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Morphology and Phylogenetic Position of *Eucomonympha imla* (Parabasalia: Hypermastigida)

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ABSTRACT. *Eucomonympha imla* is a hypermastigote parabasalian found in the gut of the wood-feeding cockroach *Cryptocercus punctulatus*. It has received little attention since its original description in 1934 as the type species of the genus *Eucomonympha* and the family Eucomonymphidae. We used light and scanning electron microscopy to characterize surface morphology and organelles, with particular attention to the form of the rostrum, operculum, nucleus, and parabasals. Two previously unrecognized groups of bacterial ectobionts were observed—spirochetes that associate with the flagella and one or more types of rod-shaped bacteria that adhere to the cell surface. The small subunit rRNA (SSU rRNA) sequence was determined from manually isolated cells, and phylogenetic analyses place *E. imla* in a strongly supported clade with the genera *Teranympha* and *Pseudotrichonympha* and three sequences from formally undescribed termite symbionts provisionally assigned to *Eucomonympha*. Unexpectedly, the *Eucomonympha* isolates from termites are more closely related to *Teranympha* than to the type species, suggesting these should not be classified as species of *Eucomonympha*, despite their morphological similarity to *E. imla*. Eucomonymphidae fall within a strongly supported Trichonymphida (also including Hoplonymphidae, Trichonymphidae, and Staurojoeninidae), but this clade branches separately from other hypermastigote groups (lophomonads and spirotrichonymphids), suggesting that hypermastigotes are polyphyletic.

Key Words. Bacterial symbionts, *Cryptocercus punctulatus*, excavata, *Pseudotrichonympha*, scanning electron microscopy, symbiosis, *Teranympha mirabilis*, Trichonymphida.

PARABASALIA comprise a large and extremely diverse group of anaerobic protists, including flagellate, amoeboflagellate, and amoeboid forms, distinguished by the presence of hydrogenosomes (and the associated absence of canonical aerobic mitochondria), parabasal bodies (Golgi vesicles with an associated complex of fibres), closed mitosis with an external spindle, and a pelta-axostyle complex (Brugerolle and Lee 2002). Parabasalia traditionally comprise two classes—Trichomonadida and Hypermastigida. The former consist mostly of small and structurally relatively simple polymastigotes, although a few have attained large size, sometimes through multiplication of the karyomastigont apparatus (Brugerolle and Lee 2002; Honigberg 1963). In contrast, hypermastigotes are complex and typically large cells, with flagella numbering in the hundreds or thousands, but their karyomastigont system has not replicated and is associated with a single nucleus. Hypermastigotes are found only in symbiotic association with termites and wood-feeding cockroaches of the genus *Cryptocercus*, where they aid in digestion of ingested wood particles in the hindgut (Cleveland 1923, 1924; Ohtoko et al. 2000; Trager 1932), a symbiotic relationship that is of enormous ecological importance in forest ecosystems, both in the decomposition of lignocellulose and in forming an important part of the terrestrial food chain (Ohkuma 2003).

The monophyly of both trichomonads and hypermastigotes has recently been questioned. Analyses of molecular data support multiple origins of the hypermastigote cell form and paraphyly of trichomonads, although some important nodes are weakly supported (Hampl et al. 2006; Ohkuma et al. 2005). Reflecting this, in a recent classification of eukaryotes, Adl et al. (2005) assigned hypermastigote taxa to three different groups, one of which also contains some of the more structurally complex trichomonads (e.g. Calonymphids).

Eucomonympha imla, a hypermastigote found exclusively in the gut of the wood-feeding cockroach *Cryptocercus punctulatus*, is a striking example of the extreme morphological complexity exhibited by parabasalians. First described by Cleveland et al. (1934), *E. imla* is a large cell (over 100 microns in length) consisting of two roughly spheroidal portions: an anterior rostrum and a much larger posterior post-rostral area. With the exception of the extreme anterior and posterior ends, the cell is completely covered in flagella, estimated to number over 52,000 in some specimens (Cleveland et al. 1934). Most swim freely in the lumen of the hindgut where they endocytose wood particles, while a minority remains attached to the wall of the hindgut and obtains nutrients through unknown means (Cleveland et al. 1934). *Eucomonympha imla* is the type species of the genus *Eucomonympha* and the genus is the type of the family Eucomonymphidae. The only other known occurrence of members of the genus *Eucomonympha* is in the hindgut of the termite *Hodotermopsis* (Brugerolle and Bordereau 2004; Kitade, Maeyama, and Matsumoto 1997; Ohkuma et al. 2000). Cleveland et al. (1934) hypothesized a close relationship with the genus *Pseudotrichonympha* from the termite *Coptotermes*, based on the presence of flagella covering the entire body surface and the manner of cell division. This hypothesis is supported by recent molecular phylogenies (Ohkuma et al. 2000, 2005), the former of which claims that Eucomonymphidae may be the sister group to the rest of the parabasalians.

