Journal of Environmental Biology
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July 2008, 29(4) 485-491 (2008)
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Notes on freshwater and terrestrial algae from Ny-Ålesund, Svalbard (high Arctic sea area)

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(Received: February 14, 2007; Revised received: December 07, 2007; Accepted: December 13, 2007)

Abstract: *Field survey of algae and cyanobacteria from terrestrial and freshwater habitats in the vicinity of arctic Ny-Ålesund, Svalbard (79°N) (high Arctic sea area) was performed in June 2006. Species diversity and abundance were evaluated by using epifluorescence microscopy and culturing methods. In total, 29 taxa in 25 genera were identified, of which Leptolyngbya spp., Trichormus sp. and Chlamydomonas nivalis were abundantly present in almost every sample. In several locations, blooms were formed by species C. nivalis, Scotiellopsis sp., Klebsormidium flaccidum, Zygnema sp., Meridion circulare, Tabellaria fenestrata and Fragilaria sp. Eleven new species from this locality are described.*

Key words: *Algae, Cyanobacteria, High Arctic sea area, Ny-Ålesund, Species composition, Svalbard*
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Introduction

Cyanobacteria and algae occur in practically all earth's environments and some species are capable of growing in dramatic ranges of temperature from thermal waters at 80°C and below 0°C in the polar regions. In northern terrestrial habitats including Arctic and Antarctic, extremes of environmental stress occur, principally freezing (Walton, 1982; Davey and Clarke, 1991) and desiccation (Warren, 2001). Nonetheless, they are inhabited by numerous cyanobacteria and eukaryotic algae (Antipina, 1986; Mueller *et al.*, 2001; Säwström *et al.*, 2002; Pocock *et al.*, 2004; Kaštovská *et al.*, 2005, 2007; Stibal *et al.*, 2006), which are indispensable in soil formation and stabilization against wind and water erosion increasing its organic matter and nitrogen content and preparing substrates for subsequent colonizers. During spring thawing, the spores of cyanobacteria and algae adapted to survive on glacier surfaces and embedded in ice, snow, and frozen soil are transported by wind, melt-water, and biological agents (birds and mammals) and would germinate in freshwater and soil and on any surface that holds moisture. The cyanobacteria and algae are also known as primary colonizers after deglaciation, and heterotrophic bacteria and fungi totally depend on them for fixed carbon (Wynn-Williams, 1990; Ohtonen *et al.*, 1999).

The high Arctic settlement Ny-Ålesund located on the seashore on northwest Spitsbergen is the northernmost human settlement on earth (79°N). The studies on freshwater and terrestrial cyanobacteria and algae in the vicinity of this distant area progress at a slow pace because of harsh weather conditions and difficulties in transportation and accommodation. Moreover, one-time seasonal collections were typically reported (Mueller *et al.*, 2001; Säwström *et al.*, 2002; Kaštovská *et al.*, 2005) and therefore, a comprehensive picture of flora still needs to be established.

The present study reports on the community structure and abundance of freshwater and terrestrial cyanobacteria and algae in the vicinity of Ny-Ålesund during our field survey in June 2006 and discusses possible existing yearly/monthly fluctuations when different groups of algae/individual species pre-dominate in this area.

Materials and Methods

Collection of plant material and laboratory culture: The samples of water and wet soil containing algae were collected daily from 13 to 18 June, 2006. Typical weather conditions during that time were -4°C at night, 0-4°C at daytime, average humidity 46%, wind 22 km/hr, daylight for 24 hr. Collecting sites included shallow puddle on the wayside near Korean research base 'Dasan' in Ny-Ålesund (hereafter: NÅ), Svalbard (79°N), peat bog and small freshwater lake near NÅ, ice- and snow-fed streamlets and puddles located at a distance of several kilometers ashore from NÅ (Figs. 1A-B, 2A-B). Soils were sampled mostly from sites with vegetative cover (vascular plants and mosses) and from several barren sites. Water-saturated soil samples and algal materials submerged in freshwater collected from the same site were placed in sterile plastic containers and kept outdoors on ice before being transported to the laboratory in Korea. Each container was labeled and sealed with a Parafilm to avoid cross-contamination.

