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Taxonomic re-examination of the toxic armored dinoflagellate *Pyrodinium bahamense* Plate 1906: Can morphology or LSU sequencing separate *P. bahamense* var. *compressum* from var. *bahamense*?

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ABSTRACT

Pyrodinium bahamense Plate 1906 is a tropical to subtropical dinoflagellate that can cause paralytic shellfish poisoning (PSP). Based on differences in the morphology of the motile stage, as well as geographic distribution, this species was separated into two varieties, the toxic var. *compressum* and the non-toxic var. *bahamense* by Steidinger et al. (1980). Thereafter, Balech (1985) carefully reinvestigated the two varieties and concluded there were no significant morphological differences between them. We re-examined the motile cell and cyst morphology of these two varieties, concurring with the arrangement of the sulcal plates, but demonstrating the plate overlap for the first time. The observed size-frequency spectra of cell body diameter, cyst body diameter and cyst process length were unimodal. Overall, we agree with Balech (1985) that there is no consistent criterion to unequivocally separate both varieties based on morphology. We therefore recommend ceasing the use of these varieties (and forma). In addition, we suggest that observations of both varieties in a single plankton sample should be interpreted as the occurrence of different life stages at the sampling time. However, the phylogenetic analysis using partial LSU rDNA sequence data revealed two clearly separated ribotypes within the *Pyrodinium* clade, an Indo-Pacific and Atlantic-Caribbean ribotype, suggesting that *Pyrodinium bahamense* is a species complex. The genetic distance between these ribotypes is short, which suggests a late Quaternary separation. Geochemical analyses of the cyst walls also show differences between specimens from both geographical regions.

1. Introduction

The armored dinoflagellate, *Pyrodinium bahamense* Plate 1906 is one of the most important harmful algal bloom (HAB) organisms in South Asian coastal waters (e.g., Usup et al., 2012). In 1972, paralytic shellfish poisoning (PSP) occurred near Port Moresby (Papua New Guinea) where *P. bahamense* was considered to be the causative organism for that event (Maclean, 1973; Worth et al., 1975). This was the first recognition of a PSP incident caused by *P. bahamense* in Southeast Asia. Since then, toxic blooms associated

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with PSP have been reported throughout Southeast Asia, in particular Malaysia (e.g., Roy, 1977), Papua New Guinea (e.g., Maclean, 1989), the Philippines (e.g., Gonzales, 1989), Brunei (e.g., Jaafar et al., 1989) and Indonesia (e.g., Wiadnyana et al., 1996) as well as the Pacific coast of Central America (e.g., Guatemala, Rosales-Loessener, 1989).

Pyrodinium bahamense was originally described from the Atlantic, specifically New Providence Island (Bahamas) by Plate (1906). Later, Böhm (1931) described from one *P. bahamense* cell from the Red Sea as forma *compressa*, based upon the fact that its body was wider than longer, and that it had only a long "antapical spine." Since then, it has been widely accepted that the Indo-Pacific populations would fall into forma *compressa*, while the Atlantic populations would correspond to the forma *bahamense*, or the "form" originally described by Plate. It was not until the first PSP outbreak in Papua New Guinea in the early 1970s caused by *P. bahamense* (Maclean, 1973) that toxicity was added to the "apparent" differences between the two *P. bahamense* forms. Steidinger et al. (1980) elevated the form status to variety on the basis of morphological criteria for the motile stage and the capability of PSP toxin production. This separation was supported at the time by the biogeographic distribution of both varieties: var. *compressum* was apparently endemic to the Pacific and Indian oceans, while var. *bahamense* occurred in the Caribbean Sea and the Atlantic Ocean. Both var. *bahamense* (Wall and Dale, 1969) and var. *compressum* (Matsuoka, 1989) produce resting cysts that preserve in the sediment, and Matsuoka (1989) reported that process length and body diameter showed significant differences between both varieties.

However, differentiation between the two varieties based on morphological criteria is not unequivocal as shown by Balech (1985) in a detailed morphological analysis of thecae comprising populations from Papua New Guinea, the Philippines, Jamaica and Puerto Rico. Moreover, the physiological criterion of toxin production versus non-production is no longer applicable because cultures isolated from Florida by Landsberg et al. (2006) showed that PSP causing toxins, *in casu* saxitoxins, can be produced by var. *bahamense*. Finally, the segregated biogeography is no longer supported as both varieties have been reported to co-occur in several locations such as Costa Rica (Vargas-Montero and Freer, 2003), the Pacific coast of Mexico (Gárate-Lizárraga and González-Armas, 2011) and the Arabian Gulf (Glibert et al., 2002).

