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Molecular Phylogeny of the Sand-dwelling Dinoflagellate *Planodinium striatum* and *Chrysodinium* gen. nov. for *Plagiodinium ballux* (Dinophyceae)

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Abstract. The molecular phylogeny of the sand-dwelling dinoflagellate *Planodinium striatum* was investigated from isolates collected in the NE English Channel. In the SSU rRNA gene phylogeny, the sequences of *Planodinium* clustered with the type species of *Plagiodinium*, *P. belizeanum*, and more distantly related to *Plagiodinium ballux*. Although the SSU rRNA gene sequences of *Planodinium* showed a high percentage of identity (96%) with partial sequences of species of *Podolampas* (~1200 base pairs), the SSU rRNA gene phylogenies did not show a relationship with the clade of the Podolampadaceae (*Podolampas*, *Blepharocysta*, *Roscoffia*), neither with the sequences of the sand-dwelling genus *Cabra*. The SSU rRNA gene sequences of *Plagiodinium belizeanum* and *P. ballux* showed a low percentage of identity (96%) clustering in separate clades in the SSU rRNA gene phylogeny with *Planodinium*. The morphology of *P. ballux* and its generic type differed in the plate arrangement and the morphology of the cingulum, amongst other features. Based on the morphological and molecular differences, we propose the new genus *Chrysodinium* gen. nov. for *P. ballux*, with the re-interpreted thecal plate formula Po 3 ' 1 " 7c 2s 5 "' 1 "".

Keywords: benthic Dinophyta, microphytobenthos, molecular phylogeny, new genus, Podolampadaceae, psammophilic Dinophyceae.

INTRODUCTION

Numerous sand-dwelling dinoflagellates have been reported from the English Channel and the North Sea where macrotidal regime results in important emersion periods affecting wide sandy beaches. During ebb tide, the exposed substratum becomes green or golden brown in color in irregular areas of the intertidal sand-flats (Gómez et al. 2011, Gómez and Artigas 2014). From the sandy beaches of the British coasts, Saunders and Dodge (1984) described the new genus and species *Planodinium striatum* as a laterally flattened heterotrophic dinoflagellate with a small epitheca and horizontal cingulum. Saunders and Dodge (1984) considered *Thecadinium petasatum*, reported from the coasts of British Columbia by Baillie (1971), as an earlier record of *P. striatum*. Hoppenrath et al. (2014) compiled records of *P. striatum* from the North, Mediterranean and Japan Seas, that suggest a wide distribution in cold and temperate waters.

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Saunders and Dodge (1984) described *Planodinium striatum* with the plate formula 3*'* 7*"* 6c 3*'"* 1*""*. They reported: 'there are some similarities between this organism and Balech's (1956) *Roscoffia capitata*'. The hypotheca of the sand-dwelling genus *Roscoffia* shows five postcingular plates (5*'"*) and a single antapical plate (1*""*) (Horiguchi and Kubo 1997, Hoppenrath and Elbrächter 1998). In the original description, Saunders and Dodge (1984) omitted two narrow longitudinal plates (two postcingular plates) that surrounded the sulcus, which sutures can be easily mistaken for the longitudinal ridges in the hypothecal plates. Hoppenrath et al. (2014) redefined the plate formula of *P. striatum* as 3*'* 1a 8*"* 7?c 5*'"* 1*""* being closer to *Roscoffia*. This hypothecal tabulation (5*'"* 1*""*) is also found in pelagic dinoflagellates such as *Archaeosphaerodiniopsis* (Carbonell-Moore 2017) and the Podolampadaceae (*Podolampas*, *Blepharocysta* and allied genera) (Carbonell-Moore 2004). Saldarriaga et al. (2003) suggested affinities of *Roscoffia* with the podolampadaceans, confirmed with the first molecular data of *Podolampas* and *Blepharocysta* (Gómez et al. 2010). The hypothecal plate formulae 5*'"* 1*""* have been also reported for other sand-dwelling genera such as *Cabra*, *Madanidinium*, *Pileidinium*, *Plagiodinium*, *Rhinodinium* or *Sabulodinium* (see a review in the tabulation in Yamaguchi et al. 2018). Wakeman et al. (2018) reported the first molecular data of the type species of *Plagiodinium*, *P. belizeanum*, and they did not find support for affinities with *Roscoffia* in the SSU rRNA gene phylogeny. Yamada et al. (2018) described a new species of *Plagiodinium*, *P. ballux*, and they provided other rRNA gene sequences identified as *Plagiodinium belizeanum*. The three SSU rRNA gene sequences of *Plagiodinium* did not show a phylogenetic relationship to *Roscoffia* (Yamada et al. 2018). In the original description of the genus *Cabra*, Murray and Patterson (2004) suggested a phylogenetic relationship with the podolampadaceans. Yamaguchi et al. (2018) reported the first molecular data of the type species of the genus *Cabra*, *C. matta*, which sequences clustered with *Roscoffia*, *Podolampas* and *Blepharocysta.* In contrast to the previous phylogenies, Yamaguchi et al. (2018) reported *Plagiodinium belizeanum* as the most basal lineage of the clade of the podolampadaceans. The relationships between *Plagiodinium* and the podolampadaceans (*Roscoffia*, *Podolampas*, *Blepharocysta*) varied among these three publications with common co-authors. In the present study, we provide the first molecular data of the type species of the genus *Planodinium*.

MATERIALS AND METHODS

Source, isolation and microscopy observations

This study was undertaken in the soft sandy sediments of the shore of Wimereux, France (50º 46*'* 12*"* N, 1º 36*'* 42*"* E) in June 2012. The upper centimeter of sand smaller pools and moist sands with a faint brownish discoloration was collected with a spoon and deposited into a bottle. There, the sand was rinsed with seawater and stirred vigorously, and the suspension settled in a composite settling chamber. The settled material was examined with an inverted microscope (Eclipse TE2000-S, Nikon, Tokyo, Japan) and photographed with a digital camera (Digital Sight DS-2M, Nikon, Tokyo, Japan). For scanning electron microscopy, aliquots of the agitated sand samples were fixed with glutaraldehyde (5%) and filtered onto a 0.8 µm pore size Nuclepore membrane filter, washed with distilled water, fixed with osmium, dehydrated with a graded series of ethanol and critical-point-dried with CO_2. Filters were mounted on stubs, sputter-coated with gold and viewed using a scanning electron microscope (S4800, Hitachi, Tokyo, Japan).

