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Morphology and phylogenetic relationships of some psychrophilic polar diatoms (Bacillariophyta)

by

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With 8 figures and 1 table

Abstract: Nine psychrophilic polar diatom species within six genera (*Chaetoceros*, *Fragilaria*, *Navicula*, *Nitzschia*, *Porosira* and *Stellarima*) were found near King Sejong Station, Maxwell Bay, King George Island, Antarctica and near Dasan Station, Ny-Ålesund, Svalbard, in the Arctic, in November 1998 and January 2005, respectively. These psychrophilic diatoms all thrived at approximately 2ºC under 25 µmol photons m^{-2} s^{-1}. We attempted to access the diversity of psychrophilic polar diatoms cultivated in the Korea Polar Research Institute (KOPRI) Culture Collections for Polar Microorganisms (KCCPM) and to establish the phylogenetic relationships among diverse diatoms based on morphological and molecular data. We determined 14 nuclear SSU rDNA and 18 plastid *rbc*L sequences and the data were deposited in GenBank. Our SSU tree recovered very similar divergence patterns to that described for previously published SSU trees. In the SSU tree, the species were generally placed in the deep branches of each clade. Although the phylogeny inferred from *rbc*L data was somewhat different from that from SSU data at higher taxonomic levels within the diatoms, *rbc*L data were more useful than SSU data for addressing phylogenetic affinities among closely related taxa.

Key words: psychrophilic polar diatoms, morphology, *rbc*L, SSU, phylogenetic relationships.

Introduction

The diversity of diatoms, which are found in the sea and freshwater and on damp rocks, soil and on ice, is exceptionally high, and includes some 285 genera with approximately 12,000 recognised species (Round et al. 1990, van den Hoek et al. 1995, Norton et al. 1996). In the polar region, where extensive permafrost, glaciers and sea ice limit the amount of photosynthesis possible on land and in marine environments, polar diatoms thrive under conditions of low temperature and high salinity. As a result, they are responsible for a significant proportion of polar primary productivity (Janech et al. 2006).

Most psychrophilic polar diatoms can live within a temperature range of -1.8 to 8 °C (Fiala & Oriol 1990) and can grow at temperatures as low as -15 °C under conditions of high salinity and extreme light limitation (Mock & Gradinger 1999). Most psychrophilic polar diatoms, however,

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thrive in open water and at the sea-ice-water interface at temperatures of approximately -1.8 to 5 °C (Mock & Valentin 2004).

Since 1989, we have collected psychrophilic polar diatoms near King Sejong Station, Maxwell Bay, King George Island, Antarctica, and more recently near Dasan Station, Ny-Ålesund, Svalbard, in the Arctic. From these collections, we have cultivated over 100 strains in the 2 °C culture room of the Korea Polar Research Institute (KOPRI) Culture Collections for Polar Microorganisms (KCCPM). In this study, we assessed the diversity of psychrophilic polar diatoms cultivated in the KCCPM and attempted to establish phylogenetic relationships among these diverse diatoms based on light microscopic and scanning electron microscopic observations as well as molecular investigations.

Materials and Methods

Cell isolation and cultivation

Strains of psychrophilic polar diatoms in the KCCPM were obtained from Marian Cove, Maxwell Bay, King George Island, Antarctica, and Ny-Ålesund, Svalbard, in the Arctic in November 1998 and January 2005, respectively (Table 1). The strains were isolated using sterile Pasteur pipettes, the cells repeatedly rinsed and grown in modified f/2 medium (Guillard & Ryther 1962). They were cultivated at approximately 2 °C under 25 µmol photons m^{-2} s^{-1} (24h light) in a low-temperature culture room.

Microscopy and taxonomic identification

All strains were characterised morphologically by observation under an Olympus BX-51 microscope (Tokyo, Japan) with Nomarski DIC optics and a JSM-5600 LV scanning electron microscope (JEOL, Japan). Samples for scanning electron microscopy (SEM) were fixed in 2% glutaraldehyde for 3 h at 4 °C, post-fixed in 1% osmium tetroxide at 4 °C for 2 h and rinsed with sodium cacodylate buffer (pH 7.5) after fixation. Specimens were then dehydrated through an ethanol series (30, 50, 70, 80, 90, 100, 100 and 100%; each stage for 10 min) and dried in hexamethyldisilazane. Finally, specimens were coated with gold-palladium using an MSC-101 (JEOL, Japan).

The terminology followed Anonymous (1975), Ross et al. (1975), Mann (1981) and Round et al. (1990). The classification followed those of Hasle & Syvertsen (1997) and Medlin & Kaczmarska (2004).

DNA extraction, amplification, sequencing, alignment and phylogenetic analyses

Collection information for the molecular investigations is provided (Table 1). Samples of 10 mL of medium containing growing cells were harvested by centrifugation at 12,000 rpm for 3 min and the pellets were washed with 1 mL of PBS buffer. Genomic DNA from samples was extracted using an Accuprep genomic DNA extraction kit (Bioneer, Daejeon, Korea).

The nuclear SSU rDNA and plastid *rbc*L were amplified from total genomic DNA using polymerase chain reaction (PCR) and the primer combinations of Saunders & Kraft (1994, 1996: E' [G01/G14] and F [G04/G07]) for SSU and Jung et al. (2006: A [Dia-F1/DiaR1], B [Dia-F2/DiaR2] and C [Dia-F3/DiaR3]) for *rbc*L. Direct purification using an Accuprep PCR purification kit (Bioneer, Daejeon, Korea) was used to purify the PCR products. DNA purified using this method was sequenced using the BigDye™ terminator cycle sequencing ready reaction kit (PE

Table 1. Collection locations and GenBank accession numbers for species used in this study. Psychrophilic polar diatoms detailed in this study are in bold. Biogeography information, although sketchy, was gathered from GenBank and Culture Collections (CCMP and CCAP). (–) indicates no data available.

Classification	Strain	Location	GenBank Accession No.	
			SSU rDNA	*rbc*L
BOLIDOPHYCEAE				
Bolidomonas pacifica	–	–	AF123596	AF372696
COSCINODISCOPHYCEAE				
Actinocyclus actinochilus	CCMP107	Antarctica	AY485506	–
Aulacoseira ambigua	FL8	–	AY569583	–
Aulacoseira ambigua	FR10	–	–	AY569606
Corethron criophilum (as *Corethron hystrix*)	p418	–	AJ535179	AY604696
Coscinodiscus radiatus	CCMP309	–	X77705	–
Guinardia delicatula	p146	–	AJ535192	–
Melosira varians	MV13	–	AJ243065	–
Podosira stelligera (as *Hyalodiscus stelliger)*	CCMP454	Wellington Harbor, New Zealand	AY485507	–
Proboscia alata	p433	Antarctica	AJ535181	–
Rhizosolenia setigera	CCMP1330	Martha's Vineyard, Mass., USA	AY485461	AF015568
Stellarima microtrias	**AnM003**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090011**	**EU090032**
Stellarima microtrias	CCMP1471	McMurdo Sound, Antarctica	AY485477	–
Stephanopyxis palmeriana	CCMP814	Gulf of Mexico	AY485527	–
MEDIOPHYCEAE				
Chaetoceros calcitrans f. *pumilus*	PCC537	–	AY485449	–
Chaetoceros curvisetus	ch.5	–	AY229895	–
Chaetoceros debilis	ch.4	–	AY229896	–
Chaetoceros didymus	–	–	X85392	–
Chaetoceros gracilis	UTEX LB 2375	–	AY625895	–
Chaetoceros gracilis	Schutt LB2658	Panama	–	AY604697
Chaetoceros muellerii	CCMP1316	Hawaii, USA	AY485453	–
Chaetoceros neogracile	**AnM0002**	**Near Ice-cliff, Marian Cove, King George Island, Antarctica**	**EU090012**	**EU090033**
Chaetoceros rostratus	–	–	X85391	–
Chaetoceros socialis	RR	–	AY485446	–

Classification	Strain	Location	GenBank Accession No.	
			SSU rDNA	*rbc*L
***Chaetoceros* sp.**	**ArM0004**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090013**	–
***Chaetoceros* sp.**	**ArM0005**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090014**	**EU090034**
Chaetoceros sp.	–	–	X85390	–
Cymatosira belgica	CCAP1018	Devon, England	X85387	–
Detonula confervacea	–	–	AF525672	AB018006
Ditylum brightwellii	CCAP1022/2	–	X85386	–
Eucampia antarctica	CCMP1452	McMurdo Sound, Antarctica	AY485503	–
Extubocellulus spinifer	CCMP393	Falmouth, Mass., USA	AY485504	–
Lauderia borealis	CCAP 1044/1	–	X85399	–
Leptocylindrus danicus	CCMP469	–	AY485501	–
Lithodesmium undulatum	–	–	Y10569	–
Minutocellus polymorphus	CCMP497	Bermuda	AY485478	–
Odontella aurita	CCMP1108	–	AY485522	–
Odontella sinensis	–	–	–	Z67753
Papiliocellulus elegans	–	–	X85388	–
Pleurosira laevis	–	–	AF525670	–
Porosira pseudodelicatula	CCMP1433	McMurdo Sound, Antarctica	AY485469	–
Porosira pseudodenticulata	AnM0008	Near shore, Marian Cove, King George Island, Antarctica	DQ436461	DQ108387
Porosira pseudodenticulata	**AnM0010**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090015**	**EU090035**
Porosira pseudodenticulata	R.M.C. 22	–	X85398	–
Skeletonema costatum	CCAP1077/4	Strait of Georgia, British Columbia, Canada	AY684946	–
Skeletonema costatum	CCMP1332	Milford, Connecticut USA	–	AF015569
Thalassiosira nordenskioeldii	CCMP997	Tromso, Norway	DQ093365	AB018007
Thalassiosira weissflogii	CCMP1049	Amityville, New York , USA	AY485445	–
BACILLARIOPHYCEAE				
Araphids				
Asterionellopsis glacialis	CCAP1009/1	–	X77701	–
Fragilaria islandica	p362	–	AJ535190	–
Fragilaria striatula	CCMP1094	Kachemak Bay, Alaska USA	AY485474	–

