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Morphological Characterization of the Diatom *Pseudo-Nitzschia* Species (Bacillariophyceae) from Malaysian Waters

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ABSTRACT

The diatom *Pseudo-nitzschia* has been known for domoic acid poisoning, or better known as Amnesic Shellfish Poisoning (ASP). Accumulation of domoic acid (DA) in commercial bivalves during the bloom of this toxic diatom has caused economic losses to the industry. To clarify the status of ASP and the diversity of toxic or potentially toxic *Pseudo-nitzschia* species in the Malaysian waters, plankton samples were collected from 17 locations using 20 µm-mesh plankton net haul. Samples were subjected to acid wash treatment before detail observation under transmission electron microscope (TEM). Identification of *Pseudo-nitzschia* species was based on the frustules morphology with the morphometric measurements. In total, 22 species were identified, including 18 new records in Malaysia. *Pseudo-nitzschia brasiliana*, *P. caciantha*, *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. multistriata*, *P. pseudodelicatissima*, *P. pungens* and *P. turgidula* are known to be toxic and previously reported to associate with ASP events worldwide. This study provides useful information on the harmful algae species inventory of the country.

INTRODUCTION

Amnesic Shellfish Poisoning (ASP) is a type of shellfish poisoning that caused by the diatom, *Pseudo-nitzschia*. The species has been getting many attentions after the first outbreak of ASP in Canada due to the blooms of *P. multiseries* in 1987 (Perl *et al*., 1990). This type of poisoning is caused by the neurotoxin, domoic acid (DA).

Studies of *Pseudo-nitzschia* in Malaysian waters were only taken place in 2007, where Su et al. (2010) investigated the temporal distribution of *Pseudo-nitzschia* spp. in the Kuching estuaries, and subsequently two species, *P. pungens* and *P. brasiliana* were reported from Borneo (Lim *et. al*. 2011). Two years later, a newly described species, *P. circumpora* (Lim et. at., in press) with three species viz. *P. micropora*, *P. cuspidata*, and *P. dolorosa* were documented from Malaysian Borneo.

There are 40 species of *Pseudo-nitzschia* been described thus far, and 16 are known to be toxic (Lelong *et al.* 2012) Precise identification of the toxic *Pseudo-nitzschia* becomes crucial in harmful algal blooms (HABs) species monitoring purpose. This study aims to document the occurrence of *Pseudo-nitzschia* species in Malaysian waters and to provide a species inventory for future monitoring uses. Field samplings had been undertaken at 17 locations from the coasts of Malaysia, and the preserved samples were underwent detailed morphological investigation under transmission electron microscopy (TEM). The occurrence of the species encountered in this study was presented herein.

MATERIALS AND METHODS

Field samplings were undertaken at Santubong, Gerigat, Kabong, Bintulu, Miri, Kuala Penyu, Kota Belud, Kota Kinabalu, Kudat, Pulau Banggi, Sempurna, Johor, Muar, Port Dickson, Teluk Batik, Queen Bay, and Kuala Terengganu (Fig. 1). Plankton samples were collected by using 20 µm mesh plankton net. Samples were filtered using a 0.2 µm nylon membrane filter and preserved with modified saline ethanol (Miller and Scholin 2000) in 50 mL centrifuge tubes.

Figure 1: Malaysia map showing sampling locations in this study.

In the laboratory, environment samples were rinsed by distilled water several times to remove the saline ethanol. Organic materials of the samples were acid-cleaned by adding 96% H_2SO_4, saturated $KMnO_4$, and 10% oxalic acid (Bargu *et al.* 2002). The cleaned samples were mounted to a 100 square-mesh formvar-coated copper grib, and air-dried overnight. Samples were examined under a JEOL JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan). TEM micrographs were taken using Gatan Digital Micrograph (DM) software with Erlangshen ES500W camera (Gatan USA).

RESULTS AND DISCUSSION

In total, 22 species of *Pseudo-nitzschia* were found in Malaysian waters. They are *P. americana*, *P. brasiliana*, *P. caciantha*, *P. calliantha*, *P. circumpora*, *P. cuspidata*, *P. decipien*, *P. delicatissima*/*P. arenysensis*, *P. dolorosa*, *P. inflatula*, *P. linea*, *P. lineola*, *P. mannii*, *P. micropora*, *P. multistriata*, *P. pseudodelicatissima*, *P. pungens*, *P. sinica*, *P. subfraudulenta*, and *P. turgidula*.

