ICES Identification Leaflets for Plankton

Fiches d'Identification du Plancton

LEAFLET NO. 185

Potentially Toxic Phytoplankton
4. The diatom genus *Pseudo-nitzschia*
(Diatomophyceae/Bacillariophyceae)

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Diatomophyceae/Bacillariophyceae

Introduction

Members of the algal class Diatomophyceae Rabenhorst, 1864/Bacillariophyceae Haeckel, 1878 (diatoms) are unicellular or colony forming. The cells appear yellow, yellowish green, golden brown, or dark brown. Cell morphology and number of chloroplasts are highly variable. The cells contain the pigments chlorophyll *a*, c_2 (or sometimes c_1 or c_3), β-carotene, fucoxanthin, diatoxanthin, and diadinoxanthin. The cell wall, or frustule, is mainly composed of silica and is divided into an upper part (epitheca) and a lower part (hypotheca). The structure and ornamentation of the frustule form the basis for classification of diatoms. The classification in this plankton leaflet refers to Christensen (1993)/ Simonsen (1979). Terminology follows Anonymous (1975) and Ross *et al.* (1979).

Systematically, the diatoms are divided into two orders. The centric diatoms, Eupodiscales Bessey, 1907/ Centrales Karsten, 1928, often have a circular outline, and the structure of the valve is radially symmetrical. The pennate diatoms, Bacillariales Hendey, 1937/ Pennales Karsten, 1928 are elongate or wedge-shaped, and the valves are bilaterally symmetrical.

To date, all planktonic diatoms that have been confirmed toxic are marine and pennate and belong to the family Bacillariaceae Ehrenberg, 1831 and the genus *Pseudo-nitzschia* H. Peragallo, 1900 (Fig. 1).

Toxic incidents: Toxic events associated with diatoms are relatively recent. The first incident appeared in late autumn 1987, when numerous persons were intoxicated after eating blue mussels (*Mytilus edulis*) cultivated in Cardigan Bay, Prince Edward Island, Canada (Bates *et al.*, 1989). The causative agent was identified as the neurotoxin domoic acid (Wright *et al.*, 1989). Three persons died and there were at least 104 other incidents of intoxication (Perl *et al.*, 1990; Teitelbaum *et al.*, 1990; Todd, 1993).

At the time of the poisoning, a plankton survey revealed that the pennate diatom *Pseudo-nitzschia multiseries* (Hasle) Hasle (= *P. pungens* f. *multiseries*), contributed 98–100% of the total cell number in the plankton. The concentration of cells reached 15×10^6 cells per litre and the concentration of domoic acid 1.7 pg per cell (Bates *et al.*, 1989). Mussels harvested in the area contained numerous frustules of *P. multiseries* in the digestive system (Wright *et al.*, 1989), and toxin from the diatoms accumulated in the mussel tissue. The concentration of domoic acid in the mussels was up to 1500 µg per g wet-weight in the digestive glands. No other major source of domoic acid was present in the area (Bates *et al.*, 1989).

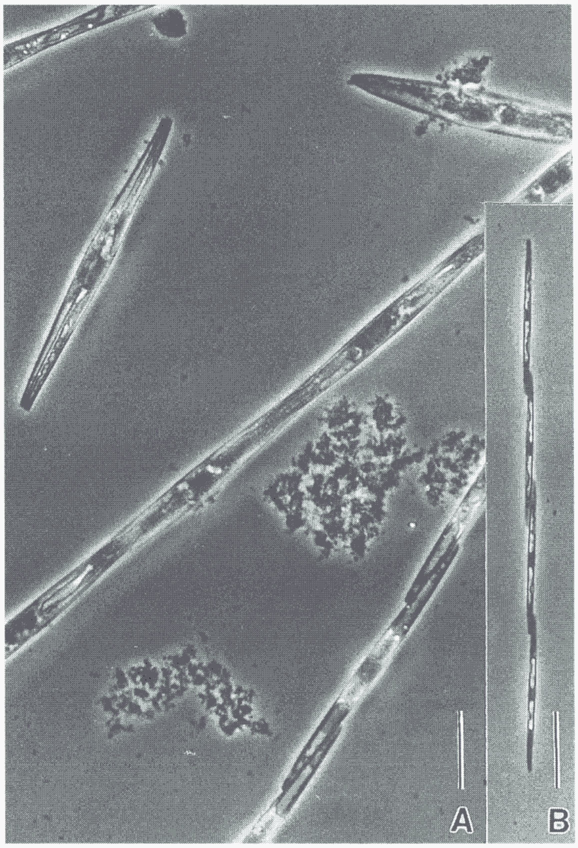

Figure 1A–B. Colonies. A: *P. seriata*, B: *P. delicatissima*. Phase contrast, Danish material (culture). Scale bars: 20 µm.

Another producer of domoic acid, *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle, was discovered in Passamaquoddy Bay, New Brunswick, Canada, in the autumn of 1988. Domoic acid had accumulated in both soft-shell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*). In late September, *P. pseudodelicatissima* constituted 99% of the phytoplankton, and the maximum concentration of cells at the surface was 1.2×10^6 per litre (Gilgan *et al.*, 1990; Martin *et al.*, 1990; Haya *et al.*, 1991).

A third species of *Pseudo-nitzschia*, *P. australis* Frenguelli, was found to produce domoic acid in September 1991 in Monterey Bay, California, USA. Intoxication from this species resulted in the death of more than a hundred Brandts cormorants (*Phalacrocorax penicillatus*) and brown pelicans (*Pelecanus occidentalis*), which fed on anchovies (*Engraulis mordax*) (Buck *et al.*, 1992; Roelke *et al.*, 1992; Work *et al.*, 1993a, b). Remnants of the diatoms and high concentrations of domoic acid were found in the viscera and muscles of anchovies and in the stomachs of the birds (Fritz *et al.*, 1992; Work *et al.*, 1993a, b). The maximum abundance of *P. australis* in Monterey Bay in November 1991 was in excess of 6.7 × 10^5 cells per litre, representing up to 87% of the total biomass of autotrophs in the phytoplankton (Buck *et al.*, 1992). The incident in California illustrates the path and possible effects of a toxic diatom bloom in the food chain.

Off the coasts of Oregon and Washington, and the west coast of Alaska, domoic acid was reported in razor clams (*Siliqua patula*) at levels as high as 160 µg per g in edible parts (Horner *et al.*, 1993), and in Dungeness crabs (*Cancer magister*) in November and December 1991 (Wright *et al.*, 1992). The causative organism has never been definitively identified (Wright, 1992; Cembella, 1993), but *P. australis* is a likely candidate (Horner and Postel, 1993; Taylor, 1993). While the toxin accumulated offshore in Dungeness crabs and razor clams, blue mussels and oysters near the coast did not become toxic (Horner and Postel, 1993), stressing the necessity of choosing monitoring organisms carefully. At least 24 people suffered from ASP and 13 had mild neurological problems (Wright *et al.*, 1992; Horner and Postel, 1993; Todd, 1993). Samples of canned clams confirmed the presence of domoic acid dating back to 1985. In the waters off Vancouver Island, British Columbia, domoic acid was found in mussels and crabs from 1992 to 1994 (Forbes and Chiang, 1994).

There has been an increasing number of reports of domoic acid in various shellfish during the last few years, caused either by *P. multiseries*, e.g., in Japan (Kotaki *et al.*, 1996), and The Netherlands (Vrieling *et al.*, 1996) or *P. australis*, e.g., in New Zealand (Rhodes *et al.*, 1996) and Portugal (Sampayo *et al.*, 1997). There have also been incidents around the world where domoic acid was detected but the causative organism was not identified, e.g., the East Coast of the United States (Maine, Massachusetts) in 1988 (Addison and Stewart, 1989), New Zealand in 1993 (Chang *et al.*, 1993), Australia in 1993 (Hallegraeff, 1994), and Mexico in 1996 (Sierra Beltrán *et al.*, 1997).

Domoic acid and Amnesic Shellfish Poisoning (ASP): Domoic acid is a naturally occurring water-soluble heat-stable amino acid (Fig. 2A) (Wright *et al.*, 1989; Pocklington *et al.*, 1990). In addition to diatoms, domoic acid has been isolated from three red algae of the family Rhodomelaceae, *Alsidium corallinum* C. Agardh (Impellizzeri *et al.*, 1975), *Chondria armata* Okamura (Takemoto and Daigo 1958), and *Chondria baileyana* Montagne (Laycock *et al.*, 1989; Wright *et al.*, 1989). The former two species occur in warmer waters (Japan and the Mediterranean), the latter in Canada (Todd, 1990). The Japanese name for *Chondria armata* is ‘domoi’ – hence the name domoic acid (Daigo, 1959).

Intoxication with domoic acid has been termed Amnesic Shellfish Poisoning (ASP). Within 24 hours of eating poisoned mussels, victims develop gastrointestinal symptoms such as nausea, vomiting, anorexia, diarrhoea, abdominal cramps and gastric bleeding. This may be followed by neurological symptoms like confusion, loss of memory, disorientation, seizures, and coma (Perl *et al.*, 1990; Todd, 1993). The main persistent symptom is loss of memory, and after the incident in 1987 some patients were unable to carry out even simple tasks. There was a close connection between age and memory loss: those most likely to develop diarrhoea were under 40 years of age, while those with loss of memory were over 50. The most seriously ill patients had still not recovered 5 years after the incident (Perl *et al.*, 1990; Todd, 1993). Twelve people were hospitalized in intensive care, and three of those died within 3 weeks (Perl *et al.*, 1990). There was a close correlation between the amount of domoic acid ingested and the severity of the symptoms. Consumption of less than 75 mg of domoic acid caused mild intoxications, while

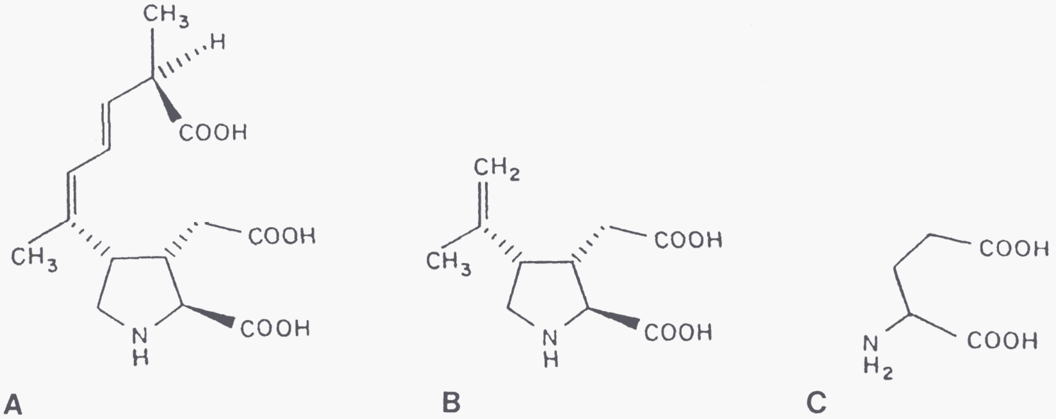

Figure 2A–C. A: domoic acid, B: kainate, C: glutamate.

