**Pseudo-nitzschia seriata** f. *obtusa* (Bacillariophyceae) raised in rank based on morphological, phylogenetic and distributional data

**GRETIE RYTTER HASEL**¹ and **NIWA LUNDHOLM**²

¹Department of Biology, University of Oslo, PO Box 1066, Blindern, NO-0316 Oslo, Norway
²Marine Biological Laboratory, Strandpromenaden 5, DK-3000 Helsingør, Denmark

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**INTRODUCTION**

The marine, planktonic diatom genus *Pseudo-nitzschia* H. Peragallo in H. Peragallo & M. Peragallo has received much attention during the last two decades, mainly because some species are potentially able to produce the neurotoxin, domoic acid (DA). Published studies have resulted in description of new species (Takano 1993; Lundholm & Moestrup 2002; Lundholm et al. 2002a, 2003; Priisholm et al. 2002), transfer of Nitzschia Hassall species to *Pseudo-nitzschia* (Hasle 1993; Takano 1995; Lundholm et al. 2002a), and numerous records of *Pseudo-nitzschia* spp. from all over the world (Hasle 2002), as well as studies of sexual reproduction (Fryxell et al. 1991; Davidovich & Bates 1998; Kaczmarska et al. 2000), physiology (Smith 1994; Bates et al. 2001; Maldonado et al. 2002; Fehling et al. 2004a; Lundholm et al. 2004) and phylogeny (Lundholm et al. 2002a, b, 2003; Orsini et al. 2002).

The distribution patterns of the various species are far from clear. However, based on the literature, some of the species seem to be restricted to particular latitudinal zones or temperature regions, whereas others seem to be cosmopolitan (Hasle & Syvertsen 1997; Hasle 2002). Further studies may reduce the number of cosmopolitan species, however, as occurred with *P. seriata* (Cleve) H. Peragallo (= *Nitzschia seriata*) when electron microscopy was first applied to *Pseudo-nitzschia* (Hasle 1965, 1972) and with other species when molecular approaches were introduced (Lundholm et al. 2003).

Hasle (1965) distinguished two forms within *P. seriata*, morphologically differentiated by valve outline, dimensions and the fine structure of the striae. These are f. *seriata* and f. *obtusa* (Hasle) Hasle. Published records indicate that the two forms may appear together in the North Atlantic Ocean, although in Arctic water f. *obtusa* is prevalent. There are apparently no well-documented records of f. *seriata* from the Pacific Ocean, but f. *obtusa* has been found in the North Pacific (Hasle 2002).

The difference in stria structure found in the two forms of *P. seriata* – two rows of poroids in f. *obtusa* but usually four in f. *seriata* – parallels the difference between *P. pungens* (Grunow ex Cleve) Hasle and *P. multiseries* (Hasle) Hasle, which was originally described as a form of *P. pungens*. Observations on the structure of the girdle and molecular studies demonstrated further differences between *P. multiseries* and *P. pungens* besides stria structure and so *P. pungens* *f. multiseries* was raised in rank to species (Hasle 1995; Manhart et al. 1995). *Pseudo-nitzschia multiseries* was the first diatom shown to produce DA, and still ranks among the most important DA producers. *Pseudo-nitzschia pungens* also produces DA (e.g. Trainer et al. 1998), but not to the same extent as *P. multiseries*. *Pseudo-nitzschia seriata* f. *seriata* is known to produce DA in cultures (Lundholm et al. 1994; Fehling et al. 2004b) and has also been implicated in the accumulation of high amounts of DA in mussels in several bays of northern Prince Edward Island, Canada, in spring 2002, leading to the closure of mollusc harvesting (Bates et al. 2002). *Pseudo-nitzschia seriata* f. *obtusa* has never been examined with respect to production of DA.

The valve and girdle structure, phylogenetic relationships and possible toxicity of *P. seriata* f. *obtusa* are examined in the present paper, and we discuss whether differences between f. *obtusa* and f. *seriata* justify elevation of f. *obtusa* to species rank. More records supporting the status of an Arctic distribution are added. We also make comparisons with the morphologically similar or phylogenetically related species, *P. americana* (Hasle) G.A. Fryxell, *P. australis* Frenguelli, *P. brasiliana* Lundholm, Hasle & G.A. Fryxell, *P. multistriata*...
Table 1. Strains used for the phylogenetic analyses.

