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ORIGINAL PAPER

Comprehensive comparisons of three pennate diatoms, *Diatoma tenuae*, *Fragilaria vaucheriae*, and *Navicula pelliculosa*, isolated from summer Arctic reservoirs (Svalbard 79°N), by fine-scale morphology and nuclear 18S ribosomal DNA

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Abstract Here we report morphological and molecular characteristics of dominant freshwater diatoms in summer Arctic reservoirs of Svalbard (Norway), using four culture isolates, when we collected the samples in the field on 15 August 2005. Analyses of morphology and BLAST searches with 18S rDNA sequences identified them to *Diatoma tenue* (HYNP006, HYNP013), *Navicula pelliculosa* (HYNP021), and *Fragilaria vaucheriae* (HYNP022), respectively. Comparative studies of morphology revealed that the body shapes of the three polar diatoms were nearly identical to the known morphology of each species; however, they were considerably shorter in body length than previously described identical species from other locations. The 18S rDNA sequences of the diatoms were nearly identical to the same species from temperate and other regions. Phylogenetic analysis showed that the polar diatoms each formed a clade with their identical species and genera according to their taxonomic positions. This suggests that the polar diatoms may possess little or no genetic or morphological variation compared to more temperate strains.

Keywords Svalbard · Arctic · Diatom · Freshwater · *Diatoma* · *Fragilaria* · *Navicula* · Morphology · Phylogeny

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Introduction

The Arctic is now experiencing some of the most rapid and severe climate change on earth (Arctic Climate Impact Assessment 2005; Richter-Menge et al. 2006). Kongsfjorden, located on the northwestern coast of Svalbard (Spitsbergen, Norway), has received a lot of research attention in recent years as a high-latitude monitoring site for the impacts of climate change (Hop et al. 2002; Wiencke et al. 2004) and for the study of environmental change in the High Arctic resulting from anthropogenic activities. Because it is located at the Arctic (79°N) and geographically isolated from the large industrial areas of Europe, Asia, and North America, local development is minimal (Rose et al. 2004). To date, considerable works have been conducted on freshwater diatoms in Svalbard (e.g. Foged 1964; Picinska-Faltynowicz 1988; Beyens and Van de Vijver 2000), which generally revealed a low species richness, and the majority of sites are dominated by benthic diatom genera such as *Fragilaria*, *Navicula*, and *Achnanthes,* based on sediment analyses (Picinska-Faltynowicz 1988; Jones and Birks 2004).

Aside from sediment diatom records, many limnological and phycological works (e.g. Miller et al. 1992; Sorvari et al. 2002; Laybourn-Parry and Marshall 2003; Cremer et al. 2005; Brutemark et al. 2006) have been done from the Arctic areas, particularly including Alaska, Greenland and Siberia; however, morphological studies of the polar diatoms are rare from the high-latitude regions of the Arctic (e.g. Douglas and Smol 1993, 1995) and Antarctic. Most of the previous studies have been based on ecological monitoring and diatom surveys under a light microscope. Some examples include: high Arctic stream diatom assemblages from Cornwallis Island, Canada (Antoniades and Douglas 2002), the distribution of diatoms in lakes of the Bunger Hills, East Antarctica (John et al. 2006), and diatom

communities in small water bodies at H. Arctowski Polish Antarctic Station (Kawecka et al. 1998). Many uncertainties remain regarding the proper identities of the diatoms from such studies, however, because of morphological similarities and the microscopic size of cells within closely related species such as *Cymbella, Fragilaria,* and *Navicula*. Furthermore, many morphological features are known to vary in response to changing environmental conditions and different growth stages, and are therefore considered unreliable for correct taxonomic identification.

The above ambiguities have been resolved in part by employing molecular techniques (e.g. PCR, immuno-assay, DNA sequencing) for proper identification and comparisons (Bates et al. 1993; Ki et al. 2004; Ki and Han 2005, 2006; Evans et al. 2007). Commonly, the ribosomal DNA (rDNA) sequences are used in taxonomic and diagnostic signatures of microorganisms, including diatoms (Karsten et al. 2006). So far, only few DNA sequences (e.g. 18S, 28S, mitochondrial genes) from polar freshwater diatoms have been revealed in the public database (e.g. GenBank). Owing to a deficiency of the genetic and morphological data, diatom identities sometimes remain ambiguously in polar environments.

In this paper, we established four culture isolates (three different species) of diatoms dominant in summer temporary reservoirs of Svalbard, and then described their fine morphological features by means of light and scanning electron microscopy. In addition, molecular analyses were performed with 18S rDNA sequences obtained from the strains to clarify their taxonomic identities, and to improve understanding of genetic variations within the diatoms. Finally, we examined the morphological and genotypic characteristics of these polar freshwater diatoms in comparison with the same species from temperate regions.

Materials and methods

Study site

The Arctic reservoirs on Spitsbergen Island, Svalbard, may be covered by ice and snow from the end of September until the beginning of August the following year. Low water temperature and prolonged darkness constrain yearly organic production. Furthermore, drainage from the glaciers in summer carries a lot of sediment particles, which may cause the primary production in these lakes to be the lowest in the world (Lund 1983). At our study area (Fig. 1), the Kongsfjord (Spitsbergen, Svalbard, Norway), covered ice regularly breaks up between April and early June, often within a few days (Gerland et al. 1999). Water samples for microalgal species were collected from the temporary reservoirs of the study areas (78.55.5N, 11.56.0E) located in the Kongsfjord (Ny-Alesund, Spitsbergen, Norway) on 15 August 2005. At

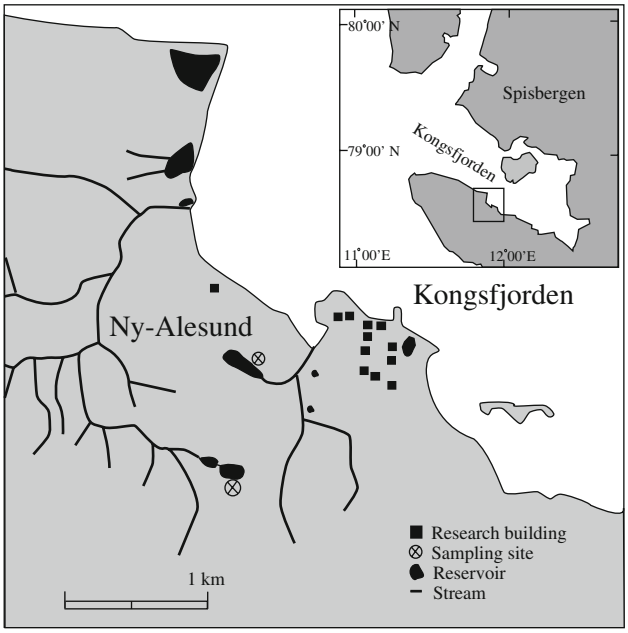

Fig. 1 Sampling sites in the Kongsfjorden at Ny-Alesund, Norway

that time, water temperature, salinity and conductivity ranged from 5 to 10°C, 0.1 to 0.3 psu and 0.21–0.36 μS cm^{-1}, respectively (Ki et al. 2006). Diatom medium (DM) (Beakes et al. 1988) was added to the collected waters and samples were kept at 4°C in a cold chamber incubator.

