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To cite this version:

Kenneth Neil Mertens, Masao Adachi, Donald M. Anderson, Christine J. Band-Schmidt, Isabel Bravo, et al.. Morphological and phylogenetic data do not support the split of Alexandrium into four genera. Harmful Algae, 2020, 98, pp.101902. 10.1016/j.hal.2020.101902 . hal-03411046

HAL Id: hal-03411046
https://hal.umontpellier.fr/hal-03411046v1

Submitted on 21 Sep 2022

Morphological and phylogenetic data do not support the split of *Alexandrium* into four genera

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Abstract

A recently published study analyzed the phylogenetic relationship between the genera *Centrodinium* and *Alexandrium*, confirming an earlier publication showing the genus *Alexandrium* as paraphyletic. This most recent manuscript retained the genus *Alexandrium*, introduced a new genus *Episemicolon*, resurrected two genera, *Gessnerium* and *Protogonyaulax*, and stated that: "The polyphyly [*sic*] of *Alexandrium* is solved with the split into four genera". However, these reintroduced taxa were not based on monophyletic groups. Therefore this work, if accepted, would result in replacing a single paraphyletic taxon with several non-monophyletic ones. The morphological data presented for genus characterization also do not convincingly support taxa delimitations. The combination of weak molecular phylogenetics and the lack of diagnostic traits (i.e., autapomorphies) render the applicability of the concept of limited use. The proposal to split the genus *Alexandrium* on the basis of our current knowledge is rejected herein. The aim here is not to present an alternative analysis and revision, but to maintain *Alexandrium.* A better constructed and more phylogenetically accurate revision can and should wait until more complete evidence becomes available and there is a strong reason to revise the genus *Alexandrium.* The reasons are explained in detail by a review of the available molecular and morphological data for species of the genera *Alexandrium* and *Centrodinium.* In addition, cyst morphology and chemotaxonomy are discussed, and the need for integrative taxonomy is highlighted.

Keywords: taxonomy, phylogenetics, paraphyletic, saxitoxin, spirolides, harmful algal blooms

Highlights

- Morpho-molecular data do not support the split of *Alexandrium* into four genera.
- The genera *Episemicolon, Gessnerium*, and *Protogonyaulax* should not be used.
- A proposal to conserve *Alexandrium* against *Centrodinium* will be submitted.

Introduction and aims

The genus *Alexandrium* includes many species that have caused extensive economic and human health impacts worldwide (e.g., Anderson et al., 2012). *Alexandrium* currently encompasses 34 accepted species, with *A. camurascutulum* considered invalid (Guiry in Guiry and Guiry, 2020). Of these species, 14 are known to produce paralytic shellfish toxins (PSTs) (Moestrup et al., 2009), which have caused extensive damage to aquaculture industries. The wide range of toxins produced by *Alexandrium* species, belong to four families – PSTs (saxitoxin (STX) and its derivatives), spiroimines (spirolides and gymnodimines), goniodomins (e.g., Lassus et al., 2016), and lytic compounds (e.g., Tillmann and John, 2002; Blossom et al., 2019). The toxins with the most recognized potential for economic impact are the PSTs, which are responsible for outbreaks of paralytic shellfish poisoning (PSP), one of the most widespread harmful algal bloom (HAB)-related shellfish poisoning syndromes. PSP outbreaks can cause human illness and death from contaminated shellfish or fish, loss of wild and cultured seafood resources, impairment of tourism and recreational activities, alterations of marine trophic structure, and death of marine mammals, fish, and seabirds (Anderson et al., 2012). Symptoms of PSP in humans range from spreading numbness and tingling sensations, headache and nausea to more extreme fatal cases due to respiratory paralysis (Hallegraeff, 2003). Blooms of species such as *Alexandrium catenella*, *A. minutum*, and *A. pacificum* regularly cause losses of tens of millions of dollars to aquaculture industries in North and South America, Europe, Asia (e.g., Trainer and Yoshido, 2014; Sanseverino et al., 2016), and Australia and New Zealand (e.g., Jin et al., 2008; MacKenzie, 2014). For example, in late 2012, a single bloom of *A. catenella* along the east coast of Tasmania (Australia) resulted in ~AUD$ 23 million loss to the wild harvest and aquaculture industries (Condie et al., 2019). Monitoring of *Alexandrium* cells in the water column and of toxins in shellfish is therefore critical for avoidance of adverse effects on human health (e.g., Nagai et al., 2019; EFSA, 2009) and nationally and internationally standardized methods have been established to guide PSP testing (e.g., FAO Marine Biotoxins, 2004; [U.S.] National Shellfish Sanitation Program, 2017; Australian Shellfish Quality Assurance Program, 2019; Turner et al., 2019). The European Union requires all its member states to monitor coastal waters for toxin-producing plankton and toxins in mussels (Directive 91/492d/EC and Commission Decision 2002/225/EC). In parallel, research on *Alexandrium* species is vigorous: since 1975, there have been 2,768 published studies that include the word *Alexandrium*, which have been cited 70,322 times, for an average of about 150 publications per year over the last 10 years (Clarivate Analytics search on Web of Science Core Collection on 20 August 2020).

The taxonomic history of the genus *Alexandrium* is complex, and nomenclatural stability was not attained for some time, as detailed by Balech (1995, pp. 1–3) and Taylor and Fukuyo (1998). The genus *Alexandrium* was erected by Halim (1960) with the PST-producing *Alexandrium minutum* as its type. A few years later, Halim (1967) erected *Gessnerium* with *Gessnerium mochimaense* Halim as its type; this species had a pentagonal first apical (1′) plate not in contact with the pore plate (Po). Loeblich III and Loeblich (1979) considered *Alexandrium minutum* to be inadequately described, left it in the genus *Alexandrium* and transferred seven *Gonyaulax* species and two *Goniodoma* species into the genus *Gessnerium*. At the same time, Taylor (1979) erected *Protogonyaulax*, with *P. tamarensis* as the type species and defined it as having a 1′ plate directly contacting the Po of the apical pore complex, and transferred eight species of the genus *Gonyaulax* and one *Pyrodinium* species into *Protogonyaulax*. Taylor's proposal was followed by Fukuyo et al. (1985), who described two new *Protogonyaulax* species (*P. affinis* and *P. compressa*). After a detailed examination of samples from the type locality of *A. minutum* (the type species of *Alexandrium*), Balech (1989) noted that plate 1′ does not necessarily directly contact the Po in this species (the same applies to *A. fraterculus* and *A. kutnerae*). He therefore re-established the genus *Alexandrium,* considering *Protogonyaulax* a junior synonym of *Alexandrium*. All *Protogonyaulax* and *Gessnerium* species were thus transferred to *Alexandrium* (Balech 1985, 1995) and *Gessnerium* was retained as a subgenus of *Alexandrium* for species in which plate 1′ is not rhomboidal and does not contact the Po (Balech, 1995). Since Balech (1989), the consensus has been to only use the genus name *Alexandrium*. The currently accepted plate formula for *Alexandrium* is Po, $4'$, $6''$, 6c, 9–10s, $5'''$, $2''''$ (Balech, 1980, 1995; Balech and Tangen, 1985).

