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 codiolas 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 *Bldingia 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
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. aurea* (C. Agardh) Setchell & N.L. Gardner.

In addition to *C. groenlandicus*, the genus *Capsosiphon* currently contains only *C. fulvescens* and *Capsosiphon aureolum* 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 μm diam. in the former, 12-16 μm 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 μmol photons m⁻²s⁻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, 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 I2-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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Source</th>
<th>GenBank No.</th>
</tr>
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<tr>
<td><em>Acrochaete endozoica</em> (W.M. Goldberg, J.C. Makemson &amp; S.B. Colley) M.J. Wynne</td>
<td>O’Kelly et al. 2004b</td>
<td>Endozoic in <em>Pseudoplexaura</em> sp. (gorgonian coral)</td>
<td>AY205327 (18S)</td>
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<td><em>Acrosiphonia caolita</em> (Ruprecht) Scagel, Garbary, Golden &amp; M.W. Hawkes</td>
<td>Lindstrom &amp; Hanic 2005</td>
<td>Baker Beach, California (CA)</td>
<td>AY455943 (ITS)</td>
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<tr>
<td><em>Acrosiphonia duriuscula</em> (Ruprecht) Yendo</td>
<td>Watanabe et al. 2001</td>
<td>Okitu, near Kushiro, Hokkaido, Japan</td>
<td>AB049418 (18S)</td>
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<td><em>Bolbocoleon piliferum</em> Pringsheim</td>
<td>O’Kelly et al. 2004a</td>
<td>Mar Vista, San Juan L, WA; Nobska Pt, Woods Hole, MA</td>
<td>AY303598 (18S); AY303599 (18S)</td>
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<td><em>Capsosiphon fulvescens</em> (C. Agardh) Setchell &amp; N.L. Gardner</td>
<td>Hayden &amp; Waaland 2002; this study</td>
<td>Monks Head Beach, Nova Scotia (NS); Kattegat, Denmark</td>
<td>EU541503 (ITS1)</td>
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<td><em>Chlorothrix Berger-Perrot</em></td>
<td>Lindstrom &amp; Hanic 2005</td>
<td>Ucluelet, Vancouver L, BC</td>
<td>AY653740 (18S &amp; ITS)</td>
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<td><em>Collinsiella tuberculata</em></td>
<td>O’Kelly et al. 2004b</td>
<td>Cattle Point, San Juan Island, Washington (WA)</td>
<td>AY198125 (18S &amp; ITS)</td>
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<tr>
<td><em>Desmochloris halophila</em> (Guillard, H.C. Bold &amp; MacEntee)</td>
<td>Friedl &amp; O’Kelly 2002</td>
<td>SAG 222</td>
<td>AJ416104 (18S)</td>
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<td><em>Gayralia oxysperma</em> (Kützing)</td>
<td>Woolcott &amp; King unpublished</td>
<td>No data</td>
<td>AY016306 (ITS)</td>
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<td><em>Gloeotilopsis sarcinoidea</em> (Groover &amp; H.C. Bold) Friedl</td>
<td>Bhattacharya et al. 1996</td>
<td>UTEX 1710</td>
<td>Z47998 (18S &amp; ITS)</td>
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<tr>
<td><em>Halochlorococcum moorei</em> (N.L. Gardner) Kornmann &amp; Sahling</td>
<td>O’Kelly et al. 2004b</td>
<td>Friday Harbor Labs, San Juan L, WA</td>
<td>AY198122 (18S)</td>
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<td><em>Halochlorococcum porphyrae</em> (Setchell &amp; N.L. Gardner) J.A. West</td>
<td>This study</td>
<td>Carmel, CA</td>
<td>DQ821520 (18S)</td>
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<td><em>Kornmannia leptoderma</em> (Kjellman) Bliding</td>
<td>Su Q., Luan R. and An L., unpublished</td>
<td>No data</td>
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<td><em>Monostroma grevillei</em> (Thuret) Wittrock</td>
<td>Tan I. and Sluiman H.; Su Q., Luan R. and An L., unpublished</td>
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<td><em>Monostroma grevillei var. vahlii</em> (J. Agardh) Rosenvinge</td>
<td>Su Q., Luan R. and An L., unpublished</td>
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<td><em>Monostroma nitidum</em> Wittrock</td>
<td>Su Q., R. Luan and An L., unpublished</td>
<td>No data</td>
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<td><em>Ochlochaete hystrix</em> Thwaites ex Harvey</td>
<td>O’Kelly et al. 2004c</td>
<td>Quissett Estuary, Woods Hole, MA</td>
<td>AY454428 (18S)</td>
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<td><em>Phaeophila dendroides</em> (P. Crouan &amp; H. Crouan) Batters</td>
<td>O’Kelly et al. 2004c</td>
<td>Lime Kiln Pt, San Juan L, WA</td>
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<td><em>Planophila laetevirens</em> Gerneck</td>
<td>Friedl &amp; O’Kelly 2002</td>
<td>Dolomites, South Tyrol, Italy</td>
<td>AJ416102 (18S)</td>
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<tr>
<td><em>Protomonostroma undulatum</em> (Wittrock) K.L. Vinogradova</td>
<td>This study; Su Q., Luan R. and An L., unpublished</td>
<td>Shaw Island, Katmai National Park, AK; China</td>
<td>DQ821517 (18S &amp; ITS); AF415169 (ITS)</td>
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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.

