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Research Note

Visualizing the complex substructure of euglenid pellicle strips with SEM

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Comparative analysis of cytoskeletal diversity within the Euglenophyceae has provided important context for understanding the phylogenetic relationships and major evolutionary transitions within the group (e.g. switches in modes of nutrition and motility). Some ultrastructural characters used in earlier cladistic analyses of euglenids involved different states for the lateral projections that extend from the frame of each pellicle strip in photosynthetic lineages. Previously, the overall structure of 'strip projections' in different lineages was (arduously) reconstructed from a series of ultra-thin sections viewed with transmission electron microscopy (TEM). In this study, we were able to determine the structure of strip projections with greater precision, and without the laborious protocols associated with TEM (e.g. ultramicrotomy), by examining disrupted pellicles from three photosynthetic euglenids (*Lepocinclis fusiformis*, *Phacus longicauda* var. *tortus*, and *P. segretii*) using scanning electron microscopy (SEM). The structure of the strip projections observed here demonstrated that either (1) previous TEM studies of the pellicle overlooked certain ultrastructural features in some taxa or (2) the (prearticular) strip projections in *L. fusiformis*, *P. segretii*, and *P. longicauda* var. *tortus* represent a novel character state that could be phylogenetically informative.

Key Words: Character evolution, Cytoskeleton, Euglenids, Morphology, Pellicle, Scanning electron microscopy, Ultrastructure

INTRODUCTION

Euglenids comprise a diverse group of flagellates that includes lineages with different modes of nutrition: some feed on bacteria or microeukaryotes (phagotrophs), some absorb nutrients directly from the environment (osmotrophs), and some are photosynthetic (phototrophs). Euglenids share a novel cytoskeleton, referred to as the 'pellicle', consisting of the plasma membrane, a taxon-specific number of proteinaceous strips that extend from the anterior end of the cell to the posterior end, longitudinal microtubules that subtend the strips, and an underlying network of endoplasmic reticulum. The ultrastructure of the proteinaceous strips varies considerably between taxa, and detailed analyses of pellicle characters have significantly improved our understanding of euglenid behaviour, development, and evolution (e.g. Leander *et al.* 2001, 2007; Leander 2004). While surface characters, such as relative strip length, can be observed rather straightforwardly with scanning electron microscopy (SEM; e.g. Brosnan *et al.* 2005; Esson & Leander 2006, 2008), other characters, such as the shape and thickness of pellicle strips in transverse section, must be viewed with TEM. This involves more time consuming fixation, staining, and sectioning protocols.

One of the characters previously recognized using TEM is the presence and morphology of lateral strip projections, defined by Leander & Farmer (2001a) as 'any proteinaceous extension branching from the heel [of the strip]'. These projections extend either below the arch (the portion of the strip visible on the cell surface) of the same strip (i.e. 'postarticular' projections) or beneath the overhang and arch of the adjacent strip (i.e. 'prearticular' projections); terms used here to describe strip ultrastructure are defined in Leander & Farmer (2001a). Strip projections are absent in phagotrophic euglenids, are delicately structured in 'plastic' photosynthetic euglenids (cells capable of euglenoid movement), and tend to be more robust in rigid photosynthetic euglenids (cells that are not capable of euglenoid movement) (Dragos *et al.* 1997; Leander *et al.* 2001; Leander 2004). However, some rigid photosynthetic euglenids, such as *Monomorphina aenigmatica*, apparently lack robust strip projections, indicating that there is not a complete correlation between strip projection morphology and the degree of euglenoid movement (Nudelman *et al.* 2006).

We were able to determine the structure of strip projections in disrupted cells of three rigid photosynthetic euglenids using SEM. This approach eliminated the need to perform three-dimensional reconstructions of strip substructure from thin sections viewed with the TEM. Here we describe the morphology of prearticular strip projections in *Lepocinclis fusiformis* (Carter) Lemmermann, *Phacus longicauda* (Ehrenberg) Dujardin var. *tortus* Lemmermann, and *Phacus segretii* Allorge & Lefèvre for the first time, and compare these findings with previous descriptions of strip projections, derived from TEM, in other euglenid taxa.