Cultures

After 5 days, a portion of the incubated waters was subsequently inoculated into fresh DM. The sample was then incubated at 4°C for a week. The culture was then inoculated again with DM and incubated at 10°C under 30–50 μE s^{-1} m^{-2} with a 12:12 light:dark cycle. A portion of the culture was poured onto solidified DM medium with 1.2% agar. The agar plate was incubated for a month under dim light (10–30 μE s^{-1} m^{-2}) at 10°C. Brownish small colonies were picked under a binocular scope (SZX12, Olympus, Tokyo, Japan) and incubated in 96 well plates containing 200 μl of DM. The grown cells, confirmed as unialgal cultures under an inverted light microscope (Axiovert 100, Zeiss, Jena, Germany), were again inoculated into liquid culture. In the end, we obtained four strains of diatoms (HYNP006, 013, 021, and 022). The diatom cells had reached exponential growth stage in 7–10 days after inoculation (e.g. Katano et al. 2007). All the isolates were subcultured with fresh DM at about 20-day intervals.

Morphological observation

Morphological characteristics were observed with a light microscope (LM) (Axioplan, Zeiss, Jenna, Germany)

equipped with a differential interference contrast and CCD camera. The diatom cells (HYNP006, 021, 022) in the exponential growth stage were examined for body length, chloroplast shape, and nucleus position. Average body length and width were calculated by measuring more than 40 cells. Digital images were taken with a cooled CCD camera (Sensys Photometrics, München, Germany), and analyzed with Image-pro Plus 3.1 software (Media Cybernetics, Silver Springs, MD).

To observe the whole shape and fine internal structures of the diatoms with scanning electron microscopy (SEM), diatom cultures were fixed in 2% glutaraldehyde for 24 h at 4°C, followed by post-fixing in 1% osmium tetroxide at 4°C for 30 min. After fixation, the cells were rinsed with phosphate buffers (pH 7.2). Specimens were then dehydrated by a graded ethanol series (30, 50, 70, 90, 100 and 100%; each stage for 30 min) and dried with a Critical-Point Dryer (SPI-DRY CPD, SPI, USA). Finally, specimens were coated with gold for 3 min, and then examined in the SEM (JSM-5600 LV, Jeol, Japan). In addition, organic compounds were removed from fixed cells by concentrating samples to near dryness, adding an equal amount of HNO_3 and three times the sample amount of H_2SO_4, and finally boiling for 3 min. Next, the samples were rinsed with distilled water to completely remove the acid. Finally, the specimens were observed with the above SEM method.

DNA extraction

Approximately 50 ml of clonal cultures at mid-logarithmic phase were harvested by centrifugation at 2,000 rpm for 10 min. The concentrated cells on the mesh were transferred to 1.5 ml micro tubes, 100 µl of TE buffer (10 mM Tris–HCl, pH 8.0 and 1 mM EDTA) was added and the tubes were stored at −20°C until DNA extraction. Genomic DNA was isolated from the stored cells using the DNeasy Plant mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

PCR and DNA sequencing

PCR primers, based on conserved sequences among several microalgal species, were developed. A set of PCR primers was used for amplification of 18S rDNA (forward AT18F01, 5′-YACCTGGTTGATCCTGCCAGTAG-3′ and reverse AT18R01, 5′-GCTTGATCCTTCTGCAGGTT CACC-3′ [International Union of Pure and Applied Chemistry degeneracy symbols were used]) from the genomic DNA of each diatom.

PCR reactions were carried out in 1 × PCR buffer (10 mM Tri–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), to which <0.1 µg genomic DNA template, 200 µM each of four dNTPs, 0.5 µM each of primers, and 0.2 Units *Taq* polymerase (Promega, Madison, WI) per 10 µl reaction were added. Using a thermoblock (iCycler, Bio-Rad), PCR thermocycling parameters were as follows: 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s. After the cycles, final extension was completed at 72°C for 5 min. The PCR-amplified products were analyzed by agarose gel electrophoresis according to standard methods.

For direct DNA sequencing, PCR amplicons were purified with QIAquick PCR purification Kit (Qiagen GmbH, Germany). DNA sequencing reactions were performed in the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, CA) using the PCR products (2 µl) as the template and 10 picomoles of identical PCR and nested flanking primers. Labeled DNA fragments were analyzed on a Model 3700 automated DNA sequencer (Applied Biosystems, CA).

Editing and contig assembly of the DNA sequence fragments were carried out with Sequencher 4.1.4 (Gene Codes, MI). Their molecular identities were briefly determined by comparisons with the available DNA sequences from each genus and high-scoring rDNA sequences in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) search algorithm. All sequences determined here were deposited to GenBank as accession numbers EU260466 to EU260469.

Phylogenetic analysis

For sequence comparison, nearly compete 18S rDNA sequences determined in the present study (Table 1) were aligned with related and pennate diatom sequences retrieved from GenBank/DDBJ/EMBL using ClustalW (Thompson et al. 1994). The aligned sequences were trimmed at the 5′ and 3′ ends to the same length. Various regions were further aligned manually, and regions that could not be aligned unambiguously were excluded from the analysis. The remaining alignment sites were 1,660 out of the total 1,903 alignment positions for the subsequent analyses. In a phylogeny of diatoms, Modeltest 3.07 (Posada and Crandall 1998) was used in order to find the optimal model of DNA substitution for maximum-likelihood construction. Substitution models were selected by both hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC). As the best-fit model for the above dataset, the Tamura and Nei (1993) model with invariable sites and gamma distribution (TrN + I + G) was chosen by hLRT and the GTR + I + G model was chosen by AIC. A Bayesian

