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Katodinium glaucum (Dinophyceae) revisited: proposal of new genus, family and order based on ultrastructure and phylogeny

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Abstract: Since its description in 1917, the taxonomy of *Katodinium glaucum*, a cosmopolitan heterotrophic marine dinoflagellate, has been in a state of flux. We added to this by arguing that *Kat. glaucum* is synonymous with both *Amphidinium extensum* and *Gymnodinium vestifici*. We presented a brief historical account of its shifting positions in *Katodinium* and *Gyrodinium* and a comparison with *Gym. vestifici* and *A. extensum*. The conclusions from our experimental work on a *Kat. glaucum* culture were based on both pheno- and genotypic characters obtained by light and electron microscopy and DNA sequencing. A nuclear-encoded large-subunit rDNA-based phylogeny revealed that *Kat. glaucum* and species of *Torodinium* are closely related and form a highly supported monophyletic lineage. The ultrastructural examination showed the apical structure complex of *Kat. glaucum* to comprise three rows of amphiesmal vesicles located beneath the cap-like projection on the anterior part of the cell. *Torodinium*, in comparison, possessed a counterclockwise spiral apical structure complex positioned on top of its bill-like apical projection. Species of both genera were longitudinally striated. The type species of *Katodinium*, *Kat. nieuportense*, was illustrated without any apical structures and without longitudinal striations, and because both morphological and molecular analysis also rejected relationship to *Amphidinium*, *Gymnodinium* and *Gyrodinium*, we proposed to erect a new genus for this species, *Kapelodinium gen. nov*. As the species name *vestifici* had priority over *glaucum*, the new name for the species was *Kapelodinium vestifici comb. nov*. To accommodate for the distinct difference in morphology of the apical structure complex in *Torodinium* and *Kapelodinium*, we further proposed two new monotypic families, Torodiniaceae *fam. nov*. and Kapelodiniaceae *fam. nov*. of the order Torodiniales *ord. nov*., which in the molecular trees was not closely related to other dinoflagellates. The ultrastructural study of *Kap. vestifici gen. & comb. nov*. also revealed a number of characteristic features, such as a novel lip-like suture within the sulcus, cross-striated filaments beneath the apical projection and atypical nuclear chambers with pores towards both the nucleoplasm and the cytoplasm.

Key Words: *Amphidinium extensum*, *Gymnodinium vestifici*, *Gyrodinium glaucum*, Kapelodiniaceae, *Kapelodinium vestifici*, Torodiniaceae, Torodiniales, *Torodinium*

INTRODUCTION

In 1895, Schütt illustrated a new heterotrophic species of *Gymnodinium*, *Gym. vestifici* Schütt, from somewhere between the Atlantic Ocean and the Mediterranean Sea. The illustrations were accompanied with a short legend. Cells had a fusiform shape with a large, longitudinally striated hypocone and a small, smooth epicone, separated by a displaced cingulum. Unfortunately, he failed to give any account of the position and direction of the flagella. Following an expedition in 1913, Wulff (1919) described the species *Amphidinium extensum* Wulff from the Barents Sea, which revealed strong similarities to *Gym. vestifici*. Wulff was aware of this close resemblance, but as he believed that his material lacked a sulcus, he erected it as a new species. At about the same time, Lebour (1917) described an unarmoured marine dinoflagellate from Plymouth Sound, *Spirodinium glaucum* Lebour, failing to recognise its striking similarity to *Gym. vestifici* if the illustrations of the latter were turned upside down. The genus *Spirodinium* had been described some years before by Schütt (1896) to include species of *Gymnodinium* with a steeply spiralling cingulum, the ends of which were displaced more than 20% of the total cell length. Kofoid & Swezy (1921) changed the genus name *Spirodinium* to *Gyrodinium*, as *Spirodinium* had been used for a ciliate parasite in 1890 and was therefore illegitimate. Lebour (1925) accepted the change to *Gyrodinium* and was the first to notice that *Gym. vestifici* and *Gyr. glaucum* (Lebour) Kofoid & Swezy most likely were conspecific. The following year, Conrad (1926) described the new genus *Massartia*, with two new species: *M. nieuportensis* Conrad and *M. ruppiae* Conrad & Kufferath. The genus was described in the same entry as *M. nieuportensis*, and this species was later designated as type species of *Massartia* by Fott (1957). Long before this, however, Schiller (1933) used the placement of the cingulum, previously used to distinguish *Gymnodinium* from *Amphidinium*, to also define the genus *Massartia*. According to Schiller (1933) *Massartia* was characterised by the cingulum being located below the posterior third of the cell. As this applied to *Gyr. glaucum*, it was renamed *Massartia glauca* (Lebour) Schiller (Schiller 1933). Unfortunately, the name *Massartia* had already been used for a zygomycete by De Wilderman (1897) and was therefore a homonym. In consequence, Fott (1957) suggested the name *Katodinium* to replace *Massartia*. He transferred all species, except the type, invalidly by using incomplete basionym references, and Loeblich corrected this in 1965,

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when *M. glauca* became *Katodinium glaucum* (Lebour) Loeblich.

Subsequently, the species has been included in *Gyrodinium* (Dodge 1982; Takayama 1985; Paulmier 1992; Okolodkov 1998a; Daugbjerg *et al.* 2000; Escobar-Morales & Hernández-Becerril 2015; Guiry & Guiry 2015) or *Katodinium* (Drebes 1974; Elbrächter 1979; Fukuyo *et al.* 1990; Hansen & Larsen 1992; Konovalova 1998; Okolodkov 1998b; Bérard-Therriault *et al.* 1999; Kim & Kim 2007), as it meets the taxonomic requirements of both genera. It agrees with *Gyrodinium* in having a cingulum displacement of more than 20% of the body length (Kofoid & Swezy 1921), and cells are longitudinally striated, which is part of the updated definition of *Gyrodinium* proposed by Daugbjerg *et al.* (2000). It agrees with *Katodinium* in the cingulum being placed below the posterior third of the cell (Schiller 1933).

Using scanning and transmission electron microscopy, a number of morphological traits supported by genetic data have recently been used to redefine the genera of naked dinoflagellates. Thus, the path of the apical structure complex was found to be a taxonomically reliable character (Daugbjerg *et al.* 2000), and more recently the structure of the eyespot was also found to be taxonomically informative (Moestrup & Daugbjerg 2007). Below we argue that the cap-like anterior projection of what has been called *Kat. glaucum* and the bill-like projection of the genus *Torodinium* (Gomez 2009) are also taxonomically significant, indicating a common ancestry.

Several species of the broadly recognized artificial genus *Katodinium* have now been transferred to new or other genera based on updated morphological characters. Thus, seven species were transferred to *Opisthoaulax* due to the presence of a type C eyespot characteristic of the Tovelliaceae (Calado 2011) and one species to *Heterocapsa* because of plate pattern tabulation and presence of organic scales on the cell body (Hansen 1995).

The type species of *Katodinium*, *Kat. nieuportense* (Conrad) Fott, has not been found since its original description in 1926; although, attempts have been made to search for it at the type locality (Calado 2011). This has prevented efforts to circumscribe the genus in detail. However, we consider that sufficient evidence has now been accumulated to allow the transfer of what has been called *Kat./Gyr. glaucum*, *A. extensum* and *Gym. vestifici* to a separate genus, *Kapelodinium gen. nov.* As the species name *vestifici* has priority, this transfer results in the new combination *Kapelodinium vestifici* (Schütt) Boutrup, Moestrup & Daugbjerg *comb. nov.*

The aim of this study was to conduct a taxonomic investigation of what will be known in the following as *Kap. vestifici gen. & comb. nov.*, using modern methods to examine the morphology and ultrastructure of the cell and to perform a molecular-based phylogeny to update the systematics of this widely distributed heterotrophic dinoflagellate.

MATERIAL AND METHODS

A culture identified as *Kat. glaucum* was established in August 2012 by single-cell isolation from samples collected in the Atlantic Ocean close to Iceland (water temperature of 10°C –12°C) during the ARCHEMHAB cruise onboard the research vessel *Maria S. Merian* in August 2012. The culture was grown in a 75-ml plastic culture flask on a slow rotating plankton wheel (1 round min^{-1}) under a photon flux density of 5–10 μmol m^{-2} s^{-1} on a 16:8 light:dark photocycle in a temperature-controlled growth chamber at 15°C using *Azadinium poporum* Tillmann & Elbrächter strain UTH C5 (Tillmann *et al.* 2011) as food.

Live cultures were examined using a Carl Zeiss Axio Imager.M2 with a 63× oil immersion lens, and photo documentation was done using a Zeiss AxioCam digital camera (Zeiss, Oberkochen, Germany).

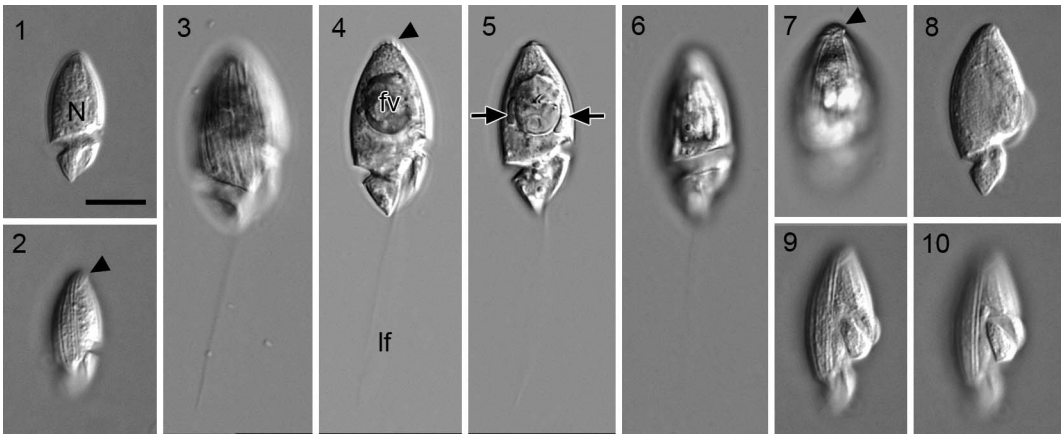
For scanning electron microscopy (SEM), part of the culture was fixed in 1.3% OsO_4 (final concentration) in 30-psu sterile filtered seawater for 1 h. The fixation cocktail was then transferred to a Swinnex filter holder (Millipore, Darmstadt, Germany) containing a 5-μm Millipore filter. Cells were rinsed in the holder by addition of filtered seawater for 1.5 h, followed by a final rinse in distilled water, and they were subsequently dehydrated in a graded ethanol series: 10 min in each of 30%, 50%, 70%, 90%, 96% and 99.9%, followed by two steps of 30 min in 100% ethanol-containing molecular sieves. Cells were critical-point dried in a Baltec CPD 030 critical point drier (Balzers, Liechtenstein), mounted on stubs and coated with palladium-gold in a Jeol JFC-2300HR sputter coater before examination in a Jeol JSM-6333F field emission scanning electron microscope (Jeol Ltd, Tokyo, Japan).

For transmission electron microscopy (TEM), we never obtained a really satisfactory preservation of cells despite four different fixation schedules being attempted: Fix 1–Fix 4.

Fix 1: Cells were fixed in a mixture of 1.7% glutaraldehyde and 0.4% OsO_4 (final concentration) in f/2 medium (Guillard & Ryther 1962) for 30 min at 4°C. After centrifugation for 5 min at 2300 rpm ($\approx 1000 \times g$), the resulting pellet was rinsed in three changes of f/2 medium, 10 min in each change, and postosmicated at 4°C in 1% OsO_4 in f/2 medium for 1.5 h. The material was rinsed briefly in distilled water and dehydrated in a graded ethanol series (30%, 50%, 70% and 90%), 10 min in each change at room temperature. It was finally placed in 100% ethanol containing molecular sieves (two rinses of 15 min each). Dehydration was completed in propylene oxide (two rinses of 5 min each). Cells were left overnight in a 1:1 mixture of propylene oxide and Spurr's resin. This mixture was replaced in the morning by a change of 100% embedding medium and 5 h later embedded in Spurr's resin in an embedding dish and placed in an oven overnight at 70°C. This method gave the best results of the four fixations and preserved internal structures and most of the amphiesma well.

Fix 2: Cells were fixed in a mixture of 2.3% glutaraldehyde and 0.01% OsO_4 (final concentrations) in f/2 medium for 1 h at room temperature. After centrifugation for 5 min at 2300 rpm ($\approx 1000 \times g$), the pellet formed was rinsed in f/2 medium three times for 10 min each. After a rinse in distilled water, cells were dehydrated and embedded as described above. This method preserved the pusule, mitochondria and trichocysts well, but other internal structures were lost.

