ARCTIC
VOL. 64, NO. 1 (MARCH 2011) P. 25–31

Freshwater and Terrestrial Algae from Ny-Ålesund and Blomstrandhalvøya Island (Svalbard)

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(*Received 20 February 2010; accepted in revised form 29 July 2010*)

ABSTRACT. A field survey of algae and cyanobacteria from terrestrial and freshwater habitats in the vicinity of Ny-Ålesund and on Blomstrandhalvøya Island (Svalbard) was performed in June 2009, and results were compared with data from our fieldwork in June 2006. In total, we identified 30 taxa belonging to 23 genera from the specimens collected near Ny-Ålesund (26 taxa) and on Blomstrandhalvøya Island (24 taxa). Five species previously unrecorded from this locality are depicted, including astaxanthin-containing *Haematococcus* sp. collected from Blomstrandhalvøya Island. This is the first report on a *Haematococcus* species from the High Arctic.

Key words: algae, cyanobacteria, *Haematococcus*, Ny-Ålesund, species composition, Svalbard

RÉSUMÉ. Une étude sur le terrain portant sur les algues et les cyanobactéries d'habitats terrestres et dulçaquicoles dans les environs de Ny-Ålesund et de l'île Blomstrandhalvøya (Svalbard) a été réalisée en juin 2009, et les résultats ont été comparés aux données de notre travail sur le terrain effectué en juin 2006. En tout, nous avons identifié 30 taxons appartenant à 23 genres à partir de spécimens prélevés près de Ny-Ålesund (26 taxons) et de Blomstrandhalvøya (24 taxons). Cinq espèces qui n'avaient jamais été répertoriées dans cette localité sont illustrées, dont l'*Haematococcus* sp. contenant de l'astaxanthine recueillie à l'île Blomstrandhalvøya. Il s'agit de la première fois que l'espèce *Haematococcus* a été signalée dans l'Extrême-Arctique.

Mots clés : algues, cyanobactéries, *Haematococcus*, Ny-Ålesund, composition des espèces, Svalbard

Traduit pour la revue *Arctic* par Nicole Giguère.

INTRODUCTION

In the terrestrial habitats of the Arctic, extremes of environmental stress occur, including freezing, desiccation, and high ultraviolet radiation during polar days. Nonetheless, these habitats support numerous cyanobacteria and eukaryotic algae (e.g., Antipina, 1986; Mueller et al., 2001; Säwström et al., 2002; Kaštovská et al., 2005, 2007; Holzinger et al., 2006, 2009; Lenzenweger and Lütz, 2006; Stibal et al., 2006; Elster et al., 2008; Kim et al., 2008). The northernmost Arctic settlement, Ny-Ålesund, located on the seashore on northwest Spitsbergen, has become a model ecosystem for studies on Arctic environments (Hop et al., 2002). The freshwater and terrestrial cyanobacteria and algae have been addressed in several studies (e.g., Kubečková et al., 2001; Säwström et al., 2002; Kaštovská et al., 2005; Komárek et al., 2006; Lenzenweger and Lütz, 2006; Kim et al., 2008; Holzinger et al., 2009). Since most studies have typically reported one-time seasonal collections, results that describe species diversity for different years do not necessarily match. Also, several works provided species lists, but without describing species morphology or providing images of the microalgae.

Kubečková et al. (2001) cited 16 diatom species from the glacial streams in the vicinity of Ny-Ålesund. Kaštovská et al. (2005), who performed quantitative analyses of algae and cyanobacteria in different soil habitats near Ny-Ålesund in August 2002, listed 57 taxa from 23 genera of cyanobacteria and algae. Cyanobacteria comprised the major proportion of the algal assemblages, and algae (mainly green algae) were present only as accessory organisms. Lenzenweger and Lütz (2006) studied algae from very humid moss patches along the rims of small ponds near the shore of the Kongsfjorden in July 2004 and reported 58 taxa of desmids. Kim et al. (2008) performed a field survey in June 2006 and reported 29 taxa in 25 genera of freshwater and terrestrial algae; diatoms and green algae comprised the major proportion of the algal assemblages. Each study reflected only a portion of the algal species diversity from this High Arctic environment.

