

## Life History and Systematic Studies of *Pseudothrix borealis* gen. et sp. nov. (=North Pacific *Capsosiphon groenlandicus*, Ulotrichaceae, Chlorophyta)

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We cultured a tubular marine green alga, originally identified as *Capsosiphon groenlandicus* (J. Agardh) K.L. Vinogradova, from Amaknak Island, Alaska. The alga had an alternation of heteromorphic generations in which tubular monoecious fronds produced quadriflagellate zoospores and/or biflagellate isogametes. The gametes fused to produce cysts or Codiolum-like zygotes with long, tortuous stalks. Cysts and codiolum produced 8-16 aplanospores, which germinated *in situ* to yield upright fronds. Fronds arising from both aplanospores and zoospores displayed a distinctive development in which non-septate colorless rhizoids from the base of the initially uniseriate, *Ulothrix*-like filament were transformed into septate uniseriate *Ulothrix*-like photosynthetic filaments. These transformed filaments then developed new basal non-septate rhizoids. This pattern of rhizoids becoming filaments, which then produced new rhizoids, was repeated to yield a tuft of up to 50 fronds. Periclinal and longitudinal divisions occurred in each filament, starting basally, until the mature tubular thallus was achieved. Pyrenoid ultrastructure revealed several short inward extensions of chloroplast lamellae, each of which was surrounded by pyrenoglobuli. Analysis of ribosomal SSU and ITS sequences placed this alga in the family Ulotrichaceae, order Ulotrichales, together with but as a distinct species from North Atlantic *Capsosiphon groenlandicus*. Analysis of a partial ITS sequence from authentic *Capsosiphon fulvescens*, the current name of the type of the genus *Capsosiphon*, indicated that neither our material nor *C. groenlandicus* belongs in that genus, and we propose a new genus, *Pseudothrix*, to accommodate both species. We propose *P. borealis* for the North Pacific entity formerly called *C. groenlandicus* and make the new combination *P. groenlandica* for the Atlantic species.

**Key Words:** *Capsosiphon fulvescens*, *Capsosiphon groenlandicus*, life history, morphology, *Protomonostroma undulatum*, *Pseudothrix* gen. nov., Ulotrichales, 18S rRNA gene

### INTRODUCTION

*Monostroma groenlandicum* J. Agardh (1883) was first recorded from the North Pacific Ocean by Saunders (1901) from Kukak Bay, Alaska, and by Setchell and Gardner (1903) from Amaknak Island, Alaska. Yendo (1909) noted its occurrence in Japan. The species is considered part of the cold water flora of the North Pacific and has been documented from the western Gulf of Alaska (northern Alaska Peninsula – Saunders 1901), the eastern Aleutian Islands (Setchell and Gardner 1903), the Commander Islands (Sinova 1940; Selivanova and Zhigadlova 1997), the Kamchatka Peninsula (Klochkova 1998), the Okhotsk Sea and Kurile Islands (Nagai 1940; Vinogradova 1979), and eastern Hokkaido (Yoshida 1998). However, it has not been recorded from the Bering

Sea coast of Alaska or the Arctic coasts of Alaska or Siberia.

*Capsosiphon groenlandicus* (J. Agardh) K.L. Vinogradova, as the species is now known, does not fit well in any of the genera to which it has been assigned. It differs from *Monostroma* in that the tubular thallus never ruptures longitudinally to form a *monostromatic* blade. Because of this, Setchell and Gardner (1920) assigned the species to *Enteromorpha*, as *E. groenlandica* (J. Agardh) Setchell & N.L. Gardner, but noted that it did not fit easily in that genus either since the cells of the thallus were not set sufficiently close together to make the thallus appear parenchymatous, as is characteristic of species of *Enteromorpha* (now included in *Ulva* – Hayden *et al.* 2003). In 1968, Vinogradova made the combination *Bidingia groenlandica* (J. Agardh) K.L. Vinogradova. The following year, she included the species in *Capsosiphon*, noting the loose arrangement of cells, similar to other species of that genus (Vinogradova 1969, without

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basionym; 1974). The species has remained in *Capsosiphon* since.

The Pacific specimens identified as *Capsosiphon groenlandicus* fit uneasily in that species. From its first record in the North Pacific, Saunders (1901) noted that his specimens had cells little more than half the size of North Atlantic specimens despite their similarities in morphology, a comment reiterated by Collins (1909), who included comparative dimensions of the two entities.

Japanese *Capsosiphon groenlandicus* was studied by Tatewaki (1969, 1972, as *Monostroma groenlandicum*), who reported that a cylindrical or tubular, monoecious gametophyte alternated with a one-celled cyst-like sporophyte; the gametophyte produced biflagellate gametes that were liberated in a hyaline sac through a linear pore in the gametangium, and the sporophyte produced 4-8 aplanospores. Hori (1973) compared the pyrenoid ultrastructure of Japanese *C. groenlandicus* (as *M. groenlandica*) to other species of *Monostroma* and related genera and noted its similarity to *C. fulvescens* (C. Agardh) Setchell & N.L. Gardner.

In addition to *C. groenlandicus*, the genus *Capsosiphon* currently contains only *C. fulvescens* and *Capsosiphon aurea* V.J. Chapman. *Capsosiphon aureolum* (C. Agardh) Gobi, the type of the genus, is considered a synonym of *C. fulvescens*. *Capsosiphon fulvescens* is distinguished by cells arranged in distinct vertical to spiral files with the cells often grouped in twos to fours and retained in a common mucilaginous envelope (Bliding 1963). In contrast to this, the cell arrangement in *C. groenlandicus* is mainly scattered, not in distinct vertical files (Vinogradova 1979).

*Capsosiphon fulvescens* has been well-studied (Bliding 1963, 1968; Chihara 1967; Migita 1967; Yoshida 1970; Kornmann and Sahling 1978). Little if anything has been published on *C. aurea* since its description. Collins (1909) provided a detailed description of Massachusetts material of *C. groenlandicus* and noted that cells of Pacific material were "decidedly smaller than in specimens from the Atlantic; 8-10  $\mu\text{m}$  diam. in the former, 12-16  $\mu\text{m}$  diam. in the latter, seen superficially."

Because of the uncertainty as to the generic as well as the specific placement of the North Pacific entity known as *Capsosiphon groenlandicus*, we have examined this taxon using newly collected material from Amaknak Island, Alaska. We cultured the material through its life cycle and subjected it to DNA sequencing. Below, we report the results.

## MATERIALS AND METHODS

Specimens were collected from mid intertidal boulders near the Dutch Harbor airport on the west shore of Amaknak Island, Alaska (53°53'38.2" N 166°32'35.5" W), 4 August 2004, by S.C. Lindstrom (UBC A84928). They were kept cool and damp until returned to the laboratory.

Field thalli were initially placed in liquid culture and on agar plates at 2 and 10°C on a 14:10 h L:D cycle under Sylvania F48T full spectrum fluorescent tubes (Sylvania Osram, Danvers, MA) providing 20-40  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  of photosynthetically active radiation. Liquid culture medium and agar petri plates were prepared as described by Hanic (2005).

Zooids from fertile field thalli were grown in liquid culture for 3 mo at 8°C 14:10h LD cycle and then for 2 wk at 2°C 6:18h LD cycle. All material was scraped off the substrate, dispersed in test tubes by vortexing, pelleted by centrifugation, washed, resuspended, pelleted, washed again, and finally resuspended in 1 mL of medium and spread on several agar petri plates. Zygotes were removed individually and placed in separate liquid cultures at 8°C on a 14:10h LD cycle.

Observations on gamete morphology, behavior, and matings were made in a temperature-controlled environmental chamber using the hanging drop method (Hanic 2005). Osmic acid-fixed gametes and zoospores were drawn at 1000 times magnification with the aid of a camera lucida over 30-60 minutes after fixation, and measurements were made from these drawings to the nearest mm. Light micrographs were taken using a Zeiss Axioplan II microscope with a Q-imaging digital camera or using an Olympus dissecting microscope with a Sony Cybershot 5.1 MPEG digital camera. Thalli were sectioned with a freezing microtome and stained with either eosine or I<sub>2</sub>-KI or both.

Electron microscopy was carried out on gametophytic thalli fixed at 4°C for 2 hr in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Material was post-fixed in 2% osmium tetroxide, dehydrated in an ethanol series, infiltrated with Spurr's resin, polymerized at 60°C, sectioned at 70 nm with a diamond knife, stained in aqueous uranyl acetate and Reynolds's lead citrate and mounted on formvar-coated grids. Viewing was done with a Hitachi electron microscope Model H 7600.