Fix 3: Cells were fixed for 30 min at 4°C in 2% glutaraldehyde (final concentration) in 0.2 M Na-cacodylate buffer containing 0.5 M sucrose. As the culture contained



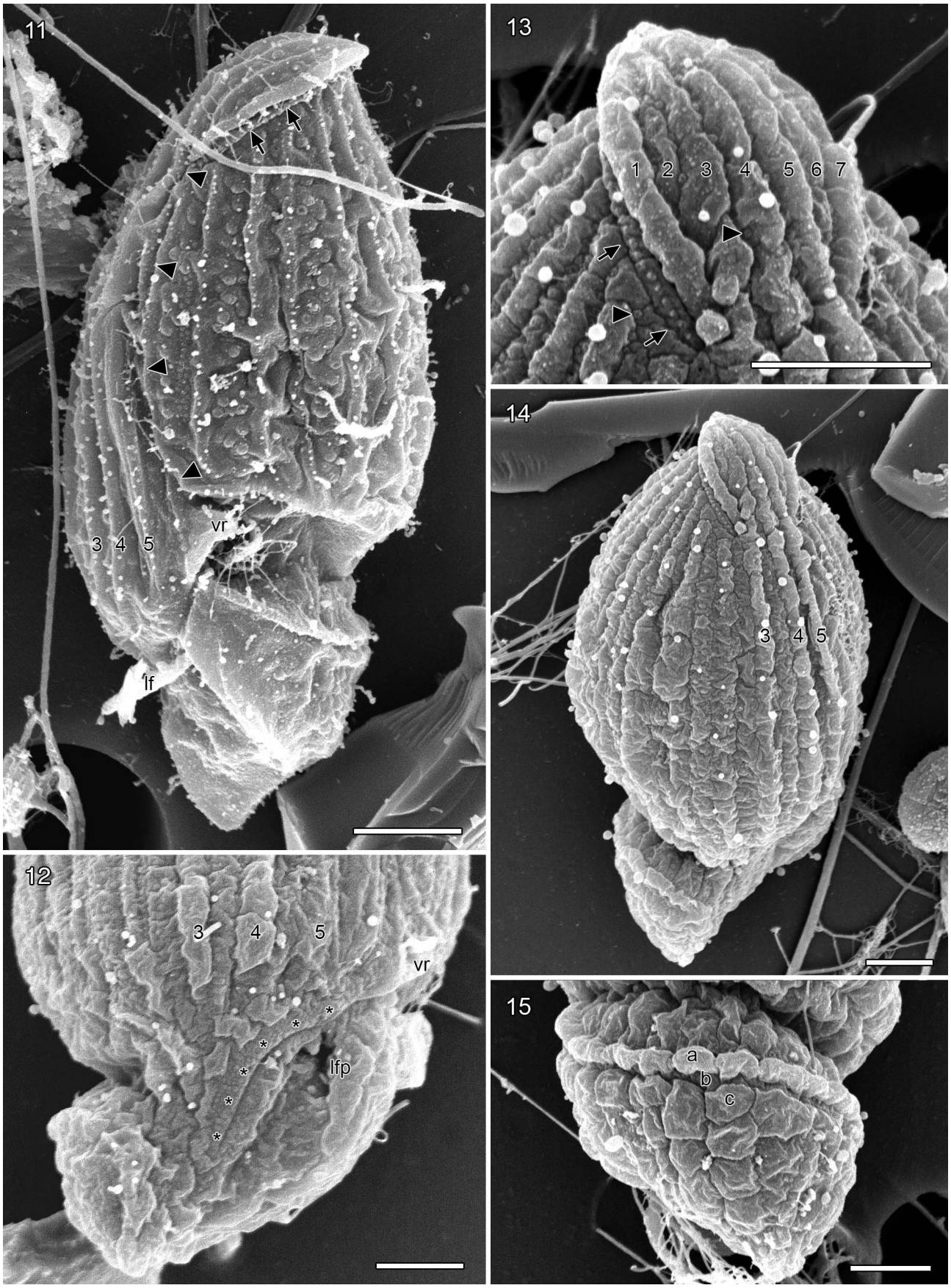
Figs 1–10. Light microscopy of live cells of *Kapelodinium vestifici*.
Figs 1, 2. Ventral view of the same cell at different focal levels.
Fig. 1. Deep focal level showing the nucleus (N) in the epicone.
Fig. 2. Cap-like structure on the apex (arrowhead), surface striations and sulcus widening posteriorly.
Figs 3–7. Same cell in different orientations and focal levels.
Fig. 3. Right ventral view showing longitudinal surface striations and end of the cingulum.
Fig. 4. Displaced cingulum and long longitudinal flagellum (lf). Striations on the apex (arrowhead) and the very broad sulcus. In the food vacuole (fv), two pyrenoids are visible in the prey organism *Azadinium poporum*.
Fig. 5. Ventral view with intact prey cell of *Azadinium poporum* in opposite orientation. In the prey cell, cingulum, sulcus and a pyrenoid are visible. Two refractive rods line the food vacuole (arrows).
Fig. 6. Dorsal view showing the broad descending cingulum.
Fig. 7. Ventral left view of the cap-like structure (arrowhead).
Figs 8–10. Dividing cells.
Fig. 8. Swollen appearance of cell.
Figs 9, 10. Daughter cell protruding from the centre of the dividing cell. Two of the longitudinal striations running from the cap to the cingulum stand out clearly.
Scale bar = 10 μm (all figures).

relatively few cells, we added 1 ml of *Pyramimonas parkeae* (R.E.Norrise & B.R.Pearson) to obtain a visible pellet. After centrifugation for 5 min at 2300 rpm, we rinsed in cacodylate buffer with decreasing contents of sucrose (50%, 25% and 0%), 30 min in each change. Postosmication was in 1% OsO_4 in distilled water for 1 h at 4°C. The fixed material was rinsed briefly in buffer and dehydrated in a graded ethanol series (30%, 50%, 70%, 90% and 96%), 20 min in each rinse, the first four at 4°C and the last at room temperature. Dehydration was completed in 100% ethanol with molecular sieves for 25 min, followed by three changes of propylene oxide (5 min in each change). Cells were left overnight in a 1:1 mixture of propylene oxide and Spurr's resin. The material was embedded as described above. Overall, cells were poorly preserved using this method, and all amphiesma and cell membranes were lost. However, trichocysts and cross-striated filaments were well preserved (Fig. 21).

Fix 4: Single cells were fixed in a mixture of 1% glutaraldehyde and 0.5% OsO_4 (final concentration) in 0.2 M Na-cacodylate buffer, pH 7.9, in 0.5 M sucrose for 10 min at 4°C. They were rinsed in buffer with 0.5 M sucrose for 30 min and embedded in agar. Postfixation was in 1% OsO_4 in 0.2 M Na-cacodylate buffer in 0.5 M sucrose for 2.5 h at 4°C. The material was subsequently rinsed in distilled water for 15 min and dehydrated in a graded ethanol series (15%, 30%, 50%, 70% and 90%), 15 min in each concentration, and dehydration was completed in 100% alcohol containing molecular sieves (two times 10 min), followed by two rinses of propylene oxide (10 min in each change). Cells were left on a rotating platform overnight in a 1:1 mixture of propylene oxide and Spurr's resin. The material was transferred to new resin the next morning, and 6 h later it was embedded in a new change of resin for curing at 70°C for 24 h. Cells did not respond well to this method, as cell membranes were lost. Trichocysts, vesicles and microtubules were relatively well preserved (Fig. 26).

All material was sectioned with a diamond knife mounted on a Leica, Super Nova, Reichert-Jung ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Sections were collected on slot grids and stained for 10 min with 2% uranyl acetate at 70°C, rinsed and stained for 10 min in Reynold's lead citrate at 70°C. They were examined in a JEM-1010 electron microscope (Jeol Ltd). Micrographs were taken using a Gatan Orius SC1000 digital camera (Gatan, Pleasanton, CA, USA).

For molecular sequencing single cell with no visible food, vacuoles were isolated using hand-drawn glass pipettes under an inverted Olympus SZX12 microscope (Olympus, Tokyo, Japan). Each cell was rinsed two to three times in autoclaved sea water and transferred to PCR tubes containing 8 μl dd H_2O and frozen at –18°C for 24 h. Further physical disruption of cells was obtained by adding an acid-washed glass bead (size 0.71–1.18 mm) to each tube and vortexing for 1 min using a Vortex Labnet VX100 (MO BIO Laboratories, Carlsbad, CA, USA) and heating of the tubes to 94°C for 10 min. To amplify ~ 1800 base pairs of the nuclear-encoded large-subunit (LSU) rDNA of single cells of *Kap. vestifici*, we used a forward primer D1R-F (Scholin *et al.* 1994) and a reverse primer DINO-ND (Hansen & Daugbjerg 2004). PCR amplification was performed using



Figs 11–15. SEM of *Kapelodinium vestifici*.

the EmeraldAmp GT PCR Master Mix following the manufacturer's recommendations (TaKaRa BIO Inc., Shiga, Japan). PCR thermal conditions were one initial cycle of denaturation at 98°C for 3 min followed by 35 cycles each consisting of denaturation at 98°C for 10 s, annealing at 54°C for 30 s and extension at 72°C for 50 s. A final extension step was run at 72°C for 2 min. This primary PCR was followed by secondary PCR seminested and nested amplifications. For seminested we used D1R-F and reverse primer D3B (Nunn *et al*. 1996) and for nested D3A (Nunn *et al*. 1996) and 28-1483R (Daugbjerg *et al*. 2000). The PCR conditions were as above; however, this time we ran only 18 cycles. Fragment lengths were confirmed by electrophoresis using 1.5% agarose-casted gels run for 20 min at 150 V. DNA fragments loaded into the gel were stained with GelRed and visualized using the gel documentation XR + System from BioRad (Hercules, CA, USA). Fragment lengths were compared to the DNA marker 100-base-pair (bp) RAINBOW eXtended DNA ladder (BIORON GmbH, Ludwigshafen, Germany). Amplified PCR products were purified by ultrafiltration using the NucleoFast 96 PCR kit from Macherey-Nagel (GmbH & Co. KG, Düren, Germany) following the manufacturer's recommendations. Partial LSU rDNA sequences were determined by the sequencing service provided by Macrogen.

The LSU rDNA sequence of *Kap. vestifici gen. & comb. nov*. was added to an alignment comprising 86 other species of dinoflagellates representing 58 genera. The alignment was based on Clustal W as incorporated in the Jalview v14 sequence editor (Waterhouse *et al*. 2009). The data matrix comprised 1657 base pairs (including introduced gaps) and included domains D1–D6. The phylogeny was inferred using Bayesian (BA) and maximum likelihood (ML) analyses. MrBayes v3.2.5 x64 (Ronquist & Huelsenbeck 2003) was used for BA analyses, and for ML we applied PhyML v3.0 (Guindon & Gascuel 2003). In BA we used 5 million generations, and a tree was sampled every 1000 generations. This analysis was performed on a desktop computer. To evaluate the burn-in value, the LnL scores were plotted as a function of generations. The burn-in occurred after 501,000 generations (conservative estimate). Hence, 501 trees were discarded, leaving 4500 trees for generating the 50% majority-rule consensus tree in PAUP (Swofford 2003). For ML analysis, we used the parameter settings obtained from jModelTest v2.1.3 (Darriba *et al*. 2012), which, among 88 models examined, chose GTR+I+G as the best-fit model for our data matrix (gamma = 0.349 and p-invar = 0.038). PhyML used the online version available on the Montpellier bioinformatics platform at http://www.atgc-montpellier.fr/phyml. The robustness of the tree topologies was evaluated using bootstrapping with 500 replications. Bootstrap support values were mapped onto the tree topology obtained from BA.

Ultrastructural characters and molecular data for phylogenetic inference (e.g. van de Peer *et al*. 1996) have indicated that ciliates and apicomplexans form sister groups to the dinoflagellates. Hence, we used four ciliates [*Euplotes aediculatus* Pierson, *Tetrahymena thermophile* (Nanney) McCoy, *T. pyriformis* (Ehrenberg) Lwoff, *Spathidium amphoriforme*], four apicomplexans (*Hammondia hammondi*, *Neospora canium*, *Toxoplasma gondii* Nicolle & Manceaux, *Sarcocystis neurona*) and the perkinsozoan *Perkinsus andrewsi* Coss, Robledo, Ruiz & Vasta to polarize the in-group of dinoflagellates.

