Molecular phylogeny of the dinoflagellates *Podolampas* and *Blepharocysta* (Peridiniales, Dinophyceae)

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In contrast to the typical peridinioid dinoflagellates, the podolampadaceans are characterised by the absence of both a cingulum and a depressed sulcus. Until now, gene sequences for typical core podolampadaceans were not available. Despite this, two genera that appeared related in small subunit rRNA gene (SSU rDNA) trees, the sand-dwelling *Roscoffia* and *Lessardia*, which was previously considered an unarmoured taxon, were ascribed to this group since they showed coincidences in the plate formula with the podolampadaceans. However, the classification of *Lessardia* and *Roscoffia* as podolampadaceans remains controversial. To test whether these two genera are actually podolampadaceans, we obtained the first SSU rDNA sequences of four species of *Podolampas* (the type *P. bipes*, and *P. elegans*, *P. palmipes* and *P. spinifera*) and a *Blepharocysta* species, morphologically similar to the type *B. splendor-maris*, from the type locality, western Mediterranean Sea. SSU rDNA phylogenies showed that podolampadaceans and *Roscoffia* formed a well-supported monophyletic group composed of two subclades: one for *Roscoffia* and *Blepharocysta* and one other for *Podolampas* species. The whole podolampadacean clade branched in the lineage comprising Gymnodiniales, Peridiniales and Prorocentrales. By applying different methods and taxon sampling, we obtained alternative obtained alternative branching topologies. Either the podolampadaceans branched with low statistical support with *Heterocapsa*, *Scrippsiella* and *Peridinium*, while *Lessardia* branched relatively far with other peridinioids, or *Lessardia* branched as sister clade of the podolampadaceans with moderate support. The position of *Lessardia* appeared to be very instable, largely depending on the taxonomic sampling used. Although members of the *Diplopsalis* group have been suggested as ancestors of the podolampadaceans, our SSU rDNA phylogenies did not support a close relationship between both clades.

Key Words: Dinoflagellata, Diplopsalid, Gymnodiniales–Peridiniales–Prorocentrales lineage, Heterotrophic dinoflagellate, *Lessardia*, *Roscoffia*, SSU rDNA phylogeny

INTRODUCTION

The members of the family Podolampadaceae Lindemann have unique characteristics among the thecate dinoflagellates, such as a cryptic cingulum and the absence of an apparent sulcus (Carbonell-Moore 1994a). Because of these peculiarities, the plate formula of the podolampadaceans has been described in many different ways, depending on whether the authors consider the three large plates in the lower cell half to be the three cingular plates found in peridinioids (Balech 1963, 1988) or as postcingular plates (Abé 1966). Carbonell-Moore (1994a) redefined the plate formula of the podolampadaceans based on the plate homology comparison with the hypotheca of members of the *Diplopsalis* group with one antapical plate. The plate formula was written as Po, Pt, X, 3′, 1a, 5″, 3C 4-5S, 4-5‴, 1⁗, instead of 2-3‴, 3″. This author suggested that the diplopsalids 'are ancestors' of the podolampadaceans.

Three genera have traditionally been included in the Podolampadaceae: *Blepharocysta* Ehrenberg, *Pod lampas* Stein and *Lissodinium* Matzenauer. The latter was considered as a synonym of *Blepharocysta* by some authors

(Taylor 1976; Sournia 1986). *Blepharocysta* cells are spherical without spines and *Podolampas* cells are pyriform and dorso-centrally compressed and have pronounced antapical spines; whereas, *Lissodinium* cells are pebble-like, laterally compressed and usually lacking, or having very small, antapical spines. The first podolampadacean, *Blepharocysta splendor-maris* (Ehrenberg) Ehrenberg, was described from the western Mediterranean Sea, and the species name was later misused (Elbrächter *et al.* 2004). Nearly all the Mediterranean records of *Blepharocysta* have been assigned to this type species (Gómez, 2003). For example, Margalef (1969) grouped under *B. splendor-maris* the records of this taxon and of *B. paulsenii* Schiller due to the difficulties to differentiate both species. In contrast, the species of the genus *Podolampas* are highly distinctive, mainly by their cell body shape and their strong antapical spines. Stein (1883) described the type *P. bipes* Stein and another species, *P. palmipes* Stein, from the locality of Quarnero (northwestern Mediterranean Sea). The same year, Gourret (1883) described *Parrocelia ovata*, which corresponded to *P. bipes*, from the Bay of Marseille (northwestern Mediterranean Sea). Two other distinctive species, *P. elegans* Schütt and *P. spinifera* Okamura, were described later. These are oceanic, generally warm tropical

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species, although some can be occasionally found in temperate waters. A fifth species, *P. antarctica*, which resembles *P. spinifera*, is only known from the cold waters of the southern hemisphere (see a review in Carbonell-Moore 1994b). There are no taxonomical difficulties for the identification of *Podolampas* at the species level other than discrepancies in the consideration of *Podolampas bipes* var. *reticulata* (Kofoid) F.J.R. Taylor as a form or as an independent species (*P. reticulata* Kofoid), and for the validity of *P. curvatus* Schiller, tentatively described from an aberrant or deteriorate specimen. In contrast to the distinctive *Podolampas* species, an accurate species identification of *Blepharocysta* and *Lissodinium* is rather difficult to achieve during routine examinations of freshly collected phytoplankton by light microscopy and, in the case of preserved material, they may be mistaken for invertebrate eggs or cysts (Balech 1963, 1988). Carbonell-Moore paid attention to the 'dark spheres' from net oceanic samples and with the aid of scanning electron microscopy, she emended the description of many of the known podolampadaceans and described the new genera *Mysticella*, *Heterobractum* and *Gaarderia* that differed on the apical pore types, cell compression and cell bilateral asymmetry (Carbonell-Moore 1991, 1992, 1993, 1994a).

Balech (1956) described the sand-dwelling thecate dino-flagellate *Roscoffia capitata* but he did not mention any relation to the planktonic podolampadaceans that he also intensively investigated (Balech 1963, 1988). *Roscoffia* has been placed either within the family Oxytoxaceae Linde-mann (Dodge 1982; Sournia 1986) or as *incertae sedis* within the Peridiniales (Fensome *et al.* 1993). Horiguchi & Kubo (1997) described a second *Roscoffia* species, *R. minor*, that they considered to possess a thecal plate arrangement somewhat intermediate between those exhibited by members of the subfamily Diplopsalioideae, which have one antapical plate (*Diplopsalis* Bergh, *Preperidinium* Mangin, *Boreadinium* Dodge & Hermes), and the podolampadaceans. Horiguchi & Kubo (1997) reported that 'because the family Podolampadaceae is well defined by its distinct external morphology (no apparent cingulum), it is not appropriate to place *R. minor* in this family even though the plate tabulation is basically the same'. Hoppenrath & Elbrächter (1998) re-examined the morphology of *Roscoffia bicapitata* and they did not suggest any relation to podolampadaceans. Two recently described benthic genera, *Cabra* Sh. Murray & Patterson and *Rhinodinium* Sh. Murray, Hoppenrath, Yoshimatsu, Toriumi & Larsen, have been related to *Roscoffia* and the podolampadaceans based on the plate formula similarities (Murray & Patterson 2004; Murray *et al.* 2006).