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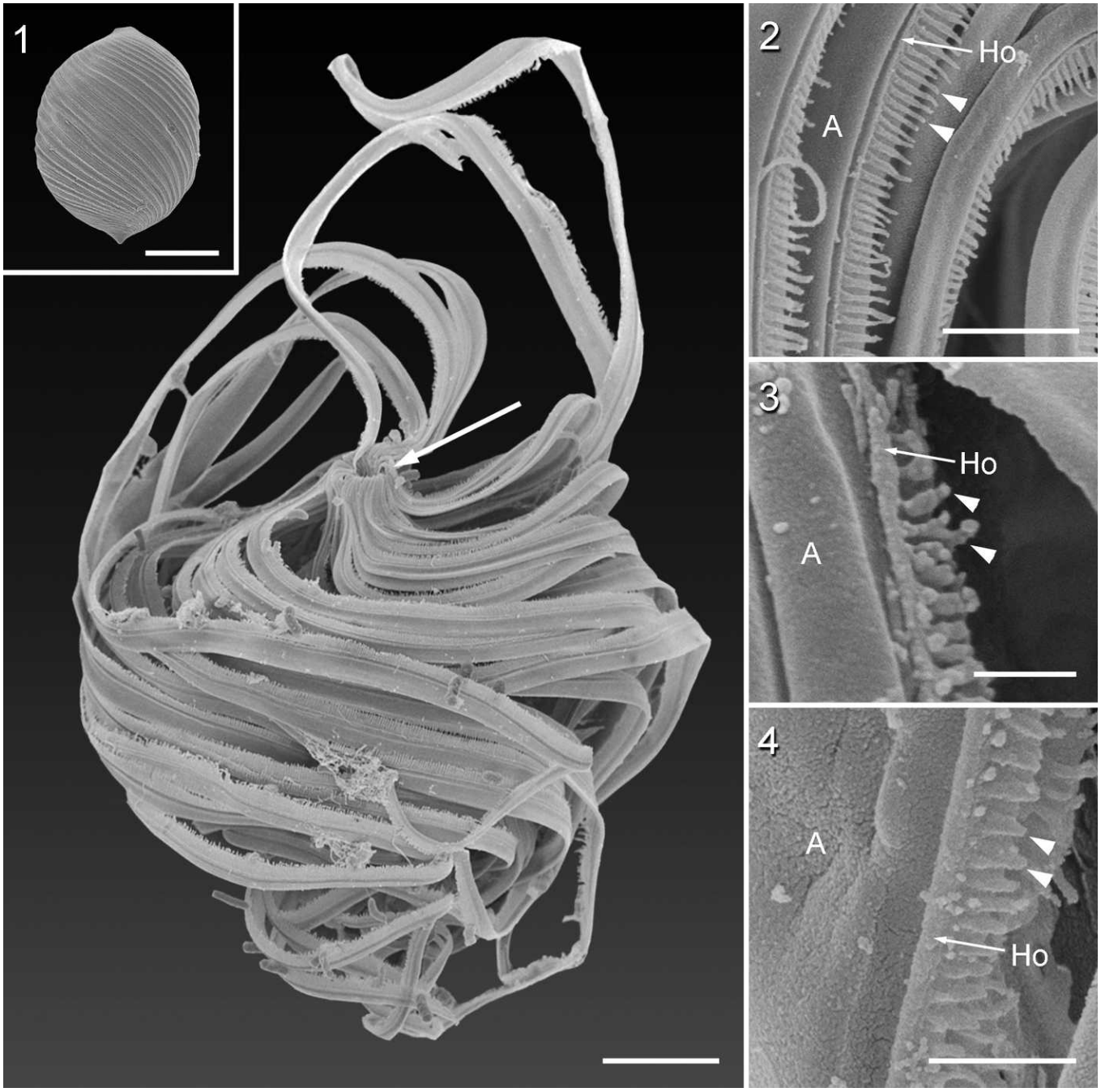

Figs 1–4. Scanning electron micrographs (SEMs) of rigid photosynthetic euglenids showing strip projections.

Fig. 1. A disrupted cell of *Lepocinclis fusiformis* (ACOI 1025) showing separated pellicle strips that originate in the anterior canal region (arrow) and extend in a helical fashion toward the posterior end of the cell. Bar = 5 μm. Inset: An intact cell of *L. fusiformis*, Bar = 10 μm.

