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DESCRIPTION OF *TYRANNODINIUM* GEN. NOV., A FRESHWATER DINOFLAGELLATE CLOSELY RELATED TO THE MARINE *PFIESTERIA*-LIKE SPECIES[1]

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On the basis of morphological (light and electron microscopy) as well molecular data, we show that the widely distributed freshwater dinoflagellate presently known as *Peridiniopsis berolinensis* is a member of the family Pfiesteriaceae, an otherwise marine and estuarine family of dinoflagellates. *P. berolinensis* is a close relative of the marine species, which it resembles in morphology, mode of swimming, food-uptake mechanism, and partial LSU rRNA sequences. It differs from all known genera of the family in plate tabulation. *P. berolinensis* is only distantly related to the type species of *Peridiniopsis*, *P. borgei*, and is therefore transferred to the new genus *Tyrannodinium* as *T. berolinense* comb. nov. *T. berolinense* is a very common freshwater flagellate that feeds vigorously on other protists and is able to consume injured metazoans much larger than itself. Production of toxins has not been reported.

Key index words: **dinoflagellates; *Peridiniopsis berolinensis*; *Pfiesteria*; Pfiesteriaceae; *Tyrannodinium***

Abbreviations: **apc, apical pore complex; BA, Bayesian analysis; cp, closing platelet; LC, layered connective; LMR, longitudinal microtubular root; ML, maximum likelihood; p, peduncle; pc, peduncle cover plate; Po, pore plate; PP, posterior probabilities; sa sd, sm, sp, ss, anterior, right, medium, posterior, and left sulcal plates, respectively; SMR, single-stranded microtubular root; TB, transverse basal body; TMR, transverse microtubular root; TMRE, transverse microtubular root extension; TSR, transverse striated root; TSRM, transverse striated root microtubule; x, canal plate**

The family Pfiesteriaceae was formally described in 1996 to accommodate the new genus *Pfiesteria*, a purportedly fish-killing dinoflagellate that attracted unprecedented attention from the general public. The original defining features of the family were centered on a complex, multiphasic life cycle, which included flagellate, amoeboid, and cyst stages (e.g., Burkholder et al. 1992, Steidinger et al. 1996). This set of characters made the group rather exclusive in the sense that other dinoflagellates with features that would fit the new group could not be found. However, the following years saw the description of new species of Pfiesteriaceae for which a multiphasic life cycle could not be demonstrated, placing the emphasis on the characters of the flagellate stage, especially the feeding mode and the tabulation of the theca (Parrow and Burkholder 2003b, Jeong et al. 2005, Steidinger et al. 2006). The flagellate, thinly thecate cells became known as ''cryptoperidiniopsoids,'' in allusion to the peridinioid type of tabulation occurring in members of *Peridiniopsis* (Parrow and Burkholder 2003a). The recently demonstrated affinity of the parasitoid marine dinoflagellate genus *Paulsenella* to *Pfiesteria* and *Amylodinium* (Kühn and Medlin 2005) highlights the feeding mode as a good phylogenetic marker for the group. The pfiesteriaceans are phylogenetically related to the calcareous dinoflagellates, such as *Thoracosphaera heimii* and species of *Scrippsiella* (Gottschling et al. 2005, Meier et al. 2007, Elbrächter et al. 2008).

As shown in the present study, the family Pfiesteriaceae is not restricted to the marine environment. On the basis of ultrastructural observations and supported by partial sequencing of LSU rRNA, we show that the species presently known as *P. berolinensis* is a close relative of the marine members of the Pfiesteriaceae. *P. berolinensis* was first described from Germany just over 100 years ago (Lemmermann 1900, as *Peridinium berolinense*), and it is a common dinoflagellate in freshwater ponds and lakes, reported from Europe, Africa, North America, and Japan (e.g., West 1907, Thompson 1951, Senzaki and Horiguchi 1994, Calado and Moestrup 1997). It feeds

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vigorously on other organisms, including algae, injured nematodes and other metazoans, even cells of its own kind (Calado and Moestrup 1997). It shares the feeding mechanism employed with its marine relations and is attracted to its prey by a chemosensory mechanism (Calado and Moestrup 1997) as in the marine species (e.g., Spero 1985). *P. berolinensis* differs from the marine species in details of the plate pattern and in LSU rRNA sequence. It constitutes a separate genus and species, described here as *T. berolinense* gen. et comb. nov.

Notes on the generic names used. (1) There is some discussion whether *Pseudopfiesteria* and *Pfiesteria* constitute separate genera (Marshall et al. 2006, Place et al. 2008). Pending additional evidence, we have followed Place et al. (2008) and retained the two genera, which differ in epithecal tabulation. (2) The genus *Stoeckeria* was published in a journal with a zoological tradition and fulfills the requirements for availability (validity of publication) under the International Code of Zoological Nomenclature (Jeong et al. 2005). However, the use of the term ''Dinophyceae'' in the title is an incongruous link to botanical nomenclature, under which the name would be invalidly published for lack of Latin. We assume from the lack of a Latin diagnosis that it was not the authors' intention to publish the new genus under the Botanical Code and therefore accept the name.

MATERIALS AND METHODS

Biological material. *T. berolinense* was collected in small lakes and ponds (incl. artificial lakes and fish ponds) in Portugal, Denmark, and Poland. Cells used for LM and SEM were collected in February 2008 from the artificial lake in ''Baixa de Santo António,'' Aveiro, Portugal, where it appears throughout the year. Cells for TEM were collected in April 1995 from ponds in Portugal and Denmark (Calado and Moestrup 1997). Cells for DNA sequencing originated at Pieskowa Skała near Cracow, Poland, collected 22 August 2007.

Light and electron microscopy. Light micrographs were taken with a Zeiss Axiophot light microscope (Carl Zeiss GmbH, Jena, Germany) using Kodak Technical Pan film (Eastman Kodak Company, Rochester, NY, USA) and a Zeiss Axioplan 2 imaging equipped with a DP70 Olympus camera (Olympus Corp., Tokyo, Japan).