| Species                        | Strain designation | Origin                          | Provided by                  | Accession number |
|--------------------------------|--------------------|---------------------------------|------------------------------|------------------|
| Pseudo-nitzschia seriata australis | au43               | Monterey Bay, California        | P. Miller and C. Scholin     | DQ062661         |
| P. australis                    | ØM1                | Aveiro, Portugal                | Ø. Moestrup                  | AY257842         |
| P. brasiliana                   | Xt3C               | Van Phong Bay, Vietnam          | J. Skov                      | DQ062662         |
| P. calliantha                   | DS2                | Do Son, North Vietnam           | J. Skov                      | AY257856         |
| P. delicatissima                | Larsø5             | Larso, Kattegat, Denmark        | N. Lundholm                  | AY257849         |
| P. fraudulenta                  | Limens1            | Monterey Bay, California        | K. Grostbol and N. Lundholm  | AY257840         |
| P. multiseriata                 | mu3                | Monterrey, California           | P. Miller and C. Scholin     | AY257844         |
| P. multiseries                  | OFPm984            | Ofunato Bay, Japan              | Y. Kotaki                    | DQ062664         |
| P. multistriata                 | KoreaA             | Chinhai Bay, Korea              | E. Cho                       | DQ062667         |
| P. seriata f. obtusa            | T5                 | Tromsø, Norway                  | H. Hegseth and N. Lundholm   | DQ062665         |
| P. pseudodelicatissima          | P-11               | Gafahna, Portugal               | N. Lundholm                  | AY257854         |
| P. pungens                      | P-24               | Costa Nova, Portugal            | N. Lundholm                  | AY257845         |
| P. pungens                      | Mex18              | near Tuxpam, Mexico             | Ø. Moestrup                  | AY257846         |
| P. pungens                      | KBH2               | Khan Hoa Bay, Vietnam           | Ø. Moestrup                  | DQ062665         |
| P. seriata                      | Lynæs 6            | Lynæs, Isefjord, Denmark        | N. Lundholm                  | DQ062663         |
| P. seriata                      | Lynæs 8            | Lynæs, Isefjord, Denmark        | N. Lundholm                  | DQ062666         |
| P. seriata                      | Nissum3            | Nissum Bredning, Denmark        | N. Lundholm                  | AY257841         |
| P. cf. subpacifica              | RdA8               | Ria de Arousa, Spain            | N. Lundholm                  | AY257860         |
| P. turgiduloides                | 3–19               | Ross Sea                        | N. Lundholm                  | AY257839         |

1 Sequences from the present study in bold.

(Takano) Takano, P. multiseries, P. pungens and P. turgidula (Hustedt) Hasle.

MATERIAL AND METHODS

Cultures and field samples

Two cultures of P. seriata f. obtusa (T5 and T26) were isolated from water samples from Sandnessundet, Tromsø, on 11 April 2002, and a Canadian culture was obtained from the Gulf of St Lawrence, Anticosti gyre (49°43’N, 66°15’W), in April 2003. Further information on the identities and origin of strains of all species is given in Table 1. Seven strains were sequenced for the present study (au43, Xt3C, OFPm984, T5, KBH2, Lynæs 6 and Lynæs 8), 12 strains had been sequenced previously (Lundholm et al. 2003). Only one strain of P. obtusa was sequenced because the two other cultures unfortunately died before material had been harvested for DNA extraction. All strains were clonal and nonaxenic. They were cultivated in L1-medium (Guillard & Hargraves 1993), based on autochlorivated seawater with a salinity of 32 psu. Stock cultures of P. seriata f. obtusa and f. seriata were cultivated at 4–5°C and other species at 15°C, with 15–25 μmol photons m⁻² s⁻¹ and a light : dark cycle of 16 h : 8 h. For morphological characters, more than 72 valves of P. seriata f. obtusa (37 of T5, 13 of T26 and 22 of the Canadian isolate) and more than 30 cingula (12 of T5, 6 of T26 and 12 of the Canadian isolate) were studied in detail from cultures. Fixed material or cleaned frustules of the strains ØM1, Limens1, OFPm984, T5, P-24, Mex18, Lynæs 6, Lynæs 8, Nissum3, RdA8 and 3–19 are deposited at the Botanical Museum in Copenhagen (Phycusat für Diatomeenkunde, the Alfred-Wegener-Institut für Polar-und Meeresforschung, Bremerhaven. The original uncleaned and acid cleaned samples are stored in the Department of Biology, University of Oslo.

Microscopy

The diatoms were cleaned and mounted in Hyrax or Naphrax (Bruel Microscopes, Wiltshire, UK), following the protocols of Hasle (1978) or Lundholm et al. (2002b). Light microscope observations (LM) and photography were done in Oslo using a Nikon Optiphot microscope (Nikon, Tokyo, Japan) equipped with phase contrast and Nomarski interference contrast optics and in Copenhagen using an Olympus BX60 microscope with an Olympus DP10 (Olympus, Hamburg, Germany) camera and Olympus DP-soft version 3.0. For transmission electron microscopy (TEM), drops of cleaned material were placed on Formvar-coated copper grids, dried and studied in R.C.A. EMU 2A (USA), JEOL – JEM 100C and 100CX (JEOL, Tokyo, Japan) electron microscopes at the EM Unit for Biological Sciences, University of Oslo, and in JEOL-100SX (JEOL) and JEM-1010 (JEOL) electron microscopes in Copenhagen. Terminology follows Hasle et al. (1996).