The present study adds new information on the freshwater and terrestrial cyanobacteria and algae of Ny-Ålesund and Blomstrandhalvøya Island. The data were collected during our second field survey in June 2009 and compared to our previous data from June 2006 (Kim et al., 2008). Here we report on five previously unrecorded species from

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this area, including a commercially important, astaxanthin-containing *Haematococcus* species.

MATERIALS AND METHODS

Samples of freshwater, wet soil, and snow containing algae were collected daily from 15 to 21 June 2009. Typical weather conditions during that time were temperatures from -1.6˚C to 7.9˚C, humidity of 66–92%, wind 3.3–16.9 km/hr, and 24-hr daylight. Collecting sites included shallow puddles in Ny-Ålesund (78˚56′ N, 11˚56′ E), ice- and snow-fed streamlets and puddles 1–3 km away from Ny-Ålesund, and snow crust, ice- and snow-fed streamlets and puddles on Blomstrandhalvøya Island (78˚59′ N, 12˚03′ E). Blomstrandhalvøya Island is located in Kongsfjorden, about 5 km north of Ny-Ålesund, which is located on the Broggerhalvøya peninsula. Water-saturated vegetated soil samples, snow, and algal materials submerged in freshwater were placed separately in sterile plastic containers, labeled, and sealed with Parafilm® to avoid cross-contamination.

Mixed cultures were first established by adding materials to a modified liquid ATCC Medium 625 (Klochkova et al., 2006) in 90 × 15 mm and 90 × 60 mm plastic Petri dishes at 4–10˚C, 15 µmol photons m^{-2} s^{-1} cool-white fluorescent lighting, and a 24-hr light regime. Wherever possible, unialgal strains were isolated.

Micrographs were taken with an Olympus DP50 digital camera affixed to an Olympus BX50 microscope, using Viewfinder Lite and Studio Lite computer programs. The organisms were mainly identified following the publications of Ettl and Gärtner (1995), Krammer and Lange-Bertalot (1997), Brook and Johnson (2002), John and Tsarenko (2002), Johnson and Merritt (2002), Whitton (2002), and Rajaniemi et al. (2005).

RESULTS

Thirty taxa in 23 genera of terrestrial and freshwater algae and cyanobacteria were identified from specimens collected near Ny-Ålesund (26 taxa) and on Blomstrandhalvøya Island (24 taxa). Table 1 lists these taxa and their habitats, and some examples are shown in Figures 1 and 2. We collected five species previously unrecorded in this area: a yellow-green alga *Tribonema* sp., two green algae, *Desmotetra* sp. and *Haematococcus* sp., a pennate diatom *Pinnularia* sp., and a naked dinoflagellate *Gymnodinium* sp. (Figs. 1G, 2). Three species, *Desmotetra*, *Haematococcus*, and *Gymnodinium*, were collected from Blomstrandhalvøya Island, and *Pinnularia* and *Tribonema* were collected in the vicinity of Ny-Ålesund. Also, *Tribonema*, *Desmotetra*, and *Gymnodinium* were not observed in microscopy at first and became noticeable only after several months in culture.

Algae Found in Snow

A sample of snow crust approximately 20 cm thick was collected (Fig. 1C and D), brought to the laboratory, and melted for further investigation of inhabiting microalgae. Upon first observation under a microscope, thick-walled ornamented cysts (Fig. $1F_{1–3}$), one filament of a green alga consisting of four cells (cell length 8–9.6 µm, width 3.2 µm), sarcinoid alga (four cells), and palmelloid aggregations of unicellular green algae were seen. Approximately 10 thick-walled ornamented cysts were present in 1 ml of freshwater from the melted snow (Fig. $1F_{1–3}$). The cysts were empty, 8.9–9.6 µm in size, thick-walled, with long or short spines and pentagonal ornamentation.

In the freshwater from melted snow, which was kept in a culture chamber at 5˚C, we later noted the development of six species: *Chlorococcum* sp. (9.6–11 µm in diameter, Fig. 1I), sarcinoid alga *Desmotetra* sp. embedded in mucilage (individual cells 3.2–4.8 µm in size; Fig. 1G), *Chlamydomonas nivalis* (Fig. $1H_{1–4}$), *Klebsormidium* sp. (cell width 7.3–8 µm; Fig. 1J), *Leptolyngbya foveolarum* (Fig. 1K), and unknown xanthophyceaen alga (Fig. 1L), which were not observed in microscopy at first and became noticeable only after two months in culture. *Desmotetra* sp. is newly recorded from this area.

