Calonympha*. These are all large cells with many karyomastigonts and/or akaryomastigonts, each associated with one of the axostyles branching off from a central axostylar bundle. The three genera are distinguished from one another by a few features (Brugerolle & Lee, 2000): in *Calonympha*, there are karyomastigonts organized in anterior spirals, but the anterior-most repeated units are akaryomastigonts (Foà, 1905); in *Stephanonympha* there are no akaryomastigonts, only anterior spirals of complete karyomastigonts (Janicki, 1911); finally, in *Snyderella* all the flagella are organized in akaryomastigonts, and several free nuclei are dispersed in the cytoplasm (Dolan et al., 2000; Kirby, 1929).

In all three collected *Rugitermes* species, we observed cells matching the description of *Snyderella* (and confirmed this identification with molecular data, see below). All cells had hundreds of flagella organized in many akaryomastigonts arranged around the anterior region. The posterior region of each cell was dominated by wood particles, and covered in bacterial ectosymbionts rather than flagella. In several cases, the axostylar bundle either distended the cytoplasm at the posterior end or protruded more sharply, but it was not clear whether this morphological feature was the result of oxygen or salinity stress from removing the cells from their environment.

Snyderella valdivia sp. nov. cells from *R. laticollis* (length: 50–75 μm, average 57 μm; width: 35–55 μm, average 43 μm; $N=8$) tended to be round or ellipsoid (Figure 1A) rather than pear-shaped with a tapering anterior end, which is otherwise more common in the genus (Kirby, 1929). Due to its superficial similarity to symbionts classified as *Stephanonympha* or *Calonympha*, we observed the distribution of nuclei using Hoechst stain (Figure 1B), which confirmed the presence of 40–70 free, roughly spherical nuclei distributed in the mid-cytoplasm, which is consistent with neither of those genera but a diagnostic feature of *Snyderella*. The "belt" of nuclei separated the anterior half of the cell, covered by short (9%–14% the cell length) flagella, from the posterior region. The latter contained wood particles in the cytoplasm and was covered by bacteria of at least two morphotypes: some short, rod-shaped, and immobile, other motile and spirochete-like.

In *Rugitermes bicolor*, we observed two different *Synderella*-like organisms: *Snyderella chachapoya* sp. nov. (Figure 1C,D) and *Snyderella caral* sp. nov. (Figure 1E). *Snyderella chachapoya* cells (length: 80–130 μm, average 109 μm; width: 47–65 μm, average 53 μm; $N=9$) had a classical *Snyderella* morphology with a pear shape and a pronounced, narrower apical zone. The central axostylar bundle was very prominent throughout the length of