DNA extraction, amplification and sequencing

The ITS1, 5.8S and ITS2 regions of the nuclear ribosomal DNA (rDNA), which constitute some of the most variable regions of the rDNA, were sequenced or acquired from GenBank (Table 1). Extraction of DNA and polymerase chain reaction (PCR)–amplification followed Lundholm et al. (2002). The PCR-products were purified using QIA-QUICK (Qiagen, Hilden, Germany) as recommended by the manufacturer. A total of 20–40 ng of PCR-product was used in each 20 μl sequencing reaction. Nucleotide sequences were determined using Dye Terminator Cycle Sequencing Ready Reaction Kits (Perkin Elmer, Wellesley, MA, USA) as recommended by the manufacturer. The sequences of both PCR-amplification and sequencing primers were as given by Lundholm et al. (2003).
Alignment and phylogenetic analyses

New sequences were aligned manually with the sequences already published by Lundholm et al. (2003); strain designations, origins and accession numbers in Table 1) using BioEdit (Hall 1999). The alignment comprised 1082 aligned nucleotide positions, of which 691 were considered unambiguous and analysed; the remainder were excluded. The data were analysed using maximum parsimony (MP), maximum likelihood (ML) and distance methods, using PAUP* version 4.0b8 (Swofford 2001). All analyses performed were unrooted. Parsimony analyses were done using heuristic searches with random addition of sequences (1000 replicates) and a branch-swapping algorithm (tree-bisection reconnection). Gaps were treated as missing data and characters treated as multistate and unordered. Distance analyses were performed using neighbour joining (NJ) (1000 replicates), with the same parameters as in ML. The optimal model for ML analyses was found with a 99% level of significance using Modeltest version 3.06 (Posada & Crandall 1998). The significantly most optimal model was a Tamura–Nei substitution model (a b a e a) (Tamura & Nei 1993) with equal base frequencies. It included parameters for rate heterogeneity between sites. The proportion of invariable sites was estimated (0.483) and on the remaining sites a gamma distribution with a shape of 0.690 with four rate categories was applied. ML analyses were performed doing heuristic searches with 10 random addition replicates and the TBR branch-swapping algorithm. The exact parameters were estimated by doing consecutive heuristic searches and re-optimising parameters until the values of the parameters converged (Swofford et al. 1996).

Bootstrap analyses were used to determine the robustness of the acquired tree topologies (Felsenstein 1985). One thousand bootstrap replicates were performed in MP and NJ and 100 in ML. For the MP, ML and NJ bootstrap analyses, settings were as above mentioned for the respective analyses.

The phylogenetic analyses for the present tree were all performed as unrooted analyses because no rooting within Pseudo-nitzschia can be deduced from previously published trees because the basal branches within Pseudo-nitzschia are not well resolved (see Lundholm et al. 2002a, b, 2003; Orsini et al. 2002). Alignment with an outgroup taxa like Nitzschia frustulum (Kützing) Grunow (Lundholm et al. 2002b) was not possible because the sequences were too divergent.

Growth experiment and toxin analyses

Prior to the growth experiment, strain T5 of P. seriata f. obtusa was acclimated to the experimental conditions for 4 days. The inoculum used, was taken from an exponentially growing culture. Initial experimental concentrations were 2000 cells ml⁻¹ and the experiment was carried out in triplicate in 260 ml nucloon bottles (containing 260 ml medium) at 4–5°C. The cultures were grown in L-medium to which silicate was added.

Table 2. Records of Pseudo-nitzschia seriata f. obtusa.

| Number (IMBB) | Date         | Location                                      | Source                                      |
|--------------|--------------|-----------------------------------------------|---------------------------------------------|
| 1941         | 17 Sep. 1980 | 82°20’N, 45°56’E, Polar Sea                  | L. Edler, Sweden Ymer²-80-Exp.              |
| 565          | 10 Aug. 1973 | 80°20’N, 28°00’E, Barents Sea, Ice           | T. Benjaminsen, IMR¹, Norway                |
| 570          | 14 Aug. 1973 | 80°20’N, 26°30’E, Barents Sea, Under-surface of ice | T. Benjaminsen, IMR, Norway                 |
| 2088         | Aug./ Sep. 1981 | 80°05’N, 30°44’E, Barents Sea              | C.F. Forsberg, Norw. Polar Institute        |
| 1785         | 19 Jul. 1979 | 77°11’N, 33°28’E, Barents Sea, G.O.Sars St 659 | E.E. Syvertsen, University of Oslo          |
| 1784         | 18 Jul. 1979 | 77°02’N, 33°14’E, Barents Sea, G.O.Sars St 658 | E.E. Syvertsen, University of Oslo          |
| 1782         | 12 Jul. 1979 | 76°45’N, 30°04’E, Barents Sea, G.O.Sars St 551, at ice-edge | E.E. Syvertsen, University of Oslo          |
| 2449         | 10 Jun. 1984 | 76°45’N, 45°00’E, Barents Sea, G.O.Sars St 733 | S Kristiansen Pro Mare¹                      |
| 2546         | 6 May 1985   | 76°21’N, 20°39’E, Barents Sea, Polarbjørn St 41, under | C.H. von Quillfeldt, University of Oslo       |
| 2444         | 31 May 1984  | 75°15’N, 18°00’E, Barents Sea, G.O.Sars St 628 | S. Kristiansen Pro Mare                      |
| 527          | 30 Apr. 1962 | 70°30’N, 25°35’E, Hjellnes, Porsangen, Asterias, Norway | P. Hognestad, Tromsø Museum                  |
| Not known    | 1 Apr. 1965  | 69°42’N, 18°49’E, Tromsø, Norway             | not known                                   |
| Not known    | 31 Mar. 1976 | 69°42’N, 18°49’E, Tromsø, Norway             | not known                                   |
| Not known    | 23 Mar. 1962 | Vatterfjord, Norway                          | not known                                   |
| Not known    | 21 Mar. 1962 | 68°13’N, 14°30’E, Kabelvåg, Norway           | not known                                   |
| Not known    | 31 Mar. 1968 | 68°05’N, 15°10’E, Vestfjorden, Norway        | P. Campbell                                 |
| Not known    | 18 Mar. 1986 | Inner Trondheimsfjord, Norway                | not known                                   |
| Not known    | 28 Mar. 1968 | 63°08’N, 07°48’E, Kristiansund, Norway G.O.Sars St 565 | P. Campbell                                 |
| 1390         | 11 Apr. 1976 | 51°30’N, 54°00’W, New Foundland, Canada 2 m below | IMR, Norway                                 |
| Not known    | 25 Jul. 1950 | 61°05’N, 69°34’W, Hudson Strait             | not known                                   |
| 2039         | Not known    | c. 76°N, 85°W, Jones Sd, Canadian Arctic     | P. Lawrence                                 |
| 418          | 4 Aug. 1965  | 71.33°N, 156.30°W, Point Barrow, Alaska      | R. Horner, University of Seattle            |
| 432          | 20 Jul. 1970 | 71.33°N, 156.30°W                           | R. Horner, University of Seattle            |
| 2550         | 16 May 1955  | Kamchatka, Russia                            | V.V. Zernova, Institute of Oceanology, Moscow |
| 2551         | 17 May 1955  | 49°47’N, 155°34’E, Sea of Okhotsk, Russia    | V.V. Zernova, Institute of Oceanology, Moscow |