Freshwater Haematococcus sp.

Haematococcus sp. was found on Blomstrandhalvøya Island, in a small freshwater basin in the rock fed with melted snow. Spherical, non-motile cells of *Haematococcus* sp. were 19.2–44.8 µm in diameter (28.2 ± 1.9 µm on average). Some cells were ellipsoidal. Motile, biflagellated cells with distinct morphology were rarely seen in field-collected samples (four biflagellated cells among several hundred non-motile astaxanthin-containing red cells). Accompanying species were filamentous blue-green algae (*Leptolyngbya* spp.). In this *Haematococcus* sp., efficient cell division was induced by a high temperature shock. The spherical, non-motile cells grown at 4˚–6˚C were placed in 15˚–20˚C temperatures overnight. They first became motile and then divided after one day of incubation in high temperature, releasing four to eight daughter cells each.

DISCUSSION

Our second survey of terrestrial and freshwater habitats in northwestern Spitsbergen took place three years after the first survey near Ny-Ålesund (Kim et al., 2008). New field collections were performed in the same summer season (15–21 June in 2009 vs. 13–18 June in 2006), which allowed comparison of yearly seasonal data. In 2009, the June weather conditions (-1.6˚ to 7.9˚C, humidity 66–92%, wind 3.3–16.9 km/hr) were somewhat warmer than in 2006 (-4˚ to 4˚C, humidity 46%, wind 22 km/hr). However, in June 2009, the snow crust was still largely present on the

TABLE 1. Taxa and habitats of cyanobacteria and algae found in the vicinity of Ny-Ålesund and on Blomstrandhalvøya Island.

Taxon	Location[1]	Habitat[2]	Study	
			Kim et al., 2008	This study
Chlorogloea sp.	NÅ	Terrestrial	+	
Komvophoron sp.	NÅ, B	Terrestrial		+
Leptolyngbya boryana (Gomont) Anagnostidis et Komárek	NÅ, B	Terrestrial		+
Leptolyngbya foveolarum (Montagne ex Gomont) Anagnostidis et Komárek	NÅ, B	Terrestrial, on snow		+
Leptolyngbya tenuis (Gomont) Anagnostidis et Komárek	NÅ, B	Terrestrial		+
Leptolyngbya sp. 1 (Pale bluish, cell length/width 7.5 × 5 µm, large oil droplets)	NÅ, B	Terrestrial	+	+
Leptolyngbya sp. 2 (Pale greenish or grayish, cell length/width 2.5–5 × 10 µm, large oil droplets)	NÅ, B	Terrestrial	+	+
Microcoleus vaginatus (Vaucher) Gomont et Gomont	NÅ	Terrestrial		+
Nostoc sp.	NÅ, B	Terrestrial	+	+
Phormidium sp. (Pale bluish or grayish, cell length/width 2.5–3.7 × 3.7 µm)	NÅ, B	Terrestrial	+	+
Trichormus sp. (Pale bluish, cell length/width 2.5–3.7 × 2.5 µm)	NÅ, B	Terrestrial	+	+
Chlorella minutissima Fott et Novakova	NÅ, B	Terrestrial	+	+
Chlorella vulgaris Beijerinck	NÅ, B	Terrestrial	+	+
Chlamydomonas nivalis (Bauer)	NÅ, B	Terrestrial, on snow	+	+
Chlorococcum sp.	NÅ, B	Terrestrial, on snow	+	+
Cladophora sp.	NÅ	Freshwater	+	
Coleochlamys cf. *cucumis*	NÅ, B	Terrestrial		+
Cosmarium subundulatum Wille	NÅ, B	Terrestrial	+	+
Cylindrocystis sp.	NÅ, B	Terrestrial	+	+
Desmotetra sp.[3]	B	Terrestrial, on snow		+
Haematococcus sp.[3]	B	Freshwater		+
Klebsormidium flaccidum (Kützing) Silva, Mattox et Blackwell	NÅ, B	Terrestrial	+	+
Klebsormidium sp. (Yellow-green, slimy, cell length/width 10–12.5 × 5–5.5 µm)	NÅ	Terrestrial	+	+
Klebsormidium sp. (Snow alga, cell width 7.3–8 µm)	B	Terrestrial, on snow		+
Muriella terrestris Petersen	NÅ	Terrestrial	+	
Scotiellopsis sp.	NÅ, B	Terrestrial	+	+
Zygnema sp.	NÅ, B	Freshwater	+	+
Achnanthes cf. *minutissima*	NÅ	Terrestrial	+	
Cymbella arctica (Lagerstedt) Schmidt	NÅ	Terrestrial	+	
Fragilaria sp.	NÅ	Terrestrial	+	
Hannaea arcus (Ehrenberg) Patrick	NÅ	Terrestrial	+	
Meridion circulare (Greville) Agardh	NÅ, B	Freshwater	+	+
Navicula sp.	NÅ	Terrestrial	+	
Nitzschia sp. (Needle-like, cell length/width 35–55 × 3.7–5 µm)	NÅ, B	Terrestrial	+	+
Pinnularia sp.[3] (Cell length/width 48–51.2 × 9.6–12.8 (at cell center) µm)	NÅ	Terrestrial		+
Tabellaria fenestrata Kützing	NÅ	Terrestrial	+	
Gymnodinium sp.[3]	B	Freshwater		+
Cryptomonas sp.	NÅ	Freshwater	+	
Tribonema sp.[3]	NÅ	Terrestrial		+
Vaucheria borealis Hirn	NÅ	Terrestrial	+	+

