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Studies on *Peridinium aciculiferum* and *Peridinium malmogiense* (=*Scrippsiella hangoei*): comparison with *Chimonodinium lomnickii* and description of *Apocalathium gen. nov.* (Dinophyceae)

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Abstract: The fine structure of the freshwater *Peridinium aciculiferum* and the closely related Baltic Sea species currently known as *Scrippsiella hangoei* were examined in serial sections. The species name *Peridinium malmogiense* is shown to be an earlier synonym of *Scrippsiella hangoei* and is restored as the name of the species. Although both species have been included in the genus *Peridinium*, their phylogenetic positions are within the Thoracosphaeraceae, close to the specialized predators known as the pfiesteriaceans and the photosynthetic freshwater *Chimonodinium lomnickii*. The fine-structural features of the two species proved to be very similar, including the details of flagellar bases and roots, and the type of pyrenoid, which consisted of dilated areas of the chloroplast crossed by two-thylakoid lamellae and not associated with starch sheaths. Comparison with *Chimonodinium* revealed significant differences, in particular the absence of an eyespot and any trace of microtubules associated with a peduncle, which contrast with the multilayered eyespot and the distinct microtubular basket (MB) of *C. lomnickii*. The absence of a MB in *P. aciculiferum* and *P. malmogiense* is regarded as a character loss within a group of species hypothesized to be derived from a MB-containing ancestor. A phylogenetic analysis based on concatenation of nuclear-encoded small subunit rDNA, internal transcribed spacers 1 and 2 including 5.8S sequences agreed with published phylogenies based on genes of the ribosomal operon in closely grouping *P. aciculiferum*, *P. malmogiense* and two other species of peridinioids with a similar amphiesmal plate arrangement: *P. euryceps* and *P. baicalense*. The four species are regarded as members of the same genus. While one of the closest known relatives of these four species is *C. lomnickii*, the variable association of this species to several other groups of species in published phylogenies and the differences in fine-structure revealed in the present work advise against transferring the studied species to *Chimonodinium*. The new genus *Apocalathium* is described with *P. aciculiferum* as type species.

Key Words: *Apocalathium*, *Chimonodinium lomnickii*, *Peridinium aciculiferum*, *P. baicalense*, *P. euryceps*, *P. malmogiense*, phylogeny, *Scrippsiella hangoei*, ultrastructure

INTRODUCTION

Recent work on phylogenetic relationships within peridinioids has led to the revision of generic boundaries with the consequent reassignment of several species to newly described genera (Calado *et al.* 2009; Craveiro *et al.* 2009, 2011). The genus *Chimonodinium* was described to accommodate *Peridinium lomnickii* Wołoszyńska, a species much more closely related to the predatory pfiesteriaceans than to the type species of *Peridinium*, *Peridinium cinctum* (O.F.Müller) Ehrenberg (Calado *et al.* 1999, 2009; Craveiro *et al.* 2011). *Chimonodinium lomnickii* (Wołoszyńska) Craveiro, Calado, Daugbjerg, Gert Hansen & Moestrup has the plate formula po, cp, x, 4′, 3a, 7′′, 6c, 5s, 5′′′, 2′′′′. The same amphiesmal plate arrangement is found in most species currently placed in *Scrippsiella*. However, typical species of *Scrippsiella* show several starch-enveloped pyrenoids (absent in *Chimonodinium*), and the fine-structural examination of *Scrippsiella trochoidea* (F.Stein) A.R.Loeblich (a synonym of *Scrippsiella acuminata* [Ehrenberg] Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling; see Kretschmann *et al.* 2015) showed no trace of microtubular strands associated with a peduncle, in contrast with the small but distinct microtubular basket and peduncle in *C. lomnickii* (Craveiro *et al.* 2011).

Peridinium aciculiferum Lemmermann is a cold-water species with the same general plate arrangement as *Chimonodinium lomnickii* and was therefore an obvious candidate to integrate into *Chimonodinium*; however, it was not transferred to the new genus by Craveiro *et al.* (2011) because in Bayesian analysis of partial large subunit (LSU) rDNA sequences the species formed an early diverging lineage in relation to the clade formed by *C. lomnickii*, the pfiesteriaceans and *Thoracosphaera* (the species *P. aciculiferum* and *C. lomnickii* switching places in maximum likelihood analysis). In addition, preliminary fine-structural observations of a *P. aciculiferum* cell showed neither a microtubular basket nor the well-defined pusular tubes found in *C. lomnickii* (but see below). It was the objective of the present study to examine *P. aciculiferum* in greater detail to resolve its relationship to *Chimonodinium* and related genera.

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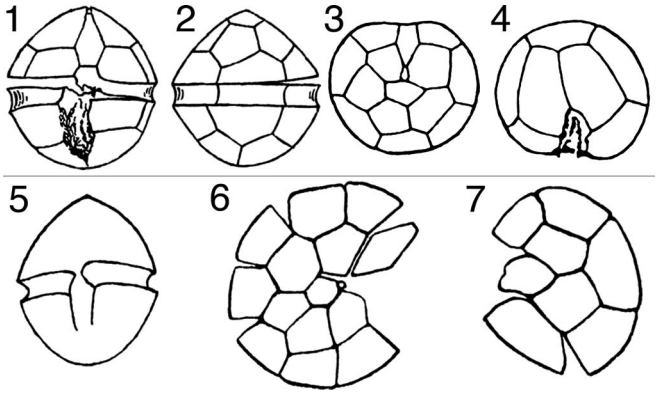
The species currently known as *Scrippsiella hangoei* (J.Schiller) J.Larsen is an important member of Baltic plankton that can grow in brackish to marine salinities up to 30‰ (Logares *et al*. 2007). It shares the same general plate arrangement with *Chimonodinium*, typical *Scrippsiella* and *Peridinium aciculiferum*. Moreover, *S. hangoei* and the freshwater *P. aciculiferum* are closely related (Gottschling *et al*. 2005), and they have identical internal transcribed spacer 1 and 2 (ITS1, ITS2), 5.8S, LSU (D1–D2) and small subunit (SSU) ribosomal DNA sequences (Logares *et al*. 2007). However, the species are morphologically distinct; *P. aciculiferum* is larger and more elongated than *S. hangoei,* and cells of *P. aciculiferum* display antapical spines and a somewhat projected apical pore region; whereas, *S. hangoei* is very round and without spines (Larsen *et al*. 1995; Logares *et al*. 2007). These two cold-water species were regarded as an example of a recent diversification from a common ancestor that spread to environments with different salinities (Logares *et al*. 2008).

The species now called *Scrippsiella hangoei* entered the literature when K.M. Levander, (Helsinki, Finland) sent fixed material from Finland to Erich Lindemann in Berlin, who illustrated and described it as *Peridinium gracile sp. nov*. (Lindemann 1924). Realizing that the name had already been used for another species by Meunier (1910), Schiller (1935a) renamed the species *Peridinium hangoei*, after the town Hangö in south-western Finland where the material originated. In the inner parts of the Baltic this is one of the most numerous species of the spring bloom (Heiskanen 1993), and Larsen *et al*. (1995), after a careful study of the amphiesmal plates, described it in detail, transferring it to *Scrippsiella*; although, it differed from typical *Scrippsiella* in producing non-calcified cysts. Cells of the Finnish material described by Lindemann (1924) were *c*. 32 μm long and *c*. 28 μm wide, and those examined by Larsen *et al*. (1995) were 27–30 μm long and 25–26 μm wide. The cysts were seen by both Heiskanen (1993) and Larsen *et al*. (1995) but apparently mixed with cysts of *Biecheleria baltica* Moestrup, K.Lindberg & Daugbjerg (Kremp *et al*. 2005, as *Woloszynskia halophila*). The cysts of *S. hangoei* were reported to be spherical to somewhat oval, 18–30 μm in the longest dimension, with a smooth wall and an orange–red accumulation body (Kremp *et al*. 2005).

A few years ago, Gertrud Cronberg, University of Lund, Sweden, directed our attention to a little-cited article in Swedish by Sjöstedt (1921) in which he described the new species *Peridinium malmogiense* from the 2 ha pond or lake Slottsparkdammen in central Malmö, in southern Sweden. The species was present in high numbers, colouring the water brown. The number of cells reached a maximum of 8.3 million per litre on 11 March 1921, the number decreasing in April and the species disappearing in May. The lake had been cleared from vegetation the previous winter and had nearly dried out. Brackish water was then let in from a nearby brackish-water canal, making the lake water slightly brackish (5.5 psu). Sjöstedt gave cell length of his new species as 28–32 μm, width 24–28 μm. In early April resting spores formed. They were drawn by Sjöstedt as slightly ovoid, with a thick, somewhat slimy membrane, and many cysts were observed together in the lake, kept together by the mucilage.



Figs 1–7. *Peridinium gracile* (=*Peridinium hangoei*) and *Peridinium malmogiense,* original illustrations.
Figs 1–4. *Peridinium gracile* Er.Lindemann, *nom. illeg*. (*Peridinium hangoei* J. Schiller). Reproduced from Lindemann (1924, pl. I, figs 3–6), slightly reduced. Ventral, dorsal, apical and antapical views, respectively.
Figs 5–7. *Peridinium malmogiense* G.Sjöstedt. Reproduced from Sjöstedt (1921, figs 1–3), slightly enlarged to facilitate comparison with *P. hangoei*. Ventral outline, and plates on the epi- and hypocone, respectively.