As most parabasalians, especially hypermastigotes and the other inhabitants of the complex wood-digesting insect gut environment, are typically not cultivated, nearly all of the recent work on these organisms has used molecular approaches to address questions related to systematics and evolution (Keeling 2002; Ohkuma et al. 2000, 2005), their role in termite and cockroach gut metabolism (Nakashima, Watanabe, and Azuma 2002; Ohtoko et al. 2000), and their symbioses with ecto- and endosymbiotic bacteria (Noda et al. 2005, 2006). However, the extreme structural diversity of these extraordinary but still poorly studied organisms serves to emphasize the need for new morphological data as well. Cleveland et al. (1934) originally described *E. imla* with illustrations based on light microscopy (LM), and Hollande and Caruette-Valentin (1971) subsequently added ultrastructural data based on transmission electron micrographs. Here we seek to complement data from previous studies by examining surface morphology with scanning electron microscopy (SEM), as well as inferring its phylogenetic position based on SSU rRNA sequence data, with the goal of providing an integrated view of this species and the implications of its phylogenetic placement.

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MATERIALS AND METHODS

Sampling. Individuals of *C. punctulatus* Scudder were collected by C. Nalepa from several locations in the Appalachian mountain range of the eastern United States. Insects were killed by severing the head with a razor blade, and the entire gut was removed by gently pulling on the posterior-most segment of the abdomen with forceps. The hindgut was then removed, immersed in a several drops of Trager Medium U, and opened with a scalpel under a dissecting microscope. Hindgut contents were then collected using a 1,000-µl micropipette. The hindgut contents of 10 individuals were examined using LM and SEM, but *E. imla* was not universally present. Data presented here come from a single individual from Mountain Lake Biological Station, Giles County, VA and one from South Mountains, Burke County, NC.

Light microscopy (LM). Samples of hindgut contents were examined and photographed live with a Zeiss Axioplan 2 compound microscope with Plan Apochromat objectives and Q-imaging Micro Imager II digital camera using differential interference contrast (DIC) illumination.

Scanning electron microscopy. Samples of hindgut contents were placed in plastic Petri dishes, diluted with Trager Medium U to a total volume of approximately 5 ml, and fixed with 4% (w/v) osmium tetroxide (OsO_4) vapours for 30 min. Five or six drops of 4% OsO_4 were then added directly to the samples, which were left for an additional 30 min to complete fixation. Approximately 200 µl of the fixed contents were pipetted onto a Millipore Isopore membrane filter with 5-µm pore size (Billerica, MA) held in a Millipore Swinnex plastic cartridge (Billerica) affixed to a 10-ml plastic syringe. This was repeated (i.e. using additional filter/cartridge/syringe assemblies) until all of the contents were exhausted. The material was then rinsed with 10 ml of Trager Medium U and dehydrated in an ethanol series (10 ml each of 50%, 70%, 90%, and two changes of 100%) for a minimum of 10 min at each stage. Filters were CO_2 critical point dried with a Balzers CPD 020 critical point drying apparatus (Liechtenstein), affixed to aluminum SEM stubs with Ted Pella double stick carbon adhesive (Redding, CA), and sputter coated with gold–palladium in a Nanotech (Manchester, United Kingdom) SEM Prep 2 sputter coater. Stubs were examined and photographed with a Hitachi S4700 field emission scanning electron microscope (Tokyo, Japan) at 1.5–5.0 kV.

DNA extraction, amplification, and sequencing. Cell suspensions in Trager's Medium U were placed in a cavity slide and individual cells matching the description of *E. imla* were manually isolated using a micropipette, as described by Keeling (2002). A pool of 30 *E. imla* cells was isolated in one tube, and three cells were isolated into three individual tubes. DNA was isolated from all four samples using a single chloroform extraction followed by ethanol precipitation, as described (Keeling 2002). The SSU rRNA genes were amplified by rehydrating the DNA directly in a 10-µl reaction volume using the primers 5′-GCGCTACCTG GTTGATCCTGCC-3′ and 5′-TGATCCTTCTGCAGGTTCACC TAC-3′ and amplifying for 35 cycles with an annealing temperature of 45 °C and an extension time of 1.5 min. Products were separated by electrophoresis and cloned. Multiple clones were sequenced on both strands. As the level of variation between clones was low, a single clone was submitted to GenBank as Accession Number DQ923125.

Phylogenetic analysis. The *E. imla* SSU rRNA sequence was added to an alignment including all known parabasalian SSU rRNA sequences. Manual inspection and phylogenetic analyses of this global alignment were carried out to identify sequences that were either nearly identical to other sequences, truncated, or highly divergent. These were removed, along with environmental clones of unknown origin that fell in well-supported clades represented by named species, resulting in an alignment of 49 sequences and 1,236 alignable sites. This alignment was analysed using Bayesian, maximum likelihood, and distance methods. Maximum likelihood trees and 1,000 ml bootstrap replicates were inferred by PHYML 2.4.4 (Guindon and Gascuel 2003) using the GTR substitution model and rate between sites modelled on a γ distribution with eight variable categories and invariable sites. The proportion of invariable sites and the α shape parameter were estimated from the data (0.22 and 0.56, respectively). Bayesian analyses were carried out using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) with the same substitution and rate between sites parameters; 1,000,000 generations were run with three hot chains and one cold chain sampled every 10,000 generations with a burnin of 100,000 generations. Distances were calculated using TREE-PUZZLE 5.2 (Schmidt et al. 2002) with the settings described for ML analysis and 1,000 distance bootstraps calculating using the shell script puzzleboot (by M. Holder and A. Roger: www.tree-puzzle.de). Trees were inferred from distances using weighbor 1.2 (Bruno, Socci, and Halpern 2000). Bootstrap analyses restricted to the Eucomonymphidae (six sequences) were also carried out using PhyML with the same parameters and 1,000 replicates.

