ACTA
PROTOZOOLOGICA

Acta Protozool. (2016) 55: 239–248
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.16.026.6095

Molecular Phylogeny of the Marine Planktonic Dinoflagellate *Oxytoxum* and *Corythodinium* (Peridiniales, Dinophyceae)

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Abstract. The dinoflagellate genera *Oxytoxum* and *Corythodinium* that account for more than fifty species are widespread in warm oceans. These genera have been considered synonyms and thecal plate designations varied among authors. Several planktonic and sand-dwelling genera have been placed within the Oxytoxaceae. We obtained the first molecular data based on small subunit (SSU) rRNA gene sequences of *Oxytoxum* and *Corythodinium*, including the type species (*O. scolopax* and *C. tessellatum*) and *C. frenguellii* and *C. cristatum*. The three species of *Corythodinium* branched together a strong support [bootstrap (BP) of 98%]. This formed a sister clade with moderate support (BP 75%) with *O. scolopax* that supported the generic split. Oxytoxaceae should exclusively remain for *Oxytoxum* and *Corythodinium*, as an independent group, unrelated to any other known dinoflagellate. *Oxytoxum* was characterized by spindle-shaped cells with an anterior narrow epitheca, an apical spine and little cingular displacement. *Corythodinium* exhibits relatively broad cell shapes, with wider epitheca and greater cingular displacement, and an obovate or pentangular anterior sulcal plate that noticeably indented the epitheca. This suggested the need of new combinations for species that were described as *Oxytoxum* and possessed the characteristics of *Corythodinium*.

Key words: 18S ribosomal RNA, armored Dinophyta, Atlantic Ocean, Mediterranean Sea, phytoplankton, SSU rDNA phylogeny, taxonomy, thecate Dinoflagellata.

INTRODUCTION

The genera *Oxytoxum* and *Corythodinium* account for more than fifty species, most often reported in the phytoplankton assemblages of warm temperate and tropical seas (Kofoid 1907, Schiller 1937; Rampi 1939, 1941, 1951; Gaarder 1954; Balech 1954, 1971a,b; Taylor 1976, Gómez *et al*. 2008). The genera *Oxytoxum* and *Pyrgidium* were described by Stein (1883) having five postcingular plates and the first one (1*‴*) being shorter and narrower than the others. He proposed *Oxytoxum* for four elongated species with a pointed apex and antapex or spines, and *Pyrgidium* for broader cells with more blunt ends. However, the generic split was not clear cut and further authors merged both genera into *Oxytoxum* (Schiller 1937). Based on plate dissections, Balech (1954, p. 115) reported the tabulation of *O. constrictum* as 3*′*, 2a, 6*″*, 5c, 4–5s, 5*‴*, 1*⁗*, and *Oxytoxum scolopax* as 3*′*, 2a, 3*″*, 5c, 4–5s, 5*‴*, 1*⁗*. Unfortunately, there

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was an error in the plate formula that was reported as 3*”* instead of 6*”*. Loeblich and Loeblich (1966) noted that *Pyrgidium* was a posterior homonym of the lichen *Pyrgidium* Nylander, and without making new observations they proposed the name *Corythodinium*. *Oxytoxum scolopax* and *Corythodinium tessellatum*, stand as type species for their respective genera.

Taylor (1976) amended the diagnosis of *Corythodinium* and supported the generic split, due to 1) the presence of a large obovate or pentagular anterior sulcal region that deeply invades the epitheca in *Corythodinium*, whereas the anterior sulcal plate of *Oxytoxum* only slightly invades the epitheca, 2) *Corythodinium* possesses a high, left-handed cingular displacement, while the cingular displacement is low in *Oxytoxum*, and 3) members of *Oxytoxum* possess a relatively small epitheca, compared to the relatively large epitheca found in *Corythodinium* (Taylor 1976). Sournia (1986) maintained the split of *Oxytoxum* and *Corythodinium*, and reported the tabulation Po, 3*’*, 2a, 3*”*, 5c, 4–5s, 5*”’*, 1*””* for *Oxytoxum*, and ?Po, 3*’*, 2a, 6*”*, 5c, ?s, 5*”’*, 1*””* for *Corythodinium*. The presence of only three precingular plates (3*”*) in *Oxytoxum* could be explained as a mistake in Balech (1954, p. 116). Balech (1988) also maintained the split between *Corythodinium* and *Oxytoxum*. Steidinger and Tangen (1997) reported the plate formula Po, 5*’*, 6*”*, 5c, 4s, 5*”’*, 1*””* for *Oxytoxum* and Po, 3*’*, 2a, 6*”*, 5c, ?4s, 5*”’*, 1*””* for *Corythodinium*. Taylor (1976, his figure 512) carried out the first SEM study. He showed the ventral cingular area of *O. scolopax*. More complete studies were reported by Burns and Mitchell (1982) and Dodge and Saunders (1985). The latter authors proposed the plate formula Po, 5*’*, 6*”*, 5c, 4s, 5*”’*, 1*””* and re-established *Corythodinium* as a synonym of *Oxytoxum* (Dodge and Saunders 1985). Fensome *et al*. (1993) also considered *Corythodinium* as a synonym of *Oxytoxum*.

