On *Levanderina fissa* gen. & comb. nov. (Dinophyceae) (syn. *Gymnodinium fissum*, *Gyrodinium instriatum*, *Gyr. uncatenatum*), a dinoflagellate with a very unusual sulcus

Ovind Møestrup1*, Paivi Hakanen2, Gert Hansen1, Niels Daugbjerg1, and Marianne Elleegaard3

1 Marine Biological Section, Department of Biology, University of Copenhagen, Universitetsparken 4, DK-2100 Copenhagen Ø, Denmark
2 Marine Research Centre, Finnish Environment Institute, P.O. Box 140, FI-00251 Helsinki, Finland
3 Department of Plant and Environmental Sciences (PLEN), University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

**ABSTRACT:** *Gymnodinium fissum* was described by Levander in 1894 from the Baltic Sea near Helsinki, and we argue, on the basis of morphological and molecular studies of material from the type locality, and on cultures from the Åland islands, Puerto Rico, Portugal and United States identified as *Gyrodinium instriatum, Gymnodinium instriatum, Gyrodinium uncatenatum* and *Gyrodinium sp.*, that all these taxa are conspecific. They are morphologically and genetically distinct from *Gymnodinium* and are described here as *Levanderina fissa* gen. & comb. nov. This species also includes *Gyrodinium pavillardii*. Levander observed chloroplasts in the cell and on some occasions diatoms, probably the first report of mixotrophy in a dinoflagellate. Biecheler in 1952 described the process of food uptake in *Gyr. pavillardii*, feeding it with ciliates and other dinoflagellates. Prey was taken up through the posterior part of the sulcus, some prey items being almost as large as the host. Our observations showed that the longitudinal flagellum, in contrast to what has been described in all other dinoflagellates possessing a longitudinal sulcal furrow, is not located in the furrow but in a separate, internal tube beneath the sulcal furrow. The tube opened to the exterior dorsally near the posterior end of the cell, and the sulcus appeared to be used for food uptake only. The cytoskeleton of *L. fissa* was complex and included a large number of muscle-like fibres. Food uptake using the sulcus involved major changes of cell shape, which requires the presence of a highly flexible cytoskeleton. *Levanderina fissa* was not morphologically or genetically close to any other dinoflagellate for which molecular sequences were available. The detailed structure of the apical furrow or acrobase comprised three rows of elongate vesicles, one row forming the bottom of a furrow. The new term apical structure complex (ASC) is introduced as a general term to replace apical furrow or acrobase, none of which adequately describes all the many known types. The ASC in *Levanderina* may be characteristic of most if not all species of the Gymnodiniinae (an apomorphy of the order?) and different from the types present in the Suesiales, the other order of mainly thin-walled dinoflagellates.

**KEY WORDS:** Dinoflagellates, Mixotrophy, Phylogeny, Ultrastructure

**INTRODUCTION**

In 1894 Levander described a new unarmoured dinoflagellate from coastal waters near the island of Lövö in southern Finland, naming it *Gymnodinium fissum* Levander (Levander 1894). Shortly afterward it was transferred by Lemmermann (1900) to the genus *Spirodinium* [S. *fissum* (Levander) Lemmermann], and subsequently Kofoid & Swezy (1921) moved it to their new genus *Gyrodinium* [G. *fissum* (Levander) Kofoid & Swezy]. The latter change was due to the cingular displacement exceeding 1/5 of the body length, the generic definition of *Gyrodinium*. Levander’s description was quite detailed with respect to cell shape, cingulum, sulcus and internal structures. He mentioned, but did not illustrate, delicate surface striations that were present on a few cells. It was therefore not in accordance with his description when Kofoid & Swezy (1921) depicted cells with distinct striae from offshore La Jolla, California, USA, which they claimed to belong to Levander’s species. Kofoid & Swezy were even able to count the striae: ‘24 across the ventral surface of the epicone, and twice as many on the hypocone’. When Biecheler found material from Étang de Thau, France, which was very similar to Levander’s material, the lack of striation and presence of metabolic activity probably led her to describe it as a new species, *Gyrodinium pavillardii* ’[pavillardii]’ Biecheler (Biecheler 1952), although these features were not mentioned in her first, more incomplete report of the species (Biecheler 1934). The lack of striations finally led Freudenthal & Lee (1963), who seemed to be unaware of Biecheler’s descriptions of *Gyr. pavillardii*, to establish the new species *Gyr. instriatum* Freudenthal & Lee on the basis of material from Long Island, New York, USA. They stressed its strong resemblance to Levander’s *Gyr. fissum* and to *Gyr. uncatenatum* Hulburt, a species described a few years before from Uncatena Island near Woods Hole, Massachusetts, USA (Hulburt 1957).

*Gyrodinium instriatum* has subsequently been found in fully saline and brackish waters from many areas of the world. It was transferred to *Gymnodinium* as *Gymnodinium instriatum* (Freudenthal & Lee) Coats because of the similarity between its apical furrow and that of *Gymnodinium* (Coats & Park 2002). Hållfors (2004) regarded *Gyr. instriatum* and *Gyr. pavillardii* as junior synonyms of *Gyr. fissum*, and did not agree that *Gyr. fissum sensu* Kofoid & Swezy (1921) represented Levander’s species. His conclusion was very
Table 1. List of dinoflagellate cultures examined in the present study. The strains with CCMP codes in parentheses were originally deposited in the NCMA culture collection, but were used for this study from the SCCAP collection.

<table>
<thead>
<tr>
<th>Species (as indicated in culture collection)</th>
<th>Origin</th>
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<td>Gymnodinium sp.</td>
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* Strain from the type locality of Gymnodinium fissaum.

plausible but has not been generally accepted. Our purpose was therefore, by combining molecular and ultrastructural analyses, to investigate the possible synonymy of Gyr. fissaum and Gyr. instriatum, on the basis of material collected from several localities in the Baltic Sea, including the type locality of Gyr. fissaum and material of Gyr. instriatum from several different geographical localities.

Furthermore, with the advent of the molecular era, numerous studies on dinoflagellate phylogeny have consistently shown that Gymnodinium instriatum and Gyr. uncatenum form a well-supported clade, distinct from Gyr. spirale, the type species of Gymnodinium, and from all other unarmoured dinoflagellates studied so far (e.g. Saldarriaga et al. 2004; Kim & Kim 2007), suggesting that it belongs in a genus of its own. In the present study, material considered to represent Levander’s species is compared with the taxonomic criteria introduced to define unarmored dinoflagellates by Daugbjerg et al. (2000). The system proposed by these authors, and supported by molecular data, was based primarily on ultrastructural details such as the structure of the apical groove, the nuclear envelope, and details of the flagellar apparatus. The findings reported during the present study have led us to propose a new genus, Levanderina gen. nov. with a single known species, Levanderina fissa comb. nov.

MATERIAL AND METHODS

Cultures used during the present study were retrieved from culture collections or established from water or sediment samples collected in the Baltic region. The cultures were established by single-cell or cyst isolations during the period July 2010–July 2011; the cultures included several strains from the Åland Archipelago as well as strain K-1769 from Lövö, the type locality of Gymnodinium fissaum. The cultures were grown at 16°C in f/2 medium (salinity = 6). Two strains from Åland and one from Lövö were deposited in the Scandinavian Culture Collection for Algae and Protozoa (SCCAP) at the University of Copenhagen (Table 1). Other cultures were obtained from SCCAP (K strain codes) and Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) (CCMP strain code). Growth conditions for the different K strains are available at the home page of SCCAP (http://www.sccap.dk) and for the CCMP strains at the home page of NCMA (https://ncma.bigelow.org). Strain CCMP1310 originates less than 20 km from the type locality of Gymnodinium uncatenum and strain K-0641 around 300 km from the type locality of Gyr. instriatum.

