Hindawi
Journal of Marine Biology
Volume 2019, Article ID 1284104, 17 pages
https://doi.org/10.1155/2019/1284104

Research Article

Redefinition of the Dinoflagellate Genus *Alexandrium* Based on *Centrodinium*: Reinstatement of *Gessnerium* and *Protogonyaulax*, and *Episemicolon* gen. nov. (Gonyaulacales, Dinophyceae)

Fernando Gómez [1,2] **and Luis Felipe Artigas** [2]

[1] *Carmen Campos Panisse 3, E-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*

Correspondence should be addressed to Fernando Gómez; fernando.gomez@fitoplancton.com

Received 17 September 2019; Revised 26 October 2019; Accepted 23 November 2019; Published 31 December 2019

Academic Editor: Punyasloke Bhadury

The genus *Centrodinium* contains oceanic and predominantly tropical species that have received little attention. Three species of *Centrodinium* were examined using thecal plate dissociation, scanning electron microscopy, and molecular sequences. The apical horn of *Centrodinium intermedium* and *C. eminens* is formed by the elongation of the fourth apical plate, and a second apical split into two plates. In *C. punctatum* two apical plates (2′ and 4′) almost completely encircle the apical pore plate (Po), while the contact with the plate 1′ in the ventral side is much reduced, and the plate 3′ does not reach the Po. Moreover, its left posterior lateral sulcal plate is longer than its right pair, while reversed in the typical *Centrodinium* spp. The sulcal posterior plate of *C. punctatum* is located in the left-ventral side below the plates 1′′′and 2′′′, while the sulcal posterior plate located in the right face below the plates 4′′′ and 5′′′ in the typical *Centrodinium* spp. Phylogenetic analyses based on the small and large subunit of the rRNA gene showed that *Centrodinium* spp. and *Alexandrium affine*/*A. gaarderae* clustered as a sister clade of the *Alexandrium tamarense*/*catenella*/*fraterculus* groups. The clade of the subgenus *Gessnerium*, and the clade of the type species of *Alexandrium, A. minutum*, with four divergent species, clustered in more basal positions. The polyphyly of *Alexandrium* is solved with the split into four genera: (1) *Alexandrium* sensu stricto for the species of the clade of *A. minutum* and four divergent species; (2) the reinstatement of the genus *Gessnerium* for the species of the clade of *A. monilatum*; (3) the reinstatement of genus *Protogonyaulax* for the species of the *tamarense*/*catenella*/*fraterculus* groups, and (4) the new genus *Episemicolon* gen. nov. for *A. affine* and *A. gaarderae.* New combinations in the genera *Gessnerium*, *Protogonyaulax,* and *Episemicolon* are proposed.

1. Introduction

Dinoflagellates are ubiquitous protists that play diverse roles in marine ecosystems. Numerous studies are focused on species that are responsible for harmful algal blooms (HABs) in coastal waters. Paralytic shellfish poisoning (PSP) is generally regarded as the most well-known and widespread HAB syndrome, and is associated with toxins produced by certain dinoflagellate species in the genus *Alexandrium* [1]. Whilst neritic species of the planktonic *Alexandrium* or the epiphytic *Gambierdiscus*, responsible for toxic events, have received considerable attention, other open-ocean gonyaulacoid dinoflagellates remain under-investigated because of the paucity of material due to their low densities. The neritic HAB species of *Alexandrium* are typically non- or slightly compressed species, without horns or spines [2], while the oceanic gonyaulacoid dinoflagellates have horns and spines (*Ceratocorys* spp., *Gonyaulax taylorii*, etc.), and/or the cells are often flattened (i.e. *Gonyaulax pacifica*, [3]). Kofoid [3] described the genus *Centrodinium* for oceanic species characterized by a high laterally flattened cell body with an apical and an antapical horn. Kofoid [3] also described the genus *Murrayella* for three types of species: globular, biconical, and laterally compressed species. An account of the taxonomy of *Centrodinium* and *Murrayella* is reported in the Appendix S1 part 1–4, 7 as Supplementary material. Balech [4–6] carried out studies on *Centrodinium* and the laterally flattened species *Murrayella* and in 1967 commented on the general resemblance between *Centrodinium* and his new species *Murrayella mimetica*, but he maintained the split of both genera due to the differences in the plate formula following a strict Kofoidian scheme of tabulation. The classification of *Centrodinium* has been a matter of controversy ([7–9]; see Appendix S1 part 8 as Supplementary material).

In 2012, Gómez [10] classified *Centrodinium* in the same subfamily of *Alexandrium* within the Gonyaulacales. Li et al. [11] reported that *Centrodinium punctatum* unexpectedly clusters with *Alexandrium affine*, and consequently the genus *Alexandrium* was polyphyletic. Li et al. [11] did not propose the split of the genus *Alexandrium* because they were based only on *C. punctatum*. The species *C. punctatum* differs from the typical species of *Centrodinium* that are fusiform, with an elongated and high flattened body, and a smooth thecal surface. Li et al. [11] submitted the sequences as *Alexandrium* sp. (GenBank accession numbers MF043217–20), and they did not propose the split of *Alexandrium* as *C. puntactum* does not represent the typical morphology of the genus *Centrodinium*. Li et al. [11] remarked the need of the study for the typical species of *Centrodinium* before considering the generic split of *Alexandrium*.

In this study, we investigate the morphology of two highly laterally flattened species with apical and antapical horns, *C. eminens* and *C. intermedium*, and also *Centrodinium punctatum*. We provide the first molecular data (SSU and LSU rRNA gene sequences) for the typical species of *Centrodinium*. The new morphological and molecular data confirm the polyphyletic character of *Alexandrium*. We propose the split of *Alexandrium* into four genera that reconciles with the molecular and morphological data, and requires fewer taxonomical innovations. No taxonomical innovations are needed for the species comprising the clade that contains the type species of *Alexandrium*, *A. minutum*, which remains as *Alexandrium* s.s. The species of the clades that contain the type species of *Protogonyaulax* and *Gessnerium* are placed in the revived genera *Protogonyaulax* and *Gessnerium*, respectively. The species *A. affine* and *A. gaarderae*, closely related to *Centrodinium*, need to be transferred into a new erected genus.

2. Materials and Methods

2.1. Sampling, Isolation, and Microscopy. Sampling was performed with a phytoplankton net (20 μm mesh size) on the surface waters of the South-Eastern Bay of Biscay, North Atlantic, in August 2017. Samples from two stations at 43°36′ N–1°57′ W and 43°36′ N–2°03′ W are described here. The plankton concentrate was preserved with acid Lugol′s iodine solution to a final concentration of 4% (vol:vol), and kept refrigerated (~3°C). The material was examined with an inverted microscope (Nikon Eclipse TE2000-S, Tokyo) and photographed with a Nikon Digital Sight DS-2 M camera. After observing the presence of *Centrodinium* in these two sampling stations, subsamples of the plankton concentrate were treated with small amounts (150–200 μl) of 10% (weight/volume) sodium thiosulfate for removing the iodine. The cells of each species of *Centrodinium* were micropipetted individually with a fine capillary into a clean chamber filled with autoclaved Milli-Qultrapure water. The same procedure was repeated twice in order to remove any source of contamination. Finally, 30–40 cells of each species were deposited in a 0.2 ml Eppendorf tube filled with absolute.

For plate dissociation, each cell was individually isolated and placed in an Utermöhl chamber with distilled water. Drops of a solution of 5% sodium hypochlorite (commercial bleach solution, 1 : 1 mixture of sodium hypochlorite and Milli-Q water) were added until the split of the thecal plates. In other cases, the theca was squashed by touching it with a fine capillary tube to split the thecal plates. The cell was repeatedly photographed at different stages during the process of splitting the theca with the inverted microscope at 600x magnification.

For analyses using scanning electron microscopy, a subsample was filtered through a 3 μm pore size polycarbonate membrane (Millipore Ltd., Middlesex, U.K.). The filter was rinsed three times in Milli-Q water, dehydrated through graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and two steps in 100%). Then, the protocol was to immerse the filter in HMDS (Hexamethyldisilazane, Molekula Limited, Newcastle, U.K.) for 30 minutes (twice). The HMDS was evaporated by placing the sample overnight under the fume hood. Filters were mounted on an aluminium stub, sputter-coated with Au/Pd (Polaron SC7620, Quorum Technologies Ltd., Ashford, U.K.) and observed at 15 kV with a SEM LEO 438 VP (Carl Zeiss AG, Oberkochen, Germany). Images were presented on a black background using Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA, USA).

2.2. DNA Extraction, PCR Amplification of rRNA Gene and Sequencing. Prior to PCR, the sample tube was centrifuged, and ethanol was evaporated by placing the tube overnight in a desiccator at room temperature. Genomic DNA was extracted using Chelex (InstaGeneTM Matrix; Bio-Rad, Hercules, CA, USA) following protocols adapted from Richlen and Barber [12], as outlined in Gómez et al. [13]. The SSU rRNA gene was amplified using two sets of primers: EukA and 1055R; and 570F and EukB [14]. The D1–D3 domains of the LSU rRNA gene were amplified using primers D1R and D3Ca [15]. PCR amplifications were performed in a 25 μl reaction volume containing 1 μl of template DNA (supernatant from each Chelex extraction), 1 × PCR buffer (500 mM KCl and 100 mM Tris–HCl, pH 8.3), 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 mM of each primer, and 0.5 U of AmpliTaq DNA Polymerase (Applied Biosystems Inc., Foster City, CA, USA). Hot start PCR amplifications were performed in a Mastercycler Nexus thermal cycler (Eppendorf, Hamburg, Germany) with the following cycling conditions for both primer sets: initial denaturation (95°C/5 min); 35 cycles of denaturation (95°C/30 s), annealing (55°C/1 min), and extension (72°C/2 min); final extension (72°C/10 min). PCR products were visualized on a 1% agarose gel stained with GelRed (Biotium, Hayward, CA, USA).

Positive PCR products were cloned into vector PCR 2.1 using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Clones were screened for inserts by PCR amplification with plasmid primers M13F and M13R, and positive clones from each PCR amplicon were purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany), and sequenced in both the forward and reverse direction (Eurofins MWG Operon, Ebersberg, Germany). Sequence reads were aligned and assembled in Geneious Pro 11.1.2 (Biomatters, Auckland, New Zealand). The newly generated consensus sequences were deposited in DDBJ/EMBL/GenBank under accession numbers MK714074–MK714082.

2.3. Phylogenetic Analyses. SSU– and LSU rRNA gene sequences of *Centrodinium* spp. were analysed using Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) against databases in GenBank. The closest matches to these searches were sequences in the genus *Alexandrium* (primarily *A. affine*) and the sequences reported as "*Alexandrium* sp. ZL2017" that were later identified as *Centrodinium punctatum* in Li et al. [11]. Based on these results, rRNA gene sequence data were compiled from similar sequences identified using BLAST. Sequence alignments of available SSU– and D1-D2 LSU rRNA gene sequences of *Centrodinium* spp., representatives of each species of *Alexandrium*, other gonyaulacoid dinoflagellates, and other dinokaryotic dinoflagellates were accomplished by Clustal W [16] and the evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model with Gamma distributed with Invariant sites and the default settings in MEGA7 software [17]. Bootstrap values were obtained after 1000 replications. The apicomplexan *Eimeria tenella* (AF026388) was used as an out group in the SSU– and LSU rRNA gene phylogenies.

3. Results

3.1. Morphology of Centrodinium punctatum. The species *Centrodinium punctatum*, *C. intermedium,* and *C. eminens* were the most abundant (in that order) in the sampling stations in the South-Eastern Bay of Biscay (Figure 1(a)), providing material for the morphological (plate dissociation and SEM) and molecular analyses. A few individuals of *Centrodinium maximum* were also found, but in an insufficient abundance for detailed studies. The sea surface temperatures in the two sampling stations ranged from 23.4°C to 23.8°C and the salinity from 34.5 to 34.6. *Centrodinium punctatum* was the most abundant species compared with other congeneric taxa. The cells were slightly laterally flattened with a rhomboid shape. Cell dimensions were 65–90 μm long, 35–42 μm depth (dorso-ventral diameter), and 24–34 μm wide (length between the right and left lateral sides) (Figure 1(a)). The epitheca was conical with a blunt apex. The hypotheca was conical with a pointed antapex directed towards the ventral side. In addition to the size variability, the individuals showed a different degree of development the pointed antapex (Figure 1(b) and 1(c)). The theca was ornamented with poroids (Figures 1(d)–1(f), 1(l)–1(q)). *Centrodinium punctatum* had a plate formula Po, 4′, 6′′, 6c, 8s+, 5′′′, and 2′′′′. A more detailed description of the plate arrangement of *C. punctatum*, *C. intermedium,* and *C. eminens* is available in the Appendix S2 as Supplementary material. We describe here the apical, sulcal, and antapical plate series.

