

## Rediscovery of the *Ochromonas* type species *Ochromonas triangulata* (Chrysophyceae) from its type locality (Lake Veysove, Donetsk region, Ukraine)

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**ABSTRACT:** *Ochromonas triangulata*, the type species for a genus with over 125 taxa, was collected for only the second time, again from the type locality. Cell morphology, cell division, palmelloid stage and cyst structure generally agreed with the original description. Molecular phylogenetic analysis based on the 18S rRNA gene revealed 13 clades of *Ochromonas*-like flagellates as well as the clade represented by our *O. triangulata* strain and the nearly identical strain RCC-21/AC025. We also conducted a concatenated analysis using the 18S rRNA and the *rbcL* genes, and we recovered the same 14 clades. One clade, containing strains CCAP 933/27 and CCMP1861, previously named *Ochromonas tuberculata*, was re-identified as *Chrysastrrella paradoxa* and *Chrysastrrella brevipendiculata*, respectively. One clade included the *Poterioochromonas* strains but we were unable to convincingly connect species names to the strains because authentic strains were unknown or not examined. Organisms in the clade that included the well-known *Ochromonas danica* were assigned to *Chlorochromonas*. The generic name *Melkoniana* gen. nov. was proposed for one distinct clade of marine flagellates. These changes addressed some of the issues associated with the polyphyletic *Ochromonas sensu lato* but many problems remained.

**KEY WORDS:** *Chlorochromonas*, *Chrysastrrella*, Chrysophyceae, *Melkoniana*, Nomenclature, *Ochromonas triangulata*, Phylogeny, *Poterioochromonas*

### INTRODUCTION

The chrysophyte flagellates dominate a large, diverse class. The oldest recognized name is the colonial flagellate *Volvox vegetans* O.F.Müller (1786) [now known as *Anthophysa vegetans* (O.F.Müller) F.Stein (1878)]. The single-celled naked flagellates date from *Monas* O.F.Müller (1773), especially *Monas mica* O.F.Müller (1773). As Silva (1960) points out, the identity of *M. mica* is problematic; however, the genus is anchored with *M. mica* because Kent (1880–81) removed the other two species (see Silva 1960). Müller (1773) describes the organism as colourless, which is an important point for this paper; interestingly, he describes a vibrating cilium and an eyespot, and the presence of an eyespot strongly suggests the presence of a second, short flagellum that probably could not be seen with Müller's microscope using his No. 3 lens. Whether one recognizes *Monas* and/or *Spumella* Cienkowski, these colourless flagellates are interwoven with *Ochromonas sensu lato* because of the polyphyletic nature of both the pigmented and nonpigmented single-celled flagellate organisms (see Stoeck & Boenigk 2008; Findenig *et al.* 2010; Grossmann *et al.* 2016).

The first two genera of photosynthetic naked, single-cell flagellates were *Chromulina* (Cienkowski 1870) and *Chrysonomonas* (Stein 1878), assuming that the pigmented single cells assigned to *Monas sensu* Ehrenberg (1838) are excluded. Both genera are explicitly described as uniflagellate organ-

isms. We know today that *Chromulina*-type organisms have two flagella (e.g. Fauré-Frémiet & Rouiller 1957; Hibberd 1976; Andersen 1986; Andersen 1991) but the short flagellum is almost always unobserved when viewed in the light microscope (LM). Because Cienkowski (1870) described the biflagellate colourless *Spumella* and the uniflagellate photosynthetic *Chromulina* in the same paper, we know that Cienkowski was able to distinguish these two basic flagellar types. Similarly, Stein (1878) illustrated many uniflagellate and biflagellate chrysophytes, so he too was able to distinguish the two flagellar types. Vysotskii (1887) was the first to describe a biflagellate, pigmented single-cell chrysophyte, which he called *Ochromonas*. The description of Vysotskii (1887) was exceptionally thorough: he described vegetative swimming cells, palmelloid cells, cysts, mitosis, cytokinesis, an unusual budding stage and flagellar transformation, even though transformation was not fully described until 100 yr later (Melkonian *et al.* 1987; Wetherbee *et al.* 1988).

The difference between uniflagellate and biflagellate swimming cells of the Chrysophyceae became the basis for two major phylogenetic lineages (e.g. Pascher 1913; Bourrelly 1957). After electron microscopy revealed the second, short flagellum on 'uniflagellates', chrysophyte literature included considerable discussion regarding the stability of flagellar number as a taxonomic and phylogenetic character (e.g. Hibberd 1976; Andersen 2007). Based on current molecular phylogenetic analyses, there are discrete clades with uniflagellate or biflagellate taxa but the two flagellar types *in toto* are polyphyletic assemblages.

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This paper focuses on the biflagellate polyphyletic assemblages, which are problematic because the phylogenetic position for the type species *Ochromonas triangulata* Vysotskii [lectotypified by Bourrelly (1957)] remained unknown. This type species was never reported again, even though *Ochromonas* became a very large genus (c. 125 species and varieties; see the Index Nominum Algarum: <http://ucjeps.berkeley.edu/INA.html>). Here, we report the rediscovery of *O. triangulata* from its type locality, Lake Veysove, a salt lake in the Donetsk region, Ukraine. We established cultures of *O. triangulata*, and here we report the phylogenetic position for the type species of this large genus.

## MATERIAL AND METHODS

A plankton sample was collected 3 November 2015 from the shore of Lake Veysove, Ukraine (48°52.046'N, 37°37.533'E). The lake was approximately twice as salty as the ocean, with a salinity of 60. Other physico-chemical parameters were not measured but see Barinova *et al.* (2015). The sample was enriched with L1 medium (Guillard & Hargraves 1993) containing 50 µM ammonium chloride (salinity = 34). *Ochromonas triangulata* was first observed on 19 December 2015, and on 26 December 2015 single cells were isolated into culture using L1 medium described above. Four strains grew and were given the strain numbers: A14,647, A14,648, A14,649 and A14,651. The strains have been deposited in three public collections (National Center for Marine Algae and Microbiota = CCMP; Culture Collection of Algae at the University of Göttingen = SAG; Culture Collection of Algae at the University of Texas - Austin = UTEX): A14,651 = CCMP3471, SAG 2530, UTEX 3030; A14,649 = SAG 2556, UTEX 3028; A14,648 = CCMP3472, SAG 2531, UTEX 3029; A14,647 = SAG 2557, UTEX 3027. After initial isolation, cultures were often maintained by adding a single, dry rice grain to each 20 mm diameter test tube containing approximately 15 ml of L1 medium. Palmelloid and cyst stages were prepared from L1 agar plates, and sometimes the four culture strains were experimentally mixed together in an effort to investigate possible sexual reproduction among the strains. Loricas were stained with either Vilh. Jensen mordant and stain (Petersen & Hansen 1961) or a filtered aqueous saturated solution of fuchsine.

Most light microscopic observations were made using a Leica DMRB light microscope equipped with differential interference contrast (DIC), phase contrast, brightfield and darkfield optics (Leica Microsystems, <http://www.leica-microsystems.com/home/>, Wetzlar, Germany). Photographs and videos were taken with a Canon EOS T6i or T2i Rebel single lens reflex camera (Canon USA, Inc, <https://www.usa.canon.com/internet/portal/us/home>). Images were captured as raw files and normally converted to tagged image file (TIF) documents using the Canon Digital Photo Professional software; however, some TIF files were created using Contenta Converter Premium software (2017). Images were further processed and assembled using Adobe Photoshop (Adobe Systems Inc. 2017). Cysts were prepared by cleaning with bleach. Scanning electron microscopic images

were taken using a Zeiss Supra 25 scanning electron microscope (Zeiss – Carl Zeiss, Göttingen, Germany). LM images for CCMP2718 were obtained using an Olympus BH2 microscope and an Olympus MagnaFire digital camera system (Olympus Corp., Tokyo, Japan).