Classification	Strain	Location	GenBank Accession No.	
			SSU rDNA	*rbc*L
Fragilaria striatula	CCAP1029/18	Gwynedd, Wales	X77704	–
Fragilaria striatula	**AnM0005**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090016**	**EU090036**
Fragilaria striatula	**AnM0006**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090017**	**EU090037**
Fragilaria striatula	**AnM0011**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090018**	**EU090038**
Fragilaria sp.	p437	–	AJ535141	–
***Fragilaria* sp. 1**	**ArM0001**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090019**	**–**
***Fragilaria* sp. 1**	**ArM0006**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090020**	**EU090039**
***Fragilaria* sp. 1**	**ArM0007**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090021**	**EU090040**
***Fragilaria* sp. 2**	**AnM0017**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090022**	**–**
***Fragilaria* sp. 2**	**AnM0018**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090023**	**EU090041**
***Fragilaria* sp. 2**	**AnM0019**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090024**	**EU090042**
***Fragilaria* sp. 2**	**AnM0033**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090025**	**EU090043**
***Fragilaria* sp. 2**	**AnM0034**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090026**	**EU090044**
***Fragilaria* sp. 2**	**AnM0035**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090027**	**EU090045**
***Fragilaria* sp. 2**	**AnM0036**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090028**	**EU090046**
Grammatophora oceanica	CCMP410	Hobsons Bay, Victoria, Australia	AY485466	–
Rhaphoneis cf. *belgica*	–	–	X77703	–
Synedra ulna (as *Fragilaria ulna*)	FULN1	Clerve river, Luxembourg	AJ866993	–
Synedropsis hyperborea (as *Synedra hyperborea*)	CCMP1423	Baffin Bay, Arctic	AY485464	–
Tabularia tabulata	CCMP846	Kachemak Bay, Alaska, USA	AY485475	–

Classification	Strain	Location	GenBank Accession No.	
			SSU rDNA	*rbc*L
Thalassionema frauenfeldii	–	–	–	AY604698
Thalassionema nitzschioides	CCAP 1084/1	–	X77702	–
Raphids				
Achnanthes brevipes	CCMP101	Redondo Beach, CA, USA	AY485476	–
Achnanthidium cf. *longipes*	CCMP101	–	AY485500	–
Amphiprora alata	CCAP1003/3	Cumbria, England	AY485497	–
Amphora coffeaeformis	CCAP1008/1	–	AY485498	–
Bacillaria paxillifer	Mull Hend.	–	M87325	–
Cylindrotheca closterium	MGB0402	–	AY866418	AY866415
Cylindrotheca closterium	JZB-3C	–	–	DQ143047
Entomoneis alata	p540	–	AJ535160	–
Fragilariopsis cylindrus	CCMP1102	–	AY485467	–
Gyrosigma limosum	–	–	AY485516	–
Haslea ostrearia	UN	–	AY485523	–
Navicula atomus	NAPE1	Lembaach river , Luxembourg	AJ867024	–
Navicula cf. *duerrenbergiana*	–	–	–	AY571749
Navicula cryptocephala var. *veneta*	–	Hungary	AJ297724	–
Navicula diserta	p750	Germany	AJ535159	–
Navicula gelida	**ArM0002**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090029**	**EU090047**
Navicula gelida	**ArM0003**	**Near shore, Ny-Ålesund, Svalbard, Arctic**	**EU090030**	**EU090048**
Navicula glaciei	Nav. I	–	AY485460	–
Navicula glaciei	GGM-2004a	–	AY485513	–
Navicula lanceolata	–	–	AY485484	–
Navicula pelliculosa	CCMP 543	Martha's Vineyard, Mass., USA	AY485454	–
Navicula phyllepta	HP	–	AY485456	–
Navicula salinicola	–	–	–	AY604699
Navicula saprophila	NSAP2	–	AJ867025	–
Navicula sclesviscensis	–	–	AY485483	–
Navicula subminuscula	NSBM1	Alzette river, Luxembourg	AJ867026	–
Navicula ulvacea (as *Dickieia ulvacea)*	BH-sed-13	–	AY485462	–
Nitzschia acicularis	NACI1	Alzette river, Luxembourg	AJ867000	–

Classification	Strain	Location	GenBank Accession No.	
			SSU rDNA	*rbc*L
Nitzschia amphibia		Osceola Co., Rush Lake, IA, USA	AJ867277	–
Nitzschia closterium	RUG	–	AY485455	–
Nitzschia communis	–	–	AJ867278	–
Nitzschia constricta (as *Nitzschia apiculata)*	–	–	M87334	–
Nitzschia dissipata		Saone river, France	AJ867018	–
Nitzschia filiformis	NFIL1	Saone river, France	AJ866999	–
Nitzschia fonticola	–	Saone river, France	AJ867022	–
Nitzschia inconspicua	–	Casemates of Olivia, Ukraine	AJ867021	–
Nitzschia longissima	–	–	–	AY881967
Nitzschia palea	–	–	DQ288289	–
Nitzschia paleaeformis		Saone river, France	AJ866997	–
Nitzschia pusilla	NIPU1	Rollingerbaach river, Luxembourg	AJ867015	–
Nitzschia sigma	–	–	AJ867279	–
Nitzschia sp.	**AnM0026**	**Near shore, Marian Cove, King George Island, Antarctica**	**EU090031**	**EU090049**
Nitzschia sp.	MBIC11128	–	AB183668	–
Nitzschia supralitorea		Alzette river, Luxembourg	AJ867019	
Nitzschia thermalis	HP	–	AY485458	–
Nitzschia vitrea	FDCC L1276	–	AJ867280	–
Pauliella taeniata	CCMP1115	Baffin Bay, Arctic	AY485528	–
Phaeodactylum tricornutum	RUG	–	AY485459	AY819643
Pleurosigma intermedium	–	–	AY485489	–
Pseudo-nitzschia pungens	F310	–	U18240	–
Pseudogomphonema kamschaticum	E3460			AY571748
Stauroneis constricta	CCMP1120	Central Pacific, equatorial upwelling zone	AY485521	–

Applied Biosytems [ABI], Foster City, CA). For sequencing of the SSU, we used the primers G01, G02, G10 and G14 for the E' fragment and G04, G06 and G07 for the F fragment (Saunders & Kraft 1994). For sequencing of the *rbc*L, we used the same primers which were used in the PCR. Sequence data were collected using an ABI Prism 3100 genetic analyser. Sequence data were edited using the SeqEd DNA sequence editor (ABI) software package. The edited sequences were aligned relative to one another using the SeqPup multiple alignment program (Gilbert 1995).

The final alignment for SSU consisted of 115 taxa, including 94 previously published SSU sequences of species (Table 1) that were considered taxonomically related to the psychrophilic polar diatoms detailed in this study. The 1887 aligned nucleotide positions of SSU data were edited to remove the 5' and 3' PCR primer regions (G01 and G07; Saunders & Kraft 1994), as well as ambiguously aligned regions, to yield 1753 base pairs for phylogenetic inference.

The final alignment for *rbc*L consisted of 36 taxa, including 18 previously published *rbc*L sequences of species (Table 1) that were considered closely related to the psychrophilic polar diatoms detailed in this study. The 1408 aligned nucleotide positions of *rbc*L data were edited to remove the 5' and 3' PCR primer regions (Dia-F1 and Dia-R3; Jung et al. 2006), as well as ambiguously aligned regions, to yield 1325 base pairs for phylogenetic inference.

Maximum likelihood, distance and parsimony analyses were performed using PAUP* 4.0*b*10 for Macintosh (Swofford 2002). The SSU and *rbc*L data were also used to generate trees by a method related to the likelihood method, i.e., the Bayesian inference of phylogeny, using the program MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). The GTR+Γ+I model was used and 1,000,000 generations of four chains (burn-in: 62,300 and 17,400 for SSU and *rbc*L data, respectively) with sampling every 100 generations were used as the parameters for the analysis. The measure of nodal support is the probability of the presence for each node computed from the 624th and 175th trees to the 10,001st tree for SSU and *rbc*L data, respectively. Modeltest 3.06 (Posada & Crandall 1998) was used for maximum likelihood and distance analyses to determine the best model for the data. The best model was a general time reversible (GTR) model, with a gamma correction for among-site variation (Γ) and invariant sites (I). Distance analyses were completed using the neighbor-joining method (Saitou & Nei 1987) and were subjected to 1,000 rounds of bootstrap re-sampling (Felsenstein 1985). Maximum likelihood and parsimony analyses (unweighted, gaps treated as missing data) were completed under a heuristic search (5 and 100 random additions, respectively) with TBR branch swapping in effect. To estimate the robustness of internal nodes, bootstrap re-sampling was completed for the parsimony analysis (1,000 replicates; random additions set to 10). In all analyses, unrooted trees were calculated and the ingroup taxa were subsequently rooted with *Bolidomonas pacifica* in the Bolidophyceae as the designated outgroup (Guillou et al. 1999, Kooistra et al. 2003, Damsté et al. 2004, Medlin & Kaczmarska 2004, Sims et al. 2006).

Results

We found at least nine different species within six genera from Antarctica and the Arctic (Table 1). These findings were based on light and scanning electron microscopic observations of the strains cultivated in the KCCPM, as well as molecular investigations. We provide morphological descriptions of all species, with the exception of *Nitzschia* sp., from which we did not obtain any light microscopy (LM) or SEM images.