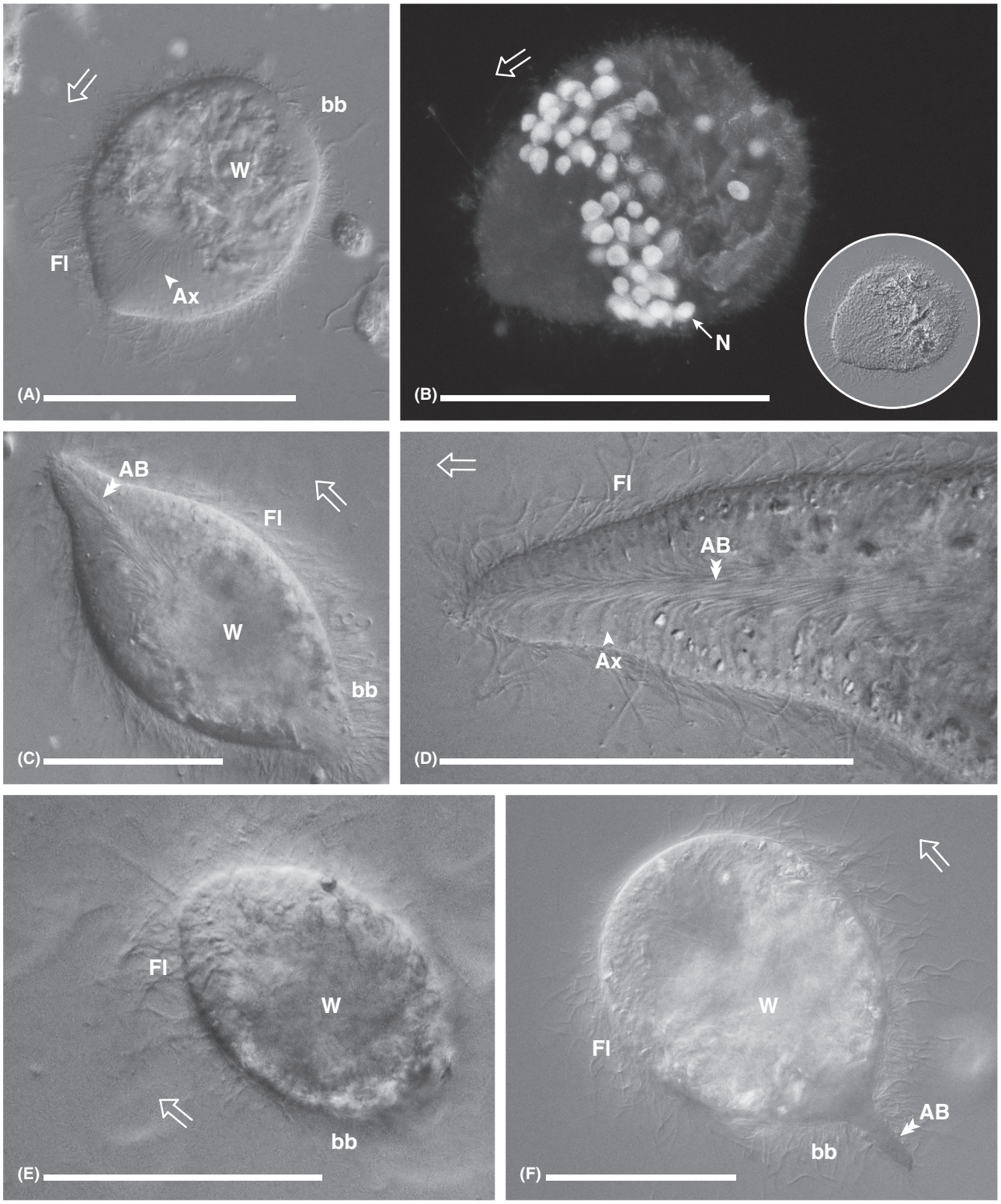

FIGURE 1 Morphological features of the four newly described *Snyderella* species. (A) *Snyderella valdivia* from *Rugitermes laticollis*. (B) *S. valdivia*, light (insert) and fluorescent microscopy of a cell treated with Hoechst stain. (C) *Snyderella chachapoya* from *Rugitermes bicolor*. (D) Details of the anterior end, flagella, and axostylar system of *S. chachapoya*. (E) *Snyderella caral* from *Rugitermes bicolor*. (F) *Snyderella nazca* from *Rugitermes aridus*. All size bars stand for 50 μm. The hollow arrow in each panel points towards the anterior pole of the cell. AB, axostylar bundle; Ax, individual axostyle; bb, bacteria; Fl, flagella; N, nucleus; W, wood particles.

the cell, and many individual axostyles branched from it toward the short (10%–15% the cell length) akaryomastigont flagella. The flagellated area extended about two thirds the length of the cell. The posterior-most region was covered by motile and immobile bacteria, most of which were longer and thicker than the flagella. *Snyderella caral* cells were rare in the host, much more rounded in shape than *S. chachapoya*, and markedly smaller (length: 58 μm; width: 39 μm; $N = 1$). Relatively long (up to 40% the cell length) flagella covered the anterior-most third of the organism, while shorter, sparser bacteria occupied the posterior surface.

