Diversity and phylogeny of Gymnodiniales (Dinophyceae) from the NW Mediterranean Sea revealed by a morphological and molecular approach

Albert Reñé *, Jordi Camp, Esther Garcés

Institut de Ciències del Mar (CSIC) Pg. Marítim de la Barceloneta, 37-49 08003 Barcelona (Spain)

* Corresponding author. Tel.: +34 93 230 9500; fax: +34 93 230 9555.
E-mail address: albertrene@icm.csic.es

Abstract
The diversity and phylogeny of dinoflagellates belonging to the Gymnodiniales were studied during a 3-year period at several coastal stations along the Catalan coast (NW Mediterranean) by combining analyses of their morphological features with rDNA sequencing. This approach resulted in the detection of 59 different morphospecies, 13 of which were observed for the first time in the Mediterranean Sea. Fifteen of the detected species were HAB producers; four represented novel detections on the Catalan coast and two in the Mediterranean Sea. Partial rDNA sequences were obtained for 50 different morphospecies, including novel LSU rDNA sequences for 27 species, highlighting the current scarcity of molecular information for this group of dinoflagellates. The combination of morphology and genetics allowed the first determinations of the phylogenetic position of several genera, i.e., *Torodinium* and many *Gyrodinium* and Warnowiacean species. The results also suggested that among the specimens belonging to the genera *Gymnodinium*, *Apicoporus*, and *Cochlodinium* were those representing as yet undescribed species. Furthermore, the phylogenetic data suggested taxonomic incongruences for some species, i.e., *Gyrodinium undulans* and *Gymnodinium agaricoides*. Although a species complex related to *G. spirale* was detected, the partial LSU rDNA sequences lacked sufficient resolution to discriminate between various other *Gyrodinium* morphospecies.

Keywords: dinoflagellates; HAB; naked; phylogeny; rDNA; single-cell PCR

1. Introduction

The diversity of living marine dinoflagellates is estimated at 2,500 species (Gómez 2005; Sournia 1995). Traditionally, dinoflagellate taxonomy has been based on the morphological features of the different groups, with the major orders established accordingly (Fensome et al. 1993; Taylor 1987). However, the interrelationships among the different lineages remain unresolved and many orders have proved to be polyphyletic (Moestrup and Daugbjerg 2007; Murray et al. 2005; Saldarriaga et al. 2004). The coupling of morphological features with molecular phylogenetic data has enabled analyses of the relationships between many genera and species (Handy et al. 2009; Murray et al. 2009; Taylor 2004). Unfortunately, most of the organisms that have been sequenced are cultivable photosynthetic species whereas there is little information for a large number of genera, such as those that are mixo- and heterotrophic. One method to avoid cell culture bias is single-cell PCR (Ruiz-Sebastián and O'Ryan 2001), which has been successfully and recurrently applied to dinoflagellates (Lynn and Pinheiro 2009).

Most dinoflagellates that lack a theca are included in the order Gymnodiniales Apstein. These "unarmoured" or "naked" dinoflagellates, which comprise about 600 species (about 25% of the described dinoflagellate species), belong mainly to the genera *Amphidinium*, *Cochlodinium*, *Gyrodinium*, and the highly diverse *Gymnodinium*, for which about 250 species have been described thus far (Gómez 2005, 2012; Guiry and Guiry 2013). However, the identification of unarmoured species is challenging because several of their key characters are difficult to observe. In addition, they show a high phenotypic plasticity, their life cycle consists of morphologically different stages, and the lack of a theca often results in their deformation when fixed for microscopy studies. Therefore, routine samplings using fixatives are not suitable for species identifications, which instead must rely on live specimens. Moreover, the original descriptions of some species are incomplete and doubtful, with a large number of species never observed again since their original description. This includes 40% of the species belonging to the genus *Gymnodinium* (Thessen et al. 2012). Some of these unarmoured taxa have been studied in detail, especially harmful algal bloom producers, whereas studies on the diversity and distribution of many other unarmoured species and genera are scarce and incomplete, in addition to being hindered by a lack of molecular data (Gómez 2014).

Molecular sequences of unarmoured dinoflagellates provide highly valuable information, as they usually allow both the discrimination of similar morphospecies and the characterization of specimens that cannot be easily assigned to a genus based on morphology alone, together with a determination of their phylogenetic position. Because many species of unarmoured dinoflagellates are heterotrophic or mixotrophic, efforts to obtain viable and dense cultures are time consuming but often unsuccessful. The available phylogenetic information is therefore scarce and generally restricted to photosynthetic cultured species. Early phylogenetic studies noted the polyphyletic nature of unarmoured dinoflagellates (Fensome et al. 1999; Lenaers et al. 1991). Subsequently, the taxonomy of this group underwent major revisions, beginning with the work of Daugbjerg et al. (2000), who redefined the genera *Gymnodinium* and *Gyrodinium* and erected the genera *Akashiwo*, *Karenia*, and *Karlodinium* by combining the morphological features, mainly the shape of the apical groove, with the ultrastructural features of these organisms with their phylogeny. The phylogenetic clade containing *Gymnodinium fuscum* (Ehrenberg) Stein 1878, the type species of the genus, was called *Gymnodinium sensu stricto* (*s.s.*). Further phylogenetic studies led to the

inclusion of other unarmoured genera within this clade: *Lepidodinium* Watanabe, Suda, Inouye, Sawaguchi et Chihara (Saunders et al. 1997); *Barrufeta* Sampedro et Fraga (Sampedro et al. 2011); *Paragymnodinium* Kang, Jeong, Moestrup et Shin (Kang et al. 2010); and *Gyrodiniellum* Kang, Jeong et Moestrup (Kang et al. 2011). Furthermore, non-*Gymnodinium*-like families, such as Polykrikaceae (Hoppenrath and Leander 2007a), Chytriodiniaceae (Gómez et al. 2009b; Kim et al. 2008), and Warnowiaceae (Gómez et al. 2009a; Hoppenrath et al. 2009a), also cluster within this clade. Accordingly, as this cluster contains *G. fuscum*, the first unarmoured species described, but also many other genera and families, it is considered as the Order Gymnodiniales *sensu stricto* clade (Gómez et al. 2009a; Hoppenrath and Leander 2007a, 2010; Yamaguchi et al. 2011). In parallel, the phylogenetic relationships of many other unarmoured genera and species have been studied, resulting in the erection of more than ten new genera (e.g. de Salas et al. 2003, Flø Jørgensen et al. 2004b, Sparmann et al. 2008). Using single-cell PCR, a powerful tool to study organisms that are difficult to culture because of their heterotrophic requirements or because they are usually found in their natural environment at low abundances, several authors have obtained phylogenetic data on Gymnodiniales, increasing the available molecular data for heterotrophic and not easily cultivable species (Hansen and Daugbjerg 2004; Hoppenrath et al. 2009a; Reñé et al. 2014; Takano and Horiguchi 2004). Based on this new phylogenetic information, the order Gymnodiniales has been recognized as either polyphyletic, and thus artificial with respect to single rRNA genes (Daugbjerg et al. 2000; Gómez et al. 2011; Saldarriaga et al. 2001), or paraphyletic when multiple genes have been used to reconstruct phylogenies (Orr et al. 2012). In light of the polyphyly or paraphyly of the order, genera and clades that do not cluster within the Gymnodiniales *s.s.* clade are still included in the order Gymnodiniales, but since they are not phylogenetically related they are considered as Gymnodiniales *sensu lato* members.

The diversity of unarmoured dinoflagellates was intensely studied in several locations in the Mediterranean Sea during the early 20th century (Gómez 2003 and references therein), but those studies were based only on morphology and relied on fixed specimens, whereas phylogenetic studies were lacking. The first detailed studies on the specific composition of dinoflagellates from the Catalan coast (NW Mediterranean Sea) were carried out during the 1940s (Margalef 1945) and were continued for some time thereafter (Estrada 1979, 1980; Margalef 1969). However, problems in the species-level characterization of unarmoured dinoflagellates impeded their unequivocal identification, resulting in a probable underestimation of their biodiversity. In the following years, the harmful effects of a few unarmoured species found off the Catalan coast led to their intensive investigation (Delgado et al. 1995; Garcés et al. 2006; Reñé et al. 2011; Sampedro et al. 2011; Vila et al. 2001), typically by combining morphological and phylogenetic information.

The aim of this work was to combine both morphological and rDNA sequence information of Gymnodiniales in order to resolve uncertainties in their diversity, distribution, and phylogenetic relationships. rDNA sequences were obtained using single-cell PCR, as following morphological observation of the organism the genetic region of interest can be sequenced without the need for successful culture. To obtain a high diversity of target dinoflagellates, the studies were conducted using samples obtained from environmentally heterogeneous regions of the Catalan coast, i.e. rocky beaches, open and semi-enclosed sandy beaches, river-influenced areas, and small and relatively large harbours.

2. Results

2.1 Morphospecies detected

During this study, 59 different species belonging to the Gymnodiniales were detected along the Catalan coast (Fig. 1). All morphospecies observed are listed in Table 1, which also includes comments on the detections, and, when quantified, physicochemical parameters (water temperature and salinity) and cellular abundances. The use of sieved samples and detailed microscopic observations yielded both abundant and rare species. Some species were detected recurrently at high abundances; others were rarely detected but the abundances were significant; and many species were rarely detected and only at very low abundances (Table 1). Most organisms were identified by their characteristic morphological features, others were only unequivocally identified at the species level when their molecular phylogeny was determined, and some could not be confidently identified even though their rDNA sequences were obtained. Supplementary Table S1 lists the correspondence of rDNA sequences and images shown in Figs. 2–5 and Supplementary Material Figs. S1–S2. Detailed morphological and molecular information about some of the organisms detected during this study was previously published; this was the case for *Polykrikos tanit* (Reñé et al. 2014), *Cochlodinium polykrikoides* (Reñé et al. 2013b), and Ceratoperidiniaceae members (Reñé et al. 2013a). These species have been included in the list of detected species, but their morphological details and phylogeny are not discussed in this work. A detailed morphological description is thus provided only for organisms not unequivocally identified at the species level:

- *Amphidinium* cf. *operculatum* Claparède & Lachmann (Fig. 2F)

One fixed specimen was observed and tentatively identified based on Murray et al. (2004). The green-brown cell was 38 µm long and 28 µm wide, ovoid, and dorsoventrally compressed. The epicone was small and overlaid the anterior part of the hypocone, bent to the left side of the cell. Its right side formed an angle of 90° and the left side deflected to the left. The hypocone was oval and symmetrical, and the antapex was round and slightly flattened. The cingulum formed a V on ventral view, its distal end descending to one-third of the cell length. The nucleus was large, occupying almost the posterior half of the hypocone. Although a round structure seemed to be present just above the nucleus, it could not be unequivocally distinguished as a pyrenoid or stigma.

- *Apicoporus* sp. (Fig. 2G; Suppl. Fig. S1C–S1G)

The specimens were 34–44 µm long and 22–29 µm wide. The cells were dorsoventrally flattened and their surfaces were smooth. The epicone was short, wide, and beak-shaped. The cingulum was deep and descending, but its distal end was not connected with the sulcus, which was narrow and ran through the hypocone, where it formed a semicircular indentation. The sulcus penetrated the epicone, reaching the apex, where a protuberance was present. The shape of the hypocone varied from rectangular to ovoid and the antapex was roundish but slightly asymmetrical. No horns or protrusions in the antapical end were observed. The nucleus was large and rectangular, occupying almost the entire hypocone, from the ends of the cingulum to the end of the sulcus. The cells were colourless and chloroplasts were absent.

- *Cochlodinium* sp. (Fig. 3B)

Only one cell was thoroughly observed. It was 49.5 µm long, 33.5 µm wide, and ovoid in shape, with its widest transdiameter in the centre of the cell. Both the apex and the

antapex were round. The cingulum more than twice encircled the orange-pigmented cell but the sulcus was not unequivocally observed. The oval nucleus was situated in the upper half of the cell, slightly displaced to the right.

- *Gymnodinium* sp. 1 (Fig. 3I; Suppl. Fig. S1J)
Light microscopy observations did not reveal any conspicuous cellular features of these organisms. The oval-shaped, yellow-brownish densely pigmented cells were 33.5–36.5 µm long and 26.5–29 µm wide. The epicone was conical and the hypocone was round. The cingulum was located medially, descending for a distance two to three times its width. The sulcus was observed near the apex and reached the antapex, where it widened. A round body was present in the hypocone.

- *Gymnodinium* sp. 2 (Fig. 3J)
These cells characteristically formed two-cell chains, although three-cell chains were also observed. No single cell could be reliably assigned to this morphospecies. Cells in chains were pigmented, 13–18 µm long, 10–13 µm wide, almost equal in size, and slightly dorsoventrally compressed. The epicone of the anterior cell was conical, with a rounded apex, while the hypocone was quadrangular, with an antapex that was completely flattened and slightly wider than the epicone. The posterior cell was ovoid, with a roundish apex and antapex. The cinguli were broad, median, and descended a distance of 1–2 times their width. The sulci were narrow and deep in the epicone, where the horseshoe-shaped acrobase joined, running anticlockwise around the apex and with its distal end not touching the sulcus. The sulcus of the anterior cell broadened in the hypocone, reaching the antapex and forming a cavity that sheltered the epicone of the posterior cell. The sulcus of the posterior cell was less broad and shallower but also reached the antapex.

- *Gyrodinium* cf. *britannicum* Kofoid & Swezy (Fig. 3K)
A few specimens were detected. The greenish cells were 120–134 µm long and 45–52 µm wide. They were fusiform in shape, widest in the middle, and tapering towards both apices, which were blunt. The highly vacuolated hypocone was larger than the epicone and the antapex less pointed. The descending cingulum began anteriorly and was displaced about one-third of the cell length. The sulcus ran from the apex to the antapex, widening in the cingular region. The nucleus was round and central. The cells had a striated surface, with bright elongated granules following the striae in the epicone. The specimens were tentatively identified based on available morphological descriptions (Elbrächter 1979; Kofoid and Swezy 1921).

- *Gyrodinium* cf. *ochraceum* Kofoid & Swezy (Fig. 3O; Suppl. Fig. S1N–S1O)
This morphospecies was isolated recurrently throughout the year. The large, elongated, brownish cells were 97–133 µm long and 36–55.5 µm wide, with numerous thin striations. Granulation was usually observed in the epicone. The apex was round. The hypocone was round posteriorly but the antapex was pointed. The severe displaced cingulum descended for a distance of about one-third the cell length. The distal end formed a 45º angle with the sulcus, which deeply penetrated the epicone and descended to reach the hypocone, where it formed an excavation. The sulcus twisted towards the left. The large nucleus was spherical to subspherical and situated in the epicone. A long extension in the antapex, probably the feeding apparatus, was observed in several specimens. The tentative identification was based on Elbrächter (1979) and Kofoid and Swezy (1921).

- cf. *Gyrodinium undulans* Hulburt (Fig. 4C)
Three specimens were observed. The cells were 32–36 µm long and 24–32 µm wide. The epicone and hypocone were almost equal in size. The epicone was sub-spherical and the apex was round. The hypocone was trapezoidal, with slightly concave sides. The antapex was flattened. The cingulum was deep and wide, descending for a distance slightly less than its width and overhanging slightly. The sulcus was bi-sigmoid, curving from left to right in the epicone, then to the left again in the intercingular area, where it widened, and to the right in the hypocone, forming a lobe. The colourless cells contained several granules. Their features agreed with those in available morphological descriptions (Drebes and Schnepf 1998; Hulburt 1957).

- *Gyrodinium* sp. 1 (Fig. 4E)
The greenish, vacuolated cells were 36.5–41.5 µm long and 27–30 µm wide, with a conical epicone and rounded apex. The hypocone was hemispherical and both wider and slightly longer than the epicone. The cingulum was wide and located medially, descending about a quarter of the cell length. The intercingular area was protuberant. The sulcus was narrow, running from the apex to the antapex. The round nucleus was situated in the epicone.

- *Gyrodinium* sp. 2 (Fig. 4F)
The colourless, fusiform cell was 112 µm long and 48 µm wide. The apex was pointed but the antapex was more rounded, with a pointed protuberance. The surfaces of the epicone and hypocone were striated, with seven or eight striae in the epicone on ventral view. The cingulum was deep, descending, highly displaced, and slightly overhanging. The distal end of the cingulum joined the sulcus perpendicularly. The sulcus, which ran in a straight line from the epicone to the antapex, was not clearly apparent. A ridge extending from the apex to the proximal end of the cingulum was prominently visible. The oval nucleus was situated on the right side of the hypocone.

- *Gyrodinium* sp. 3 (Fig. 4G)
The fusiform cell was 39.5 µm long, 13 µm wide, and had slender striations. The apex was pointed. The descending cingulum extended from the epicone to almost the antapex, with the distal end joining the sulcus to form a 45º angle. The sulcus was hardly apparent and it widened near the antapex, slightly penetrating the dorsal side of the cell. It was slightly displaced to the left of the hypocone, thus causing an asymmetry in the latter. The left side was round and the right side less developed and pointed. The nucleus was spherical, dorsal, and situated in the centre of the cell. The yellow-greenish cell lacked chloroplasts.

- *Gyrodinium* sp. 4 (Fig. 4H; Suppl. Fig. S2C)
The ovoid and spherical cells were 42.5–52.5 µm long and 29–31.5 µm wide. The epicone was conical and the apex flattened. The hypocone was slightly elongated and the antapex was round. The surface was thinly striated. The cingulum was median, descending, and highly displaced, with its distal end curved toward the antapex. The sulcus ran from the apex to the antapex. It was straight in the epicone, then turned slightly to the left to join the distal end of the sulcus, finally widening in the hypocone. The large nucleus was sub-spherical and located in the centre of the cell. The cells were colourless, but a large ingested body was visible in the hypocone of one of them.