Morphological observations

Coscinodiscophyceae

***Stellarima microtrias* (Ehrenberg) G.R. Hasle & P.A. Sims** (1986: 111, figs 18–27)
(Fig. 1)
BASIONYM: *Coscinodiscus symbolophorus* Grunow (1884: 82, pl. 4, figs 3–6)
SYNONYM: *Coscinodiscus adumbratus* Østrup (1895: 461, pl. 8, fig. 90), *Coscinodiscus furcatus* Karsten (1905: 82, pl. 4, fig. 7), *Coscinodiscus pentas* (Ehrenberg) A. Mann (1907: 256), *Coscinosira stellaris* var. *symbolophora* (Grunow) Heiden, in Heiden & Kolbe (1928: 471), *Coscinodiscus signatus* A. Mann (1937: 46, pl. 4, fig. 8), *Podosira liotardii* Manguin (1960: 241, pl. 21, figs 252–254), *Symbolophora furcata* (Karsten) Nikolajev (1983: 1126, pl. 1, figs 8, 9).
MATERIALS EXAMINED: AnM0003 (water temperature, 2.7 °C; salinity, 30 psu; Nov. 1998, J.-S. Kang)
DESCRIPTION: Vegetative cells solitary or chain-forming, discoid to lenticular, attached to sibling cells at the center or off-center (Figs 1A,B). Valves flat to slightly convex, circular (Figs 1C,D), 15–20 µm; center of valve hyaline with two or three labiate processes (Figs 1D,E); processes slit-like externally (Fig. 1C), but internally raised and elongated (Figs 1D,E); marginal processes absent. Areolae arranged in furcated radial striae, 11–16 in 10 µm, loculate (not shown), externally simple (Fig. 1C) or with cribra, internally with foramina (Fig. 1E). Plastids numerous, irregular (Fig. 1B).
DISTRIBUTION: Type locality, Antarctic sea-ice, 78°10'S 162°W; Haakon VII Sea (Hustedt 1958), Scotia Sea (Garrison et al. 1987), Weddell Sea (Fryxell 1989), near Syowa Station, East Antarctica (Watanabe et al. 1990, Ishikawa et al. 2001), southern cold waters (Hasle & Syvertsen 1997), Antarctica (Scott & Thomas 2005), King George Island (Janech et al. 2006).
MOLECULAR DATA: The SSU rDNA sequence of AnM0003 is identical to that of *S. microtrias* (CCMP1471, McMurdo Sound, Antarctica; AY485477), except for a G in place of an indel at the 590th position and an indel instead of a G at the 1721st position in a sub-alignment.

Specimens (AnM0003) from King George Island, photographed by LM and SEM here, fit the descriptions of *S. microtrias*, but they are much smaller in size (15–20 µm in apical axis) than reported by other authors (35–105 µm in apical axis).

Mediophyceae

***Chaetoceros neogracile* S.L. VanLandingham** (1968: 733)
(Fig. 2A)
BASIONYM: *Chaetoceros gracile* F. Schütt (1895: 42, pl. 5, fig. 13)
SYNONYM: *Chaetoceros septentrionalis auct. non.* Østrup (1895), *sensu* Cleve (1896: 9, pl. 2, fig. 8).
MATERIALS EXAMINED: AnM0002 (water temperature, 1.0 °C; salinity, 28 psu; near ice-cliff, Nov. 1998, J.-S. Kang)
DESCRIPTION: Cells solitary, rectangular in girdle view, isovalvate (Fig. 2A); pervalvar axis 3–10 µm. Valves elliptical; apical axis 5–10 µm. Setae fine, smooth, diverging at 60–90° to the pervalvar axis (Fig. 2A). Plastids two per cell.
DISTRIBUTION: Weddell-Scotia Sea (Buck & Garrison 1983, Garrison et al. 1987), Ross Sea (Watanabe 1982), Southern Ocean (Priddle & Fryxell 1985); Bransfield Strait (Priddle 1985); Antarctica (Scott & Thomas 2005); King George Island (Janech et al. 2006).
MOLECULAR DATA: There is a substitution of C ↔ G at the 1248th position and an additional base C instead of an indel at the 1506th position between the SSU rDNA sequence of *C. neogracile* (AnM0002) and that of *Chaetoceros* sp. (X85390) in a sub-alignment.

That materials that we examined fit the description of *C. neogracile*, but are somewhat smaller in size (apical axis 5–10 µm) than reported by other authors (apical axis 6–10 µm).

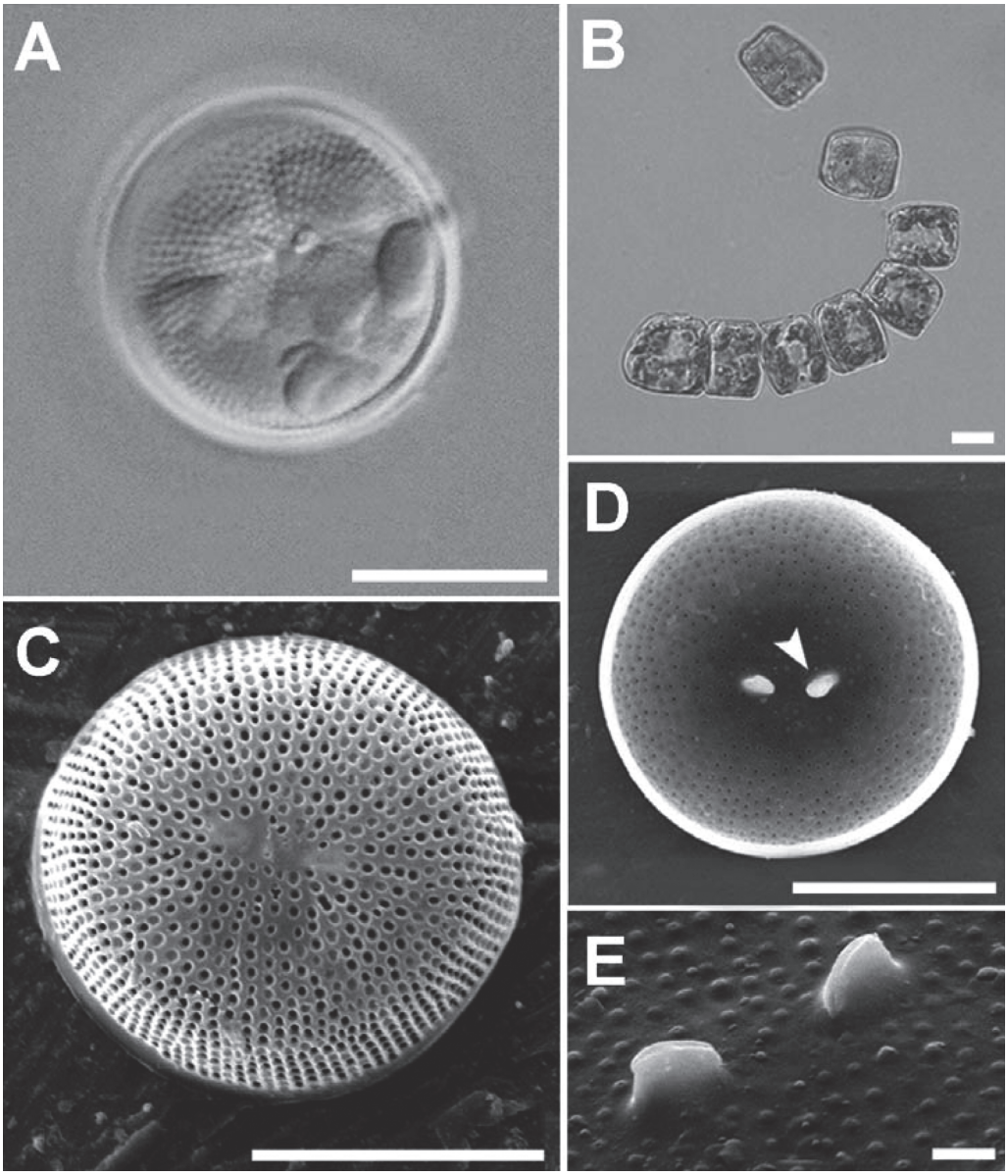


Fig. 1. *Stellarima microtrias*. **A.** Entire valve, LM. **B.** Chain of cells, LM. **C.** Valve external view, SEM. **D.** Valve internal view with two central labiate processes (arrow), SEM. **E.** Detail of labiate processes, SEM. Scale bars: A–D = 10 μm; E = 1 μm.

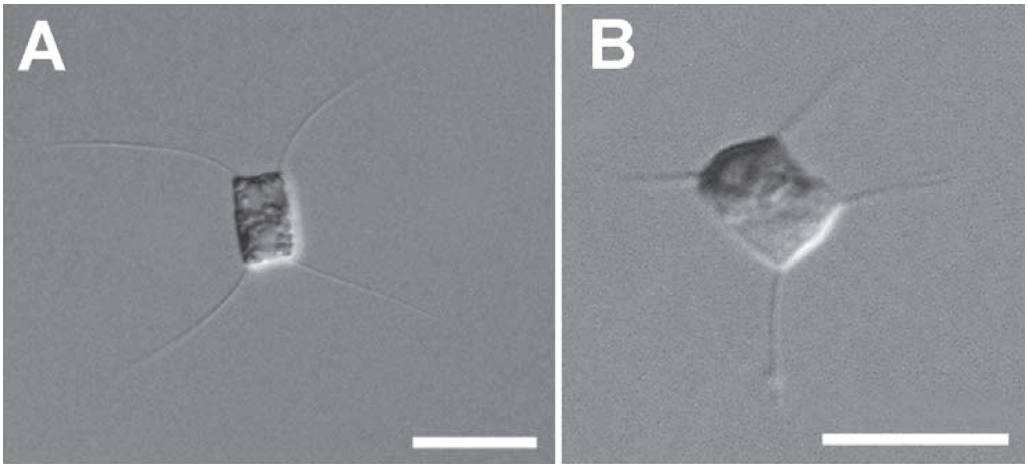


Fig. 2. *Chaetoceros* species. **A.** *Chaetoceros neogracile*, entire valve in LM. **B.** *Chaetoceros* sp., entire valve in LM. Scale bars = 10 µm.

