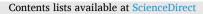
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Phylogeny and ultrastructure of a non-toxigenic dinoflagellate *Amphidoma fulgens* sp. nov. (Amphidomataceae, Dinophyceae), with a wide distribution across Asian Pacific

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Amphidoma languida, a marine thecate dinoflagellate that produces the lipophilic toxin azaspiracids (AZAs), is primarily found in the Atlantic. Although this species has not been recorded in the Asian Pacific, environmental DNAs related to Am. languida have been widely detected in the region by metabarcoding analysis. Their morphology and AZA production remain unclear. In this study, the morphology, ultrastructure, phylogeny, and AZA production of nine Amphidoma strains isolated from Japan, Malaysia, and Philippines were investigated. Phylogenetic trees inferred from rDNAs (SSU, ITS, and LSU rDNA) showed monophyly of the nine Pacific strains and were sister to the Am. languida clade, including the toxigenic strains from the Atlantic. Cells were ellipsoid, 8.7-16.7 µm in length and 7.4-14.0 µm in width, with a conspicuous apical pore complex. A large nucleus in the hyposome, parietal chloroplast with a spherical pyrenoid in the episome, and refractile bodies were observed. Thecal tabulation was typical of Amphidoma, Po, cp, X, 6', 6", 6C, 5S, 6''', 2"". A ventral pore was located on the anterior of 1' plate, beside the suture to 6' plate. The presence of a ventral depression, on the anterior of anterior sulcal plate, was different from Am. languida. A large antapical pore, containing approximately 10 small pores, was observed. Cells were apparently smaller than Am. trioculata, a species possessing three pores (ventral pore, ventral depression, and antapical pore). TEM showed the presence of crystalline structures, resembling guanine crystals, and cytoplasmic invaginations into the pyrenoid matrix. Flagellar apparatus lacking the striated root connective is similar to peridinioids and related dinoflagellates. AZAs were not detected from the Pacific strains by LC-MS/MS. This non-toxigenic Amphidoma species, here we propose as Amphidoma fulgens sp. nov., is widely distributed in the Asian Pacific. Moreover, molecular comparison also suggested that most of the environmental DNA sequences previously reported as Am. languida or related sequences from the Asian Pacific were attributable to Am. fulgens.

1. Introduction

The marine thecate dinoflagellate family Amphidomataceae consists of two genera, *Amphidoma* Stein and *Azadinium* Elbrächter et Tillmann, which include species producing lipophilic shellfish toxin azaspiracids (AZAs). Production of AZAs has so far been detected from three *Azadinium* species, *Azadinium* spinosum Elbrächter et Tillmann, *Az*. *dexteroporum* Percopo et Zingone, and *Az. poporum* Tillmann et Elbrächter (Tillmann et al., 2009, 2011, 2017b; Percopo et al., 2013; Kilcoyne et al., 2014; Krock et al., 2015; 2019; Rossi et al., 2017), and an *Amphidoma* species, *Amphidoma* languida Tillmann, Rafael Salas et Elbrächter (Tillmann et al., 2012). Since the discovery of the first AZA producer, *Az. spinosum* from the North Sea off the coast of Scotland (Tillmann et al., 2009), a rigorous survey of small amphidomatacean

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