- *Gyrodinium* sp. 5 (Fig. 4I)
The colourless cells were 20–25 µm long, 10–15 µm wide, ovoid, and slightly dorsoventrally compressed. Slender striations were present in the epicone and hypocone. The epicone was about three times smaller than the ovoid, tapering hypocone and conical in shape. A knob was observed in the apex. The antapex was roundish but asymmetrical, with its right side slightly pointed. The descending cingulum was deeply impressed in its pre-median portion, with its distal end joining the sulcus approximately in the centre of the cell. A narrow sulcus ran from the apex to the antapex, where it widened. The nucleus was ellipsoidal, occupying the intercingular area. Ingestion bodies were commonly seen in the posterior part of the cell.

- *Gyrodinium* sp. 6 (Fig. 4J)
The colourless cells were 60–70 µm long and 20–25 µm wide, fusiform, wider in their centres, and marked with slender striations in the equally sized epicone and hypocone. Both apices were pointed, but the antapex was more elongated. The cingulum was displaced, beginning and ending at a distance from the apex and antapex that was about one quarter of the cell length. It had a slight overhang and the distal end joined the sulcus at an angle of 45º. The sulcus was narrow, running from near the apex to the antapex, with a slight curvature in the intercingular zone. The ellipsoid nucleus was situated on the left side of the cell, just below the proximal end of the cingulum.

- *Karenia* cf. *papilionacea* Haywood & Steidinger (Fig. 4L)
Two fixed specimens and two live specimens were observed during the study. The tentative identification was based on Haywood et al. (2004). The fixed cells were 20–30 µm long, 30–40 µm wide, and dorsoventrally compressed. The epicone was flattened, with an apical process in the apex. The hypocone was bilobed. The cingulum was slightly displaced. The sulcus extended into the epicone and reached the antapex. The nucleus was round, situated on the left side of the hypocone. Many roundish chloroplasts were seen in the cell periphery.

- *Warnowia* sp. 1 (Fig. 5K; Suppl. Fig. S2N)
The cells were 58–67 µm long and 39–42 µm wide, ovoid, and elongated, but they could only be observed in lateral view. The epicone and hypocone were almost equal in size. The apex and antapex were round. The cingulum was medially located, with the two ends joined ventrally, but neither their junction nor the sulcus was observed. The position of the ocelloid varied in the studied specimens, as it was located near the antapex in one cell and in the hypocone near the cingulum in the two others. The lens was elongated and spherical at its end, located in the upper part of the ocelloid. The large, round, nucleus was centrally positioned in the epicone. The colourless cells were covered by a hyaline membrane.

- *Warnowia* sp. 2 (Fig. 5L; Suppl. Fig. S2O–S2P)
The cells were 47–54.5 µm long, 20.5–27 µm wide, fusiform, and elongated. The apex was blunt. The cingulum encircled the cell 2–2.5 times. The sulcus was not unequivocally observed. The antapex was asymmetrical, forming an elongated protuberance. The elongated nucleus was situated in the centre of the cell, and the elongated ocelloid posteriorly. The lens was thin and long. Numerous nematocysts were present in the anterior part of the cell, radiating from the centre to the periphery.

- *Warnowia* sp. 3 (Fig. 5M)

The 39-µm long and 26.5-µm wide cell was only observed in lateral view and its outline was similar to that of *Warnowia* sp. 1. However, the cingulum encircled the cell twice. The nucleus was elongated, occupying 75% of the cell length. The reddish ocelloid was small and elongated, situated on the ventral side of the cell. The colourless cell was covered by a hyaline membrane.

- *Warnowia* sp. 4 (Fig. 5N; Suppl. Fig. S2Q–S2S)
The cells were 44.5–54 µm long and 28.5–33.5 µm wide, roundish in shape, and slightly compressed dorsoventrally. The epicone and hypocone were almost equal in size. The epicone was conical. The asymmetrical hypocone was round on its right side and less developed on its left side. The antapex was flattened. The cingulum was medially located, descending for a distance three to four times its width. The sulcus was not observed in detail but it widened in the hypocone and accounted for the asymmetry of the latter. Numerous nematocysts were present, mainly radiating from the cell centre. The large, round nucleus had an irregular outline and was located in the epicone. The ocelloid was situated in the hypocone. The lens was round, almost equal in size to the melanosome, and situated to its left. A posterior cell 'extension' was observed in some specimens.

- *Warnowia* sp. 5 (Fig. 5O; Suppl. Fig. S2T)
The cells were ovoid and slightly dorsoventrally flattened, although amorphous cells were also observed. The epicone was ovoid and the apex round. The hypocone was asymmetrical, with the right side more pointed than the left side. The cingulum was median, displaced about twice its width. The sulcus was shallow and not clearly observed. The ocelloid was situated at the posterior end of the right side of the hypocone, in which dark bodies were scattered. An elongated refractive body was often present in the upper half of the cell. The cells had a pale yellow-greenish colour and were usually covered by a hyaline membrane.

2.2 Phylogenetic analyses:
Partial rDNA sequences were successfully obtained for 50 of the 59 detected morphospecies (Suppl. Table S1). The constructed LSU rDNA (Fig. 6) and SSU rDNA phylogenies (Fig. 7) explored the diversity of the dinoflagellates included within the Gymmodiniales order. Because the unarmoured dinoflagellates are not a monophyletic group, the Gymnodiniales order is not supported phylogenetically.
The species included in the so-called Gymnodiniales *s.s.* clade (97% bootstrap/1 BPP for LSU rDNA phylogeny) were split in two clades, although weakly supported (Fig. 8). The first contained several *Polykrikos* species (*P. hartmannii*, *P. tanit*, *P. schwartzii*, *P. kofoidii*, and *P. geminatum* (=*Cochlodinium* cf. *geminatum*), but other polykrikoid species as *Polykrikos lebourae*, *P. herdmanae*, and *Pheopolykrikos beauchampii* clustered independently. Three different *Polykrikos* species, *P. kofoidii*, *P. herdmanae*, and *P. tanit*, were detected along the Catalan coast. Several, almost identical, partial LSU rDNA sequences of the first species were obtained (only three are shown in this study). They were consistent with the *P. kofoidii* sequences from GenBank and clearly distant from those of *P. schwartzii*. The sequences of *P. tanit* clustered with that of *P. hartmannii* although with weak support. The LSU rDNA sequence of *P. herdmanae* clustered with that of *P. lebourae* and the two identical partial SSU rDNA sequences of *P. herdmanae* agreed with those available in GenBank (99.5% similarity) (Fig. 7).
The second group contained the remaining species, although their phylogenetic positions are mostly unresolved (Fig. 8). The *Barrufeta bravensis*, *Gymnodinium*

aureolum, *G. impudicum*, *G. litoralis*, and *Lepidodinium viride* sequences obtained in this study agreed with those available in GenBank. The phylogenetic position of *G. litoralis* based on its SSU rDNA sequence was consistent with that based on the LSU rDNA region (Fig. 7). Sequences of *Gymnodinium* sp. 1 (three identical partial LSU rDNA sequences), *Gymnodinium* sp. 2 (two identical partial LSU rDNA sequences), and cf. *Gyrodinium undulans* did not match any other previously available sequence.
A highly supported clade contained all the Warnowiaceae species (99%/1), which formed four main clades (Fig. 8). The first branch contained only *Warnowia* sp. 4 (two partial LSU rDNA sequences with 99.8% similarity). In a sister branch, there were two sub-clades. The first one contained *Warnowia* sp. 1 (two identical LSU rDNA sequences), *Warnowia* sp. 2 (three identical LSU rDNA sequences), and *Warnowia* sp. 3 (100%/1), with *Warnowia* sp. 1 and *Warnowia* sp. 3 clustering together (100%/1). Finally, sequences of *Warnowia* sp. 5 clustered with those previously available from GenBank and were strongly related to the *Warnowia* sp. BSL-2009a sequence. The SSU rDNA sequences of some warnowiacean specimens (*Warnowia* sp. 4 and *Warnowia* sp. 5) were also analysed (Fig. 7). All warnowiacean representatives formed a monophyletic group and the available sequences grouped in several clades. Unfortunately, some of the sequences available in GenBank were the result of environmental sequencing such that morphological descriptions for the sequenced organisms are not available (GenBank codes with no species name in Fig. 7). The two '*Nematodinium*' sequences grouped together (100%/1), as did the sequence of *Warnowia* sp. 5 from this study and that of *Warnowia* sp. (BC), which were identical (100%/1). Another clade contained '*Proterythropsis*' sp. sequences, the *Warnowia* sp. 4 sequence obtained in this study, and an environmental sequence (100%/1). Finally, another branch was obtained (95%/1) that included most of the environmental sequences and a cluster (98%/1) comprising the sequences of *Erythropsidinium agile*, *Warnowia* sp. and a sub-clade containing the *Warnowia* sp. (Florida) sequence and two environmental sequences (96%/1).

Besides the well characterized Gymnodiniales *s.s.* clade, several other groups or genera form well-supported clades.
All *Gyrodinium* species (Fig. 9) clustered together (100%/1) and split into two main branches. The first branch (100%/1) contained *G.* cf. *britannicum*, *Gyrodinium* sp. 2, and the group of specimens identified as *G.* cf. *spirale*, which clustered with the *G. fissum* sequence from GenBank. Up to 16 partial LSU rDNA sequences were obtained, but only six are shown in this study (99.1% similarity). Despite the morphological variability of *G.* cf. *spirale*, the molecular data confirmed that all specimens belonged to the same species. Five partial SSU rDNA sequences were also obtained for organisms identified as *G.* cf. *spirale* (Fig. 7). Four were identical and one slightly differed (99.9% similarity). Those sequences clustered with the available, nearly identical sequences of *G. spirale* and *G. fusiforme* (97%/1).
The second branch (99%/1) contained the remaining sequences of *Gyrodinium* species (Fig. 9). A monophyletic clade was obtained for *Gyrodinium* sp. 5, *G. moestrupii*, and *G. dominans* (100%/1). *Gyrodinium* sp. 4 and *G. spirale* clustered together (98%/1). The remaining sequences from this study (*Gyrodinium corallinum*, two sequences for *G.* cf. *ochraceum* with 99.0% similarity, two sequences for *G. viridescens* with 99.3% similarity) did not show any supported relationship, except *Gyrodinium* sp. 3, *G. heterogrammum* (three sequences with 99.9% similarity), and *Gymnodinium agaricoides*, whose LSU rDNA sequences were identical. Three SSU rDNA sequences (99.9% similarity) were obtained for *G. heterogrammum* (Fig. 7). They did not match

any other sequence available and clustered (100%/1) with sequences of *G. moestrupii*, *G. dominans*, *G. rubrum*, and *G. helveticum*.

Members of the Kareniaceae family (*Karenia*, *Karlodinium*, and *Takayama*) clustered together (Fig. 6), although there was good support only for branches representing some of the genera. All obtained sequences of species belonging to this family (two identical sequences for *Karenia mikimotoi*, *K. umbella*, *Takayama tasmanica*, *Karlodinium armiger*, *K. veneficum*, and *K. decipiens*) were almost identical to those previously available in GenBank. The LSU rDNA sequence of *A. glaber* and our three identical sequences of *Apicoporus* sp. also clustered within this clade (100%/1). The three identical partial SSU rDNA sequences of *Apicoporus* sp. clustered with *A. parvidiaboli* and *A. glaber* but were closely related to the latter (Fig. 7).

The LSU rDNA sequences of *Ceratoperidinium* and related species formed a clade with high support (91% /1) (Reñé et al. 2013a) (Fig. 6). Two partial SSU rDNA sequences (99.7% similarity) of *C. falcatum* were obtained in this study. They clustered independently of any other unarmoured dinoflagellates, given that no SSU rDNA sequence of any other member of Ceratoperidiniaceae has been reported to date (Fig. 7). *Cochlodinium polykrikoides*, *C. fulvescens*, and *Cochlodinium* sp. clustered together (95%/1) and independently of *Cochlodinium* species included in the Ceratoperidiniaceae family. Different ribotypes were obtained for *C. polykrikoides* (Iwataki et al. 2008; Reñé et al. 2013b) but also for *C. fulvescens* (Fig. 6).

The so-called *Amphidinium s.s.* clade (88%/1) included the sequences of *A. carterae* determined herein (Fig. 6). However, the sequence of *A. crassum* clustered independently. The two *Akashiwo sanguinea* sequences (97.5% similarity) and *Levanderina fissa* (two identical partial LSU rDNA sequences) were not phylogenetically related to any other unarmoured dinoflagellates sequenced to date. Finally, *Torodinium teredo*, the two identical partial LSU rDNA sequences of *T. robustum* and *K. glaucum* clustered together (84%/1). The clade containing both *Torodinium* species was highly supported (100% /1). Two identical partial SSU rDNA sequences of *Torodinium robustum* specimens were also obtained (Fig. 7). Unfortunately, the SSU rDNA of *T. teredo* specimens could not be sequenced, impeding the characterization of its phylogenetic relationship with *T. robustum*. As for the LSU rDNA phylogeny, the genus *Torodinium* showed a close phylogenetic relationship with *Katodinium glaucum* (three identical partial SSU rDNA sequences) based on their SSU rDNA sequences, although the relationship was weakly supported (29%/0.99).

3. Discussion

The combination of morphological observations with the phylogenetic information of the studied specimens allowed their unequivocal identification at least at the genus level. However, in some cases the limited morphological observations and the similarity of several unarmoured species prevented species-level determinations. In this study we detected 59 species belonging to the Gymnodiniales order and present along the Catalan coast (Table 1). Thirteen of the detected species were reported for the first time in the Mediterranean Sea (Table 1), confirming that the diversity of unarmoured species in the studied area is only partially known. The scarcity of genetic information available in the databases for some genera limited interpretation of the phylogenetic data. Partial rDNA sequences were determined for 50 of them (Suppl. Table S1). Of these, 27 sequences are the first available for the species and they will allow comparisons with further

detections of morphotypes not easily identifiable based solely on morphological characteristics.

Although many common or abundant species were detected, almost half of the collected species could be considered as rare in the study area (Table 1). Detailed studies on their morphology and phylogeny are of great importance because the diversity of Gymnodiniales includes many species that were either only rarely or never again reported after their initial description. This is the case for 40% of the approximately 270 existing *Gymnodinium* species (Thessen et al. 2012). In addition to the problem of poor and ambiguous original descriptions, the scarcity of the data on rare species has resulted in serious gaps in our knowledge of their diversity and distribution. Consequently, the total diversity of Gymnodiniales cannot be assessed with certainty, given that it is unclear whether these gaps are due to the lack of studies or the limited geographical distributions of these organisms, or whether their diversity has been overestimated because of the misinterpretation of intraspecific morphological variability.

3.1 Identification of unarmoured species:

The combination of morphology and molecular data allowed the unequivocal identification and characterization of *B. bravensis*, *G. litoralis*, *G. impudicum*, *A. sanguinea*, *L. fissa*, and some *Gyrodinium* species. However, for some morphospecies the morphology slightly differed from the original description such that they were identified by comparing their rDNA sequences with those available in databases.

The external morphology of *Lepidodinium viride* agreed with the original description (Watanabe and Suda 1990), except that in the latter the epicone was reported as slightly conical in shape while in our specimen it was completely round. The morphology of *Gymnodinium aureolum* also slightly differed from available species descriptions (Hansen et al. 2000; Hulburt 1957). The cells were round but, in contrast to available descriptions, the epicone was slightly shorter and narrower than the hypocone. The differences observed for both species could reflect the plasticity of unarmoured dinoflagellates.

Three different *Polykrikos* species were detected in the study, including the benthic *P. herdmanae*. Most of its morphological features agreed with the available morphological descriptions (Hoppenrath and Leander 2007b). However, the pseudocolonies were not symmetrical and the apical zooids were smaller and more pointed than the antapical ones. The nuclei could not be observed unequivocally and none of the pseudocolonies showed ingestion bodies. To the best of our knowledge, this is the first detection in the Mediterranean Sea. In the literature, both *P. kofoidii* and *P. schwartzii* have been widely reported along Catalan shores (Estrada 1979; Margalef 1969; Vila et al. 2005). The identification of these two species is problematic because they are similar in their external shape but distinguishable by other features, e.g., the longitudinal furrows on the hypocone of the zooids and the numbers of zooids developed in *P. kofoidii* (Matsuoka et al. 2009). In our study, *Polykrikos* specimens were commonly detected at many locations albeit always at low abundances. The presence of the furrows on the hypocone usually allowed the unequivocal identification of *P. kofoidii* specimens, while in other specimens we were unable to observe the furrows; however, according to their LSU rDNA sequences, all the sequenced organisms belong to *P. kofoidii* because they matched with sequences from a detailed morphological study (Matsuoka et al. 2009). Therefore, our inability to detect *P. schwartzii* suggests either the misidentification of this species in the literature or its absence from our samplings.

Torodinium specimens were detected several times during this study. Some agreed morphologically with *T. robustum* and others with *T. teredo* descriptions. However, our *T. teredo* specimens were shorter (59–76.5 µm in length and 20–24 µm in width) than their commonly reported dimensions (100–130 µm long) (Gómez 2009; Kofoid and Swezy 1921).