The variability in the synonymy and the plate arrangement of *Oxytoxum* and *Corythodinium* is also accompanied with differences in the classification. These genera have been placed within the family Oxytoxaceae together with other planktonic genera such as *Centrodinium* and *Schuettiella*, and the sand-dwelling genera *Amphidiniopsis*, *Planodinium*, *Pseudadenoides* (formerly *Adenoides*), *Roscoffia*, *Sabulodinium* and *Thecadinium* (Loeblich 1982, Dodge 1984, Sournia 1986, Taylor 1987, Chrétiennot-Dinet *et al*. 1993, Fensome *et al*. 1993, Steidinger and Tangen 1997). The family Oxytoxaceae has been placed within the order Gonyaulacales (Taylor 1987, Steidinger and Tangen 1997) or as an uncertain order within the subclass Peridiniphycidae (Fensome *et al*. 1993).

Currently, there is no molecular data representing *Oxytoxum* and *Corythodinium*, despite both genera being represented with the combined descriptions of more than fifty species, and being widely distributed. The lack of molecular data hinders the ability to solve the discrepancies in the synonymy and classification among members of the Oxytoxaceae. In this study, we provide the first molecular data representing the type species of *Oxytoxum* and *Corythodinium*, as well as additional sequences of other two species.

MATERIALS AND METHODS

Sampling and microscopy

Cells of *Oxytoxum* and *Corythodinium* were observed from water samples collected at four coastal sites in the north-western Mediterranean Sea (Marseille, Banyuls sur Mer, Villefranche sur Mer, Valencia), and open-water stations in the Mediterranean Sea, as reported in Gómez *et al*. (2016), and the Appendix S1 in the Supporting Information. In the South Atlantic Ocean, sampling continued after March 2013 in São Sebastião Channel (23°50′4.05″S, 45°24′28.82″W), and from December 2013 to December 2015 off Ubatuba (23°32′20.15″S, 45°5′58.94″W). The Brazilian cells were obtained using a phytoplankton net (20 µm mesh size) in surface waters. The living concentrated samples were examined in Utermöhl chambers at magnification of × 200 with inverted microscopes (Diaphot-300, Nikon Inc. at São Sebastião, and Eclipse TS-100, Nikon Inc. and Olympus IX73, Olympus Inc. at Ubatuba), and photographed with a digital camera (Cyber-shot DSC-W300, Sony, Tokyo, Japan) mounted on the microscope’s eyepiece. Cells of *O. scolopax* from Brazil that showed higher pigmentation were isolated with the aim to establish cultures. Individuals were isolated using a micropipette and placed in 12-well tissue culture plate with 0.2 µm-filtered seawater collected that day from the same locality, and they were supplemented with f/2 medium without silicates. Two days later, the healthy cells were re-isolated and placed into a 6-well tissue culture plate with f/2 medium made with filtered and sterilized seawater. The culture plates were placed in an incubator used for microalgae culturing, at 23°C, 100 µmol photons · m^{-2} s^{-1} from cool-white tubes and photoperiod 12:12 L:D.

In addition to the coastal sampling, a net sample was collected in the open South Atlantic on June 23, 2015 (25°32′43.8″S; 44°57′52.4″W, bottom depth of 1000 m). The sample was collected in a vertical haul with a phytoplankton net (20 µm mesh size) from 500 m depth to the surface. The concentrate was transferred into a 500-ml plastic container and preserved with neutral Lugol’s iodine. The sample was analyzed within 2 weeks.

PCR amplification of small subunit rRNA gene, sequencing and phylogenetic analyses

Novel sequences were obtained from cells collected in Brazil (Table 1). Each cell of *Oxytoxum* or *Corythodinium* was micropipetted individually with a fine capillary into a clean chamber and washed several times in serial drops of 0.2-µm filtered and sterilized seawater. Finally, 1–5 cells of each species were deposited in a 0.2-ml Eppen-

Table 1. List of sequences of *Oxytoxum* and *Corythodinium*.

Taxa	GenBank no.	Sampling date	Geographic origin	Bottom depth	Latitude South	Longitude West	Figure
O. scolopax FG11	KY421376	7 Aug 2013	São Sebastião Channel	40 m	23°50′4.05″	45°24′28.82″	Fig. 2A
O. scolopax FG43	KY421375	1 Nov 2015	off Ubatuba	15 m	23°32′20.15″	45°5′58.94″	Fig. S1A
C. tessellatum FG9	KY421378	15 May 2013	São Sebastião Channel	40 m	23°50′4.05″	45°24′28.82″	Fig. 2C
C. tessellatum FG40	KY421377	22 Aug 2013	São Sebastião Channel	40 m	23°50′4.05″	45°24′28.82″	Fig. 2H
C. tessellatum FG42	KY421379	27 Nov 2015	off Ubatuba	15 m	23°32′20.15″	45°5′58.94″	Fig. S2, B–C
C. frenguellii FG7	KY421380	2 May 2013	São Sebastião Channel	40 m	23°50′4.05″	45°24′28.82″	Fig. 2K
C. frenguellii FG8	KY421382	13 Jun 2013	São Sebastião Channel	40 m	23°50′4.05″	45°24′28.82″	Fig. 2L
C. frenguellii FG46	KY421381	19 Jun 2015	off Ubatuba	15 m	23°32′20.15″	45°5′58.94″	Fig. S1D
C. cristatum FG28	KY421383	23 Jun 2015	open South Atlantic	1000 m	25°32′43.8″	44°57′52.4″	Fig. 2M–N
C. cristatum FG30	KY421374	23 Jun 2015	open South Atlantic	1000 m	25°32′43.8″	44°57′52.4″	Fig. S1E–F

