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Taxonomic reappraisal of the freshwater brown algae *Bodanella*, *Ectocarpus*, *Heribaudiella*, and *Pleurocladia* (Phaeophyceae) on the basis of *rbc*L sequences and morphological characters

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This study examines the phylogenetic relations among freshwater species of brown algae using RUBISCO large subunit (*rbc*L) sequences, in conjunction with diagnostic morphological and developmental traits. We focused on the phylogenetic and taxonomic placement of five freshwater species, *Bodanella lauterborni*, *Ectocarpus siliculosus*, *Heribaudiella fluviatilis*, *Pleurocladia lacustris*, and *Porterinema fluviatile*, each of which has traditionally been assigned to the Ectocarpales by most authors. These freshwater species were compared with 26 marine taxa of brown algae from nine orders, with particular emphasis on families within the Ectocarpales. Results of BLASTn searches and later phylogenetic analyses placed all freshwater taxa within the Phaeophyceae, but phylogenetic inferences do not support their traditional classifications at the ordinal level. There was no support for the inclusion of *H. fluviatilis* or *B. lauterborni* in the Ectocarpales, but instead formed a well-supported clade intermediate between the Syringodermatales and Sphacelariales. Although morphologies of these two species are relatively distinct and remain stable in culture, *B. lauterborni* nested between two populations of *H. fluviatilis* (on the basis of *rbc*L data) from British Columbia and Germany, making a reclassification of these two species necessary. Molecular and morphological data clearly place two other freshwater taxa, *Pleurocladia lacustris* and (freshwater) *E. siliculosus* in the Ectocarpales, each nesting among marine members of the Ectocarpaceae. When compared with sequences from 13 species from several families within the Ectocarpales, *Pleurocladia* is most closely related to members of the Ectocarpaceae and may be a sister taxon to the Scytosiphonaceae. DNA sequences from a freshwater isolate of *E. siliculosus* from Australia were nearly identical to those from a marine isolate from Germany. Molecular data did not resolve the ordinal status of a freshwater *Porterinema fluviatile*, but indicated that this species should not be classified within the Ectocarpales.

KEY WORDS: *Bodanella*, Brown algae, Ectocarpales, *Ectocarpus*, Freshwater, *Heribaudiella*, Phaeophyceae, Phylogeny, *Pleurocladia*, *rbc*L, RUBISCO

INTRODUCTION

The Phaeophyceae is a diverse class of multicellular algae ranging in size from microscopic forms to huge kelps and occur from arctic to tropical biomes (Bold & Wynne 1978; Van den Hoek *et al.* 1995). Members of this class exhibit a wide range of morphologies, reproduction, and ultrastructure, and comprise roughly 265 genera and between 1500 and 2000 species (Van den Hoek *et al.* 1995; De Reviers & Rousseau 1999). Of all these species, $< 1\%$ (seven species) has been documented to occur in freshwater environments (Starmach 1977; Bourrelly 1981; Wehr 2003).

Although the study of freshwater brown algae has had a long history, with several genera known for > 100 years, their classification and phylogeny is unclear. Well-documented populations of several species are few, making thorough examinations of their morphology, reproduction, and phylogeny difficult (Wehr 2003). To date, studies have relied on morphological, reproductive, and ultrastructural characters to determine the classification of freshwater members of the class. These schemes place most or all freshwater taxa in the Ectocarpales or Sphacelariales largely on the basis of their filamentous growth forms (Starmach 1977; Bold & Wynne 1978; Bourrelly 1981; Pueschel & Stein 1983; Van den Hoek *et al.* 1995).

Five freshwater species are most often classified within the Ectocarpales *sensu lato*, although placements within families vary a great deal. *Pleurocladia lacustris* A. Braun (Family: Ectocarpaceae or Chordiariace) was the first "true" freshwater brown alga described in the literature (Braun 1855). It is a creeping, infrequently branched filament (with one or rarely two parietal chloroplasts) that occasionally produces upright, centrifugal (arched), multiply branched filaments, creating small cushion-like thalli. Filaments frequently produce unilocular sporangia (plurilocular sporangia rarely reported) and hairs (under P-limitation; Wehr 2003), and grow on rocks or aquatic plants in lakes and rivers (occasionally endophytic) in Europe and North America (Waern 1952; Wilce 1966; Bourrelly 1981). The most commonly reported species, *Heribaudiella fluviatilis* (Areschoug) Svedelius (Family undetermined), is a thick-walled, crust-forming alga that colonizes rocks in streams. Cells have multiple discoid

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chloroplasts, and thalli are composed of multiply branched prostrate filaments and erect, mostly dichotomously branched filaments that bear unilocular or plurilocular sporangia (Starmach 1977; Wehr & Perrone 2003). It has been reported from several hundred (mostly stream and river) locations worldwide (Wehr & Stein 1985; Wehr 2003). *Bodanella lauterborni* Zimmerman (Family undetermined) is a thick-walled, prostrate (creeping) branched filament with multiple chloroplasts, and unknown reproductive structures (Zimmerman 1928; Bourrelly 1981). It has been reported to colonize rocks in a few lakes in Europe (Wehr 2003). *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Family: Ectocarpaceae) is primarily a marine or brackish species, but one freshwater population has been described from Australia (West & Kraft 1996) and another inland population was reported from a river polluted by potassium mine wastes (Geissler 1983). This alga exhibits typical *Ectocarpus* morphology with infrequent branching and ribbon-like parietal or spiral chloroplasts (with pyrenoids). Plurilocular sporangia have been observed in the Australian population, but unilocular sporangia are documented only in marine populations (West & Kraft 1996).

Porterinema fluviatile (Porter) Waern is an enigmatic species that inhabits marine, estuarine, and freshwater habitats and is classified in the Ectocarpales by some authors (Waern 1952; Bourrelly 1981; Wehr 2003) and in the Ralfsiales by others (Wilce *et al.* 1970). It is a simple, rarely branched alga with creeping filaments (with 1–3 lobed, parietal chloroplasts) that may form loosely arranged monostromatic discs on plants or rocks, or live endophytically in some aquatic plants or other algae (Waern 1952). Plurilocular sporangia are apparently common and characteristically form four-celled terminal clusters or crowns (Waern 1952; Dop 1979). In general, the classification of freshwater taxa, on the basis of at least some morphological features, agrees with the circumscription of the Ectocarpales described by De Reviers & Rousseau (1999), with the possible exception of the genus *Heribaudiella*, whose combined multiply branched prostrate and upright forms are more complex than is seen in most taxa placed in this order (Wehr 2003).

Two freshwater members of the Sphacelariales are *Sphacelaria fluviatile* Jao and *S. lacustris* Schloesser & Blum (Family: Sphacerilariaceae). Both are characterized by apical growth and heterotrichy, having both uniseriate and multiseriate branched filaments, as is characteristic of the order (Jao 1943; Starmach 1977; Schloesser & Blum 1980). In fresh waters, both form small pale brown tufts or cushions on rocks, with filaments creeping along the substratum. Filaments contain many small discoid chloroplasts lacking pyrenoids. Freshwater species are currently distinguished by branching patterns (*S. fluviatile* has opposite branching; *S. lacustris* has alternate or irregular branching) and frequency of lateral cell divisions (Wehr 2003). Unilocular sporangia are known from *S. lacustris*, whereas plurilocular sporangia are not found in either. Details of the morphology, reproduction, classification (including synonymies), and ecology of all known freshwater taxa are provided elsewhere (Wehr 2003). It is worth noting that *Ectocarpus*, *Porterinema*, and *Sphacelaria* all have marine counterparts (species within each genus), whereas the three other genera do not.

