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A practical guide to new nomenclature for species within the "*Alexandrium tamarense* species complex"

For several decades, the "*Alexandrium tamarense* species complex" included three morphologically defined species, *A. catenella*, *A. fundyense*, and *A. tamarense* [1]. Worldwide, the group is one of several responsible for paralytic shellfish poisoning, a potentially life-threatening syndrome that occurs following the consumption of shellfish contaminated with paralytic shellfish toxins (saxitoxin and analogs). Morphologically, *Alexandrium catenella* was distinguished by rounded, slightly anterior-posteriorly compressed cells, chain formation, and a 1' plate lacking a ventral pore [1, 2]. *Alexandrium fundyense* and *A. tamarense* shared the same Kofoidian plate tabulation as *A. catenella*, but typically exhibited less compressed cellular morphologies [1, 3]. *Alexandrium tamarense* and *A. fundyense* were distinguished based on the presence of a ventral pore on the 1′ plate in *A. tamarense* and its absence in *A. fundyense* [1].

Field and culture studies over the past 20 years often described "*A. tamarense* species complex" cells exhibiting intermediate morphologies when compared to the original descriptions of all three species (see John et al. [4] for a summary). Phylogenetic studies of rDNA gene sequences obtained from "*A. tamarense* complex" isolates fall into one of five distinct ribotype groups. These do not correlate with the original morphologically defined species and were initially designated as Groups I–V [5-8]. The genetic distances among the ribotypes are typical of those separating other dinoflagellate species. Together, these observations indicate that the original species descriptions depicted a series of "morphotypes" shared by various species in the *A. tamarense* complex rather than actual species. Consequently, researchers set out to confirm that the five ribotypes represented separate species (e.g., [5, 8-11]). The most comprehensive of these efforts by John et al. [8] assembled diverse lines of evidence, including detailed morphological analyses, rDNA phylogenies, mating incompatibility assessments, ITS1/5.8S/ITS2 rDNA uncorrected genetic distances, ITS2 complementary base pair changes, saxitoxin production and the presence or absence of a key gene involved in saxitoxin synthesis. These combined data fully support the conclusion that the Group I–V ribotypes are distinct species.

Equally important, the morphological analysis by John et al. [8] showed ***no single morphological trait, or suite of traits,*** **corroborated the original morphospecies descriptions, nor could any combination of morphological traits distinguish the ribotype groups from one another.** There was simply too much overlap in the morphologies expressed by each of the ribotype groups for morphological characters to prove useful. For example, Group I populations in the entire north Atlantic and north Pacific including Alaska exhibit both "*A. fundyense*" and "*A. tamarense*" morphology. In contrast, those in the eastern Pacific from San Francisco Bay south to Chile, the southeast Atlantic coast of South Africa and many areas in the western Pacific from Australia to Japan, frequently form multi-cell chains, a signature of the original "*A. catenella*" morphological description. These latter Group I populations also display the typical "*A. catenella*" morphology and are indistinguishable from the Group IV populations found in the Western Pacific (South Japan, Korea and China) and the Mediterranean Sea [5, 9, 10, 12].

Based on their analyses, John et al. [8] assigned the following species designations to each ribotype group: *A. fundyense* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) and *A. australiense* (Group V). John et al. [8] took further steps to provide exhaustive morphological and genetic descriptions and submitted holotype and epitype material, as appropriate, to the Herbarium Senckenbergianum (FR) in the Centre of Excellence for Dinophyte Taxonomy (Wilhelmshaven, Germany). Most of these nomenclatural reassignments raised minimal concerns among taxonomists. The primary exception was the designation of the Group I ribotype as *A. fundyense*.

The controversy regarding the Group I designation centers on whether the cells used for the original *A. catenella* description were from Group I or IV, given that populations of both species in the Pacific are known to exhibit the classic "*A. catenella*" morphotype. Molecular analyses of "*A. catenella*" cells collected from the coast of California at Redondo Beach [13] and Monterey Bay [14], south of the type location, indicated that these cells belonged to Group I (Fig. 1). Further, there is no evidence that any species except Group I occurs in all of the Americas [5, 13-17]. Use of species-specific molecular assays in other parts of the world, however, have shown Group I and IV cells matching the *A. catenella* morphotype co-occur (see Fig. 1 and associated references). The lack of any extant type material for molecular testing and the overlapping distributions of Group I and IV cells exhibiting the "*A. catenella*" morphology in other regions creates uncertainty about which ribotype was described originally. Based partially on this uncertainty, John et al. [4] submitted a formal proposal to *Taxon* for rejection of the name *Alexandrium catenella* in favor of *A. fundyense*.