[1] NÅ = algae collected in the vicinity of Ny-Ålesund; B = algae collected from Blomstrandhalvøya Island.

[2] Organisms were considered as terrestrial if they grew on the soil and in its upper layers, in soil and mud sediments on the bottom of small puddles (at depths of less than 5 cm), or in minute streamlets and larger water bodies fed by meltwater.

[3] Algae first recorded in this study.

ground, and the streams from the melting glaciers were almost absent, whereas in June 2006 the snow had almost melted, except on the mountain slopes (Fig. 1A-B). Therefore, despite the warmer air temperatures in June 2009, the flowering plants and grasses were sparse compared to those observed in June 2006.

Of the 30 taxa identified in 2009, *Leptolyngbya* spp., *Trichormus* sp., *Klebsormidium* spp., and *Cylindrocystis* sp. were present in almost every sample, whereas in June 2006, *Cylindrocystis* sp. was so rare that it was not observed in microscopy at first and became noticeable only after several months in culture. On the other hand, we did not find diatoms *Tabellaria fenestrata* and *Fragilaria* sp. in June 2009, although in 2006 these species made almost unialgal blooms in the streams fed by melting snow. Different numbers of diatoms have been recorded from the vicinity of Ny-Ålesund in different years and months. For example, Kubečková et al. (2001) listed 16 diatoms, whereas Kaštovská et al. (2005) found five and Kim et al. (2008) found eight. Taxa *Scotiellopsis* sp., *Meridion circulare*, *Chlorella* spp., and *Cosmarium subundulatum*, which were common in June 2006, occurred only sporadically in soil and freshwater samples in June 2009. Also, in 2009 we did not find several algae (e.g., *Chlorogloea* sp., *Muriella terrestris*, *Cryptomonas* sp.) that were present in June 2006. As suggested by Kim et al. (2008), dynamic changes in species composition and diversity can occur in different years.

During this survey, we also collected four other species previously unrecorded from this locality: *Desmotetra* sp. and *Gymnodinium* sp. from Blomstrandhalvøya Island and *Pinnularia* sp. and *Tribonema* sp. from the vicinity of Ny-Ålesund. *Desmotetra* sp. was collected from the snow,