Table 1 Taxa and GenBank accession numbers utilized in this investigation

Taxonomic position	Species	Strain	Isolation locality	GenBank acc. no.
Bacillariales	Cylindrotheca closterium	MGB0501		DQ019446
Bacillariales	*Cylindrotheca closteriva*			M87326
Bacillariales	*Cylindrotheca fusiformis*	CCMP339		AY485457
Bacillariales	*Fragilariopsis curta*		Antarctica: Mertz glacier	EF140623
Bacillariales	*Fragilariopsis cylindrus*		Antarctica: Mertz glacier	AY672802
Bacillariales	*Fragilariopsis sublineata*			AF525665
Bacillariales	*Nitzschia communis*	FDCC L408		AJ867278
Bacillariales	*Nitzschia longissima*			AY881968
Bacillariales	*Nitzschia palea*			DQ288289
Bacillariales	*Nitzschia sigma*	FDCC L1546		AJ867279
Bacillariales	*Nitzschia thermalis*	HP		AY485458
Bacillariales	*Pseudo-nitzschia australis*	POMX		AM235384
Bacillariales	*Pseudo-nitzschia multiseries*	tka2		U18241
Bacillariales	*Pseudo-nitzschia pungens*	F310		U18240
Cymbellales	*Cymbella cymbiformis*	L254	USA:	AJ535156
Cymbellales	*Encyonema triangulatum*	L1313	USA:	AJ535157
Cymbellales	*Gomphonema parvulum*		Hungary:	AJ243062
Cymbellales	*Gomphonema pseudaugur*	M-0130	Japan: Tama River	AB085833
Eunotiales	*Eunotia cf. pectinalis f. minor*	L474	USA:	AJ535146
Eunotiales	*Eunotia formica var. smatrana*	A-0045	Japan: Saino-ko Pond	AB085830
Fragilariales	*Asterionella formosa*			AF525657
Fragilariales	*Asterionella japonica*	CCMP138		AY485463
Fragilariales	*Asterionellopsis glacialis*	CCAP 1009/1		X77701
Fragilariales	*Asterionellopsis kariana*	CCAP 1009/2		Y10568
Fragilariales	*Diatoma hyemalis*		Japan: Tanbara mire	AB085829
Fragilariales	*Diatoma tenue*	HYNP006	Norway: Ny-Alesund	EU260466
Fragilariales	*Diatoma tenue*	HYNP013	Norway: Ny-Alesund	EU260467
Fragilariales	*Diatoma tenue*	L1251	USA:	AJ535143
Fragilariales	*Fragilaria barbararum*	ROS D99	Norway: Kongsfjorden	AJ971376
Fragilariales	*Fragilaria cf. islandica*			AJ535190
Fragilariales	*Fragilaria cf. striatula*	ROS D125	Norway: Kongsfjorden	AJ971377
Fragilariales	*Fragilaria crotonensis*			AF525662
Fragilariales	*Fragilaria striatula*	CCMP1094		AY485474
Fragilariales	*Fragilaria vaucheriae*	HYNP022	Norway: Ny-Alesund	EU260469
Fragilariales	*Fragilariforma virescens*	V18	USA:	AJ535137
Fragilariales	*Synedra acus*			DQ250712
Fragilariales	*Synedra fragilaroides*			EF193001
Fragilariales	*Synedra hyperborea*	CCMP1423		AY485464
Naviculales	*Haslea crucigera*			AY485482
Naviculales	*Haslea ostrearia*			AY485523
Naviculales	*Navicula cryptocephala var. veneta*	NCB	Hungary	AJ297724
Naviculales	*Navicula diserta*	p750	Germany	AJ535159
Naviculales	*Navicula lanceolata*			AY485484
Naviculales	*Navicula pelliculosa*	HYNP021	Norway: Ny-Alesund	EU260468
Naviculales	*Navicula pelliculosa*	SAG 1050-3	USA	AJ544657
Naviculales	*Navicula phyllepta*	HP		AY485456
Naviculales	*Navicula ramosissima*			AY485512

Table 1 continued

Taxonomic position	Species	Strain	Isolation locality	GenBank acc. no.
Naviculales	*Phaeodactylum tricornutum*	CCAP 1055/1		EF553458
Naviculales	*Pinnularia cf. interrupta*	TE1		AJ544658
Naviculales	*Sellaphora laevissima*	US	USA: Iowa	AJ544656
Naviculales	*Sellaphora pupula*	BLUNT	UK: Threipmuir Reservoir	AJ544654
Striatellales	*Grammatophora gibberula*	WK48		AF525656
Striatellales	*Grammatophora marina*	WK43		AY216906
Striatellales	*Grammatophora oceanica*	CCMP410		AY485466
Striatellales	*Hyalosira delicatula*			AF525654
Coscinodiscales	*Coscinodiscus granii*			AY485495
Coscinodiscales	*Coscinodiscus radiatus*	CCMP 309		X77705
Thalassiosirales	*Cyclotella meneghiniana*	CCMP 337		DQ093371
Thalassiosirales	*Skeletonema costatum*	CCMP 781		EF433519
Thalassiosirales	*Skeletonema menzellii*	CCMP 787	USA: Maine	AJ536450
Thalassiosirales	*Thalassiosira nordenskioeldii*	CCMP 997		DQ093365
Thalassiosirales	*Thalassiosira weissflogii*	CCMP1587		EF585582

Table 2 Comparison of morphological characteristics of *D. tenue*, *F. vaucheriae,* and *N. pelliculosa* isolated from Ny-Alesund, Norway

Characters	*D. tenue* #HYNP006	*N. pelliculosa* #HYNP021	*F. vaucheriae* #HYNP022
Length	19.2∼23.2 μm (avg. 21.2 μm, $n = 48$)	7.1∼8.9 μm (avg. 8.1 μm, $n = 51$)	13.8∼9.6 μm (avg. 11.8 μm, $n = 63$)
Width	4.5∼6.9 μm (avg. 5.5 μm, $n = 48$)	4.1∼5.5 μm (avg. 4.8 μm, $n = 51$)	7.0∼4.8 μm (avg. 5.9 μm, $n = 63$)
Costae	Present	Present	Present
Raphe	Absent	Present	Absent
Chloroplast shape	Disc form	Disc form (with pyrenoid)	Disc form
Colony	Linked at corners by mucilage pads	Chain form linked at corners by mucilage pads or stellate form	Chain form

Diatoms were cultured in DM at 10°C

avg. average

analysis was implemented with MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001) using the molecular model selected by AIC, namely a GTR nucleotide substitution model, with among-site rate variation modeled with a proportion of sites being invariable, while the rates for variable sites were drawn from a gamma distribution. The Markov chain Monte Carlo (MCMC) process was set to four chains, and 1,000,000 generations were conducted. The sampling frequency was assigned as every 100 generations. After analysis, the first 1,000 trees were deleted as burn-in and the consensus tree was constructed. Bayesian posterior probabilities (PP) (>0.50) were indicated at each branch node. The centric diatoms were used as outgroups. The phylogenetic trees were visualized with TreeView ver.1.6.6 (Page 1996).

