Gómez, F., Artigas, L.F., Gast, R.J. 2021. Molecular phylogeny and synonymy of *Balechina gracilis* comb. nov. (=*Gymnodinium gracile*), a widespread polymorphic unarmored dinoflagellate (Dinophyceae). Journal of Phycology, https://doi.org/10.1111/jpy.13135

MOLECULAR PHYLOGENY AND SYNONYMY OF *BALECHINA GRACILIS* COMB. NOV. (=*GYMNODINIUM GRACILE*), A WIDESPREAD POLYMORPHIC UNARMORED DINOFLAGELLATE (DINOPHYCEAE)

Fernando Gómez[1,2], Luis Felipe Artigas[2] and Rebecca J. Gast[3]*

[1]Carmen Campos Panisse 3, 11500 Puerto de Santa María, Spain, [2]Université du Littoral Côte d'Opale, Université de Lille, CNRS, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, 32 Av. Foch, 62930 Wimereux, France, and [3]Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1049, USA

*Author for correspondence: email fernando.gomez@fitoplancton.com

Shortened/running title: *Gymnodinium gracile*, a polymorphic dinoflagellate

***Gymnodinium gracile*, described from the coasts of Denmark in 1881, is one of the first described unarmored dinoflagellates. Individuals which morphologically fit with the original description were isolated from the English Channel (North-East Atlantic). The SSU rRNA gene sequences were identical to the sequences identified as *Balechina pachydermata* and *Gymnodinium amphora* from the Mediterranean Sea and Brazil. We propose the transfer of *Gymnodinium gracile* into the genus *Balechina* as *B. gracilis* comb. nov. These sequences constitute an independent lineage, clustering with numerous environmental sequences from polar to tropical waters. The widespread distribution, the high plasticity in size, shape and coloration and the difficulties in discerning the fine longitudinal striae have contributed to the description of numerous synonyms: *Amphidinium vasculum*, *Balechina pachydermata* (=*Gymnodinium pachydermatum*), *Gymnodinium achromaticum*, *G. abbreviatum*, *G. amphora*, *G. dogielii*, *G. lohmannii* (=*G. roseum* sensu Lohmann 1908), *G. situla* and *Gyrodinium cuneatum* (=*G. gracile* sensu Pouchet 1885).**

***Key index words:* Dinophyta; naked Dinoflagellata; Gymnodiniales; new combination, taxonomy; molecular phylogenetics**

Abbreviations: ML, Maximum Likelihood.

Bergh (1881) described two species of the dinoflagellate genus *Gymnodinium* from the coasts of Denmark: *Gymnodinium gracile* and *Gymnodinium spirale*. The latter is one of the most common and widespread unarmored dinoflagellates currently known as *Gyrodinium spirale*. *Gymnodinium gracile* appears more rarely in the literature (see Appendix S1 as supporting information). Despite the fact that *G. gracile* was described from a region with a long tradition of taxonomic plankton studies, its classification within the current circumscription of *Gymnodinium* remains unresolved. Briefly, Elbrächter (1979) reported *Gymnodinium lohmannii* (=*Gymnodinium roseum* Lohmann), *G. abbreviatum*, and *Gyrodinium cuneatum* (=*G. gracile* sensu Pouchet 1885) as junior synonyms of *Gymnodinium gracile* (excluding *G. gracile* sensu Kofoid & Swezy 1921). Hansen and Larsen (1992) illustrated the variability of the shape of *G. gracile* from individuals collected near the type locality. Gómez et al. (2015) provided SSU rRNA gene sequences for *Balechina pachydermata* from the Mediterranean Sea and *Gymnodinium amphora* from Brazil. Based on the SSU rRNA gene phylogeny and morphological variability of the individuals, Gómez et al. (2015) proposed *Amphidinium vasculum*, *Gymnodinium amphora*, *Gymnodinium dogielii* and *Gymnodinium gracile* sensu Kofoid & Swezy as synonyms of *B. pachydermata*. It is unresolved whether *Gymnodinium gracile* or *Gyrodinium cuneatum* and *Gymnodinium achromaticum*, the latter two described from off the coast of Brittany (Pouchet 1885, Lebour 1917) are related to *Balechina pachydermata.*

This study resolves the synonymy of *Gymnodinium gracile* with the first LSU rRNA and ITS and the complete SSU rRNA gene sequences of the genus *Balechina*. Observations were carried out during the research cruise with fifty-one stations sampled from Dunkirk (North Sea) through French coastal and offshore waters of the English

Channel, to Brest on 16–31 July 2018 (Fig. S1). Plankton samples were collected from surface waters with a phytoplankton net (20-µm mesh size). Aliquots were left to settle in a composite settling chamber, examined on-board with an inverted microscope (Eclipse TS-100, Nikon Inc., Tokyo, Japan) and photographed with a digital camera (Nikon D5000). Molecular methods are similar to Gómez et al. (2019) and are reported in Appendix S2 as supporting material.

Morphotypes of *Gymnodinium gracile* were observed in the sampling stations off Normandy and Brittany, with the highest number of cells observed in the French offshore waters between Brest and Plymouth. The species was distinctive, and the only unarmored heterotrophic dinoflagellates within the same range of size were *Gyrodinium spirale* and *Syltodinium listii*. *Gymnodinium gracile* had a premedian cingulum, descending about 3–4 cingular widths, and the cells were wider at the cingulum level. Cell dimensions ranged from 75–140 µm long, and 45–70 µm wide at the cingulum level (Fig. S2), although dividing cells were wider (Fig. S2l, m). The main difference between the individuals was the shape of the episome. Numerous individuals fit well with the original description of *G. gracile* showing a pyramidal episome with a blunt apex or a truncated horn (Fig. S2a–c). The most common morphotype in our samples corresponded to the description of *Gymnodinium abbreviatum* that is characterized by a lower episome (Fig. S2d–g). When the apex was contracted, the cell morphology was similar to *Gymnodinium amphora* or *Balechina pachydermata* (Fig. S2h). Prior to division, the cell was wider showing the morphology of *Gymnodinium situla* (Fig. S2h). During oblique cell division (Fig. S2j), the daughter cell with an incomplete episome that received most of the hyposome from the mother cell, initially presented a morphology of *Amphidinium vasculum*, but later of *Gymnodinium pachydermatum*, *G. amphora* or *G. achromaticum*, the last described from the English Channel at Plymouth

(Fig. S2k). The other daughter cell with the complete episome showed a thinner hyposome that corresponded to *Gyrodinium cuneatum* (=*G. gracile* sensu Pouchet 1885) that was first described in the coast of Brittany (Fig. S2l). Cell shape also influenced other morphological features, such as cingular displacement that is lower in the *Gymnodinium amphora* morphotype (Fig. S2h) and higher in the *Gyrodinium cuneatum* morphotype (Fig. S2m). All morphotypes showed the characteristic double-layer thick cell covering. The cell surface lacked prominent ridges or furrows, and it was embossed with fine longitudinal striae difficult to observe with the ship-board optical resources (Fig. S2e). Individuals were hyaline, and colorations (pinky or yellow-greenish) were not observed, except for the vacuoles. Food vacuoles were spherical with a brown color and located in the middle of the cell (Fig. S2h, m). In other cases, the vacuoles were smaller and greenish (Fig. S2m). The nucleus was spherical, encapsulated and located in the hyposome. The presence of a large vacuole may displace the nucleus towards the antapex (Fig. S2h). Individuals of *G. gracile* swam slowly with gracile movements, but they sometimes experienced sudden accelerations. In contrast to other unarmored dinoflagellates, cells of *G. gracile* did not lyse easily when manipulated and may react with a contraction of the cell body.

We obtained DNA sequences from two samples collected on 27 July 2018 from offshore waters between Brest and Plymouth (49° 05' 28.9" N, 4°11' 24.4" W) and the English Channel waters off Roscoff (48° 57' 17.6" N, 4° 09' 28.8" W) (Fig. S1). The SSU rDNA sequences (1716, 1712, 1359 base pairs) of the three isolates were identical. The percent similarity of *G. gracile* from the English Channel, *Balechina pachydermata* from the Mediterranean Sea (KR139789) and *Gymnodinium amphora* from the South Atlantic at Brazil (KR139790–92) ranged from 99.5–99.9%. All these sequences clustered together with full (100%) bootstrap support as a monophyletic lineage distantly related to any other dinoflagellate sequence (Fig. 1). *Gymnodinium gracile* is the earliest available name for the members of this lineage, but its generic placement is unsupported because it is distantly related to the *Gymnodinium fuscum*, type species of *Gymnodinium* (Fig. 1). In a second tree, we added the closer environmental sequences after a BLAST search. The sequences of *Gymnodinium gracile*/*Balechina pachydermata*/*Gymnodinium amphora* clustered with environmental sequences from southern California (KJ763266, KJ763198, KJ763422), Caribbean Sea (GU825009, GU825612, GU825613, GU825090), Black Sea (HM749926), North Sea (DQ310213, EF526874) and Canadian Arctic (JQ956083) (see inset in Fig. 1).

