J. Eukaryot. Microbiol., 54(2), 2007 pp. 137–145

DOI: 10.1111/j.1550-7408.2007.00244.x

Description of *Rhynchopus euleeides* n. sp. (Diplonemea), a Free-Living Marine Euglenozoan

JOANNIE ROY,[a] DRAHOMÍRA FAKTOROVÁ,[b,1] OLDŘICH BENADA,[c] JULIUS LUKEŠ[b] and GERTRAUD BURGER[a,d]

[a]*Centre Robert Cedergren, Bioinformatics & Genomics, Département de biochimie, Université de Montréal, Montréal, QC, Canada H3T 1J4, and*

[b]*Biology Centre, Institute of Parasitology, Czech Academy of Science, and Faculty of Biology, University of South Bohemia, 37005 České Budějovice (Budweis), Czech Republic, and*

[c]*Institute of Microbiology, Czech Academy of Sciences, 142 20 Prague 4, Czech Republic, and*

[d]*Canadian Institute for Advanced Research, Program in Evolutionary Biology*

ABSTRACT. We describe *Rhynchopus euleeides* n. sp., using light and electron microscopy. This free-living flagellate, which was isolated earlier from a marine habitat, can be grown axenically in a rich medium based on modified seawater. In the trophic stage, cells are predominantly elliptical and laterally flattened, but frequently change their shape (metaboly). Gliding is the predominant manner of locomotion. The two flagella, which are typically concealed in their pocket, are short stubs of unequal length, have conventional axonemes, but apparently lack a paraxonemal rod. Swarmer cells, which form only occasionally, are smaller in size and carry two conspicuous flagella of more than 2 times the body length. Cells are decorated with a prominent apical papillum. Both the flagellar pocket and the adjacent feeding apparatus seem to merge together into a single sub-apical opening. The mitochondrion, which is most likely single, is located peripherally. It is reticulated in shape and contains only a few lamellar cristae. Mitochondrial DNA is abundant and evenly distributed throughout the organelle. Morphological synapomorphies confirm the affiliation of the species with the genus *Rhynchopus* (Diplonemea, Euglenozoa). We discuss the characters that distinguish *Rhynchopus* from *Diplonema* corroborating the validity of the two genera.

Key Words. Diplonemea, Euglenozoa, flagellates, morphology, taxonomy, ultrastructure.

THE genus *Rhynchopus* was first described in 1948, based on the observation of a single species, *Rhynchopus amitus* (Skuja 1948; see also Al-Qassab et al. 2002). This free-living flagellate was isolated from pelagic water in the Erken Lake of the east coast of Sweden. Up to now, the only member of this genus that has been characterized ultrastructurally is the parasitic *Rhynchopus coscinodiscivorus*, which feeds on the cytoplasm of planktonic diatoms (Schnepf 1994). Otherwise, only sketchy descriptions are available for a few additional isolates, some reported as parasites of clams and lobster larvae, and others free-living (Bodammer and Sawyer 1981; Kent et al. 1987; Simpson 1997; Vickerman 2000). Given that these reports provide only scarce details about cell size, cell shape, and length of flagella, it is difficult to verify whether these specimens are *Rhynchopus* spp. or *Diplonema* spp.

Rhynchopus was considered initially an unusual euglenid and placed in the Euglenida (Skuja 1948). Since then, classification of this group has undergone numerous modifications (in the following, we will use the taxonomical scheme of Adl et al. 2005). Most importantly, the genus *Rhynchopus* has been removed from the order Euglenida and placed, together with the genus *Diplonema*, which now also comprises species previously named *Isonema* (Patterson and Brugerolle 1988; Schuster, Goldstein, and Hershenov 1968; Triemer and Ott 1990), into the Diplonemea sensu Cavalier-Smith (1993) (Simpson 1997). Diplonemea and Euglenida, together with the Kinetoplastea (commonly named diplonemids, euglenids, and kinetoplastids), now constitute the phylum Euglenozoa. A higher-level assemblage unites Euglenozoa and Heterolobosea into the Discicristata (Cavalier-Smith 1998; Patterson 1994). Furthermore, Euglenozoa, Heterolobosea, and jakobids, together with several amitochondrial groups, have been merged into Excavata (Adl et al. 2005; Simpson and Patterson 2001). Still, solid phylogenetic evidence for the monophyly of these higher order groupings remains to be demonstrated.

Corresponding Author: G. Burger, Université de Montréal, 2900 Boul, Édouard-Montpetit, Montréal, QC, Canada H3T 1J4—Telephone number: +514 343 7936; FAX number: +514 343 2210; e-mail: gertraud.burger@umontreal.ca

[1]Previous name: Drahomíra Novotná.

Here, we describe in detail the morphology of a *Rhynchopus* species, determined by light and electron microscopy. Based on several features that set this organism apart from the other members of the genus, we propose the creation of a new species, *Rhynchopus euleeides* n. sp. With this report, we aim to fill in a critical lack of morphological information about *Rhynchopus* and diplonemids in general, a lack already underlined by others over a decade ago (Schnepf 1994; Simpson 1997). A detailed morphological description of this group is most timely. Owing to the recent discovery of a unique mitochondrial gene and genome structure in *Diplonema papillatum* (Marande, Lukeš, and Burger 2005), diplonemids are now attracting attention beyond the protistology community.

MATERIALS AND METHODS

Isolation and cultivation. *Rhynchopus euleeides* n. sp. (previously designated ''*Rhynchopus* sp.1'' – ATCC 50226) was obtained from the American Type Culture Collection, where it was deposited by T. Nerad in 1986. Cells were cultivated axenically in modified artificial seawater consisting of 3.3% (w/v) sea salts (Instant Ocean), vitamins (0.5 μg/ml biotin, 0.5 μg/ml B12, 100 μg/L thiamine-HCl), and trace metal elements (4.36 μg/L Na_2 EDTA, 3.15 μg/L $FeCl_3 \cdot 6H_2O$, 9.8 μg/L $CuSO_4 \cdot 5H_2O$, 22 μg/L $ZnSO_4 \cdot 7H_2O$, 10 μg/L $CoCl_2 \cdot 6H_2O$, 18 μg/L $MnCl_2 \cdot 4H_2O$, 6.3 μg/L $NaMoO_4 \cdot 2H_2O$), supplemented with 10% horse (HS) or foetal bovine (FBS) serum. Cultures were grown without shaking at room temperature in adhesion-treated flasks (Corning, Corning, NY). A cell scraper (Sarstedt, Newton, NC) was used for harvesting the cells from the flask bottom. A mix of antibiotics (160 μg/ml streptomycin, 160 μg/ml kanamycin, 280 μg/ml penicillin-G [Wisent, St-Bruno, QC]) was added for long-term maintenance of the strain, but not for cultures used in experiments. Stocks were conserved under liquid nitrogen in modified artificial seawater containing 5% (v/v) dimethyl sulphoxide. For the test of phagotrophy, the serum was replaced by either 0.1%–1.0% (w/v) crystallized egg yolk or $\sim 10^5$/ml bacteria (a mix of *Enterobacter aerogenes*, *Silicibacter* sp., *Bacillus* sp., and *Pseudomonas* sp.). The titre of bacteria was determined by counting the cells in a haemacytometer.