A species known in the past as the unarmoured 'Gymnodinium elongatum Hope' was redescribed under a new thecate genus as *Lessardia elongata* Saldarriaga & F.J.R. Taylor by Saldarriaga *et al.* (2003). They found that *Lessardia* coincided in the thecal plate pattern with *Roscoffia* and the Podolampadaceae, with the exception of three antapical plates in *Lessardia*. Using molecular data, Saldarriaga *et al.* (2003) related *Lessardia* and *Roscoffia*, though with very weak statistical support, and suggested their ascription to the Podolampadaceae. However, molecular data for any of the core genera of the Podolampada-

ceae (i.e. *Podolampas* and *Blepharocysta*) were missing. Carbonell-Moore (2004) did not include either *Roscoffia* or *Lessardia* in the family Podolampadaceae. She considered that *Lessardia* showed many differences from the podolampadaceans (plate formula, appearance, apical pore and other minor features), interrupting the homogeneity that characterizes the Podolampadaceae. This author preferred to assign the monotypic genus *Lessardia* to its own family, the Lessardiaceae Carbonell-Moore.

To solve the controversy on the relationships between *Roscoffia*, *Lessardia* and the podolampadaceans, and the speculation about the tentative origin of this family from a diplopsalid ancestor, it was essential to obtain sequences of the core podolampadacean species. In this study, we present the first phylogenetic analyses based on SSU rDNA sequences of the genera *Blepharocysta* and *Podolampas* from single specimens collected from the western Mediterranean Sea, the type locality of both genera. These include the four species of *Podolampas* known from warm and temperate waters and specimens of the type genus *Blepharocysta* that resemble the type species, *B. splendor-maris*. In addition, to address the different phylogenetic questions concerning the core podolampadaceans and the putatively related genera (*Roscoffia* and *Lessardia*), our study also intends to evaluate whether the tabulation is a reliable taxonomic character for the identification and classification of these dinoflagellates.

MATERIAL AND METHODS

Sampling and isolation

Specimens were collected by slowly filtering surface sea water taken from the pier of the Station Marine d'Endoume at Marseille (43°16′48″N, 5°20′57″E, bottom depth 3 m) from October 2007 to September 2008. A strainer of 20-, 40- or 60-μm netting aperture was used to collect planktonic organisms from water volumes ranging between 10 and 100 litres, depending on particle concentration. In addition, we also studied samples collected during several monitoring research cruises to the SOMLIT (Service d'Observation en Milieu LITtoral) station in the Bay of Marseille (43°14′30″N, 05°17′30″E, bottom depth 60 m). Seawater samples were collected with a 12-litre Niskin bottle at 40 and 55 m depth and filtered as described above. The plankton concentrate was scanned in settling chambers at ×100 magnification with a Nikon Eclipse TE200 inverted microscope (Nikon, Tokyo, Japan). Cells were photo-graphed alive at ×200 or ×400 magnification with a Nikon Coolpix E995 digital camera. Sampling continued from October 2008 to December 2008 in the surface waters of the port of Banyuls sur Mer, France (42°28′50″N, 3°08′09″E, bottom depth 3 m). The samples were prepared with the same procedure as described above. The specimens were observed with an Olympus IX51 inverted microscope (Olympus, Tokyo, Japan) and photographed with an Olympus DP71 digital camera. Each specimen was individually micropipetted with a fine capillary into another chamber and washed several times in serial drops of 0.2-μm filtered and sterilized seawater. Finally, the specimen was

picked up and deposited into a 0.15-ml Eppendorf tube filled with several drops of 100% ethanol. The sample was kept at laboratory temperature and in darkness until the molecular analyses could be performed.

PCR amplification of small subunit rRNA genes (SSU rDNAs) and sequencing

The specimens fixed in ethanol were centrifuged gently for 5 min at 3000 rpm. Ethanol was then evaporated in a vacuum desiccator and single cells were resuspended directly in 25 µl of Ex TaKaRa (TaKaRa, distributed by Lonza Cia., Levallois-Perret, France) PCR mix containing 10 pmol of the eukaryotic-specific SSU rDNA primers EK-42F (5′-CTCAARGAYTAAGCCATGCA-3′) and EK-1520R (5′-CYGCAGGTTCACCTAC-3′). The PCR amplifications were performed under the following conditions: 2 min denaturation at 94°C; 10 cycles of 'touch-down' PCR (denaturation at 94°C for 15 s; a 30 s annealing step at decreasing temperature from 65 down to 55°C employing a 1°C decrease with each cycle-, extension at 72°C for 2 min); 20 additional cycles at 55°C annealing temperature; and a final elongation step of 7 min at 72°C. A nested PCR amplification was then carried out using 2–5 µl of the first PCR amplification in a GoTaq (Promega, Lyon, France) polymerase reaction mix containing the eukaryotic-specific primers EK-82F (5′-GAAACTGCGAATGGCTC-3′) and EK-1498R (5′-CACCTACGGAAACCTTGTTA-3′) and similar PCR conditions as above. A third, seminested, PCR was carried out using the dinoflagellate specific primer DIN464F (5′-TAACAATACAGGGCATCCAT-3′) (Gómez *et al.* 2009). Amplicons of the expected size (~1200 bp) were then sequenced bidirectionally using primers DIN464F and EK-1498R (Cogenics, Meylan, France). The sequences were deposited in GenBank with accession numbers FJ888593–FJ888597.