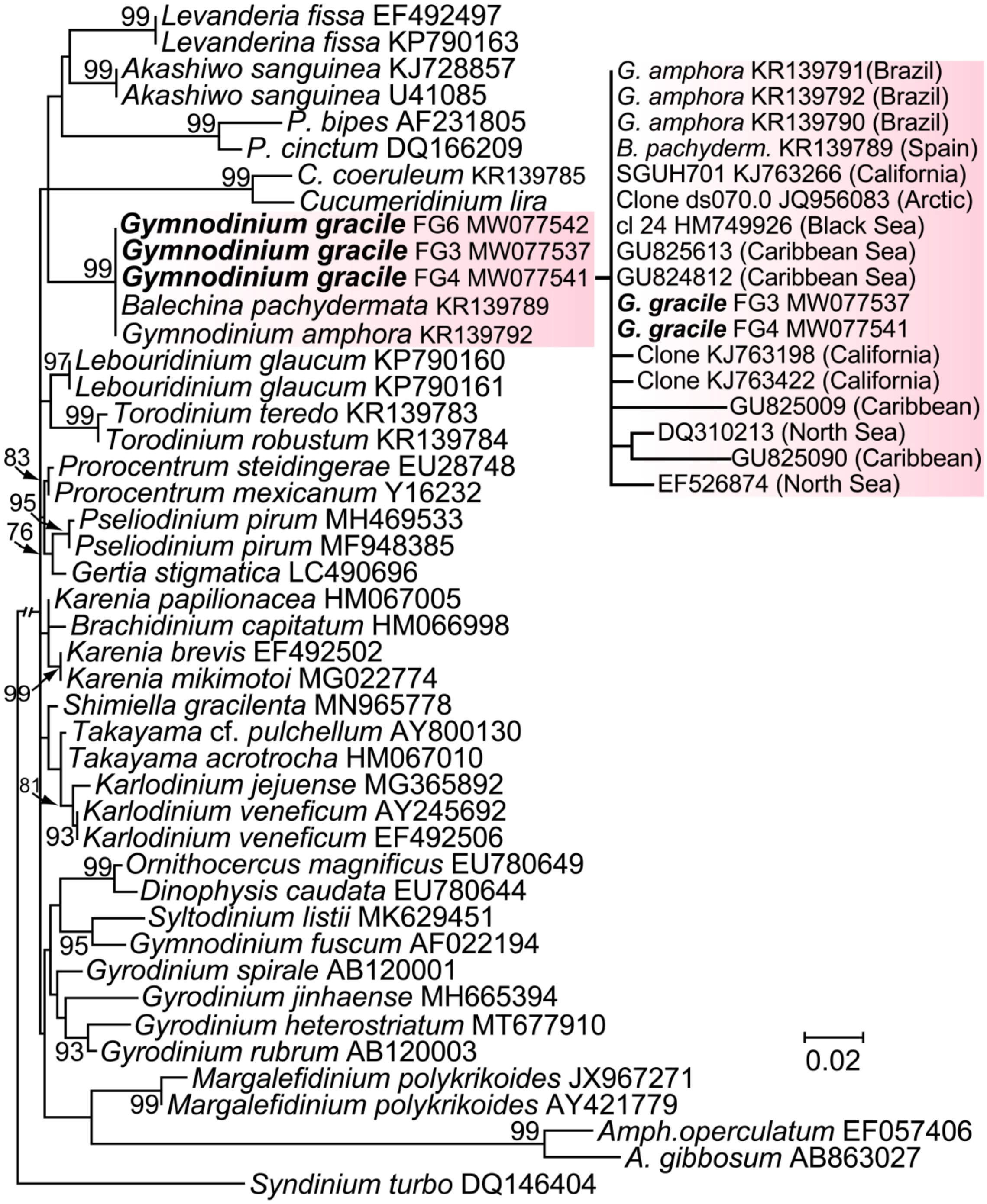


Fig. 1. Phylogenetic tree based on SSU rRNA gene sequences, showing the position of the sequences of *Gymnodinium gracile* by Maximum Likelihood (ML). Numbers near branches denote ML bootstrap probability value. Bootstrap values <70 are omitted. The inset shows the environmental sequences closer to the new sequences. The geographic origin is placed between parentheses. Scale bar denotes 0.02 substitutions per site.

The D1–D2 domains of the LSU rRNA gene sequences of two isolates from distinct locations (off Roscoff, off Finisterre) were identical, and also identical with two undocumented sequences retrieved from GenBank as 'Karenia sp. (in: Dinophyceae) strain DAB07 and DAB08)' isolated from the Black Sea (KU999990-1). These sequences diverged from the sequences of *Karenia* and its relatives by more than 13%. The two sequences of *Gymnodinium gracile* from the English Channel and the isolates from the Black Sea formed a strong supported lineage distantly related to any other dinoflagellate sequence (Fig. S3).

The observations of *Gymnodinium gracile* showed individuals that fit well with the original description (Fig. S2a–c), while other individuals were closer to other allied species (Fig. S2d–m). The individuals found in the English Channel off Brittany were hyaline, while it is more common to find individuals with yellow or greenish coloration in warm seas (Kofoid and Swezy 1921, Gómez et al. 2015). The form described as *Gymnodinium abbreviatum* is the most extended morphotype in the English Channel (Fig. S2m), while in warm waters it is more common to find individuals with the shape of *Balechina pachydermata* or *Gymnodinium amphora* (Gómez et al. 2015).

There are numerous taxa described from the morphotypes of *Gymnodinium gracile* (see Appendix S1 as supporting information). They were described from the observation of one or a few individuals, ignoring the fast changes of shape that characterize the distinct morphotypes of this species (https://www.youtube.com/watch?v=FDytvHEJsFg). The environmental sequencing data based on the SSU rRNA gene sequences reveals that this species is widely distributed from Arctic waters to tropical waters (Fig. 1). This wide geographical spread and the differences between environmental conditions (e.g. polar vs tropical seas) also contributed to the proliferation of new species descriptions. Another feature is the difficulty of observing the longitudinal striae (Fig. S2e). The presence or absence of striae was used to propose new species, although this character largely depends on the optical resources. There were anomalies such as the description of *Gyrodinium cuneatum* with striae in the episome but absent in the hyposome (Kofoid and Swezy 1921).

In the LSU rRNA gene phylogeny, the D1–D2 domains of the two sequences of *Gymnodinium gracile* from distinct isolates were identical to two undocumented sequences from the Black Sea (KU999990–1) retrieved from GenBank as 'Karenia sp. (in: Dinophyceae) strain DAB07 and DAB08)'. Although the term 'strain' may suggest that the individuals were cultured, the methodology was the direct PCR of isolates. Baytut et al. (2016, p. 55) reported: "we conclude that DAB07 and DAB08 are probably the members of a new genus in the family Kareniaceae", and they did not provide any micrographs. Baytut et al. (2016, their figure 3) placed the KU999990–1 in a phylogenetic tree where the only representative of unarmored dinoflagellates were sequences of the Bachidiniaceae (*Karenia*, *Karlodinium* and *Takayama*). In a phylogenetic tree with a wider representation of unarmored dinoflagellates, there is no support to consider the sequences KU999990–1 belonging to the Brachidiniaceae (Fig. S3). There are records of *Gymnodinium gracile* (Gómez and Boicenco 2004) and the environmental sequences (HM749926) that support the presence of *G. gracile* in the Black Sea (Fig. 1).

The molecular data confirm the synonymy of *Gymnodinium gracile* and *B. pachydermata*, generitype of *Balechina*. We propose the transfer of *Gymnodinium gracile* into *Balechina*, as *B. gracilis*.

Balechina gracilis (Bergh) F.Gómez, Artigas & Gast, comb. nov.

Basionym: *Gymnodinium gracile* Bergh (1881, *Morph. Jahrb*. **7**: 251, figs 68–69).

Heterotypic synonyms: *Amphidinium vasculum* Kofoid & Swezy (1921: 156), *Balechina pachydermata* (Kofoid & Swezy) Loeblich & A.R.Loeblich (1968: 210), (=*Gymnodinium pachydermatum* Kofoid & Swezy (1921: 239)), *Gymnodinium abbreviatum* Kofoid & Swezy (1921: 180), *Gymnodinium achromaticum* M.Lebour (1917: 190), *Gymnodinium amphora* Kofoid & Swezy (1921: 185), *Gymnodinium dogielii* Kofoid & Swezy (1921: 205), *G. lohmannii* Paulsen (1908: 99), (=*Gymnodinium roseum* Lohmann (1908: 263)), *Gymnodinium situla* Kofoid & Swezy (1921: 257), *Gyrodinium cuneatum* Kofoid & Swezy (1921: 297), (=*Gymnodinium gracile* sensu Pouchet 1885).

Acknowledgements

F.G. was supported by the convention #2101893310 between CNRS INSU and the French Ministry of Ecology (MTES) for the implementation of the Monitoring Program of the European Marine Strategy Framework directive (MSFD) for pelagic habitats and the descriptor ‘biodiversity’. Samples were collected during the ECOPEL Manche 2018 spring and summer cruises (CNRS-LOG) onboard R/V "*Antea*" (IRD, Institut de Recherche pour le Développement) in the frame of the cited convention and the CPER ‘Hauts de France’ project MARCO supported by the French state, the ‘Hauts de France’ French Region and the European Regional Development Founds (ERDF).

Supporting information

Fig. S1. Map of the sampling stations during the ECOPEL-2 cruise.

Fig. S2. Light micrographs of live cells of *Gymnodinium gracile* from the English Channel in July 2018.

Fig. S3. Phylogenetic tree based on LSU rRNA gene sequences.

Appendix S1. Historical account of *Gymnodinium gracile* and allied species names.
Appendix S2. Molecular methods.

References

Baytut, Ö., Gürkanli, C. T., Deniz, E., Özkoç, I. & Gönülol, A. 2016. First molecular records of potentially harmful planktonic dinoflagellates from the southern Black Sea. *Turk. J. Bot.* 40:546–56.

Bergh, R. S. 1881. Der organismus der Cilioflagellaten. Eine phylogenetische studie. *Morphol. Jahrb.* 7:177–288.

Elbrächter, M. 1979. On the taxonomy of unarmored dinophytes (Dinophyta) from the Northwest African upwelling region. *Meteor Forsch.-Ergebnisse, Reihe D*, 30:1–22.

Gómez, F. & Boicenco, L. 2004. An annotated checklist of dinoflagellates in the Black Sea. *Hydrobiologia* 517:43–59.

Gómez, F., López-García, P., Takayama, H. & Moreira, D. 2015. *Balechina* and the new genus *Cucumeridinium* gen. nov. (Dinophyceae), unarmored dinoflagellate with thick cell coverings. *J. Phycol.* 51:1088–105.

Gómez, F., Artigas, L. F. & Gast, R. J. 2019. Molecular phylogeny of the parasitic dinoflagellate *Syltodinium listii* (Gymnodiniales, Dinophyceae) and generic transfer of *Syltodinium undulans* comb. nov. (=*Gyrodinium undulans*). *Eur. J. Protistol.* 71:125636.