The original drawings of Lindemann's *Peridinium gracile* (*Scrippsiella hangoei*) are reproduced here as Figs 1–4, and those of Sjöstedt's *Peridinium malmogiense* as Figs 5–7. Lindemann was apparently not aware of Sjöstedt's article, as there is little doubt that the two taxa are identical. They agree in both morphology and ecology. The same conclusion was reached some years ago by Finnish colleagues (Guy Hällfors, personal communication) but it was not formally published. Nevertheless, the combination '*Scrippsiella malmogiense*' has within the last 10 years appeared occasionally in publications (e.g. Tomczak *et al*. 2009; Klais *et al*. 2013), sometimes clearly taken as a synonym of *Scrippsiella hangoei* (Olli & Trunov 2010). As mentioned below, *P. malmogiense* does not belong in *Scrippsiella*, and there is therefore no reason to formally make the transfer. The name *Peridinium malmogiense* will be used throughout the text below.

MATERIAL AND METHODS

Cultures of *Peridinium aciculiferum* and *Peridinium malmogiense* used for transmission electron microscopy were both obtained from the Scandinavian Culture Collection of Algae & Protozoa. *Peridinium aciculiferum* (strain SCCAP K-0998, isolated from freshwater Lake Tovel, Trentino Province, northern Italy) was growing at 4°C in medium DY-V and *P. malmogiense* (SCCAP K-0979, isolated from brackish water in Tvärminne, Finland, as *Scrippsiella hangoei*), was growing in medium TL5 at 4°C.

Live cells of *Peridinium aciculiferum* (strain SCCAP K-0998) and *Peridinium malmogiense* (strain SCCAP K-0979) were examined using a Zeiss Axio Imager.M2 light microscope with differential interference contrast optics and epifluorescence microscopy (Carl Zeiss, Göttingen, Germany). Micrographs were taken using Zeiss Axiocam digital cameras (models MRm and HRc). To view the arrangement of thecal plates we stained live material with

calcofluor white and used filter set 49 from Zeiss (excitation 365 nm, emission 445 nm).

Swimming cells of *Peridinium aciculiferum* were picked up and fixed according to two slightly different schedules. The first protocol (schedule 1) consisted of the following: cells were fixed in a mixture of 1% glutaraldehyde and 0.5% osmium tetroxide (final concentrations) in 0.1 M phosphate buffer, pH 7.2, at 4°C, for 40 min, washed twice in the same buffer, and embedded in 1.5% agar blocks. Post-fixation was made overnight, at 4°C, in 0.5% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2. After being washed with the same buffer and distilled water, the agar blocks were dehydrated through a graded ethanol series and propylene oxide and finally embedded in Spurr's resin. Blocks were polymerized at 75°C for *c*. 11 h. In schedule 2 cells were fixed in a mixture of 1% glutaraldehyde and 0.5% osmium tetroxide (final concentrations) in 0.2 M phosphate buffer, pH 7.4, at 4°C, for 55 min. Washing and inclusion in agar blocks was similar to schedule 1. Post-fixation was in 1% osmium tetroxide in 0.2 M phosphate buffer, pH 7.4 for 1h 45 min. The agar blocks were washed, dehydrated and embedded as in schedule 1. Blocks were polymerized at 70°C for *c*. 24 h.

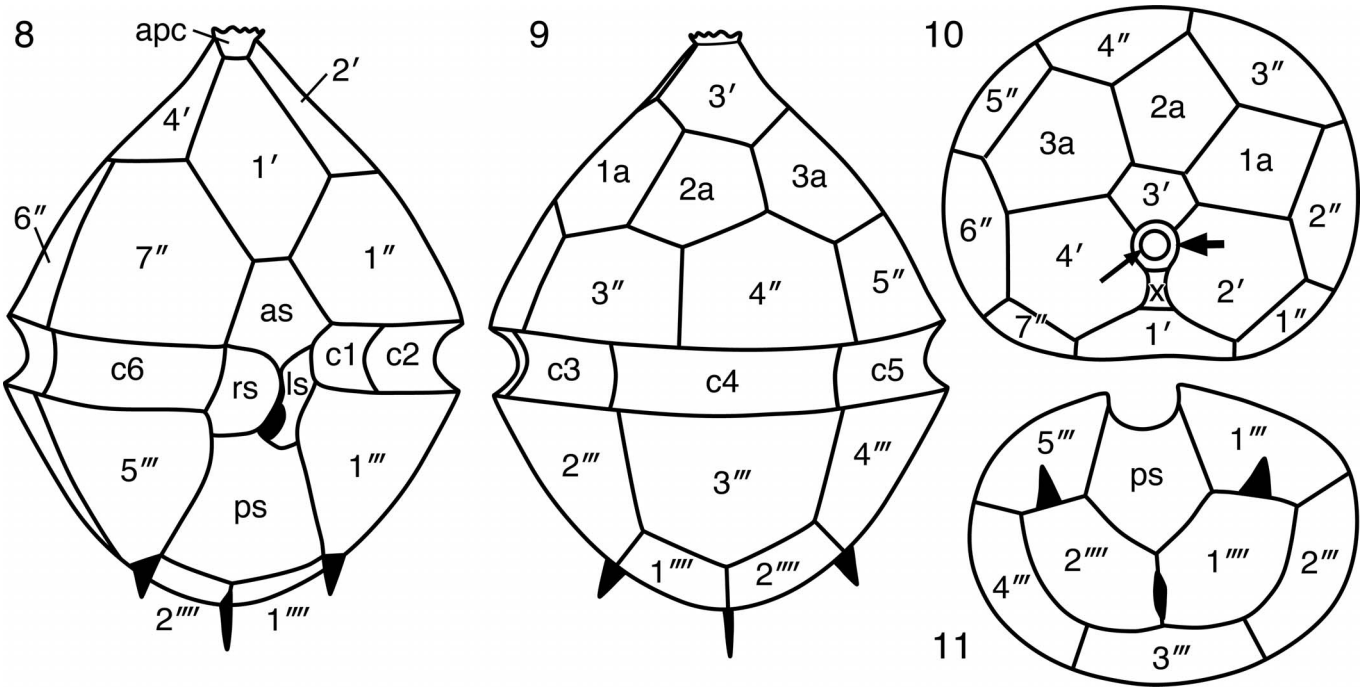
Fixation of *Peridinium malmogiense* was also prepared with swimming cells picked up from the culture and fixed with two different schedules; either (1) cells were fixed in 2% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4, at 4°C, for 35 min or (2) cells were fixed in a mixture of 1% glutaraldehyde and 0.5% osmium tetroxide (final concentrations) in 0.2 M phosphate buffer, pH 7.4, at 4°C, for 30 min. After the first fixative both groups of cells continued the same path: cells were washed with the same buffer, included in agar blocks and fixed overnight at 4°C in 0.5% osmium tetroxide in 0.2 M phosphate buffer, pH 7.4. The procedure for both fixations was then similar to procedure 1 for *Peridinium aciculiferum*. Polymerization of the resin blocks was at 70°C for 48 h.

Four cells of *Peridinium aciculiferum* and three of *Peridinium malmogiense* were serial-sectioned with a diamond knife on an EM UC6 ultramicrotome (Leica Microsystems, Wetzlar, Germany). Ribbons of sections, 70 nm thick, were picked up with slot grids, placed on Formvar film and allowed to dry. The grids were individually stained for 12 min in uranyl acetate followed by 7 min in lead citrate. The sections were observed with a JEM 1010 electron microscope (JEOL Ltd., Tokyo, Japan) and photographed with a Gatan Orius digital camera (Gatan, Inc., Pleasanton, California USA) at the Department of Biology, University of Copenhagen.

Total genomic DNA of *Chimonodinium lomnickii* (SCCAP K-1151) extracted by Craveiro *et al*. (2011) was used here to determine the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rDNA gene located in between. For this we used two primers: ITS1 and ITS4 (White *et al*. 1990). Polymerase chain reaction (PCR) amplification used the 5X HOT FIREPol Blend Master Mix following the recommendations of the manufacturer (Solis BioDyne, Tartu, Estonia). The PCR profile included the following steps: 12 min at 95°C for one cycle; 15 s at 95°C, 40 s at 55°C and 30 s at 72°C for 35 cycles and 5 min at 72°C for one final cycle. The expected DNA fragment length was confirmed by electrophoresis using a 1.5% agarose gel stained with GelRed and a molecular size marker (100 bp RAINBOW eXtended DNA ladder, BIORON GmbH, Ludwigshafen, Germany). PCR fragments were viewed using a gel documentation XR + System from BioRad (Hercules, California USA). PCR amplified ITS fragments were purified using the NucleoFast 96 PCR kit from Macherey-Nagel (GmbH & Co. KG, Düren, Germany). The purification procedure followed the manufacturer's recommendations. Some 60 ng of PCR products were sent to the sequencing service provided by Macrogen (Amsterdam, Holland). The internal transcribed spacers and 5.8S rDNA were determined in both directions using the amplification primers ITS1 and ITS4.

To infer the phylogeny of *Peridinium aciculiferum*, *Chimonodinium lomnickii* and closely related species in greater detail we established a concatenated data matrix comprising SSU rDNA, ITS1 and ITS2 (including 5.8S). All sequences are available in Genbank and accession numbers are provided in Fig. 54 and Table S1. The data matrix, which contained 15 genera and 22 species of dinoflagellates, was edited with JALVIEW (Waterhouse *et al*. 2009) and aligned with Mafft (default settings), which is incorporated in the sequence editor. The final alignment consisted of 2657 base pairs including introduced gaps. We used *Tintinnophagus acutus* as the outgroup because a study by Craveiro *et al*. (2013) showed that this species formed a sister taxon to a clade that included some of the dinoflagellate species of particular interest in this study (i.e. *Peridinium aciculiferum* and *Peridinium malmogiense*).