The advent of molecular approaches provided significant contributions to the circumscription of species within this important genus. Morpho-molecular studies suggested that species placed in the subgenus *Gessnerium* do not form a monophyletic group (John et al., 2003; MacKenzie et al., 2004; Kim et al., 2005; Rogers et al., 2006; Penna et al., 2008; Gu et al., 2013). More recently, a detailed study encompassing the morphology of vegetative cells, phylogenies based on multiple molecular markers, mating compatibility and presence/absence of genes coding for STX and analogues has shown that morphological characters used to identify species within the *Alexandrium tamarense* complex (*A. tamarense* and related species) were not consistent, but that molecular markers were able to delineate unambiguous species boundaries (John et al., 2014; but see Fraga et al., 2015 and Litaker et al., 2018).

Recently, Li et al. (2019) showed that the fusiform dinoflagellate *Centrodinium punctatum* forms a clade nesting within *Alexandrium*. Through a morpho-molecular study of two other *Centrodinium* species (*C. eminens* and *C. intermedium*), but without a re-investigation of the type, *C. elongatum*, Gómez and Artigas (2019) proposed to retain *Centrodinium* and subdivide the species formerly included in the genus *Alexandrium,* sharing a common thecal plate pattern, into four distinct genera, namely *Alexandrium sensu stricto* (*s.s.*) (emended diagnosis), the re-introduced genera *Gessnerium* and *Protogonyaulax*, and the new genus *Episemicolon*. Here, the data presented in Gómez and Artigas (2019) are critically discussed, and it is shown that they do not support such a drastic taxonomic rearrangement. The aim of this study is not to present an alternative analysis and revision, but to maintain the genus *Alexandrium*. Furthermore, it is emphasized to use integrative taxonomy which delimits taxa using multiple and complementary perspectives (Dayrat, 2005), including the consideration of cyst morphology in recognizing taxa (as previously suggested by Taylor and Fukuyo, 1998, p. 6) and chemotaxonomy, amongst other criteria.

The presented phylogenetic trees do not support the proposed subdivision of Alexandrium.

The finding that species of *Centrodinium* nest phylogenetically in *Alexandrium* (Li et al., 2019; Gómez and Artigas, 2019) makes the latter paraphyletic. Gómez and Artigas (2019) proposed to taxonomically split *Alexandrium* into four separate genera (not including *Centrodinium*, which never belonged to *Alexandrium*). This proposal establishes a new genus *Episemicolon*, while the diagnosis of three other genera (*Alexandrium, Gessnerium,* and *Protogonyaulax*) was emended to group subsets of former *Alexandrium* species. The authors stated that "The polyphyly [*sic*] of *Alexandrium* is solved with the split into four genera". Notably, *Alexandrium* was not polyphyletic, but paraphyletic. The suggestion that their proposal 'solved' this problem is the basis for our critique, and thus it is therefore worth evaluating the phylogenetic information presented by Gómez and Artigas (2019) in detail. Our question was: Does the data justify splitting a phylogenetically well-defined group (e.g., John et al., 2003; Rogers et al., 2006; Orr et al., 2011; Anderson et al., 2012; Baggesen et al., 2012; Gu et al., 2013; Murray et al 2015; Menezes et al., 2018; Kretzschmar et al., 2019), sharing the same Kofoidian plate tabulation, into segregate genera?

Gómez and Artigas (2019) presented two phylogenetic trees, Fig. 4 a phylogenetic analysis of alignments of the SSU (18S) region of the rRNA operon and Fig. 5 the D1–D2 region of the LSU (28S) region of the rRNA operon. Each alignment was analyzed using a maximum likelihood (ML) approach and clades were given with bootstrap support. There was no information on the number of base pairs analyzed, the number of informative sites, and the alignments were not made available for evaluation. These phylogenetic analyses are problematic for several reasons:

1. In their Fig. 5, the phylogeny using the D1–D2 regions of rRNA, which are generally ~600 bp in length, the proposed *Alexandrium s.s.* and *Protogonyaulax* are polyphyletic. In their Fig. 4, the phylogeny using (presumably) partial SSU regions of rRNA, of an unknown length, *Protogonyaulax, Episemicolon* and *Alexandrium s.s.* are all para- or polyphyletic. In other words, based on their own analysis, the authors propose the replacement of a single paraphyletic taxon (*Alexandrium*) with several non-monophyletic ones. The generic concepts are not substantiated by the molecular data. Thus, rather than solving the taxonomic problems, they exacerbate them.

2. There is very little genetic difference between species of *Centrodinium* and *Episemicolon.* In a comparison of sequences of *C. punctatum* in the NCBI database, a difference of < 1% and < 5% was found between it and *A. affine* in aligned sequences of SSU and LSU rRNA, which was less than the genetic differences among species of *Centrodinium*. Species that are highly similar in molecular genetic sequences of rRNA genes, with identical tabulation when considering plate homologies, need exceptionally different other autapomorphies in order to be placed in separate genera, and little evidence of this is found (see below).

3. The Gómez and Artigas (2019) phylogenies have used short alignments with too few characters and taxon information to accurately infer deeper, clade level branchings within the genus *Alexandrium* which will be stable into the future. Of the major factors that impact the accuracy of phylogenetic inference, two are particularly important: the length of aligned sequence/number of genes used, and the taxon sampling. Past studies of *Alexandrium* phylogenetics were reviewed (Table 1, John et al., 2003; Leaw et al., 2005; Rogers et al., 2006; Orr et al., 2011; Anderson et al., 2012; Baggesen et al., 2012; Gu et al 2013; Murray et al., 2014; Murray et al., 2015; Menezes et al., 2018; Gottschling et al., 2020), and this showed that clades within *Alexandrium* differed depending on gene and taxon sampling (exemplified by basal clades shown in Table 1). All else being equal, more sequence data and greater taxon sampling generally leads to more accurate and well-supported phylogenies. Murray et al. (2015) conducted an analysis of *Alexandrium* using a concatenated alignment of eight genes, with a total length of 7308 bp. The position of several of the major clades of *Alexandrium* differed in that analysis, compared to the analysis presented by Gómez and Artigas (2019). Similarly, new ribotypes are still being reported, such as *Alexandrium fragae* (Branco et al., 2020) and three new phylotypes nested within the genus (Nishimura et al., in review). It is likely that these new discoveries and longer alignments/greater gene sampling will lead to more strongly supported phylogenies than those of Gómez and Artigas (2019), whose phylogenies appear to be too weak to support taxonomic rearrangements.