Hansen, G. & Larsen, J. 1992. Dinoflagellater i danske farvande. *In* Thomsen H. A. [Eds.] *Plankton i indre danske farvande*. vol. 11, Miljøstyrelsen, Havforskning fra Miljøstyrelsen, vol. 11, pp. 45–155.

Kofoid, C. A. & Swezy, O. 1921. The free-living unarmored Dinoflagellata. *Mem. Univ. Calif.* 5:1–562.

Lebour, M. V. 1917. The Peridiniales of Plymouth Sound from the region beyond the Breakwater. *J. Mar. Biol. Ass. U.K.* 11:183–200.

Pouchet, G. 1885. Contribution à l’histoire des Péridiniens marins. *J. Anat. Physiol. Norm. Pathol. Homme Anim.* 21:28–88.

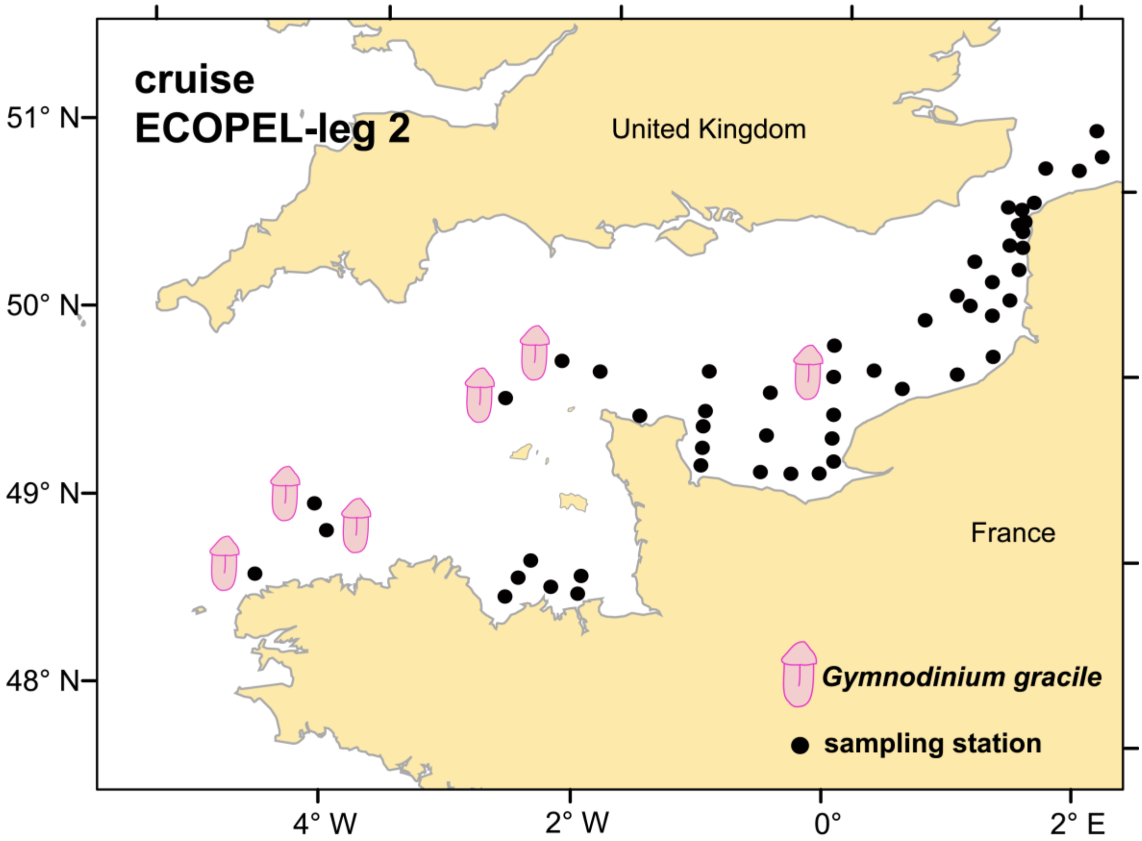


Fig. S1. Map of the sampling stations in the ECOPEL-2 cruise in July 2018.

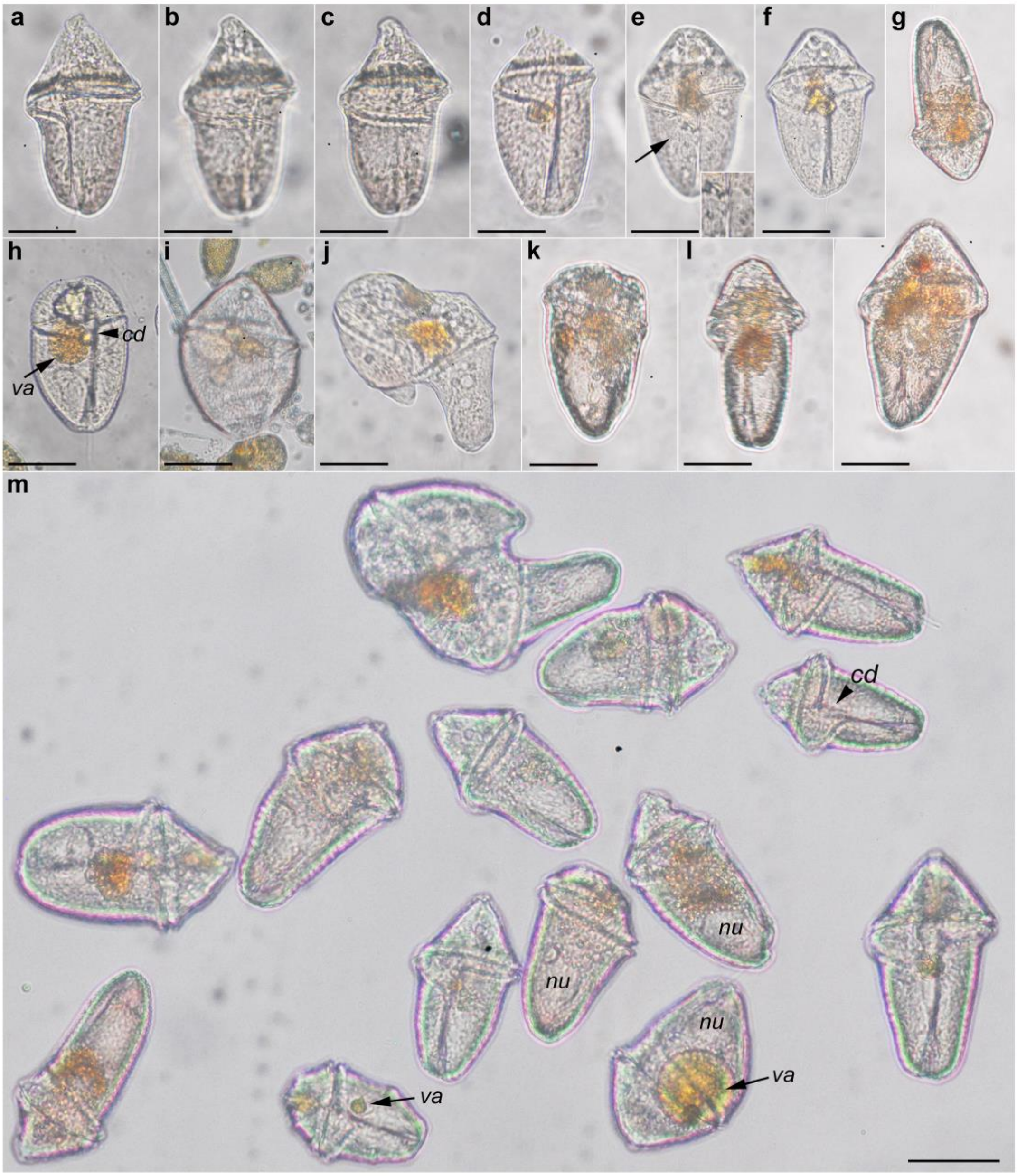


Fig. S2. Light micrographs of live cells of *Gymnodinium gracile* from the English Channel in July 2018. **a–c**. Individual of *Gymnodinium gracile* in different focal planes. **d–g**. Different individuals of the form *Gymnodinium abbreviatum*. **e**. The arrow and the inset indicate the fine longitudinal striae. **g**. Note the different sizes. **h**. Form *Gymnodinium amphora*. Note the low cingular displacement. **i**. Form *Gymnodinium situla*. **j**. Dividing cell. **k**. Recently divided individual with the form of *Amphidinium vasculum*. **l**. Recently divided individual with the form of *Gyrodinium cuneatum*. **m**. Different forms of *Gymnodinium gracile*. Abbreviations: *cd* = cingular displacement; *nu* = nucleus; *va* = vacuole. Scale bar = 50 µm

