Harmful Algae 46 (2015) 18–33

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Morphological and genetic analysis of the *Coolia monotis* species complex with the introduction of two new species, *Coolia santacroce* sp. nov. and *Coolia palmyrensis* sp. nov. (Dinophyceae)

Sarah Karafas *, Robert York, Carmelo Tomas

University of North Carolina Wilmington, Marine Biotechnology Facility, 5600 Marvin K. Moss Ln., Wilmington, NC 28403, USA

ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 7 May 2015
Accepted 7 May 2015
Available online 22 May 2015

Keywords:
Coolia santacroce
Coolia palmyrensis
Coolia monotis
Coolia malayensis
Cytotoxic

ABSTRACT

The dinoflagellate genus *Coolia* Meunier is an important epi-benthic organism that is commonly found in association with other dinoflagellates known to cause ciguatera. Two closely related taxa, *Coolia monotis* and *Coolia malayensis*, make up the *C. monotis* species complex. In this study we introduce two new toxic species that should be included in that complex, *Coolia palmyrensis* Karafas, Tomas, York sp. nov. and *Coolia santacroce* Karafas, Tomas, York sp. nov., collected from the Palmyra Atoll in the Pacific Ocean and Saint Croix, US Virgin Islands, respectively. These two species can be distinguished morphologically by size, pore shape, pore density, and the relative size of the apical pore complex. The ITS1/5.8S/ITS2 and the D1/D2 regions of the LSU rDNA were used to provide molecular support of morphological observations using maximum likelihood and Bayesian analyses. Furthermore, *C. palmyrensis* and *C. santacroce* both showed cytotoxic effects on human derived cells *in vitro*.

1. Introduction

Species in the genus *Coolia* Meunier are epi-benthic dinoflagellates found in both temperate and tropical waters worldwide. It was described in 1919 before being transferred to *Ostreopsis* in 1928, then to *Glenodinium* in 1952, and finally reinstated as its own genus in 1956 (Guiry and Guiry, 2014). Currently there are five species that are largely accepted in the literature. The type species *Coolia monotis* Meunier (Meunier, 1919) was the only *Coolia* known for many years. It was not until much later that *Coolia tropicalis* Faust (Faust, 1995) was described, followed by *Coolia areolata* Ten-Hage, Turquet, Quod and Couté (Ten-Hage et al., 2000), *Coolia canariensis* Fraga (Fraga et al., 2008), and most recently *Coolia malayensis* Leaw, P.-T. Lim and Usup (Leaw et al., 2010).

The species *Coolia monotis*, *Coolia areolata*, and *Coolia tropicalis* were originally described based solely on morphological data (Meunier, 1919; Faust, 1995; Ten-Hage et al., 2000). The first, *C. monotis* was described as a small anteroposteriorly compressed cell with a narrow and oblong 1′ plate and a suture between 1′ and 6″ runs straight down the center of the cell (Penna et al., 2005; Dolapsakis et al., 2006; Laza-Martínez et al., 2011; David et al., 2014). The species *C. tropicalis* has a centrally located 1′ plate that is the largest in the epitheca. This plate was originally described as wedge-shaped by Faust (1995), but Mohammad-Noor et al. (2013) revised the description, with the 1′ plate as having left and right sutures (those with 2″ and 6″) running nearly parallel with one another and fanning outward slightly on the ventral side. The species *C. areolata* was collected from Indian Ocean sediment samples and its 1′ plate had similarities with *C. tropicalis*, but with an areolated surface except for a smooth 1′ plate (Ten-Hage et al., 2000).

Both *Coolia canariensis* and *Coolia malayensis* were described from morphological and molecular data. *C. canariensis* had a central 1′ plate that is the largest in the epitheca, like *Coolia tropicalis* and *Coolia areolata*, but its shape varied slightly and the cell had partial pitting on some plates in the hypotheca (Fraga et al., 2008). The most recent *Coolia* described, *C. malayensis*, was very similar to *Coolia monotis* but smaller in overall size and APC dimensions. Furthermore, it is distinguished phylogenetically by the sequence data of the internal transcribed spacer (ITS) and D1/D2 regions of the large subunit (LSU) of the ribosomal DNA and in the secondary structure of the ITS2 (Leaw et al., 2010). During the discovery and analysis of the two latter species, as well as the increased public availability of sequence data, the identities of *C. monotis* and *C. tropicalis* were solidified phylogenetically (Penna et al., 2005; Dolapsakis et al., 2006; Fraga et al., 2008; Leaw et al., 2010; Laza-Martínez et al., 2011;