In other cases, morphological observations were insufficient to characterize the organisms, such that the rDNA information was crucial for their species-level characterization. Some *Karenia* species were detected during the study, most commonly *K. mikimotoi*. Other specimens were tentatively identified as *Karenia* cf. *papilionacea*, based on Haywood et al. (2004). Unfortunately their rDNA sequences were not obtained. Due to morphological plasticity, they could be confused with *K. brevis*. However, the few specimens observed were very similar morphologically and agreed with *K. papilionacea*. Finally, *K. umbella* was only identified after its rDNA sequence was obtained, as some of its key characters could not be observed. In addition to *Karlodinium armiger* and *K. veneficum*, whose blooms were previously reported in the study area (Garcés et al. 2006), specimens agreeing morphologically and genetically with *K. decipiens* were detected (de Salas et al. 2008), representing the first report in the Mediterranean Sea. Specimens attributed to the genus *Takayama* were also detected during the study, but again several of the key features needed to identify them at the species level could not be observed in detail, e.g., the presence of a ventral pore, the acrobase outline, the shape of the nucleus, and the presence of a pyrenoid. However, sequences with a 99.5% and 99.3% similarity to *T. tasmanica* and *T. tuberculata*, respectively, were obtained. The two species are easily distinguishable by their size, the presence of irregularities on the cell surface, and the shape of the sulcal intrusion (de Salas et al. 2008). Accordingly, the studied specimens were identified as *T. tasmanica*. This is the first report of this species in the Mediterranean Sea. *Takayama pulchella* was reported between 1998 and 1999 along the Catalan coast, in Fangar Bay, at abundances of $<10^4$ cells·L^{-1} (Delgado et al. 1999; Vila et al. 2001). But as *T. pulchella* was not detected during the course of this study, we cannot rule out that those proliferations were actually caused by *T. tasmanica*.

The combination of morphological and molecular data suggested that some of the organisms detected in this study represent undescribed species. *Apicoporus* sp. specimens were obtained from sediment samples. Two different *Apicoporus* species are known; *Apicoporus parvidiaboli* Sparmann, Leander & Hoppenrath, observed only in British Columbia, Canada (Sparmann et al. 2008), and *A. glaber* (Hoppenrath & Okolodkov) Sparmann, Leander & Hoppenrath, only from far northern and polar areas (Hoppenrath and Okolodkov 2000) and Kuwait's intertidal sand flats (Saburova et al. 2009). Therefore, this is the first report of the presence of any member of this genus in the Mediterranean Sea. In the studied specimens, the non-parallel sides of the hypocone and the asymmetry of the antapex were in concordance with the respective features of *A. parvidiaboli*. However, posterior horns in the hypocone were absent and some specimens resembled *A. glaber*. The LSU rDNA sequences obtained in this study differed from those available for *A. glaber* (sequences for *A. parvidiaboli* are not available). The SSU rDNA sequences differed from those of both *A. parvidiaboli* and *A. glaber* and thus probably represent an undescribed species. The partial LSU rDNA sequences of two *Gymnodinium*-like individuals (*Gymnodinium* sp. 1 and *Gymnodinium* sp. 2) were successfully sequenced. They were placed within the Gymnodiniales *s.s.* clade but did not coincide with any other available sequence. *Gymnodinium* sp. 1 could

not be identified at the species level because morphological observations were limited and distinctive morphological features were lacking. The morphology of *Gymnodinium* sp. 2 was studied in detail and, as far as we know, its morphology does not correspond to that of any other described species. The fish-killing species *Cochlodinium fulvescens* and *C. polykrikoides* were seldom detected, but a third species was also observed. It clustered with *C. polykrikoides* and *C. fulvescens*, but at a significant genetic distance. Since it did not seem to agree with any other *Cochlodinium* species, it may represent an as yet undescribed species.

For some specimens, morphological and genetic characterizations were not sufficient to identify them at the species level. Morphological similarities with close species, limited morphological observations, and the lack of rDNA sequences available in databases impeded their unequivocal assignment. This was the case of the organisms initially identified as cf. *Gyrodinium undulans*, an ectoparasite with a *Gymnodinium*-like stage in its life cycle (Drebes and Schnepf 1998). Taxonomic identification of the observed specimen according to the existing literature was not possible because of its morphological similarities with *Syltodinium listii* Drebes, another ectoparasite only known from the waters off Sylt, German Bight (Drebes 1988; Hoppenrath et al. 2009b). The lack of observations of either the organism's host or the infection process prevented us from confirming the affiliation of the studied specimen. Regardless, this is the first detection of either *G. undulans* or *S. listii* in the Mediterranean Sea.
The Warnowiaceae family is included within the Gymnodiniales *s.s.* clade. During this study, members of this family were observed rarely and always at very low cell abundances. Six different morphospecies were distinguished. The only member of Gymnodiniales *s.s.* whose rDNA sequence could not be obtained was the specimen identified as *Erythropsidinium* cf. *minor*. It was only seen in dorsal view as it collapsed during microscopy. Its tentative identification was based on Kofoid and Swezy (1921). Because the taxonomy of this group is extremely challenging, we were unable to identify any of the other observed morphospecies at the genus level and thus refer to the different species as *Warnowia* sp. The most commonly detected species was *Warnowia* sp. 2. Although it strongly resembled *Nematodinium torpedo* Kofoid et Swezy, we refrained from conferring this name because the size of our specimens was almost half that described in the literature. Our specimens of *Warnowia* sp. 4 were morphologically similar to those identified as '*Proterythropsis*' sp. by Hoppenrath et al. (2009a). None of the other Warnowiacean detected could be confidently assigned to a known species. Fourteen different *Gyrodinium* species were distinguished during this study and the partial LSU rDNA of 13 of them was successfully sequenced. Our morphological observations were limited, because many morphospecies were only detected once, impeding their species-level identification. This difficulty was further compounded by the lack of sequences in the databases; at the time of this study, GenBank contained LSU rDNA sequences corresponding only to five identified *Gyrodinium* species. Consequently, six of the morphospecies detected were not identified at the species level and are thus referred to as *Gyrodinium* sp. Nevertheless, some of the detections were the first reported for the Mediterranean Sea (Table 1). *Gyrodinium corallinum* specimens observed in this study slightly differed from the original description (Kofoid and Swezy 1921) in that the apex was less pointed and the cingulum was overhanging. Those features were similar to *Gyrodinium rubrum* (Kofoid & Swezy) Takano & Horiguchi, but our specimens shared with *G. corallinum* its typical red pigments and apices that were more pointed than those of *G. rubrum* (Kofoid and Swezy 1921). Also, the sides of the hypocone were not concave and the nucleus was round in ventral view. A high

plasticity was observed for *G. rubrum* (Takano and Horiguchi 2004), while *G. corallinum* was described from a few specimens and it is rarely detected (Gollasch et al. 2009; Kofoid and Swezy 1921; Okolodkov 1998). The partial LSU rDNA sequence of *G. rubrum* was previously available and had a 94.2% similarity with our sequence, which rules out the possibility that our specimens belong to that species. The specimens we identified as *G.* cf. *britannicum* agreed with available morphological descriptions (Elbrächter 1979; Kofoid and Swezy 1921) but the typical carmine-coloured granules following the striae were lacking; however, colourless cells were described in an early study of that species (Lebour 1925). The specimens identified as *G.* cf. *ochraceum* differed from the original description (Kofoid and Swezy 1921) in the absence of a pointed apex and the presence of an antapical loop of the sulcus. Although some variability in those characters was reported (Elbrächter 1979), this was not the case for colouration. A comparison of the morphology of our specimens with that of the closely related *G. contortum* (Schütt) Kofoid & Swezy showed that whereas the shape and the coloration of the cells agreed, other characters, such as the distance between the apex and the anterior end of the cingulum, the shape and position of the nucleus, and the shape of the sulcus, were clearly different. Therefore, we stand by the identifications of our specimens as *G.* cf. *ochraceum*.

Unfortunately, we were unable to obtain the rDNA sequences for all detected morphospecies, as in some cases they would have constituted the first available for the species. We report for the first time the presence *Balechina coerulea* along the Catalan coast. Members of this genus are characterized by their rigid amphiesma, in contrast to *Gymnodinium* members. Although the presence of *Balechina coerulea* is commonly reported worldwide, this genus is poorly known and the validity of the transfer of this species, previously included within the genus *Gymnodinium*, to the genus *Balechina* is dubious (Gómez 2007). Furthermore, molecular information is not available for any of its species and its phylogenetic position thus remains unclear. Organisms belonging to the closely related *Asterodinium* and *Brachidinium* genera were detected. Given that *B. capitatum* clusters with Kareniaceae species (Henrichs et al. 2011), it would have been of great interest to determine the phylogenetic position and relationships of *Asterodinium*, which have yet to be resolved.

3.2 Phylogenetic relationships:
By combining rDNA sequences and morphological observations we were able to identify the studied organisms or confirm their previous identification. Since the obtained sequences were often the first available for the studied species, they allowed determination of both the phylogenetic position and the relationships of poorly studied species. In some cases, they confirmed incorrect assignments to a genus and unexpected or unknown phylogenetic relationships.

- Gymnodiniales *sensu stricto*
This clade contains most of the Gymnodiniales species sequenced so far. The majority are pigmented and some are bloom-forming. However, the phylogenetic relationships of most species within the clade remain unresolved, with a few exceptions such as the clusters formed by *G. catenatum* / *microreticulatum* / *trapeziforme*, or the Warnowiaceae and Polykrikaceae families.

There is a strong phylogenetic relationship among all *Polykrikos* species, but there are discrepancies between the SSU and LSU rDNA phylogenies for *P. lebourae*, which

clusters independently with respect to the latter (Hoppenrath et al. 2009a; Hoppenrath and Leander 2007a). The LSU rDNA sequence of *P. herdmanae* obtained in this study is the first available for the species and it confirmed its close relationship with *P. lebourae*, consistent with the SSU rDNA results (Hoppenrath and Leander 2007b). The LSU rDNA sequence of one specimen identified as cf. *Gyrodinium undulans* was the first to be successfully obtained and it allowed the assignment of this organism to the Gymnodiniales *s.s.* clade. Some ectoparasitic dinoflagellates, such as *Dissodinium* and *Chytriodinium*, are also included in the Gymnodiniales *s.s.* clade (Gómez et al. 2009b). All of them produce *Gymnodinium*-like cells during their life cycles. Therefore, the phylogenetic position of the sequenced representative is in agreement with those of related ectoparasites, although our sequence did not cluster with the *Dissodinium pseudolunula* sequence available from GenBank. Since a close phylogenetic relationship between *G. undulans* and *S. listii* can be expected (if not representing the same species), *G. undulans* can be rejected as a member of the *Gyrodinium* genus because it is included within the Gymnodiniales *s.s.* clade. However, since our specimen could not be precisely identified, any systematic change would be premature.

Despite a previous report describing the presence of several species of the genus *Erythropsidinium* along the Catalan coast (Margalef 1995), we detected only one specimen, identified as *Erythropsidinium* cf. *minor*, belonging to this genus. However, our sequencing attempts failed. Gómez et al. (2009a) demonstrated that, based on the SSU region, *Erythropsidinium* specimens form a monophyletic clade with other warnowiacean genera. *Warnowia* sp. 1, *Warnowia* sp. 2, and *Warnowia* sp. 3 formed a strongly supported clade and none of the respective cells were pigmented. Nematocysts were not observed for *Warnowia* sp. 1 and *Warnowia* sp. 3 but they were present in *Warnowia* sp. 2. *Warnowia* sp. 4 clustered independently, but agreed with the SSU sequence of '*Proterythropsis*' sp. from GenBank. We are confident that the '*Proterythropsis*' genus is valid, as it clusters independently of the other species in the LSU rDNA phylogeny and forms a highly supported clade with the SSU rDNA phylogeny. The partial LSU and SSU rDNA sequences of *Warnowia* sp. 5 agreed with both the *Warnowia* sp. (BC) sequences available from GenBank and the morphological features of *Warnowia* (Hoppenrath et al. 2009a). The morphological characters currently used to classify warnowiacean specimens into different genera seem inconsistent in most cases, a conclusion confirmed by the phylogenetic results of this study. However, our data do not allow further clarification of this challenging taxonomy. While a taxonomic reorganization is still needed within the Warnowiaceae family, the molecular and morphological data provided herein will strongly aid in further efforts to reclassify its members.

- Gymnodiniales *sensu lato*

As previously discussed, genera not clustering within the Gymnodiniales *s.s.* clade are of polyphyletic nature. In this study, several independent clades were obtained.
A cluster (100%/1) within the *Gyrodinium* clade contained three different morphotypes (*G. heterogrammum*, *Gyrodinium* sp. 3, and *Gymnodinium agaricoides*) with almost identical partial LSU rDNA sequences. Although *G. agaricoides* and *Gyrodinium* sp. 3 were only detected once, *G. heterogrammum* was recurrently detected and remarkable morphological variations were never observed. Furthermore, there were no common morphological features for the three morphospecies. Consequently, we reject the possibility that they are morphological variants of the same species. In this case, the LSU fragment proved to be useless in discriminating these species. Additionally,

Gymnodinium agaricoides was placed within the *Gyrodinium* group. It lacked some of the key characters of the genus (surface striation, acrobase shape) but a detailed morphological study is still needed; however, its phylogenetic position suggests its incorrect assignment to the genus *Gymnodinium* and that it instead belongs to the genus *Gyrodinium*. A species complex comprises a group of species that are difficult to differentiate morphologically and was accordingly confirmed for *Gyrodinium spirale*. The most commonly detected *Gyrodinium* morphospecies was initially identified as *G. spirale*, which showed a high morphological plasticity. Cells whose features agreed with those of *G. spirale* were 110–130 µm long and 20–30 µm wide. In other specimens the morphologies differed; in most cases, the cingular displacement and surface striations were maintained, but the cells were completely round or had a short and round hypocone or were spindle-shaped (Fig. 4B; Suppl. Fig. S1O-1R). All specimens were confirmed to belong to the same species based on their molecular sequences; but, surprisingly, their partial LSU rDNA sequences were identical to that of *G. fissum* from GenBank and only 86% similar to that of *G. spirale*. No morphological description or image was provided in the report of the sequenced representative of *G. fissum* (Kim and Kim 2007), and the overall morphology of *G. fissum* (=*Levanderina fissa*) is not close to that of *G. spirale*. According to Moestrup et al. (2014), the specimen was probably misidentified. For the *G. spirale* specimen used to obtain the GenBank sequence (Hansen and Daugbjerg 2004), there is a lack of remarkable morphological differences with our specimens. The partial SSU sequence of our specimens had a 99.9% similarity with the available sequences of *G. fusiforme* and *G. spirale*. The available sequenced specimens could be differentiated only by their size (Takano and Horiguchi 2004), but our measurements overlapped with the dimensions of both species. In parallel, the high morphological plasticity of the species suggests that some of the described *Gyrodinium* species represent anomalous forms of the same species, as previously discussed and suggested by Elbrächter (1979).

The Ceratoperidiniaceae clade includes several species, such as *C. margalefii*, *C. falcatum*, *Cochlodinium* cf. *convolutum*, cf. *Cochlodinium* sp. 1, and a *Gymnodinium*-like species (Reñé et al. 2013a). Other *Cochlodinium* species, however, including *C. polykrikoides*, *C. fulvescens*, and *Cochlodinium* sp. from this study, do not show any phylogenetic relationship with *Cochlodinium* species included within the Ceratoperidiniaceae family. Consequently, they should be separated into different genera (Reñé et al. 2013a).

As pointed out by Flø Jørgensen et al. (2004a), several *Amphidinium* species are not phylogenetically included within the *Amphidinium sensu stricto* clade and they could represent different genera. Some new genera have already been erected, such as *Togula* (Flø Jørgensen et al. 2004b), *Apicoporus* (Sparmann et al. 2008), *Ankistrodinium* (Hoppenrath et al. 2012) or *Nusuttodinium* (Takano et al. 2014). Based on the partial LSU rDNA sequence obtained in this work, the first available for the species, *A. crassum* also clusters independently of other *Amphidinium* species and must be considered as a member of *Amphidinium sensu lato*. Unfortunately, *A. bipes* has yet to be sequenced, and whether it belongs to *Amphidinium s.s.* remains unknown.

The genus *Torodinium* is commonly detected worldwide, but it had never been sequenced. The SSU and LSU sequences of *Torodinium* obtained in this study clustered with those of *Katodinium glaucum*, although with low support, and independently of those of any other unarmoured dinoflagellate. The SSU rDNA sequences of *K. glaucum*

agree with many of the available but unidentified environmental sequences. *Torodinium* species and *K. glaucum* are characterized by the post-median position of the cingulum and a common ancestor seems probable. We observed two different *Torodinium* morphotypes (*T. robustum* and *T. teredo*) and their occurrence was also reflected in the respective LSU rDNA sequences. *Katodinium glaucum* is a widespread cosmopolitan species commonly found in estuarine waters and detected sporadically along the Catalan coast. This species is included within the *Katodinium* genus, an artificial genus previously named *Massartia* Conrad. It comprises marine, brackish, and freshwater species characterized by a maximum hypocone length that is one-third the total length of the cell (Conrad 1926). Based on this criterion, several unrelated species have been assigned to this genus but some were later assigned to other genera. For example, Calado (2011) transferred some of them to *Opisthoaulax* Calado and Murray et al. (2007) transferred *K. dorsalisulcum* to the genus *Gymnodinium*. Nonetheless, *Katodinium* remains an artificial genus (Calado 2011).

3.3 Harmful species:

The harmful effects of many unarmoured dinoflagellate species are well-known. Some species recurrently produce high-biomass blooms that cause ecological and economic problems in affected areas, while others are toxin producers or ichthyotoxic. In this study we detected seven bloom-forming species and eight toxic or fish-killing species. We also detected two harmful species never previously reported in the Mediterranean Sea (*Karenia umbella* and *Cochlodinium fulvescens*) and four harmful species detected for the first time along the Catalan coast (*Karenia mikimotoi, K.* cf. *papilionacea, Cochlodinium polykrikoides,* and *Lepidodinium viride*). Detailed basic studies on the taxonomy and distribution of toxic and noxious unarmoured species in the Mediterranean Sea are needed to enable their earlier detection and control and thereby avoid possible economic losses by the aquaculture industry.