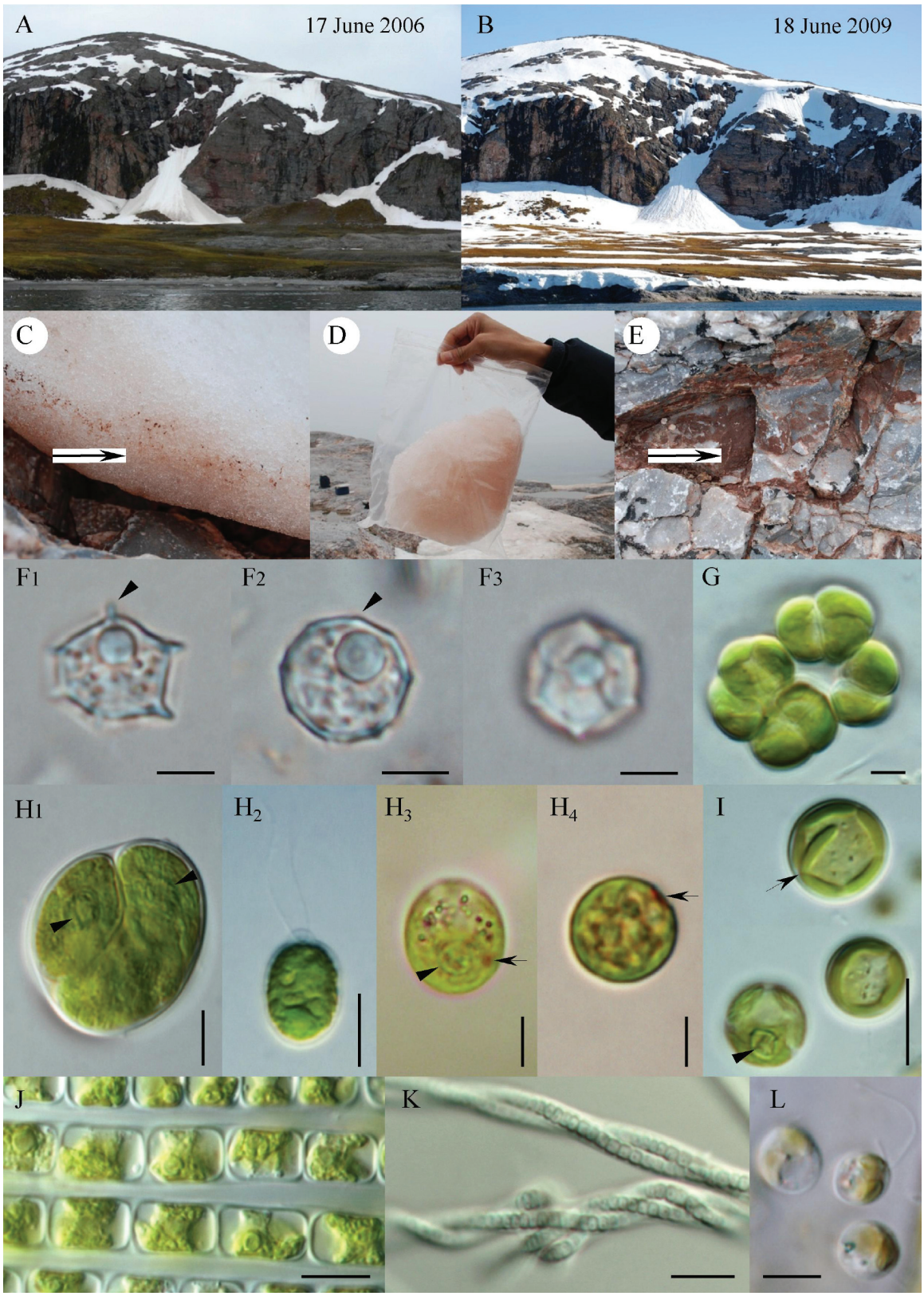

FIG. 1. A-B. Same seashore of Blomstrandhalvøya Island, as photographed on 17 June 2006 and 18 June 2009. In 2009, less snow had melted than in 2006. C. Thick snow crust saturated with a red-colored substance. D. Collected snow brought to laboratory. E. Further microscopic observation revealed that the source of red color on the snow was debris from the ground rock (arrow). F–L. Algae collected from snow. F_1–F_3. Through-focus images of thick-walled undetermined cysts with long (F_1, arrowhead) or short (F_2, arrowhead) spines and pentagonal ornamentation (F_3). G. Sarcinoid alga *Desmotetra* sp. embedded in mucilage. H_1–H_4. *Chlamydomonas nivalis*. Zoosporangium (H_1), motile biflagellated cells (H_2–H_4). Arrowheads and arrows point to pyrenoid and eye spot, respectively. I. *Chlorococcum* sp. Arrowhead and arrow point to pyrenoid and lobbed chloroplast, respectively. J. *Klebsormidium* sp. K. *Leptolyngbya foveolarum*. L. Unknown xanthophyceaen alga. Scale bars: 5 µm (F_1–F_3, G, H_3, H_4, L), 10 µm (H_1, H_2, I, J, K).

