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Observations of eleven *Pseudo-nitzschia* species in Tokyo Bay, Japan

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Abstract: Appearance of *Pseudo-nitzschia* species was observed in samples collected biweekly in Tokyo Bay during April 2008—August 2009. Species identification was based on morphological characteristics observed under light microscope (LM) and transmission electron microscope (TEM). This is the first time that a periodic survey of this genus at species level was done in the area. The following 11 species were found: *P. americana, P. brasiliana, P. caciantha, P. calliantha, P. delicatissima, P. galaxiae, P. fraudulenta, P. multistriata, P. multiseries, P. pseudodelicatissima and P. pungens*. The presence of five species, *P. americana, P. brasiliana, P. caciantha, P. calliantha* and *P. galaxiae* is reported for the first time in Tokyo Bay.

Keywords: *diatom, Pseudo-nitzschia species, Tokyo Bay*

Introduction

Currently there are 32 species known in the genus *Pseudo-nitzschia* (AMATO and MONTRESOR, 2008; CLEVE, 1897b; HASLE, 1965; HASLE, 1993; LUNDHOLM *et al.,* 2002a; LUNDHOLM and MOESTRUP, 2002; LUNDHOLM *et al.,* 2003; PRIISHOLM *et al.,* 2002; SKOV *et al.,* 1999; TAKANO, 1995). The type species of the genus, *P. seriata* was renamed from *Nitzschia seriata* CLEVE by PERAGALLO in (1897–1908). But it was only in 1993 that half, *i.e.,* 14 of the species were transferred from *Nitzschia* to *Pseudo-nitzschia* (HASLE, 1993).

Studies on this genus are numerous particularly in Europe and North America. In contrast studies of *Pseudo-nitzschia* in Southeast Asia remain to be scant. Notable are taxonomic works by LARSEN and NGUYEN (2004) that identified *Pseudo-nitzschia* species in Vietnam waters and PRIISHOLM *et al.* (2002) that described a new species, *P. micropora* found in Thailand. TAKANO and KUROKI (1977) found seven species of *Pseudo-nitzschia,* still under *Nitzschia* that time, in various coastal waters of Japan. These are *P. delicatissima* (*N. actydrophila*), *P. fraudulenta, P. multiseries* (*N. pungens* forma *multiseries*), *P. pungens, P. pseudodelicatissima, P. subfraudulenta,* and *P. turgidula.* Regular surveys of phytoplankton in Tokyo Bay have reported the presence of *Pseudo-nitzschia,* but their identification and enumeration have been done only at the genus level. As of this year 2010, there is no clear assessment of *Pseudo-nitzschia* species composition in Tokyo Bay. Thus the purpose of this study is to elucidate species composition of the genus *Pseudo-nitzschia* in Tokyo Bay. Descriptions and documentations put forth here are for morphometric characterizations of local populations and baseline data for future investigations of toxic or newly discovered taxa to further ecological studies of *Pseudo-nitzschia* in the area.

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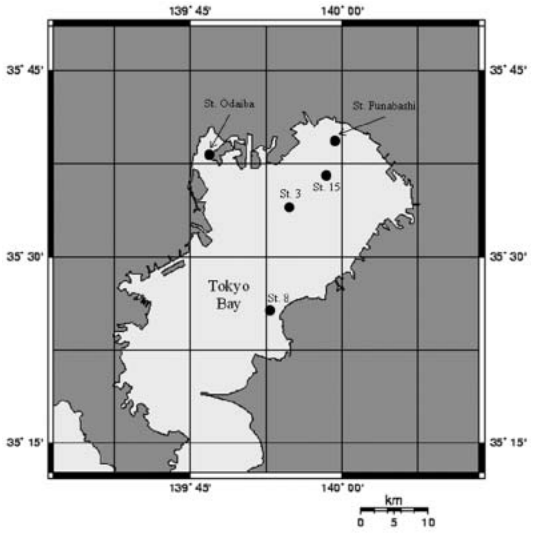


Fig 1. Map of sampling stations. (http://gmt.soest.hawaii.edu/)

Material and Methods

Field sampling

Samples collected by bucket from Odaiba station (35° 36′55″N, 139° 46′32″E) or by Van Dorn Water Sampler from Stations 3 (35° 33′16″N, 139° 54′29″E), 8 (35° 25′24″N, 139° 51′42″E), 15 (35° 36′24″N, 139° 57′48″E) and Funabashi (35° 38′43″N, 139° 59′19″E) in Tokyo Bay (Fig. 1) during April 2008—August 2009 were assessed for presence of *Pseudo-nitzschia*. One liter of sample was preserved with neutralized formaldehyde at a final concentration of approximately 1 %. Another liter of sample remained unfixed for culture strains establishment.

Culture establishment

Pseudo-nitzschia cells were isolated by picking up single chains by capillary method (THRONDSEN, 1978). These were washed at least three times with sterilized sea water under a compound microscope and placed in 24-well plates filled with f/2 media (GUILLARD, 1975). When cells are numerous enough, these were then transferred to screw capped test tubes with the same media. The culture conditions were as follows: temperature 15℃ (and/or 20℃), light intensity 100 μmol photons $m^{-2}s^{-1}$ (daylight fluorescent lamps), salinity at 30 and 12L: 12D photoperiod.

