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## The genus *Pseudo-nitzschia* (Bacillariophyceae) in Malaysia, including new records and a key to species inferred from morphology-based phylogeny

Abstract: Species of the diatom Pseudo-nitzschia are known to produce domoic acid that is responsible for amnesic shellfish poisoning (ASP). To investigate the potential risk of ASP and species occurrence of toxic or potentially toxic Pseudo-nitzschia in Malaysian waters, plankton samples were collected from 17 locations. Samples were examined through transmission electron microscopy. Species of Pseudo-nitzschia were identified on the basis of the frustule morphology and morphometric measurements. Twentytwo well-described species were recorded, of which 14 are new records for Malaysia. A new morphotype, closely resembling species in the pseudodelicatissima complex, was also discovered. The morphotype differs from other species in the complex by its lower densities of fibulae and striae. Nine of the species have previously been associated with ASP events worldwide. Our study recorded for the first time high species richness of *Pseudo-nitzschia* in the confined coasts of Malaysia. In addition, we performed a morphology-based phylogeny and proposed a key to Pseudo-nitzschia species, with a special emphasis on the poroid structure of the striae, to aid in species identification.

**Keywords:** diatom; Malaysia; morphology; new morphotype; *Pseudo-nitzschia*.

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## Introduction

Pseudo-nitzschia H. Peragallo, a genus of pennate chainforming diatoms, has drawn significant attention and scientific interest after an incidence of amnesic shellfish poisoning (ASP) in Prince Edward Island, Canada, in 1987 (Bates et al. 1989). Identification of Pseudo-nitzschia species based on morphology requires detailed examination of the frustule ultrastructure, which is only feasible through electron microscopy. Morphological characteristics such as shape; length and width of the valve; presence or absence of a central nodule; and number of fibulae, striae, and poroids are among the detailed features used for species delineation. Even though morphometric characterizations of Pseudo-nitzschia have been well documented (e.g., Hasle et al. 1996), it is challenging to distinguish some closely related pseudo-cryptic species such as P. cuspidata-P. pseudodelicatissima (Lundholm et al. 2003) and cryptic species such as P. delicatissima-P. arenysensis (Quijano-Scheggia et al. 2009). This taxonomic complexity could be a significant barrier to the early detection and mitigation of harmful algal blooms (HABs) particularly in the genus Pseudo-nitzschia.

The recent detection of the ASP toxin, domoic acid (DA), in shellfish from Southeast Asian countries (Bajarias et al. 2006, Dao et al. 2006, 2009a, Takata et al. 2009) indicates the potential risk for ASP events in the region. In Malaysia, harmful algal research and monitoring are confined to locations with known incidences of paralytic shellfish poisoning toxins produced by the dinoflagellates *Pyrodinium bahamense* Plate (Usup et al. 2012) and *Alexandrium* spp. (Lim et al. 2004, 2005). Very little attention has been given to other groups of HAB species, especially species of *Pseudo-nitzschia*.

The present study was conducted to remedy the insufficient number of background studies on the occurrence of *Pseudo-nitzschia* species in Malaysian waters, in particular on Peninsular Malaysia, which is virtually lacking such information. Several studies were previously undertaken but mainly to focus on selected sites along the coasts of Malaysian Borneo (Lim et al. 2010, 2012a). Detailed morphology and genetics of *P. brasiliana* Lundholm, Hasle *et* Fryxell, *P. micropora* Priisholm, Moestrup *et* Lundholm, *P. dolorosa* Lundholm *et* Moestrup, *P. pungens* 

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