We extracted DNA from silica-gel-dried field material and from live cultures using published protocols

**Table 1.** Sources of sequences and GenBank No. for taxa used in phylogenetic analyses of the 18S rRNA gene and ITS regions.

Species	Reference	Source	GenBank No.
<i>Acrochaete endozoica</i> (W.M. Goldberg, J.C. Makemson & S.B. Colley) M.J. Wynne	O'Kelly <i>et al.</i> 2004b	Endozoic in <i>Pseudoplexaura</i> sp. (gorgonian coral)	AY205327 (18S)
<i>Acrosiphonia</i> J. Agardh	Zechman F.W. & Chapman R.L., unpublished	No data	U03757 (18S)
<i>Acrosiphonia coalita</i> (Ruprecht) Scagel, Garbary, Golden & M.W. Hawkes	Lindstrom & Hanic 2005	Baker Beach, California (CA)	AY455943 (ITS)
<i>Acrosiphonia duriuscula</i> (Ruprecht) Yendo	Watanabe <i>et al.</i> 2001	Okitu, near Kushiro, Hokkaido, Japan	AB049418 (18S)
<i>Blidingia dawsonii</i> (Hollenberg & I.A. Abbott) S.C. Lindstrom, L.A. Hanic & Golden	Lindstrom <i>et al.</i> 2006	Seppings I., Barkley Sound, British Columbia (BC)	DQ001138 (18S & ITS)
<i>Bolbocoleon piliferum</i> Pringsheim	O'Kelly <i>et al.</i> 2004a	Mar Vista, San Juan I., WA; Nobska Pt, Woods Hole, MA	AY303598 (18S); AY303599 (18S)
<i>Capsosiphon fulvescens</i> (C. Agardh) Setchell & N.L. Gardner	Hayden & Waaland 2002; this study	Monks Head Beach, Nova Scotia (NS); Kattegat, Denmark	AF499664 (18S); EU541503 (ITS1)
<i>Chlorothrix</i> Berger-Perrot	Lindstrom & Hanic 2005	Ucluelet, Vancouver I., BC	AY653740 (18S & ITS)
<i>Collinsiella tuberculata</i> Setchell & N.L. Gardner	O'Kelly <i>et al.</i> 2004b	Cattle Point, San Juan Island, Washington (WA)	AY198125 (18S & ITS)
<i>Dangemannia microcystis</i> (P.J.L. Dangeard) Friedl & O'Kelly	Friedl & O'Kelly 2002	SAG 2022	AJ416104 (18S)
<i>Desmochloris halophila</i> (Guillard, H.C. Bold & MacEntee) Shin Watanabe, Kuroda & Maiwa	Watanabe <i>et al.</i> 2001	UTEX 2073	AB049416 (18S)
<i>Gayralia oxysperma</i> (Kützing) K.L. Vinogradova ex Scagel <i>et al.</i>	Woolcott & King unpublished	No data	AY016306 (ITS)
<i>Gloeotilopsis sarcinoidea</i> (Groover & H.C. Bold) Friedl	Bhattacharya <i>et al.</i> 1996	UTEX 1710	Z47998 (18S & ITS)
<i>Halochlorococcum moorei</i> (N.L. Gardner) Kornmann & Sahling	O'Kelly <i>et al.</i> 2004b	Friday Harbor Labs, San Juan I., WA	AY198122 (18S)
<i>Halochlorococcum porphyrae</i> (Setchell & N.L. Gardner) J.A. West	This study	Carmel, CA	DQ821520 (18S)
<i>Kornmannia leptoderma</i> (Kjellman) Bliding	Su Q., Luan R. and An L., unpublished	No data	AF415168 (ITS)
<i>Monostroma grevillei</i> (Thuret) Wittrock	Tan I. and Sluiman H.; Su Q., Luan R. and An L., unpublished	No data	AF015279 (18S); AF428049 (ITS)
<i>Monostroma grevillei</i> var. <i>vahliei</i> (J. Agardh) Rosenvinge	Su Q., Luan R. and An L., unpublished	No data	AF428051 (ITS)
<i>Monostroma nitidum</i> Wittrock	Su Q., R. Luan and An L., unpublished	No data	AF415170 (ITS)
<i>Ochlochaete hystrix</i> Thwaites ex Harvey	O'Kelly <i>et al.</i> 2004c	Quissett Estuary, Woods Hole, MA	AY454428 (18S)
<i>Oltmannsiellopsis viridis</i> (Hargraves & R.L. Steele) Chihara & I. Inouye	Nakayama <i>et al.</i> 1996	No data	D86495 (18S)
<i>Phaeophila dendroides</i> (P. Crouan & H. Crouan) Batters	O'Kelly <i>et al.</i> 2004c	Lime Kiln Pt, San Juan I., WA	AY454431 (18S)
<i>Planophila laetevirens</i> Gerneck	Friedl & O'Kelly 2002	Dolomites, South Tyrol, Italy	AJ416102 (18S)
<i>Protomonostroma undulatum</i> (Wittrock) K.L. Vinogradova	This study; Su Q., Luan R. and An L., unpublished	Shaw Island, Katmai National Park, AK; China	DQ821517 (18S & ITS); AF415169 (ITS)
<i>Pseudoneochloris marina</i> Shin Watanabe, A. Himizu, L.A. Lewis, G.L. Floyd & P.A. Fuerst	Watanabe <i>et al.</i> 2000	UTEX 1445	U41102 (18S)

**Table 1.** (continued)

Species	Reference	Source	GenBank No.
<i>Pseudothrix borealis</i> sp. nov. (=North Pacific <i>Capsosiphon groenlandicus</i> )	This study	Amaknak Island, Unalaska Island, Alaska (AK)	DQ821514 (18S & ITS)
<i>Spongomorpha aeruginosa</i> (Linnaeus) van den Hoek	Van Oppen 1995	Iceland	Not accessioned (ITS)
<i>Ulothrix</i> sp. (as <i>Chlorothrix</i> 47SI)	Lindstrom & Hanic 2005	Ucluelet, BC	AY476827 (18S & ITS)
<i>Ulothrix</i> sp. (X1)	This study	Amaknak Island, AK	DQ821515 (18S & ITS)
<i>Ulothrix</i> sp. (X3)	This study	Amaknak Island, AK	DQ821516 (18S & ITS)
<i>Ulothrix</i> sp. (U179)	This study	Amaknak Island, AK	Not accessioned (ITS)
<i>Ulothrix zonata</i> (Weber & Mohr) Kützing	O'Kelly <i>et al.</i> 2004b; Friedl 1996	Lake Michigan	AY278217 (18S); Z47999 (ITS)
<i>Ulva intestinalis</i> Linnaeus [as <i>Enteromorpha intestinalis</i> (Linnaeus) Nees]	Tan I. & Sluiman H., unpublished	No data	AJ005413
<i>Urospora neglecta</i> (Kornmann) Lokhorst & Trask	Lindstrom & Hanic 2005	Seward, AK	AY476821 (18S & ITS)
<i>Urospora penicilliformis</i> (Roth) Areschoug	Lindstrom & Hanic 2005	Point No Point, Vancouver I., BC	AY476808 (18S & ITS)
<i>Urospora wormskioldii</i> (Mertens ex Hornemann) Rosenvinge	Lindstrom & Hanic 2005	Louisbourg, NS	AY476816 (18S & ITS)

(Lindstrom and Fredericq 2003). We amplified and sequenced the DNA using the same procedures as Lindstrom and Hanic (2005). Since the identification of our material also depended on the molecular signature of *Capsosiphon fulvescens*, the current name of the type species of the genus *Capsosiphon*, we amplified the relatively short (~250 bp) ITS1 region in specimen No. 804, Algae Marinae Danicae Exsiccatae (UBC A70644) using primers ITS1 and ITS2 (White *et al.* 1990). For the ITS2 primer, we modified the sequence to fit that region of the genome in the Ulotrichales: 5'-GCTGCGTTCTTCATCG TTGC-3'. This specimen of *C. fulvescens* was collected and determined by Ruth Nielsen, 1 Sep 1988, at Hirsholm, Kattegat, Denmark (57° 29' N 10° 38' E). This location is in the same region as the type locality of *C. fulvescens*: Landskrona, Sweden, just south of the Kattegat.

