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

2

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85

86 **Abstract**

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

104

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

106

107 **Highlights**

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

111

112 *Introduction and aims*

113

114 The genus *Alexandrium* includes many species that have caused extensive economic and human
115 health impacts worldwide (e.g., Anderson et al., 2012). *Alexandrium* currently encompasses 34
116 accepted species, with *A. camurascutulum* considered invalid (Guiry in Guiry and Guiry, 2020). Of
117 these species, 14 are known to produce paralytic shellfish toxins (PSTs) (Moestrup et al., 2009), which
118 have caused extensive damage to aquaculture industries. The wide range of toxins produced by
119 *Alexandrium* species, belong to four families – PSTs (saxitoxin (STX) and its derivatives), spiroidines
120 (spiroclides and gymnodimines), goniodomins (e.g., Lassus et al., 2016), and lytic compounds (e.g.,
121 Tillmann and John, 2002; Blossom et al., 2019). The toxins with the most recognized potential for
122 economic impact are the PSTs, which are responsible for outbreaks of paralytic shellfish poisoning
123 (PSP), one of the most widespread harmful algal bloom (HAB)-related shellfish poisoning syndromes.
124 PSP outbreaks can cause human illness and death from contaminated shellfish or fish, loss of wild and
125 cultured seafood resources, impairment of tourism and recreational activities, alterations of marine
126 trophic structure, and death of marine mammals, fish, and seabirds (Anderson et al., 2012). Symptoms
127 of PSP in humans range from spreading numbness and tingling sensations, headache and nausea to
128 more extreme fatal cases due to respiratory paralysis (Hallegraeff, 2003). Blooms of species such as
129 *Alexandrium catenella*, *A. minutum*, and *A. pacificum* regularly cause losses of tens of millions of
130 dollars to aquaculture industries in North and South America, Europe, Asia (e.g., Trainer and Yoshido,
131 2014; Sanseverino et al., 2016), and Australia and New Zealand (e.g., Jin et al., 2008; MacKenzie,
132 2014). For example, in late 2012, a single bloom of *A. catenella* along the east coast of Tasmania
133 (Australia) resulted in ~AUD\$ 23 million loss to the wild harvest and aquaculture industries (Condie
134 et al., 2019). Monitoring of *Alexandrium* cells in the water column and of toxins in shellfish is
135 therefore critical for avoidance of adverse effects on human health (e.g., Nagai et al., 2019; EFSA,
136 2009) and nationally and internationally standardized methods have been established to guide PSP
137 testing (e.g., FAO Marine Biotoxins, 2004; [U.S.] National Shellfish Sanitation Program, 2017;
138 Australian Shellfish Quality Assurance Program, 2019; Turner et al., 2019). The European Union
139 requires all its member states to monitor coastal waters for toxin-producing plankton and toxins in

140 mussels (Directive 91/492d/EC and Commission Decision 2002/225/EC). In parallel, research on
141 *Alexandrium* species is vigorous: since 1975, there have been 2,768 published studies that include the
142 word *Alexandrium*, which have been cited 70,322 times, for an average of about 150 publications per
143 year over the last 10 years (Clarivate Analytics search on Web of Science Core Collection on 20
144 August 2020).

145 The taxonomic history of the genus *Alexandrium* is complex, and nomenclatural stability was not
146 attained for some time, as detailed by Balech (1995, pp. 1–3) and Taylor and Fukuyo (1998). The
147 genus *Alexandrium* was erected by Halim (1960) with the PST-producing *Alexandrium minutum* as its
148 type. A few years later, Halim (1967) erected *Gessnerium* with *Gessnerium mochimaense* Halim as its
149 type; this species had a pentagonal first apical (1') plate not in contact with the pore plate (Po).

150 Loeblich III and Loeblich (1979) considered *Alexandrium minutum* to be inadequately described, left
151 it in the genus *Alexandrium* and transferred seven *Gonyaulax* species and two *Goniodoma* species into
152 the genus *Gessnerium*. At the same time, Taylor (1979) erected *Protogonyaulax*, with *P. tamarensis* as
153 the type species and defined it as having a 1' plate directly contacting the Po of the apical pore

154 complex, and transferred eight species of the genus *Gonyaulax* and one *Pyrodinium* species into
155 *Protogonyaulax*. Taylor's proposal was followed by Fukuyo et al. (1985), who described two new
156 *Protogonyaulax* species (*P. affinis* and *P. compressa*). After a detailed examination of samples from
157 the type locality of *A. minutum* (the type species of *Alexandrium*), Balech (1989) noted that plate 1'
158 does not necessarily directly contact the Po in this species (the same applies to *A. fraterculus* and *A.*
159 *kutnerae*). He therefore re-established the genus *Alexandrium*, considering *Protogonyaulax* a junior
160 synonym of *Alexandrium*. All *Protogonyaulax* and *Gessnerium* species were thus transferred to
161 *Alexandrium* (Balech 1985, 1995) and *Gessnerium* was retained as a subgenus of *Alexandrium* for
162 species in which plate 1' is not rhomboidal and does not contact the Po (Balech, 1995). Since Balech
163 (1989), the consensus has been to only use the genus name *Alexandrium*. The currently accepted plate

164 formula for *Alexandrium* is Po, 4', 6'', 6c, 9–10s, 5''', 2'''' (Balech, 1980, 1995; Balech and Tangen,
165 1985).

