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FINE STRUCTURE AND TAXONOMY OF *MONOMORPHINA AENIGMATICA* comb. nov. (EUGLENOPHYTA)[1]

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The euglenoid genus *Monomorphina* was defined by Mereschowsky in 1877 to include rigid euglenoids that were pyriform in lateral view, had a hyaline spine at the posterior end, and one to few parietal chloroplasts typically without pyrenoids. The genus included taxa previously assigned to *Phacus* Dujardin or *Euglena* Ehrenberg. The general structure of *Monomorphina aenigmatica* comb. nov. is described on the basis of light microscopy and scanning and transmission electron microscopy. Cells were pear-shaped in lateral view, rounded at the anterior end and narrowed posteriorly, tapering into a long twisted tail. The pellicle had helically arranged strips spiralled in a counterclockwise fashion. A distinctive feature of *M. aenigmatica* was the presence of a single chloroplast bearing a pyrenoid, capped with a paramylon plate. The large parietal chloroplast extended along most of the cell with three prominent cup-shaped paramylon caps on the external face. In transverse section, the chloroplast appeared C-shaped. Because of the ambiguity surrounding the original descriptions used to diagnose this taxon, we designated an epitype for *Monomorphina aenigmatica*. Morphological features of this species were compared to other members of the genus.

Key index words: **chloroplast; Euglenida; Euglenophyta; Euglenozoa; *Monomorphina*; *Phacus*; pyrenoid; taxonomy; ultrastructure**

Euglena, *Phacus*, *Lepocinclis*, and *Monomorphina* share a complicated taxonomic history. Ehrenberg created the genus *Euglena* (1830, 1838), and shortly afterward, Dujardin (1841) transferred many rigid *Euglena* species into a newly erected genus, *Phacus*. Dujardin (1841) did not provide full descriptions of morphological characters in his text, but his detailed illustrations (*P. pleuronectes* Dujardin and *P. longicauda* Dujardin, Figs. 5 and 6) show flattened cells with numerous discoid chloroplasts and paramylon disks. In 1849, Perty established *Lepocinclis* to include rigid photosynthetic taxa, which were more pear-shaped or rounded than the typical flattened *Phacus* species, had spiraling pellicle strips, and bore a terminal spine. By 1849, taxa that were originally assigned to the genus *Euglena* were separated into three genera, *Euglena*, *Phacus*, and *Lepocinclis*.

Twenty-five years later, Mereschowsky (1877) also suggested that the genus *Euglena* should be split into three genera. He recognized *Euglena* and *Phacus* and erected a new genus, *Monomorphina*. Mereschowsky recommended that the genus *Euglena* should consist solely of taxa, capable of metaboly, and suggested that the rigid forms be divided among *Phacus* and *Monomorphina*. He placed flattened forms into *Phacus* and forms that were not as flattened into *Monomorphina*. Interestingly, it does not appear that Mereschowsky recognized the genus *Lepocinclis* (Perty) described a few decades earlier (1849).

Recently, Marin et al. (2003) emended the diagnosis of *Monomorphina* to be consistent with morphological and molecular data. Previous molecular studies (Linton et al. 1999, 2000, Leander and Farmer 2001b, Leander et al. 2001, Müllner et al. 2001, Brosnan et al. 2003) showed that the genus *Phacus*, as

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redefined by Pochmann 1942, was paraphyletic with taxa forming at least two clades. Subsequently, Nudelman et al. (2003) suggested that *Phacus* should be divided into two distinct genera. Marin et al. (2003) came to a similar conclusion and formalized this proposal. Marin et al. (2003) retained the flattened species in *Phacus* and transferred those species that were "not or just slightly compressed" into the genus *Monomorphina* (Mereschowsky) Marin et Melkonian. The emended *Monomorphina* contained taxa, which were pyriform in lateral view with a hyaline spine at the posterior end, with one to few large, parietal chloroplasts not lens–shaped, and pyrenoids typically absent (Marin et al. 2003, p. 102). The genus *Phacus* contained only the typical flattened, leaf-like species with multiple discoid chloroplasts.

In this paper, we describe the ultrastructure of *Monomorphina aenigmatica*, a species known mostly from light microscopic descriptions. Electron microscopy was necessary to: first, test Pochmann's proposal that *M. aenigmatica* (then known as *Phacus splendens*) contained only two chloroplasts and possibly a pyrenoid; and second, compare ultrastructural features of this taxon with other *Monomorphina* species. The only ultrastructural data available on *Monomorphina aenigmatica* (=*Phacus splendens* Pochmann) was by Mignot (1965) who included a partial description of the pellicle. However, from the outline of the strips, it seems that the cell he described was undergoing division and therefore, did not have the same pellicle structure as a typical interphase cell. In this study, we demonstrate several new morphological features of this taxon and compare the fine structural features of *M. aenigmatica* to other members of *Monomorphina*. We also review the taxonomy of this taxon and conclude that *Monomorphina striata* Marin et Melkonian 2003 (=*Phacus aenigmaticus* Drezepolski 1922, = *Phacus striatus* Francé 1897) should be renamed as *Monomorphina aenigmatica* and we designate an epitype.