Taxonomy of *Pseudo-nitzschia*, likes most of the diatoms, is mainly inferred from the frustules morphology (Hasle *et al.* 1996, Lundholm *et al.* 2002). The morphological characteristics used to identify the species of *Pseudo-nitzschia* include valve shape, striae, and poroids morphology (Hasle *et al.* 1996, Lundholm *et al.* 2002, Lim *et al*. 2012). Four common valve shape of *Pseudo-nitzschia* were found in this study *viz.* linear (Fig. 2A), linear to lanceolate (Fig. 2B), lanceolate with inflated end of the tips (Fig. 2C), and lanceolate (Fig. 2D). Most of the species found possess valve shape with lanceolate, 50%, follows by linear, 27.27%, linear to lanceolate, 18.18%, and lanceolate with inflated at both end, 4.55%.

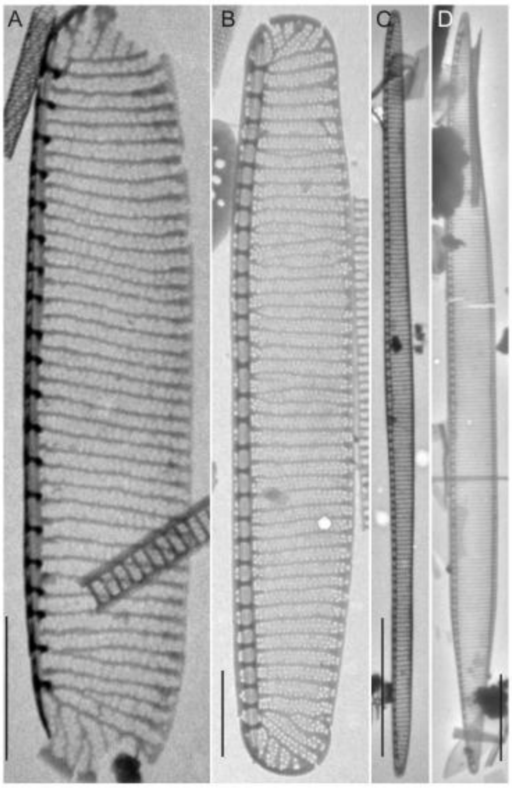

Figure 2: Valve shape of *Pseudo-nitzschia* species. TEM. (A) Linear valve of *P. linea*. (B) Linear to lanceolate valve shape observed in *P. americana*. (C) Lanceolate and inflated at the both end of tips in *P. inflatula*. (D) Lanceolate valve of *P. subfraudulenta*.

There are 3 types of striae observed, i.e. one row of poroid (Fig. 3A & B), 2 rows (Fig. 3C), and 2-3 rows of poroids (Fig. 3D). Sectors perforation is one of the important features in *Pseudo-nitzschia* identification. In this study, several types of sectors perforation were observed in the samples. Sector perforation is mainly in 2 sectors, 2-4 sectors or >7 sectors. The known toxic *Pseudo-nitzschia* spp. was distributed throughout the 17 stations investigated, with the number of toxic species ranged from two to nine species (Fig. 4). Among the 17 locations 6 locations were considered as the potential ASP hot spots, with >5 toxic *Pseudo-nitzschia* spp. found. They are Bintulu, Pulau Banggi, Sempurna, Johor Bahru, Port Dickson, and Teluk Batik (Fig. 4). Bintulu has the highest species richness with 17 species observed, followed by Port Dickson (15 spp.). The lowest number was recorded at 5 locations (2 spp.). *Pseudo-nitzschia pungens* and *P. brasiliana* were encountered in all the locations, the former was reported as cosmopolitan species (Hasle 2002), and the latter was commonly reported in the South East Asia regions (Lim *et al*. 2011; Lim *et al*. 2012;).

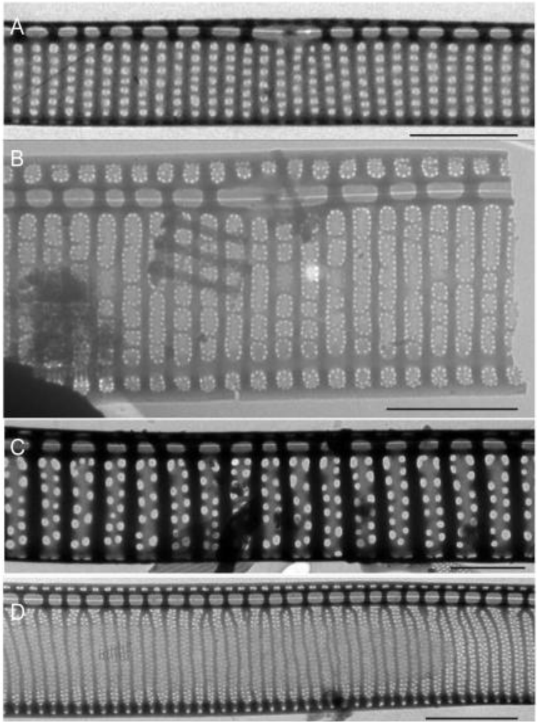

Figure 3: Poroid morphology of *Pseudo-nitzschia* species. TEM. (A) one rows of poroids perstriae showed 2-4 poroids sectors perforation. (B) One row of minute poroids per striae. The poroids have >7 sectors perforation. (C) Two row of poroids per striae. (D) 2-3 row of poroids per striae.

