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# Characterization and identification of *Pseudo-nitzschia* species in Lampung Bay, Indonesia

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ABSTRACT

*Pseudo-nitzschia* species have received more attention in recent decades due to increasing Amnesic Shellfish Poisoning (ASP) cases in many Asian countries caused by the toxic domoic acid they produced. However, information on morphological characters of *Pseudo-nitzschia* species in Indonesia was very limited, which hinders the attempt to quickly identify the species during Harmful Algal Blooms (HABs) in the coastal waters. Thus, this study aimed to identify and characterize the *Pseudo-nitzschia* species found in Lampung Bay, Indonesia. Phytoplankton samples used in this study were taken from the reference collection for plankton (RCP) in the Research Center for Oceanography, National Research and Innovation Agency (RCO-BRIN). The original samples were collected from Hurun Bay, Padang Cermin, Lampung, during high tide in 2005. In this research, morphology and morphometry of *Pseudo-nitzschia* were observed using a light microscope (LM) and transmission electron microscope (TEM). The *Pseudo-nitzschia* species found in Lampung was labelled as LMP3. Jaccard cluster analysis, using the simple average link, showed that LMP3 was *Pseudo-nitzschia pungens*. The morphology and morphometry of LMP3 were matched perfectly with *P. pungens* (100% similarity) and differed from other *Pseudo-nitzschia* species in Seriata complex. Unfortunately, it was not known whether the *P. pungens* LMP3 in this study was the toxin producer strain as this study used preserved sample, thus it was not possible to detect any trace of domoic acid in there.



## 1. Introduction

Chain-forming, pennate marine diatom genus *Pseudo-nitzschia* Peragallo is a typical component of marine phytoplankton communities, which can be found abundant in inshore, offshore and oceanic ecosystems in all biogeographic zones (Hernández-Becerril, 1998; Hasle *et al.*, 1996; Orsini *et al.*, 2002; Casteleyn *et al.*, 2008). Recently, the genus has received more attention, mainly due to the ability of some *Pseudo-nitzschia* species that are capable on produce domoic acid, a neurotoxin responsible for amnesic shellfish poisoning (ASP) (Casteleyn *et al.*, 2008; Hasle *et al.*, 1996; Orsini *et al.*, 2002).

In the past, most phytoplankton taxonomists classify *Pseudo-nitzschia* as part of genus *Nitzschia* Hassal due to the limitation of microscopy equipment available at that time. However, observation using an electron microscope managed to separate *Pseudo-nitzschia* into different genera due to its differences in morphological and morphometrical characters with *Nitschia* (Skov, *et al.*, 1999; Hasle *et al.*, 1996). A similar case also occurred in Indonesia, where most of the researchers classify *Pseudo-nitzschia* as *Nitzschia* genus, which was also commonly found in coastal ecosystems (Sidabutar, 2010; Thoha, 2010).

In general, species of *Pseudo-nitzschia* was divided into 2 major groups based on the cell width, which were: the Seriata complex and the Delicatissima complex (Hasle *et al.*, 1996; Skov *et al.*, 1999). The Seriata complex has a valve width of around 3- 4 μm or more, while the Delicatissima complex has a valve width less than 3 – 4 μm (Hasle *et al.*, 1996; Skov *et al.*, 1999).

*Pseudo-nitzschia* characters that could be observed under a light microscope (LM) were: (1) cell width, (2) cell length, (3) cell shape, (4) shape of cell or valve, (5) central nodule, (6) visibility of fibulae and interstriae, (7) cell overlap in the chain (Hernández-Becerril, 1998; Casteleyn *et al.*, 2008; Hasle *et al.*, 1996; Skov *et al.*, 1999; UNESCO/IOC/WESTPAC, 2011). However, identification of *Pseudo-nitzschia* at species level using only LM was difficult. Some species such as *Pseudo-nitzschia pungens*, *P. seriata*, *P. multiseries*, *P. australis*, and *P. pungiformis* have similar morphological characters which were difficult to distinguish under light microscopy (Hernández-Becerril, 1998; Hasle *et al.*, 1996). *P. pungens* was often confused with *P. seriata* when seen in girdle view due to highly similar fibulae structure (Hasle *et al.*, 1996). In girdle view, *P. pungens* also can hardly be distinguished from *P. multiseries*. Meanwhile, *P. pungiformis* has a similar valve outline as *P. pungens* and *P. multiseries*, but it has a larger central interspace and has a central nodule (Hasle *et al.*, 1996). Variation of cell's morphological characters in natural samples due to vegetative cell division, or due to environmental stress, could also lead to misidentification of *Pseudo-nitzschia* species. (Cerino *et al.*, 2005; Thessen *et al.*, 2005).