The concatenated data matrix was applied as input for Bayesian analysis (BA) using Mr Bayes (v 3.2.2 x64) by Ronquist & Huelsenbeck (2003) and maximum likelihood (ML) using PhyML v 3.0 by Guindon *et al*. (2010). Attempting to provide a more accurate model of sequence evolution, the genetic markers were divided into four data partitions (SSU rDNA, ITS1, 5.8S rDNA and ITS2). This approach allowed each of the regions to evolve under different models of evolution by using the 'unlink' option in Mr Bayes. Two independent Markov Chain Monte Carlo analyses each comprising one cold and three heated chains were run for 10 million generations on a local computer. Parameter values and trees were sampled and saved every 1000th generation. Using a spreadsheet we plotted the log likelihood values as a function of generations. The ln L values converged at *c*. –11,940 after 501,000 generations (conservative estimate). This left 9500 trees, and to produce a 50% majority rule consensus tree these were imported into PAUP* (Swofford 2002). Posterior probability (pp) values obtained were plotted onto the tree topology (Fig. 54). For ML analysis we applied the parameter settings obtained from jModelTest (v 2.1.7) by Darriba *et al*. (2012). Among 88 models examined jModelTest chose general time-reversible with proportion of invariable sites and gamma distribution (GTR+I+G) as the best-fit model for the ITS1-5.8S rDNA-ITS2 data matrix with gamma shape = 0.295 and proportion of invariable sites = 0.381. PhyML was run on the South of France bioinformatics platform, and the robustness of the tree topologies was evaluated using bootstrapping with 1000 replications. Bootstrap values were added to the tree topology obtained by BA.



Figs 8–11. *Peridinium aciculiferum,* diagrammatic view of morphology and plate arrangement. Plate numbering follows Kofoidian notation. Modified from Wołoszyńska (1916, pl. 12, figs 11, 12, 13, 15). The three flat posterior spines are represented in black.
Fig. 8. Ventral view. apc, apical pore complex; as, anterior sulcal; ls, left sulcal; ps, posterior sulcal; rs, right sulcal plate.
Fig. 9. Dorsal view.
Fig. 10. Apical view. Thick arrow, apical pore plate; thin arrow, cover plate; x, canal plate.
Fig. 11. Antapical view.

RESULTS

General morphology

The general features of the amphiesma of *Peridinium aciculiferum* are shown in Figs 8–11, a schematic representation modified from Wołoszyńska (1916) to include all known amphiesmal plates. Several aspects of live cells of *P. aciculiferum* SCCAP K-0998 are shown in Figs 12–18; Figs 19–25 show live cells of *Peridinium malmogiense* SCCAP K-0979, for comparison. Cells of both strains had the nucleus in a central position and most chloroplast lobes near the periphery (Figs 13, 20). However, the amount of oil was visibly larger in *P. aciculiferum* (Figs 12, 13, 15) than in *P. malmogiense* (Figs 19–22). Perhaps related the chloroplast network in *P. malmogiense* was more extensive, with chloroplast lobes more tightly packed near the cell surface (Figs 22–24) than in *P. aciculiferum* (Figs 15, 16).

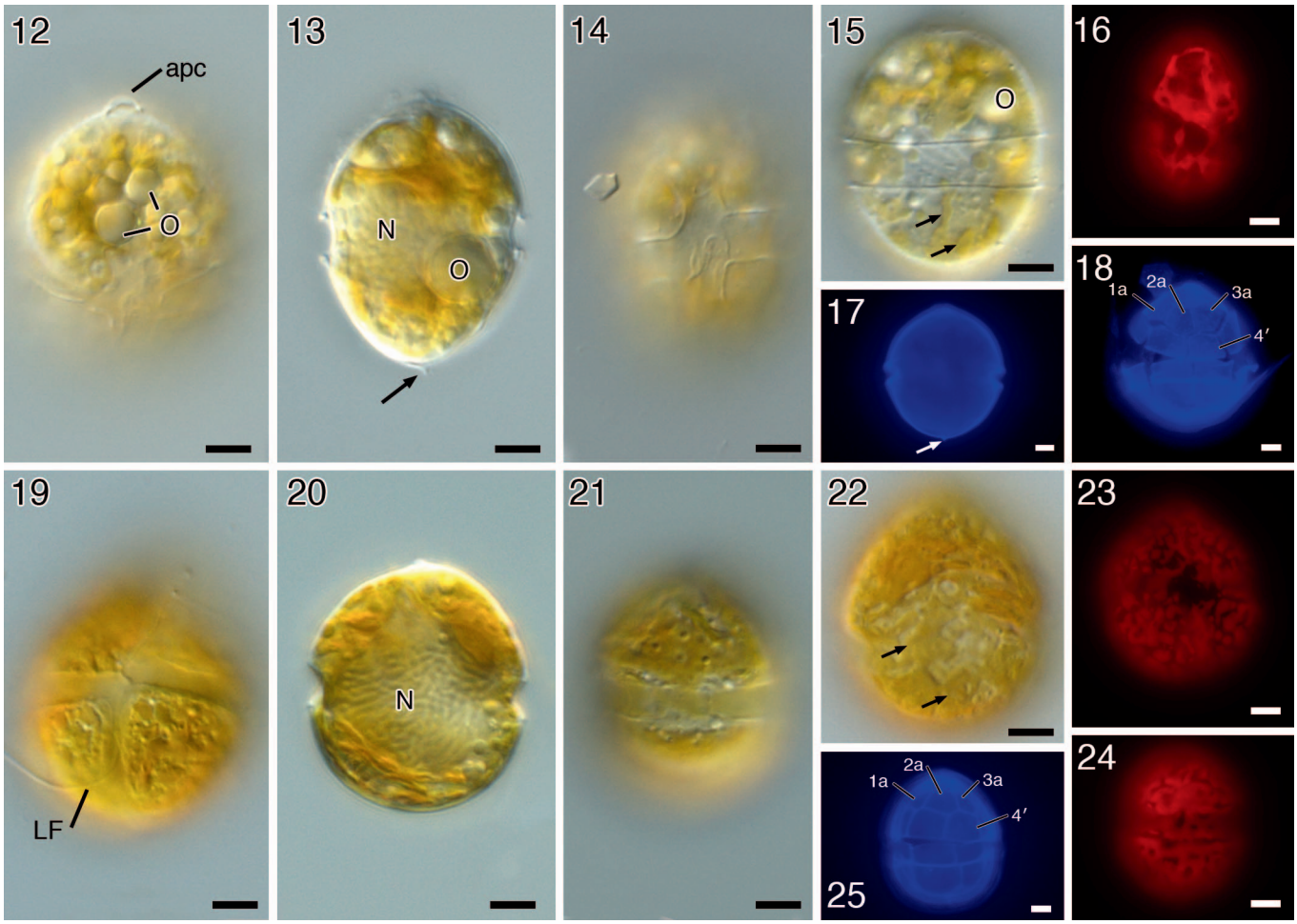
The characteristic flat hypothecal spines of *Peridinium aciculiferum* were present in most cells but were in general quite reduced (lowermost antapical spine marked in Figs 13 and 17), as commonly happens in cultures maintained over an extended period (e.g. Craveiro *et al.* 2009). Specimens with spines of regular length from the field populations where our cultured strain originated were well documented by Hansen & Flaim (2007, figs 17A–C). The three intercalary plates were clearly visible in both species with the aid of calcofluor staining and, in both, plate 1a was placed on the left-dorsal side, separated from the dorsal, precingular plate 4, which contacted with 2a and 3a (Figs 18, 25).

General ultrastructure of *Peridinium aciculiferum*

Main ultrastructural features of *Peridinium aciculiferum* are shown in Figs 26–30. The nucleus (N) was in the dorsal side at the cingulum level (Fig. 26). Oil droplets (O) filled up a large portion of the cytoplasm. Starch granules (S) and trichocysts (T) were scattered in the cell (Figs 26, 28). Chloroplast lobes were located mainly in the peripheral cytoplasm with some extending to the central region (Fig. 26). Chloroplasts had three thylakoids per lamella (not shown) except in the pyrenoid matrix, which was penetrated by two-thylakoid lamellae (Fig. 27). One or two small pyrenoids (P) were seen per cell (Fig. 26). No starch sheaths surrounded the pyrenoids. The pusular system (pu) was not ramified and consisted of at least two pusular tubes (one connected to each flagellar canal) that extended from the ventral area to the centre of the cell (Fig. 26). The width of the pusular tubes was *c.* 250 nm in cells fixed according to protocol 2, while in cells fixed according to schedule 1 the pusular tubes were somewhat collapsed and consequently thinner (Fig. 28). The pusular structure of the tubes is clearly visible in Fig. 30, with the enveloping vesicle surrounding the tube and two points of contact between the outer and inner membrane of the surrounding vesicle (arrows). No eyespot and no microtubular basket or microtubular strand that could be related to a peduncle or feeding structure was observed in any of the cells sectioned.