Species observation and species confirmation by TEM

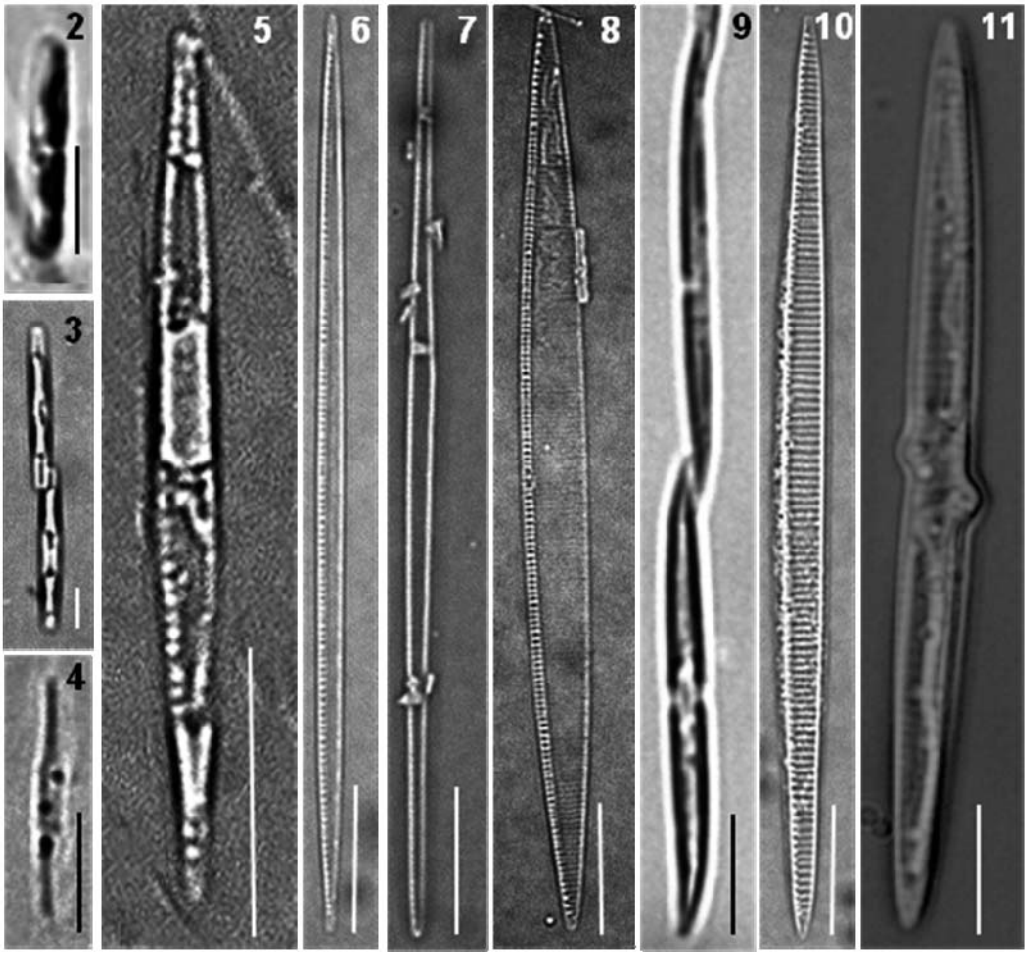
Light microscope (LM) photomicrographs were taken of live cells from the field, in culture and preserved cleaned valves devoid of organic material. Organic material was removed from the cells as follows:

Aliquots of fixed *Pseudo-nitzschia* cells from field and live cells from cultures were transferred to 15 ml centrifuge tubes. The live cells were fixed with neutralized formaldehyde. Cells were washed with milliQ water at least three times prior to acid washing. Centrifugations were done at 35,000 rpm for 10 minutes. Organic material was dissolved following LUNDHOLM *et al.* (2002b). The condensed samples (1 ml) were treated with 0.2 ml of 30% sulfuric acid and 0.5–1 ml of saturated potassium permanganate ($KMnO_4$) solution and were left for 24 hours. And then 1–2 ml saturated oxalic acid ($(COOH)_2$) was added until the purple color turned out before the valves were washed several times with distilled water to wash out the acids. Drops of cleaned cells were mounted on formvar coated copper grids and allowed to dry. These were then observed under a JEM 2000EX JEOL Transmission Electron Microscope (TEM). General shape of a cell, its length and width, number of poroid rows, number of poroid in 1 μm, number of interstriae in 10 μm, number of fibulae in 10 μm and inner poroid features were measured and noted. *Pseudo-nitzschia* species were identified the following several manuals and recent articles (HASLE and SYVERTSEN, 1997; SKOV *et al.,* 1999; LUNDHOLM *et al.,* 2002a and 2003; AMATO and MONTRESOR, 2008).

Results

Culture strains

Ten species out of the 11 species of *Pseudo-nitzschia* found in Tokyo Bay from sampling period starting April 2008—May 2009 were established into culture (Table 1). These were the following: *P. americana* (4), *P. brasiliana* (3), *P. caciantha* (1), *P. calliantha* (6), *P. delicatissima* (1), *P. fraudulenta* (6), *P. galaxiae* (1), *P. multistriata* (2), *P. multiseries* (2) and *P. pungens* (7). Numbers in parenthesis are the number of culture strains, all non-axenic, for



Figs. 2–11. LM micrographs of 10 species.
Fig. 2. *P. americana,* Fig. 3. *P. brasiliana,* Fig. 4. *P. galaxiae,* Fig. 5. *P. caciantha,* Fig. 6. *P. calliantha,* Fig. 7. *P. delicatissima,* Fig. 8. *P. fraudulenta,* Fig. 9. *P. multistriata,* Fig. 10. *P. multiseries,* Fig. 11. *P. pungens.* Figs. 2, 3, 9: LM micrographs of live samples; Figs. 4–8, 10: LM micrographs of cleaned valves of cells in culture. Fig. 11. DIC micrograph of cell in culture. Scale bar = 10 μm.

each species. These were useful for morphometric measurements of species that rarely occurred e.g.: *P. delicatissima* and *P. galaxiae* and for tidy TEM photomicrographs. Dates of isolation provide information on the days a particular species appeared in the bay during the sampling period.

Species Descriptions

Descriptions of each species in alphabetical order follow herein. A summary of morphological characteristics of these species in Tokyo Bay is shown in Table 2. Except for *P. delicatissima* and *P. galaxiae* morphometric measurements of which are from newly established cultures, all measurements are from field samples in Tokyo Bay.

***Pseudo-nitzschia americana* (HASLE) FRYXELL in HASLE**

Figs. 2, 12

LUNDHOLM *et al.,* 2002a. pp. 483–484, Figs. 1–20. ORLOVA and SHEVCHENKO, 2002, pp. 336–339, Fig. 1. KACZMARSKA *et al.,* 2005, pp. 4–7, Figs. 2–13, Table 1.

Synonym: *Nitzschia americana* HASLE

Table 1. Strains of *Pseudo-nitzschia*and their corresponding origin and dates of isolation.