dorf tube filled with several drops of absolute ethanol. The sample was kept at room temperature and in darkness until the molecular analysis could be performed. Prior to DNA extraction, the 0.2-ml Eppendorf tubes were centrifuged for 10 min at 14462 *g* (TOMY MX-201, Tokyo, Japan). Ethanol was then evaporated in a vacuum desiccator. Cells were resuspended in 10 µl of QuickExtract FFPE DNA Extraction Kit (Epicenter Biotechnologies, Madison, WI, USA) and incubated at 56°C for 1 h and 98°C for 2 min in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, Foster City, CA, USA). The extracted product (1 µl) was used as DNA template for polymerase chain reaction (PCR). In order to amplify SSU rDNA, three rounds of PCR were performed. In the first round, the primers SR1 5*'*-TACCTGGTTGATCCTGCCAG-3*'* and SR12 5*'*-CCTTCCGCAGGTTCACCTAC-3*'* were used in a reaction with Econotaq (Lucigen, Middleton, WI, USA) and 1 µl of extracted DNA. For this initial reaction, the following program was used on a thermocycler: Initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 2 min; final extension at 72°C for 7 min. In the second round of PCR, 1 µl of the first PCR product was used as DNA template and the specific forward 5*'*-CGAACGAATCGCATGGCATCC-3*'* and reverse primers 5*'*-GGAACCGAACACTGCTTCAG-3*'*, were paired with SR1 and SR12, respectively. PCR conditions for the second round of PCR was the same as the first, except that the cycling extension time was shortened to 1 min 20 s. PCR products were directly sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and a DNA autosequencer ABI PRISM3100 Genetic Analyzer (Applied Biosystems).

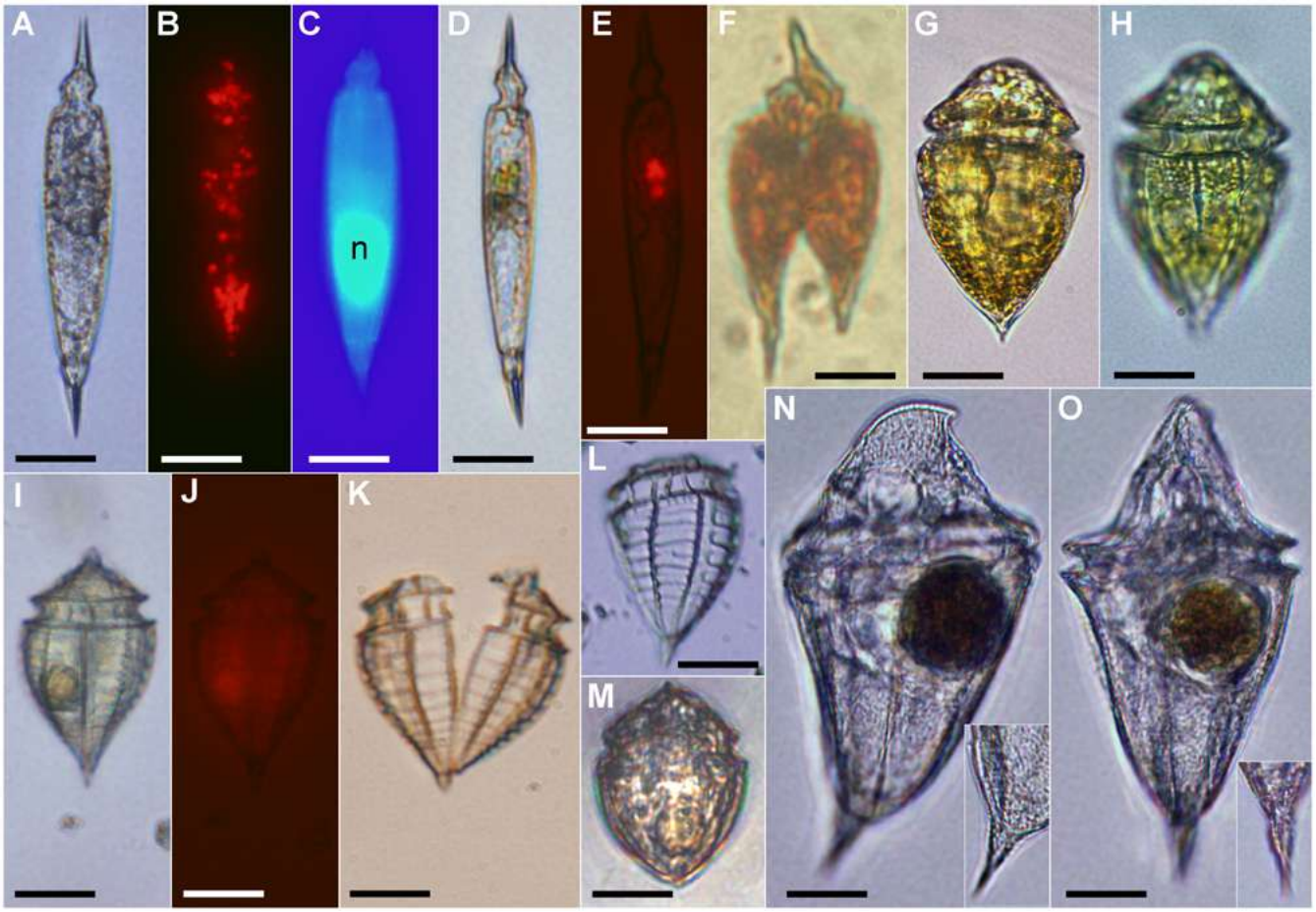
The ten sequences generated in this study were aligned with MUSCLE (Edgar 2004) with 61 sequences representing a diverse set of dinokaryotic lineages; alignments were subsequently fine-tuned manually. Sequences of the basal dinoflagellate *Hematodinium* and *Syndinium* (GenBank accession numbers EF065717 and DQ146404) were used as an outgroup. Aligned sequences were examined using Maximum Likelihood (ML) analyses with Garli-GUI under a GTR+I+Γ model of evolution selected by JModelTest 2.1.10 (proportion of invariable sites = 0.4289, gamma shape = 0.3970). Bootstrap analysis was calculated for 500 pseudoreplicates. Our sequences were deposited in DDBJ/EMBL/GenBank under accession numbers KY421374–KY421383 (Table 1).