As any taxonomic rearrangement of *Alexandrium* will potentially impact hundreds or thousands of scientists, government regulators, and the seafood industry, it is crucial that the phylogenetic basis for such a change be exceptionally clear, highly stable, and reproducible by other scientists. However, this has not occurred in this case. Gómez and Artigas (2019) have not provided access to their alignment or details of their analytical output. Dinoflagellate lineages display major differences in evolutionary rates in ribosomal RNA genes, particularly in the gonyaulacoid lineage. Examples of this can be seen in Orr et al. (2012), Gu et al. (2013) and Gottschling et al. (2020), in which the species of the Gonyaulacales are generally present on much longer branches than most other clades of dinoflagellates, a difference not seen in analyses using nuclear genes (e.g., Kretzschmar et al., 2019). While Gómez and Artigas (2019) selected a potentially suitable model for their ML analyses, inclusion of divergent taxa can still lead to misplacement of taxa/clusters, with high support values, due to various long-branch effects (Kück et al., 2012). No phylogeny-testing (such as leave-one-out testing and jackknifing by species/clusters) apart from the bootstrap support was used to determine whether the branch order of taxa/clusters was stable or unaffected by long-branch artifacts.

The morphological concepts of the reinstated and emended genera proposed by Gómez and Artigas (2019) have little taxonomic value

As mentioned in the introduction, *Protogonyaulax* was described by Taylor (1979) as having a plate 1′ in contact with the Po. As remarked by Balech (1989, p. 210) for the type species of *Alexandrium*, *A. minutum*, as well as for *A. fraterculus* and *A. kutnerae*, this feature is variable, and the plate 1′ can have an indirect contact with the Po through a filiform prolongation of plate 1′ (*Alexandrium acatenella, A. andersonii, A. ostenfeldii, A. tropicale* are additional examples; Balech, 1995). Gómez and Artigas (2019) considered species exhibiting only a direct contact of plate 1′ to the Po as belonging to their emended concept of *Protogonyaulax*, and species with direct or indirect contact (presumably meaning displaying both types) as belonging to their emended genus *Alexandrium*. However, when reviewing the literature, it is clear that there is no consistency with the application of both genus concepts (Table 2); furthermore, the high variability of the contact between plate 1′ and Po within *Alexandrium* species speaks against considering this as a reliable taxonomic character. In addition, Gómez and Artigas (2019) describe the 6″ plate as being "usually narrow" in *Alexandrium s.s.*, however, this is not so in *A. insuetum, A. ostenfeldii*, and *A. tamutum*, species which are retained in *Alexandrium s.s.* in their proposed phylogeny. Finally, the posterior sulcal plate (Sp) of the emended genus *Protogonyaulax* is longer than wide, and in *Alexandrium s.s.* wider than long, but the Sp of *A. leei*, included in *Protogonyaulax*, is wider than long and in *A. margalefii*, included in *Alexandrium s.s.*, longer than wide with an extremely oblique anterior margin (Balech, 1995). In summary, the considerations of Balech (1995) which support synonymization of *Protogonyaulax* with *Alexandrium*, still stand.

The reinstated genus *Gessnerium* also presents problems. Gómez and Artigas (2019) included within this genus species with a pentagonal plate 1′ not in contact with the Po. They excluded three species with a plate 1′ not in contact with the Po from their concept of *Gessnerium*: *A. insuetum,* which they included in *Alexandrium s.s.* despite the fact that this species has a pentagonal 1′ plate that does not touch the Po (Balech, 1995, Plate XVII, Figs. 1–23), and *A. pohangense* and *A. margalefii*, which were not formally attributed to any genus, although were assigned to the *Alexandrium s.s.* clade in their Fig. 4. The authors considered the quadrangular plate 1′ of *A. pohangense* and *A. margalefii* as a unique character distinguishing them from the other *Gessnerium*. However, the plate 1′ in *A. pohangense* has a short suture with plate 2′ and can therefore be considered pentagonal (Lim et al., 2015, their Fig. 4B), and such a short suture can also be observed in *A. balechii* and *A. foedum* according to Balech (1995, p. 103), which were classified as *Gessnerium* by Gómez and Artigas (2019). Within *A. taylorii,* the plate 1′ is known to vary between a quadrangular and pentagonal shape (Delgado et al., 1997). The infraspecific variability of the shape of this plate indicates that it cannot be used as a diagnostic character at the genus level (Table 2). Finally, the Sp of the emended genus *Gessnerium* is longer than wide and extending obliquely, but in *A. monilatum,* which is included in this genus*,* the Sp is rhomboid (Balech, 1995).

The main diagnostic character of the new genus *Episemicolon* is the presence of an anterior attachment pore placed on the dorsal side of the apical pore plate. However, in *A. gaarderae* the anterior attachment pore is defined as semi-dorsal (Larsen and Nguyen-Ngoc, 2004) and in *A. monilatum,* included in *Gessnerium*, the anterior attachment pore is slightly to the right of the dorsal side (Balech, 1995). Moreover, *A. concavum*, which also has a semi-dorsal attachment pore (Larsen and Nguyen-Ngoc, 2004) was placed in *Gessnerium* by Gómez and Artigas (2019). There is presently insufficient evidence to accept the location of the anterior attachment pore in the apical pore plate as a diagnostic character to separate these taxa at the generic level from other *Alexandrium* species. In addition, Gómez and Artigas (2019) claim that the shape of the apical pore of *Episemicolon* is unique because it is "oval or bullet" (their Table 1, as "Shape of apical pore plate") which is incorrect because it is comma-shaped (Larsen and Nguyen-Ngoc, 2004), just like other *Alexandrium* species.

The tabulation of the genus *Centrodinium*, as displayed in *Centrodinium punctatum*, is identical to that of *Alexandrium* when taking into account plate homologies (Li et al., 2019). It can not be excluded that some of the differences listed by Gómez and Artigas (2019) such as the shape of the apical pore, the presence of a pore in the anterior sulcal plate, etc., could be sufficient to separate this taxon on a subgeneric level. More detailed morphological information on the type species of *Centrodinium*, *C. elongatum*, is however required. The large variation in tabulation reported in species of *Centrodinium* by Hernández-Becerril et al. (2010, see their Table 1 for a summary) indicates that further investigation into this genus is needed to properly report on its phylogenetic placement.

In summary, the morphological concepts used to separate the reinstated genera from *Alexandrium s.s.* are highly variable and insufficient to justify a split of the genus *Alexandrium*. There is also insufficient morphological evidence to decide whether *Episemicolon* and *Centrodinium* warrant separate generic names.

The cyst morphology does not support the new genera

Cysts of *Alexandrium* are morphologically diverse (Table 2) and different from cysts of closely related genera, such as *Pyrodinium*, which has process-bearing cysts (e.g., Wall and Dale, 1968, pp. 102–103) or *Fragilidium,* which has a very thick layer of mucus (12–18 µm; Owen and

Norris, 1985). Cyst morphology can serve to subdivide genera, as has been proposed for the genus *Protoperidinium* (Harland, 1982). Because cysts are well-known within the genus *Alexandrium* (e.g., Bolch et al., 1991; Matsuoka and Fukuyo, 2000; Bravo et al., 2006), they should be taken into account for the best possible integrative taxonomy. Since there is no consistent cyst morphology that can be associated with any of the genera proposed by Gómez and Artigas (2019), cyst morphology also does not unambiguously support the subdivision of *Alexandrium* into these genera.