¹ IMBB (= Institute of Marine Biology, B) numbers refer to the diatom collection of Department of Biology, the University of Oslo.
² Ymer, name of a ship.
³ IMR, Institute for Marine Research, Norway.
⁴ Pro Mure, Norwegian Research Program for Arctic Marine Ecology.
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Fig. 1. Pseudo-nitzschia seriata, chain in girdle view, LM; culture isolated from Niva Bay, the Sound.

Fig. 2. Pseudo-nitzschia obtusa, chain in girdle view, LM; culture isolated from Tromsø, April 2002.

Fig. 3. Pseudo-nitzschia seriata, part of three perforated girdle bands, TEM; net sample, Toppsund, southern Norway, February 1976.

Fig. 4. Pseudo-nitzschia obtusa, part of valve and one perforated and two unperforated bands, arrows indicate the different girdle bands, TEM; culture as in Fig. 2.

at three times the amount prescribed, to ensure that the species was not silicate limited; the medium was adjusted to pH 8.0 by adding drops of 1 mM NaOH. The irradiance was 100 μmol photons m⁻² s⁻¹ and the light:dark cycle 16:8 h. Illumination was provided by cool fluorescent lamps and the irradiance measured using a Li-1000, Li-Cor sensor equipped with a Li-193SA spherical quantum probe (LI-COR, Lincoln, NE, USA).

Subsamples for cell counting (5 ml) and toxin analyses (2 × 10 ml) were taken at approximately the same time of the day at intervals of 1–4 days. Fresh medium was added to compensate for the subsample withdrawal. Samples for enumeration were fixed in Lugol’s solution (final concentration 2%) and live cells were counted in a Sedgewick–Rafter chamber. Differentiating between live and dead cells is difficult and was based on whether the cells still contained observable plastids or not. Each count was based on approximately 400 cells, corresponding to 95% confidence limits ± 10% (Utermöhl 1958). Maximum growth rate (μ) was calculated as μ = ln (Nt₂/Nt₁)/(t₂−t₁), where Nₜ₀ and Nₜ₁ are the cell numbers at the time t₁ and t₂. Three successive cell counts were used in the calculation and dilutions due to subsampling were adjusted for in the calculation of the growth rate.

Samples for determination of toxin content in the whole culture (cells and medium) were immediately frozen in plastic vials at −20°C and kept frozen until analyses for DA. The samples were thawed, sonicated under cool conditions (<10°C) and centrifuged. The supernatant was analysed using a modified version of Pocklington et al. (1990) as described in Kotaki et al. (2000) with a detection limit of 0.3 ng ml⁻¹.

RESULTS

Morphology

In girdle view, Pseudo-nitzschia seriata f. obtusa differs conspicuously from P. seriata f. seriata. Forma seriata has distinctly pointed ends (Fig. 1), whereas those of f. obtusa are truncate (Fig. 2). The overlap between cells in chains of f. obtusa is smaller (c. one-seventh to one-ninth of total cell length) than in f. seriata (c. one-third to one-fourth of total cell length) (Figs 1, 2). The two taxa also differ in number and structure of the striated bands. In f. seriata, there are three striated bands, which decrease in width towards the abvalvar edge of the cingulum (Fig. 3) and there is occasionally also a narrow unperforated band attached (not illustrated), whereas f. obtusa has one striated band (= valvocopula) next to the valve and one or two wide, unperforated bands abvalvarly (Fig. 4). The valvocopula striae of f. seriata are higher than wide, with two or three poroids transversely and four to six longitudinally (Fig. 3); by contrast, those of f. obtusa are square, with a 2 × 2 poroid arrangement (Figs 4, 5). As in all Pseudo-nitzschia species known so far, the width of the
bands decreases towards the distal ends and there is an accompanying reduction in the size and complexity of the band poroids (Fig. 6, Table 3).