The most commonly detected harmful species are *Gymnodinium*-like species that recurrently produce high-biomass blooms. High abundances ($>10^6$ cells$\cdot L^{-1}$) of *G. litoralis* have been reported recurrently at several beaches on the northern coast of Catalonia between May and September (Reñé et al. 2011). *Gymnodinium impudicum* forms blooms that extend for several kilometres and is often reported in harbours, especially in Tarragona Harbour from June to September (Vila et al. 2001) and near beaches (Delgado et al. 1996). Despite the low abundances detected during this study, *Barrufeta bravensis* is frequently present at beaches at high abundances during the summer months (Sampedro et al. 2011). The potentially bloom-forming species *G. aureolum* and *L. viride* were also detected in the studied area, although only once and at low cell abundances. However, while *G. aureolum* was previously detected in Catalan waters (Margalef 1995), our *L. viride* detection was the first in the sampling area although it has been reported from other locations in the NW Mediterranean (Siano et al. 2009). Finally, we found high abundances of *Akashiwo sanguinea* and *Levanderina fissa* along the study area, mainly during the summer months.

We identified three toxic *Karenia* species during this study. The most frequently detected species was *K. mikimotoi*. Its presence has been reported in several Mediterranean sites, such as the Tyrrhenian Sea (Zingone et al. 2006) and the Aegean Sea (Ignatiades and Gotsis-Skretas 2010), but never along the NW Mediterranean. Some of the other observed specimens were morphologically similar to *K. papilionacea*, but all sequencing attempts failed. This species was previously detected in the NW

Mediterranean (Puigserver et al. 2010; Zingone et al. 2006) but never along the Catalan coast. Finally, we detected *K. umbella*, which to the best of our knowledge had previously only been detected in Australian, Tasmanian, and New Zealand waters (de Salas et al. 2004; Guiry and Guiry 2013). Therefore, despite the low cell abundances, the detection of these species is of great importance as they are responsible for serious health problems in humans and negatively impact the aquaculture industry.

Two fish-killing *Karlodinium* species (*K. armiger* and *K. veneficum*) were detected during this study. Blooms of both species have occurred during the winter months in Alfacs Bay (Garcés et al. 2006), killing off fish and damaging the regional aquaculture industry (Fernández-Tejedor et al. 2003). We detected this species in habitats other than the estuarine waters where they are commonly detected in the Catalan coast.

Other potentially toxic species were those belonging to the genus *Amphidinium*, including *Amphidinium carterae*, which was seldom detected in studied planktonic samples. Most *Amphidinium* species are benthic but those communities were not sampled along the study. Thus, some other toxic or fish-killing *Amphidinium* species may be present in the study area.

The fish-killing species *Cochlodinium polykrikoides* was isolated several times from harbour sampling stations, with maximum abundances of 10^4 cells·L^{-1} (Reñé et al. 2013b). These were the first reports of this species along the Catalan coast and the first rDNA sequences obtained from Mediterranean specimens. Moreover, two different ribotypes were detected, one comprising only specimens from the Catalan coast and referred to as group II, following Reñé et al. (2013b), and the other included within what was formerly called the Philippine ribotype, now renamed as group IV (Reñé et al. 2013b). The fish-killing species *C. fulvescens* was observed once, representing the first detection in the Mediterranean Sea. Consequently, this group of specimens remains poorly studied in the Mediterranean.

4. Conclusions

In this study, we identified 59 different species belonging to the order Gymnodiniales, representing 22% of the species previously detected throughout the Mediterranean Sea and about 10% of the total diversity of Gymnodiniales. This study was conducted by intensively sampling a relatively small area of the Mediterranean Sea and it confirms the importance of detailed regional studies for assessing diversity and distribution of dinoflagellates. The environmental heterogeneity of the studied habitats allowed the detection of a relatively high number of species, including most planktonic unarmoured genera. Even though the Mediterranean Sea is a thoroughly studied area, 13 species were reported for the first time and others presumably are as yet undescribed species. This demonstrates the scarcity of detailed studies on the diversity and distribution of unarmoured dinoflagellates. However, the number of species present along the Catalan coast could be much larger than currently estimated, given that we mainly sampled coastal locations, largely excluding benthic communities and those of brackish and offshore waters.

Using genetic information we were able to provide an accurate and robust picture of species diversity. Clearly, the combination of morphological and molecular techniques can increase the detection and improve the identification of unarmoured dinoflagellates. The rDNA sequences (LSU and/or SSU) reported herein are the first available for 27 of these morphospecies. Our data allowed the first determinations of the phylogenetic

position and relationships of several genera and species, but also confirmed the current lack of molecular information for others and the bias towards cultured species. Nonetheless, using single-cell PCR, we were able to significantly expand upon what is known about unarmoured dinoflagellates. However, it has several limitations. Only those species detected and distinguished by microscopy are selected for sequencing, and most importantly, perhaps, it only allows one attempt to obtain the sequence of the isolated specimen. If this attempt fails, it is impossible to repeat the process. Furthermore, the morphological description and the sequence are obtained from the same cell. Therefore, observations are limited to one specimen and the morphological variability of the species, which is common in unarmoured dinoflagellates, remains unrecognized. Other techniques, as high-throughput sequencing, allow exhaustive studies of the diversity of the target groups. However, interpretation of the results is based on the existing molecular information available. While this method can lead to new insights into the diversity of the target group, it does not provide crucial morphological, functional, and physiological information (Caron 2013). Accordingly, the results obtained in this study provide basic information to supplement current databases, which in turn will facilitate the interpretation of environmental sequencing data.

5. Material and methods

5.1 Observation, isolation, single-cell PCR amplification, and sequencing:

Samples collected in 2011–2013 from different nearshore coastal stations, including beaches and harbours, along the Catalan coast (Fig. 1) were observed either live or following fixation in Lugol's fixative. Occasionally, samples from offshore and coastal sediments were also taken. Sub-surface water samples were collected weekly to monthly or in some cases sporadically, depending on the season and station. Sediment samples were filtered using a 200-µm mesh and cleaned with seawater from the same locality. Further steps were as follows. For fixed samples, 50 ml were settled in a settling chamber for 24 h and then aliquots thereof were examined under an inverted microscope. For live samples, a random volume was concentrated using a 10-µm mesh and observed under a Leica-Leitz DM-Il inverted microscope (Leica Microsystems, Wetzlar, Germany). Organisms were filmed and photographed with a Sony NEX-3 camera (Sony, Tokyo, Japan) and their morphological features were analysed. Each cell was then transferred, using Pasteur pipettes, into filtered seawater drops multiple times and, after these washing steps, into a 200-µl PCR tube. Several fixed cells were also isolated for sequencing using the same method. Single-cell PCR was directly conducted with a PCR mixture containing 5 ml of 10× buffer (Qiagen), 1.25 U of Taq DNA polymerase (Qiagen), 0.2 mM of each dNTP, and 0.8 mM of the primers D1R and D2C (Scholin et al. 1994) for the partial LSU region and the primers EUK A (Medlin et al. 1988) and 1209R (Giovannoni et al. 1988) for the partial SSU region. The LSU PCR conditions were as follows: initial denaturation for 5 min at 95 °C, 40 cycles of 20 s at 95 ºC, 30 s at 55 ºC, and 1 min at 72 ºC, followed by a final extension step for 7 min at 72 ºC. The SSU PCR conditions were: initial denaturation for 5 min at 95 °C, 30 cycles of 45 s at 95 °C, 1 min at 55 °C, and 3 min at 72 °C, followed by a final extension step for 10 min at 72 °C. Ten µl of the PCR products were electrophoresed for 20–30 min at 120 V in a 1.2% agarose gel and then visualized under UV illumination. The remainder of each sample was frozen at -20 °C and later used for sequencing. Purification and sequencing were carried out by an external service (Genoscreen, France). Sequencing was done using both forward and reverse primers and a 3730XL DNA sequencer.

5.2 Phylogenetic analyses

The sequences obtained (Suppl. Table S1) were aligned with those from GenBank using the MAFFT v.6 program (Katoh et al. 2002) under FFT-NS-i. The alignments were manually checked with BioEdit v. 7.0.5 (Hall 1999). A phylogenetic tree was constructed including all LSU rDNA sequences; the highly variable regions of the alignment were removed using Gblocks v.0.91b (Castresana 2000) under the less stringent options, resulting in a final alignment of about 600 positions for the D1–D2 region of the LSU rDNA. Two independent analyses were run for the Gymnodiniales *s.s.* and *Gyrodinium* clades. In these cases the entire alignment of 820 and 710 positions respectively was used. The SSU rDNA alignment contained 1340 positions.

In all cases, phylogenetic relationships were determined using maximum-likelihood (ML) and Bayesian inference methods. For the former, the GTRGAMMA evolution model was used on RAxML (Randomized Axelerated Maximum Likelihood) v. 7.0.4 (Stamatakis 2006). All model parameters were estimated by RAxML. Repeated runs on distinct starting trees were carried out to select the tree with the best topology (the one with the greatest likelihood of 1000 alternative trees). Bootstrap ML analysis was done with 1000 pseudo-replicates and the consensus tree was computed with the RAxML software. The Bayesian inference was performed with MrBayes v.3.2 (Ronquist et al. 2012), run with a GTR model in which the rates were set to gamma. Each analysis was performed using four MCMC chains, with one million cycles for each chain. The consensus tree was created from post-burn-in trees and the Bayesian posterior probabilities (BPP) of each clade were examined. The trees were represented using the web-based tool iTOL (Letunic and Bork 2011).

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Figure 1: Sampling sites from the Catalan coast. Dots and numbers indicate harbours, and triangles and letters beaches. 1) L'Estartit; 2) Palamós; 3) Arenys; 4) Olímpic; 5) Barcelona; 6) Vilanova; 7) Tarragona; 8) Cambrils; 9) L'Ametlla; A) La Muga River mouth; B) L'Estartit; C) La Fosca; D) Blanes Bay; E) Llavaneres; F) offshore Barcelona; G) Castelldefels; H) Fangar Bay; I) Platjola.

Figure 2: Light micrographs. A) *Akashiwo sanguinea*, B) *Amphidinium bipes*, C) *A. carterae*, D) *A. crassum*, E) *A. incoloratum*, F) *A.* cf. *operculatum* (arrows mark the ends of the cingulum), G) *Apicoporus* sp., H) *Asterodinium gracile*, I) *Balechina coerulea*, J) *Barrufeta bravensis*, K) *Brachidinium* sp., L) *Ceratoperidinium falcatum*, M) *C. margalefii*, N) *Cochlodinium* cf. *convolutum*, O) *C. fulvescens*. Nuclei (n) and vacuoles (v) are indicated. Scale bars = 10 µm.

Figure 3: Light micrographs. A) *Cochlodinium polykrikoides*, B) *Cochlodinium* sp., C) *Erythropsidinium* cf. *minor*, D) '*Gymnodinium*' sp., E) *Gymnodinium agaricoides*, F) *G. aureolum*, G) *G. impudicum*, H) *G. litoralis*, I) *Gymnodinium* sp. 1, J) *Gymnodinium* sp. 2, K) *Gyrodinium* cf. *britannicum*, L) *G. corallinum*, M) *G. dominans*, N) *G. heterogrammum*, O) *G.* cf. *ochraceum*. Nuclei (n) are indicated. Scale bars = 10 µm.

Figure 4: Light micrographs. A), B) *Gyrodinium* cf. *spirale*, C) cf. *Gyrodinium undulans*. The arrows show the characteristic outline of the sulcus. D) *G. viridescens*, E) *Gyrodinium* sp. 1, F) *Gyrodinium* sp. 2, G) *Gyrodinium* sp. 3, H) *Gyrodinium* sp. 4, I) *Gyrodinium* sp. 5, J) *Gyrodinium* sp. 6, K) a fixed specimen of *Karenia mikimotoi*, L) a fixed specimen of *K.* cf. *papilionacea*, M) *K. umbella*, N) *Karlodinium armiger*, O) *K. decipiens*. Scale bars 10 = µm.

Figure 5: Light micrographs. A) *Karlodinium veneficum*, B) *Katodinium glaucum*, C) *Lepidodinium viride*, D) *Levanderina fissa*, E) lateral view of *Polykrikos herdmanae*, F) *P. kofoidii*, G) *P. tanit*, H) *Takayama tasmanica*, I) *Torodinium robustum*, J) *T. teredo*, K) lateral view of *Warnowia* sp. 1, L) *Warnowia* sp. 2, M) lateral view of *Warnowia* sp. 3., N) *Warnowia* sp. 4 and O) *Warnowia* sp. 5. Scale bars = 10 µm.

Figure 6: Maximum-likelihood phylogenetic tree of selected species based on the D1–D2 domain of LSU rRNA. Numbers on the nodes are the bootstrap (%) values and the Bayesian posterior probability (BPP). Only bootstrap values >80% and BPP values >0.9 are shown. The *Perkinsus marinus* sequence was used as the outgroup. Organisms sequenced in this study are shown in bold. Nodes with support equal to 100%/1 are indicated with a thick line.

Figure 7: Maximum-likelihood phylogenetic tree of selected species based on the partial SSU rRNA. Numbers on the nodes are the bootstrap (%) and the Bayesian posterior probability (BPP) values. Only bootstrap values >80% and BPP values >0.9 are shown. The *Polarella glacialis* sequence was used as the outgroup. Organisms sequenced in this study are shown in bold. Nodes with support equal to 100%/1 are indicated with a thick line.

Figure 8: Maximum-likelihood phylogenetic tree of the Gymnodiniales *sensu stricto* clade based on the D1-D2 domain of LSU rRNA. Numbers on the nodes are the bootstrap (%) values and the Bayesian posterior probability (BPP). Only bootstrap

values >80% and BPP values >0.9 are shown. *Polarella glacialis*, *Akashiwo sanguinea*, *Karenia brevis*, and *Gyrodinium spirale* sequences were used as the outgroup. Organisms sequenced in this study are shown in bold. Nodes with support equal to 100%/1 are indicated with a thick line.

Figure 9: Maximum-likelihood phylogenetic tree of *Gyrodinium* clade based on the D1-D2 domain of LSU rRNA. Numbers on the nodes are the bootstrap (%) values and the Bayesian posterior probability (BPP). Only bootstrap values >80% and BPP values >0.9 are shown. *Gymnodinium fuscum*, *G. impudicum*, *G. litoralis*, and *G. catenatum* sequences were used as the outgroup. Organisms sequenced in this study are shown in bold. Nodes with support equal to 100%/1 are indicated with a thick line.

Supplementary Material

Figure S1: Light micrographs. A) *Akashiwo sanguinea*, B) *Amphidinium crassum*, C) – G) *Apicoporus* sp., H) and I) *Ceratoperidinium falcatum*, J) *Gymnodinium* sp. 1, K) – M) *Gyrodinium heterogrammum*, N) and O) *Gyrodinium* cf. *ochraceum*, P) – X) *Gyrodinium* cf. *spirale*. Scale bars = 10 µm.

Figure S2: Light micrographs. A) and B) *Gyrodinium viridescens*, C) *Gyrodinium* sp. 4, D) *Levanderina fissa*, E) – G) *Polykrikos herdmanae*, H) and I) *Polykrikos kofoidii*, J) – M) *Torodinium robustum*, N) *Warnowia* sp. 1, O) and P) Fixed specimens of *Warnowia* sp. 2, Q) – S) *Warnowia* sp. 4, T) *Warnowia* sp. 5. Scale bars = 10 µm.

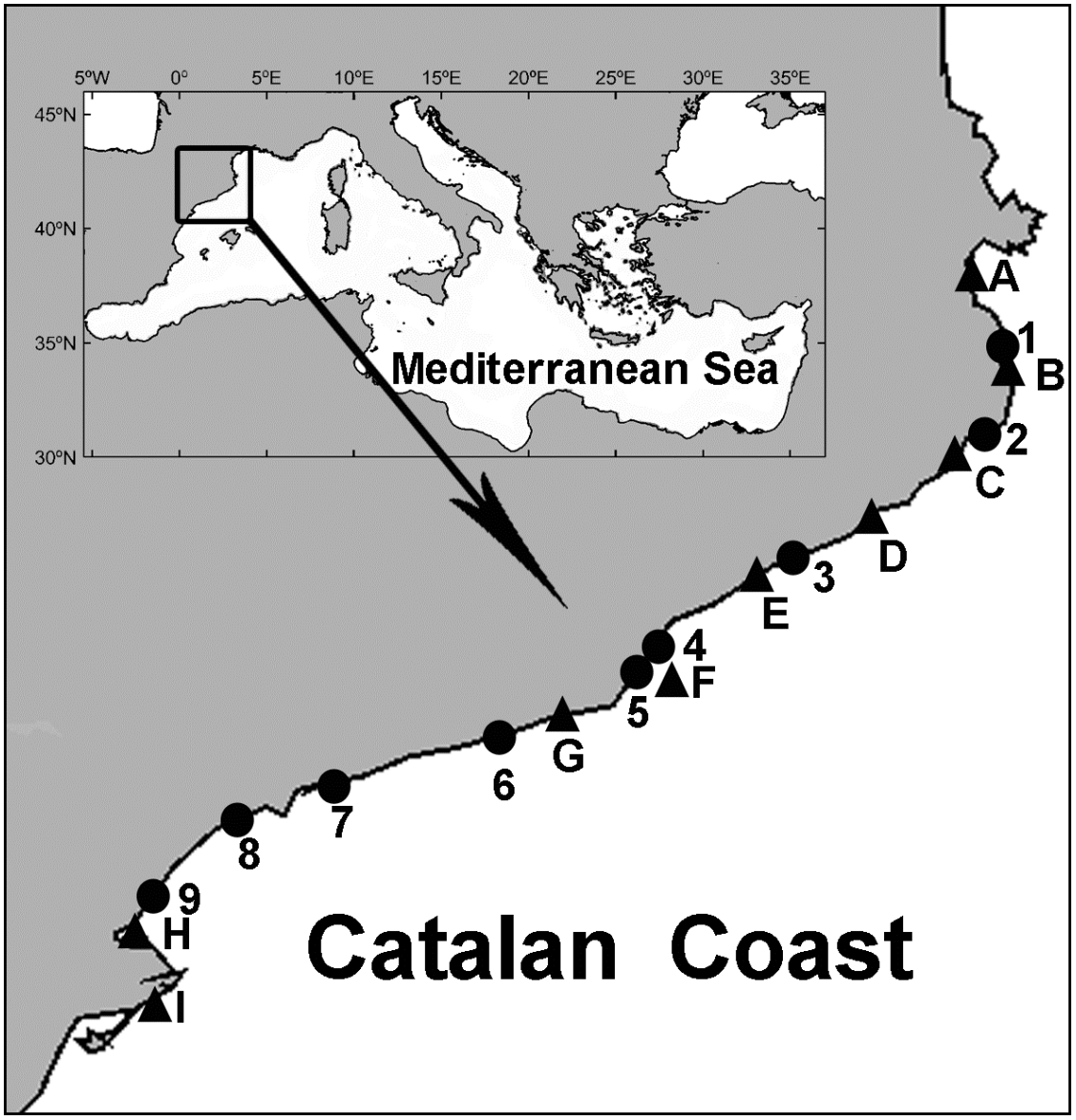
Table 1: Morphospecies detected during this study along the Catalan coast. Qualitative assessments and range, from very rare (*) to very common (****), are indicated under the Occurrence column. Detection periods, abundances, distribution, water temperature (Temp), and salinity are reported when available. First detections in the Mediterranean Sea (Med) or along the Catalan coast (CC) are indicated. Asterisks indicate that the type location of the species is in the Mediterranean Sea. Previous detections of organisms not identified at the species level are unknown and are represented by grey boxes. The correspondence with the images provided in Figs. 2-5 is shown in the last column. [a] Information previously reported in Reñé et al. (2013a); [b] Reñé et al. (2013b); [c] Reñé et al. (2014).