The monophyly of *Eucomonympha* was specifically tested using the approximately unbiased (AU) test (Shimodaira and Hasegawa 2001). The monophyly of *E. imla* and the three sequences from *Eucomonympha*-like cells in termites were constrained, and the ML tree optimized according to this constraint using PAUP 4.0 β 10 using the site-to-site rate variation parameters estimated above, the GTR substitution model, and a heuristic search. The resulting tree differed only in two nodes from the ML tree (both in the trichomonads, far from the eucomonymphids). Site likelihoods were calculated for both trees by TREE-PUZZLE using the wsl option with the settings described above. AU tests were carried out using CONSEL 1.19 (Shimodaira 2002).

RESULTS

Distribution. From 10 cockroaches investigated, *E. imla* was found in a single individual from each of two different populations, and in individuals where it was present, it was never as abundant as other hypermastigotes, such as *Trichonympha* or *Barbulanympha*.

Morphology. Under LM, *E. imla* appears as a large (>100 µm long), slowly swimming, densely flagellated cell exhibiting minimal plasticity of shape. *E. imla* cells are composed of two distinct regions: an anterior hemispherical rostrum and an oblate-spheroidal to slightly elongate post-rostral portion (Fig. 1–7), which occasionally displays an encircling constriction or furrow (Fig. 5). Measurements taken from SEM micrographs indicate a mean cell length of 135.7 µm, including both rostral and post-rostral portions, and a mean cell width of 118.3 µm (Table 1). Except for a small, anterior-most portion called the operculum (Fig. 8–10), and the extreme posterior portion, the entire cell is covered with a very dense complement of flagella. Rostral flagella appear to be considerably longer than flagella on the post-rostral portion in LM (Fig. 2, 9). With SEM, however, it is extremely difficult to determine unequivocally where even a single flagellum originates and terminates, especially on the post-rostrum, making accurate measurements and statistically significant comparisons of flagellar length impractical. This difficulty is caused by the great density of flagella on this organism and the fact that, while rostral flagella tend to project outward from the cell in a more or less straight line (Fig. 8), post-rostral flagella often appear to run more parallel to the cell surface where they become entangled among neighbouring flagella and, in some areas, spirochetes (Fig. 12–17). It may be that the apparent difference in length between rostral and post-rostral flagella observed with LM and also noted by Cleveland