115–290 mg caused neurological problems (Todd, 1990). Domoic acid is known to be an extremely strong insecticide (Hashimoto, 1979; Maeda *et al*., 1984, 1986). It has been used in smaller doses (20 mg) as an anthelmitic in Japan without observable side effects (Daigo, 1959; Wright *et al*., 1989).

Domoic acid has an effect similar to thc structurally related neurotoxic amino acid kainate (Fig. 2B). Both are agonists (competitors) to glutamate (a neurotransmitter in the central nervous system) (Fig. 2C). They activate special receptors, kainate receptors, in the hippocampus (the part of the brain concerned with memory) (Debonnel *et al*., 1989). When domoic acid binds to the kainate receptors, the neurons are continuously stimulated, overexcited and eventually destroyed. This leads to brain lesions (Quilliam and Wright, 1989). Extensive damage to the hippocampus and limited injuries of the thalamus and parts of the forebrain were found by autopsy (Teitelbaum *et al*., 1990) and confirmed by experiments with rats and monkeys (Debonnel *et al*., 1989; Iverson *et al*., 1989; Stewart *et al*., 1990; Todd, 1990; Tryphonas *et al*., 1990a, b). In animal experiments, domoic acid was 30–100 times more potent than glutamate (Biscoe *et al*., 1975; Debonnel *et al*., 1989; Olney, 1990). Subtoxic doses of domoic acid together with the excitatory amino acids aspartate and glutamate normally present in mussels may have synergistic effects leading to toxicity (Tasker *et al*., 1991; Novelli *et al*., 1992). No chemical compound is known which can block the receptors selectively, and there is presently no antidote for ASP, although kyurenic acid has been reported by Pinsky *et al*. (1989, 1990) to have a protecting effect on mice.

Acceptance limits: In July 1988 the acceptance limit for domoic acid in Canadian mussels was set to 20 μg per g wct weight (Gilgan *et al*., 1990; Todd, 1990). The concentration of domoic acid in shellfish during the incident in 1987 in Canada was 300–1000 μg per g and it is believed that individuals may have ingested 1–2 mg domoic acid per kg body weight (Wright and Quilliam, 1995). The effect of chronic low ingestion of domoic acid is unknown. The detection limit using HPLC (High Performance Liquid Chromatography) is about 0.2 μg per g (Gilgan *et al*., 1990). More sensitive methods are necessary to detect domoic acid in a water sample. One is the highly sensitive 9-fluorenyl-methoxycarbonyl chloride (FMOC) pre-column derivation method for amino acids followed by reversed phase HPLC with fluorescence detection. It has a detection limit of 15 pg per ml (Pocklington *et al*., 1990). Another technique is the capillary electrophoresis (CE), which is simple, rapid, and has a detection limit similar to the HPLC methods (Wright and Quilliam, 1995).

A staining method for detection of domoic acid, using vanillin, has been developed in Germany (Dallinga-Hannemann *et al*., 1995). It is suitable for routine monitoring. An easy-to-use thin-layer chromatographic (TLC) separation has been developed as a fast and cheap method for identifying harmful concentrations of domoic acid in extracts of shellfish meat (Dallinga-Hannemann *et al*., 1997).

Production of domoic acid: In batch culture, *P. multiseries* and *P. seriata* produce domoic acid mainly in the stationary growth phase (Bates *et al*., 1989; Subba Rao *et al*., 1990; Lundholm *et al*., 1994). During the stationary phase, the toxin concentration per cell rises to a certain level and then slowly decreases. In the stationary phase, domoic acid leaks out of the cell and the external concentration may reach higher levels than in the cells (Bates *et al*., 1989). Production of domoic acid in batch culture requires that: (1) cell divisions have ceased (e.g., by Si limitation), (2) extracellular N is available (e.g., as nitrate or ammonium), and (3) sufficient light is present (Bates *et al*., 1991).

Experiments with *P. multiseries* in silicate-limited continuous cultures have demonstrated an inverse correlation between division rates and the production of domoic acid. This implies that toxin production is not necessarily associated with complete cessation of cell division. When the silicate limitation becomes severe, domoic acid production is enhanced while cell division declines (Bates *et al*., 1996; Pan *et al*., 1996b). The results may explain the observations from the field. In nature, periods of nutrient limitations are often followed by pulses of silicate from land run-off. The subsequent population growth and suspension of domoic acid production until further depletion of silicate may account for the observed persistence of blooms for several months (Bates *et al*., 1996; Pan *et al*., 1996a, b). The results also support the contention that domoic acid is a secondary metabolite associated with physiological stress (Bates *et al*., 1996; Pan *et al*., 1996a). Furthermore, experiments with phosphate-limited continuous cultures of *P. multiseries* demonstrate enhanced domoic acid production (Pan *et al*., 1996c).

When photosynthesis is blocked chemically or by darkness, production of domoic acid ceases (Bates *et al*., 1991; Bates *et al*., 1993c).

The production rate of domoic acid in *P. multiseries* has been shown to increase with temperature from 5°C to at least 25°C, possibly because of temperature stress (Lewis *et al*., 1993). Cultures of *P. seriata* (P. T. Cleve) H. Peragallo, however, produced more domoic acid at 4°C than at 15°C (Lundholm *et al*., 1994).

Non-axenic cultures of *P. multiseries* produce domoic acid in the same amounts or up to 20 times more than axenic cultures, indicating that bacteria are not required for production of domoic acid but, if present, the domoic acid production may be enhanced (Douglas and

Bates, 1992; Bates *et al.*, 1993a; Douglas *et al.*, 1993). Laflamme and Bates (1994) have shown that an increase in the number of bacteria resulted in increased production of domoic acid.

Addition of purified domoic acid to non-toxic mussel extract has the same neurotoxic effect on rat cerebellar neurons as the same amount of domoic acid in intoxicated mussels. This implies that domoic acid is the only causative substance of ASP in the cell (Novelli *et al.*, 1992). A biosynthetic pathway for domoic acid has been proposed by Douglas *et al.* (1992).

Another pennate diatom, *Amphora coffeaeformis* (Agardh) Kützing (Fig. 3), has been connected with domoic acid. After the food-poisoning event in Canada in 1987, cultures of *Amphora coffeaeformis* were established and one of these was found to produce domoic acid (Maranda *et al.*, 1990), while others appeared to be non-toxic (Bates *et al.*, 1989).

The species so far implicated in toxic incidents are *P. multiseries*, *P. pseudodelicatissima*, and *P. australis*. In addition, *P. delicatissima* (P.T. Cleve) Heiden, *P. seriata*, and *P. pungens* (Grunow *ex* P.T. Cleve) Hasle have been shown to produce domoic acid in culture (Smith *et al.*, 1990b, 1991; Lundholm *et al.*, 1994; Rhodes *et al.*, 1997; Trainer *et al.*, 1997). The six species are morphologically very similar and undoubtedly closely related. It appears likely that other species of *Pseudo-nitzschia* may also be toxin producers, e.g., *P. turgidula* (Hustedt) Hasle isolated from a bloom in New Zealand. The species identification is uncertain, however (L. Rhodes pers. comm.). In all six verified species, both toxic and non-toxic isolates are known (Bates *et al.*, 1989; Martin *et al.*, 1990; Smith *et al.*, 1991; Garrison *et al.*, 1992; Villac *et al.*, 1993a; Villareal *et al.*, 1994; Lundholm *et al.*, 1994; Rhodes *et al.*, 1997; Trainer *et al.*, 1997), and toxin production often varies between isolates (Villac *et al.*, 1993a; Villareal *et al.*, 1994). Investigations using rRNA sequencing identified distinctly different strains of *P. australis*, *P. pungens*, and *P. multiseries* (Douglas *et al.*, 1994; Scholin *et al.*, 1994). Furthermore, several clones of *P. pseudodelicatissima* were identified using isozyme analysis (Skov *et al.*, 1997). Whether this means that toxic and non-toxic strains occur together is not known.

Various methods are available for identifying *Pseudo-nitzschia* species. One method is based on polyclonal antibodies directed against *Pseudo-nitzschia* cells, which are visualized by immunofluorescence. It has been developed against *P. multiseries* and *P. pungens* (Ross and Bates, 1996). Another method based on species-specific ribosomal RNA targeted oligonucleotide probes has been developed against eight different species and has been used on both cultures and field samples. The use of these probes is promising and the means by which field samples could be screened rapidly (Miller and Scholin, 1996; Scholin *et al.*, 1996a, b).

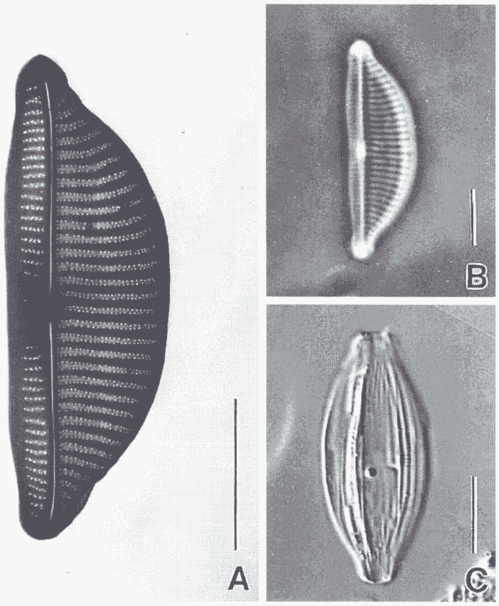

Figure 3A–C. *Amphora coffeaeformis*. A: TEM, B: phase contrast, C: DIC, micrograph kindly provided by A. Witkovski. Danish material (culture). Scale bars: 5 μm.

Description of the genus

Pseudo-nitzschia H. Peragallo, 1900

Lectotype: Pseudo-nitzschia seriata (P.T. Cleve) H. Peragallo, 1900 (according to the International Code of Botanical Nomenclature the name should be spelled with a hyphen; Art. 60.9. Note 2., ICBN 1994, Greuter *et al.*, Koeltz, Königstein, 389 pp.).

Based in part on morphological investigations by Mann (1986), Hasle (1993, 1994) reinstalled *Pseudo-nitzschia* as a separate genus rather than as a section of *Nitzschia* Hassall. This was supported by analysis of the small subunit of rRNA of *Pseudo-nitzschia*, *Nitzschia*, and other diatom species (Douglas *et al.*, 1994). The following description is based on the diagnosis by Hasle (1993).