Name	Strain No.	Origin	Date of Isolation	Collector/Isolator
P. americana	TBaM01	Tokyo Bay, JP	16 March 2009	L. Yap-Dejeto
P. americana	TBaA01	Tokyo Bay, JP	04 April 2009	L. Yap-Dejeto
P. americana	TBaA02	Tokyo Bay, JP	04 April 2009	L. Yap-Dejeto
P. americana	TBaA03	Tokyo Bay, JP	04 April 2009	L. Yap-Dejeto
P. brasiliana	TBbA01	Tokyo Bay, JP	09 April 2008	L. Yap-Dejeto
P. brasiliana	TBbS01	Tokyo Bay, JP	24 September 2008	L. Yap-Dejeto
P. brasiliana	TBbS02	Tokyo Bay, JP	24 September 2008	L. Yap-Dejeto
P. caciantha	TBcc03	Tokyo Bay, JP	18 November 2008	L. Yap-Dejeto
P. calliantha	TBcl1101	Tokyo Bay, JP	11 May 2009	L. Yap-Dejeto
P. calliantha	TBcl1102	Tokyo Bay, JP	11 May 2009	L. Yap-Dejeto
P. calliantha	TBcl2502	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. calliantha	TBcl2503	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. calliantha	TBcl2507	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. calliantha	TBcl2508	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. delicatissima	TBd05	Tokyo Bay, JP	07 July 2008	L. Yap-Dejeto
P. fraudulenta	TBfJ02	Tokyo Bay, JP	07 July 2008	L. Yap-Dejeto
P. fraudulenta	TBf1101	Tokyo Bay, JP	11 May 2009	L. Yap-Dejeto
P. fraudulenta	TBf1102	Tokyo Bay, JP	11 May 2009	L. Yap-Dejeto
P. fraudulenta	TBf2504	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. fraudulenta	TBf2505	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. fraudulenta	TBf2506	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. galaxiae	TBg01	Tokyo Bay, JP	25 May 2009	L. Yap-Dejeto
P. multiseries	Kums	Kushimoto, JP	5 March 2008	T.OMURA/L. Yap-Dejeto
P. multiseries	TBms01	Tokyo Bay, JP	15 June 2008	L. Yap-Dejeto
P. multistriata	TBma01	Tokyo Bay, JP	21 October 2008	L. Yap-Dejeto
P. multistriata	TBma02	Tokyo Bay, JP	21 October 2008	L. Yap-Dejeto
P. pungens	TBpuA00	Tokyo Bay, JP	15 April 2008	L. Yap-Dejeto
P. pungens	TBpuA01	Tokyo Bay, JP	15 April 2008	L. Yap-Dejeto
P. pungens	TBpuA03	Tokyo Bay, JP	15 April 2008	L. Yap-Dejeto
P. pungens	TBpuJ01	Tokyo Bay, JP	07 July 2008	L. Yap-Dejeto
P. pungens	TBpuJ02	Tokyo Bay, JP	07 July 2008	L. Yap-Dejeto
P. pungens	TBpuJ03	Tokyo Bay, JP	07 July 2008	L. Yap-Dejeto
P. pungens	TBpu03	Tokyo Bay, JP	16 March 2009	L. Yap-Dejeto

HASLE, 1964, pp. 41–44, Pl. 1, Fig. 4, Pl. 14, Figs. 13–19, Pl. 15, Figs. 7–10.
HASLE, 1974, p. 427. HASLE and SYVERTSEN, 1997, p. 324, Pl. 73.

Morphological Description:

Cells are linear to lanceolate with broadly rounded ends with no central interspace in valve view and almost linear with cut ends in girdle view. This cell's apical axis is between 15–21 μm and transapical axis about 2.3–2.9 μm. Two rows of poroids, sometimes three are present in striae (circled with arrow, Fig. 12b). Poroids in 1 μm are about 8–10. Interstriae in 10 μm are about 38–43, and fibulae in 10 μm are from 19–23.

Occurrence in Tokyo Bay:

This species was observed in March, April, June, July, August and September at temperatures of 14.5–27.5℃ and salinity at 31–34. *P. americana* is reported to for the first time here. LUNDHOLM *et al.* (2002a) reported *P. americana* from Japan (34°50′, 139°31′) to be epiphytic to *Chaetoceros*. We also found *P. americana* to be epiphytic to some *Chaetoceros* species e.g.: *C. affinis, C. lorenzianous, C. socialis* among others from Tokyo Bay (Fig. 23).

Remarks:

This species was first described as *Nitzschia americana* by HASLE in 1964. It was recently redefined by LUNDHOLM *et al.* (2002a) to separate two species within *P. americana* complex, *P. brasiliana* and *P. linea*.

Table 2. Morphometric characteristics of the eleven species observed during April 2008- August 2009 in Tokyo Bay. Numbers in italics found below an entry are from original descriptions of the species. (*AMATO *et al.*, 2007; ** based from photomicrograph of original description)

	Shape	central interspace	length (μm)	width (μm)	rows of poroids	poroids in 1 μm	Interstriae in 10 μm	Fibulae in 10 μm	poroid sectors
P. americana (n=18)	linear	absent	15–21 *16–24*	2.3–2.9 *2.5–4.5*	2–3 *2–3*	8–10 *8–10*	38–43 *26–31*	19–23 *18–24*	
P. brasiliana (n=14)	linear	absent	23–52 *12–65*	2.4–3 *1.8–1.6*	2–3 *2–3*	7–8.5 *7–10*	24–26 *20–26*	19–24 *20–26*	
P. caciantha (n=6)	lanceolate	present	32–33 *53–75*	2.1–2.2 *2.7–3.5*	1 *1*	3–5 *3.5–5*	32–35 *28–31*	16–19 *15–22*	2–6 *2–6**
P. calliantha (n=50)	linear	present	69–87 *44–86*	1.3–2.3 *1.4–4.7*	1 *1*	3–5 *4–6*	32–39 *34–39*	15–20 *17–22*	4–13 *several*
P. delicatissima (n=20)	linear	present	50–65 *40–78*	1.3–1.5 *1.1–2*	2 *2*	11–12 *10–12*	36–38 *36–41*	23–25 *19–25*	
P. fraudulenta (n=26)	spindle-shaped	present	55–89 *50–119*	3.4–10 *4.5–10*	2–3 *2–3*	4.5–7 *5–7*	21–27 *18–24*	19–25 *12–24*	
P. galaxiae (n=20)	lanceolate	present	24–25 *25–41*	1.5–1.6 *1.2–1.7*	—	—	55–57 *56–64*	15–16 *16–26*	
P. multistriata (n=44)	linear	absent	47–53 *55–65*	2.3–3.2 *3.1–3.6*	2 *2*	9–12 *11***	40–42 *37–42*	24–29 *23–26*	
P. multiseries (n=22)	linear-lanceolate	absent	76–89 *68–140*	2.9–5.6 3.4–6	3–6 3–4	5–6 4–6	11–16 10–19	12–16 10–19	
P. pungens (n=32)	linear-lanceolate	absent	70–92 *74–174*	2.9–5 *2.4–5.3*	2 *1–2*	3–5 *3–4*	11–17 *9–16*	12–17 *6–16*	
P. pseudodelicatissima (n=1)	linear	present	— *50–140*	1.8 *0.9–1.6*	1 *1*	4–4.8 *4–6*	30 *30–46*	16 *14–16*	2 *2*

Type locality of *P. americana* is in Atlantida, Uruguay (LUNDHOLM *et al.,* 2002a). *P. americana* is a common epiphyte on *Chaetoceros, Odontella* (LUNDHOLM *et al.,* 2002a) and sometimes *Bacteriastrum* (FRYXELL *et al.,* 1990; HASLE and SYVERTSEN 1997). It has been found in tropical to temperate waters in the world (LUNDHOLM *et al.,* 2002a).

***Pseudo-nitzschia brasiliana* LUNDHOLM, HASLE and FRYXELL**

Figs. 3, 13

LUNDHOLM *et al.,* 2002a, pp. 484–487, Figs. 21–45.