Species	Occurrence	Detection period	Abundances	Distribution	Temp	Salinity	First detection	Figure
Akashiwo sanguinea (Hirasaka) Hansen & Moestrup	****	Throughout the year	10^3–10^4 cells·L^{-1}	Catalan coast			-	2A
Amphidinium bipes Herdman	*	July 2012	Single specimen	Sediment from beaches	19.7	37.5	Med	2B
Amphidinium carterae Hulburt	***	Summer months	< 10^2 cells·L^{-1}	Sporadically at beaches			-	2C
Amphidinium crassum Lohmann	***	May–October	< 10^2 cells·L^{-1}	Several harbours	21.1-25	36.6-37.8	-	2D
Amphidinium incoloratum	*	May 2012	Single specimen	Palamós Harbour	17.9	34.8	Med	2E
Amphidinium cf. *operculatum* Claparède & Lachmann	*	July 2011	Single specimen	L'Alguer Beach	22.5	38.2	-	2F
Apicoporus sp.	**	June 2013	Several specimens	Sediments from Castelldefels Beach	19.7	37.5	Med	2G
Asterodinium gracile Sournia	*	January 2009	Three fixed specimens	Offshore Barcelona			-	2H
Balechina coerulea (Dogiel) Taylor	*	August 2011	One fixed specimen	Montjoi Beach	23.3	37.9	CC	2I
Barrufeta bravensis Sampedro & Fraga	**	June 2012	10^5-10^6 cells·L^{-1}	La Fosca Beach	22.4	38.3	*	2J
Brachidinium sp.	*	November 2010	One fixed specimen	Offshore Barcelona				2K
Ceratoperidinium falcatum (Kofoid & Swezy) Reñé & de Salas [a]	**	October 2012	< 10^3 cells·L^{-1}	Fangar Bay			-	2L
Ceratoperidinium margalefii Margalef ex Loeblich III [a]	*	July 2011	Single specimen	Mouth of La Muga River	21.2	30.9	-	2M
Cochlodinium cf. *convolutum* Kofoid & Swezy [a]	**	October 2012 November 2012	Single specimens	Barcelona Harbour Palamós Harbour	21.6 16.4	38.4 38.1	-	2N

Species	Occurrence	Detection period	Abundances	Distribution	Temp	Salinity	First detection	Figure
Cochlodinium fulvescens Iwataki, Kawami et Matsuoka	*	October 2013	Single specimen	Blanes Bay			Med	2O
Cochlodinium polykrikoides Margalef [b]	**	June–September	Up to 10^4 cells·L^{-1}	Several harbours	23.4-24.8	31.4-38.2	CC	3A
Cochlodinium sp.	*	November 2012	Several specimens	Palamós Harbour	16.4	38.1		3B
Erythropsidinium cf. m*inor* (Kofoid & Swezy) Silva	*	October 2012	Single specimen	Fangar Bay			-	3C
'*Gymnodinium*' sp. [a]	*	November 2012	Single specimen	Palamós Harbour	16.4	38.1		3D
Gymnodinium agaricoides Campbell	*	November 2011	Single specimen	Tarragona Harbour	20.5	35.9	CC	3E
Gymnodinium aureolum (Hulburt) Hansen	*	June 2012	Single specimen	Offshore Barcelona			-	3F
Gymnodinium impudicum (Fraga & Bravo) Hansen & Moestrup	***	June–September	Up to 10^6 cells·L^{-1}	Tarragona Harbour			*	3G
Gymnodinium litoralis Reñé	****	May–September	Up to 10^6 cells·L^{-1}	Northern beaches			*	3H
Gymnodinium sp. 1	**	December 2011 June 2012	< 10^2 cells·L^{-1}	Olímpic Harbour Arenys Harbour	16 20.1	37.5 37.3		3I
Gymnodinium sp. 2	*	April 2013	< 10^3 cells·L^{-1}	Arenys Harbour	17.8	36.5		3J
Gyrodinium cf. *britannicum* Kofoid & Swezy	*	March 2012 June 2012	Some specimens	Barcelona Harbour Tarragona Harbour	14.3 23.8	38.4 37.6	Med	3K
Gyrodinium corallinum Kofoid & Swezy	*	May 2012	Three specimens	Barcelona Harbour	18.6	38.4	Med	3L
Gyrodinium dominans Hulburt	***	Recurrently throughout the year	< 10^2 cells·L^{-1}	Some harbours and offshore Barcelona			-	3M
Gyrodinium heterogrammum Larsen	***	Summer to autumn	< 10^2 cells·L^{-1}	Some harbours			Med	3N
Gyrodinium cf. o*chraceum* Kofoid & Swezy [c]	***	Recurrently throughout the year	< 10^2 cells·L^{-1}	most harbours and beaches			CC	3O
Gyrodinium cf. s*pirale* (Bergh) Kofoid & Swezy	****	Commonly throughout the year	< 10^3 cells·L^{-1}	most harbours and beaches			-	4A, B
cf. *Gyrodinium undulans* Hulburt	**	February 2012 June 2012 June 2012	Single specimens	Palamós Harbour Vilanova Harbour Offshore Barcelona	11.1 25.0	38.6 37.8	Med	4C

Species	Occurrence	Detection period	Abundances	Distribution	Temp	Salinity	First detection	Figure
Gyrodinium viridescens Kofoid & Swezy	**	May 2012 July 2012 June 2013	Several specimens	Castelldefels Beach L'Estartit Beach L'Estartit Beach	25.4 22.2 17.1	38.4 38.3 37.1	Med	4D
Gyrodinium sp. 1	*	October 2011	Several specimens	Tarragona Harbour	21.1	36.6		4E
Gyrodinium sp. 2	*	December 2011	Single specimen	Barcelona Harbour	16.5	37.6		4F
Gyrodinium sp. 3	*	December 2011	Single specimen	Tarragona Harbour	15.3	37.2		4G
Gyrodinium sp. 4	*	December 2011 March 2012	Single specimen	Tarragona Harbour Arenys Harbour	15.3 14.5	37.2 36.8		4H
Gyrodinium sp.5	***	Commonly throughout the year	Several specimens	Several harbours and beaches				4I
Gyrodinium sp.6	**	May 2011 December 2012	Several specimens	La Muga river mouth Tarragona Harbour	20.2 14.8	34.7 38.0		4J
Karenia mikimotoi (Miyake & Kominami ex Oda) Hansen & Moestrup	**	Sporadically throughout the year	$< 10^2$ cells·L^{-1}	Several harbours and beaches			CC	4K
Karenia cf. *papilionacea* Haywood & Steidinger	*	June 2010 August 2010 January 2012 June 2012	Single specimens	La Fosca Beach La Fosca Beach Cambrils Harbour Offshore Barcelona	21.0 24.0 11.9	37.8 38.3 37.1	CC	4L
Karenia umbella de Salas, Bolch & Hallegraeff	*	October 2012	Single specimen	Olímpic Harbour	21	38.4	Med	4M
Karlodinium armiger Bergholtz, Daugbjerg & Moestrup	***	February 2011	$< 10^2$ cells·L^{-1}	Offshore Barcelona			-	4N
Karlodinium decipiens de Salas & Laza-Martínez	**	May 2011	Several specimens	L'Estartit Beach	20.2	37.3	Med	4O
Karlodinium veneficum (Ballantine) Larsen	***	Throughout the year	$< 10^2$ cells·L^{-1}	Several northern beaches			-	5A
Katodinium glaucum (Lebour) Loeblich III	**	Throughout the year	$< 10^2$ cells·L^{-1}	Several harbours and beaches			CC	5B
Lepidodinium viride Watanabe, Suda, Inouye, Sawaguchi & Chihara	*	October 2012	Single specimen	Fangar Bay			CC	5C