Lastly, from *Rugitermes aridus* we observed one morphotype, *Snyderella nazca* sp. nov. (length: 61–93 μm, average 83 μm; width: 61–71 μm, average 67 μm; $N = 5$; Figure 1F), also rounded in shape and often with a pronounced posterior protrusion of the axostylar bundle. The flagellated area (flagellar length: 15%–22% the cell length) extended to about half the cell, while large and long spirochete-like ectosymbiotic bacteria (some of which might additionally be interspersed with flagella) colonized the rest.

Morphology of *Daimonympha friedkini* gen. et sp. nov.

In the hindgut of *Rugitermes laticollis*, we also observed a calonymphid-like organism that had a different nuclear organization from *Snyderella*. These cells (length: 50–77 μm, average 61 μm; width: 39–54 μm; average: 46 μm; $N = 15$) had an anterior region dominated by hundreds of long (20%–30% of cell length) flagella and a posterior region rich in ingested wood particles. The axostylar bundle was not as visible as in some *Snyderella* species, but it did occasionally protrude sharply from the posterior pole. Through DIC microscopy and observation of Hoechst-stained cells, 65–70 ellipsoid nuclei appeared organized in 3–4 concentric whorls, with the number of nuclei decreasing in each more distal whorl. The position and orderly arrangement of the nuclei was consistent with an organization of the flagella into apical karyomastigonts (Figure 2), rather than the apical akaryomastigonts and free nuclei typical of *Snyderella*. Furthermore, the nuclear whorls appeared separate rather than forming one continuous helix as found in *Stephanonympha* or in the metacoronympha form of the genus *Coronympha* (Harper et al., 2009; Kirby, 1939). Indeed, the overall shape of the organism did not match any currently described genus of Cristamonadea or Parabasalia more generally, and we therefore classify it as *Daimonympha friedkini* gen. et sp. nov. The novelty of this species is reinforced by the cells' noteworthy behavior of rotating their anterior third relative to the posterior region. The movement involves the flagella with their associated cytoskeletal structures (including the long individual axostyles), and by extension the cell membrane surrounding the anterior third of the cell, as well as the karyomastigont nuclei (Movie S1). The non-motile region corresponds to the part of the cell filled by wood particles and covered by at least two types of bacteria (some of which were motile

