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Species of the planktonic diatom genus *Pseudo-nitzschia* of the Pacific coasts of Mexico

David U. Hernández-Becerril
Laboratorio de Ecología de Pesquerías, Instituto de Ciencias del Mar y Limnología, UNAM, Apdo. postal 70-305, México, D.F. 04510 Mexico

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Abstract

Species of the diatom genus *Pseudo-nitzschia* are common in the marine phytoplankton world-wide. Some species of this genus have been proved to be source of domoic acid (DA), a powerful toxin causing Amnesic Shellfish Poisoning (ASP) in humans and probably mass mortality in sea birds and mammals. Net plankton samples obtained during several cruises and seasons from the Pacific coasts of Mexico: western coasts of Baja California, Gulf of California, coasts of the tropical Pacific of Mexico (including the Gulf of Tehuantepec), were analyzed to study the species of the diatom genus *Pseudo-nitzschia*. Four species (*P. australis*, a presumed toxic species, *P. fraudulenta*, *P. lineola*, *P. pungens*) and one former species of the genus, *Nitzschia americana* were recorded and studied by light and electron microscopy. The most common species was *P. pungens*, widely distributed along the Pacific coasts of Mexico. All other species appeared occasionally and in low relative abundances. The probable misidentification of *P. australis* as *P. seriata* is discussed, as well as the presence of another potentially toxic species, *P. delicatissima*, in the Gulf of California. No case of toxicity (ASP) has been fully documented and therefore related to toxic *Pseudo-nitzschia* species in the Gulf of California.

Introduction

The diatom genus *Pseudo-nitzschia* Peragallo was formally recently reinstated (Hasle, 1993, 1994) to accomodate former *Nitzschia* species, which are marine and planktonic forms, delicate and weakly silicified cells, usually forming chains connected by the gradually attenuated apices of the fusiform, elongate or lanceolate valves (Hasle, 1994; Hasle & Syvertsen, 1996). Therefore, as expressed by Hasle (1994), the main argument to separate the genus from *Nitzschia* is the combination of morphologic and ecological features.

Species of the genus *Pseudo-nitzschia* are distributed worldwide (Takano & Kuroki, 1977; Rivera, 1985; Hallegraeff, 1994; Hasle et al., 1996), sometimes occurring in considerable densities. Very few species have a more restricted distribution (e.g. *P. seriata* Cleve, Hasle, 1972). Recent interest of studying species of this genus has grown, for at least five species of *Pseudo-nitzschia* (Lundholm et al., 1994) are now recognized to produce domoic acid (DA), a powerful toxin which causes the Amnesic Shellfish Poisoning (ASP) in humans. This toxin has been found in clams and mussels in several places such as Prince Edward Island (Subba Rao et al., 1988), Bay of Fundy, Canada (Martin et al., 1990), California (Buck et al., 1992; Lange et al, 1994) and western Washington, U.S.A. (Horner & Postel, 1993). Recent reports show that potentially dangerous species have been also found in European waters (Lundholm et al., 1994; Míguez et al., 1996; Vrieling et al., 1996a, 1996b). Unofficial reports of poisoning and mass mortality of sea birds and mammals in coasts of the Gulf of California are suspected of having been caused by blooms of domoic acid producing *Pseudo-nitzschia* species, but not sufficient evidence has been provided.

Traditional positive identification at species level is difficult and requires in most cases combined ob-