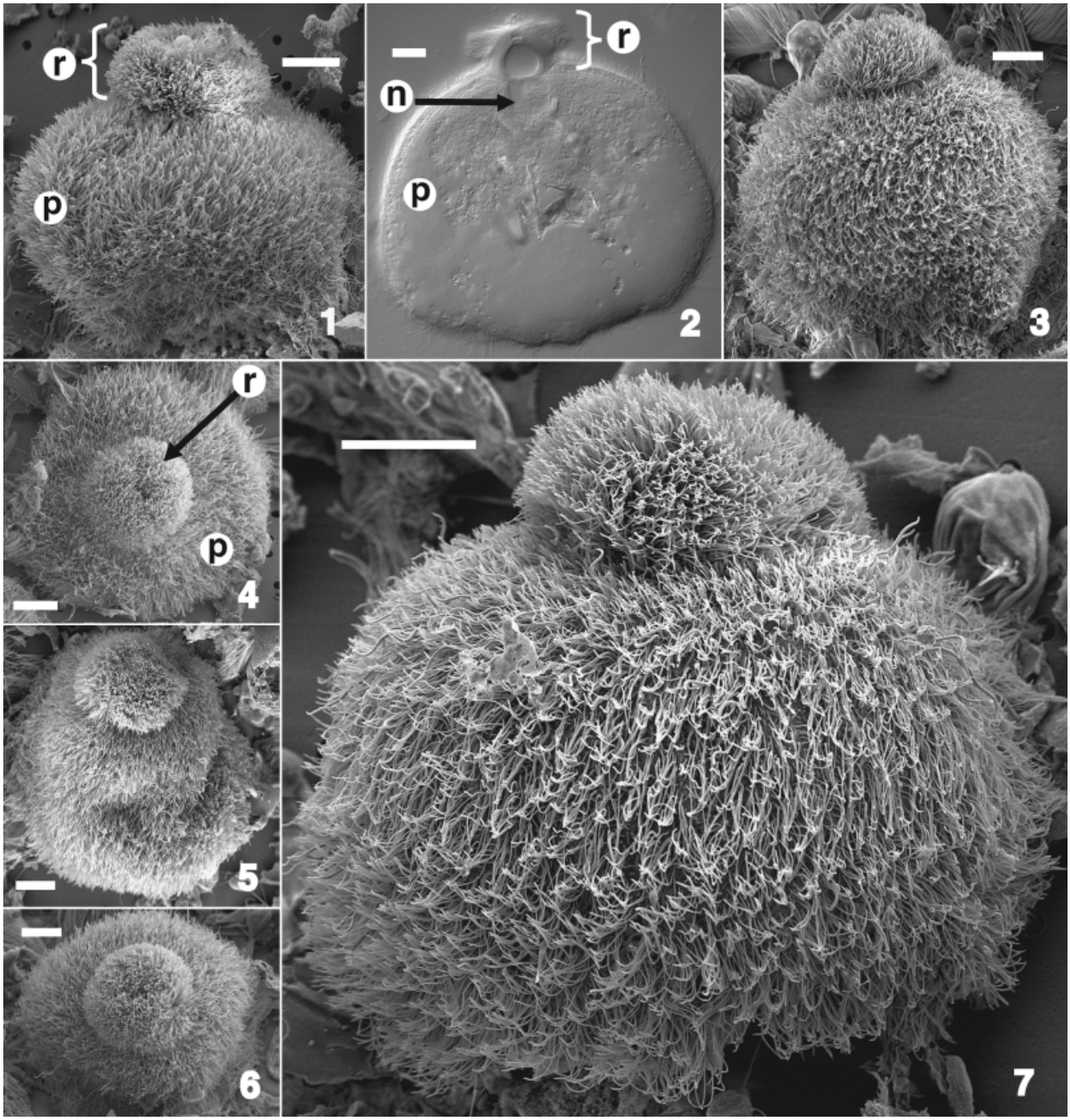

Fig. **1–7.** The whole cell of *Eucomonympha imla* (SEM and LM micrographs). **1.** SEM micrograph, side view showing rostrum (r) and post-rostral area (p), South Mountains population. **2.** LM micrograph, side view showing nucleus (n), Mountain Lake Biological Station population. **3–7.** SEM micrographs, Mountain Lake Biological Station population. **3.** Side view. **4.** Top view. **5.** Oblique view. **6.** Top view. **7.** Side view. Bars = 20 μm.

et al. (1934) is exaggerated due to these properties, which are visible only under SEM.

The operculum was often obscured by rostral flagella in SEM but was visible in some of the individuals from the South Mountains cockroach (Fig. 1, 8, 10). The operculum appears hemispherical in side view (Fig. 10), while in face view it is circular and bears approximately 20 clavate appendages more or less regularly spaced around its circumference (Fig. 8). A circular to ovoid nucleus is visible directly posterior to the rostrum (Fig. 2, 9). In the original description of *E. imla*, Cleveland stated that parabasals were not observed (Cleveland et al. 1934), but we observed numerous parabasals radiating from just under the rostrum using Nomarski DIC microscopy (Fig. 11), likely dictating the distribution of flagella. Endocytosed wood fragments are also visible scattered throughout the post-rostral region (Fig. 2).

Bacterial ectobionts. Examination of *E. imla* with SEM and DIC microscopy revealed that it harbours at least two different populations of bacterial ectobionts. The most conspicuous are spirochetes that associate with flagella and are clearly discernable even with LM (Fig. 12). They were not observed on all cells, but

Table 1. Measurements (means with ranges) of cell features of *Eucomonympha imla* (n = sample size)

Length ($n = 8$)	135.7 (110.9–161.2) μm
Width ($n = 8$)	118.3 (105.4–128.2) μm
Length of rostrum ($n = 8$)	29.9 (22.5–48.9) μm
Width of rostrum ($n = 8$)	56.6 (51.8–63.8) μm
Length of spirochaetes ($n = 21$)	11.5 (8.5–16.7) μm
Length of rod-shaped bacteria ($n = 18$)	1.3 (0.6–2.7) μm
Diameter of operculum ($n = 2$)	6.2 (5.9–6.5) μm
Length of opercular projections ($n = 3$)	0.6 (0.5–0.7) μm

when present they are abundant. Moreover, they are not evenly distributed over the surface of the cell; we observed them to be common on the anterior half of the post-rostral region (Table 1 and Fig. 12–17), rare on the posterior of the post-rostral region, and completely absent on the rostrum, although they were observed right up to the junction between the rostrum and post-rostral region (Fig. 13). We did not observe any spirochetes to be anchored to the cell surface, but instead they seem only to associate with the flagella, in some cases appearing to be entangled in them. Whether they are bound to the flagella is not clear, but their association appears to be specific, as the flagella were not observed to attract other entanglements (Fig. 1, 3–7; 12–17), and also relatively robust, as they were not observed to dislodge when cells were manipulated or isolated. Spirochetes were observed swimming freely in the gut contents, as is also common in termite gut environments, but were never observed associated with any of the other large, densely flagellated protists occurring in the gut of *C. punctulatus* (e.g. *Trichonympha*, *Spirotrichonympha*, *Barbulanympha*). The abundance of these spirochetes on *E. imla* cells and their corresponding absence on any other hypermastigote, together with their specific and restricted distribution to a post-rostral collar on *E. imla*, all suggest this association is not by chance.

In addition to the spirochetes, SEM also revealed small rod-shaped bacteria sparsely distributed over the cell surface of the post-rostral region between flagellar emergence points (Table 1 and Fig. 15–17).