RESULTS

Kapelodinium Boutrup, Moestrup & Daugbjerg *gen. nov.*

DIAGNOSIS: Athecate dinoflagellates with cap-like apical projection. Apical structure complex comprising three rows of vesicles positioned below the rim of the cap, following its shape from beginning to end. Epicone longitudinally striated. Nuclear chambers with pores both to the nucleoplasm and the cytoplasm. Chloroplasts absent.

TYPE SPECIES: *Kapelodinium vestifici* (Schütt) Boutrup, Moestrup & Daugbjerg comb. nov.

BASIONYM: *Gymnodinium vestifici* Schütt (1895). Die Peridineen der Planktonexpedition. I. Theil. Studien über die Zellen der Peridineen. Ergebnisse der Plankton-Expedition der Humboldt Stiftung. IV, p. 168, figs. 85.1–85.2a. Leipzig: Lipsius & Tischler.

TAXONOMIC SYNONYMS: *Spirodinium glaucum* Lebour 1917, pp. 196–197, fig. 13; *Amphidinium extensum* Wulff 1919, pp. 104, 119, Taf. 1, figs 8a–d; *Gyrodinium glaucum* (Lebour) Kofoid & Swezy 1921, p. 308, pl. 9, fig. 94, text fig. DD 16; *Massartia glauca* (Lebour) Schiller 1933, p. 436, fig. 462; *Katodinium glaucum* (Lebour) Loeblich III 1965, p. 15.

ETYMOLOGY: Prefix *kapelo-* (from modern Greek καπελο), 'cap', referring to the cap-like apical structure. The termination *-dinium*, originally from Greek δινη, 'vortex', is commonly applied to dinoflagellates.

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Fig. 11. Cell in ventral view. Ridges on the surface of the epicone continue onto the cap-like structure, which extends upwards from the cell's left. The apical groove with knobs releasing material is seen below the thick rim of the cap (arrows). A delicate furrow extends from the cingulum to the ASC (arrowheads). Ridges running from the top of the cap are numbered 3–5. The lobed ventral ridge (vr) almost covers the pore of the transverse flagellum. The wide cingulum spirals downwards in a left spiral. Part of the longitudinal flagellum (lf) is visible.

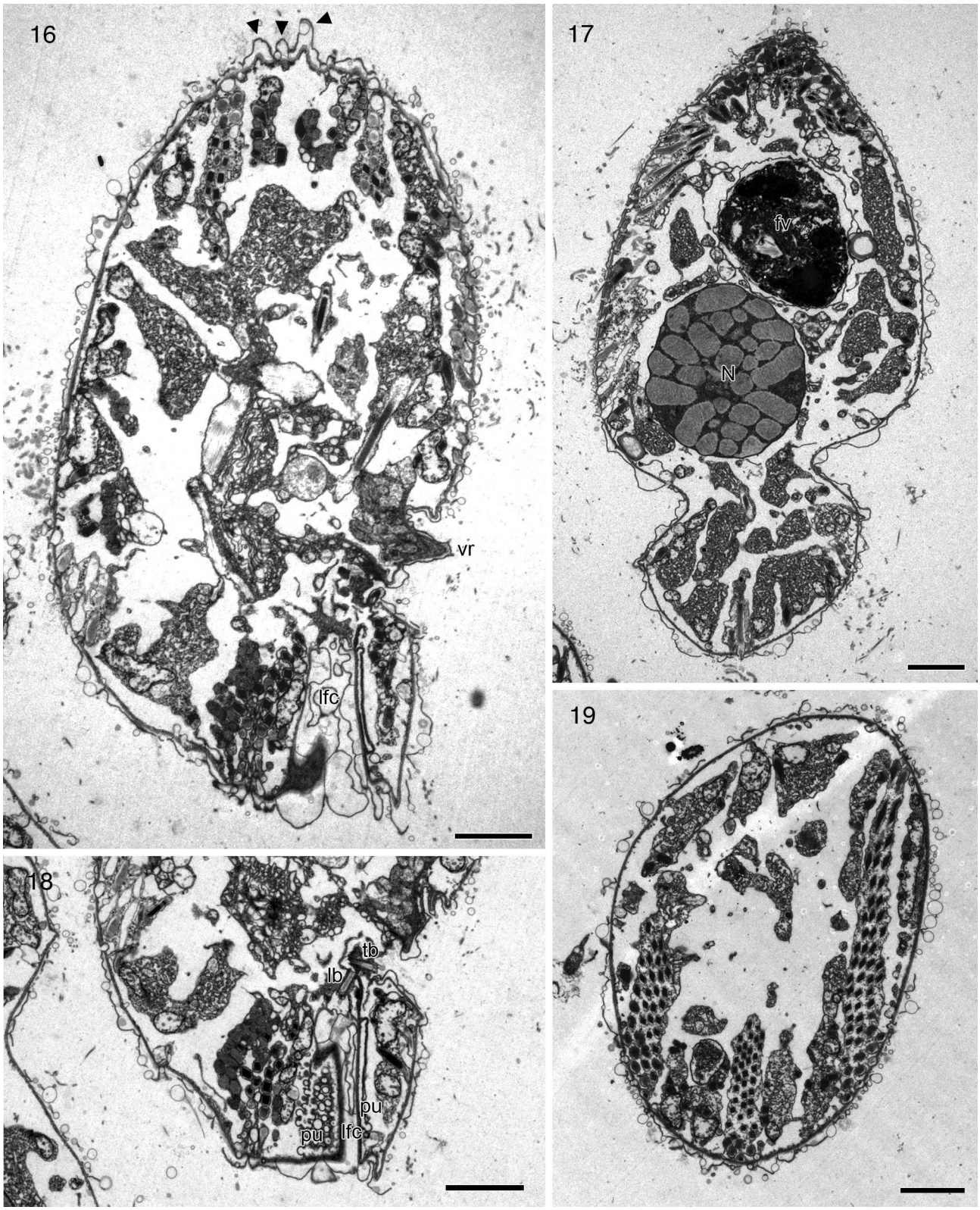
Fig. 12. Sulcal area with the longitudinal flagellar pore (lfp). The ventral ridge (vr) continues into a lip-like suture, surrounded by small rectangular amphiesmal vesicles (asterisks). Ridges running from the cap are numbered 3–5.

Fig. 13. Higher magnification of the cell in Fig. 14 to show the construction of the cap-like structure. The ASC consists of several amphiesmal vesicles with 'knobs' which extend below the cap (arrows). The five ridges on the cap (numbered 2–6) are lined by a thick rim (numbered 1 and 7). Numerous pores are present between the ridges (arrowheads).

Fig. 14. Cell seen in right lateral view, showing the thick rim of the lower cingulum border, the very wide sulcus and the distinctive ridges running from the cap to the sulcal area (numbered 3–5).

Fig. 15. Left view of the hypocone showing the thick rim on the lower border of the cingulum (a), below which is a depression (b). The amphiesmal vesicles on the hypocone are polygonal (c).

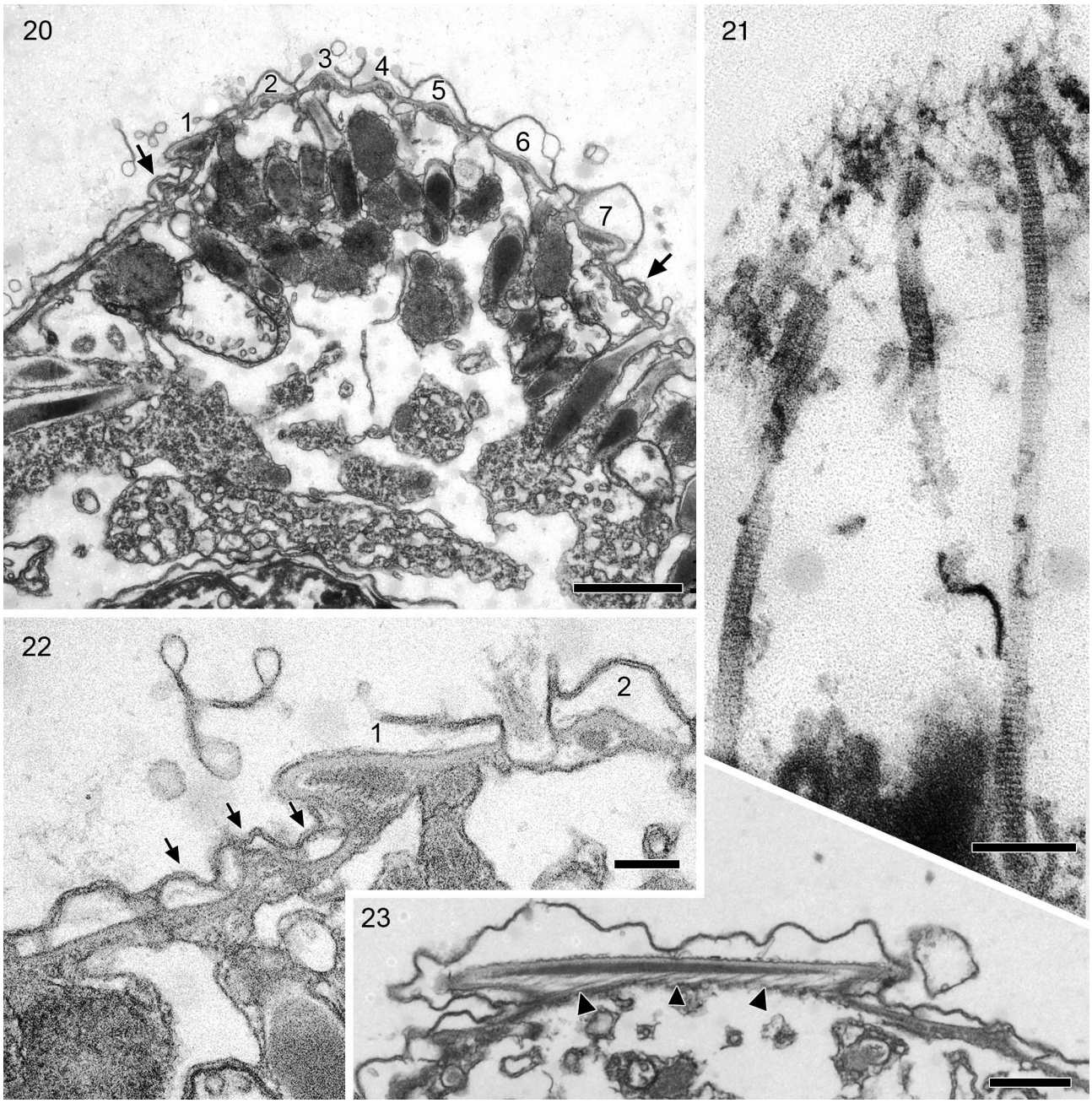
Scale bars: 2 μm.



Figs 16–19. Transmission electron microscopy of *Kaplodinium vestifiri*. Cell overview. Fix 1.

Fig. 16. Longitudinal section through the outmost ventral area of the cell showing a small part of the hypocone. The amphiesma possesses apical striations (arrowheads), a peduncle-like structure is present in the ventral ridge (vr) and the longitudinal flagellar canal (lfc) is visible. Scale bar = 2 μm.

Fig. 17. Cell in longitudinal section. The nucleus (N) is located in the lower part of the epicone, and the deeply incised displaced cingulum is also visible. A large food vacuole is present above the nucleus (fv). Numerous trichocysts line the cell periphery. Scale bar = 2 μm.



Figs 20–23. Details of the apical area of the cell in *Kapelodium vestifici*.

Fig. 20. Dorsoventral section of the apical area illustrating the cap with its thick rim (labelled 1 and 7) and five ridges (labelled 2–6) each with a small cross-striated filament beneath. On each side of the cap are the amphiesmal vesicles that constitute the ASC (arrows). Fix 1. Scale bar = 1 μm.

Fig. 21. Longitudinal section through three of the cross-striated filaments beneath the ridges on the cap-like apical structure. Fix 3. Scale bar = 0.2 μm.

Fig. 22. Close-up of the cross-striated filaments beneath the thick rim (1) and ridge (2) on the cap and the three vesicles (arrows) that constitute the ASC. Same cell as in Fig. 20. Scale bar = 0.2 μm.

Fig. 23. Electron-dense structure within the cap, probably from the rim (1 or 7) attached to the cell by thin fibres (arrowheads). Fix 1. Scale bar = 0.5 μm.