There is no evident relationship of the proposed genera to toxin production (chemotaxonomy). Toxin production has long been considered to be a character independent of chemical taxonomy because the same toxins have been described in very distantly related dinoflagellate genera, e.g., okadaic acid in *Prorocentrum* spp. and *Dinophysis* spp., STX or analogs in *Alexandrium* spp., *Gymnodinium catenatum*, *Pyrodinium bahamense* as well as in several cyanobacterial species (e.g., *Aphanizomenon flosaquae* and *Lyngbya wollei*), or domoic acid which is produced in several diatom genera of *Pseudo-nitzschia* and *Nitzschia*, as well as in the macroalga *Chondria armata*.

For the genera in question here, three toxin groups are worth considering for chemotaxonomy: saxitoxins (STXs), spiroimines (spirolides and gymnodimines) and goniodomins. Based on John et al. (2014), Murray et al. (2015), Lassus et al. (2016), Lugliè et al. (2017), and Branco et al. (2020), STX or analogs are produced by 14 *Alexandrium* species (*A. acatenella*, *A. affine*, *A. andersonii*, *A. australiense*, *A. catenella*, *A. cohorticula*, *A. fragae*, *A. leei*, *A. minutum*, *A. ostenfeldii*, *A. pacificum*, *A. tamarense*[1], *A. tamiyavanichii*, *A. taylorii*), which do not form a clear monophyletic cluster (Murray et al., 2015, Fig. 1). Due to the spread of STX-production across a range of phylogenetically different *Alexandrium* species, STX-production in this genus appears to be very common but it is not clear whether it should be considered a coherent taxonomic feature for this genus. The increasing number of STX analogues should be systematically re-verified in a large number of geographically diverse strains, with the limits of detection (LOD) and quantification (LOQ) provided.

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[1] Following the taxonomic concept of *A. tamarense* of John et al. (2014), only one strain of *A. tamarense* is currently considered to produce low amounts of gonyautoxins (Lugliè et al., 2017).

Spiroimines are solely known to be produced by *Alexandrium ostenfeldii* (= *Alexandrium peruvianum*; Kremp et al., 2014; Zurhelle et al., 2018).

Goniodomins have been reported to be produced by *Alexandrium monilatum*, *Alexandrium hiranoi* and *Alexandrium pseudogonyaulax* (Harris et al., 2020).

Overall, it should be noted that many papers only report positive presence of toxins in a strain but not the LOD or LOQ for those analogues that were not discovered. There are few studies systematically reporting comparative presence of analogues in a wide range of species, e.g. Wiese et al. (2010), for STX group toxins. However, spirolides have not been systematically searched in most *Alexandrium* species and goniodomins have been largely overlooked other than in the three species mentioned above.

Conclusions

The data presented by Gómez and Artigas (2019) are insufficiently robust to form the basis on which to subdivide species of the genus *Alexandrium* into four different genera, and maintain *Centrodinium*. Resolving consistent generic-level clusters within the genus *Alexandrium* and across the gonyaulacoids more generally, would require additional detailed morphological re-investigations and more extensive multigene phylogenies, with careful attention to rigorous testing of taxon sampling effects, branching order stability, long-branch effects, and careful selection of appropriate multiple outgroups for rooting local versus global dinoflagellate phylogenies. A secondary structure analysis of a more expanded dataset could also be beneficial. Inclusion of cyst morphology and chemotaxonomic information should also be strived for. There is an extensive literature on *Alexandrium* species and this is a very active area of research. If the proposal of Gómez and Artigas (2019) is adopted there will be considerable disruption and confusion to this field of study.

Therefore, here it is recommended to continue using the generic name *Alexandrium* for species of this complex, and to refrain from using *Protogonyaulax, Gessnerium,* and *Episemicolon.* The proposals by Gómez and Artigas (2019), if adopted, would introduce taxonomical instability into this group of species. A proposal to conserve *Alexandrium* against *Centrodinium* will be submitted to the International Nomenclature Committee for Algae (INA) in parallel to this note. The nomenclatural stability has particular importance as many species of *Alexandrium* cause harmful algal blooms and produce potent biotoxins. In addition to the biological scientific community the generic name *Alexandrium* is used also by chemists, medical scientists such as toxicologists, veterinarians, seafood safety regulators, fisheries and aquaculture industry personnel, administrators, and environmental and fisheries policy makers as outlined by Litaker et al. (2018). Furthermore, *Alexandrium* species are an important component of planktonic assemblages and taxonomic changes can create confusion for climate change studies and interpretations of long-term data sets. Finally, it is recommended that morphological criteria used to separate taxa are unambiguous and leave no room for doubt in the attribution of taxa (cf. paragraph 1 of the preamble of the ICN, Turland et al., 2018), that authors make their alignments freely available to allow for coherent progress in the field, and that authors strive for integrative taxonomy (Dayrat, 2005). Conservation of taxon names has been promoted across all organisms, to avoid taxonomic anarchy (Garnett and Christidis, 2017). As such, taxonomists should aim to conserve original names as much as possible and new taxa and combinations should only be created when robust morpho-molecular data obliges it (cf. paragraph 12 of the preamble of the ICN, Turland et al., 2018).

Acknowledgements

Support to DMA from the NOAA ECOHAB program (Grant #NA15NOS4780181) is gratefully acknowledged. Support to EG, AR, NS from the COPAs project (CTM2017-86121-R) is acknowledged. Marc Gottschling is acknowledged for interesting and civil discussions. Two anonymous reviewers are acknowledged for their useful comments.

References

Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. J. Phycol. 14, 224–234.

Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae 14, 10–35.

The Australian Shellfish Quality Assurance Program Operations Manual. 2019. Available at: http://safefish.com.au/reports/manuals-and-guidelines/the-australian-shellfish-quality-assurance-program-manual (last accessed 2 March 2020).

Baggesen, C., Moestrup, Ø., Daugbjerg, N., Krock, B., Cembella, A.D., Madsen, S., 2012. Molecular phylogeny and toxin profiles of *Alexandrium tamarense* (Lebour) Balech (Dinophyceae) from the west coast of Greenland. Harmful Algae 19, 108–116.

Balech, E., 1980. On thecal morphology of dinoflagellates with special emphasis on cingular and sulcal plates. An. Inst. Cienc. del Mar y Limnol. Univ. Nal. Autón. México 7(1), 57–68.

Balech, E., 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic Dinoflagellates. Elsevier Publishers B.V., Amsterdam, pp. 33–38.

Balech, E., 1989. Redescription of *Alexandrium minutum* Halim (Dinophyceae) type species of the genus *Alexandrium*. Phycologia 28(2), 206–211.

Balech, E., (Ed.) 1995. The genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Co. Cork, 151 p.

Balech, E., Tangen, K., 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae) *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. Sarsia 70, 333–343.