RESULTS

Morphology

Oxytoxum and *Corythodinium* were occasionally encountered during the sampling between 2007 and 2011 along the Mediterranean coasts at Marseille, Banyuls sur Mer, Villefranche sur Mer and Valencia, and samples collected from the open Mediterranean Sea during the BOUM cruise in July 2008 (see Appendix S1 in the Supporting Information). The genera never reached high abundances, and when present, observations were restricted to a few cells per sample. In the open Mediterranean Sea, the highest abundances were sixteen cells l^{-1} of *O. scolopax* and *O. variabile*, in the Gulf of Lions and south of Crete. Other species such as *C. constrictum* and *O. sceptrum* reached abundances of eight cells l^{-1} in the open Mediterranean Sea. The most common species observed, while examining live cells along the Mediterranean coastal sites were *O. scolopax* (Figs 1A–E) and *C. constrictum* (Figs 1G–H). Other, less frequently encountered species included *C. tessellatum* (Figs 1J–L), *C. reticulatum*, *C. diploconus*, *O. coronatum*, *O. cribrosum*, *O. sceptrum* and *O. crassum*. Other species such as *C. frenguellii* (Fig. 1M) and *C. cristatum* (Figs 1N–O) were rare, especially the latter species that was only observed in the vertical hauls in the Bay of Villefranche sur Mer.

Oxytoxum scolopax is the most common species encountered within the genus. The cells were sharply elongated, spindle-shaped and measured 85–105 µm long and 12–17 µm wide (n = 24). The epitheca was triangular in outline, with a prominent apical spine. The cingulum was situated a quarter of the cell length from



Figs 1A–O. Light micrographs of *Oxytoxum* and *Corythodinium* from the Mediterranean Sea. **A–E** – *O. scolopax* from Banyuls sur Mer. **B–C, E, J** – Epifluorescence microscopy. **B, E, J** – Note the autofluorescence of the chloroplasts. **C** – Nucleus stained by DAPI. **F** – Dividing cells of *O. sceptrum*. **G–H** – *C. constrictum* from Villefranche sur Mer. **I–L** – *C. tessellatum* from Banyuls sur Mer. **K–L** – Empty thecae. **M** – *C. frenguellii* from Villefranche sur Mer. **N–O** – *C. cristatum* from Villefranche sur Mer. The insets show the antapical spine. n – nucleus. Scale bars: 20 µm.

the anterior. The elongated hypotheca showed convex margins. The antapical spine showed a bladder-like swelling at the base (Figs 1A–E; 2A–B). The recently collected cells of *O. scolopax* were weakly covered with small and dispersed globular chloroplasts, as revealed by epifluorescence microscopy (Figs 1A–B). Other cells contained chloroplasts restricted to the middle of the hypotheca (Figs 1D–E). The nucleus was ellipsoidal and oriented along the anteroposterior axis, in the middle of the posterior half of the hypotheca (Fig. 1C). During the cell division of *Oxytoxum*, tentatively *O. sceptrum*, the dividing cells joined at the anterior half of the hypotheca. One daughter cell maintained the pointed antapex, formed by the first antapical plate of the mother cell, while the other daughter kept the apical spine (Fig. 1F). The species *C. constrictum* (Figs 1G–H) usually appeared more pigmented than *O. scolopax*. *Corythodinium tessellatum* measured 50–60 µm long (n = 26). The epitheca was wide and low, with a short apical spine. The hypotheca was slightly wider than the epitheca, with convex contour and a short antapical spine. In addition to the longitudinal ridges, the most distinctive feature of this species was the transversal tessellation of the hypotheca (Figs 1I–L). The cells were slightly pigmented, often restricted to a spherical structure in the hypotheca (Figs 1I–J). *Corythodinium frenguellii* was about 50 µm long (Fig. 1M). When compared to *C. tessellatum*, the cells of *C. frenguellii* were more broad, without apical and antapical spines, with a more posterior cingulum and lacking the transversal tessellation that characterized *C. tessellatum* (Fig. 1L). *Corythodinium cristatum* was

a large species (115–120 µm long, 60 µm wide, n = 2) with a laterally compressed cell body. The epitheca was helmet-shaped with a dorsally recurved apex. The hypotheca showed slightly convex margins, the dorsal margin broadly rounded and the ventral margin with a ventrally deflected antapical spine. The cells were hyaline, except with a dark-green granule located in the anterior half of the hypotheca (Figs 1N–O).