SEM preparations followed two schedules of fixation: one to retain the flagella and the peduncle (1), and the other to remove the outermost membrane to show the plates (2). (1) Swimming cells were picked up with a micropipette into 1 mL of filtered lake water. They were fixed by addition of 0.5 mL of a fixative comprising a 1:3 mixture of saturated $HgCl_2$ and 2% OsO_4. Cells were collected on 8 μm pore-size Isopore polycarbonate membrane filters in a Swinnex filter holder (Millipore Corp., Billerica, MA, USA), washed with distilled water for 30 min, dehydrated through a graded ethanol series, and critical-point-dried. The dried filters were glued onto stubs, sputter-coated with gold-palladium, and examined in a Hitachi S-4100 scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan). (2) Swimming cells were transferred to 25% ethanol and fixed for 45 min. After collecting the cells in the filters, dehydration was completed, and the schedule followed as in (1).

TEM of feeding and nonfeeding cells was performed as described in Calado and Moestrup (1997).

Single-cell PCR and LSU rRNA sequencing. Cells of *T. berolinense* from field samples collected in Poland were isolated by pipetting and double rinsed in distilled water. Individual cells were transferred to 0.2 mL PCR tubes containing 8 μL of ddH_2O. Prior to performing single-cell PCR, 5 μL of 10X Taq buffer [67 mM Tris-HCl, pH 8.5, 2 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, and 10 mM β-mercaptoethanol] was added to each tube, and the material heated to 94°C for 11 min. The tubes were placed on ice, and PCR reagents added to perform a 50 μL reaction (see Daugbjerg et al. 2000, Hansen et al. 2000, Hansen and Daugbjerg 2004 for PCR reagents, reaction conditions, and primer sequences). Despite using a dinoflagellate-specific primer (''Dino-ND''), the primary PCR reaction provided no visible DNA fragments when loaded onto a 1.5% agarose gel containing ethidium bromide and viewed on a UV light table. Therefore, nested and seminested PCR was performed using 1 μL from the first PCR reaction as template in two new PCR reactions with different sets of primer combinations (D1R-D3B and D3A-28-1483, respectively). The volume, PCR reagents, and temperature profile were identical to those of the primary PCR reactions. However, the nested and seminested PCR reactions only used 18 cycles. Nested and seminested PCR resulted in DNA fragments of correct size based on a molecular marker (viz. Phi X175 HAEIII). All DNA fragments were purified using NucleoFast 96 PCR Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), following the manufacturer's recommendations. PCR product (500 ng) was air-dried overnight and sent to the sequencing service at Macrogen (Seoul, Korea) for determination in both directions using five primers (see Table 1 in Hansen et al. 2007 for primer sequences).

Phylogenetic analyses. A data matrix comprising nuclear-encoded LSU rRNA sequences from 46 species of dinoflagellates was assembled to examine the phylogeny of *T. berolinense*. A diverse assemblage of outgroup taxa were included to polarize the dinoflagellate ingroup. Hence, four ciliates, five apicomplexans, and *Perkinsus* formed the outgroup. GenBank accession numbers for all species analyzed phylogenetically are given in Figure 7. The data matrix excluded the highly divergent domain D2 (sensu Lenaers et al. 1989) as this DNA fragment was too variable to allow unambiguous alignment among all of the 56 alveolates included. The remaining 1,176 base pairs incorporated information from the secondary structure of the mature RNA molecule forming stems and loops as suggested by de Rijk et al. (2000). The final data matrix was edited manually in MacClade ver. 4.08 (Maddison and Maddison 2003) and analyzed phylogenetically using Bayesian analysis (BA) and maximum likelihood (ML) as implemented in PhyML 3.0 (Guindon and Gascuel 2003). Bayesian analysis (BA) was performed using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003) with 2 million generations (Ronquist and Huelsenbeck 2003). Every 50th generation, a tree was sampled, and the burn-in was evaluated by plotting the LnL values as a function of generations in a spreadsheet. The burn-in occurred after 20,050 generations, and, therefore, 401 trees were discarded, leaving 39,600 trees for estimating posterior probabilities (PP). The PP values were obtained from a 50% majority-rule consensus of the saved trees using PAUP* (Swofford 2003). In ML, we used the parameter settings suggested by MrModeltest ver. 2.3 (Nylander 2004). The ML analysis was run using the online version available on the Montpellier bioinformatics platform located at http://www/atgc-montpellier.fr/phyml. To evaluate the robustness of the tree topology in ML, we used bootstrapping with 100 replications.

RESULTS

General morphology and tabulation. Cells of *T. berolinense* are generally round, slightly compressed

dorsoventrally, and mostly in the range of 20–37 μm long. A large nucleus occupies most of the hypocone, and food items are often seen in the epicone (Figs. 1a and 2a). Chloroplasts and eyespot are absent. Nonswimming, aflagellate stages, during which the theca detaches from the protoplast (so-called temporary cysts), are common. Cells divide into two in the temporary cyst stage, usually before exiting the parent theca. The theca opens along the cingular-epithecal sutures with the undivided sulcus linking the two halves (Fig. 1b).

Details of cell morphology and plate arrangement are shown in Figures 3–5. The longitudinal flagellum and the peduncle (feeding tube) protrude from within a cavity in the upper half of the sulcus, lined externally by an unusual plate called the peduncle cover plate (pc; Figs. 2a; 3, a and b; and 4a). Five other plates are visible in the sulcal area, three of them partially hidden behind the pc (Figs. 4a and 5a). The transverse, undulating flagellum extends to the cell's left along a nearly circular groove (the cingulum), which is lined by six plates (Figs. 3, a–c; 4a; and 5). Large cells with well-developed thecae usually show two granular or spiny flanges, one antapical and another on the posterior left side of the sulcus (Fig. 3, a and e, arrows). An apical pore complex (apc), which is slightly deviated toward the cell's left, tops the epitheca; it comprises three platelets (Figs. 3, a and d; and 4b). Four so-called apical plates are asymmetrically arranged around the apc, with the dorsal, 3′ plate much smaller than its neighbors and somewhat displaced to the cell's left (Figs. 3d and 5c). Six precingular plates complete the epitheca (Figs. 3d and 5c). The hypotheca is made up of the five postcingular and two antapical plates that are typical of peridinioids but is marked by the presence of a wavy suture between the antapical plates (Figs. 3e and 5d).

The flagellar apparatus. Four microtubule-containing roots associate with the flagellar bases (Fig. 6). One microtubule extends from the apical surface of the transverse basal body (TB) and nucleates a dome-shaped row of microtubules directed toward the cell's left (TMR/r3 and TMRE in Fig. 6, a–e). One multistranded root extends longitudinally beneath the sulcus, and its proximal part is linked through fibrous material to the TB and to a layered structure; the apical part of this layered connective associates with the TB and with a transverse fibrous root that runs along a microtubule (Fig. 6, c–e). A single microtubule associates with the right-hand side of the longitudinal basal body and arches in a dorsal-posterior direction for about 1 μm (Fig. 6, f and g; for further information on the arrangement and nomenclature of peridinioid flagellar roots, see Calado and Moestrup 2002).