The plates 2′ and 4′ almost completely encircled the apical pore plate (Po), while the contact with the plate 1′ in the ventral side was much reduced, and the plate 3′ did not reach the Po (Figures 1(d)–1(f)). Scanning electron microscopy revealed a horseshoe-shaped apical pore surrounded by a rim of small marginal pores (Figures 1(q)–1(s)). The sulcal plates are placed between the anterior sulcal plate (S.a.) in the epitheca and the posterior sulcal plate (S.p.) near the antapex (Figures 1(g)–1(l), 1(p)). Two small plates known as the anterior and posterior median plates (S.m.a. and S.m.p.)—one above the other—occurred below the anterior sulcal and the left and right anterior lateral plates (S.s.a. and S.d.a.). Two lateral pairs of plates were located below, the left and right posterior lateral plates (S.s.p. and S.d.p.), with the left plate being longer than the right pair (Figures 1(h)–1(k)). The sulcal posterior (S.p.) was an irregular pentagon with length approximately equal to the width (Figures 1(f), 1(h)). The S.p. plate was displaced towards the left side below the plates 1′′′ and 2′′′ and the left margin joining to the plate 1′′′′ (Figures 1(h), 1(l) and 1(m)). There were two antapical plates with a triangular shape that conformed a pointed antapex directed towards the ventral side. The first antapical (1′′′′) in the left face (Figures 1(h), 1(l)–1(m)) was slightly smaller than the second antapical plate (2′′′′) in the right face (Figures 1(f), 1(i), 1(n)–1(o)). The plate 1′′′′ was in contact to S.p. and 2′′′′ plate (Figures 1(h), 1(l)–1(n)).

3.2. Morphology of Centrodinium intermedium. The lateral flattening of *C. intermedium* is probably the highest of the genus. The species also differed from the congeneric species in the contour of the hypotheca being oval to semicircular (Figures 2(a)–2(b)), while conical in the other species (Figure 1(a)). The apical horn of *C. intermedium* was usually shorter than the other species of *Centrodinium*. Cells were 130–175 μm long. The depth of the cells (dorso-ventral distance) was 55–80 μm. The width between the left and right sides is difficult to measure in these highly laterally flattened cells, with values of about 25–35 μm wide at the cingulum level (Figures 2(a)–2(c)). The dense poroid ornamentation of the theca observed in *C. punctatum* was missing in *C. intermedium*, with only scattered pores, more abundant in the right face of the apical horn (Figure 2(d)). The apical horn (~20 μm long) of *C. intermedium* was a short truncated cone (Figures 2(a)–2(d)). The antapical horn was longer (>50 μm) and directed towards the left-ventral side. Consequently, the antapical horn was in a different same plane than the main body and the apical horn (Figure 2(c)). The antapical horn had a triangular section with a slight anticlockwise torsion, and three terminal spinules (Figures 2(m)–2(o)). Each face of the antapical horn had a row of sunken areas with 3-4 small pores (Figure 2(n)).

The molecular data revealed a very close phylogenetical relationship between *C. punctatum* and *C. intermedium* (see below in Figures 4 and 5). It is commonly assumed that congeneric species share a similar plate formula. The epithecal plate formula of *C. punctatum* is Po, 4′, 6′′ or alternatively 3′,

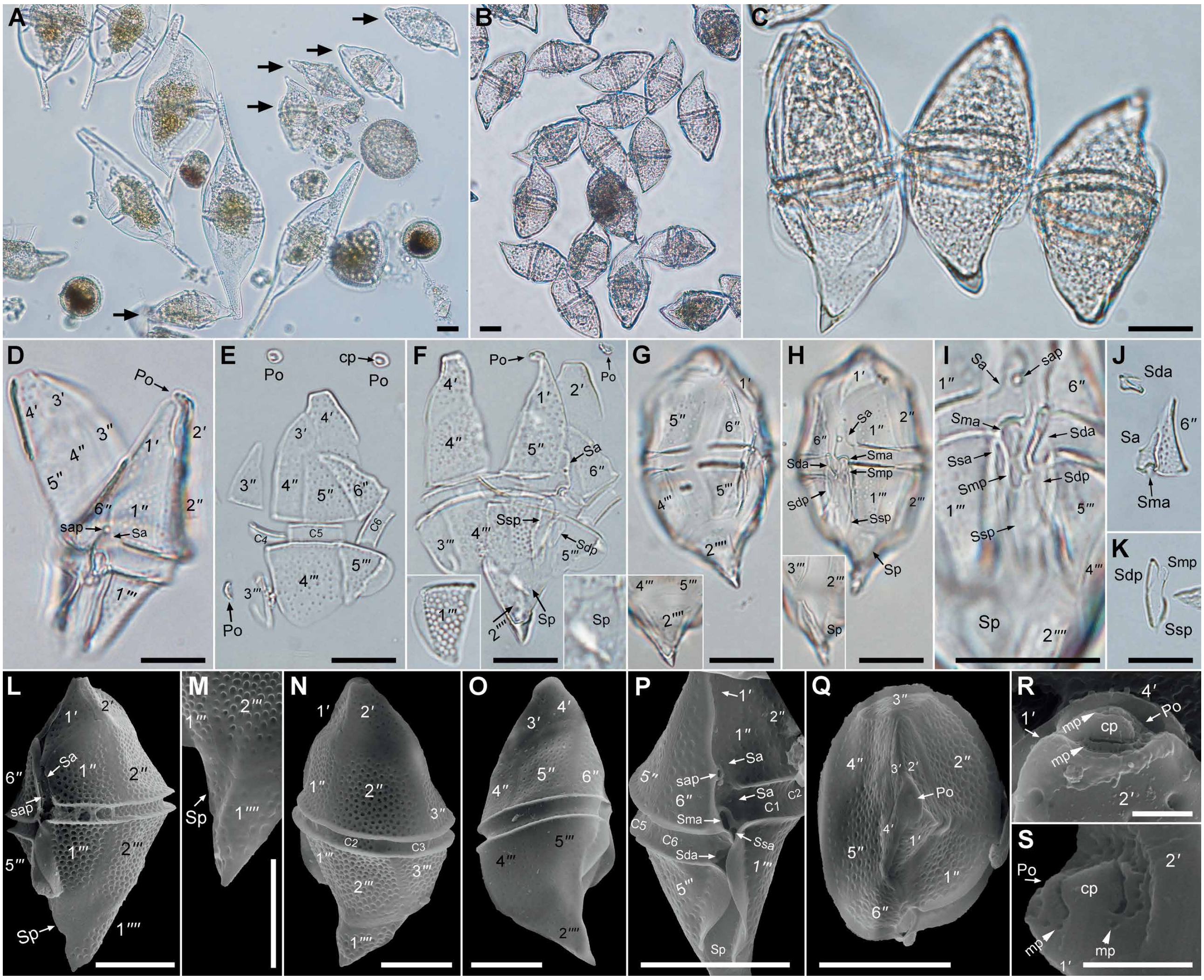


Figure 1: Light (a–j) and scanning electron (l–s) micrographs of *Centrodinium punctatum*. (a) Plankton sample with *Centrodinium* spp. The arrows point the cells of *C. punctatum*. (b–c) Individuals used for molecular analyses. (d) Partially dissociated theca in ventral view. (e–f) Epitheca. The insets show the first postcingular plate and the posterior sulcal plate. (g) Right-ventral face. The inset shows the right antapex. (h) Left-ventral view. The inset shows the dorsal antapex. (i) Ventral view of the sulcal area. (j-k) Dissociated sulcal plates. (l) Left-ventral view. (m) Left antapex. (n) Left face. (o) Right face. (p) Ventral view. (q) Apical view. (r–s) Apex. 1′–4′ = apical plates; 1″–6″ = precingular plates; 1‴–5‴ = postcingular plates; 1⁗–2⁗ = antapical plates; C1–C6 = cingular plates; c.p. = closing, cover platelet or canopy; mp = marginal pores surrounding the apical pore plate; Po = apical pore plate; S.a. = anterior sulcal plate; s.a.p. = pore of the anterior sulcal plate; S.d.a. = right (dexter) anterior lateral sulcal; S.d.p. = right posterior lateral sulcal; S.m.a. = anterior median sulcal; S.m.p. = posterior median sulcal; S.p. = posterior sulcal plate. S.s.a. = left (sinister) anterior lateral sulcal; S.s.p. = left posterior lateral sulcal. Scale bars (a–q) = 20 μm, (r–s) = 2 μm.

1a, 6″ in a strict Kofoidian scheme. The species *C. intermedium* has an additional plate in the left face of the epitheca, and the plate formula in a strict Kofoidian scheme is Po, 2′, 2a, 7″. In contrast to *C. punctatum*, the apical plates of *C. intermedium* were larger than the precingular plates (Figure 2(d)). The first apical plate of *C. punctatum* reached the apical pore (insert 1′), while in *C. intermedium* it does not reach the apex (exsert 1′). When compared to *C. punctatum*, the main modifications of *C. intermedium* were the elongation of the plates 4′ and 2′ (the latter split into two plates) to conform the apical horn, the different length of the posterior lateral sulcal plates, and the formation of a tubular antapical horn supported at its ventral basis by two triangular plates. The apical plates 2′ and 4′ of *C. intermedium* have extended anteriorly to conform the apical horn, and the development of these plates hindered that the plates 1′ and 3′ reached the apex (Figures 2(d), 2(o)–2(q)). While the plate 4′ was narrow and long, the elongation of the plate 2′ resulted in the split into two plates. The formula of the epitheca of *C. punctatum* and *C. intermedium* is similar (Po, 4′, 6″), using the labelling 2′ ($\alpha+\beta$) to denote the split of the second apical plate in *C. intermedium*. The right side of the epitheca was essentially similar to *C. punctatum*, where 4′ plate has expanded anteriorly, and then the 3′ plate did not reach the apex (Figure 2(d)). During the plate dissociations, the Po remained attached to the plate 2′β as a circular structure of about 1 μm in diameter (Figure 2(d)). The tiny membranous Po platelet was poorly conserved in the SEM preparations. The very thin plate 2′β appeared crushed against