* Corresponding author. Tel.: +1 910 962 3359.
E-mail address: hutchinsons@uncw.edu (S. Karafas).

http://dx.doi.org/10.1016/j.hal.2015.05.002
1568-9883/

Jeong et al., 2012; Mohammad-Noor et al., 2013; Momigliano et al., 2013; David et al., 2014; Rhodes et al., 2014).

Currently only *Coolia malayensis* and *Coolia tropicalis* are thought to be toxic (Penna et al., 2005; Fraga et al., 2008; Laza-Martínez et al., 2011; Mohammad-Noor et al., 2013; Rhodes et al., 2014). There are a few reports of toxicity in *Coolia monotis* (Holmes et al., 1995; Rhodes et al., 2010), however these organisms have either been reclassified subsequent to the description of *C. malayensis* in 2010 (Mohammad-Noor et al., 2013) or were from areas where similar clones were already reclassified (Rhodes et al., 2014). Rhodes et al. (2014) suggested that any positive toxicity studies performed on *C. monotis* prior to the description of *C. malayensis* should be carefully considered if there is not DNA data to accompany it. Nonetheless, all *Coolia* are found in association with other genera, such as *Gambierdiscus*, *Prorocentrum*, and *Ostreopsis*, that are associated with ciguatera.

In this study two new species are introduced, *Coolia palmyrensis* and *Coolia santacroce*, which morphologically are closely related, yet molecularly distinct from *Coolia monotis* and *Coolia malayensis*. Using cultured clones of *C. monotis*, *C. malayensis*, *C. palmyrensis*, and *C. santacroce* we present a detailed morphological analysis of typical distinguishing characters among *Coolia* species, as well as propose new characters diagnostic of these four closely related taxa that make up the *Coolia monotis* species complex (Leaw et al., 2010; Laza-Martínez et al., 2011). The ITS1/5.8S/ITS2 and the D1/D2 regions of the ribosomal DNA were shown to effectively elucidate species relationships in eukaryotes (Pin et al., 2001; Litaker et al., 2007; Sonnenberg et al., 2007; Wylezich et al., 2010; Leliaert et al., 2014; Stoeck et al., 2014) and were used in this study to elucidate the phylogenetic relationships of these taxa and lend support to the morphological distinctions reported.

2. Methods

2.1. Collection, isolation, and growth

Seven of the nine samples presented in this study were collected at different locations including the Palmyra Atoll in the Pacific Ocean (Cp1208-1; 5.8903 N, 162.0870 W; Fig. 1A), St. Croix in the U.S. Virgin Islands (Cm1303-1 and Cs1303-1; 17.7781 N, 64.7910 W; Fig. 1B), the Dominican Republic (Cp1412-1, Cm1412-1, and Cm1412-2; 19.822 N, 70.728 W; Fig. 1C), and offshore North Carolina (Cm0607-1). The Palmyra Atoll is a 12 km^2 unincorporated territory of the U.S. located in the equatorial North Pacific almost due South of Hawaii. St. Croix is a 214.6 km^2 island of the U.S. Virgin Islands, and the Dominican Republic is a Caribbean nation on the island of Hispaniola. Additionally, two clones (CCMP304 and CCMP2582) were acquired from the National Center for Marine Algae and Microbiota (NCMA) and integrated into the Algal Resources Collection (ARC) at the University of North Carolina Wilmington (Table 1). Samples collected by ARC were obtained using screen traps according to the protocols outlined by Tester et al. (2014). Subsequently, individual cells were isolated into 96-well microtiter plates, each well containing 200 μl of filtered seawater, and stepped up serially into 250 mL Erlenmeyer flasks of modified K medium (Keller and Guillard, 1985; Keller et al., 1987) with F/2 trace metals (Guillard and Ryther, 1962; Guillard, 1975) at 39 salinity. Clones were maintained at ARC in 25 °C culture collection chambers and exposed to a 14:10 light/dark cycle.