Phylogenetic analyses

The new sequences were aligned to a large multiple sequence alignment containing 890 publicly available complete or nearly complete (> 1300 bp) dinoflagellate SSU rDNA sequences using the profile alignment option of MUSCLE 3.7 (Edgar 2004). The resulting alignment was manually inspected using the program ED of the MUST package (Philippe 1993). Ambiguously aligned regions and gaps were excluded in phylogenetic analyses. Preliminary phylogenetic trees with all sequences were constructed using the Neighbour Joining (NJ) method (Saitou and Nei 1987) implemented in the MUST package (Philippe 1993). These trees allowed identifying the closest relatives of our sequences, which were selected together with a sample of other dinoflagellate species to carry out more computationally intensive Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. ML analyses were done with the program TREEFINDER (Jobb *et al.* 2004) applying a GTR + Γ + I model of nucleotide substitution, taking into account a proportion of invariable sites, and a Γ-shaped distribution of substitution rates with four rate categories. Bootstrap values were calculated using 1000 pseudoreplicates with the same substitution model. The BI analyses

were carried out with the program PHYLOBAYES applying a GTR + CAT Bayesian mixture model (Lartillot and Philippe 2004), with two independent runs and 1,000,000 generations per run. After checking convergence (maximum difference between all bipartitions < 0.01) and eliminating the first 1500 trees (burn-in), a consensus tree was constructed sampling every 100 trees.

A preliminary phylogenetic analysis of our *Podolampas* and *Blepharocysta* sequences included in an exhaustive dinoflagellate SSU rDNA sequence alignment showed that the species of these genera branched close to representatives of the thecate dinoflagellates (not shown). Their phylogeny was further investigated by applying Maximum Likelihood (ML) and Bayesian Inference (BI) methods upon a more restricted taxonomic sampling, including 99 taxa representing different peridinioids, especially sequences of members with three or four cingular plates such as *Lessardia, Roscoffia, Diplopsalis* group and *Protoperidinium* Bergh.

RESULTS

The records of *Podolampas* were sporadic along one-year sampling in the coast of Marseille. Most of these records corresponded to *Podolampas palmipes*, *P. elegans* and, more rarely, *P. bipes*. Only three specimens of *P. spinifera* were observed during the year. All the *Podolampas* species were colourless, except for *P. bipes* that sometimes presented green granules similar to those described as endocytobionts by Schweikert & Elbrächter (2004). From samples collected in Marseille, we obtained the first SSU rDNA sequences for the type species *P. bipes* and also for *P. palmipes* and *P. elegans* (Figs 1, 2, 4). From specimens collected in the port of Banyuls sur Mer, we obtained the sequences of two specimens of *P. spinifera* and an additional sequence of *P. elegans* (Figs 3, 5, 6). The two sequences of *P. spinifera* were identical, as well as the other two of *P. elegans*, so that we only used one from each species in our phylogenetic analyses. While the identification of *Podolampas* at the species level was very easy, the diagnostic characters used for the identification of the different *Blepharocysta* species were difficult to determine under routine inverted microscopy observations of the actively swimming living specimens. The specimens of *Blepharocysta* illustrated here presented the morphology usually reported as the type species, *Blepharocysta splendor-maris*, in the studies based on fixed material from the coastal Mediterranean Sea (Margalef 1969). Nevertheless, despite this similarity, the uncertainty linked to the observation of living material led us to classify these specimens as *Blepharocysta* sp. (Figs 7–9). The SSU rDNA sequences of the three specimens, two collected from the Bay of Marseille and one from the port of Banyuls sur Mer, were identical.

We studied the phylogenetic position of our *Podolampas* and *Blepharocysta* species using a data set including a variety of dinoflagellate SSU rDNA sequences and rooted using perkinsozoan and syndinean sequences as the outgroup (Fig. 10). All the new sequences formed a well-supported clade [bootstrap proportion (BP) of 99% and posterior probability (PP) of 1], which also included
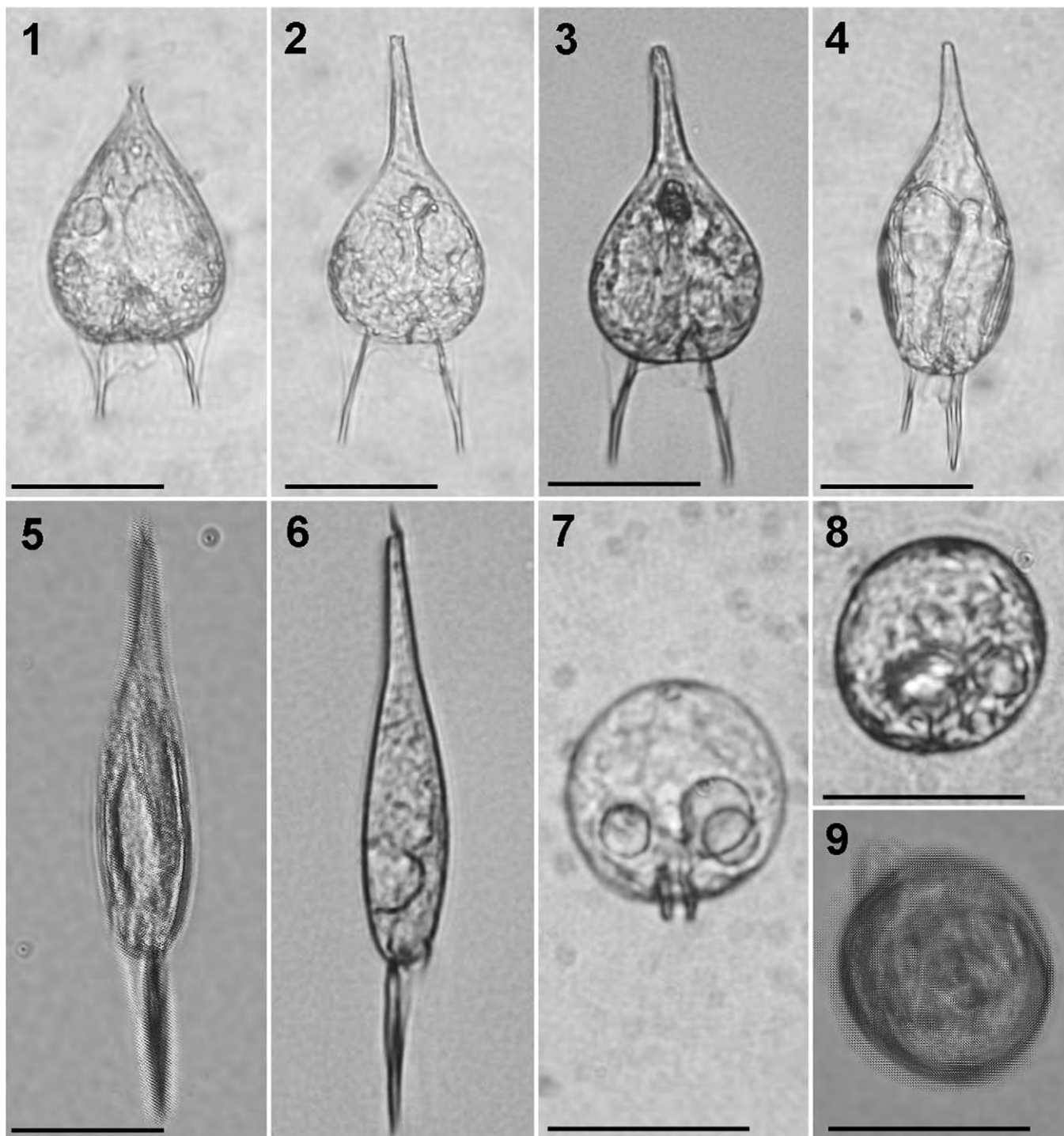
Figs 1–9. Light micrographs of the specimens of *Podolompas* and *Blepharocysta* used for the single-cell PCR analysis. Scale bar = 50 μm.
Fig. 1. *Podolompas bipes* (Endoume, 11 June 2008).
Fig. 2. *P. elegans* (Bay of Marseille, 2 September 2008).
Fig. 3. *P. elegans* (Banyuls, 27 November 2008).
Fig. 4. *P. palmipes* (Endoume, 14 December 2007).
Fig. 5. *P. spinifera* (Banyuls, 30 November 2008).
Fig. 6. *P. spinifera* (Banyuls, 7 December 2008).
Fig. 7. *Blepharocysta* sp. (Bay of Marseille, 2 September 2008).
Fig. 8. *Blepharocysta* sp. (Bay of Marseille, 2 September 2008).
Fig. 9. *Blepharocysta* sp. (Banyuls, 1 December 2008).