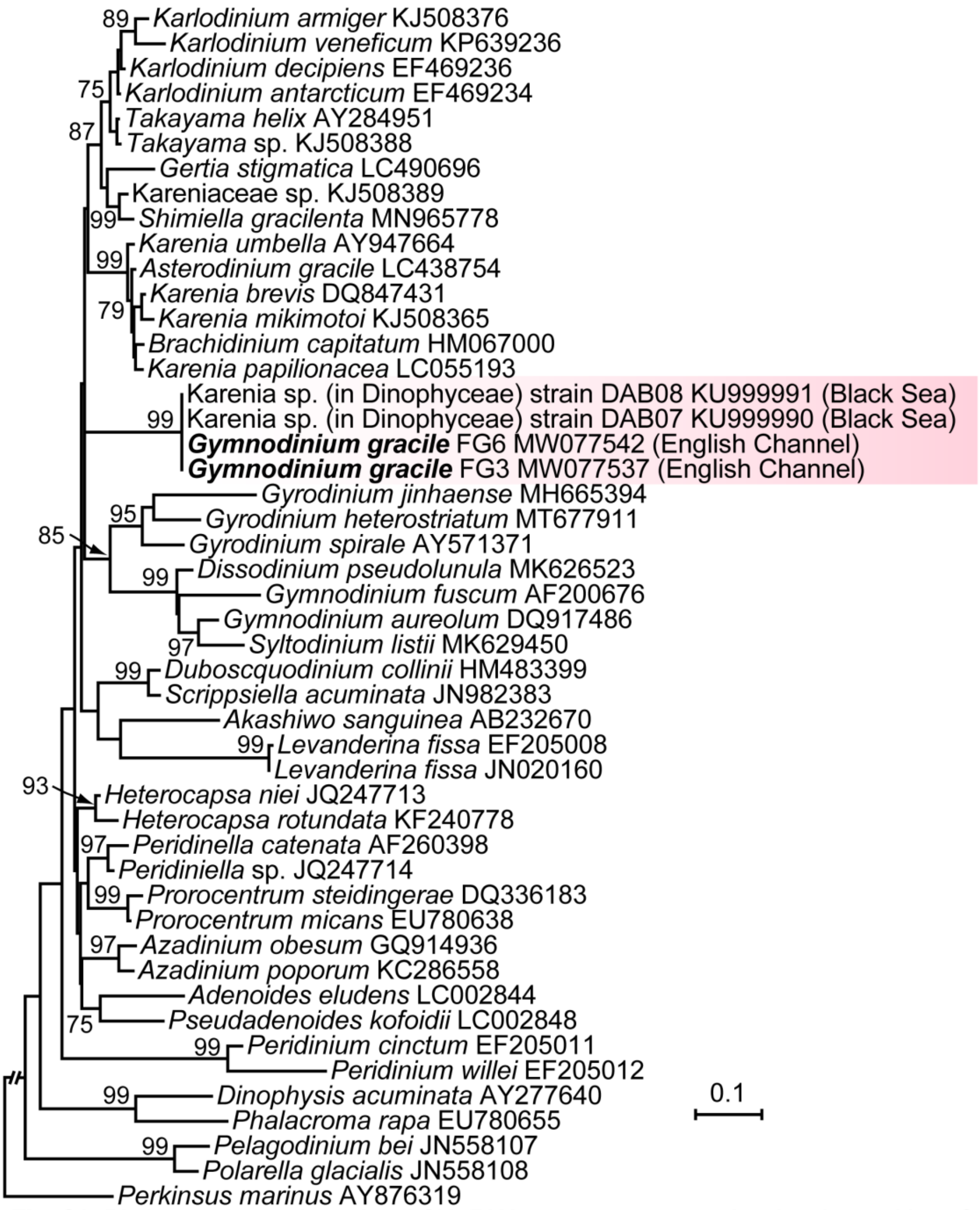


Fig. S3. Phylogenetic tree based on LSU rRNA gene sequences, showing the position of the sequences of Gymnodinium gracile by Maximum Likelihood (ML). Numbers near branches denote ML bootstrap probability value. Bootstrap values <70 are omitted. The geographic origin is placed between parentheses. Scale bar 0.1 substitutions per site.

Molecular phylogeny and synonymy of *Balechina gracilis* comb. nov. (=*Gymnodinium gracile*), a widespread polymorphic unarmored dinoflagellate (Dinophyceae)

Fernando Gómez, Luis Felipe Artigas, Rebecca J. Gast

Appendix S1

Historical account of *Gymnodinium gracile* and allied species names (Fig. 1, Table 1)

From the coast of the Baltic Sea at Denmark, Bergh (1881, figs 68–69) described *Gymnodinium gracile* with illustrations in ventral and dorsal view. The cell body was elongate, twice as long as broad. The cingulum was premedian, with a low displacement. The episome was pyramidal with a slightly notched apex, and the hyposome drawn out and rounded. The sulcus was straight and extended into the antapex, and there was apparently a slight intrusion into the episome. The cell lacked chloroplasts and the cytoplasm showed a pinkish color. The nucleus was spherical and located in the hyposome. Contrary to other heterotrophic unarmored dinoflagellates such as *G. spirale* that continuously swims and changes direction, Bergh (1881) remarked that the cell moved regularly, with spasmodic jerky displacements. Bergh reported dimensions of 90 µm long and 24/34 µm wide; although very likely, he might have underestimated the value of the cell width based on the relative proportions of his line drawings.

The taxonomical confusion soon followed. From the coasts of Brittany, Pouchet (1883) found an abundant species that he identified as *Gymnodinium gracile*. The cell shape differed from Bergh's *G. gracile*, the species was photosynthetic, the cingulum was median, the antapex bifurcated, and the cells were highly active (Pouchet, 1883; his fig. 39). Pouchet probably provided the earlier description of *Akashiwo sanguinea*, that was later described as *Gymnodinium splendens* Lebour in the same region (Lebour, 1925). Pouchet (1883, fig. 40) also described a new variety, *G. gracile* var. *exiguum* Pouchet, with several line drawings. Pouchet (1883, p. 447) reported this variant as a small cell of 30 µm enclosed inside a hyaline membrane. He commented that the cells have a red granule, and one of his illustration showed it. This suggests the melanosome of the warnowiid dinoflagellate (too big to be the stigma of former *Cochlodinium* taxa such as *Margalefidinium*). It is uncertain why Pouchet (1883) labelled with –g- the figure 40 because this is a source of confusion with species of the figure 37 labelled from –a- to –g-.

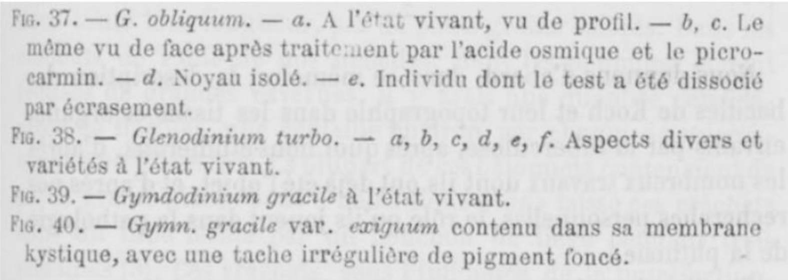
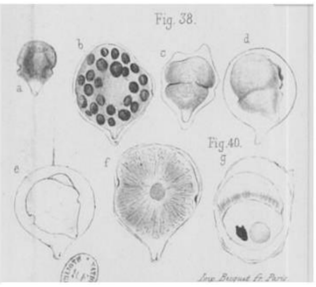


FIG. 37. — *G. obliquum.* — *a.* A l'état vivant, vu de profil. — *b, c.* Le même vu de face après traitement par l'acide osmique et le picro-carmin. — *d.* Noyau isolé. — *e.* Individu dont le test a été dissocié par écrasement.

FIG. 38. — *Glenodinium turbo.* — *a, b, c, d, e, f.* Aspects divers et variétés à l'état vivant.

FIG. 39. — *Gymdodinium gracile* à l'état vivant.

FIG. 40. — *Gymn. gracile* var. *exiguum* contenu dans sa membrane kystique, avec une tache irrégulière de pigment foncé.



It is uncertain which species corresponds to Pouchet's figure 40, but unequivocally it is unrelated to *Gymnodinium gracile*. Pouchet (1883, p. 448) described a new variety, *Gymnodinium spirale* var. *nobilis* Pouchet. He described it as « Toutefois les exemplaires que nous avons observés étaient plus fusiformes. La couleur de ces êtres est généralement la nuance chamois ; nous en avons trouvé plusieurs exemplaires offrant de fines traînées de carmin, aboutissant d'une part au aboral acuminé… ». Pouchet (1883) did not provide any illustration, but the fusiform shape and the carmine-colored granules suggest that it was *Gyrodinium spirale* or allied species. Later Pouchet (1885, p. 69) modified his identifications. He considered that *Gymnodinium gracile* was the taxon previously described as *Gymnodinium spirale* var. *nobilis*. The cells identified as *G. gracile* in the figures 32–33 by Pouchet (1885) do not fit with the description of the fusiform cells of *Gymnodinium spirale* var. *nobilis* in Pouchet (1883, p. 448).

Pouchet (1885) considered *G. gracile* var. *exiguum* a new species, unrelated to *G. gracile*, but he did not propose a species name and there are no new illustrations. Pouchet (1885, fig. 32) reported longitudinal striae in the episome of *Gymnodinium gracile* and a higher cingular displacement than in Bergh's original illustration. It is anomalous to find an unarmored dinoflagellate with longitudinal striae in the episome, while the surface of the hyposome is smooth. Although Pouchet's figure 32 unequivocally corresponded to *G. gracile*, Kofoid and Swezy (1921) considered it as a distinct species and they proposed *Gyrodinium cuneatum*. After the observations by Pouchet (1885) in the coast of Brittany, the species name *Gymnodinium gracile* disappeared from the region between France and England. In the British waters of the English Channel, Lebour (1917) reported the new species *Gymnodinium achromaticum* from the observation of a single individual. She illustrated longitudinal striae along the entire cell. From her own observations, Lebour (1925) reported *Gymnodinium abbreviatum* Kofoid & Swezy 1921. In her monograph of the North Atlantic dinoflagellates, she cited the historical records in the *Gymnodinium gracile* by Bergh and *Gyrodinium cuneatum* by Pouchet (=Pouchet 1885's *G. gracile*). Without own observations, Dodge (1982) illustrated *Gymnodinium achromaticum*, *G. abbreviatum* and *Gyrodinium cuneatum* in his book of the dinoflagellates from the British Isles. In the French coast of the Gulf of Biscay, Paulmier (1994) reported a line drawing of *Gymnodinium achromaticum*. The species name *G. gracile* disappeared from the English Channel and the observations were identified as *Gymnodinium abbreviatum*, *G. achromaticum* or *Gyrodinium cuneatum*.