In this study, we provide a multi-approach investigation into whether *Pyrodinium bahamense* can be unambiguously separated

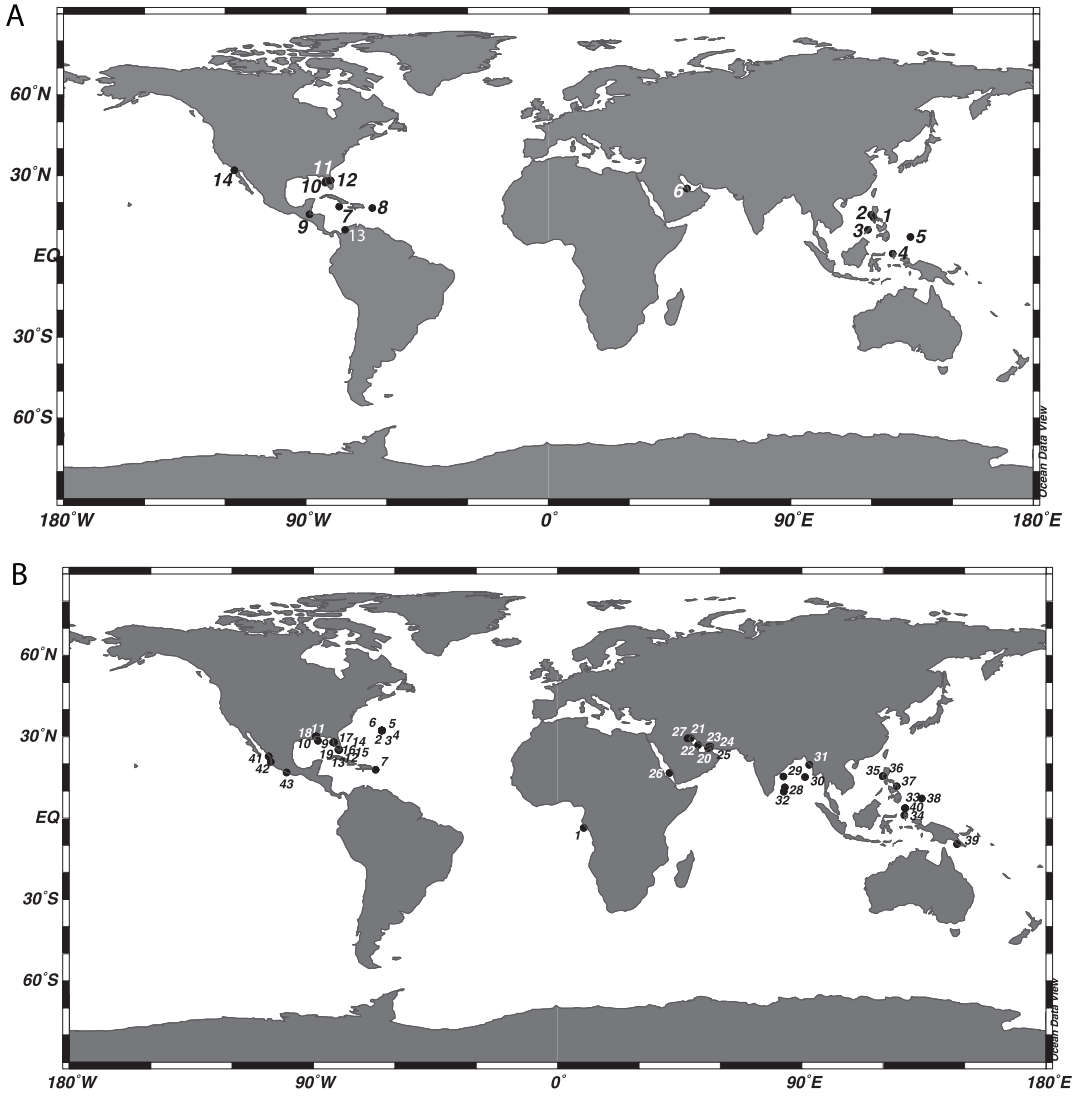

Fig. 1. Sampling locations of motile stage (A) and resting cysts (B) of *Pyrodinium bahamense* studied in the present study. Numbers on the maps correspond to numbers in Tables 1 and 2.