The sulcal cavity and the peduncle cover plate are shown in oblique section in Figure 6h; the pc is linked along the right edge of the sulcus by a normal plate suture, whereas along the left side, the connection involves numerous thin fibers that extend between the plasma membrane areas covering the parts in contact (Fig. 6i).

LSU rRNA-based phylogeny. Figure 7 illustrates the phylogenetic tree inferred from a Bayesian analysis of nuclear-encoded LSU rRNA sequences from 46 species of dinoflagellates and 10 outgroup taxa comprising ciliates, Apicomplexa, and *Perkinsus*. The

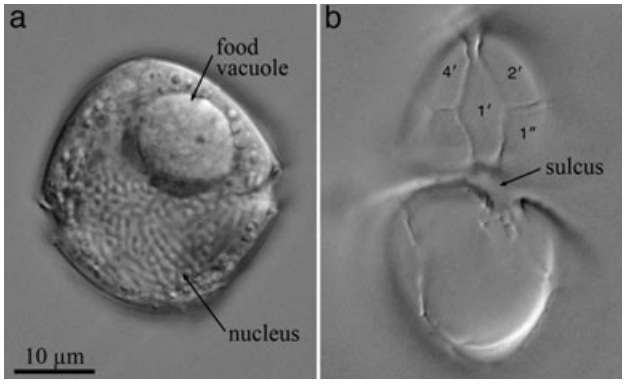

FIG. 1. LM of *Tyrannodinium berolinense*. The scale bar applies to both panels. (a) Optical section through a cell containing a food vacuole with apparently digested contents. (b) Ventral view of open theca showing the epi- and hypotheca linked by the unbroken sulcus.

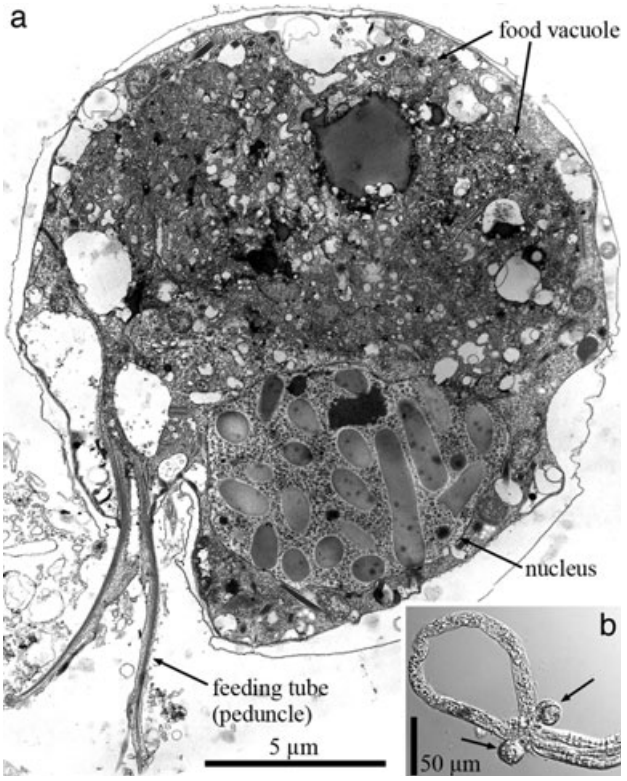

FIG. 2. Feeding *Tyrannodinium berolinense*. (a) TEM of cell fixed while feeding, viewed from the left. (b) Two cells (arrows) feeding on an experimentally injured nematode (details in Calado and Moestrup 1997).