Thereafter, mixed cultures were first established by adding materials to a modified liquid ATCC Medium 625 (Klochkova *et al.*, 2006) in 90 x 15 mm and 90 x 60 mm plastic Petri dishes at 4-6°C, 15 μmol photons m^{-2} s^{-1} cool-white fluorescent lighting and 24 hr light-dark regime. Wherever possible, unialgal strains were isolated and transferred into agar medium (agar 10 g, ATCC Medium 1 liter) to obtain pure cultures. The strains are currently held at Kongju National University (KNU), Korea.

Field materials were observed with Leica bright field and epifluorescence microscope under the 20 and 40x objective lens in NÅ. Micrographs were taken with Olympus DP50 digital camera

Special Issue - **Marine Environmental Biology**
Guest Editor - **H.W. Shin, Korea**

equipped to microscope Olympus BX50 using Viewfinder Lite and Studio Lite computer programs in KNU. When necessary, the materials were fixed in 2% formaldehyde diluted in ATCC medium for immobilizing the cells to facilitate microscopic examination. The organisms were identified following the publications of Krammer and Lange-Bertalot (1997), Brook and Johnson (2002), John (2002), John and Tsarenko (2002), Johnson and Merritt (2002), Novarino (2002), Whitton (2002) and other research publications and Internet resources on the subject.

Results and Discussion

A total of 29 taxa in 25 genera were identified in the vicinity of NÅ (Table 1), of which *Leptolyngbya* spp., *Trichormus* sp. and *Chlamydomonas nivalis* were abundantly present in almost every sample. Taxa *Scotiellopsis* sp., *Klebsormidium flaccidum, Zygnema* sp., *Meridion circulare*, *Tabellaria fenestrata*, and *Fragilaria* sp. were very abundant and bloomed in some locations. Taxa *Phormidium* sp., *Chlorogloea* sp., *Nostoc* sp., *Chlorella* spp., *Cosmarium subundulatum*, *Achnanthes* sp., and *Vaucheria borealis* were commonly present. Two species, *Cryptomonas* sp. and *Cylindrocystis* sp., were not observed in microscopy at first and became noticeable only after several months in culture.

In August 2002, Kaštovská *et al.* (2005) performed quantitative analyses of algae and cyanobacteria in different soil habitats (cryonite, barren, subglacial, and vegetated soils) in the vicinity of five glaciers on the Brøggerhalvøya (northwest Spitsbergen), areas located at a distance ca. 1~2 km from NÅ across the fjord, and at three sites near NÅ, including peat bog. They recorded 57 taxa of 23 genera of cyanobacteria and algae, with cyanobacteria comprising the major proportion of the algal assemblages and algae (mainly greens) being present only as accessory organisms. However, previous study did not record 11 species most of which were common during the time of our study (Figs. 1,2).

The species found by us often composed >90% of the algal assemblages (*e.g. Zygnema* sp., *Tabellaria fenestrata*, *Fragilaria* sp., and *Chlamydomonas nivalis*). Interestingly, snow flagellate *C. nivalis* common in Svalbard (Mueller *et al.*, 2001; Säwström *et al.*, 2002; Stibal *et al.*, 2006; Kaštovská *et al.*, 2007) was not reported in a previous study (Kaštovská *et al.*, 2005), and was by far one of the most abundant species in NÅ and surrounding areas during our study in June 2006. Also, previous study did not record two very distinct algae, *Zygnema* sp. and *Vaucheria borealis.*

Table - 1: Cyanobacteria and algae collected in the vicinity of Ny-Ålesund in June 2006 and their habitats