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

176 Recently, Li et al. (2019) showed that the fusiform dinoflagellate *Centrodinium punctatum*
177 forms a clade nesting within *Alexandrium*. Through a morpho-molecular study of two other
178 *Centrodinium* species (*C. eminens* and *C. intermedium*), but without a re-investigation of the type, *C.*
179 *elongatum*, Gómez and Artigas (2019) proposed to retain *Centrodinium* and subdivide the species
180 formerly included in the genus *Alexandrium*, sharing a common thecal plate pattern, into four distinct
181 genera, namely *Alexandrium sensu stricto* (*s.s.*) (emended diagnosis), the re-introduced genera
182 *Gessnerium* and *Protogonyaulax*, and the new genus *Episemicolon*. Here, the data presented in Gómez
183 and Artigas (2019) are critically discussed, and it is shown that they do not support such a drastic
184 taxonomic rearrangement. The aim of this study is not to present an alternative analysis and revision,
185 but to maintain the genus *Alexandrium*. Furthermore, it is emphasized to use integrative taxonomy
186 which delimits taxa using multiple and complementary perspectives (Dayrat, 2005), including the
187 consideration of cyst morphology in recognizing taxa (as previously suggested by Taylor and Fukuyo,
188 1998, p. 6) and chemotaxonomy, amongst other criteria.

189

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

191 The finding that species of *Centrodinium* nest phylogenetically in *Alexandrium* (Li et al.,
192 2019; Gómez and Artigas, 2019) makes the latter paraphyletic. Gómez and Artigas (2019) proposed to
193 taxonomically split *Alexandrium* into four separate genera (not including *Centrodinium*, which never
194 belonged to *Alexandrium*). This proposal establishes a new genus *Episemicolon*, while the diagnosis of
195 three other genera (*Alexandrium*, *Gessnerium*, and *Protogonyaulax*) was emended to group subsets of
196 former *Alexandrium* species. The authors stated that “The polyphyly [*sic*] of *Alexandrium* is solved
197 with the split into four genera”. Notably, *Alexandrium* was not polyphyletic, but paraphyletic. The
198 suggestion that their proposal ‘solved’ this problem is the basis for our critique, and thus it is therefore
199 worth evaluating the phylogenetic information presented by Gómez and Artigas (2019) in detail. Our
200 question was: Does the data justify splitting a phylogenetically well-defined group (e.g., John et al.,
201 2003; Rogers et al., 2006; Orr et al., 2011; Anderson et al., 2012; Baggesen et al., 2012; Gu et al.,
202 2013; Murray et al 2015; Menezes et al., 2018; Kretzschmar et al., 2019), sharing the same Kofoidian
203 plate tabulation, into segregate genera?

204 Gómez and Artigas (2019) presented two phylogenetic trees, Fig. 4 a phylogenetic analysis of
205 alignments of the SSU (18S) region of the rRNA operon and Fig. 5 the D1–D2 region of the LSU
206 (28S) region of the rRNA operon. Each alignment was analyzed using a maximum likelihood (ML)
207 approach and clades were given with bootstrap support. There was no information on the number of
208 base pairs analyzed, the number of informative sites, and the alignments were not made available for
209 evaluation. These phylogenetic analyses are problematic for several reasons:

210

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

218

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

226
227 3. The Gómez and Artigas (2019) phylogenies have used short alignments with too few characters and
228 taxon information to accurately infer deeper, clade level branchings within the genus *Alexandrium*
229 which will be stable into the future. Of the major factors that impact the accuracy of phylogenetic
230 inference, two are particularly important: the length of aligned sequence/number of genes used, and
231 the taxon sampling. Past studies of *Alexandrium* phylogenetics were reviewed (Table 1, John et al.,
232 2003; Leaw et al., 2005; Rogers et al., 2006; Orr et al., 2011; Anderson et al., 2012; Baggesen et al.,
233 2012; Gu et al 2013; Murray et al., 2014; Murray et al., 2015; Menezes et al., 2018; Gottschling et al.,
234 2020), and this showed that clades within *Alexandrium* differed depending on gene and taxon
235 sampling (exemplified by basal clades shown in Table 1). All else being equal, more sequence data
236 and greater taxon sampling generally leads to more accurate and well-supported phylogenies. Murray
237 et al. (2015) conducted an analysis of *Alexandrium* using a concatenated alignment of eight genes,
238 with a total length of 7308 bp. The position of several of the major clades of *Alexandrium* differed in
239 that analysis, compared to the analysis presented by Gómez and Artigas (2019). Similarly, new
240 ribotypes are still being reported, such as *Alexandrium fragae* (Branco et al., 2020) and three new
241 phylotypes nested within the genus (Nishimura et al., in review). It is likely that these new discoveries
242 and longer alignments/greater gene sampling will lead to more strongly supported phylogenies than
243 those of Gómez and Artigas (2019), whose phylogenies appear to be too weak to support taxonomic
244 rearrangements.