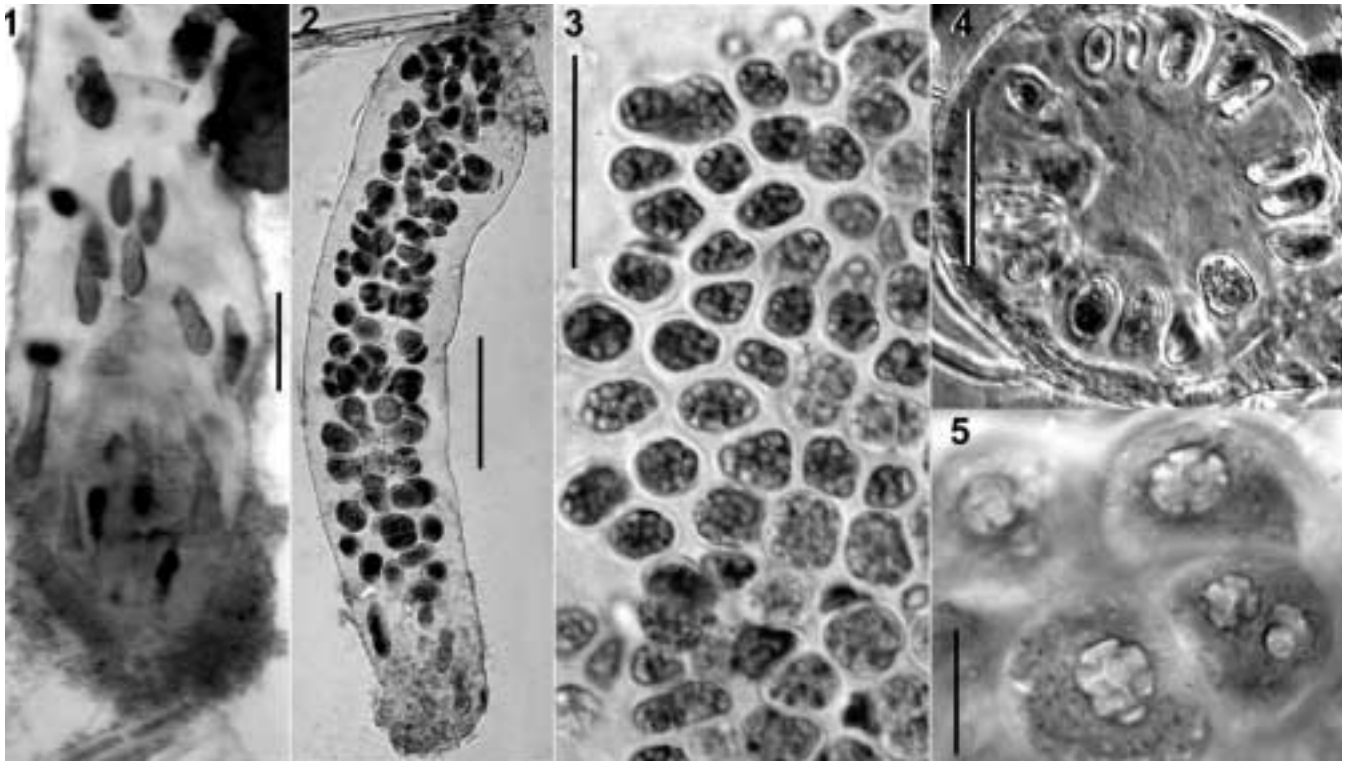
Sequences were aligned with GenBank sequences of other ulvophycean taxa (Table 1) using BioEdit (Tom Hall, Department of Microbiology, North Carolina State University, Raleigh, NC, USA). Phylogenetic analyses were performed using the maximum parsimony (MP) and maximum likelihood (ML) algorithms of the computer program PAUP\*4.0b10 (Swofford 2002) as implemented by Lindstrom and Fredericq (2003). ML analyses were carried out after determining the appropriate evolutionary model inferred by Modeltest v.3.7 (Posada and Crandall 1998). Bootstrap proportions were determined based on 20000 replicates for MP and 100 for ML. *Oltmannsiellopsis viridis* (Hargraves & R.L. Steele)

Chihara & I. Inouye and *Dangemannia microcystis* (P.J.L. Dangeard) Friedl & O'Kelly, early diverging species in the Ulvophyceae (Nakayama *et al.* 1996; Friedl and O'Kelly 2002), were used as outgroup for the 18S rRNA gene sequences, and *Kornmannia leptoderma* (Kjellman) Bliding and *Blidingia dawsonii* (Hollenberg & I.A. Abbott) S.C. Lindstrom, L.A. Hanic & Golden served as outgroup for the nuclear ribosomal ITS sequences.

In addition to North Pacific *C. groenlandicus*, we sequenced the 18S rRNA gene and the ITS regions of an Alaskan specimen *Protomonostroma undulatum* (Wittrock) K.L. Vinogradova to verify its identity. We obtained a partial 18S rDNA sequence for field material of *Halochlorococcum porphyrae* (Setchell & N.L. Gardner) J.A. West, an endophyte in *Porphyra schizophylla* Hollenberg. We also obtained 18S rDNA and ITS sequences for two *Ulothrix*-like species that appeared in the initial cultures from which our North Pacific *C. groenlandicus* was isolated. These species were isolated into unialgal culture. Since the taxonomic position of all of these species is relevant to the present study, their sequences are included in this paper. Dr. Charley O'Kelly kindly provided an unpublished ITS sequence of North Atlantic *Capsosiphon groenlandicus* from Maine, USA, for comparison with our North Pacific material.

## RESULTS

Field thalli were filiform, tubular, and dark green, up to 10 mm long and 1.2 mm wide. They formed extensive



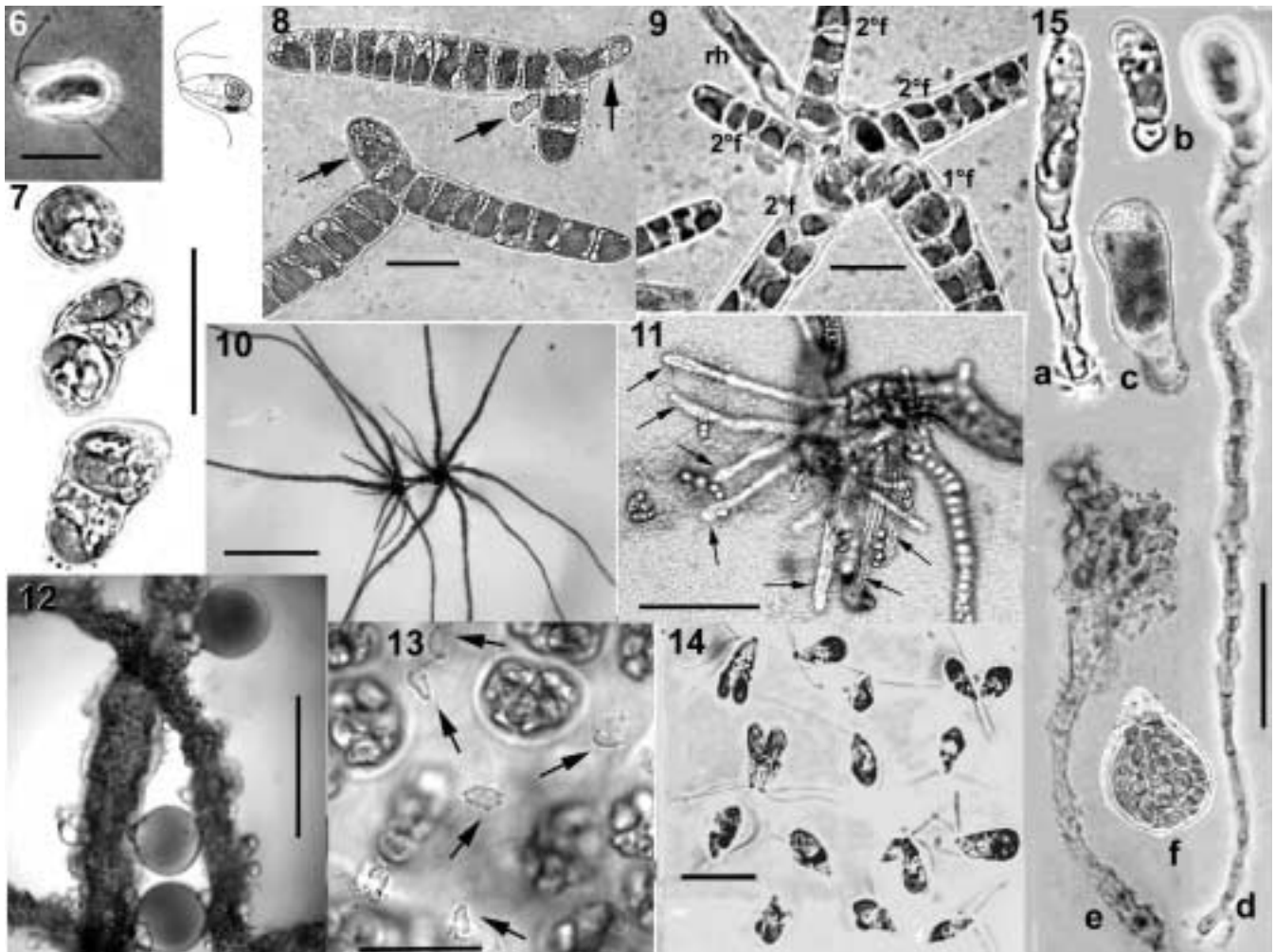
**Figs 1-5.** *Pseudothrix borealis* (=North Pacific *Capsosiphon groenlandicus*). 1. Holdfast of a mature field thallus showing several rhizoids with narrow tops and broader bases forming a blunt rounded structure. The dark basal area consists of substrate debris, unicellular algae and bacterial filaments. Scale bar = 20  $\mu\text{m}$ . 2. Small field thallus showing widely scattered cells in gelatinous wall material, cells often grouped in twos to fours in common mother envelope. Scale bar = 20  $\mu\text{m}$ . 3. Fertile area of thallus showing relatively closely spaced gametangial cells. Scale bar = 50  $\mu\text{m}$ . 4. Cross section of field thallus showing hollow center filled with a solid gel. Scale bar = 20  $\mu\text{m}$ . 5. Vegetative cells containing a single outward-facing chloroplast with a central pyrenoid. Scale bar = 10  $\mu\text{m}$ .