No.	Species	Habitat
1	*Chlorogloea* sp.	Terrestrial
2	*Leptolyngbya* sp. 1 (Pale bluish, cell length/width 7.5 x 5 μm, large oil droplets)	Terrestrial
3	*Leptolyngbya* sp. 2 (Pale greenish or grayish, cell length/width 2.5-5 x 10 μm, large oil droplets)	Terrestrial
4	*Nostoc* sp.	Terrestrial
5	*Phormidium* sp. (Pale bluish or grayish, cell length/width 2.5-3.7 x 3.7 μm)	Terrestrial
6	*Trichormus* sp. (Pale bluish, cell length/width 2.5-3.7 x 2.5 μm)	Terrestrial
7	*Chlorella minutissima* Fott et Novakova	Terrestrial
8	*Chlorella vulgaris* Beijerinck	Terrestrial
9	*Chlamydomonas nivalis* (Bauer) Wille *	Terrestrial
10	*Chlorococcum* sp.	Terrestrial
11	*Cladophora* sp. *	Freshwater
12	*Cosmarium subundulatum* Wille *	Terrestrial
13	*Cylindrocystis* sp. (Long chains of dark green cells)	Terrestrial
14	*Klebsormidium flaccidum* (Kützing) Silva, Mattox et Blackwell	Terrestrial
15	*Klebsormidium* sp. (Yellow-green, slimy, cell length/width 10-12.5 x 5-5.5 μm)	Terrestrial
16	*Muriella terestris* J.B. Petersen	Terrestrial
17	*Scotiellopsis* sp.	Terrestrial
18	Unidentified green coccoid (cell diam. 5-8.7 μm)	Terrestrial
19	*Zygnema* sp. *	Freshwater
20	*Achnanthes* cf. *minutissima*	Terrestrial
21	*Cymbella arctica* (Lagerstedt) Schmidt *	Terrestrial
22	*Fragilaria* sp. *	Terrestrial
23	*Hannaea arcus* (Ehrenb.) Patr.	Terrestrial
24	*Meridion circulare* (Greville) Agardh *	Freshwater
25	*Navicula* sp. *	Terrestrial
26	*Nitzschia* sp. (Needle-like, cell length/width 35-55 x 3.7-5 μm)	Terrestrial
27	*Tabellaria fenestrata* Kützing *	Terrestrial
28	*Cryptomonas* sp. *	Freshwater
29	*Vaucheria borealis* Hirn *	Terrestrial