Although no classification scheme for the Phaeophyceae has been fully accepted by all workers, recent syntheses that combine molecular and traditional characters have emerged. One such classification characterizes members of the Ectocarpales as simple, uniseriate filamentous forms whose cells have one or few large band-like chloroplasts and a stalked, pyriform pyrenoid (Rousseau & De Reviers 1999). These and other details have not been fully assessed in the freshwater taxa. Grouping of marine taxa is also strongly supported by molecular studies, which show that several taxa traditionally classified within the Ectocarpales, Chordariales, Dictyosiphonales, and Scytosiphonales form a strongly supported monophyletic group (Rousseau & De Reviers 1999; Draisma *et al.* 2001; Rousseau *et al.* 2001). De Reviers & Rousseau (1999) also excluded the crust-forming *Ralfsia* (classified with the Ectocarpales or Ralfsiales) from the Ectocarpales on the basis of both molecular data and the absence of characteristic pyrenoids. Tan & Druehl (1994) showed that *Ralfsia* was more closely associated with members of the Desmarestiales, Dictyotales, Fucales, Laminariales, Sphacelariales, and Syringodermatales, rather than with members of the Ectocarpales, on the basis of 18S rDNA sequence data. These latter findings with marine taxa are of importance for the present study, because most freshwater taxa have been classified within the Ectocarpales, and none of the recent classification schemes or molecular approaches has considered the freshwater genera or species (Starmach 1977; Bourrelly 1981; Wehr 2003).

Molecular studies using the 18S rDNA and RUBISCO large subunit (*rbc*L) genes have proven useful for inferring phylogenetic relations among orders, families, and genera of marine brown algae (e.g. Assali *et al.* 1990; Andersen *et al.* 1998; Müller *et al.* 1998; Peters & Ramírez 2001; Draisma *et al.* 2001, 2002; Burrowes *et al.* 2003) and so should be considered in efforts to understand the evolutionary history of freshwater taxa. Determining phylogenetic relations and proper taxonomic placement of these poorly known species may also prove to be important in future biogeographic and ecological studies. Links between biogeographic and molecular analyses are especially needed with freshwater representatives of the Phaeophyceae, as many species are apparently disjunct, and their origin and modes of dispersion are poorly understood (Wehr & Stein 1985).

Our goal was to characterize the phylogenetic relations and taxonomic placement of five freshwater species (*B. lauterborni*, *E. siliculosus*, *H. fluviatilis*, *Pleurocladia lacustris*, *Porterinema fluviatile*) that are currently assigned to the Phaeophyceae. Field specimens and cultures of freshwater taxa from a wide range of locations were assessed and compared with 26 marine brown algal species from nine orders whose phylogenetic positions have previously been determined. Specifically, our aims were (1) to verify, using molecular evidence, that freshwater populations of presumed phaeophyte species are truly members of the Phaeophyceae; (2) to determine the correct taxonomic placement of freshwater species within the brown algae; and

Table 1. Brown algal specimens used in the present study for DNA extraction and molecular analysis.

Species and authority	Origin	Strain ID	Collection site	Year of collection	Environment[6]	NCBI # *rbc*L
Bodanella lauterborni Zimmerman	culture[1]	LB 2190	Lake Konstanz, Germany	1977	FW	AY307407
	culture[1]	LB 2191	Lake Konstanz, Germany	1977	FW	AY307408
Ectocarpus siliculosus (Dillwyn) Lyngbye	culture[2]	3477	Hopkins River Falls, Victoria, Australia	1995	FW	AY307410
Ectocarpus sp. Lyngbe	culture[1]	LB 1433	not given	unknown	Mar	AY307411
Heribaudiella fluviatilis (Areschoug) Svedelius	culture[3]	13.90	River Aach, Baden Wurtemberg, Germany	1966	FW	AY307406
	field[4]	Ashnola 1	Ashnola River, British Columbia, Canada	2002	FW	AY307402
	field[4]	Ashnola 2	Ashnola River, British Columbia, Canada	2002	FW	AY307403
	field[4]	Granite	Granite Creek, British Columbia, Canada	2002	FW	AY307404
	field[4]	Smith	Smith Creek, British Columbia, Canada	2002	FW	AY307405
Pleurocladia lacustris A. Braun	culture[5]	Fischer	Fischer River, Austria	1991	FW	AY307309
Porterinema fluviatile (Porter) Waern	culture[3]	124.79	ditch in Bad Sooden, Germany	1964	FW	AY307412
Sphacelaria sp. Lyngbe	culture[1]	LB 800	not given	unknown	Mar	AY307413

[1] Culture Collection of Algae at the University of Texas at Austin (UTEX; Starr and Zeikus 1987).
[2] Provided by Dieter G. Müller (University of Konstanz), who obtained the sample from John West (University of Melbourne).
[3] Culture collection at Sommlung von Algenkulturen at Gottingen (SAG).
[4] Collected by Andrew Martin (Fordham University).
[5] Culture from Elsa Kusel-Fetzmann (University of Vienna).
[6] Abbreviations: FW = freshwater; Mar = marine.

(3) to determine the phylogenetic relations specifically among these freshwater taxa.

METHODS

Strategy

Brown algal specimens were obtained whenever possible from live samples, either freshly collected field material or laboratory cultures. Live material was obtained for freshwater *Ectocarpus*, *Heribaudiella*, *Pleurocladia*, *Bodanella*, and *Porterinema* (Table 1). Repeated efforts to obtain live samples of either freshwater species of *Sphacelaria* (divers sampling in western Lake Michigan [*S. lacustris*]; requests to the Chinese Academy of Sciences [*S. fluviatilis*]) were unsuccessful. Intact DNA could not be extracted from dried herbarium material of *S. lacustris* (National Herbarium, Smithsonian Institution). Thus the present study focused mainly on those five freshwater taxa for which we had live material. Marine sister taxa of *Sphacelaria* were used to assess ordinal placements of all freshwater taxa that were sampled.

Field and culture specimens

A list of source material (new specimens and cultures) is provided in Table 1. Algal crusts were collected from rocks from three freshwater locations (Ashnola River, Granite Creek, Smith Creek) in British Columbia, Canada (described in Wehr & Stein 1985). These rocks were air dried, labeled, maintained at ambient temperature, and returned to the laboratory (Louis Calder Center, Armonk, NY). Upon arrival ($<$ 7 d), rocks were wetted with sterile deionized water and separate crusts were scraped into sterile 12-well plates containing approximately 1 ml of Allen's medium (Allen 1968). Observations (inverted microscope) determined which wells contained *H. fluviatilis*. Plates were maintained in semicontinuous culture (media changes semimonthly) for several months in a lighted incubator (20°C; 100 μmol m^{-2} s^{-1} photons irradiance; 12:12 photoperiod) until used for DNA extraction. Pure cultures of freshwater brown algae were obtained from colleagues (freshwater: *Ectocarpus*, *Heribaudiella*, *Pleurocladia*) and the University of Texas Culture Collection (freshwater: *Bodanella*, *Porterinema*; marine: *Ectocarpus*, *Sphacelaria*), and maintained in Allen's medium (Allen 1968), Basal medium (ES), or Desmidiacean medium (Pringsheim 1946) under conditions described above. Every 1–3 months a portion of the cultures was transferred to a sterile flask with fresh medium for maintenance.