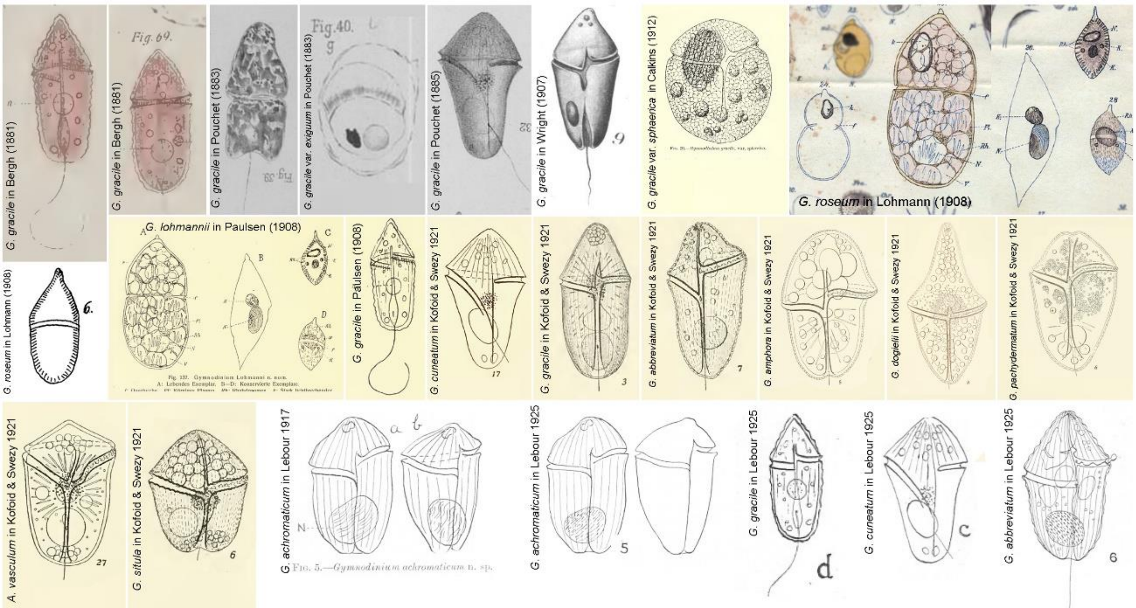


Figure 1. Illustrations *Gymnodinium gracile* and allied taxa names from the literature before 1926.

On the coasts of North America at Woods Hole, Calkins (1902) described a new variety, *Gymnodinium gracile* var. *sphaericum*. Based on his illustration, that taxon was unrelated to *G. gracile*. Based on Calkins' description, Kofoid and Swezy (1921) ranked the variety at the species level as *Gymnodinium sphaericum* Kofoid & Swezy 1921. *Gymnodinium sphaericum* disappeared from the literature, and even Hulburt (1957) did not report it in his study of the unarmored dinoflagellates near Woods Hole. In the Canadian Atlantic waters, Wright (1907) reported an illustration *Gymnodinium gracile* that unequivocally correspond to the species described by Bergh (1881). The species was further reported in the same region (Béard-Therriault *et al.*, 1999)

At Kiel, very close to the type locality of *Gymnodinium gracile*, Lohmann (1908) described the new species *Gymnodinium roseum*. The color was close to the pinky individuals in Bergh's original description. Lohmann illustrated cells from preserved material and they were consequently highly distorted. The species name *Gymnodinium* *roseum* was already used for a parasitic dinoflagellate described by Dogiel (1906), and Paulsen (1908) renamed it as *Gymnodinium lohmannii*. In this monograph of the North Atlantic dinoflagellates. Paulsen (1908) reproduced the original illustrations of *Gymnodinium lohmannii* (=*Gymnodinium roseum* sensu Lohmann) and *G. gracile* as independent species. Later, Elbrächter (1979) considered *Gymnodinium lohmannii* as a junior synonym of *G. gracile*.

Kofoid and Swezy (1921) published a monograph of the unarmored dinoflagellates from their observations from the coasts of California, and the previous species descriptions in the literature. Kofoid was a splitter taxonomist who proposed new species from the observation of a single or few individuals, admitting little intraspecific variability, and using diagnostic characters such as morphometric values or even cell coloration. Kofoid and Swezy (1921) also interpreted the illustrations of other authors as new species. Under the name *Gymnodinium gracile,* Kofoid and Swezy (1921, p. 214–216, plate 2, fig. 19) reported cells that were larger (105–130 μm long and 50–61 wide) than in Bergh's description (80 μm long and 24/32 μm wide). Kofoid and Swezy illustrated longitudinal striae on the cell surface, a kind of radial rodlets (ejectile bodies), and a marked intrusion of the sulcus in the episome that were missing in the Bergh's original description. Kofoid and Swezy reported a yellow-greenish pigmentation, while pinky in the Bergh's original description. There was a clear environmental and geographical split between the coasts of southern California, and the Baltic Sea. Therefore, there is doubt whether the observations by Kofoid and Swezy correspond to Bergh's *Gymnodinium gracile*.

Kofoid and Swezy (1921) described almost each distinct individual as a new species based on the shape of the episome (pyramidal or round), smooth surface, surface striation on the episome, or surface striation on all the cell body, differences on the cingular displacement or the intrusion of the sulcus into the episome. Kofoid and Swezy (1921, fig. Z, 9; plate 6, fig. 63) described *Gymnodinium abbreviatum*. The morphology is similar to Bergh's *Gymnodinium gracile*, including the pinky pigmentation. The main differences are the higher cingular displacement, and the sulcus extended into the apex and the longitudinal striae. For *G. abbreviatum*, Kofoid and Swezy (1921, p. 216) reported: "its nearest counterpart in *G. abbreviatum* sp. nov., differing from it, however, in its lack of a distinct alveolar layer". The description of *G. abbreviatum* was more complete than the original description of *G. gracile*, and Drebes (1974) used the name *G. abbreviatum* described from California for his records in the North Sea, not far from the type locality of *G. gracile*. Elbrächter (1979) considered that these records of *G. abbreviatum* from the North Sea corresponded to *G. gracile,* and *G. abbreviatum* is a junior synonym.

In Brittany, Pouchet (1885) described cells correctly identified as *Gymnodinium gracile*. Pouchet (1885, fig. 32) illustrated a cell with striae on the episome, and higher cingular displacement that Bergh's original illustrations. Without additional observations, Kofoid and Swezy (1921) considered that Pouchet's figure 32–33 correspond to a distinct species, and they proposed *Gyrodinium cuneatum*. As mentioned before, it is anomalous to find a cell with striae in the episome, while the surface of the hyposome is smooth. Therefore, Elbrächter (1979) considered *G. cuneatum* as a junior synonym of *G. gracile*.

Kofoid and Swezy (1921, p. 297) from the observation of single or few individuals described *Amphidinium vasculum*, *Gymnodinium amphora*, *G. dogielii*, *G. pachydermatum* and *G. situla*. These species were similar to *Gymnodinium gracile*, with the spherical nucleus in the hyposome, premedian cingulum, and sometimes even the radial rodlets (ejectile bodies). Kofoid and Swezy (1921) used diagnostic criteria such as the color, position of the food vacuoles, cingular displacement and the different shape of the episome to propose these new species. Years later, Loeblich and Loeblich (1968), based upon these descriptions, proposed the new genus *Balechina* for only the species *Gymnodinium pachydermatum*. The morphology of the cell in the line drawings of *G. pachydermatum* in Kofoid and Swezy (1921) differed from that in Schiller (1933).

In the English Channel off Plymouth, Lebour (1917) described the new species *Gymnodinium achromaticum* from the observation of a single individual as a pellucid cell of 78 µm long covered by longitudinal striae and very low episome. Later, in her monograph of the dinoflagellates of the North Atlantic, Lebour (1925) reproduced the Bergh's illustrations of *Gymnodinium gracile* and *Gyrodinium cuneatum* in Kofoid and Swezy 1921 (= *Gymnodinium gracile* sensu Pouchet 1885), and her illustrations of *G. achromaticum*, considering these three taxa as independent species.

On the coasts of Florida, Steidinger and Williams (1970) presented a micrograph of a cell identified as *Gymnodinium* J that corresponds to *Gymnodinium gracile*. Drebes (1974, his figure 72) reported *G. abbreviatum* from the North Sea. Elbrächter (1979) reported line drawings of *G. gracile* from the subtropical Atlantic, and he commented on his observations from the North Sea. Elbrächter (1979, p. 7) reported for *G. gracile*: "Surface with longitudinal, equidistant striae, about 15 in ventral view, the same number on epicone and hypocone. The surface of the body is mamillated and this gives the characteristic appearance of the species". Elbrächter (1979) reported the following synonyms for *G. gracile*: "Syn: *Gymnodinium spirale* var. *nobilis* Pouchet 1883, p. 448 (identical with *G. gracile* Bergh in Pouchet 1885, p. 69, Pl. 4 fig. 32), non *Gymnodinium spirale* Bergh; *Gymnodinium roseum* Lohmann 1908, p. 263, Pl. 17, figs 24–28, non *Gymnodinium roseum* Dogiel 1906; *Gymnodinium lohmannii* Paulsen 1908, p. 99–100, fig. 137 = nomen novum pro *G. roseum* Lohmann; *G. abbreviatum* Kofoid &

Swezy 1921, p. 180–181, Pl. 6, fig. 63, textfig. CC, 17 = nomen novum pro *Gymnodinium gracile* sensu Pouchet 1885, Pl. 4, fig. 32; *Gymnodinium* J Steidinger & Williams 1970, p. 52, fig. 72; *Spirodinium gracile* Entz jr., fide Kofoid & Swezy 1921, p. 214; – non *Gymnodinium gracile* Bergh sensu Pouchet 1883, p. 446–447, Pl. 20–21 fig. 40; non *G. gracile* Bergh sensu Kofoid & Swezy 1921, p. 214–216, Pl. 2, fig. 19 text. Z,3.W".