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Observation with Scanning Electron Microscope (SEM) or Transmission Electron Microscope (TEM), revealed more specific characters that could be used to identify *Pseudo-nitzschia* species up to species level. Characters that could be observed under TEM microscopy were: (1) density of fibulae per 10 µm, (2) density of interstriae per 10 µm, (3) number or poroids row, (4) structure of poroid (Hernández-Becerril, 1998; Casteleyn *et al.*, 2008; Hasle *et al.*, 1996; Skov *et al.*, 1999; UNESCO/IOC/WESTPAC, 2011). A combination of characters that were observed under LM and TEM were commonly used in *Pseudo-nitzschia* identification at the species level. However, more recent phylogenetic studies using DNA sequence was frequently favoured to identify the *Pseudo-nitzschia* up to sub-species level (Casteleyn *et al.*, 2008).

Specific studies of *Pseudo-nitzschia* species in Indonesia were rare and most studies were focused on the distribution and abundance of the genus in marine ecosystems. Similarly, taxonomic studies of *Pseudo-nitzschia* species were rarely conducted, therefore information on *Pseudo-nitzschia* species found in Indonesian water was very limited. Thus, this study was aimed to (1) identify a common *Pseudo-nitzschia* species found in the preserved samples from Lampung Bay; and (2) understand the morphological and morphometrical character of *Pseudo-nitzschia* found in Lampung Bay, Indonesia.

## 2. Materials and Method

### 2.1 Sample Origins

*Pseudo-nitzschia* specimens were taken from preserved samples stored in the Reference Collection for Plankton (RCP) of the Plankton Productivity Laboratory, Research Center for Oceanography, National Research and Innovation Agency (RCO-BRIN). The samples were collected in fieldwork in 2005 during high tide, using Kitahara plankton net (mesh size 80 µm), in Hurun Bay, Padang Cermin district, Lampung, Indonesia (Figure 1). All samples were preserved in 4% formaldehyde (v/v). The samples from Hurun Bay, Padang Cermin, Lampung were selected by taking into account the high density of *Nitzschia* (*Pseudo-nitzschia*) cells in the observation data of samples from the adjacent areas within the Lampung Bay during 2005 field sampling.

### 2.2 Sample Preparation and Observation

Morphological and morphometrical analysis of *Pseudo-nitzschia* species found in samples was conducted with a light microscope (LM) and transmission electron microscope (TEM), using non-cleaned and acid cleaned materials. The non-cleaned material was obtained directly from the preserved sample, then was transferred to Sedgewick Rafter Counting Chamber (SRCC) for observation. Observation of non cleaned *Pseudo-nitzschia* species by light microscopy was conducted using an inverted microscope Nikon Diaphot model 108 under 200 – 400X magnification. The phase-contrast illumination technique was used to increase the contrast of the non-cleaned *Pseudo-nitzschia* samples. LM images taken with Canon EOS 500D mounted on Nikon Diaphot using Nikon F to Canon EF mount adapter. On the other hand, the acid cleaned material was obtained after cleaning all organic material in the sample. The acid cleaning process was conducted following the diatom cleaning and electron microscopy preparation methods described by G.R. Hasle in Sournia (1976). The acid cleaned sample was then placed on a copper grid coated with a Formvar film. JEOL JEM-12 TEM microscope in Universiti Malaysia Sarawak, Malaysia was used to observe *Pseudo-nitzschia* nanostructures under 10.000 – 300.000X magnification. As a note, some images shown in the Result section have been published in Rachman (2013) (Figure 2A, 3A, 3B, 3D) and Bayu *et al.* (2020) (Figure 3A, 3D). For this study, those images were then modified by adding arrows, lines, and letters to point out some specific morphological and morphometrical characters of the *Pseudo-nitzschia* LMP3 frustule.

Specific taxonomic characters, as described earlier in the Introduction section, were used for species-level identification of the *Pseudo-nitzschia* cells in this study (Hernández-Becerril, 1998; Casteleyn *et al.*, 2008; Hasle *et al.*, 1996). The most common *Pseudo-nitzschia* species found in the sample of