2.2. Microscopy

Samples were observed live or as lugol's fixed material using a Zeiss Axio Imager Z.1 Microscope equipped with a 100× oil objective and AxioCAM and MRc5 cameras. Cells were also prepared for scanning electron microscopy as follows: 1 mL of cultured cells was treated with 4% Triton and washed with seawater through 5 μm Nucleopore filters (Whatman) to remove external membranes. They were then fixed with a 2% gluteraldehyde solution overnight at 4 °C, rinsed, and then dehydrated with two rinses each of 30%, 50%, 75%, 95%, and 100% ethanol over a period of two days. The filters containing cells were processed with a critical point dryer, placed on stubs, and coated with 10 nm of platinum. Samples were viewed on a Philips XLS-FEG scanning electron microscope. Cell and plate measurements were obtain from both light microscope and SEM images.

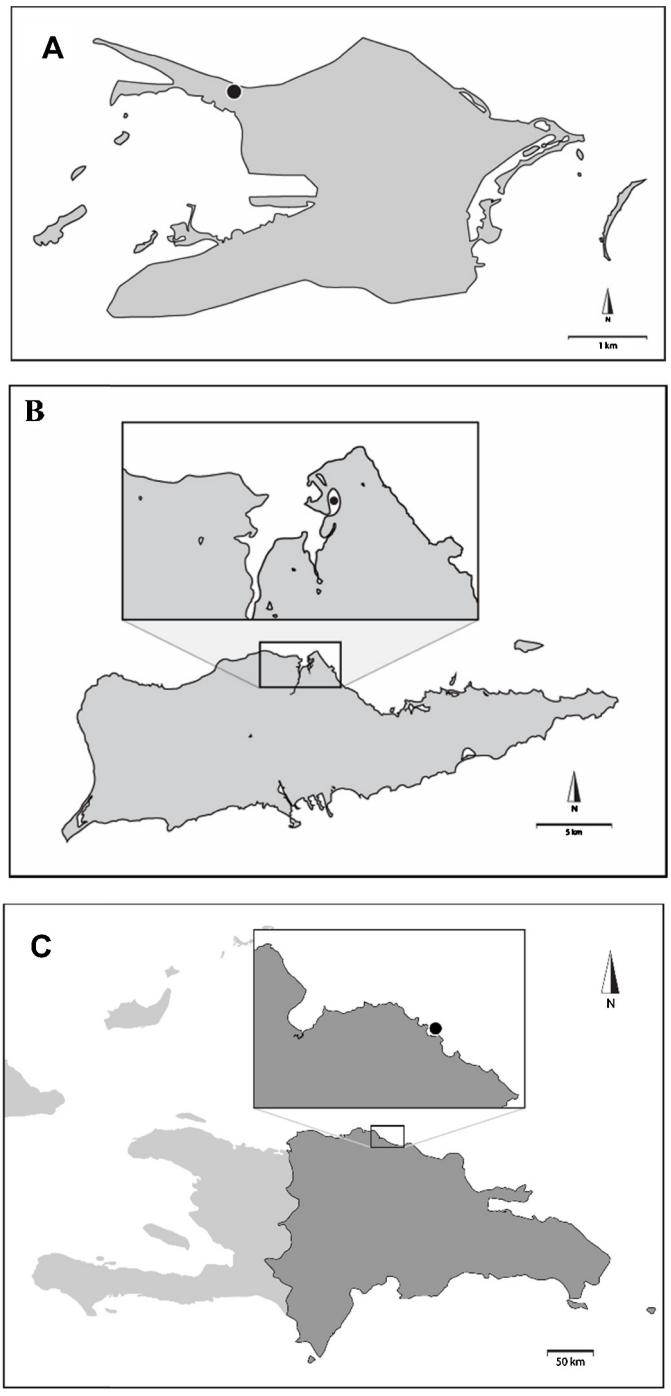

Fig. 1. Map of (A) Palmyra Atoll, (B) St. Croix, USVI, and (C) Dominican Republic. Dots indicate collection locations at 5.8903N, 162.0870W in Palmyra, 17.7781 N, 64.7910 W in St. Croix, and 19.822 N, 70.728 W in the Dominican Republic.