### Table 1. (continued)

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<tr>
<td><em>Pseudothrix borealis sp. nov.</em> (=North Pacific <em>Capsosiphon groenlandicus</em>)</td>
<td>This study</td>
<td>Amaknak Island, Unalaska Island, Alaska (AK)</td>
<td>DQ821514 (18S &amp; ITS)</td>
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<td><em>Spongopomorpha aeruginosa</em> (Linnaeus) van den Hoek</td>
<td>Van Oppen 1995</td>
<td>Iceland</td>
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<td><em>Ulothrix</em> sp. (as <em>Chlorothrix</em> 47SI)</td>
<td>Lindstrom &amp; Hanic 2005</td>
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<td><em>Ulothrix</em> zonata (Weber &amp; Mohr) Kützing</td>
<td>O’Kelly et al. 2004b; Friedl 1996</td>
<td>Lake Michigan</td>
<td>AY278217 (18S); Z47999 (ITS)</td>
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<td><em>Urospora neglecta</em> (Kornmann) Lokhorst &amp; Trask</td>
<td>Lindstrom &amp; Hanic 2005</td>
<td>Point No Point, Vancouver I., BC</td>
<td>AY476808 (18S &amp; ITS)</td>
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<td><em>Urospora penicilliformis</em> (Roth) Areschoug</td>
<td>Lindstrom &amp; Hanic 2005</td>
<td>Louisbourg, NS</td>
<td>AY476816 (18S &amp; ITS)</td>
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<td><em>Urospora wormskioldii</em> (Mertens ex Hornemann) Rosenvinge</td>
<td>Lindstrom &amp; Hanic 2005</td>
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RESULTS

Field thalli were filiform, tubular, and dark green, up to 10 mm long and 1.2 mm wide. They formed extensive
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 µm 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 ± 1.59)-8.0 × 3.0-(3.6 ± 0.96)-7.0 µm (n=30; numbers in parentheses the means of the measurements + one standard deviation); at mid thallus, 6.0-(7.5 ± 1.66)-11 × 3.0-(5.0 ± 0.94)-7.0 µm (n=19), and in distal fertile areas of the thallus, 12-(17 ± 2.51)-22 × 6.0-(12.4 ± 2.30)-20 µm (n=75). Cells were randomly arranged and 15-(22)-30 µm deep (n=9); they were more widely spaced, up to two cell widths, near the base of the thallus (Figs 1, 2) compared 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 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 ± 0.86)-11.0 µm × 4.0-(5.0 ± 0.48)-5.8 µm (length × diameter; n=15), with flagella 9.0-(11.2 ± 1.43)-12.0 µm 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
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 multituboidal 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-
sured 5.5-(7.6 ± 1.08)-11 × 2-(2.8 ± 0.49)-4.3 µm, with fla-
gella 8-(11.8 ± 1.28)-14 µm 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 spheri-
tical to slightly ovate, reaching 50 µm diam. A few cysts
also developed within empty gametangia, presumably
due to germination of parthenogametes. Initially, the
codiola 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 µm long. The codio-
la were sausage-shaped and ca 30 µm long. Only a few
cysts and codiola frozen for 30 days at -18°C and
returned to culture at 5°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 ulti-
mately 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 Ulotrichaceae (Fig. 17), as did analy-
ses of the ITS data, including the 5.8S rRNA gene (Fig.
18). In the SSU analysis, North Pacific *Capsosiphon groen-
landicus* occurred within a clade containing other mem-
bers of the Ulotrichaceae: Acrosiphonia, Chlorothrix,
Urospora, Protomonostroma and Ulothrix. It occurred more
distally than *Acrosiphonia* within the clade but with weak
support; the remaining taxa of Ulotrichaceae 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 dis-
tal 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 Ulotrichales in the
ML analysis; in the MP analysis, it appeared on its own
branch at the base of the Ulotrichaceae. In both analyses,
it clearly belonged to the Ulotrichales since the subtend-
branch, separating the Ulotrichales 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 Ulotrichales
(*Proto monostroma 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 *Halochloroccycum*, includ-
ing *H. porphyrae* (which was identical to *H. moorei* in our
SSU analyses—Fig. 17), is clearly something else. This
latter “*Capsosiphon fulvescens*” and *Halochlorocycum* 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 Ulotrichaceae in the ITS.