Along the coasts of the São Paulo State in Brazil, the genera *Oxytoxum* and *Corythodinium* never reached high abundances. The most common species were *O. scolopax* (Figs 2A–B), *C. tessellatum* (Figs 2B–I), *C. constrictum* (Figs 2I–J), and more rarely *C. frenguellii* (Figs 2K–L). The species *C. cristatum* was only observed in one sample collected from a vertical haul between 500 m depth and the surface (Figs 2M–N). As *Oxytoxum* (Fig. 1F), *Corythodinium* divided by desmoschisis (Figs 2E–H, J). One daughter cell maintained the apical plates and lacked the pointed, first antapical plate. The other daughter cell had a relatively smaller epitheca and maintained the antapical spine, formed by the antapical plate (Figs 2E–H, J). We attempted to culture chloroplast-containing species of *Oxytoxum* and *Corythodinium*. However, the cells did not survive more than three days under laboratory conditions.

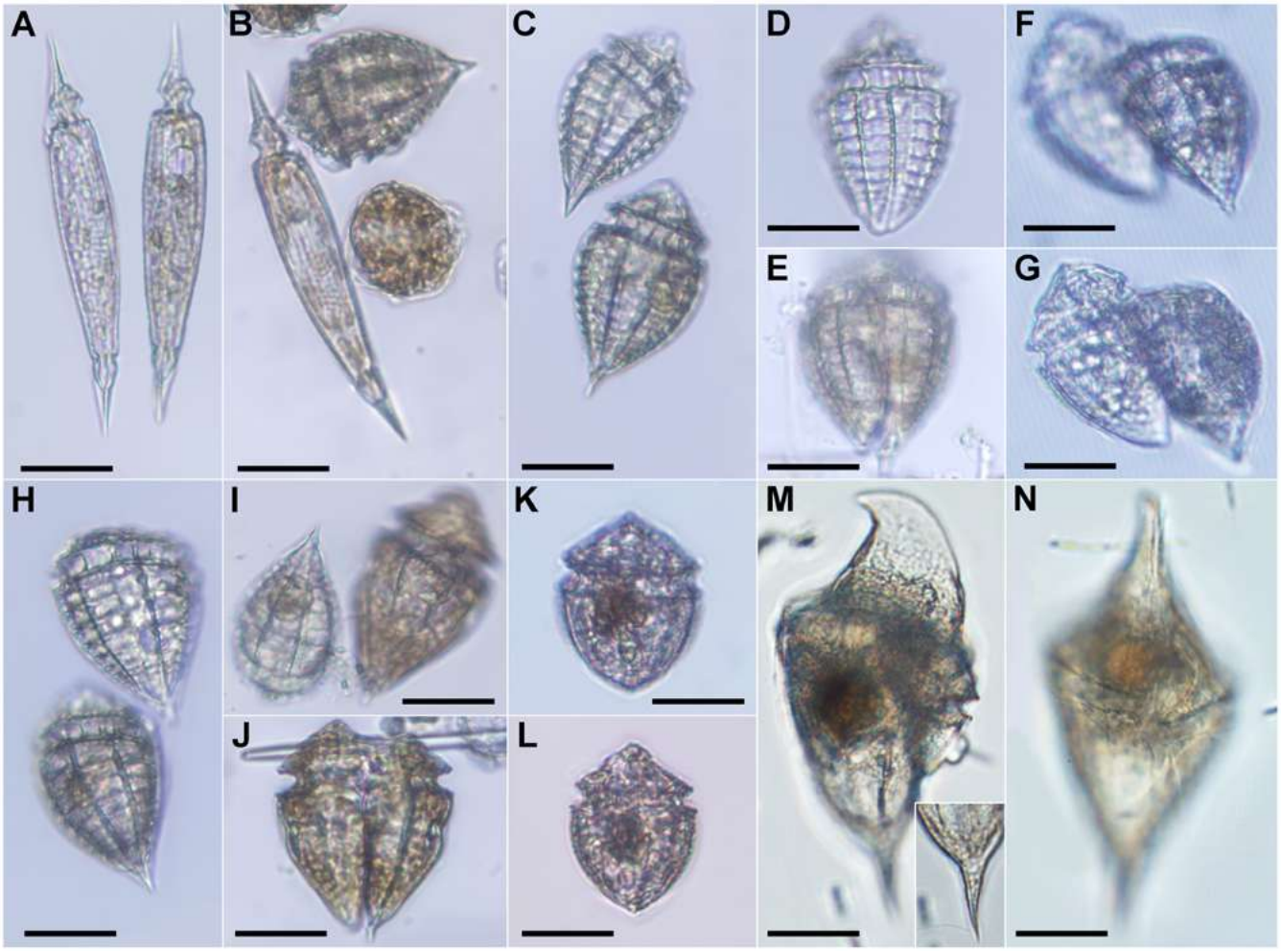
Molecular phylogeny

Two novel SSU rDNA sequences representing the type species of *Oxytoxum*, *O. scolopax*, were generated from cells collected at São Sebastião Channel in 2013 and off Ubatuba in 2015 (Figs 2A–B; S1A), three sequences representing the type species of *Corythodinium*, *C. tessellatum*, were also generated. Samples of *C. tessellatum* were also collected from different periods and locations (Figs 2C–H; S1B–C). Three sequences of *C. frenguellii* (Figs 2K–L; S1D) were obtained from cells collected from two different sites, and two sequences of *C. cristatum* (Figs 2M–N; S1E–F) were obtained from cells collected from a vertical haul from 500 m depth to the surface (Table 1). The sequence identity between the type species of the two genera, *O. scolopax* and *C. tessellatum*, was 97% across 1,654 base pairs of 18S rDNA. We examined the phylogenetic position of *Oxytoxum* and *Corythodinium* using a dataset that included a variety of dinoflagellate SSU rDNA sequences with focus on Peridiniales, and including the genera that have been classified as members of the Oxytoxaceae (*Amphidiniopsis*, *Pseudadenoides*, *Roscoffia*, *Sabulodinium* and *Thecadinium*).

The analysis showed that *Oxytoxum* and *Corythodinium* sequences were subdivided into two clades that formed a monophyletic group with moderate support [(bootstrap (BP) of 75%] in the maximum-likelihood phylogenetic tree (Fig. 3). One clade comprised the sequences of the three species of *Corythodinium* with a high support (BP, 98%), while the second clade contained the sequences of *O. scolopax* (Fig. 3). Our data supported the splitting of *Oxytoxum* and *Corythodinium* into two distinct genera based on the evolutionary distance of their respective SSU rDNA sequences. Our new sequences did not show any particularly close affiliation to any known dinoflagellate present in public sequence databases, and branched within the large lineage comprising the short-branching Gymnodiniales, Peridiniales, Dinophysales and Prorocentrales, but with poor support, making it difficult to infer the affinity of *Oxytoxum* and *Corythodinium* in the context of these orders. The new sequences branched with peridinioid dinoflagellates such as *Heterocapsa* and podolampadaceans such as *Roscoffia*, without support to infer a phylogenetic relationship to the order Peridiniales. Our new sequences did not branch with the long-branching members of the order Gonyaulacales.