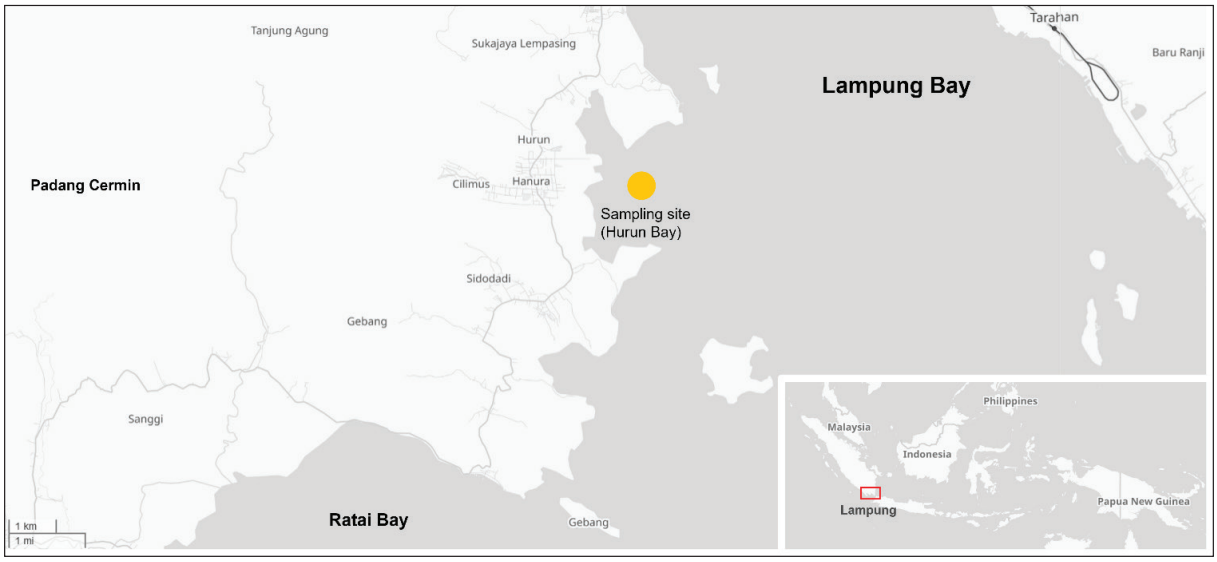


**Figure 1.** Location of the sampling site from which the preserved Pseudo-nitzschia samples were originated. Samples were collected during fieldwork in 2005.

this study was labelled as *Pseudo-nitzschia* LMP3 or LMP3. Taxonomic characters of LMP3 were then compared to other *Pseudo-nitzschia* species in both Seriata and Delicatissima complex (Hasle *et al.*, 1996; Skov *et al.*, 1999; UNESCO/IOC/WESTPAC, 2011). Taxonomic information, particularly, systematic classification follows the most recent information on Algaebase website (Guiry & Guiry, 2020).

### 2.3 Data Analysis

Taxonomic data, consisting of morphology and morphometric characters of *Pseudo-nitzschia*, were then analyzed with Jaccard cluster analysis (simple average link) to determine the similarity of the characters with other species within the Seriata and Delicatissima complex. The clustering analysis was done using BioDiversity Professional Ver.2 statistic program (Guiry & Guiry, 2020).

## 3. Result

### 3.1 Systematic account and descriptions of *Pseudo-nitzchia pungens*

***Pseudo-nitzschia pungens*** (Grunow ex. Cleve) Hasle,

(Hasle, 1993; Hallegraeff, 1994; Hasle *et al.*, 1996; Skov *et al.*, 1999)

**Synonym:** *Nitzschia pungens* Grunow ex. Cleve

(Cupp, 1943; Rivera, 1985)

**Systematic classification**

(Guiry & Guiry, 2020)

| | |
|---|---|
| Empire | : Eukaryota |
| Kingdom | : Chromista |
| Phylum | : Bacillariophyta |
| Subphylum | : Bacillariophytina |
| Class | : Bacillariophyceae |
| Subclass | : Bacillariophycidae |
| Order | : Bacillariales |
| Family | : Bacillariaceae |
| Genus | : *Pseudo-nitzschia* |
| Species | : *Pseudo-nitzschia pungens* (Grunow ex. Cleve) Hasle |

#### 3.1.1 Light microscopy characters

Cells formed a chain-shaped colony. Cells in the chain overlapped ± 1/3 of cell length. The valve is symmetric, with a linear to lanceolate shape. The ends of the valve were pointed. The cells were strongly silicified, thus the interstriae and fibulae were visible in fresh and acid cleaned material. However, the interstriae is often coarsely silicified so it is difficult to differentiate fibulae from interstriae. In fresh material, two chloroplasts sometimes could be seen inside the cell. Cell length around 24-121 µm, and cell width around 2.4 – 4.2 µm (Skov *et al.*, 1999; UNESCO/IOC/WESTPAC, 2011). The central nodule is absent.