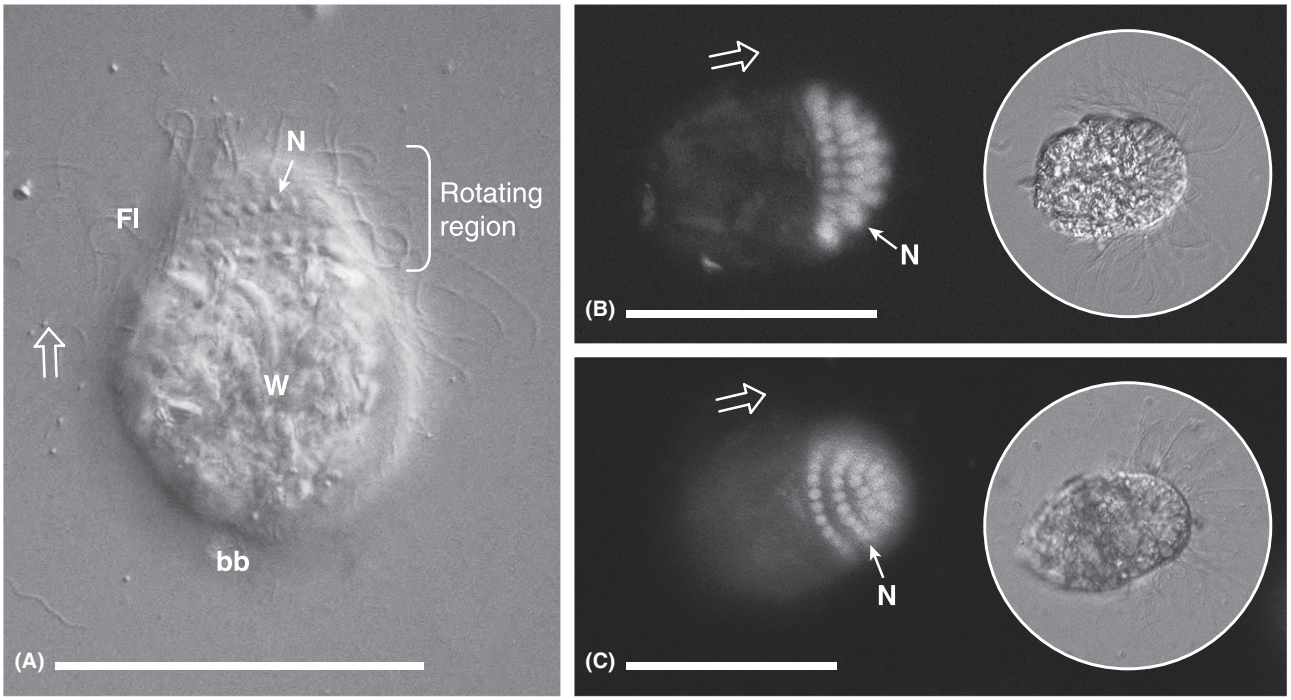

FIGURE 2 Morphological features of the newly described genus and species, *Daimonympha friedkini*, from *Rugitermes laticollis*. (A) Light microscopy of a living cell. (B and C) Anterior nuclei whorls as shown by the Hoechst stain with fluorescent microscopy. All size bars stand for 50 μm. The hollow arrow in each panel points towards the anterior pole of the cell. bb, bacteria; Fl, flagella; N, nucleus; W, wood particles.

and spirochete-like). In the observed cells, the rotatory movement was smooth and continuous, and the direction of rotation was counter-clockwise (when looking down at the anterior apex of the cell).

This "rotating head" behavior in a cell is very rare in nature, indeed only observed in other Parabasalia. The best-known case is the Devescovinidae, *Caduceia versatilis*, from the Florida termite *Cryptotermes cavifrons*, long referred to informally as "rubberneckia" due to the rotation of the anterior portion of the cell relative to the posterior (Kirby, 1947; Tamm & Tamm, 1974). The species was eventually assigned to the genus *Caduceia* based on its morphology (d'Ambrosio et al., 1999), although it is the only *Caduceia* species with rotational movement and the only one found in any species of *Cryptotermes*.

The behavior in *Caduceia* is thought to arise from rotation around its central axostyle (Tamm, 1978; Tamm & Tamm, 1974), leading to motion of one cell pole relative to the other, and a boundary zone where shearing of the lipid bilayer is inferred (and also supported by the fact that this zone is free of bacteria, while the head and the body are covered by ectosymbionts). The striking movement of part of the cell membrane in rubberneckia relative to the rest was in fact hailed as direct visual evidence for the fluid membrane paradigm (Tamm & Tamm, 1974). Two other genera of Devescovinidae from Australian termites have also been reported to exhibit variations on rotary motion (Tamm & Tamm, 1976): one species of *Hyperdevescovina* performs a smooth rotational movement akin to that of *Caduceia versatilis*, and which seems based on the same internal mechanism; in contrast, one species of *Devescovina* where rotation has been observed is thought to be an observational artifact, where a jerky and intermitted head rotation seems to arise from the papilla and anterior flagella.