Blossom, H.E., Markussen, B., Daugbjerg, N., Krock, B., Norlin, A., Hansen, P.J., 2019. The cost of toxicity in microalgae: direct evidence from the dinoflagellate *Alexandrium*. Front. Microbiol. 10, 1065.

Bolch, C.J., Blackburn, S.I., Cannon, J.A., Hallegraeff, G.M., 1991. The resting cyst of the red-tide dinoflagellate *Alexandrium minutum* (Dinophyceae). Phycologia 30(2), 215–219.

Branco, S., Oliveira, M.M.M., Salgueiro, F., Vilar, M.C.P., Azevedo, S.M.F.O., Menezes, M., 2020. Morphology and molecular phylogeny of a new PST-producing dinoflagellate species: *Alexandrium fragae* sp. nov. (Gonyaulacales, Dinophyceae). Harmful Algae 95, 101793.

Bravo, I., Garcés, E., Diogène, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum.* Eur. J. Phycol. 41(3), 293–302.

Condie, S.A., Oliver, E.C.J., Hallegraeff, G.M., 2019. Environmental drivers of unprecedented *Alexandrium catenella* dinoflagellate blooms off eastern Tasmania, 2012–2018. Harmful Algae 87, 101628.

Dayrat, B., 2005. Towards integrative taxonomy. Biol. J. Linnean Soc. 85, 407–415.

Delgado, M., Garcés, E., Vila, M., Camp, J., 1997. Morphological variability in three populations of the dinoflagellate *Alexandrium taylori*. J. Plankton Res. 19(6), 749–757.

EFSA, 2009. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. The EFSA Journal 1019, 1–76.

FAO, 2004. *Marine biotoxins*. Food & Agriculture Organization of the United Nations 80.

Fraga, S., Sampedro, N., Larsen, J., Moestrup, Ø., Calado, A.J., 2015. Arguments against the proposal 2302 by John & al. to reject the name *Gonyaulax catenella* (*Alexandrium catenella*). Taxon 64(3), 634–635.

Fukuyo, Y., 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. Bull. Mar. Sci. 37(2), 529–537, 1 pl.

Fukuyo, Y., Inoue, H., 1990. *Alexandrium affine* (Inoue et Fukuyo) Balech. In: Fukuyo, Y., Takano, H., Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic guide. Uchida Rokakuho Co. Ltd., Tokyo, pp. 84–85.

Fukuyo, Y., Pholpunthin, Y., 1990a. *Alexandrium cohorticula* (Balech) Balech. In: Fukuyo, Y., Takano, H., Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic guide. Uchida Rokakuho Co. Ltd., Tokyo, pp. 88–89.

Fukuyo, Y., Pholpunthin, Y., 1990b. *Alexandrium leei* (Balech) Balech. In: Fukuyo, Y., Takano, H., Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic guide. Uchida Rokakuho Co. Ltd., Tokyo, pp. 92–93.

Fukuyo, Y., Yoshida, K., Inoue, H., 1985. *Protogonyaulax* in Japanese coastal waters. In: Anderson, D.M., White, A.W., Baden, D.G. (Eds.), Toxic dinoflagellates. Elsevier, New York, p. 27–32.

Gaarder, K.R., 1954. Dinoflagellates from the Michael Sars North Atlantic Deep-Sea Expedition 1910. In: Report on the scientific results of the "Michael Sars" north Atlantic deep-sea expedition 1910, carried out under the auspices of the Norwegian government and the superintendence of Sir John Murray, K.C.B., and Dr. Johan Hjort, Volume 2, 1–62.

Garnett, S.T., Christidis, L., 2017. Taxonomy anarchy hampers conservation. Nature 546, 25–27.

Garrett, M.J., Wolny, J.L., Williams, B.J., Dirks, M.D., Brame, J.A., Richardson, R.W., 2011. Methods for sampling and analysis of marine microalgae in ship ballast tanks: a case study from Tampa Bay, Florida, USA. Algae 26, 181–192.

Gómez, F., Artigas, L.F., 2019. Redefinition of the dinoflagellate genus *Alexandrium* based on *Centrodinium*: Reinstatement of *Gessnerium* and *Protogonyaulax*, and *Episemicolon* gen. nov. Hindawi Journal of Marine Biology, 1284104.

Gottschling, M, Chacón, J, Žerdoner Čalasan, A, Neuhaus, S., Kretschmann, J., Stibor, H., John, W., 2020. Phylogenetic placement of environmental sequences using taxonomically reliable databases helps to rigorously assess dinophyte biodiversity in Bavarian lakes (Germany). Freshw. Biol. 65, 193–208.

Gu, H., Zeng, N., Liu, T., Yang, W., Müller, A., Krock, B., 2013. Morphology, toxicity, and phylogeny of *Alexandrium* (Dinophyceae) species along the coast of China. Harmful Algae 27, 68–81.

Guiry, M.D. in Guiry, M.D., Guiry, G.M., 2020. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 24 February 2020.

Halim, Y., 1960. *Alexandrium minutum*, n. gen. n. sp. dinoflagellé provocant des "eaux rouges". Vie et Milieu 11, 102–105.

Halim, Y., 1967. Dinoflagellates of the south-east Caribbean sea. Int. Rev. gesamten Hydrobiol. 52(5), 701–755.

Hallegraeff, G.M., 2003. Harmful algal blooms: a global overview. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds), Manual on Harmful Marine Microalgae, Vol. 11, 2nd ed. IOC-UNESCO, Paris, pp. 25–49.

Harland, R., 1982. A review of Recent and Quaternary organic-walled dinoflagellate cysts of the genus *Protoperidinium*. Palaeontology 25(2), 369–397, pl. 38–42.

Harris, C.M., Reece, K.S., Stec, D.F., Scott, G.P., Jones, W.M., Hobbs, P.L.M., Harris, T.M., 2020. The toxin goniodomin, produced by *Alexandrium* spp., is identical to goniodomin A. Harmful Algae 92, 101707.

Hernández-Becerril, D.U., Bravo-Sierra, E., Ceballos-Corona, J.G.A, Esqueda-Lara, K., Escobar-Morales, S., Parra-Toriz, D., 2010. Morphology and taxonomy of the marine planktonic dinoflagellate *Centrodinium pulchrum* Bohm (Dinophyta) from the tropical Mexican Pacific, with comments on the taxonomy and distribution of the genus *Centrodinium*. Phycologia 49(5), 461–470.

Jin, D., Thunberg, E., Hoagland, P., 2008. Economic impact of the 2005 red tide event on commercial shellfish fisheries in New England. Ocean Coast. Manag. 51(5), 420–429.

John, U., Fensome, R.A., Medlin, L.K., 2003. The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* “species complex” (Dinophyceae). Mol. Biol. Evol. 20(7), 1015–1027.

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014. Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: The Introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165, 779–804.