DNA extraction, PCR amplification and sequencing

For molecular analysis, each cell of *Planodinium striatum* was individually picked up with a fine capillary into a clean chamber and washed several times in serial drops of 0.2 µm filtered and sterilized seawater. Finally, 3–4 cells were deposited in a 0.2 ml Eppendorf tube filled with several drops of absolute ethanol. The sample was kept at room temperature and in darkness until the molecular analysis was performed. Prior to DNA extraction, the 0.2 ml Eppendorf tubes were centrifuged for 10 min at 14462 × g in a centrifuge (MX-201, TOMY, Tokyo, Japan). Ethanol was then evaporated in a vacuum desiccator. Cells were resuspended in 50 µL of guanidine-containing extraction buffer (GITC buffer). After thermal shocks (preserved in –80 °C overnight and heated at 70 °C for 60 min), 100 µL of isopropanol was added to precipitate the DNA following the protocol described in Nakamura et al. (2015). The precipitated DNA was rinsed with 70% ethanol and stored in 20 µL of Tris-EDTA buffer. To amplify SSU rRNA gene fragments, the primers EukA1 and EukB2 (Medlin et al. 1988) were used in a reaction with KAPA3G Plant DNA Polymerase (Kapa Biosystems, U.S.A.). The following thermocycler program was performed: initial denaturation at 95 °C for 10 min; 45 cycles of denaturation at 95 °C for 20 s, annealing at 52 °C for 15 s, and extension at 72 °C for 40 s; then final extension at 72 °C for 7 min. The amplified PCR products were purified with AMPure XP Kit (Beckman Coulter, U.S.A.). Sequencing reactions were conducted using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, U.S.A.) and a DNA autosequencer, ABI PRISM 3130xl Genetic Analyzer (ABI, Foster City, U.S.A.). The newly generated consensus sequences were deposited in DDBJ/EMBL/GenBank under accession numbers LC507455 –LC507456.

Phylogenetic analyses

The alignment used the same sequences and outgroup in the SSU rRNA gene phylogeny by Yamaguchi et al. (2018). We added the two identical sequences of *Planodinium striatum* obtained from two different samples collected in the beach of Wimereux on 21 and 23 June 2012, two environmental sequences (accession num-

bers KJ761640, KJ761122) belonging to the Podolampadaceae, the sequence of *Plagiodinium ballux* (LC054938) and another sequence identified as *Plagiodinium belizeanum* LC054937 after Yamada et al. (2018). We kept the two sequences of *Cabra matta* (LC333936–7) in the alignment despite they were anomalously long (1858 and 1856 base pairs). This is due to numerous insertions (at the positions 180–210, 250–290, and others) that were missing in other dinoflagellate sequences. Sequence alignments were accomplished by ClustalW (Larkin et al. 2007). The general time-reversible model with gamma-distributed rate variation and a proportion of invariable sites (GTR + G + I) was selected, and the phylogenetic tree was constructed by Maximum Likelihood method with the default settings in MEGA7 software (Kumar et al. 2016). Bootstrap values were obtained after 1000 replications. The sequence of apicomplexan *Neospora caninum* (L24380) was used as outgroup as in Yamaguchi et al. (2018).

RESULTS

The observations of cells of *Planodinium striatum* were scarce and they were never abundant as usually occur with heterotrophic species. The cells were active swimmers that makes difficult the detection from live samples or they may be mistaken for *Sabulodinium undulatum*. We observed up to ten cells per day during several days in June 2012 associated with a proliferation of benthic diatoms and photosynthetic dinoflagellates such as *Togula* spp., *Amphidinium* spp., *Polykrikos lebouriae*, among others. Cells of *P. striatum* were colorless with a food vacuole of brown coloration in the lower half of the hypotheca (Fig. 1A–M), and often a large pusule in the upper half of the hypotheca (Fig. 1A–B). The cells were laterally compressed with an oval shape. Dimensions were 28–35 μm long, 23–26 μm depth (dorsoventral length) and 11–15 μm wide. The cingulum was horizontal and the epitheca was much smaller and narrower than the hypotheca (Fig. 1A–M). The surface of the hypotheca showed longitudinal ridges (Fig. 1L–M). Most of the collected cells were used for molecular analysis. Only two cells were observed by SEM, but the epitheca of the cells showed attached debris and apparently mucous (Fig. 1N) or were covered by trichocysts (Fig. 1O) that rendered the observations. There was an elongated and slightly sigmoidal plate, the first postcingular plate 1*'''*, adjacent to the left side of the sulcus, while a large rectangular plate, the second postcingular plate 2*'''*, occupied most of the left lateral hypotheca (Fig. 1N). This 1*'''* plate and other narrow plate (5*'''*) adjacent to the right side of the sulcus were missing in the original description as probably the sutures were mistaken for longitudinal ridges. The hypothecal plates showed large pores arranged in longitudinal rows (Fig. 1O). Each pore was surrounded by 5–8 small pores. Trichocysts emerged from the large pores. All these characteristics fit the original description confirming that our isolates corresponded to *Planodinium striatum*.