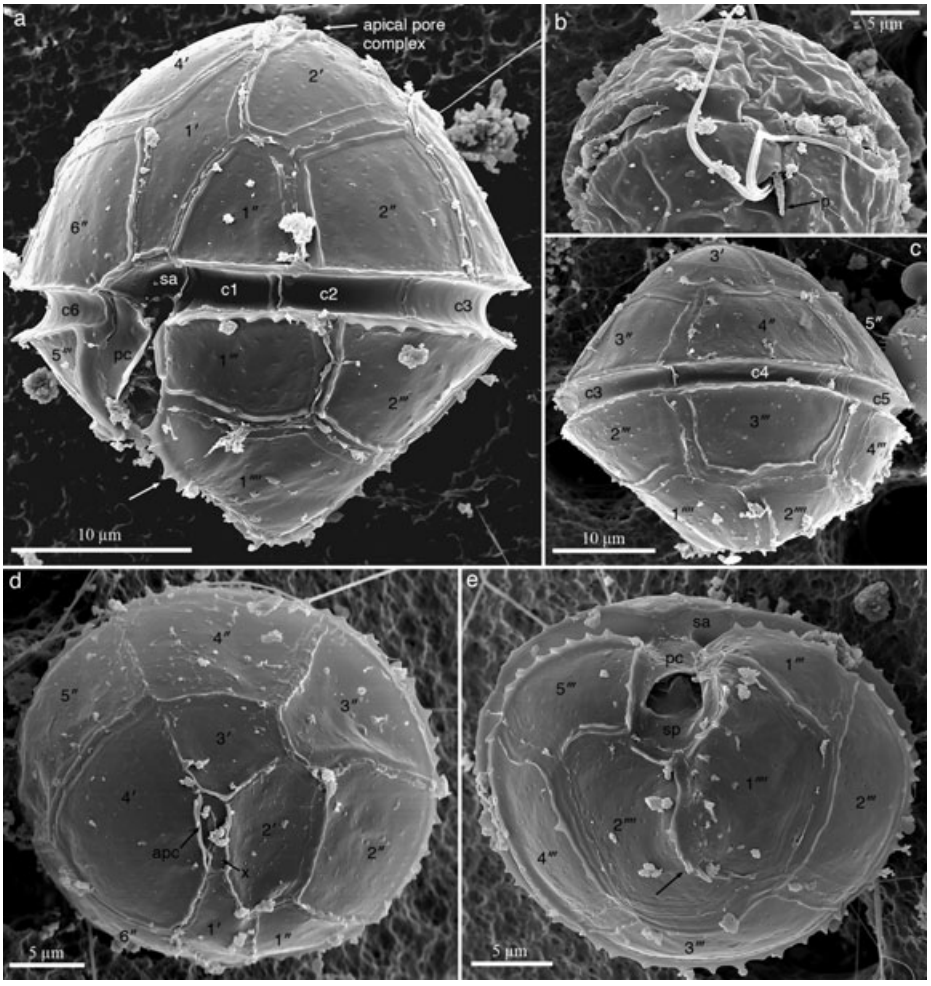

FIG. 3. Morphology and plate arrangement of *Tyrannodinium berolinense* (SEM). (a) Left-ventral view. The peduncle cover plate (pc) is detached on its left edge, probably as an artifact. The arrow points to the spiny flange on the left edge of the sulcus. (b) Ventral-posterior view of a planozygote showing the two longitudinal flagella and the peduncle (p) emerging from the sulcal cavity (preparation schedule 1). (c) Dorsal view. (d) Apical view. (e) Antapical view. The antapical flange is indicated by the arrow. apc, apical pore complex; sa, anterior sulcal plate; sp, posterior sulcal plate.

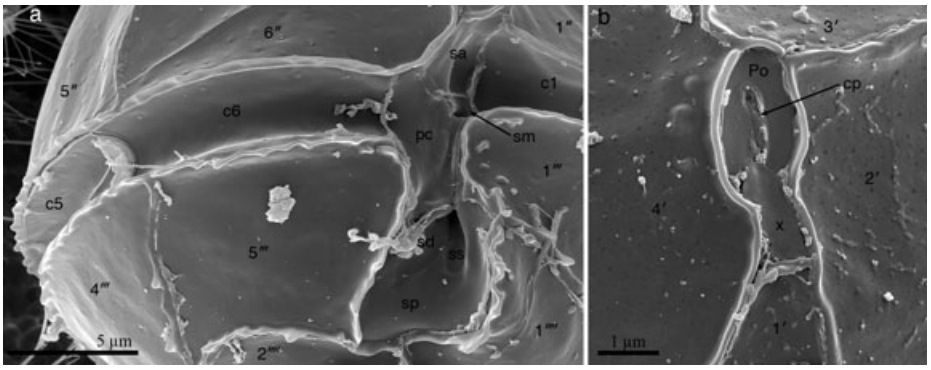

FIG. 4. Details of the sulcal and apical areas (SEM). Plate abbreviations as in Figure 5. (a) Sulcus and ventral-right part of the cingulum. (b) Apical pore complex. cp, closing platelet; pc, peduncle cover plate; Po, pore plate; sa, sd, sm, sp, ss, respectively anterior, right, medium, posterior, and left sulcal plates; x, canal plate.

phylogenetic analysis revealed that *T. berolinense* formed a highly supported sister taxon to the clade with *Pfiesteria* and *Cryptoperidiniopsis* (PP = 1.0). In ML bootstrap analysis (BS), this relationship was also highly supported (BS = 100%). The coccoid species *Thoracosphaera heimii* formed a highly supported sister to *Tyrannodinium* and the two pfiesteriacean species (PP = 1.0, BS = 93%). *Scrippsiella trochoidea* and *Peridiniopsis polonica* made a highly supported sister group to the clade comprising *Thoracosphaera*, *Tyrannodinium*, and the pfiesteriaceans (PP = 1.0, BS = 95%). The type species of the genus *Peridiniopsis*, *P. borgei*, was also included in this study, and it was related to three species of *Peridinium* (*P. willei*, *P. cinctum*, and *P. palatinum*). However, this topology was poorly supported by the posterior probability in BA (PP = 0.59) and not at all in ML bootstrap analysis (<50%). Yet *P. borgei* was distantly related to *T. berolinense* (=*P. berolinensis*). The most divergent branches for the dinoflagellate ingroup

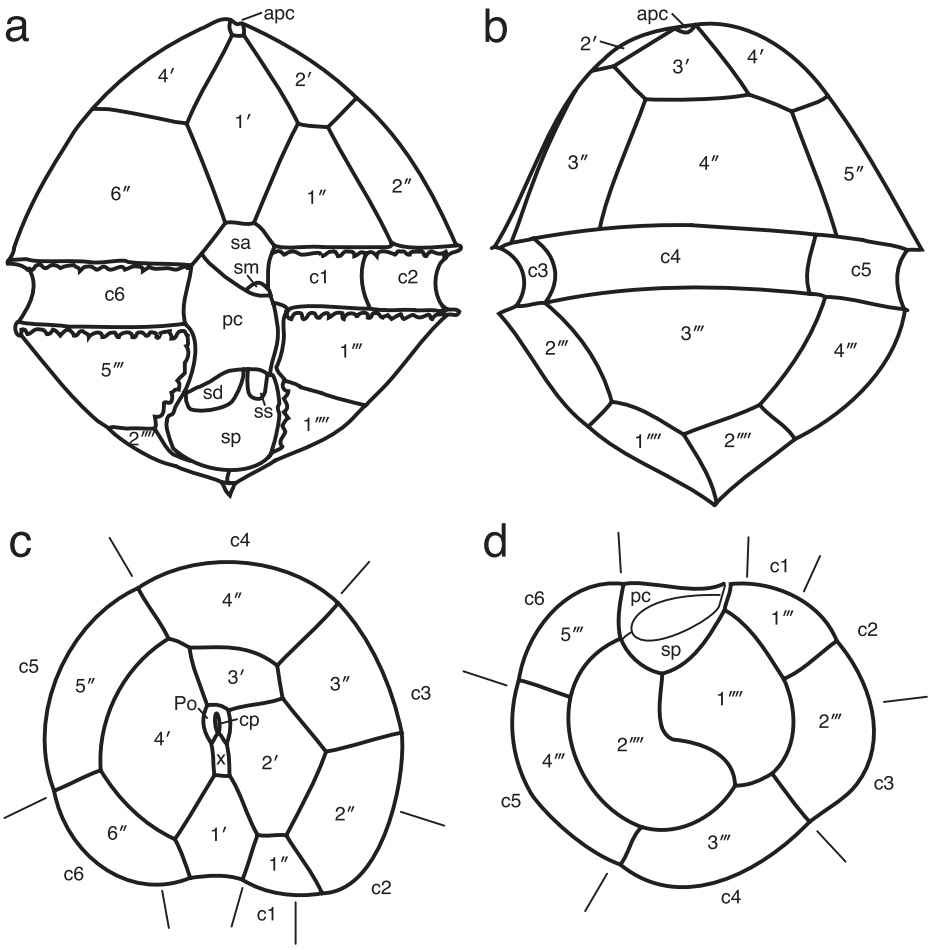

FIG. 5. Diagrammatic view of morphology and plate arrangement. Plate numbering follows Kofoidian notation. Modified from Wołoszyńska (1916, pl. 13, figs. 22, 23, 25, 26). (a) Ventral view. apc, apical pore complex; pc, peduncle cover plate; sa, sulcal anterior; sd, sulcal right; sm, sulcal medium; sp, sulcal posterior; ss, sulcal left. (b) Dorsal view. (c) Apical view. Po, pore plate; cp, closing platelet; x, canal plate. (d) Antapical view.

formed a large polytomy (i.e., no support for the tree topology).