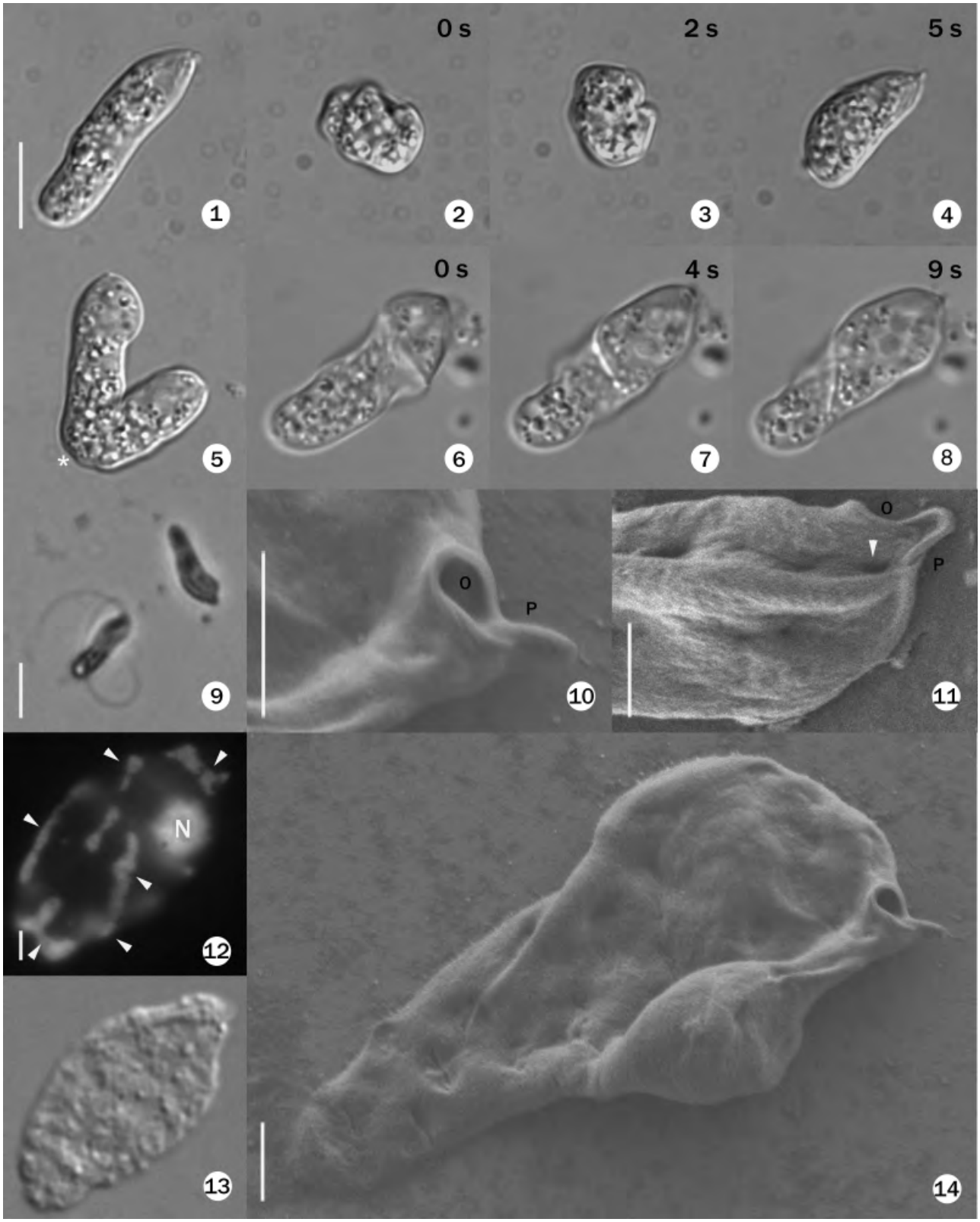
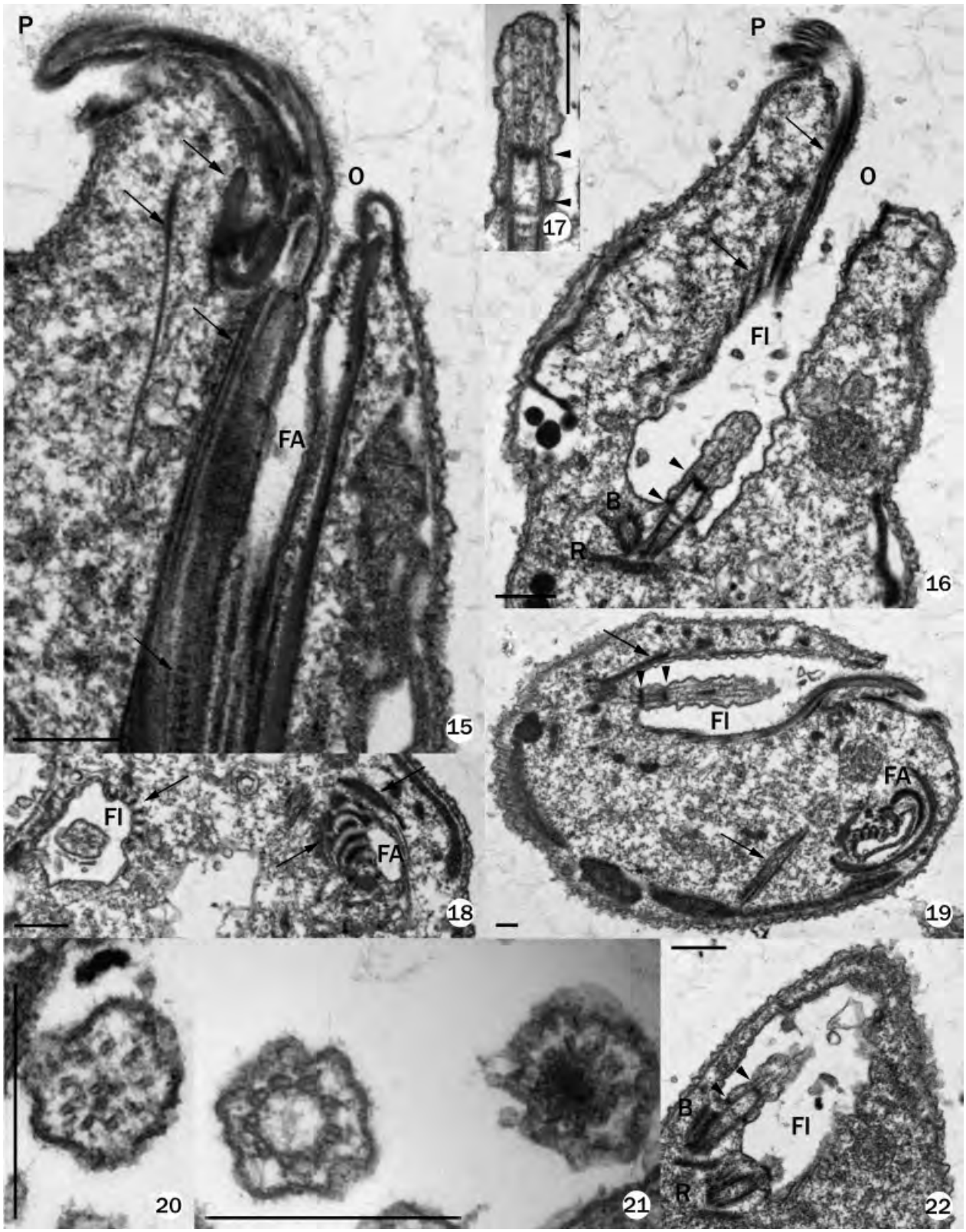