Species	Occurrence	Detection period	Abundances	Distribution	Temp	Salinity	First detection	Figure
Levanderina fissa (Levander) Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard	****	Throughout the year (blooms in summer)	Up to 10^6 cells·L^{-1}	Most harbours and beaches			-	5D
Polykrikos herdmanae Hoppenrath & Leander	*	June 2013	< 10^2 cells·L^{-1}	Sediments from L'Estartit Beach	17.1	37.1	Med	5E
Polykrikos kofoidii Chatton	****	Spring to autumn	< 10^3 cells·L^{-1}	Several harbours and beaches			-	5F
Polykrikos tanit Reñé [c]	**	April–June	< 10^3 cells·L^{-1}	Several harbours and beaches	14-22	31.2-37.8	*	5G
Takayama tasmanica de Salas, Bolch & Hallegraeff	**	July 2012 October 2012	Single specimen 10^3 cells·L^{-1}	Llavaneres Beach Fangar Bay	23.4	38.1	Med	5H
Torodinium robustum Kofoid & Swezy	**	Spring to autumn	< 10^2 cells·L^{-1}	Several beaches			-	5I
Torodinium teredo (Pouchet) Kofoid & Swezy	**	Spring to autumn	< 10^2 cells·L^{-1}	Several beaches			-	5J
Warnowia sp. 1	*	December 2011 October 2012	Three specimens	Barcelona Harbour	16.5 21.6	37.6 38.4		5K
Warnowia sp. 2	**	December 2011 June 2012	< 10^2 cells·L^{-1}	Tarragona Harbour	15.7 23.8	36.7 37.6		5L
Warnowia sp. 3	*	June 2012	Single specimen	Vilanova Harbour	25	37.8		5M
Warnowia sp. 4	*	August 2012	< 10^2 cells·L^{-1}	Vilanova Harbour	27	37.8		5N
Warnowia sp. 5	*	November 2012 April 2013	< 10^2 cells·L^{-1}	Arenys Harbour	17.1 17.8	37.8 36.5		5O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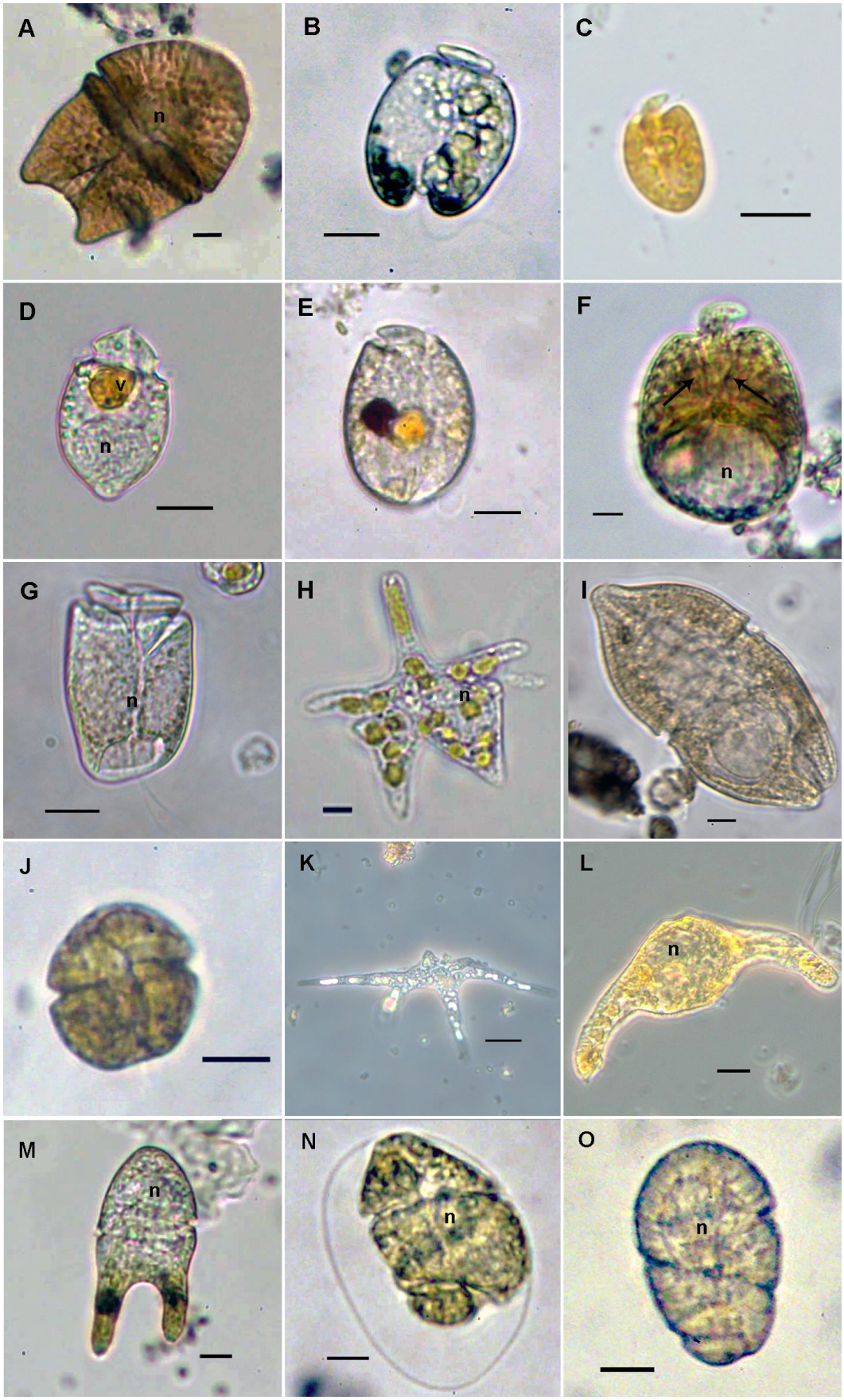
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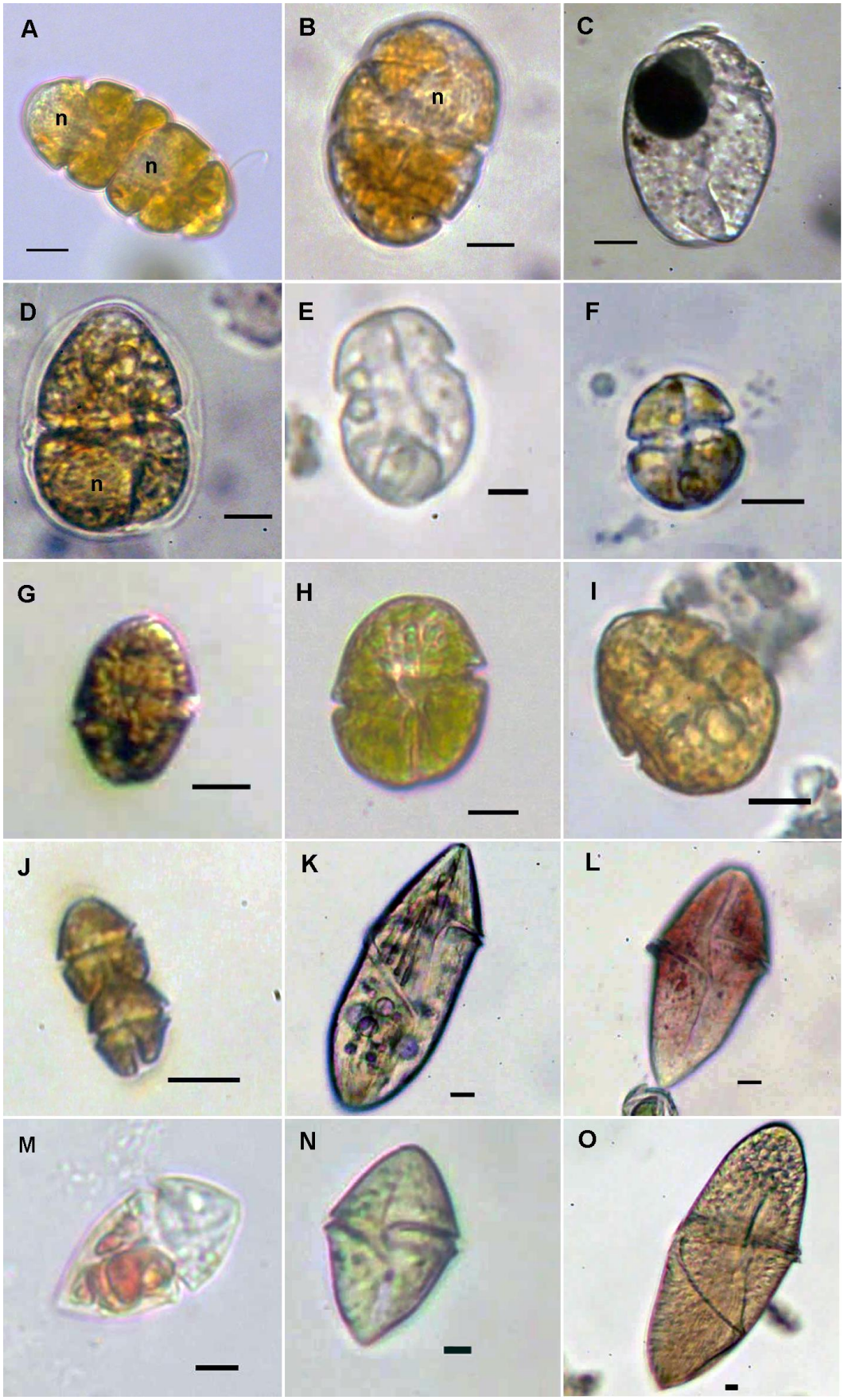
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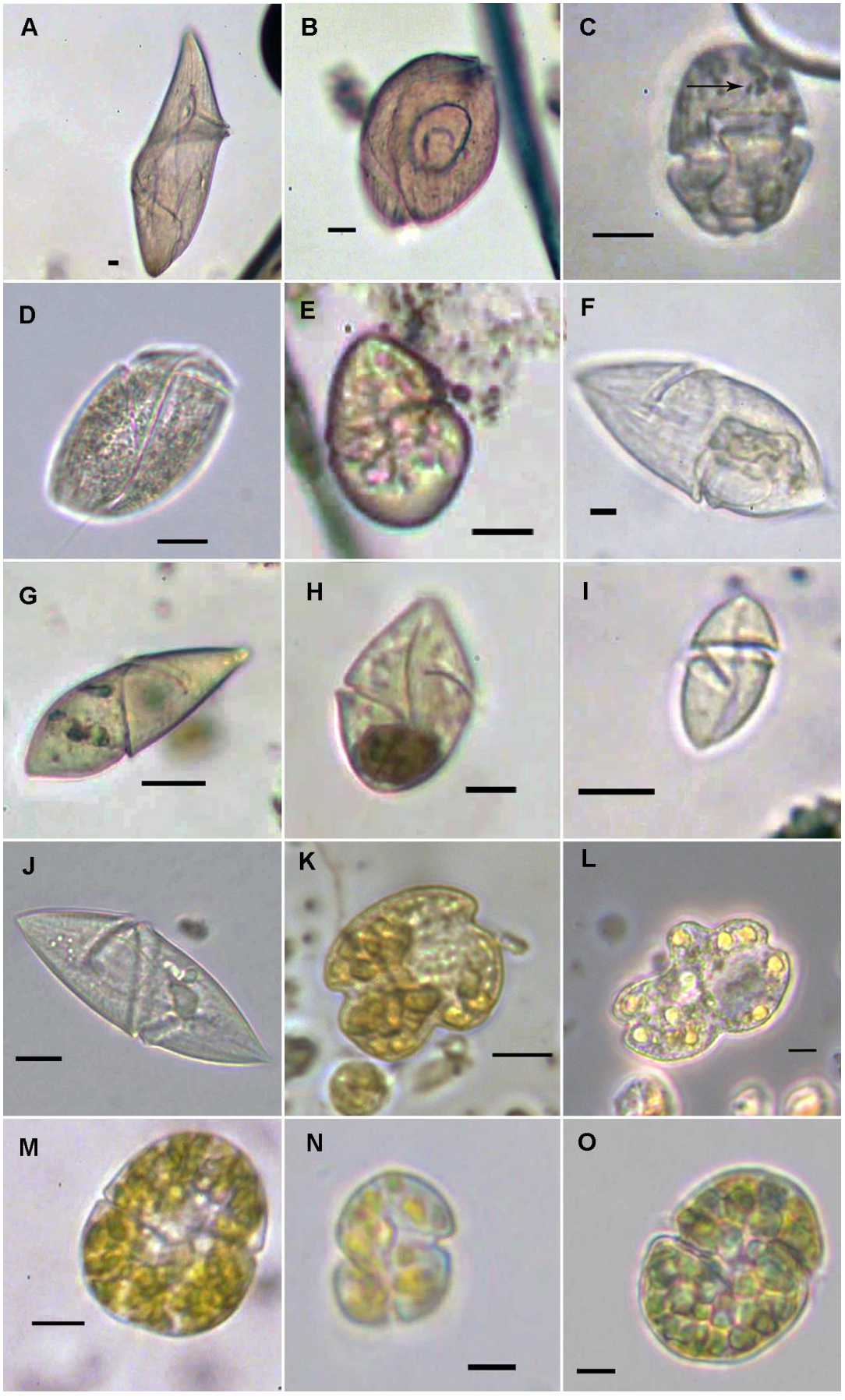
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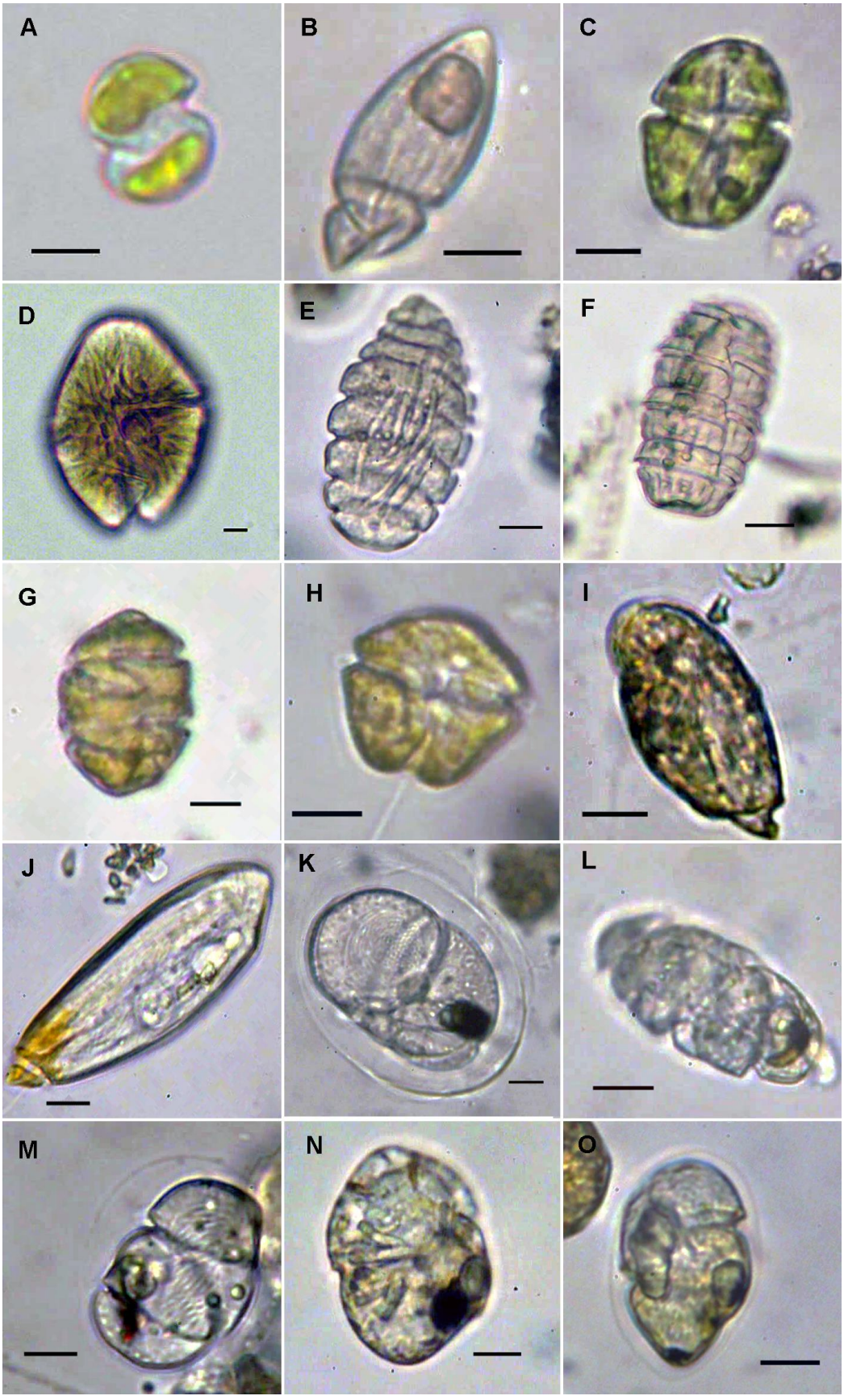
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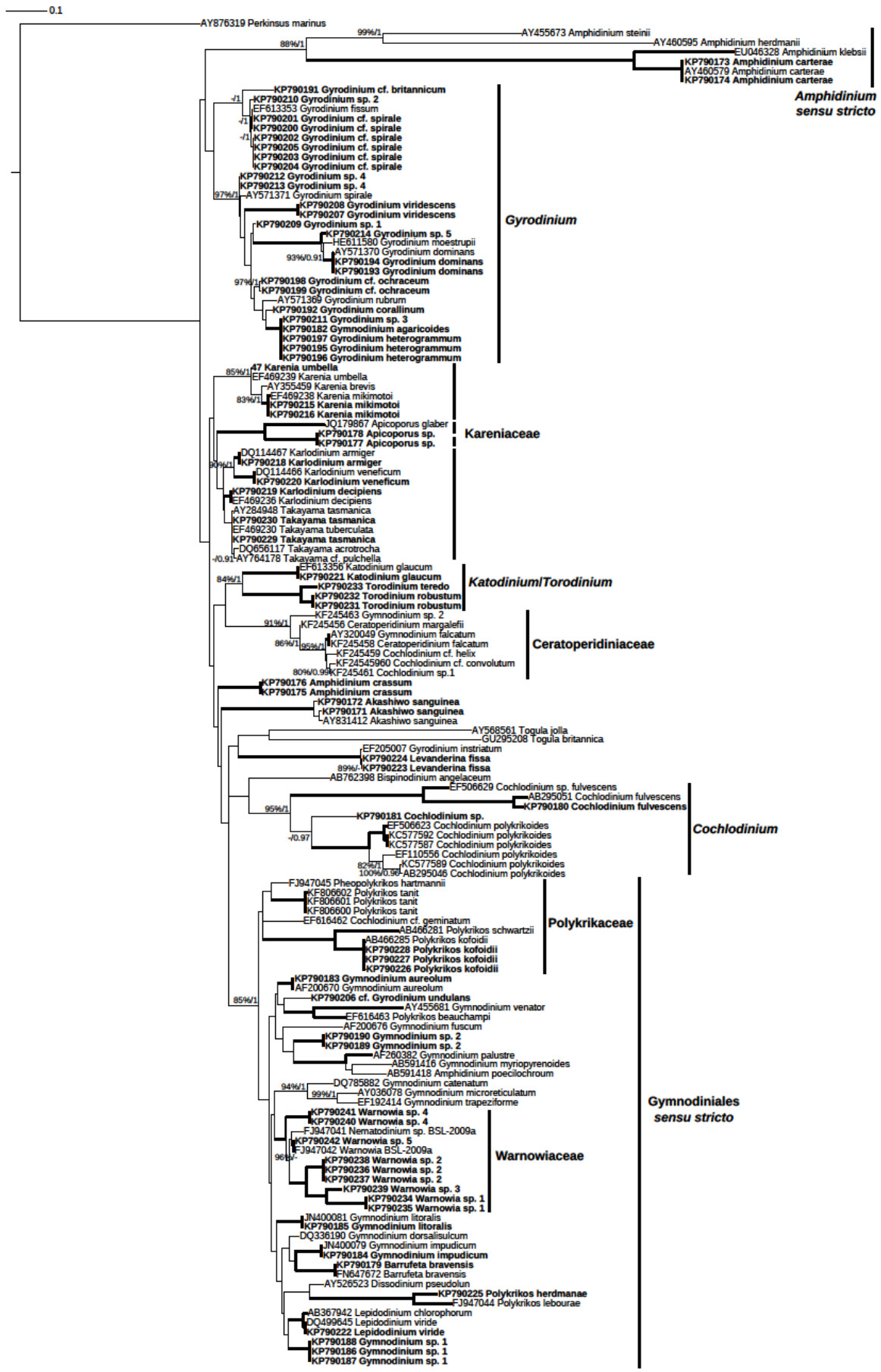
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KP790173 Amphidinium carterae
AY460579 Amphidinium carterae
KP790174 Amphidinium carterae
Amphidinium sensu stricto
KP790191 Gyrodinium cf. britannicum
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KP790210 Gyrodinium sp. 2
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-/1
KP790201 Gyrodinium cf. spirale
KP790200 Gyrodinium cf. spirale
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97%/1
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KP790208 Gyrodinium viridescens
KP790207 Gyrodinium viridescens
KP790209 Gyrodinium sp. 1
KP790214 Gyrodinium sp. 5
HE611580 Gyrodinium moestrupii
93%/0.91
AY571370 Gyrodinium dominans
KP790194 Gyrodinium dominans
KP790193 Gyrodinium dominans
97%/1
KP790198 Gyrodinium cf. ochraceum
KP790199 Gyrodinium cf. ochraceum
AY571369 Gyrodinium rubrum
KP790192 Gyrodinium corallinum
KP790211 Gyrodinium sp. 3
KP790182 Gymnodinium agaricoides
KP790197 Gyrodinium heterogrammum
KP790195 Gyrodinium heterogrammum
KP790196 Gyrodinium heterogrammum
Gyrodinium
85%/1
47 Karenia umbella
EF469239 Karenia umbella
AY355459 Karenia brevis
83%/1
EF469238 Karenia mikimotoi
KP790215 Karenia mikimotoi
KP790216 Karenia mikimotoi
JQ179867 Apicoporus glaber
KP790178 Apicoporus sp.
KP790177 Apicoporus sp.
Kareniaceae
DQ114467 Karlodinium armiger
90%/1
KP790218 Karlodinium armiger
DQ114466 Karlodinium veneficum
KP790220 Karlodinium veneficum
KP790219 Karlodinium decipiens
EF469236 Karlodinium decipiens
AY284948 Takayama tasmanica
KP790230 Takayama tasmanica
EF469230 Takayama tuberculata
KP790229 Takayama tasmanica
DQ656117 Takayama acrotrocha
-/0.91
AY764178 Takayama cf. pulchella
EF613356 Katodinium glaucum
84%/1
KP790221 Katodinium glaucum
KP790233 Torodinium teredo
KP790232 Torodinium robustum
KP790231 Torodinium robustum
Katodinium/Torodinium
KF245463 Gymnodinium sp. 2
91%/1
KF245456 Ceratoperidinium margalefii
AY320049 Gymnodinium falcatum
86%/1
95%/1
KF245458 Ceratoperidinium falcatum
KF245459 Cochlodinium cf. helix
KF24545960 Cochlodinium cf. convolutum
80%/0.99
KF245461 Cochlodinium sp.1
Ceratoperidiniaceae
KP790176 Amphidinium crassum
KP790175 Amphidinium crassum
KP790172 Akashiwo sanguinea
KP790171 Akashiwo sanguinea
AY831412 Akashiwo sanguinea
AY568561 Togula jolla
GU295208 Togula britannica
EF205007 Gyrodinium instriatum
KP790224 Levanderina fissa
89%/-
KP790223 Levanderina fissa
AB762398 Bispinodinium angelaceum
EF506629 Cochlodinium sp. fulvescens
AB295051 Cochlodinium fulvescens
95%/1
KP790180 Cochlodinium fulvescens
KP790181 Cochlodinium sp.
EF506623 Cochlodinium polykrikoides
-/0.97
KC577592 Cochlodinium polykrikoides
KC577587 Cochlodinium polykrikoides
EF110556 Cochlodinium polykrikoides
82%/1
KC577589 Cochlodinium polykrikoides
100%/0.96
AB295046 Cochlodinium polykrikoides
Cochlodinium
FJ947045 Pheopolykrikos hartmannii
KF806602 Polykrikos tanit
KF806601 Polykrikos tanit
KF806600 Polykrikos tanit
EF616462 Cochlodinium cf. geminatum
AB466281 Polykrikos schwartzii
AB466285 Polykrikos kofoidii
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KP790227 Polykrikos kofoidii
KP790226 Polykrikos kofoidii
Polykrikaceae
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AF200670 Gymnodinium aureolum
85%/1
KP790206 cf. Gyrodinium undulans
AY455681 Gymnodinium venator
EF616463 Polykrikos beauchampi
AF200676 Gymnodinium fuscum
KP790190 Gymnodinium sp. 2
KP790189 Gymnodinium sp. 2
AF260382 Gymnodinium palustre
AB591416 Gymnodinium myriopyrenoides
AB591418 Amphidinium poecilochroum
94%/1
DQ785882 Gymnodinium catenatum
99%/1
AY036078 Gymnodinium microreticulatum
EF192414 Gymnodinium trapeziforme
KP790241 Warnowia sp. 4
KP790240 Warnowia sp. 4
FJ947041 Nematodinium sp. BSL-2009a
KP790242 Warnowia sp. 5
FJ947042 Warnowia BSL-2009a
96%/-
KP790238 Warnowia sp. 2
KP790236 Warnowia sp. 2
KP790237 Warnowia sp. 2
KP790239 Warnowia sp. 3
KP790234 Warnowia sp. 1
KP790235 Warnowia sp. 1
Warnowiaceae
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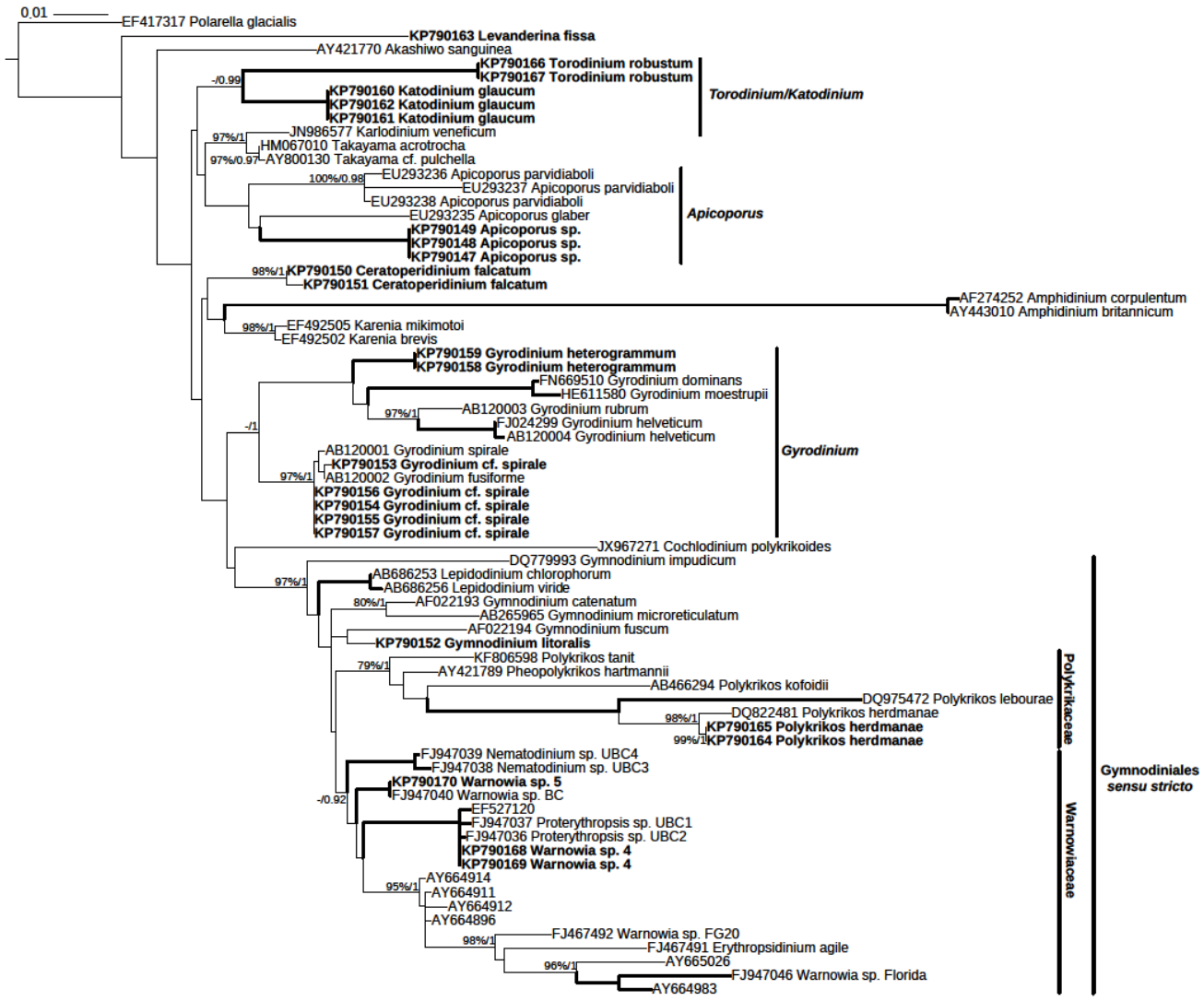
KP790185 Gymnodinium litoralis
DQ336190 Gymnodinium dorsalisulcum
JN400079 Gymnodinium impudicum
KP790184 Gymnodinium impudicum
KP790179 Barrufeta bravensis
FN647672 Barrufeta bravensis
AY526523 Dissodinium pseudolun
KP790225 Polykrikos herdmanae
FJ947044 Polykrikos lebourae
AB367942 Lepidodinium chlorophorum
DQ499645 Lepidodinium viride
KP790222 Lepidodinium viride
KP790188 Gymnodinium sp. 1
KP790186 Gymnodinium sp. 1
KP790187 Gymnodinium sp. 1
Gymnodiniales sensu stricto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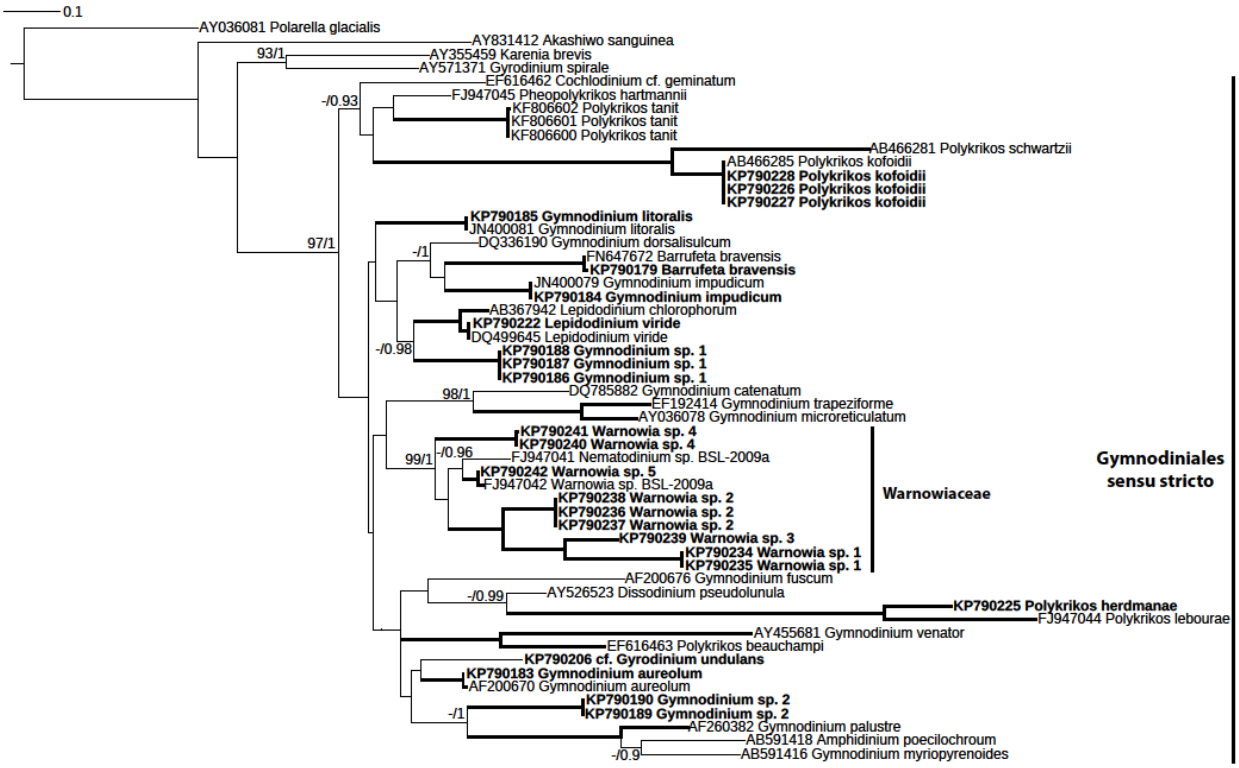
EF417317 Polarella glacialis
KP790163 Levanderina fissa
AY421770 Akashiwo sanguinea
KP790166 Torodinium robustum
KP790167 Torodinium robustum
-/0.99
KP790160 Katodinium glaucum
KP790162 Katodinium glaucum
KP790161 Katodinium glaucum
Torodinium/Katodinium
97%/1
JN986577 Karlodinium veneficum
HM067010 Takayama acrotrocha
97%/0.97
AY800130 Takayama cf. pulchella
100%/0.98
EU293236 Apicoporus parvidiaboli
EU293237 Apicoporus parvidiaboli
EU293238 Apicoporus parvidiaboli
EU293235 Apicoporus glaber
KP790149 Apicoporus sp.
KP790148 Apicoporus sp.
KP790147 Apicoporus sp.
Apicoporus
98%/1
KP790150 Ceratoperidinium falcatum
KP790151 Ceratoperidinium falcatum
AF274252 Amphidinium corpulentum
AY443010 Amphidinium britannicum
98%/1
EF492505 Karenia mikimotoi
EF492502 Karenia brevis
KP790159 Gyrodinium heterogrammum
KP790158 Gyrodinium heterogrammum
FN669510 Gyrodinium dominans
HE611580 Gyrodinium moestrupii
97%/1
AB120003 Gyrodinium rubrum
-/1
FJ024299 Gyrodinium helveticum
AB120004 Gyrodinium helveticum
AB120001 Gyrodinium spirale
KP790153 Gyrodinium cf. spirale
97%/1
AB120002 Gyrodinium fusiforme
KP790156 Gyrodinium cf. spirale
KP790154 Gyrodinium cf. spirale
KP790155 Gyrodinium cf. spirale
KP790157 Gyrodinium cf. spirale
Gyrodinium
JX967271 Cochlodinium polykrikoides
DQ779993 Gymnodinium impudicum
97%/1
AB686253 Lepidodinium chlorophorum
AB686256 Lepidodinium viride
80%/1
AF022193 Gymnodinium catenatum
AB265965 Gymnodinium microreticulatum
AF022194 Gymnodinium fuscum
KP790152 Gymnodinium litoralis
KF806598 Polykrikos tanit
79%/1
AY421789 Pheopolykrikos hartmannii
AB466294 Polykrikos kofoidii
DQ975472 Polykrikos lebourae
98%/1
DQ822481 Polykrikos herdmanae
99%/1
KP790165 Polykrikos herdmanae
KP790164 Polykrikos herdmanae
Polykrikaceae
FJ947039 Nematodinium sp. UBC4
FJ947038 Nematodinium sp. UBC3
KP790170 Warnowia sp. 5
-/0.92
FJ947040 Warnowia sp. BC
EF527120
FJ947037 Proterythropsis sp. UBC1
FJ947036 Proterythropsis sp. UBC2
KP790168 Warnowia sp. 4
KP790169 Warnowia sp. 4
95%/1
AY664914
AY664911
AY664912
AY664896
98%/1
FJ467492 Warnowia sp. FG20
FJ467491 Erythropsidinium agile
AY665026
96%/1
FJ947046 Warnowia sp. Florida
AY664983
Warnowiaceae
Gymnodiniales
sensu stricto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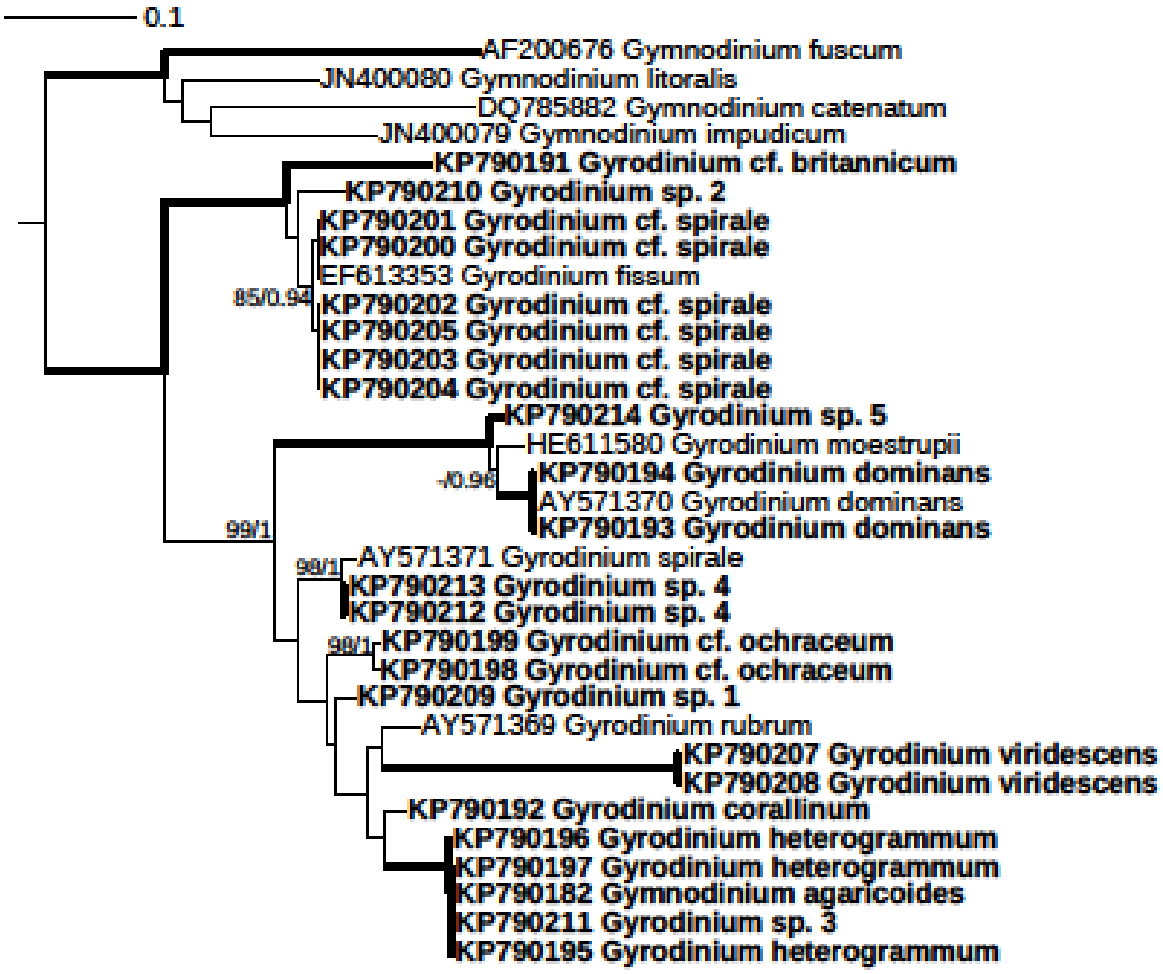
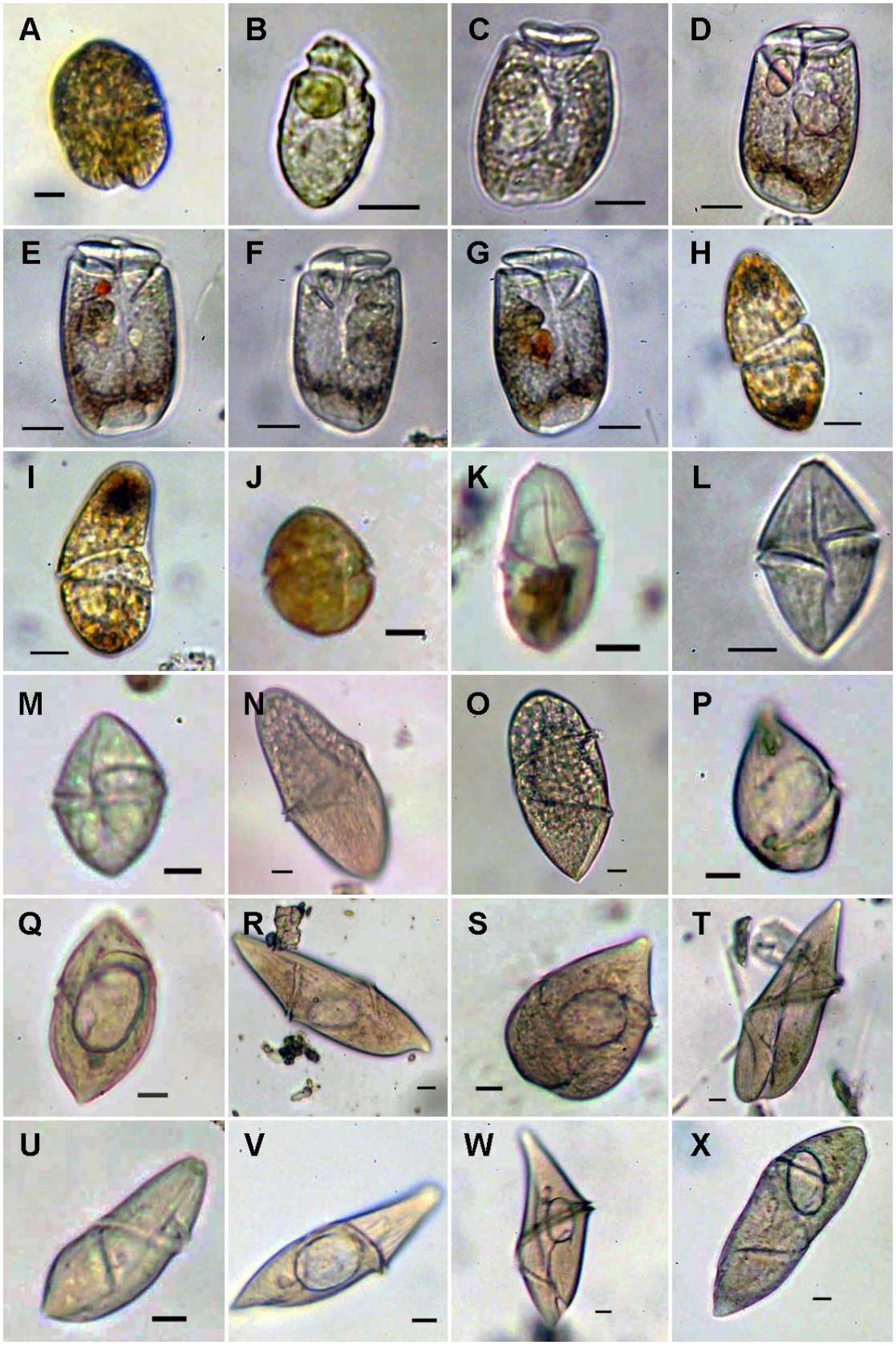
AY036081 Polarella glacialis
AY831412 Akashiwo sanguinea
93/1
AY355459 Karenia brevis
AY571371 Gyrodinium spirale
EF616462 Cochlodinium cf. geminatum
-/0.93
FJ947045 Pheopolykrikos hartmannii
KF806602 Polykrikos tanit
KF806601 Polykrikos tanit
KF806600 Polykrikos tanit
AB466281 Polykrikos schwartzii
AB466285 Polykrikos kofoidii
KP790228 Polykrikos kofoidii
KP790226 Polykrikos kofoidii
KP790227 Polykrikos kofoidii
KP790185 Gymnodinium litoralis
JN400081 Gymnodinium litoralis
97/1
DQ336190 Gymnodinium dorsalisulcum
-/1
FN647672 Barrufeta bravensis
KP790179 Barrufeta bravensis
JN400079 Gymnodinium impudicum
KP790184 Gymnodinium impudicum
AB367942 Lepidodinium chlorophorum
KP790222 Lepidodinium viride
DQ499645 Lepidodinium viride
-/0.98
KP790188 Gymnodinium sp. 1
KP790187 Gymnodinium sp. 1
KP790186 Gymnodinium sp. 1
98/1
DQ785882 Gymnodinium catenatum
EF192414 Gymnodinium trapeziforme
AY036078 Gymnodinium microreticulatum
KP790241 Warnowia sp. 4
KP790240 Warnowia sp. 4
99/1
-/0.96
FJ947041 Nematodinium sp. BSL-2009a
KP790242 Warnowia sp. 5
FJ947042 Warnowia sp. BSL-2009a
KP790238 Warnowia sp. 2
KP790236 Warnowia sp. 2
KP790237 Warnowia sp. 2
KP790239 Warnowia sp. 3
KP790234 Warnowia sp. 1
KP790235 Warnowia sp. 1
Warnowiaceae
AF200676 Gymnodinium fuscum
-/0.99
AY526523 Dissodinium pseudolunula
KP790225 Polykrikos herdmanae
FJ947044 Polykrikos lebourae
AY455681 Gymnodinium venator
EF616463 Polykrikos beauchampi
KP790206 cf. Gyrodinium undulans
KP790183 Gymnodinium aureolum
AF200670 Gymnodinium aureolum
KP790190 Gymnodinium sp. 2
KP790189 Gymnodinium sp. 2
-/1
AF260382 Gymnodinium palustre
AB591418 Amphidinium poecilochroum
-/0.9
AB591416 Gymnodinium myriopyrenoides
Gymnodiniales
sensu stricto