As reported above, it is doubtful whether the description of *Gymnodinium spirale* var. *nobilis* in Pouchet (1883) can be assigned to *G. gracile*. Elbrächter (1979) reported "*G. abbreviatum* Kofoid & Swezy 1921= nomen novum pro *Gymnodinium gracile* sensu Pouchet 1885, Pl. 4, fig. 32)". However, Kofoid & Swezy (1921) did not report that *G. abbreviatum* corresponded to *Gymnodinium gracile* sensu Pouchet 1885's figure 32. In the description of *G. abbreviatum*, Kofoid & Swezy (1921, p. 180–181) did not cited *G. gracile*. Kofoid and Swezy (1921) reported that the new species name *Gyrodinium cuneatum* for *Gymnodinium gracile* sensu Pouchet 1885's fig. 32. Elbrächter (1979) omitted *Gyrodinium cuneatum* in the list of synonyms, but he reported in the text: "*Gyrodinium cuneatum* is a later synonym of *G. gracile* Bergh". Elbrächter (1979) considered that *Gymnodinium abbreviatum* is a synonym of Bergh's *G. gracile*, while *G. gracile* sensu Kofoid and Swezy (1921) is a distinct taxon from Bergh's *G. gracile*. Elbrächter (1979) did not comment on the relationship of *G. gracile* with the species *Amphidinium vasculum*, *Gymnodinium amphora*, *G. dogielii*, *G. pachydermatum* or *G. situla*.

From the Danish coasts, near the type locality of *Gymnodinium gracile*, Hansen and Larsen (1992, their figure 4.41a–d) reported line drawings and light micrographs of individuals identified as *G. gracile*. These images showed the variability in the shape of the episome (from conical to hemispherical). One the line drawings in page 85 showed the cell with mamillated surface, while the figure 4.43b in page 87 showed a line drawing of the cell with 14 longitudinal striae in ventral view. These striae were not clearly visible in the light micrographs in page 85. The line drawings of *G. gracile* showed that the sulcus extended into the apex, but this feature is hardly visible in the micrographs. These observations explain the morphological variability reported the literature. Depending on the optical resources and/or physiological stage of the individuals, the cells can be reported as having a smooth surface, striae in the episome or striae in all the cell body, or the sulcus slightly invading the episome or the sulcus that reaches the apex (see figure 1 for the original drawings in the literature).

From the coast of Japan, Takayama (1998) reported light and scanning electron micrographs of *Gymnodinium abbreviatum* (his plate 28, figs 1–7) and light micrographs of *Gymnodinium situla*. The cell surface was of *G. abbreviatum* was mamillated, but the longitudinal striae were not visible after the preparation for electron microscopy, the sulcus extended into the apex, and there was an apical groove.

From the Mediterranean Sea and Brazil, Gómez *et al*. (2015) reported the co-occurrence of individuals with different shapes of the episome that corresponded to *Amphidinium vasculum*, *Gymnodinium amphora*, *Gymnodinium dogielii* and *G. gracile* sensu Kofoid and Swezy (Gómez *et al*. 2015, their figures 6 and 7). A single individual changed shape during the observations, and when the individual was disturbed it suddenly contracted and the episome showed other different shapes (see video at https://www.youtube.com/watch?v=FDytvHEJsFg). When observed at a high magnification, the cells showed fine longitudinal striae (Gómez *et al*. 2015, their figure 8). Gómez *et al.* (2015) provided the first molecular information on one individual that corresponded to the morphology of *Gymnodinium pachydermatum* (GenBank accession number KR139789) from the Mediterranean Sea, and several individuals with the morphology of *G. amphora* (KR139790–2) from Brazil. The sequences were almost identical, confirming the intraspecific morphological variability, and the wide distribution in warm seas. Gómez *et al*. (2015) identified the individuals as *Balechina pachydermata* (=*G. pachydermatum*), and they considered *Amphidinium vasculum*, *G. amphora*, *G. dogielii* and *G. gracile* sensu Kofoid and Swezy as synonyms. This avoids introducing any taxonomical innovation because if *Gymnodinium gracile* is considered as a synonym of *Balechina pachydermata*, then this taxon should be transferred into *Balechina* (the earlier available genus name). However, it is not easy to make that decision because data on *G. gracile* from high latitudes in Europe is missing to assure that Bergh's *G. gracile* from the Baltic Sea is the same species found in warm seas. Consequently, Gómez *et al*. (2015) in agreement with Elbrächter (1979) considered that *G. gracile* sensu Kofoid & Swezy from California as a distinct species from Bergh's *G. gracile* from the Baltic Sea. Molecular data of *G. gracile* from high latitudes in Europe is needed to verify the synonymy, and to support the transfer of *Gymnodinium gracile* into *Balechina*, which is provided now with the present study.

Table 1. Records of *Gymnodinium gracile* and allied taxa.

Amphidinium vasculum	California (Kofoid and Swezy, 1921); Australia (Wood, 1963a), Mediterranean Sea (Skolka *et al*., 1986), Mediterranean (Gómez et al. 2015).
Balechina pachydermata (=Gymnodinium pachydermatum)	California (Kofoid and Swezy, 1921); Gulf of Mexico (Steidinger et al. 2009); Mediterranean, Brazil (Gómez et al. 2015), Korea (Lee and Kim, 2017)

Gymnodinium abbreviatum	California (Kofoid and Swezy, 1921); Alaska (Bursa (1963), Canada Pacific (Wailes 1939); Russian Pacific (Konovalova 1998); Japan (Kofoid, 1931; Yamaji, 1980; Takayama, 1998); Korea (Lee and Kim, 2017); New Zeland (Kospartov et al. 2019); North Sea (Drebes, 1974; Round 1986); oceanic (Steidinger and Tangen 1997) Chesapeake Bay, 1997; Mediterranean (Ignatiades and Gotsis-Skretas 2013); Canary Islands (Afonso-Carrillo, 2014)
Gymnodinium achromaticum	English Channel (Lebour, 1917; Lackey & Lackey 1963)), North Sea (Conrad & Kufferath 1954); Woods Hole (Lackey, 1936), California (Lackey & Clendenning, 1965); Australia (Wood 1963), North Sea (van Meel, 1984), Mediterranean (Dangeard 1932; Margalef 1969; Gotsis-Skretas et al. 2008, https://doi.org/10.1594/PANGAEA.690564)
Gymnodinium amphora	California (Kofoid and Swezy, 1921); Australia (Wood, 1963b); Mediterranean Sea (Innamorati et al., 1989), Gulf of Mexico (Steidinger et al., 2009), Mediterranean, Brazil (Gómez et al., 2015)
Gymnodinium dogielii	California (Kofoid and Swezy, 1921); Mediterranean (Gómez et al. 2015)
Gymnodinium gracile	Baltic Sea (Bergh, 1881; Hällfors, 2004), Brittany (Pouchet, 1885); Canadian Atlantic (Wright, 1907; Béard-Therriault et al. 1999), North Sea (Ostenfeld, 1913, Hoppenrath, 2004), California (Kofoid and Swezy, 1921); Pacific Mexico (Okolodkov & Gárate-Lizárraga 2006); Arctic (Hsiao, 1963), Britain (Parke and Dixon, 1976); Russian Pacific (Konovalova 1998); Japan (Yamaji, 1980;

	Omura et al., 2012), Mediterranean Sea (Drira et al. (2009, Skolka *et al.*, 1986), Arctic (Okolodkov 1998); Korea (Lee and Kim, 2017), Canary Islands (Gil-Rodriguez et al., 2003; Afonso-Carrillo, 2014); Pacific Russia (Bessudova et al., 2014); Baja California (Gárate-Lizárraga, 2014); Gulf of California (Escobar-Morales and Hernández-Becerril, 2015); Black Sea (Gómez and Boicenco, 2004); Brazil (Odebrecht 2010).
G. lohmannii (=*G. roseum* sensu Lohmann)	Baltic Sea (Lohmann, 1908); Japan (Yamaji, 1980); Mediterranean Sea (Skolka et al., 1986); Arctic (Okolodkov 1998), Mexico (Okolodkov & Gárate-Lizárraga, 2006); Chesapeake Bay, 1992
Gymnodinium situla	California (Kofoid and Swezy, 1921); Australia (Wood (1963), Mediterranean (Skolka et al., 1986); Japan (Takayama, 1998); Gulf of Mexico (Steidinger et al., 2009); South Africa (Marangoni et al., 2001); Korea (Lee and Kim, 2017)
Gyrodinium cuneatum (=*G. gracile* sensu Pouchet 1885)	Brittany (Pouchet 1885, as *G. gracile*), Lebour (1925), Dodge 1982), Mediterranean (Innamorati et al., 1989); South-Africa (Marangoni *et al.*, 2001)

References

Afonso-Carrillo, J. 2014. Lista actualizada de las algas marinas de las islas Canarias. Elaborada para la Sociedad Española de Ficología (SEF), Las Palmas.

Bergh, R. S. 1881. Der organismus der Cilioflagellaten. Eine phylogenetische studie. *Morphologisches Jahrbuch* **7**: 177–288.

Bessudova, A. Y., Sorokovikova, L. M. Firsova, A. D., Kuz'mina, A. Y., Tomberg, I. V. and Likgoshway, Y. V. 2014. Changes in phytoplankton community composition along a salinity gradient from lower Yrnisei River to the Kara Sea, Russia. *Botanica Marina* **57**: 225–239.

Bérard-Therriault, L., Poulin, M. and Bossé, L. 1999. Guide d'identification du phytoplancton marin de l'estuaire et du Golfe du Saint-Laurent incluant également certains protozoaires. *Publication spéciale canadienne des sciences halieutiques et aquatiques* **128**: 1–387.

Bursa, A. 1963. Phytoplankton in coastal waters of the Arctic Ocean at Point Barrow, Alaska. *Arctic* **16**: 239–262.

Calkins, G. N. 1902. Marine protozoa from Woods Hole. *Bulletin of the United States Fisheries Commission* **21**: 413–468.

Chesapeake Bay, 1992. A Comprehensive List of Chesapeake Bay Basin Species. U.S Environmental Protection Agency for the Chesapeake Bay program.

Chesapeake Bay, 1997. A Comprehensive List of Chesapeake Bay Basin Species. U.S Environmental Protection Agency for the Chesapeake Bay program.

Conrad W, Kufferath H (1954) Recherches sur les eaux saumatres des environs de Lilloo. 2. Partie descriptive. Alges et protistes. Consdérations écologiques. Mém Inst Roy Sci Nat Belgique 127: 1–346.