* = Algae described in this study

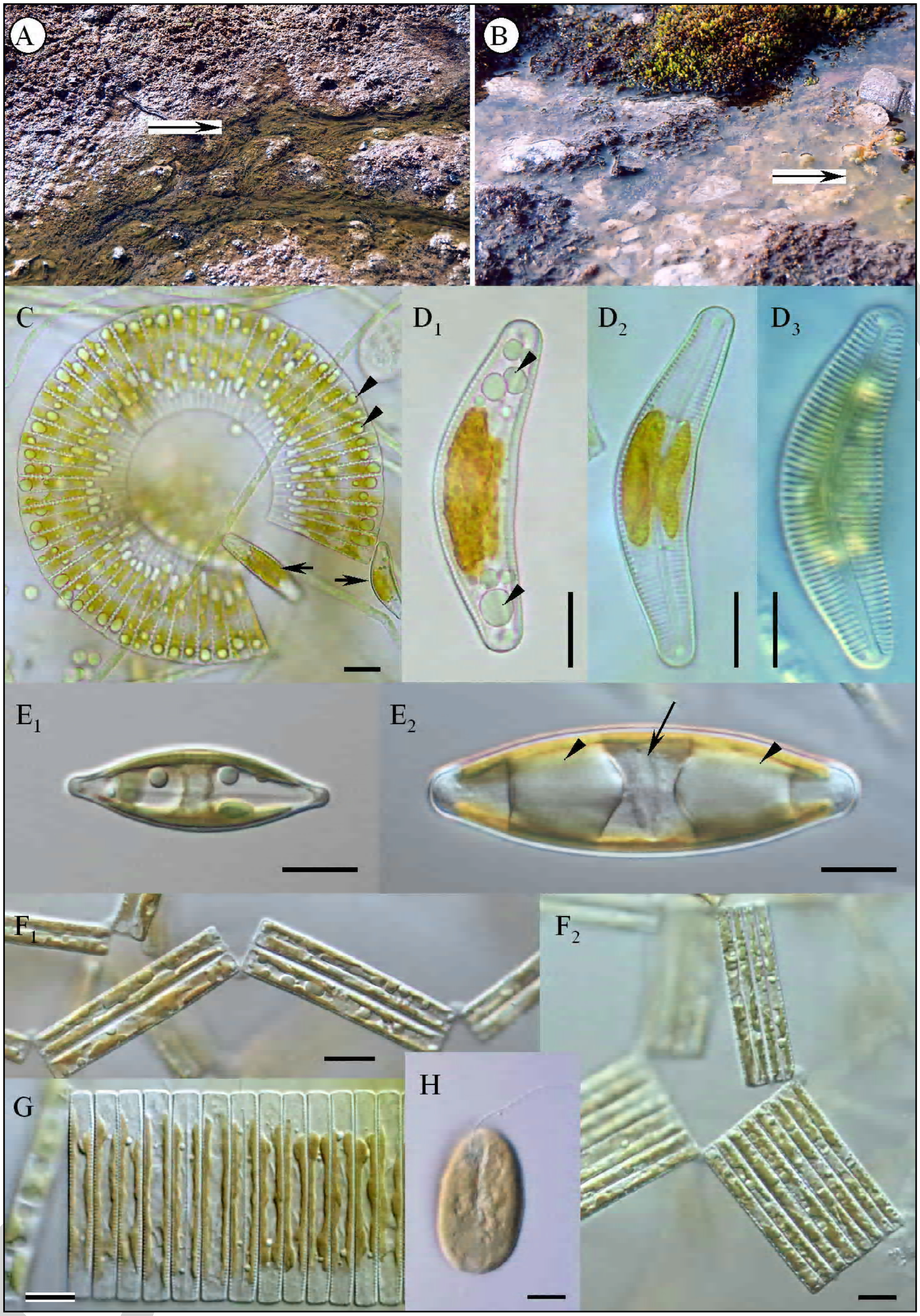

Fig. 1. Sample collection sites and collected materials. A. Diatom assemblage consisting of almost only *Tabellaria fenestrata*. B. Diatom assemblage consisting of *Meridion circulare* and *C. arctica*. C. *Meridion circulare* (Greville) Agardh. Arrows point to *Cymbella* cells. D_1-D_3. *Cymbella arctica* (Lagerstedt) Schmidt (arrowheads point to oil globules). E_1-E_2. *Navicula* sp. Visible, across the center of the cell, is the protoplasmic bridge, which contains the nucleus (arrow). Oil globules to each side of the nuclear bridge were always present (arrowheads). F_1-F_2. *Tabellaria fenestrata.* G. *Fragilaria* sp. H. *Cryptomonas* sp. Scale bars: 10 µm

Characteristics of species newly recorded from this locality are given below. Seven algae were considered as terrestrial (Table 1) as they grew on the soil and in its upper layers, in soil and mud sediments on the bottom of small puddles (< 1-5 cm at depth), and in minute streamlets and larger water bodies from melt-water. Species *Meridion circulare*, *Cryptomonas*, *Zygnema*, and *Cladophora* were considered as freshwater, although moisture was present intermittently in their growth habitats. In times when water supply is insufficient, the algae might persist in a state of anabiosis and start growing again in the presence of water.

***Meridion circulare* (Greville) Agardh:** This species was abundant and dominated in NÅ and surrounding areas. It was present in water and soil sediments in ice and snow-fed shallow puddles and streamlets. Accompanied by other diatoms, coccoid greens, and *Klebsormidium flaccidum*, it often formed long light-brown filaments of chains of cells floating in water (Fig. 1B). Cell colonies were fan- or spiral-shaped (Fig. 1C). In some samples, the cells contained pale olive chloroplasts, whereas in other samples the chloroplast color was olive-brown. The cells always contained several large oil droplets. Cell length 25-42.5 μm, cell width 2.5-5 μm (narrowest part) and 5-10 μm (broadest part).

***Cymbella arctica* (Lagerstedt) Schmidt:** This species was common and often present together with other diatoms (*M. circulare, T. fenestrata*), green algae (*Zygnema*, *Klebsormidium*) and cyanobacteria (*Leptolyngbya*, *Phormidium*). In some samples, the cells contained pale olive chloroplasts, whereas in other samples the chloroplast color was light brown (Fig. 1D). Most cells contained several large oil droplets (Fig. $1D_1$). Cell length 30-70 μm, cell width (central broadest part) 10-17.5 μm.

***Navicula* sp. (Fig. 1E):** This species was not abundant, but often present in small numbers together with other diatoms and blue-greens in soil and water samples. When collected, the cells were pail olive in color and contained numerous oil droplets, along with two large oil globules to each side of the nuclear bridge typical for the representatives of this genus. Cell length 27.5-82.5 μm, cell width 7.5-17.5 μm (in the broadest central part).

***Tabellaria fenestrata* Kützing:** This species was common, dominant in some shallow and deeper streamlets, forming long filaments of zig-zag chains of cells (Fig. 1F). It also grew on water-saturated vegetative soil and in soil sediments in ice- and snow-fed streamlets (Fig. 1A). The cells contained pale olive or light brownish chloroplasts. Cell length 17.5-42.5 μm, cell width 3-6.3 μm.