Taxonomic considerations

Molecular data gathered in this study support the separation between the genera *Oxytoxum* and *Corythodinium*. The clade of *Corythodinium* showed a close phylogenetic relationship among its species, such as the relationship between the type species, *C. tessellatum* (Figs 1I–L; 2C–H; S1B–C), and other species that largely differed with regard to size, the general appearance, and cell compression and shape such as *C. cristatum* (Figs 1N–O; 2M–N; S1E–F). However, these species maintained diagnostic characteristics of the genus *Corythodinium* such as the broad cell body, wider epitheca, higher cingular displacement, and an indentation of the sulcus in the epitheca, which is distinct, compared to *Oxytoxum*. Nonetheless, there are species described under the genus *Oxytoxum* that have been proposed, prior to the establishment of the genus *Corythodinium* in 1966; moreover, other species have been described by authors that considered *Corythodinium* a synonym of *Oxytoxum*. The next species are candidates to be transferred into *Corythodinium*: *Oxytoxum adriaticum*, *O. areolatum*, *O. brunellii*, *O. crassum*, *O. cribrosum*, *O. criophilum*, *O. depressum*, *O. ligusticum*, *O. milneri*, *O. minutum*, *O. mitra*, *O. ovale*, *O. ovum*, *O. punctulatum*, *O. pyramidale*, *O. radiosum*, *O. robustum*, *O. strophalatum* and *O. viride*.



Figs 2A–N. Light micrographs of *Oxytoxum* and *Corythodinium* from Brazil. **A** – *Oxytoxum scolopax*, isolated cell FG11. **B** – *O. scolopax* and *C. tessellatum*. **C–D** – *C. tessellatum*. **C** – Isolated cell FG9. **E–H** – Dividing cells of *C. tessellatum*. **H** – Isolated cell FG40. **I** – *C. tessellatum* and *C. constrictum*. **J** – Diving cells of *C. constrictum*. **K–L** – *Corythodinium frenguellii*. **K** – Isolated cell FG7. **L** – Isolated cell FG8. **M–N** – *C. cristatum* from the open South Atlantic Ocean, isolated cell FG28. **M** – The inset focuses on the antapical spine. Scale bars: 20 µm.

DISCUSSION

Molecular data largely aided in solving the discrepancies in the relationship between *Oxytoxum* and *Corythodinium*, and the classification of the members within the family Oxytoxaceae. Previous classifications have placed *Amphidiniopsis*, *Pseudoadenoides*, *Roscoffia*, *Sabulodinium* and *Thecadinium* in the Oxytoxaceae (Loeblich 1982, Dodge 1984, Sournia 1986, Taylor 1987, Chrétiennot-Dinet *et al*. 1993, Fensome *et al.* 1993, Steidinger and Tangen 1997). However, the sequences of these genera did not branch within the clade of *Oxytoxum* and *Corythodinium* (Fig. 3). Consequently, the family Oxytoxaceae should be restricted to *Oxytoxum* and *Corythodinium* that forms their own clade within the dinokaryotic dinoflagellates.