Roscoffia capitata. The clade formed by *Blepharocysta*, *Podolampas* and *Roscoffia* (which we will hereafter name the podolampadacean clade, assuming that *Roscoffia* is a *bona fide* podolampadacean) branched within the lineage comprising Gymnodiniales, Peridiniales and Prorocentrales (Fig. 10). Nevertheless, it was difficult to establish the placement of the podolampadaceans within this group. Their closest relatives appeared to be species of the genera *Heterocapsa*, *Scrippsiella* and *Peridinium*, although with weak support (BP of 56% and PP of 0.67). The phylogenetic position of *Lessardia* was particularly instable, largely depending on the taxonomic sampling used. In a relatively taxon-sampling rich phylogenetic tree (Fig. 10), *Lessardia* and an environmental SSU rDNA sequence from the Sargasso Sea (SMC28C10), branched as sister group of the core podolampadaceans, with a moderate support (BP 81% and PP 0.87), which agreed with the proximity between this species and *Roscoffia* observed by Saldarriaga *et al.* (2003), also with moderate support. However, in some phylogenies with other taxonomic samplings, *Lessardia* branched far from the podolampadaceans, close to peridinioid representatives (*Cachonina* A.R. Loeblich III and *Heterocapsa* Stein), whereas the podolampadaceans branched close to Scrippsiella Balech or Pentapharsodinium Indelicato & Loeblich III, though always with very low support (BP < 60% and PP < 0.70) (data not shown). Therefore, the possible relationship between *Lessardia* and the podolampadaceans remains an open question, although the fact of retrieving it in the trees with the richest taxonomic sampling suggests that it may be the correct position. In contrast with this instability, in all our phylogenies the sequences of protoperidiniaceans and diplopsalids (represented by species of *Protoperidinium*, *Diplopsalis*, *Oblea* Balech and *Gotoius* Abé) formed a weakly supported clade (BP < 50% and PP < 0.60) that branched with gonyaulacoid dinoflagellates (such as *Ceratium* Schrank and *Gonyaulax* Diesing). Therefore, our SSU rDNA phylogeny appeared to provide no evidence for a close relationship between the diplopsalids and the podolampadaceans. Nevertheless, all diplopsalid species displayed very long branches in the SSU rDNA phylogenies, so that we cannot discard that they emerged at an artefactual position due to a long branch attraction artefact, especially taking into account that all species in that region of the tree had also very long branches (Fig. 10).

We have carried out a more detailed analysis of the internal phylogeny of the podolampadaceans using as outgroup several short-branching GPP sequences (Fig. 11). In agreement with the result of the more general phylogenetic trees, this analysis showed the podolampadaceans subdivided into two subclades: a very well-supported group (BP 97% and PP 1) containing the four *Podolampas* species, and a second group joining *Blepharocysta* and *Roscoffia* (BP 86% and PP 0.92). *Podolampas bipes* branched with *P. elegans*, and *P. palmipes* with *P. spinifera*, though the support for these internal nodes within the *Podolampas* group was extremely low. Nevertheless, this result agreed with the general morphological appearance of these species: *P. bipes* and *P. elegans* having globular cell bodies with two highly developed antapical spines of similar length (Figs 1–3), and *P. palmipes* and *P. spinifera* having
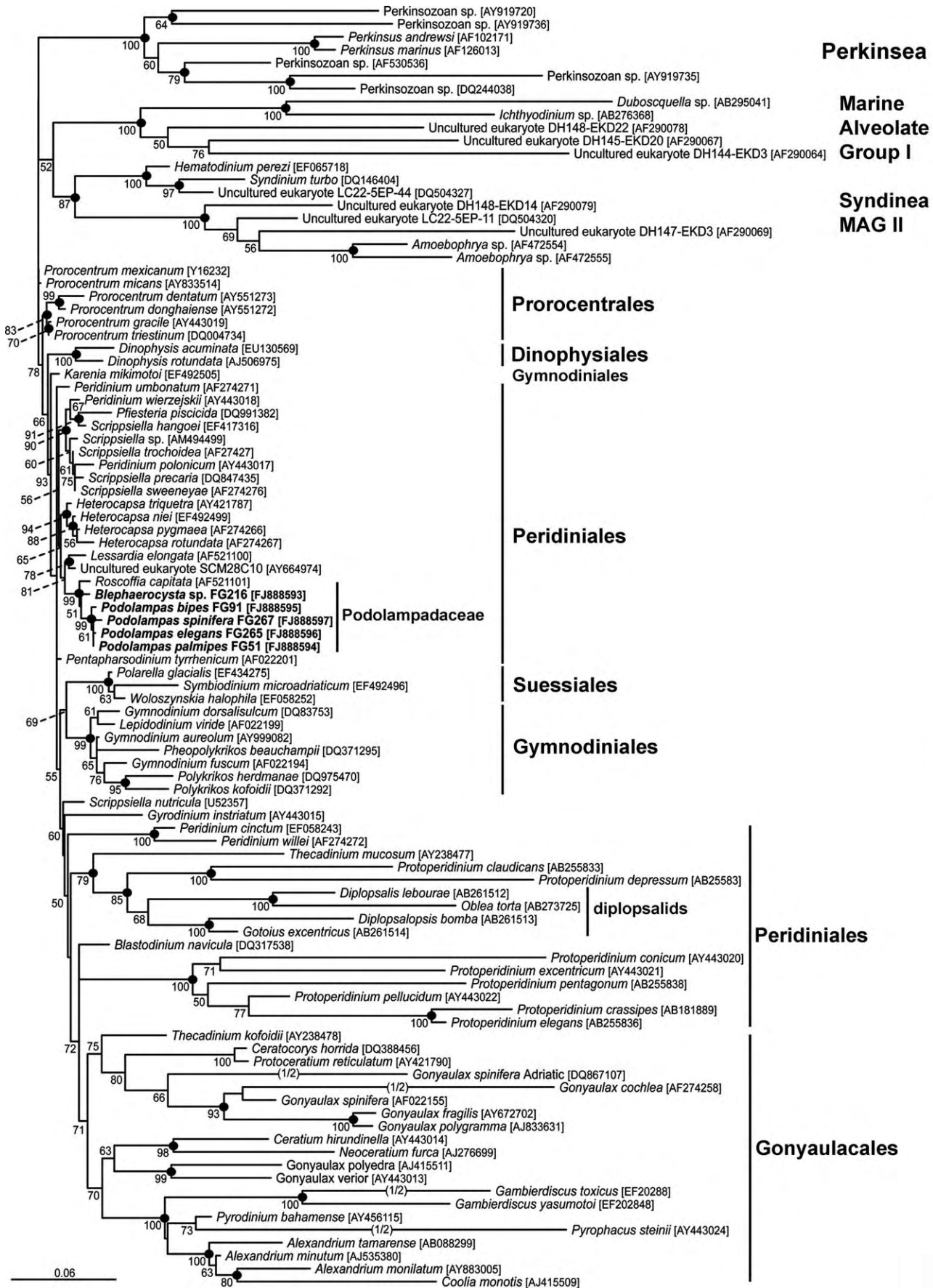
slender cell bodies and two antapical spines of different shapes and lengths (Figs 4–6).