Fig. 2. High magnification SEM of the *L. fusiformis* pellicle shown in Fig. 1; the anterior end of the cell is at the top of the micrograph. Prearticular projections consist of regularly spaced, tooth-like structures or 'ridges' (arrowheads) that are attached to the strip hook (Ho) and lie on top of a plate. The arch (A) of the strip lies to the left of the projections when the anterior end of the cell is oriented upwards. Bar = 1 μm.

Fig. 3. High magnification SEM showing the prearticular strip projections in *Phacus segretii* (ACOI 1337). Ridges (arrowheads) extend from the strip hook (Ho) and over an underlying plate. A = arch; Bar = 0.25 μm.

Fig. 4. High magnification SEM showing the prearticular strip projections in *P. longicauda* var. *tortus* (ACOI 1139). Ridges (arrowheads) extend beyond the edge of an underlying plate, similar to the projections in *L. fusiformis*. Ho = hook; A = arch; Bar = 0.50 μm.

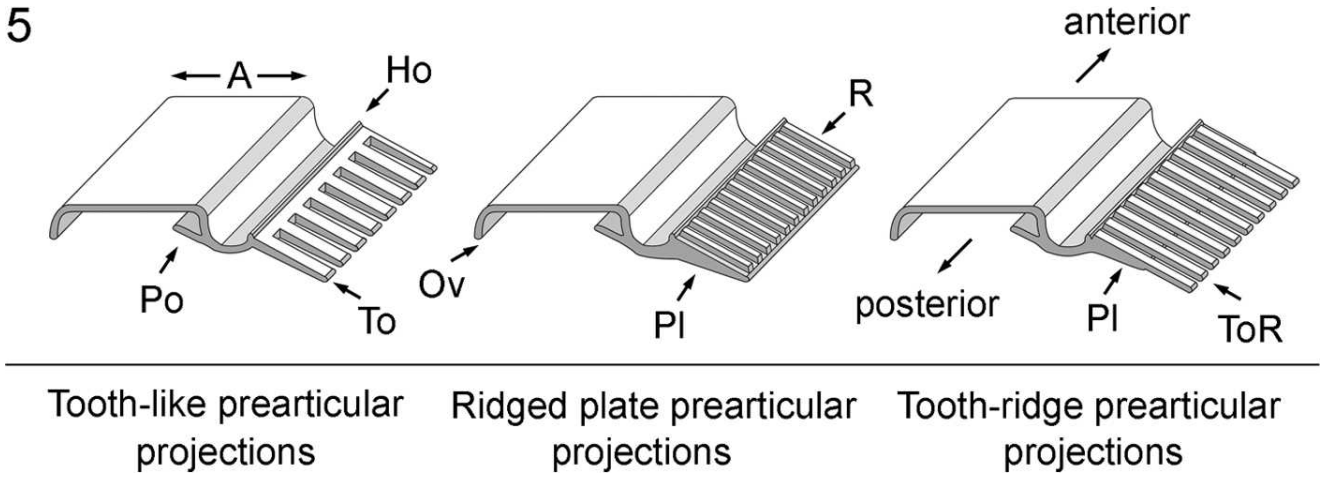

Fig. 5. Summary of three character states for prearticular strip projections described in *Lepocinclis* and *Phacus*. Strips are depicted so that their posterior end is oriented toward the lower left of the figure. The leftmost drawing illustrates tooth-like strip projections (To) previously described for members of the genus *Phacus*. The middle drawing shows plate-like projections (Pl) with regularly spaced ridges (R), such as those described for some *Lepocinclis* species and observed in *P. segretii*. The drawing on the right illustrates the plate-like projections (Pl) with overlying tooth-like ridges that extend beyond the plate (ToR), like those observed in *L. fusiformis* and *P. longicauda* var. *tortus*. A = arch; Ho = hook; Ov = overhang; Po = postarticular projection.