Species of *Pseudo-nitzschia* form colonies characterized by overlapping cells (Fig. 1). Each cell contains two chloroplasts, one at each end, and a central nuclear area. Both individual cells and colonies are capable of moving, sliding in the longitudinal direction. The cells move in one direction for a few seconds, followed by movement in the opposite direction. Species differ in valve view and girdle view

(Fig. 4). The girdle consists of a number of linear bands (Figs. 5, 6). For identification, the cells should be seen in *valve view* (Figs. 4, 5). The frustules are often linear to lanceolate in valve and girdle view, but some species (e.g., *P. seriata*) are asymmetrical along the apical axis in valve view (Fig. 7). The number of interstriae equals or is approximately twice the number of fibulae (Fig. 7), and one or more rows of poroids are present between the interstriae (Figs. 5, 7, 8). The raphe is eccentric and flush with the valve surface, and the wall of the raphe canal has no poroids. The two raphes of one cell are diagonally opposed (Fig. 5). Some species possess a central nodule between the central raphe endings. Using LM this is seen as a larger space (=central interspace) between the central fibulae (Fig. 7). Using EM, the raphe is seen as a fine slit (Fig. 7). Hasle (1965) and Hasle and Syvertsen (1997) divided *Pseudo-nitzschia* into two subgroups (Tables 1, 2): (1) the *seriata* group (valve width > 3 µm) and (2) the *delicatissima* group (valve width < 3 µm). In this text, only toxic species and morphologically similar non-toxic ones are treated.

Description of species

Several references are not included in the species descriptions below. These include investigations made without electron microscopy, which we consider necessary for critical identification. In other papers, photographic documentation was not included. New morphological and taxonomic characters are discussed by Hasle *et al.* (1996).

In the following text, measurements by Buck *et al.* (1992) and measurements from Danish waters (Table 3) are given as mean ± SD.

Pseudo-nitzschia australis Frenguelli, 1939
Synonym: *Nitzschia pseudoseriata* Hasle, 1965

Fig. 8A–D

Description: *Light microscopy:* Frustules linear to lanceolate in valve and girdle view. The length is 75–144 µm (Hasle, 1965), 105.5 ± 3.8 µm ($n = 21$, Buck *et al.*, 1992), the width 6.5–8 µm (Hasle, 1965), 7.5 ± 0.4 µm ($n = 21$, Buck *et al.*, 1992). The ends are rounded and slightly rostrate (Hasle, 1965). According to Hasle (1965), valves are symmetric in the apical plane and the middle third of the valves have parallel or nearly parallel sides. According to Buck *et al.* (1992), valves are slightly asymmetric in the apical plane. Most cells from a sample off the coast of Chile were distinctly asymmetric, but in a sample from Monterey Bay both symmetric and asymmetric valves were seen (own obs.)

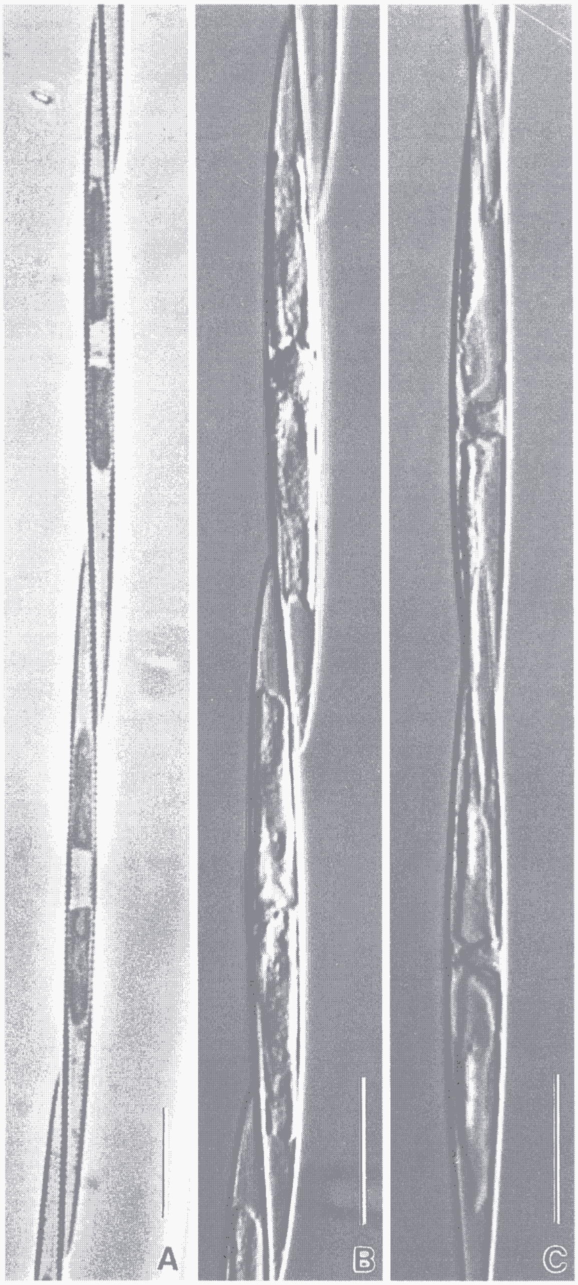

Figure 4A–C. Valve and girdle views of *Pseudo-nitzschia* species. A: Girdle view of *P. pungens*, glutaraldehyde-preserved, phase contrast, B: Girdle view of *P. australis*, glutaraldehyde-preserved, DIC, C: Valve view of *P. australis*, glutaraldehyde-preserved, DIC. Figure 4A: Danish material (culture); Figure 4B–C: material from Monterey Bay, USA, kindly provided by K. Buck. Scale bars: 20 µm.

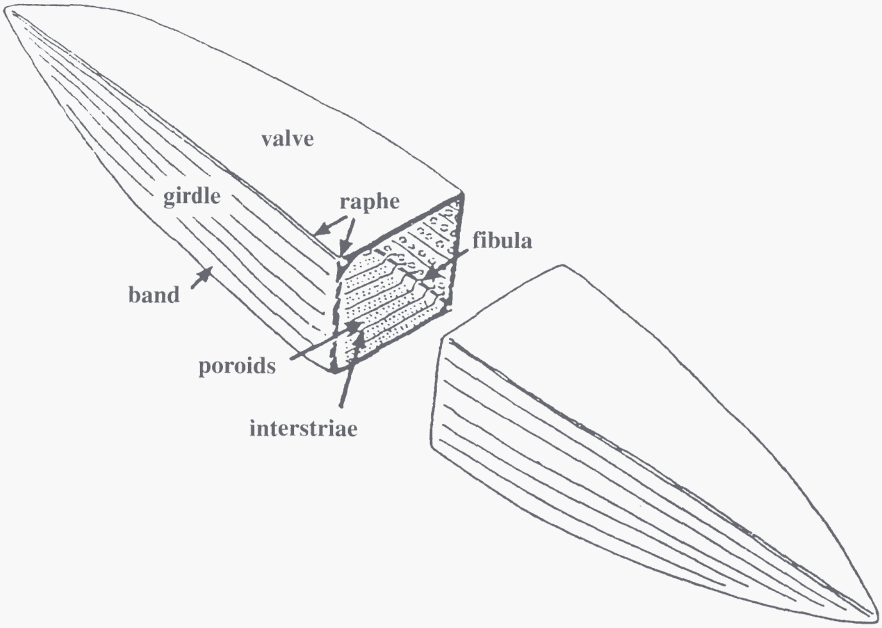

Figure 5. Frustule of *Pseudo-nitzschia* sp. Redrawn after MacPhee *et al.* (1992).

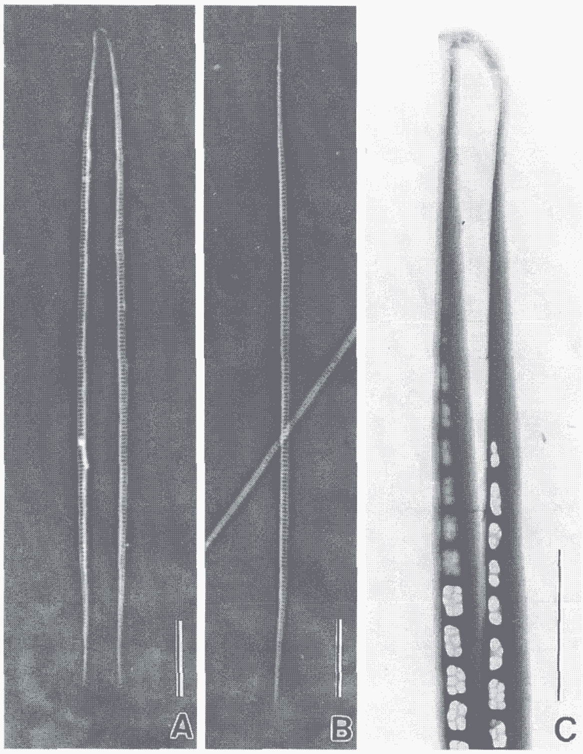

Figure 6A–C. Girdle bands. A: *P. australis*, phase contrast, B: *P. australis*, phase contrast, C: *P. australis*, TEM. Figure 6A–C: material from Chile. Scale bars in Figure 6A, B: 10 µm; Figure 6C: 2 µm.

(Fig. 8). In the original description, Frenguelli (1939) does not comment on the valve symmetry. The valve lacks a central interspace and the number of interstriae and fibulae is 12–18 in 10 µm (Hasle, 1965). The cells overlap by about 1/3 to 1/4 of the total cell length ($n = 40$) in material from Monterey Bay and Chile examined by us.

Description: *Electron microscopy:* Two rows of fairly large poroids, 4–5 transversely in 1 µm (Hasle, 1965).

Taxonomic notes: *P. australis* belongs to the *seriata* group (Table 1). It differs from *P. fraudulenta* (P.T. Cleve) Hasle, *P. subfraudulenta* (Hasle) Hasle, *P. heimii* Manguin, and *P. subpacifica* (Hasle) Hasle in the lack of a central interspace and in slightly rostrate valve ends (Hasle, 1965). It differs from *P. pungens*, *P. multiseries*, and *P. pungiformis* (Hasle) Hasle in the width and shape of the valve ends. The valve of *P. australis* is wider and has rounded ends, while *P. pungens*, *P. multiseries*, and *P. pungiformis* are more pointed. *P. australis* and *P. seriata* are very similar in form and structure, but *P. australis* has rostrate ends and *P. seriata* may be more asymmetric with prolonged ends (Hasle, 1972; G.R. Hasle pers. comm). It is, however, difficult to distinguish the two species by light microscopy. In *P. australis*, electron microscopy shows two rows of poroids; *P. seriata* has 3–5 (Hasle, 1965). Rivera (1985) merged the two species and claimed the existence of intermediate forms according to both symmetry and the number of poroids. We observed asymmetric and symmetric valves in *P. australis*, but did not find any intermediate forms with regard to rows of poroids or shape of the valve ends.