DNA extraction and amplification

Genomic DNA was extracted from field and culture material (Table 1) using the DNeasy® Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions with minor modifications. Algal material (*c.* 40 mg) was disrupted in a microcentrifuge tube with a micropestle (USA Scientific, Ocala, FL) and incubated 30 min to lyse cells. After the final elution step, DNA was diluted 5-, 10-, 20-, 50-, or 100-fold for use in polymerase chain reaction (PCR) amplification.

A dilution of extracted DNA was used to amplify roughly a 1000–1450-base pair (bp) fragment of the *rbc*L gene (Siemer *et al.* 1998; Burrowes *et al.* 2003). PCR was performed using the *Taq* PCR Core Kit (Qiagen, Valencia, CA) using 1 μl of diluted DNA template, PCR buffer (containing 1.5 mM of Mg^{2+}), 200 μM of each dNTP, 0.5 μM of each primer, 2.5 units of *Taq* DNA polymerase,

Table 2. Amplification primers used for PCR or sequencing.

Primer	Direction[1]	Sequence (5′–3′)	Approximate annealing position	Reference
LM*rbc*L17F	F	ATG CCT GAA GAT GTG CA	*rbc*L 1–17	present study
BLS*rbc*L23F	F	ATG CCT GAA GAT GTG CAR AAT CG	*rbc*L 1–23	Siemer et al. (1998)
LM*rbc*L130F	F	ACT GAT ATT CTA GCT CTT T	*rbc*L 111–130	present study
rbc-F2.5	F	TTC CAA GGC CCA GCA ACA GGT	*rbc*L 454–474	Kawai et al. (2000)
rbc-R3	R	CCT TTA ACC ATT AAG GGA TC	*rbc*L 1040–1021	Kawai et al. (2000)
LM*rbc*L1207R	R	TAC CAC CAC CAA ATT GTA GA	*rbc*L 1207–1187	present study
LM*rbc*L1378R	R	CCA TAA ATC TAA AGC CGC T	*rbc*L 1397–1378	present study
LM*rbc*L1387R	R	TAA TAT CTT TCC ATA AAT CT	*rbc*L 1406–1387	present study
BLS*rbc*L1433R	R	TTG CAA CTT CTA CGA AAT CAG	*rbc*L 1453–1433	Siemer et al. (1998)

[1] F, forward; R, reverse.

and water to 50 µl. Amplification was performed as follows: an initial denaturation step of 94°C (5 min), followed by 40 cycles of 94°C for 45 s, 48°C for 45 s, and 72°C for 2 min, with a final 7-min extension of 72°C. Amplification primers (Table 2) were synthesized on the basis of previously reported primers designed primarily for marine brown algae (Siemer *et al.* 1998; Kawai *et al.* 2000), or designed by the authors.

New primers were designed since PCR amplification was rarely successful (except using DNA isolated from freshwater and marine *Ectocarpus* spp.) using the following primers designed by Siemer *et al.* (1998): BLS*rbc*L23F, BLS*rbc*L596R, BLS*rbc*L773F, BLS*rbc*L977F, BLS*rbc*L 1395F, and BLS*rbc*L1433R. The primer LM*rbc*L17F was designed on the basis of a shortened version of the primer BLS*rbc*L23F of Siemer *et al.* (1998). The primers LM*rbc*L130F, LM*rbc*L1207R, LM*rbc*L1378R, and LM*rbc* L1387R were designed by aligning sequences of two marine species, *E. siliculosus* (NCBI accession number X52503) and *Sphacelaria cirrosa* (Roth) C. Agardh (NCBI accession number AJ287865), with the sequence of a freshwater population of *E. siliculosus* from Australia (this study), and selecting conserved regions among the three sequences. To amplify the *rbc*L gene typically a nearly complete (1000–1450 bp) region was amplified as one piece using various combinations of the following primers: LM*rbc*L17F, BLS*rbc*L23F, LM*rbc*L130F, LM*rbc*L1207R, LM*rbc*L 1378R, LM*rbc*L1387R, and BLS*rbc*L1433R (Siemer *et al.* 1998; present study). A middle portion (bp 454–1040) of the *rbc*L gene was also amplified using the primers *rbc*-F2.5 and *rbc*-R3 (Kawai *et al.* 2000). The middle portion was amplified and later used for sequencing because sequencing in the forward and reverse directions from larger PCR products did not always overlap in this region. The PCR products were checked on 0.8% agarose gels with ethidium bromide. Post-PCR cleaning was performed on samples that gave positive results, using Geneclean® Turbo for PCR Kit (Qbiogene, Carlsbad, CA).

DNA sequencing

Nucleotide sequences of 10 species or populations of freshwater brown algae and two cultures of marine brown algae (listed in Table 1) were determined using the dideoxy chain termination method, with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and primers listed in Table 2. PCR products were sequenced using the same primers as used for PCR amplification. Occasionally internal primers were used when these primers failed. Sequencing reactions were run on an ABI Prism 3700 automated sequencer (Applied Biosystems, Foster City, CA) at the American Museum of Natural History, NY, NY.

BLASTn search

A nucleotide–nucleotide BLASTn (basic local alignment search tool) was used to search the nucleotide database of the National Center for Biotechnology Information website (NCBI; www.ncbi.nlm.nih.gov/BLAST; Altschul *et al.* 1990) to align and compare the sequences we obtained with published sequences. From these searches the closest two matches were recorded to verify that our specimens were truly members of the Phaeophyceae and to assure that DNA used for sequencing was not a contaminant. This step was necessary because (1) some freshwater species have rarely been collected or isolated (e.g. *Porterinema*), (2) several taxa were described 100 or more years previously, and (3) all have thus far been identified using only morphological data.

Other sources of DNA sequences and outgroups

To understand the taxonomic position of freshwater species within the Phaeophyceae, sequences from a diverse range of (marine) phaeophyte taxa were obtained from NCBI. Our *a priori* plan was (1) to sample a few members from most orders in the Phaeophyceae and (2) to heavily sample members from within several families in the Ectocarpales, because nearly all freshwater species have traditionally been classified in that order. It was not our intention to examine the phylogeny of all groups within this class, since this broader question has been addressed by others (e.g. De Reviers & Rousseau 1999; Draisma *et al.* 2001). Here, *rbc*L sequences from a total of 24 brown algal species from nine orders within the Phaeophyceae were used (Table 3), plus two obtained in this study (Table 1). This included 13 taxa in five or six families from within the Ectocarpales (Peters & Ramírez 2001; Rousseau *et al.* 2001). In addition, sequences from three species of Chrysophyceae (three orders), one from the Tribophyceae and two members of the Phaeothamniophyceae, were selected (Table 3) as outgroups (Daugbjerg & Andersen 1997; Saunders *et al.* 1997; Andersen *et al.* 1998; Bailey *et al.* 1998).