***Chaetoceros* sp.**
(Fig. 2B)
MATERIALS EXAMINED: ArM0004 and ArM0005 (water temperature, 4.4 °C; salinity, 31 psu; May 2002, J.-S. Kang)
DESCRIPTION: Cells solitary, rectangular in girdle view, isovalvate (Fig. 2B); pervalvar axis 5–7 µm. Valves elliptical; apical axis 6–8 µm. Setae fine, smooth, diverging at 60–90° to the pervalvar axis (Fig. 2B). Plastids two per cell.
DISTRIBUTION: Ny-Ålesund, Svalbard, the Arctic (this study)
MOLECULAR DATA: The SSU rDNA sequence of ArM0004 is identical to that of ArM0005. There are 15–17 substitutions (0.84–0.96% divergence) between SSU rDNA sequences of ArM0004/ArM0005 and those of *C. neogracile* (AnM0002) and *Chaetoceros* sp. (X85390), which allied to ArM0004/ArM0005 with strong support (100% Bayesian posterior probabilities and replicates in distance and maximum parsimony analyses).

We did not obtain any SEM images of the material and thus defer taxonomic treatment of the species until such time that ultrastructural data of taxonomic relevance become available.

***Porosira pseudodenticulata* (Hustedt) Jousé**, in Kozlova (1962: 10, fig. 3, no. 2)
(Fig. 3)
BASIONYM: *Coscinodiscus pseudodenticulatus* Hustedt (1958: 117, figs 20, 21)
SYNONYM: ?*Podosira adeliae* Manguin (1960: 240, pl. 1, figs 10–12; pl. 21, figs 250, 251)
MATERIALS EXAMINED: AnM0010 (water temperature, 2.9 °C; salinity, 31 psu; Dec. 2000, J.-S. Kang)
DESCRIPTION: Cells solitary or attached by mucilaginous threads from the valve surfaces to form loose chains (Fig. 3B), discoid to cylindrical. Valves circular, 15–40 µm in diameter; pervalvar axis 10–30 µm. Areolation radial (Fig 3A); striae spiraling; areolae 20–30 in 10 µm, loculate, with external foramina and internal cribra (not shown). Strutted processes scattered over valve face, although sometimes sparse in the center (Figs 3A,C), 3–4 in 10 µm, simple or with short tubes from which mucilaginous threads emerge (not shown). Labiate process solitary, inside margin (Fig. 3C). Girdle bands with areolae similar to valves (not shown). Plastids small, discoid, numerous (Fig. 3B).

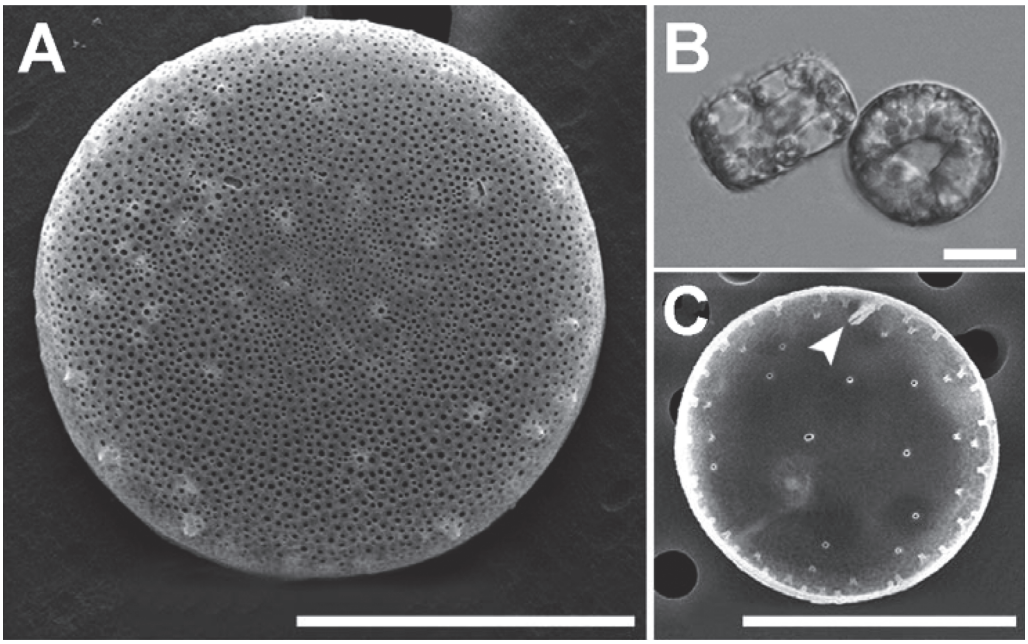


Fig. 3. *Porosira pseudodenticulata*. **A.** Entire valve, SEM. **B.** Entire cell, LM. **C.** Internal view of valve with a single labiate process (arrow). Scale bars = 10 µm.

DISTRIBUTION: Type locality, South Atlantic Ocean and Antarctic, *ca.* 44–69°S and 0–6°30' E (Hustedt 1958); Weddell-Scotia Confluence (Garrison et al. 1987); Antarctic circumpolar (Hasle 1973); Terra Nova Bay, Ross Sea (Andreoli et al. 1995); Lützow-Holm Bay (Tanimura et al. 1990); Sea-ice near Davis Station, East Antarctica, and the Southern Ocean, south of Australia (Scott & Thomas 2005).
MOLECULAR DATA: The SSU rDNA sequence of AnM0010 is identical to that of *P. pseudodenticulata* (DQ436461). Two substitutions, i. e., T ↔ C at the 1176th position and G ↔ T at the 1177th position, and an additional base G instead of an indel at the 576th position were found between the SSU rDNA sequence of AnM0010 and that of *P. pseudodenticulata* (X89398) in a sub-alignment. The *rbc*L sequence of AnM0010 was also identical to that of *P. pseudodenticulata* (DQ108387).

Specimens from King George Island, photographed under LM and SEM, fit the descriptions of *P. pseudodenticulata*, but are somewhat smaller in size and can have a higher density of areolae (20–30 in 10 µm) than reported by other authors (10–16 in 10 µm).

Bacillariophyceae – araphids

***Fragilaria striatula* Lyngbye** (1819: 183, pl. 63)
(Fig. 4)
MATERIALS EXAMINED: AnM0005, AnM0006 and AnM0011 (water temperature, 2.9 °C; salinity, 31 psu; Dec. 2000, J.-S. Kang)
DESCRIPTION: Cells united to form flat ribbon-like colonies (Fig. 4A); cells and colonies rectangular in girdle view (Fig. 4A); apical axis *ca.* 25–40 µm; transapical axis 7–9 µm. Valves lanceolate, with subacute apices (Figs 4B–D). Transapical striae fine, 17–19 in 10 µm, with a narrow hyaline axial area in the center (Figs 4B–D). Striae uniseriate; areolae poroid, *ca.* 40 in 10 µm (Figs 4B–D).

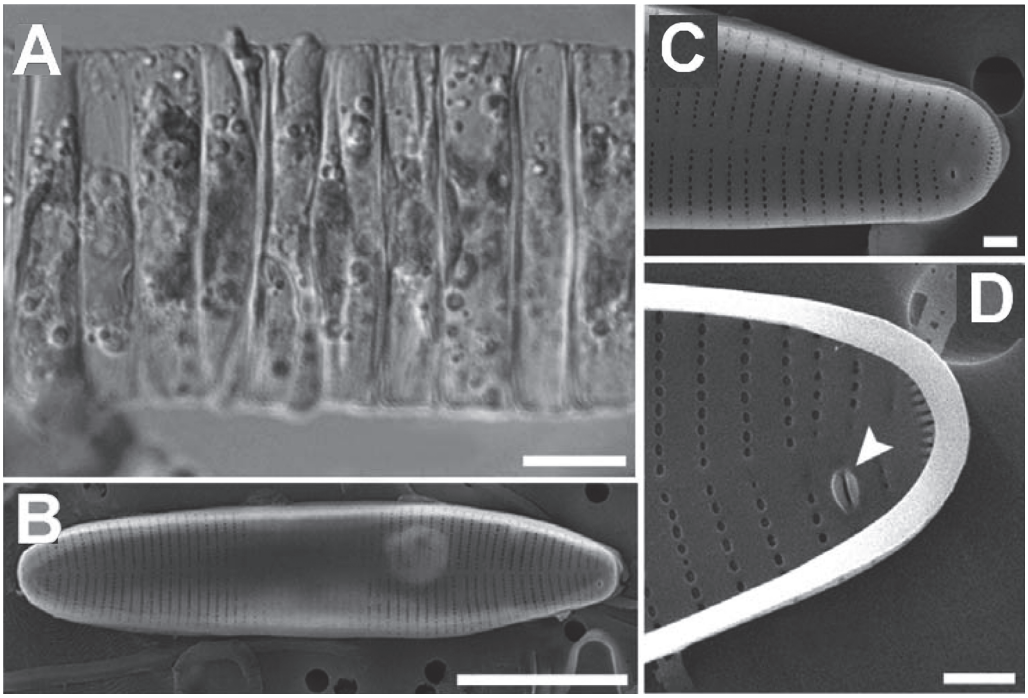


Fig. 4. *Fragilaria striatula*. **A.** Ribbon colonies, LM. **B.** Valvar view, SEM. **C.** Valve apex in external view, SEM. **D.** Single labiate process (arrow) in internal view. Scale bars: A–B = 10 µm; C–D = 1 µm.

Apical pore field comprising short rows of areolae. Labiate process slit-like, located at valve pole (Fig. 4D). Plastids two narrow plates lying along the valve (Fig. 4A).

DISTRIBUTION: Antarctica and the sub-Antarctic islands, Wilkes Land (Cremer et al. 2003); North America, Kachemak Bay, Alaska, USA (CCMP1094, http://ccmp.bigelow.org), Nova Scotia (Kim et al. 2004); Chile (Rivera & Cruces 2002).