MATERIALS AND METHODS

Cultures. *Monomorphina aenigmatica* was originally obtained from the Culture Collection of Algae at the University of Texas at Austin, under the name of *Phacus megalopsis* Pochmann (UTEX LB 1284). The cultures were grown in soil-water (GR+) medium and maintained at 22° C under a 16:8 LD photoperiod at 25–35 μmol photons $\cdot m^2 \cdot s^{-1}$. Light and electron microscope observations confirmed that the organism in the culture (UTEX LB 1284) did not conform to the diagnosis of *P. megalopsis*, but fit the description of what is now named as *M. aenigmatica*. When we recently reordered the UTEX LB 1284 strain, we found that it was neither *P. megalopsis* nor *M. aenigmatica* but is another taxon entirely. This study was based on the original UTEX LB1284 culture.

Scanning electron microscopy (SEM). Samples were gently pelleted and fixed in the same fixatives used for TEM. Cells were rinsed twice with distilled water and filtered onto polycarbonate membranes (3 μm pore size). The cells adsorbed to the membranes and were dehydrated in a graded ethanol series (30%–100%, 15 min each) and then, critical-point dried in liquid CO_2. The filters were sputter-coated with Au–Pd and examined in a LEO 982 Field Emission Scanning Electron Microscope (University of Georgia, Athens, GA, USA).

Transmission electron microscopy (TEM). Cells were concentrated by centrifugation and fixed in 2% glutaraldehyde, followed by 1% OsO_4, both buffered with 0.05 M sodium cacodylate, at pH 7.4, for 1 h at 4° C. Cells were gently pelleted and washed several times with 0.05-M cacodylate buffer. Samples were dehydrated through a graded ethanol series (20%–100%, 15 min each), washed with propylene oxide, infiltrated with epoxy resin, and polymerized overnight at 60° C. Blocks were sectioned serially and stained with uranyl acetate and lead citrate, prior to viewing in a JEOL 100CX-II transmission electron microscope (University of Georgia, Athens, GA, USA and Universidad Nacional del Sur, Bahía Blanca, Argentina). The terminology used to describe pellicle morphology is that defined by Leander and Farmer (2001a).

RESULTS

Morphology. Cells of *M. aenigmatica* were pear-shaped in lateral view, rounded at the anterior end, and narrowed posteriorly, tapering into a long twisted tail (Fig. 1a, b). In lateral view, the anterior margins were asymmetric ranging from undulate to serrate. Cells were about 24 μm long × 14 μm wide and were never observed to undergo metaboly (Fig. 1a–d). The chloroplast was lobed and had at least one haplopyrenoid, that is, a pyrenoid with a single cap of paramylon located on the inner surface of the chloroplast (Fig. 1c, d). Two haplopyrenoids were found in some cells (not shown). The flagellum was about the length of the body or slightly longer (Fig. 1b).

Electron microscopy was required to further elucidate the structure of the pellicle and determine the shape of the chloroplast (Figs. 1e–g, 2a–g, and 3a–e). The pellicle consisted of helically arranged strips, the majority of which terminated before the end of the tail (Figs. 1e–g and 2f). The maximum number of pellicle strips was 16, and at the anterior, all of them entered the canal, indicating no reduction (see Fig. 1f). At the posterior end of the cell, the strips undergo two whorls of reduction in strip number. The first reduction occurs at the base of the cell prior to the tail (Fig. 1g, arrows), bringing the total number of strips to eight. The second reduction occurs in the tail, which is finally reduced to four strips (Fig. 2f).

In transverse section, the arch of each pellicle strip was markedly concave (Fig. 2a) and articulation zones were raised (Fig. 2a–c). Four microtubules were located under each pellicular frame, one below the heel (M_1), two others at each side of the articulation zone, that is, one under the overhang (M_2) and one (M_3) under the keel (Fig. 2b), and the last microtubule (M_4) approximately in the middle of the arch (Fig. 2c). Fibrillar projections extended from each side of the heel (Po, Pr in Fig. 2a, b). The nucleus was positioned toward the posterior of the cell, the chromatin was permanently condensed, and one or more large endosomes were present (Figs. 2e and 3c, d).