Flagellar apparatus of *Peridinium aciculiferum*

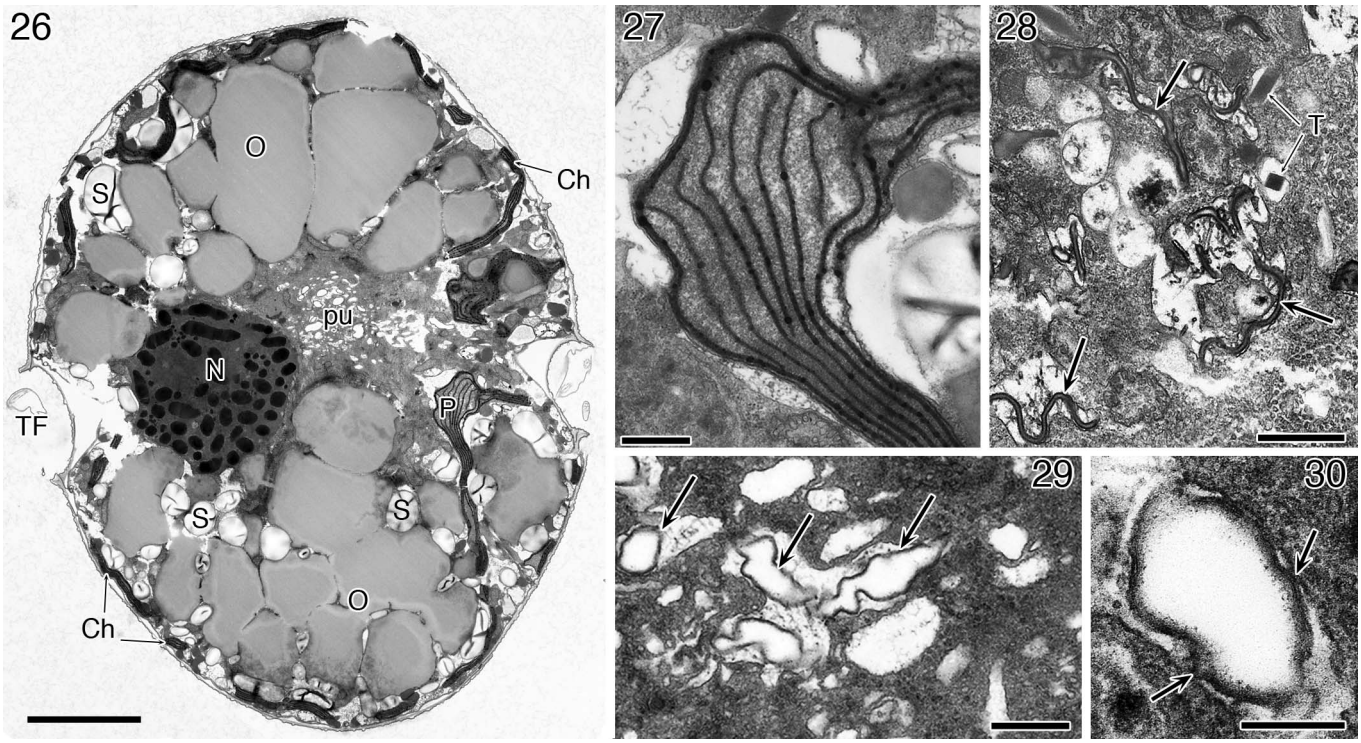
The main components of the flagellar apparatus are shown in serial, longitudinal sections progressing from left to right of



Figs 12–25. *Peridinium aciculiferum* strain SCCAP K-0998 and *Peridinium malmogiense* strain SCCAP K-0979, LM. All scale bars=4 μm.
Figs 12–18. *Peridinium aciculiferum*.
Fig. 12. Ventral–apical view with slightly projecting apical pore complex (apc).
Fig. 13. Optical section with the nucleus (N) at cingulum level, oil droplets (O) and the central, antapical spine (arrow).
Fig. 14. Ventral focus showing slightly displaced cingulum ends and the anterior part of the sulcus invading the epicone.
Fig. 15. Dorsal view. The cell was slightly squashed, forcing the cingulum to flatten out. Note chloroplast lobes (arrows) and abundant oil droplets (O) near the surface.
Fig. 16. Epifluorescence microscopy showing the somewhat loose chloroplast network.
Fig. 17. Theca stained with calcofluor white, optical section. The arrow marks the central antapical spine.
Fig. 18. Theca stained with calcofluor white, dorsal view. Note the position of the three intercalary plates (1a, 2a, 3a).
Figs 19–25. *Peridinium malmogiense*.
Fig. 19. Ventral view showing the slight displacement of the cingulum ends and the relatively narrow sulcus. LF, longitudinal flagellum.
Fig. 20. Optical section with central nucleus (N) and chloroplasts in the periphery.
Fig. 21. Dorsal view.
Fig. 22. Surface focus showing clearly interconnected, relatively large chloroplast lobes.
Figs 23–24. Epifluorescence microscopy. Two views of the relatively dense peripheral chloroplast network.
Fig. 25. Theca stained with calcofluor white, dorsal view. The three intercalary plates are marked (1a, 2a and 3a).

one cell (Figs 31–35) and in nearly transverse sections of another cell progressing from apex to antapex (Figs 36, 37). The angle made by the basal bodies was *c*. 90° as estimated in three-dimensional reconstructions from series of sections. The transverse basal body (TB) was in a dorsal position in relation to the longitudinal basal body (LB) and *c*. 170 nm distant from it. Both flagella emerged from their respective flagellar canals through openings bordered by complete striated collars (longitudinal striated collar, LSC, and transverse striated collar, TSC), which were connected to each other by a small fibrous extension (not shown).

Each basal body was associated with two roots. The TB connected on its anterior surface with a single microtubule (transverse microtubular root, TMR; r3 in Moestrup 2000) that extended upward along the ventral-anterior side of the transverse flagellar canal (TFC) and nucleated *c*. 30 microtubules (transverse microtubular root extension; TMRE). The TMRE curved around the TFC toward the dorsal side (Figs 31–35). Some electron-opaque vesicles were detected between the dorsal edge of the TFC and the TMRE (arrows in Figs 35, 36). A striated fibre with an embedded microtubule associated with the basal portion of the TB (transverse striated root and



Figs 26–30. *Peridinium aciculiferum,* general ultrastructural features, transmission electron microscopy (TEM).
Fig. 26. General view of a longitudinal section of a cell seen from the right side, showing the nucleus (N) in the dorsal side, the chloroplast lobes (Ch) and the distribution of large oil droplets (O) and some starch grains (S). A pyrenoid (P) is also visible. Pusular tubes (pu) are seen in the central part of the cell. The transverse flagellum is located in the sulcus. Scale bar = 5 μm.
Fig. 27. Pyrenoid transversed by thylakoid lamellae. Scale bar = 500 nm.
Fig. 28. Collapsed pusular tubes (arrows) and trichocysts (T). Scale bar = 2 μm.
Fig. 29. Pusular tubes (arrows). Scale bar = 500 nm.
Fig. 30. Higher magnification of a pusular tube and the enveloping vesicle with contact regions between membranes marked with arrows. Scale bar = 200 nm.

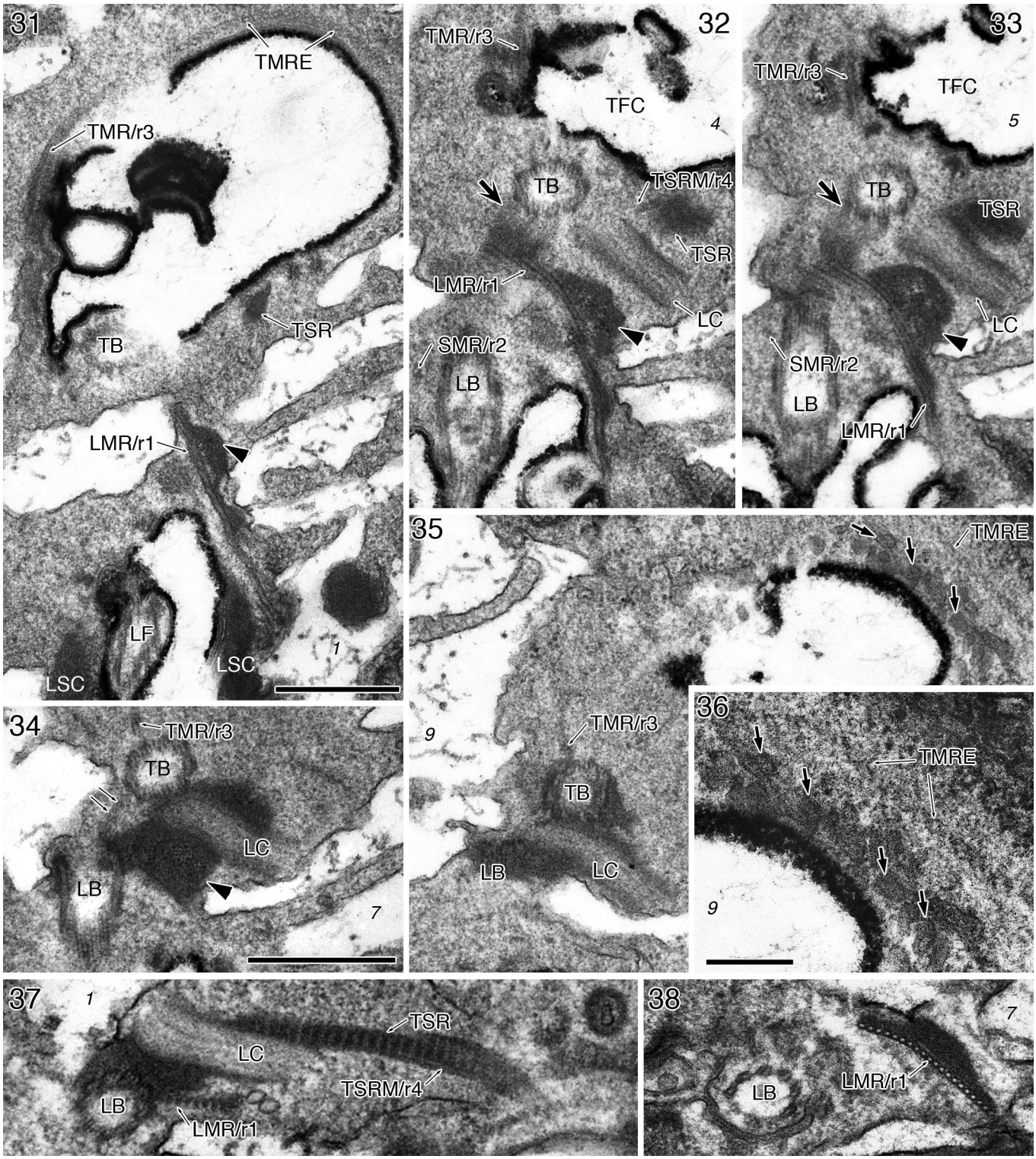
associated microtubule; TSR and TSRM, designated root 4 in Moestrup 2000). The TSRM/r4 connected with both the posterior surface of the TB and the anterior face of the layered connective (LC, see below). Both TSRM/r4 and TSR extended for about 1.5 μm along the posterior side of the TFC (Figs 31–34, 37).