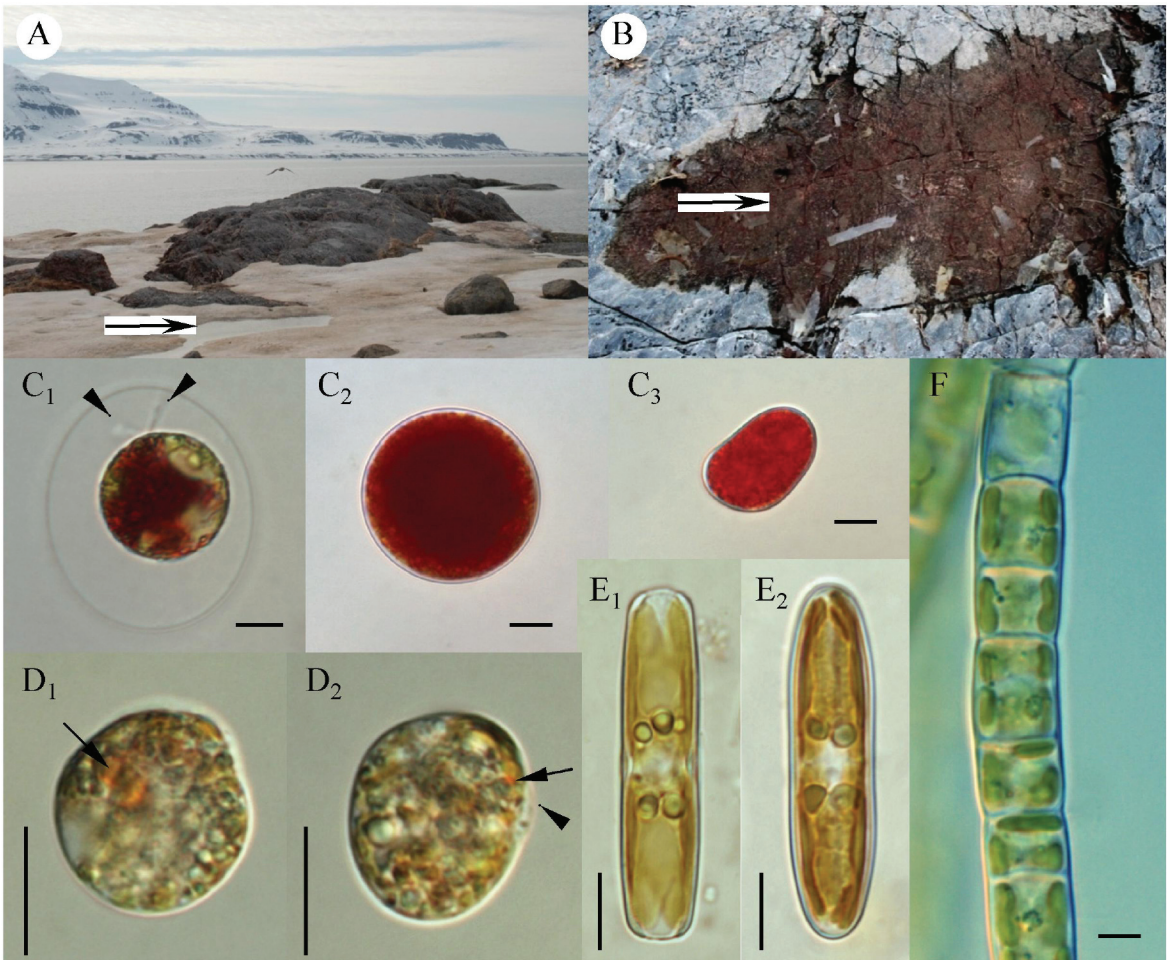

FIG. 2. Previously unrecorded algae collected from Blomstrandhalvøya Island (A–D) and in the vicinity of NÅ (E and F). A. Site where *Haematococcus* sp. and *Gymnodinium* sp. were collected. Arrow points to a snow-fed stream, which supplied water to a small shallow basin in the rock (B, arrow) where *Haematococcus* sp. grew abundantly, covering the bottom with a red-colored film. C_1–C_3. *Haematococcus* sp. C1. Motile biflagellated cell (arrowheads point to flagella). C_2, C_3. Non-motile spherical and ellipsoidal cells of different sizes. D_1, D_2. Cells of naked dinoflagellate *Gymnodinium* sp. collected from soil sediments. Arrows point to eyespots and arrowhead points to flagellum. E_1, E_2. Diatom *Pinnularia* was rare, present in soil sediments in ice- and snow-fed shallow puddles and streamlets. F. *Tribonema* sp. Cells with several chloroplasts without pyrenoids and H-shaped cell wall. Scale bars: 10 µm.