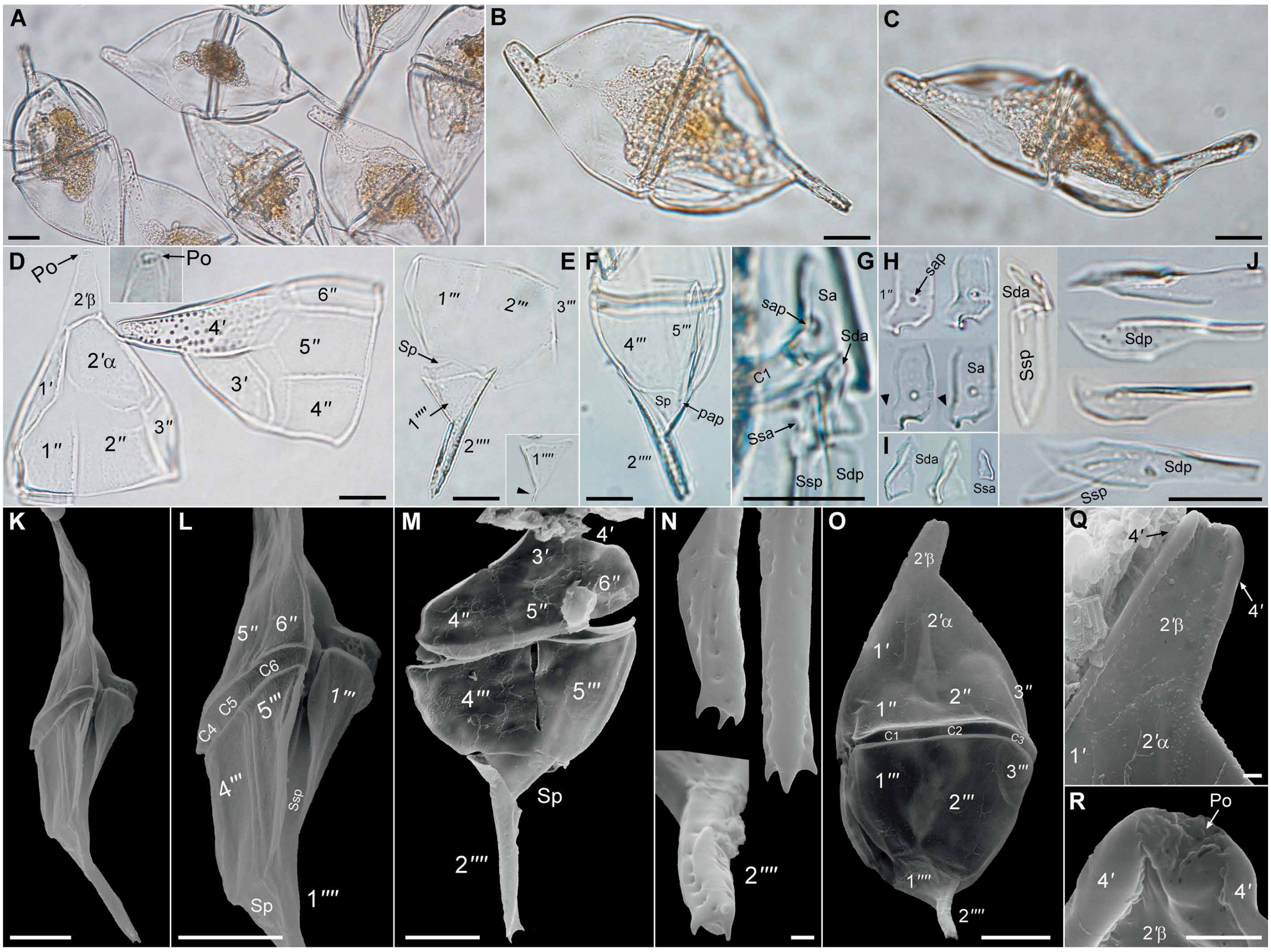


Figure 2: Light (a–j) and scanning electron (k–r) micrographs of *Centrodinium intermedium*. (a) Several individuals. (b) Left face. (c) Left-ventral view. Note the antapical horn oriented toward the left side. (d) Left and right faces of the same epitheca. The inset shows the apex. (e) Left hypotheca. The inset shows the first antapical plate. The arrowhead points a filiform extension. (f) Right hypotheca. (g) Detail of the sulcal area. (h) Dissociated anterior sulcal plates. The arrowheads point a membranous flange. (i) Anterior lateral sulcal plates. (j) Dissociated posterior lateral sulcal plates. (k–l) Ventral view. (m) Right face. (n) Different antapical horns. (o) Left face. (q–r) Detail of the apex. $1'$–$4'$ = apical plates; $1''$–$6''$ = precingular plates; $1'''$–$5'''$ = postcingular plates; $1''''$–$2''''$ = antapical plates; C1–C6 = cingular plates; Po = apical pore plate; S.a. = anterior sulcal plate; s.a.p. = pore of the anterior sulcal plate; S.d.a. = right (dexter) anterior lateral sulcal; S.d.p. = right posterior lateral sulcal; S.m.a. = anterior median sulcal; S.m.p. = posterior median sulcal; S.p. = posterior sulcal. S.s.a. = left (sinister) anterior lateral sulcal; S.s.p. = left posterior lateral sulcal plate. Scale bars (a–m, o) = 20 μm, (n, q–r) = 2 μm.

the thick plate $4'$ (Figures 2(q)–2(r)). The antapex of *C. punctatum* and *C. intermedium* showed differences. The posterior hypotheca of *C. intermedium* was composed of three plates: a tubular plate that conforms the antapical horn and two plates in the ventral side that acted as a counterfort or backstay. These two triangular plates were slightly laterally inclined towards the left face, and the antapical horn was directed towards the left and ventral sides (Figures 2(e)–2(f), 2(k)–2(o)). The most immediate interpretation was that the antapex consists one antapical plate that conforms the horn, and two posterior intercalary plates that support the ventral basis of the antapical horn. This implies that the posterior sulcal plate (S.p.) was missing in *C. intermedium*. In *C. punctatum*, the S.p. was an irregular pentagon located in the left-ventral side below the plates $1'''$ and $2'''$ (Figures 1(f), 1(i), 1(l)–1(m)), while the S.p. of *C. intermedium* was triangular and located in the right face below the plates $4'''$ and $5'''$ (Figures 2(f), 2(k)–2(m)). There was a pore, the posterior attachment pore, located in this triangular plate in the right face (Figure 2(f)), which is a characteristic of the posterior sulcal plate of chain-forming gonyaulacoid dinoflagellates. In the left face, the first antapical ($1''''$) was a triangular plate that often showed a posterior filiform extension (Figure 1(e)). The second antapical plate ($2''''$) emerged from the dorsal side to conform a tubular antapical horn (Figures 2(e)–2(f)), with a slight anticlockwise torsion and three terminal spinules (Figures 2(m)–2(n)).

In the sulcal plate series, the anterior sulcal plate (S.a.) was part of the epitheca, enclosed between the plates $6''$, $1'$, and $1''$ and the first cingular plate (Figures 2(g)–2(h)). There was a prominent pore in the middle of the plate connected to the right border by a narrow canal. In some cells, the right posterior corner of the S.a. showed a membranous flange that connected with the first cingular plate (Figure 2(h)). The right anterior lateral sulcal plate (S.d.a.) was larger than its left pair, with the shape of an irregular right triangle that resembled the shape of the Sicily Island (Figures 2(g), 2(i)). In *C. punctatum*, the left posterior lateral sulcal plate (S.d.p.) was longer than its right pair (Figures 1(i), 1(k)), while reversed in *C. intermedium* (Figure 2(j)). The right posterior sulcal plate (S.d.p.) of *C. intermedium* was the longest of the sulcal series and showed the shape of a knife, with a reinforcement in the left margin (Figure 2(j)). The left posterior sulcal plate (S.s.p.) was smaller, like a very elongated pentagon that fit in the knife handle formed by the anterior left margin of the S.d.p. (Figure 2(j)). The morphology of these plates suggests that the overlap growth of the S.d.p. has hindered the posterior development of the S.s.p.

3.3. Morphology of Centrodinium eminens. In lateral view, the cells of *C. eminens* were fusiform and slightly sigmoid because the apical horn was slightly directed towards the dorsal side, and the antapical horn towards the ventral side. The ventral margin of the epitheca was almost straight. The dorsal margin was curved in the posterior half and almost straight in the anterior half where the apical horn with a brunt apex was slightly directed towards the dorsal side (Figure 3(a)). The cells of *C. eminens* were 182–239 μm long, and 31–47 μm in depth (dorso-ventral distance), being less robust (lower depth), and less flattened than *C. intermedium*. The apical and antapical horns of *C. eminens* were longer (Figure 3(a)) than in *C. intermedium* (Figure 2(a)). The antapical horn of *C. intermedium* was very inclined towards the face (Figure 2(c)), while the inclination was almost absent in *C. eminens* (Figures 3(a)–3(h)). The plate arrangement of *C. eminens* and *C. intermedium* was similar, with more anterior-posteriorly elongated plates, especially in the apical series in *C. eminens* (Figures 3(b)–3(l), 3(t)–3(v)). The two plates, $2'$ $(\alpha+\beta)$, resulting of the split of the second apical plate remained joined (Figure 3(d)). The distal antapical horn also showed three spinules (Figure 3(u)). The sulcal series was fully similar (Figures 3(m)–3(q)). The triangular first antapical and the posterior sulcal plates showed a filiform posterior extension (Figures 3(r)–3(s)). The posterior sulcal plate showed a posterior attachment pore (Figures 3(r)–3(s)). In the SEM preparations, some individuals of *C. eminens* were in better preservation stage than those of *C. intermedium*, and some details of the apex were revealed (Figures 3(w)–3(z)). The apex of *C. eminens* also collapsed in the SEM preparations but in some individuals the membranous apical pore platelet and the thin second antapical were not crushed against the thicker four apical plate. In these cases, a large pore of 1–1.5 μm in diameter was observed devoid of the cover platelet (Figures 3(w)–3(x)). This membranous cover platelet remained in few individuals, with the apical pore surrounded by a few tiny pores (Figures 3(y)–3(z)).

3.4. Molecular Phylogeny. The SSU and LSU rRNA gene sequences were obtained from three species of *Centrodinium*: *C. punctatum* that is the first described laterally flattened species of the former genus *Murrayella*; *C. intermedium* that is the most flattened species of this genus with an oval hypotheca, and *C. eminens* which morphology is close to the type species, *C. elongatum*. It should be noted that the type species remains unreported since the original description in 1907. It seems likely that *C. elongatum* corresponds to a recently divided cell of *C. maximum* or *C. eminens* (see Appendix S1 part 2 in the Supplementary material).

In the SSU rRNA gene phylogeny, the three species of *Centrodinium* clustered together with high support with *C. punctatum* in a basal position. The *Centrodinium* spp. clade clustered with *Alexandrium affine*, with strong support (BP 100%) (Figure 4). In the LSU rRNA gene phylogeny, *Centrodinium* spp. also clustered with sequences retrieved from GenBank as *Alexandrium affine* and *A. concavum* (Figure 5). In an additional LSU rRNA tree more reference sequences were added from GenBank within the *A. affine* clade to include sequences identified as *A. affine*, *A. tamarense,* and *A. concavum* (Figure S1 as Supplementary material). The strains CAWD51-52 diverged from the other sequences of *A. affine*. In the SSU- and LSU rRNA gene phylogenies (Figures 4–5), the species of the *tamarense/catenella/fraterculus* groups of *Alexandrium* clustered with high support as a sister group to the *Centrodinium* spp. and *A. affine* clades. The clade of *Alexandrium* sensu stricto (s.s.) containing the type, *A. minutum*, and four divergent species (*A. diversaporum*, *A. leei*, *A. margalefii*, *A. pohangense*). The species of the subgenus *Gessnerium*, *A. monilatum* and allied species, formed other clade (Figures 4–5).

4. Discussion

4.1. Affinities between Centrodinium and Alexandrium. The molecular data reveal that *Centrodinium* clusters with strong support amongst the clades of *Alexandrium* (Figures 4–5; [11]). Species such as *C. punctatum* have the same plate formula of *Alexandrium* (Figures 1, 6(e)). The most typical apical pore plate of *Alexandrium* has a comma-shaped pore surrounded by marginal pores, and the chain-forming species have an anterior attachment pore [18]. The apical pore plate of *Alexandrium* is larger (>6 μm), and we can easily observe an oval or comma-shaped pore. The formation of the apical horn of *Centrodinium* implies a reduction of the surface available for the apical pore plate (<2 μm), and the horseshoe-shaped could be a result of the constriction of the oval or comma-shaped pore (Figures 1(r)–1(s), 3(y)–3(z)).

The chain-forming species of *Alexandrium* have an attachment pore (a.a.p.) in the apical pore plate, and an attachment pore (p.a.p.) in the posterior sulcal plate. The cells of a chain are interconnected by these pores [18]. In *Centrodinium*, the anterior attachment pore is more difficult to observe due to the small size and fragility of the membranous apical platelet, or it may be confused with marginal pores. Hernández-Becerril et al. ([9], their Figure 33) reported a pore in the apex that could be the apical pore devoid of the foramen, or alternatively the anterior attachment pore. The posterior attachment pore in the posterior sulcal plate is evident in *C. intermedium* and *C. eminens* (Figures 2(f), 3(r)–3(s)), and *C. pulchrum* ([9], their Figure 37).