The two SSU rRNA gene sequences of *Planodinium striatum* isolated from two samples collected on 21 and 23 June 2012 were identical for 1739 base pairs (bp) (LC507455–6). The sequences of *P. striatum* showed a percentage of similarity of 96% when compared to the partial sequence of *Podolampas elegans* (accession number FJ888596, for only 1227 bp). The comparison with full SSU rRNA gene sequences (>1700 bp) showed percentages of similarities of 93% with those of *Plagiodinium belizeanum* (KX008973) and *Roscoffia capitata* (AF521101). In the SSU rRNA gene molecular phylogeny, the sequences of *Planodinium striatum* clustered with *Plagiodinium belizeanum* (KX008973) as a sister group of *P. ballux* (LC054938) and a sequence identified as *P. belizeanum* (LC054937) (Fig. 2). The sequences of *Podolampas* spp., *Blepharocysta* sp. and *Roscoffia capitata* and two environmental sequences (KJ761122, KJ761640) formed the high supported clade of the Podolampadaceae that was distantly related to the sequences of *P. striatum* and *Plagiodinium* spp. The anomalous sequences of *Cabra matta* (LC333936–7) clustered in a basal position of the dinokaryote core (Fig. 2).

DISCUSSION

The pelagic thecate dinoflagellates are usually globular, with a well-defined equatorial cingulum constituted of horizontally elongated plates with depression harboring the transversal flagellum, and an apex with a conspicuous apical pore plate. Using the cingulum and the apical pore plate as landmarks, we can establish the tabulation of the epitheca after considering the plates above the cingulum as the precingular series, and the plates surrounding the apical pore as the apical series. However, these reference points are missing in numerous sand-dwelling dinoflagellates. The cingulum is usually very anterior that resulted in a small epitheca, and it is difficult to discern the tiny epithecal plates which morphologies are also altered due to the cell flattening. The uncertainties in the assignment of the epithecal plates increase when the

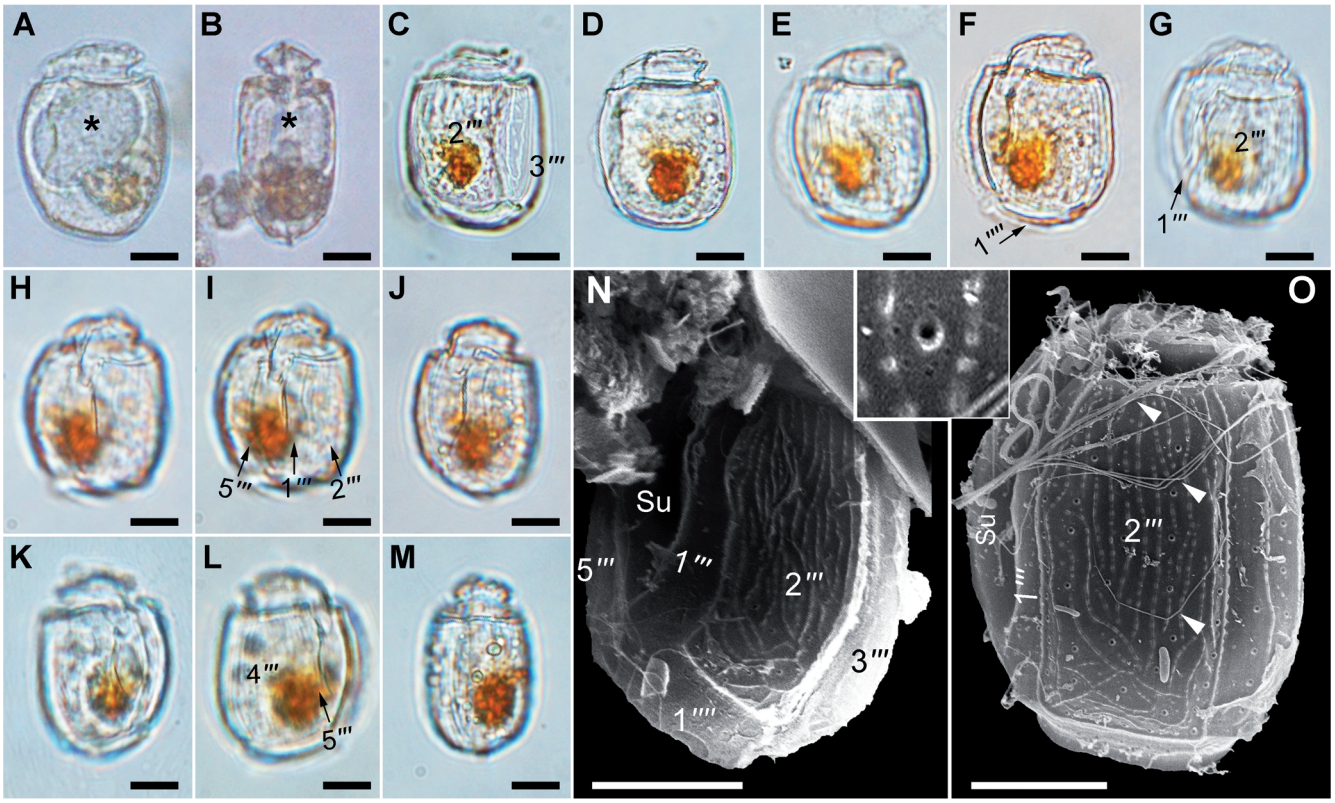
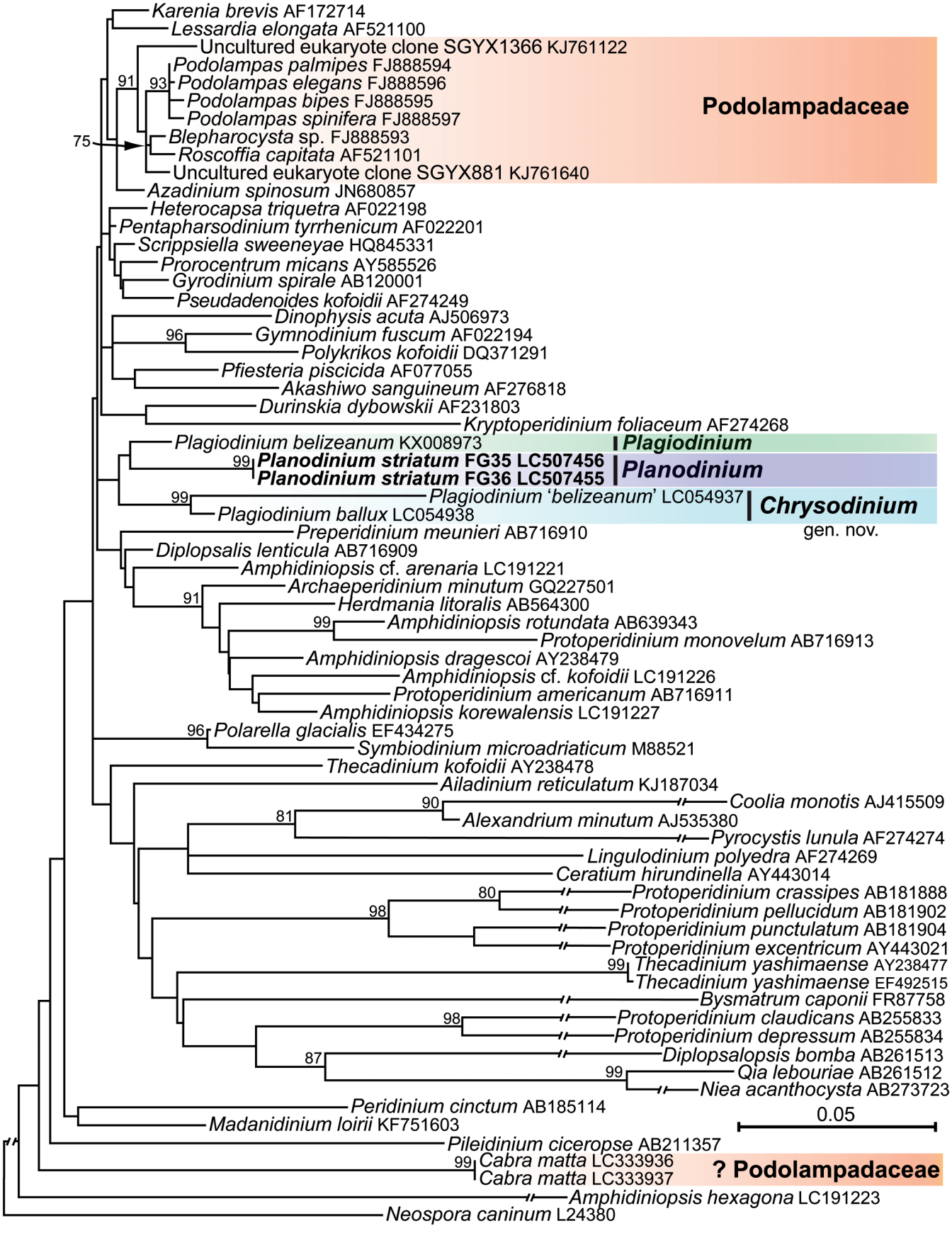