The LB associated, on the left side of its proximal end, with a row of seven microtubules (longitudinal microtubular root, LMR; r1 in Moestrup 2000). The LMR/r1 bent slightly toward the dorsal side and extended to the antapex of the cell, with the number of microtubules gradually increasing to about 20 (Figs 31–33, 37, 38). A second root associated with the right-hand side of the LB consisted of a single microtubule (single-stranded microtubular root, SMR; r2 in Moestrup 2000), which described a rather short longitudinal arc approximately parallel to LMR/r1 (Figs 32, 33).

Further structures connected the basal bodies to the roots and to each other. Near the proximal end of the LMR/r1, on the dorsal side, a layer of electron-opaque material some 120 nm thick connected to three thin fibres nearly 130 nm long that were linked to three contiguous triplets of the TB (arrows in Figs 32, 33). Posterior to this layer, and separated from it by a narrow gap, a more extensive layer of electron-opaque material covered the LMR/r1 on its dorsal side (arrowhead, Figs 31–34). This material contacted the posterior face of the LC (Figs 32–35, 37). The LC was about 600 nm long, 400 nm wide and 300 nm thick. The layered aspect was due to the presence of electron-opaque layers separated by electron-transparent ones that are only clearly distinguished in perfect cross-section (not shown). A single striated thin fibre *c*. 250 nm long attached one triplet of the proximal part of the TB to the base of the LB (double thin arrows in Fig. 34).

General ultrastructure of *Peridinium malmogiense*

The main ultrastructural features of *Peridinium malmogiense* are presented in Figs 39–43. In a longitudinal section of a cell seen from the right side, the nucleus (N) was located dorsally at the cingulum level (Fig. 39). Chloroplast lobes (Ch) were placed near the cell surface, and some extended internally to the centre of the cell (Fig. 39). Up to five pyrenoids (P) were present in these more internal chloroplast lobes (Fig. 39). They consisted of an enlargement of the matrix area (Figs 42, 43). The chloroplast lobes had three thylakoids per lamella in all their extensions, except in the pyrenoids where they were in groups of two (Figs 42, 43). There were no starch sheaths surrounding the pyrenoids. Starch globules (S) and oil droplets (O) were dispersed in the cell but oil predominated in the epicone and starch predominated in the hypocone (Fig. 39). At least one elongated pusular tube connected to each flagellar canal and extended along the ventral and central

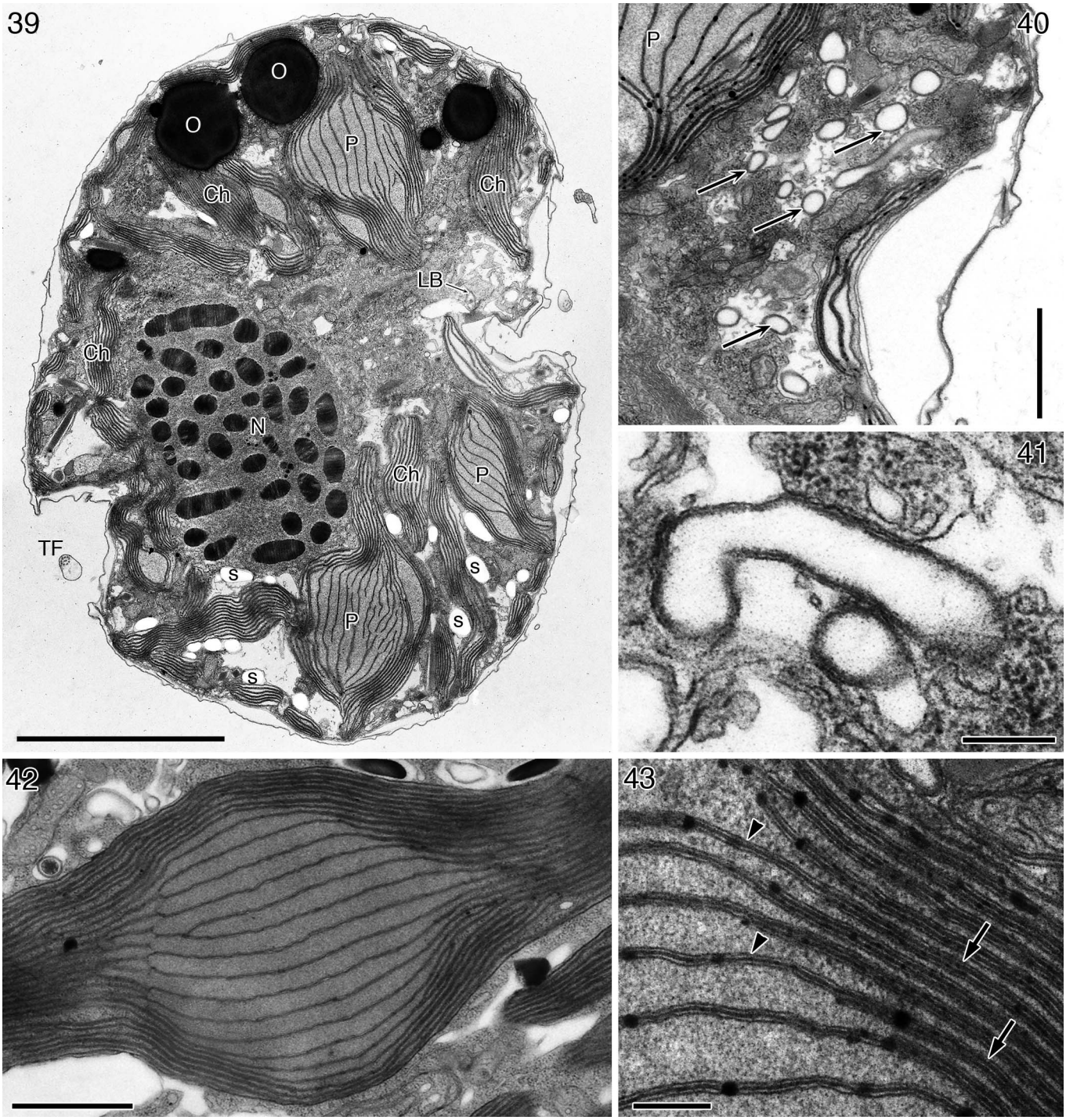


Figs 31–38. *Peridinium aciculiferum,* flagellar apparatus, TEM. Slanted numbers represent section numbers.

Fig. 31–35. Non-adjacent serial sections proceeding from the left side of the cell. The transverse microtubular root extension (TMRE) is visible around the upper side of the transverse flagellar canal (TFC). The transverse microtubular root (TMR/r3) converges to the transverse basal body (TB). A connection between three triplets of the TB and the longitudinal microtubular root (LMR/r1) is marked with an arrow. A layer of electron-opaque material covers the proximal-dorsal side of the LMR (arrowhead). Both basal bodies are linked through a thin fibre (two thin arrows in Fig. 34). The longitudinal basal body is disappearing in Fig. 35, and the TMRE and vesicles with electron-opaque content are seen close to the TFC (arrows). LB, longitudinal basal body; LC, layered connective; LF, longitudinal flagellum; LSC, longitudinal striated collar; TSR, transverse striated root; TSRM/r4, transverse striated root microtubule. Fig. 31, scale bar = 500 nm. Figs 32–35, all to the same scale as Fig. 34; scale bar = 500 nm.

Fig. 36. Higher magnification of Fig. 35. Scale bar = 200 nm.

Figs 37–38. Non-adjacent serial sections of another cell, apical view. Detail of LB and its connection with the LMR/r1 and LC. The striated aspect of the TSR is visible as well as the TSRM/r4 present along it. Same scale as Fig. 34.



Figs 39–43. *Peridinium malmogiense,* general ultrastructural features, TEM.