LARSEN and NGUYEN, 2004, pp. 30–31, Pl. III, Figs. 1, 3–4. VILLAC *et al.,* 2005, pp. 139–145, Figs.2–3. QUIJANO-SCHEGGIA *et al.,* 2009a, pp. 100–107, Fig. 2.

Morphological Description:

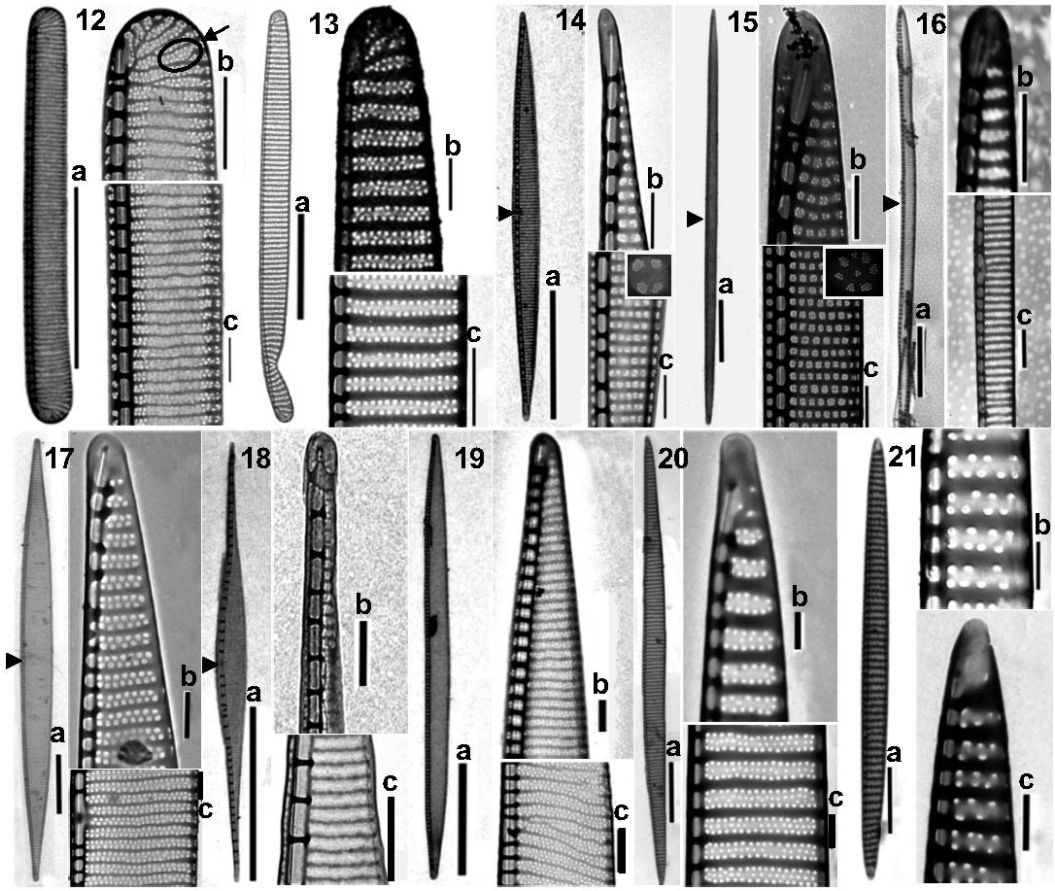
Cells are broadly linear, cigarette-shaped with broadly rounded tips in valve view and linear with truncate ends in girdle view. Apical axis is from 23–52 μm and transapical axis at 2.4–3 μm. No central interspace is found. Two rows of poroids, sometimes three near the border between valve face and mantle can be clearly seen under TEM (Fig. 13b). Poriods in 1 μm are about 7–8.5 small and closely spaced. Interstriae in 10 μm are about 24–26 and the fibulae in 10 μm are in the range of 19–24. Overlap of chains is within 1/8–1/11 of the cell's length.

Occurrence in Tokyo Bay:

This species was observed during the months of April, July, and September at temperatures of 14.5–26.4℃ and salinity at 33–34. *P. brasiliana* is reported for the first time here.

Remarks:

The approximately equal number of fibulae and striae, the coarser silicification, the single row of poroids in the girdle bands, the slightly longer length, the formation of stepped colonies and the planktonic mode of living are



Figs. 12–21. TEM micrographs of species observed in Tokyo Bay brought in culture.
A set of micrographs per species consists of: the whole valve (a), valve tip (b), and a section of the valve showing pores (c). Fig. 12. *P. americana* (arrow point to circled striae with 3 rows of poroids), Fig. 13. *P. brasiliana* Fig. 14. *P. caciantha,* Fig. 15. *P. calliantha,* Fig. 16. *P. delicatissima,* Fig. 17. *P. fraudulenta,* Fig. 18. *P. galaxiae,* Fig. 19. *P. multistriata,* Fig. 20. *P. multiseries,* Fig. 21. *P. pungens.* Scale bars: (a) = 10 μm; valve section showing pores and tip (b and c) = 1 μm. Arrowheads point to central interspaces, respectively.

features distinguishing *P. brasiliana* from *P. americana* under LM. *P. brasiliana* differs from *P. americana* under EM by the morphometric measurements found in Table 2.

Type locality of this species is in Sepetiba Bay, Brazil (LUNDHOLM *et al.,* 2002a). *P. brasiliana* is distributed mainly in warmer waters. Brazil, Vietnam, Gulf of Panama, Gulf of Mexico, Gulf of California, Vietnam, Indonesia (Jakarta Harbour), Thailand and Jinhae Bay, and S. Korea (LUNDHOLM *et al.,* 2002a).

***Pseudo-nitzschia caciantha* LUNDHOLM, HASLE and FRYXELL**

Figs. 5, 14

LUNDHOLM *et al.,* 2003, pp. 806–809, Figs. 5 A-F, Table 2.

Synonym: *P. pseudodelicatissima* (HASLE) HASLE 1993 pro parte

Morphological Description:

Cells at valve view are lanceolate tapering from the middle towards the tips and appear almost asymmetrical. A central interspace is present (Fig. 14a, arrowhead). Its apical axis is around 32–33 μ m and around 2.1–2.2 μ m

transapical axis. One row of poroid is found in striae. Poroids are large and are 3–5 in every 1 μm partly closed by a membrane with 2–6 sectors of poroid hymen (Fig. 14c). Interstriae in 10 μm are about 32–35, while fibulae in 10 μm are about 16–19.

Occurrence in Tokyo Bay:

This species was observed in Tokyo Bay during the months of July and November at temperatures of 16.9–24.1℃ and salinity at 31–33. *P. caciantha* is reported for the first time here.