MOLECULAR DATA: The SSU rDNA sequences of AnM0005, AnM0006, AnM0011, AnM0018 and AnM0036 were identical to those of *F. striatula* (AY485474) from Alaska and *F. islandica* (AJ535190), except for a mixed base Y (C or T instead of C in others) at the 628th position of AY485474 in a sub-alignment. The SSU data of *F. striatula* (X77704) from Wales, however, showed a 2-bp (0.11%) divergence from those of AnM0005, AnM0006, AnM0011, AnM0018, AnM0036, *F. striatula* (AY485474) from Alaska and *F. islandica* (AJ535190). The divergence of *rbc*L sequence data ranged from 1 to 4 bp (0.08–0.30%) among AnM0005, AnM0006 and AnM0011.

The specimens AnM0005, AnM0006 and AnM0011 from King George Island, photographed under LM and SEM, fit the descriptions of *F. striatula* by Hasle & Syvertsen (1981) and Rivera & Cruces (2002), having sizes of intermediate values to those reported by other authors (apical axis *ca.* 25–64 µm; transapical axis 6–10 µm).

***Fragilaria* sp. 1**
(Fig. 5A–C)
MATERIALS EXAMINED: ArM0001, ArM0006 and ArM0007 (water temperature, 4.4 °C; salinity, 31 psu; May 2002, J.-S. Kang)
DESCRIPTION: Cells united to form flat ribbon-like colonies (Fig. 5B); cells and colonies rectangular in girdle view (Fig. 5B); apical axis *ca.* 13–18 µm; transapical axis 5–7 µm. Valves ovate (Figs 5A,C). Transapical striae fine, 30–35 in 10 µm, with a narrow hyaline axial area in the center (Figs 5A,C). Striae uniseriate; areolae poroid, *ca.* 40 in 10 µm (Figs 5A,C). Apical pore field comprising short rows of areolae. Labiate process slit-like, located at valve pole (Fig. 5C). Plastids two narrow plates lying along the valve (Fig. 5B).
DISTRIBUTION: Ny-Ålesund, Svalbard, the Arctic (this study)
MOLECULAR DATA: The SSU sequence of ArM0001 was identical to that of ArM0007, and the *rbc*L sequence of ArM0006 was also identical to that of ArM0007. SSU data of ArM0001 and ArM0007, however, showed a 7-bp (0.40%) divergence from those of AnM0005, AnM0006, AnM0011, AnM0018, AnM0036, *F. striatula* (AY485474) from Alaska and *F. islandica* (AJ535190). The divergence of *rbc*L sequence data ranged from 64 to 72 bp (4.83–5.44%) among *Fragilaria* sp. 1 (ArM0001 and ArM0007), *F. striatula* (AnM0005, AnM0006 and AnM0011) and *Fragilaria* sp. 2 (AnM0018, AnM0019 and AnM0033-36).

Our specimens from Ny-Ålesund, photographed under LM and SEM, fit the descriptions of the genus *Fragilaria* by Williams & Round (1987) and Round et al. (1990). They are much smaller in size (apical axis 13–18 µm) than specimens of *F. striatula* from Maxwell Bay (AnM0005, AnM0006 and AnM0011; apical axis 25–40 µm).

***Fragilaria* sp. 2**
(Fig. 5D–G)
MATERIALS EXAMINED: AnM00018, ArM0019 and AnM0033-0036 (water temperature, 3.2 °C; salinity, 30 psu; Jan. 2002, J.-S. Kang)
DESCRIPTION: Cells united to form flat ribbon-like colonies (Fig. 5E); cells and colonies rectangular in girdle view (Fig. 5E); apical axis *ca.* 20–25 µm; transapical axis 7–10 µm. Valves lanceolate, with subacute apices (Figs 5D,F). Transapical striae fine, 13–17 in 10 µm, with a narrow hyaline axial area in the center (Fig. 5F). Striae uniseriate; areolae poroid, 45–50 in 10 µm (Fig. 5F). Apical pore field comprising short rows of areolae. Double labiate process slit-like, located at each valve pole (Fig. 5F). Plastids two narrow plates lying along the valve (Fig. 5E).
DISTRIBUTION: Maxwell Bay, King George Island, the Antarctic (this study)
MOLECULAR DATA: The SSU sequence of AnM00018 was identical to that of AnM0036 and the *rbc*L sequences of AnM00018, ArM0019 and AnM0033-0036 were identical. The divergence of *rbc*L sequence data ranged from 16 to 19 bp (1.21–1.44%) between these specimens and *F. striatula* (AnM0005, AnM0006 and AnM0011).

Our specimens from Maxwell Bay, photographed under LM and SEM, fit the descriptions of the genus *Fragilaria* by Williams & Round (1987) and Round et al. (1990), but are somewhat smaller in size (apical axis 20–25 µm) and have a higher density of areolae (45–50 in 10 µm) than specimens of *F. striatula* from Maxwell Bay (AnM0005, AnM0006 and AnM0011; apical axis 25–40 µm; areolae *ca.* 40 in 10 µm).

Bacillariophyceae – raphids

***Navicula gelida* Grunow** (1884)
(Fig. 6)
MATERIALS EXAMINED: ArM0002 and ArM0003 (water temperature, 4.4 °C; salinity, 31 psu; May 2002, J.-S. Kang)

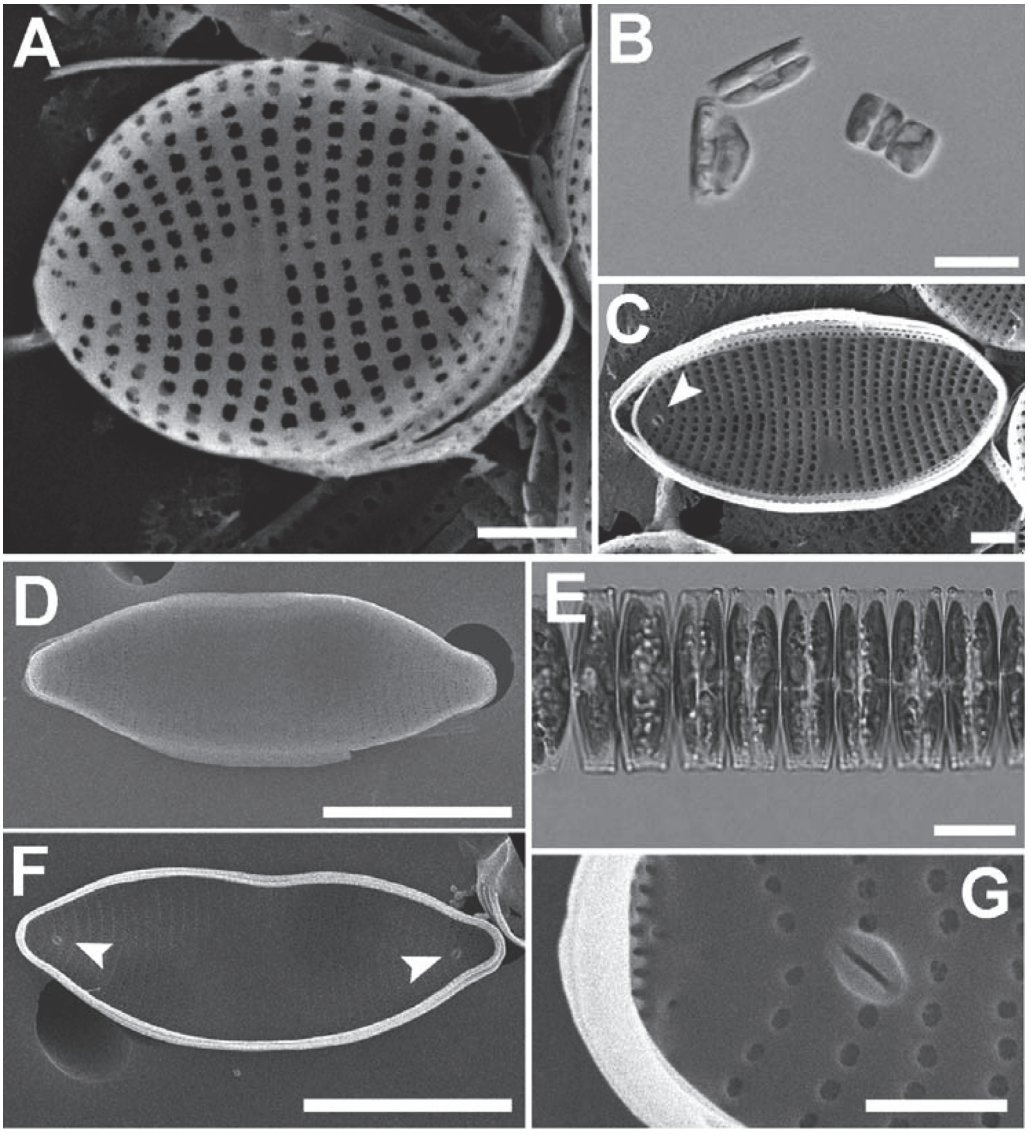


Fig. 5. *Fragilaria* spp. **A–C.** *Fragilaria* sp. 1. **A.** Valvar view, SEM. **B.** Colonies in LM. **C.** Single labiate process (arrow) in internal view. **D–G.** *Fragilaria* sp. 2. **D.** Valvar view, SEM. **E.** Ribbon colonies in LM. **F.** Internal view with double labiate processes (arrows). **G.** Detail of one labiate process, SEM. Scale bars: B, D–F = 10 µm; A, C, G = 1 µm.