For molecular work, the sources of genes are listed in Table S1. Cells were harvested by centrifugation at 16,800 × *g* for 20 min and manually ground using sterilized glass beads and a mortar. Genomic DNA extraction was carried with a GeneAll Exgene Plant SV mini Kit (GeneAll Biotechnology, Seoul, Korea) following manufacturer's instructions. Polymerase chain reactions (PCRs) were performed to amplify the nuclear encoded 18S rRNA small subunit (SSU) and the plastid encoded RuBisCo large subunit gene (*rbcL*) using various combinations of published and newly designed primers. For the SSU sequence they were the SSU1F (CCT GGT TGA TCC TGC CAG T) forward primer (Medlin *et al.* 1988) with the SSU1295R (TCA GCC TTG CGA CCA TAC) reverse primer (Yang *et al.* 2012) and the SSU1065F (TCA GAG GTG AAA TTC TTG GAT T) forward primer (Yang *et al.* 2012) with the SSU1954R (CCT TGT TAC GAC TTC TCC TTC C) reverse primer (Yang *et al.* 2012). For the *rbcL* sequence they were the *rbcL*46F (CGT TAY GAA TCT GGT GTA ATH CC) forward primer with the *rbcL*1425R (GTA TCT GTT GAW GWA TAG TCR AA) reverse primer. PCR amplifications, PCR purification and sequencing were all performed following protocol described in Wynne *et al.* (2014).

The SSU and *rbcL* sequences were compared to published sequences using the Basic Local Alignment Search Tool Nucleotide-Nucleotide (BLASTN; Altschul *et al.* 1990) available on the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/>). The first 500 sequences producing significant alignment were used to build a dataset for phylogenetic reconstruction. The original datasets were trimmed to exclude short sequences, environmental sequences and sequences obtained from uncultured organisms. Furthermore, a thorough search for sequences containing the label 'Ochromonas' was performed, and sequences missing from the BLAST search were added to the final datasets. The two datasets were aligned using MAFFT v7.305b (Katoh & Standley 2013). For the SSU alignment, the highly divergent regions of the alignment were further trimmed out with Gblocks v0.91b allowing for gaps in the final alignment (Talavera & Castresana 2007). Finally, both alignments were carefully refined by eye using Se-AL version 2.0 (<http://tree.bio.ed.ac.uk/software/seal/>). The *rbcL* alignment was translated into amino acid for further analysis. Maximum likelihood phylogenies were reconstructed with IQ-TREE version 1.4.4 (Nguyen *et al.* 2015) using the evolutionary model selected with the IQ-TREE build-in model selection function. It was the general time reversible (GTR) with a four-class gamma distributed rate of heterogeneity (G4) with a proportion of invariable sites (I) for the SSU rRNA alignment and the LG (Le and Gascuel 2008) + G4 + I for the *rbcL* alignment. For the concatenated tree reconstruction, an independent model for each partition of the concatenated data was used. Support values were estimated using 1000 replicates of the ultrafast bootstrap implemented in IQ-TREE (Minh *et al.* 2013).

## RESULTS

### Light microscopy: *Ochromonas triangulata*

Cells were highly variable in shape, from the stereotypical triangulate outline to nearly spherical shapes (Figs 1–7). Triangulate cells changed shape over short periods as they were swimming (Movie S1). Overall, cell morphology varied but generally cells were 6–9  $\mu\text{m}$  wide and 8–12  $\mu\text{m}$  long (Figs 1–7). The long flagellum was 1 to 1.5 $\times$  the length of the cell; this flagellum pulled the cell while swimming. The short flagellum was  $\frac{1}{4}$  to  $\frac{1}{3}$  the cell length. Internally, cells had a single plate-like parietal bilobed chloroplast, and a pyrenoid was sometimes located in the centre where the lobes joined (Figs 4, 5, 7). The chloroplast had a large, red eyespot (Fig. 3). A transparent nucleus was at the anterior end near the flagellar basal bodies (Fig. 5). Lipid droplets and a chrysolaminarin vacuole were present in cells from older, stationary phase cultures (Fig. 3).

Pseudopods were formed and absorbed as the cells swam. Triangular cells frequently formed small pseudopods from the posterior cell end, especially from one ‘corner’ of the triangle beneath the eyespot (Fig. 2). Non-triangulate cells produced pseudopods, usually from the lateral surface beneath the short flagellum/eyespot complex. A continually changing pseudopodial band or ribbon below the eyespot was observed on several occasions (Figs 8–9). Also, fine, branching pseudopods from the same region below the eyespot were observed as cells were swimming (Figs 10–11).

The flagellate cells fed phagotrophically on bacteria that were captured near the short flagellum (Figs 12–13). The food vacuoles moved down the lateral side of the cell beneath the eyespot, and the food particles were digested in the vacuoles near the posterior end of the cell (Fig. 5).

Cysts were formed in mixed strain cultures that were kept in the dark at 4°C for several days. The cysts were not ornamented, and the pore was filled with a small plug (Figs 14–16).

Cells grown on agar flooded with L1 medium showed some unusual stages. The cells often grew as a palmelloid layer on the agar (Fig. 17). These cells lacked flagella and food vacuoles; the chloroplast filled the cell, and the eyespot was absent. Some palmelloid cells had two chloroplasts, and some resembled recently divided cells; however, cell division was not documented for the palmelloid cells. On one occasion, cells growing on agar produced a ribbon-like or band-like structure (Figs S1–S4). The band was flexible, and it appeared to be one cell layer thick (compare Figs S2, S3). Cells were sometimes located in cavities made of organic matter, and bacteria were abundant (Fig. S4). The cells were flagellate but shaped only as spheroidal cells.

Cell division occurred most frequently at the end of the photoperiodic light phase but some cells tried to divide at various times during the light phase. Cell division began with flagellar transformation (not shown) that eventually produced the second set of flagella (Fig. 18). A second eyespot appeared quite early, often before the second flagellar set was fully replicated. Although difficult to see, the nucleus divided (Fig. 19), and after nuclear division, the flagellar pairs separated. The flagellar pairs moved to opposite poles, and the cell was typically spherical (Fig. 20). Cells then elongated (Fig. 21), and

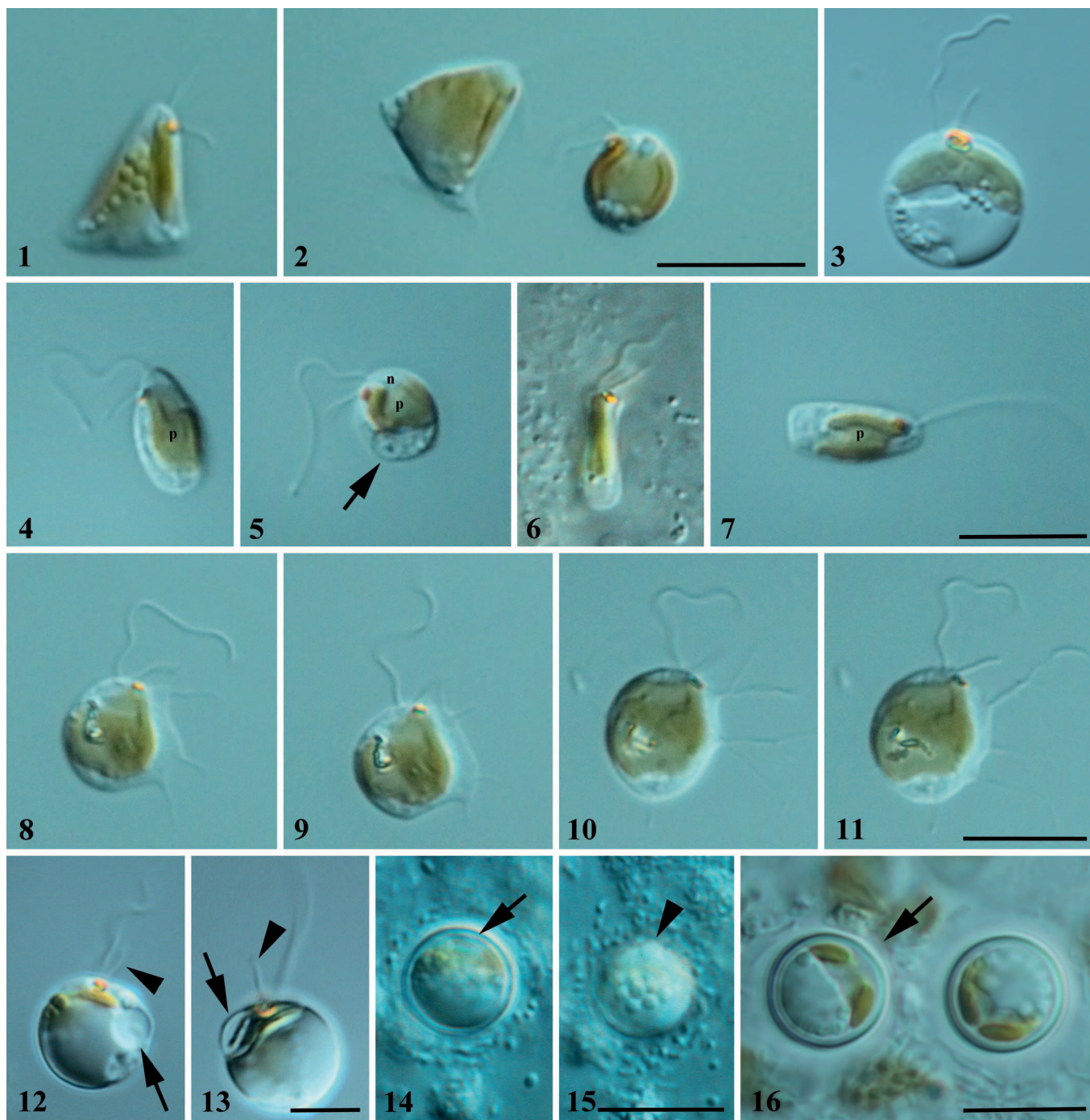
during this period plastid division was completed. Once the plastid divided, the two daughter cells began to separate, and they formed a ‘dumbbell’ shape (Fig. 22, Movie S2). The cytoplasmic connection quickly elongated to a thin strand and then broke off (Figs. 23–25). Within a few minutes, the remnant thin strands were reabsorbed, and the daughter cells appeared like normal vegetative cells. Fine pseudopods were often produced during cell division (Fig. 25). Some cells had trouble with chloroplast division, and the elongated cell was retracted back to a more spherical cell before the process was repeated (Movie S3).

