

## ***Pseudo-nitzschia* (Bacillariophyceae) in Malaysia: a record of taxa from field investigations**

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### **Abstract**

Different diatoms species of the genus *Pseudo-nitzschia* have been associated with Amnesic Shellfish Poisoning (ASP). In recent years, domoic acid contamination in seafood has been increasingly reported in Southeast Asian countries. To assess the potential risk of ASP and determining species diversity of toxic or potentially toxic *Pseudo-nitzschia* in Malaysia, plankton samples were collected from 17 locations throughout the coasts of Malaysia using a 20 µm-mesh plankton net. Samples were subjected to acid wash treatment prior to observation under transmission electron microscope (TEM). Identification of *Pseudo-nitzschia* species was based on the frustules morphology and morphometric measurements. In total, 22 species were identified, including 14 new records in Malaysia. Occurrence of nine known toxic species which were previously associated with ASP events worldwide are documented. This study reports for the first time a high species richness of *Pseudo-nitzschia* along the coastal waters of Malaysia.

**Keywords:** *Pseudo-nitzschia*, Malaysia, Malacca Straits, South China Sea

### **Introduction**

Amnesic shellfish Poisoning caused by shellfish contamination with a neurotoxin, domoic acid (DA), was first reported at Prince Edward Island, Canada in 1987; the causative organism for the event was identified later as *Pseudo-nitzschia multiseries* (Bates *et al.* 1989). Research on *Pseudo-nitzschia* have gained wide attention after this incidence and numerous studies have been conducted worldwide. Thus far, 37 species of *Pseudo-nitzschia* has been documented (Lelong *et al.* 2012, Lundholm *et al.* 2012), with a few species known to be cosmopolitan (Hasle 2002).

Contamination of domoic acid in shellfish mollusks in Southeast Asia has been confirmed in at least two countries from the region: Vietnam (Dao *et al.* 2009) and Philippines (Takata *et al.* 2009). However, the status of ASP or domoic acid levels in shellfish from neighboring countries remained unclear. *Pseudo-nitzschia* species are common in the phytoplankton assemblages in Malaysian waters. Five *Pseudo-nitzschia* species have been recorded from northeastern Borneo, i.e. *P. brasiliensis*, *P. calliantha*, *P. delicatissima*, *P. micropora*, and *P. multistriata* (Skov pers. comm.; Larsen and Nguyen

2004) but there is a lack of supporting information. Recently, the presence of these species, with new records of *P. cuspidata*, and *P. dolorosa* and *P. pungens* (Lim *et al.* 2012) was confirmed. A new morphotype was described as *P. circumpora* based on morphological and molecular evidence (Lim *et al.* 2012) (Lim *et al.* 2012). However, these studies were limited to few locations in Borneo. Hence, a study on *Pseudo-nitzschia* distribution was initiated in 2009 to document the presence of species of this genus along the Malacca Straits and South China Sea. This study was also aimed to assessing the potential risk of ASP in Malaysia, in particular in areas with extensive shellfish mariculture.

### **Material and Methods**

Plankton samplings were conducted at 17 locations along the coastal waters in Malaysia particularly the areas with shellfish farming industry (Figure 1). Samples were collected using 20 µm-mesh plankton net and preserved with acidic Lugol's solution. Plankton samples were rinsed with distilled water before acid washed to remove organic material. Cleaned plankton samples were mounted on the Formvar-coated copper grid, air-dried, and examined

