



## Original Article

Six marine thecate *Heterocapsa* (Dinophyceae) from Malaysia, including the description of three novel species and their cytotoxicity potential

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## ABSTRACT

Thirty-four strains of *Heterocapsa* were established from Malaysian waters and their morphologies were examined by light, scanning, and transmission electron microscopy. Three species, *H. bohaiensis*, *H. huensis*, and *H. rotundata*, and three new species, *H. borneoensis* sp. nov., *H. limii* sp. nov., and *H. iwatakii* sp. nov. were described in this study. The three species were differentiated morphologically by unique characteristics of cell size, shape, displacement of the cingulum, shape and position of nucleus, the number and position of pyrenoids, and body scale ultrastructure. The species delimitations were robustly supported by the molecular data. A light-microscopy-based key to species of *Heterocapsa* is established, with two major groups, i.e., species with a single pyrenoid, and species with multiple pyrenoids. Bioassays were conducted by exposing *Artemia* nauplii to *Heterocapsa* densities of  $1-5 \times 10^5$  cells mL<sup>-1</sup>, and treatments exposed to *H. borneoensis* showed naupliar mortality, while no naupliar death was observed in the treatments exposed to cells of *H. bohaiensis*, *H. huensis*, *H. limii*, and *H. iwatakii*. Naupliar death was observed during the initial 24 h for both tested *H. borneoensis* strains, and mortality rates increased up to 50% after 72-h exposure. This study documented for the first time the diversity and cytotoxic potency of *Heterocapsa* species from Malaysian waters.

## 1. Introduction

The genus *Heterocapsa* F.Stein is one of the cosmopolitan marine thecate dinoflagellates (Dinophyceae, Peridinales). Some species are infamous for causing harmful algal blooms (HABs) worldwide, notably in the Western Pacific region (Matsuyama et al., 1995; Horiguchi, 1995; Iwataki, 2008; Choi and Kim, 2021). As a case in point, blooms of *H. circularisquama* Horiguchi in Japan caused mass mortality of cultured and wild bivalve mollusks in the late 1980s (Matsuyama et al., 1995; Okaichi, 2003). The impact caused by this species on the local bivalve culture industry, particularly on the pearl oyster industry of Japan, was tremendous (as reviewed in Matsuyama, 2012). It is noteworthy that, however, only two out of 23 currently described *Heterocapsa* species were confirmed to cause harm to shellfish mollusks. The second toxic species, *H. bohaiensis* Xiao et al. (2018), has been reported to

heavily hit the aquaculture industries of prawns (*Panaeus japonicus*) and Chinese mitten-handed crabs (*Eriocheir sinensis*) in China (Yang et al., 2015; Xiao et al., 2018). Other *Heterocapsa* species, such as *H. steinii* Tillmann, Gottschling, Hoppenrath, Kusber & Elbrächter (formerly designated as *H. triquetra*), *H. rotundata* (Lohmann) Gert Hansen, and *H. minima* A.J.Pomroy are known to cause massive blooms (Hansen, 1989; Lindholm and Nummelin, 1999; Litaker et al., 2002; Shahi et al., 2015; Millette et al., 2015; Jaschinski et al., 2015; Mohd Razali et al., 2022), but these blooms were not associated with mortality of marine organisms. Numerous studies have been conducted to examine the toxicology and shellfish killing mechanisms of *Heterocapsa* species; some *in situ* and laboratory studies have revealed toxic effects of *H. circularisquama* on bivalves (pearl oyster *Pinctada fucata martensii*, Nagai et al., 1996; manila clam *Ruditapes philippinarum*, Basti and Segawa, 2010) and gastropods (juvenile abalones, *Haliotis discus* and

Abbreviations: HAB, Harmful algal bloom; rDNA, ribosomal DNA; ITS, Internal transcribed spacer region.

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