The sequences of *Centrodinium* clustered as a sister group to *Alexandrium affine* (Figures 4 and 5; [11]). That clade includes sequences retrieved from GenBank under the names

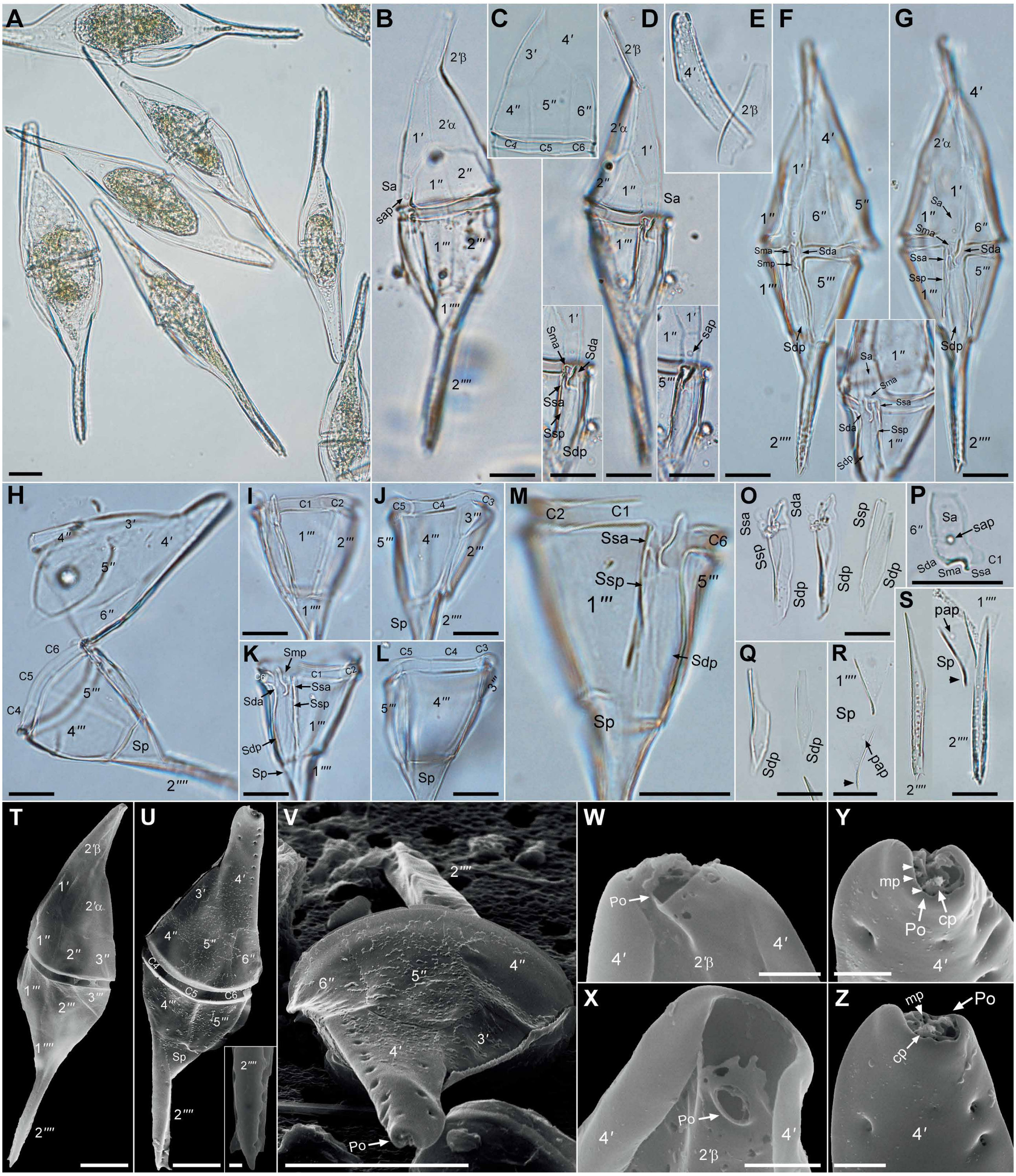


Figure 3: Light (a–s) and scanning electron (t–z) micrographs of *Centrodinium eminens*. (a) Several individuals. (b) Left face. (c) Right epitheca. (d) Left-ventral view. The insets show the sulcus. (e) Dissociated plates of the apical horn. (f–g). Ventral views. The inset shows the sulcus. (h) Dissociated epitheca and hypotheca. (i–m) Several views of the same epitheca. (o, q) Dissociated posterior lateral sulcal plates. (p) Anterior sulcal. (r) Posterior sulcal and first antapical plate. The arrowhead points a filiform extension. (s) Antapical horn. (t) Left face. (u–v) Right face. (w–z) Apex. 1′–4′ = apical plates; 1′′–6′′ = precingular plates; 1′′′–5′′′ = postcingular plates; 1′′′′–2′′′′ = antapical plates; C1–C6 = cingular plates; c.p. = closing, cover platelet or canopy; mp = marginal pores surrounding the apical pore plate; Po = apical pore plate; S.a. = anterior sulcal plate; s.a.p. = pore of the anterior sulcal plate; S.d.a. = right (dexter) anterior lateral sulcal; S.d.p. = right posterior lateral sulcal; S.m.a. = anterior median sulcal; S.m.p. = posterior median sulcal; S.p. = posterior sulcal; S.s.a. = left (sinister) anterior lateral sulcal; S.s.p. = left posterior lateral sulcal plate; Scale bar (a–v) = 20 *μ*m, (w–z) = 2 *μ*m.

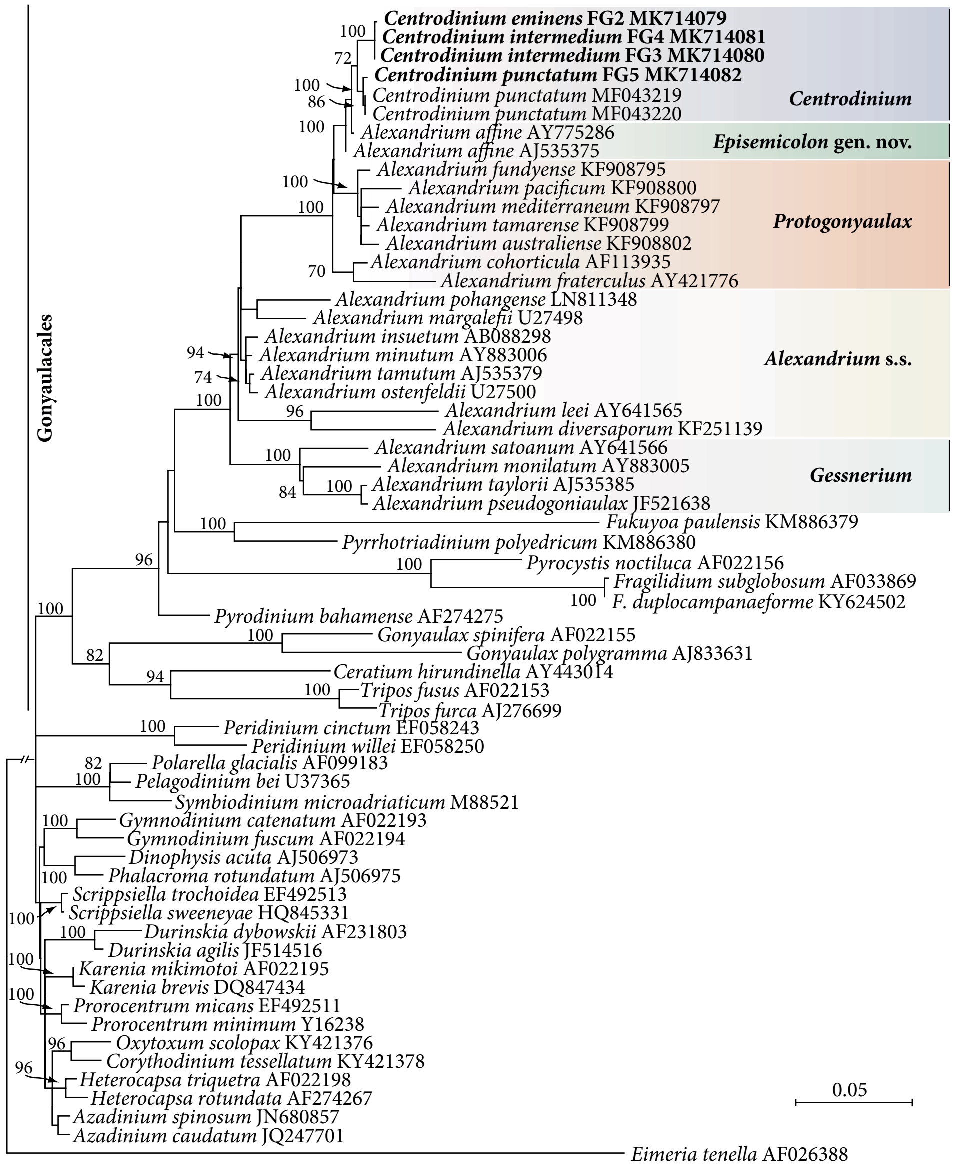


Figure 4: Maximum-likelihood phylogenetic tree of the SSU rRNA gene. Bootstrap support values (BP) >70 are shown. New sequences are highlighted in bold. The scale bar represents the number of substitutions for a unit branch length.

A. affine, *A. tamarense*, and *A. concavum*. Two sequences from New Zealand, the strains CAWD51 named *A. affine* (accession number AY338753) and CAWD52 named *A. concavum* (accession number AF032348) were identical and diverged from the main group of *A. affine*. The latter subdivided into two groups, one for strains isolated exclusively from Japan and China, and other group for strains from diverse world regions (Figure S1). The cells of the strain CAWD52 illustrated in MacKenzie et al. [19] corresponded to *A. gaarderae* as defined by Larsen and Nguyen-Ngoc [20]. The species *Alexandrium affine* was first described as *Protogonyaulax affinis* [21], and since the earlier molecular phylogenies the sequences of *A. affine* have always diverged from the members of the *tamarense*/*catenella* group [22]. The species *A. affine* and *A. gaarderae* (non *A. concavum* emend. Nguyen-Ngoc & Larsen) clustered as a sister group of *Centrodinium* and more distantly related to the clade of *Protogonyaulax* (Figure 5, S1).The members of the *tamarense*/*catenella* group are responsible for paralytic shellfish poisoning (PSP) events. The sxtA gene (saxitoxin biosynthesis pathway protein A domain) has been detected in the members of the *tamarense*/*catenella* group or *A. fraterculus*. In contrast, PSP toxicity or the presence of the sxtA gene have not been detected in *A. affine* [23] and *Centrodinium punctatum* [11]. *Alexandrium affine* is distinguished primarily by the apical pore plate and other differences in the sulcal plates. Balech [18] reported that the apical pore platelet is narrow, long, and fundamentally bullet-shaped. The foramen does not form a true comma because it is oval and relatively small; it is located in the ventral half of the plate and a large and almost circular connecting pore is dorsal [18]. *Alexandrium gaarderae* (reported as *A. concavum*) also has a dorsal connecting pore [24]. The location of the anterior attachment pore at the dorsal

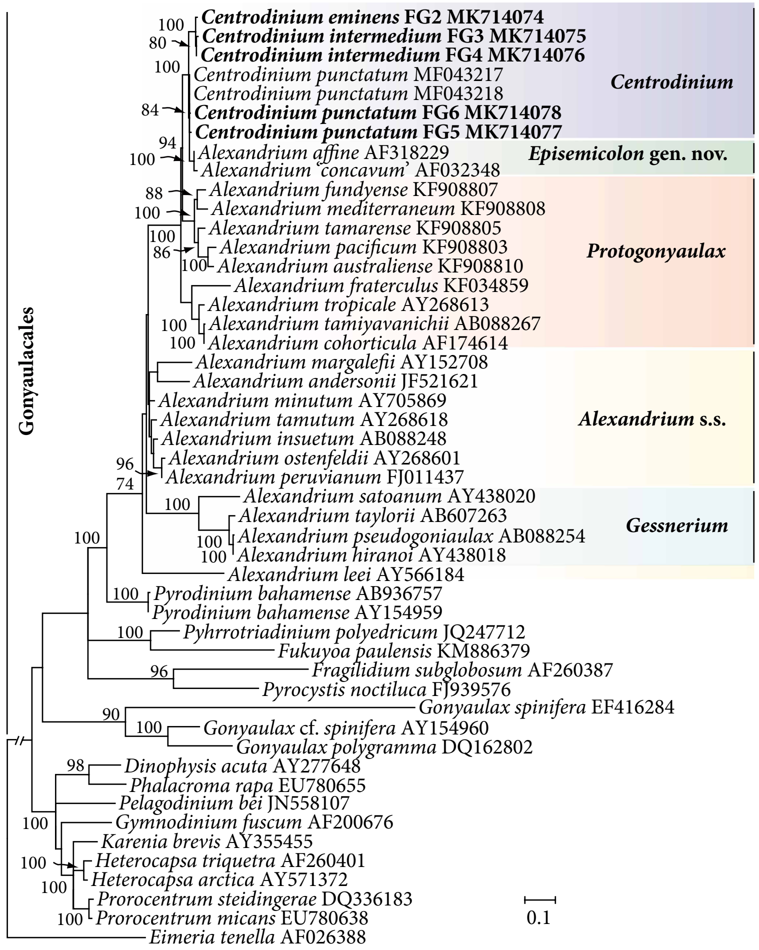
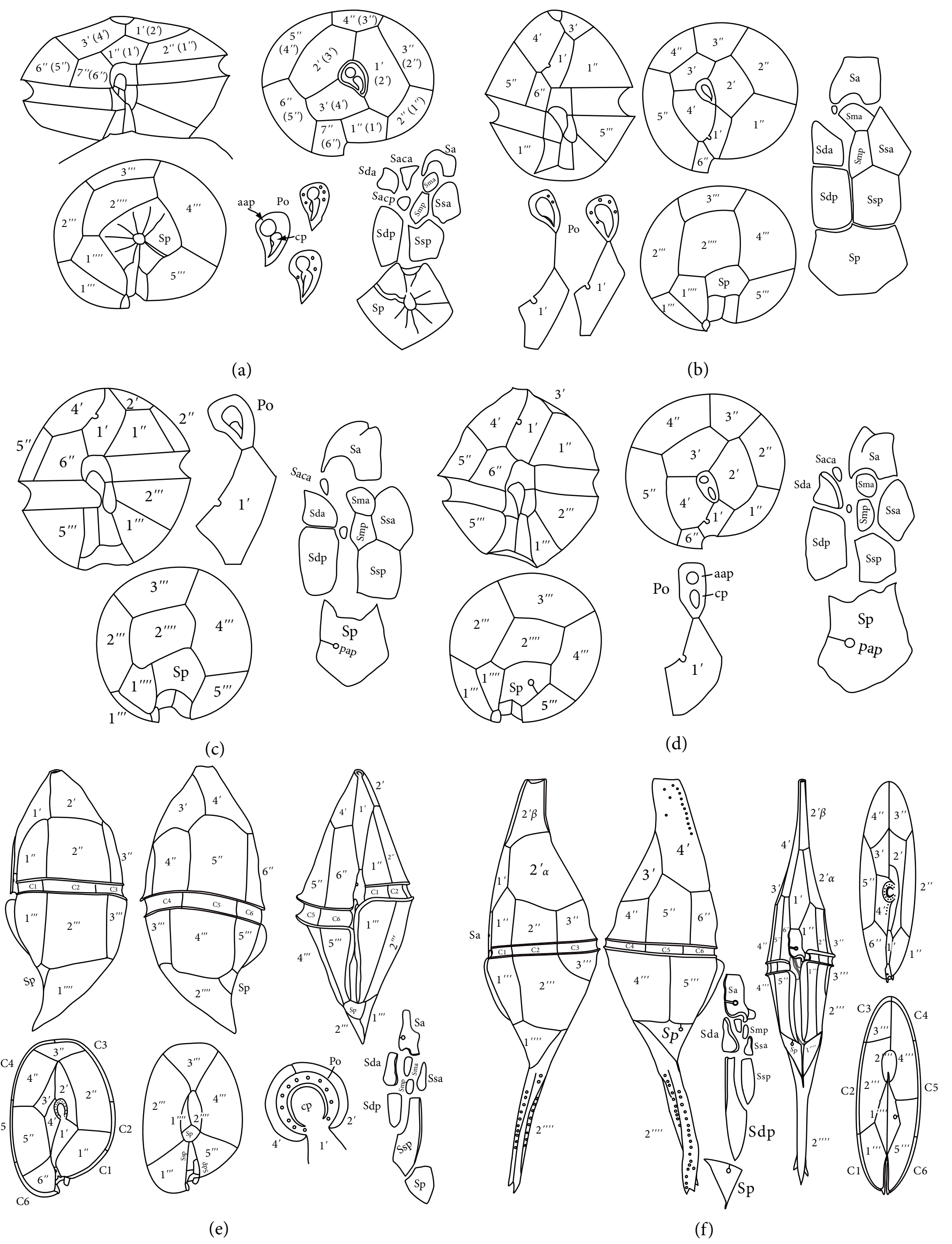


