



## Co-occurrence of *Alexandrium minutum* (Dinophyceae) ribotypes from the Chinese and Malaysian coastal waters and their toxin production

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### ABSTRACT

The bloom-forming dinophyte *Alexandrium minutum* comprises biogeographic inferred, global and Pacific clades with both toxic and nontoxic strains reported. *A. minutum* has a wide distribution in the Western Pacific, but to date only a few strains have available DNA sequences. To fully understand its genetic diversity, sampling was undertaken from the Yellow Sea, the East and South China Sea, and five strains of *A. minutum* and two strains of its sister species, *A. tamutum*, were established. Their morphology was examined by light and scanning electron microscopy. In addition, sequences were obtained from both large subunit (LSU) ribosomal DNA and/or internal transcribed spacer (ITS) region. Strains of *A. minutum* are morphologically indistinguishable, characterized by a smaller cell size and a narrow sixth precingular plate. In contrast, *A. tamutum* has a wider sixth precingular plate. High nucleotide divergences of LSU (D1–D3) rDNA and ITS were revealed amongst strains of *A. minutum* (10% and 25%, respectively), and *A. tamutum* (3% and 13%, respectively). Molecular phylogenies based on LSU rDNA and ITS revealed three ribotypes (B–D) of *A. minutum*, and two ribotypes of *A. tamutum* in the Western Pacific. Seasonal sampling in the East China Sea to detect *A. minutum* using the DNA metabarcoding targeting ITS1 region was also performed. Our results showed that the ribotypes B and C of *A. minutum* co-occurred in the water. Paralytic shellfish toxin (PSTs) of all seven strains was analysed using liquid chromatography with tandem mass spectrometry (LC–MS/MS). PSTs were detected only in *A. minutum* ribotypes B and C with predominance of gonyautoxins 1/4. Our results suggest high diversity and risk potential of this toxic species in this region.

### 1. Introduction

The dinophyte genus *Alexandrium* Halim encompasses 33 taxonomically accepted species (Guiry and Guiry, 2022) and many of them are toxic and bloom forming (Anderson et al., 2012). The type species of *Alexandrium*, *A. minutum* Halim was originally described from the Alexandria Harbour, Egypt (Halim, 1960). Its morphological details, however, remained unclear until the re-description of materials from the type locality (Balech, 1989). Prior to this, many *Alexandrium* species were classified within *Gonyaulax* Diesing, including *G. tamarensis* Lebour (Lebour, 1925), and *G. catenella* Whedon & Kofoid (Whedon and Kofoid, 1936). Once the identity of *A. minutum* was settled, *Gonyaulax tamarensis*

was transferred into *Alexandrium* as *A. tamarensis* (Lebour) Balech together with many other species (Balech, 1995).

The taxonomic criteria to differentiate *Alexandrium* species are subtle; these include size and shape of cells, the connection between the first apical plate and apical pore complex, the shape of the first apical plate, presence or absence of the ventral pore, the shape of the sixth precingular plates, anterior and posterior sulcal plates (Balech, 1995). *A. tamarensis*, *A. catenella* (Whedon & Kofoid) Balech and *A. fundyense* Balech are three morphologically similar species and used to form the *A. tamarensis* species complex. Recently, these species were redefined and five species were identified based on evidence from morphological, molecular and crossing data (John et al., 2014; Fraga et al., 2015).

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