***Fragilaria* sp. (Fig. 1G):** This species was dominant in some shallow and deeper water bodies, forming comb-like colonies of 690~800 μm at length. It also grew on water-saturated vegetative soil and in soil sediments in ice- and snow-fed streamlets. The cells contained light brown chloroplasts; several oil droplets were often present. Cell length (10)30-47.5 μm, cell width 2.5-5 μm.

***Cryptomonas* sp. (Fig. 1H):** This species was not noticed in field samples due to its rarity and was later discovered in cultured samples. The original sample was mud collected from the bottom of small puddle located in the vicinity of NÅ. In mixed culture, typical accompanying species were *Nostoc*, *Leptolyngbya*, *Chlorogloea*, *Chlorella*, and *Cladophora* (Fig. 2F). Cell length 38-42 μm, cell width 20-22 μm.

***Zygnema* sp. (Fig. 2C):** Large bundles of filaments were found in a shallow puddle (< 3 cm at depth) on the wayside in NÅ, and in several shallow streamlets and on the surface of water-saturated soil and stone particles several kilometers ashore from NÅ (Fig. 2A-B). In filaments with short cells protoplasmic content was very condensed and was more dispersed in filaments with longer cells so that chloroplasts, nucleus, and numerous small bubbles and lipid droplets were clearly distinguishable (Fig. $2C_1$-C_2). Most cells had 2 stellated chloroplasts (Fig. $2C_2$); some cells were under division and contained 4 chloroplasts but no developing cell plate (Fig. $2C_3$). Conjugating filaments and spores were not observed in field samples, and conjugation did not occur in laboratory culture in over 1 year. Cell length 25-100 μm, cell width 32.5 μm.

***Cosmarium subundulatum* Wille (Fig. 2D):** The sporadic cells of *C. subundulatum* were present along with *Vaucheria borealis*, some diatoms, and *Zygnema*. Cells ovoid in shape, with deep closed sinus. Margins have about 14-18 undulations, cell apices slightly flattened. Cell length 35-50 μm, cell width 20-30 μm.

***Chlamydomonas nivalis* (Bauer) Wille:** This species was present in almost every sample and made blooms in several muddy puddles in the vicinity of NÅ. Non-motile cells (Fig. $2E_1$), biflagellated motile cells (Fig. $2E_2$), aplano and zoosporangia, and released zoospores were present. Resting cells with warty cell surface, mostly empty, were abundant on/in the wet soil and mud samples. Common accompanying species were *Chlorococcum*, *Scotiellopsis*, *Chlorella*, and *Klebsormidium*. Cell length 12.5-15 μm, cell width 8.7-10 μm (in motile cells); 22.5 x 17.5 μm (in non-motile cells).

***Cladophora* sp. (Fig. 2F):** This species was found in the mud collected from the bottom of small puddle located in the vicinity of NÅ. It had band- and saucer-shaped chloroplasts with pyrenoids, cross-walls at points of branching. The cell walls of collected winter material appeared black brown, very thick; cultured filaments developed less rigid walls, cross walls were slantwise or almost vertical. Cultured plants reached 1.5~2 cm at length. The main axial cells were 5~6 times longer than wide, and side cells were 2~3 times longer than wide.

***Vaucheria borealis* Hirn (Fig. 2G):** Short filaments growing from spores were collected from the peat bog and wet soil covered with *Sphagnum* and other higher plants in the vicinity of NÅ. Typical accompanying species were *Leptolyngbya* and *Trichormus*. Cultured plants reached 1~3 cm at length. Filaments 30-75 μm wide, monoecious. Oogonia essentially sessile or scarcely stalked, single or paired, with antheridium positioned next and usually at a close distance, or between two oogonia. Oogonia ovoid to oblique, often with a horizontal beak; oospores ovoid or kidney-shaped; antheridia bent laterally and downward.