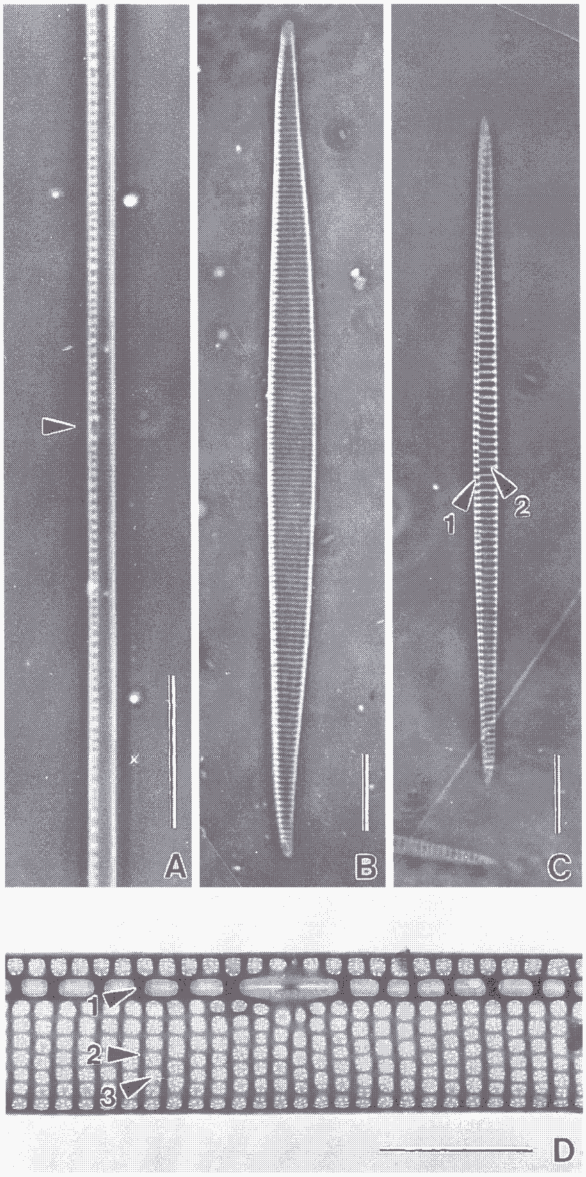

Figure 7A–D. Valve structures in *Pseudo-nitzschia* species. A: *P. pseudodelicatissima*, middle part of the valve, phase contrast, arrow shows central interspace, B: *P. seriata*, asymmetrical valve, phase contrast, C: *P. pungens*, valve, phase contrast, arrow 1 shows fibula, arrow 2 interstria, D: *P. pseudodelicatissima*, middle part of the valve with central interspace, arrow 1 shows fibula, arrow 2 interstria, arrow 3 poroids, TEM. Figure 7A–D: Danish material; Figure 7A–C: cultures. Scale bars in Figure 7A–C: 10 µm; Figure 7D: 2 µm.

Ecology and distribution: Until recently, *P. australis* was reported only from the temperate zone of the South Pacific and the South Atlantic, i.e., the Southern Hemisphere, apart from two questionable records from the North Pacific (Hasle, 1972). Following the bloom in Monterey Bay in 1991, its presence has been confirmed also in older samples from the area. It is now known from several sites along the west coast of the U.S.A. (Buck *et al.*, 1992; Garrison *et al.*, 1992; Villac *et al.*, 1993a, b; Lange *et al.*, 1994). *P. seriata*, on the other hand, has not been reported from the Southern Hemisphere.

P. australis is a common species of the phytoplankton all year in the open sea and in coastal waters (Hasle, 1972). The high numbers of *P. australis* in Monterey Bay were associated with high temperature (13–14°C) and upwelling, presumably enriching the water with nitrogen. There was a high organic content from the excretion of birds and fish present at the time (Buck *et al.*, 1992; Work *et al.*, 1993a, b).

In February 1993 *P. australis* was found in large numbers (8×10^8 cells per litre) in a marine inland sea area in Chile, but ASP problems were not reported (A. Clement, pers. comm., material examination in the EM by us). In September 1994, a bloom in Ría de Muros, Galicia, NW of Spain was reported with concentrations of up to 4.5×10^5 cells per litre, followed by detection of domoic acid in mussels (Míguez *et al.*, 1996).

Toxicology: Concentrations of domoic acid in Monterey Bay in the autumn of 1991 were 3–31 pg per cell (Buck *et al.*, 1992). A maximum concentration of 37 pg per cell was shown in culture studies (Garrison *et al.*, 1992). This is significantly higher than in *P. multiseries*, and may be explained by the greater cell volume of *P. australis* (Buck *et al.*, 1992).

Pseudo-nitzschia delicatissima (P. T. Cleve) Heiden, 1928 Fig. 9A–J

Basionym: *Nitzschia delicatissima* P. T. Cleve, 1897
Synonym: *Nitzschia actydrophila* Hasle, 1965

Description: *Light microscopy:* Valves very narrow, linear to lanceolate. The ends are cut off straight, rather than pointed, in both valve and girdle view. Valve length 40–76 µm (Hasle, 1965), 64 ± 14 µm (Table 3) and width ca. 2 µm (Hasle, 1965), 1.3 ± 0.2 µm (Table 3). The cells overlap by 1/9 (Hasle, 1965), 1/7 to 1/10 (Table 3) of the total cell length.

Rivera (1985) found cells up to 3.9 µm wide, or almost twice the width given by Hasle (1965). He described the species as "bastante variable en sus características morfológicas" (Rivera, 1985, p. 18). This suggests possible confusion with another species, e.g., *P. turgidula*?

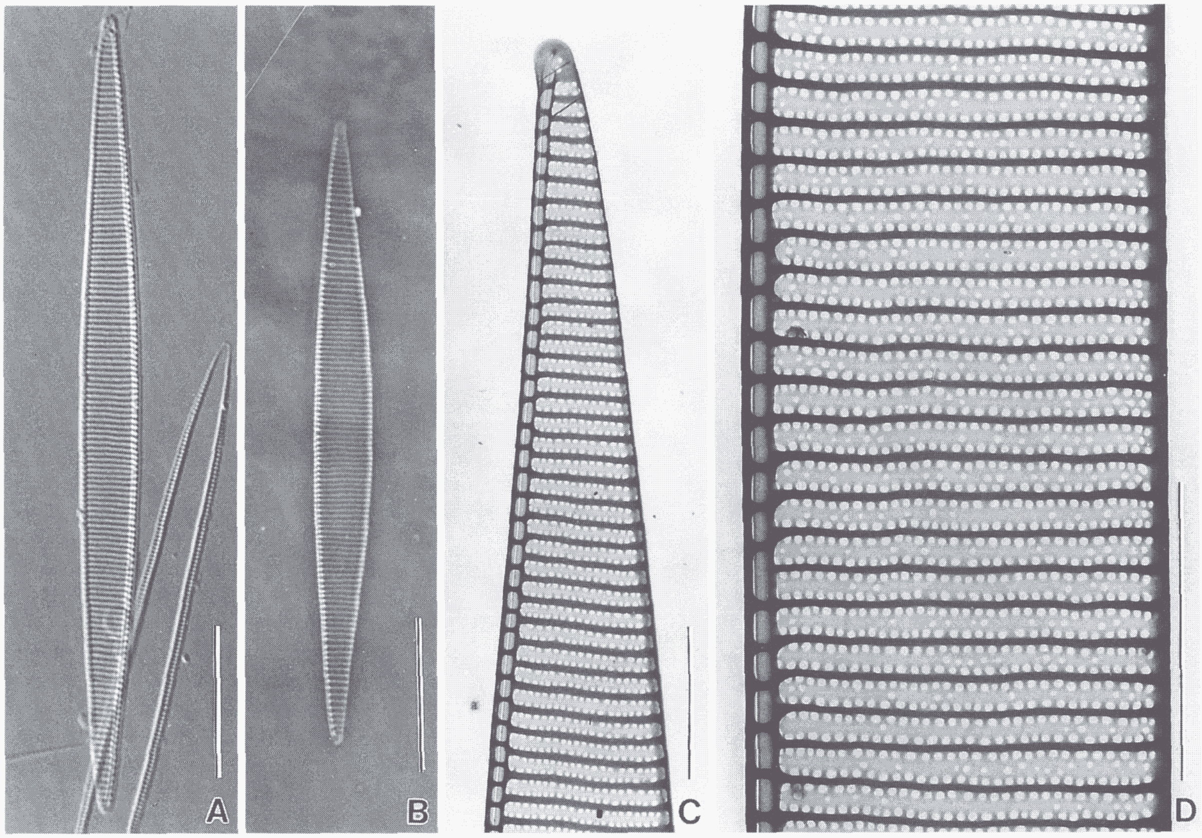

Figure 8A–D. *P. australis.* A: almost symmetrical valve, and girdle band, DIC, B: asymmetrical valve, phase contrast, C: tip of the valve, TEM, D: middle part of the valve, TEM. Figure 8A–D: material from Chile. Scale bars in Figure 8A–B: 20 μm; Figure 8C–D: 5 μm.

Description: *Electron microscopy:* The number of interstriae in 10 μm is 36–40 (Hasle, 1965), 39.9 ± 1.3 (Table 3) and the number of fibulae 19–25 (Hasle, 1965), 23.0 ± 1.4 (Table 3). There are two rows of small, circular poroids; and 10–12 (Hasle, 1965), 10.6 ± 1.0 (Table 3) poroids transversely in 1 μm. A central interspace is present.

Taxonomic notes: *P. delicatissima* belongs to the *delicatissima* group (Table 2). All seven species treated in this paper have a central interspace. Three of these, *P. inflatula* (Hasle) Hasle, *P. turgiduloides* (Hasle) Hasle, and *P. turgidula* can be distinguished from *P. delicatissima* by the bloated middle part of the valve. The others can be distinguished as follows: The valve of *P. cuspidata* (Hasle) Hasle is broader, the overlap in the chains is greater and the striae have one row of poroids compared to two in *P. delicatissima* (Hasle, 1965). *P. pseudodelicatissima* is linear in the middle part of the valve and has pointed ends, while *P. delicatissima* is more lanceolate and ends are cut off straight (Hasle and Medlin, 1990). *P. delicatissima* has two rows of small poroids; *P. pseudodelicatissima* has one row of larger poroids; *P. lineola* (P. T. Cleve) Hasle is slightly wider and not as delicate as *P. delicatissima*. It has 1–2 rows of fairly large poroids compared to the two rows of small poroids of *P. delicatissima*. *P. lineola* has 22–28 interstriae in 10 μm compared to 36–41 in *P. delicatissima* (Hasle, 1965). Critical identification requires electron microscopy.

Takano (1995) described a new species, *P. multistriata*, from southern Japan. It is similar to *P. delicatissima*, except for the lack of a central interspace (Hasle, 1997).