Dangeard P (1932) Phytoplancton recueilli a Banyuls-sur-Mer. Arch Zool Exp Gen 74 (18) : 317–355.

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

Dogiel, V. 1906. Beiträge zur Kenntnis des perininen. *Mittheilungen aus der Zoologischen Staatsinst zu Neapel* **18**: 1–45.

Drebes, G. 1974. *Marines Phytoplankton*. Georg Thieme Verlag, Stuttgart.

Drira Z, Hassen MB, Hamza A, Rebai A, Bouain A, et al. (2009) Spatial and temporal variations of microphytoplankton composition related to hydrographic conditions in the Gulf of Gabes. J Mar Biol Assoc UK 89: 1559–1569

Elbrächter, M. 1979. On the taxonomy of unarmored dinophytes (Dinophyta) from the Northwest African upwelling region. *Meteor Forsch.-Ergebnisse, Reihe D*, **30**: 1–22.

Escobar-Morales, S. and Hernández-Becerril, D.U. 2015. Free-living marine planktonic unarmored dinoflagellates from Gulf of Mexico and the Mexican Pacific. *Botanica Marina* **58**: 9–22.

Gil-Rodriguez, M.C., Haroun, R., Ojeda Rodríguez, A., Berecibar Zugasti, E., Domínguez Santana, P., Herrera Morán B. (2003) Proctoctista. In Lista de Especies Marinas de Canarias (Algas, Hongos, Plantas y Animales) (Moro, L., Martin, J.L., Garrido, M.J, and Izquerdo, I. Eds.). Consejería de Política Territorial y Medio Ambiental del Gobierno de Canarias, Las Palmas, pp. 5–30.

Gómez, F. and Boicenco, L. 2004. An annotated checklist of dinoflagellates in the Black Sea. *Hydrobiologia* **517**: 43–59.

Gómez, F., López-García, P., Takayama, H. and Moreira, D. 2015. *Balechina* and the new genus *Cucumeridinium* gen. nov. (Dinophyceae), unarmored dinoflagellate with thick cell coverings. *Journal of Phycology* **51**: 1088–1105.

Gotsis-Skretas O, Pagou K (2008) Microplankton abundance in the euphotic zone of the Aegean Sea in October-November 1991. p. doi:10.1594/PANGAEA.692046.

Hällfors, G. 2004. Checklist of Baltic Sea phytoplankton species (including some heterotrophic protistan groups). Baltic Sea Environment Proceeding No. **95**: 1–208.

Hansen, G. and Larsen, J. 1992. Dinoflagellater i danske farvande. In H.A. Thomsen (Ed.), Plankton i indre danske farvande. vol. 11, Miljøstyrelsen, Havforskning fra Miljøstyrelsen, vol. 11, pp. 45–155.

Hoppenrath, M. 2004. A revised checklist of planktonic diatoms and dinoflagellates from Helgoland and List (Sylt), German. *Helgoland Marine Research* **58**: 243–251.

Hulburt, E. M. (1957) The taxonomy of unarmored Dinophyceae of shallow embayments on Cape Cod, Massachusetts. *Biological Bulletin (Woods Hole)* 112: 196–219

Hsiao, S. I. 1983. A checklist of marine phytoplankton and sea ice microalgae recorded from Arctic Canada. *Nova Hedwigia* 37: 225–313.

Hulburt, E. 1957. The taxonomy of unarmored Dinophyceae of shallow embayments on Cape Cod, Massachusetts. *Biological Bulletin (Woods Hole)* **112**: 196–219.

Ignatiades, L. and Gotsis-Skretas, O. 2013. The contribution of rare species to coastal phytoplankton assemblages. *Marine Ecology* 35: 132–145.

Inglis, G., Kospartov, M., Peacock, L., Middleton, C. and Johnston, O. 2019. Taharoa Terminal First baseline survey for non-indigenous marine species (Research Project ZBS2005-19). Biosecurity New Zealand Technical Paper No: 2019/01. ISBN 978-1-98-857160-7 ISSN 2624-0203.

Innamorati, M., L. Albertotanza, M. De Pol, M. Mannucci, C. Nuccio, G. Rargion, G. Innicenti, G. Mori, L. Lazzara (1986) Popolamenti fitoplanctonici e condizioni idrologiche nel Mar Ligure. *Resoconti dei Rilevamenti in mare, Università di Firenze* **2**: 1–56.

Innamorati, M., C. Nuccio, C. Lenzi Grillini, M. De Pol and M. Mannucci. 1989. Biomassa, produzione e specie fitoplantoniche nel mare antistante lo scarico termico della centrale elettrica di Torre del Sale (Golfo di Follonica). *Resoconti dei Rilevamenti in mare, Università di Firenze* 5: 1–43.

Innamorati, M., L. Lazzara, C. Nuccio, M. De Pol, M. Mannucci and G. Mori. 1989. Popolamenti fitoplanctonici e condizioni idrologiche nell´arcipelago Toscano. *Resoconti dei Rilevamenti in mare, Università di Firenze* **6**: 1–117.

Kofoid, C. A. and Swezy, O. 1921. The free-living unarmoured Dinoflagellata. *Mem. Univ. Calif*. **5**: 1–562.

Kofoid, C. A. 1931. Report of the biological survey of Mutsu Bay. 18. Protozoan Fauna of Mutsu Bay. Subclass Dinoflagellata; Tribe Gymnodinioidae. *Science Reports of the Tohoku Imperial University. Fourth Series, Biology* **6**(1): 1–43.

Konovalova, G.V. 1998. Dinoflagellates (Dinophyta) of the Far East seas of Russia and adjacent areas of the Pacific Ocean. Dalnauka Press, Vladivostok.

Lackey, J. 1936. Some freshwater protozoa with blue chromatophores. *Biological Bulletin (Woods Hole)* **71**: 492-497.

Lackey JB, Lackey EW (1963) Microscopic algae and protozoa in the waters near Plymouth in August 1962. J Mar Biol Assoc UK 43: 797–805

Lackey, J.B. & Clendenning, K.A. 1965. Ecology of the microbiota of San Diego Bay. *Transactiond of the San Diego Society of Natural History* 14(2): 9-40

Lalli, C. and Parsons, T. R. 1997. Biological Oceanography: An Introduction Elsevier. 2nd Edition.

Lebour, M. V. 1917. The Peridiniales of Plymouth Sound from the region beyond the Breakwater. *Journal of the Marine Biological Association of the United Kingdom* **11**: 183–200.

Lebour, M. V. 1925. *The dinoflagellates of northern seas*. Marine Biological Association of the United Kingdom, Plymouth.

Lee, J. B. and Kim, G. B. 2017. New records of five unarmored genera of the family Gymnodiniaceae (Dinophyceae) in Korean waters. *Korean J. Environ. Biol.* 35: 273–288.

Lohmann, H. 1908. Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. *Wissenschaftliche Meeresuntersuchungen, Abteilung Kiel, Neue Folge* 10: 129–370.

Marangoni, C., Pienaar, R. N. and Sym, S. O. 2001. Possible introduction of alien phytoplankton via shipping ballast water: A South African perspective. *South African Journal of Botany* 67: 465–474.

Margalef R (1969) Small scale distribution of phytoplankton in western Mediterranean at the end of July. Publ Staz Zool Napoli 37: 40–61.

Meel, L. van. 1984. Les eaux saumâtres de Belgique : approches, progrès, perspectives. *Institut royal des sciences naturelles de Belgique*, no. 179.

Odebrecht, C. 2010. Dinophyceae. In Catálogo de Plantas e Fungos do Brazil. Vol. 1 (Forzza, R.C., Ed.). Andrea Jakobsson Estúdio, Instituto de Pesquisa Jardim Botánico do Rio de Janeiro. Rio de Janeiro, pp. 366–383.

Okolodkov, Y. B. 1998. Unarmoured dinoflagellates collected during the cruise of the r/v Oceania to the European Arctic, in June-August 1992 and 1993. *Polish Polar Research* **19**: 211–218.

Okolodkov, Y. B. 1998. A checklist of dinoflagellates recorded from the Russian Arctic seas. *Sarsia* **83**: 267–292.

Okolodkov, Y.B., Gárate-Lizárraga, I. (2006) An annotated checklist of dinoflagellates (Dinophyceae) from the Mexican Pacific. *Acta Botanica Mexicana* 74: 1–154.

Omura, T., Iwataki, M, Borja, V.M., Takayama, H. and Fukuyo, Y. 2012. Marine Phytoplankton of the Western Pacific. Kouseisha Kouseikaku, Tokyo.

Parke, M. and Dixon, P. S. 1976. Checklist of British marine algae - third revision. *Journal of the Marine Biological Association of the United Kingdom* **56**: 527–594.

Paulmier, G. 1994. Les dinophycées pélagiques et benthiques du Golfe de Gascogne sud de la Bretagne à Arcachon. *Annales de la Société des Sciences Naturelles de la Charente-Maritime* **8**: 289–357.

Paulsen, O. 1908. XVIII. Peridiniales. In: Nordisches Plankton (Brandt, K. & Apstein, C., Eds). Verlag von Lepsius & Tischer, Keil und Leipzig, pp. 1–124.

Peters, N., 1930. Peridinea. In Grimpe, Die Tierwelt der Nord- u. Ostsee, Teil II d2. Akad. Verlagsges, Leipzig. Rabenhorst, Kryptogamenflora, Bd. 10, 11. Akad.

Pouchet, G. 1883. Contribution à l'histoire des Cilio-Flagelles. *Journal de l'Anatomie et de la Physiologie Normales et Pathologiques de l'Homme et des Animaux* **19**: 399–455.

Pouchet, G. 1885. Contribution à l'histoire des Peridiniens marins. *Journal de l'Anatomie et de la Physiologie Normales et Pathologiques de l'Homme et des Animaux* **21**: 28–88.

Round, F. E. 1984. *The Ecology of Algae*. Cambridge University Press, 653 pp.