Fig. 1. Light **(A–M)** and scanning electron microscopy **(N–O)** images of *Planodinium striatum* isolated in June 2012 at Wimereux, France. (A–B) A cell in left lateral and dorsal views. Asterisk (*) indicates the pusule. (C–M) Different views of another cell. The arrows indicate hypothecal plates. (N) Cell in ventro-left lateral view. (O) Another cell in left lateral view. The arrowheads indicate the trichocysts. The inset shows a large pore surrounded by several small pores. Scale bar = 10 µm.

Fig. 2. The phylogenetic position of *Planodinium striatum* based on Maximum Likelihood analysis inferred from SSU rRNA gene sequences. The new sequences are indicated in bold face. Each species name is followed by an accession number. The bootstrap values (>70) are indicated at each node. Scale bar denotes 0.05 substitutions per site.

cingulum, as a latitudinal series of depressed plates, is apparently missing or incomplete. Then, it is difficult to interpret whether a plate belongs to the cingular, precingular, intercalary and apical series. Successive re-interpretations of the tabulation of the epitheca often occurred in sand-dwelling dinoflagellates such as *Cabra* (Murray and Patterson 2004, Selina et al. 2015, Yamaguchi et al. 2018). In addition, the criterion of the consideration of the depressed plates as the cingular plate series is variable. For example, the typical pelagic podolampadaceans (*Podolampas*, *Blepharocysta*) do not possess a cingulum with depressed plates, but they are considered to have three cingular plates in the position where the transversal flagellum encircles the cell (Carbonell-Moore 2004). The close phylogenetic relationship between the sand-dwelling *Roscoffia* with the typical depressed cingular plates and the pelagic podolampadaceans (Fig. 2) evidences that the presence of depressed or non-depressed cingular plates is not a diagnostic character at a suprageneric rank. An affinity of the genus *Cabra*, described with an incomplete cingulum, and the podolampadaceans has been suggested since the original description (Murray and Patterson 2004). Yamaguchi et al. (2018) showed that the SSU rRNA gene sequences of *Cabra* clustered within the Podolampadaceae. We have built phylogenetic trees with the same taxonomic sampling in Yamaguchi et al. (2018), but we did not find the same topology (Fig. 2). The alignment of the sequences of *Cabra* (accession numbers LC333936–7) was difficult due to numerous insertions. Yamaguchi et al. (2018) reported *Plagiodinium belizeanum* as a basal lineage in the clade of the