Ecology and distribution: *P. delicatissima* occurs from the Arctic to Northwest Africa (Hasle, 1965). In the Northern Hemisphere it has been recorded from Norway (Nordkapp, Espegrend, Drøbak, and Grønsfjord) and Northwest Africa (Hasle, 1965); Denmark and Nuuk (Greenland) (own obs.), Italy (the Adriatic Sea) (Caroppo *et al.*, 1997), Spain (Galician waters)

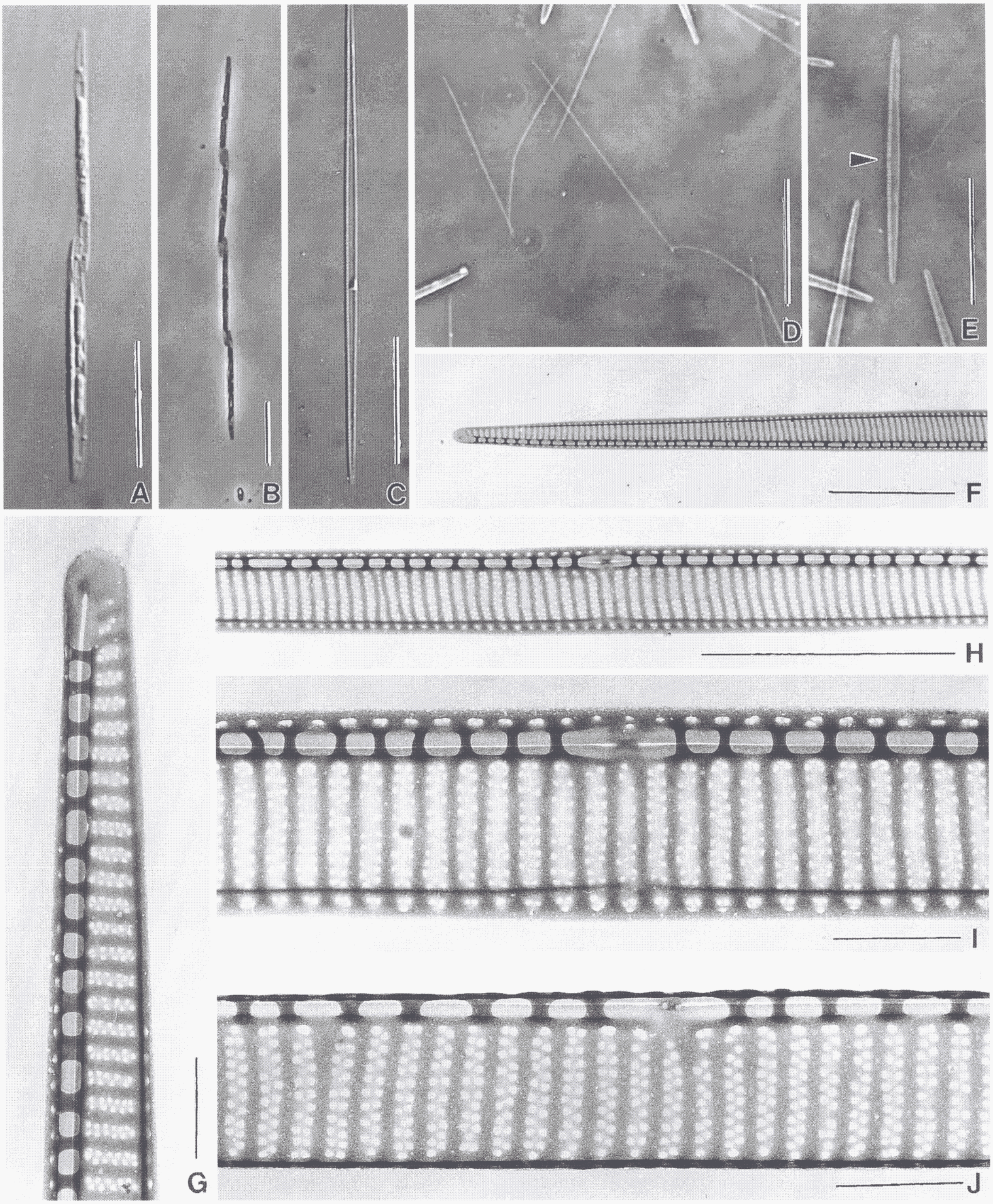

Figure 9A–J. *P. delicatissima*. A: lugol-preserved colony, chloroplasts visible, DIC, B: glutaraldehyde-preserved colony, phase contrast, C: single valve, DIC, D: girdle bands, phase contrast, E: valve, phase contrast, arrow shows central interspace, F, G: tip of the valve, TEM, H, I, J: middle part of the valve, I and J: note the different poroid patterns, TEM. Material from Denmark; Figure 9A–E: cultures. Scale bars in Figure 9A–E: 20 µm; Figure 9F, H: 5 µm; Figure 9G, I–J: 1 µm.

Table 1. *Pseudo-nitzschia seriata* group (frustule width > 3 µm *in valve view*). The table is based mainly on Hasle (1965), on own observations (Table 3), and the authors referred to in the text.

	P. seriata f. *seriata*	*P. seriata* f. *obtusa*	*P. australis*	*P. pungens*	*P. multiseries*	*P. pungiformis*	*P. fraudulenta*	*P. subfraudulenta*	*P. subpacifica*	*P. heimii*
Valve view	Linear to lancet-shaped. Asymmetric along the longitudinal axis	As the species, but more obtuse valve ends	Linear to lanceolate. Asymmetric. Tips rounded	Linear to lanceolate. Symmetric	Linear to lanceolate. Symmetric	Lanceolate. Tapering towards slightly pointed ends	Spindle-shaped. Symmetric	Linear in the middle part. Pointed ends. Symmetric	One straight and one convex side. More or less pointed ends. Asymmetric	Linear to lanceolate (one margin straight). Broadly rounded obtuse ends
Girdle view	Linear to lanceolate. Symmetric	Linear to lanceolate. Symmetric	Linear to spindle-shaped	Symmetric, linear to lanceolate	Symmetric, linear to lanceolate			Sigmoid	Ends sigmoid, obliquely truncated	Ends sigmoid, obliquely truncated
Central interspace	–	–	–	–	–	+	+	+	+	+
Length (µm)	91–160	61–100	75–144	74–174	68–140	96–145	50–119	65–106	33–70	67–120
Width (µm)	5.5–8	4.5–5.5	6.5–8	2.4–5.3	3.4–6	4–5	4.5–10	4.6–7	5–7	4–6
Chains: overlap of cell length	1/3–1/4	1/3–1/4	1/3–1/4	1/3–1/4					1/5–1/6	1/4–1/5
Rows of poroids	3–5, often 2 × 2 rows	2	2	1–2 visible in LM	(2)3–4	2	2–3	2	2	1–2
Poroids in 1 µm	7–8	7–8	4–5, large	3–4	4–6	5–6	5–7, with star-shaped membrane	5–6, with star-shaped membrane	9–10, small	5–6 (7–8)
Interstriae in 10 µm	14–18	15–20	12–18	9–16	10–19	14–20	18–24	23–26	28–32	19–26
Fibulae in 10 µm	14–18	15–20	12–18	9–16	10–19	12–18	12–24	12–17	15–20	11–16
Recorded from	77°N–45°N. Northern Hemisphere. Cold-water species	Arctic, coastal regions	6°S–43°S in Southern Hemisphere, also some reports from the Northern Hemisphere	Neritic, cosmopolitan	Cosmopolitan	24°N–33°S. Only few reports	Coastal and oceanic, cosmopolitan	43°N–34°S. Mainly in inshore warmer waters. Warm-water species	51°N (Atlantic Ocean) to 43°S (Pacific Ocean). Oceanic. Warm-water species	Only a few reports, from both Southern and Northern Hemisphere

Table 2. *Pseudo-nitzschia delicatissima* group (frustule width < 3 µm *in valve view*). The table is based mainly on Hasle (1965), on own observations (Table 3), and the authors referred to in the text.

	P. delicatissima	*P. pseudodelicatissima*	*P. cuspidata*	*P. lineola*	*P. turgidula*	*P. turgiduloides*	*P. inflatula*
Valve view	Narrow, linear to lanceolate. Straight cut ends	Narrow and linear. Tapering towards pointed ends	Linear to lanceolate. Pointed ends	Linear to lanceolate	Variable species: rounded ends, middle part inflated	Parallel sides. Slightly inflated in the middle part. Broad and rounded ends	Linear to lanceolate. Inflated in the middle part and near the ends. Pointed ends
Girdle view	Linear. Straight cut ends	Linear. Pointed ends	Linear to lanceolate. Slightly sigmoid, pointed ends	Linear to lanceolate. Slightly sigmoid, pointed ends	Parallel sides. Tapering towards truncated ends	Linear. Truncated ends	Linear. Pointed ends
Central interspace	+	+	+	+	+	+	+
Length (µm)	40–78	50–140	30–90	56–112	30–80	63–126	60–100
Width (µm)	1.1–2	1.5–3.4	2.5–3.9	1.8–2.7	2.5–3.5	1.8–2.7	1.5–2.5
Chains: overlap of cell length	1/7–1/10	1/5–1/6	1/5–1/6	1/5–1/6	1/6		1/6–1/7
Rows of poroids	2	1	1	1	2	1–2	1
Poroids in 1 µm	10–12, small	4–6, partly closed by a membrane	4–6, large, partly closed by a membrane	3–6, with star-shaped membrane in some specimens	7–9	8–10	5, almost squared and divided
Interstriae in 10 µm	36–41	30–46	29–39	22-28	23–28	17–21	32–35
Fibulae in 10 µm	19–25	14–26	14–22	11–16	13–18	10–13	18–21
Recorded from	Inshore waters, e.g. the Oslofjord, Kattegat, Nordkapp and NW Africa	North of 53°S	Reported from NW Africa, Canary Islands, Chile and Washington State	Cosmopolitan	62°S–61°N	Confined mostly to the Antarctic zone	Sporadic reports from Northern and Southern Hemisphere. Mostly cold waters

Table 3. Measurements of six species of *Pseudo-nitzschia* found in Danish coastal waters. The measurements are given as mean ± SD, n = number of observations and the observed range of measurements as (minimum–maximum).