Fig. **1–14.** Light and scanning electron microscopy of *Rhynchopus euleeides* n. sp. Cell morphology and DNA distribution. **1.** Cell of elliptical shape with a conical apical end. **2–4.** Time series of a cell changing its shape (metaboly). Time interval is 5 s. **5.** Intermediate stage of cell division. Posterior end is indicated by an asterisk. **6–8.** Time series of peristaltic and contortive movement. Time interval is 9 s. **9.** Swarmer cell with two long flagella (left) and a trophic-starved cell (right). **10.** Anterior part of a cell showing the sub-apical pocket (O) and the apical papillum (P). **11.** Lateral view of the anterior part of a cell. Sub-apical pocket (O), apical papillum (P). The cavity (arrowhead) close to the apical papillum is not interpreted as a second (small) opening of the cell, because several such cavities are seen at the surface of this cell. **12.** 4′,6′-Diamidino-2-phenylindole-stained cell revealing nuclear (N) and mitochondrial (arrowheads) DNA. **13.** Same cell as in Fig. 7. **14.** Whole cell (same cell as in 10). Note the smooth surface. Scale bar = 10 μm (Fig. 1–9) and 2 μm (Fig. 10–14).

Light and fluorescent microscopy. Live cells were observed with an inverted Nikon Eclipse TE2000-U microscope (Nikon, Tokyo, JP) using a Nomarski differential interference contrast (DIC) filter, with the exception of Fig. 9, which was taken in phase contrast. Pictures were recorded with MetaMorph 6.3r3 (Molecular Devices, Sunnyvale, CA). For 4′,6′-diamidino-2-phenylindole (DAPI) staining, cells were fixed for 10 min at room temperature in 4% (w/v) paraformaldehyde diluted in 3.3%



artificial seawater. Fixation was stopped by spinning the cells down and resuspending them in 3.3% artificial seawater. After the cells were allowed to adhere onto poly-L-lysine-coated slides for 2 h in a humidity chamber, the slides were stained with 1 μg/ml DAPI in phosphate-buffered saline (PBS) pH 7.2 for 5 min. The stained cells were washed, mounted with the antifade reagent (0.233 g 1,4-diazabicyclo-(2,2,2)octane; 1 ml 0.2 M Tris-HCl, pH 8.0; 9 ml glycerol), and examined with a Zeiss Axioplan 100 microscope (Jena, Germany) in DIC mode.

Electron microscopy. For transmission electron microscopy, cells were washed twice in artificial seawater, centrifuged at low speed ($\sim$ 1,200 *g*), and fixed following two different protocols: (i) 2% (v/v) glutaraldehyde (GA) in 0.25 M phosphate buffer (PB), pH 7.2, overnight at 4 °C; or (ii) same as (i) but the concentration of 0.1 M PB. After fixation, cells were washed in the respective buffer supplemented with 4% (w/v) glucose, pelleted, and embedded in 2% (w/v) agarose. Post-fixation was done with 2% (w/v) OsO_4 in PB for 2 h at room temperature, followed by a washing step with PB. After dehydration in graded series of ethanol, the cells were embedded in Epon-Araldite. Ultra-thin sections were stained with lead citrate and uranyl acetate and examined under a JEOL JEM 1010 microscope (Tokyo, JP).

For scanning electron microscopy observations, actively growing cells were placed in a dish and allowed to adhere to coverslips for a period of time, fixed in 5% GA in 0.25 M PB pH 7.2 for 5 min at 37 °C in a PELCO 3440 Max laboratory Microwave Oven (Ted Pella; Reddin, CA), washed in the same buffer, followed by a post-fixation step with 1% osmium tetroxide in 0.1 M PBS, pH 7.2 for 5 min and dehydrated in graded series of ethanol. Coverslips were air dried, mounted on aluminium stubs with conductive carbon paint (SPI supplies, West Chester, PA), and observed without any further treatment with a JEOL JSM-7400F high-resolution field emission scanning electron microscope in the secondary mode at 1.5 kV.

RESULTS

Light microscopical observations. The feeding behaviour of *R. euleeides* n. sp. depends on available nutrients. In a serum-based medium, cells feed osmotrophically; at high concentrations of serum ($\sim$ 10%), the culture turns blackish due to an unidentified compound secreted by the cells once they reach the stationary phase ($\sim 10^7$ cells/ml). Alternatively, in a medium containing crystallized egg yolk or bacteria, the flagellates will feed by phagotrophy. Generation time of axenic cultures in modified artificial seawater supplemented with 10% serum is approximately 8 h. Division occurs longitudinally, from the anterior to the posterior end (Fig. 5).

Trophic cells are typically 15–27 μm long and 4–8 μm wide in the centre, of almost symmetrical elliptical shape, yet flattened laterally (Fig. 1, 4–8). Generally, cells are attached to a surface, but with increasing cell density, a small percentage of them will float and cluster together. When cells move by gliding, the anterior portion of the cell appears to be responsible for motion and steering. Peristaltic-like contractions, expansions, and contortions of the body (termed ''metaboly'') are radical and frequent (Fig. 2–4, 6–8). Cysts, which form readily in aging cultures, are smaller and somewhat rounder than trophic cells. Flagella are invisible in both the trophic and cyst stages. Free-swimming cells are rare, and have been observed only on a few occasions in starved cultures. These ''swarmers'' are significantly shorter (up to $\sim$ 15 μm) than trophic cells. Flagella of the swarmer stage are about 2.5 times longer than the body and used for fast propulsion (Fig. 9).