Table 1 summarizes information on strain numbers and origin of cultures for which molecular sequences were determined during the present study.

Live cultures were examined using Olympus BX51/Provis AX70; Olympus Corp., Tokyo, Japan) or Zeiss Axioskop (Carl Zeiss, Göttingen, Germany) light microscopes equipped with differential interference contrast optics. Photodocumentation was performed using Zeiss Axio Cam HR or Olympus DP72 digital cameras, or a SVCam 085 digital video camera (SVS-Vistek Cameras, Seefeld, Germany). Images from video sequences were grabbed using Video Savant Pro (IO Industries Inc., Ontario, Canada). Chloroplast autofluorescence was studied in live cells using an inverted Olympus IX81 microscope equipped with a disk-spinning unit. Micrographs were taken with a black and white digital camera FViewII (Olympus Soft Imaging System, Tokyo, Japan).

For scanning electron microscopy (SEM) of the vegetative stage, cells were fixed for 20 min in a final concentration of 2% OsO₄ and 0.1% glutaraldehyde in distilled water. Strains K-0675 and K-1067 were fixed only in 2% OsO₄ in 0.2-μm filtered seawater. After fixation, cells were coated onto an Isopore membrane filter (8 μm pore size) (Millipore) and washed with distilled water for 30 min. They were dehydrated in an ethanol series: 10 min in each concentration of 30%, 50%, 70%, 90%, 96% and 99.9%, followed by two steps of 30 min each in absolute ethanol at 4°C. Cells were critical-point-dried in a Bal-Tec CPD 030 (Bal-Tec AG, Balzers, Liechtenstein) and sputter coated with platinum-palladium or gold in a JEOL JFC-2300HR sputter (JEOL Ltd., Tokyo, Japan) before examination in a JEOL JSM-6333F field-emission scanning electron microscope operated at 7 kV. For SEM of the resting stage, c. 40 cysts of the K-1727 culture were isolated and fixed in acid Lugol’s solution for 30 min. They were subsequently treated and examined as described above.

For transmission electron microscopy (TEM), strain K-1727 was fixed using two different schedules. Schedule 1: equal volumes were mixed of culture and 4% glutaraldehyde made up in 0.2 M Na-cacodylate buffer and containing 0.1 M sucrose at pH 7.8, and the cells were fixed for 2 h at 4°C. They were pelleted by centrifugation, and washed with 0.2 M...
species and indicated sequences, respectively.

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<th>GUSW00a/</th>
<th>GUDE000/</th>
<th>K-1727/</th>
<th>K-1067/</th>
<th>K-1769/</th>
<th>K-675/</th>
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amplification was in 25 μl of a solution containing 2.5 units of PuReTaq DNA polymerase, 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂, 200 μM each of deoxynucleotide triphosphate, stabilizers, and bovine serum albumin (BSA) (illustra PuReTaq Ready-To-Go™ PCR beads, GE Healthcare Bio-Sciences Corp., Piscataway, New Jersey, USA), 1 μl of extracted sample and 1 μl of each 10 μM primer. PCR conditions comprised an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 2 min, annealing at 54°C for 4 min, extension at 72°C for 2 min, and a final extension at 72°C for 7 min. DNA fragments were checked in a 2% agarose gel containing ethidium bromide and visualized with ultraviolet light. Successful PCR products were sent for sequencing to the Institute of Biotechnology (University of Helsinki, Finland). PCR products were purified here by MultiScreen PCR-XL (Merck-Millipore Corp., Billerica, Massachusetts, USA) before sequencing using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA), following the protocol recommended by the manufacturer. The sequencing reactions were cleaned using an Agencourt® CleanSEQ kit (Beckman Coulter Inc., Brea, California, USA) and run with an ABI 3130XL Genetic Analyzer (16 capillaries) or an ABI 3730 DNA Analyzer (48 capillaries) (Applied Biosystems). Primers used in sequencing were D1R, D2R and D2C (Nunn et al. 1996).

For strain CCMP 1310, amplification conditions and temperature profiles were as described in Hansen and Daugbjerg (2011). PCR products were purified following the instructions of the QiAquick PCR purification Kit (Qiagen). Nucleotide sequences were determined using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, California, USA), according to the recommendation of the manufacturer. The cycle sequencing reactions were run on an ABI PRISM 377 DNA Sequencer (Perkin Elmer). We aimed at determining the LSU rDNA sequence from a number of dinoflagellates that morphologically resembled Gymnodinium uncatenum, Gym. intristiun and Gymnodinum fiscum, and we included sequences of eight strains, in addition to the strains from Finland. The sequences and
the LSU rDNA sequence from a single isolate of *Gyr. instriatum* and two isolates of *Gyr. uncatenum* available in GenBank were added to a data matrix recently compiled to describe the dinoflagellate genus *Moestrupia* (Hansen & Daugbjerg 2011). This alignment comprised 71 taxa, and due to ambiguous alignment of the highly divergent domain D2 (sensu Lenaers et al. 1989) this domain was omitted, leaving 746 base pairs. Of the 71 taxa, 62 were dinoflagellates, the rest being ciliates (three taxa), apicomplexans (five taxa) and the perkinsid *Perkinsus*. The latter groups comprised the outgroup. The sequence data matrix was edited using SeaView (ver. 4.3.2) by Gouy et al. (2010). For phylogenetic
Figs 13–17. SEM of *Levanderina fissa* comb. nov. (K-1769) from the type locality of *Gymnodinium fissum* at Lövö, Finland.

**Fig. 13.** Ventral view, ASC, transverse (tf) and longitudinal flagellum (lf). The longitudinal furrow appears empty as it does not contain the longitudinal flagellum. This flagellum runs in an internal tube that opens in the posterior part of the cell; compare with Fig. 23. Scale bar = 1 μm.

**Fig. 14.** Apical view, showing the long U-shaped ASC. Scale bar = 1 μm.

**Fig. 15.** Planozygote in right lateral view. Notice the duplicated transverse flagellum (arrows) and faint striations (arrowhead). Scale bar = 10 μm.

**Fig. 16.** ASC consists of three rows of rectangular amphiesmal vesicles (arrows), the topmost row located at a slightly deeper level in the cell. Fine fibrils extrude from the central row (arrowhead). Scale bar = 1 μm.

**Fig. 17.** Cingulum comprises eight horizontal rows of amphiesmal vesicles (asterisks). Arrow indicates the amphiesmal vesicle on the epicone. Scale bar = 1 μm.
inference we used two approaches: Bayesian analysis (BA) as implemented in MrBayes (ver. 3.1.2 by Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) analysis as implemented in PhyML (ver. 3.0 by Guindon & Gascuel 2003). BA used a general time-reversible substitution model with base frequencies and substitution rate matrix estimated from the data. It was run for two million Markov chain Monte Carlo generations with four parallel chains. For every 50th generation a tree was sampled, and the burn-in was evaluated by plotting the LnL values as a function of generations using Microsoft Excel. The graph revealed a burn-in after 20,050 generations, leaving 39,600 trees for

Figs 18–21. SEM of *Levanderina fissa* comb. nov. Scale bars = 10 μm.