Figure 5: Maximum-likelihood phylogenetic tree of the D1-D2 domains of the LSU rRNA gene. Bootstrap support values (BP) >70 are shown. New sequences are highlighted in bold. The scale bar represents the number of substitutions for a unit branch length.

margin of the apical pore plate is the main diagnostic character of the species *A. gaarderae* and *A. affine* [24]. In the other species of *Alexandrium*, the apical pore is comma-shaped and the anterior attachment pore lying in the right side. The two posterior lateral sulcal plates are more or less similar in length in the members of the *tamarense*/*catenella* group, while the right posterior sulcal plate is longer than the left posterior sulcal plate in *A. affine* (Figure 6(d)). This feature is variable in *Centrodinium* spp. (Figures 6(e) and 6(f)). The cingulum and the sulcus of *Centrodinium* spp. and *A. affine* are deeply incised and bordered by pronounced list, and the posterior left margin of the plate 6′′ is reinforced, long and concave (Figures 1–3, 6(d)–6(f); [20, 21]).

4.2. Reclassification of the Subgenus Gessnerium. An historical account of the taxonomy and nomenclature of *Alexandrium* s.l., including *Gessnerium* and *Protogonyaulax*, is reported in the Appendix S3 as Supplementary material. The plate formula of *Alexandrium* is usually reported as Po, 4′, 6′′, 6c, 8s+, 5′′′, 2′′′′ [25]. It is similar to the plate formula of *C. punctatum* and differs from the more flattened species of *Centrodinium* in the anterior elongation of the plates 4′ and 2′, and the split in the latter plate. Li et al. [11] reported the plate formula of *C. punctatum* as Po, 3′, 1a, 6′′, 6c, 8s, 5′′′, 1p, 2′′′′. These authors follow a strict Kofoidian scheme of tabulation of the epitheca, and labelled the apical plate that does not touch the apical pore plate as an intercalary plate. Li et al. [11] misidentified the sulcal and hypothecal plates. Li et al. ([11], p. 177, their Figure 8(c)) illustrated the right (S.d.p.) and left posterior sulcal (S.s.p.) plates with a similar length. Li et al. [11] did not carry out a study using plate dissection, and the sulcal lists were hiding the morphology of the sulcal plates. These plates have very different length as revealed in this study (Figure 1(k)) and the plate dissections of *C. punctatum* by Balech [5, 6]. They labelled the left lateral posterior sulcal as the posterior plate, and this induces the subsequent errors in the tabulation of the hypotheca (see Appendix S1 part 6 as Supplementary material).

The genus *Alexandrium* is currently a pool of species with significant differences in the plate arrangement [26]. Balech [18] reported that the species of the subgenus *Gessnerium* were closer to *Pyrrhotriadinium* than *Alexandrium*. The apical pore plate in *Pyrrhotriadinium* is totally transverse orientated, while oblique in *Gessnerium* [18, 27]. *Pyrrhotriadinium* lacks the accessory sulcal plates, and the two median sulcal plates are separated, while in *Gessnerium* the accessory plates are prominent and the two median sulcal plates are in contact [18].



(a) (b) (c) (d) (e) (f)

FIGURE 6: Line drawings of the ventral, apical and antapical views, apical pore plate and sulcal plates of *Alexandrium* sensu lato and *Centrodinium*. (a) *Gessnerium monilatum* redrawn and modified from Balech (1995). (b) *Alexandrium minutum* redrawn and modified from Balech (1989, 1995). (c) *Protogonyaulax tamarensis* redrawn and modified from Balech (1995). (d) *Episemicolon affine* gen. & comb. nov. (formerly *A. affine*) redrawn and modified from Balech (1995). (e) *Centrodinium punctatum*. (f) *Centrodinium eminens*. $1'$–$4'$ = apical plates; $1''$–$6''$ = precingular plates; $1'''$–$5'''$ = postcingular plates; $1''''$–$2''''$ = antapical plates; a.a.p. = anterior attachment pore; C1–C6 = cingular plates; c.p. = closing, cover plate or canopy; p.a.p. = posterior attachment pore; Po = apical pore plate; S.a. = anterior sulcal plate; S.a.c.a. = anterior accessory sulcal; S.a.c.p. = posterior accessory sulcal; S.d.a. = right (dexter) anterior lateral sulcal; S.d.p. = right posterior lateral sulcal; S.m.a. = anterior median sulcal; S.m.p = posterior median sulcal; S.p. = posterior sulcal; S.s.a. = left (sinister) anterior lateral sulcal; S.s.p. = left posterior lateral sulcal.

In *Pyrrhotriadinium*, the first precingular plate, equivalent to the first gonyaulacoid apical plate, does not contact the left apical plate [18]. The $1''$ plate is pentagonal in *Gessnerium* (Figure 6(a)) and quadrangular in *Pyrrhotriadinium*, while rhomboidal in nearly all the species of the subgenus *Alexandrium* (Figures 6(b)–6(c)). This is a precingular plate based on its shape and position. The posterior sulcal plate of *Gessnerium* is large, longer than wide and prolonged obliquely towards the posterior right (Figure 6(a)). In the species of the subgenus *Alexandrium*, the posterior sulcal plate is relatively smaller and non-oblique ([18]; Figures 6(b)–6(d)). PSP toxicity has not been reported in species of *Gessnerium*.

The species *Alexandrium margalefii* and *A. pohangense* have the first apical plate disconnected from the apical pore plate, which suggests an affinity with *Gessnerium*, but this plate is quadrangular in these species while pentagonal in *Gessnerium* [18, 23]. The position of *A. margalefii* and *A. pohangense* in the molecular phylogenies is unstable, typically is represented as divergent species of the clade of the type, *A. minutum* [23]. These two species, and other two divergent species (*A. diversaporum*, *A. leei*) need further research before to propose a change of genus.

4.3. The Generic Split of the Subgenus Alexandrium. Previous morphological and molecular phylogenetic studies including sequences of *Pyrodinium* already suggested the reinstatement of the *Gessnerium* at the genus level ([28, 29], Appendix S3 as Supplementary material). With the inclusion of *Centrodinium* spp. in the molecular phylogenies, *Alexandrium* can no longer be considered as a monophyletic genus (Figures 4 and 5). The morphological differences amongst species of the subgenera *Gessnerium* (Figure 6(a)) and *Alexandrium* (Figures 6(b)–6(d)) are evident, but a split between species of the subgenus *Alexandrium* based on morphology is less conspicuous. In the molecular phylogenies, the sequences of the subgenus *Alexandrium* are divided into two major groups. A group that contains the type species of *Alexandrium*, *A. minimum*, and another group split into two sister clades: one major clade that contains the type species of *Protogonyaulax*, *P. tamarensis*, with members of the *tamarense/catenella/fraterculus* groups, and other major clade for *Centrodinium* and *Alexandrium affine/A. gaarderae* (Figures 4–5). Sequences of the members of the subgenus *Alexandrium* do not cluster as a monophyletic group, unless we consider placing all the species of the subgenus *Alexandrium* into *Centrodinium* because *Centrodinium* Kofoid 1907 has the priority over *Alexandrium* Halim 1960. This implies numerous taxonomical innovations, and requires merging species with different morphologies into a single genus. Splitting members of the subgenus *Alexandrium* into three genera reconciles the molecular and morphological data, and requires fewer taxonomical innovations.

The clade *Alexandrium* s.s. contains the type species, *A. minutum*, and other species in which the first apical plate is rhomboidal and may connect directly or indirectly through a thread-like prolongation with the apical pore plate (Figure 6(b), Table 1). This feature may vary intraspecifically as reported for *A. minutum* [30]. The posterior sulcal plate is relatively small, symmetrical or asymmetrical, and wider than long. The anterior and posterior attachment pore is a common feature in chain-forming species, but few species of *Alexandrium* s.s. forms chains, and the attachment pores are absent (Figure 6(b), Table 1; [18, 24]).

The genus *Protogonyaulax* contains species where the first apical plate is rhomboidal and directly connects to the apical pore plate. The posterior sulcal plate is reversed pentagonal, symmetrical, and longer than wide. There are numerous chain-forming species, and the presence of anterior and posterior attachment pores is a common feature (Figure 6(c), Table 1; [18, 24]).

The new scenario derived on the close relationship of *Centrodinium* and the species of *Alexandrium* s.l. suggests the reinstatement of the genera *Gessnerium* and *Protogonyaulax*, and the erection of a new genus for *A. affine* and *A. gaarderae*. The diagnoses of the genera *Centrodinium*, *Alexandrium*, *Gessnerium,* and *Protogonyaulax* need to be amended. The species *Peridinium splendor-maris*, type of the genus *Blepharocysta*, has been interpreted to correspond to an earlier description of *Alexandrium balechii*. Carbonell-Moore [31] submitted a proposal to conserve the name *Peridinium splendor-maris* as a species *Blepharocysta*, avoiding the possible transfer of all the species of *Alexandrium* into *Blepharocysta*. If the proposal is rejected, the change does not affect *Alexandrium* because *A. balechii* is now a species of *Gessnerium*. If the proposal is recommended, no change is applied to *Alexandrium*.

4.4. Emended Diagnosis of Centrodinium (Figures 6(e)–6(f), Table 1)

4.4.1. Centrodinium Kofoid emended Gómez & Artigas. Gonyaulacoid dinoflagellates with different degree of lateral flattening, an elongated brunt apex or an apical horn. Cingulum deep, median, descending about one cingular width, without overhanging. The cingular list at both upper and lower margins are prominent. The sulcus with list at both right and left margins. The apical pore plate with a horseshoe-shaped pore surrounded by small marginal pores. The plate formula is Po, $4'$ ($2'$ $\alpha+\beta$), $6''$, 6c, ≥8s, $5'''$, $2''''$, and the more flattened species showed a split of the second apical plate, $2'$ $(\alpha+\beta)$. The apical pore plate is mainly surrounded by the second and fourth apical plates, while the third apical plate does not reach the apex. In the less compressed species, the first apical plate ($1'$) reaches the apex, whereas in the more flattened species the $1'$ plate does not reach the apex, and the $2'$ plate is divided into two plates. In all the species, the anterior sulcal plate has a distinct pore. The sulcus contains at least 8 plates, the two lateral posterior sulcal plates are long. The left plate is longer than the right one in the less compressed species, and vice versa in the more compressed species. In less compressed species, the antapex is pointed, while in the more flattened species the antapical horn derived from a tubular second antapical plate has terminal spinules. The antapical horn is supported by two triangular plates, the posterior sulcal in the right face, and first antapical plate in the left face. The posterior sulcal plate may contain a posterior attachment pore near the anterior margin. The species of *Centrodinium* typically inhabit in warm oceans and have chloroplasts. The species *C. punctatum* is not toxic.