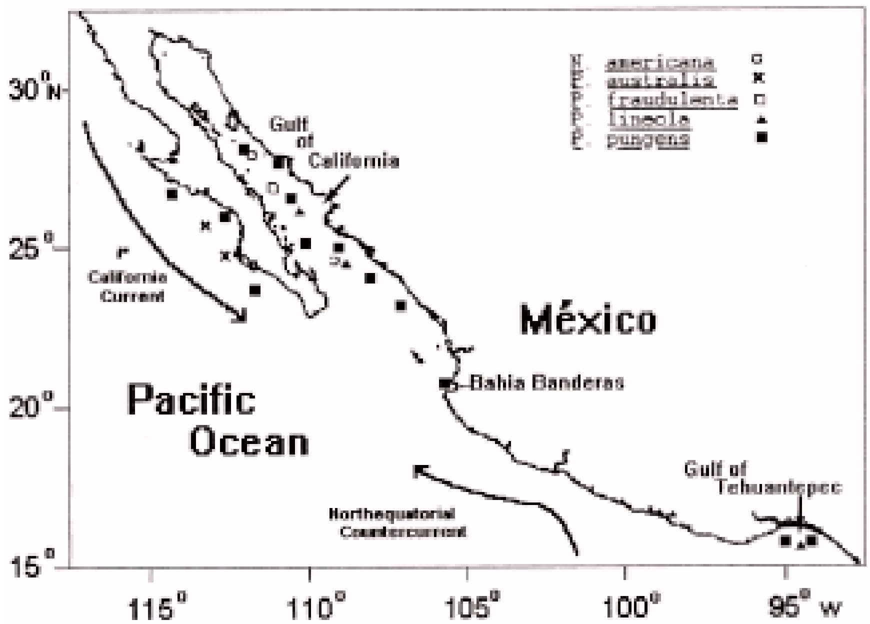


Figure 1. Map of the Mexican Pacific showing points where species of *Pseudo-nitzschia* and *Nitzschia americana* were found.

servations by light and electron microscopy (mainly TEM). Useful taxonomic characters include: valve outline, length (proportion) of overlap of cell ends, width of valves, density of interstriae and fibulae, and presence of interspace (Hasle & Syvertsen, 1996). However, new molecular techniques (mainly using large-subunit ribosomal RNA-targeted oligonucleotides) have been recently developed to identify suspected toxic *Pseudo-nitzschia* species in environmental samples (e.g. Scholin et al., 1996; Vrieling et al., 1996). These methods seem to be promising for routine and rapid enumeration of *Pseudo-nitzschia* cells.

The purpose of this work is contribute to the knowledge and to provide a guide of the genus in a tropical-subtropical region, using collection of samples from the Pacific coasts of Mexico and taking into account the current taxonomic criteria in identifying and describing species of *Pseudo-nitzschia*.

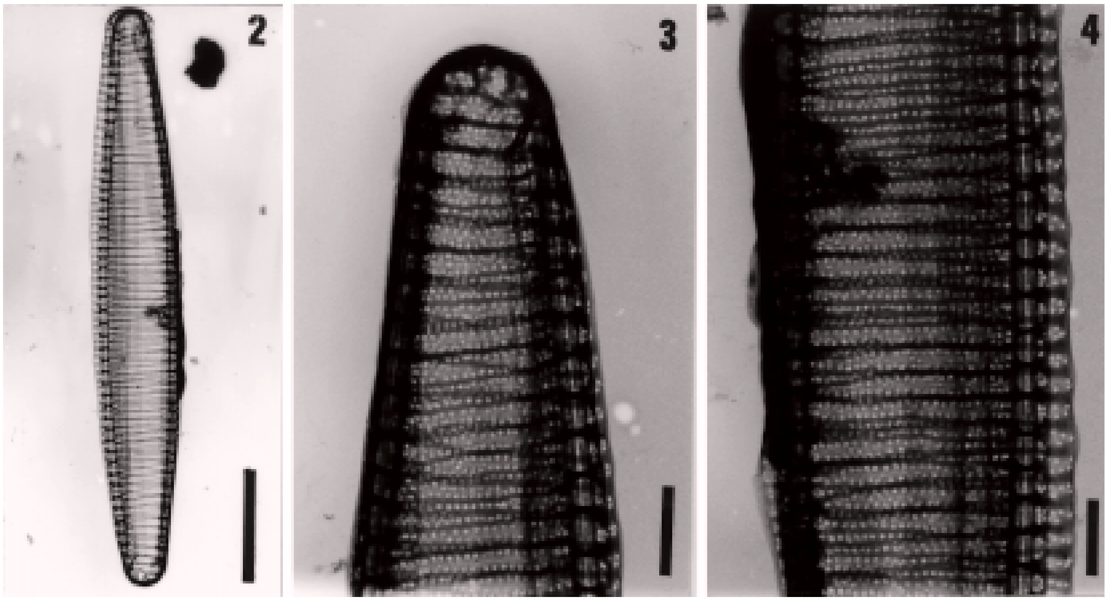
Material and methods

Material studied was collected by net (24, 50, 54 μm mesh) from several locations along the coasts of the Mexican Pacific: Gulf of California, western coasts of Baja California and the Gulf of Tehuantepec, during various cruises and different seasons (1984–1989) (Figure 1). In general, the western coasts off Baja California are considered a temperate region (with strong influence by the California Current), whereas the Gulf of California is a subtropical area and the waters of the Gulf of Tehuantepec and the rest of the Mexican Pacific are tropical-subtropical (with an important flow by the Northequatorial Countercurrent) (Figure 1). All samples were preserved in formalin 4%.