Table 3. Sequences retrieved from the NCBI nucleotide sequence database, which served as outgroups and additional marine brown algal taxa for phylogenetic analyses (listed alphabetically by order and family; groupings within Phaeophyceae after Peters & Ramírez 2001, Rousseau et al. 2001).

Class Order	Family	Genus species	Habitat	NCBI # *rbc*L
Chrysophyceae				
Chromulinales		*Chromulina nebulosa* Cienkowski	FW	AF155876
Hibberdiales		*Chrysocapsa venralis* Starmach	FW	AF155877
Ochromonadales		*Epipyxis pulchra* Hilliard & Asmund	FW	AF015571
Tribophyceae				
Tribonematales		*Tribonema intermixum* Pascher	FW	AF015588
Phaeothamniophyceae				
Phaeothamniales		*Phaeothamnion confervicola* Lagerheim	FW	AF064746
		Phaeoschizochlamys mucosa Lemmermann	FW	AF064747
Phaeophyceae				
Cutleriales	Cutleriaceae	*Cutleria multifida* (J.E. Smith) Greville	Mar	AY157692
Desmarestiales	Desmarestiaceae	*Desmarestia ligulata* (Lightfoot) J.V. Lamouroux	Mar	AJ287848
Dictyotales	Dictyotaceae	*Dictyota dichotoma* (Hudson) Lamouroux	Mar	AJ287852
Ectocarpales	Acinetosporaceae	*Geminocarpus austro-georgiae* Skottsberg	Mar	AJ295830
	Acinetosporaceae[1]	*Pylaiella littoralis* (Linnaeus) Kjellman	Mar	X55372
	Adenocystaceae	*Adenocystis utricularis* (Bory) Skottsberg	Mar	AJ295823
	Chordariaceae	*Chordaria flagellifornis* (Müller) C. Agardh	Mar	AY095324
	Chordariaceae[2]	*Dictyosiphon foeniculaceus* (Hudson) Greville	Mar	AF055397
	Chordariaceae	*Myrionema strangulans* Greville	Mar	AY079435
	Ectocarpaceae	*Ectocarpus siliculosus* (Dillwyn) Lyngbe	Mar	X52503
	Ectocarpaceae	*Hincksia mitchelliae* (Harvey) Silva	Mar	U38753
	Ectocarpaceae	*Kuckuckia spinosa* (Kützing) Kuckuck	Mar	AF207804
	Ectocarpaceae	*Streblonema maculans* (Hamel) South & Titley	Mar	AY157694
	Incertae sedis	*Asteronema rhodochortonoides* (Børgesen) Müller & Parodi	Mar	AJ295825
	Scytosiphonaceae	*Petalonia fascia* (O.F. Müller) O. Kuntze	Mar	AF385845
	Scytosiphonaceae	*Scytosiphon lomentaria* (Lyngbe) Link	Mar	AB022238
Fucales	Fucaceae	*Fucus distichus* Linnaeus[3]	Mar	AF195515
	Sargassaceae	*Sargassum muticum* (Yendo) Fensholt	Mar	AJ287854
Laminariales	Chordaceae	*Chorda filum* (Linnaeus) Stackhouse	Mar	AB035784
Tilopteridales	Phyllariaceae	*Saccorhiza polyschides* (Lightfoot) Batters	Mar	AB045256
Scytothamnales	Scytothamnaceae	*Scytothamnus australis* (J. Agardh) Hooker & Harvey	Mar	AJ295833
Sphacelariales	Sphacelariaceae	*Sphacelaria cirrosa* (Roth) C. Agardh	Mar	AJ287865
	Sphacelariaceae	*Sphacelaria nana* Nägeli ex Kützing	Mar	AJ287875
Syringodermatales	Syringodermataceae	*Syringoderma phinneyi* Henry & Müller	Mar	AJ287868

[1] Classified in Acinetosporaceae by Rousseau *et al.* (2001), in Ectocarpaceae by Peters & Ramírez (2001).
[2] Classified in Dictyosiphonaceae by Rousseau *et al.* (2001), in Chordariaceae by Peters & Ramírez (2001).
[3] Now named *Fucus gardneri* Silva.

Sequence alignment and phylogenetic analysis

Individual sequences were imported into Sequencher™ 3.0 (Gene Codes, Ann Arbor, MI), a DNA sequence editor program, for editing and to assemble consensus sequences. Consensus sequences were next exported into MacClade 4 (Maddison & Maddison 2001), where they were aligned to existing *rbc*L sequences. The alignment included sequences from six freshwater phaeophytes, 26 marine species (two from this study and 24 from the NCBI database), and sequences from six outgroups (NCBI), and was 1467 bp long. Four Canadian *Heribaudiella* consensus sequences were identical; thus only one consensus sequence was used in the alignment (to avoid biasing the phylogenetic analysis); for the same reason, only one *Bodanella* consensus sequence was used in the analysis. The alignment was then imported into PAUP (v.4.0b10; Swofford 2002) for phylogenetic analysis.

Maximum parsimony analyses were conducted under a heuristic search using the tree bisection–reconnection branch-swapping algorithm, with 100 random additions and gaps treated as missing data. Neighbor-joining (NJ) distance analyses were also performed using a GTR+I+G model selected under the AIC criterion in Modeltest (v. 3.7; Posada & Crandall 1998). Bootstrapping (Felsenstein 1985) was completed as a measure of support using 2000 replicates under NJ and 1000 for parsimony (10 random sequence additions). Bayesian analyses were performed in Mr. Bayes (v. 3.1.2; Huelsenbeck & Ronquist 2001) with two independent trials (each with parallel runs) of inference under the GTR+I+G model (but with parameters estimated during the runs). Parallel runs of four Markov chains were completed with 1,000,000 generations and sampling each 1000 generations. Data were partitioned by codon and parameters were unlinked during the analyses. Log-likelihood values for the sampled trees stabilized before 30,000 generations in all analyses and the final 750 trees (250 discarded as burn-in) were used to calculate posterior probabilities.

Reconciling morphological and sequence data

To assess the usefulness of phylogenetic analyses performed here, a collection of putatively diagnostic morphological and developmental variables (based on Waern 1952; Starmach 1977; Bourrelly 1981; Wehr 2003) were compared against the *rbc*L-based phylogenetic groupings. These attributes included (1) type of cell division (location of

meristem and uni- or multiseriate cell division); (2) branching pattern; (3) presence/absence of perennial system of prostrate and erect filaments; (4) cell dimensions and shape; (5) form of chloroplasts and presence/absence of pyrenoids; and (6) characteristics of the pluri- and unilocular sporangia. Such comparisons between morphological and molecular groupings have proven useful in resolving the taxonomic placement of other species within the Phaeophyceae (Burrowes *et al.* 2003; Kawai *et al.* 2005). Because two freshwater *Sphacelaria* species (but not considered here) were clearly members of the Sphacelariales (Jao 1943; Schloesser & Blum 1980), an additional diagnostic trait was assessed in two of the freshwater species (*B. lauterborni*, *H. fluviatilis*) studied here (7): a temporary blackening of cell walls when treated with hypochlorite bleach (full strength and 20% dilution) as described by Draisma *et al.* (2002). These results were compared against the marine *Sphacelaria* sp. (UTEX 800) as a positive control (after Henry 1987).