Phylogenetic analysis. The SSU rRNA gene was amplified from 30 manually isolated *E. imla* cells and eight clones were sequenced. All clones were nearly identical (<1% variation was observed and always in a single clone) and similar to sequences from *Teranympha mirabilis* and undescribed termite symbionts assigned to the genus *Eucomonympha*. To confirm the identity of the isolated cells, three additional cells were isolated individually, and the SSU rRNA was amplified, cloned, and sequenced from each. Once again, all three cells yielded sequences nearly identical to those amplified from the 30 cells and showed about the same level of variation (at all variable sites these clones were identical to the consensus). Since the variation was so low, a single clone was selected for use in phylogenetic analysis.

Phylogenetic analysis consistently placed *E. imla* within a strongly supported clade (100%) including the eucomonymphid *Pseudotrichonympha grassii*, three sequences from *Eucomonympha*-like cells from termites, and the teranymphid *T. mirabilis* (Fig. 18). Within the larger tree, the Eucomonymphidae/Teranymphidae group falls within a strongly supported (97%–98%) clade that corresponds to the order Trichonymphida (Brugerolle and Lee 2002), which also includes Hoplonymphidae, Trichonymphidae, and Staurojoenidae. Within the Eucomonymphidae/Teranymphidae group, *T. mirabilis* and the *Eucomonympha*-like cells from termites consistently formed a strongly supported (100%) group to the exclusion of *E. imla* and *P. grassi*. *E. imla* consistently shares a common ancestor with *T. mirabilis* and the *Eucomonympha*-like cells from termites, to the exclusion of *P. grassii*, although this node is not very strongly supported (Fig. 18). This suggests *E. imla* and the *Eucomonympha*-like cells from termites are not monophyletic, despite their similarity in overall appearance. We tested this in a reduced data set of Eucomonymphidae/Teranymphidae and it was again supported at 100%. We also used AU tests to compare the ML tree with an alternative where the monophyly of *E. imla* and the *Eucomonympha*-like cells from termites was enforced and found this alternative was rejected with a P-value of 6×10^{-6}.

DISCUSSION

Morphology. Many of the morphological characters observed here were also noted in earlier descriptions of *E. imla* based on LM or transmission electron microscopy (TEM) (Cleveland et al. 1934; Hollande and Caruette-Valentin 1971). The one major exception to this is the association between *E. imla* and various prokaryotes. Ecto- and endosymbiotic bacteria are commonly found in association with numerous different termite and cockroach gut protists not only with parabasalians (Brugerolle and Lee 2002; Noda et al. 2005, 2006), but also with oxymonads (Leander and Keeling 2004). Noda et al. (2006) determined that Gram-negative, rod-shaped bacterial ectosymbionts found in gut protists belong to three different lineages of Bacteroidales. One of these lineages is characteristic of the order Trichonymphida (strongly supported as monophyletic in the present study), to which *E. imla* belongs. Although Noda et al. (2005) found that bacterial endosymbionts (thought to be derived from ectosymbionts) of the genus *Pseudotrichonympha* (which is closely related to *E. imla*) actually belong to a different group than these, they are still members of Bacteroidales. Thus, it is possible that the rod-shaped bacterial ectobionts of *E. imla* may also be members of Bacteroidales, but this will have to be tested directly.

Spirochetes, on the other hand, have been found to have less specific relationships with host protists; more than one species of spirochete may associate with a given host protist and any given species of spirochete may associate with more than one protist host species. Although the nature of their interactions with the host remains unknown, it seems clear that such bacterial symbionts are of some importance to their protist hosts and the functioning of the gut metabolism (see Noda et al. 2005, 2006). It seems likely that this is true for *E. imla* as well, although the exact nature of this symbiosis remains to be investigated. At very least, the morphological evidence argues that the observed association of spirochetes with *E. imla* is more than the result of mere accidental entanglement: when present, spirochetes are consistently restricted to the upper portion of the post-rostral area; they remain attached when *E. imla* cells are manipulated; and they are not seen associated with any of the other large, densely flagellated protists present in the same gut environment. Thus, we hypothesize that the spirochetes are genuine ectobionts.

Phylogeny. Our analysis of SSU rRNA places *E. imla* in a clade with *Teranympha*, *Pseudotrichonympha*, and sequences obtained from unidentified *Eucomonympha*-like cells from a termite. Ohkuma et al. (2005) inferred a relationship between these *Eucomonympha*-like cells and *T. mirabilis*, but with the addition of *E. imla* it is clear that the *Eucomonympha*-like isolates from termites share a more recent common ancestor with *Teranympha* than they do with *E. imla*. As *E. imla* is the type species of the genus *Eucomonympha* and the genus *Teranympha* (Koidzumi 1921) predates *Eucomonympha* (Cleveland et al. 1934), we suggest that when the currently undescribed *Eucomonympha*-like organisms from termites are formally named, they should not be included in the genus *Eucomonympha* because it would make the genus paraphyletic. Instead, they could be included in *Teranympha*, if they fit its description, or in a new genus.