Karenia brevis AF172714
Lessardia elongata AF521100
Uncultured eukaryote clone SGYX1366 KJ761122
Podolampas palmipes FJ888594
Podolampas elegans FJ888596
Podolampas bipes FJ888595
Podolampas spinifera FJ888597
Blepharocysta sp. FJ888593
Roscoffia capitata AF521101
Uncultured eukaryote clone SGYX881 KJ761640
Podolampadaceae
Azadinium spinosum JN680857
Heterocapsa triquetra AF022198
Pentapharsodinium tyrrhenicum AF022201
Scrippsiella sweeneyae HQ845331
Prorocentrum micans AY585526
Gyrodinium spirale AB120001
Pseudadenoides kofoidii AF274249
Dinophysis acuta AJ506973
Gymnodinium fuscum AF022194
Polykrikos kofoidii DQ371291
Pfiesteria piscicida AF077055
Akashiwo sanguineum AF276818
Durinskia dybowskii AF231803
Kryptoperidinium foliaceum AF274268
Plagiodinium belizeanum KX008973
Plagiodinium
Planodinium striatum FG35 LC507456
Planodinium striatum FG36 LC507455
Planodinium
Plagiodinium 'belizeanum' LC054937
Plagiodinium ballux LC054938
Chrysodinium
gen. nov.
Preperidinium meunieri AB716910
Diplopsalis lenticula AB716909
Amphidiniopsis cf. arenaria LC191221
Archaeperidinium minutum GQ227501
Herdmania litoralis AB564300
Amphidiniopsis rotundata AB639343
Protoperidinium monovelum AB716913
Amphidiniopsis dragescoi AY238479
Amphidiniopsis cf. kofoidii LC191226
Protoperidinium americanum AB716911
Amphidiniopsis korewalensis LC191227
Polarella glacialis EF434275
Symbiodinium microadriaticum M88521
Thecadinium kofoidii AY238478
Ailadinium reticulatum KJ187034
Coolia monotis AJ415509
Alexandrium minutum AJ535380
Pyrocystis lunula AF274274
Lingulodinium polyedra AF274269
Ceratium hirundinella AY443014
Protoperidinium crassipes AB181888
Protoperidinium pellucidum AB181902
Protoperidinium punctulatum AB181904
Protoperidinium excentricum AY443021
Thecadinium yashimaense AY238477
Thecadinium yashimaense EF492515
Bysmatrum caponii FR87758
Protoperidinium claudicans AB255833
Protoperidinium depressum AB255834
Diplopsalopsis bomba AB261513
Qia lebouriae AB261512
Niea acanthocysta AB273723
Peridinium cinctum AB185114
Madanidinium loirii KF751603
0.05
Pileidinium cicerope AB211357
Cabra matta LC333936
Cabra matta LC333937
? Podolampadaceae
Amphidiniopsis hexagona LC191223
Neospora caninum L24380

Podolampadaceae, while previous molecular studies did not show a relationship with podolampadaceans such as *Roscoffia* (Wakeman et al. 2018, Yamada et al. 2018). The SSU rRNA gene sequences of *P. striatum* show a relative high percentage of similarity (~93%) with *Roscoffia* and *Plagiodinium*, and even higher (96%) when compared to the partial sequences of *Podolampas*, but the phylogenetic closeness between the Podolampadaceae and *Planodinium* was not proven by SSU rRNA gene analysis using the taxonomic sampling by Yamaguchi et al. (2018) (Fig. 2). However, we observed an affinity using other taxonomic samplings. This suggests that *Planodinium* and *Plagiodinium* share a recent common ancestor with the Podolampadaceae, although the resolution of the rRNA gene markers is insufficient to provide statistical support. The clade of the Podolampadaceae (*Podolampas*, *Blepharocysta*, *Roscoffia*), also including *Cabra*, contain species with three cingular plates, while *Planodinium striatum* and *Plagiodinium* spp. possess five or more cingular plates. Discussions of the interpretation of the tabulation of the genera *Plagiodinium* and *Cabra*, and their relationship with the Podolampadaceae are largely reported in recent literature (Wakeman et al. 2018, Yamada et al. 2018, Yamaguchi et al. 2018).

Planodinium and its allied genera *Plagiodinium* share the same tabulation of the hypotheca than the podolampadaceans (5*‴* 1*⁗*) (Fig. 3A–B, D–E). That hypothecal plate arrangement is also present in other sand-dwelling genera such as *Madanidinium*, *Pileidinium* or *Sabulodinium* that do not show any phylogenetic relationship to the podolampadaceans or any other dinoflagellate in the SSU rRNA gene phylogenies (Fig. 2, Yamada et al. 2018, Yamaguchi et al. 2018). Our results support a close phylogenetic relationship between *Planodinium* and *Plagiodinium* that always cluster together in molecular phylogenies using different taxonomic samplings, although without robust statistical support (Fig. 2).

The species of *Plagiodinium* are photosynthetic with a short sulcus, while *Planodinium* is heterotrophic and the sulcus is long. It should be noted that the prey, tentative amphidinioid dinoflagellates, is ingested through the sulcus. The plate arrangement of the species of *Planodinium* and *Plagiodinium* are subjected to an unstable interpretation of the epithecal tabulation. In addition, *Planodinium striatum* is a heterotrophic species, and the available material for detailed morphological studies is more limited that in a photosynthetic and cultivable genus such as *Plagiodinium*. *Planodinium* possesses a relatively well-defined cingulum, but there is no evidence of the apical pore plate. We have distinct situations in the two described species of *Plagiodinium*, with especial difficulties for *P. ballux* where the cingulum is considered incomplete and the epitheca is very small, being easily overlooked as a *Prorocentrum* cell during routine light microscopy observations (Yamada et al. 2018). An incomplete cingulum means that depressed plates do not completely encircled the cell at the cingulum level. Then, the interpretation could be that the cingular plates really do not completely encircle the cell and there is an intrusion of the precingular plates at the cingulum level or, alternatively, that the cingulum also contains plates that are not depressed. If we admit non-depressed plates as a part of the cingulum of the typical Podolampadaceae (*Podolampas*, *Blepharocysta*), we have to be opened to the same interpretation in *Plagiodinium*. On the other hand, an absence of cingular plates has been reported in other sand-dwelling dinoflagellates such as *Adenoides* where there is no depressed plate at the cingulum level (Gómez et al. 2015).