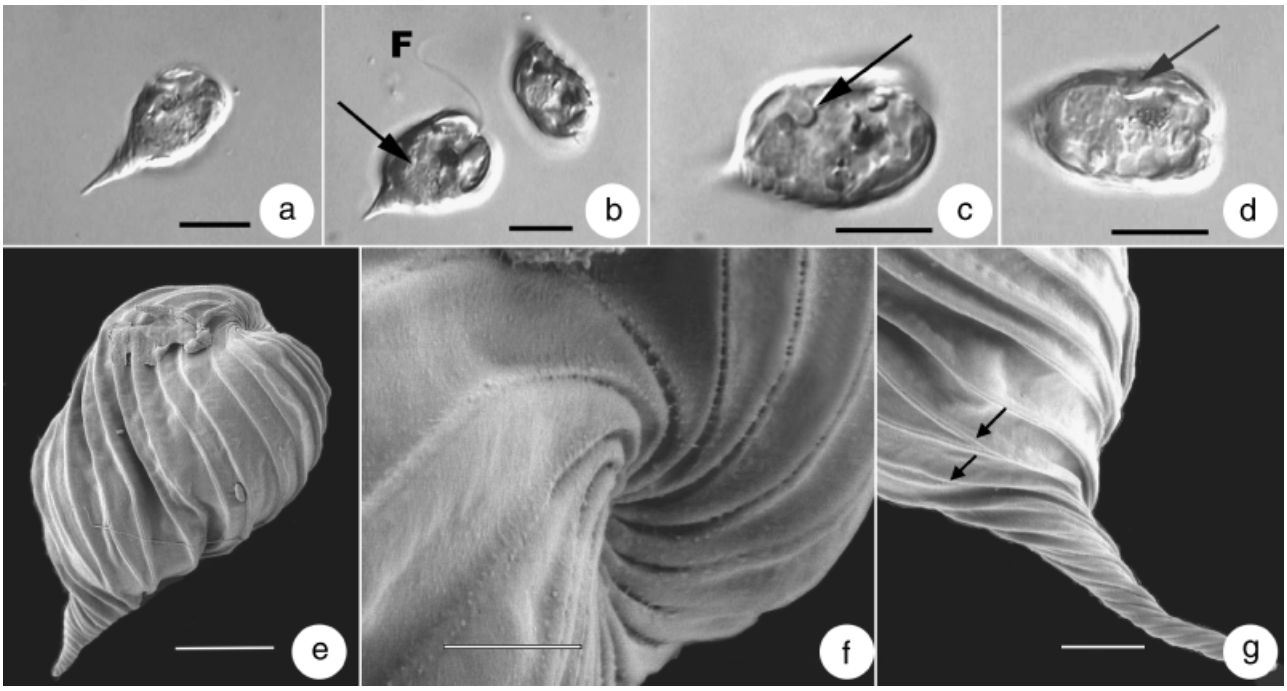


FIG. 1. Light and scanning electron micrographs of *Monomorphina aenigmatica*. (a–d) Light micrographs. Scale bar, 10 μm. (a) General overview of a living cell showing the long twisted tail. (b) Light micrograph showing the flagellum. The large nucleus (arrow) is partially covered by the chloroplast. (c) Optical section through the cell, showing the lobed chloroplast and haplopyrenoid (arrow). (d) Near median optical section showing the cup-shaped chloroplast and haplopyrenoid (arrow). (e-g) SEM micrographs. (e) General overview of the cell surface, showing the strips and twisted tail. Scale bar, 5 μm. (f) High magnification view of the anterior end of the cell showing the pellicular strips that continue inward to the canal. Scale bar, 1 μm. (g) Posterior view of the cell with twisting in the caudal portion, and the strips that extend along the entire tail. The first whorl of strip reduction is indicated by arrows. Scale bar, 2 μm. F, flagellum.

Longitudinal and tangential sections revealed a single, parietal cup-shaped chloroplast. At the posterior end, the chloroplast tapered to adopt the shape of the cell, while at the anterior end, it was irregularly lobed (Fig. 2d, e). In transverse sections through the anterior-most portion of the cell, the lobed chloroplast appeared to be two chloroplasts (Fig. 3a). However, serial sectioning down into the "cup" towards the posterior end of the cell confirmed a C-shaped chloroplast (Fig. 3b, c). At the base of the cup, the edges of the chloroplast met as shown by the circular profile toward the extreme posterior end (Fig. 3d). Three prominent curved paramylon plates were positioned between the chloroplast and the plasma membrane (Fig. 2d,e, g). Two were located anteriorly and the other was positioned toward the cell posterior (Figs. 2d, e and 3a–d). At least one haplopyrenoid with a small inner paramylon cap was present (Figs. 1c, d and 3e). Some smaller paramylon grains also were scattered throughout the cytoplasm (Fig. 3a–d). A diagrammatic representation of the single chloroplast is shown in Fig. 3f.

DISCUSSION

Taxonomy of the genus Monomorphina. In our earlier molecular study (Nudelman et al. 2003), we recognized that the genus *Phacus* was not monophyletic and must be subdivided. The choice of the name *Monomorphina* (Mereschkowsky 1877), which Marin et al. (2003) later applied to the "mixed *Phacus/Lepocinclis* clade," is appropriate and recognizes Mereschkowsky's and Popova's (1947) contributions. The *Monomorphina* clade in the Marin et al. (2003) phylogenies included three taxa that were formerly assigned to *Phacus* [*P. striata* Francé, *P. pseudonordstedtii* Pochmann, *P. pyrum* (Ehrenberg) Stein] and two formerly assigned to *Lepocinclis* [*L. ovata* (Playfair) Conrad, *L. reeuwykiana* Conrad]. The authors examined these taxa and confirmed that all have one to few parietal chloroplasts and subsequently transferred twenty taxa from *Phacus* and *Lepocinclis* into the genus *Monomorphina*. However, nearly all of these taxa are described in the literature as having multiple discoid chloroplasts without pyrenoids (Perty 1852, Stein 1878, Skuja 1926, Pochmann 1942, Hüber-Pestalozzi 1955, Leedale 1967, Kim et al. 2000). As noted by Popova (1947), Schmitz (1884) was the first to describe the chloroplasts of *M. pyrum* [=*Phacus pyrum* (Ehrenberg) Stein] and noted two large parietal chloroplasts each capped with a large paramylon plate on the side facing the pellicle. However, Dangeard (1901) disagreed with Schmitz and described many small discoid plastids. Subsequent authors such as Van Goor (1925) and Krichenbauer (1938) confirmed the presence of the multiple discoid plastids,