Fluorescent microscopy of DAPI-stained cells reveals the nucleus, enclosing a single, less fluorescent nucleolus. A large amount of mitochondrial DNA (mtDNA) is seen peripherally, apparently distributed uniformly throughout the reticulated organelle (Fig. 12, 13). Clearly, a kinetoplast-like structure is absent.

Electron microscopical observations. Scanning microscopy shows an elliptical, laterally flattened cell shape. Although the fixation protocol used may cause dehydration, the flattened shape and the folds of the cell surface are not artefacts, because light microscopy of live cells recovered the same morphology (see Fig. 1–9). The cell surface is smooth without pellicles strips. A conspicuous opening is located sub-apically, with its rim folded into a lip, which protrudes at the dorsal side to form a conical, flattened apical papillum (Fig. 10, 11, 14). None of the >100 cells inspected had more than a single opening. Flagella remain concealed in trophic cells from young and axenic cultures, but a structure resembling a single, short, barely emerging flagellum has sometimes been observed in older, non-axenic cultures (data not shown).

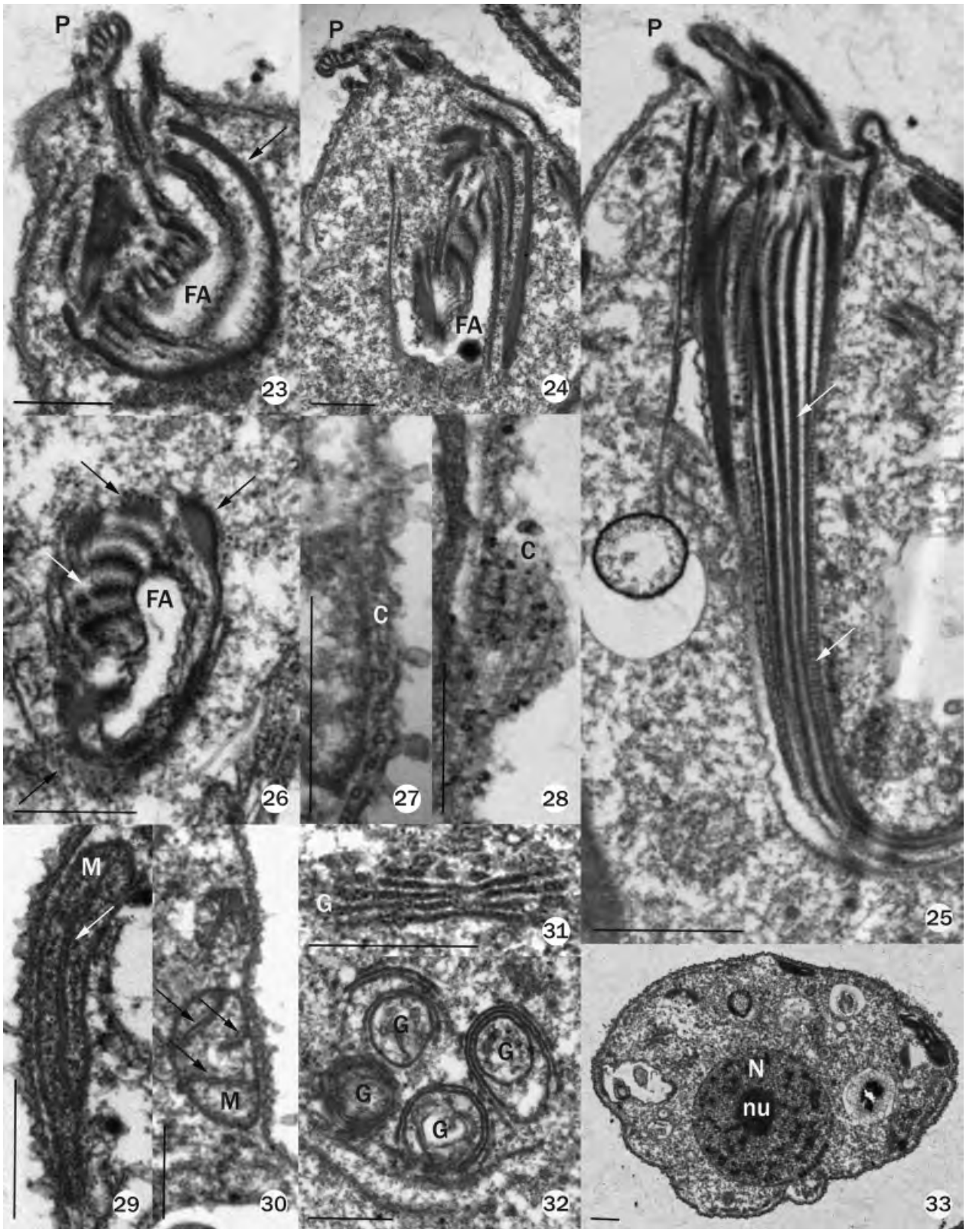
Transmission microscopy of this organism proved to be difficult, as the cell collapsed easily during fixation, probably due to their poor resistance to osmotic change. Among the different protocols tested, the fixation with GA diluted in 0.25 M PB yielded the best results. The images show that cells possess a sub-apical cavity that encloses the feeding apparatus and the flagellar apparatus. Longitudinal sections of the whole cell reveal that both apparatuses occupy up to half of the total body length (Fig. 15, 16, 25, see scale bar), and are generally oriented parallel to one another (Fig. 18), but depending on the cell shape, they may appear as well in perpendicular orientation (Fig. 19). The two apparatuses appear to merge peripherally to form the single sub-apical opening observed in scanning microscopy.

The two flagella, which are inserted in their pocket in parallel orientation (Fig. 16, 21, 22), consist of conventional 9+2 axonemes and lack a paraxonemal rod (Fig. 17–21). Transversal sections often show only one flagellum within the pocket, indicating that the two flagella are of unequal length (Fig. 18, 20). The flagellar root system features a transitional zone bounded by two plates (Fig. 16, 17, 19, 22). The two basal bodies are supported by surrounding microtubules (Fig. 16, 22).

The feeding apparatus, which is positioned adjacent to the flagellar pocket, displays a cytopharyngal component that is coated on the interior with vanes (ribs) and supporting rods on one side, and with a row of multidirectional microtubules on the opposite side (Fig. 18, 19, 23–26). In a longitudinal view, the cytopharynx appears as a horn-like structure through which nutrients appear to enter the cell (Fig. 25).