**Fig. 16.** *Pseudothrix borealis* (=North Pacific *Capsosiphon groen-
landicus*). Electron micrograph of cell of upright thallus
showing pyrenoid surrounded by starch plates and pene-
trated by invaginations of chloroplast lamellae, which are
surrounded by pyrenoglobuli. Scale bar = 1 µm.
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
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 codiolum 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 codiolum 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 codiolum-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

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**Fig. 18.** Maximum likelihood tree (-lnL = 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.
predominant or only form of reproduction observed in European material of that species (Bliding 1963; Kornmann and Sahling 1978), where quadriflagellate zooids were $6.0 \times 9.0 \, \mu m$, significantly smaller than the $9 \times 11.7 \, \mu m$ 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 \textit{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 \textit{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 \textit{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 \textit{C. fulvescens}.

Our material shares with species of \textit{Acrosiphonia} and some species \textit{Ulothrix} a distinctive pattern of early development of rhizoids and uprights not observed in \textit{C. fulvescens}. Early development of the uniseriate gametophytic generation of our plant strikingly resembles \textit{Ulothrix}. The production of secondary filaments arising from rhizoids has not been noted before in \textit{Capsosiphon} but has been described in three species of \textit{Ulothrix} (Lokhorst and Vroman 1974; Lokhorst 1978) and in species of the \textit{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 \textit{Ulva nepalitana} (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 \textit{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 \textit{Capsosiphon fulvescens}, \textit{Uloaria obscura} var. blyttii, and most species of \textit{Ulva} (including \textit{Enteromorpha}) by Bliding.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig19.png}
\caption{Life history of \textit{Pseudothrix borealis} (= North Pacific \textit{C. groenlandicus}) from Amaknak Island, Alaska. COZ = codiolum zygote, CZ = cyst zygote, M = mitosis, N = haploid, 2N = diploid, PC = parthenogamete cyst, PCO = parthenogamete codiolum, PG = parthenogamete, R! = site of meiosis, Z = zoospore.}
\end{figure}
(1963, 1968). Protomonostroma undulatum also arises as a uniseriate filament, but perical (longitudinal) divisions begin when the filament is only 3-6 cells long, and the thallus then becomes blade-like (Kornmann and Sahling 1962; Tatewaki 1969, as Monostroma undulatum Wittrock; Golden and Garbary 1984, as M. undulatum). Gyaerula oxyysperma (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 oxyyspermum (Kützing) Doty; Golden and Garbary 1984, as M. oxyyspermum]. 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 codiola 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 codiola, 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 Acrasisiphonia coalita (Hollenberg 1958; Fan 1959, both as Spongomorpha coalita), in other members of the Spongomorpha-Acrasisiphonia complex (Kornmann 1961, 1962, 1964a, 1964b, 1965), and in some Ulothrix species (Lokhorst and Vromen 1974; Lokhorst 1978). Although endophytic codiola often appear to have short and smooth (non-tortuous) stalks, codiola in the process of infecting a Petrocelis crust displayed long, tortuous stalks (Fan 1959). Codiola 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 codiola or cysts in foliose or crustose species collected where the tubular phase has been observed.

In Acrasisiphonia, 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.
**Capsoplithon 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 basalio non septata e filo uniseriato orta in fila uniseriata septata transeunt, qua fila dein emittunt rhizoidea basalio non septata, qua iterum in fila transeunt, iterum et iterum donec caespitem frondum usque ad 50 faciunt. Ab algis viridibus tubularibus alteris differunt structura primaria geni nucleo notati pro RNA monadis parvae ribosomatis et regionibus ITS.

Gametophytic thallus tubular, consisting of small, rounded, irregularly arranged, widely spaced cells, often paired or in fours; monoecious, isogamous. Sporophytic thallus codiolum- or cyst-like. Tubular thallus 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 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 codiola (Golden and Garbary 1984), like other members of the Ulotrichaceae, although their figure of purported codiola 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 Ulotrichaceae 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 chlorocythrium-) like sporophyte that overwinters. *Pseudothrix borealis*, occurring in the cold waters of the North Pacific, clearly fits within this radiation.

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**REFERENCES**


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