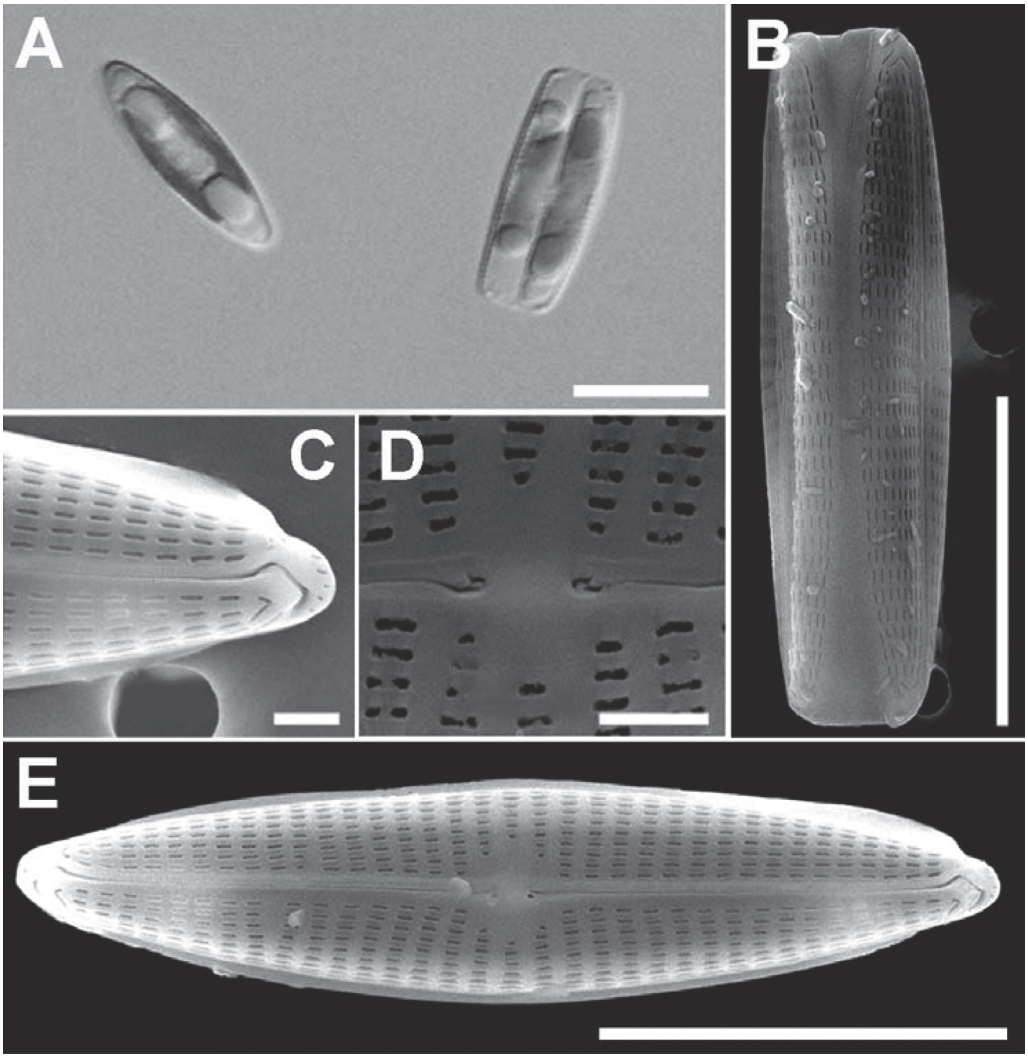


Fig. 6. *Navicula gelida.* **A.** General view, LM. **B.** Lateral view, SEM. **C.** Detail of terminal raphe ending, SEM. **D.** Central raphe endings. **E.** Cell in valve view, SEM. Scale bars: A, B, E = 10 µm; C, D = 1 µm.

DESCRIPTION: Cells solitary or joined together at valve surface, lanceolate (Fig. 6A); apical axis 17–29 µm (Fig. 6A,B,E); transapical axis 4–7 µm (Fig. 6A,E); pervalvar axis *ca*. 2.9 µm. Transapical striae 14–20 in 10 µm, straight but slightly radiating toward the cell apices (Fig. 6E). Raphe central; endings expanded into pores (Fig. 6D); terminal endings hooked in the same direction (Fig. 6C).

DISTRIBUTION: British Isles (Hendey 1974), Ny-Ålesund, Svalbard, Arctic (Hellum 1989; Hasle & Quillfeldt 1996; this study).

MOLECULAR DATA: The SSU and *rbc*L sequences of ArM0002 were identical to those of ArM0003. Ten substitutions (0.58 % of 1753 bp) were found between SSU rDNA sequences of AnM0002/

ArM0003 and that of *N. lanceolata* (AY485484), and were grouped together with strong support (100% Bayesian posterior probabilities and replicates in distance and maximum parsimony analyses).

The specimens from Ny-Ålesund (ArM0002 and ArM0003), photographed under LM and SEM, fit the descriptions of *N. gelida* by Hendey (1974) and *N. gelida* var. *parvula* Heiden by Scott & Thomas (2005), but they have a somewhat higher density of areolae (14–20 in 10 µm) than reported by Scott and Thomas (2005; 12–19 in 10 µm).

Phylogenetic relationships of psychrophilic polar diatoms based on molecular data

The 14 SSU and 18 *rbc*L sequences were newly determined. No ambiguities were observed in the SSU and *rbc*L data, and sequence data have been deposited in GenBank (Table 1).

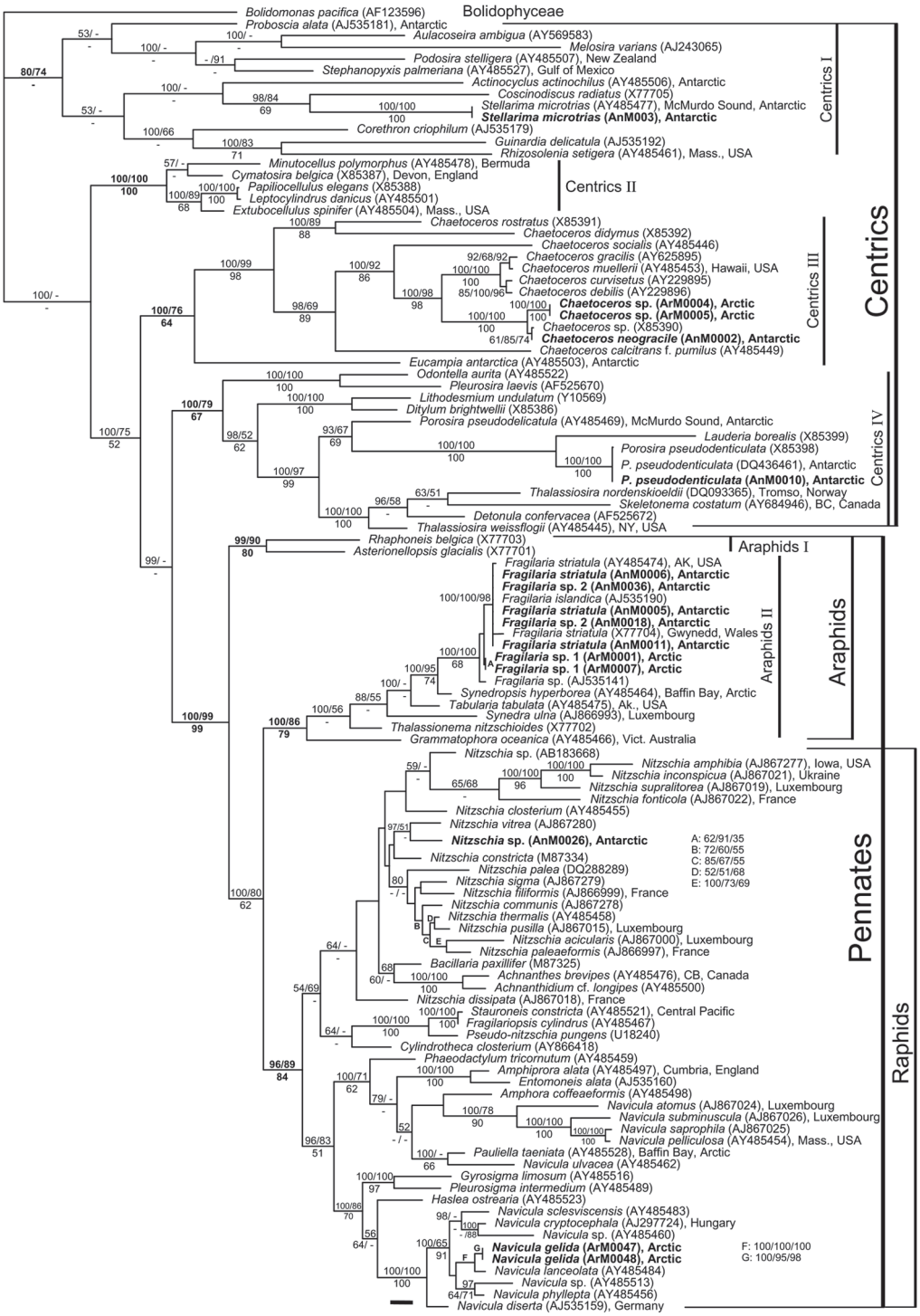
A tree for the SSU data, including 109 taxa and 1753 bp, was generated by Bayesian inference with posterior probabilities (BP) and bootstrap results from the distance (NJ) and maximum parsimony (MP) analyses appended (Fig. 7). Our analyses resolved four clades for the paraphyletic centric diatoms (centrics I–IV) and a single clade for the monophyletic pennate diatoms with moderate to strong support of BP values (80–100% in bold numbers in Fig. 7).