Table 1: Comparison of the morphological and ecological characters of the genera *Gessnerium* emend., *Alexandrium* emend., *Protogonyaulax* emend., *Episemicolon* gen. nov., and *Centrodinium* emend. Gómez & Artigas. Data based on Balech (1995), and this study.

	Gessnerium emend.	*Alexandrium* emend.	*Protogonyaulax* emend.	*Episemicolon* gen. nov.	*Centrodinium* emend.
Cell compression	Non or slightly compressed	Non or slightly compressed	Non or slightly compressed	Non compressed or slightly compressed	Moderate to highly lateral flattening
Horn or spines	No	No	No	No	Variable
Epitheca plate formula	Po, 3′(4′), 7″ (6″)	Po, 4′ (3′), 6″ (7″)	Po, 4′, 6″	Po, 4′, 6″	Po, 4′, 6″
Shape of apical pore plate	Comma or fishhook	Comma	Comma	Oval or bullet	Horse-shoe
Anterior attachment pore (when present)	Right to Po	Right to Po	Right to Po	Dorsal to Po	Unreported
Shape of the equivalent gonyaulacoid first apical plate	Pentagonal	Rhomboidal	Rhomboidal	Rhomboidal	Rhomboidal
Pore in anterior sulcal	No	No	No	No	Yes
Precingular part in sulcal anterior	No	Sometimes	Sometimes	No	No
Left cingular part in the anterior sulcal plate	No	No	No	No	Yes
Median sulcal plates	Large	Small	Small	Small	Small
Accessory sulcal plates	Large	Small	Small	Small	Small
Lateral posterior sulcal plates	Right longer than left	Right and left of similar length	Similar or right longer than left	Right longer than left	Variable
Posterior sulcal plate	Large	Short, wider than long	Short, longer than wide	Short, longer than wide	Polygonal or triangular
Sulcal list	Moderate	Absent or inconspicuous	Moderate	Moderate or prominent	Very prominent
Posterior sulcal attachment (connecting) pore	Typically present in chain-forming species	Typically absent or inconspicuous	Present in chain-forming species	Present in chain-forming species	Present in chain-forming species
V-shaped anterior margin of the sulcal posterior plates	Prominent or not	Inconspicuous	Prominent	Prominent	No
Chain-forming	Variable	Rarely	Common	Variable	Variable
Habitat	Plankton, tropical to temperate seas	Plankton, cosmopolitan, bloom-forming in neritic waters	Plankton, cosmopolitan, bloom-forming in neritic waters	Plankton, tropical to temperate seas	Plankton, low abundance in open warm to tropical seas
Paralytic shellfish poisoning	No	Yes	Yes	No	No

(1) Type species: *Centrodinium elongatum* Kofoid 1907.

(2) Other species:

- (i) *Centrodinium biconicum* (G. Murray & Whitting 1899) F.J.R. Taylor 1976 [=*Murrayella biconica* (G. Murray & Whitting) Pavillard 1931, *Pavillardinium biconicum* (G. Murray & Whitting) Rampi 1948].
- (ii) *Centrodinium complanatum* (Cleve 1903) Kofoid 1907 (=*Steiniella complanata* Cleve).
- (iii) *Centrodinium deflexoides* Balech 1962.
- (iv) *Centrodinium deflexum* Kofoid 1907.
- (v) *Centrodinium eminens* Böhm 1933.
- (vi) *Centrodinium expansum* Kofoid & J. R. Michener 1911.
- (vii) *Centrodinium intermedium* Pavillard 1930.
- (viii) *Centrodinium maximum* Pavillard 1930.
- (ix) *Centrodinium mimeticum* (Balech 1967) F.J.R. Taylor 1976 (=*M. mimetica* Balech).
- (x) *Centrodinium ovale* (Pavillard 1930) Hernández-Becerril in Hernández-Becerril et al. 2010 [=*M. ovalis* Pavillard, *P. ovale* (Pavillard) G. De Toni 1936].
- (xi) *Centrodinium pacificum* (Rampi 1950) F. J. R. Taylor 1976 (=*P. pacificum* Rampi).
- (xii) *Centrodinium pavillardii* F. J. R. Taylor 1976 [=*P. intermedium* (Pavillard 1916) G. de Toni 1936, *M. intermedia* Pavillard, non *C. intermedium* Pavillard 1930].

(xiii) *Centrodinium porulosum* Kofoid & J. R. Michener 1911.

(xiv) *Centrodinium pulchrum* Böhm 1933 [=*C. eminens* f. *pulchrum* (Böhm) J. Schiller 1933].

(xv) *Centrodinium punctatum* (Cleve 1900) F. J. R. Taylor 1976 [=*Steiniella punctata* Cleve, *M. punctata* (Cleve) Kofoid 1907, *P. punctatum* (Cleve) G. De Toni 1936, *M. splendida* Rampi 1941, *P. splendidum* (Rampi) Rampi 1950].

4.5. Emended Diagnosis of Alexandrium (Figure 6(b), Table 1)

4.5.1. Alexandrium Halim 1960 emended Gómez & Artigas. Gonyaulacoid dinoflagellates without or with scarce cell compression, and lacking horn or spines. Cingulum deep, median, descending about one cingular width, without overhanging. The theca is usually smooth, and only ornamented in few species. The plate formula is Po, 4′, 6″, 6c, >8s, 5‴, 2⁗. The plate 1′ is rhomboidal, narrow, and asymmetrical and can be either in direct contact with the apical pore plate or indirectly connected via a thin suture (thread-like process). *Alexandrium insuetum*, has severely reticulated thecal plates and the exsert 1′. The plate 6″ is usually narrow. The posterior sulcal is relatively small, wider than long. The apical pore plate contains a comma-shaped pore. Relatively few chain-forming species, and the attachment pore, if present, is in the lateral right to the apical pore plate. A posterior connecting pore is usually absent. The species are typically bloom-forming in eutrophic and/or confined neritic waters. PSP toxicity has been reported in numerous species.

(1) Type species: *Alexandrium minutum* Halim 1960 (=*A. ibericum* Balech 1985, *A. lusitanicum* Balech 1985, *A. angustitabulatum* F. J. R. Taylor 1995 nom. illeg.)

(2) Other species of the genus *Alexandrium*:

- (i) *Alexandrium andersonii* Balech 1990.
- (ii) *Alexandrium insuetum* Balech 1985.
- (iii) *Alexandrium ostenfeldii* (Paulsen 1904) Balech & Tangen 1985 [=*Goniodoma ostenfeldii* Paulsen, *Gonyaulax ostenfeldii* (Paulsen) Paulsen 1949, *Protogonyaulax ostenfeldii* (Paulsen) S. Fraga & F. J. Sánchez 1985, *Heteraulacus ostenfeldii* (Paulsen) A. R. Loeblich 1970, *Gessnerium ostenfeldii* (Paulsen) A. R. Loeblich & L. A. Loeblich 1979, *Triadinium ostenfeldii* (Paulsen) J. D. Dodge 1976, ?*Gonyaulax globosa* (Braarud 1945) Balech 1971 nom. illeg., ?*Protogonyaulax globosa* (Braarud) F. J. R. Taylor 1979, ?*Gonyaulax trygvei* M. Parke & J. D. Dodge in Parke & Dixon 1976, ?*Gonyaulax tamarensis* M. Lebour var. *globosa* Braarud 1945, ?*Gonyaulax dimorpha* Biecheler 1952].
- (iv) *Alexandrium peruvianum* (Balech & B.R. Mendiola 1977) Balech & Tangen 1985 [=*Gonyaulax peruviana* Balech & B. R. Mendiola, *Protogonyaulax peruviana* (Balech & B. R. Mendiola) F. J. R. Taylor 1979, ?*A. ostenfeldii* Paulsen 1904) Balech & Tangen 1985].
- (v) *Alexandrium tamutum* Montresor, Beran & U. John 2004.

(3) The placement in *Alexandrium* s.s. needs further research for the species: *Alexandrium diversaporum* Sh. Murray et al. 2014, *A. leei* Balech 1985, *A. margalefii* Balech 1994, and *A. pohangense* A. S. Lim & H. J. Jeong in Lim, Jeong, Kim, & Lee 2015.

4.6. Reinstatement of the Genus Gessnerium (Figure 6(a), Table 1)

4.6.1. Gessnerium Halim 1967 ex Halim 1969 emended Gómez & Artigas. Gonyaulacoid dinoflagellates without or scarce cell compression, without spines or horns. Cingulum deep, median, descending about one cingular width, without overhanging. Apical pore plate is longitudinally oriented. The typical gonyaulacoid first apical plate, narrow and rhomboidal of *Alexandrium* s.l. is absent. The anterior right margin of the first apical is an anterior margin in *Gessnerium*, and the equivalent plate is pentagonal and never reaches the apical pore plate. This plate is considered the first apical plate (Po, 4′, 6″, 6c, 10s, 5‴, 2⁗) or a precingular plate in a strict Kofoidian scheme (plate formula Po, 3′, 7″, 6c, 10s, 5‴, 2⁗). The left anterior sulcal plate (S.s.a) is large and superficial, while in *Alexandrium* s.l. is small and sunk into the sulcus. There are two relatively large accessory sulcal plates that are absent or hardly visible in *Alexandrium* s.l. The right posterior lateral sulcal plate (S.d.p.) is long and narrow. The posterior sulcal plate (S.p.) is longer than wide, extending obliquely towards the posterior right. The second antapical plate of *Gessnerium* is lateral, while this plate is more dorsal than lateral in *Alexandrium* s.l. The formation of chains is variable amongst the species. The species are more common in warm waters, and rarely reported in cold waters. Paralytic shellfish poisoning has not been associated with the presence of *Gessnerium*, but several species are known as fish-killers that produce goniodomin A, spirolide, or hemolytic toxins that may be involved in mixotrophy.

(1) Type species: *Gessnerium mochimaense* Halim 1967 *ex* Halim 1969 [=*Gessnerium monilatum* (J. F. Howell 1953) A. R. Loeblich 1970].

(2) Other species of *Gessnerium*:

- (i) *Gessnerium balechii* (Steidinger 1971) A. R. Loeblich & L.A. Loeblich 1979 [=*Gonyaulax balechii* Steidinger, *Pyrodinium balechii* (Steidinger) F. J. R. Taylor 1976, *Alexandrium balechii* (Steidinger) Balech 1995].
- (ii) *Gessnerium concavum* (Gaarder 1954) A. R. Loeblich & L. A. Loeblich 1979 [=*Goniodoma concava* Gaarder, *A. concavum* (Gaarder) Balech 1985 emend. Nguyen-Ngoc & Larsen 2004, non *Gonyaulax concava* (Gaarder) Balech 1967, nec *A. concavum* (Gaarder) Balech 1985, auct. non Balech 1995 (=*A. gaarderae* Nguyen-Ngoc & J. Larsen 2004)].
- (iii) *Gessnerium monilatum* (J. F. Howell 1953) A. R. Loeblich 1970 [=*Gonyaulax monilata* J. F. Howell, *Pyrodinium monilatum* (J. F. Howell) F. J. R. Taylor 1976, *Alexandrium monilatum* (J. F. Howell) Balech 1985, *Gessnerium mochimaensis* Halim 1967 *ex* Halim 1969].

4.6.2. New Combinations of Gessnerium:

(i) *Gessnerium camurascutulum* (L. MacKenzie & K. Todd 2002) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium camurascutulum* L. MacKenzie & K. Todd (2002, Harmful Algae, 1: 296, Figure 1). MacKenzie & Todd [32] designed as holotype the Figures 1, 7, and 13. The Figure 13 also includes *A. minutum*, and the Figures 1 and 7 correspond to cells from different geographical origins (see Article 8.1 of International Code of Nomenclature (I.C.N.) for Algae, Fungi, and Plants). The Figure 1 (cell from Marlborough Sounds) is designed as type.

(ii) *Gessnerium hiranoi* (T. Kita & Fukuyo 1988) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium hiranoi* T. Kita & Fukuyo (1988, Bull. Plankt. Soc. Jap. 35: 2, pl. 1 a–k, Figures 1(a)–1(f)).