Observations of *Pseudo-nitzschia* species were carried out using rinsed and cleaned material, by light (LM) and electron microscopy (both scanning, SEM, and transmission, TEM). Fresh or permanent slides were used for LM (Olympus CH, phase contrast, and Reichert Diastar, phase contrast, with attached camera, objetives 40X and 100X), and conventional methods (already described earlier, Hernández-Becerril & Tapia Peña, 1995) were followed for SEM and TEM (Phillips 501, at 10–12 kv, and JEOL 1200 EX, respectively). Specific taxonomic characters were used for identification, as mentioned earlier (Hasle & Syvertsen, 1996) and specific terminology was used (Hasle et al., 1996). Typical measurements were made on the available number of specimens on the LM: apical axis, transapical axis, density of striae and fibulae in 10 μm.

Results and observations

Four species of *Pseudo-nitzschia* were found in the samples: *P. australis*, *P. fraudulenta*, *P. lineola* and



Figures 2–4. Nitzschia americana, TEM. *Figure 2.* Complete frustule (two valves). *Figure 3.* Detail of one end of the valve, there are three rows of poroids between two interstriae. *Figure 4.* Middle part of the valve showing interstriae, poroids and coarse fibulae (right hand). Scale bars = 5 μm (Figure 2), = 1 μm (Figures 3, 4).

Table 1. Typical measurements of the species of *Pseudo-nitzschia* and *Nitzschia americana*. The numbers in brackets are the measurements given by Hasle & Syversten (1996). A = Absent, P = Present; Cosmop = Cosmopolitan; Subt-Temp = Subtropical to temperate.

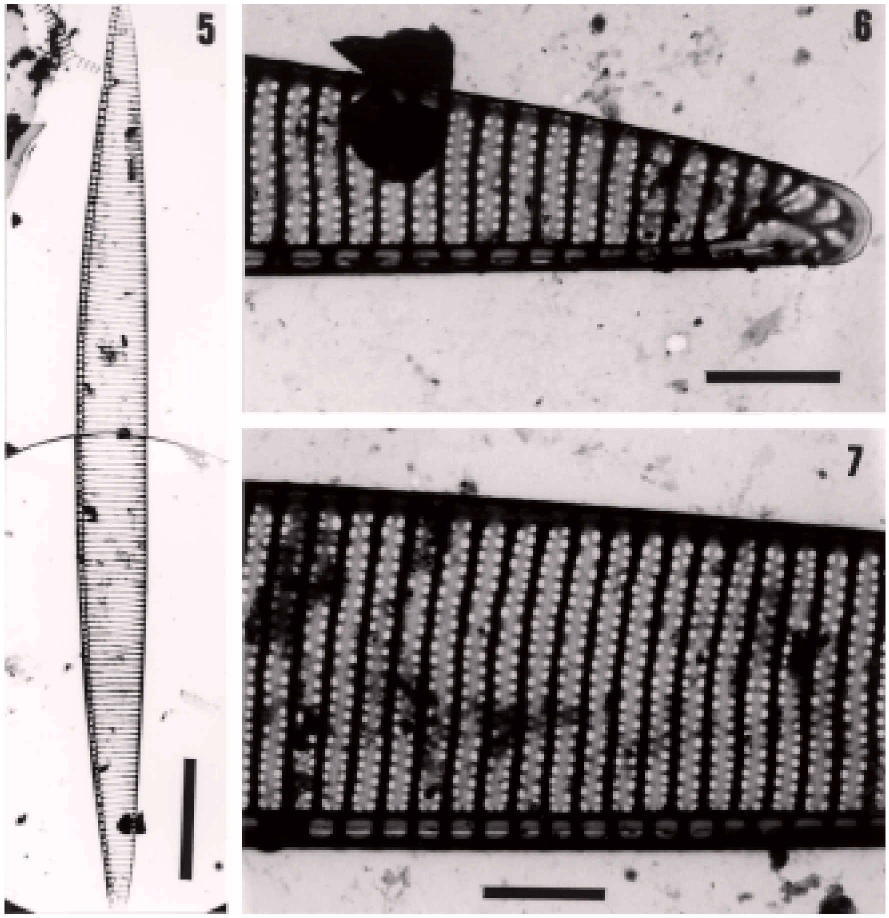
Species	Lenght (μm)	Width (μm)	Central nodule	No.Interst/ 10 μm	No. Fib./ 10 μm	Distr.
N. americana	18–26	3.5–4	A	28–30	20–21	Cosmop
P. australis	68–73 (75–144)	5–6 (6–8)	A	15–16	14–15	Subt–Temp
P. fraudulenta	63–99	3–4.5	P	14–15	15–17	Cosmop
P. lineola	86–111	2–2.5	P	24–26	12–14	Cosmop
P. pungens	94–109	3–4	A	10–12	10–11	Cosmop