The family Oxytoxaceae is characterized by one antapical plate and five postcingular plates. The first postcingular (1*'''*) was shorter and narrower than the others and contacts with the last postcingular plate (5*'''*) (Stein 1883, Taylor 1976, Dodge and Saunders 1985). The affinities of the Oxytoxaceae and the plate arrangement of peridinioid and gonyaulacoid dinoflagellates have been discussed in Fensome *et al.* (1993). The family Oxytoxaceae has been placed within the Gonyaulacales (Taylor 1987, Steidinger and Tangen 1997), and authors that do not accept the order Gonyaulacales have placed it between gonyaulacoid genera (Sournia

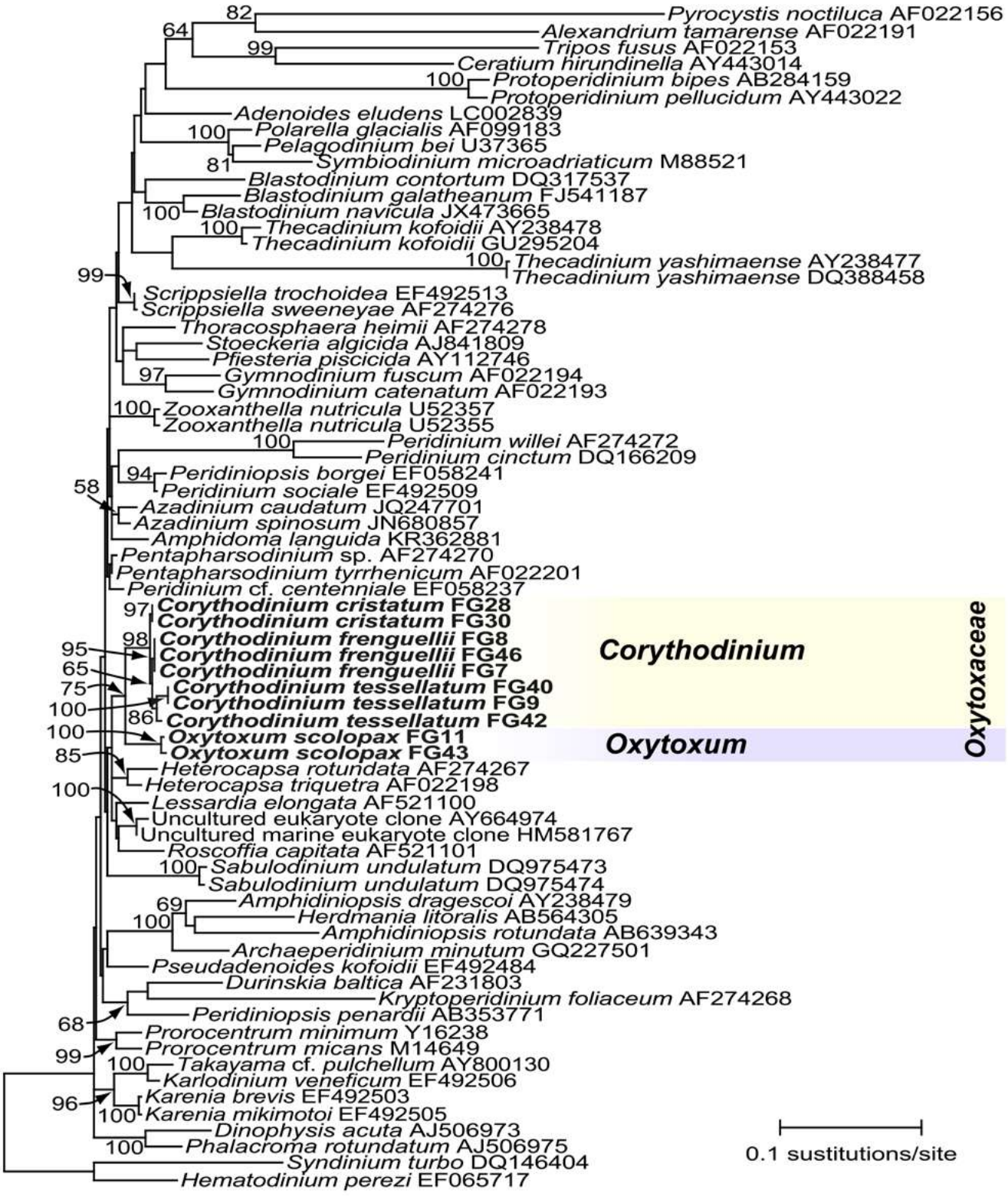


Fig. 3. Maximum Likelihood (ML) phylogenetic tree of *Oxytoxum scolopax* and *Corythodinium* spp. with other dinoflagellates inferred from SSU rDNA sequences based on 1,654 aligned positions. The species newly sequenced in this study are highlighted in bold. The numbers at each node represent bootstrap support (only values above 50% are indicated). The scale bar represents inferred evolutionary distance in substitutions/site.