Species	Length (µm)	Width (µm)	Cell overlap	Striae in 10 µm	Fibulae in 10 µm	Poroids in 1 µm
P. delicatissima	64.0 ± 14.0 $n = 68$ (37.5–77.5)	1.3 ± 0.2 $n = 38$ (0.9–1.8)	1/7–1/10 $n = 73$ (1/5–1/10)	39.3 ± 1.3 $n = 48$ (37–43)	23.0 ± 1.4 $n = 40$ (20–26)	10.6 ± 1.0 $n = 45$ (8–12)
P. fraudulenta	–	$n = 3$ (4.0–4.2)	–	$n = 3$ (23–26)	$n = 3$ (23–26)	$n = 3$ (5–6)
P. multiseries	–	4.2 ± 0.3 $n = 41$ (3.5–4.7)	–	11.7 ± 0.9 $n = 52$ (10–14)	11.8 ± 0.9 $n = 40$ (10–14)	5.6 ± 0.6 $n = 50$ (4–7)
P. pseudodelicatissima	76.6 ± 8.1 $n = 90$ (62.5–97.5)	2.0 ± 0.2 $n = 77$ (1.5–2.4)	1/6 $n = 51$ (1/5–1/7)	35.9 ± 2.3 $n = 119$ (29–41)	18.1 ± 1.6 $n = 107$ (14–23)	4.5 ± 0.5 $n = 109$ (4–6)
P. pungens	116.1 ± 13.2 $n = 74$ (100–155)	2.9 ± 0.5 $n = 60$ (1.8–4.0)	1/4 $n = 70$ (1/3–1.5)	12.0 ± 1.1 $n = 82$ (10–14)	12.3 ± 1.6 $n = 78$ (10–20)	3.3 ± 0.4 $n = 81$ (2–4)
P. seriata	107.0 ± 9.2 $n = 114$ (75–130)	5.9 ± 0.4 $n = 114$ (4.8–6.9)	1/3–1/4 $n = 38$ (1/3–1/5)	16.0 ± 1.3 $n = 93$ (13–20)	15.3 ± 1.1 $n = 79$ (13–19)	7.6 ± 0.7 $n = 81$ (6–11)

(Fraga *et al.*, 1997), the Pacific coasts of Mexico (Hernández-Beccrril, 1997), and Canada (Bates and Douglas, 1993). Off Nordkapp, *P. delicatissima* was found as single cells associated with *Phaeocystis pouchetii* (Hariot) Lagerheim (Hasle, 1965). In the Southern Hemisphere it is recorded from the Bay of Valparaiso, Chile (Avaria and Munoz, 1982).

Toxicology: *P. delicatissima* has never been implicated in a known toxic event. In culture it has once been reported to produce 5 fg domoic acid per cell (Smith *et al.*, 1991).

Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle, 1993 Fig. 10A–G

Basionym: *Nitzschia pseudodelicatissima* Hasle, 1976
Synonym: *Nitzschia delicatula* Hasle, 1965

Description: *Light microscopy:* Valves narrow, symmetric along the apical axis. The length is 59–140 µm (Hasle, 1965), 50–109 µm (Rivera, 1985), 76.6 ± 8.1 µm (Table 3) and the width 1.5–2.5 µm (Hasle, 1965), 1.5–3.4 µm (Rivera, 1985), 1.8–3.0 µm (Takano and Kuroki, 1977), 2.0 ± 0.2 µm (Table 3). The valves are linear in the middle part and have more or less pointed ends (Hasle, 1965). A central interspace is present in each valve. The cells overlap by 1/5 to 1/6 of the cell length (Rivera, 1985), 1/6 (Table 3).

Description: *Electron microscopy:* The number of interstriae in 10 µm is 30–46 (Hasle, 1965), 31–38 (Takano and Kuroki, 1977), 32–42 (Rivera, 1985), 35.9 ± 2.3 (Table 3). The number of fibulae in 10 µm is 16–26 (Hasle, 1965), 14–21 (Takano and Kuroki, 1977), 18–24 (Rivera, 1985), 18.1 ± 1.6 (Table 3). The cribrum (a perforated membrane covering the poroids) (Fig. 10F) often resembles a flower, and is more distinct than in the other species. There is one row of round or square poroids, 4–6 transversely in 1 µm (Hasle, 1965), 5–6 (Takano and Kuroki, 1977), 5–6 (Rivera, 1985), 4.5 ± 0.5 (Table 3).

Taxonomic notes: *P. pseudodelicatissima* belongs to the *delicatissima* group (Table 2). *P. pseudodelicatissima* and *P. delicatissima* differ from *P. inflatula* by the inflated valve of the latter, and from *P. turgidula* and *P. turgiduloides* by a larger number of interstriae. *P. pseudodelicatissima* is distinguished from *P. lineola* by the coarser structure and fewer interstriae of the latter species (Hasle, 1965). See *P. delicatissima* for distinguishing further between *P. delicatissima* and *P. pseudodelicatissima*. *P. pseudodelicatissima* and *P. cuspidata* are very similar, but *P. cuspidata* is lanceolate and the valves taper towards the ends, while *P. pseudodelicatissima* is more linear (Hasle, 1965). A distinction between *P. pseudodelicatissima* and *P. pungens* can sometimes be difficult in very narrow specimens of the latter. We have observed valves of *P. pungens* only 2.2 µm wide (Table 3). The two species may be distinguished by the

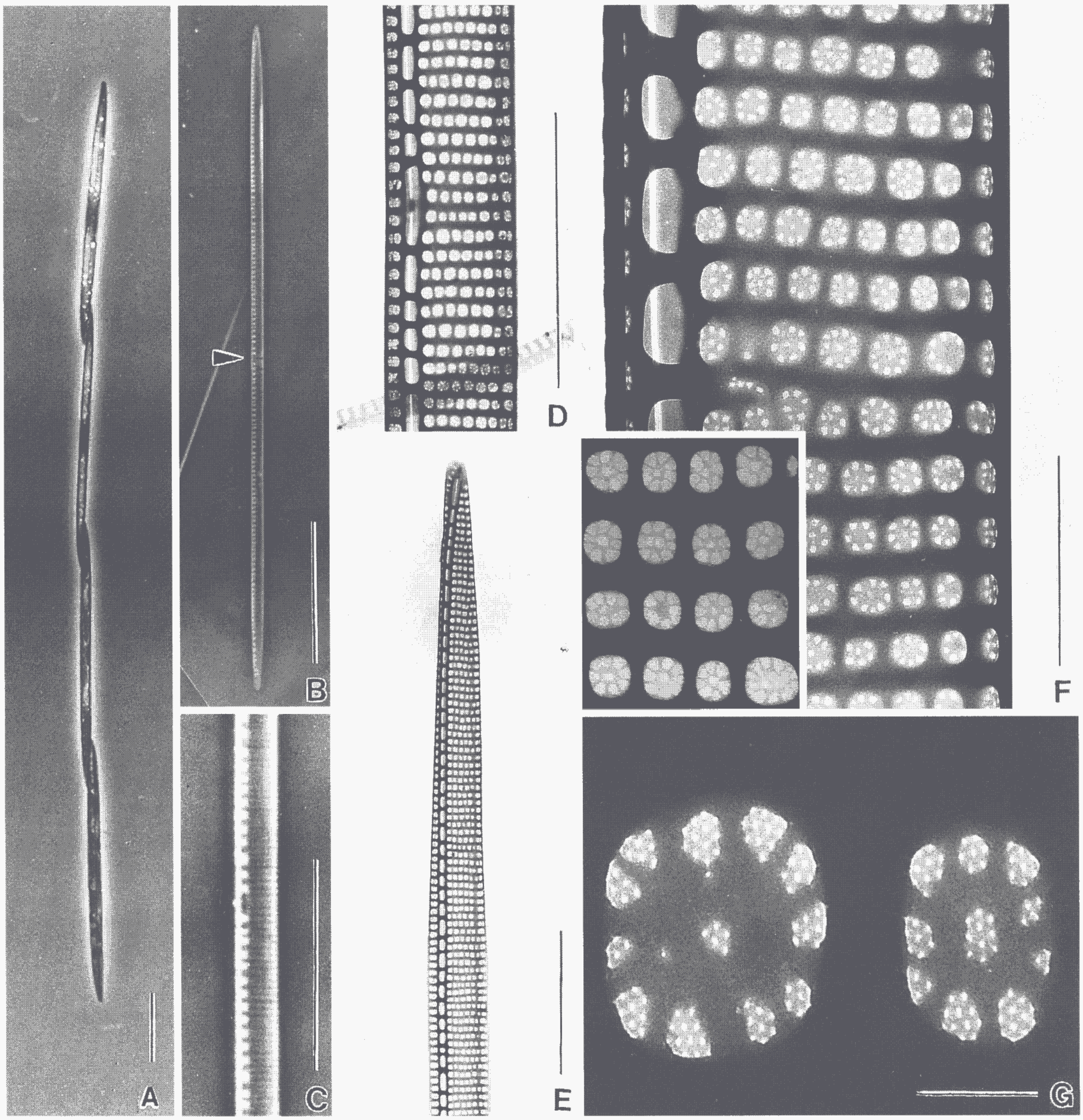

Figure 10A–G. *P. pseudodelicatissima*. A: colony in girdle view, phase contrast, B: valve, arrow shows central interspace, phase contrast, C: middle part of the valve with visible interstriae and central interspace, TEM, D: middle part of the valve with central interspace, TEM, E: tip of the valve, TEM, F: middle part of the valve, showing the perforated membrane of the poroids. Inset: Same with different perforation, TEM, G: each perforation is further perforated, TEM. Danish material; Figure 10A–E: cultures. Scale bars in Figure 10A–C: 20 µm; Figure 10D–E: 5 µm; Figure 10F: 1 µm; Figure 10G: 0.2 µm.

appearance of the ends of the interstriae. These are visible in *P. pungens* in girdle view, like dots along the edge of the valve (Fig. 4A), but not visible in *P. pseudodelicatissima* (Fig. 10A).

Qi (1994) described a new species, *P. sinica*, from China which is similar to *P. pseudodelicatissima*, but it is wider and has more interstriae.

Isozyme analysis of isolates of *P. pseudodelicatissima* revealed nine putative loci. Six of those were polymorphic, showing variation within the species from the same locality, indicating that several clones of the species are present in Danish waters (Skov *et al.*, 1997).

Ecology and distribution: According to Hasle (1965) and Rivera (1985) the geographic distribution of *P. pseudodelicatissima* is the Northern Hemisphere and the Southern Hemisphere north of 53°S. In the Bay of Fundy it occurs throughout the year, but the highest concentrations appear at higher water temperature (June–September, 10–18°C) (Martin *et al.*, 1993). In a culture experiment using a Danish isolate, growth rates were salinity and temperature dependent. Growth optimum was at 25‰ and 25°C or higher. No growth was seen in 5 and 10‰. The lower temperature limit for growth depended on salinity (Lundholm *et al.*, 1997).

Toxicity in *P. pseudodelicatissima* has been reported once in 1988 in Passamaquoddy Bay (the southwestern Bay of Fundy), New Brunswick, Canada (Martin *et al.*, 1990; Haya *et al.*, 1991). *P. pseudodelicatissima* has been sighted in the area every year from 1976 onwards and bloomed regularly March–September in the 1930s (Martin *et al.*, 1990). In 1988, cells of *P. pseudodelicatissima* were, as a result of vertical mixing, distributed in high concentrations at all depths, and the bloom lasted for 2 months (Martin *et al.*, 1990).