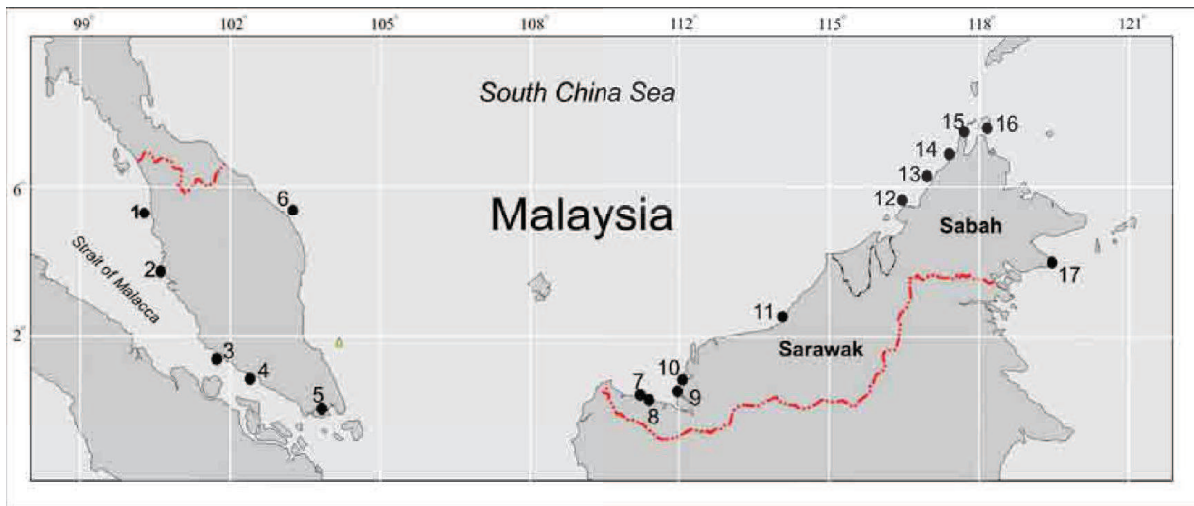


Fig. 1: Sampling locations along the Malaysian coast.

under a JEOL JEM1230 transmission electron microscope (Tokyo, Japan).

### Results and Discussion

In this study, a total of twenty two species of *Pseudo-nitzschia* were documented from samples collected in locations shown in Table 1. Generally, at least two potentially toxic *Pseudo-nitzschia* species were observed at each station. Locations such as Port Dickson (Station 3), Bintulu (Station

11), Pulau Banggi (Station 16) and Semporna (Station 17) were suggested as potential ASP hotspots, with at least five known toxic species found in the samples (Table 1). Bintulu recorded the highest diversity of *Pseudo-nitzschia*, with nine known to be toxic, and seven non-toxic *Pseudo-nitzschia* species; it was followed by Port Dickson, with 13 species, nine of which are known to be toxic (Table 1). Five and six potentially toxic species were recorded at Pulau Banggi and Semporna respectively (Table 1).

Table 1: *Pseudo-nitzschia* species found at each sampling location.

Sta.	Species	Total # species	Potential toxic species	Potential ASP risk
1	Queen Bay, Penang	b,r	2	Low
2	Teluk Batik	a,b,n,r,t	5	Low
3	Portdickson	a,b,d,e,h,i,j,k,n,o,p,r,t	13	High
4	Muar	b,r,	2	Low
5	Johore	a, b, c, g, r	6	Low
6	Kuala Terengganu	b, r	2	Low
7	Santubong	b, r	3	Low
8	Semariang	b, r	2	Low
9	Kabong	b, r	2	Low
10	Gerigat	b, r, t	3	Low
11	Bintulu	a, b,c,d,e,f,g,h,j,k,l,m,n,,p,r,t,u	16	High
12	Kuala Penyu	b,i,l,r	4	Low
13	Kota Belud	b,f,q,r	4	Low
14	Kota Kinabalu	b,g,h,r	4	Low
15	Kudat	b,c,r	3	Low
16	Pulau Banggi	b,c,d,e,g,h,i,k,n,r,s,t	11	Medium
17	Semporna	b,d,e,g,h,i,k,r,u	9	Medium

a. *P. americana*; b. *P. brasiliiana* c. *P. caciantha* d. *P. calliantha*. e. *P. circumpora* f. *P. cuspidata* g. *P. decipiens* h. *P. delicatissima* /arenysensis i. *P. dolorosa* j. *P. hasleana* k. *P. inflatula* l. *P. linea* m. *P. lineola* n. *P. mannii* o. *P. micropora*. p. *P. multistriata* q. *P. pseudodelicatissima*. r. *P. pungens*. s. *P. sinica*. t. *P. subfraudulenta*. u. *P. turgidula*.

Thirteen species from the *delicatissima*-group belonging to three main complexes, viz. *pseudodelicatissima*, *delicatissima* and *americana* complex, were documented in this study. Eight species from the *pseudodelicatissima* complex were observed including three species, i.e. *P. cacialantha*, *P. calliantha*, and *P. pseudodelicatissima* (Fig. 2), known to produce domoic acid or associated with ASP events elsewhere (Álvarez *et al.* 2009, Moschandreu *et al.* 2010) Moschandreu *et al.* 2010). Four species from the *delicatissima* complex (Fig. 2), with only *P. delicatissima* reported as a toxic species (Larsen and Nguyen 2004), were documented. Three species from the *americana* complex (Fig. 2), of which only *P. brasiliana* is reported as toxic (Sahraoui *et al.* 2011) Sahraoui *et al.* 2011), were also described. Only three species from the *seriata*-group, i.e., *P. pungens*, *P. turgidula* and *P. subfraudulenta*, were

documented (Fig. 2). Among these, *P. pungens* and *P. turgidula* are known to be toxic (Rhodes *et al.* 1996) Rhodes *et al.* 1996). Interestingly, species such as *P. turgidula*, *P. lineola*, and *P. decipiens*, previously reported from cold climate regions (Almandoz *et al.* 2008, Marchetti *et al.* 2008, Lundholm *et al.* 2012) were observed in this study (Fig. 2). Further studies are needed to clarify the origin of these species.