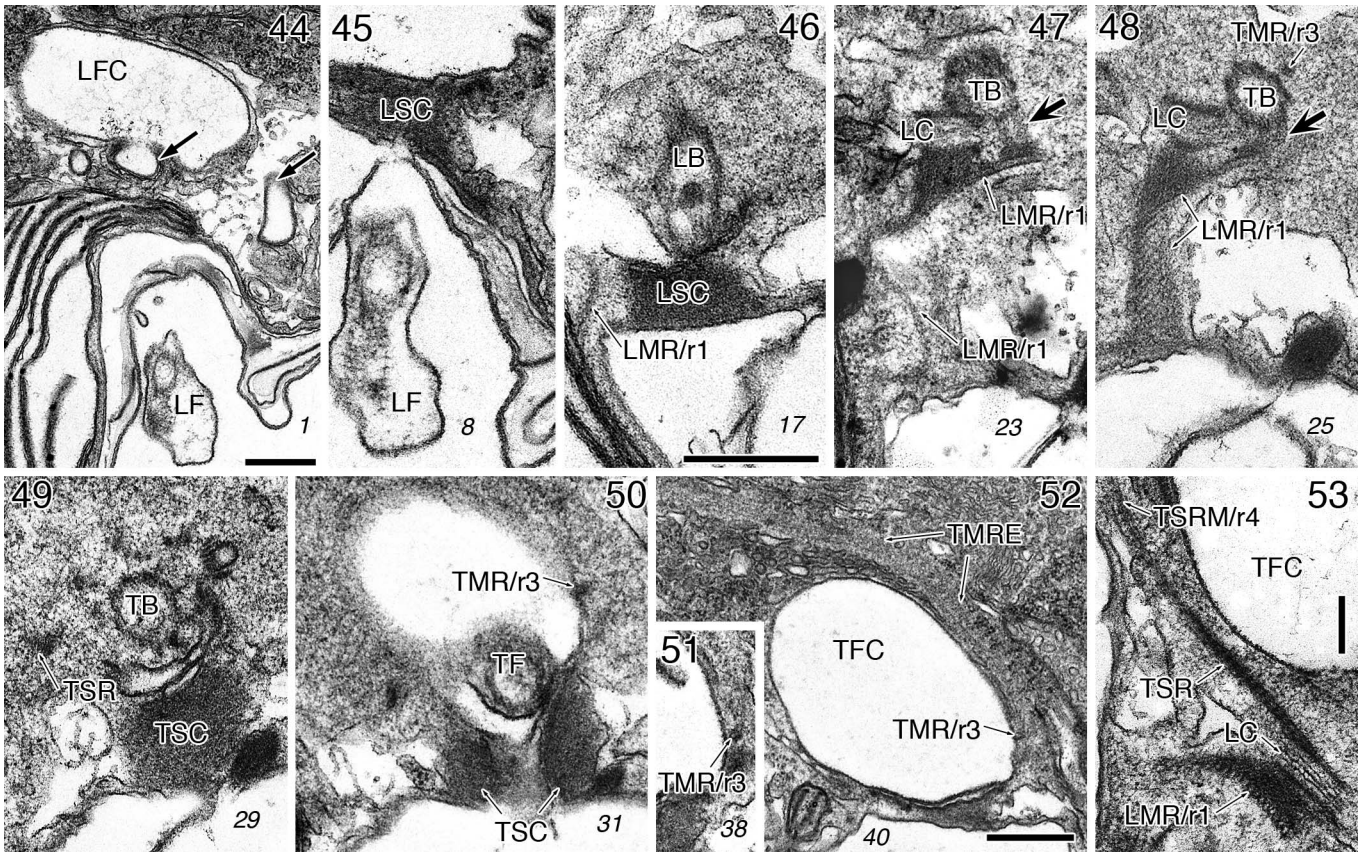
Fig. 39. General view of a longitudinal section of a cell seen from the right side, showing the nucleus (N) in the dorsal side, the chloroplast lobes (Ch) and the pyrenoids (P). Oil droplets (O) appear in the epicone and some starch grains (s) in the hypocone. LB, longitudinal basal body. Scale bar = 5 μm.

Fig. 40. Ventral area with pusular tubes (arrows) and pyrenoid (P). Scale bar = 1 μm.

Fig. 41. Higher magnification of a pusular tube and the enveloping vesicle. Scale bar = 200 nm.

Fig. 42. Pyrenoid traversed by thylakoid lamellae. Scale bar = 1 μm.

Fig. 43. Higher magnification of pyrenoid matrix traversed by two-thylakoid lamellae (arrowheads). The same lamellae have three thylakoids in the more external portion of the chloroplast (arrows). Scale bar = 200 nm.



Figs 44–53. *Peridinium malmogiense,* flagellar apparatus, TEM. Slanted numbers represent section numbers.

Figs 44–52. Non-adjacent serial sections proceeding from the right side of the cell. A pusular tube (arrow in Fig. 44) connects to the longitudinal flagellar canal (LFC). Three (?) triplets of the transverse basal body (TB) connect to the longitudinal microtubular root (LMR/r1). The transverse microtubular root (TMR/r3) and the extension (TMRE) are also visible near the transverse flagellar canal (TFC). LB, longitudinal basal body; LC, layered connective; LF, longitudinal flagellum; LSC, longitudinal striated collar; TF, transverse flagellum; TSC, transverse striated collar; TSR, transverse striated collar. Fig. 44, scale bar = 500 nm. Figs 45–50, same scale bar as Fig. 46, scale bar = 500 nm. Fig. 51, same scale bar as Fig. 52, scale bar = 500 nm.

Fig. 53. Dorsal view of a section of a different cell, showing the longitudinal microtubular root (LMR/r1) contacting the layered connective (LC) that connects on its apical face, to the transverse striated root (TSR). TSRM/r4, transverse striated root microtubule. Scale bar = 200 nm.

areas of the cell (Figs 39–41, 44). Pusular tube width varied between 130 and 230 nm. Eyespots were not observed, nor were microtubular baskets or peduncle-associated microtubular strands.

Flagellar apparatus of *Peridinium malmogiense*

The main components of the flagellar apparatus are seen in serial sections of one cell sectioned from right to left (Figs 44–52) and in details of another cell seen from the dorsal side (Fig. 53). The angle made by both basal bodies was *c*. 90°. Both flagellar canals, LFC and TFC, had complete striated collars (LSC and TSC) surrounding the exit points of the flagella (Figs 45, 46, 49, 50). As in *Peridinium aciculiferum*, *Peridinium malmogiense* also showed four roots associated to the basal bodies. The LB associated on its left-anterior side with six microtubules of the longitudinal microtubular root (LMR/r1). The number of microtubules increased gradually along the root until *c*. 20 (Fig. 53). The second root associated with the LB, the single-stranded microtubular root (SMR/r2), was very short (not shown). The transverse microtubular root (TMR/r3) started near the anterior-proximal end of the TB and extended upwards around the TFC (Figs 50–52), nucleating a strand of microtubules around the anterior surface of the TFC (Fig. 52). The fourth root, the transverse striated root and associated microtubule (TSR and TSRM/r4), extended ventrally for about 1.4 μm (Fig. 53). The proximal part of the TSR attached to the anterior side of the LC (Fig. 53). The LC was located between the posterior face of the TB and a layer of electron-opaque material that covered the anterior-dorsal side of the LMR/r1 (Figs 47, 48). The LC, as estimated from serial sections, was *c*. 450 nm long, 300 nm wide and 150–180 nm thick.

A connection was seen between the TB and the LMR/r1: two thin fibres, some 95 nm long, extended from two triplets of the TB to a narrow 55 nm layer of electron-opaque material on the anterior-dorsal end of the LMR/r1 (Figs 47, 48, arrow). A third fibre was observed in some cells, connecting directly one triplet of the proximal end of the TB to the base of the LB (not shown).

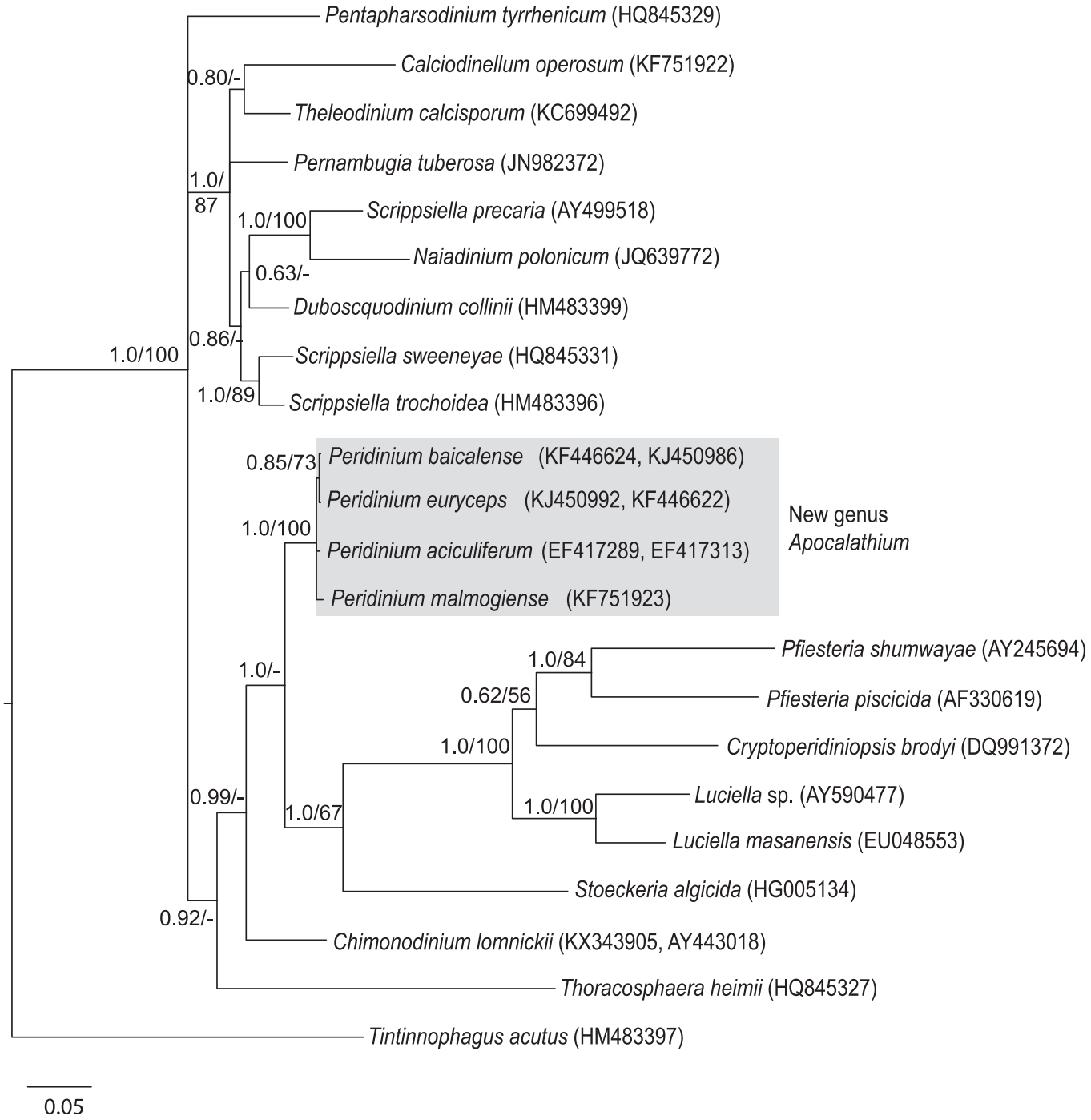


Fig. 54. Bayesian analysis of four species of *Apocalathium* and 15 related dinoflagellate genera based on nuclear-encoded ITS1-5.8S rDNA-ITS2 sequences. The data matrix comprised 824 base pairs including introduced gaps. *Peridinium cinctum* formed the outgroup taxon. Posterior probabilities ≥ 0.5 from Bayesian analyses and bootstrap values $\geq 50\%$ from maximum likelihood analyses are given at internodes. Branch lengths are proportional to the number of changes per site.