All the aforementioned species are Devescovinidae, which have a single large axostyle wrapped around the nucleus and associated to a single apical karyomastigont; Cristamonadea like *Daimonympha*, instead, have many individual axostyles eventually converging into an axostylar bundle (Figure 2A; Movie S1). Consequently, there must be profound mechanical and possibly functional differences in these rotatory motions: for example, in *Caduceia versatilis* the movement itself is less striking since the single nucleus and the four basal bodies are all close to the rotation axis, while in *Daimonympha* the corresponding structures move considerably in the three-dimensional space during the rotation. On the other hand, it is less clear how the single axostyle of *C. versatilis* transmits its motion to the entire cellular apex, which is instead reached by many axostyles in *Daimonympha*. It will be interesting to examine this behavior further to see whether these perhaps superficially similar (and very rare) cell movements arose from any underlying homologous structures or mechanisms, or evolved entirely independently.

Phylogenetic analysis of SSU rRNA gene sequences from the new species of *Snyderella* and *Daimonympha*

To examine the phylogenetic position of the new taxa, single cells were manually isolated and used to amplify SSU rRNA genes for phylogenetic analysis. Multiple clones were obtained for each morphotype, and sequence similarities within each were well within the range of variation observed for other species, or indeed single cells, of Parabasalia termite symbionts (Gile et al., 2018; Table 1).

A phylogenetic tree including a wide range of Parabasalia was reconstructed with ML and Bayesian methods, and consistently recovered all new taxa within a strongly supported Cristamonadea clade. Within Cristamonadea (Figure 3), the new species all clustered in a strongly supported subgroup containing other Calonymphidae, as expected based on their overall morphology. The four new *Snyderella* species branched with available *Snyderella* sequences in a clade that was only partially supported, as observed in previous trees (Gile et al., 2011; Singh et al., 2021). That the two morphotypes from *R. bicolor* represent distinct species was confirmed by the tree since the two respective sequences fell in different major subdivisions of the *Snyderella* lineage. Neither *Calonympha* nor *Stephanonympha* are monophyletic in our analysis.

In Parabasalia, morphological traits classically used to distinguish species within a genus have often proven unreliable, and tend to lump together separate lineages (Harper et al., 2009; James, Tai, et al., 2013). In fact, there is extensive evidence for a strong (albeit not perfect) host-specificity in the phylum, suggesting that the identity and geographical range of the termite host are useful diagnostic characters, especially in combination with molecular sequences (James, Burki, et al., 2013; Jasso-Selles et al., 2020; Michaud et al., 2019; Noda et al., 2018). There are only three formally described *Snyderella* species without an associated SSU rRNA sequence, and of these only one, *Snyderella ypiranga* (de Mello, 1954), was found in *Rugitermes*. However, *S. ypiranga*'s type host is *Rugitermes rugosus*, it was only collected from southern Brazil, and it was characterized by axostyles joining several, rather than individual, akaryomastigonts, a feature that we did not observe in the reported morphotypes. Hence, none of the *Snyderella* described here is likely to belong to a previously established species.

With the exception of its "rotating head" behavior, the overall morphology of *Daimonympha friedkini* is consistent with that of Calonymphidae, and indeed in the molecular tree it branched within the group.

TABLE 1 Number of cells and clonal sequences of the SSU rRNA gene obtained in this work, including ranges of pairwise sequence similarities within each species.

Species	Number of individual cells	Number of clones	Intraspecies similarity	Accession numbers
Snyderella valdivia	3	5	98.95%–99.34%	OQ883714–OQ883718
Snyderella chachapoya	3	4	99.03%–99.29%	OQ883704–OQ883707
Snyderella caral	2	3	99.16%–99.42%	OQ883701–OQ883703
Snyderella nazca	3	6	98.14%–99.36%	OQ883708–OQ883713
Daimonympha friedkini	3	3	97.95%–98.85%	OQ883698–OQ883700

Daimonympha friedkini was however not closely related to sequences from any known genus of the same family (i.e., *Snyderella*, *Calonympha*, or *Stephanonympha*), and instead branched, albeit with moderate statistical support, with two sequences from uncharacterized Parabasalia from the Asian termite, *Neotermes koshunensis* (Ohkuma et al., 2000). The phylogenetic position and the inferred molecular distance from other taxa (Figure 3) are altogether consistent with the establishment of a new genus. Moreover, the tree showed quite clearly that *Daimonympha friedkini* is not closely related to the Devescovinidae *Caduceia versatilis* (Figure 3), consistent with the conclusion that these two species have evolved their unusual "rotating head" behavior in parallel.