Fig. 18. Strain K-1768 (*Gymnodinium fissum*) from Åland, Finland, in ventral view.

Fig. 19. Strain K-0641 (*Gyrodinium instriatum*) from the Rhode River, USA, planozygote in ventral view. Both the longitudinal and the transverse flagella have duplicated (arrowhead and arrow, respectively).

Fig. 20. Strain K-1067 (*Gyrodinium instriatum*) from Gulf of Mexico, Texas, in ventral view.

Fig. 21. Strain K-0675 (*G. instriatum*) from Portugal, vegetative cell (small arrow) and planozygote (large arrow), both in ventral view. ASC, apical structure complex in all figures.
estimating posterior probabilities. Hence, 401 trees were discarded. Posterior probability values were obtained from a 50% majority-rule consensus of the 39,600 saved trees using PAUP* (ver. 4b10 of Swofford 2003). Before running the ML analysis, we used Modeltest (ver. 3.7 by Posada & Crandall 1998) to choose the best among 56 predefined models for our data set (results not shown). To understand the robustness of the ML tree topology we used bootstrapping with 100 replications. BA was run on a desktop computer, whereas we used the South of France bioinfor-

Figs 22–25. SEM of *Levanderina fissa* comb. nov., all showing strain K-1273 from Puerto Rico.

Fig. 22. Ventral view. Scale bar = 10 μm.
Fig. 23. Dorsal view showing the deeply incised and the dorsal emergence of the longitudinal flagellum from the sulcus (arrow). Scale bar = 10 μm.
Fig. 24. Details of the ASC. The amphiesmal vesicles of the central row possess small knobs (arrows). Scale bar = 1 μm.
Fig. 25. Planozygote in apical view. The transverse flagellum has duplicated (arrow). Scale bar = 1 μm.
matics platform at http://www.atgc-montpellier.fr/phylm/ for PhyML analyses.

To elucidate in greater detail the relationship between the dinoflagellates of particular interest we compiled a second alignment comprising the boxed-in species in Fig. 75. Since these dinoflagellates had very similar LSU rDNA sequences we were able to include the divergent domain D2. For this phylogenetic inference we used the type species of Gymnodinium (viz. Gym. fuscum) as outgroup. Posterior probabilities in BA and bootstrap values (100 replications) in ML were performed to obtain an understanding of the robustness of the tree topology (Fig. 76).

We used PAUP* to estimate the divergence between unique sequences (Table 2). Since some of the determined LSU rDNA sequences were identical, only a single representative sequence was included in all pair-wise comparisons (indicated in Table 2). Estimates of pair-wise comparisons were based on the Kimura two-parameter model. The sequence divergence estimates included 963 base pairs.

RESULTS

**Levanderina gen. nov.**

**DESCRIPTION:** Unarmoured dinoflagellates with U-shaped apical groove surrounding the cell apex and opening on the ventral side of the cell. Apical groove with three rows of vesicles. Nuclear envelope without vesicular chambers. Nucleus connected to the flagellar apparatus via a finger-like projection. Chloroplasts present. The sulcus divided into an inner tube containing the longitudinal flagellum and an outer, open furrow. Cell division in the motile stage.

**TYPE SPECIES:** Levanderina fissa (Levander) comb. nov.


**ETYMOLOGY:** The genus is named after Prof. Kaarlo Mainio Levander (1867–1943), primus motor in marine plankton research in Finland. Prof. Levander found and described the type and presently only species of the genus.

All strains studied by us had essentially the same morphology and although some variation was noted, no consistent differences were observed (Figs 1–12). Cells were characteristically ovoid and varied from spherical to slightly laterally or dorsoventrally compressed. All strains exhibited considerable variation in cell size. Dividing cells and planozygotes with two longitudinal flagella were frequent in the cultures. *Levanderina fissa* strain K-1769 from the type locality (Figs 1–4) measured 21.9–49.4 μm in length and...
16.5–34.8 \( \mu \text{m} \) in width \( (n = 50) \). The epicone of *L. fissa* was rounded (Figs 1, 4, 9) or truncated (Figs 7, 11), a variation also observed within the strains. The hypocone was bilobed as the sulcus extended to the antapex (e.g. Figs 1, 8, 9). Cingulum was equatorial, spiralling steeply down on the right ventral side, with a cingular displacement of approximately 1/2–1/3 the cell length. The transverse flagellum extended in the cingulum (Figs 7, 12) and the longitudinal flagellum emerged from the excavate hypocone. In the light microscope, the apical groove was discernible in all strains (Figs 1, 3, 5, 7–9, 11, 12), although not in every cell. The U-shaped apical groove encircled the apex and will be described in more detail in SEM (see below). In some specimens deep, longitudinal furrows were observed on the cell (Fig. 10). The large, spherical nucleus was located in the epicone (Fig. 4). Cells were yellow-green or yellow-brown in colour, and epifluorescence microscopy revealed elongate chloroplasts radiating from the centre of the cell (Fig. 6). Orange or red assimilation bodies were present in the circular area or in the hypocone (Fig. 10), particularly in old cultures.

**Figs 33–36.** *Levanderina fissa* comb. nov.

Fig. 33. Longitudinal section of the cell showing anterior nucleus (N), profiles of chloroplasts, cingulum, and the longitudinal flagellar canal. The transverse flagellum (tf), longitudinal flagellum (lf) and finger-like extension of the nucleus (Ne) are also indicated. Scale bar = 2 \( \mu \text{m} \).

Fig. 34. Nucleus with typical nuclear pores. Scale bar = 100 nm.

Fig. 35. From the same series of sections as Fig. 33, illustrating the sulcus opening through narrow canal into the flagellar canal. Scale bar = 2 \( \mu \text{m} \).

Fig. 36. ASC, formed by three rows of amphiesmal vesicles (arrows), the middle row with pores through which mucilage is extruded (compare with Fig. 24). The row on the right on the figure is located at a deeper level in the epicone and forms the groove. Scale bar = 0.5 \( \mu \text{m} \).
Figs 37–39. *Levanderina fissa* comb. nov. Three sections from a series of transverse sections through the cell at the level of the cingulum. The cell is seen from above, i.e. the cell’s left is on the viewer’s right. Scale bars = 2 μm.

Fig. 37. Section through the cingulum and the sulcal furrow, showing the transverse flagellum (tf).
SEM revealed the finer details of the cell surface, cingular displacement and apical groove (Figs 13–25). No major differences were observed between the different strains, although strain K-1273 from Puerto Rico had a longer apical groove (see below), and the epicone of this strain generally had more concave sides and a more flat apex. However, the latter features were also observed in cells of the other strains. A few specimens of the strain from Løvø (K-1769) showed faint longitudinal striations (Fig. 15).

The intercingular region, i.e. the right part of the cell between the two ends of the cingulum, was very prominent in all strains. It was triangular in shape, displaced to the left. A characteristic feature was the deep dorsal incision of the sulcus into the hypocone, and the emergence of the longitudinal flagellum from this dorsal location (Fig. 23).

Many cells formed planozygotes as shown by their duplicated transverse and longitudinal flagella (Figs 15, 19, 21, 25). They were generally considerably larger than the vegetative cells (compare with Fig. 21).