(iii) *Gessnerium foedum* (Balech 1990) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium foedum* Balech (1990, Helgol. Meeresunters. 44: 392, Figures 19–33). Synonym: *Goniodoma pseudogoniaulax* sensu Kita et al. 1985.

(iv) *Gessnerium globosum* (Nguyen-Ngoc & J. Larsen in Larsen & Nguyen-Ngoc 2004) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium globosum* Nguyen-Ngoc & J. Larsen in Larsen and Nguyen-Ngoc (2004, Opera Bot., 140: 93, pl. 7, Figure 8). Non *Protogonyaulax globosa* (Braarud 1945) F. J. R. Taylor 1979.

(v) *Gessnerium pseudogoniaulax* (Biecheler 1952) F. Gómez & Artigas, comb. nov. Basionym: *Goniodoma pseudogoniaulax* Biecheler (1952, Bull. Biol. Fr. Belg., Suppl. 36: p. 55, Figures 30–32). Synonyms: *Triadinium pseudogoniaulax* (Biecheler) J.D. Dodge 1981, *Alexandrium pseudogoniaulax* (Biecheler 1952) Horiguchi 1983 *ex* T. Kita & Fukuyo 1992. The epithet is often reported as “pseudogonyaulax”.

(vi) *Gessnerium satoanum* (K. Yuki & Fukuyo 1992) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium satoanum* K. Yuki & Fukuyo (1992, J. Phycol., 28: 396, Figures 1–12).

(vii) *Gessnerium taylorii* (Balech 1994) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium taylorii* Balech (1994, Trans. Amer. Microscop. Soc., 113: 219, Figures 7–11).

4.6.3. The Next Species Do not Belong to Gessnerium:. *Gessnerium acatenella* (Whedon & Kofoid 1936) A. R. Loeblich & L. A. Loeblich 1979 (accepted as *Protogonyaulax acatenella*). *Gessnerium catenella* (Whedon & Kofoid 1936) A. R. Loeblich & L. A. Loeblich 1979 (accepted as *Protogonyaulax catenella*). *Gessnerium cohorticula* (Balech 1967) A. R. Loeblich & L. A. Loeblich 1979 (accepted as *Protogonyaulax cohorticula*). *Gessnerium fraterculus* (Balech 1964) A. R. Loeblich & L. A. Loeblich 1979 (accepted as *Protogonyaulax fraterculus*). *Gessnerium tamarense* (M. Lebour 1925) A. R. Loeblich & L. A. Loeblich 1979 (accepted as *Protogonyaulax tamarensis*).

4.7. Reinstatement of the Genus Protogonyaulax (Figure 6(c), Table 1)

4.7.1. Protogonyaulax F. J. R. Taylor 1979 emended Gómez & Artigas. Gonyaulacoid dinoflagellates without or with scarce cell compression, and lacking horn or spines. Cingulum deep, median, descending about one cingular width, without overhanging. The theca is usually smooth, and very rarely ornamented. The plate formula is Po, 4′, 6′′, 6c, ≥8s, 5′′′, 2′′′′. The first apical plate (1′) plate is rhomboidal, narrow and asymmetrical and always directly connected to the apical pore plate (Po). The plate 6′′ is usually wide. The posterior sulcal plate is longer than wide, with usually two ventrally directed anterior prolongations and a connecting pore. The Po plate contains a comma-shaped pore, and usually an anterior attachment pore in the right lateral side of the apical pore plate. Relatively many chain-forming species. The species are bloom-forming in eutrophic and/or confined neritic waters. Cosmopolitan distribution with a few species reported from cold waters. Paralytic shellfish poisoning toxicity events have been reported in numerous species.

(1) Type species: *Protogonyaulax tamarensis* (M. Lebour 1925) F. J. R. Taylor 1979. Basionym: *Gonyaulax tamarensis* M. Lebour. Homotypic synonyms: *Alexandrium tamarense* (M. Lebour) Balech 1985, *Gessnerium tamarense* (M. Lebour) A. R. Loeblich & L. A. Loeblich 1979. Heterotypic synonyms: *Gonyaulax tamarensis* var. *excavata* Braarud 1945, *Gonyaulax excavata* (Braarud) Balech 1971, *Alexandrium excavatum* (Braarud 1945) Balech & Tangen 1985.

(2) Other species of the genus *Protogonyaulax*:

  (i) *Protogonyaulax acatenella* (Whedon & Kofoid 1936) F. J. R. Taylor 1979 [=*Gonyaulax acatenella* Whedon & Kofoid, *A. acatenella* (Whedon & Kofoid) Balech 1985].

  (ii) *Protogonyaulax catenella* (Whedon & Kofoid 1936) F. J. R. Taylor 1979 [=*Gonyaulax catenella* Whedon & Kofoid, *A. catenella* (Whedon & Kofoid) Balech 1985, *Gonyaulax washingtonensis* Hsu 1967, *Gessnerium catenella* (Whedon & Kofoid) A. R. Loeblich & L. A. Loeblich 1979]. John et al. [33] proposed to reject the name *Gonyaulax catenella*, the basionym of *A. catenella*, in order to permit usage of the more recent name *A. fundyense*. The proposal (2302) was not recommended, and Prud′homme van Reine [34] reported: “*Alexandrium fundyense* and *A. catenella* are certainly conspecific, and then “*catenella*” has nomenclatural priority”.

  (iii) *Protogonyaulax cohorticula* (Balech 1967) F. J. R. Taylor [=*Gonyaulax cohorticula* Balech nom. inval., *Gessnerium cohorticula* (Balech) A. R. Loeblich & L. A. Loeblich 1979, *A. cohorticula* (Balech 1967) Balech 1985]. Balech described the basionym lacking Latin description and designation of type. Balech described the species under the Zoological Nomenclature (see Article 45 of I.C.N.).

(iv) *Protogonyaulax compressa* Fukuyo, K. Yoshida & H. Inoue 1985 [=*Alexandrium compressum* (Fukuyo, K. Yoshida & H. Inoue) Balech 1995].

(v) *Protogonyaulax fraterculus* (Balech 1964) F. J. R. Taylor 1979 [=*Gonyaulax fraterculus* Balech 1964 as *G. "fratercula"*, nom. inval.; *Gessnerium fraterculus* (Balech) A. R. Loeblich & L. A. Loeblich 1979, *Alexandrium fraterculus* (Balech) Balech 1985, nom. inval.]. Balech [35] described the basionym lacking Latin description and designation of type. Balech described the species under the Zoological Nomenclature (see Article 45 of I.C.N.).

(vi) *Protogonyaulax kutnerae* (Balech 1979) Sournia 1984 (=*Gonyaulax kutnerae* Balech 1979).

(vii) *Protogonyaulax leei* (Balech 1985) Fukuyo, Pholpunthin & K. Yoshida 1988 (=*Alexandrium leei* Balech).

(viii) *Protogonyaulax phoneus* (Wołoszyńska & W. Conrad 1939) F. J. R. Taylor 1979 [=*Pyrodinium phoneus* Wołoszyńska & W. Conrad, *Gonyaulax phoneus* (Wołoszyńska & W. Conrad) F. J. R. Taylor 1975, *Gonyaulax phoneus* (Wołoszyńska & W. Conrad) Loeblich & A. R. Loeblich 1975, ?*A. ostenfeldii* (Paulsen 1904) Balech & Tangen 1985]. The epithet "*phoneus*" is masculine, while the genus is feminine.

4.7.2. New Combinations of Protogonyaulax

(i) *Protogonyaulax australiensis* (Sh. Murray in John et al. 2014a) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium australiense* Sh. Murray in John et al. 2014a (Protist, 165: 797–798, Figure 8). Synonym: *Alexandrium australis* Wang et al. 2014 nom. inval.

(ii) *Protogonyaulax fundyensis* (Balech 1985) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium fundyense* Balech in Anderson et al. (1985, Toxic dinoflagellates. Proceedings of the Third International Conference on Toxic dinoflagellates. Elsevier, New York, p. 37, Figure 18). Prud′homme van Reine [34] reported: "*Alexandrium fundyense* and *A. catenella* are certainly conspecific, and then "*catenella*" has nomenclatural priority".

(iii) *Protogonyaulax mediterranea* (U. John in John et al. 2014a) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium mediterraneum* U. John in John et al. 2014a (Protist 165: 795–797, Figure 7). Synonym: *Alexandrium mediterranis* Wang et al. 2014 nom. inval.

(iv) *Protogonyaulax pacifica* (Litaker in John et al. 2014a) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium pacificum* Litaker in John et al. 2014a (Protist, 165: 793–795, Figure 6). John et al. ([36], p. 794) reported "As it is no longer possible to establish the identity of the material originally assigned to *A. catenella*, we have designated a new species name for Group IV isolates - *A. pacificum* - and have submitted a proposal to reject the name *Alexandrium catenella* (John et al., 2014b)". The proposal (2302) in John et al. [33] was not recommended [34].

(v) *Protogonyaulax tamiyavanichii* (Balech 1974) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium tamiyavanichii* Balech (1994, Trans. Amer. Microscop. Soc., 113: 217, Figures 1–6). Synonym: *Protogonyaulax cohorticula* (Balech) F. J. R. Taylor 1979 sec Kodama et al. 1988.

(vi) *Protogonyaulax tropicalis* (Balech 1985) F. Gómez & Artigas, comb. nov. Basionym: *Alexandrium tropicale* Balech in Anderson et al. [1985, Toxic dinoflagellates. Proceedings of the Third International Conference on Toxic dinoflagellates. Elsevier, New York, p. 37, Figure 7]. Synonym: *Gonyaulax excavata* Braarud 1945 sensu Balech (1971, Serv. Hydrogr. Naval, Buenos Aires, H 654: 28, pl. 7: Figures 119–124).

4.7.3. The Next Species Do not Belong to Protogonyaulax

(i) *Protogonyaulax affinis* H. Inoue & Fukuyo 1985 (placed in a new genus, see below). *Protogonyaulax dimorpha* (Biecheler 1952) F. J. R. Taylor 1979 (accepted as *Gonyaulax dimorpha* Biecheler, may be related to *A. ostenfeldii*). *Protogonyaulax globosa* (Braarud 1945) F. J. R. Taylor 1979 [accepted as *Gonyaulax globosa* (Braarud) Balech 1971 nom. illeg., and it may be related to *A. ostenfeldii*]. *Protogonyaulax ostenfeldii* (Paulsen 1904) S. Fraga & F. J. Sánchez 1985 (accepted as *Gessnerium ostenfeldii*). *Protogonyaulax peruviana* (Balech & B.R. Mendiola 1977) F. J. R. Taylor 1979 (accepted as *Alexandrium peruvianum* or synonym of *A. ostenfeldii*).

4.8. New Erected Genus for the Clade of Alexandrium affine (Figure 6(d), Table 1)

4.8.1. Episemicolon F. Gómez & Artigas, gen. nov. (1) *Diagnosis:* Gonyaulacoid dinoflagellate without or scarce cell compression, without spines or horns. Cingulum deep, median, descending about one cingular width, without overhanging. The cingular lists at both upper and lower margin are prominent. The sulcus with list at both right and left margins. Plate formula Po, 4′, 6′′, 6c, ≥8s, 5′′′, 2′′′′. The first apical plate is rhomboidal and reaches the apical pore plate. The apical pore plate contains an oval or bullet-shaped apical pore, with an attachment pore lying at the dorsal side. The sulcus contains at least eight plates, the two lateral posterior plates are long, and with the right one longer than the left pair. The posterior sulcal plate is right displaced and may contain a marginal attachment pore. Paralytic shellfish poisoning toxicity has not been reported.

(2) *Etymology:* epi- from Ancient Greek "epi" (= on top of); semicolon: the punctuation mark (;) from Latin "semi" (= half), and Greek "kolon" (= verse, a part of a strophe, column) and a mark of punctuation (:). The apical pore and the dorsal attachment pore in the apical pore plate resemble the typographic symbol (;). The gender is neuter.

(3) *Type species: Episemicolon affine* (H. Inoue & Fukuyo 1985) F. Gómez & Artigas, gen. & comb. nov., *hic designatus*. Basionym: *Protogonyaulax affinis* H. Inoue & Fukuyo in Fukuyo et al. (1985, Proceedings of the Third International Conference on Toxic dinoflagellates. Elsevier, New York, p. 30, Figure 3(a)–3(c)). Synonyms: *A. affine* (H. Inoue & Fukuyo) Balech 1985 nom. inval., *A. fukuyoi*

Balech in Anderson et al. 1985 nom. inval., *A. affine* (H. Inoue & Fukuyo) Balech 1995.