0.1
AF200676 Gymnodinium fuscum
JN400080 Gymnodinium litoralis
DQ785882 Gymnodinium catenatum
JN400079 Gymnodinium impudicum
KP790191 Gyrodinium cf. britannicum
KP790210 Gyrodinium sp. 2
KP790201 Gyrodinium cf. spirale
KP790200 Gyrodinium cf. spirale
EF613353 Gyrodinium fissum
85/0.94
KP790202 Gyrodinium cf. spirale
KP790205 Gyrodinium cf. spirale
KP790203 Gyrodinium cf. spirale
KP790204 Gyrodinium cf. spirale
KP790214 Gyrodinium sp. 5
HE611580 Gyrodinium moestrupii
-/0.96
KP790194 Gyrodinium dominans
AY571370 Gyrodinium dominans
KP790193 Gyrodinium dominans
99/1
98/1
AY571371 Gyrodinium spirale
KP790213 Gyrodinium sp. 4
KP790212 Gyrodinium sp. 4
98/1
KP790199 Gyrodinium cf. ochraceum
KP790198 Gyrodinium cf. ochraceum
KP790209 Gyrodinium sp. 1
AY571369 Gyrodinium rubrum
KP790207 Gyrodinium viridescens
KP790208 Gyrodinium viridescens
KP790192 Gyrodinium corallinum
KP790196 Gyrodinium heterogrammum
KP790197 Gyrodinium heterogrammum
KP790182 Gymnodinium agaricoides
KP790211 Gyrodinium sp. 3
KP790195 Gyrodinium heterogrammum