The apical groove and adjacent rows of amphiesmal vesicles, from now on termed the apical structure complex (ASC; for discussion of terminology, see below), was more or less U-shaped and originated from or near a rather indistinct sulcal indentation (Figs 13, 18–20, 22). It continued over the cell apex and about one-third down the dorsal part of the epicone, before terminating 2–3 μm below and to the right of its starting point (Figs 13–25). However, this distance was considerably longer in strain K-1273 from Puerto Rico, usually c. 8 μm (Fig. 22), occasionally up to c. 12 μm (not shown). The ASC was long, often reaching 30 μm or more from one end of the U to the other. It was composed of three parallel rows of rectangular amphiesmal vesicles. The outermost row comprised rather short vesicles, typically 1.3–1.5 μm long, in which the inner margin was straight, adjoining the second row of vesicles, whereas the outer margin was more irregular, the narrowest parts adjoining the adjacent vesicles.

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**Figs 40, 41. Levanderina fissa comb. nov.**

**Fig. 40.** Centrally located complex of pyrenoids (py), a so-called compound pyrenoid, from which extend numerous chloroplasts into the cytoplasm. st, starch grain. Scale bar = 2 μm.

**Fig. 41.** Clone of *Levanderina* used for fixation and embedding contained numerous bacteria in the cytoplasm, each bacterium (B) enclosed in a vacuole. Some of the bacteria were apparently dividing (arrow). Scale bar = 500 nm.

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**Moestrup et al.: Levanderina fissa gen. & comb. nov. Dinophyceae** 275
The middle row was formed by elongate vesicles with parallel sides. The vesicles bore numerous small ‘knobs’, and delicate fibrillar, probably mucilaginous, material often extruded from the knobs (Figs 16, 24). The length of these vesicles was very variable, from less than 1 to several micrometers, judging from the apparent lack of transverse sutures in the micrographs. The innermost row was more depressed than the other two and comprised the groove per se (compare with Fig. 36). Vesicles of this row were elongate with more or less parallel sides, the length very variable from less than 0.5 to c. 3 μm, occasionally longer.

The amphialval vesicles were not generally visible but covered by membranous material or mucilage. Only in a few cells were they exposed. The vesicles were then observed to be polygonal and measured only about 1 μm across (Fig. 17). The vesicles of the cingulum were more or less quadrangular and arranged in eight rows (Fig. 17).

TEM

A longitudinal section through the cell is shown in Fig. 33, which illustrates the nucleus, located in the epicone, profiles of chloroplasts, and part of the transverse (circular) and longitudinal (sulcal) furrows with the flagella. The nucleus extends in a several-micrometers-long, finger-like projection toward the flagellar bases, with which it is connected. It is barely visible at the low magnification in Figs 33 and 35, but shown at higher magnification below (Figs 39–61, 64). The nuclear envelope has typical nuclear pores (Fig. 34), providing direct connection between the nucleoplasm and the cytoplasm. The figures also show the most unusual feature of *Levanderina*, the division of the sulcus into two parallel canals, an inner, longitudinal sulcal tube that is closed at one end and contains the longitudinal flagellum (Fig. 39), and an outer, open longitudinal furrow (s in Fig. 38). The latter is the sulcal furrow visible in the light and scanning electron micrographs, whereas the inner tube is not visible from the outside. The two canals merge at some distance from the antapex (Fig. 35). The apical structure complex is illustrated in transverse section in Fig. 36. The outer row of amphialval vesicles is level with the cell surface, whereas the middle row bends over the edge of the apical furrow, part of it forming the side of the furrow. The inner row forms the bottom of the furrow. Adjacent amphialval vesicles form the opposite side of the furrow, and curve over the other rim of the furrow.

Transverse sections of the cell provided additional information about cell shape and the furrow system (Figs 37–39). The cingulum did not show unusual morphological features. The longitudinal flagellum in its tube is visible in Fig. 39, several micrometers from the cell surface, whereas the more peripherally located furrow on the ventral side of the cell corresponds to the furrow observed in the TEM. This furrow was usually open to the exterior (Figs 37, 38) but occasionally closed (Fig. 39). A peduncle was not observed in the TEM, but microtubules probably forming part of a peduncle system were always present on the right rim of the sulcal furrow (Fig. 38, in more detail below).

The pusule system was extensive and comprised at least two large canals apparently discharging their contents into the sulcal tube (in detail in Figs 71, 72). Branches of the chloroplast system were observed almost throughout the cell. Centrally the cell contained numerous aggregated pyrenoids (Fig. 40). They formed a compound pyrenoid system. The pyrenoids lacked penetrating thylakoid lamellae (Fig. 40). Generally cultures of *Levanderina* grew well, although large numbers of bacteria were often present in the cells,
Figs 47–53. *Levanderina fissa* comb. nov. From series of sections at the junction of the transverse and longitudinal furrows. Numbers in circles indicate section number, the direction of sectioning being toward the antapex. The invagination from the exterior labeled s is occasionally seen in this position, and probably represents the longitudinal flagellum furrow, connected to the cingulum (c). The most conspicuous fiber of this region is the cross-banded strand (cs), compare with Figs 42–44. It proceeds along the proximal part of the cingulum, gradually becoming...
was distinctly cross-banded with a periodicity of \(c\) 80 nm, eventually took up most of (Fig. 42) and eventually the entire area between the proximal part of the sulcal tube and the sulcal furrow (Figs 42, 44). It continued along the proximal part of the sulcal furrow into the cingulum, along the surface of the cingular canal, gradually becoming thinner (Figs 42, 44, 47–51). It passed close to a very distinctly cross-banded fibre near the sulcal surface (Fig. 52). The fibre was slightly longer than 1 \(\mu\)m long, and the surface of the sulcus was often deeply convoluted outside the fibre, indicating that contraction of the fibre had occurred (Figs 49–52).

**Peduncle**

A structure that we interpreted as a peduncle in a withdrawn state within the cell was always present on the right-hand side of the sulcal furrow (Fig. 45), closely appressed to the cell surface. At higher magnification the peduncle proved to contain a single row of microtubules, located next to vesicles with opaque contents (Fig. 45), and as many as 18 microtubules were counted (Fig. 46), a rather small number for such a relatively large species. The peduncle microtubules extended along the right-hand side of the sulcal furrow but the ends of the peduncle were not identified. We did not observe the peduncle outside the cell, either in the light microscope or in the SEM, but Gaines & Elbrächter (1987, fig. 6.14c) noted a partly emergent peduncle in SEM. On a rare occasion we observed an opaque bulbous structure outside the peduncle microtubules within the cell (Fig. 73), and this may represent part of the emergent part of a peduncle.

**Flagellar apparatus (Fig. 74 diagrammatic illustration)**

Two flagella were inserted at an oblique angle of \(c\) 145° to one another, the basal bodies being mutually attached by opaque material (Fig. 55). The transverse flagellum was readily identified by its wing-like extension, which started as soon as the flagellum emerged from the cell, extending in the cingulum (Fig. 54). A band of rather stiff hairs was present on the flagellum, but the structure of the hairs and their insertion on the flagellum were not examined in detail. The wing was supported by an opaque rod (perhaps visible in Fig. 38). The longitudinal flagellum, which from its emergence extended into the sulcal tube, carried a short, rather thick wing. This wing was also supported by an opaque rod, and by a thin cross-banded fibre next to the axoneme (Figs 42, 43, also 71 and 72). A tuft of hairs was occasionally seen on the wing, but the hairs were not examined any further.