RESULTS

Position of freshwater species using BLASTn

Our first goal was to determine if molecular data obtained from the freshwater taxa (based on morphology) placed all these organisms in the Phaeophyceae. A BLASTn search compared all newly obtained *rbc*L sequences with those in the NCBI database to determine which sequence they most closely matched. In all cases the freshwater sequences matched most closely to the sequences of well-recognized marine members of the Phaeophyceae. Similarities ranged from 89 to 99%, and averaged ≈ 92.2%. The greatest similarity for any freshwater specimen was obtained for the freshwater *E. siliculosus* collected from Australia, which was most similar in its sequences to a marine *E. siliculosus* collected from Europe (99%). The lowest similarities obtained from the existing NCBI database were for *B. lauterborni* (89% with *Sphacelaria caespitula* Lyngbye) and *P. fluviatile* (89% with *Alethocladus corymbosus* [Dickie] Sauvageau). BLASTn searches between these same freshwater taxa using the NCBI database with 18S rDNA data produced identical or similar matches (McCauley 2002).

Phylogenetic analysis

The analysis of the *rbc*L alignment resulted in the formation of three large clades, with *Porterimena* and *Dictyota* falling outside of these (Fig. 1). The first (clade 1) included members of six different orders, Tilopteridales, Laminariales, Cutleriales, Fucales, Desmarestiales, and Scytothamnales. The second clade, which had high internal support, included all members of the Ectocarpales (16 taxa) including two of the freshwater species, *E. siliculosus* and *Pleurocladia lacustris*. The alignment revealed a slight separation between the three *Ectocarpus* samples considered, with *Kuckuckia spinosa* (Kützing) Kuckuck falling in between. The marine (European) and freshwater (Australian) *E. siliculosus* sequences were nearly identical, whereas *K. spinosa* was a sister taxon to these two strains. A third marine *Ectocarpus* sp. (UTEX #1433) formed a sister to *K. spinosa*. Freshwater *P. lacustris* was clearly placed within the Ectocarpales, and more closely associated with the *Ectocarpus* subgroup (family Ectocarpaceae) than with members of any of the other five Ectocarpalean families represented, and was positioned as a sister to this grouping. However, although more closely related to members of the Ectocarpaceae, *Pleurocladia* was positioned between the Ectocarpaceae and Scytosiphonaceae. Other families within the Ectocarpales (Acinetosporaceae, Adenocystaceae, Chordariaceae) were more distantly related to *Pleurocladia*. One marine species, *Chorda filum* (Linnaeus) Stackhouse, grouped outside of the Ectocarpales clade, forming a sister. None of the remaining freshwater taxa proved to be closely related to the Ectocarpales.

A third large clade included two smaller subclades, with one member of the Syringodermatales as a sister taxon to the two. One subclade (termed clade 3a) included all (marine) *Sphacelaria* and the other (clade 3b) included the freshwater species of *H. fluviatilis* and *B. lauterborni*, with *Bodanella* intermediate between *Heribaudiella* populations from British Columbia and Germany. Although the *Heribaudiella* and *Bodanella* had much closer affinities with *Sphacelaria*, these subclades were fairly distinct from one another, differing by roughly 4.6% (≈ 68 bp). Furthermore, the *Heribaudiella–Bodanella* subclade was genetically distant from the Ectocarpales, in which these genera are currently classified, by at least 11.6% (≈ 170 bp), which is only somewhat less than the number of bases by which it differs from *Fucus distichus* (Linnaeus) (e.g. 14.5%, ≈ 213 bp) or from any other species in the first large clade. Similarly, the distance between either *Heribaudiella* population and any *Ectocarpus* sequence is 13.6% (≈ 200 bp). The *Heribaudiella–Bodanella* subclade is in fact more closely related to *Syringoderma phinneyi* Henry & Müller (differed by 6.5%, ≈ 96 bp), as a sister to this lower clade. The freshwater-brackish *P. fluviatile* did not group closely with any of the species tested or show a strong association with any of the recognized brown algal orders. However, sequencing this species proved to be more difficult than for the other taxa, given that only a portion of this sequence was determined (814 bp). Therefore, its position was not clearly established.

Reconciling molecular and morphological data

As the principal focus of the present study was to resolve the phylogenetic placement of four freshwater taxa, *B. lauterborni*, *E. siliculosus*, *H. fluviatilis*, and *P. lacustris*, the *rbc*L-based groupings were compared with the morphological features used in current taxonomic schemes for diagnostic keys and confirmed in ultrastructural studies. The first group (placed in clade 2, Ectocarpales) included the freshwater *Ectocarpus* and *Pleurocladia*; both species are uniseriate filaments with second or irregular branching arising (from basal or intercalary meristems) from a minimal (*Ectocarpus*) or loosely spreading (*Pleurocladia*) prostrate structure. Cells were isodiametric or somewhat swollen (Table 4). Cells were typically thin-walled and cylindrical (although dimensions were quite dissimilar in the two taxa), and had one or few large band-like chloroplasts and