Normal cell division did not always succeed, failing in at least two ways. In one case, a bud-like projection was produced, and one flagellar pair was present at the end of that projection. No nucleus was observed in the projection, and the chloroplast, although divided, did not move into the projection (Fig. 26, Fig. S5). One cell was observed for over 45 min, and the projection was repeatedly formed and decreased, as though the flagellar apparatus were attempting to connect to the nucleus–chloroplast complex (Fig. S5). This cell was observed in the morning but the start of cell division for this cell was not observed (i.e. the cell may have started the division process much earlier). Budding cells were observed several times in cultures but they were rare. The second, rare, abnormal cell division resulted in one daughter cell without a chloroplast (Figs 27–29). These colourless cells were approximately 5  $\mu\text{m}$  in diameter, smaller than typical cells. The colourless cells contained a spherical structure that resembled a nucleus. The fate of the colourless cells was not determined.

### Light microscopy: *Poterioochromonas*

Molecular phylogenetic analyses showed that two strains, tentatively identified as *Ochromonas* cf. *gloeopara* Skuja, fell into the *Poterioochromonas* clade (see below). Therefore, we carefully examined strains CCMP2060 and CCMP2718. Strain CCMP2060 was predominately free-swimming flagellates (Figs S6–S8). The cells were approximately 10–13  $\mu\text{m}$ . They appeared to have two chloroplasts but it may have been a single chloroplast connected by a very narrow bridge (Figs S6, S7). Cells had a single contractile vacuole (Fig. S7). An extended search revealed the presence of loricas on less than 1% of the cells (Figs S9, S10). The loricas were delicate and easily missed (Fig. S9) but after staining the loricas were clearly defined (Fig. S10). The lorica ‘cup’ was broad and shallow (Figs S9, S10), and when stained, there was a septum across the base of the cup where it attached to the stalk (Fig. S10). The cysts produced by strain CCMP2060 had a raised collar, and there was a flange around the upper region (Figs S11–S13).

Strain CCMP2718 was sequenced and photographed in 2006 but when we re-examined the strain in 2016, a different organism was present. Therefore, the comments and figures were based on the 2006 observations and gene sequences. The vegetative cells were without loricas, cells had both a short and long flagellum and the chloroplast was bilobed (Figs S14–S16). The cysts had a bi-winged collar and a flange that was sometimes segmented (Figs S17–S19). A netlike pattern was observed above the flange (Fig. S18). The outer cyst surface was smooth or slightly punctate.



**Figs 1–16.** *Ochromonas triangulata*.

**Fig. 1.** Mixed strains. Typical triangulate cell morphology showing the two flagella, a single parietal chloroplast with a red eyespot and lipid droplets. (See Fig. 2 scale bar).

**Fig. 2.** Mixed strains. Comparison of a large triangulate cell and a small spherical cell. Note the short pseudopod on the triangulate cell. Scale bar = 10  $\mu$ m.

**Fig. 3.** Strain A14,651. Spherical cell showing the long and short flagella, chloroplast with a large red eyespot and a large chrysolaminarin vacuole. (See Fig. 7 scale bar).

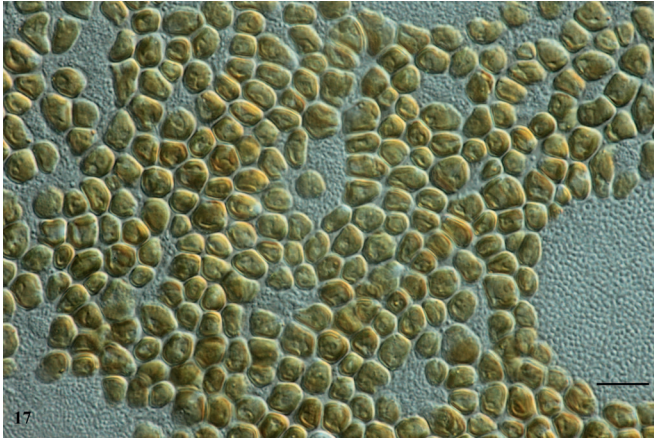
**Fig. 4.** Strain A14,651. Elongate cell showing the close association of the short flagellum and the eyespot. Note the pyrenoid (p). (See Fig. 7 scale bar).

**Fig. 5.** Strain A14,651. Spherical cell showing the nucleus (n), pyrenoid (p) and a food vacuole (arrow). (See Fig. 7 scale bar).

**Fig. 6.** Strain A14,651. Elongate, slightly hourglass-shaped cell. (See Fig. 7 scale bar).

**Fig. 7.** Strain A14,651. Elongate cell showing the long flagellum at approximately 1.5 $\times$  cell length and a pyrenoid (p) in the trough of the parietal chloroplast. Scale bar = 10  $\mu$ m.

**Fig. 8.** Strain A14,647. Cell with a lateral band-like pseudopod formed beneath the eyespot. (See Fig. 11 scale bar).



**Fig. 17.** *Ochromonas triangulata*. Mixed strains. Palmelloid cells grown on agar flooded with medium. Note the cells were almost entirely filled with the chloroplast, which lacked an eyespot; chrysolaminarin vacuoles and lipid droplets were greatly reduced or absent. Scale bar = 20  $\mu\text{m}$ .

Strain CCMP1862 was previously identified as *Poterioochromonas stipitata* Scherffel, and we re-examined this strain as well. Cells were 7–12  $\mu\text{m}$  in size and had two chloroplasts (Figs S20–S24). The lorica was relatively broad and shallow, and a septum was barely visible without staining (Fig. S20). The cells ate bacteria by phagocytosis (Fig. S23). An elongate bud-like structure was observed on one cell but it was not investigated over time (Fig. S21). Cells without chloroplasts were sometimes observed, and one was found still occupying a small lorica (Fig. S22).

### Molecular phylogeny

We generated a 1755 bp partial sequence of the nuclear rRNA SSU and a 1254 bp partial sequence of the plastid *rbcL* gene for the *Ochromonas triangulata* strain A14,651 (GenBank accession numbers KY575274 and KY575275, respectively; see Table S1 for other accession numbers). The SSU sequence was aligned with 165 sequences representing the diversity of the class Chrysophyceae. Two sequences of the class Synchromophyceae were used as outgroup based on the current understanding of the stramenopile phylogeny (Yang *et al.* 2012). The final SSU alignment comprised 168 taxa and 1655 nucleotides and was used to determine the phylogenetic position of *Ochromonas triangulata* within the class Chrysophyceae.

