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 amphimesial 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 hypcone 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, Wulf (1919) described the species Amphidinium extensum Wulf from the Barents Sea, which revealed strong similarities to Gym. vestifici. Wulf was aware of this close resemblance, but as he believed that his material lacked a sulcus, he erected it as a new species. At 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,
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* (Dredes 1974; Elbrächter 1979; Fukuyo *et al.* 1990; Hansen & Larsen 1992; Konovalova 1998; Okolodkov 1998b; Béard-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 have recently been used to redefine the genera of naked dinoflagellates. 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 *Opisthostoxa* due to the presence of a type C eyespot characteristic of the Tovelliaeae (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. nieuporteense* (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. extentum* 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 ARCHEMHB 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 photocyte 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% OsO₄ (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% OsO₄ (final concentration) in f/2 medium (Guillard & Ryther 1962) for 30 min at 4°C. After centrifugation for 5 min at 2300 rpm (≈ 1000 × 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% OsO₄ 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% OsO₄ (final concentrations) in f/2 medium for 1 h at room temperature. After centrifugation for 5 min at 2300 rpm (≈ 1000 × 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...
Glutaraldehyde and 0.5% OsO4 cross-striated filaments were well preserved (Fig. 2). However, trichocysts and amphiesma were poorly preserved using this method, and all amphiesma material was embedded as described above. Overall, cells were relatively few cells, we added 1 ml of Pyramimonas parkeae (R.E. Norris & 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% OsO4 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 95%), 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 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. 2).

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 H2O 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*. 

**Fig. 11.** Detail of the apical region showing the apical pore (arrow).

**Fig. 12.** Close-up view of the apical pore (arrow).

**Fig. 13.** Demonstration of the pore opening (arrow).

**Fig. 14.** Side view of the apical region showing the pore opening (arrow).

**Fig. 15.** Close-up view of the apical region showing the pore opening (arrow).
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) RAINTOx 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 v1.4.5 sequence editor (Waterhouse et al. 2012), which, among other things, 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.

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Ultraceutural 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, Tetrarhymena thermophile (Nannen) McCoy, T. pyriformis (Ehrenberg) Lwoff, Spathidium amphorfiforme], four apicomplexans (Hammondia hammondi, Neospora canum, Toxoplasma gondii Nicolle & Manceaux, Sarcozystis 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.


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

ETYMOLOGY: Prefix kapelo- (from modern Greek καπέλο), ‘cap’, referring to the cap-like apical structure. The termination -dinium, originally from Greek δίνη, ‘vortex’, is commonly applied to dinoflagellates.

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 amphimesial 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 amphimesial 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 cingulum border of the cingulum (a), below which is a depression (b). The amphimesial vesicles on the hypocone are polygonal (c). Scale bars: 2 μm.

**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 *Kapeldium 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.

**Fig. 18.** Longitudinal section from the same cell as Fig. 17. The two basal bodies are inserted perpendicular to each other, \( \text{tb} = \) the transverse flagellum base, \( \text{lb} = \) 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 trichocysts 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). Ifc = 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.
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 dorsosuturally 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, dino-
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 (≥ 0.5) from BA, and the last numbers are bootstrap values (≥ 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.
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 *Kaploidinium* 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 stratified like the epicone. In fact, in one of our light micrographs, the hypocone appeared to be stratified 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 striae 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*.

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; IF, 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).
if turned upside down. She also noted that Gymn. 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 glaucaum 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 (Gosselek 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 been no 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 Hållfors (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.

**Kapetidinium**: As the type species of Kapetidinium, 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 Kapetidinium. 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 Gymrodinium 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 Gymnodinium 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 Reá et al. (2015), using both SSU and LSU rDNA gene sequences, also found a close phylogenetic relationship between Torodinium and Kapelodinium (as Kapetidinium).

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;
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 Kapelodinium vestifici is shown by its known synonyms in Fig. 42. The LSU rDNA sequences of Kapelodinium vestifici provided by Kim & Kim (2007) from coastal waters of Korea, and by René 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


However, morphological features separate the two genera. On cells of Kapelodinium 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 Kapelodinium vestifici, the sulcus terminates at the lower end of the cingulum. Finally, unlike Kapelodinium 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-Theriault 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 Kapelodinium vestifici (as Gymnodinium 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 Bøstrup, Moestrup & Daugaard ord. nov.

**Diagnosis:** Aethecate dinoflagellates with or without chloroplasts. With hat-like apical projection. Epicone considerably larger than hypocone.
reported such material in the pusule of auxotrophically grown Hansen & Daugbjerg 2004), and Dodge & Crawford (1968) also

Similar contents were found in the pusule of collecting tubes and discharged into the longitudinal flagellar canal. membranous material, which appeared to be emptied into the serial sectioning of the pusule is required to determine whether it what Dodge (1972) described as a 'complex tubular pusule with

PUSULE Ultrastructure and food uptake

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.


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 FILAMENT: 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 (Robertson et al. 1987). This 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 Axadinium 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 & Moestrup 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 Esotroplidium genmaa 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 (Lemmerrmann) Bourrelly] and Esotroplidium genmaa (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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southeastern Gulf of Mexico, Mexico.

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Prey selection in free-living dinoflagellates. 

K.-Y. & K

Gyrodinium spirale

katodinium rotundatum

Gyrodinium fuscum

microscopical observations on the type species


1898–1901


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G. & D

A.J. 1999. Phagotrophic mechanisms and

Katodinium glaucum

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G. spirale

Gyr. uncatenum

G. rubrum


On Levanderina fissa gen. & comb. nov. (Dinophyceae) (syn. Gymnodinium fuscum, Gymnodinium intristium, Gym. uncatenum), a dinoflagellate with a very unusual sulcus.


Gyrodinium spirale

Gyrodinium uncatenum

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