Remarks:

This species was described by LUNDHOLM *et al.* (2003) from *P. pseudodelicatissima* complex. *P. caciantha* is distinguished from the "true" *P. pseudodelicatissima* by morphometric measurement observed under EM (Table 2).

The morphometric measurements of *P. caciantha* from this study matched up more with a newly described species, *P. mannii* (AMATO and MONTRESOR, 2008) than with *P. caciantha* in terms of length: measurements for this species was at 32–38 μm, *P. manni* was at 33–130 μm while *P. caciantha* was at 53–75 μm; width: this study at 2.1–2.2 μm, *P. manni* at 1.7–2.6 μm, *P. caciantha* at 2.7–3.5 μm; interstriae in 10 μm: this study at 32–38, *P manni* at 30–40, *P. caciantha* at 28–31 and fibulae in 10 μm: this study at 16–19, *P. manni* at 17–19, *P. caciantha* at 15–22. But according to AMATO and MONTRESOR (2008), *P. manni* is primarily differentiated from *P. caciantha* in three aspects: (1) the valve shape which is linear in *P. manni* while lanceolate in *P. calliantha,* (2) cell width, and (3) the pattern of sectors in *P. caciantha* where the poroids do not bear a central sector while *P. manni* has 3.6% poroids bearing central sectors. Our strain is lanceolate and all poroids do not have a central sector. Thus this was identified as *P. caciantha.*

Type locality of this species is Off Tuxpam, Mexico (LUNDHOLM *et al.,* 2003). This was found in Mexico, Andaman Sea, near Phuket Island Thailand (LUNDHOLM *et al.,* 2003).

***Pseudo-nitzschia calliantha* LUNDHOLM, MOESTRUP and HASLE**

Figs. 6, 15

LUNDHOLM *et al.,* 2003, pp. 801–804, Figs. 2, A-G, Table 2.
BARGU *et al.,* 2002, pp. 480–482, Figs. 1–2.
LARSEN and NGUYEN, 2004, pp. 32–33, Figs. 5–8.
CAROPPO *et al.,* 2005, pp. 763–774, Figs. 3a and c. FEHLING *et al.,* 2006, pp. 32–33, Figs. 5–8.
BESIKTEPE *et al.,* 2008, p. 440, Figs. 2–3.
Synonym: *P. pseudodelicatissima* (HASLE) HASLE 1993 pro parte
HASLE *et al.,* 1996, pp. 149–150, Figs. 26–29, 76–81, Tables 1, 2, 8.
SKOV *et al.,* 1999, pp. 13–15, Figs. 10A-G. PHLIPS *et al.,* 2004, pp. 41–42, Fig. 2.

Morphological Description:

Cells at valve view are straight and linear and abruptly taper to rounded tips. A central interspace is present (Fig. 15a, arrowhead). A single row of poroids and about 3–5 large poroids in 1 μm are found in striae. Each poroid is divided by hymen to form "flower (calliantha) like appearance, with one "central disc" and about four to 13 "petals" around it (Fig. 15c). Interstriae in 10 μm are about 32–39; fibulae in 10 μm are from 15–20. Its apical axis is runs from 69–87 μm and transapical axis at 1.3–2.3 μm.

Occurrence in Tokyo Bay:

This species was observed during the months of May, June, July, August, September and November at temperatures of 17.8–27.5℃ and salinity at 31–33. *P. calliantha* is reported for the first time here.

Remarks:

P. calliantha is distinguished from the "true" *P. pseudodelicatissima* by the following features observed under EM: Poroids in 1 μm for *P. calliantha* is 4–6 while for *P. pseudodelicatissima,* it was 5–6. Interstriae in 10 μm for *P. calliantha* are 34–39 while *P. pseudodelicatissima* is at 36–43. Fibulae in 10 μm for *P. calliantha* are at 17–22 while *P. pseudodelicatissima* is at 20–25. Inner poroid sectors of *P. calliantha* are 7–10 with central poroid sector while *P. pseudodelictassima* has only two sectors.

Type locality of this species is in Ejby, Isefjorden, Sealand, Denmark (LUNDHOLM *et*

al., 2003). This is cosmopolitan (SKOV *et al.* 1999, LUNDHOLM *et al.,* 2003).

Pseudo-nitzschia delicatissima (**CLEVE**) **HEIDEN**
Figs. 7, 16
HEIDEN and KOLBE, 1928.
HASLE *et al.,* 1996, pp. 147–149, Figs. 23–25, 70–75, Tables 1, 2, 7. SKOV *et al.,* 1999, pp. 8–13, Figs. 9A-J. STEHR, *et al.,* 2002, p. 59, Figs. 3G and H. CUSACK *et al.,* 2004, pp. 58–71, Pl. V. LARSEN and NGUYEN, 2004, pp. 35–36, Pl. IV, Figs 2, 4, 8. CAROPPO *et al.,* 2005, pp. 765, Fig. 3C. KACZMARSKA *et al.,* 2005, p. 8, Figs. 14–23. FEHLING *et al.,* 2006, pp. 95–97, Fig. B. LUNDHOLM *et al.,* 2006, pp. 467–470, Figs. 1A-G, Table 4. SCHNETZER *et al.,* 2007, pp. 377–378, Figs. 4c and d.
Synonym: *Nitzschia delicatissima* CLEVE
CLEVE 1897 b. p. 24.
Nitzschia actydrophila HASLE
HASLE, 1965, pp. 35–37, Pl. 2, Fig. 10, Pl. 15, Figs. 19–23, Pl. 16, Figs. 3–7.
Pseudo-nitzschia arenyesis QUIJANO-SCHEGGIA, GARCÉS, LUNDHOLM
QUIJANO-SCHEGGIA *et al.*, 2009b, pp. 506–508, Figs. 30–34.

Morphological Description:

Cells in valve view are narrow and linear-lanceolate with straight cut tips while linear with straight cut ends in girdle view. A central interspace is present (Fig. 16a, arrowhead). There are two rows of poroids (Fig. 16b) and about 11–12 small poroids in every 1 μm. Interstriae in 10 μm are about 36–38; fibulae in 10 μm are about 23–25. Apical axis around 50–65 μm and transapical axis at 1.3–1.5 μm. Overlap of chains is within 1/7–1/10 of the cell's length.

Occurrence in Tokyo Bay:

This species was observed once on the months July, 2008 at temperature of 24.1℃ and salinity at 31.

Remarks:

P. delicatissima can be easily distinguished from *P. pseudodelicatissima, P. caciantha* and *P. calliantha* by having two rows of poroids instead of one as the other three species have. It is more linear than *P. caciantha* and has wider transapical axis compared to *P. pseudodelicatissima* and *P. calliantha.* Its ends seem cut-off when viewed under LM (Fig. 7).