Yamada et al. (2018) described a second species *Plagiodinium*, *P. ballux* that showed important genetical and morphological differences when compared to the type species, *P. belizeanum*. The SSU rRNA gene sequence of *P. ballux* (LC054938) showed a percentage of similarity of 95.18% and 96.37% with respect to the sequences identified as *Plagiodinium belizeanum* from Mageshima Island (LC054937) and *P. belizeanum* from Okinawa Island (KX008973), respectively. This is a too low percentage of similarity for two dinoflagellate species placed under the same genus. In addition, these two SSU rRNA gene sequences identified as *P. belizeanum* (KX008973, LC054937) showed a percentage of similarity of 95.24% that evidence that the sequences do not belong to the same species, and very likely to distinct genera. Yamada et al. (2018, p. 8) reported: "The two registered sequences of *P. belizeanum* were found to differ more than one might reasonably expect for one species. The morphology of the strain linked with the registered sequence KX008973 (Wakeman et al. 2018) was found to be morphologically identical to the original description of *P. belizeanum*". The strain of *P. belizeanum* from Mageshima Island (LC054937) very likely correspond to an undescribed species, but there is no information on its morphology (Yamada et al. 2015, 2018). The isolate of *P. belizeanum* from Okinawa KX008973 corresponded to the morphology of the type species (Faust and Balech 1993, Wakeman et al. 2018). The low percentage of similarity (96.37%) be-

tween *P. belizeanum* from Okinawa and *P. ballux* is also accompanied by important morphological differences that do not support the placement under the same genus (Fig. 3D–I, Table 1). Yamada et al. (2018, p. 5) reported: "The main difference between *P. belizeanum* and *P. ballux* lies with the number of sulcal plates and lack of complete cingulum in the latter. Another difference is that in *P. belizeanum* the presence of a distinct apical pore was demonstrated (Wakeman et al. 2018), while in *P. ballux*, the pore identified as apical pore is rather obscure".

Wakeman et al. (2018) proposed the plate formula Po 1*'* 5*"* 5(6)c 5*'"* 1*""* for *Plagiodinium belizeanum* with other three alternative interpretations of the epithecal tabulation as Po 3*'* 3*"*, Po x 0*'* 5*"* and Po x 5*'* 0*"*. The interpretation of the thecal plate arrangement of *P. belizeanum* is relatively easy because the cingulum is complete and contains depressed thecal plates. In contrast, the interpretation of the plate formula of *P. ballux* is controversial, especially the assignation of the plates located at the cingulum level that were labelled as 4*"* and 5*"* in the original description (Yamada et al. 2018). Taking into account the phylogenetic relationship between *Plagiodinium* and the Podolampadaceae as shown in Yamaguchi et al. (2018), we have to admit non-depressed plates as a part of the cingulum as occurred in the typical members of the Podolampadaceae (Carbonell-Moore 2004). We can interpret the plates 4*"* and 5*"* in the original description of *P. ballux* as cingular plates. Then, the modified plate formula of *P. ballux* (Po 3*'* 1*"* 7c 2s 5*'"* 1*""*) differed from that of *P. belizeanum* [Po 1*'* 5*"* 5(6)c 4s 5*'"* 1*""*]. In that case, the members of the Podolompadaceae (with *Roscoffia* and tentatively *Cabra*) are characterized by three cingular plates, while the members of the clade of *Planodinium striatum* and *Plagiodinium* spp. possess five to eight cingular plates. The two species of *Plagiodinium* are characterized by a low number (≤6) of epithecal plates (Fig. 3F, I), while *Planodinium* shows more epithecal plates, but very tiny precingular plates in the dorsal epitheca (Fig. 3C).

In the molecular phylogeny including the sequences of *Planodinium*, the placement of *Plagiodinium ballux* in the genus *Plagiodinium* is unsupported as the type species, *P. belizeanum*, is closer to *Planodinium striatum* (Fig. 2). The sequence of *P. ballux* clusters with an undocumented sequence identified as *P. belizeanum* that very likely correspond to a second species of the genus of *P. ballux* (Fig. 2). This suite of morphological and molecular differences leads to the conclusion that the species *P. ballux* should be placed into another genus.

Taxonomic description

Chrysodinium F. Gómez, Y. Nakamura & Artigas, gen. nov.

LSID urn:lsid:zoobank.org:act:9637DE42-84A3-4A7A-B6BF-665619CB30B8

Diagnosis

Laterally compressed thecate dinoflagellates with a very small epitheca. The cingulum was hardly recognizable being difficult to discern the epitheca clearly. A very short sulcus could be seen near the apex of the cell. The hypotheca consisted of six plates: five postcingulars and one antapical plate (5*'"*, 1*""*). The 1*'"* plate is located directly below the sulcus. The 2*'"* and 4*'"* plates were the largest of all the plates and placed on the left and right sides, respectively. There is a single antapical plate (1*""*) that is located in the antapex. The sulcus contains an anterior and posterior plate. The interpretation of the tabulation of the epitheca is difficult. There is no a complete latitudinal series of depressed plates encircling the cell. There are five depressed plates that can be unequivocally attributed to the cingular series, and other two non-depressed plates near the sulcus in the right lateral side that are here also assigned to the cingular series. There is an inconspicuous apical pore plate surrounded by three apical plates, and one precingular plate in the left lateral side. The plate formula is interpreted as Po 3*'* 1*"* 7c 2s 5*'"* 1*""*. All the known species are photosynthetic and living in marine benthic habitats.

Type species: *Chrysodinium ballux* (N. Yamada, Dawut, R. Terada & Horiguchi) F. Gómez, Y. Nakamura & Artigas comb. nov. (designated here).

Icotype: Fig. 3H

Etymology: Chryso: From Ancient Greek χρυσός (khrusós, the color gold, golden, golden yellow). The epithet of the type species refers to the golden coloration. The suffix '–dinium', meaning 'vortex' is commonly applied to dinoflagellates. The gender is neuter. Basionym: *Plagiodinium ballux* N. Yamada, Dawut, R. Terada & Horiguchi (2018, *Phycological Research* 67, p. 8–9, figs. 1–4)

Yamada et al. (2018) designed the SEM stub SAP 115372 as holotype, but no figure is assigned to the holotype. The figures 1–4 correspond to distinct specimens. We design the specimen of the figure 2b in Yamada et al. (2018) as type (Article 8.1 of International Code of Nomenclature, I.C.N.).

Other species belonging to *Chrysodinium*: *Plagiodinium belizeanum* sensu Yamada et al. 2018 (non *P. belizeanum* sensu Wakeman et al. 2018).