patches on the tops of mid to high intertidal boulders, just below boulders dominated by either *Urospora neglecta* (Kornmann) Lokhorst & Trask and *Ulothrix* spp. or *Blidingia minima* (Naegeli ex Kützing) Kylin. The basal area of twelve thalli examined closely was blunt to rounded and consisted of elongate cells that expanded towards the base, resembling upside down rhizoids (Fig. 1). Cells of germlings were round to quadrangular, 2-2.5  $\mu\text{m}$  diam., in twos to fours, widely spaced and often in a common mucilaginous parental envelope (Fig. 2). Cells in mature thalli increased in size from base to apex. At one-third distance above the base, cells in surface view were 5.6-(6.4  $\pm$  1.59)-8.0  $\times$  3.0-(3.6  $\pm$  0.96)-7.0  $\mu\text{m}$  (n=30; numbers in parentheses the means of the measurements + one standard deviation); at mid thallus, 6.0-(7.5  $\pm$  1.66)-11  $\times$  3.0-(5.0  $\pm$  0.94)-7.0  $\mu\text{m}$  (n=19), and in distal fertile areas of the thallus, 12-(17  $\pm$  2.51)-22  $\times$  6.0-(12.4  $\pm$  2.30)-20  $\mu\text{m}$  (n=75). Cells were randomly arranged and 15-(22)-30  $\mu\text{m}$  deep (n=9); they were more widely spaced, up to two cell widths, near the base of the thallus (Figs 1, 2) com-

pared to fertile regions (Fig. 3). The thallus center was filled with a solid gel and became hollow in wider portions (Fig. 4). Vegetative cells contained a single outward-facing chloroplast with a single central pyrenoid (Fig. 5). Fertile cells contained up to 24 zooids.

Single field thalli produced both quadriflagellate zoospores and biflagellate gametes. Zoospores were ovoid, with a conspicuous posterior stigma and pyrenoid (Fig. 6); they measured 7.6-(9.3  $\pm$  0.86)-11.0  $\mu\text{m} \times$  4.0-(5.0  $\pm$  0.48)-5.8  $\mu\text{m}$  (length  $\times$  diameter; n=15), with flagella 9.0-(11.2  $\pm$  1.43)-12.0  $\mu\text{m}$  long (n=25).

Zoospore germination followed a distinctive pattern. Zoospores divided without forming a germination tube (Fig. 7) and developed into a short filament, which produced one to several short, broad, initially non-septate rhizoids at one end (Fig. 8, arrows). These rhizoids lengthened and underwent transverse cell divisions to become secondary uniseriate filaments (Fig. 9), which were *Ulothrix*-like in having a collar-shaped chloroplast and a single pyrenoid. These secondary filaments in turn

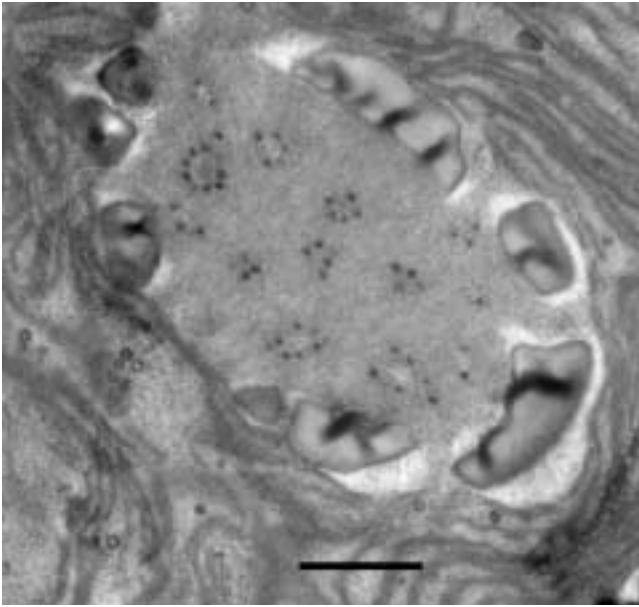


**Figs 6-15.** *Pseudothrix borealis* (=North Pacific *Capsosiphon groenlandicus*). 6. Zoospore micrograph (left) and line drawing (right). Scale bar = 10  $\mu\text{m}$ . 7. Germination of zoospores to form a short filament of broad cells. Scale bar = 20  $\mu\text{m}$ . 8. Next stage in filament development, showing initiation of rhizoids (arrows). Scale bar = 20  $\mu\text{m}$ . 9. Later stage in development in which secondary filaments ( $2^\circ\text{f}$ ) are being produced from cell divisions in former rhizoids.  $1^\circ\text{f}$  = primary filament, rh = rhizoid. Note *Ulothrix*-like collar-shaped chloroplast in filament cells. Scale bar = 20  $\mu\text{m}$ . 10. A later stage showing two thalli (originally produced by two zoospores) with each thallus having about 8 uprights. Scale bar = 1 mm. 11. Additional non-septate rhizoids (arrows) formed at the base of a thallus with multiple uprights. The larger filaments have begun periclinal cell divisions at the base. Scale bar = 100  $\mu\text{m}$ . 12. Cultured thallus with lateral bubbles filled with mucilage. Scale bar = 200  $\mu\text{m}$ . 13. Fertile area showing gametangia with 8-16 zooids and elongate oval release pores (arrows). Scale bar = 20  $\mu\text{m}$ . 14. Gametes and copulants from a single fertile cultured thallus. Scale bar = 20  $\mu\text{m}$ . 15. Codiola in various stages of development (a-e) and fertile cyst (f). Note empty spore germination and elongation of stalk in codioliola a-d. Codiolum with aplanospores (e), which have started to germinate *in situ*. Scale bar = 20  $\mu\text{m}$ .

produced short, swollen, initially non-septate, colorless rhizoids, which repeated the process to form tertiary uniseriate filaments. This process continued, yielding conjoined clusters of up to 50 upright filaments of varying lengths with a common multirhizoidal base, all arising from germination of a single zoospore (Fig. 10). All filaments underwent periclinal cell division, starting at the base and proceeding to the apex to produce upright fronds that were 50 or more cells in circumference and up to 9 cm long at maturity. As the uprights enlarged,

additional non-septate, colorless rhizoids formed from the base of the uprights (Fig. 11, arrows). Many of the cultured uprights developed lateral bubbles filled with mucilage (Fig. 12).