P. pungens, and one until recently considered a true *Pseudo-nitzschia* species: *Nitzschia americana*. Measurements of the five species are given in Table 1.

Systematic account and descriptions

Nitzschia americana Hasle, Figures 2–4
Hasle, 1964, p. 41, pl. 1, Figure 4, pl. 14, Figures 13–19, pl. 15, Figures 7-10.
Synonym: *Pseudo-nitzschia americana* (Hasle) Fryxell *in* Hasle.
Hasle, 1993, p. 318; Hallegraeff, 1994, p. 407, Figures 8a, b.
Species apparently solitary. The valves are linear, with rounded ends: 18–26 μm length, 3.5–4 μm width. Transapical interstriae parallel, 28–30 interstriae in 10 μm, having three (sometimes two) rows of poroids (striae) in between two interstriae. Fibulae are very conspicuous, with a lower density than that of the interstriae: 20–21 fibulae in 10 μm. Central interspace (central nodule) absent.

Pseudo-nitzschia australis Frenguelli, Figures 5–7
Frenguelli, 1939, p. 217, pl. 2, Figure 13; Villac et al., 1993, p. 217, Figures 3–5.
Synonym: *Nitzschia pseudoseriata* Hasle.
Hasle, 1965, p. 11, pl. 4, Figures 3, 4, pl. 5, Figures 1–6, pl. 6, Figure 1.
Most species of the genus form chains. The valves are fusiform, with ends curved or slightly rostrate, one margin of the valve (the one having the raphe)



Figures 5–7. Pseudo-nitzschia australis, TEM. *Figure 5.* A complete valve. *Figure 6.* Detail of one curved end of the valve. *Figure 7.* Middle part of a valve with interstriae, two rows of poroids in between two interstriae and fibulae. Scale bars = 10 μm (Figure 5), = 2 μm (Figures 6, 7).

curved, whereas the opposite is more straight: 68–73 μm length, 5–6 μm width. Density of interstriae (15–16 in 10 μm) and fibulae (14–15 in 10 μm) about the same. Two rows of poroids (striae) in between two interstriae. No central interspace (central nodule) present.