Fig. **15–22.** Transmission electron microscopy of *Rhynchopus euleeides* n. sp. Architecture of the flagellar and feeding apparatuses. **15.** Longitudinal section through the anterior part of the cell showing the feeding apparatus (FA), which is supported by multiple fibres (arrows) and adjacent to the apical papillum (P) anterior to the sub-apical opening (O). **16.** Longitudinal section through the anterior part of the cell showing the flagellar pocket (Fl) reinforced apically by fibres (arrows), the apical papillum (P), the sub-apical opening (O), the transitional plate (arrowheads), the basal body (B), and the flagellar root (R). **17.** Detail of Fig. 16 showing the transitional zone of the flagellum (arrowheads). **18.** Transverse section of the Fl and the FA, both supported by fibres (arrows). **19.** Section through the flagellar and feeding apparatuses. Transitional plates (arrowheads) and supporting fibres (arrows) are visible. **20.** Transverse section of a 9+2 ordered axoneme. Only a single flagellum was seen in this section. **21.** Transverse section of the FI containing two flagella. The left flagellum shows the transitional zone, characterized by a 9+0 tubular arrangement. The right flagellum shows a section through the plate in the transition zone. **22.** Section through the FI, basal bodies (B), and flagellar roots (R) of both flagella. Note the transitional plates (arrowheads). Scale bar = 500 nm; the fixation protocol (i) was used (see ''Materials and Methods'').

Beneath the cell membrane lies a single layer of tightly packed microtubules oriented longitudinally. This component of the cytoskeleton is thought to be a major player in metaboly and locomotion (Fig. 27, 28). The mitochondrion is most likely single and lies beneath the inner side of the cell membrane. It has a reticulated shape and encloses sparse and mostly longitudinally oriented lamellar cristae (Fig. 29, 30). A fibrillar array of mtDNA was not detected, corroborating the absence of a kinetoplast-like



structure. The Golgi apparatus generally assumes a linear form, but occurs also in a circularized form (Fig. 31, 32). The round nucleus contains a single centrally located dense nucleolus and chromosomes that seem to be permanently condensed (Fig. 33). No structures resembling trichocysts or extrusomes have been observed.

DISCUSSION

Rhynchopus sp. 1 (ATCC 50226) is a species widely used in molecular phylogenies that aim at the elucidation of the evolutionary history of Euglenozoa (Busse and Preisfeld 2002, 2003; Moreira, Lopez-Garcia, and Rodriguez-Valera 2001; Simpson, Lukeš, and Roger 2002; Simpson et al. 2004; von der Heyden et al. 2004). In this report, we fill a gap in the knowledge of this key flagellate by providing a detailed morphological description (Fig. 34–36). The new name we propose, *R. euleeides* n. sp., means ''maggot-like'' and is inspired by the cells' movement and inclination to congregate.

Members of the genus *Rhynchopus* bear in their trophic stage two short flagella that are barely emerging from, or completely buried in, the flagellar pocket, while in the swarmer stage, flagella are long and fully motile (Vickerman 2000; von der Heyden et al. 2004). The features that distinguish individual species within the genus are manifold (Table 1). First, the habitat varies. Many members of the genus, including *R. euleeides* n. sp., live in marine environments, whereas the type species *R. amitus* occurs in fresh water. We propose that *R. amitus* derives from a marine taxon, as the Erken Lake, from which it has been isolated, was originally part of the Baltic Sea and turned into an enclosed basin only relatively recently (Weyhenmeyer 1998). Second, the lifestyle of *Rhynchopus* species is quite diverse. Some members are free-living (*Rhynchopus* sp. 2 [ATCC50230], *R. amitus, R. euleeides* n. sp.], *R. coscinodiscivorus* is an intracellular parasite of diatoms, and several poorly described species were reported to be ectoparasites or ectocommensals (Bodammer and Sawyer 1981; Kent et al. 1987; Simpson 1997; Vickerman 2000). Still, it is possible that these latter taxa are only opportunistic parasites rather than obligate ones. Third, the flagella are quite diverse across the genus. In the trophic stage, they are of about equal length in *R. coscinodiscivorus*, while in *R. euleeides* n. sp., one flagellum appears to be shorter than the other. The axoneme of *R. euleeides* n. sp. displays a canonical organization, whereas the limited data of *R. coscinodiscivorus* suggest that its axoneme is arranged irregularly (Schnepf 1994). Finally, what sets *R. euleeides* n. sp. conspicuously apart from the well-described *R. amitus* and *R. coscinodiscivorus* is the symmetrical, elliptical shape of its resting cells, contrasting with the pear-shaped cells of the latter two species. Taken together, the particular combination of ecological, morphological, and ultrastructural characteristics observed in *R. euleeides* n. sp. qualifies this taxon clearly as a new species (Table 1).

Diplonemea, comprising *Rhynchopus* and *Diplonema*, share several features. Both genera display pronounced metaboly, feature an oval to sack-like cell shape, possess two short flagella, and contain an exceptionally large amount of mtDNA (Marande et al. 2005; Maslov, Yasuhira, and Simpson 1999; this work). In addition, the single reticulated mitochondrion of diplonemids encloses only a few lamellar, parallel-arranged cristae (with the exception of *Diplonema ambulator*, which was reported to have numerous cristae (Triemer and Ott 1990). Moreover, the flagellar and feeding apparatuses are very similar in *Rhynchopus* and *Diplonema* (Montegut-Felkner and Triemer 1994, 1996; Schnepf 1994). Members of both genera have their feeding apparatus longitudinally arranged and connected with the flagellar apparatus (Triemer and Ott 1990). This complex structure was initially classified as a type II feeding apparatus known from euglenids (Linton and Triemer 2001; Triemer and Farmer 1991a), but a detailed three-dimensional ultrastructural analysis did not support this notion (Montegut-Felkner and Triemer 1996).

Given the synapomorphies of Diplonemea described above, it is difficult to identify features that unambiguously distinguish the two genera. Morphological distinctions are usually based on the relative length of their two flagella, which is unequal in most *Rhynchopus* species, but equal in all *Diplonema* species described. Some authors consider the occurrence of a swarmer stage with fully motile flagella as an additional distinctive trait of *Rhynchopus* (von der Heyden et al. 2004), but this character is of limited practical use for taxonomic identification, as swarmers form rarely, at least in *R. euleeides* n. sp. As summarized in Table 1, the taxonomic breadth of available morphological information on *Diplonema* and *Rhynchopus* species has been too limited to rigorously distinguish the two genera, raising the question whether the two genera should be merged. However, our new results indicate that the presence of a single sub-apical opening could be a feature characteristic for *Rhynchopus*, as the *Diplonema* species examined by scanning electron microscopy were reported to have two separate openings (Porter 1973; Triemer and Farmer 1991b). Testing of our hypothesis will require a comprehensive scanning electron microscopy study of recognized *Rhynchopus* and *Diplonema* species.

Diplonemea were initially associated with the Euglenida (Griessmann 1913; Skuja 1948). Indeed, there are several features common to both groups, including the microtubule-reinforced feeding apparatus, the flagellar apparatus consisting of two basal bodies, the microtubular root system, and reticulated mitochondria. But we know today that these features are also shared with kinetoplastids, which refutes the inclusion of Diplonemea in the Euglenida (Brugerolle et al. 1979; Farmer and Triemer 1994; Pellegrini 1980; Simpson 1997; Simpson et al. 2002).