The name *Pseudo-nitzschia arenyesis* QUIJANO-SCHEGGIA, GARCÉS, LUNDHOLM is considered here as a synonym of *P. delicatissima.* Diagnosis for this name compared with *P. delicatissima* stipulates that there is no difference morphologically between *P. delicatissima* and *P. arenysensis,* but in helix I of ITS2 rDNA (QUIJANO-SCHEGGIA *et al.,* 2009b). Considering that ITS2 is not transcribed and its high intraspecific variability, make this a weak criterion for species determination. Other regions of DNA should be investigated and compared. Further studies should be made to find distinguishing morphological characteristics that separates *P. delicatissima* and *P. arenyesis.*

Type locality for *P. delicatissima* is not designated. This is widely distributed in arctic (CAROPPO *et al.,* 2005; SKOV *et al.,* 1999), temperate (TAKANO and KUROKI, 1977; FRAGA *et al.,* 1998; SKOV *et al.,* 1999; CUSACK *et al.,* 2004; KACZMARSKA *et al.,* 2005; FEHLING *et al.,* 2006, SCHNETZER *et al.,* 2007), and subtropical waters (HASLE, 1965; SKOV *et al.,* 1999; LARSEN and NGUYEN, 2004).

Pseudo-nitzschia fraudulenta (**CLEVE**) **HASLE**
Figs. 8, 17
HASLE *et al.,* 1996, pp. 144–146, Figs. 17–19, 57–61, Tables 1, 2, 6.
STEHR, *et al.,* 2002, p. 59, Figs. 3G and H. CUSACK *et al.,* 2004, pp. 58–72, Pl. IV, Figs. a-f. KACZMARSKA *et al.,* 2005, pp. 8–11, Figs. 14–23. FEHLING *et al.,* 2006, pp. 97–98, Fig. C. CONGRESTRI *et al.,* 2008, p. 201, Figs. 2a-f.
Synonym: *Nitzschia fraudulenta* P.T. CLEVE
CLEVE 1897a, p. 300, Fig. 11.
HASLE 1965, pp. 15–18, Pl.1, Figs. 2–3, Pl. 4, Figs. 8–10, Pl.6,
Figs. 5–10, Pl.8, Figs. 1 3. TAKANO and KUROKI, 1977, p. 43, Figs. 3, 14–18.
Pseudo-nitzschia seriata var. *fraudulenta* (P.T. CLEVE) H. PERAGALLO
H. PERAGALLO in H&M PERAGALLO, 1900.

Morphological Description:
Cells in valve view are symmetric and spindle-shaped with straight cut tips. A central interspace is present (Fig. 17a, arrowhead). Two or a few times three rows of poroids and about 4.5–7 poroids with star-shaped membranes in every 1 μm are in striae (Fig. 17b). Interstriae in 10 μm are about 21–27; 19–25 fibulae in 10 μm. Apical axis is around 55–89 μm and 3.4–10 μm transapical axis.

Occurrence in Tokyo Bay:
This species was observed in May, June and July at temperatures of 18.8–24.1℃ and salinity at 31.

Remarks:
P. fraudulenta, like *P. delicatissima* has striae which consisted of two rows of poroids and valves with central insterspace. But it is very different from *P. delicatissima* because it is wider more lanceolate or spindle-shaped; more heavily silicified than the latter, and has larger pores with star-patterned inner poroids within each pore.

Type locality of this species is in Plymouth Harbour, England (HASLE *et al.,* 1996). Reports of its occurrence include Atlantic Moroccan waters, N. Atlantic; Skagerrak (HASLE, 1965), and temperate waters (TAKANO and KUROKI, 1977; RINES *et al.,* 2002; CUSACK *et al.,* 2004; KACZMARSKA *et al.,* 2005; FEHLING *et al.,* 2006; ALMANDOZ *et al.,* 2007).

Pseudo-nitzschia galaxiae **LUNDHOLM and MOESTRUP**
Figs. 4, 18
LUNDHOLM and MOESTRUP, 2002, pp. 596–601, Figs. 1–22.
CERINO *et al.,* 2005, pp. 347–39, Figs. 1A-E, 2A-H.

Morphological Description:
Cells in valve view are lanceolate with rostrate apices. A central interspace is present (Fig. 18a, arrowhead). Rows of poroids are tiny, about 3–4 μm wide. Poroids are very tiny (Fig. 18c) and spread all over the valve. These are difficult to count. Interstriae in 10 μm is about 55–57 and fibulae in 10 μm is about 15–16 and barely seen in LM. This tiny cell has an apical axis of around 24–25 μm and 1.5–1.6 μm transapical axis. Chains of about 2–4 cells were observed in culture.

Occurrence in Tokyo Bay:
This species was observed once in May, 2009 at temperature of 18.8℃ and salinity at 31.

Remarks:
P. galaxiae is also a tiny species almost the same size as *P. americana* and *P. brasiliana.* Under the LM, it may be mistaken for *P. brasiliana* as it is also planktonic and form short chains. But it is very different from *P. americana* and *P. brasiliana* when viewed under the EM. *P. galaxiae* has a central interspace while the two other species do not have one. Its shape tapers to rostrate ends while the former two species are linear. *P. americana* and *P. brasiliana* have distinctly round poroids arranged in two rows. But *P. galaxiae* have very tiny poroids scattered all over the valve which are not arranged in rows.

Type locality for this species is in Off Tuxpam, Mexico (LUNDHOLM and MOESTRUP 2002). This is also found in Caribbean coast of Mexico off Tuxpam; Sydney, N.S.W. Australia; Bay of Naples, Italy (LUNDHOLM and MOESTRUP 2002; CERINO *et al.,* 2005).

Pseudo-nitzschia multistriata **(TAKANO) TAKANO**
Figs. 9, 19
TAKANO, 1995, pp. 73–74. RHODES *et al.,* 1998, pp. 463–465, Figs. 1A-F. LARSEN and NGUYEN, 2004, pp. 41–43, Pl. VII, Figs. 1–4.
Synonym: *Nitzschia multistriata*
TAKANO, 1993, pp. 39–41, Figs. A-E, Table 1.

Morphological Description:
Cells at valve view are narrow, linear, tapering near the ends for a longer or shorter distance towards rounded apices. There is no central interspace. Two rows of poroids and about 9–12 poroids are found in 1 μm (Fig. 19c). Interstriae in 10 μm are about 40–42, and fibulae in 10 μm range from 24–29. Apical axis is around 47–53 μm and transapical axis at 2.3–3.2 μm. There are also two types of *P.*

multistriata encountered here as originally described: one with uniform striations and another with uneven striations (Figs. 24 a and b).

Occurrence in Tokyo Bay:

This species was observed in June, July, August, September and October at temperatures of 19.9–27.5℃ and salinity at 31–34.