245 As any taxonomic rearrangement of *Alexandrium* will potentially impact hundreds or
246 thousands of scientists, government regulators, and the seafood industry, it is crucial that the

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

259

260 *The morphological concepts of the reinstated and emended genera proposed by Gómez and Artigas*
261 *(2019) have little taxonomic value*

262 As mentioned in the introduction, *Protogonyaulax* was described by Taylor (1979) as having a
263 plate 1' in contact with the Po. As remarked by Balech (1989, p. 210) for the type species of

264 *Alexandrium*, *A. minutum*, as well as for *A. fraterculus* and *A. kutnerae*, this feature is variable, and the
265 plate 1' can have an indirect contact with the Po through a filiform prolongation of plate 1'

266 (*Alexandrium acatenella*, *A. andersonii*, *A. ostefeldii*, *A. tropicale* are additional examples; Balech,
267 1995). Gómez and Artigas (2019) considered species exhibiting only a direct contact of plate 1' to the

268 Po as belonging to their emended concept of *Protogonyaulax*, and species with direct or indirect
269 contact (presumably meaning displaying both types) as belonging to their emended genus

270 *Alexandrium*. However, when reviewing the literature, it is clear that there is no consistency with the
271 application of both genus concepts (Table 2); furthermore, the high variability of the contact between

272 plate 1' and Po within *Alexandrium* species speaks against considering this as a reliable taxonomic

273 character. In addition, Gómez and Artigas (2019) describe the 6'' plate as being “usually narrow” in
274 *Alexandrium s.s.*, however, this is not so in *A. insuetum*, *A. ostenfeldii*, and *A. tamutum*, species which
275 are retained in *Alexandrium s.s.* in their proposed phylogeny. Finally, the posterior sulcal plate (Sp) of
276 the emended genus *Protogonyaulax* is longer than wide, and in *Alexandrium s.s.* wider than long, but
277 the Sp of *A. leei*, included in *Protogonyaulax*, is wider than long and in *A. margalefii*, included in
278 *Alexandrium s.s.*, longer than wide with an extremely oblique anterior margin (Balech, 1995). In
279 summary, the considerations of Balech (1995) which support synonymization of *Protogonyaulax* with
280 *Alexandrium*, still stand.

281 The reinstated genus *Gessnerium* also presents problems. Gómez and Artigas (2019) included
282 within this genus species with a pentagonal plate 1' not in contact with the Po. They excluded three
283 species with a plate 1' not in contact with the Po from their concept of *Gessnerium*: *A. insuetum*, which
284 they included in *Alexandrium s.s.* despite the fact that this species has a pentagonal 1' plate that does
285 not touch the Po (Balech, 1995, Plate XVII, Figs. 1–23), and *A. pohangense* and *A. margalefii*, which
286 were not formally attributed to any genus, although were assigned to the *Alexandrium s.s.* clade in
287 their Fig. 4. The authors considered the quadrangular plate 1' of *A. pohangense* and *A. margalefii* as a
288 unique character distinguishing them from the other *Gessnerium*. However, the plate 1' in *A.*
289 *pohangense* has a short suture with plate 2' and can therefore be considered pentagonal (Lim et al.,
290 2015, their Fig. 4B), and such a short suture can also be observed in *A. balechii* and *A. foedum*
291 according to Balech (1995, p. 103), which were classified as *Gessnerium* by Gómez and Artigas
292 (2019). Within *A. taylorii*, the plate 1' is known to vary between a quadrangular and pentagonal shape
293 (Delgado et al., 1997). The infraspecific variability of the shape of this plate indicates that it cannot be
294 used as a diagnostic character at the genus level (Table 2). Finally, the Sp of the emended genus
295 *Gessnerium* is longer than wide and extending obliquely, but in *A. monilatum*, which is included in
296 this genus, the Sp is rhomboid (Balech, 1995).

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

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

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

320

321 *The cyst morphology does not support the new genera*

322 Cysts of *Alexandrium* are morphologically diverse (Table 2) and different from cysts of
323 closely related genera, such as *Pyrodinium*, which has process-bearing cysts (e.g., Wall and Dale,
324 1968, pp. 102–103) or *Fragilidium*, which has a very thick layer of mucus (12–18 µm; Owen and

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

331
332 *There is no evident relationship of the proposed genera to toxin production (chemotaxonomy).*

333 Toxin production has long been considered to be a character independent of chemical taxonomy
334 because the same toxins have been described in very distantly related dinoflagellate genera, e.g.,
335 okadaic acid in *Prorocentrum* spp. and *Dinophysis* spp., STX or analogs in *Alexandrium* spp.,
336 *Gymnodinium catenatum*, *Pyrodinium bahamense* as well as in several cyanobacterial species (e.g.,
337 *Aphanizomenon flosaquae* and *Lyngbya wollei*), or domoic acid which is produced in several diatom
338 genera of *Pseudo-nitzschia* and *Nitzschia*, as well as in the macroalga *Chondria armata*.