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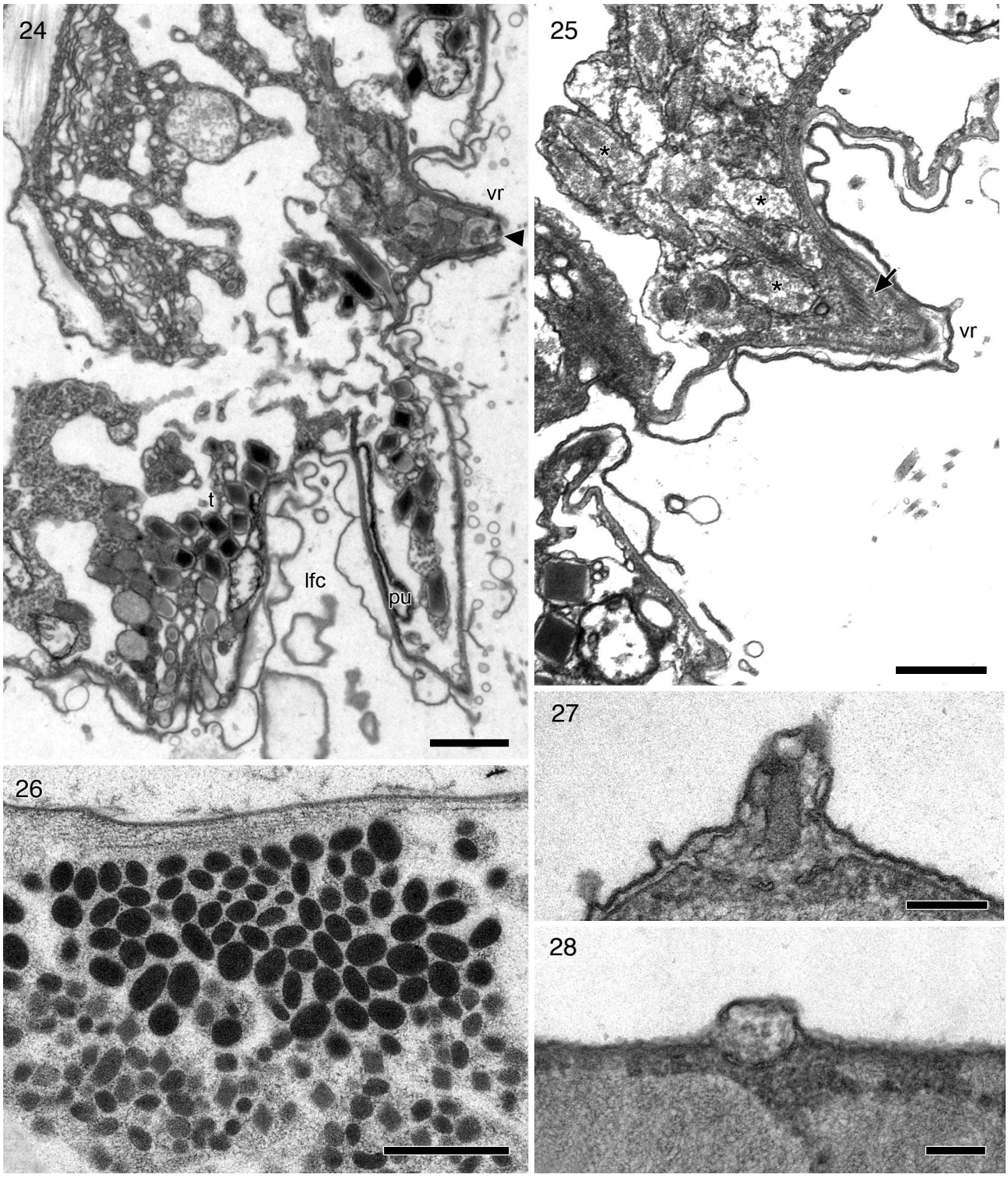
Fig. 18. Longitudinal section from the same cell as Fig. 17. The two basal bodies are inserted perpendicular to each other, tb = the transverse flagellum base, lb = the longitudinal flagellum base. The longitudinal flagellar canal (lfc) is surrounded by the extensive pusule system (pu). Scale bar = 2 μm.

Fig. 19. App. 130 trichocysts visible in a thin section of the hypocone. Many trichcysts are arranged in three to four longitudinal bands. Scale bar = 2 μm.



Figs 24–28. The ventral ridge and nuclear chambers in *Kapelodinium vestifici*.

Fig. 24. Overview of the ventral ridge (vr) with an opening (arrowhead). lfc = longitudinal flagellar canal, t = trichocysts, pu = pusule. Fix 1. Scale bar = 1 μm.

Fig. 25. Microtubular strand of the peduncle (arrow) and adjacent elongate vesicles (asterisks) in the ventral ridge (vr). Fix 1. Scale bar = 0.5 μm.

Fig. 26. Microtubules beneath the amphiesma with numerous somewhat elongate electron-opaque vesicles nearby and trichocysts further away from the outer membrane. Fix 4. Scale bar = 1 μm.

Figs 27, 28. Nuclear chambers. Three to four pores connect the nucleoplasm to the chamber, and two pores are visible between the chamber and the cytoplasm. In Fig. 27 an opaque vesicle is present in the chamber. Fix 1. Scale bars = 0.2 μm.

Light microscopy

Cells of *Kap. vestifici* (Figs 1–10) were fusiform in shape, in our culture measuring 15–33 μm in length ($n = 11$). Cells had a cap-like apical protuberance pointing towards the left (Figs 2, 7). The hypocone was about 0.3 times the total cell length, and the deeply indented cingulum was displaced 0.2 cell length in a left downward spiral (Figs 1–6). Cells were circular in cross section (not shown), with longitudinal striations restricted to the epicone. The sulcus widened posteriorly (Fig. 4) and included the longitudinal flagellum, which was longer than the cell body (Figs 3–6). A large spherical nucleus was positioned centrally in the cell (Fig. 1). If present, one or sometimes two food vacuoles were found anteriorly in the cell, above the nucleus (Figs 3–7). We observed food vacuoles with complete dinoflagellate prey, in our case *Azadinium poporum*, oriented upside down and showing cingulum, sulcus and pyrenoids (Figs 4, 5). Sometimes cells also possessed refractive rods (Fig. 5). Dividing cells had a swollen appearance in which one of the cells was protruding from the ventral side of the other, above the cingulum (Figs 8–10; in the area of the delicate furrow seen in SEM in Fig. 11). Two distinct longitudinal ridges were visible on the dividing cell (Fig. 10; see supplementary data Fig. S1).

Scanning electron microscopy

The surface of the cells showed longitudinal surface ridges on the epicone (Fig. 11). A mucilaginous substance was excreted from the longitudinal ridges and was seen as translucent spheres in the SEM. Pores were present on the cell surface in the spaces between the ridges (Fig. 13). Ventrally, the upper border of the cingulum continued as a delicate furrow upwards to the apical structure complex (ASC; Moestrup *et al.* 2014) (Fig. 11). The apex of the cell consisted of a cap-like, slightly pointed lobe, which extended upwards from the left side of the cell. Five ridges were seen on the top of the lobe (numbered 2–6 in Fig. 13), the three central ridges extending from the pointed end of the cap dorsoventrally to the sulcal area (numbered 3–5 in Figs 11–14). A thick rim bordered the cap-like structure (numbered 1 and 7 in Fig. 13). The ASC was horseshoe shaped and ran all the way below this elevated structure (Fig. 13). It consisted of rectangular elongate amphiesmal vesicles, each with a row of small knobs (Fig. 13), and fibrillar, possibly mucilaginous material often extruded from the knobs (Fig. 11). The flagellar pore of the transverse flagellum was covered slightly by a lobed ventral ridge (Fig. 11). The longitudinal flagellum was oriented perpendicular to the transverse flagellum and travelled through a flagellar canal before exiting in the anterior end of the sulcus (Fig. 11). The curve of the ventral ridge continued downwards above and to the right of the opening of the longitudinal flagellar canal and into a lip-like suture within the sulcus, lined with small square amphiesmal vesicles (Fig. 12). The lower border of the broad cingulum was marked with a thick rim of amphiesmal vesicles, followed on the antapical side by a depression (Fig. 15). The hypocone was covered by polygonal amphiesmal vesicles (Fig. 15).

Transmission electron microscopy

GENERAL ULTRASTRUCTURE: The ultrastructure of *Kap. vestifici* revealed typical dinoflagellate features, such as amphiesma, dinokaryon and mitochondria with tubular cristae (Figs 16, 17). The nucleus was located in the lower part of the epicone (Fig. 17), and an extensive pusule system took up considerable space in the hypocone (Fig. 18). The amphiesma vesicles contained only indistinct, poorly defined material (see, e.g., Fig. 22). Food vacuoles were often seen in the epicone (Fig. 17). The periphery of the cell contained large numbers of trichocysts (Fig. 17), some of which were arranged in longitudinal bands of three to four rows of trichocysts (Fig. 19). The fixation of the flagellar apparatus was not satisfactory in any of the four fixation methods used; thus, reconstruction of the flagellar root system was not attempted.

CAP AND ASC: The anterior cap-like structure of the cell is visible in two different orientations in Figs 16 and 17 and in more detail in Figs 20–23. Within each of the five ridges and the thick rim bordering the edge of the cap-like structure was a cross-striated filament, seen as opaque spots underneath the amphiesmal vesicles in Fig. 20 and numbered 1–7. In Fix 3, we measured one of these filaments to be at least 1.5 μm long (Fig. 21). We interpret an electron-opaque structure from which numerous thin fibres extend downwards towards the cytoplasm (Fig. 23, arrowheads) to be rim fibres 1 or 7. It is seen as the cross-banded structure labelled 1 in Fig. 22. Immediately below the cap-like structure was the ASC (Figs 20, 22). In cross section, it consisted of three rows of amphiesmal vesicles (Fig. 22), one raised central row, bordered by two rows of smaller vesicles indiscernible in SEM. The central vesicle in Fig. 22 had a concavity on the top, probably a pore through which mucilaginous substance was released. This was also visible in SEM (Fig. 11).

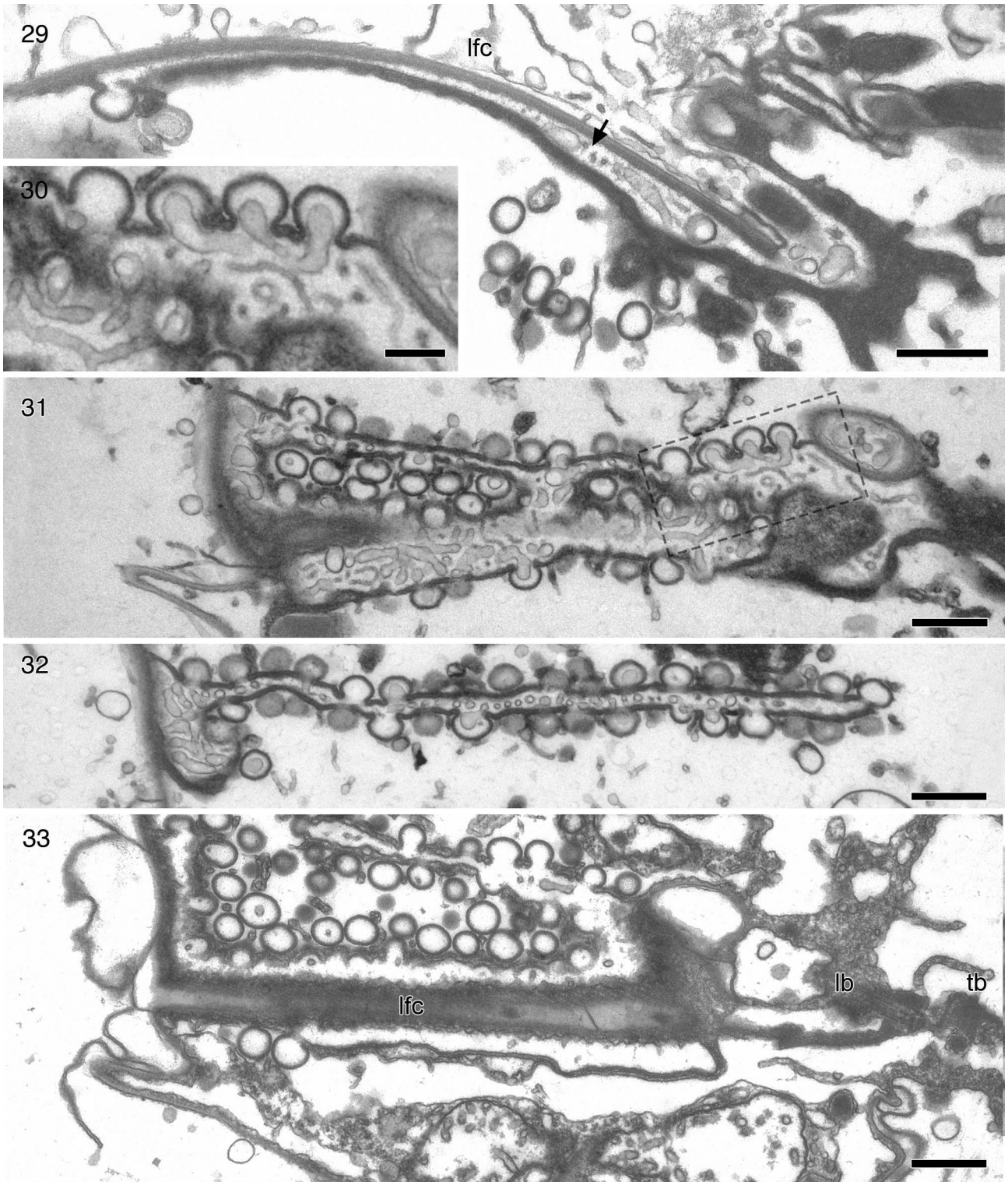
VENTRAL RIDGE: The lobed ventral ridge appeared to possess an opening distally at which point amphiesmal vesicles were absent (Fig. 24). Within the ridge the microtubular strand of the peduncle (MSP) was visible in longitudinal section (Fig. 25). Numerous elongate vesicles were present in the ventral area of the cell close to the MSP. Some of them had an electron-opaque content, the density of the contents depending on the fixation method applied (Figs 24–26). Thus, in Fig. 26, numerous electron-opaque vesicles are visible, underlain by trichocysts. Unfortunately, the bundle of microtubules, near the electron-opaque vesicles in Fig. 26, was poorly preserved.