A maximum likelihood phylogeny using SSU sequences recovered a highly polyphyletic assemblage of *Ochromonas*

*sensu lato* where *Ochromonas triangulata* formed one clade by itself and the *Ochromonas*-like taxa were split into 13 additional clades (Fig. 30). Strain A14,651 represented the type species and was sister to *Ochromonas* strain AC-25 with an Ultrafast Bootstrap Approximation (BS) of 100%. The *O. triangulata* clade was included within a much larger clade that was strongly supported (BS 95%), and this clade included colourless taxa as well as *Uroglenopsis americana* (Calkins) Lemmermann and unidentified chrysophytes. However, phylogenetic relationships within this group were poorly resolved. All the other *Ochromonas*-like taxa were distantly related to the type species *O. triangulata*, and therefore their classification must be reconsidered.

To attempt to delimit more clearly the phylogenetic relationships within the monophyletic group including *Ochromonas*, we assembled a concatenated dataset of the SSU and *rbcL* genes (Table S1). To avoid reconstruction artefacts, we excluded nonphotosynthetic lineages (i.e. *Paraphysomonas*, *Oikomonas*, *Spumella*, *Pedospumella* and *Spumella*-like). The concatenated dataset included 1655 nucleotides (SSU alignment) and 417 amino acids (*rbcL* alignment) for 127 taxa, of which 72 (i.e. 56%) were represented by both SSU and *rbcL* sequences. The analysis recovered the 13 *Ochromonas*-like clades identified in the SSU phylogeny (Fig. S25). The *Ochromonas triangulata* clade, the *Ochromonas*-like clades IV to VIII, *Uroglenopsis* and strain CCAP 909/3 formed a strongly supported monophyletic group (BS 97%). Within this group, the *O. triangulata* clade was the first to diverge but with low support (BS 57%). To avoid confusion, we replaced the name *Ochromonas* with Chrysophyceae sp. for previously identified *Ochromonas* spp. in Figs 30, S25; previous names are listed in Table S1.

### DISCUSSION

Our LM observations on *Ochromonas triangulata* from Lake Veysove, Ukraine, agreed with many of the observations reported by Vysotskii (1887). Vysotskii maintained the alga in culture for at least one year. Cells in all four of our cultures produced the triangulate shape along with ovate to spherical shapes, and these morphologies were the same as those illustrated by Vysotskii (1887, fig. 1a–1f). The single-lobed chloroplast with a prominent red eyespot, the two unequal flagella and the large, shiny chrysolaminarin vacuole were the same for both his observations and ours.

The fine pseudopods observed in our cultures (Figs 10–11) were essentially identical to those of Vysotskii (1887, fig. 1c, fig. 1c'). Vysotskii also observed a broad pseudopod-like

**Fig. 9.** Strain A14,647. Same cell approximately 15 sec later, showing changes in the pseudopodial projection. (See Fig. 11 scale bar).

**Fig. 10.** Strain A14,647. Cell with fine pseudopodia formed beneath the eyespot. (See Fig. 11 scale bar).

**Fig. 11.** Strain A14,647. Same cell approximately 30 sec later, showing changes in the pseudopodia. Scale bar = 10  $\mu\text{m}$ .

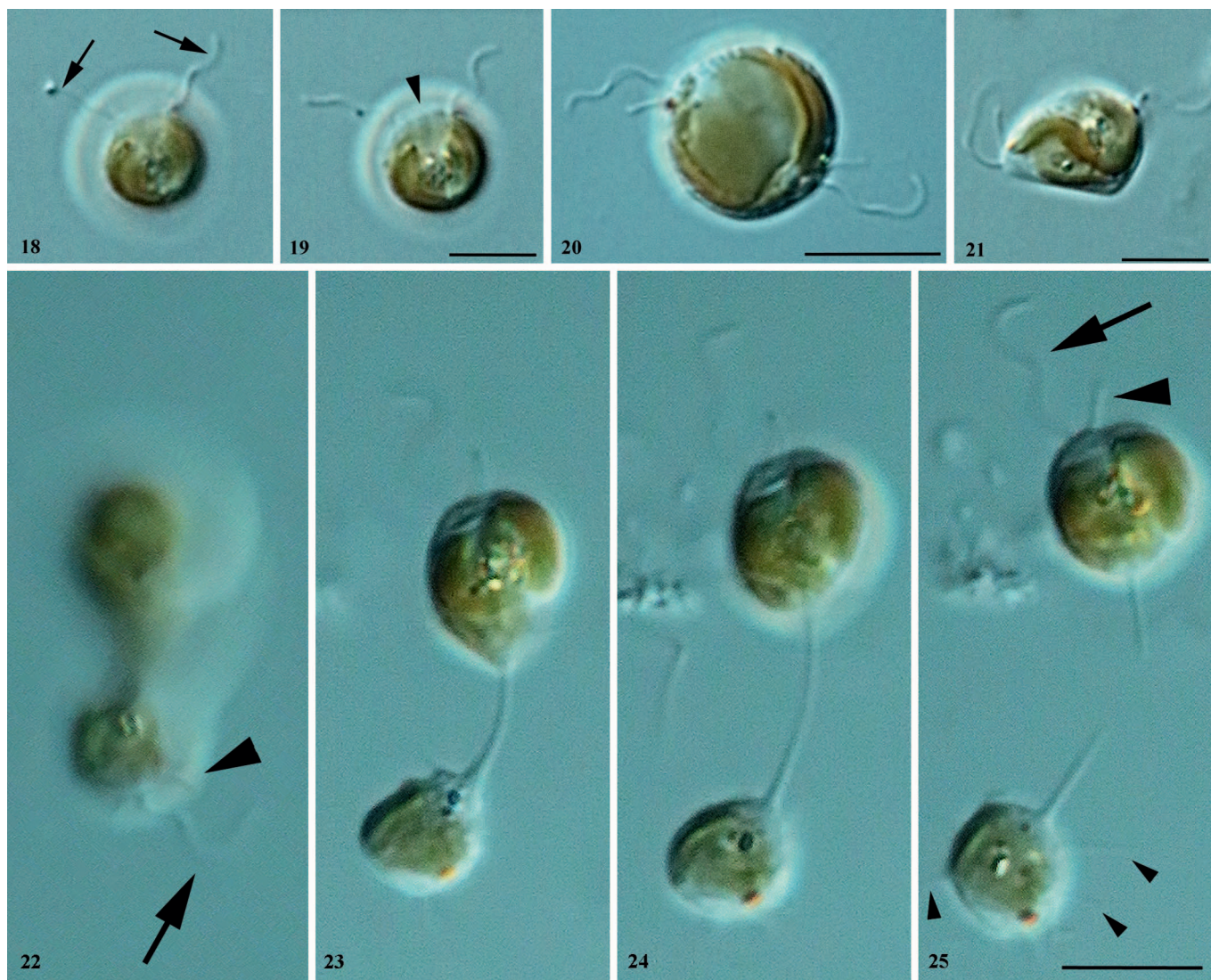
**Fig. 12.** Strain A14,649. Food vacuole (arrow) with captured bacterium. Note the short flagellum (arrowhead) and large chrysolaminarin vacuole. (See Fig. 13 scale bar).

**Fig. 13.** Strain A14,649. Cell with elongate bacterium in a food vacuole near the base of the short flagellum (arrowhead). Scale bar = 5  $\mu\text{m}$ .

**Fig. 14.** Mixed strains. Medial optical section of a cyst, showing the chloroplast, a chrysolaminarin vacuole and many lipid droplets. Note the probable pore region (arrow). (See Fig. 15 scale bar).

**Fig. 15.** Mixed strains. Tangential optical section of the same cyst showing the pore (arrowhead). Scale bar = 10  $\mu\text{m}$ .

**Fig. 16.** Mixed strains. Two cysts embedded in a gel-like layer. Note the plug (arrow), chloroplasts and large chrysolaminarin vacuole. Scale bar = 10  $\mu\text{m}$ .