#### 3.1.2 TEM microscopy characters:

Fibulae and interstriae have relative equal densities with a ratio of 1:1. The density of fibulae is 8-14 in 10 µm and the density of interstriae is 8-13 in 10 µm. There are 2 rows of poroids (striae) in between two interstriae, sometimes it has 1 additional row of poroids. The density of poroids is 2-4 poroids in 1 µm. The valve end often has fewer poroids per striae than the other. The structure of poroids is simple and hymenate, usually perforated by closely packed holes in a hexagonal array.

### 3.2 Characters and identification of *Pseudo-nitzschia* LMP3

In water mounted sample under phase-contrast light microscopy, the *Pseudo-nitzschia* cells of LMP3 appears to have a lanceolate shape and exhibit longitudinal symmetry (Figure 2A). The central nodule was absent in all cells in the sample. The bands of striae and interstriae, also the preserved chloroplast, were often observable in the non-cleaned water mount samples (Figure 2B). In LMP3 specimens, the fibulae were barely observable in the water mount at 400X magnification (Figure 2). The *Pseudo-nitzschia* cells of LMP3 form a chain-shaped colony, with each cell overlapping each other about 1/4 of cell length (Figure 2A).

The length of LMP3 *Pseudo-nitschia* cells was varied between 70.5 to 93.4 µm, while the cell width was varied between 2.4 to 3.6 µm. Those morphological and morphometric characteristics were similar to the character of *P. pungens* (Table 1). However, it is important to note that other *Pseudo-nitzschia* species, particularly *P. seriata*, also have similar morphological and morphometric characteristics when observed under LM in a water mount. Thus, it was very difficult to confidently classify

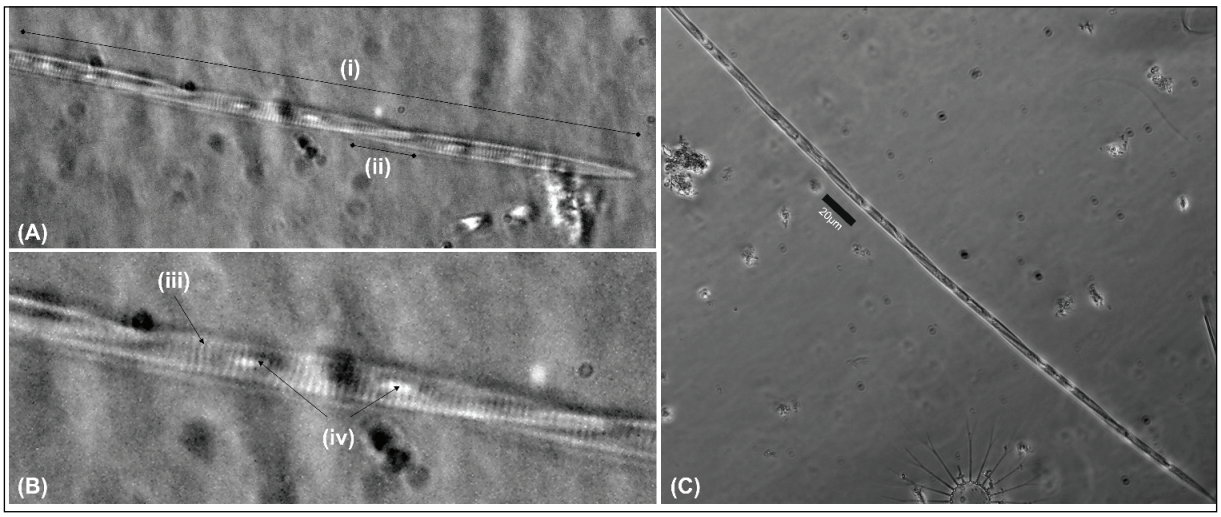


**Figure 2.** *Pseudo-nitzschia* LMP3 in water mount under the light microscope (LM) with phase-contrast lighting. (A) Cells observed at 400X magnification; (B) digitally cropped and magnified image showing details of a single cell within the chain colony; (C) chain colony viewed under lower magnification (200X). (i) whole chain colony; (ii) overlapping at cell ends; (iii) striae and interstriae (spaces between striae); (iv) preserved chloroplast. Image A and B without scale. Images A and B were taken and modified from Rachman (2013).