NUCLEUS: A two-membrane envelope lined the nucleus, and nuclear chambers (Figs 27, 28) were distributed sparingly over the envelope. The nuclear chambers were positioned with one part protruding into the nucleoplasm and the other into the cytosol. Three to four pores were visible between the nucleoplasm and each chamber. From the chamber to the cytoplasm, two pores were often visible. An electron-dense vesicle was present within one of the chambers (Fig. 27).

PUSULE: The pusule was highly branched and surrounded the longitudinal flagellar canal (Fig. 18). Details of the pusule system are illustrated in Figs 29–33. The pusule consisted of long intertwining collecting tubes lined by two membranes, with pusule vesicles attached along the sides (Figs 30–33). The vesicles often contained large amounts of tubular membranous material shown in high magnification in Fig. 30. This gave the impression of being emptied into the collecting tubes and transported to a canal parallel to the flagellar canal (Fig. 29). Similar material was present in the flagellar canal, seemingly being discharged to the exterior through the longitudinal flagellar canal and the sulcus (Fig. 29).

Molecular data

PHYLOGENY: The tree topology obtained from Bayesian analysis of 58 genera of dinoflagellates and inferred from partial nuclear-encoded LSU rDNA is illustrated in Fig. 34. In general, the deepest dinoflagellate branches were not resolved (posterior probabilities < 0.5 and bootstrap support $< 50\%$) in this analysis. However, individual evolutionary lineages equivalent to higher systematic levels, such as orders and families, were in some cases highly supported, e.g., the orders Suessiales, Dinophysiales and Gonyaulacales and the families Kareniaceae and Tovelliaceae. The Gymnodiniales *sensu lato* did not form a monophyletic group, but the species were dispersed



Figs 29–33. The pusule system in *Kapelodinium vestifici*. The cell is seen in longitudinal section, the antapical end towards the left.

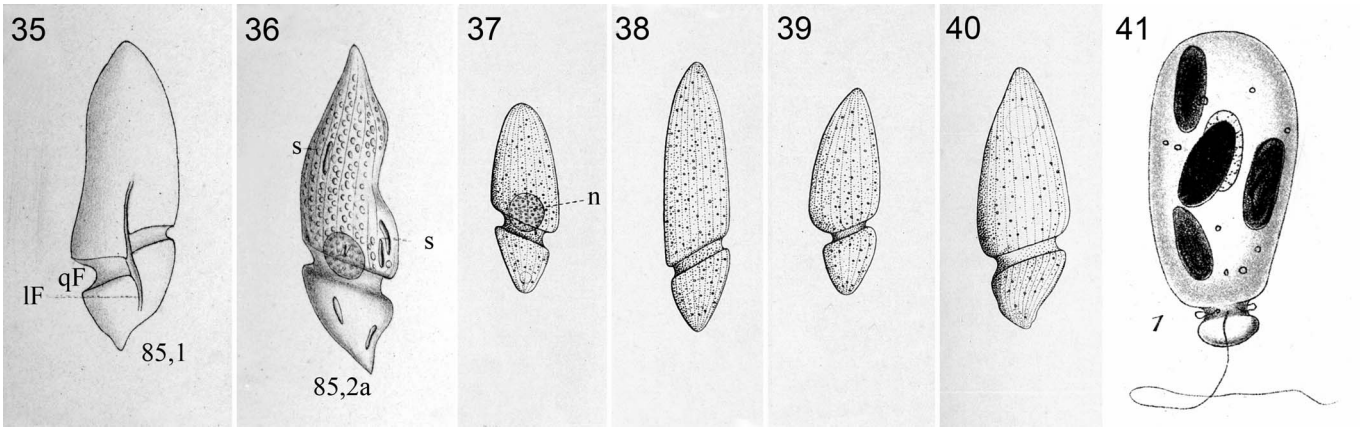
Fig. 29. Pusule collecting tube (arrow) running parallel to the longitudinal flagellar canal (lfc) and opening into the top-most part of the longitudinal flagellar canal. Fix 2. Scale bar = 0.5 μm.

Fig. 30. Higher magnification (Fig. 31, excerpt shown) of material interpreted as being released from pusule vesicles into collecting tubes. Scale bar = 0.2 μm.

Figs 31, 32. Overview showing pusule collecting tubes filled with membranous material interpreted as being released from the pusule vesicles. Fix 3. Scale bars = 0.5 μm.

Fig. 33. Longitudinal flagellar canal (lfc) surrounded by the extensive pusule complex. The longitudinal and transverse flagellar bases are also visible, lb and tb, respectively. Fix 1. Scale bar = 0.5 μm.

Fig. 34. Phylogeny of *Kapelodinium vestifici* and 86 other dinoflagellate species inferred from BA of nuclear-encoded LSU rDNA sequences. The data matrix comprised 1657 bp, including introduced alignment gaps (from domain D1 to *c*. 60 bp downstream of domain D6). The outgroup consisted of four ciliates, four apicomplexans and *Perkinsus*. The first numbers at internal nodes are posterior probabilities ($\geq$ 0.5) from BA, and the last numbers are bootstrap values ($\geq$ 50%) from ML with 500 replications. Filled circles illustrate the highest possible support in BA and ML (1.0% and 100%, respectively). GenBank accession numbers are provided in brackets. The new order Torodiniales is marked in grey.



Figs 35–41. Reproduction of original illustrations.
Figs 35, 36. *Gymnodinium vestifici* by Schütt (1895, pl. 25, figs 85,1–2a). Upside down compared to original. New lettering was added to the figure. qF, transverse furrow; lF, longitudinal furrow; s, refractive rod.
Figs 37–40. *Amphidinium extensum* by Wulff (1919, pl. 1, figs 8a–d). Upside down compared to original. New lettering was added to the figure. n, nucleus.
Fig. 41. *Massartia nieuportensis*, type species of *Katodinium*, by Conrad (1926, pl. 1, fig. 1), reproduced in grey scale. The four dark bodies are said to represent plate-like, yellowish chloroplasts (reproduction courtesy of António J. Calado).

over several evolutionary lineages. The dinoflagellate of particular interest here, *Kap. vestifici*, formed a highly supported sister taxon to two species of *Torodinium* [i.e. *T. robustum* Kofoid & Swezy and *T. teredo* (Pouchet) Kofoid & Swezy] (pp = 0.99 and bootstrap support = 73%). In BA, these three dinoflagellates formed a sister group to the remaining dinoflagellate taxa (pp = 0.98). However, ML analyses did not support this early branching lineage (bootstrap support < 50%; Fig. 34), as *Kapelodinium* and *Torodinium* was part of the basal polytomy, which grouped with clusters of single species of dinoflagellates (not shown).

DISCUSSION

Identity of the dinoflagellate

The characteristic morphology of this dinoflagellate makes species identification relatively easy when observed in a good light microscope. Features readily observed are the large epicone compared to the hypocone, the fusiform shape of the cell, the apical projection, the surface striations (especially when observed by phase contrast or interference contrast) and the displaced cingulum.

However, the taxonomy of this dinoflagellate is surprisingly complicated. One of the reasons for the confusion is undoubtedly that in the light microscope, it can be difficult to decide whether the minute hypocone is striated like the epicone. In fact, in one of our light micrographs, the hypocone appeared to be striated when observed in a certain focal plane (not shown). This difficulty may explain why the species has sometimes been drawn with striations on both epicone and hypocone (Dodge 1982; Hansen & Larsen 1992; Konovalova 1998). The same applies to the original description and illustration of *A. extensum* (reproduced here as Figs 37–40); whereas, Schütt (1895) was more accurate in his illustrations (reproduced as Figs 35, 36).

Lebour (1917) described the striations to be widely separated and drew them as internal in the cell. Kofoid & Swezy (1921) compared these striae with what they described as four or five long rodlets extending ~ 0.5–0.66 the cell length. We have not observed such rodlets. However, they may represent the longitudinal bands of trichocysts, three of which are illustrated in Fig. 19, each band containing at least three rows of trichocysts. The bands in the figure are slightly less than 2 μm wide, and they may occasionally be visible in the light microscope, perhaps in fixed material. Unfortunately, not only the striations have been illustrated incorrectly. Also, the cap and the sulcus can be difficult to see and depict correctly when using light microscopy only (Kofoid & Swezy 1921; Wailes 1939; Dodge 1982; Konovalova 1998; Okolodkov 1998a). We believe that these difficulties explain the variation seen in the historical illustrations of *Kap. vestifici.*

Species level

The first complication is orientation of the cell, which leads to addressing the similarity between *Gym. vestifici* and *Kat. glaucum*. Schütt (1895) illustrated *Gym. vestifici* in two drawings reproduced here as Figs 35 and 36 and accompanied the drawings with a short legend. The illustration is essentially identical to *Kat. glaucum* if the illustrations are turned upside down. Turned around, it has a hypocone smaller than one-third of the cell, surface striations limited to the epicone, rod-shaped refractive bodies (as seen in Fig. 5) and a leftwards-spiralling, displaced cingulum. Schütt (1895) also described that a ball filled with chromatophores was pushed out from the ventral side of the cell. This we interpret as a lost prey item. Unfortunately, Schütt (1895) failed to describe the position of the flagella and flagellar pores, which probably led to the mistake in cell orientation.

After its description in 1895, Lohmann (1908) observed *Gym. vestifici* as part of the marine plankton community in the Baltic Sea, reaching a maximum abundance of 840 cells l^{-1} in August 1905, and Hansen-Ostenfeld (1913) observed the species in the Kattegat. Kofoid & Swezy (1921) did not notice the similarities between the newly described *Kat. glaucum* and *Gym. vestifici*, and Lebour (1925) therefore became the first to comment that *Gym. vestifici* strongly resembled *Kat. glaucum*

if turned upside down. She also noted that *Gym. vestifici* was insufficiently defined by Schütt and that the flagella needed to be described. A few identifications of *Gym. vestifici* have been recorded since 1913. Thus, Parke & Dixon (1976) mention both *Kat. glaucum* and *Gym. vestifici* but for the latter note that it 'includes *Gyrodinium glaucum* in Lebour (1925)' and thus indicate conspecificity. *Gymnodinium vestifici* was also identified in a study of the gut content of lancelets from the northwest African shelf (Gosselck *et al.* 1978). Four identifications of *Gym. vestifici* were made from the Russian Arctic together with one record of *Kat. glaucum*, and it was noted that they were probably conspecific (Okolodkov 1998b). Two identifications of *Gym. vestifici* were made in May 2000 from the Baltic Sea by Jaanus (2003), who did not mention *Kat. glaucum*, and one identification of *Gym. vestifici* from the Mediterranean Sea (1989) was described as 'doubtful' by Gomez (2003), who also noted the resemblance between the two species. *Gymnodinium vestifici* has been seen sporadically in the Oslofjord (Throndsen *et al.* 2003) and illustrated with the original drawing by Schütt (1895). The origin and direction of the longitudinal flagellum of *Gym. vestifici* has never been observed, and there have not been any indisputable identifications of *Gym. vestifici* since its description 120 years ago.