In July, August, and September 1992, *P. pseudodelicatissima* bloomed in coastal waters of Denmark, Sweden, Norway, and the southwestern Baltic (Edler, 1993; Hansen and Horstmann, 1993; Lundholm and Skov, 1993; G. R. Hasle pers. comm.). In Denmark the concentration reached ca. 16.6×10^6 cells per litre (Per Andersen, pers. comm.). In some areas, high cell concentrations seemed to occur in deeper water (7–18 m) (Susanne Petersen, the county of Funen, Denmark, pers. comm.). No domoic acid was found in blue mussels (*Mytilus edulis*) or oysters (*Crassostrea gigas*) (Helle Emsholm, the Fish Inspection Service, Denmark, and Matts Hageltorn, pers. comm.; Lundholm and Skov, 1993). Isozyme analysis of isolated clones from the Danish bloom indicate that the bloom was polyclonal (Skov *et al.*, 1997). In July and August 1993, *P. pseudodelicatissima* formed extensive blooms in the Berkeley marina, Carlifornia, apparently without producing domoic acid (R. Horner, University of Washington, U.S.A., pers. comm.).

Toxicology: In culture, the production of domoic acid is 7.0×10^{-3} to 9.8×10^{-2} pg per cell (Martin *et al.*, 1990). Toxicity has been confirmed in a culture isolated from Danish coastal waters as 9.7×10^{-2} to 2.21×10^{-1} pg per cell (Lundholm *et al.*, 1997). This corresponds with the level found in *P. multiseries* when taking into account that *P. pseudodelicatissima* has a smaller cell volume (Martin *et al.*, 1990). When domoic acid was detected in shellfish and plankton, *P. pseudodelicatissima* comprised >90% of the total biomass of phytoplankton, and the concentration reached more than 10^5 cells per litre (Haya *et al.*, 1991).

Pseudo-nitzschia multiseries (Hasle) Hasle, 1995 Fig. 11A, B, G, I

Basionym: *Nitzschia pungens* forma *multiseries* Hasle, 1974
Synonym: *Pseudo-nitzschia pungens* forma *multiseries* (Hasle) Hasle, 1993

Description: *Light microscopy:* Frustules linear to lanceolate, symmetric along the apical axis in valve and girdle view. The length is 68–140 µm (Hasle, 1965), 82–135 µm (Takano and Kuroki, 1977), 97.6–115.6 µm (Fryxell *et al.*, 1990) and the width 4–5 µm (Hasle, 1965), 4.7–6 µm (Takano and Kuroki, 1977), 3.4–4.9 µm (Fryxell *et al.*, 1990), 4.2 ± 0.3 µm (Table 3). Valves are strongly silicified and interstriae and fibulae are visible in the light microscope. There is no central interspace present. The number of interstriae in 10 µm is 10–13 (Hasle, 1965), 12–19 (Fryxell *et al.*, 1990), 11.7 ± 0.9 (Table 3). The number of fibulae in 10 µm is 10–13 (Hasle, 1965), 12–19 (Fryxell *et al.*, 1990), 11.8 ± 0.9 (Table 3). The cells overlap about 1/3 of the total cell length (Hasle, 1965).

Description: *Electron microscopy:* The number of poroid rows is 3–4, rarely 2 (Hasle, 1965), 2–4 (Takano and Kuroki, 1977), 3–4 (Table 3). Each row has 4–6 poroids in 1 µm (Hasle, 1965), 5.6 ± 0.6 were seen in our material (Table 3). The girdle bands are striated (two or three rows of parallel poroids). The valve ends do not differ in structure (Hasle, 1995).

Taxonomic notes: *P. multiseries* was formerly known as *P. pungens* f. *multiseries* but is now considered a separate species, based on morphological, physiological, and genetic examinations (Hasle, 1995; Manhart *et al.*, 1995). Morphological characters separating *P. multiseries* from *P. pungens* are the different number of rows of poroids (Hasle, 1965), the distinctive structures of the bands, and the different structures of the valve ends (Hasle, 1995).

Pseudo-nitzschia multiseries belongs to the *seriata* group (Table 1). In the light microscope it differs from *P. fraudulenta*, *P. subfraudulenta*, *P. subpacifica*, *P. heimii*, and *P. pungiformis* by the lack of a central interspace. The structure of the valve is very similar to that of *P. pungiformis*. Critical identification may require electron microscopy because the interspace in *P. pungiformis* is difficult to see in the light microscope (Hasle, 1971; Simonsen, 1974).

Pseudo-nitzschia seriata, *P. australis*, and *P. pungens* also lack a central interspace. They differ from *P. multiseries* in the following characters: *P. australis* is

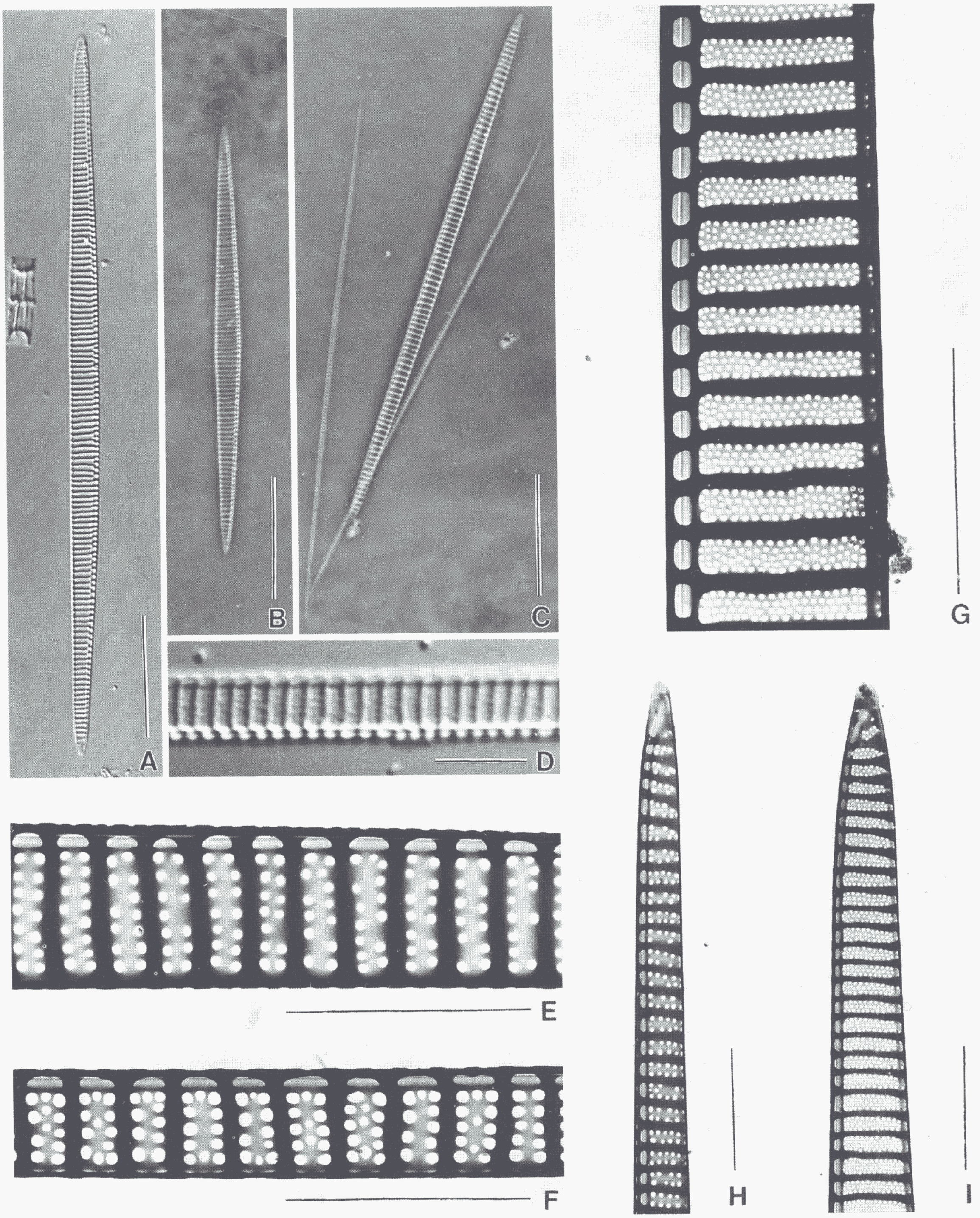

Figure 11A–I. *P. pungens* and *P. multiseries*. A: Presumably *P. multiseries*, valve, DIC, B: *P. multiseries*, valve, phase contrast, C: *P. pungens*, valve, phase contrast, D: *P. pungens*, middle part of the valve with visible poroids, DIC, E, F: *P. pungens*, middle part of the valve, TEM, G: *P. multiseries*, middle part of the valve, TEM, H: *P. pungens*, tip of the valve, TEM, I: *P. multiseries*, tip of the valve, TEM. Figure 11A, E–I: Danish material, from nature; Figure 11C–D: Danish material (cultures); Figure 11B: material from Monterey Bay (culture) kindly provided by M. C. Villac. Scale bars in Figure 11A–C: 20 μm; Figure 11D–I: 5 μm.

wider, 6.2–8 µm compared to 3.4–6 µm. *Pseudo-nitzschia seriata* is asymmetric along the apical axis and tends to be slightly wider, 5.5–8 µm. At times *P. multiseries* can be very difficult to distinguish from *P. pseudodelicatissima*. It can (like *P. pungens*, Fig. 4A), be distinguished *in girdle view*, since the interstriae of *P. multiseries* are visible in girdle view using LM, but not visible in *P. pseudodelicatissima* (G. R. Hasle pers. comm.). It may sometimes be possible to distinguish *P. pungens* from *P. multiseries* using LM, as the poroids are sometimes visible at high power light microscopy in *P. pungens* but not in *P. multiseries*. Critical identification requires electron microscopy. *P. pungens* has 1–2 rows of poroids and 3–4 poroids transversely in 1 µm, while *P. multiseries* has 3–4 rows of poroids and 4–6 poroids in 1 µm.

Ecology and distribution: Using EM, the occurrence of *P. multiseries* has been confirmed in the following areas: along the Atlantic coast of North America (Hasle, 1965; Kaczmarska *et al.*, 1986; Bates *et al.*, 1989; Fryxell *et al.*, 1990; Villareal *et al.*, 1994), the Pacific coast of North America (Hasle, 1965; Forbes and Denman, 1991; Buck *et al.*, 1992; Horner and Postel, 1993). In European waters in the Oslofjord (Hasle, 1965), Danish coastal waters (Lundholm *et al.*, 1990), the Dutch Wadden Sea (Vrieling *et al.*, 1996). In the Gulf of Annaba, the Mediterranean, Algiers (own obs.). Along the Atlantic coast of South America at Atlantida, Uruguay, and Quequen, Argentina (Hasle, 1965), and in Asia in Ofunato Bay, Japan (Takano and Kuroki, 1977; Kotaki *et al.*, 1996), Jinhae Bay, South Korea (Fryxell *et al.*, 1990), and Peter the Great Bay, the Sea of Japan, Russia (Orlova *et al.*, 1996, 1997). *P. multiseries* is associated with coastal areas (Hasle, 1965), while the closely related *P. pungiformis* occurs both in the open sea and in coastal regions (Hasle, 1971).