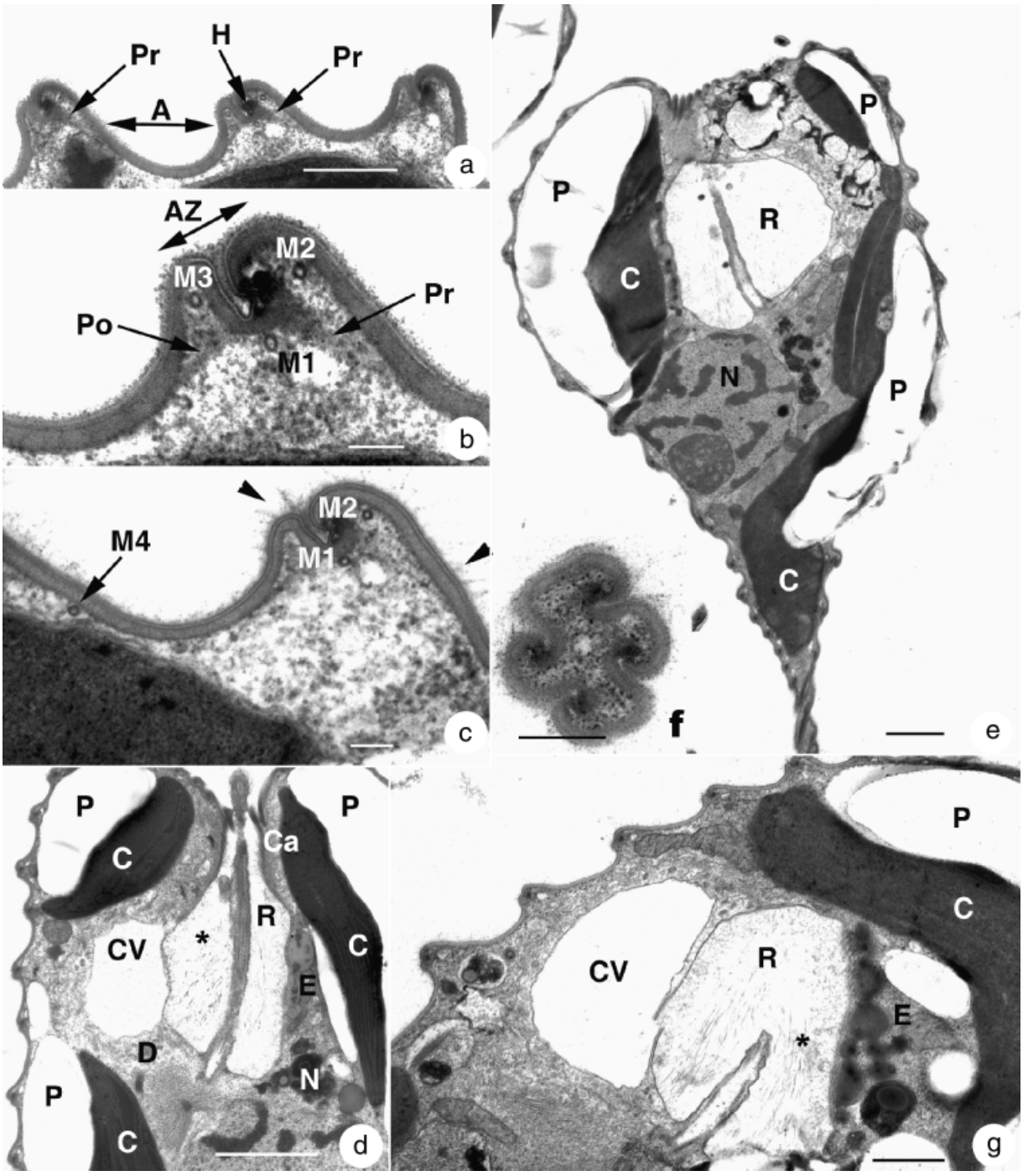


FIG. 2. TEM micrographs. (a–c) Cross-sections through the pellicle. (a) Low magnification view showing an alternating pattern of raised (keel) and depressed (arch) regions of adjacent strips. Prearticulation projections extend between the heel and the arch region of the strip Scale bar, 0.5 μm. (b) Higher magnification view of the strip, showing the plasma membrane, submembranous protein layer, and pre- and postarticulation projections that connect adjacent strips and three microtubules. Scale bar, 0.25 μm. (c) High magnification view of pellicle, showing a fourth microtubule located in the concave portion of each strip. Mucilaginous material is often seen on the cell surface (arrowheads). Scale bar, 0.25 μm. (d) Longitudinal section through the reservoir, showing a short canal, contractile vacuole, and eyespot. The two anterior lobes of the chloroplast and associated concave paramylon plates are visible at the top of the figure. Numerous flagellar hairs are observed in the reservoir (asterisk). A dictyosome is positioned near the base of the reservoir. Scale bar, 2 μm. (e) Longitudinal section of the cell, showing the parietal chloroplast with three paramylon caps. Note the latero-posterior position of the nucleus. Scale bar, 2 μm. (f). Cross-section near the extreme base of the tail, showing four strips reaching the end of the body. Scale bar, 0.25 μm. (g) Higher magnification view of reservoir region showing non-linear arrangement of eyespot globules, flagellar hairs (asterisk), and contractile vacuole. Scale bar, 1 μm. A, arch; AZ, articulation zone; Ca, canal; C, chloroplast; CV, contractile vacuole; D, dictyosome; E, eyespot; H, heel; M1, M2, M3, M4, microtubule; N, nucleus; P, Paramylon; Po, postarticular projection; Pr, prearticular projection; R, reservoir.