Supplementary Table S1: rDNA sequences obtained in this study. GenBank accession number, species name (* sequences obtained from cultured organisms, + sequences obtained from fixed organisms), rDNA region sequenced, locality and date of the isolation. The correspondence of the images provided in Figs. 2, 3, 4, 5 and Supplementary Material Figs. S1, S2 with the sequenced specimens is shown in the last column.

GenBank Accession Number	Species	rDNA	Locality	Date	Fig.
KP790171	*Akashiwo sanguinea* *	LSU	Vilanova Harbour	Aug-10	
KP790172	*Akashiwo sanguinea*	LSU	Ametlla Harbour	Jun-12	S1A
KP790173	*Amphidinium carterae* *	LSU	Llavaneres Beach	Dec-11	
KP790174	*Amphidinium carterae* *	LSU	Llavaneres Beach	Dec-11	
KP790175	*Amphidinium crassum*	LSU	Tarragona Harbour	Jul-11	S1B
KP790176	*Amphidinium crassum*	LSU	Tarragona Harbour	Oct-11	
KP790177	*Apicoporus* sp.	LSU	Castelldefels Beach	Jun-13	S1C
KP790178	*Apicoporus* sp.	LSU	Castelldefels Beach	Jun-13	S1D
KP790179	*Barrufeta bravensis*	LSU	La Fosca Beach	Jul-12	2J
KP790180	*Cochlodinium fulvescens*	LSU	Blanes Bay	Oct-13	2O
KP790181	*Cochlodinium* sp.	LSU	Palamós Harbour	Nov-12	3B
KP790182	*Gymnodinium agaricoides*	LSU	Tarragona Harbour	Nov-11	3E
KP790183	*Gymnodinium aureolum*	LSU	Offshore Barcelona	Jun-12	3F
KP790184	*Gymnodinium impudicum* *	LSU	Tarragona Harbour	-	
KP790185	*Gymnodinium litoralis*	LSU	La Muga river mouth	Jul-11	
KP790186	*Gymnodinium* sp. 1	LSU	Olímpic Harbour	Dec-11	3I
KP790187	*Gymnodinium* sp. 1	LSU	Olímpic Harbour	Dec-11	S1J
KP790188	*Gymnodinium* sp. 1	LSU	Arenys Harbour	Jun-12	
KP790189	*Gymnodinium* sp. 2	LSU	Arenys Harbour	Apr-13	3J
KP790190	*Gymnodinium* sp. 2	LSU	Arenys Harbour	Apr-13	
KP790191	*Gyrodinium* cf. *britannicum*	LSU	Barcelona Harbour	Mar-12	3K
KP790192	*Gyrodinium corallinum*	LSU	Barcelona Harbour	May-12	3L
KP790193	*Gyrodinium dominans* *	LSU	Barcelona offshore	Feb-11	
KP790194	*Gyrodinium dominans* *	LSU	Barcelona offshore	Feb-11	
KP790195	*Gyrodinium heterogrammum*	LSU	Tarragona Harbour	Oct-11	3N
KP790196	*Gyrodinium heterogrammum*	LSU	Tarragona Harbour	Dec-11	S1K
KP790197	*Gyrodinium heterogrammum*	LSU	Arenys Harbour	Dec-11	S1L
KP790198	*Gyrodinium* cf. *ochraceum*	LSU	Tarragona Harbour	Oct-11	S1N
KP790199	*Gyrodinium* cf. *ochraceum*	LSU	Tarragona Harbour	Oct-11	S1O
KP790200	*Gyrodinium* cf. *spirale*	LSU	L'Estartit Beach	Dec-11	4A
KP790201	*Gyrodinium* cf. *spirale*	LSU	L'Estartit Beach	Dec-11	S1P
KP790202	*Gyrodinium* cf. *spirale*	LSU	Barcelona Harbour	Dec-11	S1Q
KP790203	*Gyrodinium* cf. *spirale*	LSU	Tarragona Harbour	Dec-11	S1R
KP790204	*Gyrodinium* cf. *spirale*	LSU	Tarragona Harbour	Dec-11	4B
KP790205	*Gyrodinium* cf. *spirale*	LSU	Barcelona Harbour	Dec-11	S1S
KP790206	cf. *Gyrodinium undulans*	LSU	Palamós Harbour	Feb-12	4C
KP790207	*Gyrodinium viridescens*	LSU	Castelldefels Beach	May-12	S2A
KP790208	*Gyrodinium viridescens*	LSU	L'Estartit Beach	Jul-12	S2B
KP790209	*Gyrodinium* sp. 1	LSU	Tarragona Harbour	Oct-11	4E
KP790210	*Gyrodinium* sp. 2	LSU	Barcelona Harbour	Dec-11	4F
KP790211	*Gyrodinium* sp. 3	LSU	Tarragona Harbour	Dec-11	4G
KP790212	*Gyrodinium* sp. 4	LSU	Tarragona Harbour	Dec-11	4H
KP790213	*Gyrodinium* sp. 4	LSU	Arenys Harbour	Mar-12	S2C
KP790214	*Gyrodinium* sp. 5	LSU	Tarragona Harbour	Oct-11	4I
KP790215	*Karenia mikimotoi*	LSU	Fangar Bay	Oct-12	
KP790216	*Karenia mikimotoi* +	LSU	Tarragona Harbour	Jun-12	4K
KP790217	*Karenia umbella*	LSU	Olimpic Harbour	Oct-12	4M
KP790218	*Karlodinium armiger* *	LSU	Barcelona offshore	Feb-11	
KP790219	*Karlodinium decipiens*	LSU	L'Estartit Beach	May-11	4O
KP790220	*Karlodinium veneficum* *	LSU	Alfacs Bay	Jan-00	
KP790221	*Katodinium glaucum*	LSU	Tarragona Harbour	Jan-12	5B

GenBank Accession Number	Species	rDNA	Locality	Date	Fig.
KP790222	*Lepidodinium viride*	LSU	Fangar Bay	Oct-12	5C
KP790223	*Levanderina fissa*	LSU	La Muga River mouth	May-12	S2D
KP790224	*Levanderina fissa* *	LSU	Arenys Harbour	Jun-10	5D
KP790225	*Polykrikos herdmanae*	LSU	L'Estartit Beach	Jun-13	S2E
KP790226	*Polykrikos kofoidii*	LSU	L'Estartit Beach	Dec-11	S2H
KP790227	*Polykrikos kofoidii*	LSU	L'Estartit Harbour	Oct-11	S2I
KP790228	*Polykrikos kofoidii*	LSU	L'Estartit Beach	Dec-11	5F
KP790229	*Takayama tasmanica*	LSU	Llavaneres Beach	Jul-12	
KP790230	*Takayama tasmanica*	LSU	Fangar Bay	Oct-12	5H
KP790231	*Torodinium robustum*	LSU	Fangar Bay	Oct-12	S2J
KP790232	*Torodinium robustum* +	LSU	Castelldefels Beach	Aug-12	S2K
KP790233	*Torodinium teredo*	LSU	Barcelona offshore	Dec-11	5J
KP790234	*Warnowia* sp. 1	LSU	Barcelona Harbour	Dec-11	S2N
KP790235	*Warnowia* sp. 1	LSU	Barcelona Harbour	Oct-12	5K
KP790236	*Warnowia* sp. 2	LSU	Tarragona Harbour	Dec-11	5L
KP790237	*Warnowia* sp. 2 +	LSU	Tarragona Harbour	Jun-12	S2O
KP790238	*Warnowia* sp. 2 +	LSU	Tarragona Harbour	Jun-12	S2P
KP790239	*Warnowia* sp. 3	LSU	Vilanova Harbour	Jun-12	5M
KP790240	*Warnowia* sp. 4	LSU	Vilanova Harbour	Aug-12	5N
KP790241	*Warnowia* sp. 4	LSU	Vilanova Harbour	Aug-12	S2Q
KP790242	*Warnowia* sp. 5	LSU	Arenys Harbour	Nov-12	S2T
KP790147	*Apicoporus* sp.	SSU	Castelldefels Beach	Jun-13	S1E
KP790148	*Apicoporus* sp.	SSU	Castelldefels Beach	Jun-13	S1F
KP790149	*Apicoporus* sp.	SSU	Castelldefels Beach	Jun-13	S1G
KP790150	*Ceratoperidinium falcatum*	SSU	Fangar Bay	Oct-12	S1H
KP790151	*Ceratoperidinium falcatum*	SSU	Fangar Bay	Oct-12	S1I
KP790152	*Gymnodinium litoralis* *	SSU	La Muga river mouth	-	
KP790153	*Gyrodinium* cf. *spirale*	SSU	Vilanova Harbour	Mar-12	S1T
KP790154	*Gyrodinium* cf. *spirale*	SSU	Barcelona Harbour	Mar-12	S1U
KP790155	*Gyrodinium* cf. *spirale*	SSU	Arenys Harbour	May-12	S1V
KP790156	*Gyrodinium* cf. *spirale*	SSU	Ametlla Harbour	Jun-12	S1W
KP790157	*Gyrodinium* cf. *spirale*	SSU	Tarragona Harbour	Dec-12	S1X
KP790158	*Gyrodinium heterogrammum*	SSU	Tarragona Harbour	Feb-12	
KP790159	*Gyrodinium heterogrammum*	SSU	Tarragona Harbour	Feb-12	S1M
KP790160	*Katodinium glaucum*	SSU	Castelldefels Beach	Jun-13	
KP790161	*Katodinium glaucum*	SSU	Castelldefels Beach	Jun-13	
KP790162	*Katodinium glaucum*	SSU	Castelldefels Beach	Jun-13	
KP790163	*Levanderina fissa* *	SSU	La Muga River mouth	Aug-09	
KP790164	*Polykrikos herdmanae*	SSU	L'Estartit Beach	Jun-13	S2F
KP790165	*Polykrikos herdmanae*	SSU	L'Estartit Beach	Jun-13	S2G
KP790166	*Torodinium robustum*	SSU	Fangar Bay	Oct-12	S2L
KP790167	*Torodinium robustum*	SSU	Fangar Bay	Oct-12	S2M
KP790168	*Warnowia* sp. 4	SSU	Vilanova Harbour	Aug-12	S2R
KP790169	*Warnowia* sp. 4	SSU	Vilanova Harbour	Aug-12	S2S
KP790170	*Warnowia* sp. 5	SSU	Arenys Harbour	Apr-13	5O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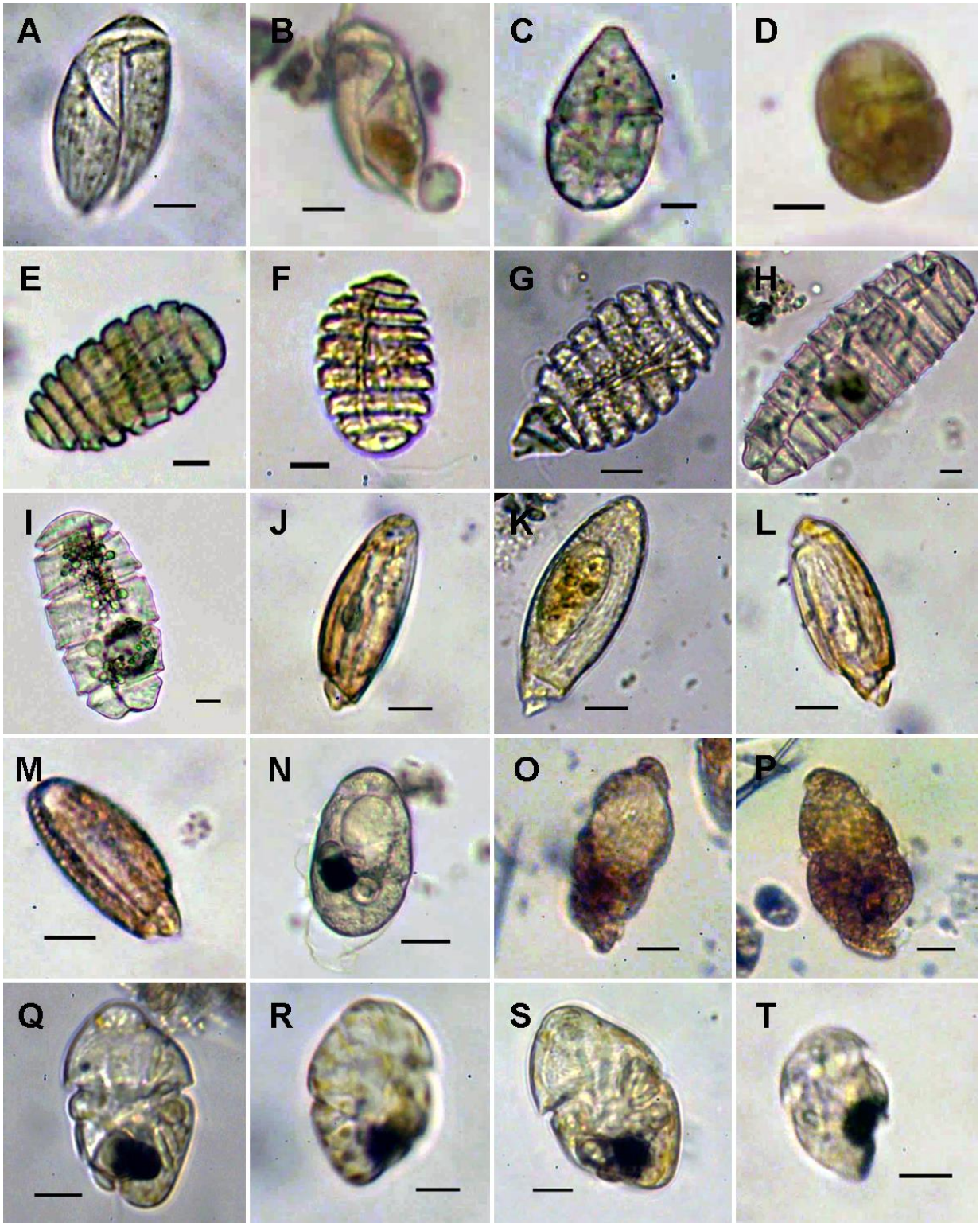
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