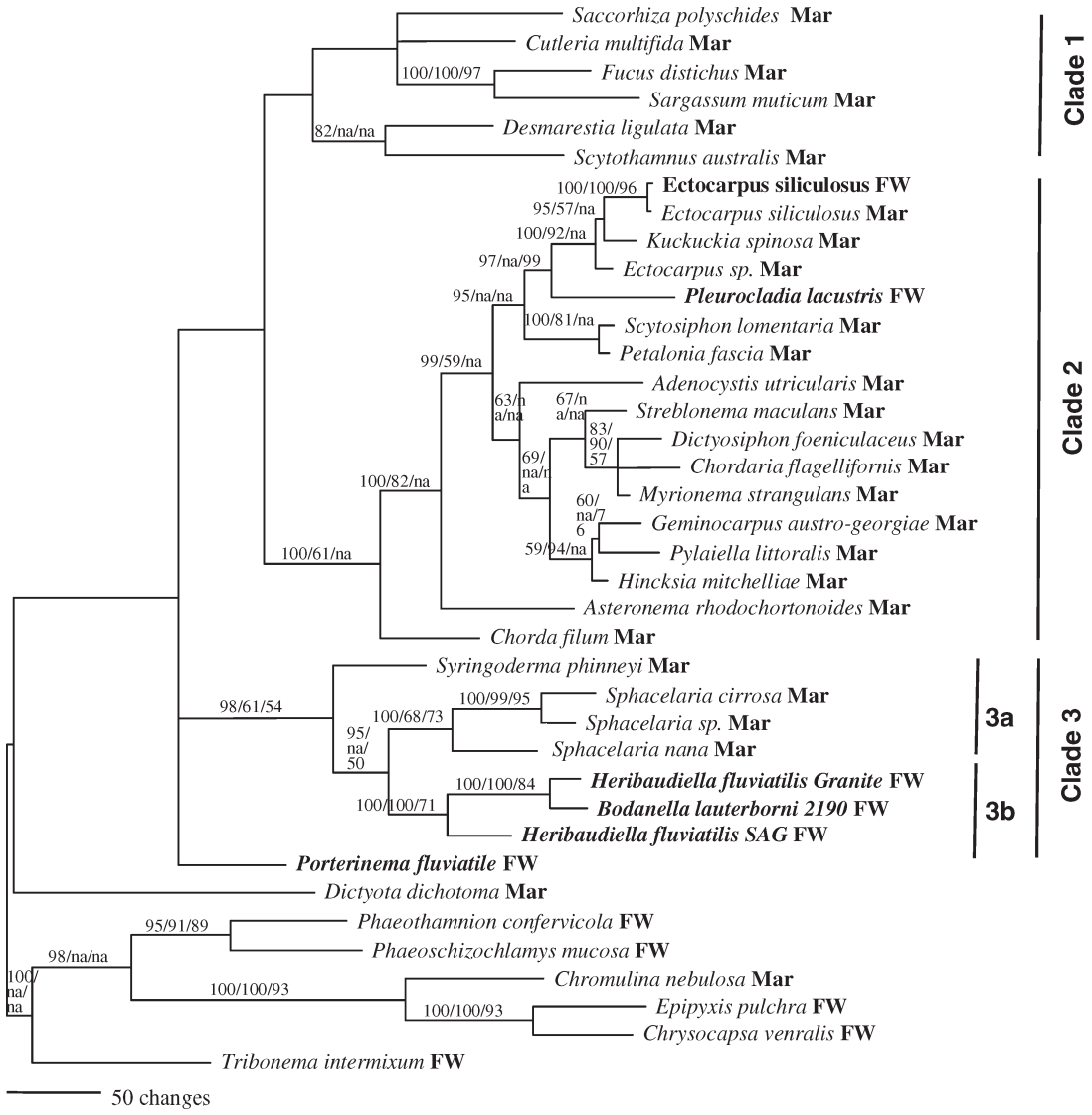

Fig. 1. Bayesian consensus tree for the alignment of *rbc*L sequences from freshwater (FW) and marine (Mar) brown algal species within the Phaeophyceae. Values at the branches indicate Bayesian posterior probabilities, maximum parsimony bootstrap, and neighbor-joining bootstrap support, respectively (na = < 50% support by that method). Clades 1, 2, 3a, and 3b are described in the text; species names of freshwater phaeophyte species are written in bold.

a single, stalked pyrenoid. Cylindrical–elongate plurilocular sporangia occur in both, whereas only *Pleurocladia* has been observed to produce unilocular sporangia.

Heribaudiella fluviatilis and *B. lauterborni* (*rbc*L-based subclade 3b) each possessed apical growth and formed a dense and spreading pseudoparenchymatous basal system. In *Heribaudiella*, erect filaments originated from this prostrate system. Cells in both taxa were thick-walled, swollen or lumpy in appearance, and possessed many (10 to 30) small, disc-shaped chloroplasts. Pyrenoids were not observed in either species. Both produce large ovoid unilocular sporangia, whereas plurilocular sporangia have been observed only (but infrequently) in *Heribaudiella*. Specimens of *Heribaudiella* (Ashnola River) and *Bodanella* (UTEX 2190) when exposed to two concentrations of sodium hypochlorite (diagnostic for Sphacelariales) were both negative. A similar test with *Sphacelaria* sp. (UTEX 800) was positive (blackened cell walls).

DISCUSSION

Classification of freshwater species

The present study was able to discern the phylogenetic positions of four freshwater taxa (*B. lauterborni*, *E. siliculosus*, *H. fluviatilis*, *P. lacustris*) currently placed in the Phaeophyceae. Thus far, most classification schemes place these freshwater taxa within the order Ectocarpales on the basis of morphology, reproductive features, and ultrastructure (Bourrelly 1981; Pueschel & Stein 1983; Wehr 2003). This study was the first to use molecular data to confirm the placement of these freshwater browns into the Phaeophyceae and to infer their phylogenetic relations within this class. We compared these sequences with those of 26 marine species from the class encompassing nine orders. Our results support their inclusion at the class level, but call into question some classifications at the ordinal and

Table 4. Analytical comparison of *Ectocarpus siliculosus*, *Pleurocladia lacustris*, *Bodanella lauterborni*, and *Heribaudiella fluviatilis* on the basis of *rbc*L groupings and morphological and reproductive attributes.

*rbc*L Grouping	Clade 2: Ectocarpales		Clade 3b: *Bodanella* + *Heribaudiella*	
Character	*Ectocarpus siliculosus*	*Pleurocladia lacustris*	*Bodanella lauterborni*	*Heribaudiella fluviatilis*
(1) Cell division				
Meristem	intercalary	basal	apical	apical
Uni-/multiseriate	uniseriate	uniseriate	uniseriate	uniseriate
(2) Branching				
Frequency	sparse	common, spreading;	common, spreading	common, dense
Pattern	secund or irregular	secund, alternate, irregular	irregular	dichotomous, alternate, irregular
(3) Prostrate/erect system				
Prostrate filaments	unknown	common, loosely organized	abundant, creeping, rarely pseudoparenchymatous	abundant; pseudoparenchymatous
Upright filaments	common	common, spreading	unknown	common, compact
(4) Cell dimensions				
Filament width (μm)	20–40	(6) 8–12	11–15	8–10 (15)
Cell length (μm)	20–80	12–30 (40)	12–25	(8) 10–15
L:W ratio	1–2	2–4	1–2	0.5–1.5
Cell shape	isodiametric	isodiametric or slightly swollen	swollen or lumpy	isodiametric, swollen or irregular
Cell wall thickness (μm)	0.1–0.2	0.1–0.2	0.5–0.7	0.5–1.5
(5) Chloroplasts				
Number/shape	one–several; large band-like, parietal	one, large band-like, parietal	many, disc-shaped, parietal	many, disc-shaped, parietal
Pyrenoids	single, stalked	single, stalked	unknown	absent
(6) Sporangia				
Plurilocular shape	elongate–cylindrical	elongate–cylindrical	unknown	uncommon, elongate
Width	20–35	8–12	NA	8–12 (15)
Length	70–160	10–12	NA	20–25
Unilocular shape	unknown	pyriform–globose	ellipsoid–ovoid	ellipsoid–ovoid
Width	NA[1]	(12) 20–30	25–30	10–25
Length	NA	20–40 (80)	30–35	15–35
(7) Cell wall blackening	ND	ND	negative	negative

[1] NA, not applicable; ND, not done.

perhaps genus level. Nucleotide sequences of the *rbc*L gene from freshwater taxa were consistently most similar to analogous sequences of marine brown algae rather than to sequences from other taxa in the NCBI database. Specifically, sequences from the *rbc*L gene showed strong support for the inclusion of the freshwater taxa into the Phaeophyceae, as analogous sequences of marine taxa were at least 89% and up to 99% identical.