**Figs 18–25.** *Ochromonas triangulata* cell division. Strain A14,647.

**Fig. 18.** Optical section of an early division cell with two pairs of flagella. Arrows indicate the two long flagella. (See Fig. 19 scale bar).

**Fig. 19.** Optical section of the same cell showing the dividing nucleus (arrowhead). Scale bar = 10 µm.

**Fig. 20.** Cell with flagellar pairs at opposite poles of the cell. Scale bar = 10 µm.

**Fig. 21.** An elongating cell that was completing chloroplast division. Scale bar = 10 µm.

**Fig. 22.** Video image showing early cytokinesis. Note the long flagellum (arrow) and short flagellum (large arrowhead). (See Fig. 25 scale bar).

**Fig. 23.** Video image mid-cytokinesis nearly complete but a short, bending cytoplasmic strand still connects the cells. (See Fig. 25 scale bar).

**Fig. 24.** Video image showing late cytokinesis where the cytoplasmic strand is thin but still bent. (See Fig. 25 scale bar).

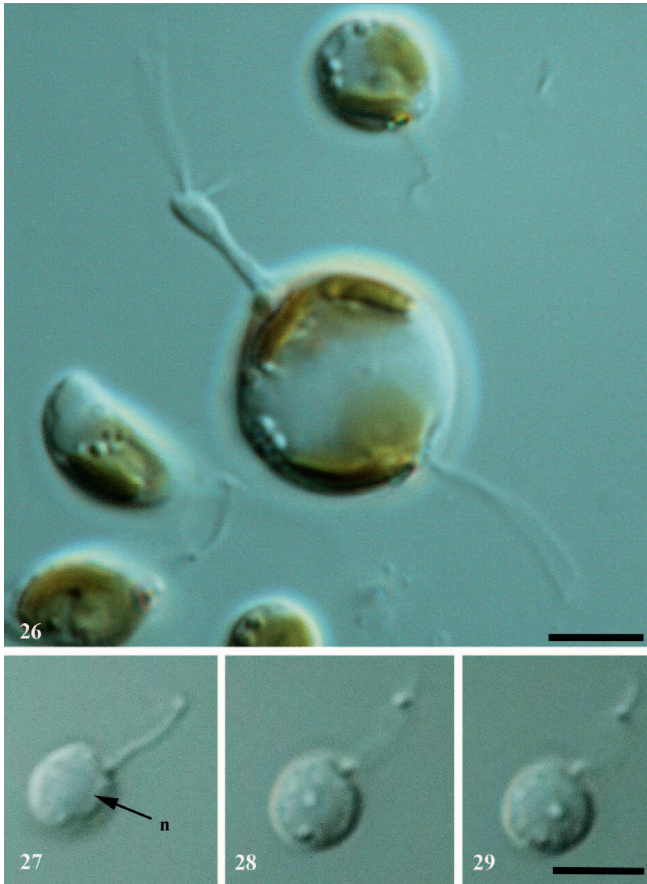
**Fig. 25.** Video image showing complete separation of the two cells with the cytoplasmic strand breaking in the area it was bent. Arrow = long flagellum, large arrowhead = short flagellum, small arrowheads = pseudopodia. Scale bar = 10 µm.

extension posterior to the eyespot (fig. 10), which by description and illustration was the same as our Figs 8–9. Vysotskii followed a cell with the broad colourless pseudopod for 21 h (noon to 9:00 AM the following day) and reports that during the morning of the second day, another flagellum appeared and the cell divided.

Vysotskii (1887) described and illustrated the capture of solid food particles, and his observations agreed with ours. Interestingly, Vysotskii added a footnote where he discussed the ability of *Chrysomonas flavicans* F.Stein to capture a variety of foods and did not understand why Bütschli (1883)

questioned the observations of Stein (1878). In reading Bütschli (1883, p. 866), Bütschli was very clear that he accepted Stein’s observations. Therefore, it may be possible that Vysotskii (1887) was referring to Bütschli’s rejection of mixotrophy for other photosynthetic flagellates. In any case, *Ochromonas triangulata* took up bacteria by phagotrophy, and in this way resembled *C. flavicans* and many other, if not all, *Ochromonas*-like flagellates (e.g. Moestrup & Andersen 1991).

Vysotskii (1887) described cell division in detail, and his observations differed somewhat from ours. Vysotskii (1887,



**Figs 26–29.** *Ochromonas triangulata* aberrant cell division stages. Strain A14,651.

**Fig. 26.** Cell with a bud-like projection terminated with one flagellar pair. In this case, the chloroplast had divided (see also Suppl. Fig. S5). Scale bar = 5  $\mu$ m.

**Fig. 27.** Cell without a chloroplast. Note the nucleus (n) (see Fig. 29 scale bar).

**Fig. 28.** Same cell with slightly different view. (See Fig. 29 scale bar).

**Fig. 29.** Same cell but nucleus no longer visible. Scale bar = 5  $\mu$ m.

fig. 2a) illustrated one stage that was like our Fig 23 but his fig. 2b differed from our Figs 24–26. That is, the flagellar pairs illustrated by Vysotskii pointed in the same direction, while our flagellar pairs rotated to the poles and pointed in opposite directions. Also, he showed one flagellar pair near the cytoplasmic strand as the two daughter cells separated (his fig. 3a,b), whereas, our flagella were at opposite ends (Figs 27–29). Interestingly, he observed the same failure to divide in some cells (his fig. 4, Movie S3). Like our observations on the budding (Fig. 31, Fig. S5), Vysotskii also described and illustrated a similar event (1887, fig. 5); however, Vysotskii said that the colourless bud gained colour and eventually produced a small swimming cell but we did not observe any plastid moving into the colourless protuberance (Fig. S5). Accepting Vysotskii's observations, it seems likely that the flagellar apparatus was able to connect to the nucleus/chloroplast complex in the cell that he described.

Vysotskii (1887) also observed and illustrated what he termed the vegetative stage (fig. 11), which was identical to

our palmelloid stage (Fig. 22). Specifically, he reported a tightly packed one-cell layer growing on the water surface; these cells lacked an eyespot, and the chloroplast filled the cell. Vysotskii also illustrated cysts (figs 8, 9), which he described with a parietal chloroplast in one half of the cyst volume and a large chrysolaminarin vacuole plus small oil droplets in the other half. He did not illustrate or describe the organic plug but given the difficulty we had in demonstrating the presence of the plug (Figs 14–16), this oversight may be dismissed. Finally, Vysotskii described what he termed division of the cyst but his description and illustrations (figs 9a, 9b) suggest to us that he was describing an early stage of the palmelloid stage. In summary, there are some minor differences in Vysotskii's descriptions and interpretations compared to ours but there can be no doubt that we both observed the same organism from Lake Veysove. Some discrepancies might be attributed to differences in microscopic technology and technique; other differences are probably due simply to the variability that this organism expresses. There were no other previous reports for *Ochromonas triangulata* other than Vysotskii (e.g. Starmach 1985; Barinova *et al.* 2015; but see reference to unpublished Massart manuscript in Conrad 1926); therefore, no further comments can be added about the type species.

The sister taxon to *Ochromonas triangulata* in our tree (Fig. 30) was strain AC-25. Using LM we examined strain AC-25 (Figs S26–S34), and it bore a strong resemblance to *O. triangulata*. Strain AC-25, known by the invalid name '*Ochromonas distigma*' (see below), frequently had two eyespots per cell (Figs S26–S28), and in some cases before division, three eyespots (Fig. S31). The strain produced triangulate cells, and pseudopodia often extended from one of the posterior 'corners' (Figs S29, S30). Cells also produced broad and thin pseudopods beneath the short flagellum/eyespot complex (Fig. S32). Cell division, while not investigated thoroughly, appeared similar to our observations of *O. triangulata* (Fig. S33). The cyst also was similar to that for *O. triangulata* (Fig. S34). Based on these morphological similarities and the molecular phylogenetic analyses (Fig. 30, Fig. S35), we have identified strain AC-25 as *O. triangulata*.