The estimated sequence divergence between *T. berolinense*, *Pfiesteria piscicida*, and *Cryptoperidiniopsis brodyi* is given in Table 1. *P. piscicida* and *C. brodyi* diverged from each other by only 3.3%, whereas *T. berolinense* diverged from both species by about 5%. This sequence divergence is also reflected in the branching topology among these dinoflagellates (Fig. 7). A significantly higher sequence divergence was estimated when comparing *Tyrannodinium*, *Pfiesteria*, and *Cryptoperidiniopsis* to *Peridiniopsis*. Here, the divergence was 16%–18%.

TABLE 1. Sequence divergence estimates. Uncorrected (''p'') distances are given above the diagonal in percentage.

	Tyrannodinium berolinense	*Pfiesteria piscicida*	*Cryptoperidiniopsis brodyi*	*Peridiniopsis borgei*
T. berolinense	–	5.0	4.9	17.8
P. piscicida		–	3.3	16.5
C. brodyi			–	17.36
P. borgei				–

DISCUSSION

Ultrastructure. Four flagellar roots with the characteristics shown here for *T. berolinense* have previously been found in the peridinioid species analyzed (Calado et al. 1999, Calado and Moestrup 2002). Roots 1, 3, and 4 are present in nearly all dinoflagellates examined, both naked and thecate, whereas the distribution of root 2 seems restricted to two thecate groups (gonyaulacoids and peridinioids; Hansen et al. 1996, Calado and Moestrup 2002) and some woloszynskioids, for example, the recently described *Baldinia anauniensis* Gert Hansen et Daugbjerg (Hansen et al. 2007). In contrast, the layered connective linking roots 1 and 4, and the proximal portion of the TB, apparently replacing the more slender and widespread striated root connective of other dinoflagellates (src; e.g., Calado et al. 2006), has only been found in peridinioids and in *Kryptoperidinium foliaceum*, a species containing a diatom type of symbiont (Dodge and Crawford 1969).

The microtubules associated with peduncle formation and the two well-defined fibers linking r1 to the TB suggest a closer relationship of *T. berolinense* to *P. borgei* than to *P. cinctum*, which lacks these