Amphidinium extensum was brought to our attention by a study of phytoplankton from the Baltic by Hällfors (2004), who indicated possible conspecificity between *A. extensum* and *Gym. vestifici*. When Wulff (1919) described *A. extensum* (from the Barents Sea), he drew it upside down – as *Gym. vestifici* – with surface striations, displaced cingulum and a bluntly rounded antapex (apex in correct orientation). Wulff noted the similarity to *Gym. vestifici* but erected a new species with the single argument that his specimen lacked a sulcus altogether. Schiller (1933) believed that the sulcus was probably short and narrow and interpreted Wulff's drawings to show only the dorsal side of the cell. The drawings are very similar to *Gym. vestifici* although less detailed, with striations throughout the cell, a centrally placed nucleus and no indication of a longitudinal flagellum. In her annotation of *A. extensum*, Lebour (1925) made the probable connection between all three species, a suggestion supported by Schiller (1933). *Amphidinium extensum* has subsequently been identified from the Russian Arctic (Okolodkov 1998b; Bessudova *et al.* 2014), the Black Sea (Cărăuş 2012; BSPC Editorial Board 2015) and a few 'doubtful' identifications from the Mediterranean (Gomez 2003). It was also mentioned from New Zealand as *Amphidinium cf. extensum* (Chang *et al.* 2012). A recent report of *A. extensum* from the Pacific coast of Mexico includes a light micrograph of a Lugol-fixed cell, which is identical to *Kap. vestifici* if turned upside down (Meave-del Castillo *et al.* 2012). This emphasises the difficulty of identifying species in fixed material if the flagella have been lost.

In conclusion, during the last 90 years, it has often been mentioned that the three taxa are conspecific. Based on our literature survey, combined with the observations reported in the present study, we have reached the same conclusion.

Generic level

AMPHIDINIUM AND GYMNODINIUM: Morphological and molecular data do not support a relationship between *Kap. vestifici* and *Amphidinium or Gymnodinium*. *Amphidinium* lacks an apical structure complex, and in the updated definition of *Gymnodinium*, the apical structure complex comprises a horseshoe-shaped groove running in a counterclockwise direction (Daugbjerg *et al.* 2000). *Kapelodinium vestifici* also has a different type of nuclear vesicular chambers (Hansen *et al.* 2000), and to the best of our knowledge, no species within *Gymnodinium* has a striated cell surface. Molecular analyses support the conclusions based on the morphological differences, as *Kap. vestifici* is widely separated from both *Amphidinium* and *Gymnodinium*.

KATODINIUM: As the type species of *Katodinium*, *Kat. nieuportense* has not been found since its original description by Conrad (1926); ultrastructural and molecular studies of this species have not been made. Hence, we have to rely on the available morphological features. The epicone of *Kat. nieuportense* was described as completely smooth, in other words, without striations and without an apical structure (*cf.* Fig. 41). This separates *Kap. vestifici* from *Katodinium*. Conrad (1926) described *Kat. nieuportense* as lacking a sulcus, but it may have been difficult to see in the microscope used. Moreover, what was described as two to four yellow plastids present in the cytoplasm of *Kat. nieuportense* could have been food vacuoles, leaving some doubt as to whether *Kat. nieuportense* is phototrophic.

GYRODINIUM: Despite the morphological similarities between *Gyrodinium* and *Kap. vestifici*, such as the striated surface and the displaced cingulum, the two genera are only distantly related in the molecular analysis. The nuclear-encoded LSU rDNA sequence of *Kat. glaucum* was first determined by Kim & Kim (2007). In their phylogenetic tree, *Kat. glaucum* was separated from *Gyrodinium*. This may have been overlooked by Escobar-Morales & Hernández-Becerril (2015), who used the name *Gyr. glaucum* in a survey of naked dinoflagellates from Mexican waters. Besides the molecular evidence, the apical construction also separates the two genera. The apex in species of *Gyrodinium* is described as having an elliptical apical groove-like structure, which in the type species *Gyr. spirale* (Bergh) Kofoid & Swezy is combined with a central surface ridge, giving it a structure resembling a 'conquistador' helmet (Hansen & Daugbjerg 2004).

As *Kap. vestifici* shows no close relationship with any of the genera in which it has been previously placed, and as the phylogenetic tree revealed a relationship to the well-known genus *Torodinium*, we erected the new genus *Kapelodinium gen. nov.* and made the new combination *Kap. vestifici comb. nov.* It should be mentioned here that Reñé *et al.* (2015), using both SSU and LSU rDNA gene sequences, also found a close phylogenetic relationship between *Torodinium* and *Kapelodinium* (as *Katodinium*).

A coherent population

The reported size ranges of *Kat. vestifici* of 16–57 μm in length (Kofoid & Swezy 1921; Lebour 1925; Schiller 1933; Hulburt 1957; Konovalova 1998; Okolodkov 1998a; Bérard-Therriault *et al.* 1999; Escobar-Morales & Hernández-Becerril 2015) and our own observations of 15–33 μm demonstrate substantial variation in cell size. This could call into question whether we are dealing with more species of *Kapelodinium*. The size variation does not appear to be linked to recent cell division or food uptake, as small cells in our culture also contained food vacuoles. The size range of unarmoured heterotrophic dinoflagellates can be very large, such as 40–200 μm in *Gyr. spirale* (Hansen & Daugbjerg 2004), 36–75 μm in *Torodinium robustum* (Hansen & Larsen 1992) and 30–100 μm in *Nematodinium armatum* (Dodge 1982).

Kapelodinium vestifici is a cosmopolitan dinoflagellate distributed from arctic (Okolodkov 1998b) to tropical regions (Wood 1963; Escobar-Morales & Hernández-Becerril 2015), and it has often been described as 'common' (Lebour 1917;

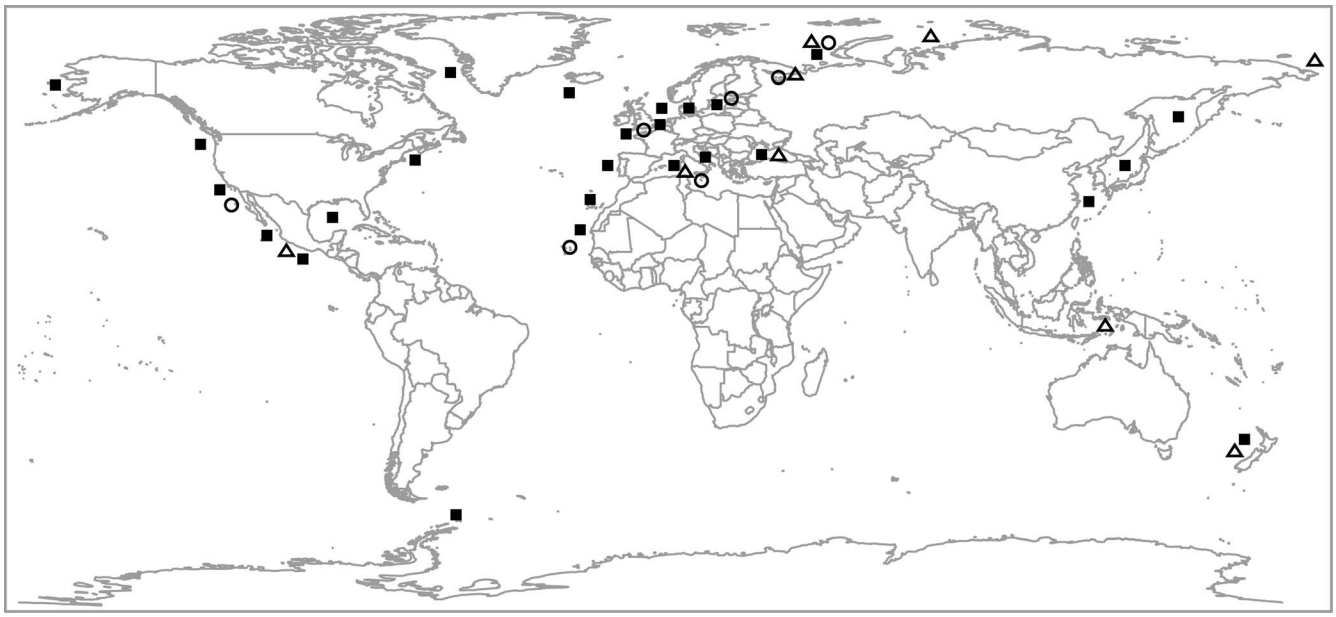


Fig. 42. World map showing the distribution of *Kapelodinium vestifici* by its known synonyms. *Amphidinium extensum* = Δ, *Gymnodinium vestifici* = ○, *Katodinium*/*Gyrodinium glaucum* = ■ (Lohmann 1908; Lebour 1917; Wulff 1919; Kofoid & Swezy 1921; Wailes 1939; Hulburt 1957; Wood 1963; Drebes 1974; Parke & Dixon 1976; Gosselck 1978; Elbrächter 1979; Hansen & Larsen 1992; Paulmier 1992; Konovalova 1998; Okolodkov 1998a, 1998b; Gil-Rodríguez *et al*. 2003; Gomez 2003; Hällfors 2004; Okolodkov & Gárate-Lizarraga 2006; Kim & Kim 2007; Gárate-Lizarraga *et al*. 2009; Sukhanova *et al*. 2009; Cărăuş 2012; Chang *et al*. 2012; Meave-Del Castillo *et al*. 2012; Merino-Virgilio *et al*. 2013; Bessudova *et al*. 2014; BSPC Editorial Board 2015; Escobar-Morales & Hernández-Becerril 2015; Ø. Moestrup, unpublished; U. Tillmann, unpublished).

Hansen & Larsen 1992; Paulmier 1992; Gárate-Lizarraga *et al*. 2009; Sukhanova *et al*. 2009) or even as a bloom-forming species (Gárate-Lizarraga 2014). The distribution of *Kap*. *vestifici* is shown by its known synonyms in Fig. 42. The LSU rDNA sequences of *Kap*. *vestifici* provided by Kim & Kim (2007) from coastal waters of Korea, and by Reñé *et al*. (2015) from the Mediterranean Sea were 100% identical. Our Atlantic Ocean isolate differed by 4 out of 967 aligned bp from the Korean sequence, making them 99.6% identical, and by 4 out of 678 bp from the Mediterranean material, making them 99.4% identical. Thus, current molecular data suggest that they probably form one coherent population.

Sister group

Lebour (1917), Kofoid & Swezy (as *Gymnodinium glaucum*, 1921 p. 390) and Fukuyo *et al*. (1990) all noticed the similarity between *Kap*. *vestifici* and *Torodinium*. The two genera resemble each other in overall cell shape, a very small hypocone and a distinct apical projection described as a cap in *Kapelodinium* (Fig. 44) and a bill in *Torodinium* (Gomez 2009) (Fig. 45). In SEM, the type species of *Torodinium*, *T*. *teredo* (Takayama 1998) and *T*. *robustum* (Bérard-Therriault *et al*. 1999), also display a longitudinally striated epicone; although, this is difficult to see in the light microscope.

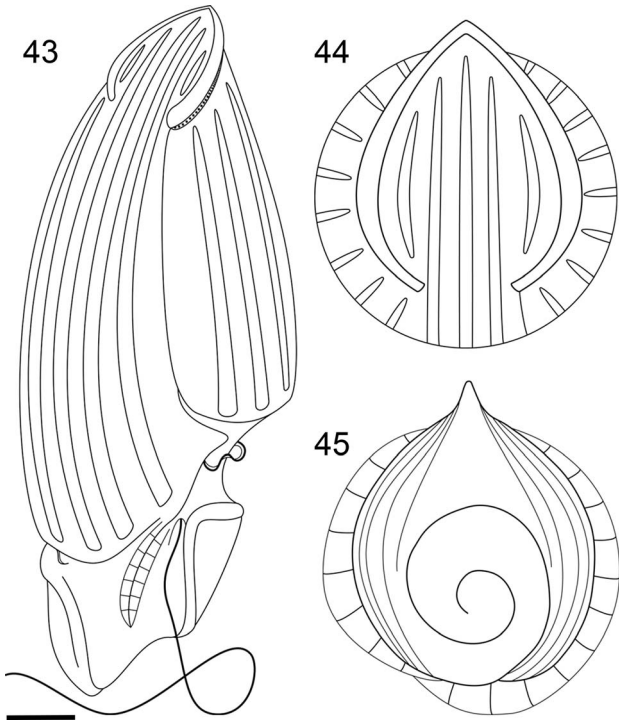
However, morphological features separate the two genera. On cells of *Kap*. *vestifici*, the apical groove is positioned below the edge of the apical cap; whereas, the apical groove on *Torodinium* is a counterclockwise inwards-spiralling loop on top of the apical projection (fig. 32.8–9 in Takayama 1998). Furthermore, both species of *Torodinium* possess a distinct sulcal extension that continues almost to the apex of the cell (Kofoid & Swezy 1921). In *Kap*. *vestifici*, the sulcus terminates at the lower end of the cingulum. Finally, unlike *Kap*. *vestifici*, the two species of *Torodinium* possess chloroplast-like structures placed in longitudinal bands, not clearly connected to the cell surface striae. They have been called rhabdosomes (Kofoid & Swezy 1921; Bérard-Therriault *et al*. 1999), a term otherwise associated with an organelle of unknown function in Dinophysiales (Vesk & Lucas 1986). Ultrastructural studies of *Torodinium* are needed to clarify the nature of the chloroplast-like structures. Light microscopy of *T*. *teredo* (Gomez 2009) revealed both a peduncle and filaments resembling those of a feeding veil; thus, *Torodinium* appears to be mixotrophic.