The original description of f. obtusa as belonging in P. seriata was mainly based on the valve outline being asymmetrical. The asymmetry is less expressed than in f. seriata, however, and the valve ends taper less and the apices are more obtuse (Figs 7–15). As in f. seriata, the flatter side can be on the side bearing the raphe (Fig. 10) or the side distal to the raphe (Figs 7–9), reflecting the fact that the frustules are nitzschioi, with the raphes diagonally opposite. Forma obtusa is generally also smaller than the nominate form, especially in valve width (Figs 7–13, Table 3). The valve striae of f. seriata have two rows of hymenate poroids, each located close to the interstriae (Figs 16, 17), whereas there are usually four rows in f. seriata (Fig. 18), comprising two rows of larger poroids located as in f. obtusa, and two rows of smaller poroids in between (Fig. 18). The structure of the valve mantle reflects that of the valve striae, f. seriata having more poroids than f. obtusa (Figs 16–18).

Like P. seriata f. seriata, P. australis differs from P. seriata f. obtusa in girdle view by the shape of the valve ends and density and structure of band striae (Figs 26, 27, Table 3). In valve view, however, P. australis may in some respects appear more similar to P. seriata f. obtusa than P. seriata does, P. australis and f. obtusa both having rounded ends and both having two rows of poroids (Figs 4, 25). In contrast to f. obtusa, P. australis has a basically lanceolate outline with somewhat protracted ends (Figs 21–24), a lower density of poroids and a larger valve width (Table 3).

Distribution

Literature records characterize P. seriata f. obtusa as an Arctic species often associated with sea-ice. In the eastern North Atlantic, it has been found in the Barents Sea, east and north of Svalbard (Von Quillfeldt 2000), in Kings Bay and Hornsund, Svalbard (Hasle & Heimdal 1998; Wiktor & Hegseth 2003), Bear Island (Metzeltn & Witkowski 1996), Ullsfjord, North Norway (Heimdal 1974) and Norwegian coastal waters southwards to c. 63°N (Hasle et al. 1996), and in a crude culture derived from a sample from Helsingør, Denmark (Hasle 1965). From the western North Atlantic, it has been recorded from the Northeast Water Polynya, Greenland (Von Quillfeldt 1997), the New Polynia and the west coast of Greenland and Resolute Bay, Canada (Von Quillfeldt 2000), the Hudson Strait (Hasle 1965) and the Gulf of St Lawrence (Bérard-Therriault et al. 1999). The only records from the Pacific Ocean are from the Kuril Islands and Kamchatka (Hasle 2002).

Our new records (Table 2) strongly support the previous findings and show that P. seriata f. obtusa is a typical cold-water diatom. The limits of the Arctic region are somewhat diffuse (Lüning 1990), but the locations mentioned above, as well as those in Table 2, can all be regarded as belonging to this region. Restriction to the northern hemisphere is a feature f. obtusa shares with f. seriata. They differ, however, in that f. seriata has a wider latitudinal distribution, being recorded from temperate as well as Arctic regions of the North Atlantic, whereas f. obtusa has been recorded from the Arctic only. A further difference is the apparent absence of f. seriata from the Pacific (Hasle 2002).

Forma obtusa is also demonstrated to be a cold-water diatom by the seasonal variation in samples from Tromsø, Norway, where it occurs in the cold season only. Significant are also the records of f. obtusa at its northernmost locations, where it occurs during summer; further south, it is found only in spring (Table 2).

Phylogenetic inference

Pseudo-nitzschia seriata f. obtusa was found to belong to a clade (clade I; as in Lundholm et al. 2003) supported by bootstrap values of 100% and comprising P. seriata f. seriata, P. australis, P. pungens, P. multiseriata and P. brasiliiana (Fig. 19). Pseudo-nitzschia fraudulenta made up a sister taxon to clade I. Pseudo-nitzschia seriata f. seriata and P. australis strains formed two monophyletic groups. There was some support for a sister group relationship between the P. seriata–P. australis clade and P. seriata f. obtusa (MP and NJ but not ML). The calculated distances based on the ML settings were 0.000 among the P. seriata f. seriata as well as among the P. australis strains. Between P. seriata f. obtusa and P. australis–P. seriata the distances were larger (0.033 and 0.030) than the distance between P. australis and P. seriata f. seriata (0.012). The position of P. brasiliiana as a sister group to the P. seriata f. obtusa–P. australis clade was not supported by bootstrap values above 50.

If these branches that are not supported are collapsed, P. brasiliiana or P. multiseriata or a clade comprising P. multiseriata and P. pungens will appear as equally likely candidates for a sister group to the P. seriata f. obtusa–P. seriata–P. australis clade.