P. multiseries has been found from September to March on the Northern Hemisphere (Hasle, 1965) but it is reported mainly from colder months (Fryxell *et al.*, 1990). *P. pungens* seems to be more abundant in warmer water (mainly in the autumn). In the estuaries of Prince Edward Island, Canada, and the waters around Galveston, Texas, U.S.A., *P. pungens* is gradually replaced by *P. multiseries* during the autumn and winter (Smith *et al.*, 1990b; Fryxell *et al.*, 1991; Dickey *et al.*, 1992). In the Skagerrak 1980–1990, Lange *et al.* (1992) found *P. pungens* most frequently from September to January and *P. multiseries* from December/January through March.

Hasle *et al.* (1996) observed a gradual replacement of *P. multiseries* by *P. pungens* in the Skagerrak in the 1980s and 1990s.

Based on growth experiments, Hargraves *et al.* (1993) showed that *P. multiseries* has greater tolerance to UV light than *P. pungens*. A global increase in UV light may thus cause a change in species composition favouring *P. multiseries*.

P. multiseries occurs at temperatures from −1 to +30°C (Hasle, 1972; Fryxell *et al.*, 1990; Forbes and Denman, 1991; Pan *et al.*, 1993; Smith *et al.*, 1993) and at salinities from 18 to 36 (Hasle, 1972; Lundholm *et al.*, 1990). In culture it grows from 15 to 48‰ (Jackson *et al.*, 1992). Blooms may last up to 3 months (Bates *et al.*, 1989; Smith *et al.*, 1990a).

Toxicology: The concentrations of domoic acid during the blooms in Canada were 1–7 pg per cell (Bates *et al.*, 1989). In culture it produced up to 21 pg per cell (whole culture) (Bates *et al.*, 1989; Subba Rao *et al.*, 1988, 1990; Reap, 1991; Douglas and Bates, 1992). Up to 7 pg per cell was found within the cell by Bates *et al.* (1991), Smith *et al.* (1993), 0.6–1.5 pg by Villac *et al.* (1993a). Recently, cultures from the Dutch Wadden Sea were found to produce domoic acid (Vrieling *et al.*, 1996).

Pseudo-nitzschia pungens (Grunow *ex* P. T. Cleve) Hasle, 1993 Fig. 11C–F, H

Basionym: *Nitzschia pungens* Grunow *ex* P. T. Cleve, 1897

Description: *Light microscopy:* Frustules linear to lanceolate and symmetric in both valve and girdle view. The length is 74–142 µm (Hasle, 1965), 81–151 µm (Takano and Kuroki, 1977), 87–174 µm (Rivera, 1985), 79.3–117.3 µm (Fryxell *et al.*, 1990), 116.1 ± 13.2 µm (Table 3). The width is 3–4.5 µm (Hasle, 1965), 2.8–5.3 µm (Takano and Kuroki, 1977), 4–6.5 µm (Rivera, 1985), 2.9–4.6 µm (Fryxell *et al.*, 1990), 2.9 ± 0.5 µm (Table 3). Valves are strongly silicified and interstriae and fibulae are visible in light microscope. A central interspace is absent. The number of interstriae in 10 µm is 9–15 (Hasle, 1965), 9–17 (Rivera, 1985), 10–14 (Fryxell *et al.*, 1990), 12.0 ± 1.1 (Table 3), and the number of fibulae in 10 µm is 9–15 (Hasle, 1965), 9–15 (Rivera, 1985), 12.6 ± 1.6 (Table 3). The cells overlap about 1/3 of total cell length (Hasle, 1965), 1/4 (Table 3).

Description: *Electron microscopy:* The number of poroid rows is 2, rarely 1 (Hasle, 1965), 2 (Takano and Kuroki, 1977), 2–3 (Rivera, 1985), 2 (Table 3). Within each row there are 3–4 large poroids in 1 µ (Hasle, 1965; Takano and Kuroki, 1977; Rivera, 1985), 3.3 ± 0.4 (Table 3). The girdle bands have single poroids. The valve ends differ in structure; one end has fewer poroids per stria than the other (Hasle, 1995).

Taxonomic notes: *P. pungens* belongs to the *seriata* group (Table 1). See Taxonomic notes of *P. multiseries* for additional details.

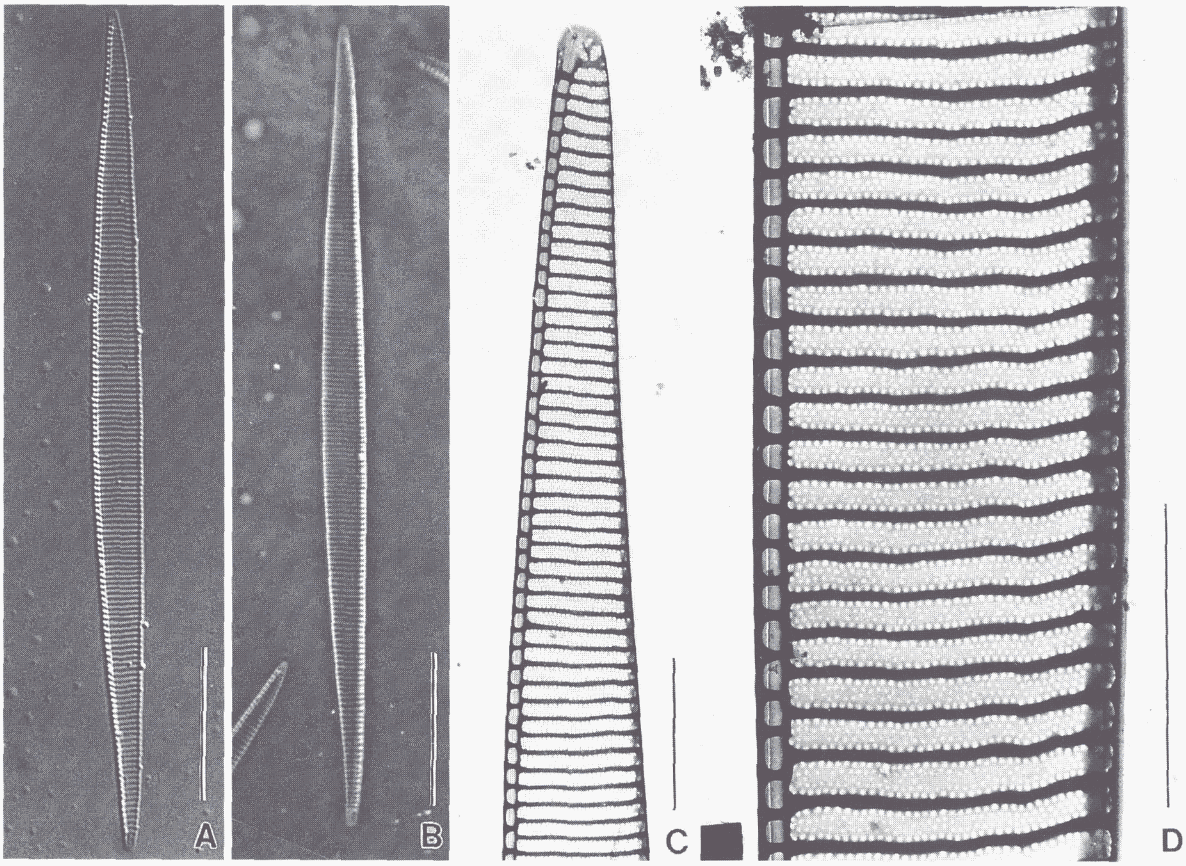

Figure 12A–D. *P. seriata*. A: valve, DIC, B: valve, phase contrast, C: tip of the valve, TEM, D: middle part of the valve, TEM. Danish material (cultures). Scale bars in Figure 12A–B: 20 µm; Figure 12C–D: 5 µm.

Ecology and distribution: *P. pungens* is a common species, confined to coastal waters (Hasle, 1972) and cosmopolitan (Hasle and Fryxell, 1995). See Ecology and distribution of *P. multiseries* for further information.

Toxicology: In culture, two isolates from U.S. coastal waters of Washington and California have been found to produce domoic acid at a concentration of up to 80 fg per cell. Domoic acid has also been detected in cultures isolated from blooms in the Marlborough Sounds and Bay of Plenty, New Zealand, November 1995 (Rhodes *et al.*, 1997).

Pseudo-nitzschia seriata (P. T. Cleve) H. Peragallo, 1990 Fig. 12A–D

Basionym: *Nitzschia seriata* P. T. Cleve, 1883

Description: *Light microscopy:* In valve view the frustules are lanceolate and asymmetric in the apical plane, narrowing towards the more or less rounded ends. The length is 91–160 µm (Hasle, 1965), 107.0 ± 9.2 µm (Table 3) and width 5.5–8 µm (Hasle, 1965), 5.9 ± 0.4 µm (Table 3). The valve lacks a central interspace and has 14–18 interstriae and fibulae in 10 µm (Hasle, 1965), 16.0 ± 1.3 interstriae and 15.3 ± 1.1 fibulae (Table 3). The cells overlap by about 1/3 to 1/4 of the cell length (Hasle, 1965), 1/3 to 1/4 (Table 3).

Description: *Electron microscopy:* The number of poroid rows is 3–5 (Hasle, 1965), 3–4 (Table 3). The two rows along the interstriae are often composed of larger poroids, with 7–8 poroids in 1 µm (Hasle, 1965), 7.6 ± 0.7 (Table 3).

Taxonomic notes: *P. seriata* belongs to the *seriata* group (Table 1). It differs from most other species of *Pseudo-nitzschia* in the lack of a central interspace and the asymmetrical shape. It may be distinguished from *P. pungens* and *P. multiseries* by its asymmetry, the wider valves and the larger number of interstriae. *P. seriata* f. *obtusa* (Hasle) Hasle has more obtuse ends than the nominal variety and has only two rows of poroids (Hasle, 1965). Rivera (1985) regards *P. seriata* f. *obtusa* to be synonymous with *P. seriata*.

Ecology and distribution: *P. seriata* is a cold-water species, both neritic and oceanic. The limits for growth have been reported as −1.6°C to 12–15°C, with an optimum between 6 and 12°C measured in an isolate from below the ice in Canada (Smith *et al.*, 1994). Reports of *P. seriata* are only confirmed from the Northern Hemisphere (Hasle, 1972, 1976). Records from the Southern Hemisphere or from warm waters are due to confusion with closely related species (Hasle, 1972).

Toxicology: In three isolates from Nivå Bugt in Denmark the production of domoic acid was shown to be up to 33 pg per cell (whole culture). This corresponds with toxin levels in *P. multiseries*, with which it compares closely in volume (Lundholm *et al.*, 1994).

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