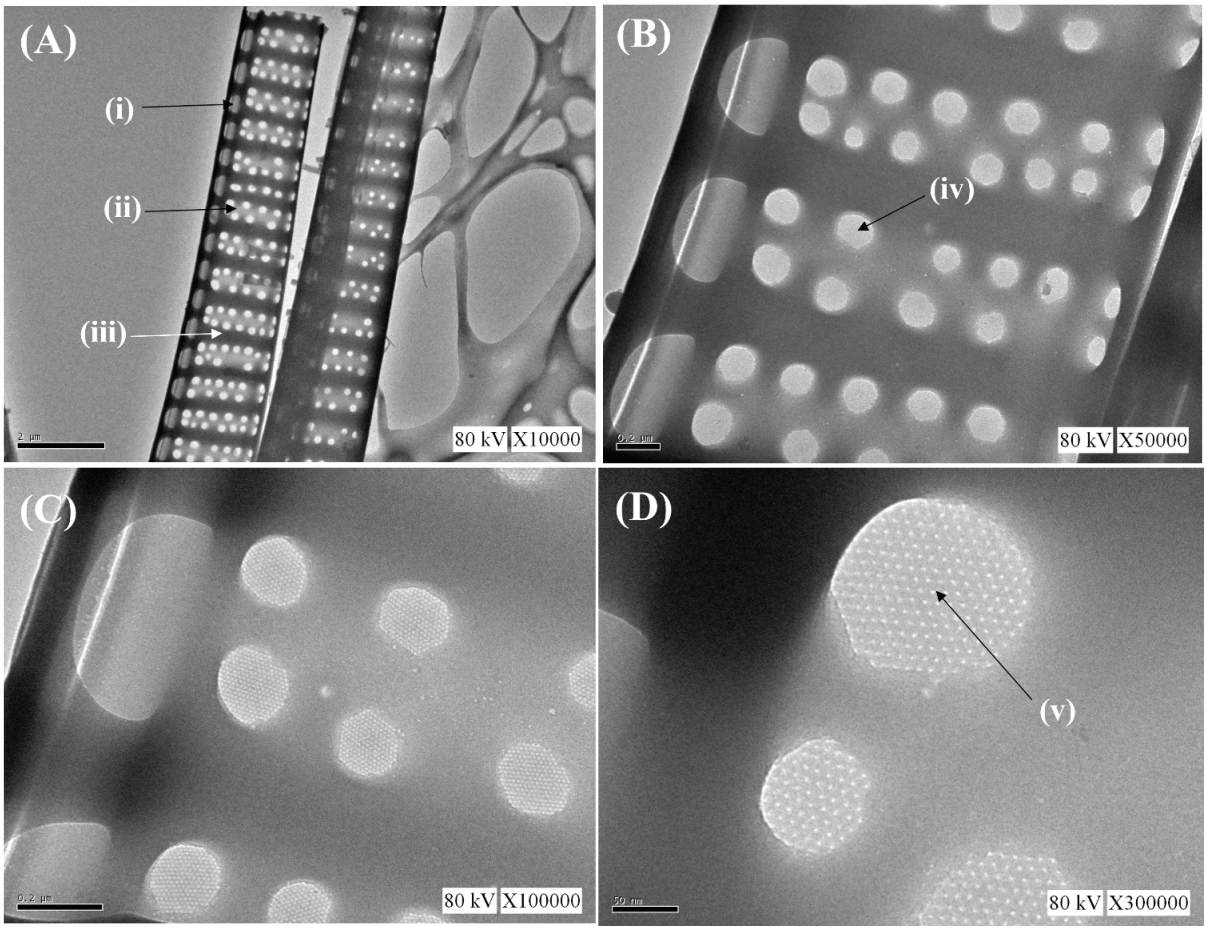


**Figure 3.** *Pseudo-nitzschia* LMP3 under electron microscope. (A) TEM image of *P. pungens* (LMP3) valve, revealing the number of poroids rows and density of poroids; (B) Details of the valve under 50.000X magnification; (D) Details of the valve under 100.000X magnification; (D) Details of poroids structure. (i) fibulae; (ii) striae with two rows of poroids; (iii) inter-striae; (iv) poroid; (v) hymen, an ultra/nanostructure within each poroid. Scale bars = (A) 2 µm; (B) 0.2 µm ;(C) 0.2 µm; 50 nm (E). Images A, B, and D were taken and modified from Rachman (2013) and Bayu et al. (2020).

*Pseudo-nitzschia* LMP3 as *P. pungens* or *P. seriata* by using only its key characters that were observable under LM.