Results

Morphological features of three Arctic diatoms

Morphological features of the three dominant diatoms *D. tenue*, *F. vaucheriae,* and *N. pelliculosa* in Arctic reservoirs are summarized in Table 2. LM SEM micrographs are shown in Figs. 2, 3, 4. The body of polar *D. tenue* HYNP006 (Fig. 2a–d) was symmetrical along the all axes of the pennates with no raphe system on either valve (Fig. 3a). The valves were linear to lanceolate in shape with subcapitate ends. Under a light microscope, the yellow-brown chloroplast appeared to be stick-like and lacked a pyrenoid region. SEM micrographs (Fig. 3a–p) showed that the valves of HYNP006 were linear or sometimes elliptical in shape. This isolate had an almost indistinct linear, but very

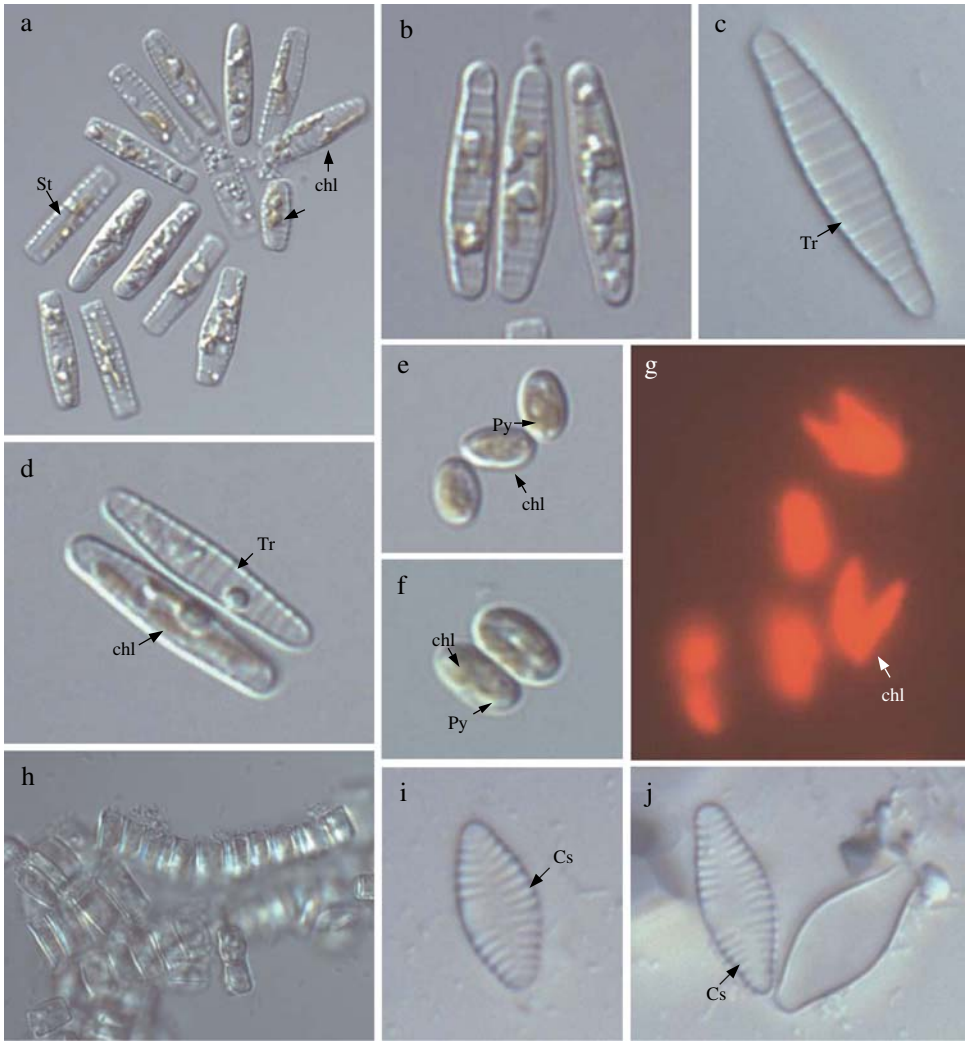

Fig. 2 Light micrographs of the three diatoms *D. tenue* HYNP006 (**a**–**d**), *N. pelliculosa* HYNP021 (**e**–**g**), and *F. vaucheriae* HYNP022 (**h**–**j**), respectively. All pictures except **g** (epifluorescence) were taken with DIC. In all the cells, chlorophylls (*chl*) were *yellow-brown* in color. A number of internal transverse ribs (*Tr*) are found in *D. tenue*. In *N. pelliculosa,* a pyrenoid (*Py*) surrounded by chlorophylls is seen; however the raphe was not visible under a light microscope. Costae (*Cs*) are clearly shown in *F. vaucheriae*

narrow, sternum, and finely punctated transapical striae consisting of single rows of areolae. Approximately 100 striae per 10 µm were observed in SEM photographs (Fig. 3a). At both tips of the valve, a well-circumscribed area of minute pores (pore field) was present (Fig. 3e, g, h). None of the spine was found on the valve. A number of internal transverse ribs could be found in *D. tenue* under light and scanning electron microscopy (Figs. 2c, d, 3b, e). At an end of the valve, a slit-like opening was seen on SEM micrographs (Fig. 3a, f–k). The algal cells were often seen as single cells, but sometimes formed chains of about 3–10 parallel cells both in fresh culture and under natural conditions.

The body of *N. pelliculosa* HYNP021 (see Figs. 2e–g, 3l–p) was symmetric along both the long and crossvalve axes. Cells were ovoid-shaped in the valve view and rectangular in the girdle view (Fig. 3o), making the frustules essentially lens-shaped. A fully developed raphe system was present on both valves (Fig. 3l–p). The two valves were usually identical. Striaes typically radiated from the center, and approximately 20 striae per 5 µm were observed on SEM photographs (Fig. 3n). Costae and stigma were not present. In culture, the cells were frequently found singly, although some cells joined at the valve face to form ribbon-like colonies. The chloroplast was shaped like a curved plate lying under one valve face and contained a pyrenoid.

F. vaucheriae HYNP022 (see Figs. 2h–j, 4a–l) was symmetrical along all axes of the pennates, with a spindle-shaped body that appeared rectangular in the girdle view (Fig. 4a). Striae were parallel, sometimes radiating slightly towards the poles. There was a narrow axial area and a central area that typically extended to a slight swelling on one side of the valve only. Apparent striaes radiated from the center, and approximately 7 striae per 5 µm were observed on SEM photographs (Fig. 4h, i). Costae could be seen under light and scanning electron microscopy (Figs. 2i, j, 4b, c); a stigma was not present. The axial area was narrow and linear with a distinct border. Approximately rectangular frustules typically linked at the valve face to form chains (Fig. 4a). The yellow-brown chloroplasts were disc-like, without a pyrenoid region. In culture, the cells were frequently found as single cells, with some joined at the valve face to form ribbon-like colonies.