TAXONOMIC SUMMARY

Taxonomic assignment. Eukaryota; Excavata (Cavalier- Smith, 2002); Parabasalia (Honigberg, 1973); Cristamonadea (Brugerolle & Patterson, 2001); Calonymphidae (Grassi & Foà, 1911; emend. Gile et al., 2011); *Snyderella* (Kirby, 1929).

Snyderella valdivia sp. nov. Hehenberger, Boscaro, and Keeling, 2023

urn:lsid:zoobank.org: act:4101C83C-54B8-411D-BF7E-11C53E74E34E

Etymology: Named for the Valdivia archeological cultures from the area of Ecuador around Quito.

Type host: *Rugitermes laticollis* (Isoptera, Kalotermitidae: barcode MF062147).

Type locality: La Carolina Park, Quito, Ecuador: 0°11′18.4″S; 78°29′09.4″W.

Host collection: University of Florida termite collection, accession number EC1465. Collectors: Mullins, Scheffrahn, and Kreček.

Description: Parabasalian flagellate with morphological characteristics of the genus *Snyderella*. Rounded, approximately spherical or ellipsoid cells about 57 μm in length and 43 μm in width. Flagellated area extending to about half the cell length. Found in the hindgut of *Rugitermes laticollis*. Distinct SSU rRNA sequence.

Holotype: Specimen in Figure 1A of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: OQ883716.

Snyderella chachapoya sp. nov. Hehenberger, Boscaro, and Keeling, 2023

urn:lsid:zoobank.org:act: 5ECA1A51-C6BE-4A28-A5A6-99DCA6FEF461

Etymology: Named for the Chachapoya archeological cultures from Andean Peru.

Type host: *Rugitermes bicolor* (Isoptera, Kalotermitidae: barcode MF062150).

Type locality: Tingo Maria, Peru: 9°08′59.1″S; 75°59′32.4″W.

Host collection: University of Florida termite collection, accession number PU946. Collectors: Chase, Mangold, and Scheffrahn.

Description: Parabasalian flagellate with morphological characteristics of the genus *Snyderella*. Pear-shaped cells about 109 μm in length and 53 μm in width, with a distinctly tapered apical zone in which many microtubular axostyles are visible, and a posterior zone filled with wood particles. Flagellated area extending to about two thirds the cell length. Found in the hindgut of *Rugitermes bicolor*. Distinct SSU rRNA sequence.

Holotype: Specimen in Figure 1C of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: OQ883705.

Snyderella caral sp. nov. Hehenberger, Boscaro, and Keeling, 2023

urn:lsid:zoobank.org:act: 8ED5DACE-0569-44CB-8DF9-62DD42BDFFE2

Etymology: Named for the Caral archeological cultures from Andean Peru.

Type host: *Rugitermes bicolor* (Isoptera, Kalotermitidae: barcode MF062150).

Type locality: Tingo Maria, Peru: 9°08′59.1″S; 75°59′32.4″W.