Kofoid & Swezy (1921) considered *Kap*. *vestifici* (as *Gym*. *glaucum*, p. 390) to be a first step of divergence from *Torodinium*, the two genera being closely related. This early insight is supported by both high-resolution morphology and molecular data. The clade comprising *Kapelodinium* and *Torodinium* is separated from other evolutionary lineages in the phylogenetic tree. As the two genera differ significantly in the position and shape of their ASC (Figs 43–45), we have chosen to erect a family for each genus, Kapelodiniaceae *fam*. *nov*. and Torodiniaceae *fam*. *nov*. Furthermore, as the clade is distinctly separated from other dinoflagellate lineages, we also erect a new order comprising the two families, Torodiniales *ord*. *nov*.

Formal descriptions

Order Torodiniales Boutrup, Moestrup & Daugbjerg *ord. nov*.

DIAGNOSIS: Athecate dinoflagellates with or without chloroplasts. With hat-like apical projection. Epicone considerably larger than hypocone.



Figs 43–45. Drawings of *Kapelodinium* and *Torodinium*.
Fig. 43. Ventral view of *Kap. vestifici* showing ASC, cap-like apical projection, striated cell surface, continuation of cingulum to ASC in a delicate furrow, wide sulcus and ventral ridge continuing into the lip-like structure in the sulcus. Scale bar = 2.5 μm.
Fig. 44. Apex of *Kapelodinium* showing cap-like apical projection with rim and ridges under which the ASC is situated.
Fig. 45. Apex of *Torodinium* (after Takayama 1998), showing counterclockwise spiraling ASC and delicate striations on top of bill-like apical structure.

Family Kapelodiniaceae Boutrup, Moestrup & Daugbjerg *fam. nov*.

DIAGNOSIS: Dinoflagellates without chloroplasts. With cap-like apical projection. Apical structure complex with three rows of vesicles positioned below the rim of the cap. Cells longitudinally striated. Nuclear chambers with pores both to the nucleoplasm and the cytoplasm.

Family Torodiniaceae Boutrup, Moestrup & Daugbjerg *fam. nov*.

DIAGNOSIS: Dinoflagellates with chloroplasts. With bill-like apical projection. Apical structure complex positioned on top of the bill-like apical projection, shaped as a counterclockwise inwards spiral. Cells longitudinally striated.

Ultrastructure and food uptake

PUSULE: The pusule was rather complex and conforms somewhat to what Dodge (1972) described as a 'complex tubular pusule with vesicles'. However, there may be more than one collecting tube, but serial sectioning of the pusule is required to determine whether it constitutes a new type of pusule. The pusule vesicles contained membranous material, which appeared to be emptied into the collecting tubes and discharged into the longitudinal flagellar canal. Similar contents were found in the pusule of *Gyr. spirale* (fig. 39 in Hansen & Daugbjerg 2004), and Dodge & Crawford (1968) also reported such material in the pusule of auxotrophically grown *Amphidinium carterae* Hulburt. From Figs 29 and 30, we interpret the material as being discharged from the cell; whereas, Klut *et al.* (1987) observed macromolecules being taken up through the flagellar canal and accumulating in the pusule. In one case, the macromolecules were observed in small vesicles no longer connected to the pusule, indicating endocytotic uptake (Schnepf & Elbrächter 1992).

NUCLEAR CHAMBERS: The nucleus did not display typical nuclear pores but had sparingly distributed nuclear chambers. The chambers of *Kap. vestifici* protruded into both the nucleoplasm and the cytoplasm. Whereas nuclear chambers and other unusual structures are not uncommon in dinoflagellate nuclei – for example, globular invaginations in *Gyr. spirale* (Hansen & Daugbjerg 2004) and the nuclear chambers of *Gymnodinium fuscum* (Ehrenberg) Stein (Hansen *et al.* 2000) with pores only towards the nucleoplasm – the closest match with the construction of the chambers found in *Kap. vestifici* are the annulated vesicles with opaque contents found in *Noctiluca scintillans* (Macartney) Kofoid & Swezy (Afzelius 1963). However, *N. scintillans* has numerous such vesicles and a fibrous layer below the nuclear envelope not observed in *Kap. vestifici*. This superficial similarity is not an indicator of common ancestry, as *Kapelodinium* and *Noctiluca* are distantly related in the LSU rDNA–based phylogeny (Fig. 34).

CROSS-STRIATED FILAMENTS: The cap of *Kap. vestifici* consisted of a slightly pointed horseshoe-shaped thick rim and five striae (Fig. 13), all of which contained cross-striated filaments below the amphiesma (Figs 20–22). This is a novel cytological feature in an athecate dinoflagellate, and its function is yet unknown. Analogous features have, however, been found immediately beneath the apical pore plate of the two thecate dinoflagellates *Heterocapsa pygmaea* Loeblich III, R.J. Schmidt & Sherley and *Scrippsiella sweeneyae* Balech *ex* A.R. Loeblich III and have been called the apical pore fibrous complex (Roberts *et al.* 1987). The complex in *H. pygmaea* is very similar to that observed in *Kap. vestifici*. It consisted of a striated fibrous C-shaped ring from which striated apical fibres (SAF) radiated posteriorly under the theca, and it appeared to terminate midway between the apical pore and the cingulum. The opening of the ring corresponded to the junction of the pore plate and the canal plate positioned on the ventral side of the cell (Roberts *et al.* 1987). While studying TEM micrographs of *Kap. vestifici*, we came across a longitudinal section of the apical pore region of the prey organism *Azadinium poporum*. It also displayed SAF in the subthecal membrane (not shown). Thus, this feature may be more common in dinoflagellates.

FEEDING: The ventral ridge of the cell contained structures which showed many similarities to peduncles described in other dinoflagellates (Calado & Moestrup 1997; Hansen & Calado 1999). The microtubular strand of the peduncle (MSP) and associated with electron-opaque vesicles are typical components of a peduncle, and in *Kap. vestifici* they were located within the ventral ridge, which is the reported position of peduncles in many other species (Hansen 1992; Schnepf & Elbrächter 1992; Calado *et al.* 1998, 2006). The ventral ridge of *Kap. vestifici* was similar to the feeding apparatus of *Esoptrodinium gemma* Javornicky, which possessed a small peduncle (Calado *et al.* 2006). The lack of amphiesma vesicles at the tip of the ventral ridge, the MSP and the electron-opaque vesicles are comparable features. However, we never observed a peduncle in light microscopy or SEM. Peduncles have been described primarily to suck out cell material from the prey (Hansen 1992); although, prey cells that are small enough to pass through the peduncle can be ingested whole as seen in, for example, *Tyrannodinium edax* (A.J. Schilling) Calado [as *Peridiniopsis berolinensis* (Lemmermann) Bourrelly] and *Esoptrodinium gemma* (Calado & Moestrup 1997; Calado *et al.* 2006). Since *Kap. vestifici* may engulf complete prey that is relatively large (Fig. 5), the peduncle-like structure observed here may have a somewhat different function related to food capture and uptake.

The lip-like suture in the sulcus has not been described in naked dinoflagellates before. It was observed in all *Kap. vestifici* cells which had been well preserved, associated with the lobed ventral ridge. Direct engulfment by heterotrophic dinoflagellates has been described to take place through the

posterior part of the sulcus (Hansen 1992; Schnepf & Elbrächter 1992; Jeong *et al*. 2010). Thus, we suspect that it has a function as an entryway of captured prey into the cell. Perhaps three of the cross-striated filaments found within the central three ridges on the cap (3–5 in Fig. 20), extend to the lip-like suture (Fig. 12), and we speculate that they may play a role in opening the suture by contracting. However, the feeding process of *Kap. vestifici* was not observed, and studies addressing food uptake are needed to fully understand the function of the lip-like structure.

SUPPLEMENTARY DATA

Supplementary data associated with this article is available online at http://dx.doi.org/10.2216/15-138.1.s1.

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REFERENCES

Afzelius B.A. 1963. The nucleus of *Noctiluca scintillans*. Aspects of nucleocytoplasmic exchanges and the formation of nuclear membrane. *Journal of Cell Biology* 19: 229–238.

Bérard-Therriault L., Poulin M. & Bossé L. 1999. *Guide d'identification du phytoplancton marin de l'estuaire et du golfe du Saint-Laurent incluant également certains protozoaires*. Publication spéciale canadienne des sciences halieutiques et aquatique 128. Les presses scientifiques du CNRC, Ottawa. 387 pp.

Bessudova A.Y., Sorokovikova L.M., Firsova A.D., Kuz'mina A.Y., Tomberg I.V. & Likhoshway Y.V. 2014. Changes in phytoplankton community composition along a salinity gradient from the lower Yenisei River to the Kara Sea, Russia. *Botanica Marina* 57: 225–239.

BSPC Editorial Board. 2015. Black Sea phytoplankton checklist. Available at http://phyto.bss.ibss.org.ua (13 November 2015).

Calado A.J. 2011. On the identity of the freshwater dinoflagellate *Glenodinium edax*, with a discussion on the genera *Tyrannodinium* and *Katodinium*, and the description of *Opisthoaulax* gen. nov. *Phycologia* 50: 641–649.

Calado A.J. & Moestrup Ø. 1997. Feeding in *Peridiniopsis berolinensis* (Dinophyceae): new observations on tube feeding by an omnivorous, heterotrophic dinoflagellate. *Phycologia* 36: 47–59.

Calado A.J., Craveiro S.C. & Moestrup Ø. 1998. Taxonomy and ultrastructure of a freshwater, heterotrophic *Amphidinium* (Dinophyceae) that feeds on unicellular protists. *Journal of Phycology* 34: 536–554.

Calado A.J., Craveiro S.C., Daugbjerg N. & Moestrup Ø. 2006.Ultrastructure and LSU rDNA-based phylogeny of *Esoptrodinium gemma* (Dinophyceae), with notes on feeding behaviour and the description of the flagellar base area of a planozygote. *Journal of Phycology* 42: 434–452.

Cărăuş I. 2012. *Algae of Romania. A distributional checklist of actual algae*, ver. 2.3, rev. 3. University of Bacău, Bacău, Romania. 809 pp.

Chang F.H., Charleston T., Mckenna P.B., Clowes C.D., Wilson G.J. & Broady P.A. 2012. Phylum Myzozoa: dinoflagellates, perkinsids, ellobiopsids, sporozoans. In: *New Zealand inventory of biodiversity. Volume Three. Kingdoms Bacteria, Protozoa, Chromista, Plantae, Fungi* (Ed. by D.P. Gordon), pp. 175–216. Canterbury University Press, Christchurch.

Conrad W. 1926. Recherches sur les Flagellates de nos eaux saumâtres. Ie partie: Dinoflagellates. *Archiv für Protistenkunde* 55: 63–100.

Darriba D., Taboada G.L., Doallo R. & Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Daugbjerg N., Hansen G., Larsen J. & Moestrup Ø. 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmored dinoflagellates. *Phycologia* 39: 302–317.

De Wilderman É. 1897. Notes mycologiques. *Annales de la Société Belge de Microscopie* 21: 5–31.

Dodge J.D. 1972. The ultrastructure of the dinoflagellate pusule: a unique osmo-regulatory organelle. *Protoplasma* 75: 285–302.

Dodge J.D. 1982. *Marine dinoflagellates of the British Isles*. Her Majesty's Stationary Office, London. 303 pp.

Dodge J.D. & Crawford R.M. 1968. Fine structure of the dinoflagellate *Amphidinium carteri* Hulburt [*sic*]. *Protistologica* 4: 231–248.