The phylogenetic relationships within the Euglenozoa remain controversial (Busse and Preisfeld 2002, 2003; Moreira, Lopez-Garcia, and Vickerman 2004; Moreira et al. 2001; Simpson and Roger 2004; Simpson et al. 2002; von der Heyden et al. 2004). Recent single-gene phylogenies provide reasonable supports for the sister relationship of Diplonemea and Kinetoplastea, with Euglenida branching before the divergence of the two former clades (Simpson and Roger 2004). Yet, *Rhynchopus* and *Diplonema* are not always recovered as sister groups. For example, an rRNA-based phylogenetic analysis including numerous diplone-

Fig. **23–33.** Transmission electron microscopy of *Rhynchopus euleeides* n. sp. Architecture of feeding apparatus, organelles, and cytoskeleton. **23.** The feeding apparatus (FA) at the very apical end of the cell. Note the prominent fibres surrounding the cytostome (arrow) and the structured apical papillum (P). **24.** Oblique view of the FA. Note the P. **25.** Longitudinal view of the P merged with the FA that forms a J-shaped tube surrounded by two sets of fibres indicated by arrows: one running parallel and the other perpendicular to the longitudinal axis of the cell. **26.** Transverse section of the FA containing a number of microtubules and plicate vanes (arrows) surrounding the central cytostome. **27.** Transverse section of the cytoskeletal corset (C) of the cell. **28.** Longitudinal section of the C. **29.** Longitudinally sectioned peripherally located mitochondrion (M) with prominent cristae (arrow). **30.** Cross-sectioned peripherally located M with scarce cristae (arrows). **31.** Linear form of the Golgi apparatus (G). **32.** Multimembraneous vesicles, interpreted as circularized G. **33.** Transverse section of the cell showing a large nucleus (N) with condensed chromosomes and a nucleolus (nu). Scale bar = 500 nm. The fixation protocols used are (i) (Fig. 19–24, 27–29) and (ii) (Fig. 25, 26); see ''Materials and Methods''.

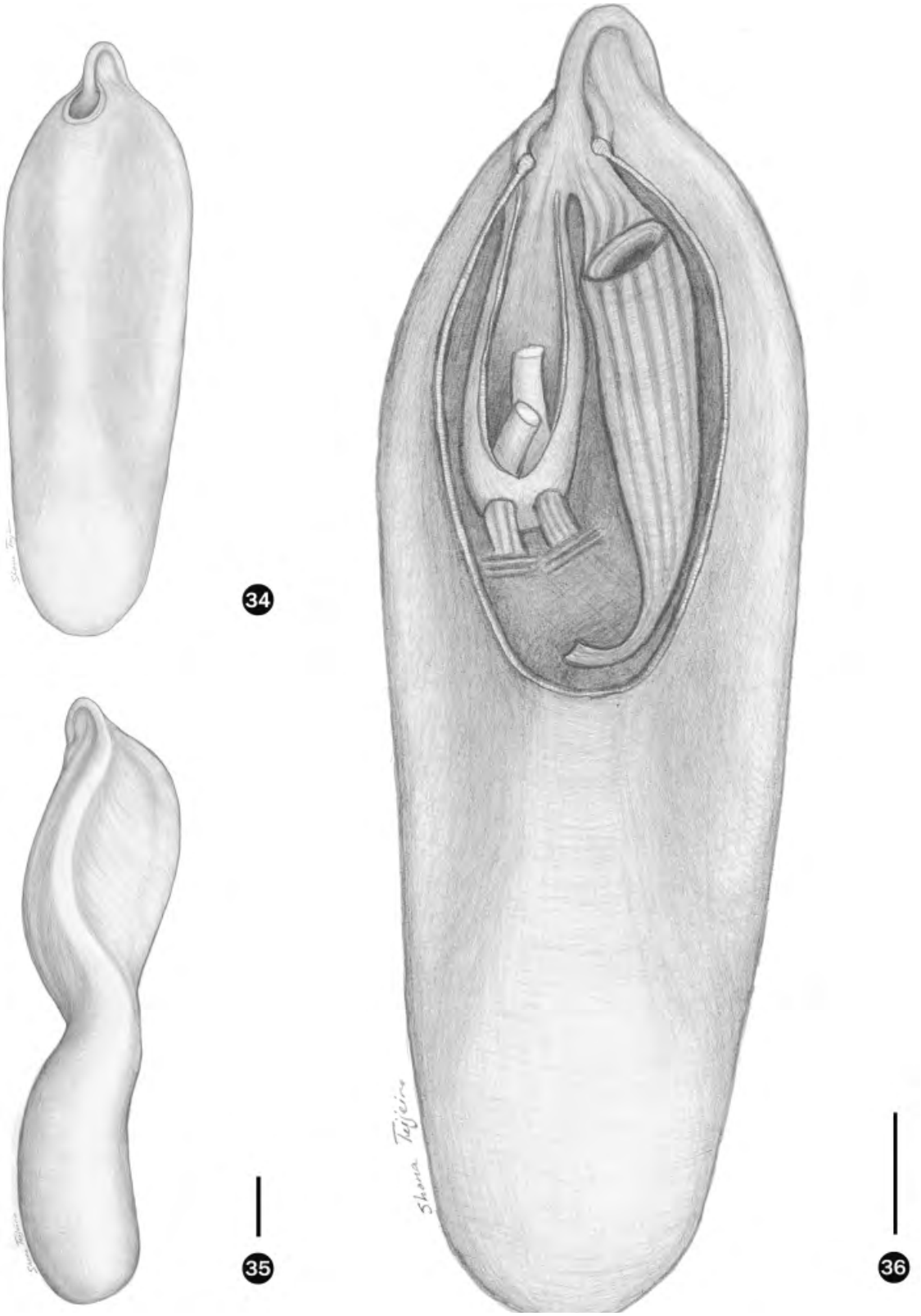


Fig. **34–36.** Schematic three-dimensional representation of *Rhynchopus euleeides* n. sp. **34.** Outer view of a trophic cell. **35.** Dorsal view of a cell in movement, illustrating its lateral flatness. **36.** Inner view of the cell represented in Fig. 34. Note that the flagellar and feeding apparatuses merge together into a single microtubule-reinforced area. Scale bar = 2 μm.

Table 1. Comparative features of species in the two Diplonemea genera *Rhynchopus* and *Diplonema*.