339 For the genera in question here, three toxin groups are worth considering for chemotaxonomy:
340 saxitoxins (STXs), spiroimines (spiroptides and gymnodimines) and goniodomins. Based on John et al.
341 (2014), Murray et al. (2015), Lassus et al. (2016), Lugliè et al. (2017), and Branco et al. (2020), STX
342 or analogs are produced by 14 *Alexandrium* species (*A. acatenella*, *A. affine*, *A. andersonii*, *A.*
343 *australiense*, *A. catenella*, *A. cohorticula*, *A. fragae*, *A. leei*, *A. minutum*, *A. ostenfeldii*, *A. pacificum*,
344 *A. tamarensis*¹, *A. tamiyavanichii*, *A. taylorii*), which do not form a clear monophyletic cluster (Murray
345 et al., 2015, Fig. 1). Due to the spread of STX-production across a range of phylogenetically different
346 *Alexandrium* species, STX-production in this genus appears to be very common but it is not clear
347 whether it should be considered a coherent taxonomic feature for this genus. The increasing number of
348 STX analogues should be systematically re-verified in a large number of geographically diverse
349 strains, with the limits of detection (LOD) and quantification (LOQ) provided.

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

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

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

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

360

361 *Conclusions*

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

373 Therefore, here it is recommended to continue using the generic name *Alexandrium* for
374 species of this complex, and to refrain from using *Protogonyaulax*, *Gessnerium*, and *Episemicolon*.
375 The proposals by Gómez and Artigas (2019), if adopted, would introduce taxonomical instability into
376 this group of species. A proposal to conserve *Alexandrium* against *Centrodinium* will be submitted to
377 the International Nomenclature Committee for Algae (INA) in parallel to this note. The nomenclatural

378 stability has particular importance as many species of *Alexandrium* cause harmful algal blooms and
379 produce potent biotoxins. In addition to the biological scientific community the generic name
380 *Alexandrium* is used also by chemists, medical scientists such as toxicologists, veterinarians, seafood
381 safety regulators, fisheries and aquaculture industry personnel, administrators, and environmental and
382 fisheries policy makers as outlined by Litaker et al. (2018). Furthermore, *Alexandrium* species are an
383 important component of planktonic assemblages and taxonomic changes can create confusion for
384 climate change studies and interpretations of long-term data sets. Finally, it is recommended that
385 morphological criteria used to separate taxa are unambiguous and leave no room for doubt in the
386 attribution of taxa (cf. paragraph 1 of the preamble of the ICN, Turland et al., 2018), that authors make
387 their alignments freely available to allow for coherent progress in the field, and that authors strive for
388 integrative taxonomy (Dayrat, 2005). Conservation of taxon names has been promoted across all
389 organisms, to avoid taxonomic anarchy (Garnett and Christidis, 2017). As such, taxonomists should
390 aim to conserve original names as much as possible and new taxa and combinations should only be
391 created when robust morpho-molecular data obliges it (cf. paragraph 12 of the preamble of the ICN,
392 Turland et al., 2018).

393

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399

400 **References**

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

403

- 404 Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The
405 globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on
406 human health. *Harmful Algae* 14, 10–35.
- 407
- 408 The Australian Shellfish Quality Assurance Program Operations Manual. 2019. Available at:
409 [http://safefish.com.au/reports/manuals-and-guidelines/the-australian-shellfish-quality-assurance-](http://safefish.com.au/reports/manuals-and-guidelines/the-australian-shellfish-quality-assurance-program-manual)
410 [program-manual](http://safefish.com.au/reports/manuals-and-guidelines/the-australian-shellfish-quality-assurance-program-manual) (last accessed 2 March 2020).
- 411
- 412 Baggesen, C., Moestrup, Ø., Daugbjerg, N., Krock, B., Cembella, A.D., Madsen, S., 2012. Molecular
413 phylogeny and toxin profiles of *Alexandrium tamarense* (Lebour) Balech (Dinophyceae) from the west
414 coast of Greenland. *Harmful Algae* 19, 108–116.
- 415
- 416 Balech, E., 1980. On thecal morphology of dinoflagellates with special emphasis on cingular and
417 sulcal plates. *An. Inst. Cienc. del Mar y Limnol. Univ. Nal. Autón. México* 7(1), 57–68.
- 418
- 419 Balech, E., 1985. The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: Anderson, D.M.,
420 White, A.W., Baden, D.G. (Eds.), *Toxic Dinoflagellates*. Elsevier Publishers B.V., Amsterdam, pp.
421 33–38.
- 422
- 423 Balech, E., 1989. Redescription of *Alexandrium minutum* Halim (Dinophyceae) type species of the
424 genus *Alexandrium*. *Phycologia* 28(2), 206–211.
- 425
- 426 Balech, E., (Ed.) 1995. The genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station,
427 Co. Cork, 151 p.
- 428
- 429 Balech, E., Tangen, K., 1985. Morphology and taxonomy of toxic species in the *tamarensis* group
430 (Dinophyceae) *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen)
431 comb. nov. *Sarsia* 70, 333–343.

432

433 Blossom, H.E., Markussen, B., Daugbjerg, N., Krock, B., Norlin, A., Hansen, P.J., 2019. The cost of
434 toxicity in microalgae: direct evidence from the dinoflagellate *Alexandrium*. *Front. Microbiol.* 10,
435 1065.

436

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

439

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

443

444 Bravo, I., Garcés, E., Diogène, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the
445 toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean
446 coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. *Eur. J. Phycol.* 41(3),
447 293–302.

448

449 Condie, S.A., Oliver, E.C.J., Hallegraeff, G.M., 2019. Environmental drivers of unprecedented
450 *Alexandrium catenella* dinoflagellate blooms off eastern Tasmania, 2012–2018. *Harmful Algae* 87,
451 101628.