Remarks:

P. multistriata may be distinguished by its curved ends or characteristic sigmoid shape when observed in girdle view under LM (Fig. 19). Valves of this species have striae which consisted of two rows of poroids similar to *P. fraudulenta* and *P. delicatissima*. Unlike these two species, *P. multistriata* has no central interspace.

Type locality for this species is in Fukuoka Bay, Japan (TAKANO, 1993). Reports of its distribution include China (QI *et al.* 1996), Gulf of Naples, Italy (SARNO and DAHLMANN, 2000), Japan, Korea, Malaysia, Vietnam (LARSEN and NGUYEN, 2004) and in Sea of Japan (ORLOVA *et al.,* 2008).

Pseudo-nitzschia multiseries **(HASLE) HASLE**

Figs. 10, 20

SKOV *et al.,* 1999, pp. 15–17, Figs. 11A, B, G, I.
STEHR, *et al.,* 2002, p. 58, Figs. 2 B and C.
KACZMARSKA *et al.,* 2005, pp. 11–12, Figs. 30–34.
Synonym: *Nitzschia pungens* f. *multiseries* G. R. HASLE 1965
HASLE, 1965, pp. 14–15, Pl. 2, Figs. 1–2, Pl.5, Figs. 10–12, Pl. 6, Fig.4, Pl. 7, Figs. 9–11.
TAKANO and KUROKI, 1977, p. 43, Figs. 2, 10, 12–13 vix 9, 11.
Pseudo-nitzschia pungens f. *multiseries* G.R. HASLE 1993
HASLE, 1993, p.39. VILLAC *et al.,* 1993, pp. 215–217, Figs. 1–2.

Morphological Description:

Cells are symmetric and linear-lanceolate in both the valve and girdle views. There is no central interspace. Apical axes are at 76–89 μm and transapical axis at 2.9–5.6 μm. Three (Fig. 20c) and up to six (not in photo) rows of poroids are found in a striae with about 5–6 poroids in every 1 μm (Fig. 20c). Interstriae in 10 μm are about 11–16, fibulae about 12–16 in 10 μm.

Occurrence in Tokyo Bay:

This species was observed in April, May, June, July, August and September at temperatures of 14.5–27.5℃ and salinity at 31–34.

Remarks:

P. multiseries was formerly called as *P. pungens* f. *multiseries* (HASLE, 1965). This is now considered as a separate species from *P. pungens* based on morphological, physiological and genetic data (HASLE, 1995). Morphological characteristics that separate *P. multiseries* from *P. pungens* are the different number of rows of poroids (HASLE, 1965). *P. multiseries* has three and as many as six rows of poroids especially near the border between valve face and mantle. *P. pungens* has three to four poroids in 1 μm while *P. multiseries* has four to six poroids in 1 μm.

Type locality of this species is in Drøbak, Oslofjord, Norway. Reports of its distribution are mainly in temperate waters (HASLE, 1965; SKOV *et al.,* 1999; TAKANO and KUROKI, 1977; KOTAKI *et al.,* 1996; ORLOVA *et al.,* 2008).

Pseudo-nitzschia pungens **(GRUNOW ex CLEVE) HASLE**

Figs. 11, 21

HASLE *et al.,* 1996, pp. 138–140, Figs. 3–6, 30–37, Table 1–3.
SKOV *et al.,* 1999, pp. 17–19, Figs. 11C-F, H.
STEHR, *et al.,* 2002, pp. 57–58, Figs. 2A and B.
CUSACK *et al.,* 2004, pp. 58–63, Pl. 1, Figs. a-j, Table 2. LARSEN and NGUYEN, 2004, pp. 43–45, Pl. VIII, Figs. 1–3.
CHEPURNOV *et al.,* 2005, pp. 382–383, Figs. 3, 4–6, Table 2. KACZMARSKA *et al.,* 2005, p. 16, Figs. 48–52.
Synonym: *Nitzschia pungens* Grunow ex P.T. CLEVE 1897b
HASLE, 1965, pp. 12–14, Pl. 1, Figs. 4–5, Pl. 5, Figs. 7–9, Pl. 6, Fig. 3, Pl. 7, Figs. 1–8.

Morphological Description:

Cells are symmetric and linear to lanceolate in both the valve and girdle views. Apical axis is around 70–92 μm and transapical axis at 2.9–

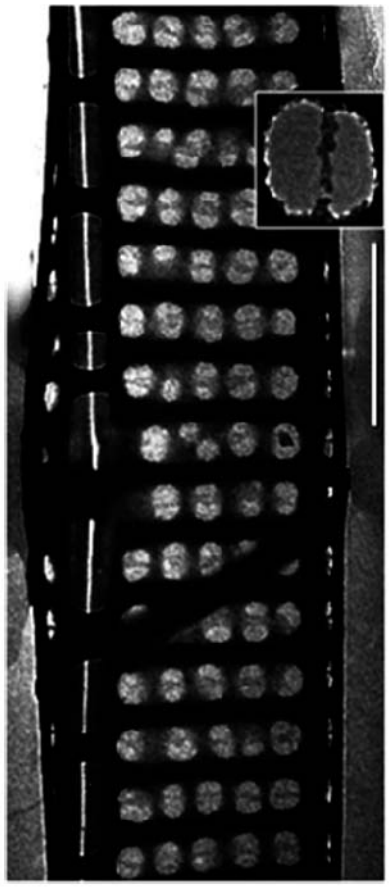


Fig. 22. TEM micrograph of a valve section of *P. pseudodelicatissima* showing pores and inner pore feature divided into two. Scale bar = 1 μm.

5 μm. No central interspace can be observed. Two rows of poroids are present (Fig. 21c) and about 3–5 poroids in every 1 μm are counted (fig. 21b) under the TEM. Interstriae in 10 μm are about 11–17; fibulae at 12–17. Overlap of chains is within 1/3–1/4 of the cell's length.

Occurrence in Tokyo Bay:

This species was observed in March, April, May, June, July, August and September at temperatures of 12.3–27.5℃ and salinity at 31–34.

Remarks:

P. pungens has larger pores and is the most heavily silicified based on sharpness of TEM micrographs among all Pseudo-nitzschia species in this study. The interstriae of this species are widely spaced as another species, *P. multiseries*. This, plus the resemblance with size and shape made these two species appear similar. The number of rows of poroids distinguishes *P. pungens* from *P. multiseries* (Figs. 20c, 21c). *P. pungens* has two rows of poroids that consisted of larger poroids while *P. multiseries* has three or more.

Type locality of this species is in Yeddo Bay (Tokyo Bay), Japan (HASLE, 1965). HASLE and FRYXELL in 1995 reported this to be a cosmopolitan species. This was further supported by CASTELEYN *et al.* (2008) who reported global distribution of one strain of *P. pungens*.