DISCUSSION

The podolampadaceans are thecate dinoflagellates in which the cingulum is not fully apparent. Carbonell-Moore (1994a) concluded that the three plates on their equatorial plane are the homologues of the three cingular plates of other peridinioids. The five plates adjacent to the three cingular plates in the hypotheca are the homologues of the five postcingular plates found in the hypotheca of other peridinioids. The plate posterior to the postcingular plate series and the sulcus is the homologue of the single antapical plate in *Diplopsalis* group. Carbonell-Moore (1994a) modified the hypothecal plate formula from 2–3‴ 3⁗ to 4–5‴ 1⁗, which corresponded to diplopsalids with one antapical plate (Dodge & Toriumi 1993). Most of the thecate dinoflagellates have five to six cingular plates, and the unusual occurrence of three cingular plates is restricted to podolampadaceans, diplopsalids and a few benthic genera (Steidinger & Tangen 1997). Taking this into account, Carbonell-Moore (1994a) considered the diplopsalids as a probable ancestor of the podolampadaceans. However, the hypothecal tabulation 4–5‴ 1⁗ is quite common among thecate dinoflagellates other than diplopsalids and the podolampadaceans (Steidinger & Tangen 1997). Recent molecular studies have confirmed the phylogenetic relationship between the diplopsalids and *Protoperidinium*, the latter with three cingular plates plus a transitional plate (Gribble and Anderson 2006, Matsuoka *et al.* 2006). Moreover, our SSU rDNA phylogenetic analyses did not support the grouping of diplopsalids and podolampadaceans (Fig. 10).

The re-interpretation of the podolampadacean hypothecal tabulation from 2–3‴ 3⁗ to 4–5‴ 1⁗ was based on the comparison with diplopsalids (Carbonell-Moore 1994a). Interestingly, despite the fact that there is no phylogenetic evidence to relate the diplopsalids and the podolampadaceans, the re-interpretation of the plate formula seems to be correct. Our study has revealed that *Roscoffia* is a *bona fide* member of the podolampadacean clade, related to the type *Blepharocysta* (Fig. 11). In contrast to the planktonic podolampadaceans, *Roscoffia* possesses a well-defined cingulum that facilitates the interpretation of the plate formula. Its hypothecal tabulation (5‴ 1⁗) justifies the re-interpretation of the tabulation in Podolampadaceae. Until now, the podolampadaceans were characterized by the absence of an apparent cingulum, but with the inclusion of *Roscoffia*, this feature is now restricted to the planktonic podolampadaceans. Since *Roscoffia* is related to *Blepharocysta* within this group, it may be deduced that the cingulum has been independently lost in *Blepharocysta* and *Podolampas*. Subsequently, being most likely a convergent character, the cingulum aspect (but not the number of cingular plates) has a low systematic value for this clade.

The diplopsalids and *Protoperidinium* coincided with the planktonic podolampadaceans in their general appearance, i.e. globular cells in *Blepharocysta* and *Diplopsalis*, habitat,
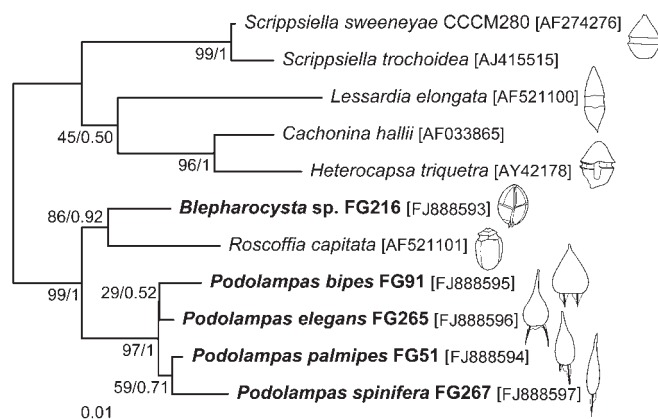
Perkinsozoan sp. [AY919720]
Perkinsozoan sp. [AY919736]
Perkinsus andrewsi [AF102171]
Perkinsus marinus [AF126013]
Perkinsozoan sp. [AF530536]
Perkinsozoan sp. [AY919735]
Perkinsozoan sp. [DQ244038]

Perkinsea

Duboscquella sp. [AB295041]
Ichthyodinium sp. [AB276368]
Uncultured eukaryote DH148-EKD22 [AF290078]
Uncultured eukaryote DH145-EKD20 [AF290067]
Uncultured eukaryote DH144-EKD3 [AF290064]