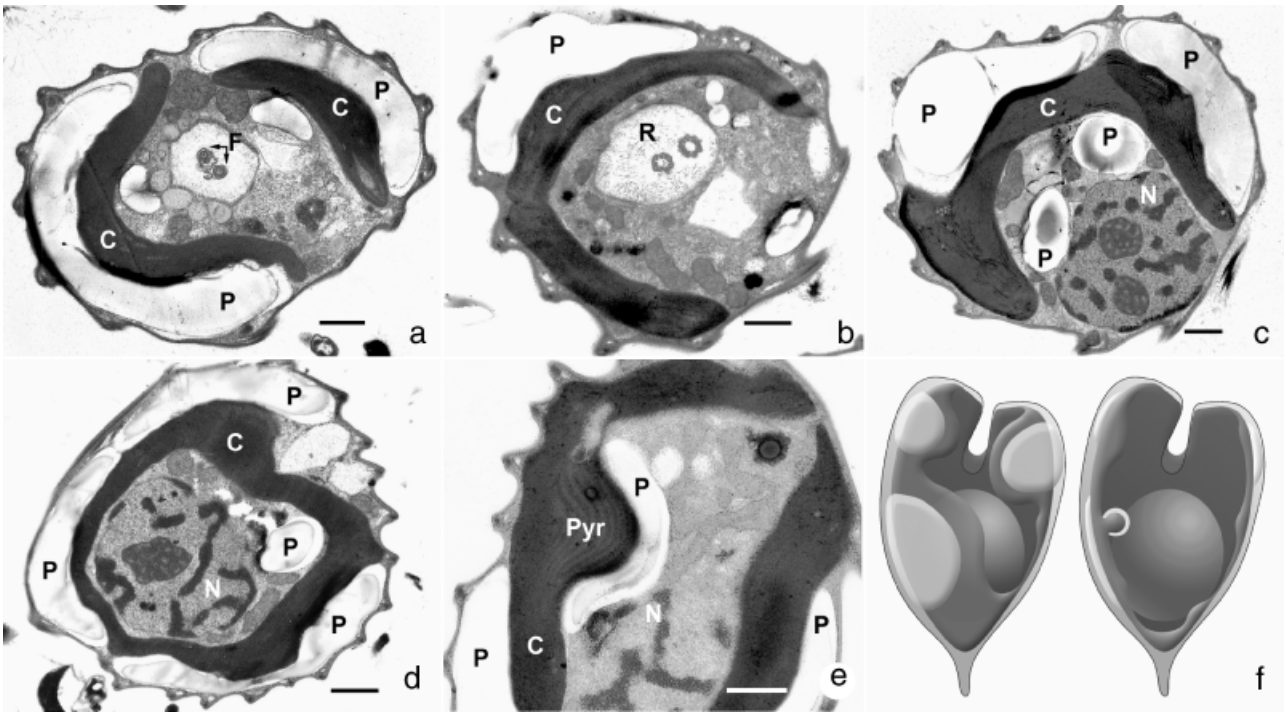


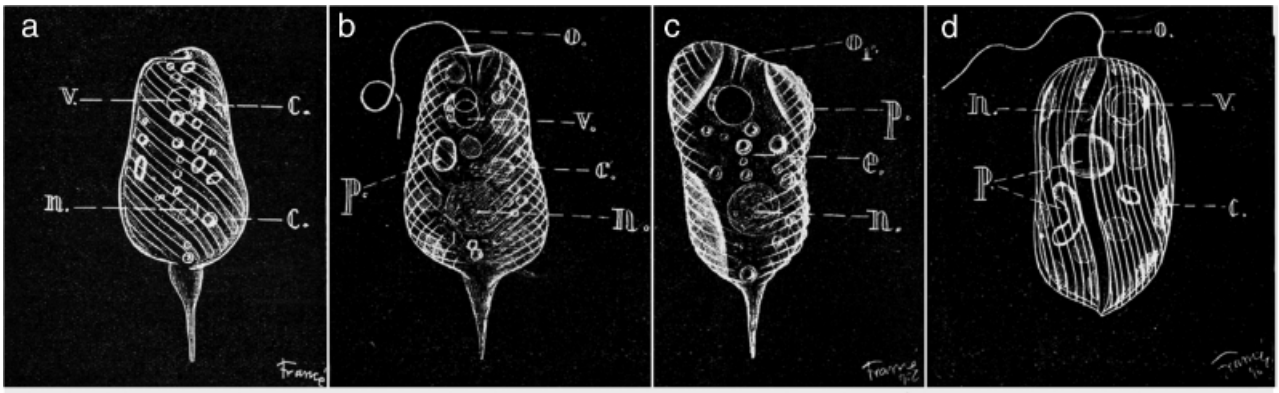
FIG 3. TEM images of the chloroplast beginning at the reservoir region and sectioned posteriorly toward the nucleus. (a) Cross section through reservoir showing cross sections of the emergent and non-emergent flagellum. At this level, the two anterior lobes of the chloroplast imply that two chloroplasts are present. Scale bar, 1 μm. (b) The chloroplast lobes merge to form a C-shape toward the base of the reservoir. One paramylon plate is visible. Scale bar, 1 μm. (c) C-shaped chloroplast with two large, concave paramylon plates. Scale bar, 2 μm. (d) Section taken at the level of the nucleus. The chloroplast lobes join to form a circular cup surrounding the nucleus. Two large paramylon plates cap the chloroplast. Additional free paramylon grains are present in the cytoplasm. Scale bar, 1 μm. (e) Near longitudinal section near the nucleus, showing the chloroplast and haplopyrenoid with inner paramylon cap. Two of the large paramylon plates are seen associated with the chloroplast. Scale bar, 1 μm. (f) Diagrammatic representation of the chloroplast, illustrating the lobes, the haplopyrenoid, and the nucleus. C, chloroplast; F, flagellum; N, nucleus; P, Paramylon; Pyr, pyrenoid; R, reservoir.

while others (Drezepolski 1925, Chadefaud 1937) argued for few large parietal chloroplasts.