The result from TEM analysis (Figure 3B – 3E) revealed more details on the morphological characters of *Pseudo-nitzschia* LMP3, as well as some of its valve's nanostructures. As seen in Figures 2B and 2C, the density of fibulae and striae were equal, with 13 fibulae and 13 striae per 10 µm. However, further examination in some other *Pseudo-nitzschia* LMP3 cells shows the unequal density of fibulae and striae. The *Pseudo-nitzschia* LMP3 cells generally have two rows of poroids, although there was a striae with only one row of poroids (Figure 3A). The density of the poroids in *Pseudo-nitzschia* LMP3 cell was 4 poroids per 1 µm (Figure 3A). It appears that the rows of poroids in one striae often didn't have the same number between the first and second row. In *Pseudo-nitzschia* LMP3 cell, the number of the poroids in one row are higher than the other rows (Figure 3A and 3B). The structure of the poroid is simple (Figure 3D) and the poroids are hymenate and perforated by closely packed holes in a hexagonal array (Figure 3D).

The result of TEM and LM observation highly suggested that the *Pseudo-nitzschia* species found in Lampung (LMP3) was *Pseudo-nitzschia pungens*. The morphological and morphometric characters of *Pseudo-nitzschia* LMP3 specimens were matched perfectly with *P. pungens* as described in Skov *et al.* (1999), Skov *et al.* (1999); Hasle *et al.* (1996), and Skov *et al.* (1999). All specific characters of *P. pungens* were observed in *Pseudo-nitschia* LMP3 and were different from other similar *Pseudo-nitzschia* species in the Seriata complex, such as *P. seriata* (Table 1).

## 3.3 Jaccard cluster analysis on *Pseudo-nitzschia* LMP3 and other *Pseudo-nitzschia* species in the Seriata complex

The result of Jaccard clustering analysis (simple average link) showed that the *Pseudo-nitzschia* LMP3 was *P. pungens*, with 100% similarity in its morphology and morphometry (Figure 4). *Pseudo-nitzschia* LMP3 also has a high similarity with *P. multiseries*, and *P. australis*, with a similarity percentage of 54.12% (Figure 4). Those similarities were mainly due to the equal density of the striae and fibulae in their valve (cells) (Table 1). The clustering analysis also showed that *P. seriata* was in the same group with *Pseudo-nitzschia* LMP3, *P. pungens*, *P. multiseries*, and *P. australis* (Figure 4). However, *P.seriata* was slightly separated from other species in the same group (Figure 4), mainly due to the higher density of poroids present in its valve (Table 1). The group of LMP3, *P. pungens*, *P. multiseries*, *P. australis*, and *P. seriata*, were separated from groups of *P. pungiformis*, *P. fraudulenta*, *P. subfraudulenta*, *P. subpasifica*, and *P. heimii* (Figure 4), mainly due to the lack of central nodule in the valve (Table 1).

**Table 1.** Comparison of key morphologic and morphometric character between *Pseudo-nitzschia* LMP3 and other *Pseudo-nitzschia* species in Seriata complex.

| Characters | LMP3 | P. Pungens* | P. seriata* | P. multiseries* | P. pungiformis* | P. australis* |
|---|---|---|---|---|---|---|
| Valve shape | Linear-lanceolate | Linear-lanceolate | Lanceolate | Lanceolate | Linear-lanceolate | Lanceolate |
| Valve symmetricity | Symmetric | Symmetric | Asymmetric | Symmetric | Symmetric | Asymmetric |
| Central nodule | - | - | - | - | + | - |
| Length (µm) | 70.5-93.4 | 24-121 | 61-100 | 67-140 | 96-145 | 82-144 |
| Width (µm) | 2.4-3.6 | 2.4-4.2 | 4-8 | 5-7 | 5-6 | 4-5 |
| Cell overlap in chain | 1/4 | 1/3-1/4 | 1/3-1/4 | *Nd* | *nd* | 1/3-1/4 |
| Number of fibulae in 10 µm | 13-14 | 8-14 | 14-18 | 10-15 | 12-18 | 12-18 |
| Number of striae in 10 µm | 12-13 | 8-13 | 14-18 | 10-15 | 14-20 | 12-18 |
| Row of poroids | 2 | 2 | 2 + 1 (-2) | 3-4 (5) | 2 | 2 |
| Number of poroids in 1 µm | 3-4 | 2-4 | 7-8 | 5-7 | 5-6 | 4-5 |
| Complexity of poroids | Simple | Simple | Simple | Simple | Simple | Simple |

Note: *nd* = no data; *Morphology and morphometry data based on Skov *et al.* (1999) and UNESCO/IOC/WESTPAC (2011)

**Table 1** (Continued). Comparison of key morphologic and morphometric character between *Pseudo-nitzschia* LMP3 and other *Pseudo-nitzschia* species in Seriata complex.