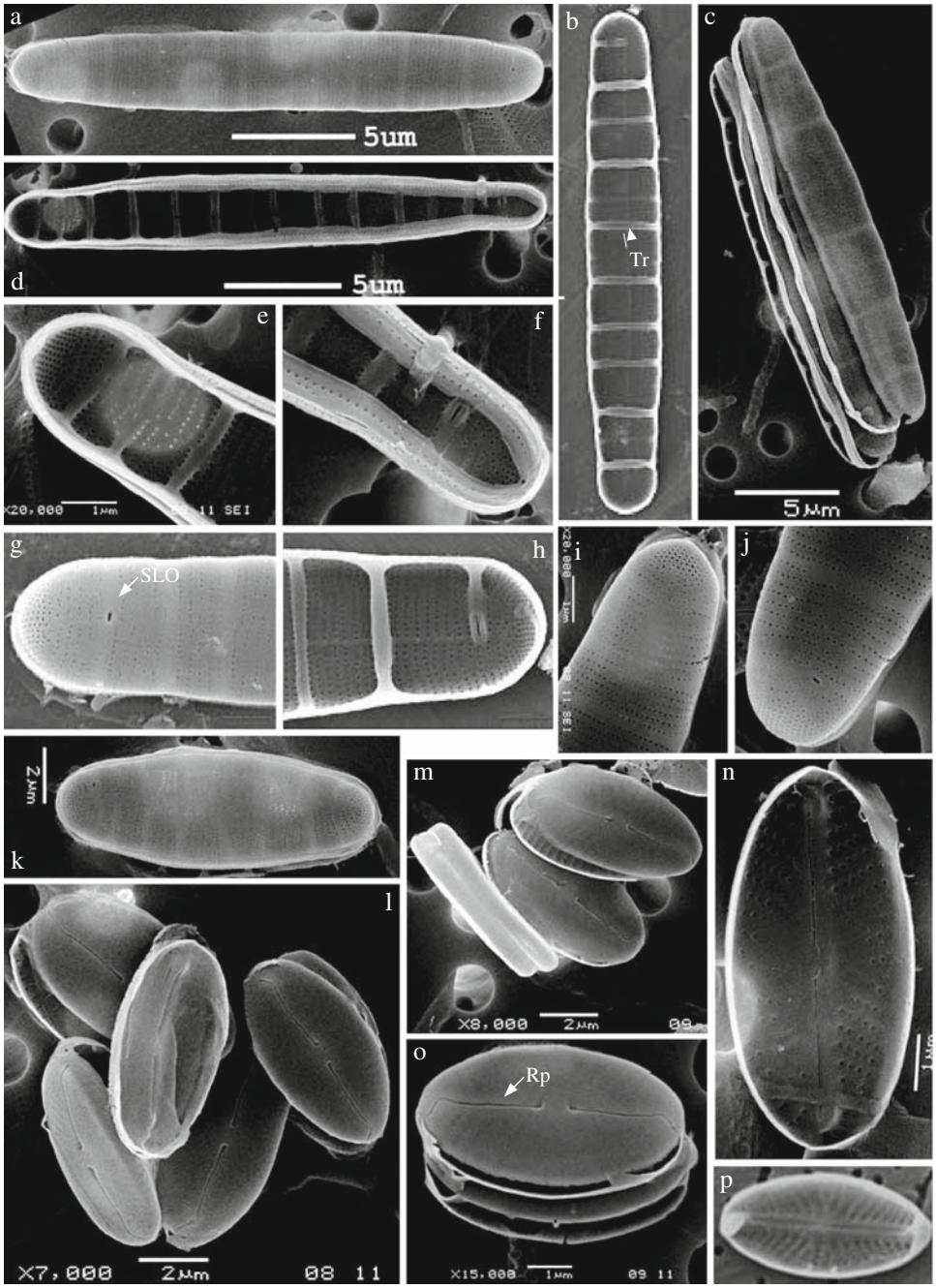

Fig. 3 SEM micrographs of *D. tenue* HYNP006 (**a**–**k**) and *N. pelliculosa* HYNP021 (**l**–**p**). In *D. tenue* HYNP006, transverse ribs (Tr) are seen inside of the frustule. A number of internal transverse ribs (Tr) were found. A transverse, slit-like opening (SLO) is seen internally and externally. In *N. pelliculosa,* there were thick raphes (Rp). A raphe slit was curved to each marginal end. Striae (St) are clearly seen (n). Stigma and costae are not present

Geometric dimensions

Body length was plotted against width (Fig. 5), and the length:width ratio [known as LAB (long as broad)] described the geometric character of the diatoms (Bellinger 1992). The lanceolate-shaped cells of *D. tenue* HYNP006 had an average diameter of 21.2 µm ($n = 48$, range 19.2–23.2 µm). The average LAB of *D. tenue* was calculated at 3.9 [standard deviation (SD) = 0.474] by measuring 48 cultured cells. This value represented clearly that the cells were linear to lanceolate in shape, as described previously. On the other hand, the ovoid-shaped body of *N. pelliculosa* HYNP021 was an average of 8.1 µm long ($n = 52$, range 7.1–8.9 µm). The LAB ratio was measured at 1.68 (SD = 0.144), representing an ovoid shape. For *F. vaucheriae* HYNP022, the spindle-shaped body was an average length of 11.8 µm ($n = 63$, range 13.8–9.6 µm). The LAB ratio was measured at 2.01 (SD = 0.246), representing a spindle-shaped cell.

Comparative sequence and phylogenetic analyses

We determined more than 1,700 bp of the 18S rDNA sequences from the three isolates (HYNP006, 021, 022) of