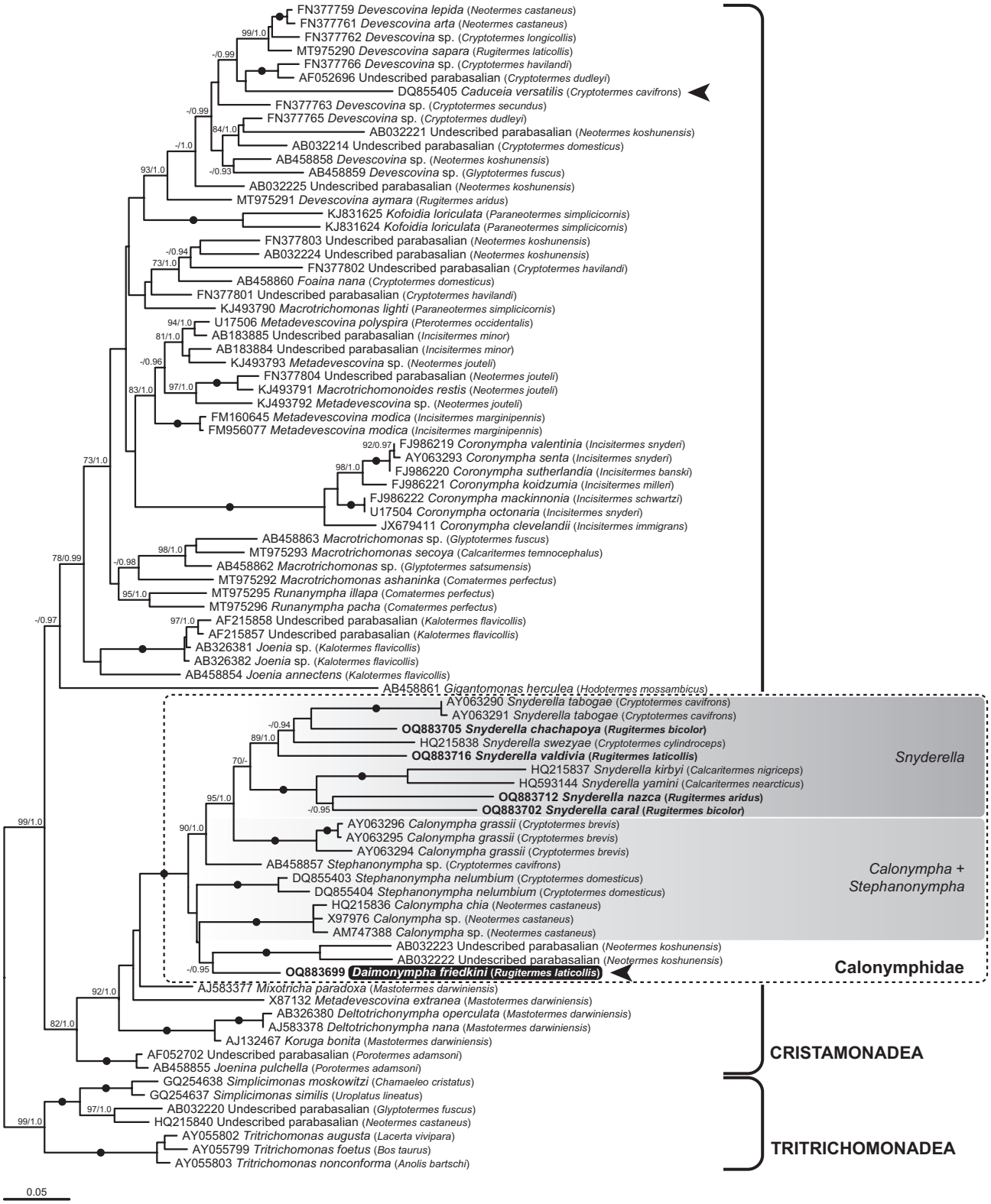

FIGURE 3 Maximum likelihood tree of Cristamonadea based on the small subunit rRNA gene, with representatives of Tritrichomonadea as the outgroup. Non-parametric bootstrap and posterior probability values are reported next to nodes, or as dots on branches for full support (100%/1.0); values below 70% (bootstrap) or 0.9 (posterior probability; also omitted if the node was not present in the Bayesian inference analysis) are not shown. The new *Snyderella* species are indicated in bold, the new genus *Daimonympha friedkini* is indicated by white text on black background. Large arrowheads point at the only two organisms with "rotating heads" with molecular data available.