The present *rbc*L sequence-based analysis clearly placed two of the freshwater taxa, *P. lacustris* and (freshwater) *E. siliculosus*, in the Ectocarpales (Fig. 1). Each species nested among marine members of the Ectocarpaceae, although *Pleurocladia* may be a sister to Scytosiphonaceae. Wilce advised in 1966 that *Pleurocladia* should remain within the Ectocarpaceae until further studies are conducted. Our consideration of the new molecular evidence in conjunction with existing morphological and cytological data (Table 4) further supports the retention of *Pleurocladia* within the Ectocarpaceae, especially given their predominantly filamentous habit, reproductive structures, ribbon-shaped chloroplast morphology, and the presence of stalked pyrenoids. Our molecular analysis demonstrated that the recently discovered freshwater *E. siliculosus* from Australia (Hopkins River Falls) is closely related to European marine conspecifics, with only five nucleotide differences in their *rbc*L genes. Its identity, previously based on an elongate plurilocular sporangium (West & Kraft 1996), seems certain based on a nearly identical *rbc*L sequence (> 99%) to that in a marine population from Germany (Fig. 1).

Our analyses provide no support for the inclusion of *H. fluviatilis* or *B. lauterborni* within the Ectocarpales. The *Heribaudiella–Bodanella* subclade 3b differs (within the *rbc*L gene) from members of traditional order (Ectocarpales) and from any *Ectocarpus* species by more than 13.6% (≈ 200 bp). Morphological characteristics also differ strongly from members of the Ectocarpales, with both *Heribaudiella* and *Bodanella* exhibiting apical growth originating from a dense system of perennial prostrate system of pseudoparenchymatous filaments and thick-walled cells with numerous discoid chloroplasts lacking obvious pyrenoids (Table 4). The *Heribaudiella–Bodanella* subclade has strong internal bootstrap support, and its relation to other sister groups is similarly strong (Fig. 1), being more closely related to members of the Sphacelariales than the Ectocarpales. Our initial BLASTn searches found the closest matches in the NCBI database using *rbc*L sequences for both species with various *Sphacelaria* species (McCauley 2002). However, present molecular and morphological data suggest that classification in this order is also inappropriate. The *rbc*L sequences of the *Heribaudiella–Bodanella* subclade differed by 4.6% (≈ 68 bp) from those of three *Sphacelaria* species that were included in the present analysis (similar to differences among other brown algal orders). Members of the Sphacelariales are character-

ized by filaments with apical growth, which is true in both *Heribaudiella* and *Bodanella*, but also transverse division in subapical cells (leading to multiseriate axes) and blackening of cell walls after treatment with bleach. The latter two traits were negative in *Heribaudiella* and *Bodanella* (Table 4), but are regarded as basal traits for members of the order (Draisma *et al.* 2002). The *Heribaudiella–Bodanella* subclade is most likely a sister group to *Syringoderma phinneyi* (differs by 6.5%, ≈ 96 bp), but both species lack dorsiventral differentiation and heteromorphic life cycle, characteristic of the Syringodermatales (Henry 1984). No other species or orders placed near this clade on the basis of *rbc*L sequences possess the suite of morphological, cytological, or reproductive traits exhibited in *H. fluviatilis* or *B. lauterborni*. (Table 4; Draisma *et al.* 2002). It is also possible that they are related to other marine crust-forming taxa in the Phaeophyceae, such as *Ralfsia or Pseudolithoderma*, but presently there are no *rbc*L sequences available for either species in the NCBI database. For these reasons both species should be removed from the Ectocarpales, and cannot be placed in either the Sphacelariales or the Syringodermatales. While it is possible that they represent a new order within the Phaeophyceae, we recommend that they remain unclassified (*incertae sedis*) until further work is completed on other crust-forming marine taxa, especially members of the Ralfsiales. While species of *H. fluviatilis* and *B. lauterborni* were distinct from the other brown algal species considered in our study, they themselves were closely related, differing by no more than 2.5% (≈ 36 bp). Populations of *B. lauterborni* consistently nested between the populations of *H. fluviatilis* from British Columbia and Germany, regardless of method of analysis. Future population studies on both species, perhaps using more variable gene regions (e.g. ITS region, RUBISCO spacer, or microsatellites) may resolve these two taxa.

Molecular data from our study were unable to resolve the ordinal classification of *P. fluviatile* (isolate 124.79 from SAG culture collection) and lend support to earlier findings that this species is quite enigmatic (Wilce *et al.* 1970). Our extraction efforts yielded a sequence of only 814 bp in length, which in part may have led to ambiguous results for this species. Here, the branching distance between *P. fluviatile* and the common node for the Ectocarpales clade was > 10.9% or ≈ 160 bp (Fig. 1). While the correct phylogenetic position of *P. fluviatile* remains ambiguous, our data provide no support for its inclusion within the Ectocarpales (as classified by Bourrelly 1981). Moreover, the shortest distance of this species to any specific taxon was *Dictyota dichotoma* (Hudson) Lamouroux (13.0%, ≈ 190 bp difference), suggesting that this species was not closely related to any of the taxa included in the present study. However, if *Porterinema* truly belongs in the Ralfsiales (as suggested by Wilce *et al.* 1970), then our data strongly suggest that *Heribaudiella* and *Bodanella* cannot also be placed in this order.

Ecological and evolutionary considerations

Ecologically, nearly all populations of *Pleurocladia lacustris* are distinctly anticoastal in their freshwater distributions (Israelsson, 1938; Waern 1952; Ekenstam *et al.* 1996), although one notable population has been collected from just above the high-tide line in the Canadian Arctic (Wilce 1966). The origin of this species from its marine relatives seems difficult to discern at this point. But the increasing number of freshwater *Pleurocladia* populations being reported substantially inland in Europe (e.g. Szymanska & Zakrys 1990; Kusel-Fetzmann 1996) and North America (Ekenstam *et al.* 1996, E.B. Young, unpublished observations [Lake Michigan]) support the suggestion by Waern (1952) that this species may be older than the postglacial history of the Northern Hemisphere. For this reason, it would be informative to compare sequences (perhaps using mtDNA) of different populations of *Pleurocladia* to test distance from the ocean or conductivity (salinity) as possible factors leading to its genetic divergence. Studies on this species are also of interest at the intrapopulation level because of its widespread but apparently disjunct geographic distribution (Waern 1952; Kirkby *et al.* 1972; Ekenstam *et al.* 1996; Kusel-Fetzmann 1996).

The ecological and biogeographic history of freshwater *Ectocarpus* is likely quite different. We have observed that in culture this isolate can grow at 0 parts per thousand (ppt) and 7 ppt seawater (unpublished data), whereas West & Kraft (1996) have shown that it grows equally well in 5, 15, and 30 ppt seawater. The authors also noted that the freshwater population was able to acclimate to much wider ranges of conditions than were isolates from fully marine sites. These findings taken together suggest that this freshwater population is likely a marine invader, and may have the potential to expand its range inland in perhaps other estuarine locations. An earlier culture study of clonal (marine) populations of *E. siliculosus* demonstrated that many geographically distant populations have a sterility barrier, and that Australian populations were infertile with nearly all strains isolated from the Northern Hemisphere (Müller 1979). Being located only 25 km from the coast and 40 m above a waterfall, the Australian freshwater population may have very recently evolved traits that enable its tolerance to fresh water. Future studies on its method of invasion from marine habitats and possible rapid evolution (using the ITS region) vs marine *Ectocarpus* could prove to be informative (e.g. Müller *et al.* 1998). Studies are also needed to clarify the phylogenetic position of the two described freshwater species of *Sphacelaria* (*S. fluviatilis* Jao, *S. lacustris* Schloesser *et* Blum), which have been collected from only three locations worldwide.