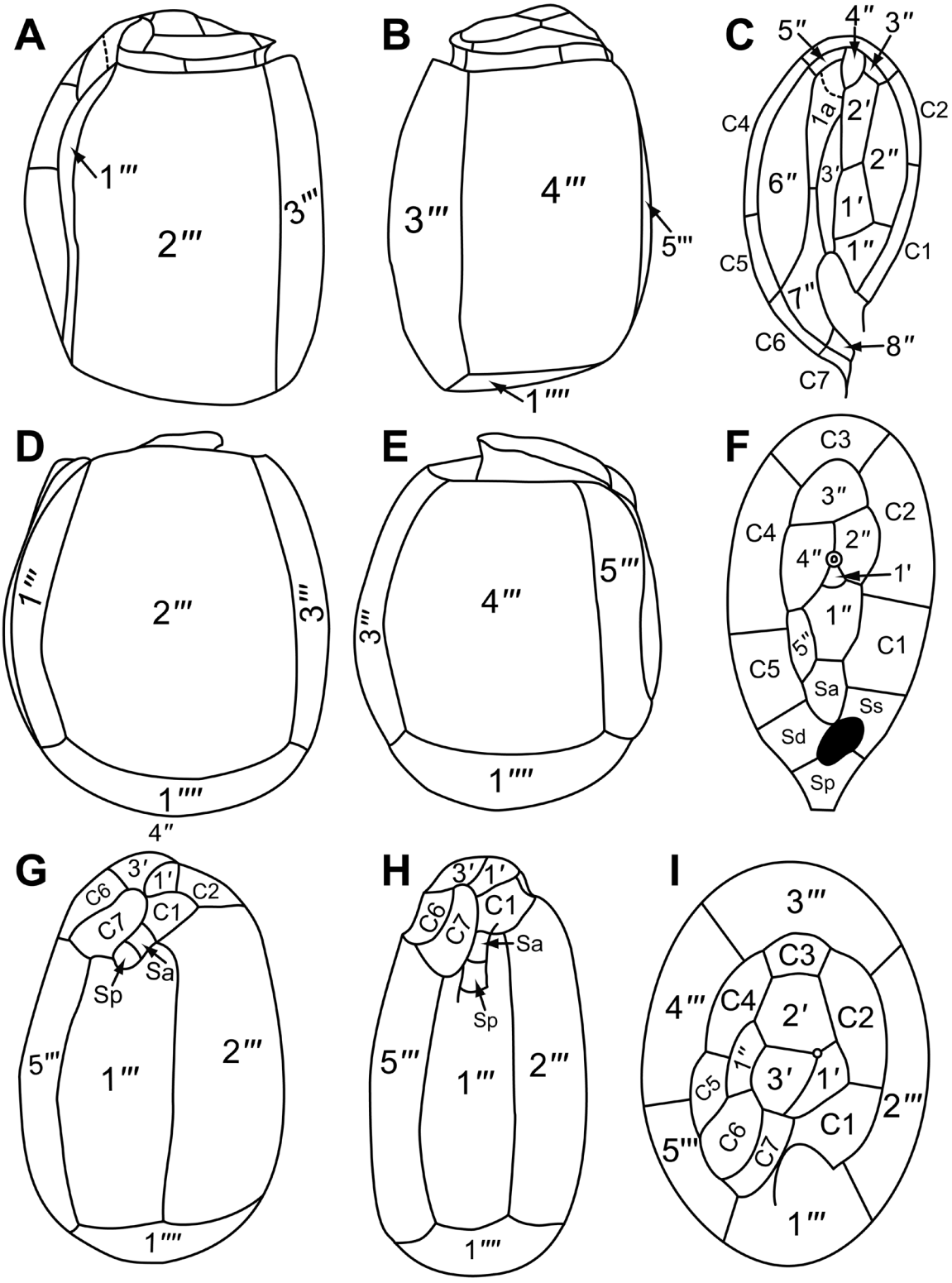


Fig. 3. Line drawings of the plate arrangement of *Planodinium striatum* **(A–C)**, *Plagiodinium belizeanum* **(D–F)** and *Chrysodinium ballux* gen. nov. & comb. nov. (=*Plagiodinium ballux*) **(G–I)**. Left lateral (A), right lateral (B), apical (C) views of *Planodinium striatum* redrawn from Hoppenrath et al. (2014). Left lateral (D), right lateral (E) and apical (F) views of *Plagiodinium belizeanum* redrawn from Wakeman et al. (2018). Left lateral (G), ventral (H) and apical (I) views of *Chrysodinium ballux* gen. & comb. nov. redrawn from Yamada et al. (2018) with a re-interpreted tabulation.

Table 1. Comparative morphological characteristics of *Planodinium striatum*, *Plagiodinium belizeanum* and *Chrysodinium ballux* gen. nov. & comb. nov. (=*Plagiodinium ballux*) based on Hoppenrath et al. (2014), Wakeman et al. (2018), Yamada et al. (2018) and this study.

	Planodinium striatum	*Plagiodinium belizeanum*	*Chrysodinium ballux* (=*Plagiodinium ballux*)
Cell dimensions (in µm)	28–35 long, 23–26 depth	13–34 long, 6–18 depth	15–23 long, 10–17 depth
Cell shape	almost rectangular	oval to oblong	oval
Cell compression	high	moderate	low
Epitheca	conspicuous	small cap-like with dorsal tapering	hardly visible
Cingulum	horizontal, with narrow depressed plates	small with wide plates	inconspicuous, with depressed and non-depressed plates
Number of cingular plates	6 or 7	5 or 6	5 (depressed) + 2 (non-depressed)
Sulcus	long	short with 4 plates	very short with 2 plates (anterior and posterior)
Apical pore plate	unreported	ring-like	inconspicuous simple pore
Plate formula	Po? 3 ' 1a 8 " 7?c 5 "' 1 ""	Po 1 ' 5 " 5(6)c 5 "' 1 ""	Po 3 ' 1 " 7c 5 "' 1 ""
First postcingular plate	narrow, adjacent to left sulcus	narrow, adjacent to left sulcus	wide, below the sulcus
Surface ornamentation	two different size pore classes, longitudinal ridges	two different size pore classes, intercalary bands with striation	small pores near the sutures
Chloroplast	heterotroph	radiating from the center with lobes	reticulate
Pyrenoid	–	multistalked	multistalked, ring-shaped
Pusule	prominent	non-observed	reported