After 3 months in culture, the upright fronds became fertile, producing abundant biflagellate gametes, which were released through elongate oval pores (Fig. 13, arrows), but no zoospores. Gametes were elongate to spindle-shaped, and each contained a conspicuous stigma and an inconspicuous posterior pyrenoid; they mea-



**Fig. 16.** *Pseudothrix borealis* (=North Pacific *Capsosiphon groenlandicus*). Electron micrograph of cell of upright thallus showing pyrenoid surrounded by starch plates and penetrated by invaginations of chloroplast lamellae, which are surrounded by pyrenoglobuli. Scale bar = 1  $\mu\text{m}$ .

sured  $5.5\text{--}(7.6 \pm 1.08)\text{--}11 \times 2\text{--}(2.8 \pm 0.49)\text{--}4.3 \mu\text{m}$ , with flagella  $8\text{--}(11.8 \pm 1.28)\text{--}14 \mu\text{m}$  long ( $n=100$ ). Gametes from single thalli (field and culture) mated readily (Fig. 14), and plasmogamy was completed within 5 min.

A mating mixture produced codiolum-like cells (Fig. 15a-e) and cysts (Fig. 15f). The cysts were mostly spherical to slightly ovate, reaching  $50 \mu\text{m}$  diam. A few cysts also developed within empty gametangia, presumably due to germination of parthenogametes. Initially, the codioli germinated by the empty-spore process, the tube growing away from the zygote wall and over time laying down a long, tortuous stalk up to  $60 \mu\text{m}$  long. The codioli were sausage-shaped and ca  $30 \mu\text{m}$  long. Only a few cysts and codioli frozen for 30 days at  $-18^\circ\text{C}$  and returned to culture at  $5^\circ\text{C}$  on a 14:10 LD cycle became fertile two weeks later and produced up to 16 aplanospores (Fig. 15e). Cultured together, the aplanospores germinated following the same distinctive pattern displayed by zoospore germination and ultimately produced sexual tubular uprights to complete the life cycle.

**Pyrenoid ultrastructure.** The pyrenoid of our material was surrounded by several starch plates and traversed by a number of intrusions of the chloroplast lamellae (Fig. 16). These lamellae were themselves surrounded by a ring of pyrenoglobuli.

**Molecular analyses.** We sequenced both field-collected

and laboratory-grown material, which had identical sequences. Analyses of the ribosomal SSU gene sequence data using the species in Table 1 revealed our material to be a member of the Ulotracheae (Fig. 17), as did analyses of the ITS data, including the 5.8S rRNA gene (Fig. 18). In the SSU analysis, North Pacific *Capsosiphon groenlandicus* occurred within a clade containing other members of the Ulotracheae: *Acrosiphonia*, *Chlorothrix*, *Urospora*, *Protomonostroma* and *Ulothrix*. It occurred more distally than *Acrosiphonia* within the clade but with weak support; the remaining taxa of Ulotracheae occurred on a more distal, strongly supported branch. In the ITS analysis (Fig. 18), North Pacific *Capsosiphon groenlandicus* clustered with North Atlantic *C. groenlandicus* with weak to moderate support. Both of these species occurred distal to *Protomonostroma undulatum*, with the remaining genera distal to them but with their relative positions lacking bootstrap support.

Neither North Pacific nor North Atlantic *Capsosiphon groenlandicus* was related to *C. fulvescens* based on the partial ITS sequence we obtained from authentic material of that species (Fig. 18). *Capsosiphon fulvescens* appeared on its own branch at the base of the Ulotracheales in the ML analysis; in the MP analysis, it appeared on its own branch at the base of the Ulotracheae. In both analyses, it clearly belonged to the Ulotracheales since the subtending branch, separating the Ulotracheales from members of the Ulvales, had 100% bootstrap support in all analyses. Comparison of our short, 242-bp sequence to other nuclear sequences in GenBank using the BlastN search algorithm also indicated that our sequence showed its highest overall similarity to members of the Ulotracheales (*Protomonostroma undulatum*, *Capsosiphon groenlandicus*, *Monostroma grevillei*, *Ulothrix* and *Chlorothrix* spp.) although it was not highly similar to any of these sequences. The published SSU sequence of *C. fulvescens*, which is related to species of *Halochlorococcum*, including *H. porphyrae* (which was identical to *H. moorei* in our SSU analyses—Fig. 17), is clearly something else. This latter “*Capsosiphon fulvescens*” and *Halochlorococcum* spp. clustered with *Desmochloris halophila*, and other members of the Ulvales. We obtained similar results with more taxon-replete analyses (up to 50 taxa).

The Alaska specimen of *Protomonostroma undulatum* had an identical ITS sequence to that published for this species from China, except for three 1-2 bp indels. *Protomonostroma undulatum* occurred as the sister taxon to the *Ulothrix* spp. in the SSU analysis (Fig. 17) but was basal to other members of the Ulotracheae in the ITS

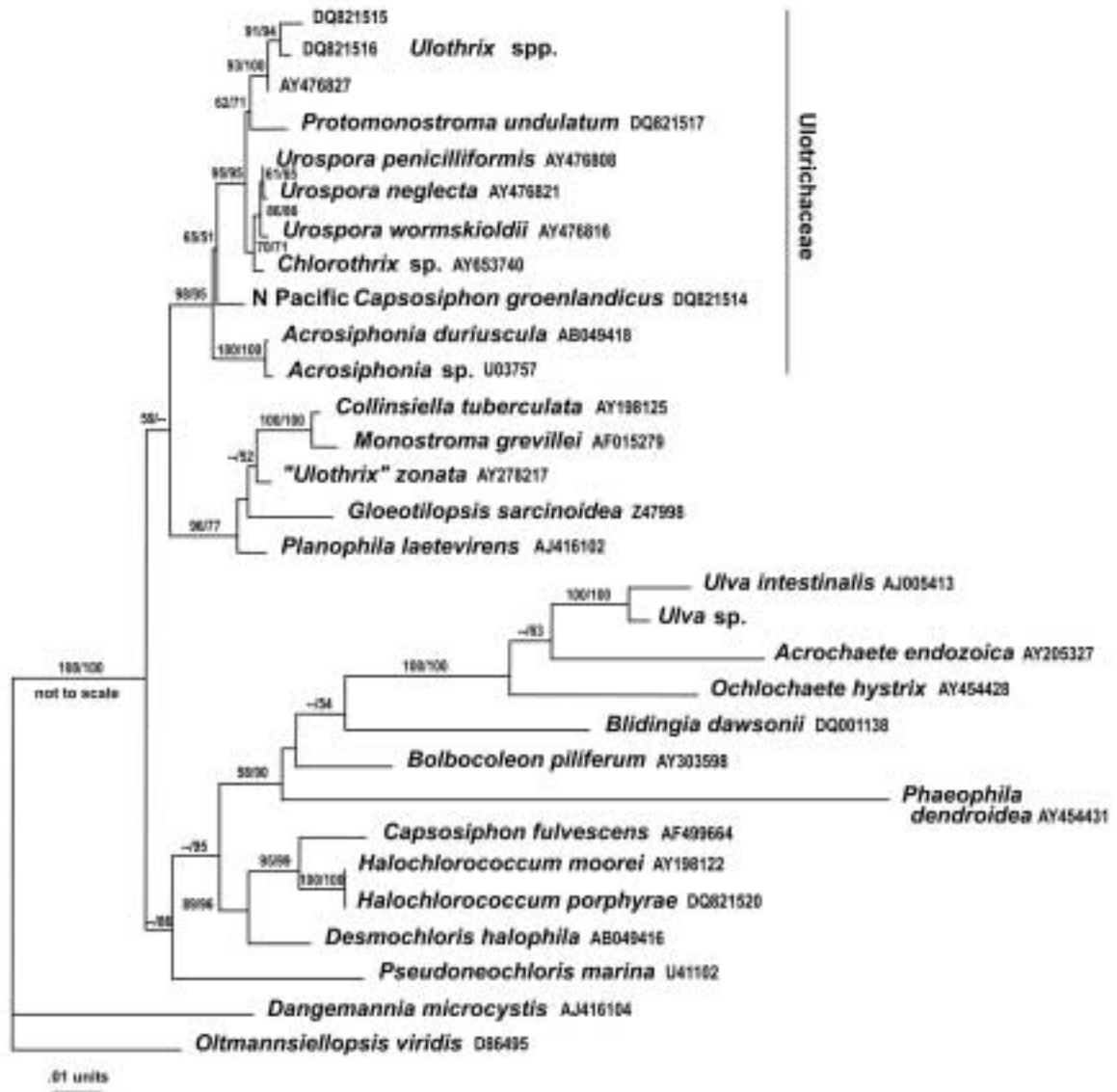


Fig. 17. Maximum likelihood tree (-lnL = 7177.3250) of 18S rRNA gene sequences of 30 species of Ulvophyceae. Numbers above branches are bootstrap proportions for maximum parsimony (left) and maximum likelihood (right) analyses. Sources of sequences are listed in Table 1.