#### Phylogeny and reorganization of *Ochromonas sensu lato*: Nomenclatural possibilities

It has been known for some time that *Ochromonas* was polyphyletic (Andersen *et al.* 1999; Boenigk *et al.* 2005; Andersen 2007). Nomenclaturally, the genus *Ochromonas* must include the type species but our results show that this clade is small (Fig. 30, Fig. S35). Therefore, what to do with the remaining *Ochromonas*-like clades that fall outside *Ochromonas*? These clades represent distinctly different genera, so we examined the literature for available names. Several generic names have been used for *Ochromonas*-like flagellates, including (by priority) *Chrysomonas* (Stein 1878), *Stylochrysalis* (Stein 1878), *Poterioochromonas* (Scherffel 1901), *Chlorochromonas* (Lewis 1913), *Chrysoglena* (Wistouch 1914), *Wellheimia* (Pascher 1917), *Chrysastrrella* (Chodat 1921), *Brehmiella* (Pascher 1928), *Didymochrysis* (Pascher



**Fig. 30.** Maximum likelihood tree of the 18S rRNA of the class Chrysophyceae inferred with IQTree. Support values evaluated are shown above the nodes and were estimated by IQTree with the Ultrafast Bootstrap Approximation algorithm. Some species previously identified as *Ochromonas* have been re-named Chrysophyceae sp. (see Table S1). Scale bar = 0.05 substitutions per site.

1929), *Ochrostylon* (Pascher 1942), *Rhizochromonas* (Nichols 1990) and *Chrysolepidomonas* (Peters & Andersen 1993).

*Chrysomonas* was described as having a single flagellum and forming colonies with radial cells (i.e. *Chromulina*-like); however, Stein (1878) showed three cells in detail and each cell had one or two food items within the cell (*Ochromonas*-

like). As mentioned earlier, Bütschli (1883), accepts Stein’s observation that *Chrysomonas* feeds by phagotrophy. Conversely, food particles are not generally (if ever) ascribed to *Chromulina*. Regarding flagellar number, Stein (1878) provided drawings of cells of *Uroglena* immediately adjacent to cells of *Chrysomonas*, and he clearly illustrated the short, second



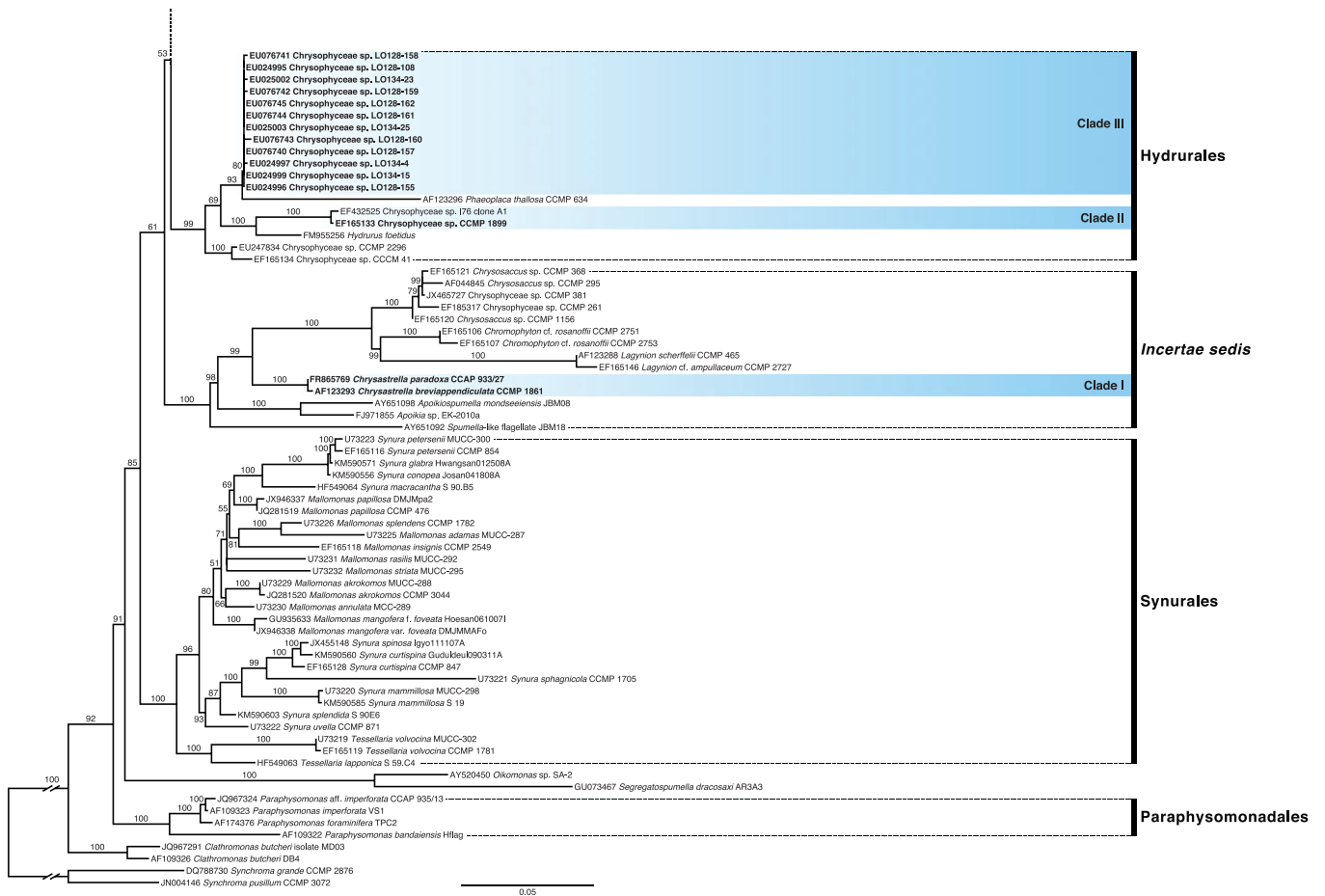


Fig. 30. Continued.

flagellum. Therefore, the second flagellum of *Chrysomonas* (which surely existed) must have been quite short, like those for *Chromulina sensu lato*. It may be possible to apply the name *Chrysomonas* to a clade that has organisms with a very short second flagellum and other features in common with the description of Stein (1878). However, at this time, we are unable to apply the name.

*Stylochrysalis* was described briefly but validly, in a figure legend (Stein 1878). The description states that there were many individuals growing radially around *Eudorina elegans* Ehrenberg; the cells were extended on stiff stalks and had golden yellow chloroplasts and two apparently equal flagella. Lemmermann (1910) adds that the contractile vacuole is in the posterior of the cell, which is not a typical chrysophycean feature (Andersen 1987). The equal flagella shown in the illustration (Stein 1878, Plate XIV, section IV) and posterior contractile vacuole are not characteristic for chrysophytes, and this contradicts the golden yellow chloroplasts. Skuja (1948) observed a distinctly chrysophycean alga that he named *Stylochrysalis parasitica* Skuja but his alga had a lorica and two pseudopods in place of flagella; Skuja's alga resembles *Stylococcus* (Chodat 1898) much more than *Stylochrysalis*. None of the taxa in our tree resembled Stein's description of *Stylochrysalis*, so we excluded this name.

*Poterioochromonas* was assigned to our Clade XIII (see below).

*Chlorochromonas* was assigned to our Clade XI (see below).

*Chrysoqlena* Wislouch was described as a uniflagellate alga with a coarse cell surface (Wislouch 1914). The cells were quite large (up to 50  $\mu\text{m}$ ), had a single-lobed chloroplast with an eyespot and anterior contractile vacuoles. Lund (1949) also gave an account of this genus, which agreed in most ways with Wislouch's description. There was no evidence that this flagellate did not agree with any of the taxa in our study, and we could not apply the name to a clade.

*Wellheimia* was proposed, almost in passing, while Pascher (1917) was describing axopodial protists. The 'axopod' of *Wellheimia pfeifferi* Pascher, the only species in the genus, superficially resembled the feeding loop of *Poterioochromonas* as illustrated by Scherffel (1901, plate 17, fig. 9 a-c). *Wellheimia* had a single flagellum, did not fully agree with any of the taxa in our study, and we could not apply the name to a clade.

*Chrysaetrella* was assigned to our Clade I (see below).