Phylogeny

The inferred phylogeny from Bayesian analysis, and based on concatenation of nuclear-encoded SSU rDNA and internal transcribed spacers and the 5.8S rDNA gene, is illustrated in Fig. 54. Both analyses (i.e. BA and ML) favoured a monophyletic origin of the four species *Peridinium aciculiferum*, *P. malmogiense*, *P. baicalense* and *P. euryceps*, and the monophyly received high statistical support (posterior probability = 1.0 and bootstrap = 100%). This was not surprising as the sequences of these four species included here were nearly or completely identical depending on the species pairs being compared. For example those of *P. baicalense* and *P. euryceps* were 100% identical (1403 base pairs compared) and those of *P. aciculiferum* and *P. malmogiense* were 99.4% identical (1783 base pairs compared). The difference between these two

species pairs was only 0.2–0.7%. In total, 1293 base pairs were included in all pairwise comparisons. The sequence divergence estimates based on these genetic markers indicated that the four species are very closely related. The pfiesteriaceans (*Stoeckeria*, *Pfiesteria*, *Cryptoperidiniopsis* and *Luciella*) formed a sister group to the four species, and this relationship received high support in BA (pp = 1.0) but not in the ML analysis. The freshwater dinoflagellate *Chimonodinium lomnickii* formed a sister taxon to a clade comprising *P. aciculiferum*, *P. malmogiense*, *P. baicalense*, *P. euryceps* and the pfiesteriaceans. This internode also received fairly high support in BA (pp = 0.90) but not in ML. *Thoracosphaera heimii* formed the earliest diverging taxon (pp = 0.92) in this part of the phylogenetic tree. In general, ML bootstrap analysis did not provide support (< 50%) for any of the deep branches in the tree (not shown). We note that the genus *Scrippsiella* appeared polyphyletic and thus is in need of taxonomic revision.

DISCUSSION

Comparison with *Chimonodinium* and other peridinioids

The present comparison between the fine structure of *Peridinium aciculiferum* and *Peridinium malmogiense* (=*Scrippsiella hangoei*), on one side, and *Chimonodinium lomnickii* on the other is justified by their well-established phylogenetic relatedness (Craveiro *et al.* 2011; Annenkova *et al.* 2015) and takes advantage of the available detailed description of the cell organization of *C. lomnickii* (Craveiro *et al.* 2011). Both *P. aciculiferum* and *P. malmogiense* showed typical dinoflagellate features, including a large nucleus with condensed chromosomes, a pusular system and trichocysts.

Chloroplast arrangements of peridinin-containing peridinioid dinoflagellates vary from a peripheral network of chloroplast lobes without conspicuous pyrenoids in typical *Peridinium*, to radial lobes extending from a central pyrenoid, as in *Palatinus* Craveiro, Calado, Daugbjerg & Moestrup (Calado *et al.* 1999; Craveiro *et al.* 2009). The presence of several starch-enveloped pyrenoids projecting from peripheral chloroplast lobes is common in peridinioids that produce calcareous cysts, as in typical *Scrippsiella* and in *Theleodinium calcisporum* Craveiro, Pandeirada, Daugbjerg, Moestrup & Calado (Balech 1959; Craveiro *et al.* 2011, 2013) and has also been demonstrated in the phylogenetically related *Naiadinium polonicum* (Craveiro *et al.* 2015). The pyrenoids of *Peridinium aciculiferum* and *Peridinium malmogiense* are essentially similar in the sense of being crossed by regularly spaced lamellae of two thylakoids and occupying inflated areas of the chloroplast; both are surrounded externally by at least one regular lamella of three thylakoids, and perhaps this somewhat internal position relates to the absence of starch sheaths. *Chimonodinium lomnickii* showed only some thylakoid-free areas surrounded by thylakoid lamellae in regular-looking chloroplast lobes (Craveiro *et al.* 2011).

An eyespot of type A *sensu* Moestrup & Daugbjerg (2007), i.e. one or more layers of lipid globules underneath the surface of a ventrally located chloroplast lobe, is common in peridinioids and was demonstrated in *Scrippsiella trochoidea*, *Naiadinium polonicum* and *Chimonodinium lomnickii* (Craveiro *et al.* 2011, 2015). However, an eyespot was not found in *Theleodinium calcisporum*, which therefore approaches *Peridinium aciculiferum* and *Peridinium malmogiense* in this respect (Craveiro *et al.* 2013).

The pusular system is an essential feature of dinoflagellates that assumes considerable variation within the group. Although a complete understanding of its functions has not been achieved, its constant presence and sometimes large development in dinoflagellate cells suggest that pusules are under evolutionary pressure and may be one of the markers of evolutionary clades, as seems to be the case of the regular, convoluted tube with diverticula found in the Tovelliaceae (Calado et al., 2006; Calado 2011; Pandeirada *et al.* 2014). However, their delicate nature makes pusules sensitive to fixation methods, and reliable description of their features is usually a demanding task that is often left unfinished. The pusular system of some peridinioids extends over a fairly large area, and descriptions that may be taken as reference points are few. The pusular systems of *Peridinium aciculiferum* and *Peridinium malmogiense*, with regular, convoluted tubules, were essentially similar to that of *Chimonodinium* and did not display the large sacs connected to the flagellar canals found in core peridinioids (Calado *et al.* 1999; Calado & Moestrup 2002; Craveiro *et al.* 2009, 2011). Among closer relatives, the flat aspect shown for pusular tubules in *Theleodinium* resembles the collapsed tubules seen in some of the *P. aciculiferum* cells and is probably of a similar type (Craveiro *et al.* 2013). More puzzling is the presence in *Naiadinium polonicum* of an elongated duct extending from a flagellar canal to an internal collecting chamber to which numerous pusular tubules connect; the apparent similarity of this pusular structure to the one described from the distantly related *Sphaerodinium* is not understood (Craveiro *et al.* 2010, 2015).

Ultrastructural examination of the flagellar base area of peridinioids has revealed a basic pattern: two microtubular roots associated with each of the basal bodies and a LC linking two roots 1 and 4, instead of the more elongated striated root connective found in other dinoflagellate groups (summarized in Craveiro *et al.* 2015). Within this general arrangement details may vary, some even in closely related species. Both *Peridinium malmogiense* and *Peridinium aciculiferum* had one connective linking the LMR/r1 to triplets of the TB and a second connective linking the two basal bodies. In *Chimonodinium lomnickii* there was also a connective linking the LMR/r1 and the TB (named TB-LMRc) but its fibres were thinner, more diffuse and not individually connected to specific triplets of the TB; a second connective linking the basal bodies was not found (Craveiro *et al.* 2011).

Predatory dinoflagellates that use a peduncle or a feeding tube to ingest parts of their prey always display a microtubular strand (usually labelled MSP) or a system of partially overlapping rows of microtubules (the microtubular basket, MB) that seems to provide support and movement to the feeding structure (Hansen & Calado 1999). Whereas the MSP is very widespread in dinoflagellates, including those with photosynthetic capacities and no known uptake of food particles, the distribution of the MB is much more restricted, and this feature may be considered a specialization typically associated with the lifestyle of the pfiesteriaceans (Calado *et al.* 2009). Although an MB has been found in all pfiesteriaceans examined (e.g. *Paulsenella*, *Tyrannodinium*, *Pfiesteria*; Schnepf

et al. 1985; Calado & Moestrup 1997; Litaker *et al*. 2002), structures unmistakably like an MB have been found in some photosynthetic members of the calcareous clade (the Thoracosphaeraceae *sensu* Elbrächter *et al*. 2008), namely, *Chimonodinium lomnickii*, *Theleodinium calcisporum* and *Naiadinium polonicum* (Craveiro *et al*. 2011, 2013, 2015). The phylogenetic positions of these MB-containing taxa and the absence of reports of MSP among their relatives suggest an origin for the MB near or at the base of the thoracosphaeracean clade. In this scenario the absence of any kind of peduncle-related microtubular strand in *Scrippsiella trochoidea*, on one side, and in both *Peridinium aciculiferum* and *Peridinium malmogiense*, on the other, would plausibly be the result of independent loss of the MB by the direct ancestors of these taxa.

Phylogeny

The close association of the species *Peridinium malmogiense* (= *Scrippsiella hangoei*), *Peridinium aciculiferum*, *Peridinium baicalense* and *Peridinium euryceps* in the phylogenetic hypothesis shown in Fig. 54 agrees with previous results of a phylogenetic analysis based on partial SSU and LSU rDNA (Annenkova *et al*. 2015; Luo *et al*. 2016). All of these species had identical SSU rDNA fragments and small differences in the LSU rDNA, ITS2 rDNA and mitochondrial cytochrome b gene markers (Annenkova *et al*. 2015; present work). These four species share the same general plate arrangement. However, external morphology (size and shape of the cells, presence and number of spines) varies widely, making them readily recognizable as different morphospecies (e.g. Hansen & Flaim 2007; Annenkova *et al*. 2015). The contrast between these clear morphological differences and the remarkable similarity of the genetic sequences examined was interpreted as adaptive radiation, and this group of taxa was considered as a species flock that evolved recently (Annenkova *et al*. 2015). Although neither *P. baicalense* nor *P. euryceps* have been analysed in fine-structural detail, the close-relatedness revealed in the phylogenetic analyses marks them as belonging to the same genus as *P. aciculiferum* and *P. malmogiense*.