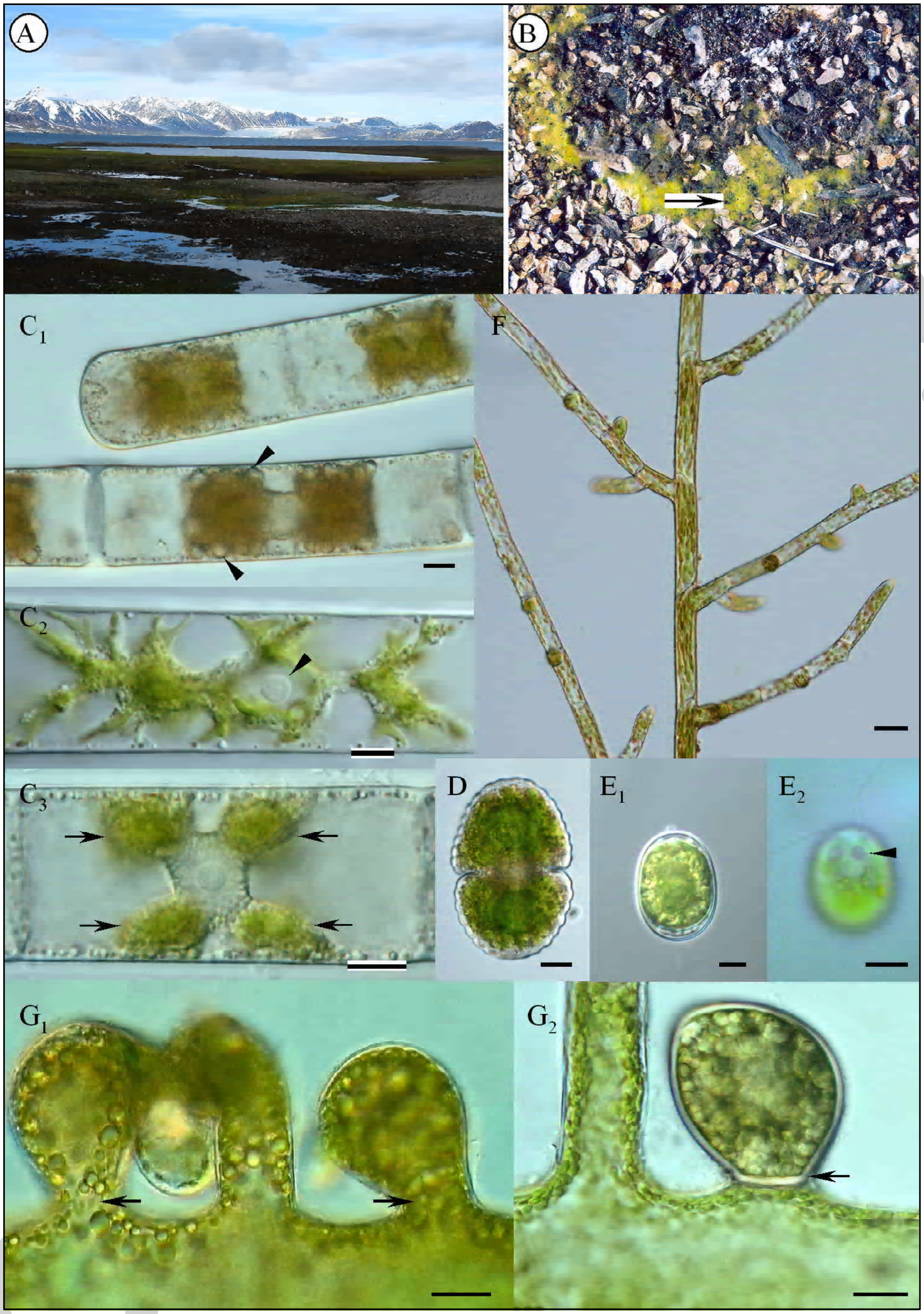

Fig. 2. Sample collection sites and collected materials. A. Shallow ice- and snow-fed freshwater streams and puddles located several km ashore from NÅ. B. Filamentous green algae *Klebsormidium* and *Zygnema* on water-saturated soil and stones. C_1-C_3. *Zygnema* sp. (arrowheads in Fig. C_1 point to oil droplets). C_2. Cell with 2 stellated chloroplasts and large nucleus (arrowhead). C_3. Cell with 4 chloroplasts (arrows). D. *Cosmarium subundulatum*. E_1-E_2. Non-motile (E_1) and motile cells (E_2) of *Chlamydomonas nivalis*. Arrowhead points to contractile vacuoles. F. *Cladophora* sp. G. *Vaucheria borealis*, oogonia and antheridium. G_2. A cross septum forms to cut-off oogonium from the main filament (arrow). Scale bars: C, G = 10 µm, D-E = 5 µm, F = 50 µm