Marine Alveolate Group I

Hematodinium perezi [EF065718]
Syndinium turbo [DQ146404]
Uncultured eukaryote LC22-5EP-44 [DQ504327]
Uncultured eukaryote DH148-EKD14 [AF290079]
Uncultured eukaryote LC22-5EP-11 [DQ504320]
Uncultured eukaryote DH147-EKD3 [AF290069]
Amoebophrya sp. [AF472554]
Amoebophrya sp. [AF472555]

Syndinea MAG II

Prorocentrum mexicanum [Y16232]
Prorocentrum micans [AY833514]
Prorocentrum dentatum [AY551273]
Prorocentrum donghaiense [AY551272]
Prorocentrum gracile [AY443019]
Prorocentrum triestinum [DQ004734]

Prorocentrales

Dinophysis acuminata [EU130569]
Dinophysis rotundata [AJ506975]

Dinophysiales
Gymnodiniales

Karenia mikimotoi [EF492505]
Peridinium umbonatum [AF274271]
Peridinium wierzejskii [AY443018]
Pfiesteria piscicida [DQ991382]
Scrippsiella hangoei [EF417316]
Scrippsiella sp. [AM494499]
Scrippsiella trochoidea [AF27427]
Peridinium polonicum [AY443017]
Scrippsiella precaria [DQ847435]
Scrippsiella sweeneyae [AF274276]
Heterocapsa triquetra [AY421787]
Heterocapsa niei [EF492499]
Heterocapsa pygmaea [AF274266]
Heterocapsa rotundata [AF274267]
Lessardia elongata [AF521100]
Uncultured eukaryote SCM28C10 [AY664974]
Roscoffia capitata [AF521101]
Blephaerocysta sp. FG216 [FJ888593]
Podolampas bipes FG91 [FJ888595]
Podolampas spinifera FG267 [FJ888597]
Podolampas elegans FG265 [FJ888596]
Podolampas palmipes FG51 [FJ888594]

Podolampadaceae

Peridiniales

Pentapharsodinium tyrrhenicum [AF022201]
Polarella glacialis [EF434275]
Symbiodinium microadriaticum [EF492496]
Woloszynskia halophila [EF058252]

Suessiales

Gymnodinium dorsalisulcum [DQ83753]
Lepidodinium viride [AF022199]
Gymnodinium aureolum [AY999082]
Pheopolykrikos beauchampii [DQ371295]
Gymnodinium fuscum [AF022194]
Polykrikos herdmanae [DQ975470]
Polykrikos kofoidii [DQ371292]

Gymnodiniales

Scrippsiella nutricula [U52357]
Gyrodinium instriatum [AY443015]
Peridinium cinctum [EF058243]
Peridinium willei [AF274272]
Thecadinium mucosum [AY238477]
Protoperidinium claudicans [AB255833]
Protoperidinium depressum [AB25583]
Diplopsalis lebourae [AB261512]
Oblea torta [AB273725]
Diplopsalopsis bomba [AB261513]
Gotoius excentricus [AB261514]

diplopsalids

Blastodinium navicula [DQ317538]
Protoperidinium conicum [AY443020]
Protoperidinium excentricum [AY443021]
Protoperidinium pentagonum [AB255838]
Protoperidinium pellucidum [AY443022]
Protoperidinium crassipes [AB181889]
Protoperidinium elegans [AB255836]

Peridiniales

Thecadinium kofoidii [AY238478]
Ceratocorys horrida [DQ388456]
Protoceratium reticulatum [AY421790]
Gonyaulax spinifera Adriatic [DQ867107]
Gonyaulax cochlea [AF274258]
Gonyaulax spinifera [AF022155]
Gonyaulax fragilis [AY672702]
Gonyaulax polygramma [AJ833631]
Ceratium hirundinella [AY443014]
Neoceratium furca [AJ276699]
Gonyaulax polyedra [AJ415511]
Gonyaulax verior [AY443013]
Gambierdiscus toxicus [EF20288]
Gambierdiscus yasumotoi [EF202848]
Pyrodinium bahamense [AY456115]
Pyrophacus steinii [AY443024]
Alexandrium tamarense [AB088299]
Alexandrium minutum [AJ535380]
Alexandrium monilatum [AY883005]
Coolia monotis [AJ415509]

Gonyaulacales

Dinokaryota

0.06

Fig. 11. Maximum likelihood phylogenetic tree of podolampadacean and closely related dinoflagellate SSU rDNA sequences, based on 1232 aligned positions. Names in bold represent sequences obtained in this study. Numbers at nodes are bootstrap proportions (left) and Bayesian analysis posterior probabilities (right). Accession numbers are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

and also type of feeding (pallium-feeders) (Dodge 1982; Sournia 1986; Carbonell-Moore 2004b; Gribble & Anderson 2006). However, it is not possible to confirm that all podolampadaceans are pallium-feeders because the mode of feeding in *Roscoffia*, which our analyses authenticate as a *bona fide* podolampadacean, is unknown. Hoppenrath & Elbrächter (1998) disagreed from the view of the diplopsalids as possible ancestors of the podolampadaceans, by stating that *Podolampas* divides by desmoschisis whereas the members of the Peridiniaceae *sensu lato*, including the diplopsalids, divide by eleutheroschisis (Hoppenrath & Elbrächter 1998). However, the type of division, difficult to observe for numerous dinoflagellates, especially the oceanic heterotrophic species, is unknown for other podolampadaceans, including *Roscoffia* (Hoppenrath & Elbrächter 1998).

The placement of *Roscoffia* within the podolampadaceans reveals that the type of habitat, benthic or planktonic, is not a constant feature for this peridinioid clade. In other peridinioid taxa, such as *Heterocapsa*, the first benthic representative has been recently described (Tamura *et al.* 2005). It opens the possibility that other benthic genera may also belong to the podolampadaceans, especially those with a low number of cingular plates. Several species of *Amphidiniopsis* Woloszyńska have been reported with three cingular plates (Table 1). In some of them, the cingulum incompletely encircles the cell and this may explain the low number of cingular plates. The monotypic genera *Cabra* and *Rhinodinium* also have a low number of cingular plates and the hypothecal formula (5″, 1‴′) coincided with the podolampadaceans and diplopsalids (Murray & Patterson 2004; Murray *et al.* 2006; Table 1). Nevertheless, only the LSU rDNA sequence of *Rhinodinium broomense* is avail-

able, so that more data, in particular SSU rDNA sequences, will be necessary to investigate their possible relationship with the podolampadaceans.