Kim, K.-Y., Yoshida, M., Kim, C.H., 2005. Molecular phylogeny of three hitherto unreported *Alexandrium* species: *Alexandrium hiranoi, Alexandrium leei* and *Alexandrium satoanum* (Gonyaulacales, Dinophyceae) inferred from the 18S and 26S rDNA sequence data. Phycologia 44(4), 361–368.

Kita, T., Fukuyo, Y., Tokuda, H., Hirano, R., 1993. Sexual reproduction of *Alexandrium hiranoi* (Dinophyceae). Bull. Plankton Soc. Japan 39, 79–85.

Kremp, A., Lindholm, T., Dreßler, N., Erler, K., Gerdts, G., Eirtovaara, S., Leskinen, E., 2009. Bloom forming *Alexandrium ostenfeldii* (Dinophyceae) in shallow waters of the Åland Archipelago, Northern Baltic Sea. Harmful Algae 8, 318–328.

Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C.P., Tomas, C., 2014. Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. J. Phycol. 50, 81–100.

Kretzschmar, A.L., Verma, A., Murray, S., Kahlke, T., Fourment, M., Darling, A.E., 2019. Trial by phylogenetics - evaluating the multi-species coalescent for phylogenetic inference on taxa with high levels of paralogy (Gonyaulacales, Dinophyceae). bioRxiv 683383; doi: https://doi.org/10.1101/683383.

Kück, P., Mayer, C., Wägele, J.-W., Misof, B., 2012. Long branch effects distort maximum likelihood phylogenies in simulations despite selection of the correct model. PLoS One 0036593.

Larsen, J., Nguyen-Ngoc, L. (Eds.), 2004. Potentially toxic microalgae of Vietnamese waters. Opera Bot. 140, 5–216 [in English and Vietnamese].

Lassus P., Chomérat, N., Hess, P., Nézan, E., 2016. Toxic and Harmful Microalgae of the World Ocean / Micro-algues toxiques et nuisibles de l'océan mondial. Denmark, International Society for the Study of Harmful Algae / Intergovernmental Oceanographic Commission of UNESCO. IOC Manuals and Guides, 68 [Bilingual English/French].

Leaw, C.P., Lim, P.T., Ng, B.K., Cheah, M.Y., Ahmad, A., Usup, G., 2005. Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and nuclear ribosomal gene sequence. Phycologia 44(5), 550–565.

Li, Z., Mertens, K.N., Nézan, E., Chomérat, N., Bilien, G., Iwataki, M., Shin, H.H., 2019. Discovery of a new clade nested within the genus *Alexandrium* (Dinophyceae): Morpho-molecular characterization of *Centrodinium punctatum* (Cleve) F.J.R. Taylor. Protist 170(2), 168–186.

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

Litaker, R.W., Fraga, S., Montresor, M., Brosnahan, M., Anderson, D.M., Hoppenrath, M., Murray, S., Wolny, J., John, U., Sampedro, N., Larsen, J., Calado, A.J., 2018. A practical guide to new nomenclature for species within the "*Alexandrium tamarense* species complex". Harmful Algae News 61, 13–15.

Loeblich III, A.R., Loeblich, L.A., 1979. The systematics of *Gonyaulax* with special reference to the toxic species. In: Taylor, D.L., Seliger, H.H. (Eds.), Toxic dinoflagellate blooms. Elsevier North. Holl., New York, pp. 41–46.

Lugliè, A., Giacobbe, M.G., Riccardi, E., Bruno, M., Pigozzi, S., Mariani, M.A., Satta, C.T., Stacca, D., Bazzoni, A.M., Caddeo, T., Farina, P., Padedda, B.M., Pulina, S., Sechi, N., Milandri, A., 2017. Paralytic Shellfish Toxins and Cyanotoxins in the Mediterranean: New Data from Sardinia and Sicily (Italy). Microorganisms 5, 72.

MacKenzie, A.L., 2014. The risk to New Zealand shellfish aquaculture from paralytic shellfish poisoning (PSP) toxins. N. Z. J. Mar. Freshwater Res. 48(3), 430–465.

MacKenzie, L., Todd, K., 2002. *Alexandrium camurascutulum* sp. nov. (Dinophyceae): a new dinoflagellate species from New Zealand. Harmful Algae 1(3), 295–300.

MacKenzie, L., de Salas, M., Adamson, J., Beuzenberg, V., 2004. The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. Harmful Algae 3(1), 71–92.

Matsuoka, K., Fukuyo, Y., 2000. Technical guide for modern dinoflagellate cyst study. WESTPAC-HAB/WESTPAC/IOC, Japan Society of the Promotion Science, Tokyo, 29 p.

Menezes, M., Branco, S., Miotto, M.C., Alves-de-Souza, C., 2018. The Genus *Alexandrium* (Dinophyceae, Dinophyta) in Brazilian Coastal Waters. Front. Mar. Sci. 5, p. 421.

Moestrup, Ø., Akselmann-Cardella, R., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Lundholm, N., Zingone, A. (Eds.), 2009 onwards. IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Accessed at http://www.marinespecies.org/hab on 2020-02-20. doi:10.14284/362

Montresor, M., Zingone, A., Marino, D., 1993. The paratabulate resting cyst of *Alexandrium pseudogonyaulax* (Dinophyceae). In: Smayda, T.J., Shimizu, Y. (Eds), Toxic Phytoplankton Blooms in the Sea. Elsevier, pp. 159–164.

Montresor, M., Zingone, A., Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. J. Plankton Res. 20, 2291–2312.

Montresor, M., John, U., Beran, A., Medlin, L.K., 2004. *Alexandrium tamutum* sp. nov. (Dinophyceae): a new nontoxic species in the genus *Alexandrium*. J. Phycol. 40, 398–411.

Murray, S.A., Hoppenrath, M., Orr, R.J., Bolch, C., John, U., Diwan, R., Yauwenas, R., Harwood, T., de Salas, M., Neilan, B., Hallegraeff, G., 2014. *Alexandrium diversaporum* sp. nov., a new non-saxitoxin producing species: Phylogeny, morphology and *sxtA* genes. Harmful Algae 31, 54–65.

Murray, S.A., Diwan, R., Orr, R.J., Kohli, G.S., John, U., 2015. Gene duplication, loss and selection in the evolution of saxitoxin biosynthesis in alveolates. Mol. Phylogenetics Evol. 92, 165–180.

Nagai, S., Itakura, S., Matsuyama, Y., Kotani, Y., 2003. Encystment under laboratory conditions of the toxic dinoflagellate *Alexandrium tamiyavanichii* (Dinophyceae) isolated from the Seto Inland Sea, Japan. Phycologia 42, 646–653.

Nagai, S., Nishitani, G., Takano, Y., Yoshida, M., Takayama, H., 2009. Encystment and excystment under laboratory conditions of the nontoxic dinoflagellate *Alexandrium fraterculus* (Dinophyceae) isolated from the Seto Inland Sea, Japan. Phycologia 48, 177–185.