MATERIAL AND METHODS

The following cultures were purchased from the Culture Collection at the University of Coimbra (ACOI): *Lepocinclis fusiformis* (strain number ACOI 1025), *Phacus longicauda* var. *tortus* (ACOI 1139), and *Phacus segretii* (ACOI 1337). Cells were prepared for SEM with osmium tetroxide vapour as previously described (Leander & Farmer 2000) with no additional steps taken to manually disrupt the cells. Fixed cells were transferred to millipore filters and critical point dried with CO_2. Filters were attached to stubs and sputter coated with gold or a mixture of gold and palladium. Samples were viewed using a Hitachi S4700 scanning electron microscope.

RESULTS AND DISCUSSION

Although most euglenid cells observed with SEM were intact (Fig. 1, inset), a few had disrupted pellicles with two or more strips that were torn apart along their articulation zones (Fig. 1). Prearticular projections could be observed where pellicle strips had disassociated (Figs 2–4). Postarticular projections, which are relatively delicate as inferred from TEM (Leander *et al.* 2001), were never observed, even when the underside of the strip arch was visible. Although postarticular strip projections might be absent in the three taxa described here, this is unlikely because postarticular strip projections are present in all previously examined lineages of *Phacus* and *Lepocinclis* (Leander & Farmer 2001a, b; Leander *et al.* 2001). It seems more probable that either (1) the postarticular projections were obscured by amorphous cytoplasmic components that remained attached to the underside of the pellicle strips, (2) delicate postarticular projections were firmly fixed to the underside of the arch making them invisible with SEM, or (3) the delicate structure of the postarticular projections was destroyed during the preparation of the cells for SEM.

Nonetheless, the prearticular strip projections were clearly visible in this study and consisted of a flat plate that extended from the strip hook and was covered with regularly spaced ridges oriented perpendicular to the longitudinal axis of the strip (Figs 2–4, 5); this configuration was similar to that observed in some other *Lepocinclis* species (Leander & Farmer 2001a, b). However, in *L. fusiformis* and *P. longicauda* var. *tortus*, the ridges extended beyond the plate to form tooth-like structures or 'tooth-ridges' (Figs 2, 4–5). The prearticular projections in *P. segretii* may also take the form of tooth-ridges, but evidence that the ridges extended beyond the underlying plate was uncertain because of lower preservation quality. Nevertheless, the tooth-ridge configuration represents a hybrid of two previously described morphologies for prearticular strip projections: ridged plates and tooth-like projections (Fig. 5). For instance, *L. helicoideus* and *L. oxyuris* have been shown to have prearticular projections in the form of ridged plates (Leedale 1964; Leander *et al.* 2001); whereas, *L. ehrenbergii* (Mikolajczyk 1975), *L. fusca* (Suzaki & Williamson 1985), *L. spirogyroides* (= *Euglena spirogyra*; Leedale 1964; Leander *et al.* 2001), *L. acus* (Dragos *et al.* 1997), *L. buetschlii*, *L. tripteris*, *Phacus acuminatus* (identified as *P. brachykentron*), and *P. oscillans* (Leander & Farmer 2001a, b; Leander *et al.* 2001) have been shown to have tooth-like prearticular projections without ridges (Fig. 5).

It is possible that these earlier reconstructions of prearticular projections, reporting the absence of ridges on top of the toothed prearticular plate in *Lepocinclis* and *Phacus*, reflect incomplete or difficult to interpret observations derived from TEM studies. It is also possible that the tooth-ridge prearticular projections represent a novel state that has not been observed until now. The latter interpretation is consistent with previous observations that specific subcomponents in other microeukaryotes show little or no difference between the substructural details observed with either TEM or SEM (Sant' Anna *et al.* 2005). Nonetheless, because *Lepocinclis fusiformis*, on one hand,

and *P. longicauda* var. *tortus* and *P. segretti*, on the other hand, are members of two different sister clades (Kosmala et al. 2005; Esson & Leander, unpublished observations), the tooth-ridge prearticular strip projections observed in these taxa (Figs 2, 4–5) are probably widespread in both genera. However, we cannot currently infer whether tooth-ridge prearticular projections evolved convergently in several different lineages within the *Phacus–Lepocinclis* clade or were secondarily lost (modified) several times independently within this clade.