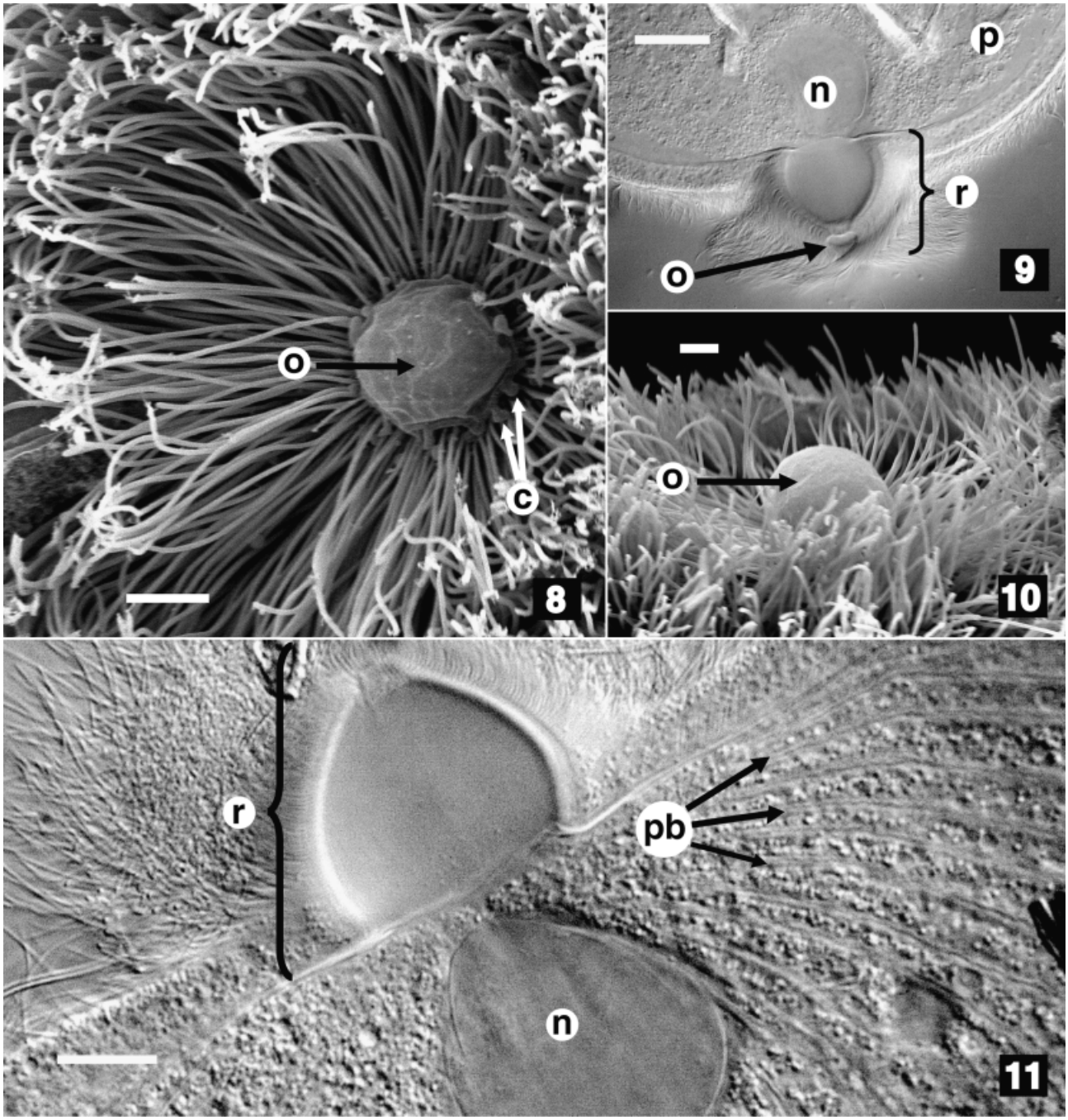

Fig. **8–11.** Micrographs (SEM and LM) of surface structures and organelles of *Eucomonympha imla*. **8.** SEM micrograph showing operculum (o) with clavate projections (c), South Mountains population, bar = 3 μm. **9.** LM micrograph showing rostrum (r), post-rostral area (p), operculum (o), and nucleus (n), Mountain Lake Biological Station population, bar = 20 μm. **10.** SEM micrograph showing operculum (o), South Mountains population, bar = 2 μm. **11.** LM micrograph showing rostrum (r), nucleus (n), and parabasals (pb), Mountain Lake Biological Station population, bar = 10 μm.

This phylogenetic placement of *E. imla* also implies that the presence of flagella covering nearly the entire cell surface is likely a synapomorphy of the Eucomonymphidae/Teranymphidae clade because all investigated members of this clade exhibit this feature except for *T. mirabilis*, which nests above *P. grassii* and *E. imla*. The *Eucomonympha*-like organisms from termites also appear to be fully flagellated in light micrographs (Ohkuma et al. 2005). In *T. mirabilis*, the arrangement of flagella in rings separated by bands of cytoplasm, both of which run perpendicular to the long axis of the cell, is thus likely to be an autapomorphy derived from the fully flagellated state in *Eucomonympha* and *Pseudotrichonympha*. Likewise, depending on the resolution of its position with respect to *E. imla*, the highly elongate cell form of *Pseudotrichonympha* may represent an autapomorphy of this genus. Attached forms of *E. imla* appear somewhat elongate (Cleveland et al. 1934), as does *T. mirabilis* (Brugerolle and Lee 2002), but not to the extreme seen in *Pseudotrichonympha* (Cleveland et al. 1934).