An alignment including only P. seriata, P. australis and P. seriata f. obtusa and one of the most closely related taxa, did not enable us to include further positions in the alignment. Only the exclusion of all taxa except P. seriata, P. australis and P. seriata f. obtusa made an alignment including further positions (actually all sequenced positions) possible. Using that alignment, we found 19 base-pair differences (10 in ITS1, 9 in ITS2) between P. seriata and P. australis. Between f. obtusa and either P. seriata or P. australis, we found 48 and 49 base-pair differences (nearly half of them in ITS1, the other half in ITS2), respectively. Of these base-pair positions where we found divergences between f. obtusa and one either P. australis or P. seriata, we observed that 38 positions were identical for P. seriata and P. australis, in three positions the bases differed for all three taxa and in seven and eight positions, respectively, f. obtusa were either identical with P. seriata or P. australis.

Growth and production of DA

The culture of P. seriata f. obtusa showed the typical growth pattern of a batch culture, with an exponential growth phase, a stationary growth phase and a senescent phase (Fig. 20). The growth rate in the exponential growth phase was 0.55 ± 0.03 day⁻¹. The pH of the medium rose from 8.0 during exponential growth phase, but stabilised at c. 9.1 when the culture entered stationary growth phase, and stayed at this level until the end of the experiment. DA was not detected in any of the triplicates in exponential, stationary or senescent phase (Fig. 20).
Table 3. Morphological characters of *P. seriata* f. *obtusa* and morphologically similar species.1

|                     | *P. seriata* f. *obtusa* | *P. seriata* | *P. australis* | *P. americana* | *P. brasiliiana* | *P. multistriata* | *P. pungens* | *P. multiseries* | *P. turgidula* |
|---------------------|---------------------------|--------------|----------------|----------------|-----------------|-------------------|--------------|----------------|-----------------|
| Valve shape         | slightly asymmetrical, tapering pointed ends | asymmetrical, tapering pointed ends | symmetrical, linear-lanceolate, rounded ends | symmetrical, linear-lanceolate, rounded ends | symmetrical, linear-lanceolate, rounded ends | symmetrical, linear-lanceolate, pointed ends | symmetrical, linear-lanceolate, pointed ends | symmetrical, linear-lanceolate, pointed ends | symmetrical, middle expansion, rounded ends |
| Girdle ends         | truncate                  | pointed      | pointed        | linear, truncate ends | truncate  | sigmoid and pointed ends | pointed      | pointed         | truncate        |
| Apical axis (μm)    | 39–81.5                  | 91–160       | 75–144         | 16–42          | 12–65         | 38–65             | 74–174       | 68–140          | 30–80           |
| Transapical axis (μm) | 2.9–5.0                | 4.6–8.0      | 6.5–8.0        | 2.5–4.0        | 1.8–3.0 (3.6) | 2.5–4.0           | 2.4–5.3      | 3.4–6.0         | 2.5–3.5         |
| Interstriae in 10 μm | 15–22                   | 14–20        | 12–18          | 26–31          | 20–26         | 37–44             | 9–16         | 10–19           | 23–28           |
| Fibulae in 10 μm    | 15–22                   | 14–20        | 12–18          | 18–24          | 20–26         | 23–32             | 9–16         | 10–19           | 10–13           |
| Central interspace  | no                       | no           | no             | no             | no             | no                | no           | yes             |                 |
| Rows of poroids per valve stria | 2                       | 2–more, usually 4 | 2              | 2 (3)          | 2 (3)         | 2 (1, 3)          | 2            | 3–4             | 2               |
| Poroids in 1 μm     | 6–8                      | 6–8          | 4–5            | 8–10           | 7–10          | 11–13             | 3–4         | 4–6             | 7–9             |
| Number of bands     | usually 3                | 3            | 4              | 3              | 3              | 3                | 3           | ND              |                 |
| Band striae in 10 μm | 28–32                   | 21–24        | 19–20          | 39–45          | 42–51         | 46–50             | 15–19       | 19–22           | ND              |
| Stria structure of bands (width × height in number of poroids) | I: 2 × 2–3, seldom 2 × 1 | I: 2–3 × 4–6 | I: 3–4 × 3–5 | I: 2 × 3 | I: 1 × 1 | I: (1) 2 × 3–4 | I: 1 × 1 | I: 2–3 × 4–5 | ND              |
|                     | II: more, bands without poroids | II: 2 × 2–3  | II: 2–4 × 2–3  | II: 1–2 × 1–2  | II: 1 × 1 | II: 1–3 poroid rows | II: 1 × 1 | II: 2 × 2       |                 |
|                     | III: 1–2 × 1–2, sometimes a fourth band without poroids | III: 2–3 × 1 | III: no striae | III: no striae | III: no striae | III: no striae | III: 1 × 1 | III: 1 × 1       |                 |

1 Based on Hasle (1965), Takano (1993), Hasle et al. (1996), Skov et al. (1999), Lundholm et al. (2002a), Orsini et al. (2002), Fryxell & Hasle (2003), Fehling et al. (2004b) and references herein.

