



## Phylogenetic analysis of the toxigenic genus *Amphidinium* (Amphidinales, Dinophyceae) revealed an unexpectedly high diversity in the Asia–Pacific region

Haifeng Gu<sup>a,b,\*</sup>, Shuning Huang<sup>a</sup>, Bernd Krock<sup>c</sup>, Chui Pin Leaw<sup>d</sup>, Po Teen Lim<sup>d</sup>, Nur Shazwani Kassim<sup>d</sup>, Hyeon Ho Shin<sup>e</sup>, Kakaskasen Andreas Roeroe<sup>f</sup>, Hao Yuan<sup>a</sup>, Shimaa Hosny<sup>g</sup>, Rimi Sasai<sup>h</sup>, Kazuya Takahashi<sup>i</sup>, Hikmah Thoha<sup>j</sup>, Faisal Hamzah<sup>j</sup>, Dao Viet Ha<sup>k</sup>, Nantapak Potisarn<sup>l</sup>, Thaithaworn Lirdwitayaprasit<sup>l</sup>, Mitsunori Iwataki<sup>m,\*</sup>

<sup>a</sup> Key Laboratory of Marine Ecological Conservation and Restoration, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China

<sup>b</sup> Nansha Islands Coral Reef Ecosystem National Observation and Research Station, Guangzhou 510300, Guangdong Province, China

<sup>c</sup> Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

<sup>d</sup> Institute of Ocean and Earth Sciences, University of Malaya, 16310 Bachok, Kelantan, Malaysia

<sup>e</sup> Division of Fisheries Life Sciences, Pukyong National University, Busan 48574, South Korea

<sup>f</sup> Sam Ratulangi University, Sulawesi Utara, Manado, 95115, Indonesia

<sup>g</sup> National Institute of Oceanography and Fisheries, NIOF, Alexandria 11516, Egypt

<sup>h</sup> Graduate School of Science and Engineering, Yamagata University, Yamagata 990-8560, Japan

<sup>i</sup> Institute of Parasitology, Biology Center CAS, Branišovská, 370 05 České Budějovice, Czech Republic

<sup>j</sup> Research Center for Oceanography, National Research and Innovation Agency, Jakarta 14430, Indonesia

<sup>k</sup> Institute of Oceanography, Viet Nam Academy of Science and Technology, Nha Trang 650000, Vietnam

<sup>l</sup> Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

<sup>m</sup> Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan

### ARTICLE INFO

#### Keywords:

Amphidinols  
Benthic dinoflagellates  
DNA metabarcoding  
Molecular phylogeny  
Morphology

### ABSTRACT

The dinoflagellate genus *Amphidinium* encompasses several toxic species known to cause harmful algal blooms. Despite their ecological significance, the diversity within this genus may be underestimated due to the morphological similarities among species. In this study, we established 82 strains of *Amphidinium* by isolating single cells from the Asia–Pacific region. We examined their morphology using light and transmission electron microscopy. Additionally, we obtained partial sequences of the large subunit ribosomal (LSU) DNA and/or internal transcribed spacer regions for all strains. Furthermore, DNA metabarcoding targeting the LSU D1–D2 region was employed to detect species in the Bohai Sea, Yellow Sea, Mediterranean Sea, and Red Sea, where strain data is limited. The 82 strains were classified into 13 *Amphidinium* species. Among these were four undescribed species, provisionally named *Amphidinium* sp. 1 to *Amphidinium* sp. 4, as well as *A. cupulatisquama*, *A. fijiensis*, *A. gibbosum*, *A. massartii*, *A. operculatum*, *A. pseudomassartii*, *A. thermaeum*, *A. tomasii*, and *A. trulla*, based on both morphological and molecular analyses. DNA metabarcoding detected nine *Amphidinium* species. While *Amphidinium gibbosum* and *A. tomasii* are confined to tropical and warm subtropical waters, the other species exhibit a broader distribution. Molecular phylogenetic analysis revealed two distinct clades within the genus *Amphidinium*. Species in clade A, including *A. uduigamense*, *A. stirisquamum*, *A. operculatum*, *Amphidinium* sp. 1, and *Amphidinium* sp. 2, share a characteristic sulcus that originates in the posterior one-third of the hypocone. In contrast, species in clade B are characterized by a sulcus that originates in the anterior or middle part of the cell. Additionally, amphidinol analysis was conducted on ten strains of five *Amphidinium* species using liquid chromatography–tandem mass spectrometry (LC–MS/MS), but amphidinols were below the detection limit. However, one strain of *A. massartii* produces a new amphidinol variant with a molecular mass of 1402.7 Da (34.47 fg cell<sup>−1</sup>) and hemolysis assays suggest the potential presence of novel amphidinols or related compounds in *A. operculatum*. Our findings underscore the significant diversity and potential risk posed by *Amphidinium* species in the Asia–Pacific region and beyond.

\* Corresponding authors.

E-mail addresses: [guhaifeng@tio.org.cn](mailto:guhaifeng@tio.org.cn) (H. Gu), [iwataki@g.ecc.u-tokyo.ac.jp](mailto:iwataki@g.ecc.u-tokyo.ac.jp) (M. Iwataki).

<https://doi.org/10.1016/j.hal.2025.102934>

Received 10 April 2025; Received in revised form 18 July 2025; Accepted 22 July 2025

Available online 23 July 2025

1568-9883/© 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.