### Conclusions

Our results showed high diversity of *Pseudo-nitzschia* species in Malaysian coastal waters, with 14 new records in the country. Coexistence of the known toxic and non-toxic species was found in most of the sampling site. At least two toxic or potentially toxic species was documented at each station. Five locations with high numbers of toxic

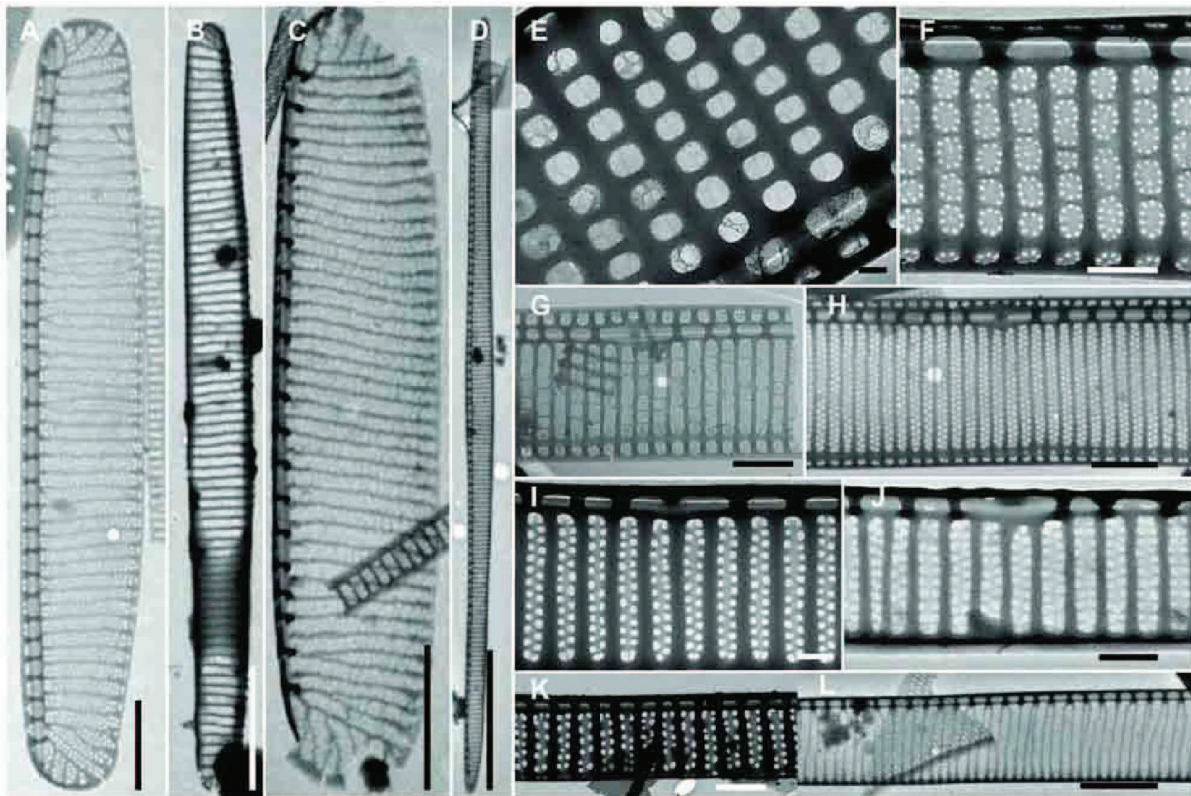


Fig. 2: TEM micrographs of selected *Pseudo-nitzschia* spp. found in this study. (A) *P. amaricana*, linear to lanceolate valve, (B) *P. brasiliana*, lanceolate valve, (C) *P. lineola*, linear valve, (D) *P. inflatula*, inflated at tip of the valve, (E) *P. pseudodelicatissima*, two hymen sector, (F) *P. caliantha*, 6-8 sector with central sector, (G) *P. circumpora*, >7 sector in one hymen poroid, (H) *P. subfraudulenta*, 2 rows with dividing sector poroid perstriae, (I) *P. turgidula*, detail of two row poroids, (J) *P. delicatissima*, detail of two row poroid, (K) *P. pungens*, detail of two row poroid, (L) *P. multistriata*, 2-3 row of poroids per striae.

species are potential hot spot area for ASP outbreak and required. Toxin analysis on culture and nature samples is required to study the toxicity of the species in Malaysia. Development of molecular probe for rapid detection of the toxic species is undergoing.

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