The flagellar apparatus comprised four microtubular flagellar roots, r1–4, which will be described separately. Root r1 is a band of microtubules that extended from the area above the basal bodies (Figs 59–61), bypassing and attaching to the basal body of the long flagellum (Fig. 63) to continue along the plasmalemma of the sulcal tube (Fig. 55, 67, 71–73). As many as 37 microtubules were observed in this root. Root r1 is a central part of the cell that connected with three major structural components of the cell: (1) On its ventral side a \(c\) 0.4-\(\mu\)m-long fibrillar, cross-banded, conical structure (Figs 57, 58) continued into a distinct cross-banded fibre, the ventral connective (vc). The conical structure comprised numerous fibres, which proximally spread out to establish contact with microtubules of the r1 (Figs 57, 58). It comprised two major bands (Fig. 58), a distal and a centrally located band and, in the area between, several less distinct ones. The distal transverse band was attached to the ventral connective, and the latter was \(c\) 2.2 \(\mu\)m long and very distinctly cross-banded (periodicity \(c\) 0.30 nm) (Fig. 56). For a short distance the ventral connective passed beneath the sulcal tube (Fig. 42), bypassing the large connecting strand (cs) (Fig. 42, also in Fig. 67), to continue in a straight line on the ventral side of the sulcal tube, and terminating next to a chloroplast and a mitochondrion (Fig. 42). (2) Arising on the opposite side of the r1 was a major, nonstriated rod complex, which extended along the plasmalemma of the sulcal furrow (Figs 54–57). Initially it proceeded as a single rod (Figs. 57, 63), connected to r1 with fine fibers (Fig. 63) but eventually it divided into several parallel opaque rods along or close to the plasmalemma (Figs 59–61, also Fig. 47). In Fig. 73, a cell in which the sulcal furrow was not visible, the group of rods is located midway between the plasmalemma and the sulcal tube (see further in the Discussion).

(3) Arising at some distance from the vc and the nonstriated rod was a large area of opaque material, into which attached a finger-like extension of the nucleus (Figs 59–61, also in Fig.
Figs 54–58. *Levanderina fissa* comb. nov. Scale bars = 200 nm.

Figs 54, 55. The two basal bodies (lb and tb) insert at an oblique angle to each other. Root r1 emerges above the level of the longitudinal flagellum base (Fig. 54, compare with Fig. 47) and attaches to the side of the longitudinal flagellum basal body (Fig. 55) before continuing to the posterior part of the cell. The opaque material in Fig. 54 (cs) is part of the large cross-banded fibre (cf. Fig. 42–44), the asterisks indicate the opaque fibres or rods between root r1 and the cingular canal. Tf, transverse flagellum.
Considering that the nucleus was located anteriorly in the cell (Fig. 33), the nuclear finger must be relatively long. The finger is visible at its emergence from the nucleus in Fig. 64, for a distance of c. 2.5 μm. Its presence in Figs 33 and 35 indicates a length of at least 7 μm. Nuclear pores are visible distally on the nuclear finger in Fig. 64. In a transverse section of the cell, the nuclear finger and the vc were visible on opposite sides of the sulcal tube (Fig. 67). This figure also illustrates that the sulcal tube at its proximal end divides into two shallow extensions. The r1 root initially passed along the ventral extension of the sulcal tube, which was longer than the other, whereas the longitudinal flagellum emerged from the dorsal-most tube (Fig. 67).

The r1 root also included a dorsal striated part, which is readily confused with the r4 root. It is seen best in Figs 69 and 70 and had a periodicity of c. 40 μm. The nuclear finger attached indirectly to r1 via the dorsal striated fibre or other fibrous material (nfc in Fig. 74), close to where the flagellar canal divided into the two extensions (Fig. 67). The root r2 was very short and we have only seen it a few times (Fig. 68 and inset). Only a single, very short microtubule was observed.

The root r3 was present but difficult to study, apparently having a somewhat irregular path. Our micrographs indicate that it began as a single microtubule on the right/apical side of the basal body of the transverse flagellum (Fig. 69). From here it extended to the cingulum, and profiles of the microtubule were seen in several places along the cingulum (Figs 68, 69). Along the microtubule were seen at least four groups of (usually) triplets of microtubules (Figs 68, also 66), apparently nucleated by r3.

The root r4 was rather short, and started proximally in a rather compact mass of opaque material on the left-hand side of the basal body of the transverse flagellum, almost opposite the emergence site of Fig. 68. A striated component with a periodicity of 30–35 nm soon became visible, as shown at higher magnification in Fig. 65. The root proceeded from the basal body to the canal of the transverse flagellum (Fig. 45), and then extended along the cingulum for some distance. A fan-shaped conical connecting fiber (TB/rkc) was present between the root and the basal body of the transverse flagellum (Fig. 70). A structure somewhat reminiscent of the striated root component of many other dinoflagellates was occasionally seen (Fig. 70 and inset). It comprised an opaque central layer, surrounded on each side by a thinner less opaque layer.

Molecular phylogenetics

A phylogenetic analysis based on partial, nuclear-encoded rDNA sequences for a diverse assemblage of dinoflagellates is shown in Fig. 75. The dinoflagellates of the tree comprised a highly supported monophyletic group (posterior probability =1 and bootstrap =100%). However, the relationship between the deepest lineages was not resolved using this data matrix, which was based on partial, nuclear-encoded LSU rDNA. Yet major groups, which may represent orders, families or genera, were strongly supported (Fig. 75). The dinoflagellate strains studied in the present work formed a clade that obtained maximum support both in terms of posterior probability and bootstrap. The branch length leading to the clade was relatively long, indicating the distinctiveness of the strains. On the basis of molecular data they were as unique as the recently described dinoflagellate genera Moestropia (Hansen & Daugbjerg 2011), Tovella and Jadwigia (Lindberg et al. 2005), the family Kareniaecae (Bergholtz et al. 2006) and even the order Dinophysiales, representing different taxonomic levels. On the basis of these results the novelty of the cultures matched at least the generic level. The branch length leading to the 11 cultures examined here were very short, indicating that their sequences differed very little. This was also supported by the sequence divergence estimates (Table 2). The relationship within this clade was generally not resolved by posterior probabilities or bootstrap values, yet posterior probabilities proposed a subgroup comprising the two strains presently identified as Gyrodinium instriatum (K-1067 and K-0675), strain K-1769 and the two isolates from Åland (K-1727 and K-1768).

Reducing the taxon sampling to Gymnodinium fuscum and the 11 dinoflagellates of particular interest allowed us to include the divergent domain D2 in the alignment. Using this as input for phylogenetic inference we obtained a second BA tree (Fig. 76). The branch lengths were still short but the two cultures of Gym. instriatum and the cultures from Lövö and Åland again formed a highly supported clade also in terms of bootstrap values (96%; posterior probability = 1.0). The other branches received low support, indicating that the proposed tree topology for these lineages was not significant (Fig. 76).

Table 2 reveals similarity estimates between LSU rDNA sequences (including the highly divergent domain D2) for the 11 dinoflagellate cultures indicated by a grey box in Fig. 75. When comparing Gymnodinium fuscum with each of the seven unique LSU rDNA sequences the divergence was found to be 23.9–25.3%. The divergence between the seven unique LSU rDNA sequence was 0.13–1.71%. The highest value was between K-1768 and K-1273 and the lowest between K-675 and K-1727/K-1067/K-1769.