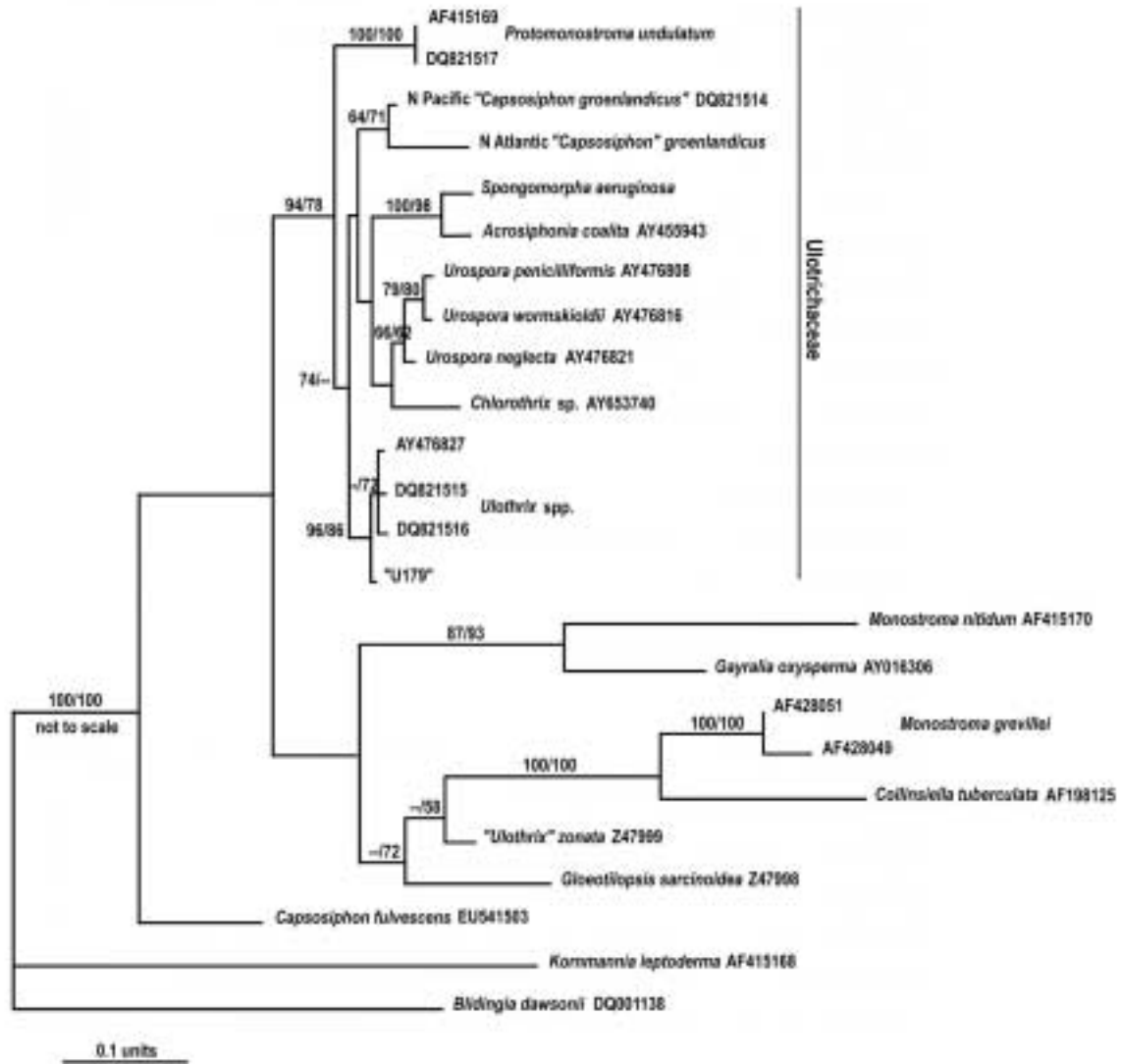
analysis (Fig. 18).

## DISCUSSION

The initial placement of our Alaskan material in *Capsosiphon* was based on thallus shape, which was cylindrical, hollow in part, with cells of the adult thallus being rounded, well-separated but enclosed in a mucilaginous substance, with daughter cells grouped in twos to fours, often contained in the mother cell envelope, as in *C. fulvescens* (the correct name of the type species, *C. aureolum*). Our material corresponded closely in cell and thallus morphology to *C. groenlandicus*, as described by Vinogradova (1979), in that the cells were

more or less scattered and not in distinct, vertical, twisted files, as in *C. fulvescens* (Bliding 1963, Kornmann and Sahling 1978). The basal region of our field material was unusual in being rounded to blunt (clublike) and consisting of several cells that were thinly drawn out at the top and swollen basally. Bliding (1963) described the holdfast of *C. fulvescens* as consisting of a ring of rounded basal cells that with their gelatinous walls attach the thallus to the substratum (Fig. 4i), and Garbary *et al.* (1982) illustrated the basal portion of *C. fulvescens* with irregularly stretched cells. These descriptions are more similar to the holdfast in our material than Yoshida's (1970), in which the cells of the holdfast of *C. fulvescens* were wide at the top and thinly drawn out basally, as normally





**Fig. 18.** Maximum likelihood tree ( $-\ln L = 4485.5687$ ) of rRNA ITS and 5.8S sequences of 24 species of Ulvophyceae. Numbers above branches are bootstrap proportions for maximum parsimony (left) and maximum likelihood (right) analyses. Sources of sequences are listed in Table 1.

occurs in species with basal holdfast (rhizoidal) cells.

Our studies indicate an alternation of heteromorphic generations consisting of two phases: (1) a monoecious multicellular tubular gametophytic generation that produces quadriflagellate zoospores, which recycle the phase, and isogamous biflagellate gametes, which produce (2) a unicellular codium or cyst-like sporophytic generation (derived from zygotes or parthenogametes). This latter phase produces aplanospores that develop into the gametophyte, which completes the life cycle (Fig. 19). The position of meiosis is assumed to be the codium zygote, which is the normal position for meiosis in the Ulotrichales.

Our results are very similar to those obtained by Tatewaki (1969, 1972) for specimens identified as

*Capsosiphon groenlandicus* from northeastern Hokkaido, Japan. Tatewaki also observed an alternation between the slender, tubular monoecious gametophyte and the codium-like sporophyte, which produced 4-8 aplanospores that germinated *in situ*. However, he did not report zoospore production by the tubular phase, as we observed, nor did he describe or illustrate the distinctive development of multiple uprights from initially rhizoid-like filaments. Thus, we are unsure whether his material represents the same species as ours.

In contrast to the monoecy of our material, Yoshida (1970) found *C. fulvescens* to be dioecious. Gametes of both species are isogamous and have a distinct stigma. Yoshida did not observe asexual quadriflagellate zoospores in Japanese *C. fulvescens* whereas that was the