Species	Description	Habitat	Parasitism[a]	Pigment production	Swarmer stage	Cell dimensions (μm)	Apical papillum	Opening	Equal length of flagella	Emerging length of flagella (μm)	Ordered axoneme	Paraxonemal rod	Trichocyst
Rhynchopus euleeides n.sp.	This report	Marine	No	Yes	Yes	10–25 × 4–8	Yes	1	No (T)	0 (T); ~ 12 (S)	Yes (Y)	No (T)	No
Rhynchopus amitus	Skuja (1948)[b]	Fresh water, marine	No[b]	?	Yes	20–25[b] × 7–9	Yes	?	?	0 (T); ~ 5–11 (S)	?	?	?
Rhynchopus coscino-discovorus	Schnepf (1994)[b]	Marine	Yes	?	?	20–25 × 10–12	Yes	?	Yes (T)	?	No (T)	No (T)	No
Rhynchopus sp.2 ATCC 50230	Simpson (1997)	Marine	?	?	Yes	?	?	?	?	?	Yes (S)	Yes (S)	?
Diplonema breviciliata	Griessmann (1914)	Marine	No	?	No	28–35 × 8–10	Yes	?	Yes	~ 8–10	?	?	?
Diplonema nigricans	Schuster et al. (1968)	Marine	No	Yes	No	40–50 × 3–7	No	?	Yes	3	Yes	No	Yes
Diplonema papillatum	Porter (1973)	Marine	No	No	No	10–24 × 4–8	Yes	2	Yes	5.5–7	Yes	No	No
Diplonema ambulatory	Larsen and Patterson (1990)[c]	Fresh water, marine	Yes	Yes	No	17–24 × 6–10	Yes	2	Yes	2–3	Yes	No	No
Diplonema metabolicum	Larsen and Patterson (1990)	Marine	?	?	No	30–48 × ?	No	?	?	~ 10	?	?	?

[a]Data are lacking to distinguish between opportunistic and obligate parasitism.
[b]See also Al-Qassab et al. (2002).
[c]See also Triemer and Ott (1990).
?, no information available; T, trophic stage; S, swarmer stage.

mean taxa places certain *Rhynchopus* species within the genus *Diplonema* (von der Heyden et al. 2004), though with low bootstrap support. Obviously, multiple-gene based phylogenetic analysis will be required to resolve the relationship of these two genera.

The relationship within Diplonemea might be revealed by comparative genomics. As we have shown recently, the mitochondrial genome of *D. papillatum* has a unique multi-partite structure with gene fragments encoded on distinct circular chromosomes (Marande et al. 2005). Interestingly, a similar genome structure is found in *R. euleeides* n. sp. (Marande, Roy, and Burger, unpubl. observ.). Work is in progress to determine how broadly these features are distributed across Diplonemea and related groups.

Taxonomic summary

Phylum Euglenozoa Cavalier-Smith (1981)
Class Diplonemea Cavalier-Smith (1993)
Genus *Rhynchopus* Skuja (1948)
Rhynchopus euleeides n. sp.

Diagnosis. We describe ''*Rhynchopus* sp.1'' (ATCC 50226) and rename it *R. euleeides* n. sp. The description is based on morphological and physiological data.

Description. In the trophic stage, cells generally attach to a surface. Cells are elongated, 15–27 μm in length by 4–8 μm in width. The cell shape is elliptical with nearly symmetrical anterior and posterior ends, and laterally flattened. Cells display conspicuous metaboly and move by gliding. Flagella are concealed. When starved, the swarmer stage appears with two fully motile flagella used for swimming. Flagellar and feeding apparatuses are arranged parallel to the longitudinal axis of the cell. The two apparatuses merge together into a single sub-apical opening, located below the apical papillum. The mitochondrion, lying beneath the cell periphery, has a few, long, longitudinally oriented cristae of lamellar shape. In serum-supplemented medium, cells secrete a black pigment as the culture ages.

In vitro cultivation. Axenic cultures can be maintained in modified artificial seawater medium, supplemented with mineral trace elements, vitamins, and serum. Cells should be grown in an adhesion-treated culture flask in horizontal position, with passage to new medium twice a week. Alternatively, serum can be replaced with bacteria or crystallized egg yolk, which results in a slower grow rate.

Type locality. Isolated from seawater, New Bedford, MA, USA (latitude 41.6379°N; longitude 70.9372°W).

Etymology. *Rhynchopus euleeides* n. sp. (from Gr. *eulê-*, ''maggots,'' ''worm'' + latinized form of Gr. suff. *-eidês*, ''in shape of,'' ''similar to'').

Type material. The type culture was deposited by T.A. Nerad in 1986 at the American Type Culture Collection, under the Accession number ATCC 50226. A slide prepared from this culture has been deposited as a hapantotype at the International Protozoan Type Slide Collection under the Accession number USNM 1091283.

ACKNOWLEDGMENTS

We thank Shona Teijeiro (Université de Montréal) for the art work and the electronic microscopy unit of Antonio Nanci (Médecine dentaire, Université de Montréal) for usage of their facility. J. R. thanks Hugo S. Doyer (Université de Montréal) and Jean-Michel Lavoie (Université Laval) for etymological advice. This research was supported by a grant (MOP-79309) from the Canadian Institutes of Health Research (CIHR) Genetics Program to G. B., by fellowships of the Agence universitaire de la francophonie

(AUF), the Ministry of Education of Québec and CIHR to J. R., by grants 5022302 and Z60220518 from the Grant Agency of the Czech Academy of Sciences and grant 2B06129 from the Ministry of Education of the Czech Republic to J. L., and by Institutional Research Concept Z50200510 to O. B. We gratefully acknowledge the Canadian Institute for Advanced Research (CIAR) Program for Evolutionary Biology, for salary and interaction support to G. B. and D. F.

LITERATURE CITED

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, O., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W. & Taylor, M. F. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.*, **52**:399–451.

Al-Qassab, S., Lee, W. J., Murray, S., Simpson, A. G. B. & Patterson, D. J. 2002. Flagellates from Stromatolites and surrounding sediments in Shark Bay, Western Australia. *Acta Protozool.*, **41**:91–144.

Bodammer, J. E. & Sawyer, T. K. 1981. Aufwuchs protozoa and bacteria on the gills of the rock crab, *Cancer irroratus* Say: a survey by light and electron microscopy. *J. Protozool.*, **28**:35–46.

Brugerolle, G., Lom, J., Nohýnková, E. & Joyon, L. 1979. Comparaison et évolution des structures cellulaires chez plusieurs espèces de bodonidés et cryptobiidés appartenant aux genres *Bodo*, *Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica*, **15**:197–221.

Busse, I. & Preisfeld, A. 2002. Phylogenetic position of *Rhynchopus* sp. and *Diplonema ambulator* as indicated by analyses of euglenozoan small subunit ribosomal DNA. *Gene*, **284**:83–91.

Busse, I. & Preisfeld, A. 2003. Systematics of primary osmotrophic euglenids: a molecular approach to the phylogeny of *Distigma* and *Astasia* (Euglenozoa). *Int. J. Syst. Evol. Microbiol.*, **53**:617–624.