The first divergence among the diatoms was that of the Coscinodiscophyceae (centrics I) from all other diatoms; these are radial centric diatoms, including members of the Aulacoseirales (*Aulacoseira*), Corethrales (*Corethron*), Coscinodiscales (*Actinocyclus*, *Coscinodiscus* and *Stellarima*), Melosirales (*Melosira*, *Podosira* and *Stephanopyxis*) and Rhizosoleniales (*Guinardia*, *Proboscia* and *Rhizosolenia*). Next, the remaining centric diatoms (the Mediophyceae) paraphyletically diverged into three major clades, centrics II–IV. Centrics II was strongly supported (100% of BP, NJ and MP value); these are bipolar centrics, including the Cymatosirales (*Cymatosira*, *Extubocellulus*, *Minutocellus* and *Papiliocellulus*) and the Leptocylindrales (*Leptocylindrus*). Centrics III are bipolar centrics composed of the Chaetocerotales (*Chaetoceros* spp.) and the Hemiaulales (*Eucampia*). Centrics IV is a bipolar/multipolar/radial centrics complex that consists of the Triceratiales (*Odontella* and *Pleurosira*; bipolar), Lithodesmidales (*Ditylum* and *Lithodesmium*; multipolar) and Thalassiosirales (*Detonula*, *Lauderia*, *Porosira* and *Thalassiosira*; radial).

For the pennate diatoms, we resolved two clades for the paraphyletic araphids (araphids I and II) and a single clade for the monophyletic raphids with moderate to strong support (79–100% of BP, NJ and MP values). Araphids I included a member of the Fragilariales (*Asterionellopsis*) and Rhaponeidales (*Rhaphoneis*); araphids II consisted of most members of the Fragilariales (*Fragilaria* spp., *Synedra*, *Synedropsis* and *Tabularia*), Striatellales (*Grammatophora*) and Thalassionematales (*Thalassionema*). Finally, raphids consisting of the Achnanthales (*Achnanthes* and *Achnanthidium*), Bacillariales (*Bacillaria*, *Cylindrotheca*, *Fragilariopsis*, *Nitzschia* and *Pseudo-nitzschia*) and Naviculales (*Amphiprora*, *Gyrosigma*, *Haslea*, *Navicula*, *Pauliella*, *Phaeodactylum*, *Pleurosigma* and *Stauroneis*) were strongly supported (96, 89 and 84 % of BP, NJ and MP values, respectively).

In the SSU tree, the specimens from near Maxwell Bay, King George Island, Antarctica and Ny-Ålesund, Svalbard, the Arctic were included in the centrics I (*Stellarima microtrias*), centrics III (*Chaetoceros* spp.), centrics IV (*Porosira pseudodenticulata*), araphids II (*Fragilaria* spp.) and raphids (*Navicula gelida* and *Nitzschia* sp.) clades. They were generally placed in the deep branches of each clade.

Our specimens of *Fragilaria* spp. in the SSU tree were resolved into two clades, the Antarctic, Alaska and Wales clade (including *F. islandica* and *F. striatula* from Alaska, Wales and the Antarctic, AnM0005 and AnM0006; and *Fragilaria* sp. 2, AnM0018 and AnM0036) and the Arctic clade (consisting of *Fragilaria* sp. 1, ArM0001 and ArM0007). *Nitzschia* sp. (AnM0026) from Antarctica was allied with *N. vitrea* (AJ867280) with weak to strong support, but the phylogenetic relationships among other *Nitzschia* spp. were unresolved.


Bolidophyceae
Bolidomonas pacifica (AF123596)
Proboscia alata (AJ535181), Antarctic
Aulacoseira ambigua (AY569583)
Melosira varians (AJ243065)
Podosira stelligera (AY485507), New Zealand
Stephanopyxis palmeriana (AY485527), Gulf of Mexico
Actinocyclus actinochilus (AY485506), Antarctic
Coscinodiscus radiatus (X77705)
Stellarima microtrias (AY485477), McMurdo Sound, Antarctic
Stellarima microtrias (AnM003), Antarctic
Corethron criophilum (AJ535179)
Guinardia delicatula (AJ535192)
Rhizosolenia setigera (AY485461), Mass., USA
Minutocellus polymorphus (AY485478), Bermuda
Cymatosira belgica (X85387), Devon, England
Papiliocellulus elegans (X85388)
Leptocylindrus danicus (AY485501)
Extubocellulus spinifer (AY485504), Mass., USA
Chaetoceros rostratus (X85391)
Chaetoceros didymus (X85392)
Chaetoceros socialis (AY485446)
Chaetoceros gracilis (AY625895)
Chaetoceros muelleri (AY485453), Hawaii, USA
Chaetoceros curvisetus (AY229895)
Chaetoceros debilis (AY229896)
Chaetoceros sp. (ArM0004), Arctic
Chaetoceros sp. (ArM0005), Arctic
Chaetoceros sp. (X85390)
Chaetoceros neogracile (AnM0002), Antarctic
Chaetoceros calcitrans f. pumilus (AY485449)
Eucampia antarctica (AY485503), Antarctic
Odontella aurita (AY485522)
Pleurosira laevis (AF525670)
Lithodesmium undulatum (Y10569)
Ditylum brightwellii (X85386)
Porosira pseudodelicatula (AY485469), McMurdo Sound, Antarctic
Lauderia borealis (X85399)
Porosira pseudodenticulata (X85398)
P. pseudodenticulata (DQ436461), Antarctic
P. pseudodenticulata (AnM0010), Antarctic
Thalassiosira nordenskioeldii (DQ093365), Tromso, Norway
Skeletonema costatum (AY684946), BC, Canada
Detonula confervacea (AF525672)
Thalassiosira weissflogii (AY485445), NY, USA
Rhaphoneis belgica (X77703)
Asterionellopsis glacialis (X77701)
Fragilaria striatula (AY485474), AK, USA
Fragilaria striatula (AnM0006), Antarctic
Fragilaria sp. 2 (AnM0036), Antarctic
Fragilaria islandica (AJ535190)
Fragilaria striatula (AnM0005), Antarctic
Fragilaria sp. 2 (AnM0018), Antarctic
Fragilaria striatula (X77704), Gwynedd, Wales
Fragilaria striatula (AnM0011), Antarctic
Fragilaria sp. 1 (ArM0001), Arctic
Fragilaria sp. 1 (ArM0007), Arctic
Fragilaria sp. (AJ535141)
Synedropsis hyperborea (AY485464), Baffin Bay, Arctic
Tabularia tabulata (AY485475), Ak., USA
Synedra ulna (AJ866993), Luxembourg
Thalassionema nitzschioides (X77702)
Grammatophora oceanica (AY485466), Vict. Australia
Nitzschia sp. (AB183668)
Nitzschia amphibia (AJ867277), Iowa, USA
Nitzschia inconspicua (AJ867021), Ukraine
Nitzschia supralitorea (AJ867019), Luxembourg
Nitzschia fonticola (AJ867022), France
Nitzschia closterium (AY485455)
Nitzschia vitrea (AJ867280)
Nitzschia sp. (AnM0026), Antarctic
Nitzschia constricta (M87334)
Nitzschia palea (DQ288289)
Nitzschia sigma (AJ867279)
Nitzschia filiformis (AJ866999), France
Nitzschia communis (AJ867278)
Nitzschia thermalis (AY485458)
Nitzschia pusilla (AJ867015), Luxembourg
Nitzschia acicularis (AJ867000), Luxembourg
Nitzschia paleaeformis (AJ866997), France
Bacillaria paxillifer (M87325)
Achnanthes brevipes (AY485476), CB, Canada
Achnanthidium cf. longipes (AY485500)
Nitzschia dissipata (AJ867018), France
Stauroneis constricta (AY485521), Central Pacific
Fragilariopsis cylindrus (AY485467)
Pseudo-nitzschia pungens (U18240)
Cylindrotheca closterium (AY866418)
Phaeodactylum tricornutum (AY485459)
Amphiprora alata (AY485497), Cumbria, England
Entomoneis alata (AJ535160)
Amphora coffeaeformis (AY485498)
Navicula atomus (AJ867024), Luxembourg
Navicula subminuscula (AJ867026), Luxembourg
Navicula saprophila (AJ867025)
Navicula pelliculosa (AY485454), Mass., USA
Pauliella taeniata (AY485528), Baffin Bay, Arctic
Navicula ulvacea (AY485462)
Gyrosigma limosum (AY485516)
Pleurosigma intermedium (AY485489)
Haslea ostrearia (AY485523)
Navicula sclesviscensis (AY485483)
Navicula cryptocephala (AJ297724), Hungary
Navicula sp. (AY485460)
Navicula gelida (ArM0047), Arctic
Navicula gelida (ArM0048), Arctic
Navicula lanceolata (AY485484)
Navicula sp. (AY485513)
Navicula phyllepta (AY485456)
Navicula diserta (AJ535159), Germany
A: 62/91/35
B: 72/60/55
C: 85/67/55
D: 52/51/68
E: 100/73/69
F: 100/100/100
G: 100/95/98
Centrics I
Centrics II
Centrics III
Centrics
Centrics IV
Araphids I
Araphids II
Araphids
Pennates
Raphids

A tree for the *rbc*L data consisting of 36 taxa and 1325 bp was generated by Bayesian inference with BP, NJ and MP analyses appended (Fig. 8). The first divergence among the diatoms was the Corethrales (*Corethron*), a member of the radial centrics belonging to the Coscinodiscophyceae, similar to the analysis of SSU data. The remainder of diatoms diverged into two major clades, with weak to moderate supports of BP values (60–88 %): centric diatoms, including the Chaetocerotales (*Chaetoceros* spp., bipolar), Coscinodiscales (*Stellarima*, radial), Rhizosoleniales (*Rhizosolenia*, radial), Triceratiales (*Odontella*, bipolar) and Thalassiosirales (*Detonula*, *Porosira* and *Thalassiosira*; radial); and pennate diatoms, consisting of araphid diatoms [the Fragilariales (*Fragilaria* spp.) and Thalassionematales (*Thalassionema*)]; and raphid diatoms [the Bacillariales (*Bacillaria*, *Cylindrotheca* and *Nitzschia*), Naviculales (*Navicula* and *Phaeodactylum*)]. Unexpectedly, the radial centric diatom *Aulacoseira ambigua* (Aulacoseirales) was grouped with the araphid diatom *Thalassionema frauenfeldii* (Thalassionematales).