A second reason for using the *A. fundyense* name in lieu of *A. catenella* is that a large volume of literature regarding Group I blooms, especially from the Gulf of Maine, has been published using the name *A. fundyense*. Continued use of *A. fundyense* would cause less disruption to the existing documentation and serve the International Code of Nomenclature (ICN) Article 14.2 goal that states: "Conservation aims at retention of those names that best serve stability of nomenclature". A third reason for rejecting *A. catenella*, and assigning Group I to *A. fundyense* and Group IV to *A. pacificum*, was to provide the scientific community a means of unambiguously distinguishing the Group I and IV cells exhibiting the same *A. catenella* morphotype, especially in regions where they are sympatric.

Based on the known distribution of Group I cells along the coast of North and South America, Fraga et al. [18] submitted a counterproposal to *Taxon* against rejecting the name *A. catenella*. The authors argued that the Group I

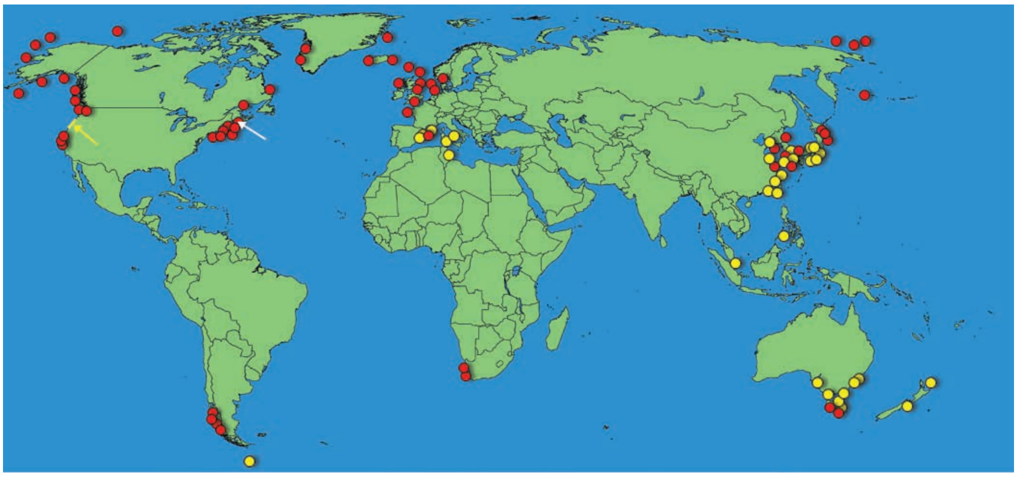

Fig. 1. Known locations where Group I A. catenella (red dots) and Group IV A. pacificum (yellow dots) cells have been collected and confirmed with molecular assays [6-8, 11, 12, 15-17, 20-37]. The yellow line and yellow arrow on the west coast of North America indicates the type locality for A. catenella. The white arrow shows the type locality for A. fundyense in the Bay of Fundy, Canada. The single report of A. catenella in the Mediterranean and A. pacificum in the Drake Passage between South America and Antarctica should be considered provisional until additional isolates were identified in both regions. No isolates of Alexandirum catenella have been obtained for the Mediterranean Sea despite extensive sampling and though Busch et al. [34] detected putative A. catenella sequences from environmental DNA samples collected from Fangar Bay (Catalan coast) using 454 pyrosequencing of LSU rDNA, the reads are so short they cannot be considered as definitive. If A. catenella does occur in the Mediterranean it may represent a recent introduction.

distribution in the type locale was sufficiently established to conclude only Group I cells were used for the original *A. catenella* description (Fig. 1). If true, the ICN rules of priority, in cases where the type material is not in question, clearly dictates retention of the name "*A. catenella*" for Group I because it was published prior to the "*A. fundyense*" Group I description [1, 2].