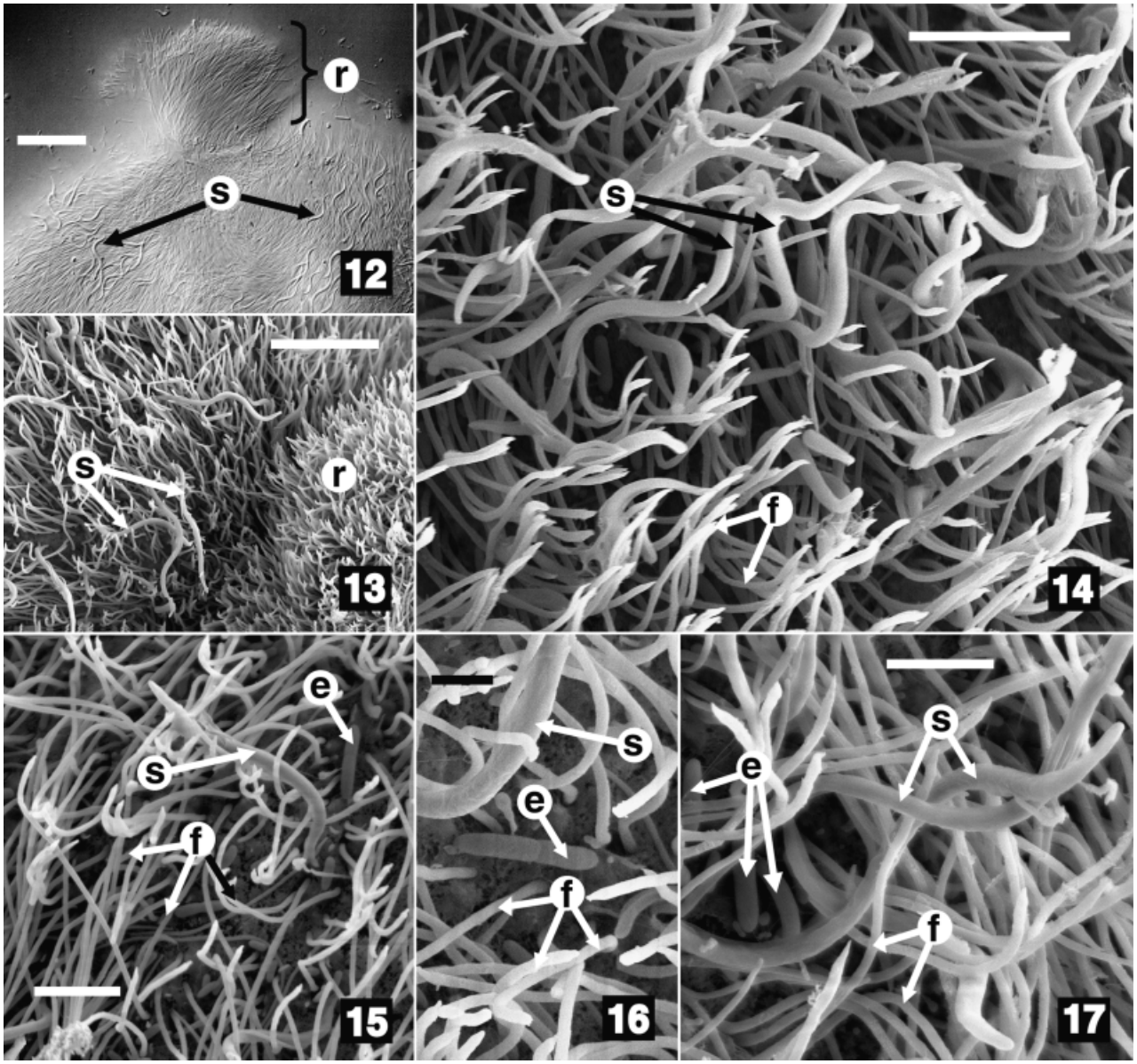

Fig. **12–17.** Micrographs (SEM and LM) of bacterial ectosymbionts of *Eucomonympha imla*, Mountain Lake Biological Station population. **12.** LM micrograph of the anterior end of cell showing spirochetes (s) associated with flagella on the post-rostral area, bar = 20 μm. **13.** SEM micrograph showing portions of the rostrum (r) and post-rostral areas with spirochetes (s) associated with flagella of the latter, bar = 10 μm. **14.** SEM micrograph of the post-rostral area showing spirochetes (s) associated with flagella (f), bar = 5 μm. **15–17.** SEM micrographs of the post-rostral area showing spirochetes (s) associated with post-rostral flagella (f) and rod-shaped bacteria (e) on the cell surface (Bars: $D = 3$ μm; $E = 1$ μm; $F = 2$ μm).

Within the parabasalian tree as a whole, our results also support the monophyly of the order Trichonymphida (including families Eucomonymphidae, Teranymphidae, Hoplonymphidae, Staurojoeninidae, and Trichonymphidae), whose putative synapomorphies include bilateral symmetry (two symmetric-flagellated areas that separate during cell division), a rostral tube and cap, and a cell body consisting of rostral and post-rostral areas (Brugerolle and Lee 2002). Thus, lack of any of these characters (e.g. the rostral tube), as seen in the families Staurojoeninidae and Hoplonymphidae, probably represents a secondary loss. Other groups previously or currently considered to be hypermastigotes that branch elsewhere include lophomonads, which branch with devescovinids and calonymphids, and spirotrichonymphids, which are sister to monocercomonads. This is consistent with other recent analyses (Ohkuma et al. 2005) and at face value supports the possibility that hypermastigotes are polyphyletic, although the branches separating Trichonymphida and spirotrichonymphids are not supported.