2 ND, no data.

3 I, valvocopula, II, III or more, other copulae, numbered outwards from the valvocopula.
DISCUSSION

*Pseudo-nitzschia obtusa* is an independent species

Differences between *P. seriata* f. *obtusa* and f. *seriata* in morphology (Table 3), and ITS sequence (Fig. 19) justify raising *P. seriata* f. *obtusa* to species rank. The two taxa also have different geographical distribution. We therefore propose:

*Pseudo-nitzschia obtusa* (Hasle) Hasle & Lundholm, 
*comb. et stat. nov.*

**Basionym:** *Nitzschia seriata* f. *obtusa* Hasle (1974, p. 426; this validated the name proposed by Hasle 1965).

**SYNONYM:** *Pseudo-nitzschia seriata* f. *obtusa* (Hasle) Hasle 1993 (p. 319).

**Holotype:** IMBB slide no. 15, 70°30’N, 25°35’E, 30 April 1962.

**Comparison with other *Pseudo-nitzschia* species**

*Pseudo-nitzschia obtusa* is similar to *P. turgidula, P. brasiliiana* and *P. americana* in gross morphology, all having truncate ends in girdle view and rounded ends in valve view (Table 3; Hasle 1965, pl. 1. fig. 11; Lundholm et al. 2002a, figs 1, 3–5, 21–30; Fryxell & Hasle 2003, fig. 17.9, e–h; Kaczmarska et al. 2005, figs 1–13). *Pseudo-nitzschia turgidula, P.
brasili ana and P. americana, however, have symmetrical valves. In addition, P. turgidula and P. americana have a larger number of interstriae than fibulae and P. turgidula a larger central interspace with a central nodule (Hasle 1965, pl. 12, figs 1–6; Lundholm et al. 2002a, figs 14–17; Kaczmarska et al. 2005, fig. 7). Pseudo-nitzschia brasili ana and P. americana differ from P. obtusa in having a larger number of band striae and P brasili ana also by having one row of po roids in the first and second girdle band, whereas P. americ ana is more similar to P. obtusa in this respect (Lundholm et al. 2002a, figs 18–20, 43–45).

The truncate shape of the cell ends in girdle view is apparently also shared with several extremely narrow species (not included in Table 3), e.g. P. prolong atoides (Hasle) Hasle (Hasle 1965, pl. 1, fig. 8), P. micropora Priisholm, Moestrup & Lundholm (Priisholm et al. 2002, figs 1, 2, 4) and P. delicatissima (Cleve) Heiden (Hasle 1965, pl. 1, fig. 10a). However, unlike P. obtusa, these species have more valve striae than fibulae and differ also in other morphological features, e.g. the rostrate apices of P. prolong atoides and P. micropora.

The finding of a monophyletic clade, here referred to as clade I (Fig. 19) and comprising the species P. obtusa, P. seriata, P. australis, P. pungens, P. multiseries, P. multistriata and P. brasili ana, is supported by studies using the D1–D3 regions of the nuclear large subunit rDNA (Lundholm et al. 2002b; Orsini et al. 2002). All these species (including also P. americana; see Lundholm et al. 2002a) are characterized by the lack both of central raphe endings and a larger central interspace and by the presence of simple hymenate pores in striae and bands (Table 3; Hasle et al. 1996; Lundholm et al. 2002a; Orsini et al. 2002). The sister taxon to clade I, P. fraudulenta, on the other hand, possesses a central nodule and has pores with hymenate velum divided into several parts (sectors) (Hasle et al. 1996, fig. 58). This latter kind of velum also appears in some other non–clade I Pseudo-nitzschia species possessing a central nodule, viz. P. calliantha Lundholm, Moestrup & Hasle and, in two parts, in P. pseudodelicatissima (Hasle) Hasle and P. cuspidata (Hasle) Hasle (Lundholm et al. 2003, fig. 7, table 2).

Simple hymenate pores are, however, also found outside clade I; e.g. the smaller P. delicatissima with a central nodule, and P. micropora without a central nodule, has simple hymenate pores (Hasle et al. 1996, figs 73–75, table 2; Priisholm et al. 2002; Orsini et al. 2004, fig. 4e). A central nodule is absent also in species outside clade I, e.g. P. subcurvata (Hasle) G. Fryxell and P. micropora (Fryxell et al. 1991; Lundholm et al. 2002a; Priisholm et al. 2002). Three girdle bands per theca, sometimes with a fourth extremely narrow one attached and a great diversity of band stria structure have been found in clade I and non–clade I Pseudo-nitzschia species examined so far (Table 3; Hasle et al. 1996; Lundholm et al. 2002a, 2003; Orsini et al. 2002; Priisholm et al. 2002). Hence, absence of a central nodule and presence of simple hymenate pores and three to four girdle bands do not discriminate between species inside and outside clade I.