DISCUSSION

The cells from the type locality of Gymnodinium fuscum agreed well with Levander’s description. The most contentious issue, the presence of very fine longitudinal lines observed in a few cells by Levander (1894, p. 46), was, as mentioned above, confirmed, but usually cells did not show any striation, either in the light or in the scanning electron microscope. Selected drawings from Levander’s work are reproduced here as Fig. 77. Very significantly, Levander did not see or illustrate the longitudinal flagellum inside the longitudinal furrow. He drew cells with an indentation at the
Figs 59–61. Sections from a series through the proximal part of r4, on the surface of the transverse flagellum basal body. Section numbers in circles. The r1 flagellar root bypasses the basal body and establishes contact with the distal end of the finger-like extension of the nucleus (Ne). Scale bars = 200 nm.
antapical end and visible also from the dorsal side, and the longitudinal flagellum is seen to emerge from this part of the cell, ‘also ziemlich weit von dem Ausgangspunkte der Quergeisels’ (rather far from the emergence point of the transverse flagellum). This is exactly as observed in the present material and illustrated in Fig. 23. In our Fig. 19 two longitudinal flagella emerge from the sulcal tube, and in fact Levander in his fig. 5 also drew two longitudinal flagella in this position (our Fig. 77a) and commented that cells with two longitudinal flagella were more common than cells with a single flagellum. This we know now indicates that fusion of gametes had taken place in Levander’s original material from 1892 to 1894 and was confirmed in our material from the same locality.

Levander’s drawings also illustrate cell division, which was followed from the beginning of nuclear division to cytokinesis, the whole process lasting c. 1½ h. The longitudinal flagellum was illustrated throughout cell division in one of the daughter cells, but no other flagella were illustrated. This agrees with the theory of flagellar replication in dinoflagellates, which postulates that the longitudinal flagellum remains as such in the next generation, whereas the transverse flagellum transforms into a longitudinal flagellum in the other daughter cell. Both daughter cells develop new transverse flagella (Heimann et al. 1995).

Mixotrophy

Our preliminary experiments with feeding failed. We therefore initially suspected that Levanderina only fed on very select organisms, a situation that has its parallel in Dinophysis (Park et al. 2006). There is no doubt, however, that L. fissa is mixotrophic. Levander (1894), when describing the species for the first time, included a drawing in which the cell contained a large pennate diatom in the cytoplasm (his fig. 12, reproduced here as Fig. 77G), probably the first demonstration of mixotrophy in dinoflagellates. The diatom is actually longer than the dinoflagellate host, which is somewhat distorted anteriorly. On another occasion Levander found an ‘elongate structure, not a diatom’, within the cell. Levander worked with mixed samples from nature. Biecheler (1952, p. 124), however, relegated of the longitudinal flagellum to a separate internal system, not a diatom’, within the cell. Levander worked with mixed samples from nature. Biecheler (1952, p. 124), however, considered to be identical to L. fissa) ingested a small phototrophic dinoflagellate that in her samples co-occurred with Gyrodinium. She was unable to see how contact between prey and predator was established but described food uptake to take place at the level of the longitudinal furrow. She subsequently discovered and described in more detail how Gyrodinium fed on the eiliate Strombidium. When a swimming Strombidium happened to hit the apical end of Gyrodinium, nothing happened, and the two cells again moved apart. However, if a passing cell of Strombidium hit the antapical end of Gyrodinium, Strombidium stopped swimming instanta-

neously, becoming and remaining immotile. Its trichocysts were not discharged. Strombidium remained attached to the antapical end of the sulcus, which then widened throughout the entire length of the sulcus to form two lips: a 50-µm Gyrodinium was capable of ingesting a Strombidium that was 40 µm long and 25 µm wide! Ingestion took some 10 min and Gyrodinium remained swimming throughout ingestion and digestion, the latter process taking 5–6 h. Our findings of peduncle microtubules close to the right-hand side of the sulcal furrow supports Levanderina as mixotrophic. Another question is, however, how feeding takes place in detail. The evidence so far indicates that the peduncle and the sulcal furrow are involved, and it supports Biecheler’s observations that the sulcal furrow plays a central role in food uptake: the sulcal furrow often appeared very irregularly wavy in our sections. The wavy appearance of the proximal part (bottom) of the furrow indicated that it may change shape during feeding, and this is supported by the finding of at least three systems of putative contractile fibres in the area. The SEM showed the bottom of the sulcal furrow to contain a longitudinal ridge (Fig. 19), whereas in some thin sections the sulcal furrow appeared to be absent, the area instead possessing several shallow invaginations. Four such invaginations are visible in Fig. 48. In the scanning micrograph in Fig. 21 the proximal part of the sulcal furrow is very narrow. This may be a response to the treatment for SEM and it proves that this whole area is highly labile. Further experimentation is required to study details of the feeding process, but the involvement of the sulcus as a feeding structure and the relegation of the longitudinal flagellum to a separate internal tube are presently unique. Considering Levander’s and Biecheler’s observations, Levanderina is not choosy when it comes to food, and feeding with diatoms, small dinoflagellates or Strombidium should be attempted for further studies on food uptake.

Phylogeny

In the present study all strains within the Levanderina clade formed a well-supported clade. The small genetic differences between the different strains suggested that they represent the same species with L. fissa as type for the monospecific genus. However, two distinct subgroups within ‘Gyrodinium instriatum’, perhaps representing two species, were recently demonstrated on the basis of internal transcribed spacer (ITS) sequences (Stern et al. 2012). One group consisted exclusively of strains from China (Shao et al. 2004), suggesting a biogeographical separation (Stern et al. 2012). A detailed morphological analysis of these strains would be very interesting, for comparison with the data presented in the present study.

It has long been clear from the molecular trees published by the two taxa identified as Gyrodinium instriatum and Gyr.
Figs 67–70. *Levanderina fissa* comb. nov.

Fig. 67. The r1 root from its association with the nuclear finger (Ne) extends along the pocket of the longitudinal flagellum canal. The nuclear finger attaches to the dorsal striated part (dsf) of the r1. The vc fibre and the large cs are also visible in this region. Scale bar = 200 nm.
uncatenum are only remotely related to Gymodinium. The two taxa, in the present work renamed Levanderina fissa, never cluster with other species of Gymodinium. Levanderina sometimes forms a sister group to the Kareniaaceae [on the basis of small subunit (SSU) rDNA, Kang et al. 2011], or to Akashiwo sanguinea, the two then forming a sister group to the Kareniaaceae (on the basis of LSU rDNA, Rehé et al. 2011). Logares et al. (2007) showed the Levanderina group as a sister to a clade comprising Glenodiniopsis steinii, Gymnodinium impatiens and A. sanguinea (on the basis of SSU rDNA). Levanderina never forms a sister group to true Gymodinium, i.e. the group that includes the type species Gym. fuscuum (SSU, Saldarriaga et al. 2001, and many others). The ultrastructural data also demonstrate considerable difference between Gymodinium and Levanderina, Levanderina lacking characteristic features of the Gymodinium group such as nuclear chambers and a distinct nuclear fibrous connective. The true phylegetic position of Levanderina is, however, still somewhat ambiguous. If the structure of the ASC proves to be shared between Gymodinium and Levanderina, a relationship to the Gymnodiniaceae is likely, although this is not supported by the molecular data. Levanderina is presently unique in its use of the sulcus, which in other dinoflagellates always contains the longitudinal flagellum. Most likely, in the ancestor of Levanderina, the sulcus was divided up to serve a dual function, the innermost part forming a tube containing the beating longitudinal flagellum. The strain created in this part of the cell by the beating of the flagellum is counterbalanced by the r1 microtubules along the tube. The outer part of the sulcal complex, here called the sulcal furrow, is no longer associated with the longitudinal flagellum but has gained a different function. Judging from its proximity to the point of insertion of the peduncle, it appears likely that it is indeed involved in food uptake as suggested by Biecheler. The morphology of the sulcus is the most exceptional feature of Levanderina and presently sets it apart from all other dinoflagellates known.