| Characters | LMP3 | P. fraudulenta* | P. subfraudulenta* | P. subpasifica* | P. heimii* |
|---|---|---|---|---|---|
| Valve shape | Linear-lanceolate | Lanceolate | Linear-lanceolate | Lanceolate | Linear-lanceolate |
| Valve symmetricity | Symmetric | Symmetric | Symmetric | Asymmetric | Symmetric |
| Central nodule | - | + | + | + | + |
| Length (µm) | 70.5-93.4 | 50-119 | 65-106 | 33-70 | 50-120 |
| Width (µm) | 2.4-3.6 | 4-7 | 4.8-7 | 9-10 | 5-8 |
| Cell overlap in chain | 1/4 | *nd* | *nd* | 1/5-1/6 | 1/4-1/5 |
| Number of fibulae in 10 µm | 13-14 | 12-24 | 12-17 | 15-20 | 11-18 |
| Number of striae in 10 µm | 12-13 | 18-24 | 23-28 | 28-32 | 19-28 |
| Row of poroids | 2 | 2-3 | 2 | 2 | 1-2 |
| Number of poroids in 1 µm | 3-4 | 4-7 | 5-6 | 9-10 | 5-8 |
| Complexity of poroids | Simple | Complex | Complex | Simple | Simple |

Note: *nd* = no data; *Morphology and morphometry data based on Skov *et al.* (1999) and UNESCO/IOC/WESTPAC (2011)

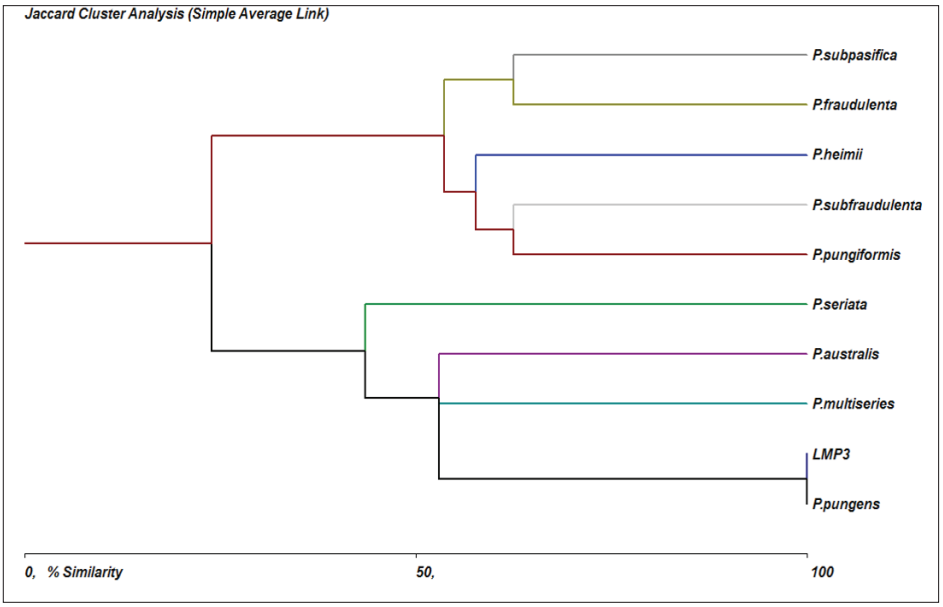


**Figure 4.** Jaccard cluster analysis (simple average link) on *Pseudo-nitzschia* LMP3 and other *Pseudo-nitzschia* species in seriata complex. Morphometric and morphological characters were used in this analysis. Binomial code (1 and 0) were used to substitute the presence or absence of characters in the specimens.

# 4. Discussion

## 4.1 Comparison of *P. pungens* LMP3 characters with other species in seriata complex

Under light microscopy (LM), it was very difficult to distinguish each species in the Seriata complex, mainly due to the high similarity in their cell's morphological characters. For example, *P. pungens* and *P. seriata* both have similar cell outlines and also have a similar density of fibulae and striae when observed under LM. Thus, in the past, those species were considered as a different form of the same species (Orsini *et al.*, 2002). Additionally, due to its morphological similarity, *Pseudo-nitzschia* used to be classified under the genus *Nitzschia* but later was separated from that genus due to the ability of most *Pseudo-nitzschia* species to form chain colonies (Lelong *et al.*, 2012).

In higher magnification using TEM, the difference in some specific cell characters serves as a base to separating the *P. pungens* and *P. seriata* as different species. Although both species appear similar under LM, the shape of *P. seriata* cell or valve was always lanceolate and appear asymmetric (Table 1) compared to *P. pungens*. Both *P. seriata* and *P. pungens* have a relatively equal number of striae and fibulae. However, the density of poroids in *P. seriata* is higher than the *P. pungens* (Table 1). On the other hand, *P. multiseries* also have a similar cell outline to *P. pungens* and was very hard to be distinguished under LM.