The *rbc*L tree differed from the SSU result in: i) the relative positioning of the Chaetocerotales (*Chaetoceros* spp.) and Thalassiosirales (*Detonula*, *Porosira* and *Thalassiosira*) among the centric diatoms; ii) failing to associate *Odontella* with the Thalassiosirales in the centric diatoms; and iii) associating *Aulacoseira* with *Thalassionema* (araphid pennate diatom), rather than within the centric diatoms. However, none of these relationships received any NJ or MP bootstrap support, except for moderate to strong support in BP values (79–96%).

In the *rbc*L tree, our specimens of *Fragilaria* spp. from the Antarctic and Arctic were resolved into three distinct clades: the *F. striatula* (AnM0005, AnM0006 and AnM0011), *Fragilaria* sp. 1 (ArM0006 and ArM0007) and *Fragilaria* sp. 2 (AnM0018, AnM0019 and AnM0033-36) clades. The *rbc*L sequence data for AnM0018, AnM0019 and AnM0033-36 are identical, and those of ArM0006 and ArM0007 were also identical. The divergence of *rbc*L sequence data, however, ranged from 16 to 19 bp (1.20–1.43%) and from 64 to 72 bp (4.81–5.41%) between each group [(*F. striatula*, AnM0005/AnM0006/AnM0011 and *Fragilaria* sp. 2, AnM0018/AnM0019/AnM0033-36) and *Fragilaria* sp. 1, ArM0006/ArM0007].

Discussion

Hasle & von Quillfeldt (1996) listed marine diatoms of Svalbard, the Arctic, including 101 species and six forms and varieties. Recently, Scott & Thomas (2005) described 196 diatom species and intraspecific taxa from Antarctic waters and the Southern Ocean. We found six species, including two only determined to genus (*Fragilaria* sp. 2 and *Nitzschia* sp.) from Maxwell Bay, King George Island and three species, including two only determined to genus (*Chaetoceros* sp. and *Fragilaria* sp. 1) from Ny-Ålesund, Svalbard, and investigated their morphologies and nuclear SSU and plastid *rbc*L sequence data. These psychrophilic diatoms all thrived at approximately 2 °C under 25 µmol photons m^{-2} s^{-1} (24h light). However, at temperatures > 10 °C their growth slowed and they died.

Our specimens of *S. microtrias*, *C. neogracile*, *P. pseudodenticulata* and *N. gelida* were much smaller in size or had a somewhat higher density of areolae than reported by other authors (Hasle & Sims 1986; Scott & Thomas 2005), indicating that cells can become smaller during subculture for 5–9 years.

Fig. 7. Tree constructed with Bayesian inference for the SSU alignment (GTR+Γ+I model). Taxonomic labels are based on the system of classification presented here (Table 1). Values at branches represent BP (top left value) and 1000 bootstrap replicates each for NJ and MP (top right and lower values, respectively). Branches lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.

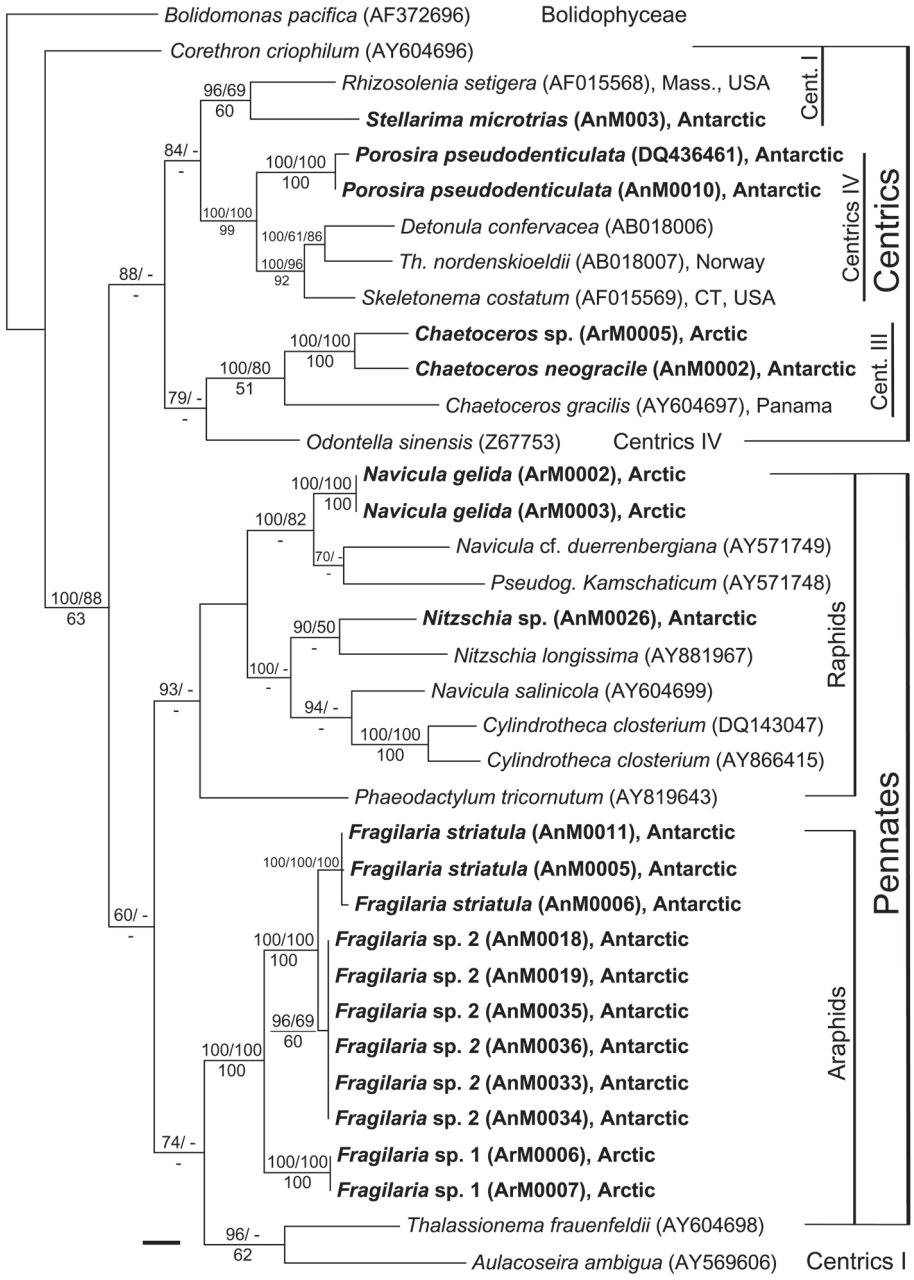


Fig. 8. Tree constructed with Bayesian inference for the *rbc*L alignment (GTR+Γ+I model). Values at branches represent BP (top left value) and 1000 bootstrap replicates each for NJ and MP (top right and lower values, respectively). Branches lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.

No SEM or LM images of *Chaetoceros* sp. and *Nitzschia* sp. materials from Ny-Ålesund were obtained. Thus, we defer taxonomic treatment of these species until such time as ultrastructural data of taxonomic relevance become available.

Specimens of *F. striatula*, *Fragilaria* sp. 1 and *Fragilaria* sp. 2 from King George Island and Ny-Ålesund, photographed under LM and SEM, fit the descriptions of the genus *Fragilaria* (Hasle & Syvertsen 1981; Williams & Round 1987), except that the genus is now regarded as an exclusively freshwater one (Williams & Round 1987; Round et al. 1990; Williams 2006). We did not have any molecular data for the type species of the genus, *F. pectinalis* (O.F. Müller) Lyngbye, thus we leave these species in this genus until such time as molecular data for proper phylogenetic affinities become available.

The SSU tree (Fig. 7) recovered very similar divergence patterns to the SSU tree of Medlin & Kaczmarska (2004): centric diatoms were resolved in more than four paraphyletic clades; pennate diatoms were also resolved as two paraphyletic araphids and a single monophyletic raphid with moderate to strong support (80–100% of BP values). In the tree, our species from near Maxwell Bay, King George Island, Antarctica and Ny-Ålesund, Svalbard, the Arctic, were generally placed in the deep branches of each clade, indicating that the species, especially *Chaetoceros* spp. and *Fragilaria* spp., from Antarctica and the Arctic have evolved relatively recently.

A topology similar to that of the SSU tree was obtained in the *rbc*L tree (Fig. 8), except for i) the relative positioning of the Chaetocerotales (*Chaetoceros* spp.) and Thalassiosirales (*Detonula*, *Porosira* and *Thalassiosira*); ii) failing to associate *Odontella* with the Thalassiosirales; and iii) associating a centric diatom *Aulacoseira* with *Thalassionema* (an araphid pennate diatom). Alhough the phylogeny inferred from *rbc*L data with the limited taxon sampling available from GenBank was somewhat different from that inferred from SSU data at higher taxonomic levels within the diatoms by long branch attraction, the *rbc*L data may be more powerful than the SSU data for determining relationships between closely related taxa (see Edgar & Theriot 2004). In the *rbc*L tree, our specimens of *Fragilaria* spp. from Antarctica and the Arctic were resolved into three distinct clades, i. e., the *F. striatula*, *Fragilaria* sp. 1, and *Fragilaria* sp. 2. with divergences ranging from 16 to 19 bp (1.20–1.43%) and from 64 to 72 bp (4.81–5.41%) between each group [(*F. striatula* and *Fragilaria* sp. 2) and *Fragilaria* sp. 1], whereas they were unresolved in the SSU data.

We found nine psychrophilic diatoms from Antarctica and the Arctic. An important consequence of our results is the establishment of a database for psychrophilic polar diatoms based on morphological observations and molecular investigations in the KOPRI culture collections for polar microorganisms.

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