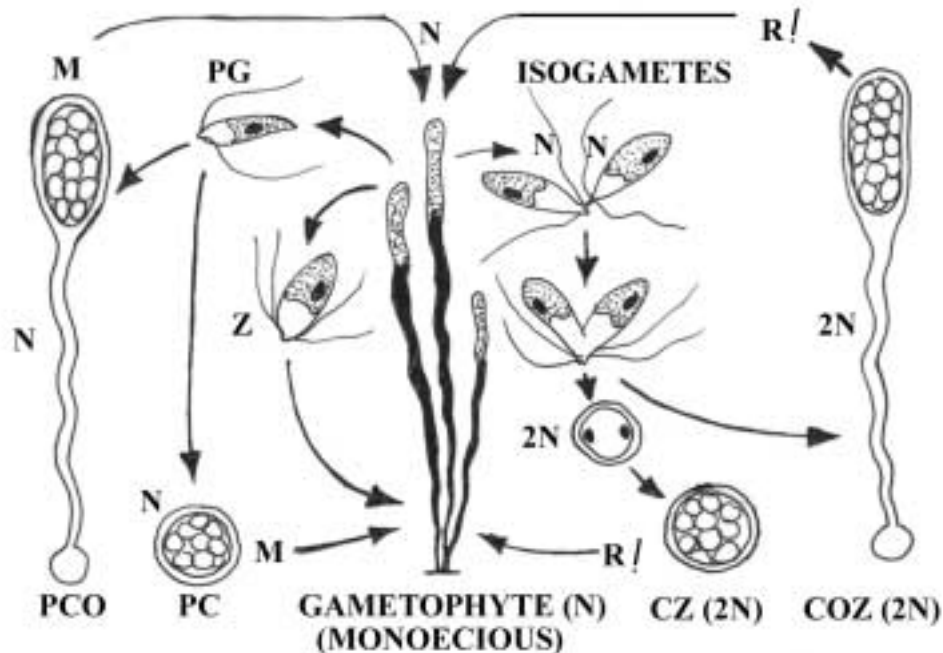


Fig. 19. Life history of *Pseudothrix borealis* (= North Pacific *C. groenlandicus*) from Amaknak Island, Alaska. COZ = codium zygote, CZ = cyst zygote, M = mitosis, N = haploid, 2N = diploid, PC = parthenogamete cyst, PCO = parthenogamete codium, PG = parthenogamete, R! = site of meiosis, Z = zoospore.

predominant or only form of reproduction observed in European material of that species (Bliding 1963; Kornmann and Sahling 1978), where quadriflagellate zooids were  $6.0 \times 9.0 \mu\text{m}$ , significantly smaller than the  $9 \times 11.7 \mu\text{m}$  we observed in our material, and where they recycled the tubular phase.

Parthenogametes produced germlings of several cells and also single-celled cysts in Yoshida's material of *C. fulvescens*. Yoshida frequently observed large cyst-like cells within the gelatinized membrane of the gametangia after liberation of the gametes; he interpreted this unicellular cyst-like stage as part of an alternation of heteromorphic generations. Chihara (1967) had earlier reported *C. fulvescens* from the Izu Peninsula to be dioecious and isogamous, with zygotes always developing into new thalli identical to their parents, thus lacking a thick-walled cyst stage. In contrast, Migita (1967) reported that zygotes of *C. fulvescens* from Nagasaki developed into unicellular cysts which, rather than developing directly into an adult thallus, produced zoospores that then produced a multicellular thallus, for an alternation of heteromorphic generations. These observations point out substantial differences in the life history of our material from that of *C. fulvescens*.

Our material shares with species of *Acrosiphonia* and some species *Ulothrix* a distinctive pattern of early devel-

opment of rhizoids and uprights not observed in *C. fulvescens*. Early development of the uniseriate gametophytic generation of our plant strikingly resembles *Ulothrix*. The production of secondary filaments arising from rhizoids has not been noted before in *Capsosiphon* but has been described in three species of *Ulothrix* (Lokhorst and Vroman 1974; Lokhorst 1978) and in species of the *Spongomorpha-Acrosiphonia* complex (Kornmann 1961, 1964a, 1964b). The process is probably a means for increasing the number of fronds from one zoospore. A similar pattern of "germlings with primary and secondary attaching cells and cells developing into branches" was also illustrated by Bliding (1968) for *Ulva neapolitana* (Figs 26D-H). Moreover, the complex basal system of these species and the ability of uprights to develop from the transformation of rhizoidal filaments may contribute to the vegetative perennation of the species; Sussmann and DeWreede (2007) showed that up to 50% of annual *Acrosiphonia* upright thalli developed from basal remnants at sites in Barkley Sound, British Columbia.

The origin of the fronds of our material as long uniseriate filaments appears to be a plesiomorphic condition. A similar uniseriate origin of fronds has been documented for *Capsosiphon fulvescens*, *Ulvaria obscura* var. *blyttii*, and most species of *Ulva* (including *Enteromorpha*) by Bliding

(1963, 1968). *Protomonostroma undulatum* also arises as a uniseriate filament, but periclinal (longitudinal) divisions begin when the filament is only 3-6 cells long, and the thallus then becomes bladelike (Kornmann and Sahling 1962; Tatewaki 1969, as *Monostroma undulatum* Wittrock; Golden and Garbary 1984, as *M. undulatum*). *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel *et al.* shows a similar pattern of blade development from a relatively short uniseriate filament, but the thallus becomes saccate [Kornmann 1964c, as *Monostroma oxyspermum* (Kützing) Doty; Golden and Garbary 1984, as *M. oxyspermum*]. Of course, the uniseriate condition is maintained in the adult form of marine species of *Ulothrix* (Lokhorst 1978) and *Chlorothrix* (Berger-Perrot 1981, as *Ulotrichella* non Iyengar).

Reproduction in the mature codiolum phase by aplanospores has also been recorded in codioli of marine species of *Ulothrix*, with concomitant *in situ* germination (Lokhorst 1978), as we and Tatewaki (1969, 1972) observed in North Pacific *Capsosiphon groenlandicus*. Since *Ulothrix* species can also release zoospores from codioli, we have to wonder whether the production of aplanospores with accompanying *in situ* germination is merely an artifact of culturing.

The long, tortuous stalk of the codiolum phase in our material resembles that obtained in cultures of *Acrosiphonia coalita* (Hollenberg 1958; Fan 1959, both as *Spongomorpha coalita*), in other members of the *Spongomorpha-Acrosiphonia* complex (Kornmann 1961, 1962, 1964a, 1964b, 1965), and in some *Ulothrix* species (Lokhorst and Vroman 1974; Lokhorst 1978). Although endophytic codioli often appear to have short and smooth (non-tortuous) stalks, codioli in the process of infecting a *Petrocelis* crust displayed long, tortuous stalks (Fan 1959). Codioli that are free-living also tend to have short, smooth stalks, as illustrated for *Urospora* by Lokhorst and Trask (1981). We hypothesize that the long, tortuous stalks we saw in our cultures indicate that the codiolum of this species is endophytic. However, we have not yet found any codioli or cysts in foliose or crustose species collected where the tubular phase has been observed.

In *Acrosiphonia*, *Spongomorpha*, and *Ulothrix*, germination of the zygote is via a germination tube, as in our material. The germination of the zygote appears to start by empty-spore formation and production of a short germ-tube, which continues to grow through internal incremental depositions of the advancing protoplast. The early stage resembles empty-spore germination in

*Blidingia* (Bliding 1963) as well as the formation of stoloniferous cells from prostrate discs of some *Blidingia* taxa (Garbary and Tam 1989; Lindstrom *et al.* 2006). The function of empty-spore germination is unknown, but its occurrence in brown and red as well as green algae suggests it is important. Perhaps it serves to elevate the germinating cell above other encrusting algae or as a "search organ" to access more suitable substrate.

The invaginated intrusion of chloroplast lamellae with associated pyrenoglobuli in the pyrenoid of our material is like that described for North Pacific *Capsosiphon groenlandicus* (as *Monostroma groenlandica*) and *C. fulvescens* (Hori 1973) and for *Ulothrix speciosa* (Carmichael) Kützing and *U. palusalsa* Lokhorst (Lokhorst 1978). The nature of pyrenoglobuli is unknown, but Lokhorst and Star (1980) postulated that they might be involved in conducting photosynthate from the photosynthetic thylakoids to areas specialized in the synthesis of storage products. Pyrenoglobuli have been reported in other green algae, including *Trebouxia* (Ascaso *et al.* 1995), *Chlorella* species with the glucan-type cell wall (Ikeda and Takeda 1995), and *Dilabifilum* (Broady and Ingerfeld 1993), where they were also associated with presumed thylakoidal tubules within the pyrenoid.

DNA sequence analysis of the nuclear ribosomal ITS1 region of *Capsosiphon fulvescens* indicates that this species is a member of the Ulotrichales but is only distantly related to North Pacific and North Atlantic *C. groenlandicus* or to any other species in the order, indicating that *C. fulvescens* is probably correctly placed in its own family, the *Capsosiphonaceae*. The morphological, ultrastructural and life history similarities of North Pacific *C. groenlandicus* to *C. fulvescens* suggest that these features may be symplesiomorphies rather than shared derived characters. This distant relationship necessitates the creation of a new genus for North Pacific and North Atlantic *C. groenlandicus*, which we describe below. These species are distinguished morphologically from *Capsosiphon* by cells not occurring in distinct longitudinal files.