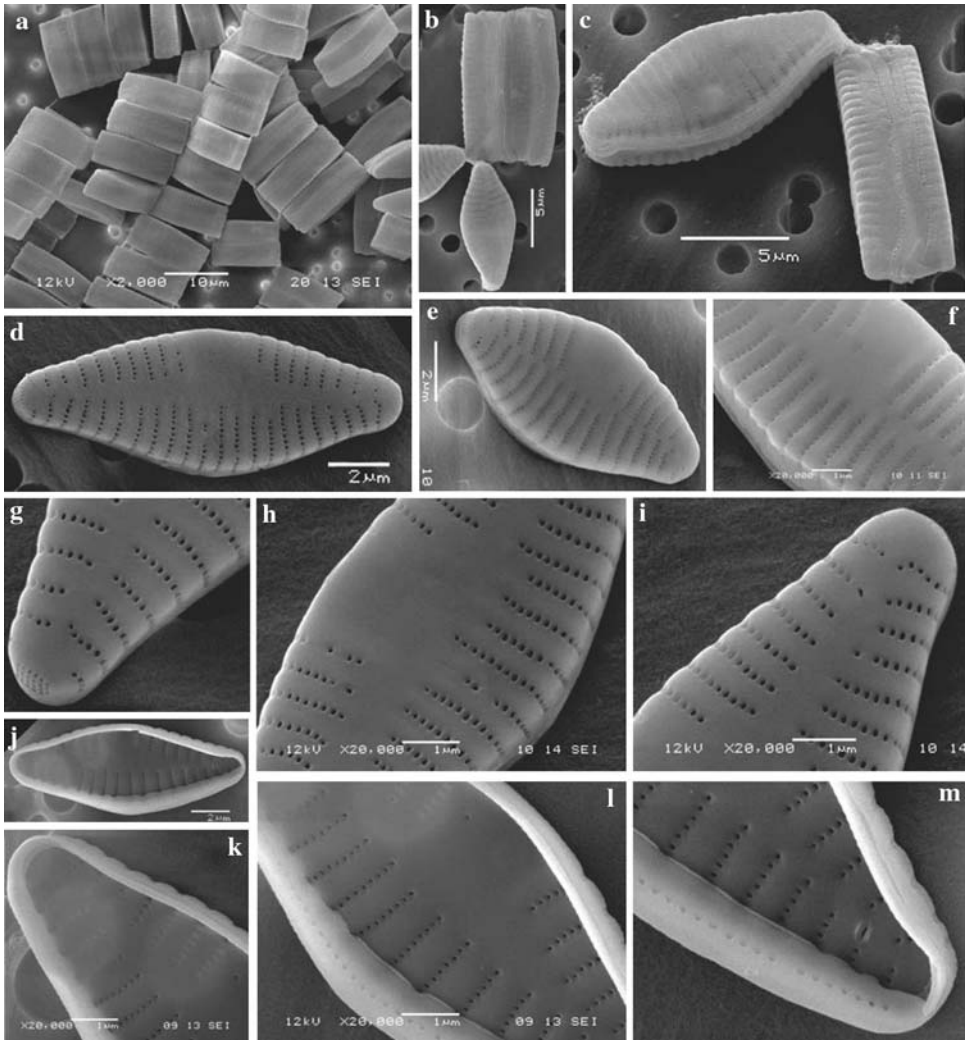

Fig. 4 SEM micrographs of *F. vaucheriae* HYNP022. Striae are clearly present; raphe and stigma are not absent

polar diatoms. Of them, HYNP006 had nearly identical genotype with HYNP013, as judged by divergences of the 18S rDNA, and so we included only the HYNP006 sequence in subsequent analyses. By BLAST searches in GenBank database, the three strains belonged to the genera *Diatoma, Navicula*, and *Fragilaria*, respectively. The results indicated that the HYNP006 strain had the highest level of similarity (0.0006 genetic distance, 99.9% identity in 1,660 compared sites) to *D. tenue* (GenBank No. AJ535143), suggesting that their genotypes were almost identical to each other. In the same manner, HYNP021 corresponded to *N. pelliculosa* (AJ544657) with a 0.0012 genetic distance and 99.8% identity, and HYNP022 to *F. crotonensis* (AF525662) with a 0.0207 genetic distance and 97.8% identity.

Phylogeneic analysis showed that the pennate diatoms were separated into two distinct lineages (i.e. raphid, araphid pennate), supported by a PP of 1.00 (Fig. 6). The five-compared orders in the Bacillariphyceae clade were generally divided according to their taxonomic position. On the other hand, in the Fragilariophyceae clade, some members of Fragilariales were genetically diverse, whereas Striatellales formed a cluster with high PP (1.00). Overall, our Bayesian tree showed that each diatom's genus (e.g. *Navicula, Diatoma*, *Flagilaria*) was grouped into each genus-cluster (0.99 PP). With regard to polar between non-polar isolates, the two polar isolates and a temperate one (GenBank No. AJ535143) of *D. tenue* formed a clade (0.96 PP), which was separated from *D. hyemalis* and others. In addition, the *N. pelliculosa* polar isolate forming a clade with the same species (AJ544657) reported from a temperate region of North America (1.00 PP). Furthermore, polar *F. vaucheriae* also formed an identical cluster with the genera *Flagilaria/Synedra* complex. These represent the lack of difference between the polar strains to more temperate strains of the three species (e.g. *D. tenue, F. vaucheriae, N. pelliculosa*).

Discussion

Most diatoms profiles in Arctic and Antarctic freshwater have been revealed through environmental surveys (Beyens

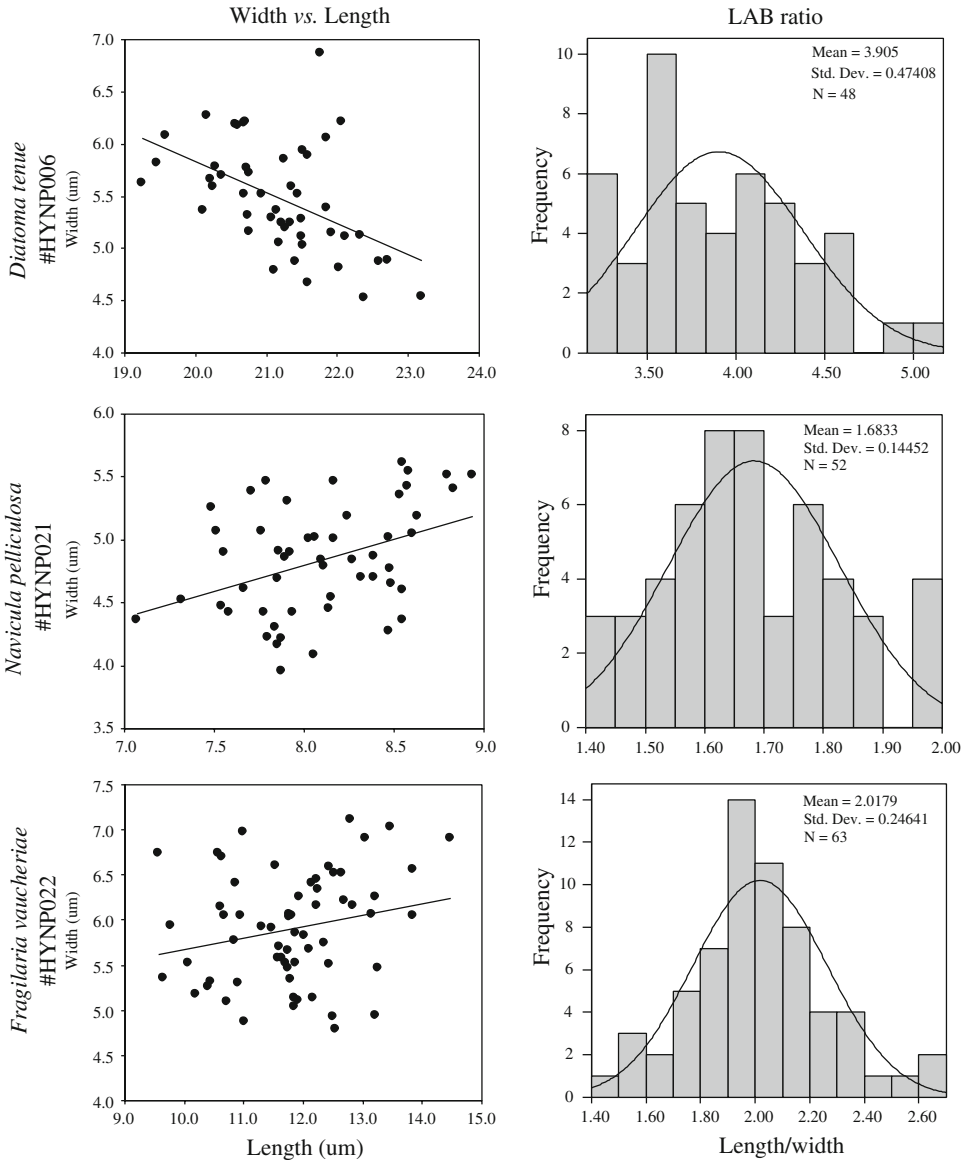

Fig. 5 Relationships between body length and width (*left*), and the LAB frequency distribution (*right*) of the diatoms *D. tenuae*, *N. pelliculosa,* and *F. vaucheriae*, respectively. The line on the histogram represents the normal curve