Nagai, S., Chen, H., Kawakami, Y., Yamamoto, K., Sildever, S., Kanno, N., Oikawa, H., Yasuike, M., Nakamura, Y., Hongo, Y., Fujiwara, A., Kobayashi, T., Gojobori, T., 2019. Monitoring of the toxic dinoflagellate *Alexandrium catenella* in Osaka Bay, Japan using a massively parallel sequencing (MPS)-based technique. Harmful Algae 89, 101660.

National Shellfish Sanitation Program. 2017. Guide for the Control of Molluscan Shellfish 2017 Revision. Available at: http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm (last accessed 2 March 2020).

Orr, R.J., Stüken, A., Rundberget, T., Eikrem, W., Jakobsen, K.S., 2011. Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach. Harmful Algae 10(6), 676–688.

Orr, R.J., Murray, S.A., Stüken A., Rhodes, L., Jakobsen, K.S., 2012. When naked became armored: an eight-gene phylogeny reveals monophyletic origin of theca in dinoflagellates. PLoS One 7(11), e50004. doi:10.1371/journal.pone.0050004

Owen, K.C., Norris, D.R., 1985. Cysts and life cycle considerations of the thecate dinoflagellate *Fragilidium*. J. Coast. Res. 1(3), 263–266.

Penna, A., Fraga, S., Masó, M., Giacobbe, M.G., Bravo, I., Garcés, E., Vila, M., Bertozzini, E., Andreoni, F., Luglie, A., 2008. Phylogenetic relationships among the Mediterranean *Alexandrium* (Dinophyceae) species based on sequences of 5.8 S gene and Internal Transcript Spacers of the rRNA operon. Eur. J. Phycol. 43(2), 163–178.

Rogers, J.E., Leblond, J.D., Moncreiff, C.A., 2006. Phylogenetic relationship of *Alexandrium monilatum* (Dinophyceae) to other *Alexandrium* species based on 18S ribosomal RNA gene sequences. Harmful Algae 5(3), 275–280.

Sanseverino, I., Conduto, D., Pozzoli, L., Dobricic, S., Lettieri, T., 2016. Algal bloom and its economic impact; EUR 27905 EN; doi:10.2788/660478.

Shin, H.H., Baek, S.H., Li, Z., Han, M.-S., Oh, S.J., Youn, S.-H., Kim, Y.S., Kim, D., Lim, W.-A. 2014. Resting cysts, and effects of temperature and salinity on the growth of vegetative cells of the potentially harmful species *Alexandrium insuetum* Balech (Dinophyceae). Harmful Algae 39, 175–184.

Taylor, F.J.R., 1979. The toxigenic gonyaulacoid dinoflagellates. In: Taylor, D.L., Seliger, H.H. (Eds.), Toxic dinoflagellate blooms. Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms, Key Biscayne, Florida, October 31–November 5, 1978, Elsevier/North-Holland, New York pp. 47–56.

Taylor, F.J.R., Fukuyo, Y., 1998. The neurotoxigenic dinoflagellate genus *Alexandrium* Halim: general introduction. In: *Physiological ecology of harmful algal blooms*. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), NATO ASI Series, vol. G 41, Springer-Verlag Berlin, Heidelberg, pp. 283–317.

Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Mar. Ecol. Prog. Ser. 230, 47–58.

Trainer, V.L., Yoshida, T. (Eds.) 2014. Proceedings of the Workshop on Economic Impacts of Harmful Algal Blooms on Fisheries and Aquaculture. PICES Sci. Rep. No. 47, 85 p.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M. J., Smith, G.F. (Eds.) 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen

Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. DOI https://doi.org/10.12705/Code.2018.

Turner, A.D., Dhanji-Rapkova, M., Fong, S.Y., Hungerford, J., McNabb, P.S., Boundy, M.J. and Harwood, D.T., 2019. Ultrahigh-Performance Hydrophilic Interaction Liquid Chromatography with Tandem Mass Spectrometry Method for the Determination of Paralytic Shellfish Toxins and Tetrodotoxin in Mussels, Oysters, Clams, Cockles, and Scallops: Collaborative Study. *Journal of AOAC International*, https://doi.org/10.5740/jaoacint.19-0240.

Walker, L.M., Steidinger, K.A., 1979. Sexual reproduction in the toxic dinoflagellate *Gonyaulax monilata*. J. Phycol. 15, 312–315.

Wall, D., Dale, B., 1969. The “hystrichosphaerid” resting spore of the dinoflagellate *Pyrodinium bahamense* Plate 1906. J. Phycol. 5, 140–149.

Wiese M., D'Agostino P.M., Mihali T.K., Moffitt M.C., Neilan B.A. 2010 Neurotoxic Alkaloids: Saxitoxin and Its Analogs. Mar. Drugs 8(7), 2185–2211.

Yoshida, M., Mizushima, K., Matsuoka, K., 2003. *Alexandrium acatenella* (Gonyaulacales: Dinophyceae): Morphological characteristics of vegetative cell and resting cyst. Plankton Biol. Ecol. 50(2), 61–64.

Yuki, K., Fukuyo, Y., 1992. *Alexandrium satoanum* sp. nov. (Dinophyceae) from Matoya Bay, central Japan. J. Phycol. 28, 395–399.

Zurhelle, C., Nieva, J., Tillmann, U., Harder, T., Krock, B., Tebben, J., 2018. Identification of Novel Gymnodimines and Spirolides from the Marine Dinoflagellate *Alexandrium ostenfeldii*. Mar. Drugs. 16(11), 446, doi: 10.3390/md16110446.

Table 1: A summary of published *Alexandrium* phylogeneties carried out with different DNA regions; reference of the study, figure number in that study, basal species mentioned (question marks denote uncertainty), and accessory notes. ND: the basal species cannot be determined because the authors chose *Alexandrium* species as the outgroup.