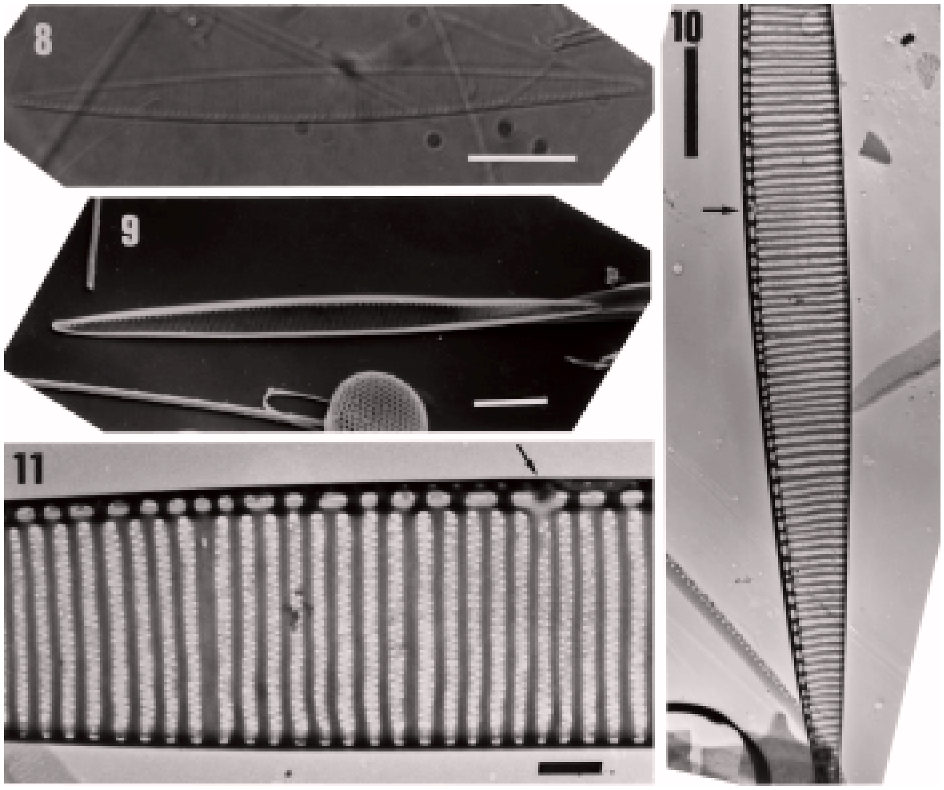
This species has been known as a domoic acid-producing diatom in other areas. Measurements (length and width) given here are slightly lower than those provided in the literature (especially in Hasle & Syvertsen, 1996) (see Table 1).

Pseudo-nitzschia fraudulenta (Cleve) Hasle, Figures 8–11

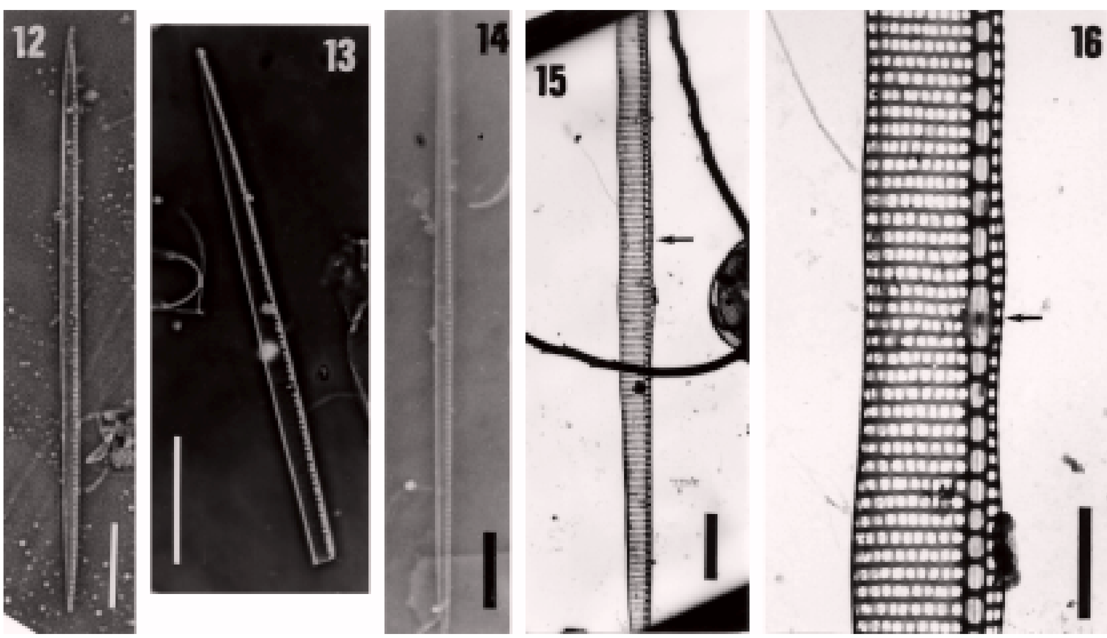
Hasle, 1993, p. 318; Hallegraeff, 1994, p. 398, Figures 2a–k; Hasle et al, 1996, p. 144, Figures 17–19, 57–61.
Synonym: *Nitzschia fraudulenta* Cleve.
Hasle, 1965, p. 15, pl. 1, Figures 2, 3, pl. 6, Figures 5–10; Rivera, 1985, p. 15, Figures 39–51.
Species also forming chains. Valves fusiform, weakly silicified, ends slightly pointed: 63–99 μm length, 3–4.5 μm width. Densities of interstriae (14–15 in 10 μm) and fibulae (15–17 in 10 μm) are about the same. Striae consisting of two rows of poroids in between two interstriae. Central interspace with central nodule present.