and Van de Vijver 2000; Ki et al. 2006), and few studies have examined the fine morphology and genetics of polar freshwater diatoms. Focusing on diatoms in Svalbard, Jones and Birks (2004) identified 182 diatom taxa from surface sediments of 23 lakes, and found that diatom species richness was generally low, with the majority of sites dominated by benthic genera such as *Fragilaria, Navicula*, and *Achnanthes*. Centric diatoms of the genus *Cyclotella* occurred at only three sites. Beyens and Van de Vijver (2000) reported that a total of 116 freshwater diatom taxa belonging to 22 genera were observed in 5 aquatic and 9 relatively dry moss samples from Hopen Island (Svalbard). *Navicula*, *Pinnularia,* and *Eunotia* had the highest species diversity. Recently, we surveyed algal flora from five temporary reservoirs around the International Polar Research Station, Norway (Ki et al. 2006), and found that diatoms (i.e. *Diatoma, Fragilaria, Navicula*) were predominant members in the temporal reservoirs during the summer, but their identities remained unclear because of morphological similarities and variations. In the present study, we isolated some dominant cells for laboratory culture in order to collect decisive morphological characteristics and genetic data. With the present morphological and molecular data (Table 2; Figs. 2–6), we were able to clearly identify them as *D. tenue*, *F. vaucheriae*, and *N. pelliculosa*.

When compared with temperate regions, Svalbard is separated from such areas, and has a harsh environment, especially in winter. During the long polar night that covers 116 days in the Kongsfjorden area, primary production of all phototrophs is completely suppressed (see Lund 1983; Gerland et al. 1999; Hanelt et al. 2001). These extreme environmental conditions could lead to unusual morphologies for polar diatoms. However, the morphology of *D. tenue* (e.g. striae, valve shape, linear valve with rounded,

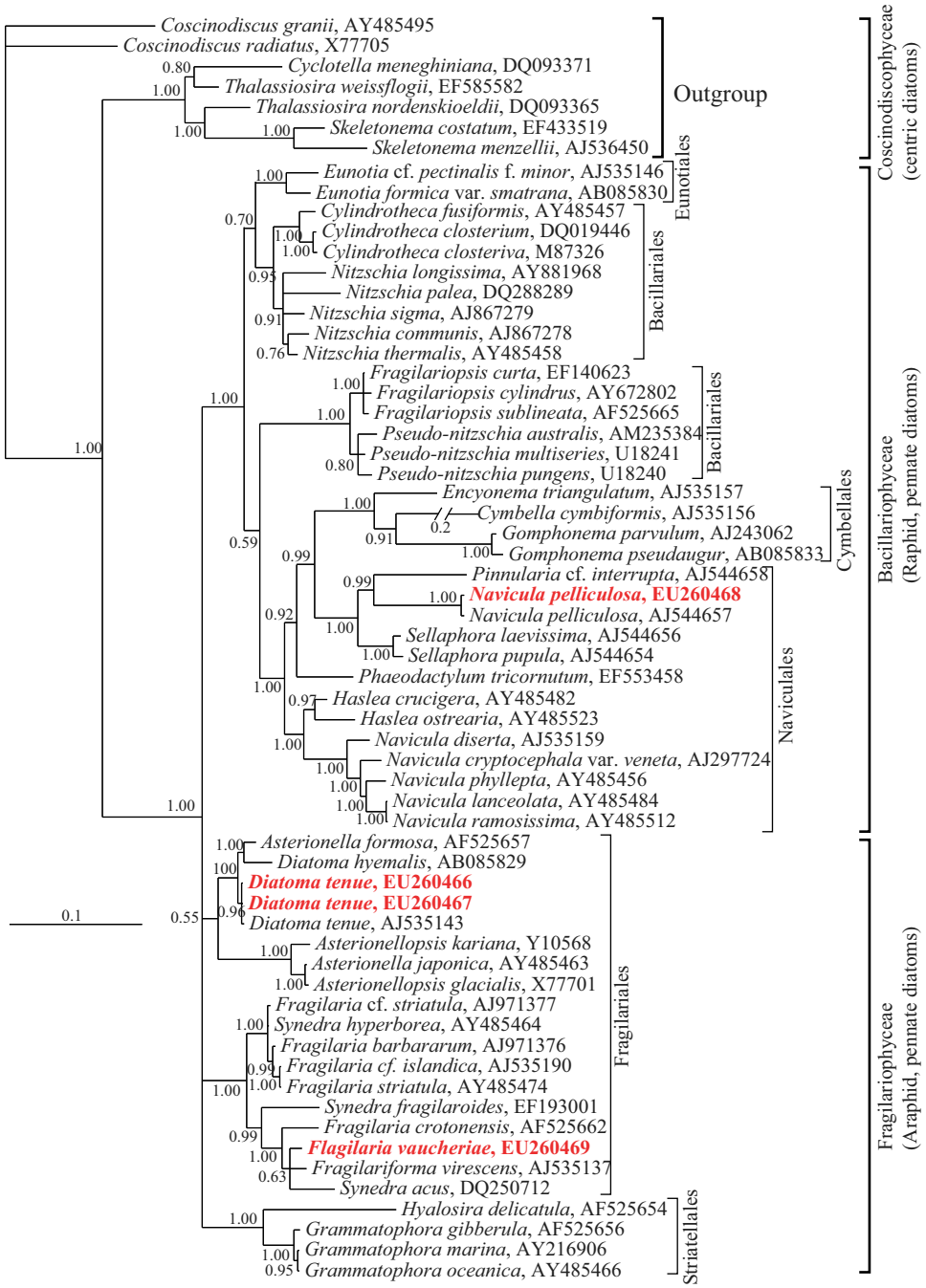

Fig. 6 Phylogeny of the diatoms based on 18S rDNA sequences (62 diatom taxa) and a Bayesian analysis, computed with MrBayes ver. 3.1.2. The numbers at the nodes are posterior probabilities (0.50). Centric diatoms were used as the outgroup. DNA sequences determined in this study are in *bold*