Cavalier-Smith, T. 1981. From eukaryotic kingdoms: seven or nine? *Biosystems*, **10**:93–116.

Cavalier-Smith, T. 1993. Kingdom protozoa and its 18 phyla. *Microbiol. Rev.*, **57**:953–994.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. *Biol. Rev. Cambridge Philos. Soc.*, **73**:203–266.

Farmer, M. A. & Triemer, R. E. 1994. An ultrastructural study of *Lentomonas applanatum* (Preisig) n.g. (Euglenida). *J. Eukaryot. Microbiol.*, **41**:112–119.

Griessmann, K. 1913. Ueber marine flagellaten. *Arch. Protistenkd.*, **32**: 1–78.

Kent, M. L., Elston, R. A., Nerad, T. A. & Sawer, T. K. 1987. An Isonema-like flagellate (Protozoa Mastigophora) infection in larval Geoduck clams, *Panope abrupta*. *J. Invertebr. Pathol.*, **50**:221–229.

Larson, J. & Patterson, D. J. 1990. Some protists from tropical marine sediments. *J. Nat. Hist.*, **24**:801:937.

Linton, E. W. & Triemer, R. E. 2001. Reconstruction of the flagellar apparatus in *Ploeotia costata* (Euglenozoa) and its relationship to other euglenoid flagellar apparatuses. *J. Eukaryot. Microbiol.*, **48**:88–94.

Marande, W., Lukeš, J. & Burger, G. 2005. Unique mitochondrial genome structure in diplonemids, the sister group of kinetoplastids. *Eukaryot. Cell*, **4**:1137–1146.

Maslov, D. A., Yasuhira, S. & Simpson, L. 1999. Phylogenetic affinities of *Diplonema* within the Euglenozoa as inferred from the SSU rRNA gene and partial COI protein sequences. *Protist*, **150**:33–42.

Montegut-Felkner, A. & Triemer, R. 1994. Phylogeny of *Diplonema ambulator* (Larsen and Patterson) 1. Homologies of the flagellar apparatus. *Eur. J. Protistol.*, **30**:227–237.

Montegut-Felkner, A. & Triemer, R. 1996. Phylogeny of *Diplonema ambulator* (Larsen and Patterson) 2. Homologies of the feeding apparatus. *Eur. J. Protistol.*, **32**:64–76.

Moreira, D., Lopez-Garcia, P. & Rodriguez-Valera, F. 2001. New insights into the phylogenetic position of diplonemids: G+C content bias, differences of evolutionary rate and a new environmental sequence. *Int. J. Syst. Evol. Microbiol.*, **51**:2211–2219.

Moreira, D., Lopez-Garcia, P. & Vickerman, K. 2004. An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. *Int. J. Syst. Evol. Microbiol.*, **54**:1861–1875.

Patterson, D. J. 1994. Protozoa: evolution and systematics. *In*: Hausmann, K. & Hülsmann, N. (ed.), Progress in Protozoology. Gustav Fischer Verlag, Stuttgart. p. 1–14.

Patterson, D. J. & Brugerolle, G. 1988. The ultrastructural identity of *Stephanopogon apogon* and the relatedness of this genus to the other kinds of protists. *Eur. J. Protistol.*, **23**:279–290.

Pellegrini, M. 1980. Three-dimensional reconstruction of organelles in *Euglena gracilis* Z. II. Qualitative and quantitative changes of chloroplasts and mitochondrial reticulum in synchronous cultures during bleaching. *J. Cell. Sci.*, **46**:313–340.

Porter, D. 1973. *Isonema papillatum* sp. n., a new colorless marine flagellate: a light- and electron microscopic study. *J. Protozool.*, **20**:351–356.

Schnepf, E. 1994. Light and electron microscopical observations in *Rhynchopus coscinodiscivorus* spec. nov., a colorless, phagotrophic euglenozoon with concealed flagella. *Arch. Protistenkd.*, **144**:63–74.

Schuster, F., Goldstein, S. & Hershenov, B. 1968. Ultrastructure of a flagellate, *Isonema nigricans* nov. gen. nov. sp., from a polluted marine habitat. *Protistologica*, **4**:141–154.

Simpson, A. G. B. 1997. The identity and composition of the Euglenozoa. *Arch. Protistenkd.*, **148**:318–328.

Simpson, A. G. B. & Patterson, D. J. 2001. On core jakobids and excavate taxa: the ultrastructure of *Jakoba incarcerata*. *J. Eukaryot. Microbiol.*, **48**:480–492.

Simpson, A. G. B. & Roger, A. J. 2004. Protein phylogenies robustly resolve the deep-level relationships within Euglenozoa. *Mol. Phylogenet. Evol.*, **30**:201–212.

Simpson, A. G. B., Lukeš, J. & Roger, A. J. 2002. The evolutionary history of kinetoplastids and their kinetoplasts. *Mol. Biol. Evol.*, **19**:2071–2083.

Simpson, A. G. B., Gill, E. E., Callahan, H. A., Litaker, R. W. & Roger, A. J. 2004. Early evolution within kinetoplastids (Euglenozoa), and the late emergence of trypanosomatids. *Protist*, **155**:407–422.

Skuja, H. 1948. Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. *Symb. Bot. Upsal.*, **9**:5–399.

Triemer, R. & Farmer, M. A. 1991a. The ultrastructural organization of the heterotrophic euglenids and its evolutionary implications. *In*: Patterson, D. & Larsen, J. (ed.), The Biology of Free-living Heterotrophic Flagellates. Clarendon Press, Oxford, U.K. p. 185–205.

Triemer, R. E. & Farmer, M. A. 1991b. An ultrastructural comparison of the mitotic apparatus, feeding apparatus, flagellar apparatus and cytoskeleton in euglenoids and kinetoplastids. *Protoplasma*, **164**:91–104.

Triemer, R. E. & Ott, D. W. 1990. Ultrastructure of *Diplonema ambulator* Larsen & Patterson (Euglenozoa) and its relationship to *Isonema*. *Eur. J. Protistol*, **25**:316–320.

Vickerman, K. 2000. Diplonemids. *In*: Lee, J. J., Leedale, G. F. & Bradbury, P. (ed.), An Illustrated Guide to the Protozoa. Allen Press, Lawrence, Kansas. p. 1157–1159.

von der Heyden, S., Chao, E. E., Vickerman, K. & Cavalier-Smith, T. 2004. Ribosomal RNA phylogeny of bodonid and diplonemid flagellates and the evolution of Euglenozoa. *J. Eukaryot. Microbiol.*, **51**: 402–416.

Weyhenmeyer, G. A. 1998. Resuspension in lakes and its ecological impact—a review. *Arch. Hydrobiol.*, **51**:185–200.

Received: 04/07/06, 07/19/06; accepted: 10/30/06