452

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

454

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

457

- 458 EFSA, 2009. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the
459 European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. The EFSA Journal 1019,
460 1–76.
461
- 462 FAO, 2004. *Marine biotoxins*. Food & Agriculture Organization of the United Nations 80.
463
- 464 Fraga, S., Sampedro, N., Larsen, J., Moestrup, Ø., Calado, A.J., 2015. Arguments against the proposal
465 2302 by John & al. to reject the name *Gonyaulax catenella* (*Alexandrium catenella*). Taxon 64(3),
466 634–635.
467
- 468 Fukuyo, Y., 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax*
469 *catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. Bull. Mar. Sci. 37(2), 529–537,
470 1 pl.
471
- 472 Fukuyo, Y., Inoue, H., 1990. *Alexandrium affine* (Inoue et Fukuyo) Balech. In: Fukuyo, Y., Takano,
473 H., Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic guide.
474 Uchida Rokakuho Co. Ltd., Tokyo, pp. 84–85.
475
- 476 Fukuyo, Y., Pholpunthin, Y., 1990a. *Alexandrium cohorticula* (Balech) Balech. In: Fukuyo, Y.,
477 Takano, H., Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic
478 guide. Uchida Rokakuho Co. Ltd., Tokyo, pp. 88–89.
479
- 480 Fukuyo, Y., Pholpunthin, Y., 1990b. *Alexandrium leei* (Balech) Balech. In: Fukuyo, Y., Takano, H.,
481 Chihara, M., Matsuoka, K. (Eds.), Red tide organisms in Japan – an illustrated taxonomic guide.
482 Uchida Rokakuho Co. Ltd., Tokyo, pp. 92–93.
483
- 484 Fukuyo, Y., Yoshida, K., Inoue, H., 1985. *Protogonyaulax* in Japanese coastal waters. In: Anderson,
485 D.M., White, A.W., Baden, D.G. (Eds.), Toxic dinoflagellates. Elsevier, New York, p. 27–32.

486

487 Gaarder, K.R., 1954. Dinoflagellates from the Michael Sars North Atlantic Deep-Sea Expedition 1910.

488 In: Report on the scientific results of the "Michael Sars" north Atlantic deep-sea expedition 1910,

489 carried out under the auspices of the Norwegian government and the superintendence of Sir John

490 Murray, K.C.B., and Dr. Johan Hjort, Volume 2, 1–62.

491

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

493

494 Garrett, M.J., Wolny, J.L., Williams, B.J., Dirks, M.D., Brame, J.A., Richardson, R.W., 2011.

495 Methods for sampling and analysis of marine microalgae in ship ballast tanks: a case study from

496 Tampa Bay, Florida, USA. *Algae* 26, 181–192.

497

498 Gómez, F., Artigas, L.F., 2019. Redefinition of the dinoflagellate genus *Alexandrium* based on

499 *Centrodinium*: Reinstatement of *Gessnerium* and *Protogonyaulax*, and *Episemicolon* gen. nov.

500 *Hindawi Journal of Marine Biology*, 1284104.

501

502 Gottschling, M, Chacón, J, Žerdoner Čalasan, A, Neuhaus, S., Kretschmann, J., Stibor, H., John, W.,

503 2020. Phylogenetic placement of environmental sequences using taxonomically reliable databases

504 helps to rigorously assess dinophyte biodiversity in Bavarian lakes (Germany). *Freshw. Biol.* 65, 193–

505 208.

506

507 Gu, H., Zeng, N., Liu, T., Yang, W., Müller, A., Krock, B., 2013. Morphology, toxicity, and

508 phylogeny of *Alexandrium* (Dinophyceae) species along the coast of China. *Harmful Algae* 27, 68–81.

509

510 Guiry, M.D. in Guiry, M.D., Guiry, G.M., 2020. *AlgaeBase*. World-wide electronic publication,

511 National University of Ireland, Galway. <http://www.algaebase.org>; searched on 24 February 2020.

512

- 513 Halim, Y., 1960. *Alexandrium minutum*, n. gen. n. sp. dinoflagellé provocant des "eaux rouges". Vie et
514 Milieu 11, 102–105.
- 515
- 516 Halim, Y., 1967. Dinoflagellates of the south-east Caribbean sea. Int. Rev. gesamten Hydrobiol. 52(5),
517 701–755.
- 518
- 519 Hallegraeff, G.M., 2003. Harmful algal blooms: a global overview. In: Hallegraeff, G.M., Anderson,
520 D.M., Cembella, A.D. (Eds), Manual on Harmful Marine Microalgae, Vol. 11, 2nd ed. IOC-UNESCO,
521 Paris, pp. 25–49.
- 522
- 523 Harland, R., 1982. A review of Recent and Quaternary organic-walled dinoflagellate cysts of the
524 genus *Protoperidinium*. Palaeontology 25(2), 369–397, pl. 38–42.
- 525
- 526 Harris, C.M., Reece, K.S., Stec, D.F., Scott, G.P., Jones, W.M., Hobbs, P.L.M., Harris, T.M., 2020.
527 The toxin goniodomin, produced by *Alexandrium* spp., is identical to goniodomin A. Harmful Algae
528 92, 101707.
- 529
- 530 Hernández-Becerril, D.U., Bravo-Sierra, E., Ceballos-Corona, J.G.A, Esqueda-Lara, K., Escobar-
531 Morales, S., Parra-Toriz, D., 2010. Morphology and taxonomy of the marine planktonic dinoflagellate
532 *Centrodinium pulchrum* Bohm (Dinophyta) from the tropical Mexican Pacific, with comments on the
533 taxonomy and distribution of the genus *Centrodinium*. Phycologia 49(5), 461–470.
- 534
- 535 Jin, D., Thunberg, E., Hoagland, P., 2008. Economic impact of the 2005 red tide event on commercial
536 shellfish fisheries in New England. Ocean Coast. Manag. 51(5), 420–429.
- 537
- 538 John, U., Fensome, R.A., Medlin, L.K., 2003. The application of a molecular clock based on
539 molecular sequences and the fossil record to explain biogeographic distributions within the
540 *Alexandrium tamarense* “species complex” (Dinophyceae). Mol. Biol. Evol. 20(7), 1015–1027.