1986, Balech 1988). The higher cingular displacement of some species of *Corythodinium* is reminiscent of the torsion that characterizes gonyaulacoid dinoflagellates. As in most of the gonyaulacoids, the members of the Oxytoxaceae have six precingular plates. However, peridinioid dinoflagellates such as *Peridinium* and some members of the *Diplopsalis*-group also possess six precingular plates (Sournia 1986, Steidinger and Tangen 1997). The molecular data does not support any relationship between Oxytoxaceae and gonyaulacoid dinoflagellates that are characterized by long branches in the SSU rDNA phylogenies (Fig. 3). Another characteristic unifying the Oxytoxaceae is the single antapical plate; most dinoflagellates possess two antapical plates (Steidinger and Tangen 1997). In our molecular phylogeny, the Oxytoxaceae branched, without support, as a sister group to *Heterocapsa* and *Roscoffia*, the latter a true member of the Podolampadaceae (Gómez *et al*. 2010a; Fig. 3). A character observed in some members of the *Diplopsalis*-group and Podolampadaceae, including *Roscoffia*, is the presence of a single antapical plate (Carbonell-Moore 1994, Steidinger and Tangen 1997).

Within the Oxytoxaceae, the sequences of *Corythodinium* formed a strongly supported clade. The size and the general appearance (lateral flattening, presence of a crest) of *C. cristatum* (Figs 1N–O; 2M–N) was quite different from species such as *C. tessellatum* and *C. frenguellii.* However, there was little genetic difference between these species. This situation is similar in other genera such as *Tripos* with species containing significant differences in the cell shape and small genetic differences (Gómez *et al*. 2010b). This might suggest that a recent speciation event has occurred, resulting in contrasting morphologies and few genetic differences. This study supports the plate arrangement as a diagnostic character for generic split. Still, care should be taken when using general appearance (size, cell shape and compression) as a tool for classification.

Acknowledgements. F.G. was supported by the Ministerio Español de Ciencia y Tecnología (contract number JCI-2010-08492) and the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant number BJT 370646/2013–14). This is a contribution to the ANR Biodiversity program (ANR BDIV 07 004–02 'Aquaparadox'). This work was supported by a Grant-in-Aid for Scientific Research (grant number 253040412 to H.N.) and funds provided to A.Y. from Kobe University, and K.C.W. from the University of Tokyo and the Hokkaido University.

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Received on 19th September, 2016; revised on 24th November, 2016; accepted on 16th December, 2016

SUPPORTING INFORMATION

Appendix S1. Methods of sampling and observations in the Mediterranean Sea

Sampling, isolation and light microscopy

Specimens were collected from the Mediterranean Sea by slowly filtering surface seawater taken from the pier of the Station Marine d'Endoume at Marseille, France (43°16′48.05″N, 5°20′56.22″E, bottom depth 3 m) from October 2007 to September 2008. A strainer of 20, 40, or 60-µm mesh size was used to collect planktonic organisms from water volumes ranging between 10 and 100 l, depending on particle concentration. The plankton concentrate was scanned in settling chambers at × 100 magnification with an inverted microscope (Nikon Eclipse TE200; Nikon Inc., Tokyo, Japan). Cells were photographed alive at × 200 or × 400 magnifications with a Nikon Coolpix E995 digital camera. Further specimens were collected using the same method from October 2008 to August 2009 in the surface waters (depth of 2 m) of the port of Banyuls sur Mer, France (42°28′50″N, 3°08′09″E). The concentrated sample was examined in Utermöhl chambers with an inverted epifluorescence microscope (Olympus IX51; Olympus Inc., Tokyo, Japan) and photographed with an Olympus DP71 digital camera. Sampling continued from September 2009 to February 2010 in the Bay of Villefranche sur Mer, France. For this location, sampling was performed at the long-term monitoring site Point B (43°41′10″N, 7°19′00″E, water column depth ~80 m). Water column samples (0–80 m) were obtained using a phytoplankton net (53 µm mesh size, 54 cm diameter, 280 cm length). Samples were prepared according to the same procedure as described above and specimens were observed with an inverted microscope (Olympus IX51, Olympus Inc.) and photographed with an Olympus DP71 digital camera. Sampling continued from May 2012 to February 2013 in the port of Valencia, Spain (39°27′38.13″N, 0°19′21.29″W, water column depth of 4 m). Specimens were obtained using a phytoplankton net (20 µm mesh size). Samples were prepared according to the same procedure as described above and specimens were observed with an inverted microscope (Nikon Eclipse T2000; Nikon Inc.) and photographed with an Olympus DP71 digital camera.

In addition, samples were collected during the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) cruise on board R/V *L'Atalante* from the south of France to the south of Cyprus (20 June–18 July 2008). Seawater samples were collected with Niskin bottles from 30 stations. At each station 6 depths were sampled between 5 and 125 m, with an additional sample at 250 m depth. These samples were preserved with acid Lugol's iodine and stored at 5°C. Samples of 500 ml were concentrated via sedimentation in glass cylinders. The top 450 ml of sample was slowly siphoned off with small-bore tubing during 6 days. The remaining 50 ml of concentrate, representing 500 ml whole water, was then settled in composite settling chambers. The sample was examined in Utermöhl chambers at × 100 magnification with a Nikon inverted microscope (Nikon Eclipse TE200) and the specimens were photographed with a digital camera (Nikon Coolpix E995).

Figure S1. Light micrographs of isolated cells of *Oxytoxum* and *Corythodinium* for molecular analysis.