Skolka, V. H., Bodeanu N. and Roban A. 1986. Contributions to the systematic study of Mediterranean phytoplankton along the Libyan coasts. *Cercetari Marine/Rech. Mar. Constanta* **19**: 23–54.

Steidinger, K. A. and Tangen, K. 1997. Dinoflagellates. In: Tomas, C.R. (Ed.), *Identifying marine phytoplankton*, pp. 387–584. Academic Press, San Diego.

Steidinger, K. A., Faust, M. A. and Hernández-Becerril, D.U. 2009. Dinoflagellates (Dinoflagellata) of the Gulf of Mexico. In: D. L. Felder, D. K. Camp (Eds.), Gulf of Mexico: Origin, Waters, and Biota. Volume 1, pp. 131–154. College Station, Tex., Texas A&M University Press.

Takayama, H. 1998. Morphological and taxonomical studies on the free-living unarmored dinoflagellates occurring in the Seto Inland Sea and adjacent waters. PhD dissertation, The University of Tokyo, Tokyo, 211 pp.

Yamaji, I. 1984. *Illustrations of the Marine Plankton of Japan*. 3rd ed. Hoikusha Publishing Co. Ltd., Osaka, Japan.

Wailes, G. H. 1939. Canadian Pacific fauna. 1. Protozoa. le. Mastigophora. Univ. Toronto Press, for Fish. Res. Bd. Can. 45 p

Wood, E.J.F. 1963. Dinoflagellates in the Australian region. II – Recent collections, *Tech. Pap. Div. Fish. Oceanogr. CSIRO* **14**: 1–55.

Wood, E. J. F. 1963. Dinoflagellates in the Australian region. III – Further collections, *Tech. Pap. Div. Fish. Oceanogr. CSIRO* **17**: 1–20.

Wood, E. J. F. 1968. *Dinoflagellates of the Caribbean Sea and adjacent areas*. University of Miami Press, Coral Gables, 142 pp.

Wright, R. R. 1907. The plankton of eastern Nova Scotia waters; an account of certain floating organisms upon which young food fishes mainly subsist. Ann. Rep. Dept. Marine and Fish., Fisheries Branch, Ottawa, **39**: 1–18.

Molecular phylogeny and synonymy of *Balechina gracilis* comb. nov. (=*Gymnodinium gracile*), a widespread polymorphic unarmored dinoflagellate (Dinophyceae)

Fernando Gómez, Luis Felipe Artigas, Rebecca J. Gast

Appendix S2. Molecular methods

PCR amplification and sequencing

For molecular analyses, twenty cells of *Gymnodinium gracile* were micropipetted with a fine capillary into a 0.2 mL microcentrifuge tube filled with absolute ethanol. The samples were kept at room temperature and in darkness until the molecular analyses could be performed. The 0.2 mL microcentrifuge tubes containing the isolates of *Gymnodinium gracile* were briefly centrifuged and then opened to allow the ethanol to evaporate overnight on the benchtop in a covered container. Cells were resuspended in 10 μL extraction buffer (final concentration: 1 mg mL^{-1} bovine serum albumin, 10 mM Tris pH 7.4, 100 mM KCl, 1 mM EDTA, 50% glycerol). A negative extraction control was 10 μL of extraction buffer in a sterile 0.2 mL microcentrifuge tube. The tubes were frozen at −80 °C for 20 min followed by rapid warming to room temperature for 20 min. A 2 μL aliquot of the extracted product was used as DNA template for polymerase chain reaction (PCR) amplification. To amplify the SSU rRNA gene fragment, the primers EukA1 and EukB2 (Table 1) were used in a reaction with GoTaq polymerase (Promega Corp., Madison, WI, *USA*). For this reaction, the following thermocycler program was performed: initial denaturation at 94 °C for 5 min; 40 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 2 min; then final extension at 72 °C for 7 min. No product was obtained, so 1 µl of the reaction was re-amplified using primer pairs Euk A1/1200R and 373F/Euk B2. The cycles were the same as before.

To amplify the ITS/5′-LSU rRNA gene region of sample FG3, primers 570F and TW14 (Table 1) were initially used with the same conditions as the first round SSU rRNA gene amplification. A nested amplification was accomplished using the primer pair 1200F/TW14 (Table 1) using the same cycling parameters and 1 µl of the prior PCR reaction as template. For sample FG6, the ITS/LSU region was amplified using the primer pair A1/TW14 followed by reamplification with 892F/TW13. This product was cloned using the TOPO® TA Cloning® Kit for Sequencing (pCR™ 4-TOPO vector) (Invitrogen, Carlsbad, CA, USA), transformed into TOP10 competent cells and 6 colonies picked for sequencing.

The PCR products were cleaned up using the MinElute PCR Purification Kit (Qiagen Inc. Germantown, MD, USA) while cloned products were isolated using alkaline lysis and ethanol precipitation. All sequencing was accomplished by *GENEWIZ* Inc. (South Plainfield, NJ, USA). Chromatograms were checked and assembled using Sequencher v.5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA), and the contigs exported as FASTA files. The sequences for samples FG3 (18S, ITS and 5' LSU) and FG4 (18S only) were assembled from direct PCR product sequences, and the sequence for sample FG6 (18S, ITS and 5' LSU) is a combination of direct PCR products and 5 plasmid clones. Final sequences were deposited in DDBJ/EMBL/GenBank under accession numbers MW077537, MW077541−MW077542.

Table 1. List of primers used for initial amplification, nested PCR, and sequencing of the isolates of *Gymnodinium gracile* from the English Channel.

Primer name	**Sequence (5´-3´)**	**Use**	**Reference**
EukA1	AAY CTG GTT GAT YCT GCC AG	Initial amplification, nested PCR, sequencing	Modified from Medlin et al. (1988)
EukB2	GAT CCT KCT GCA GG TTC ACC TA	Initial amplification, nested PCR, sequencing	Modified from Medlin et al. (1988)
373F	GAT TCC GGA GAG GGA GCC T	Nested PCR, sequencing	Gast et al. (1994)
570F	GTA ATT CCA GCT CCA ATA GC	Nested PCR, sequencing	Gast et al. (1994)
892R	CCA AGA ATT TCA CCT CTG AC	sequencing	Gast et al. (1994)

892F	GTC AGA GGT GAA ATT CTT GG	Nested PCR, sequencing	Gast et al. (1994)
1200F	CAG GTC TGT GAT GCC C	Nested PCR, sequencing	Weekers et al. (1994)
1200R	GGG CAT CAC AGA CCT G	sequencing	Weekers et al. (1994)
TW13	GGT CCG TGT TTC AAG ACG	Nested PCR, sequencing	https://nature.berkeley.edu/brunslab/tour/primers.html
TW14	GCT ATC CTG AGG GAA ACT TC	Initial amplification, sequencing	https://nature.berkeley.edu/brunslab/tour/primers.html

Phylogenetic analyses

The small and large subunit rRNA gene sequences of *Gymnodinium gracile* were analysed using Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the GenBank database. The closest matches of the SSU rRNA gene sequence were environmental sequences (KJ763198, KJ763422, KJ763407, KJ76326) with a percentage of similarity higher than 98%. The closest documented sequence was *Pentapharsodinium tyrrhenicum* (AF022201) with only 96% of identity. When the first 1/3 of the sequence is trimmed (remaining the last 1200 base pairs), the closer relatives were also the sequence of *Balechina pachydermata* (KR139789–92) with a similarity that higher than 99%. Based on these results, SSU rRNA gene sequence alignments were constructed with the new sequences and those of *Balechina pachydermata*, and representatives of the main clades of unarmoured dinoflagellates, and other dinokaryotic dinoflagellates. In a second alignment, we included all the environmental sequences closer to the new sequences of *Gymnodinium gracile* according to a BLAST search using the full sequence or fragments.

The closest matches of the LSU rRNA gene sequence were two sequences named *Karenia* sp. (in: Dinophyceae) strain DAB07 (KU999990) and DAB08 (KU999991) with a percentage of similarity of 99.8%, followed by sequence of

Takayama acrotrocha (DQ656115) with a similarity of only 82.9%. Using a trimmed sequences of species of the thecate dinoflagellate *Pentapharsodinium* spp. and *Ensiculifera* with a percentage of similarity 88%, the thecate dinoflagellate *Heterocapsa* and unarmored species of *Karenia*, *Karlodinium* and *Takayama* with similarity of 85%. The identical sequences KU999990-1 to our sequence of *Gymnodinium gracile*. These sequences published in Baytut *et al.* (2016) are not strains, they are isolates that were directly sequenced. The morphology is not documented with micrographs. A BLAST search of the sequences KU999990-KU999991 showed as closer hits the sequences of the genera *Karlodinium* and *Karenia*, with a percentage of similarity of only 86% and 85%, respectively. These percentage are too low to assign these isolates to the genus *Karenia* or allied taxa. Based on these results, LSU rRNA gene sequence alignments were constructed with the new sequences of *Gymnodinium gracile*, the closer BLAST hits, and representatives of the main clades of unarmoured dinoflagellates, and other dinokaryotic dinoflagellates. The sequence of *Perkinsus marinus* was used as outgroup.

SSU- and LSU rRNA gene sequence alignments were accomplished by ClustalW (Larkin et al. 2007) and the evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model with Gamma Distributed (G) rates in MEGA7 software (Kumar et al. 2016). Bootstrap values were obtained after 1000 replications. The sequences of *Syndinium turbo* and *Perkinsus marinus* were used for rooting the SSU- and LSU rRNA gene phylogenetic trees, respectively.

References

Baytut, O., Gurkanli, C.T., Deniz, E., Özkoç, I. & Gönülol, A. 2016. First molecular records of potentially harmful planktonic dinoflagellates from the southern Black Sea. *Turk. J. Bot*. 40:546−56.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. **33**:1870−4.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**:2947−8.

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**:491−9.

Weekers, P. H. H., Gast, R. J., Fuerst, P. A. & Byers, T. J. 1994. Sequence variations in small subunit ribosomal RNAs of *Hartmannella vermiformis* and their phylogenetic implications. *Mol. Biol. Evol*. 11:684−90.