Pseudo-nitzschia lineola (Cleve) Hasle, Figures 12–16

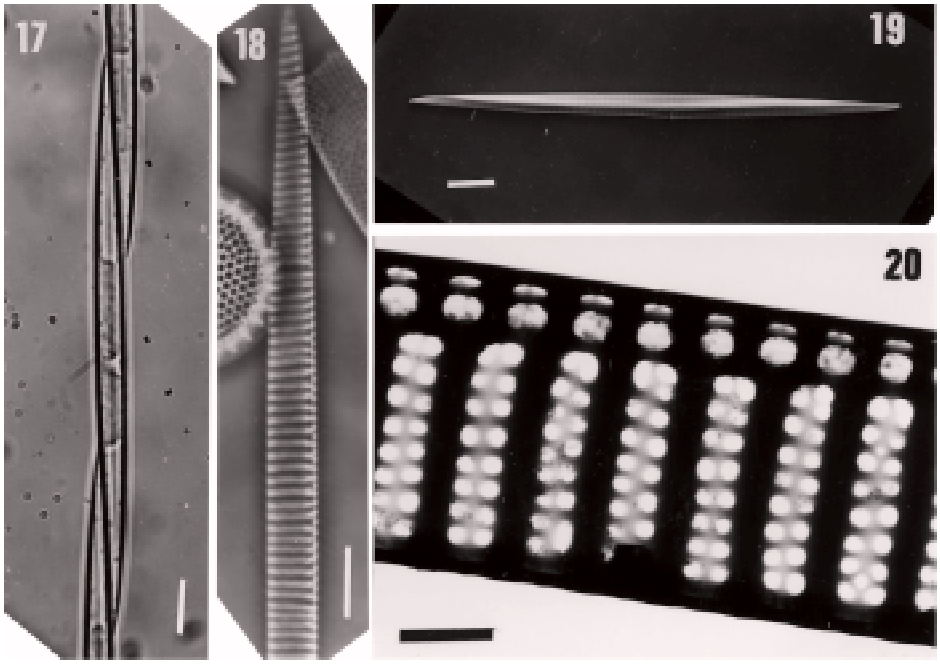
Hasle, 1993, p. 319; Hallegraeff, 1994, p. 407, Figures 7a–e.
Synonym: *Nitzschia lineola* Cleve.
Simonsen, 1974, p. 53, pl. 39, Figures 4–6.
Cells usually occuring in chains. Valves linear to lanceolate, with pointed ends: 86–111 μm length, 2–



Figures 8–11. Pseudo-nitzschia fraudulenta. *Figure 8.* One single valve, LM. *Figure 9.* One valve still connected in a chain, SEM. *Figure 10.* Part of a valve, note the central interspace (arrow), TEM. *Figure 11.* Detail of the middle part of a valve exhibiting two rows of poroids in between two interstriae, the central interspace arrowed, TEM. Scale bars = 10 μm (Figures 8, 9), = 5 μm (Figure 10), = 1 μm (Figure 11).



Figures 12–16. Pseudo-nitzschia lineola. *Figure 12.* A complete valve, LM. *Figure 13.* One valve partially broken, LM. *Figure 14.* Another complete valve, LM. *Figure 15.* Part of a valve showing the central interspace (arrow), TEM. *Figure 16.* Detail of the middle part of a valve, note one row of rather large poroids in between two interstriae, the central interspace arrowed, TEM. Scale bars = 10 μm (Figures 12–14), = 2 μm (Figures 15, 16).