slightly capitate ends) observed here was similar overall to a previous description (Patrick and Reimer 1966). At the same time, we observed a transverse, slit-like opening (Fig. 3g), which has not been reported previously, from the polar *D. tenue*. The opening was present in all *D. tenue* frustules, representing a taxonomic key. In addition, the present isolate of *D. tenue* was considerably shorter (average 21.2 µm) in body length and the number of striae (e.g. 100 per 10 µm) than previously reported. For example, the diatom present in temperate regions has a reported length of 20–55 µm and breadth of 3–5 µm (Patrick and Reimer 1966). In addition, our observations differed from previously reported ranges of striae (16–20 striaes per 10 µm) and costae (6–10 costaes per 10 µm) (Patrick and Reimer 1966). Another report described a *D. tenue* known from Cape Adare, Antarctica, and South Victoria Land (Baker 1967; Patrick and Reimer 1966) with a linear valve with rounded, slightly capitate ends, 6–10 costae in 10 µm, 16–20 striae in 10 µm, a length of 57–80 µm, and a breadth of 2–4 µm. Recently, Genkal (2004) described the ultrastructure of *D. tenue* and found high morphological variability of the valve. Transmission electron microscopy studies

revealed that qualitative (valve shape, arrangement of axial area, striae, costae, and rimoportula) as well as quantitative (valve length and width, number of costae and striae in 10 μm) characteristics vary to a great extent. Although Genkal (2004) reported that number of striae in 10 μm and valve width proved to be the most stable and valve length and number of costae in 10 μm the most labile features, we found that the number of striae without costae in polar *D. tenue* was considerably variable. These differences suggest that Arctic *D. tenue* might have a distinct morphology and body size, particularly in length. The Arctic diatom cells are more ovoid than those from other regions. According to Baker (1967), *D. tenue* is a very common diatom that seems to occur in a wide range of conditions. This taxon is often found in slightly salty waters (Patrick and Reimer 1966). However, the Arctic species was present in low salinity waters in the field (Ki et al. 2006), even in small pools (almost 0‰) near coastal waters. We tested this property of the Arctic *D. tenue* isolate on a salt gradient in media, and showed that a salt concentration for optimal growth was nearly 0‰ (Katano et al. 2007).

The diatom *F. vaucheriae* is more cosmopolitan (Castenholz 1960), and is primarily distributed in streams. It also occurs among mixed periphyton communities on rocks in reduced stream current. *F. vaucheriae* is the commonest winter diatom (Castenholz 1960). Its favored temperature (around 10°C) is generally in accordance with our data. However, the Arctic *F. vaucheriae* was significantly shorter in length when compared with the identical species (approximately 25–65 μm in length, 3–5 μm in breadth, 8–13 striae in 10 μm) from Lake Michigan (Stoermer and Yang 1969). In addition, *N. pelliculosa* is widely distributed around the world, with data reported from populations in Germany (Ettl and Gärtner 1995, Toepel et al. 2005), Australia and New Zealand (Day et al. 1995). In polar areas, the species has been identified in the Antarctic and sub-Antarctic regions (Van de Vijver and Beyens 1999).

Previous work (Jones and Birks 2004) found that *Achnanthes, Cymbella, Fragilaria, Navicula,* and *Nitzschia*, were the most abundant genera in surface Svalbard sediment samples, with *Achnanthes petersenii* as most common species. Although more than 180 diatom taxa have been recorded from fossil materials and modern surface sediments on 23 Svalbard lakes, and the three diatoms described here are dominant in summer Arctic reservoirs (Ki et al. 2006), we found that *D. tenue* and *F. vaucheriae* have been recorded only at northern Svalvard lakes and not at the present study sites. In addition, *N. pelliculosa* has not been found elsewhere. This discrepancy is presumably due to the difficulty of morphological observation of diatoms, as well as the morphological variations described previously (such as short body size). The present morphological descriptions and LAB ratios of the three diatoms should be valuable features to distinguish them from others. The LAB ratio has been used to numerically describe body shape geometry of diatoms (Bellinger 1992) and particularly it can be an important key to discriminate sediment diatoms, where dead cells only allow the observation of frustules.

As has been pointed out, the molecular characteristics of diatoms within the Arctic remain poorly understood, primarily because there are few cultured isolates for molecular study. In the present study, we revealed nearly full 18S rDNA sequences (at least 1,700 bp) from three dominant diatoms during the summer. By molecular analyses, we found that two polar diatoms (e.g. *D. tenue, N. pelliculosa*) had nearly identical genotype independently when compared to the already-reported genotype of the same species from temperate and other regions (Fig. 6), while they were considerably shorter in body length than previously described identical species. In addition, we identified a polar isolate to *F. vaucheriae*, as judged by morphological key characters, and determined their 18S rDNA sequence as well. At present, we did not compare the polar *F. vaucheriae* genotype to the same species from other regions, due to a lack of available sequence. However, phylogenetic analysis showed that all the *Fragilaria, Fragilariforma,* and *Synedra* members, including the Arctic *F. virescens*, studied here belonged to the order Fragilariales, and formed a clade (1.00 PP). Within the clade, the polar *F. vaucheriae*, *F. virescens, and S. acus* are sister species (0.63 PP), of which clade formed a cluster with *F. crotonensis* (1.00 PP). Phylogenetically, the *Fragilaria* clade included members of both *Synedra* and *Fragilariforma*, but their branch pattern was not clearly separated according to each genus. Indeed, the genus *Fragilaria* and its allies are difficult to distinguish purely on the basis of characters visible under the light microscope and, as a result, have been the subject of considerable controversy in recent years. Traditionally, *Fragilaria* is distinguished from *Synedra* due to its tendency to form long filaments; indeed, the Arctic isolate was separated from *Synedra* due to its character to form long filaments (Fig. 2h). In the present phylogeny including polar isolates, the *F. ragilaria* and *F. crotonensis* are sister species (1.00 PP), suggesting that variations in the 18S rDNA sequences among *F. ragilaria* isolates might be little, like *D. tenue* and *N. pelliculosa*. Without doubt, these represent identical genotype between the polar strains to non-polar strains of the three diatom species.

Notably, freshwater diatoms are sensitive indicators of water quality, and they have been used as proxy data examining environmental changes, particularly in polar environments (e.g. Smol and Douglas 1996; Douglas and Smol 1999; Smol et al. 2005). Because diatom valves are often well-preserved in lake sediments, variations in their populations through time can be documented, providing critical information (e.g. Sorvari et al. 2002). However, occasionally,

their identities remain equivocal because of a deficiency of morphological data and the difficulty of sample acquisition. From this study, we added detailed information about the morphology of three diatoms, e.g. *D. tenue, F. vaucheriae*, *N. pelliculosa*, that were abundantly present in temporary reservoirs of Svalbard during the summer. The *N. pelliculosa* was first recorded in the Arctic. These represent an improvement of our understanding of certain predominant diatoms in the summer Arctic reservoirs, and will permit more precise assessment of proxy data on diatoms for Arctic sediment records, as well as relevant studies of environmental variables influencing distribution of taxa. In addition, the 18S rDNA sequences of the three diatoms determined here will be used as molecular signatures and reference sequences to discriminate these organisms by molecular means when morphological distinctions are ambiguous.

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