together with species *Chlamydomonas nivalis*, *Klebsormidium* sp., *Chlorococcum* sp., *Leptolyngbya foveolarum*, and an unidentified xanthophyceaen alga. Two cryophilic *Desmotetra* species, *D. aureospora* Ling and *D. antarctica* (Fritsch) Ling, were reported as snow algae from the Windmill Islands region, Antarctica (Ling, 1996, 2001). *Klebsormidium* species are also well known for their high freezing resistance (Elster et al., 2008). Komárek and Nedbalová (2007) listed *C. nivalis*, *Desmotetra,* and *Klebsormidium* species among the main green algae from cryosestic habitats. However, other taxa that we found in the snow were well-established soil and freshwater algae that apparently had intermingled with typical snow algae (Fig. 1E).

Leya et al. (2004) reported that *Chlamydomonas* sp. and *Chloromonas nivalis* (Chodat) Hoham et Mullet were responsible for the red and green snow, and *Ancylonema nordenskiöldii* Berggren and *Mesotaenium berggrenii* (Wittrock) Lagerheim were responsible for the violet snow, on glacier fields in northwestern Spitsbergen. However, we did not find microalgae *A. nordenskioldii* and *M. berggrenii* and astaxanthin-containing resting Chlorophyceaen spores in our snow samples.

Until recently, the Kongsfjorden and Ny-Ålesund area was difficult to access and isolated enough to be considered a model ecosystem for studies on Arctic environments (Hop et al., 2002; Holzinger et al., 2009). Nowadays, however, it is visited by many tourists. Several cruise ships arrive at Ny-Ålesund almost daily in summer, and numerous tourists have short breaks in Ny-Ålesund and on Blomstrandhalvøya Island. One may speculate about their negative influences, such as importation of non-native organisms. The finding of ubiquitous mesophilic microalgae in Spitsbergen (Leya et al., 2004; this study) with dramatically different temperatures for survival and reproduction might lead one to presume their anthropogenic introduction. When defining an area of species distribution, one should consider that it is limited by the temperatures needed for maximum or optimum reproduction, and not by those needed for survival. We found *Haematococcus* sp., which reproduces efficiently at 15˚–20˚C, on Blomstrandhalvøya Island, even though

the average summer temperature of Svalbard reaches only 4˚–6˚C. Moreover, some other algae from Svalbard die after several hours in an ambient temperature (Kim et al., 2008).

The AlgaeBase (Guiry, 2010) listed 15 species of *Haematococcus*, of which seven have been currently accepted taxonomically. This genus is cosmopolitan, reported from all continents except Antarctica (Guiry, 2010). In this study, we have applied the name *Haematococcus* sp. to our isolate until more information becomes available, because its morphology and distribution do not correspond to the taxonomically accepted species. This is the first report on a *Haematococcus* species from the High Arctic; moreover, our molecular-phylogenetic data strongly suggest that this species may be new (G.H. Kim et al., unpubl. data).

H. pluvialis is the richest known natural source for astaxanthin, containing 1.5–3.0% astaxanthin by dry weight, and has gained acceptance in aquaculture and other markets as a "concentrated" form of natural astaxanthin (Todd Lorenz and Cysewski, 2000). Finding astaxanthin-producing *Haematococcus* species well adapted to a harsh Arctic environment is important from the biotechnological point of view.

In conclusion, our new data from this High Arctic area extend the knowledge on its species diversity, which appears to be more abundant than was previously thought (e.g., Skulberg, 1996; Säwström et al., 2002; Kaštovská et al., 2005; Komárek et al., 2006; Lenzenweger and Lütz, 2006; Kim et al., 2008). Our results also highlight the need for further phycological studies into snow, freshwater, and terrestrial Arctic ecosystems.

ACKNOWLEDGEMENT

This study was supported by KOPRI grant #PE09060 to Han Gu Choi and by a grant from the Marine and Extreme Genome Research Center Program of the Ministry of Land, Transportation and Maritime Affairs, Republic of Korea, to Gwang Hoon Kim.

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