Peridinium aciculiferum appeared as a sister taxon to a clade containing *Chimonodinium lomnickii*, *Thoracosphaera heimii* and the pfiesteriaceans (partial LSU rDNA phylogeny, Craveiro *et al*. 2011). More recently, *C. lomnickii* appeared as a sister taxon to a clade that included the pfisteriaceans and the group of species *P. aciculiferum*, *Peridinium malmogiense*, *Peridinium euryceps* and *Peridinium baicalense* (partial SSU–partial LSU rDNA phylogeny, Annenkova *et al*. 2015). In the present work, *C. lomnickii* was sister to a clade containing the pfiesteriaceans and *P. aciculiferum*, *P. malmogiense*, *P. euryceps* and *P. baicalense*. The somewhat variable relationship between *Chimonodinium* and *P. aciculiferum* in previously published phylogenetic analyses (Craveiro *et al*. 2011; Annenkova *et al*. 2015; Luo *et al*. 2016) and the differences in fine-structural organization between *C. lomnickii*, on one side, and both *P. aciculiferum* and *P. malmogiense*, on the other, most notably the absence of MB and eyespot in the latter two species, argue against transferring the target species to *Chimonodinium*. In the absence of any obvious closer relatives, we are placing the four species: *P. aciculiferum*, *P. malmogiense*, *P. euryceps* and *P. baicalense* in a new genus, described below.

Taxonomic descriptions and new combinations

Apocalathium Craveiro, Daugbjerg, Moestrup & Calado *gen. nov.*

DESCRIPTION: free-living, photosynthetic dinoflagellates; thecate motile cells with plates arranged in a peridinioid pattern; Kofoidian plate formula typically po, x, 4′, 3a, 7′′, 6c, 5–7s, 5′′′, 2′′′′; pyrenoids not enveloped by starch caps, consisting of an enlargement of the matrix that is crossed by lamellae of two thylakoids; microtubular basket (MB) and microtubular strand of the peduncle (MSP) absent; eyespot absent.

TYPE SPECIES: *Apocalathium aciculiferum* (Lemmermann) Craveiro, Daugbjerg, Moestrup & Calado *nov. comb.,* designated here.

ETYMOLOGY: Greek prefix *apo*-, away from; Greek *kalathos*, a basket; Greek diminutive, noun suffix *-ium*. In allusion to the absence, by presumed character loss, of the microtubular basket.

Apocalathium aciculiferum (Lemmermann) Craveiro, Daugbjerg, Moestrup & Calado *nov. comb.'*

BASIONYM: *Peridinium aciculiferum* Lemmermann 1900: 28

LEMMERMANN E. 1900. Beiträge zur Kenntniss der Planktonalgen. III. Neue Schwebalgen aus der Umgegend von Berlin. *Berichte der Deutschen Botanischen Gesellschaft* 18: 24–32.

HOMOTYPIC SYNONYMS: *Peridinium umbonatum* var. *aciculiferum* (Lemmermann) Lemmermann (1908: 181); *Glenodinium aciculiferum* (Lemmermann) Er.Lindemann (1928: 260).

NEOTYPE (designated here): Figure 9 herein. Figures 8, 10 and 11 complement Fig. 9 so that together they display all known plates of a single cell (see below).

Note on the identity of *Peridinium aciculiferum*: the original description of this species, from a lake near Berlin, was not accompanied by an illustration (Lemmermann 1900). Ostenfeld (in Ostenfeld & Wesenberg-Lund 1906) provided several figures of cells from an Icelandic population he identified with *P. aciculiferum* after checking the identification against material sent to him by Lemmermann. The figures show the cell shape and the flat antapical spines characteristic of the species but amphiesmal plates were only indicated on a ventral view (Ostenfeld & Wesenberg-Lund 1906, pl. II, fig. 18). Ostenfeld referred to the plate arrangement as 'about the same as in *P. umbonatum*' (Ostenfeld & Wesenberg-Lund 1906, p. 1127) and he made clear that he could not see the location of all plates (Ostenfeld 1907, p. 391). After examining material that he received from Ostenfeld, Lemmermann (1908) became convinced that *P. aciculiferum* had the same tabulation as *P. umbonatum* and that the two taxa differed only at variety level. The typical position of intercalary plates 2 and 3, both contacting precingular plate 4 near the mid-dorsal face (whereas plate 1a is partly concealed on the left side of a dorsal view), lends itself to such a mistake (Figs 9, 18).

Wołoszyńska (1916) provided the first accurate description and illustrations of plate arrangement in *P. aciculiferum* from material collected around Lviv, now Ukraine, during the colder months. Lindemann (1919) examined material from lakes in the Spree River system around Berlin, including the Müggelsee, from where Lemmermann followed a population of *P. aciculiferum* between February and April (Lemmermann 1903, 1910) and confirmed Wołoszyńska's observations.

Another name that has been brought up in the context of the lomnickii group of *Peridinium* is *Chalubinskia tatrica* Wołoszyńska. This species was described from Morskie Oko, in the Tatra Mountains, from a single empty theca with only three postcingular and one antapical plates (Wołoszyńska 1916, p. 276, pl. 13, figs 1–8). The very unusual tabulation of the hypotheca, which she deemed unlikely to be a deformation of a *Peridinium*, led Wołoszyńska (1916) to consider the specimen as representing a new genus, which she named *Chalubinskia*. Although the name *C. tatrica* was never applied to any specimens, Lindemann (1925, p. 171) provided an entry for *Chalubinskia* with the description of *C. tatrica* and the reproduction of three of Wołoszyńska's drawings. While he noted that the studied specimen might be an abnormal *Peridinium*, Lindemann (1925) did not suggest any particular species, thereby apparently dropping his earlier contention that it was a form similar to *Peridinium aciculiferum* or *Peridinium wierzejskii* (Lindemann 1920). About 20 years after the original description, Wołoszyńska concluded that *Chalubinskia* had been based on a mistake and that she no longer recognized the genus. This was first through Schiller (1935b, p. 166), who cited a letter from Wołoszyńska, and then in Wołoszyńska (1936, p. 195) where she stated that the name *C. tatrica* should be 'erased'. Schiller (1935b) used the provisional name *Peridinium tatrae* for the species and added that it belonged perhaps to *Peridinium lomnickii* or *P. wierzejskii*, without clarifying whether this had been suggested in Wołoszyńska's letter. In contrast, Wołoszyńska (1936) interpreted *C. tatrica* as a teratological cell of *P. aciculiferum*. In any case subsequent monographers took up the suggestion of disregarding *Chalubinskia*, and the names *C. tatrica* and *Peridinium tatrae* are absent from the main dinoflagellate floras produced over the next five decades (Huber-Pestalozzi 1950; Kisselev 1954; Starmach 1974; Matvienko & Litvinenko 1977). The name *C. tatrica* returned to floristic treatments in Popovský & Pfiester (1990), where it was regarded as a synonym of *Peridinium lomnickii*. Although the features visible in Wołoszyńska's drawings of *C. tatrica* are much more suggestive of *P. aciculiferum* than of *P. lomnickii*, the theca was too extensively modified to allow a positive identification. A list of species identified from the sample that contained *C. tatrica* included nine species of *Peridinium*, none of them *P. aciculiferum* (Wołoszyńska 1916). *Chalubinskia* is therefore a name created in error and rejected by its author, and its application would depend on the identity of its type species, which was based on the description of an empty and extensively abnormal theca. We think it is not in the interest of nomenclatural stability to apply such a name to this group of species that are well defined by modern methods.

Neotype designation

Although the contributions of Wołoszyńska (1916) and Lindemann (1919) provided the standard upon which application of the name *Peridinium aciculiferum* has been based for nearly a century, no illustration was given in the protologue, and no original material is known to exist; therefore, the name has no type. Figures 8–11 represent a cell of *P. aciculiferum* in four orientations; these were modified from Wołoszyńska's drawings (Wołoszyńska 1916, pl. 12, figs 11, 12, 13, 15) to include available information on cingular and sulcal plates. To preserve current usage we here designate Fig. 9, complemented by Figs 8, 10 and 11, as the type of *P. aciculiferum* Lemmermann.

Apocalathium baicalense (Kisselev & V. Zvetkov) Craveiro, Daugbjerg, Moestrup & Calado *nov. comb.*

BASIONYM: *Peridinium baicalense* Kisselev & V. Zvetkov 1935: 518, figs 1–14

KISSELEV J.A. & ZVETKOV V.N. [ZWETKOW W.N.] 1935. Zur Morphologie und Ökologie von *Peridinium baicalense* n. sp. *Beihefte zum Botanischen Centralblatt* 53B: 518–524.

Apocalathium euryceps (Rengefors & Barbara Meyer) Craveiro, Daugbjerg, Moestrup & Calado *nov. comb.*

BASIONYM: *Peridinium euryceps* Rengefors & Barbara Meyer 1998: 285, figs 1–31

RENGEFORS K. & MEYER B. 1998. *Peridinium euryceps sp. nov.* (Peridiniales, Dinophyceae), a cryophilic dinoflagellate from Lake Erken, Sweden. *Phycologia* 37: 284–291.

Apocalathium malmogiense (G.Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado *nov. comb.*

BASIONYM: *Peridinium malmogiense* G.Sjöstedt 1921: 184, figs 1–6

SJÖSTEDT G. 1921. Anteckningar öfver vegetationsfärgningar i saltvatten. I. En vegetationsfärgande högproduktion af *Peridinium malmogiense nov. spec. Botaniska Notiser* 1921: 181–187.

HETEROTYPIC SYNONYMS: *Peridinium hangoei* J.Schiller (1935a: 135, fig. 129), replacement name for *Peridinium gracile* Er.Lindemann (1924: 2, pl. I, figs 3–6, *nom. illeg.* (non *Peridinium gracile* Meunier 1910: 31, pl. III, fig. 51); *Scrippsiella hangoei* (J.Schiller) J.Larsen *in* Larsen *et al.* (1995: 136).

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/16-20.1.s1.

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