Figures 17–20. Pseudo-nitzschia pungens. *Figure 17.* Part of a chain with the overlap (about one-third of the length of cells) of cells, LM. *Figure 18.* Part of a linear valve, LM. *Figure 19.* One complete frustule, SEM. *Figure 20.* Detail of a valve, showing the interstriae, poroids (two rows in between two interstriae) and fibulae, TEM. Scale bars = 10 μm (Figures 17, 19), = 5 μm (Figure 18), = 1 μm (Figure 20).

2.5 μm width. Fibulae are coarse, interstriae having striae of one row of poroids in between two interstriae. Density of interstriae (24–26 in 10 μm) nearly the double of those of the fibulae (12–14 in 10 μm). A central interspace with its central nodule is present.

Pseudo-nitzschia pungens (Grunow ex. Cleve) Hasle, Figures 17–20

Hasle, 1993, p. 319; Hallegraeff, 1994, p. 401, Figures 3a–i; Hasle et al., 1996, p. 138, Figures 3–6, 30–37.

Synonym: *Nitzschia pungens* Grunow *ex*. Cleve Cupp, 1943, p. 202, Figure 156; Rivera, 1985, p. 12, Figures 1–13.

Cells arranged in chains, sometimes very long (13 cells), cells overlap in chains close to one-third of cell length. Valves linear to lanceolate with pointed ends, strongly silicified: 94–109 μm length, 3–4 μm width. Fibulae and interstriae of about the same densities: 10–11 fibulae in 10 μm, 10–12 interstriae in 10 μm. There are two rows of poroids (striae) in between two interstriae. Absence of central interspace (central nodule). Species non-toxic.

Discussion

Pseudo-nitzschia species are rather common in the plankton of the Mexican Pacific, although just four species were found in the samples observed. Only one species of the encountered is known to produce Domoic Acid: *Pseudo-nitzschia australis*. This species may be easily misidentified as *P. seriata*, as both are superficially similar in morphology (e.g. Moreno et al., 1996), although detailed observations by TEM show the presence of more than two rows of poroids in between two interstriae in *P. seriata*, whereas *P. australis* has only two (Hasle et al., 1996). Moreover, it has been shown that the distribution of *P. seriata* is restricted to higher latitudes in the North Hemisphere (Hasle, 1972). *P. australis* was present in very few samples (just in coasts off Baja California) and low relative abundances.

The most widespread and relatively abundant species was *P. pungens*, present in many samples along the coasts of Baja California, the Gulf of California and the Gulf of Tehuantepec. *P. multiseries*, a Domoic Acid-producing species, closely related to *P. pungens* was not encountered in the samples analyzed; it has been reported the distribution of *P. multiseries* in the world (Fryxell et al., 1990), being present in the Pacific Ocean, at a higher latitude than 35°. Other presumed toxic species, *P. delicatissima* (Cleve) Heiden has been reported to be present in the Gulf of California (Moreno et al., 1996). Further surveys may indicate the presence of more species of the genus.

Other species found in this study: *Nitzschia americana* and *Pseudo-nitzschia fraudulenta* may be considered as rather uncommon forms, although their distribution is more tropical-subtropical. *P. lineola* can be regarded as cosmopolitan. Most of the species treated here had measurements comparable with those of the literature, except *P. australis*, which is slightly shorter and narrower in this study (see Table 1), although its density of both interstriae and fibulae is similar to current findings (e.g. Hasle & Syvertsen, 1996).

It is not possible to relate the events of poisoning and mass mortality of sea birds and mammals in the Gulf of California to the presence of toxic *Pseudo-nitzschia* species, as no plankton samples were taken when the events occurred. Very weak evidence shows nearly undammaged sardines possibly containing *P. australis*, inside some dolphins' stomachs; this species, as shown here, is present in the area, although cells numbers data are not available. Monitoring of areas where toxic species may potentially occur is highly reccomended.

Also, as the samples used in this study were collected by net only, it is reccomended to count numbers of cells, using adequate sampling (e.g. bottle samples) and method (e.g. inverted microscope), or fitting the mentioned molecular techniques (Scholin et al., 1996; Vrieling et al., 1996) to identify and enumerate cells of particular species belonging to the genus.

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