



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

Koyo Kuwata^a, Wai Mun Lum^{a,b}, Kazuya Takahashi^a, Garry Benico^{a,c}, Kazutaka Takahashi^a, Po Teen Lim^d, Chui Pin Leaw^d, Hajime Uchida^b, Mayu Ozawa^b, Ryoji Matsushima^b, Ryuichi Watanabe^b, Toshiyuki Suzuki^b, Mitsunori Iwataki^{a,*}

^a Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo, 113-8657, Japan

^b Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa, 236-8648, Japan

^c Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120, Philippines

^d Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, 16310, Bachok, Kelantan, Malaysia

ARTICLE INFO

Edited by Ying Zhong Tang

Keywords:

Azaspiracids (AZAs)
Biogeography
Flagellar apparatus
Guanine crystals
Harmful algal blooms
Refractile body
Ventral depression

ABSTRACT

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 toxicogenic 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-toxicogenic *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

* Corresponding author.

E-mail address: iwataki@g.ecc.u-tokyo.ac.jp (M. Iwataki).

<https://doi.org/10.1016/j.hal.2024.102701>

Received 3 April 2024; Received in revised form 20 June 2024; Accepted 24 July 2024

Available online 25 July 2024

1568-9883/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).