DNA region	**Reference**	**Fig**	**Basal species**	**Note**
SSU	Gómez and Artigas (2019)	Fig. 4	*A. satoanum, A. monilatum, A. taylorii, A. pseudogoniaulax*	
	John et al. (2003)	Fig. 1	*A. taylorii*	
	Rogers et al. (2006)	Fig. 2	*A. monilatum, A. taylorii*	
	Li et al. (2019)	Fig. S1	*A. pseudogonyaulax*	
LSU	Orr et al. (2011)	Fig. 1	*A. minutum, A. insuetum, A. ostenfeldii, A. tamutum, A. leei*?	
	Li et al. (2019)	Fig. S2	*A. minutum, A. ostenfeldii, A. andersonii, A. tamutum, A. insuetum, A. pseudogonyaulax*?	
LSU D1/D2	Gómez and Artigas (2019)	Fig. 5	*A. leei*	
	John et al. (2003)	Fig. 1	*A. taylorii*	
	Leaw et al. (2005)	Fig. 2	*A. leei*	*Pyrodinium* nests within *Alexandrium*
	Anderson et al. (2012)	Fig. 1	*A. leei* (+ *A. satoanum, A. pseudogonyaulax, A. hiranoi, A. taylorii*)?	
	Baggesen et al. (2012)	Fig. 3	ND	
	Gu et al. (2013)	Fig. 5	*A. insuetum, A. minutum, A. tamutum, A. ostenfeldii, A. margalefii, A. leei*?	
	Menezes et al. (2018)	Fig. 5	ND	
ITS region	Gu et al. (2013)	Fig. 5	*A. leei*	
	Menezes et al. (2018)	Fig. 4	*A. margalefii*	
	Li et al. (2019)	Fig. S3	*A. pseudogonyaulax*	
SSU+ITS region+LSU	Orr et al. (2011)	Fig. 2	*A. ostenfeldii, A. andersonii, A. tamutum, A. leei, A. insuetum*?	
	Murray et al. (2014)	Fig. 8	*A. hiranoi, A. pseudogonyaulax, A. taylorii, A. monilatum, A. satoanum*	
	Gottschling et al. (2018)	Fig. 4	*A. margalefii*	*Coolia* nests within *Alexandrium*
SSU+ITS region+LSU+cox1+cob+hsp90	Li et al. (2019)	Fig. 10	*A. pseudogonyaulax*	
SSU+ITS region+LSU+actin+beta-tubulin+cytochrome b+cox1+hsp90	Murray et al. (2015)	Fig. 8	*A. insuetum, A. ostenfeldii, A. andersonii, A. tamutum, A. minutum, A. margalefii, A. leei, A. diversaporum*	

Table 2: Summary of characters discussed in the text: name of taxon used in Gómez and Artigas (2019), species name, contact between plate 1' and Po, shape of plate 1', and resting cyst morphology. 1 = Balech, 1995; 2 = Balech, 1989; 3 = Lim et al., 2015; 4 = Montresor et al., 2004; 5 = John et al., 2014; 6 = Murray et al., 2014; 7 = MacKenzie and Todd, 2002; 8 = Gaarder, 1954; 9 = Larsen and Nguyen-Ngoc, 2004; 10 = Yuki and Fukuyo, 1992; 11 = Li et al., 2019; 12 = Fukuyo, 1985, as *A. catenella*; 13 = Delgado et al., 1997; 14 = Montresor et al., 1998; 15 = Shin et al., 2014; 16 = Bravo et al., 2006; 17 = Bolch et al., 1991; 18 = Kremp et al., 2009; 19 = Montresor et al., 2004; 20 = Yoshida et al., 2003; 21 = Anderson and Wall, 1978; 22 = Fukuyo and Pholpuntin, 1990a; 23 = Nagai et al., 2009; 24 = Fukuyo and Pholpuntin, 1990b; 25 = Nagai et al., 2003; 26 = Fukuyo and Inoue, 1990; 27 = Garret et al., 2011; 28 = Kita et al., 1993; 29 = Walker and Steidinger 1979; 30 = Montresor et al., 1993. 31 = Branco et al., 2020. * = not all strains produce saxitoxins. # = most strains do not produce saxitoxins. & = although also placed in *Alexandrium s.s.* by Gómez and Artigas (2019).

Taxon used in Gómez and Artigas (2019)	Species name	Contact between plate 1′ and Po	Shape of plate 1′	Resting cyst morphology
Alexandrium s.s.	*A. andersonii*	Direct or indirect[1]	Pentagonal[1]	Spherical[14]
Alexandrium s.s.	*A. diversaporum*	Direct[6]	Pentagonal[6]	Spherical[6]
Alexandrium s.s.	*A. insuetum*	None[1]	Pentagonal[1]	Spherical[15]
Alexandrium s.s.	*A. margalefii*	None[1]	Quadrangular[1]	Spherical[16]
Alexandrium s.s.	*A. minutum*	Direct or indirect[2]	Pentagonal[1]	Discoid[17]
Alexandrium s.s.	*A. ostenfeldii*	Direct or indirect[1]	Pentagonal[1]	Spherical[18]
Alexandrium s.s.	*A. pohangense*	None[3]	Pentagonal[3]	-
Alexandrium s.s.	*A. tamutum*	Direct[4]	Pentagonal[4]	Discoid[19]
Protogonyaulax	*A. acatenella*	Direct or indirect[2]	Pentagonal[1]	Ellipsoidal[20]
Protogonyaulax	*A. australiense*	Direct[5]	Pentagonal[5]	Ellipsoidal[5]
Protogonyaulax	*A. catenella*	Direct[1]	Pentagonal[1]	Ellipsoidal[21]
Protogonyaulax	*A. cohorticula*	Direct[1]	Pentagonal[1]	Ellipsoidal[22]
Protogonyaulax	*A. compressum*	Direct[1]	Pentagonal[1]	-
Protogonyaulax	*A. fraterculus*	Direct or indirect[2]	Pentagonal[1]	Spherical to ovoid[23]
Protogonyaulax	*A. kutnerae*	Direct or indirect[2]	Pentagonal[1]	Ellipsoidal[16]
Protogonyaulax[&]	*A. leei*	Direct[1]	Pentagonal[1]	Spherical[24]
Protogonyaulax	*A. mediterraneum*	Direct[5]	Pentagonal[5]	Ellipsoidal[5]
Protogonyaulax	*A. pacificum*	Direct[5]	Pentagonal[12]	Ellipsoidal[12]
Protogonyaulax	*A. tamarense*	Direct[1]	Pentagonal[1]	Ellipsoidal[21]
Protogonyaulax	*A. tamiyavanichii*	Direct[1]	Pentagonal[1]	Spherical[25]
Protogonyaulax	*A. tropicale*	Direct or indirect[1]	Pentagonal[1]	-
Episemicolon	*A. affine*	Direct[1]	Pentagonal[1]	Spherical[26]
Episemicolon	*A. gaarderae*	Direct[1]	Pentagonal[1]	-
Gessnerium	*A. balechii*	None[1]	Pentagonal[1]	Spherical[27]
Gessnerium	*A. camurascutulum*	None[7]	Pentagonal[7]	-
Gessnerium	*A. concavum*	None[8]	Pentagonal[8]	-
Gessnerium	*A. foedum*	None[1]	Pentagonal[1]	-
Gessnerium	*A. globosum*	None[9]	Pentagonal[9]	-
Gessnerium	*A. hiranoi*	None[1]	Pentagonal[1]	Discoid[28]
Gessnerium	*A. monilatum*	None[1]	Pentagonal[1]	Spherical to ovoid[29]
Gessnerium	*A. pseudogonyaulax*	None[1]	Pentagonal[1]	Spherical, paratabulate[30]
Gessnerium	*A. satoanum*	None[10]	Pentagonal[10]	-
Gessnerium	*A. taylorii*	None[1]	Quadrangular-Pentagonal[13]	Spherical to discoid[16]
Not listed	*A. depressum*	Direct or indirect[8]	Pentagonal[8]	-
Not listed	*A. fragae*	Direct or indirect[31]	Pentagonal[31]	-
Centrodinium	*C. punctatum*	Direct[11]	Quadrangular[11]	-