Observation with TEM showed that *P. multiseries* have a higher number in the row of poroids and poroids density, compared to *P. pungens* and *Pseudo-nitzschia* LMP3 (Table 1). High similarity in morphological and morphometric characters was also observed between *P. pungens* and *P. australis* cell valves or frustules. Both species share similar characters and only differ in the density of poroids (Table 1). Using TEM, *P. pungens* could be easily distinguished from *P. pungiformis*, *P. fraudulenta*, *P. subfraudulenta*, *P. subpasifica*, and *P. heimii* by its lack of central nodule, which could be found in the valve of those other *Pseudo-nitzschia* species in Seriata complex.

As a note, a study done by Casteleyn *et al.* (2010) revealed that *P. pungens* can further be distinguished into three different clades by using nuclear-encoded rDNA ITS, plastid-encoded rbcL sequences, and subtle differences in the ultra/nanostructures of its frustules. The study classified the clades by their geographical distribution, in which (A) clade I (*P. pungens var. pungens*) widely distributed in temperate waters in the Atlantic and Pacific region; (B) clade II (*P. pungens var. cingulate*) that have limited distribution around northeastern Pacific; and (C) clade III (*P. pungens var. aveirensis*) which often found in tropical to warm-temperate waters within Atlantic and Pacific oceans (Casteleyn *et al.*, 2010). This study did not include any genetic analysis on the LMP3 specimen, but based on the distribution of *P. pungens* clades in Casteleyn *et al.*, (2010) paper, the *P. pungens* LMP3 most likely belonged to the clade III (*P. pungens var. aveirensis*). However, a further study that involves detailed genetic analysis and a more thorough analysis of *P. pungens* LMP3 ultra/nanostructures is required to further determine its clade.

## 4.2 Notes on the physiology and ecology of *P. pungens*

As a member of the cosmopolitan genus of *Pseudo-nitzschia*, *P. pungens* was known to have a very wide geographical distribution across different climate types that includes most of the coastal area within Europe, North America, Central America, South America, East Africa, South Africa, Mediterranean, India, East Asia, and Southwest Asia, including Indonesian waters (Hasle *et al.*, 1996; Casteleyn *et al.*, 2008; Lelong *et al.* 2012). In general, *P. pungens* is often found in high density at a warm temperature, high salinity, and interestingly, in low nutrient concentration (oligotrophic) waters (Almandoz *et al.*, 2008; Lelong *et al.* 2012). Even so, higher nutrient concentration, particularly silica (Si), is beneficial to *P. pungens* as it could increase the cell biomass. On the other hand, Si deficiency could trigger an increase in domoic acid production (Pednekar *et al.*, 2018). Similarly, a higher concentration of urea ($CH_4N_2O$) in the water was also known to boost the domoic acid production in *P. pungens* by up to two times higher compared with the response to a higher concentration of nitrate ($NO_3$-) (Lelong *et al.* 2012). Another important parameter for *P. pungens* growth and toxin production is pH, in which a lower pH level (< 8) could dramatically increase the domoic acid concentration in the cells to up to five-fold (Lelong *et al.* 2012).

In Lampung Bay, the case of *Pseudo-nitzschia* outbreak or blooms hasn't been reported so far. The blooming phytoplankton species in Lampung Bay usually belong to the dinoflagellate groups, such as *Pyrodinium* sp., *Alexandrium* sp., and, quite recently, *Cochlodinium* (*Margalefdinium*) sp., in which those species also produces a harmful toxin that is dangerous to animals and humans (Mizushima *et al.*, 2007; Thoha *et al.*, 2015, Thoha *et al.*, 2019). In general, *Pseudo-nitzschia* cells was reported to be abundant during the west monsoon or rainy season in Indonesia (Thoha *et al.*, 2019). Additionally, according to the study by Solihin *et al.* (2015), the genus also responds positively towards higher temperature, higher nutrient concentration ($PO_4$ and $NO_3$), and higher pH in the water column of Lampung Bay. Thus, with the rapid development of coastal cities, industries, and marine aquacultures in and around Lampung Bay, there was a chance that the blooming trend could be shifted from the toxic dinoflagellates to the potentially toxic diatoms, particularly, the *Pseudo-nitzschia* spp. Therefore, rapid species-level identification and monitoring on the density of *Pseudo-nitzschia* population technique in the Lampung Bay might soon become a necessity. That would be done to quickly determine the species and to mitigate any possible negative effects from its blooms in the bay.

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### Additional information