Lessardia elongata was described as a member of the family Podolampadaceae based on SSU rDNA phylogenetic analysis (Saldarriaga *et al.* 2003), despite the lack of sequences of the core podolampadacean species and the numerous morphological differences of *L. elongata* with the podolampadaceans as reviewed by Carbonell-Moore (2004). The main differences in the plate formula are found in the hypotheca: four postcingular plates and three antapical plates in *Lessardia*, but five postcingular and one antapical plate in the podolampadaceans. Saldarriaga *et al.* (2003) reported that the fact that *Lessardia* has three antapical plates rather than one is not problematic because the supposed closest peridinialean family to the Podolampadaceae, the Protoperidiniaceae, has members with both one and two antapical plates, so that this feature appears to vary easily, despite the fact that some authors considered the number of cingular and postcingular plates conservative characters (Balech 1980). Molecular phylogeny supports a close relationship between diplopsalids (one or two antapical plates) and *Protoperidinium* (two antapical plates) (Gribble & Anderson 2006; Matsuoka *et al.* 2006), but our results do not confirm a proximity between the Protoperidiniaceae and the Podolampadaceae (Fig. 10). Saldarriaga *et al.* (2003) also reported the broad flat cingulum of *Lessardia* as a characteristic of the Podolampadaceae. Nevertheless, while *Roscoffia* possesses a well-marked cingulum, its closest relative, *Blepharocysta*, apparently lacks a cingulum. As for the other characters mentioned above, this precludes to consider the cingulum shape as a stable diagnostic criterion for this peridinioid group.

In the SSU rDNA trees, the phylogenetic position of *Lessardia* appeared to be unstable, depending on the taxonomic sampling used, but in some phylogenies it appeared as a sister group of the core podolampadaceans (Fig. 10). The podolampadaceans, *Lessardia* and *Diplopsalis* group have a similar number of cingular plates. *Lessardia* and *Diplopsalis* have a similar epithecal and hypothecal plate formula, respectively, to the podolampadaceans (Table 1). Although the hypothecal plates are considered more conservative that the epithecal ones (Balech 1980), *Lessardia* seems to modify this rule because it appears closer than the diplopsalids to the podolampadaceans in the SSU rDNA phylogenies. As reported before, the hypothecal tabulation of the diplopsalids is quite common among thecate dinoflagellates (Steidinger & Tangen 1997). This may suggest a convergence between diplopsalids and podolampadaceans, rather than a common ancestor. In contrast, the epithecal plate formula of *Lessardia* and the podolampadaceans is less common (Steidinger & Tangen 1997) and probably suggests a real evolutionary relationship.

←

Fig. 10. Maximum likelihood phylogenetic tree of dinoflagellate SSU rDNA sequences, based on 1171 aligned positions. Names in bold represent sequences obtained in this study. Numbers at the nodes are bootstrap proportions (values under 50% are omitted). Nodes supported by Bayesian posterior probabilities > 0.75 are indicated by black circles. Accession numbers are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

Table 1. Plate formula of dinoflagellates with 3–4 cingular plates and *Heterocapsa*, *Scrippsiella* and *Peridinium*.

Taxon	Apical	Intercalary	Precingular	Cingular	Sulcal	Postcingular	Antapical
Podolampas[1]	3′	1a	5″	3c	4s	5‴	1⁗
Blepharocysta[1]	3′	1a	5″	3c	4s	4–5‴	1⁗
Roscoffia capitata[2]	4′	–	5″	3c	3s	5‴	1⁗
Roscoffia capitata[3]	3′	1a	5″	3c	4s	5‴	1⁗
Roscoffia capitata[4]	3′(4′)	–	5″	3c	4s	5‴	1⁗
Roscoffia minor[3]	3′	1a	5″	3c	3s	5‴	1⁗
Lessardia elongata[5]	3′	1–2a	5″	3c	6s	4‴	3⁗
Diplopsalis group[6]	4′	1–2a (1a,2a,4a)	7″	3c		5‴	1–2⁗
Protoperidinium[6]	4′	3a (1a,2a,4a)	7″	3c	6–7s	5‴	2⁗
Rhinodinium broomeense[7]	3′	1a	5″	4c	?s	5‴	1⁗
Cabra matta[8]	4′	–	4″	3c	?s	5‴	1⁗
Amphidiniopsis kofoidii[9]	4′	3a	7″	3c	?3s	5‴	2⁗
A. hexagona, A. swedmarkii[10]	4′	2a	7″	3c	4s+2	5‴	2⁗
Amphidiniopsis[11]	4′	1–3a	6–8″	5–8c	3–5s	5–6‴	2⁗
Heterocapsa[12]	5′	3a	7″	6c	5s	5‴	2⁗
Scrippsiella[6]	4′	3a	7″	6c	4–5s	5‴	2⁗
Peridinium[6]	4′	3a	7″	5–6c	5–6s	5‴	2⁗

[1] Carbonell-Moore (2004).
[2] Balech (1956).
[3] Horiguchi & Kubo (1997).
[4] Hoppenrath & Elbrächter (1998).
[5] Saldarriaga *et al.* (2003).
[6] Steidinger & Tangen (1997).
[7] Murray *et al.* (2006).
[8] Murray & Patterson (2004).
[9] Dodge & Lewis (1986).
[10] Yoshimatsu *et al.* (2000).
[11] Selina & Hoppenrath (2008).
[12] Iwataki *et al.* (2003).

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REFERENCES

ABÉ T.H. 1966. The armoured Dinoflagellata: I. Podolampidae. *Publications of the Seto Marine Biology Laboratory* 14: 129–154.

BALECH E. 1956. Étude des Dinoflagellés du sable de Roscoff. *Revue Algologique* 2: 29–52.

BALECH E. 1963. La familia Podolampadaceae (Dinoflagellata). *Boletín del Instituto de Biología Marina Mar del Plata* 2: 3–27.

BALECH E. 1980. On the thecal morphology of dinoflagellates with special emphasis on circular [sic] and sulcal plates. *Anales del Centro de Ciencias del Mar y Limnología de la Universidad Nacional Autónoma de México* 7: 57–68.

BALECH E. 1988. Los dinoflagelados del Atlántico Sudoccidental. *Publicaciones especiales del Instituto Español Oceanografía* 1: 1–310.

CARBONELL-MOORE M.C. 1991. *Lissodinium* Matzenauer, emend., based upon the rediscovery of *L. schilleri* Matz., another member of the family Podolampadaceae Lindemann (Dinophyceae). Botanica Marina 34: 327–334.