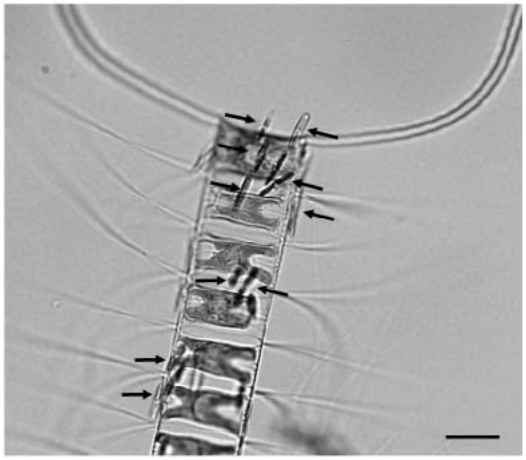


Fig. 23. LM micrograph of *P. americana* (shown by arrows) epiphytic to *Chaetoceros affinis*. Scale bar = 20 μm.

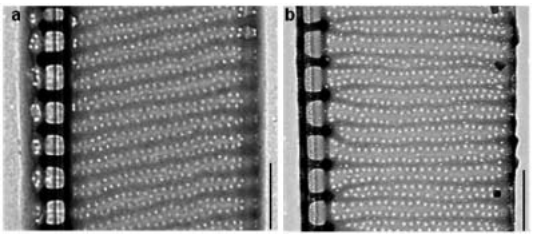


Fig. 24. TEM micrograph of *P. multistriata* (a) regular striations of interstriae, (b) irregular striations of interstriae. Scale bar = 1 μm.

Pseudo - nitzschia pseudodelicatissima **(HASLE) HASLE**

Fig. 22

LUNDHOLM *et al.,* 2003, p. 801. Figs. 1A-G, Table 2. non HASLE *et al.,* 1996, pp. 149–150, Figs. 26–29, 76–81, Tables 1, 2, 8. non SKOV *et al.,* 1999, pp. 13–15, Figs. 10A-G. non CUSACK *et al.,* 2004, pp. 58–63, Pl. VI, Figs. a-f. non PHLIPS *et al.,* 2004, pp. 41–42, Fig. 2. non FEHLING *et al.,* 2006, pp. 95–97, Fig. C. KACZMARSKA *et al.,* 2005, pp. 12–16, Figs. non 35–38 vix 39 non 10–47.

Synonym: *Nitzschia pseudodelicatissima* G.R. HASLE 1976

TAKANO and KUROKI, 1977, p. 44, vix Figs. 21–25.

Nitzschia delicatula HASLE 1965

HASLE, 1965, pp. 37–40, Pl. 4, Figs. 4 –5, Pl. 16, Figs. 8–18, Pl. 17 Figs. 1–16 non 14–16.

Morphological Description:

There was only one broken valve of this cell observed during the sampling period. It is linear in shap. A central interspace is present for this species (not found in micrograph). Transapical axis was at 4.8 μm. Apical axis was not measured since an intact valve was not observed. A single row of poroids was found in striae and poroids in 1 μm is about 4–4.8, divided by a membrane into 2 sectors of inner poroids. Interstriae in 10 μm were calculated to about 30 and fibulae in 10 μm at 16.

Occurrence in Tokyo Bay:

This species was observed on July, 2009 at temperature of 24.5℃ and salinity at 31.

Remarks:

P. pseudodelicatissima is recently emended by LUNDHOLM *et al.* (2003). This species was split in three; *P. pseudodelicatissima, P. caciantha* and *P. calliantha* based on inner poroid features among others. This publication distinguished these three species mainly by sectors dividing each poroid wherein 7–10 in *P. calliantha,* 2 in *P. pseudodelicatissima* and 4–5 or 2–6 (AMATO *et al.,* 2007) in *P. caciantha* with no central perforated area. It was only *P. calliantha* which had a central perforated area surrounded by similar sized sectors inside its poroid. *P. pseudodelicatissima* at 50–140 μm in length, 1.5–3.4 μm in width, 30–46 interstriae and 14–26 fibulae per 10 μm (HASLE, 1965) was emended by LUNDHOLM *et al.* (2003) to 54–87 μm length, 0.9–1.6 μm width, 36–42 and 20–25 in 10 μm respectively.

Identification of *P. pseudodelicatissima* complex before and even after LUNDHOLM *et al.,* 2003 need to be restudied to ascertain proper identification of species. For example, Figs. 1–16 in HASLE (1965) identifications in HASLE *et al.* (1996), SKOV *et al.* (1999) and PHLIPS *et al.* (2004) based on inner poroid features are clearly *P. calliantha.* Figures in, FEHLING *et al.* (2006) CUSACK *et al.* (2004) and KACZMARSKA *et al.* (2005) have four sectors and thus could be *P. caciantha* or *P. mannii* (AMATO and MONTRESOR, 2008). Inner poroid features of figures in TAKANO and KUROKI (1977) are not visible.

Type locality for this genus is in Denmark Strait. Other reports of it occurrence are near Iceland off Costa Nova, Portugal and Napoli, Italy (LUNDHOLM *et al.,* 2003).

Discussion

During the survey conducted in April 2008—August 2009 in Tokyo Bay, 11 species of *Pseudo-nitzschia* which were critically identified using TEM are yielded. This report of 11 species found in one bay is higher compared to reports in other bays *e.g.* eight, in Irish waters (CUSACK *et al.,* 2004) and Argentina (ALMANDOZ *et al.,* 2007) and seven in Bay of Fundy, Canada (KAKZMARSKA *et al.,* 2005). Further studies of *Pseudo-nitzschia* in this area, *e.g.*. ecological in nature should prove productive. Species previously reported to produce domoic Acid are the following: *P. calliantha* (LUNDHOLM *et al.,* 2003; BESIKTEPE *et al.,* 2008), *P. delicatissima* (SMITH *et al.,* 1991), *P. fraudulenta* (RHODES *et al.,* 1998), P. galaxiae (CERINO *et al.,* 2005), *P. multistriata* (SARNO and DAHLMAN, 2000; AMATO *et al.,* 2010), *P. multiseries* (BATES *et al.,*1989; KOTAKI *et al.,* 1996), *P. pungens* (RHODES *et al.,* 1996; TRAINER *et al.,* 1998). Most of toxic *P. pseudodelicatissima* have been found to be *P. calliantha* but there are still some unresolved toxic reports that attribute to this species (e.g. KACZMARSKA *et al.,* 2005).

Five species are reported for the first time here. These are the following: *P. americana, P.*

brasiliana, P. caciantha, P. calliantha and *P. galaxiae*. Morphometric characterizations of local populations and baseline data are provided herein for future investigations of toxic or newly discovered taxa. Methods are currently devised to aid species enumeration by LM to further ecological studies of *Pseudo-nitzschia* in the area.

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