Host collection: University of Florida termite collection, accession number PU946. Collectors: Chase, Mangold, and Scheffrahn.

Description: Parabasalian flagellate with morphological characteristics of the genus *Snyderella*. Ellipsoid cells about 58 μm in length and 39 μm in width. Flagellated area extending to about half the cell length, with relatively long (up to 40% the cell length) flagella. Found in the hindgut of *Rugitermes bicolor*. Distinct SSU rRNA sequence.

Holotype: Specimen in Figure 1E of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: OQ883702.

Snyderella nazca sp. nov. Hehenberger, Boscaro, and Keeling, 2023

urn:lsid:zoobank.org:act: 1FF8A24C-463F-437E-BF1E-2D1EA8C2CDF5

Etymology: Named for the Nazca archeological cultures from Andean Peru.

Type host: *Rugitermes aridus* (Isoptera, Kalotermitidae: barcode MT975288).

Type locality: Huánuco, Peru: 9°52′37.6″S; 76°09′50.7″W.

Host collection: University of Florida termite collection, accession number PU991. Collectors: Carrijo, Constantino, Chase, Kreček, Kuswanto, Mangold, Mullins, Nishimura, and Scheffrahn.

Description: Parabasalian flagellate with morphological characteristics of the genus *Snyderella*. Rounded, almost spherical cells about 83 μm in length and 67 μm in width, often with a sharp posterior axostylar protrusion. Flagellated area extending to about half the cell length. Found in the hindgut of *Rugitermes aridus*. Distinct SSU rRNA sequence.

Holotype: Specimen in Figure 1F of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: OQ883712.

Taxonomic assignment. Eukaryota; Excavata (Cavalier-Smith, 2002); Parabasalia (Honigberg, 1973); Cristamonadea (Brugerolle & Patterson, 2001); Calonymphidae (Grassi & Foà, 1911; emend. Gile et al., 2011).

Daimonympha gen. nov. Hehenberger, Boscaro, and Keeling, 2023

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

Etymology: Named based on an iconic scene in the film, The Exorcist, where a demon [greek=daimos] possessing a little girl causes her to rotate her head independently of her body, reminiscent of the rotating apex of the cell.

Type species: *Daimonympha friedkini* (Figure 2A).

Type host: *Rugitermes laticollis* (Isoptera, Kalotermitidae: barcode MF062147).

Description: Parabasalian flagellate with hundreds of flagella organized in 3–4 (non-spiral) whorls of karyomastigonts in the anterior portion of the cells, which rotates steadily relative to the wood-particle filled posterior portion. Found in the hindgut of *Rugitermes laticollis*. Distinct SSU rRNA sequence.

Daimonympha friedkini sp. nov. Hehenberger, Boscaro, and Keeling, 2023

urn:lsid:zoobank.org:act: 6E9C525D-FF65-43C9-ABDA-CEB876EC335D

Etymology: Named for William Friedkin, the director of the film, The Exorcist.

Type host: *Rugitermes laticollis* (Isoptera, Kalotermitidae: barcode MF062147).

Type locality: La Carolina Park, Quito, Ecuador: 0°11′18.4″S; 78°29′09.4″W.

Host collection: University of Florida termite collection, accession number EC1465. Collectors: Mullins, Scheffrahn, and Kreček.

Description: Parabasalian flagellate with morphological characteristics of the genus *Daimonympha*. Ovoid cells about 61 μm in length and 46 μm in width. Flagellated area extending to about one third the cell length. Found in the hindgut of *Rugitermes laticollis*. Distinct SSU rRNA sequence.

Holotype: Specimen in Figure 2A of the present publication.

Gene sequence: SSU rRNA gene. GenBank accession number: OQ883699.

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