541

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

545

546 Kim, K.-Y., Yoshida, M., Kim, C.H., 2005. Molecular phylogeny of three hitherto unreported
547 *Alexandrium* species: *Alexandrium hiranoi*, *Alexandrium leei* and *Alexandrium satoanum*
548 (Gonyaulacales, Dinophyceae) inferred from the 18S and 26S rDNA sequence data. *Phycologia* 44(4),
549 361–368.

550

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

553

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

557

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

561

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

566

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

- 569
- 570 Larsen, J., Nguyen-Ngoc, L. (Eds.), 2004. Potentially toxic microalgae of Vietnamese waters. *Opera*
571 *Bot.* 140, 5–216 [in English and Vietnamese].
- 572
- 573 Lassus P., Chomérat, N., Hess, P., Nézan, E., 2016. Toxic and Harmful Microalgae of the World
574 Ocean / Micro-algues toxiques et nuisibles de l'océan mondial. Denmark, International Society for the
575 Study of Harmful Algae / Intergovernmental Oceanographic Commission of UNESCO. IOC Manuals
576 and Guides, 68 [Bilingual English/French].
- 577
- 578 Leaw, C.P., Lim, P.T., Ng, B.K., Cheah, M.Y., Ahmad, A., Usup, G., 2005. Phylogenetic analysis of
579 *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and
580 nuclear ribosomal gene sequence. *Phycologia* 44(5), 550–565.
- 581
- 582 Li, Z., Mertens, K.N., Nézan, E., Chomérat, N., Bilién, G., Iwataki, M., Shin, H.H., 2019. Discovery of
583 a new clade nested within the genus *Alexandrium* (Dinophyceae): Morpho-molecular characterization
584 of *Centrodinium punctatum* (Cleve) F.J.R. Taylor. *Protist* 170(2), 168–186.
- 585
- 586 Lim, A.S., Jeong, H.J., Kim, J.H., Lee, S.Y., 2015. Description of the new phototrophic dinoflagellate
587 *Alexandrium pohangense* sp. nov. from Korean coastal waters. *Harmful Algae* 46, 49–61.
- 588
- 589 Litaker, R.W., Fraga, S., Montresor, M., Brosnahan, M., Anderson, D.M., Hoppenrath, M., Murray, S.,
590 Wolny, J., John, U., Sampedro, N., Larsen, J., Calado, A.J., 2018. A practical guide to new
591 nomenclature for species within the “*Alexandrium tamarense* species complex”. *Harmful Algae News*
592 61, 13–15.
- 593
- 594 Loeblich III, A.R., Loeblich, L.A., 1979. The systematics of *Gonyaulax* with special reference to the
595 toxic species. In: Taylor, D.L., Seliger, H.H. (Eds.), *Toxic dinoflagellate blooms*. Elsevier North.
596 Holl., New York, pp. 41–46.

597

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

602

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

605

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

608

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

612

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

615

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

618

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

623

- 624 Montresor, M., Zingone, A., Marino, D., 1993. The paratabulate resting cyst of *Alexandrium*
625 *pseudogonyaulax* (Dinophyceae). In: Smayda, T.J., Shimizu, Y. (Eds), Toxic Phytoplankton Blooms
626 in the Sea. Elsevier, pp. 159–164.
- 627
- 628 Montresor, M., Zingone, A., Sarno, D., 1998. Dinoflagellate cyst production at a coastal
629 Mediterranean site. J. Plankton Res. 20, 2291–2312.
- 630
- 631 Montresor, M., John, U., Beran, A., Medlin, L.K., 2004. *Alexandrium tamutum* sp. nov.
632 (Dinophyceae): a new nontoxic species in the genus *Alexandrium*. J. Phycol. 40, 398–411.
- 633
- 634 Murray, S.A., Hoppenrath, M., Orr, R.J., Bolch, C., John, U., Diwan, R., Yauwenas, R., Harwood, T.,
635 de Salas, M., Neilan, B., Hallegraeff, G., 2014. *Alexandrium diversaporum* sp. nov., a new non-
636 saxitoxin producing species: Phylogeny, morphology and *sxtA* genes. Harmful Algae 31, 54–65.
- 637
- 638 Murray, S.A., Diwan, R., Orr, R.J., Kohli, G.S., John, U., 2015. Gene duplication, loss and selection in
639 the evolution of saxitoxin biosynthesis in alveolates. Mol. Phylogenetics Evol. 92, 165–180.
- 640
- 641 Nagai, S., Itakura, S., Matsuyama, Y., Kotani, Y., 2003. Encystment under laboratory conditions of
642 the toxic dinoflagellate *Alexandrium tamiyavanichii* (Dinophyceae) isolated from the Seto Inland Sea,
643 Japan. Phycologia 42, 646–653.
- 644
- 645 Nagai, S., Nishitani, G., Takano, Y., Yoshida, M., Takayama, H., 2009. Encystment and excystment
646 under laboratory conditions of the nontoxic dinoflagellate *Alexandrium fraterculus* (Dinophyceae)
647 isolated from the Seto Inland Sea, Japan. Phycologia 48, 177–185.
- 648
- 649 Nagai, S., Chen, H., Kawakami, Y., Yamamoto, K., Sildever, S., Kanno, N., Oikawa, H., Yasuike, M.,
650 Nakamura, Y., Hongo, Y., Fujiwara, A., Kobayashi, T., Gojobori, T., 2019. Monitoring of the toxic