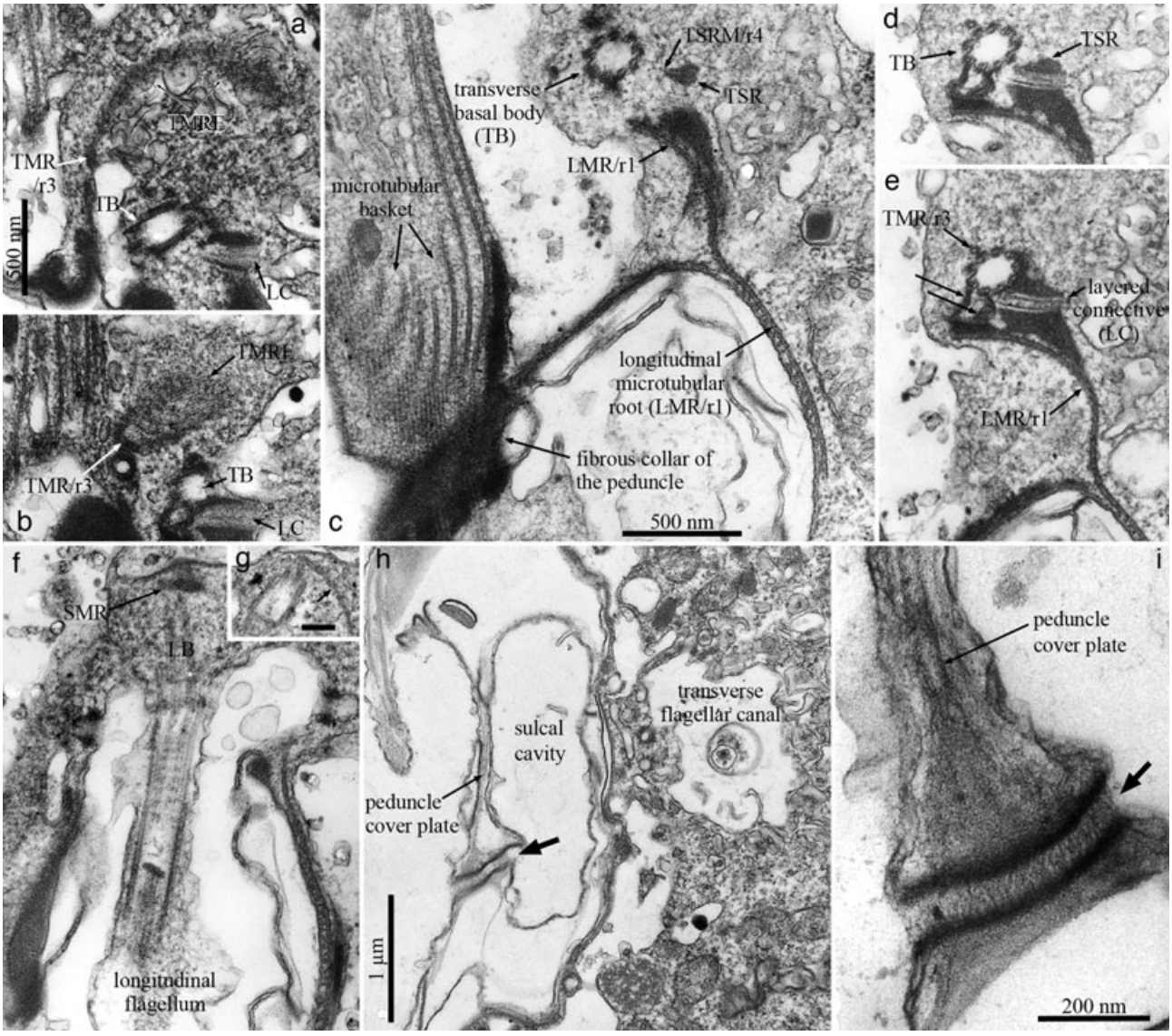

FIG. 6. Flagellar apparatus of *Tyrannodinium berolinense* (TEM). Sections from two series progressing from left to right with the cell's longitudinal axis slightly more tilted toward the observer in panels (a, b, g). (a, b) The arched microtubular extension (TMRE) of the transverse microtubular root (TMR/r3). (c–e) Roots on the left side of the basal bodies and their interconnections. Two fibrous connectives link the transverse basal body (TB) to electron-opaque material on the dorsal side of the longitudinal microtubular root (LMR) (arrows). Both the TB and the transverse striated root (TSR) are connected to the LMR through a layered connective (LC). (f, g) The single microtubule (arrows) associating with the right-hand side of the longitudinal basal body (LB). (h, i) Oblique section through the sulcal cavity showing the unusual fibrous connection along the left edge of the peduncle cover plate (thick arrows).

features (Calado et al. 1999, Calado and Moestrup 2002). However, the peduncle in *P. borgei* is a flat structure rather than a tube, and its supporting microtubules are arranged in a single row that turns around the upper-left side of the cell before dividing consecutively into two and four smaller rows, ending near a large central vesicle (Calado and Moestrup 2002). The cylindrical arrangement of 23 microtubules of the TMRE, surrounding a rod of fibrous material, is so far also known exclusively from *P. borgei* (Calado and Moestrup 2002) and contrasts with the simple and much shorter dome-shaped extension to r3 found in *T. berolinense*.

The parallel arrangement of basal bodies and some flagellar roots in a planozygote of *T. berolinense* was documented by Wedemayer and Wilcox (1984). This finding is consistent with the organization described in detail for the planozygote of *Esoptrodinium gemma* (=*Bernardinium bernardinense*; Calado et al. 2006), although it is not known whether both transverse flagella also converge to a single flagellar canal in *Tyrannodinium*.

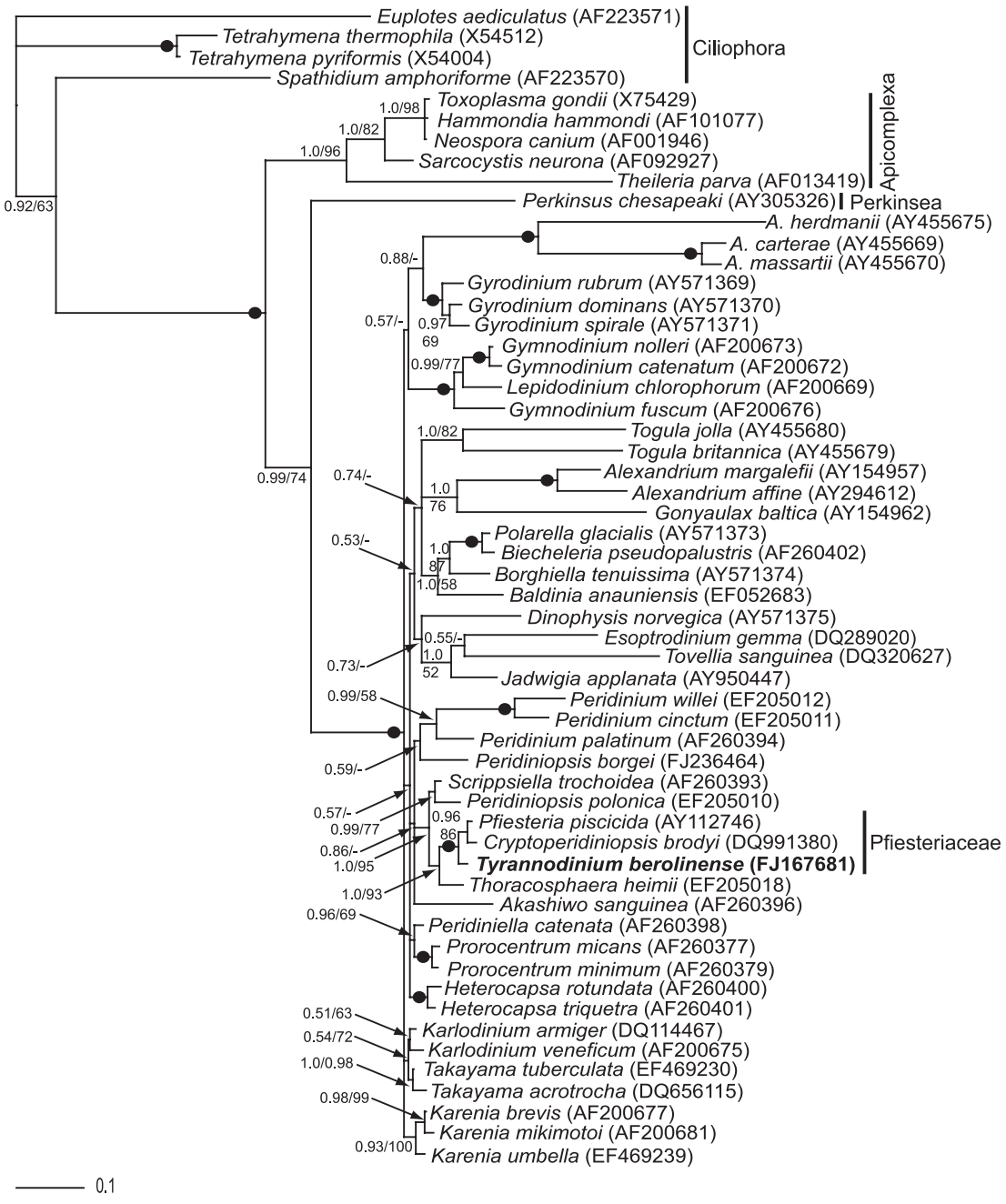

FIG. 7. Phylogeny of *Tyrannodinium berolinense* inferred from Bayesian analysis of nuclear-encoded LSU rRNA sequences from 46 species of dinoflagellates. Four ciliates, five apicomplexans, and *Perkinsus* formed the outgroup taxa. The first numbers to the left of internal nodes are posterior probabilities from Bayesian analysis. The last numbers are bootstrap values (>50%) from maximum likelihood (PhyML) with 100 replicates. GenBank accession numbers are written in parentheses. Three species belonging to the genus *Amphidinium* are listed as *A. herdmanii*, *A. carterae*, and *A. massartii* due to space limitations.