A similar problem arose in the genus *Phacus*. Kim et al. (2000) recently studied a large number of samples of *Phacus trypanon* from Korea. *Phacus trypanon* was described as having many discoid chloroplasts, but the authors observed that in starved cells maintained at 5° C, the chloroplasts aggregated giving the appearance of a single large plate-like plastid (Kim et al. 2000, Fig. 2c). Similarly, we have seen the chloroplast of a *Monomorphina* sp. from Michigan fragment into multiple (discoid?) pieces when the cells age or nutrients are limiting (personal observation). This suggests that it is likely that the discoid chloroplasts noted by the early investigators in most of the descriptions were actually fragmented chloroplasts. We have never observed "discoid" chloroplasts in healthy cells and concur that the number of chloroplasts (one to few) is a stable diagnostic character in *Monomorphina*.

Taxonomy of Monomorphina aenigmatica. There are three *Monomorphina* taxa with identical SSU rDNA sequences in GenBank. All of these taxa bear different names. The published SSU rDNA sequence (AF283313) for *Phacus aenigmaticus* ASW 08012 (Müllner et al. 2001) is identical to the sequence for *P. splendens* (AF190814) obtained from the UTEX LB 1284 strain several years ago (the strain currently in the Culture Collection of Algae at the University of Texas at Austin labeled UTEX LB 1284 is no longer that strain, but is a different species of *Phacus*). Similarly, the sequence of *Monomorphina striata* (CCAP1261/9, GenBank accession number AJ532432) nov. comb. Marin et Melkonian is also identical to that of *P. aenigmaticus* (AF283313) and *P. splendens* (AF190814). In their emended description of *Monomorphina*, Marin et al. synonymized *P. aenigmaticus* with *M. striata* and based upon sequence data, we would fully concur that they are indeed the same species. However, we argue that the species should be named *Monomorphina aenigmatica*.

Phacus striatus, the basionym for *M. striata* Marin et Melkonian, was described by Francé in 1897. *Phacus aenigmaticus* was described by Drezepolski in 1922 and *Phacus splendens* was described by Pochmann in 1942. Normally, one would accept *M. striata* as the correct name since it is based on the earlier description by Francé (1897). However, there are a number of problems with the original Francé paper that make this description ambiguous. The illustrations shown by Francé (1897) represent three different organisms



Figur 21. Phacus setosus n. sp.
Normale Schwärmzelle mit abgeworfener Geissel. Circa 610fach vergrössert. Die Membranstreifung ist nicht gezeichnet, um die spiraligen Chlorophyllbänder *(c)* besser hervortreten zu lassen; v = das Vacuolensystem mit dem anhaftenden Stigma, n = der kleine, kugelige Zellkern.

Figur 22—23. Phacus striatus n. sp.
Figur 22. Schwärmzelle, deren Paramylon nur in Form kleiner Körnchen entwickelt ist *(p)*, = v Vacuolensystem, c = Chlorophyllscheiben, n = Zellkern, o = Geissel.
Figur 23. Das Paramylon bildet drei grosse Klumpen *(p)*, o_1 = Oesophagus, n = Zellkern, c = Excretkörnchen. Circa 650fach vergrössert.

Figur 24. Phacus striatus n. sp.
Habitusbild der im Tarhanyer Moorteiche gefundenen Form. Circa 650fach vergrössert. o = Geissel, v = Vacuolensystem mit dem Augenfleck, n = Zellkern, c = Chlorophyllscheiben, p = Paramylonkörnchen.

FIG. 4. Original Francé (1897) drawings of *Phacus striatus*. (a) Francé's Figure 21 incorrectly labelled as *P. setosus*. Note, cell body is broad at the posterior and tapers toward the anterior. (b) Francé's Figure 22. Cell has same body shape as in previous figure. Note, apical position of the canal and scattered oval paramylon grains. (c) Francé's Figure 23. Cell body is broadly truncate at the anterior and tapers toward the posterior. The canal is sub-apically angled and three distinct paramylon plates are shown. (d) Francé's Figure 23. Cell body rectangular to ovoid with a longitudinal groove extending from the anterior to the posterior. Pellicle striations are nearly longitudinal as opposed to the spiral arrangement shown in the other figures. The paramylon is shown as oval or curved disks.

