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Morphological and Molecular Characterization of Three New *Azadinium* Species (Amphidomataceae, Dinophyceae) from the Irminger Sea

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Some species of the planktonic dinoflagellate genus *Azadinium* produce azaspiracids (AZAs), a group of lipophilic phycotoxins causing human poisoning after mussel consumption. We describe three new species from the North Atlantic, all of which shared the same Kofoidean plate pattern characteristic for *Azadinium*: Po, cp, X, 4′, 3a, 6″, 6C, 5S, 6‴, 2⁗. *Azadinium trinitatum* sp. nov. was mainly characterized by the presence of an antapical spine and by the position of the ventral pore at the left distal end of the pore plate in a cavity of plate 1′. *Azadinium cuneatum* sp. nov. had a conspicuously formed first apical plate, which was asymmetrically elongated and tapered on its left lateral side with a ventral pore located at the tip of this elongated 1′ plate. *Azadinium concinnum* sp. nov. was of particular small size (< 10 μm) and characterized by an anteriorly elongated anterior sulcal plate and by large and symmetric precingular plates. The ventral pore was located inside the apical pore plate on the cells' right lateral side. Molecular phylogenetics as inferred from concatenated SSU rRNA, ITS, and LSU rRNA sequence data supported the distinctiveness of the three new species. None of the new species produced any known AZAs in measurable amounts.

Key words: *Azadinium*; azaspiracids; Irminger Sea; Iceland; new species.

Introduction

A large number of marine biotoxins produced by microalgae are known to accumulate in shellfish making it harmful for human consumption. Intoxications have been categorized based on diagnostic symptoms as Paralytic, Amnesic, Diarrhetic, and Neurotoxic Shellfish Poisoning (PSP, ASP, DSP, NSP). As a fifth category, Azaspiracid Shellfish Poisoning (AZP) was recently coined to account for a toxic syndrome associated with the consumption of animals contaminated with azaspiracid toxins.

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The history of azaspiracids (AZAs) extends back to November 1995, when a harvest of blue mussels cultivated in Killary Harbour (Ireland) was implicated in the poisoning of at least eight people in the Netherlands. Three years later, the causative toxin was isolated from mussels, identified, structurally defined and named azaspiracid according to its chemical characteristics (Satake et al. 1998). The AZA-producing organism, however, remained unknown until the isolation and identification of *Azadinium spinosum* Elbrächter et Tillmann from the North Sea (Tillmann et al. 2009) as a new species in a newly erected genus.

Considering the short interval since the first identification of *Azadinium*, the knowledge about its diversity has rapidly increased. The currently encountered seven species are the type species *A. spinosum* (Tillmann et al. 2009) and further *A. obesum* Tillmann et Elbrächter (Tillmann et al. 2010), *A. poporum* Tillmann et Elbrächter (Tillmann et al. 2011), *A. polongum* Tillmann (Tillmann et al. 2012b), *A. caudatum* (Halldal) Nézan et Chomérat [(Nézan et al. 2012); occurring in two distinct varieties: *A. caudatum* var. *margalefii* (Rampi) Nézan et Chomérat and *A. caudatum* var. *caudatum*], *A. dexteroporum* Percopo et Zingone (Percopo et al. 2013), and *A. dalianense* Z.Luo, H.Gu et Tillmann (Luo et al. 2013). Moreover, a close relative was identified with the description of *Amphidoma languida* Tillmann, Salas et Elbrächter, and *Azadinium* and *Amphidoma* were subsequently placed in the family Amphidomataceae (Tillmann et al. 2012a).

Cells of *Amphidoma* and *Azadinium* are generally small and rather inconspicuous in light microscopy. Determination of diagnostic morphological characteristics, such as presence/absence of an antapical spine and distinct pyrenoid(s), or the location of a ventral pore, requires electron microscopy or tedious high resolution light microscopy (Tillmann et al. 2009, 2010, 2012a,b). Reliable identification of fixed cells of *Azadinium* from field samples is thus problematic and is further challenged by similar size and shape in comparison to a number of small species of *Heterocapsa* F. Stein. However, there is a need to unambiguously identify and quantify the toxigenic source organisms of AZAs and to distinguish these from their non-toxigenic relatives. This task is challenging because AZA-producing and non-toxigenic species are known to co-exist in the same water mass (Tillmann et al. 2010, 2011, 2012b).

Multiple strains of the type species *A. spinosum*, collected at different localities, consistently produce AZA-1, AZA-2, and AZA-33 (an AZA with the molecular mass of 715; Tillmann et al. 2012b). Other species have initially been described as non-toxigenic, as none of the known AZAs have been identified (Tillmann et al. 2010, 2011). However, the recent detection of four new AZAs in species such as *A. languida* and *A. poporum* indicates that species diversity within the Amphidomataceae is also reflected by high chemical diversity (Krock et al. 2012). Molecular probes for the first three described species (*A. spinosum*, *A. obesum*, *A. poporum*) are now available (Toebe et al. 2013) and are in the stage of being tested in field application (Tillmann et al. 2014a).

It cannot be excluded, or it is even to be expected, that there are more yet undescribed species of the Amphidomataceae. These may either include a yet not recorded primary source of AZAs, or might yield false-positive (if non-toxigenic) signals with the molecular probes already designed for toxigenic *A. spinosum* and *A. poporum*. It is therefore important to gain more information on the diversity of species present in the Amphidomataceae, on their molecular signatures, and on their geographical distribution. Both the widespread records of AZA toxins (Braña Magdalena et al. 2003; James et al. 2002; López-Rivera et al. 2009; Taleb et al. 2006; Yao et al. 2010) and the increasing number of records of species of *Azadinium* (Akselman and Negri 2012; Gu et al. 2013b; Hernández-Becerril et al. 2012; Percopo et al. 2013; Potvin et al. 2012; Salas et al. 2011) indicate a global distribution of the genus. However, species of *Azadinium* and/or the presence of azaspiracid toxins have not yet been reported for arctic or sub-Arctic areas (Poulin et al. 2011). In the present paper, we present detailed morphological descriptions and sequence data of three new species of *Azadinium* isolated from water samples collected in the North Atlantic between Greenland and Iceland.

Results

Species Descriptions

Specimens of *Azadinium* were observed in concentrated whole water samples at a number of stations between Greenland and Iceland and around the north-west coast of Iceland (Fig. 1). A total of seven different strains were established. Two strains identified as *Amphidoma languida* (isolated from station 532) and *Azadinium dexteroporum* (isolated from station 526, see Fig. 1) will be presented elsewhere. The other strains were identified to represent three different new species with three strains (4A8, 4B11, A2D11) of *Azadinium trinitatum* sp. nov., and one