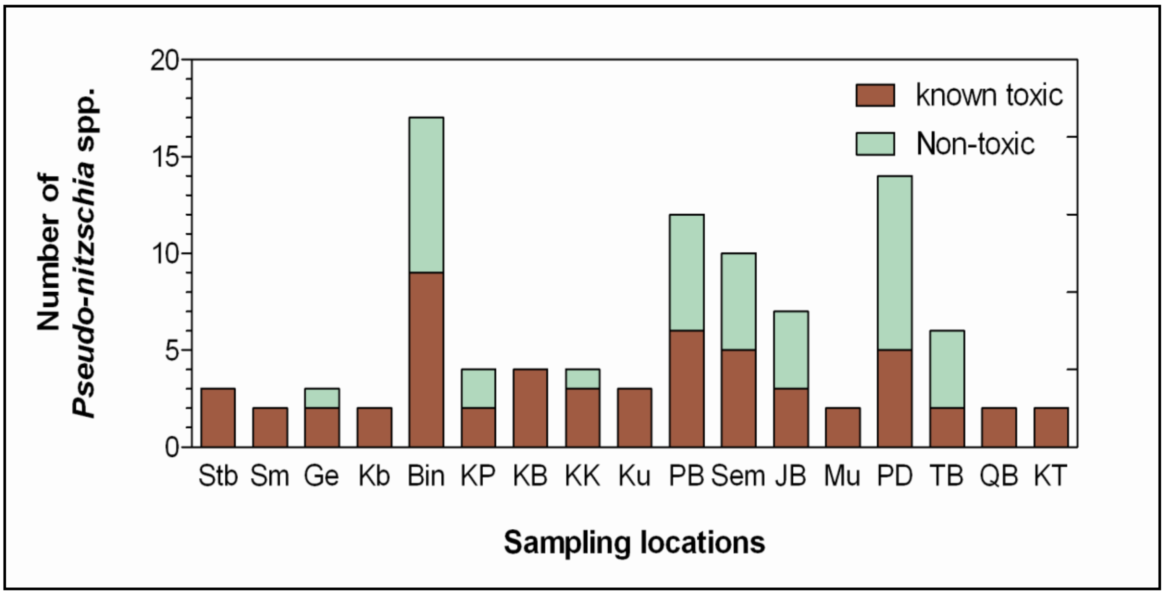

Figure 4: Species composition of known toxic and non-toxic *Pseudo-nitzschia* species found in Malaysian waters. Abbreviations of sampling locations are as: Stb, Santubong; Sm, Samariang; Ge, Gerigat; Kb, Kabong; Bin, Bintulu; KP, Kuala Penyu; KB, Kota Belud; KK, Kota Kinabalu; Ku, Kudat; PB, Pulau Banggi; Sem, Sempurna; JB, Johore Bahru; Mu, Muar; PD, Port Dickson; TB, Teluk Batik; QB, Queen Bay; KT, Kuala Terengganu.

Some species of *Pseudo-nitzschia* are restricted to certain geographical regions, which are classified as tropic, temperate and cold water species. However, our distribution data showed that some of the species considered as cold-water species and temperate species have been found in our waters. For instance, *P. turgidula* and *P. decipiens* were known to be the cool-water and temperate species, respectively. Both species were encountered in our plankton samples. The occurrence of these species in our waters may be explained by dispersal through ships' ballast waters. Further studies are required to elucidate the origin and dispersal mechanisms of this important genus.

CONCLUSION

This study showed a considerable high species diversity of *Pseudo-nitzschia* in Malaysian coastal waters, with more than half of the species described here is far from being discovered from our waters. Some of the species found are currently known as toxic species. It is noteworthy that these toxic species co-exist with the non-toxic species in most of the sampling sites. This imposed the importance and urgency to precisely and rapidly detect the toxic species in the waters. Molecular techniques such as whole-cell 'Fluorescence in situ Hybridization' (FISH) using species-specific oligonucleotide probes to detect the toxic species in our waters are currently on-going. The species inventory we gathered from this study provides further information to the related authorities for better HABs monitoring in the country.

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