(Figs. 21–24). Reproductions of the four original drawings are provided in this manuscript for clarity (Fig. 4a–d). Francé's Figures 21 and 22 (Fig. 4a, b) show nearly identical cells. Unfortunately, the legend under Figure 21 is labelled as "*Phacus setosus n*. sp." This is the species described in the paper prior to *P. striatus* and therefore may simply represent a typographical error or labelling mistake. However, his Figures 22 (Fig. 4b) and 23 (Fig. 4c) are labelled as *Phacus striatus n. sp*. and illustrate what are two distinct taxa. The body shapes are different, with Figure 22 (Fig. 4b) having a wide posterior narrowing towards the anterior, while Figure 23 (Fig. 4c) is wide at the anterior and tapers toward the posterior. The opening of the canal in Figure 22 (Fig. 4b) is apical. The canal opening in Figure 23 (Fig. 4c, labeled as *oesophagus* by Francé) is subapical and angled. Similarly, the organism in Figure 22 is described as being a swarmer (Swärmzelle) in which the paramylon is only in the form of small grains. In Francé's Figure 23 (Fig. 4c), the paramylon is described and shown as three large "lumps" (=plates). Francé's Figure 24 (Fig. 4d), also labelled as *Phacus striatus n. sp*., is nearly ovoid and lacks the prominent tailpiece found in Figures 21–23 (Fig. 4a–c). More importantly, the drawing clearly illustrates a longitudinal groove running down the middle of the cell, and the pellicle strips are nearly longitudinal and not spiral as in the previous drawings. Francé does state that this is a small form ($\sim$9 µm long) of *P. striatus* found in the bog at Tarhanyer. He notes the resemblance to *P. oscillans* and suggests that this may be a transitional form. Based upon the description given, the drawing, and the dimensions provided, we conclude that this taxon is not a transitional form of *P. striatus* but represents another taxon entirely.

Given the arguments presented above, one can conclude that three different taxa are described under the name of *P. striatus* by Francé (1897). The two larger taxa differ in their overall body shape, the type of paramylon present, and the position of the flagellar canal, while the smaller "form" is distinct from either of the large forms. The species description and illustrations given by Francé are inadequate to identify a single known taxon. However, this taxonomic problem can be rectified.

Phacus striata Francé was effectively lectotypified by Lemmermann (1913) when he chose Francé's Figure 22 for his description of *Phacus striata* (Lemmermann's figure 251). This ties Francé's Figure 22 to the description of *Phacus striata*. Marin et Melkonian cited all four of France's figures under their new combination but then, specifically note that they do not recognize the description of *Phacus striata* (Fig. 251) by Lemmermann (1913). Because Lemmermann (1913) had already lectotypified *Phacus striata*, the new combination, *Monomorphina striata* (Francé) Marin et Melkonian is invalid.

Of the remaining figures, only Figure 23 of Francé (Fig. 4c) can be clearly identified as what Drezepolski (1922) later named *Phacus aenigmaticus*. The taxon we describe in this paper matches that diagnosed by Drezepolski (1922) as *Phacus aenigmaticus*. Therefore, we transfer this taxon into the genus *Monomorphina*, create a new combination, and establish an epitype for *Monomorphina aenigmatica*. This will provide nomenclatural stability and conserve the name "*aenigmaticus*," which has long been used for the taxon described by

Drezepolski (1922) and illustrated by Francé (1897, Fig. 23).

***Monomorphina aenigmatica* (Drezepolski) Nudelman et Triemer, comb. nov.**

Basionym: Phacus aenigmaticus Drezepolski 1921/1922. Rozpr. Wiad. Muz. Dzieduszyckich 7–8, Figs. 4–4a, p. 14.

Synonyms. *Phacus striatus* Francé 1897. Result. Wiss. Erforsch. des Balatonsees 2, Fig. 23, pp. 29–32; non *Phacus striatus* Francé 1897. Result. Wiss. Erforsch. des Balatonsees 2, Figs. 21, 22, 24 pp. 29–32; non *Phacus striata* Francé in Lemmermann 1913 (in Pascher [Ed]: Die Süßwasser -Flora Deutschlands, Österreichs und der Schweiz 2) p. 141, Fig. 251; non *Phacus striata* Francé in Hüber-Pestalozzi 1955. Das Phytoplankton des Süsswassers. Systematic und Biologie. Vol. 4, Euglenophyceae. (in Thienemann [Ed.]. Die Binnengewässer. E. Schweizerbartsche Verlagsbuchandlung); *Phacus aenigmaticus* var. *monochloron* Pochmann 1942. Arch. Protistenkd. 95 Figs. 24–25, p. 137; *Phacus splendens* Pochmann 1942. Arch. Protistenkd. 95 Fig. 141, pp. 224–225; *Monomorphina splendens* (Pochmann) Popova 1947. Izv Zap Sib Fil AN SSSR Ser biol (Novosibirsk) 2, p. 56; *Monomorphina monochloron* (Pochmann) Marin et Melkonian 2003. Protist 154 p. 102; *Monomorphina striata* (Francé) Marin et Melkonian 2003. Protist 154 p. 102.

Lectotype: Here designated Fig. 4 in Rozpr. Wiad. Muz. Dzieduszyckich 7–8, 1921/1922 p. 14.

Epitype: Lyophilized sample of Monomorphina aenigmatica has been deposited at the Michigan State University Herbarium (MSC). Culture from which epitype is described is available from Algensammlung Wien (ASW), as *Phacus aenigmaticus* strain ASW08039.

Taxonomy of Monomorphina monochloron. *Phacus aenigmaticus* var. *monochloron* Pochmann (1942) has been recognized as a separate species and the name used as a basionym to create *Monomorphina monochloron* Marin et Melkonian comb. nov. Interestingly, *Phacus aenigmaticus* var. *monochloron* was the only taxon transferred to *Monomorphina* that was described as having a single chloroplast rather than many discoid chloroplasts (Pochmann 1942). Since one to few chloroplasts is now one of the major diagnostic features in the emended diagnosis of *Monomorphina* Marin et Melkonian, *Monomorphina monochloron* now becomes a junior synonym of *Monomorphina aenigmatica*.