The amoeboid *Brehmiella* was described from a tiled pool in Franzensbad, a spa town now included within the Czech Republic (Pascher 1928). Pascher illustrated 17 cells growing attached to *Rhizoclonium*, and all cells had multiple anterior pseudopods. He also reported that cells became detached, swam freely like *Ochromonas* (naked biflagellate single cells) and underwent cell division while swimming. This generic name could be used for a clade

containing attached ochromonad-like algae that produced pseudopods from the anterior end. Our tree had no organisms of this type, so we have excluded the name.

*Didymochrysis paradoxa* Pascher had the appearance of an *Ochromonas* cell that became arrested during cell division so that it has two sets of flagella, two chloroplasts, two eyepots, two to four contractile vacuoles, smooth walled cysts but only one nucleus (Pascher 1929). Cell division was described. The alga was reported from several sites, including the type locality (aus den Musikantenteichen im Teichgebiet von Hirschberg in Böhmen), which today is near Doksy, Czech Republic. We had no representatives of this distinctive genus, and therefore we excluded this name.

*Ochrostylon epiplankton* Pascher was described from freshwater lakes in northern Germany where it grew attached to diatoms (Pascher 1942). This alga looked like an *Ochromonas* cell that had attached to a substrate, and this morphological form has been widely observed for a number of ochromonad flagellates. In principle, the name could be applied in certain instances but the group or clade should be from freshwater and readily producing attachment stalks. In our study, we did not apply this name.

*Rhizochromonas* was a predominantly amoeboid alga that was restricted to loricas of *Dinobryon* (Nicholls 1990). This organism might produce more typical ochromonad flagellate cells if brought into culture but lacking any such data, we excluded this name.

*Chrysolepidomonas* had organic scales surrounding the cell, and the scales were not visible using the light microscope (Peters & Andersen 1993). The SSU sequence of the type species was sequenced using an authentic culture (Andersen *et al.* 1999), and this sequence branched with *Epipyxis* and *Chrysonephale* in our tree. Therefore, we excluded this name.

In addition to the photosynthetic species names given above, it is obvious from our tree and other published papers that nonphotosynthetic species (and genera) are widely interspersed among those having chloroplasts. We agree with Grossman *et al.* (2016), who attempted to use older names for their colourless clades (see their supplemental material). We have not attempted to apply existing colourless generic or specific names in our paper.

#### Clade I: *Chrysastrrella* Chodat

Our clade I contained two strains that were identified as *Ochromonas tuberculata* D.J.Hibberd (Hibberd 1970). Strain CCAP 933/27 was the authentic strain used by Hibberd, and it was collected at Three Tarns, Cumbria, England. Strain CCMP 1861 was isolated from Volo Bog, Illinois, USA. One distinguishing character of this species is the spiny appendages on the cyst (Hibberd 1977, Fig. S35), which closely resembles the structure of *Chrysastrrella*, a cyst genus described by Chodat (1921). Chodat provided no information about the vegetative cells and described three species, *Chrysastrrella paradoxa* Chodat, *Chrysastrrella minor* Chodat and *Chrysastrrella breviappendiculata* Chodat. Chodat did not designate a generitype; however, Deflandre (1934) lectotypified the genus by designating *C. paradoxa* as the type species. A number of additional taxa have been described (e.g. Deflandre 1934; Frenguelli 1953; Frenguelli & Orlando 1958), and the genus

*Echinochrysis* (Conrad 1926) should be included in *Chrysastrrella*. All these species were from freshwater, and they often appear to be from acidic waters (especially habitats containing *Sphagnum* moss). We identify the strain CCAP 933/27 as the type species, *C. paradoxa*, and we identify strain CCMP 1861 as *C. breviappendiculata* because it has different gene sequences and less pronounced cyst spines (Fig. S35).

#### Clade II

Strain CCMP 1899 was collected from Antarctic sea ice where it grew in salty brines. Similarly, GenBank entry EF432525 was based upon a culture established at Woods Hole Oceanographic Institution, and it also was collected from Antarctic sea ice (Dr. Rebecca Gast, pers. comm.). We were unable to find an existing generic name for taxa in clade II.

#### Clade III

These sequences were from single-celled isolates collected from Loibersbacher Teich 1, a lake in the Salzkammergut area of Austria; no further information is available.

#### Clades IV–VI

The taxa are all marine flagellates, and they are undescribed or invalidly described. For example, strain AC-22 was called ‘*Ochromonas marina*’ Billard-Haas ex Gayral & Billard (Gayral and Billard 1977) but the name *Ochromonas marina* Lackey was in existence (Lackey 1940), making the name superfluous. We found no name(s) that could be applied.

#### *Ochromonas triangulata* clade

*Ochromonas triangulata* was sister to strain AC-25, an alga collected from Estuaire de l’Odet, France. Two earlier studies attempted to describe this alga as ‘*Ochromonas distigma*’ but the descriptions were invalid (Lepailleur 1969, Gayral & Billard 1977). Based upon our phylogenetic trees (Fig. 30, Fig. S25), we identify strain AC-25 as *O. triangulata* even though it frequently has two eyepots. Other marine species, such as *Ochromonas olivacea* Büttner (1910), and possibly *Ochromonas viridis* Böcher (1945), resemble *O. triangulata* but without additional information, it is impossible to assign the taxa, especially given the extensive polyphyly of *Ochromonas*-like flagellates.

#### Clades VII and VIII

Three strains make up these two clades, although the bootstrap supports were weak for the deep branches (Fig. 30, Fig. S35). It is unclear whether they belong within *Ochromonas sensu stricto* or belong to a separate genus. Furthermore, these marine or brackish water strains are sister to the freshwater colonial flagellate *Uroglenopsis americana*. Strain CCAP 933/25 was validly named *Ochromonas villosa* K.J.Clarke & N.C.Pennick (Clarke & Pennick 1981), and therefore this name will be a basionym if a new genus is described.

#### Clade IX

This marine clade was distinct from its freshwater sister clade that contained *Epipyxis*, *Chrysonephale* and *Chrysolepidomo-*

nas. We found no existing generic name, so we have proposed the following new genus:

***Melkoniania* R.A.Andersen & H.-S.Yoon gen. nov.**

DESCRIPTION: single-celled marine flagellates without scales or theca; two unequal flagella; one or two parietal, trough-like chloroplasts; phagocytosis of food particles by feeding loop derived from near short flagellum base; 18S rRNA and *rbcL* gene sequences forming an unique clade in phylogenetic analyses.

TYPE SPECIES: *Melkoniania moestrupii* (R.A.Andersen) R.A.Andersen comb. nov.

BASIONYM: *Ochromonas moestrupii* R.A.Andersen (2011. *Phycologia* 50: 601).

ETYMOLOGY: the genus is named in honour of Prof. Dr. Michael Melkonian, the University of Cologne, Germany, who has made many significant contributions to phycology.

COMMENTS: Strains AC-24 and CCMP591 can be described as new species within *Melkoniania*. Strain AC-24, collected from the English Channel waters at Sallenelles, France, was invalidly described as '*Ochromonas aestuarii*' (Lepaillieur 1969, Gayral & Billard 1977); the name could be validated if biological material were deposited in accordance with the ICN (McNeill *et al.* 2012). Strain CCMP591, from San Juan Island, Washington, USA, requires a full description, which is beyond the scope of this paper.

**Clade X**

Three pigmented strains and a single colourless strain compose this clade, and they were all freshwater strains. The pigmented strains were isolated from North America and formed a closely related group with low genetic divergence. Two strains were previously identified as *Ochromonas sphaerocystis* Matvienko and *Ochromonas* cf. *perlata* Doflein. A new genus should be proposed for these taxa; however, this is beyond the scope of this paper.

**Clade XI**

These were all freshwater taxa, including pigmented and colourless organisms. For pigmented strains SAG 933.7 and CCMP588, the generic name *Chlorochromonas* was available and appropriate (Lewis 1913; Pringsheim 1955), so we applied the name to this clade. The clade contained *Ochromonas danica* E.G.Pringsheim, and the sequence was from Pringsheim's authentic culture (SAG 933.7). Strain CCMP588 was isolated from Lake Fayetteville, Arkansas, USA, in 1977. The GenBank accession number M32704 does not provide a strain, and in the publication it was identified as *Ochromonas danica* without reference to a strain (Gunderson *et al.* 1987).

