

## MORPHOLOGY AND MOLECULAR CHARACTERIZATION OF A NEW SPECIES OF THECATE BENTHIC DINOFLAGELLATE, *COOLIA MALAYENSIS* SP. NOV. (DINOPHYCEAE)<sup>1</sup>

Chui-Pin Leaw<sup>2</sup>

Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan, 94300 Sarawak, Malaysia

Po-Teen Lim

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

Kok-Wah Cheng, Boon-Koon Ng, and Gires Usup

Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600 Selangor, Malaysia

*Coolia* Meunier is an important component of benthic dinoflagellate assemblages in tropical and subtropical seas. In this study, detailed morphological observation of *Coolia* species from Malaysian waters was carried out using light and electron microscopy in parallel with molecular characterization of nuclear-encoded partial LSU rDNA, and internal transcribed spacer (ITS) regions. Live specimens were collected from seaweed samples and established into clonal cultures. There are significant morphological variations between the Malaysian isolates in comparison to the type species, *C. monotis* Meunier. The feature that differentiates the new species is the third postcingular plate (3<sup>'''</sup>), which is the largest hypothecal plate in the Malaysian isolates, whereas in *C. monotis*, the 3<sup>'''</sup> and 4<sup>'''</sup> plates are almost equal in size. Detailed observations of the thecal pores also revealed the presence of fine perforations within the pores of the Malaysian isolates, but these perforations are absent in *C. monotis*. Comparisons between Malaysian isolates and *C. monotis* nucleotide sequence of the ITS region showed high genetic divergence at 28%, in contrast to the 0.3%–3% divergence observed among populations of the same species. Structural comparison of the second internal transcribed spacer (ITS2) rRNA transcript between the two species showed compensatory base changes (CBCs) in the three helices of ITS2 rRNA. Based on morphological and molecular data, the Malaysian isolates are considered to represent a new species, for which the name *Coolia malayensis* is proposed.

**Key index words:** benthic dinoflagellates; *Coolia malayensis*; ITS2 rRNA transcript; morphology; phylogeny

**Abbreviations:** CBC, compensatory base change; ITS, internal transcribed spacer

Benthic marine dinoflagellates are species that live attached to sand particles, corals, seaweeds, and mangroves. Many benthic dinoflagellates are capable of producing bioactive compounds, including those that can cause seafood toxicity. The most well-known human intoxication due to benthic dinoflagellates is ciguatera fish poisoning (CFP) where the responsible toxins occur in species of the genus *Gambierdiscus*. *Coolia* species often share the same habitats with other toxic epi-benthic dinoflagellates, and it has been suggested that the benthic dinoflagellate assemblage may contribute to CFP in ciguatera endemic areas (Tindall and Morton 1998).

The genus *Coolia* was originally described by Meunier (1919) with a single species, *C. monotis*, based on specimens collected from Nieuport, Belgium. *C. monotis* was subsequently collected and described by other researchers over the last few decades from wide geographic ranges and highly varied habitats (Lebour 1925, Balech 1956, Taylor 1979, Fukuyo 1981, Besada et al. 1982, Dodge 1982, Carlson and Tindall 1985, Faust 1992).

*Coolia* remained monospecific until *C. tropicalis* M. A. Faust (Faust 1995); *C. areolata* Ten-Hage, Turquet, Quod et Couté (Ten-Hage et al. 2000); and, most recently, *C. canariensis* S. Fraga (Fraga et al. 2008) were described. These additional species are differentiated by thecal plate arrangement and ornamentation. Recently, molecular phylogenetic analyses have been widely applied in species recognition and delineation of *Coolia* (Penna et al. 2005, Dolapsakis et al. 2006). Phylogenetic analyses of rRNA genes revealed and confirmed the status and identity of European *C. monotis* (Penna et al. 2005), and *C. canariensis* as a new species (Fraga et al. 2008). Considerable morphological variability has been reported in *C. monotis*, while analyses of molecular markers have revealed genetic divergences among *C. monotis* isolates from a wide geographic range (Penna et al. 2005, Dolapsakis et al. 2006). The cosmopolitan *C. monotis*, therefore, may comprise a complex of cryptic taxa.

<sup>1</sup>Received 9 November 2008. Accepted 19 August 2009.

<sup>2</sup>Author for correspondence: e-mail cpleaw@ibec.unimas.my, chuipinl@yahoo.com.