What we can confidently state is that the tooth-ridge projections described here with SEM represent a previously unrecognized substructure of euglenid strips that will serve as a guide for future reconstructions of prearticular strip projections in other species, whether by using SEM or TEM. Although SEM observations of pellicle strip projections should be consistent with TEM observations, SEM is much less time consuming and produces micrographs that are much easier to interpret. Continued experimentation with SEM protocols associated with cell disruption and fixation (e.g. by briefly applying pressure to cells prior to preparation for SEM; Leedale 1964) will hopefully help preserve the morphology of more delicate structures (e.g. postarticular projections) and facilitate an improved appreciation for the complexity of the euglenid cytoskeleton. This in turn will encourage more extensive taxon sampling within a molecular phylogenetic context, resulting in a better understanding of euglenid diversity and pellicle character evolution.

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REFERENCES

Brosnan S., Brown P.J.P., Farmer M.A. & Triemer R.E. 2005. Morphological separation of the euglenoid genera *Trachelomonas* and *Strombomonas* (Euglenophyta) based on lorica development and posterior strip reduction. *Journal of Phycology* 41: 590–605.

Dragos N., Peterfi L.S. & Popescu C. 1997. Comparative fine structure of pellicular cytoskeleton in *Euglena* Ehrenberg. *Archiv für Protistenkund* 148: 277–285.

Esson H.J. & Leander B.S. 2006. A model for the morphogenesis of strip reduction patterns in photosynthetic euglenids: evidence for heterochrony in pellicle evolution. *Evolution & Development* 8: 378–388.

Esson H.J. & Leander B.S. 2008. Novel pellicle surface patterns on *Euglena obtusa* Schmitz (Euglenophyta), a euglenophyte from a benthic marine environment: implications for pellicle development and evolution. *Journal of Phycology* 44: 132–141.

Kosmala S., Karnkowska A., Milanowski R., Kwiatowski J. & Zakrys B. 2005. Phylogenetic and taxonomic position of *Lepocinclis fusca* comb. nov. (= *Euglena fusca*) (Euglenaceae): morphological and molecular justification. *Journal of Phycology* 41: 1258–1267.

Leander B.S. 2004. Did trypanosomatid parasites have photosynthetic ancestors? *Trends in Microbiology* 12: 251–258.

Leander B.S. & Farmer M.A. 2000. Comparative morphology of the euglenid pellicle. I. Patterns of strips and pores. *Journal of Eukaryotic Microbiology* 47: 469–479.

Leander B.S. & Farmer M.A. 2001a. Comparative morphology of the euglenid pellicle. II. Diversity of strip substructure. *Journal of Eukaryotic Microbiology* 48: 202–217.

Leander B.S. & Farmer M.A. 2001b. The evolution of *Phacus* (Euglenozoa) as inferred from pellicle morphology and SSU rDNA. *Journal of Phycology* 37: 1–17.

Leander B.S., Witek R.P. & Farmer M.A. 2001. Trends in the evolution of the euglenid pellicle. *Evolution* 55: 2215–2235.

Leander B.S., Esson H.J. & Breglia S.A. 2007. Macroevolution of complex cytoskeletal systems in euglenids. *BioEssays* 29: 987–1000.

Leedale G.F. 1964. Pellicle structure in *Euglena*. *British Phycological Bulletin* 2: 291–306.

Mikolajczyk E. 1975. The biology of *Euglena ehrenbergii* Klebs. I. Fine structure of pellicular complex and its relation to euglenoid movements. *Acta Protozoologica* 14: 233–240.

Nudelman M.A., Leonardi P.I., Conforti V., Farmer M.A. & Triemer R.E. 2006. Fine structure and taxonomy of *Monomorphina aenigmatica* comb. nov. (Euglenophyta). *Journal of Phycology* 42: 194–202.

Sant' Anna C., Campanati L., Gadelha C., Lourenço D., Labati-Terra L., Bittencourt-Silvestre J., Benchimol M., Cunha-e-Silva N.L. & De Souza W. 2005. Improvement on the visualization of cytoskeletal structures of protozoan parasites using high-resolution field emission scanning electron microscopy (FESEM). *Histochemistry and Cell Biology* 124: 87–95.

Suzaki T. & Williamson R.E. 1985. Euglenoid movement in *Euglena fusca*: evidence for sliding between pellicular strips. *Protoplasma* 124: 137–146.

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