Pringsheim himself discussed the strong similarity between *Chlorochromonas minuta* I.F.Lewis and *Ochromonas danica* and argued that (1) colour was not enough to distinguish genera, and (2) if it were not colour, he would consider *O. danica* to be the same as *C. minuta* (Pringsheim 1955, p. 152). Specifically, *O. danica* was described as 'gelblichgrün' (Pringsheim 1955) while *C. minuta* was described as 'yellowish-green' (Lewis 1913). We agree with Pringsheim that these should not be different genera, and because they fall outside of

*Ochromonas sensu stricto*, we can use the name *Chlorochromonas* and recognize two species (*C. minuta*, *C. danica*). In the future, if it is shown that these two species are synonymous, the name *C. minuta* has priority.

***Chlorochromonas* I.F.Lewis 1913**

TYPE SPECIES: *Chlorochromonas minuta* I.F.Lewis 1913.

***Chlorochromonas danica* (E.G. Pringsheim) R.A.Andersen, L.Graf, Y.Malakhov & H.S.Yoon comb. nov.**

BASIONYM: *Ochromonas danica* E.G.Pringsheim (1955, *Archiv für Mikrobiologie* 23: 193)

Clade XI also contained colourless flagellates, including the genus *Cornospumella* (Grossmann *et al.* 2016).

**Clade XII**

The clade contained both pigmented and nonpigmented taxa, including the recently proposed colourless genus *Poteriospumella* (Findenig *et al.* 2010). The clade requires further investigation, including a discussion about combining pigmented and colourless taxa in the same genus.

**Clade XIII: *Poterioochromonas***

Strains of *Poterioochromonas* were found only in Clade XIII. There are three species; although, the distinctiveness of the three species has been questioned (Péterfi 1969). *Poterioochromonas stipitata* Scherffel is the generitype, and the type material was collected from Csorba Lake, Tatra, Hungaria (Scherffel 1901, p. 147), which is currently known as Štrbské pleso in the Tatra Mountains, Slovakia. This lake is 1346 m above sea level, the water was derived from glacial ice and the lake is highly regarded for its purity; the pH and conductivity are low (Kopáček *et al.* 2006). Scherffel (1901) considered *Poterioochromonas* to be an intermediate form between *Ochromonas* and *Dinobryon*, which appears to be validated by our phylogenetic study (Fig. 30). The cells had a short second flagellum, and Scherffel provided detailed descriptions of phagocytosis. He reported, perhaps for the first time, the occurrence of colourless cells that were rare but appeared by the accidental loss of the chloroplast (see also colourless cells above). The colourless cells did not immediately lose viability, and Scherffel stated that this was evidence of the organism's dependence on phagotrophic nutrition. There was limited detail about the cell itself, although cells had one chloroplast, apparently devoid of an eyespot. Cell sizes were not directly reported but Scherffel gave the lenses and magnifications used when preparing illustrations with an Abbe drawing apparatus. Pascher (1913) translated the cell size as 7–10 µm, with the long flagellum up to 20 µm long; Starmach (1985) gave a cell size as 2–10 µm but the '2' may be a typographical error.

*Poterioochromonas nutans* Jane was added to the genus 43 yr later; the type locality was a bog in England (Jane 1944). According to Jane (1944), the lorica was broader and shallower than the lorica of *Poterioochromonas stipitata*. The

cells of *P. nutans* were 7.0–7.6 µm in diameter, and the mouth of the lorica was 8–9 µm wide; the lorica stalk was up to 30 µm long (Jane 1944). The cells had 1–3 chloroplasts, no eyespot and one contractile vacuole.

Pringsheim (1952) described *Ochromonas malhamensis* E.G.Pringsheim based upon light microscopic observations. Spherical cells were 6–8 µm in diameter and elongate cells were up to 4 × 12 µm long. Cells had a single bilobed chloroplast and no eyespot, and Pringsheim specifically stated that they lacked phototaxis. The type locality was Malham Tarn, Yorkshire, England, and the original material was collected in July 1949. Malham Tarn is the highest lake in England, it is an alkaline marl lake and the water has a pH of about 8.5. The original description and work by Pringsheim (1952) was based on a strain now known as SAG 933-1a (= CCAP933/1a). It may be significant that Pringsheim did not observe loricas.

Péterfi (1969) reclassified the species as *Poterioochromonas malhamensis* (E.G.Pringsheim) L.Ş.Péterfi after demonstrating the presence of a lorica using light and electron microscopy. Of significance, Péterfi re-examined a number of strains (CCAP933/1a – authentic strain for *Ochromonas malhamensis*; CCAP933/3 – authentic strain for *Ochromonas sociabilis* Pringsheim; strain 933; CCAP933/1b – Provasoli strain 1; CCAP 933/1c – Provasoli strain 2; CCAP933/1d – Lewin strain named *Poterioochromonas stipitata*). Unfortunately, Péterfi did not identify the strain(s) used to make the specific figures, general observations, size measurements and so forth. For example, cells are described as having two chloroplasts – but was this true for all strains? Loricas of different sizes and shapes were observed but there was no precise documentation of these occurring within one single strain. Furthermore, Péterfi (1969) suggested that *P. malhamensis* could be a synonym of *P. stipitata* but chose to recognize *P. malhamensis* until further data were available.

Strain CCMP1862 was collected from an acid bog (pH 6.5, 180 µS conductivity, RAA pers. coll. notes). Our re-examination of strain CCMP1862 suggests this strain is similar to *Poterioochromonas nutans* with regard to chloroplast numbers and lorica shape, it is similar to *Poterioochromonas stipitata* and *Poterioochromonas malhamensis* with regard to cell size and its habitat is similar to *P. stipitata* and *P. nutans*. Because of these conflicting observations, we used *Poterioochromonas* sp. for this strain.

Our observations on CCMP2060 showed it produced loricas but strain CCMP2718 lacked loricas entirely as far as we know. It may be that *Poterioochromonas* contains taxa without loricas, or it may be that under certain unknown culture conditions, the strains fail to produce loricas. Pringsheim, by most measures, was an outstanding observer and scientist, and he failed to observe loricas (Pringsheim 1952). Therefore, it seems that a lorica may not be necessary to define the genus if one emphasizes molecular phylogenetic results. As a note, genera such as *Stokesiella* Lemmermann (1910) and *Arthroxyxis* Pascher (1942) were described for colourless chrysophytes that produce loricas. Also, Lackey's (1940) *Stylochomonas* was a colourless loricate organism but it resembles a choanoflagellate more than a chrysophyte.

Until one knows where the type species (*Poterioochromonas stipitata*) branches in a phylogenetic tree, it is difficult to assign species identities to the strains used in this study. The authentic strain of *Poterioochromonas malhamensis* (SAG-

933-1a) has not been sequenced, and there are no designated holotypes and no authentic cultures for *P. stipitata* and *Poterioochromonas nutans*. Therefore, we did not place names on any of the *Poterioochromonas* isolates in our tree (Fig. 30).

Finally, Péterfi (1969) technically failed to meet the requirements of the International Code of Nomenclature regarding new combinations because the source of the basionym was not explicitly provided (Art. 41.5; McNeill *et al.* 2012). A new combination was validated here, should three species be shown to exist:

***Poterioochromonas malhamensis* (E.G.Pringsheim)  
L.Ş.Péterfi ex R.A.Andersen, L.Graf, Y.Malakhov &  
H.S.Yoon comb. nov.**

BASIONYM: *Ochromonas malhamensis* E.G.Pringsheim. *Quarterly Journal of Microscopical Science*, 93: 95 (1952).

Overall, we have anchored the genus *Ochromonas* using a culture isolate from the type locality. We used available older names *Chrysastralla* and *Chlorochromonas* for some *Ochromonas*-like species, and we proposed a new genus *Melkoniana* for some marine flagellates. We showed that additional work is necessary before species can be assigned within *Poterioochromonas*. This paper does not fully solve the problems associated with the polyphyly of *Ochromonas sensu lato* but we believe it shows a path forward as more work is completed. To this end, we provided a thorough evaluation of older names, and we hope this information will help others.

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#### SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/17-15.1.s1>.

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