651 dinoflagellate *Alexandrium catenella* in Osaka Bay, Japan using a massively parallel sequencing
652 (MPS)-based technique. *Harmful Algae* 89, 101660.

653

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

658

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

662

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

666

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

669

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

674

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

678

- 679 Sanseverino, I., Conduto, D., Pozzoli, L., Dobricic, S., Lettieri, T., 2016. Algal bloom and its
680 economic impact; EUR 27905 EN; doi:10.2788/660478.
681
- 682 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.
683 2014. Resting cysts, and effects of temperature and salinity on the growth of vegetative cells of the
684 potentially harmful species *Alexandrium insuetum* Balech (Dinophyceae). *Harmful Algae* 39, 175–
685 184.
686
- 687 Taylor, F.J.R., 1979. The toxigenic gonyaulacoid dinoflagellates. In: Taylor, D.L., Seliger, H.H.
688 (Eds.), *Toxic dinoflagellate blooms. Proceedings of the Second International Conference on Toxic*
689 *Dinoflagellate Blooms, Key Biscayne, Florida, October 31–November 5, 1978*, Elsevier/North-
690 Holland, New York pp. 47–56.
691
- 692 Taylor, F.J.R., Fukuyo, Y., 1998. The neurotoxic dinoflagellate genus *Alexandrium* Halim:
693 general introduction. In: *Physiological ecology of harmful algal blooms*. In: Anderson, D.M.,
694 Cembella, A.D., Hallegraeff, G.M. (Eds.), *NATO ASI Series, vol. G 41*, Springer-Verlag Berlin,
695 Heidelberg, pp. 283–317.
696
- 697 Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an
698 allelochemical defence mechanism independent of PSP-toxin content. *Mar. Ecol. Prog. Ser.* 230, 47–
699 58.
700
- 701 Trainer, V.L., Yoshida, T. (Eds.) 2014. *Proceedings of the Workshop on Economic Impacts of*
702 *Harmful Algal Blooms on Fisheries and Aquaculture. PICES Sci. Rep. No. 47*, 85 p.
703
- 704 Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp,
705 S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.
706 J., Smith, G.F. (Eds.) 2018. *International Code of Nomenclature for algae, fungi, and plants* (Shenzhen

707 Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017.
708 Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. DOI
709 <https://doi.org/10.12705/Code.2018>.
710
711 Turner, A.D., Dhanji-Rapkova, M., Fong, S.Y., Hungerford, J., McNabb, P.S., Boundy, M.J. and
712 Harwood, D.T., 2019. Ultrahigh-Performance Hydrophilic Interaction Liquid Chromatography with
713 Tandem Mass Spectrometry Method for the Determination of Paralytic Shellfish Toxins and
714 Tetrodotoxin in Mussels, Oysters, Clams, Cockles, and Scallops: Collaborative Study. *Journal of*
715 *AOAC International*, <https://doi.org/10.5740/jaoacint.19-0240>.
716
717 Walker, L.M., Steidinger, K.A., 1979. Sexual reproduction in the toxic dinoflagellate *Gonyaulax*
718 *monilata*. *J. Phycol.* 15, 312–315.
719
720 Wall, D., Dale, B., 1969. The “hystrichosphaerid” resting spore of the dinoflagellate *Pyrodinium*
721 *bahamense* Plate 1906. *J. Phycol.* 5, 140–149.
722
723 Wiese M., D'Agostino P.M., Mihali T.K., Moffitt M.C., Neilan B.A. 2010 Neurotoxic Alkaloids:
724 Saxitoxin and Its Analogs. *Mar. Drugs* 8(7), 2185–2211.
725
726 Yoshida, M., Mizushima, K., Matsuoka, K., 2003. *Alexandrium acatenella* (Gonyaulacales:
727 Dinophyceae): Morphological characteristics of vegetative cell and resting cyst. *Plankton Biol. Ecol.*
728 50(2), 61–64.
729
730 Yuki, K., Fukuyo, Y., 1992. *Alexandrium satoanum* sp. nov. (Dinophyceae) from Matoya Bay, central
731 Japan. *J. Phycol.* 28, 395–399.
732

- 733 Zurhelle, C., Nieva, J., Tillmann, U., Harder, T., Krock, B., Tebben, J., 2018. Identification of Novel
734 Gymnodimines and Spirolides from the Marine Dinoflagellate *Alexandrium ostenfeldii*. Mar. Drugs.
735 16(11), 446, doi: 10.3390/md16110446.