By any standards, terrestrial habitats of the Arctic are extreme environments and the inhabiting organisms must be specialized to freezing, intense UV irradiation, and repeated cycles of drying and wetting. It is noteworthy that in several locations, puddles densely populated with *C. nivalis* and *Zygnema* were dry in 3-4 days after samples were first collected. In the field, many algae, including *Zygnema*, were embedded in copious mucilage, which might contribute to their survival mechanism against desiccation because of its physico-chemical property allowing the retention of water (*e.g.* Schumann *et al.*, 2005). Also, the green algal resting cells with warty cell surface closely resembling hypnozygotes of *C. nivalis* (*e.g.* Graham and Wilcox, 2000) were quite common in the mud and soil samples. Almost all collected plants, including filamentous cyanobacteria, contained numerous small and/or large oil droplets and the plastid colors were not intense because of permanent high irradiation intensity. In laboratory samples maintained at 4~6^{o}C and in 24 hr light condition the oil droplets disappeared and the plastid colors brightened, obviously because irradiation intensity was lower (15 μmol photons m^{-2} s^{-1}) than in the field.

The *Zygnema* isolate from Svalbard had some peculiar characteristics, such as cells with 4 chloroplasts, single nucleus, and no developing cell plate, and displayed a very different pattern of cell division from Korean isolates of *Zygnema*, which never had cells with 4 chloroplasts. Also, unlike in Korean *Zygnema* that always started conjugation after being transported to the laboratory (Kim *et al.*, 2007), conjugation was never observed in the field samples of Svalbard *Zygnema* as well as in cultured samples for over 1 year. Unlike other field materials from Svalbard, the *Zygnema* isolate was very intolerant to changes of temperature and did not survive in an ambient (room) temperature even for several hours. Korean isolates of *Zygnema*, originally adapted to warm environment, could survive multiple reverse high-low temperature shocks.

In conclusion, our field observations showed opposite results to those by Getsen (1990), Elster *et al.* (1999) and Kaštovská *et al.* (2005), who proposed that Arctic terrestrial environments were dominated by green algae (Getsen, 1990; Elster *et al.*, 1999) and, on the contrary, by cyanobacteria (Kaštovská *et al.*, 2005). As stated by Kaštovská *et al.* (2005) despite the fact that the species richness of green algae was higher than that of cyanobacteria, the latter made up the dominant part of biovolume in algal communities. Since our study did not encompass cryonite, barren, and subglacial soils in the vicinity of NÅ, we cannot totally disagree with Kaštovská *et al.* (2005) about dominant organisms in these habitats. However, the supposition should not be applied to the terrestrial habitat of this area on the whole, which also includes vegetated soil, numerous freshwater bodies, and generally any surface on the land capable of holding moisture.

Although *Leptolyngbya* spp. and *Trichormus* sp. were abundantly present in almost every sample during our field survey in June 2006, the diatom species and *C. nivalis*, as well as *Klebsormidium* spp. and *Zygnema* sp. were by far more abundant and bloom-forming so that the soil surfaces discolored brown and green in locations where they bloomed.

The five diatom species, *C. nivalis*, and *Zygnema* sp. found by us were not reported from this locality by Kaštovská *et al.* (2005), as well as *Vaucheria borealis* from the vegetated soil and peat bog. We consider that the algae truly were absent or present extremely sparsely in August 2002. On the other hand, we did not find several very common algae (*Bracteacoccus* sp., *Muriella* spp., *Pseudococcomyxa simplex*, *Stichococcus* spp., *Coleochlamys cuccumis*) reported from this locality by Kaštovská *et al.* (2005). Each result (Getsen, 1990; Elster *et al.*, 1999; Kaštovská *et al.*, 2005; this study) could, therefore, be correct to a particular extent and imply dynamic changes existing per years/months when different groups of algae/individual species, *e.g.* greens (Getsen, 1990; Elster *et al.*, 1999), cyanobacteria (Kaštovská *et al.*, 2005), and diatoms and greens (this study) dominate in Arctic terrestrial environments.

Our data also extend the knowledge on species composition in NÅ, Svalbard, which appears to be more abundant than what is known up to date (*e.g.* Skulberg, 1996; Kaštovská *et al.*, 2005), and show that areas located at a close distance from NÅ (ca. 1-2 km, Brøggerhalvøya, northwest Spitsbergen) may have different species composition. More extensive field studies are necessary to fully understand possible seasonal fluctuations of diversity and abundance of terrestrial algae and cyanobacteria in this high Arctic region.

Acknowledgments

We thank Dr. Klára Kaštovská for providing several important papers discussed in this study. This work was supported by KOPRI Project PE06060.

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