Studies on previously unrecognized pfiesteriaceans. The attraction to injured organisms and the mechanisms of capture, food uptake, and the underlying ultrastructural features, as reported for *T. berolinense* (Calado and Moestrup 1997), are remarkably similar to those described from a brackish water species examined in detail by Spero in the 1980s under the name *Gymnodinium fungiforme* (Spero and Morée 1981, Spero 1982, 1985). Although it is clear that *G. fungiforme* sensu Spero is a pfiesteriacean, the lack of diagnostic tabulation features makes it impossible to decide whether it belongs to any of the other named species of the group. As originally described from Russian waters by Anisimova (1926),

G. fungiforme resembles the unnamed isolate known as ''Bullet'' (ODU034, VDH034S, Seaborn et al. 2006, fig. 1, E and F).

Comparison with other pfiesteriaceans. The single most notable feature of the pfiesteriaceans is undoubtedly their physiology. All known species are predators or ectoparasites, while autotrophy remains unknown. The genera *Pfiesteria*, *Pseudopfiesteria*, *Tyrannodinium*, *Cryptoperidiniopsis*, *Paulsenella*, *Luciella,* and *Stoeckeria* share many characteristics, including cell structure, way of swimming, and the food-uptake mechanism employed. They are morphologically very similar in average size and shape, although size varies considerably depending on how recently food uptake has taken place. The nucleus fills most of the hypocone. Cells congregate and swarm around the prey, which may be unicellular protists (algae and protozoa), injured metazoans, or fish. In culture, blood cells (fish or human blood cells) have been used as food. The species exhibit a characteristic mode of swimming, comprising a rotating movement near the prey, the axis of movement being the dorsoventral cell axis (*Paulsenella*: Drebes and Schnepf 1982; *Cryptoperidiniopsis*: Parrow and Burkholder 2003a; *Tyrannodinium*: Calado and Moestrup 1997—see also Movies S1 and S2 in the supplementary material). The way of attachment is only documented in a few cases, but an attachment filament has been shown to be present in *T. berolinensis* (Calado and Moestrup 1997) and *Luciella masanensis* (Jeong et al. 2007). Whether trichocysts are involved in prey capture remains unknown, but trichocysts are known to be present in *Pfiesteria* (Steidinger et al. 1996), *Tyrannodinium* (Calado and Moestrup 1997), and *Paulsenella* (Schnepf et al. 1985).

Cells are attracted to their prey by chemotaxis, documented so far in *Paulsenella* (Schnepf and Drebes 1986), *Tyrannodinium* (Calado and Moestrup 1997), *Pseudopfiesteria* (Vogelbein et al. 2002), *Cryptoperidiopsis brodyi* (Steidinger et al. 2006), and *Gymnodinium fungiforme* (Spero 1985). It is likely to be a characteristic of most if not all members of the family, and cells are attracted by many organic compounds. For a detailed account, see Vogelbein et al. (2002). Once contact has been established with the prey, food is sucked up through a feeding tube supported internally by overlapping rows of microtubules, also known as a ''microtubular basket.'' This structure is presently known from *Paulsenella* (Schnepf et al. 1985), *Gymnodinium fungiforme* (Spero 1982), *Tyrannodinium* (Calado and Moestrup 1997), *Pfiesteria* (Litaker et al. 2002), *Pseudopfiesteria* (Marshall et al. 2006), and *Amyloodinium* (Lom and Lawler 1973). In *Amyloodinium,* the microtubular basket develops into the ''tentacle'' or ''root-like process'' described by Brown and Hovasse (1946). The microtubular basket is probably a characteristic of the family, although it may not be confined to the Pfiesteriaceae. The term was coined for another heterotrophic species, *Crypthecodinium cohnii,* by Kubai and Ris (1969), and although the information on the phylogenetic relationships of this species is contradictory, there is no indication that it is phylogenetically related to the Pfiesteriaceae (e.g., Murray et al. 2005, Parrow et al. 2006). The microtubular basket was also illustrated in strains from South Africa identified as *Gyrodinium lebouriae* (Lee 1977), but the phylogenetic relationships of this material remain unknown.

Amyloodinium ocellatum stands out from the other members of the Pfiesteriaceae in several respects, notably its complex life cycle, which includes a pyriform, so-called trophont, attached to fish gills and skin by a basal, flattened plate from whose borders numerous rhizoids penetrate into the host (Lom and Lawler 1973). The trophont stage changes into a cyst (tomont) the contents of which divide into as many as 256 motile cells (dinospores) that serve as the infection stage. Other pfiesteriaceans appear to have a different life cycle, comprising only the motile vegetative feeding cell, gametes, and cysts. The cyst is the meiotic stage (recognized by nuclear cyclosis), and the cyst contents divide into 2, 4, or 8 cells, which are released as motile, haploid feeding cells (Litaker et al. 2002, Parrow and Burkholder 2003b, 2004). In the SSU rRNA molecular tree published by Litaker et al. (1999), *Amyloodinium* forms a sister group to the other pfiesteriaceans.

Structurally, members of the Pfiesteriaceae differ from other dinoflagellates most particularly in the presence of a distinct plate, sometimes known as the peduncle cover plate, covering the proximal part of the sulcus. It has been found in all species examined in detail, but it does not, to our knowledge, occur outside the family. We speculate that its function may be to confer structural support to the proximal part of the peduncle, which is an area of intense activity during feeding. A comparative overview of the epithecal tabulations of known pfiesteriacean genera is given in Figure 8.

We conclude that *T. berolinense* is a freshwater member of the Pfiesteriaceae. It represents yet another case of a branch of dinoflagellates having left the marine environment in which the group originated to enter freshwater (cf. Logares et al. 2007). *T. berolinense* cells may be locally numerous, and numbers as high as 600,000 cells $\cdot$ L^{-1} have been recorded (B. Meyer in Weisse and Kirchhoff 1997). It has been assessed to feed on cryptomonad cells at a rate of 0.7–0.8 cells $\cdot$ h^{-1} for moderately starved cells (Weisse and Kirchhoff 1997), making it quantitatively important for the energy budget of the freshwater systems, especially when occurring in bloom proportions. Toxicity has not been documented.