The compound chloroplast, in which all pyrenoids are concentrated centrally in the cell, was originally thought to be a rare type of chloroplast in dinoflagellates. However, in recent years this type of chloroplast has been detected in an increasing number of species, from Alexandrium catenella (Whedon & Kofoid) Balech (Hansen & Moestrup 1998), over Tovella sanguinea Moestrup, G. Hansen, Daugbjerg, Flaim & d’Andrea (Moestrup et al. 2006), to Dinophysis acuminata Claparède & Lachmann (Garcia-Cuetos et al. 2010), representing three different orders of dinoflagellates. The functional significance of this arrangement is not clear. The connection between individual pyrenoids is relatively loose, and chloroplasts under certain conditions lose contact with one another and disperse in the cell, as also reported in the euglenoid Entodinium eupharyngea Moestrup & Norris (Walne et al. 1986).

A somewhat unusual feature of Levanderina is the very long narrow finger-like extension that connects the nucleus to the flagellar apparatus, attaching to the dorsal side of the r1 root. The finger is several micrometers long. This recalls the situation described in Cochlodinium polykrikoides Margalef, in which a short rather bulky and irregular extension of the nucleus connects to the dorsal side of r1, i.e. to the same location as in Levanderina (Iwataki et al. 2010). Cochlodinium polykrikoides shares the lack of nuclear chambers with Levanderina but is otherwise very different, particularly so in the path of the ASC. Molecular trees such as Fig. 74 also do not indicate a phylogenetic relationship between Levanderina and C. polykrikoides. In typical members of the Gymnodiniaceae, represented by Gymnodinium, Lepidodinium and Polykrikos, a fibre, which in some species is cross-banded [the nuclear connecting fibre (NFC)], connects the nucleus to the r1 root. The nucleus may extend more or less into a cone at the point of attachment between the NFC and the nucleus (Lepidodinium chlorophorum; Hansen & Moestrup 2005) or, in Lep. viride, a c. 1-μm-long cylindrical extension of the nucleus is directed toward the flagellar apparatus, surrounded by the NFC fibre, which then proceeds toward the flagellar apparatus, located another c. 3 μm away (Hansen et al. 2007, fig. 33). A NFC-like structure was also observed in Biecheleria balitica of the order Suesiales (Moestrup et al. 2009), whereas in most other dinoflagellates, no direct connection has been found between the nucleus and the flagellar apparatus.

The r1 root of Levanderina possesses another feature that has been reported very rarely in dinoflagellates, a dorsal striated fibre (dsf) located on the dorsal side of the root. It is readily confused with the striated part of the r4 root located close by. The dsf is presently known in two species only, one of which is Oxyrrhis marina (Roberts 1985), presently considered to be one of the most primitive dinoflagellates (e.g. Moestrup & Daugbjerg 2007). In molecular trees, Oxyrrhis consistently branches off at the base of the phylogenetic trees, and cells show several unusual morphological features, including mitosis, which is open as in many other protists and thus unlike the closed mitosis with microtubular canals in typical dinoflagellates. Oxyrrhis has a very prominent striated component on the dorsal side of the very large (c. 50 micrometres) r1 root (labelled sc-pmr in Roberts 1985, figs 16, 17, 22). A similar structure was found by Hansen (1993) in another, very aberrant species, Actiniscus pentasterias, a species characterized by cells possessing an internal silicified skeleton (Hansen 1993). Its phylegetic relationships remain unknown, but another similarity between this species and Levanderina is the presence of a distinct, muscle-like, striated fibre (vf), which from the ventral side of r1 proceeds along the
Figs 71–73. *Levanderina fissa* comb. nov. Scale bar = 500 nm.

Figs 71, 72. Transverse sections of the cell at some distance below the insertion of the flagella, showing openings of two pusule canals (pu, and arrow) into the tube of the longitudinal flagellum opposite the insertion of microtubular root r1. The large cs is visible as an opaque structure on the side of the sulcal tube.
sulcus. The fibre is attached to r1 by very thin fibres and the
whole arrangement is strongly reminiscent of the vc of
Levanderina and its attachment to the r1. A similar structure,
an elongate (at least 5 µm long) muscle-like fibre, extends from
the ventral side of r1 in Akashiwo sanguinea (Roberts &
Robert 1991) and in Cochlodinium polykrikoides (Iwataki
et al. 2010). In Levanderina the vc initially extends toward
the sulcal tube, but soon diverges away, to terminate on the
surface of a mitochondrion or chloroplast. In Akashiwo it
apparently extends to the ventral side of the cell (Roberts
& Roberts 1991), and in C. polykrikoides it runs in an antapical
direction, parallel to the longitudinal flagellum canal. A much
less prominent structure occurs in this position in certain other
species of dinoflagellates such as Gymnodinium nolfi (Ellegaard
& Moestrup 1999, fig. 34, labeled c2) and

Borghiella dodgei (Moestrup et al. 2008) and connects to the
so-called ventral ridge of the cells.

In conclusion, some molecular and some ultrastructural
data, in particular the very-well-developed and characteristic
c, indicate a possible phylogenetic relationship between
Levanderina, Akashiwo and Cochlodinium polykrikoides. A
separate family will most likely need to be erected but it is
presently difficult to identify the characteristic features of such
as family.

**Gyrodinium fissum**, GenBank EF613353

The LSU rDNA sequence of *Gyrodinium fissum* from
GenBank, accession number EF613353, formed a highly
supported sister taxon to a clade comprising *Gyr. spirale* (the
type species) and *Gyr. rubrum* (Fig. 75). On the basis of our
identifications of *Gyr. fissum* from the type locality we
conclude that this material, which originates from coastal
waters of Korea, was misidentified. It is a ‘true’ *Gyrodinium.*

**Gyrodinium dorsum** UTEX LB 2334

Both SSU and ITS sequences place UTEX LB 2334,
deposited as *Gyrodinium dorsum* Kofoid et Swezy, together
with *Gyr. instriatum* (Saldarriaga et al. 2004; Stern et al. 2012).
*Gyrodinium dorsum* was originally described by Kofoid and
Swezy (1921) from offshore La Jolla, California. It is very
different from *Gyr. instriatum*, measuring 72 µm in length,
with a posterior nucleus, and although described as having a
yellowish colour it seems to lack chloroplasts. We were
unsuccessful in obtaining live material of the UTEX strain for
closer morphological examination, but on the basis of the
pictures of this strain at www.utex.org, there can be little
doubt that it represents a misidentified *Levanderina fissa.*

**ASC as a phylogenetic indicator**

The material of *Levanderina fissa* responded very successfully
to the preservation methods used for both SEM and TEM,
and this allowed us to obtain information on the detailed
construction of the apical furrow apparatus. *Gyrodinium
pavillardi*, which appears to be identical to *L. fissa*, was shown
by Biecheler (1952, fig. XIX), in silver-impregnated material,
to possess a U-shaped ‘acrobase’, described to be composed of
four parallel lines. It undoubtedly represents three parallel
rows of narrow amphiesmal vesicles, identical to what we
found in *L. fissa*. We introduce the term ASC for the structure
variously termed acrobase or apical furrow, as neither of these
terms is entirely satisfactory, a furrow is not always present.