Nomenclature change proposals such as those of John et al. [4] and Fraga et al. [18] are adjudicated by The ICN Nomenclature Committee for Algae. This committee met to consider the proposals and ruled the name *Gonyaulax catenella* (*Alexandrium catenella*) should not be rejected and that *A. fundyense* and *A. catenella* are conspecific with nomenclatural priority being given to *A. catenella* [19]. For the valid names and synonymy one can refer to the following checklist: *https://www.dinophyta.org/checklists/of-species/alexandrium/*.

The decision of the Nomenclature Committee has the following implications:

1. All publications involving Group I cells published using the name *A. fundyense* should now be considered *A. catenella*.
2. This nomenclatural revision affects the two most widespread toxic species (Group I and IV) within the "*A. tamarense* complex". Resource managers in much of the world need to be aware of this nomenclatural change as it will affect reporting requirements, communication with the public, and use of past literature when developing strategies for dealing with paralytic shellfish poisoning events and regulations.
3. This decision also places significant demands on future researchers as they investigate the literature. For example, with the exception of Chilean and South African research, most of the papers published on "*A. catenella*" before 2015 represent reports of the morphologically indistinguishable species *A. pacificum*. Similarly, future workers will need to recognize the extensive literature published between 1985 and 2017 regarding Group I *A. fundyense* actually refers to *A. catenella*.
4. The formal assignment of the name *A. catenella* to Group I also means this name cannot apply to Group IV (*A. pacificum*) despite Group IV exhibiting morphologies exactly matching the original *A. catenella* description.
5. Ribotype groups II, III and V species should be referred to as *A. mediterraneum*, *A. tamarense* and *A. australiense*, respectively [8].

Example scenarios clarifying how to identify and name "*Alexandrium tamarense* complex" species:

1. You work in a region where co-occurring members of the "*Alexandrium tamarense* species complex" are present. Based on their morphology, you have assigned them the names *A. catenella* and *A. tamarense*. What do you do?
    - You need to sequence barcoding marker regions from the strains, specifically, ribosomal RNA genes or use species-specific molecular assays. Only then can you truly determine the species present, i.e. *A. australiense*, *A. catenella*, *A. mediterraneum*, *A. pacificum*, or *A. tamarense*.
    - A particular case is that from Japan, where species typically identified as *A. catenella* should be called *A. pacificum*, and the species recognized as *A. tamarense* should be designated as *A. catenella.*
2. You are working on an isolate from a culture collection identified as *Alexandrium catenella*. Cells have the typical morphology of *A. catenella* as described by Whedon and Kofoid [2]. When you sequence its ribosomal RNA genes, you discover that it is the *A. pacificum* (Group IV) genotype. What do you do?
    - The isolate should be reclassified as *A. pacificum*.
3. The typical toxic bloom-forming organism that occurs in your region

is well known and has always been known as *Alexandrium fundyense* or *A. tamarense*. It has the Group I genotype. What do you do?

- You refer to the species as *A. catenella*.

4. Toxic and non-toxic strains with the morphology of *Alexandrium tamarense* coexist in your region. What do you do?
    - Molecular barcoding or sequencing is mandatory.
    - In the case of Scotland, the non-toxic strains correspond to *A. tamarense*, while the toxic ones are *A. catenella.*
    - In the case of the Mediterranean Sea, the non-toxic species *A. tamarense* and *A. mediterraneum* may coexist. The toxic *A. pacificum* also occurs in this region but typically forms chains.
5. Chain forming cells of the "*Alexandrium tamarense* complex" are observed. What do you do?
    - Start with the hypothesis that if it is a high latitude, cold-water area, the species present is *A. catenella*, but if it is a warm temperate area, it is *A. pacificum*, then confirm using molecular assays.
6. Special attention should be given to the non-toxic *A. affine*. Initially, this species was not considered part of the "*Alexandrium tamarense* species complex" because it could be morphologically identified by the position of the anterior attachment pore. However, the anterior attachment pore can be absent in old cells, making it an unreliable morphological character leaving *A. affine* morphologically indistinguishable from the "*Alexandrium tamarense* species complex".