Drebes G. 1974. *Marines Phytoplankton, Eine Auswahl der Helgoländer Planktonalgen (Diatomeen, Peridineen)*. Georg Thieme Verlag, Stuttgart. 186 pp.

Elbrächter M. 1979. On the taxonomy of unarmoured dinophytes (Dinophyta) from the Northwest African upwelling region. *Meteor Forschungs-Ergebnisse* 30: 1–22.

Escobar-Morales S. & Hernández-Becerril D.U. 2015. Free-living marine planktonic unarmoured dinoflagellates from the Gulf of Mexico and the Mexican Pacific. *Botanica Marina* 58: 9–22.

Fott B. 1957. Taxonomie drobnohledné flory našich vod. *Preslia* 29: 278–319.

Fukuyo Y., Takano H., Chihara M. & Matsuoka K. 1990. *Red tide organisms in Japan. An illustrated taxonomic guide*. Uchida Rokakuho Co., Ltd, Tokyo. 430 pp.

Gárate-Lizárraga I. 2014. Proliferation of *Levanderina fissa* and *Polykrikos hartmannii* (Dinophyceae: Gymnodiniales) in Bahìa de la Paz, Gulf of California, México. *CICIMAR Oceánides* 29: 25–35.

Gárate-Lizárraga I., Band-Schmidt C.J., Aguirre-Bahena F. & Grayeb Del Alamo T. 2009. A multi-species microalgae bloom in Bahía de La Paz, Gulf of California, Mexico (June 2008). *CICIMAR Oceánides* 24: 1–15.

Gil-Rodríguez M.C., Haroun R., Rodríguez A.O., Zugasti E.B., Santana P.D. & Morán B.H. 2003. Protoctista. In: *Lista de especies marinas de Canarias (algas, hongos, plantas y animales) 2003* (Ed. by L. Moro, J.L. Martín, M.J. Garrido & I. Izquierdo). Consejería de Política Territorial y Medio Ambiente del Gobierno de Canarias, Las Palmas de Gran Canaria, Canary Islands. 248 pp.

Gomez F. 2003. Checklist of Mediterranean free-living dinoflagellates. *Botanica Marina* 46: 215–242.

Gomez F. 2009. *Torodinium* and *Pavillardia* (Gymnodiniales, Dinophyceae): two unarmoured dinoflagellates with a body extension, collected from the open Pacific Ocean. *Protistology* 6: 131–135.

Gosselck F., Kell V. & Spittler P. 1978. On the feeding of *Branchiostoma senegalense* (Acrania: Branchiostomidae). *Marine Biology* 46: 175–179.

Guillard R.R.L. & Ryther J.H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian Journal of Microbiology* 8: 229–239.

Guindon S. & Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

Guiry M.D. & Guiry G.M. 2015. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 3 August 2015.

Hällfors G. 2004. Checklist of Baltic Sea phytoplankton species (including some heterotrophic protists). *Baltic Sea Environment Proceedings* 95: 1–208.

Hansen G. 1995. Analysis of the thecal plate pattern in the dinoflagellate *Heterocapsa rotundata* (Lohmann) comb. nov. (=*Katodinium rotundatum* (Lohmann) Loeblich). *Phycologia* 34: 166–170.

Hansen G. & Daugbjerg N. 2004. Ultrastructure of *Gyrodinium spirale*, the type species of *Gyrodinium* (Dinophyceae), including a phylogeny of *G. dominans*, *G. rubrum* and *G. spirale* deduced from partial LSU rDNA sequences. *Protist* 155: 271–294.

Hansen G. & Larsen J. 1992. Dinoflagellater i danske farvande. In: *Plankton i de indre danske farvande* (Ed. by H.A. Thomsen), pp. 45–155. Havforskning fra Miljøstyrelsen, Copenhagen.

Hansen G., Moestrup Ø. & Roberts K.R. 2000. Light and electron microscopical observations on the type species *Gymnodinium*, *G. fuscum* (Dinophyceae). *Phycologia* 39: 365–376.

Hansen P.J. 1992. Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. *Marine Biology* 114: 327–334.

Hansen P.J. & Calado A.J. 1999. Phagotrophic mechanisms and prey selection in free-living dinoflagellates. *Journal of Eukaryotic Microbiology* 46: 382–389.

Hansen-Ostenfeld C. 1913. *De danske farvandes plankton i årene 1898–1901*. Det kongelige danske videnskabernes selskabs skrifter, 7. række, afd. 9 (2). Copenhagen. 364 pp.

Hulburt E.M. 1957. The taxonomy of unarmored Dinophyceae of shallow embayments on Cape Cod, Massachusetts. *Biological Bulletin* 112: 196–219.

Jaanus A. 2003. Water environment of Haapsalu Bay in retrospect (1975–2000). *Proceedings of the Estonian Academy of Sciences, Biology and Ecology* 52: 91–111.

Jeong H.J., Yoo Y.D., Kim J.S., Seong K.A., Kang N.S. & Kim T.H. 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Science Journal* 45: 65–91.

Kim K.-Y. & Kim C.-H. 2007. Phylogenetic relationships among diverse dinoflagellate species occurring in coastal waters off Korea inferred from large subunit ribosomal DNA sequence data. *Algae* 22: 57–67.

Klut M.E., Bisalputra T. & Antia N.J. 1987. Some observations on the structure and function of the dinoflagellate pusule. *Canadian Journal of Botany* 65: 736–744.

Kofoid C.A. & Swezy O. 1921. The free-living unarmoured Dinoflagellata. *Memoirs of the University of California* 5: 1–564.

Konovalova G.V. 1998. *Dinoflagellates (Dinophyta) of the Far-Eastern seas of Russia and adjacent areas of the Pacific Ocean*. Dalnauka, Vladivostok. 300 pp.

Lebour M.V. 1917. The Peridiniales of Plymouth Sound from the region beyond the breakwater. *Journal of the Marine Biological Association of the United Kingdom* 11: 183–200.

Lebour M.V. 1925. *The Dinoflagellates of Northern Seas*. Mayflower Press, Plymouth, UK. 250 pp.

Loeblich A.R. III. 1965. Dinoflagellate nomenclature. *Taxon* 14: 15–18.

Lohmann H. 1908. Untersuchungen zur Feststellung der vollständigen Gehaltes des Meeres an Plankton. *Wissenschaftliche Meeresuntersuchungen, Abt. Kiel* 10: 131–370.

Meave-Del Castillo M.E., Zamundio-Resendiz M.E. & Castillo-Rivera M. 2012. Riqueza fitoplanctónica de la Bahía de Acapulco y zona costera aledaña, Guerrero, México. *Acta Botanica Mexicana* 100: 405–487.

Merino-Virgilio F. Del C., Okolodkov Y.B., Aguilar-Trujillo A.C. & Herrera-Silveira J.A. 2013. Phytoplankton of the northern coastal and shelf waters of the Yucatan Peninsula, southeastern Gulf of Mexico, Mexico. *Check List* 9: 771–779.

Moestrup Ø. & Daugbjerg N. 2007. On dinoflagellate phylogeny and classification. In: *Unravelling the algae: the past, present, and future of algal systematics* (Ed. by J. Brodie & J. Lewis), pp.215–230. CRC Press, Boca Raton, Florida.

Moestrup Ø., Hakanen P., Hansen G., Daugbjerg N. & Ellegaard M. 2014. On *Levanderina fissa* gen. & comb. nov. (Dinophyceae) (syn. *Gymnodinium fissum*, *Gyrodinium instriatum*, *Gyr. uncatenum*), a dinoflagellate with a very unusual sulcus. *Phycologia* 53: 265–292.

Nunn G.B., Theisen B.F., Christensen B. & Arctander P. 1996. Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda. *Journal of Molecular Evolution* 42: 211–223.

Okolodkov Y.B. 1998a. Unarmoured dinoflagellates collected during the cruise of the r/v Oceania to the European Arctic, in June–August 1992 and 1993. *Polish Polar Research* 19: 211–218.

Okolodkov Y.B. 1998b. A checklist of dinoflagellates recorded from the Russian Arctic Seas. *Sarsia* 83: 267–292.

Okolodkov Y.B. & Gárate-Lizárraga I. 2006. An annotated checklist of dinoflagellates (Dinophyceae) from the Mexican Pacific. *Acta Botanica Mexicana* 74: 1–154.

Parke M. & Dixon P.S. 1976. Check-list of British marine algae – third revision. *Journal of the Marine Biological Association of the United Kingdom* 56: 527–594.

Paulmier G. 1992. *Catalogue illustré des microphytes planctoniques et benthiques des côtes Normandes*. IFREMER, DRV-92.007-RH/Le Robert. 108 pp.

Reñé A., Camp J. & Garcés E. 2015. Diversity and phylogeny of Gymnodiniales (Dinophyceae) from the NW Mediterranean Sea revealed by a morphological and molecular approach. *Protist* 166: 234–263.

Roberts, K.R., Timpano P. & Montegut A.E. 1987. The apical pore fibrous complex: a new cytological feature of some dinoflagellates. *Protoplasma* 137: 65–69.

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Schiller J. 1933. Dinoflagellatae (Peridineae) in monographischer Behandlung. In: *Rabenhorst's Kryptogamen-flora von Deutschland, Österreich und der Schweiz*, 10 (3). 1. Teil, 3. Ausgabe, 433–617. Akademische Verlagsgesellschaft, Leipzig.

Schnepf E. & Elbrächter M. 1992. Nutritional strategies in dinoflagellates. A review with emphasis on cell biological aspects. *European Journal of Protistology* 28: 3–24.

Scholin C.A., Herzog M., Sogin M. & Anderson D.M. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology* 30: 999–1011.

Schütt F. 1895. Die Peridineen der Plankton-Expedition. *Ergebnisse der Plankton-Expedition der Humboldt-Stiftung* 4: 1–170.

Schütt F. 1896. Gymnodiniaceae, Prorocentraceae, Peridiniaceae, Bacillariaceae. In: *Die natürlichen Pflanzenfamilien* (Ed. by A. Engler & K. Prantl). Wilhelm Engelmann, Leipzig, 1ste Augabe, Teil 1, Abt B, pp. 1–153.

Sukhanova I.N., Flint M.V., Pautova L.A., Stockwell D.A., Grebmeier J.M. & Sergeeva V.M. 2009. Phytoplankton of the western Arctic in the spring and summer of 2002: structure and seasonal changes. *Deep-Sea Research II* 56: 1223–1236.

Swofford D.L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, ver. 4. Sinauer Associates, Sunderland, Massachusetts.

Takayama H. 1985. Apical grooves of unarmored dinoflagellates. *Bulletin of Plankton Society of Japan* 32: 129–140.

Takayama H. 1998. *Morphological and taxonomical studies of the free-living unarmored dinoflagellates occurring in the Seto Inland Sea and adjacent waters*. PhD thesis. University of Tokyo, 211 pp. (in Japanese). The *Torodinium* plate can be viewed online at http://www.geocities.jp/takayama_haruyoshi/HAB/Torodinium_teredo.html (5 October 2015).

Throndsen J., Hasle G. R. & Tangen K. 2003. *Norsk kystplankton flora*. Almater Forlag AS, Oslo. 341 pp.

Tillmann U., Elbrächter M., John U. & Krock B. 2011. A new non-toxic species in the dinoflagellate genus *Azadinium*: *A. poporum* sp. nov. *European Journal of Phycology* 46: 74–87.

Van De Peer Y., Van Der Auwera G. & De Wachter R. 1996. The evolution of stramenopiles and alveolates as derived by 'substitution rate calibration' of small ribosomal subunit RNA. *Journal of Molecular Evolution* 42: 201–210.

Vesk M. & Lucas I.A.N. 1986. The rhabdosome: a new type of organelle in the dinoflagellate *Dinophysis*. *Protoplasma* 134: 62–64.

Wailes G.H. 1939. *Canadian Pacific fauna. 1. Protozoa. 1(e). Mastigophora*. University of Toronto Press, Fisheries Research Board of Canada, Toronto. 45 pp.

Waterhouse A.M., Procter J.B., Martin D.M.A, Clamp M. & Barton G.J. 2009. Jalview version 2 – a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25: 1189–1191.

Wood E.J.F. 1963. Dinoflagellates in the Australian region III. Further collections. Technical paper. Division of Fisheries and Oceanography C.S.I.R.O. Australia 17: 1–20.

Wulff A. 1919. Ueber das Kleinplankton der Barentssee. *Wissenschaftliche Meeresuntersuchungen Kiel*, 13, Heft 1: 95–125.

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