4.8.2. Other Species. Episemicolon gaarderae (Nguyen-Ngoc & J. Larsen in Larsen & Nguyen-Ngoc 2004 *ex* F. Gómez & Artigas) F. Gómez & Artigas, comb. nov. Basionym: *Gonyaulax concava* Gaarder sensu Balech (1967, Rev. Mus. argent. Cienc. Nat. 'B. Rivadavia', Hidrobiol., 2, 108–111; plate 6, Figures 108–116). Synonym: *Alexandrium gaarderae* Nguyen-Ngoc & J. Larsen in Larsen & Nguyen-Ngoc 2004 *ex* F. Gómez & Artigas, *A. concavum* (Gaarder) Balech 1985, non *Goniodoma concava* Gaarder 1954, nec *Goniodoma gaarderae* Balech 1980 nom. inval. According to Index Nominum Algarum (http://ucjeps.berkeley.edu/ina/), Nguyen-Ngoc and Larsen [20] did not provide Latin description and designation of type of *A. gaarderae* (intended as a new name for *Gonyaulax concava* sensu Balech 1967, but effectively a new species). We designate as type the Figure 4, plate 6, in Larsen & Nguyen-Ngoc ([20, p. 91).

Abbreviations

a.a.p.:	Anterior attachment pore in apical pore plate
auct. non:	auctorum non (of authors [but] not....), used for misapplied names
BP:	Bootstrap probability
c.p.:	Cover or closing platelet or canopy in the apical pore plate
HAB:	Harmful algal bloom
I.C.N.:	International Code of Nomenclature for algae, fungi, and plants
LM:	Light microscopy
LSU:	Large subunit
mp:	Marginal pore in the apical pore plate
nom. inval.:	nomen invalidum, an invalid name
nom. illeg.:	nomen illegitimum, an illegitimate name.
Po:	Apical pore plate
p.a.p.:	Posterior attachment pore in the posterior sulcal plate
PCR:	Polymerase chain reaction
PSP:	Paralytic shellfish poisoning
rRNA:	Ribosomal RNA
S.a.:	Anterior sulcal plate
s.a.p.:	Pore of the anterior sulcal plate
SEM:	Scanning electron microscopy
S.d.a.:	Right (dexter) anterior lateral sulcal
S.d.p.:	Right posterior lateral sulcal
S.m.a.:	Anterior median sulcal
S.m.p.:	Posterior median sulcal
S.p.:	Posterior sulcal
s.l.:	Sensu lato
s.s.:	Sensu stricto
S.s.a.:	Left (sinister) anterior lateral sulcal
S.s.p.:	Left posterior lateral sulcal
SSU:	Small subunit.

Data Availability

The molecular sequences have been submitted to GenBank. They will be released after the formal acceptance.

Conflicts of Interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

Acknowledgments

F. G. was partly supported by a convention (no: 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 IFREMER "Etoile" cruise onboard R/V *Côtes de la Manche* (CNRS-INSU) in the frame of the Joint European Research Infrastructure network for Coastal Observatory – Novel European eXpertise for coastal observa-Tories (JERICO-Next) H2020 INFRAIA project no. 654410. We thank I. Puillat, project manager, and P. Lazure, scientific coordinator of the Etoile cruise. We thank L. Courcot for the SEM assistance.

Supplementary Materials

Supplementary 1. Appendix S1. Taxonomy, synonymy, plate arrangement and classification of *Centrodinium*.
Supplementary 2. Appendix S2. Detailed morphology of *Centrodinium* spp.
Supplementary 3. Appendix S3. Brief historical account of the taxonomy and nomenclature of *Alexandrium* sensu lato.
Supplementary 4. Figure S1. Maximum-likelihood phylogenetic trees of D1–D2 domains of the LSU rRNA gene sequences of selected species of *Alexandrium* sensu lato and *Centrodinium* spp. with especial focus on the group of *A. affine*.

References

[1] D. M. Anderson, T. J. Alpermann, A. D. Cembella, Y. Collos, E. Masseret, and M. Montresor, "The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health," *Harmful Algae*, vol. 14, no. 1, pp. 10–35, 2012.

[2] E. Balech, "The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group," *Toxic Dinoflagellates*, in *Proceedings of the 3rd International Conference on Toxic Dinoflagellates*, D. M. Anderson, A. W. White, and D. G. Baden, Eds., pp. 33–38, Elsevier, NY, 1985.

[3] C. A. Kofoid, "Reports on the scientific results of the expedition to the Eastern Tropical Pacific, in charge of Alexander Agassiz, by the U.S. fish commission steamer "Albatross" from October 1904 to March 1905, Lieut. Commander L. M. Garrett, U.S.N., commanding IX. New species of dinoflagellates," *Bulletin of the Museum of Comparative Zoology*, vol. 50, pp. 161–207, 1907.

[4] E. Balech, "Tintinnoinea and dinoflagellata from the Pacific based on materials of the Norpac y Downwind expeditions of the Scripps Institute of Oceanography," *Revista del Museo argentino de Ciencias Naturales "B. Rivadavia", Ciencias Zoológicas*, vol. 7, no. 1, pp. 1–253, 1962.

[5] E. Balech, "New or interesting dinoflagellates from the Gulf of Mexico and Caribbean Sea," *Revista del Museo argentino*

de Ciencias Naturales "B. Rivadavia", Hidrobiología, vol. 2, pp. 77–126, 1967.

[6] E. Balech, "Microplankton from the west Equatorial Atlantic Ocean (Equalant I)," *Armada Argentina, Servicio Hidrográfico Naval H*, vol. 654, pp. 1–103, 1971.

[7] F. J. R. Taylor, "Dinoflagellates from the international Indian Ocean expedition: a report on material collected by the R. V. "Anton Bruun"," *Bibliotheca Botanica*, vol. 132, pp. 1–234, 1976.

[8] E. Balech, "Dinoflagellates from the south-west Atlantic Ocean," *Publicaciones Especiales de Instituto Español de Oceanografía*, vol. 1, pp. 1–310, 1988.

[9] D. U. Hernández-Becerril, E. Bravo-Sierra, J. G. A. Ceballos-Corona, K. Esqueda-Lara, S. Escobar-Morales, and D. Parra-Toriz, "Morphology and taxonomy of the marine planktonic dinoflagellate *Centrodinium pulchrum* Böhm (Dinophyta) from the tropical Mexican Pacific, with comments on the taxonomy and distribution of the genus *Centrodinium*," *Phycologia*, vol. 49, no. 5, pp. 461–470, 2010.

[10] F. Gómez, "A checklist and classification of living dinoflagellates (Dinoflagellata, Alveolata)," *CICIMAR Océanides*, vol. 27, no. 1, pp. 65–140, 2012.

[11] Z. Li, K. N. Mertens, E. Nézan et al., "Discovery of a new clade nested within the genus *Alexandrium* (Dinophyceae): morpho-molecular characterization of *Centrodinium punctatum* (Cleve) F. J. R. Taylor," *Protist*, vol. 170, no. 2, pp. 168–186, 2019.

[12] M. L. Richlen and P. H. Barber, "A technique for the rapid extraction of microalgal DNA from single live and preserved cells," *Molecular Ecology Notes*, vol. 5, no. 3, pp. 688–691, 2005.

[13] F. Gómez, M. L. Richlen, and D. M. Anderson, "Molecular characterization and morphology of *Cochlodinium strangulatum*, the type species of *Cochlodinium*, and *Margalefidinium* gen. nov. for *C. polykrikoides* and allied species (Gymnodiniales, Dinophyceae)," *Harmful Algae*, vol. 63, no. 1, pp. 32–44, 2017.

[14] L. Medlin, H. J. Elwood, S. Stickel, and M. L. Sogin, "The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions," *Gene*, vol. 71, no. 2, pp. 491–499, 1988.

[15] C. A. Scholin, M. Herzog, M. Sogin, and D. M. Anderson, "Identification of group specific and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae): II. Sequence analysis of a fragment of the LSU ribosomal RNA gene," *Journal of Phycology*, vol. 30, no. 6, pp. 999–1011, 1994.

[16] M. A. Larkin, G. Blackshields, N. P. Brown et al., "Clustal W and clustal X version 2.0," *Bioinformatics*, vol. 23, no. 21, pp. 2947–2948, 2007.

[17] S. Kumar, G. Stecher, and K. Tamura, "MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets," *Molecular Biology and Evolution*, vol. 33, no. 7, pp. 1870–1874, 2016.

[18] E. Balech, *The genus Alexandrium Halim (Dinoflagellata)*, Sherkin Island Marine Station Publication, Cork, Ireland: Sherkin Island Co, 1995.

[19] L. MacKenzie, M. de Salas, J. Adamson, and V. Beuzenberg, "The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics," *Harmful Algae*, vol. 3, no. 1, pp. 71–92, 2004.

[20] J. Larsen and L. Nguyen-Ngoc, "Potentially toxic microalgae of Vietnamese waters," *Opera Botanica*, vol. 140, pp. 5–216, 2004.

[21] Y. Fukuyo, K. Yoshida, and H. Inoue, "*Protogonyaulax* in Japanese coastal waters," in *Toxic Dinoflagellates*, in *Proceedings of the 3rd International Conference on Toxic Dinoflagellates*, D. M. Anderson, A. W. White, and D. G. Baden, Eds., pp. 27–32, Elsevier, NY, 1985.

[22] M. Adachi, Y. Sako, and Y. Ishida, "Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions," *Journal of Phycology*, vol. 32, no. 3, pp. 424–432, 1996.

[23] A. S. Lim, H. J. Jeong, J. H. Kim, and S. Y. Lee, "Description of the new phototrophic dinoflagellate *Alexandrium pohangense* sp. nov. from Korean coastal waters," *Harmful Algae*, vol. 46, pp. 49–61, 2015.

[24] M. Yoshida, "The taxonomic study of the genus *Alexandrium*," University of Tokyo, Tokyo, 2000.

[25] K. A. Steidinger and K. Tangen, "Dinoflagellates," in *Identifying Marine Phytoplankton*, C. R. Tomas, Ed., pp. 387–584, Academic Press, San Diego, 1997.

[26] K. A. Steidinger and O. Moestrup, "The taxonomy of *Gonyaulax*, *Pyrodinium, Alexandrium*, *Gessnerium*, *Protogonyaulax,* and *Goniodoma*," *Toxic Marine Phytoplankton*, E. Granéli, B. Sundström, L. Edler, and D. M. Anderson, Eds., pp. 522–523, Elsevier, NY, 1990.

[27] E. Balech, "The genus *Goniodoma* Stein (Dinoflagellata)," *Lilloa*, vol. 35, no. 1, pp. 97–109, 1979.

[28] C. Leaw, P. T. Lim, B. K. Ng, M. Y. Cheah, A. Ahmad, and G. Usup, "Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and nuclear ribosomal gene sequence," *Phycologia*, vol. 44, no. 5, pp. 550–565, 2005.

[29] G. Usup, A. Ahmad, K. Matsuoka, P. T. Lim, and C. P. Leaw, "Biology, ecology and bloom dynamics of the toxic marine dinoflagellate *Pyrodinium bahamense*," *Harmful Algae*, vol. 14, pp. 301–312, 2012.

[30] E. Balech, "Redescription of *Alexandrium minutum* Halim (Dinophyceae) type species of the genus *Alexandrium*," *Phycologia*, vol. 28, no. 2, pp. 206–211, 1989.

[31] M. C. Carbonell-Moore, "(2608) Proposal to conserve the name *Peridinium splendor-maris* (*Blepharocysta splendor-maris*) (Dinophyceae) with a conserved type," *Taxon*, vol. 67, no. 3, pp. 633–635, 2018.

[32] L. MacKenzie and K. Todd, "*Alexandrium camurascutulum* sp. nov. (Dinophyceae): a new dinoflagellate species from New Zealand," *Harmful Algae*, vol. 1, no. 3, pp. 295–300, 2002.

[33] U. John, W. Litaker, M. Montresor, S. Murray, M. L. Brosnahan, and D. M. Anderson, "Proposal to reject the name *Alexandrium catenella* (Dinophyceae)," *Taxon*, vol. 63, no. 4, pp. 932–933, 2014.

[34] W. F. Prud′homme van Reine, "Report of the nomenclature committee for algae: 15," *Taxon*, vol. 66, no. 1, pp. 191–192, 2017.

[35] E. Balech, "Plankton of Mar de Plata Sea during the period 1961-62 (Buenos Aires, Argentina)," *Boletín del Instituto de biología marina, Mar del Plata*, vol. 4, no. 1, pp. 1–49, 1964.

[36] U. John, R. W. Litaker, M. Montresor, S. Murray, M. L. Brosnahan, and D. M. Anderson, "Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification," *Protist*, vol. 165, no. 6, pp. 779–804, 2014a.