In conclusion, the "*Alexandrium tamarense* complex" currently includes the following five species: *A. catenella* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) and *A. australiense* (Group V). Multiple analyses clearly show these species cannot be distinguished based on morphology. Instead, species-specific molecular assays are required for reliable identification. Vandersea et al. [17] provided an overview of the various assays that can be used for this purpose. It is further recommended that the harmful algae research community work to identify and standardize a set of molecular assays to be used when screening samples for these species. All publications regarding these species should include both the Group I–V ribotype designations and species names. This will ensure unambiguous species identifications and reduce further confusion in the literature. The routine use of multiple, standardized, species-specific molecular assays will also allow better definition of species ranges, avoid any biases regarding which species are present in a region and identify when new introductions have occurred.

References

1. *Balech E 1995. Sherkin Island Marine Station Publication, Sherkin Island, Co., Cork, Ireland*
2. *Whedon WF & CA Kofoid 1936. University of California Publications in Zoology 41: 25-34*
3. *Balech E 1985. In: Toxic Dinoflagellates (Elsevier, New York), pp 33–38*
4. *John U et al 2014. Taxon 63: 932-933*
5. *Lilly EL et al 2007. J Phycol 43: 1329-1338*
6. *Penna A et al 2008. Eur J Phycol 43: 163-178*
7. *Miranda LN et al 2012. Harmful Algae 16: 35-48*
8. *John U et al 2014. Protist 65: 779-804*
9. *Scholin CA et al 1994. J Phycol 30: 999-1011*
10. *John U et al 2003. Mol Biol Evol 20: 1015-1027*
11. *Wang L et al 2014. Harmful Algae 31: 100-113*
12. *Gao Y et al 2015. Mar Pollut Bull 96: 210-219*
13. *Garneau ME et al 2011. Appl Environ Microbiol 77: 7669-7680*
14. *Jester R et al. 2009. Harmful Algae 8: 291-298*
15. *Jedlicki A et al 2012. AoB Plants. 12. doi: 10.1093/aobpla/pls033*
16. *Varela D et al 2011. Harmful Algae 15: 8-18*
17. *Vandersea et al 2017. Phycologia 56(3): 303-320*
18. *Fraga S et al 2015. Taxon 64: 634-635*
19. *Prud'homme van Reine WF 2017. Taxon 6: 197-198*
20. *Bolch CJS & MF de Salas 2007. Harmful Algae 6: 465-485*
21. *Penna A & L Galluzzi 2008. In: Algal Toxins: Nature, Occurrence, Effect and Detection (NATO Science for Peace and Security Series A-Chemistry and Biology (Springer Netherlands),pp. 261-283*
22. *Masseret E et al 2009. Appl Environ Microbiol 75: 2037-2045*
23. *Chambouvet A et al 2011. Res Microbiol 62: 959-968*
24. *Genovesi B et al. 2011. J Plank Res 33: 405-414*
25. *Anderson DM et al. 2012. Harmful Algae 14: 10-35*
26. *Baggesen C et al 2012. Harmful Algae 19: 108-116*
27. *Murray SA et al 2012 Harmful Algae 18: 96-104*
28. *Gu HF et al 2013. Harmful Algae 27: 68-81*
29. *Bolch C et al 2014. In: 16th ICHA, Wellington, New Zealand 27-31 October 2014. Book of Abstracts p 29*
30. *Fertouna-Bellakhal M et al 2014. Mar Pollut Bull 84: 347-362*
31. *Zou C et al 2014. Mar Pollut Bull 89: 209-219.*
32. *Dias PJ et al 2015. Bioinvasions Rec 4: 233-241*
33. *Genovesi B et al 2015. Mar Pollut Bull 98: 95-105*
34. *Busch JA et al. 2016. Harmful Algae 55: 191-201*
35. *Shin HH et al 2017. Harmful Algae 68: 31-39*
36. *Subong BJ et al 2017. Philipp J Sci 146:421-423*
37. *Fabro E et al 2017. J Phycol 53: 1206-1222*

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