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REFERENCES

Baillie K. D. (1971) *A taxonomic and ecological study of intertidal sand-dwelling dinoflagellates of the north eastern Pacific Ocean* M.S. Thesis, University of British Columbia, Vancouver

Balech E. (1956) Étude des Dinoflagellés du sable de Roscoff. *Rev. Algol*., *n. s.* **2:** 29–52

Carbonell-Moore M. C. (2004) On the taxonomical position of *Lessardia* Saldarriaga et Taylor within the family Podolampadaceae Lindemann (Dinophyceae). *Phycol. Res*. **52:** 340–345

Carbonell-Moore M. C. (2017) The rediscovery of *Archaeosphaerodiniopsis* Rampi (Dinophyceae). *Eur. J. Phycol.* **52:** 57–63

Faust M. A., Balech E. (1993) A further SEM study of marine benthic dinoflagellates from a mangrove island, Twin Cays, Belize, including *Plagiodinium belizeanum* gen. et sp. nov. *J. Phycol.* **29:** 826–832

Gómez F., Moreira D., López-García P. (2010) Molecular phylogeny of the dinoflagellates *Podolampas* and *Blepharocysta* (Peridiniales, Dinophyceae). *Phycologia* **49:** 212–220

Gómez F., López-García P., Moreira D. (2011) Molecular Phylogeny of the sand-dwelling dinoflagellates *Amphidiniopsis hirsuta* and *A. swedmarkii* (Peridiniales, Dinophyceae). *Acta Protozool*. **50:** 255–262

Gómez F., Artigas L. F. (2014) High diversity of dinoflagellates in the intertidal sandy sediments of Wimereux (north-east English Channel, France). *J. Mar. Biol. Ass. U.K.* **94:** 443–457

Gómez F., Onuma R., Artigas L. F., Horiguchi T. (2015) A new definition of *Adenoides eludens*, an unusual marine sand-dwelling dinoflagellate without cingulum, and *Pseudadenoides kofoidii* gen. & comb. nov. for the species formerly known as *Adenoides eludens*. *Eur. J. Phycol.* **50:** 125–138

Horiguchi T., Kubo F. (1997) *Roscoffia minor* sp. nov. (Peridiniales, Dinophyceae); a new, sand-dwelling, armored dinoflagellate from Hokkaido, Japan. *Phycol. Res*. **45:** 65–69

Hoppenrath M., Elbrächter M. (1998) *Roscoffia capitata* (Dinophyceae) refound: notes on morphology and biology. *Phycologia* **37:** 450–457

Hoppenrath M., Murray S. A., Chomérat N., Horiguchi T. (2014) *Marine benthic dinoflagellates – unravelling their worldwide biodiversity.* Kleine Senckenberg-Reihe Vol. 54. V. Mosbrugger & E. Schweizerbartsche Verlagsbuchhandlung, Frankfurt am Main & Stuttgar

Larkin M. A., Blackshields G., Brown N. P., Chenna R., McGettigan P. A., McWilliam H., Valentin, F., Wallace I. M., Wilm A.,

Lopez R., Thompson J. D., Gibson T. J., Higgins D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23:** 2947–2948

Medlin L., Elwood H., Stickel S., Sogin M. (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA coding regions. *Gene* **71:** 491–499

Murray S., Patterson D. J. (2004) *Cabra matta*, gen. nov., sp. nov., a new benthic, heterotrophic dinoflagellate. *Eur. J. Phycol*. **39:** 229–234

Nakamura Y., Imai I., Yamaguchi A., Tuji A., Not F., Suzuki N. (2015) Molecular phylogeny of the widely distributed marine protists, *Phaeodaria* (Rhizaria, Cercozoa). *Protist* **166:** 363–373

Saldarriaga J. F., Leander B. S., Taylor F. J. R., Keeling P. J. (2003) *Lessardia elongata* gen. et sp. nov. (Dinoflagellata, Peridinales), Podolampaceae and the taxonomic position of the genus *Roscoffia*. *J. Phycol*. **39:** 368–378

Saunders R. D., Dodge J. D. (1984) An SEM study and taxonomic revision of some armoured sand-dwelling marine dinoflagellates. *Protistologica* **20:** 271–283

Selina M., Chomérat N., Hoppenrath M. 2015. Morphology and spatial distribution of *Cabra* species (Dinophyceae, Peridiniales) from Peter the Great Bay (northwestern Sea of Japan), including the description of *C. levis* sp. nov. *Eur. J. Phycol*. **50:** 80–91

Wakeman K. C., Hoppenrath M., Yamaguchi A., Gavelis G. S., Leander B. S., Nozaki, H. (2018) Ultrastructure of the marine benthic dinoflagellate *Plagiodinium belizeanum* (Dinophyceae) from the Southeast Pacific island of Okinawa, Japan. *Phycologia* **57:** 209–222

Yamada N., Tanaka A., Horiguchi T. (2015) Pigment compositions are linked to the habitat types in dinoflagellates. *J. Plant Res*. **128:** 923–932

Yamada N., Dawut M., Terada R., Horiguchi T. (2018) *Plagiodinium ballux* sp. nov. (Dinophyceae), a deep (36 m) sand dwelling dinoflagellate from subtropical Japan. *Phycol. Res*. **67:** 12–20

Yamaguchi A., Wakeman K. C., Hoppenrath M., Horiguchi T., Kawai H. (2018) Molecular phylogeny of the benthic dinoflagellate *Cabra matta* (Dinophyceae) from Okinawa, Japan. *Phycologia* **57:** 630–640

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