TAXONOMIC DESCRIPTIONS

***Tyrannodinium* Calado, Craveiro, Daugbjerg et Moestrup gen. nov.**

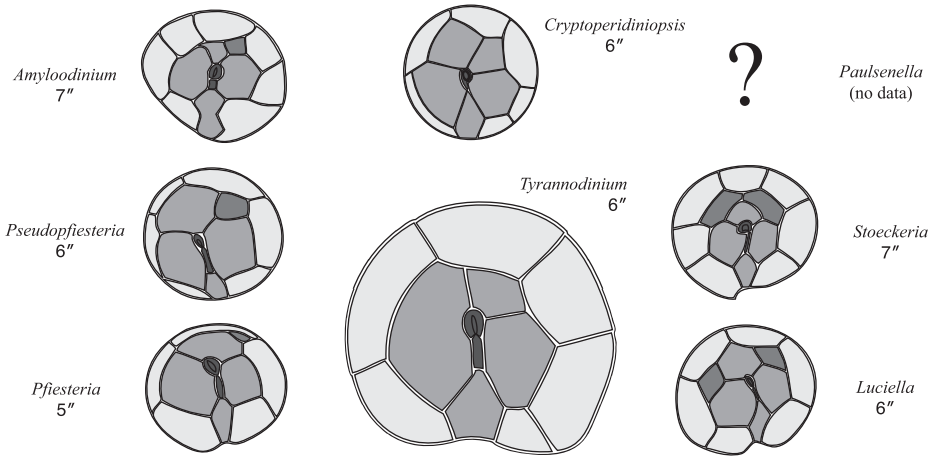

FIG. 8. Pfiesteriacean epithecal tabulations. Kofoidian series of plates are shown in different gray tones. Adapted from Landsberg et al. (1994, *Amyloodinium*), Steidinger et al. (2006, *Cryptoperidiniopsis*), Mason et al. (2007, *Luciella*), Litaker et al. (2005, *Pfiesteria* and *Pseudopfiesteria*), Jeong et al. (2005, *Stoeckeria*), and Wołoszyńska (1916, *Tyrannodinium*).

Dinoflagellati heterotrophi, aquae dulcis, cibum haurientes nutritorio canale sustento microtubulorum ordinibus impositis. Nucleus commune dinokaryon est atque fere omnem hypoconum occupat. Cellulae theca satis subtili tectae, formula kofoidiana thecarum Po, cp, x, 4′, 0a, 6″, 6c, pc, 5+ s, 5‴, 0p, 2⁗. Cingulum equatoriale, quasi circulare, leviter motum. Cellulae divisio in cystis caducis fit. Theca iuxta cinguli superiorem marginem patet. Per sexum procreatio planozygotae duobus flagellis longitudinalibus gignit. Hypnozygota ignota.

Freshwater, heterotrophic dinoflagellates that ingest food through a feeding tube supported by overlapping rows of microtubules. Nucleus a typical dinokaryon, occupying most of the hypocone. Cells covered by a relatively thin theca with the Kofoidian plate formula Po, cp, x, 4′, 0a, 6″, 6c, pc, 5+ s, 5‴, 0p, 2⁗. Cingulum equatorial, nearly circular, with small displacement. Cell division in temporary cysts, theca opening along upper edge of the cingulum. Sexual reproduction resulting in planozygotes with two longitudinal flagella. Hypnozygote unknown.

Type species: *Tyrannodinium berolinense* (Lemmermann) Calado, Craveiro, Daugbjerg et Moestrup comb. nov., designated here.

Etymology: Latin *tyrannus* (from Greek τυραννος), ''tyrant,'' in allusion to the ruthless feeding behavior. The termination–*dinium*, originally from Greek δινη, ''vortex,'' is commonly applied to dinoflagellates.

***Tyrannodinium berolinense* (Lemmermann) Calado, Craveiro, Daugbjerg et Moestrup comb. nov.**

Basionym: Peridinium berolinense Lemmermann 1900 *Ber. Deutsch. Bot. Ges.* 18, p. 308 (no figure).

Neotype: Since Lemmermann (1900) did not provide an illustration and no original material is extant, the name *P. berolinense* has no type. The illustrations provided by Lemmermann (1910, figs. 17–20 on p. 672) included a ventral and an antapical view of the cell but did not show the epithecal tabulation. Wołoszyńska (1916, pl. 13, figs. 22–26) provided a group of figures accurately representing morphology and tabulation of the species. Identification guides have repeatedly reproduced Wołoszyńska's drawings, solidly linking them to our concept of the species. Figure 5 is based on Wołoszyńska's figures, modified to include all the plates as shown by modern methods. We therefore designate Figure 5 as the type of *P. berolinense*.

Homotypic synonyms: Glenodinium berolinense (Lemmermann) Er. Lindemann (1925, pp. 162, 164); *Peridiniopsis berolinensis* (Lemmermann) Bourrelly (1968, p. 9).

***Tyrannodinium berolinense* var. *apiculatum* (Lemmermann) Calado, Craveiro, Daugbjerg et Moestrup comb. nov.**

Basionym: Peridinium berolinense var. *apiculatum* Lemmermann in West (1907, p. 188, pl. 9, fig. 3).

Note: This rarely reported form was described with a more conical epicone than the type and a concave antapex provided with two spines. Although the stability of these characters is uncertain, the size range given by Lemmermann (West 1907), 41–42 × 40–41 μm, exceeds the dimensions we found in large planozygotes. Wołoszyńska (1916), who also recognized the taxon, reported a tabulation similar to the type, but represented a cell with a pointed, rather than concave, antapex.

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Supplementary Material

The following supplementary material is available for this article:

Movie S1. *Tyrannodinium berolinense* precapture and feeding behavior. Cells exhibiting precapture rotation and attaching to an injured rotifer (0–50 s). Cell attaching to punctured spot on nematode, deploying the feeding tube and repeatedly pulling at it, revealing an apparently empty vesicle in the epicone before any visible uptake of food takes place (50–1′51 s).

Movie S2. *Tyrannodinium berolinense* feeding on punctured nematode. Food uptake in ventral view (0–26 s). Cell feeding on tip of extruded nematode gut, showing feeding tube flexibility (26–1′17 s). Food leaking out of feeding tube and being pulled back in, ventral view (1′17–1′45 s).

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