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

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
<i>Alexandrium</i> s.s.	<i>A. andersonii</i>	Direct or indirect ¹	Pentagonal ¹	Spherical ¹⁴
<i>Alexandrium</i> s.s.	<i>A. diversaporum</i>	Direct ⁶	Pentagonal ⁶	Spherical ⁶
<i>Alexandrium</i> s.s.	<i>A. insuetum</i>	None ¹	Pentagonal ¹	Spherical ¹⁵
<i>Alexandrium</i> s.s.	<i>A. margalefii</i>	None ¹	Quadrangular ¹	Spherical ¹⁶
<i>Alexandrium</i> s.s.	<i>A. minutum</i>	Direct or indirect ²	Pentagonal ¹	Discoid ¹⁷
<i>Alexandrium</i> s.s.	<i>A. ostenfeldii</i>	Direct or indirect ¹	Pentagonal ¹	Spherical ¹⁸
<i>Alexandrium</i> s.s.	<i>A. pohangense</i>	None ³	Pentagonal ³	-
<i>Alexandrium</i> s.s.	<i>A. tamutum</i>	Direct ⁴	Pentagonal ⁴	Discoid ¹⁹
<i>Protogonyaulax</i>	<i>A. acatenella</i>	Direct or indirect ²	Pentagonal ¹	Ellipsoidal ²⁰
<i>Protogonyaulax</i>	<i>A. australiense</i>	Direct ⁵	Pentagonal ⁵	Ellipsoidal ⁵
<i>Protogonyaulax</i>	<i>A. catenella</i>	Direct ¹	Pentagonal ¹	Ellipsoidal ²¹
<i>Protogonyaulax</i>	<i>A. cohorticula</i>	Direct ¹	Pentagonal ¹	Ellipsoidal ²²
<i>Protogonyaulax</i>	<i>A. compressum</i>	Direct ¹	Pentagonal ¹	-
<i>Protogonyaulax</i>	<i>A. fraterculus</i>	Direct or indirect ²	Pentagonal ¹	Spherical to ovoid ²³
<i>Protogonyaulax</i>	<i>A. kutnerae</i>	Direct or indirect ²	Pentagonal ¹	Ellipsoidal ¹⁶
<i>Protogonyaulax</i> &	<i>A. leei</i>	Direct ¹	Pentagonal ¹	Spherical ²⁴
<i>Protogonyaulax</i>	<i>A. mediterraneum</i>	Direct ⁵	Pentagonal ⁵	Ellipsoidal ⁵
<i>Protogonyaulax</i>	<i>A. pacificum</i>	Direct ⁵	Pentagonal ¹²	Ellipsoidal ¹²
<i>Protogonyaulax</i>	<i>A. tamarensis</i>	Direct ¹	Pentagonal ¹	Ellipsoidal ²¹
<i>Protogonyaulax</i>	<i>A. tamiyavanichii</i>	Direct ¹	Pentagonal ¹	Spherical ²⁵
<i>Protogonyaulax</i>	<i>A. tropicale</i>	Direct or indirect ¹	Pentagonal ¹	-
<i>Episemicolon</i>	<i>A. affine</i>	Direct ¹	Pentagonal ¹	Spherical ²⁶
<i>Episemicolon</i>	<i>A. gaarderae</i>	Direct ¹	Pentagonal ¹	-
<i>Gessnerium</i>	<i>A. balechii</i>	None ¹	Pentagonal ¹	Spherical ²⁷
<i>Gessnerium</i>	<i>A. camurascutulum</i>	None ⁷	Pentagonal ⁷	-
<i>Gessnerium</i>	<i>A. concavum</i>	None ⁸	Pentagonal ⁸	-
<i>Gessnerium</i>	<i>A. foedum</i>	None ¹	Pentagonal ¹	-
<i>Gessnerium</i>	<i>A. globosum</i>	None ⁹	Pentagonal ⁹	-
<i>Gessnerium</i>	<i>A. hiranoi</i>	None ¹	Pentagonal ¹	Discoid ²⁸
<i>Gessnerium</i>	<i>A. monilatum</i>	None ¹	Pentagonal ¹	Spherical to ovoid ²⁹
<i>Gessnerium</i>	<i>A. pseudogonyaulax</i>	None ¹	Pentagonal ¹	Spherical, paratabulate ³⁰
<i>Gessnerium</i>	<i>A. satoanum</i>	None ¹⁰	Pentagonal ¹⁰	-
<i>Gessnerium</i>	<i>A. taylorii</i>	None ¹	Quadrangular-Pentagonal ¹³	Spherical to discoid ¹⁶
Not listed	<i>A. depressum</i>	Direct or indirect ⁸	Pentagonal ⁸	-
Not listed	<i>A. fragae</i>	Direct or indirect ³¹	Pentagonal ³¹	-
<i>Centrodinium</i>	<i>C. punctatum</i>	Direct ¹¹	Quadrangular ¹¹	-