The path of the ASC on the epicone was used by Daugbjerg
et al. (2000) to redefine the genera of several gymnodinialean
dinoflagellates, and although this has generally worked
satisfactorily, it has not contributed toward determining the
higher levels of classification: the number of families within the
Gymnodiniales, or the definition of the order Gymnodiniales
itself. Present molecular evidence indicates that all genera of the
‘Gymnodiniales s.s. clade’ (Hoppenrath et al. 2009; Kang et
al. 2011) constitute a single family, for which the name
Gymnodiniaceae Lankester 1885 is available. The molecular

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**Fig. 73.** Area between the cell surface and the sulcal tube in this cell contains a group of opaque rods, some of which are marked with
asterisks, but there is no sign of a sulcal furrow. Outside the cell is a large opaque structure, perhaps part of the peduncle (arrow in Fig. 73).
Bayesian phylogeny of a diverse selection of dinoflagellates (62 species) on the basis of partial, nuclear-encoded LSU rDNA (746 base pairs). Ciliates, apicomplexans and *Perkinsus* form the outgroup taxa. The dinoflagellate cultures of particular interest to this study are boxed (11 in total). The new genus *Levanderina* proposed in this work replaces the names of all the strains indicated by the vertical line. Posterior probabilities from BA and bootstrap values from ML analyses (100 replications) are noted to the left of internodes. Bootstrap values below 50% are indicated by a ‘-’. Filled circles indicate maximum posterior probabilities (1.0) and bootstrap values (100%). Strain and GenBank accession numbers are written in brackets.
evidence indicates that this may also include the genera usually included in the Warnowiaceae Lindemann 1928 (Hoppenrath et al. 2009), although we prefer, for the time being, to retain this as a separate family within the Gymnodiniales. The molecular data do not support inclusion of Levanderina in the Gymnodiniaceae, and this, together with the very unusual path of the longitudinal flagellum in a tube, justifies the creation of a new family. The structure of the ASC indicates phylogenetic relationship to other members of the Gymnodiniales, and although there is insufficient and sometimes contradictory information on the structure of the ASC in species of the Gymnodiniales, recent studies indicate similarities. Before going into detail, we notice that in the Suessiales the ASC has been examined in several species, and considerable diversity has been found (presently four types), all of which are different from what has been reported in the Gymnodiniales and in the Tovelliaceae. The latter is a family of somewhat uncertain phylogenetic affinity, in which the species possess an ASC comprising a single row of elongate vesicles (Moestrup et al. 2009). Is there a uniform type of ASC in the Gymnodiniales? Sampedro et al. (2011), in Barrufeta bravensis, a marine gymnodinioid, reported the presence of an ASC comprising three elongated vesicles, the middle row with a line of knobs. The low-magnification micrographs included do not allow further conclusions, but a series of unpublished micrographs sent to ØM for further scrutiny showed three rows of vesicles, sometimes with indication of a fourth row, each row almost certainly comprising several amphiesmal

Fig. 76. Bayesian phylogeny of 11 dinoflagellate cultures identified as Gymnodinium instriatum, Gymnodinium uncatenum, Gymnodinium uncatenum or Gymnodinium fissum. Gymnodinium fissum was included to polarize the ingroup. Posterior probabilities from BA and bootstrap values from ML analyses (100 replications) are noted at internodes. Bootstrap values below 50% are indicated by a '-'.

Solid circles indicate maximum posterior probabilities and bootstrap values (i.e. 1.0 and 100%, respectively). The alignment included 962 base pairs.

Fig. 77. Selected original drawings of Gymnodinium fissum from Levander (1894). (A) A planozygote seen from the dorsal side, the flagella leaving the cell through an invagination on the antapical–dorsal side; (B–F) dividing cells; the cells are dividing in the motile stage; (G) a cell containing an ingested diatom, which is longer than the dinoflagellate, causing some distortion of the cell.
vesicles. The outermost row was a furrow, and although thin sections of the apical apparatus were not included, the evidence indicates an apical apparatus identical to that of *Levanderina*. In *Cochlodinium polykrikoides* the ASC also comprises three rows of vesicles (G. Hansen, unpublished observations). Kang et al. (2011) illustrated a ‘central ridge’ of five elongate amphiasomal vesicles on the epicone of *Gyrodinieum shiwaense*, another gymnodioid, and the presence of a row of small knobs on each vesicle, extruding mucilaginous material, indicates it to be homologous to the central row of amphiasomal vesicles in the ASC of *Levanderina*. Whether *Gyrodinieum* also possesses two additional rows as in *Levanderina* will require additional SEM combined with thin sections, but Kang et al. (2011) mentioned that the central ridge was surrounded on each side by an indistinct groove. U-shaped apical grooves were illustrated very recently in ‘*Proerythropsis*’ and *Gymnodinium literalis* (Hoppenrath et al. 2009; Reike et al. 2011), and in the latter species the presence of three elongate vesicles was reported. There is a clear similarity to *Levanderina* in this respect, but additional studies are required to determine whether the three single vesicles reported in *Gym. literalis* actually comprise three rows of amphiasomal vesicles. Biecheler (1952), using stained cells and light microscopy, drew a single vesicle with knobs in *Gyrodinium vorax*. It was lined on each side by a row of narrow elongate vesicles. This species may belong to the newly described genus *Gyrodinieum* and, if this can be confirmed, more likely possesses a row of amphiasomal vesicles. On the basis of these studies we hypothesize the existence of a common type of three-rowed apical apparatus (ASC) in many gymnodioids, including *Levanderina*. Whether it characterizes the entire order Gymnodinioida must await additional data.

**Cyst morphology**

Recent work has shown that the morphology of the resting cyst (hynnocyst) can be important for distinguishing between genera (e.g. Lindberg et al. 2005; Moestrup et al. 2009). Descriptions of the cyst of *Gyrodinium inustriatum* (Matsukawa 1985; Orlova et al. 2003) agree with *L. fissa* in the slightly variable circular to ovoid shape, the transparent, unstructured cyst wall, the cyst content and the surrounding thick mucus layer. Light microscopy images and cyst size of Russian material by Orlova et al. (2003, figs 1a, b) also appear identical to our observations on Finnish cells of *L. fissa* (Figs 26–32), whereas they are somewhat wider than Japanese cysts measured by Matsukawa (1985). Light microscopy images of *Gyr. uncatum* cysts (Tyler et al. 1982, figs 9D, E; Coats et al. 1984, fig. 21) look indistinguishable from our cysts, which also agree in size (48 × 39 μm: Coats et al. 1984). The data available thus support the idea that the many taxa belong to the same species.

The pattern of the inner cyst wall observed in the present study does not correspond to any previously reported cyst type. Cysts of true *Gyrodinium* (sensu Daughberg et al.) are apparently not known. However, the type species of *Gymnodinium*, *Gym. fuscum*, has a very different type of cyst (e.g. Hansen & Moestrup 2000). As presently understood, the morphology of the cyst may represent a unique feature of *Levanderina* and supports it forming a separate genus.

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