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

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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* *pacificca*, [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. punctatum* 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 reconcile 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 sub-sample 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 x PCR buffer (500 mM KCl and 100 mM Tris–HCl, pH 8.3), 2 mM MgCl₂, 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 deep (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.p.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 spines (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 epithelial plate formula of C. punctatum is Po, 4′, 6′′ or alternatively 3′,
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 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 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 2β appeared crushed against 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, 3'. 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' (α+β) 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 2β 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 2β appeared crushed against

![Figure 1](image-url)
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), 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 Centrodinium 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' (α + β), 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/praterculus* 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. diversisporum*, *A. lei*, *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)). *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 μm, (w–z) = 2 μm.
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. affine [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 Centrodiunum 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 Centrodiunum 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
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 *Centrodiinium* spp. (Figures 6(e) and 6(f)). The cingulum and the sulcus of *Centrodiinium* 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 Pyrrhotriadinium, is reported in the Appendix S3 as Supplementary material. The plate formula of Alexandrium is usually reported as Po, 1a, 6′, 6c, 6s, 5′′, 1p, 2′′′ [25]. It is similar to the plate formula of *C. punctatum* and differs from the more flattened species of *Centrodiinium* 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, 6s, 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 plate as an intercalary plate. Li et al. [11] misidentified the sulcal and hypotheal 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 posterior sulcal as the posterior plate, and this induces the subsequent errors in the tabulation of the hypotheal (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].

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![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.](image-url)
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) *Episecoton colon affinis* gen. & comb. nov. (formerly *A. affinis*) 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.a.s. = 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′th 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 margalefi_ 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. margalefi_ 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. diversivorum_, _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, _A. margalefi_ and _A. pohangense_ and the type species of the genus _Blepharocysta_, type of the genus _Gessnerium_, 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 circular 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 horsehoe-shaped pore surrounded by small marginal pores. The plate formula is Po, 4′(2′a + β), 6′, 6c, ≥8s, 5′′′, 2′′′′, and the more flattened species showed a split of the second apical plate, 2′ (α + β). 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., Protagonyaulax emend., Episemicolon gen. nov., and Centrodinium emend. Gómez & Artigas. Data based on Balech (1995), and this study.

|                      | Gessnerium emend. | Alexandrium emend. | Protagonyaulax 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′, 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* Kofoed 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) Kofoed 1907 (=Steiniella complanata Cleve).

(iii) *Centrodinium deflexoides* Balech 1962.

(iv) *Centrodinium deflexum* Kofoed 1907.

(v) *Centrodinium eminens* Böhm 1933.

(vi) *Centrodinium expansum* Kofoed & 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) *Centroductinium porosum* Kofoid & J. R. Michener 1911.
(xiv) *Centroductinium pulchrum* Böhm 1933 [=C. eminens f. pulchrum (Böhm) J. Schiller 1933].
(xv) *Centroductinium 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 insutetum*, 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. Istitanicum* Balech 1985, *A. angustitubulatum* F. J. R. Taylor 1995 nom. illeg.)
2. Other species of the genus *Alexandrium*:
   (i) *Alexandrium andersonii* Balech 1990.
   (ii) *Alexandrium insutetum* Balech 1985.
   (iii) *Alexandrium ostenfeldii* (Paulsen 1904) Balech & Tangen 1985 [= *Goniomera 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, *Triandrum 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 peruvianum* Balech & B. R. Mendiola, *Protogonyaulax peruvianum* (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, >8s, 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 [= *Goniomera concavum* Gaarder, *A. concavum* (Gaarder) Balech 1985 emend. Nguyen-Ngoc & Larsen 2004, non *Gonyaulax concava* (Gaarder) Balech 1967, nec *A. concavum* (Gaarder) Balech 1985, aut. 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 mochimaense* 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: Goniadoma 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: Goniadoma 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 “pseudogoniaulax”.

(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 tamarense).

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

4.7.1. Protogonyaulax F. J. R. Taylor 1979 emended Gómez & Artigas. Gonyaulacoïd dinoflagellates without or with scarce cell compression, and lacking horn or spines. Cingulum deep, median, descending about one circular 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 tamarense (M. Lebour 1925) F. J. R. Taylor 1979. Basionym: Gonyaulax tamarense 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.).
4.7.2. New Combinations of Protogonyaulax

(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. "fraterculus", 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 lee** (Balech 1985) Fukuyo, Pholpunthin & K. Yoshida 1988 (=*Alexandrium lee* Balech).

(viii) **Protogonyaulax phoneus** (Woloszyńska & W. Conrad 1939) F. J. R. Taylor 1979 (=*Pyrodinium phoneus* Woloszyńska & W. Conrad, *Gonyaulax phoneus* (Woloszyńska & W. Conrad) F. J. R. Taylor 1975, *Gonyaulax phoneus* (Woloszyńska & 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 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 Aubert in Aubert & J. Larsen in Larsen & Nguyen-Ngoc 2004 108–116).

The molecular sequences have been submitted to GenBank.

**Conflicts of Interest**

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

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**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.

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