A more thorough assessment of character evolution in Eucomonymphidae and Teranymphidae awaits investigation with both of *T. mirabilis* SEM and TEM and the *Eucomonympha*-like termite flagellates that are only known from SSU rRNA sequences and light micrographs at present. Likewise, the task of understanding morphological character evolution in all parabasalians awaits improved sampling of taxa with both molecular and morphological methods and improved support for important nodes.

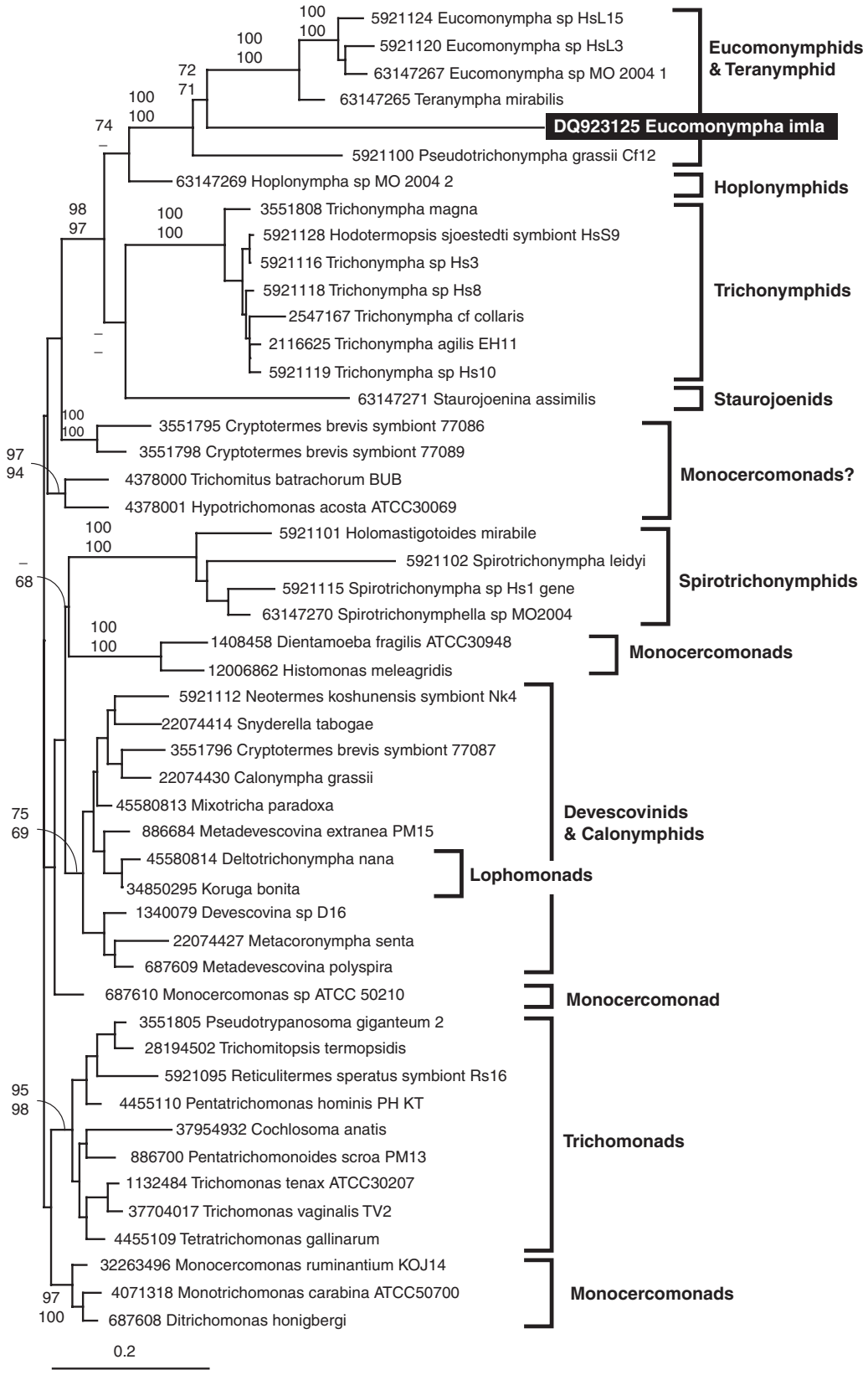

Fig. **18.** Small subunit rRNA phylogeny of Parabasalia. The tree shown is a Bayesian topology with maximum likelihood branch lengths. Subgroups are named and bracketed to the right. Taxon names are preceded by GenBank locus indicators except in the case of *Eucomonympha imla* where the GenBank Accession Number is used. In cases of unidentified symbiont gene the name of the host is used with a strain designation. Numbers at nodes correspond to bootstraps from maximum likelihood (top) and distance (bottom).

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