



Research Paper

An evaluation of the genus *Amphidinium* (Dinophyceae) combining evidence from morphology, phylogenetics, and toxin production, with the introduction of six novel species



Sarah Karafas^{a,*}, Sing Tung Teng^b, Chui Pin Leaw^c, Catharina Alves-de-Souza^d

^a Algal Resources Collection, University of North Carolina Wilmington, Marine Biotechnology Facility, 5600 Marvin K. Moss Ln., Wilmington, NC 28403, USA

^b Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

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

^d Algal Resources Collection, University of North Carolina Wilmington, Marine Biotechnology Facility, 5600 Marvin K. Moss Ln., Wilmington, NC 28403, USA

ARTICLE INFO

Article history:

Received 4 May 2017

Received in revised form 1 August 2017

Accepted 1 August 2017

Available online 29 August 2017

This manuscript is dedicated to Dr. Theodore J. Smayda (1932–2017) in honor of his lifetime dedication to phytoplankton ecology and service as former editor of Harmful Algae.

Keywords:

Secondary structure

Compensatory base change

Cytotoxicity

Scales

28S

ITS

ABSTRACT

The genus *Amphidinium* is an important group of athecated dinoflagellates because of its high abundance in marine habitats, its member's ability to live in a variety of environmental conditions and ability to produce toxins. Furthermore, the genus is of particular interest in the biotechnology field for its potential in the pharmaceutical arena. Taxonomically there is a history of complication and confusion over the proper identities and placements of *Amphidinium* species due to high genetic variability coupled with high morphological conservatism. Thirteen years have passed since the most recent review of the group, and while many issues were resolved, some remain. The present study used microscopy, phylogenetics of the 28S region of rDNA, secondary structure of the ITS2 region of rDNA, compensatory base change data, and cytotoxicity data from *Amphidinium* strains collected world-wide to elucidate remaining confusion. This holistic approach using multiple lines of evidence resulted in a more comprehensive understanding of the morphological, ecological, and genetic characteristics that are attributed to organisms belonging to *Amphidinium*, including six novel species: *A. fijiensis*, *A. magnum*, *A. paucianulatum*, *A. pseudomassartii*, *A. theodori*, and *A. tomasii*.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

The genus *Amphidinium* is a group of athecated dinoflagellates that are incredibly diverse in that, while being highly conserved morphologically, they thrive world-wide in a wide variety of habitats (Dodge, 1982; Dolapsakis and Economou-Amilli, 2009; Flø Jørgensen et al., 2004a, 2004b; Larsen, 1985; Larsen and Patterson, 1990), temperatures (Murray and Patterson, 2002), and trophic modes (Flø Jørgensen et al., 2004; Murray et al., 2004; Murray and Patterson, 2002). They also produce toxins and bioactive compounds that can have both harmful effects. Reports indicate that *Amphidinium* blooms cause fish kills and that the toxins they produce may increase the effects of Ciguatera Fish Poisoning as they are often found in association with *Gamberdiscus* in affected

areas (Baig et al., 2006; Rhodes et al., 2010; Tindall and Morton, 1998). They also have beneficial effects, producing compounds that exhibit antifungal or antimicrobial properties (Echigoya et al., 2005; Kobayashi and Kubota, 2007; Kobayashi et al., 1991; Kobayashi, 2008; Meng et al., 2010; Nuzzo et al., 2014; Satake et al., 1991; Washida, 2006). They grow easily in culture, and can be scaled up to mass culturing volumes appropriate for chemical analyses.

Presently the genus includes both heterotrophic and autotrophic forms possessing a characteristically minute epicone that is deflected towards the left. The autotrophic *Amphidinium* consist of two clades (Flø Jørgensen et al., 2004a, 2004b) that are sister to one another. The first will be referred to as the Herdmanii Clade and includes: *A. steinii* Lemmerman, *A. mootonorum* Murray and D. J. Patterson, *A. herdmanii* Kofoid and Swezy, and *A. cupulatisquama* M. Tamura and T. Horiguchi. The second is the Operculatum Clade and includes: *A. carterae* Hulburt, *A. massartii* Biecheler, *A. gibbosum* (L. Maranda and Y. Shimizu) Flø Jørgensen and Murray, *A. trulla* Shauna Murray, Rhodes, and Flø Jørgensen, *A. operculatum* Claparède & Lachmann, and, most recently, *A. thermaeum*

* corresponding author.

E-mail addresses: hutchinsons@uncw.edu (S. Karafas), stteng@unimas.my (S.T. Teng), cpleaw@um.edu.my (C.P. Leaw), desouzac@uncw.edu (C. Alves-de-Souza).