Ultrastructure of Monomorphina aenigmatica. Few ultrastructural studies have been conducted on taxa that are now included in the genus *Monomorphina*. Previous studies focused on general ultrastructure (Dynesius and Walne 1975), pellicle structure (Mignot 1965, Bourrelly and Couté 1981, Leander and Farmer 2001a), and structure of the flagellar apparatus (Dynesius and Walne 1975, Shin and Boo 2001). As noted by Kim et al. (2000), the taxonomy of *Phacus*, and now *Monomorphina*, is difficult at the species level because of the limited data available on pellicular features and intracellular components.

Several unusual features of *Monomorphina aenigmatica* were revealed in this study. In *M. aenigmatica*, as in *M. pyrum* or *M. ovata* (=*Phacus pyrum* and *Lepocinclis ovata* in Leander and Farmer 2001b), the maximum number of pellicle strips was 16. There was no clear reduction of the strip number at the anterior end of the cell, but at the posterior end, strip number decreased exponentially across two whorls going from 16 strips to 8 and then 8 to 4. The terminal four strips extended to form the pronounced tail. Although only three taxa have been examined, this pattern of strip reduction is consistent among them and may prove to be characteristic of the genus.

Similarly, the overall pellicle structure of *M. aenigmatica* is similar to that of other rigid euglenoids (Leander and Farmer 2001a, b). However, the pellicle submembraneous layer is not as thick as described in *Phacus*, and the prominent struts that are oriented perpendicular to the strips in euglenoids, which are rigid (e.g. *P. triqueter*, *P. oscillans*, *P. brachykentron*, Leander and Farmer 2001b; *P. horridus* Bourrelly and Couté 1981; *P. platalea* and *P. alatus*, personal observation) or capable only of bending as *Lepocinclis acus* (= *Euglena acus* Ehrenberg), *L. spirogyra* (=*Euglena spirogyra* Ehrenberg), *L. oxyuris* (=*Euglena oxyuris* Schmarda), Suzaki and Williamson 1985, 1986; *L. helicoideus* (=*Euglena helicoideus*), *L. buetschlii* Lemmerman, Leander and Farmer 2001a; *L. tripteris* [=*Euglena tripteris* (Dujardin) Klebs], Leander and Farmer 2001b or *L. salina* (personal observation)) are not evident.

In *M. pyrum*, the submembraneous layer is not thickened. The pellicle does have robust prearticular projections, but postarticular projections were not evident (Leander and Farmer 2001a, b). In *M. aenigmatica*, the prearticular and postarticular projections are fibrillar and delicate, more like those found in some of the metabolic species (Suzaki and Williamson 1986, Leander and Farmer 2001a, b). Leander and Farmer (2001a) suggest, "in general, it does appear that taxa with thread-like projections tend to be capable of more metabolic movement than taxa with tooth-like and plate-like projections." *Monomorphina aenigmatica* bears only delicate fibrillar prearticular and postarticular projections but remains rigid. Therefore, the rigidity may come from another source.

An important finding of this study was the demonstration of a single parietal chloroplast with large paramylon caps, contrary to earlier works that reported two chloroplasts (Pochmann 1942, Hüber-Pestalozzi 1955). The occurrence of one parietal chloroplast per cell has been reported in *Euglena archaeoplastidiata* Chadefaud, *E. cingula* Godjics, (Godjics 1953) and *Cryptoglena pigra* Ehrenberg (Rosowski and Lee 1978). The presence of two parietal chloroplasts also is rare and was reported in *E. agilis* Carter, (Godjics 1953), *Phacus agilis* Skuja (now, *Cryptoglena skujai*, Marin et Melkonian, 2003), and *P. atraktoides* Pochmann (now *Monomorphina atraktoides*, Weik 1967). The earlier

reports of two parietal chloroplasts in *P. splendens* (now *Monomorphina aenigmatica*) by Pochmann (1942) are not surprising considering that even modern methods of light microscopy and observation of videotaped living cells cannot clearly resolve whether the cells have one or two chloroplasts. Apical views of the cell further confuse the issue since the anterior portion of the chloroplast is lobed, giving the impression that multiple chloroplasts are present. It is likely that future electron microscopic studies of other species reported as having two chloroplasts may also demonstrate the presence of only one chloroplast as well.

Another unusual feature of *Monomorphina aenigmatica* is the presence of at least one pyrenoid. The diagnosis of the genus *Monomorphina* states that the cells have "one to few large, parietal chloroplasts" with pyrenoids "typically absent" (Marin et al. 2003). Pochmann (1942) had suspected that a pyrenoid might be present in what was then called *Phacus splendens* but was not able to confirm this. Later, Popova (1947) transferred *Phacus splendens* to *Monomorphina splendens*. The present investigation is the first to demonstrate the presence of a pyrenoid in the genus confirming the earlier suspicions of Pochmann (1942). Given the difficulty of seeing the pyrenoid in these small cells, ultrastructural studies may be required to determine if pyrenoids are present in other taxa currently assigned to *Monomorphina*.

In summary, recent molecular and morphological data indicated that *Phacus* was paraphyletic and the resurrection of the genus *Monomorphina* was warranted. As demonstrated by the current study, features such as chloroplast number and presence of pyrenoids are difficult to be determined with light microscopy alone. Moreover, characters such as chloroplast type/number may be dependent upon age and nutrients (Pringsheim 1956, Zakrys et al. 2001, 2002), reinforcing the importance of conducting culture studies and the need for healthy, growing cells when trying to address these taxonomic issues.

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