Several authors (Saunders 1901; Collins 1909) have noted a size difference in vegetative cells of North Pacific and North Atlantic *Capsosiphon groenlandicus*. We did not observe these differences consistently as older parts of North Pacific thalli had cells as large as those reported for North Atlantic thalli. However, isolates from the two oceans have distinctly different ITS sequences (ca 5%). Moreover, the ontogeny of North Pacific *C. groenlandicus* differs from that of North Atlantic *C. groenlandicus* (O'Kelly, pers. comm., 18 Oct. 2006). Furthermore, *C.*

*groenlandicus* is not reported from the Arctic Ocean and thus is widely disjunct between the Pacific and Atlantic Oceans. We interpret these observations to indicate that the North Pacific entity should be considered a distinct species, which we describe as new below.

*Pseudothrix* gen. nov.

Thalli gametophytici filiformes, tubulares et cylindrici, cellulis ad basin thalli laxe binatim et quaternatim dispositis, non distincte longitudinaliter seriatis.

Gametophytic thalli filiform, tubular and cylindrical. Cells loosely arranged in twos and fours at base of thallus, not in distinct longitudinal files.

Etymology: From the Greek, meaning false filament. The thallus arises as a uniseriate filament, but soon becomes multiseriate and eventually hollow, although still having the macroscopic appearance of a filament because of its narrow diameter. The name also differentiates it from but still allies it to its close relatives, *Chlorothrix* and *Ulothrix*.

Type species: *Pseudothrix borealis* sp. nov.

Figs 1-20.

Thalli gametophytici tubularis constants e cellulis parvis rotundatis irregulariter dispositis late dispersis, saepe binis et quaternis; monoecii, isogami. Thallus sporophyticus codiolo vel cysta similis. Thalli tubulares ex aplanosporis et zoosporis orientes et crescentes more proprio a quo rhizoidea basalia non septata e filo uniseriato orta in fila uniseriata septata transeunt, qua fila dein emittunt rhizoidea basalia non septata, qua iterum in fila transeunt, iterum et iterum donec caespitem frondum usque ad 50 faciunt. Ab algis viridibus tubularibus alteris differt structura primaria geni nucleo notati pro RNA monadis parvae ribosomatis et regionibus ITS.

Gametophytic thalli tubular, consisting of small, rounded, irregularly arranged, widely spaced cells, often paired or in fours; monoecious, isogamous. Sporophytic thallus codiolium- or cyst-like. Tubular thalli arising from both aplanospores and zoospores with a distinctive development in which basal, non-septate rhizoids from the initially uniseriate filament are transformed into septate uniseriate filaments, which then develop basal non-septate rhizoids; this pattern repeated to yield a tuft of up to 50 fronds. From other tubular green algae it differs in the primary structure of the nuclear encoded small subunit rRNA gene and the ITS regions.

Holotype (lower half of Fig. 20): UBC A84928, field thalli collected on mid intertidal boulders near the Dutch Harbor airport on the west shore of Amaknak Island, Alaska, 4 August 2004. Upper half of Fig. 20, which is

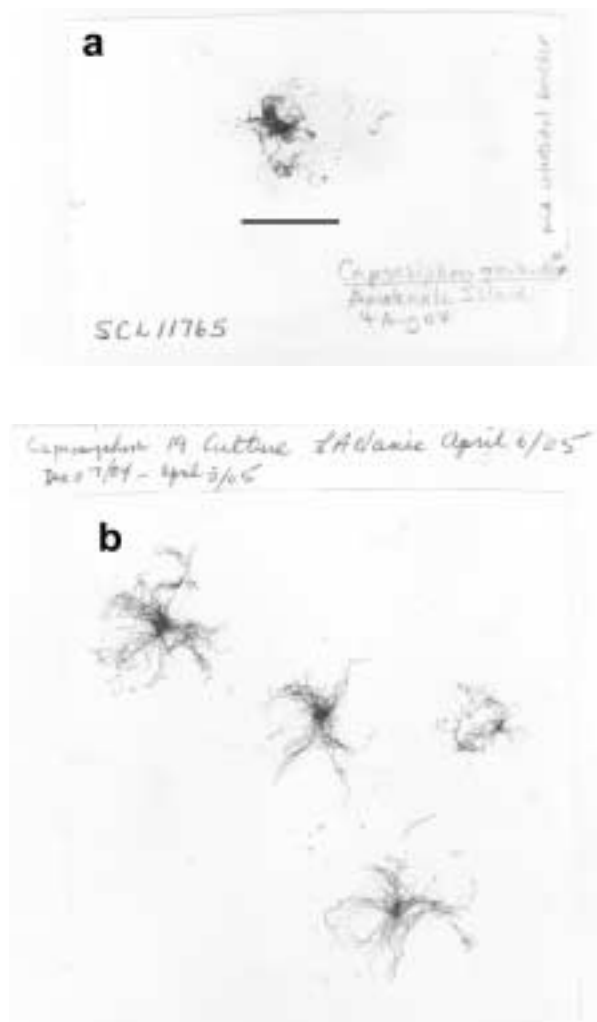


Fig. 20. a, Holotype of *Pseudothrix borealis* (= North Pacific *Capsosiphon groenlandicus*); b, cultured thalli. UBC A84928. Scale bar = 2 cm.

included with the holotype on a single herbarium sheet, is from cultured thalli of the holotype collection, as described herein.

Geographical distribution: In addition to our material from Amaknak Island, we have also observed specimens from lower Cook Inlet, Alaska (ALA 188; ABFL 752 in ALAJ; unaccessioned). Thus, the distribution of the species in the North Pacific extends from Cook Inlet, Alaska, through eastern Russia to northern Japan.

Additional species: *Pseudothrix groenlandica* (J. Agardh) Hanic et S.C. Lindstrom comb. nov.

Basionym: *Monostroma groenlandicum* J. Agardh 1883: 107, Pl. 3, figs 80-83.

DNA sequence analysis also places *Protomonostroma undulatum* in the Ulotrichaceae although its relationship to other genera is equivocal. *Protomonostroma* had been placed in the Gayraliaceae by Vinogradova (1969). In our

ITS analysis, *Gayralia oxysperma*, the type of the genus, clusters with *Monostroma nitidum* outside the Ulotrichaceae (Fig. 18). *Protomonostroma* has been reported to have squat codiolaria (Golden and Garbary 1984), like other members of the Ulotrichaceae, although their figure of purported codiolaria resembles the zoospore germination in our Fig. 7 rather than codiolum germination.

The observation that many of the clades in the 18S rRNA gene tree are not strongly supported by bootstrap analysis raises the question of ordinal classification in the Ulvophyceae. Previous studies have shown that recognition of separate orders Ulotrichales and Ulvales is not universally supported by molecular data, and different authors have observed that different taxa cluster with one or the other of these orders depending on the analysis and the included taxa (Watanabe *et al.* 2001, Hayden and Waaland 2002, O'Kelly *et al.* 2004a, 2004c). Whether a multi-ordinal solution is required or the simpler solution of recognizing all taxa as members of the order Ulotrichales *sensu lato* is followed, as suggested by Gabrielson *et al.* 2000, remains to be decided.

Lack of strong support for branching order among genera of the Ulotrichaceae may represent a real radiation associated with ocean cooling in the late Miocene. Van Oppen (1995) estimated a divergence of 13-14 Ma for the clade containing *Acrosiphonia*, *Spongomorpha*, *Urospora* and marine *Ulothrix* (all members of the Ulotrichaceae and part of the radiation seen in the ITS analyses). All of these genera occur in colder waters of the world's oceans. Species of Ulotrichaceae share a heteromorphic life history in which a macroscopic, initially uniseriate gametophyte, which appears on shores in spring or summer, alternates with a microscopic codiolum- (or chlorochytrium-) like sporophyte that overwinters. *Pseudothrix borealis*, occurring in the cold waters of the North Pacific, clearly fits within this radiation.

## ACKNOWLEDGEMENTS

We thank Dr. Charley O'Kelly, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, for sharing his unpublished sequence of *Capsosiphon groenlandicus* and for informative discussions, and Dr. Kim Rensing, UBC BioImaging Facility, for preparing the electron micrograph. Mark A. Garland, Scientific Latin Translations, Micanopy, Florida, provided the Latin translation. This research was supported by a NSERC grant to SCL.

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Received 1 November 2007

Accepted 10 March 2008