The species phylogenetically most closely related to P. obtusa, viz. P. australis, P. seriata and P. brasili ana, along with P. multiseries and P. pungens, share another morphological feature with P. obtusa, viz. approximately equal numbers of fibulae and striae (Fig. 19, Table 3). One could suppose that Pseudo-nitzschia multiseries and P. americana, which belong to clade I but have different numbers of fibulae and striae, appeared at the base of clade I. The basal branches in clade I are not supported, however, and the position of P. multiseries
and *P. americana* in clade I is therefore uncertain. Similarly, one cannot determine which taxa make up the sister group to the *P. obtusa*–*P. seriata*–*P. australis* clade. *Pseudo-nitzschia pungens* and *P. multiseries* differ from *P. obtusa* by having valves that are symmetrical with respect to the apical axis and pointed cell ends (Table 3). Further differences between *P. obtusa* and *P. pungens* are the coarser structure of the valve and the single row of poroids in the bands of *P. pungens* (Hasle et al. 1996, figs 30–35), but both species have valve striae with two rows of poroids (Table 3). The valve striae of *P. multiseries* have more than two rows of poroids, but *P. multiseries* resembles *P. obtusa* in possessing bands with more than one row of poroids (Table 3). *Pseudo-nitzschia multiseries* differs by appearing in sigmoid colonies in girdle view and by possessing more striae than fibulae (Takano 1993). In addition, the densities of the band striae are higher in *P. multiseries* (Orsini et al. 2002) than in *P. obtusa*.

*Pseudo-nitzschia seriata* and *P. australis* constitute the sister group to *P. obtusa*. The two species differ from *P. obtusa* in cell size, especially in valve width (Figs 7–13, 21–24, Table 3). Further morphological differences between *P. obtusa* and *P. australis*–*P. seriata* are found in the structure of the valve mantle and the bands, as well as the density of the poroids (Figs 3–5, 16, 18, 25–27, Table 3).

One of the other differences between *P. seriata* and *P. obtusa* is the number of rows of poroids. Most often four rows of poroids per valve stria are seen in *P. seriata*, but as seen in samples from Scotland and in laboratory cultures from Denmark grown at high temperatures (15°C), the middle rows of smaller poroids can be reduced to scattered poroids in between the two main rows (Fehling et al. 2004b, N. Lundholm, unpublished observations). In laboratory experiments with *P. seriata* grown at different temperatures, this was the only morphological character found to vary (N. Lundholm, unpublished observations).

The sequences of *P. seriata* (three Danish strains) and *P. australis* (one Portuguese and one Californian strain) differed by 19 positions in ITS1 and ITS2. Exactly the same differences are found in two Scottish strains of *P. seriata* and two Scottish strains of *P. australis*. Hence, presently there are no indications of intraspecific variability of the two species, unlike the large variation detected in *P. delicatissima* (Lundholm et al. 2000; Orsini et al. 2004).

The occurrence of *P. obtusa* with *P. seriata* or *P. australis*, in the northernmost parts of the Atlantic and the Pacific Oceans, respectively, shows that it is important to identify the various features, including band structure, that distinguish *P. obtusa* from these two other species. The reduced structure of the valve striae found in *P. seriata* valves from Scottish waters is an example of difficulties in identification of *Pseudo-nitzschia* species and it emphasizes the importance of using several morphological characters, here especially including the number and structure of the bands, for discrimination of the three species.

**Growth, pH and domoic acid production**

The growth of *P. obtusa* was similar to that of *P. seriata*, with growth rates of 0.55 and 0.65 day⁻¹, respectively, when grown under the same conditions (Fig. 20 and see Lundholm et al. 2004). In the growth experiment, nutrients were added in amounts that ensured that pH and not nutrients limited growth. The upper pH limit for growth of *P. obtusa* was 9.1, which is similar to the limit in *P. seriata* (9.0), *P. australis* (8.9) and several other *Pseudo-nitzschia* species (8.7–9.3) (Lundholm et al. 2004). DA was not detected during any growth phase in *P. seriata*, which is an example of difficulties in identification of *Pseudo-nitzschia* species and it emphasizes the importance of using several morphological characters, here especially including the number and structure of the bands, for discrimination of the three species.

**Fig. 19.** Phylogeny of *Pseudo-nitzschia* species inferred with maximum likelihood (unrooted analyses) using ITS1, 5.8S and ITS2 rDNA. The numbers are bootstrap values obtained by maximum likelihood, neighbour joining and parsimony analyses, respectively.

**Fig. 20.** Growth, pH and domoic acid in cells and medium as a function of time. Data points are mean ± SE, n = 3.
(e.g. Bates et al. 1989; Villareal et al. 1994). These differences in toxin production may be caused by genetic variation or by differences in environmental conditions. Several isolates of *P. seriata* from Danish coastal waters have all been found to produce DA in amounts comparable to *P. multiseries* (Lundholm et al. 1994; N. Lundholm, unpublished observations) and several toxic strains of *P. australis* have been detected (e.g. Garrison et al. 1992; Cusack et al. 2002). If these differences in toxin production have a genetic basis and are not caused purely by different environmental conditions, it is worth noting that closely related species like *P. obtusa* and *P. seriata*—*P. australis* apparently have different abilities with respect to production of DA and therefore only the toxic species would contain any genes responsible for production of DA. The most obvious explanation for the scattered presence of toxic and nontoxic species within the genus, however, is differential expression of genes due to environmental regulation of the genes responsible for production of DA. In that case, all the species could contain the genes responsible for the toxin production. At present, however, the gene(s) responsible for production of DA has not been identified.

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