CARBONELL-MOORE M.C. 1992. *Blepharocysta hermosillai*, sp. nov. A new member of the family Podolampadaceae Lindemann (Dinophyceae). *Botanica Marina* 35: 273–281.

CARBONELL-MOORE M.C. 1993. Further observations on the genus *Lissodinium* Matz., emend., Carbonell-Moore (Dinophyceae), with descriptions of seventeen new species. *Botanica Marina* 36: 561–587.

CARBONELL-MOORE M.C. 1994a. On the taxonomy of the family Podolampadaceae Lindemann (Dinophyceae) with descriptions of three new genera. *Review of Palaeobotany and Palynology* 84: 73–99.

CARBONELL-MOORE M.C. 1994b. On the biogeography of the family Podolampadaceae (Dinophyceae): vertical and latitudinal distribution. *Review of Palaeobotany and Palynology* 84: 23–44.

CARBONELL-MOORE M.C. 2004. On the taxonomical position of *Lessardia* Saldarriaga *et* Taylor within the family Podolampadaceae Lindemann (Dinophyceae). *Phycological Research* 52: 340–345.

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

DODGE J.D. & LEWIS J. 1986. A further SEM study of armoured sand-dwelling marine dinoflagellates. *Protistologica* 22: 221–230.

DODGE J.D. & TORIUMI S. 1993. A taxonomic revision of the *Diplopsalis* group (Dinophyceae). *Botanica Marina* 36: 137–147.

EDGAR R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.

ELBRÄCHTER M., KUSBER W.-H., JAHN R. & HOPPENRATH M. 2004. *Peridinium splendor-maris* Ehrenberg is an *Alexandrium*-species, but not *Blepharocysta splendor-maris* sensu Stein, 1883! Abstract Book of the XI International Conference on Harmful Algal Blooms, pp. 142. Cape Town, South Africa.

FENSOME R.A., TAYLOR F.J.R., NORRIS G., SARJEANT W.A.S., WHARTON D.I. & WILLIAMS G.L. 1993. *A Classification of Living and Fossil Dinoflagellates*. American Museum of Natural History, Sheridan Press, Hanover, Pennsylvania. 351 pp.

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

GÓMEZ F., LÓPEZ-GARCÍA P. & MOREIRA D. 2009. Molecular phylogeny of the ocelloid-bearing dinoflagellates *Erythropsidinium* and *Warnowia* (Warnowiaceae, Dinophyceae). *Journal of Eukaryotic Microbiology* 56: 440–445.

GRIBBLE K.E. & ANDERSON D.M. 2006. Molecular phylogeny of the heterotrophic dinoflagellates, *Protoperidinium*, *Diplopsalis* and *Preperidinium* (Dinophyceae), inferred from large subunit rDNA. *Journal of Phycology* 42: 1081–1095.

HORIGUCHI T. & KUBO F. 1997. *Roscoffia minor* sp. nov. (Peridiniales, Dinophyceae): a new, sand-dwelling, armored dinoflagellate from Hokkaido, Japan. *Phycological Research* 45: 65–69.

IWATAKI M., BOTES L., SAWAGUCHI T., SEKIGUCHI K. & FUKUYO Y. 2003. Cellular and body scale structure of *Heterocapsa ovata* sp. nov. and *Heterocapsa orientalis* sp. nov. (Peridiniales, Dinophyceae). *Phycologia* 42: 629–637.

JOBB G., VON HAESELER A. & STRIMMER K. 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* 4: 18.

LARTILLOT N. & PHILIPPE H. 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution* 21: 1095–1109.

MARGALEF R. 1969. Composición específica del fitoplancton de la costa catalano-levantina (Mediterráneo Occidental) en 1962–1967. *Investigaciones Pesqueras* 33: 345–380.

MATSUOKA K., KAWAMI H., FUJII R. & IWATAKI M. 2006. Further examination of the cyst-theca relationship of *Protoperidinium thulesense* (Peridiniales, Dinophyceae) and the phylogenetic significance of round brown cysts. *Phycologia* 45: 632–641.

MURRAY S. & PATTERSON D.J. 2004. *Cabra matta*, gen. nov. sp. nov., a new heterotrophic benthic dinoflagellate. *European Journal of Phycology* 39: 229–234.

MURRAY S., HOPPENRATH M., PREISFELD G., LARSEN J., YOSHIMATSU S., TORIUMI S. & PATTERSON D.J. 2006. Phylogenetics of *Rhinodinium broomeense* gen. et sp. nov., a peridinioid, sand-dwelling dinoflagellate (Dinophyceae). *Journal of Phycology* 42: 934–942.

PHILIPPE H. 1993. MUST, a computer package of management utilities for sequences and trees. *Nucleic Acids Research* 21: 5264–5272.

SAITOU N. & NEI M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.

SALDARRIAGA J.F., LEANDER B.S., TAYLOR F.J.R. & KEELING P.J. 2003. *Lessardia elongata* gen. et sp. nov. (Dinoflagellata, Peridiniales, Podolampadaceae) and the taxonomic position of the genus *Roscoffia*. *Journal of Phycology* 39: 368–378.

SCHWEIKERT M. & ELBRÄCHTER M. 2004. First ultrastructural investigations of the consortium between a phototrophic eukaryotic endocytobiont and *Podolampas bipes* (Dinophyceae). *Phycologia* 43: 614–623.

SELINA M. & HOPPENRATH M. 2008. An emended description of *Amphidiniopsis arenaria* Hoppenrath 2000, based on material from the Sea of Japan. *European Journal of Protistology* 44: 71–79.

SOURNIA A. 1986. *Atlas du phytoplancton marin. Introduction Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées.* Vol. I. Editions du CNRS, Paris. 219 pp.

STEIDINGER K.A. & TANGEN K. 1997. Dinoflagellates. In: *Identifying marine phytoplankton* (Ed. by C.R. Tomas), pp. 387–584. Academic Press, London.

TAMURA M., IWATAKI M. & HORIGUCHI T. 2005. *Heterocapsa psammophila* sp. nov. (Peridiniales, Dinophyceae), a new sand-dwelling marine dinoflagellate. *Phycological Research* 53: 303–311.

TAYLOR F.J.R. 1976. Dinoflagellates from the International Indian Ocean Expedition. A report on material collected by the R.V. ''Anton Bruun'' 1963–64. *Bibliotheca Botanica* 132: 1–234.

YOSHIMATSU S., TORIUMI S. & DODGE J.D. 2000. Light and scanning microscopy of two benthic species of *Amphidiniopsis* (Dinophyceae), *Amphidiniopsis hexagona* sp. nov. and *Amphidiniopsis swedmarkii* from Japan. *Phycological Research* 48: 107–113.