The ecology of *Heribaudiella* is fairly well established, being a species apparently restricted to very clear mountain streams with rapid current velocity, slightly alkaline pH (7–8.5), moderate dissolved P (10–60 $\mu g\ l^{-1}$), NH_4^+ (15–65 $\mu g\ l^{-1}$ N), and Ca (≈ 10–70 $mg\ l^{-1}$), but relatively high specific conductance (≈ 600–1000 $\mu S\ cm^{-1}$) (Wehr & Stein 1985, Wehr & Perrone 2003). Its global distribution includes a few hundred known populations, but many are hundreds or thousands of kilometres from the next nearest population (Wehr 2003). Because of its locally abundant but patchy distribution, further efforts are needed to characterise its genetic, reproductive, and morphological variation. In addition, the morphologies of *H. fluviatilis* and *B. lauterborni*, while quite distinct in field populations and laboratory cultures, are genetically quite closely

related. Nonetheless, the morphologies of each remain stable and distinct in laboratory culture (Wehr & McCauley, unpublished data), their ecological and geographic patterns (thus far) do not overlap (Bourrelly 1981, Wehr 2003), and no reports of new localities for *Bodanella* are known. Whether the production of upright filaments in *Heribaudiella* is a fixed trait and cannot be induced in *Bodanella* remains to be seen. These findings suggest that more extensive biogeographic and molecular studies on these two species are warranted. Like *Pleurocladia*, their distinctly anticoastal distributions (Wehr & Stein 1985, Wehr 2003) suggest that their freshwater origins are also quite old. Further efforts to understand their biogeographic patterns may also help to better understand their evolutionary origins.

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REFERENCES

Allen M.M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *Journal of Phycology* 4: 1–4.

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.

Andersen R.A., Potter D., Bidigare R.R., Latasa M., Rowan K. & O'Kelly C.J. 1998. Characterization and phylogenetic position of the enigmatic golden alga *Phaeothamnion confervicola*: ultrastructure, pigment composition and partial SSU rDNA sequence. *Journal of Phycology* 34: 286–298.

Assali N.E., Mache R. & Loiseaux-de Goer S. 1990. Evidence for a composite phylogenetic origin of the plastid genome of the brown alga *Pylaiella littoralis* (L.) Kjellm. *Plant Molecular Biology* 15: 307–315.

Bailey J.C., Bidigare R.R., Christensen S.J. & Andersen R.A. 1998. Phaeothamniophyceae *classis nova*: a new lineage of chromophytes based upon photosynthetic pigments, *rbc*L sequence analysis and ultrastructure. *Protist* 149: 245–263.

Bold H.C. & Wynne M.J. 1978. *Introduction to the algae.* Prentice-Hall, Englewood Cliffs, New Jersey, 706 pp.

Bourrelly P. 1981. *Les algues d'eau douce. Vol. II. Les algues jaunes et braunes, Chrysophycees, Phaeophycees, Xanthoophycees, et Diatomees*, rev. ed. Soc. Nouv des Editions Boubee, Paris. 438 pp.

Braun A. 1855. Algarum unicellularum genera nova et minus cognita, praemissis obervationibus de algis unicelularibus in genere. Pp. 1–111, pls. 1–6, Leipzig.

Burrowes R., Rousseau F., Müller D.G. & de Reviers B. 2003. Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the *rbc*L and 28S nrDNA sequences. *Cryptogamie Algologie* 24: 63–73.

Daugbjerg N. & Andersen R.A. 1997. A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbc*L sequence data. *Journal of Phycology* 33: 1031–1041.

De Reviers B. & Rousseau F. 1999. Towards a new classification of the brown algae. *Progress in Phycological Research* 13: 107–195.

Dop A.J. 1979. *Porterinema fluviatile* (Porter) Waern (Phaeophyceae) in the Netherlands. *Acta Botanica Neerlandica* 28: 449–458.

Draisma S.G.A., Olsen J.L., Stam W.T. & Prud'homme van reine W.F. 2002. Phylogenetic relationships within the Sphacelariaceae (Phaeophyceae): *rbc*L, RUBISCO space and morphology. *European Journal of Phycology* 37: 385–401.

Draisma S.G.A., Prud'homme van Reine W.F., Stam W.T. & Olsen J.L. 2001. A reassessment of phylogenetic relationships within the phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. *Journal of Phycology* 37: 586–603.

Ekenstam D., Bozniak E.G. & Sommerfeld M.R. 1996. Freshwater *Pleurocladia* (Phaeophyta) in North America. *Journal of Phycology Supplement* 32: 15.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Geissler U. 1983. Die salzbelastete flubstrecke der werra - ein binnenlandstandort fur Ectocarpus confervoides (Roth) Kjellman. *Nova Hedwigia* 37: 193–217.

Henry E.C. 1984. Syringodermatales ord. nov. and *Syringoderma floridana* sp. nov. (Phaeophyceae). *Phycologia* 23: 419–426.

Henry E.C. 1987. Morphology and life histories of *Onslowia bahamensis* sp. nov., and *Verosphacela ebrachia* gen. et sp. nov., with a reassessment of the Choristocarpaceae (Sphacelariales, Phaeophyceae). *Phycologia* 26: 182–191.

Huelsenbeck J.P. & Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Israelsson G. 1938. Über die Süsswasserphaeophycéen Schwedens. *Botaniska Notiser* 1938: 113–128.

Jao C.C. 1943. Studies on the freshwater algae of China. XI. *Sphacelaria fluviatilis*, a new freshwater brown alga. *Sinensia* 14: 151–154.

Kawai H., Sasaki H., Maeda Y. & Arai S. 2000. Morphology, life history, and molecular phylogeny of *Chorda rigida*, sp. nov. (Laminariales, Phaeophyceae) from the Sea of Japan and the genetic diversity of *Chorda filum*. *Journal of Phycology* 37: 130–142.

Kawai H., Sasaki H., Maeba S. & Henry E.C. 2005. Morphology and molecular phylogeny of *Phaeostrophion irregulare* (Phaeophyceae) with a proposal for Phaeostrophiaceae fam. nov., and a review of Ishigeaceae. *Phycologia* 44: 169–182.

Kirkby S.M., Hibberd D.J. & Whitton B.A. 1972. *Pleurocladia lacustris* A. Braun (Phaeophyta) – a new British Record. *Vasculum* 57: 51–56.

Kusel-Fetzmann E.L. 1996. New records of freshwater Phaeophyceae from lower Austria. *Nova Hedwigia* 62: 79–89.

Maddison D.R. & Maddison W.P. 2001. *MacClade 4: Analysis of phylogeny and character evolution*, Version 4.03. Sinauer Associates, Sunderland, MA.

McCauley L.A.R. 2002. *Phylogenetic relationships of freshwater brown algae (Phaeophyceae) based on Rubisco large subunit and ribosomal DNA sequences.* Master's thesis, Fordham University.

Müller D.G. 1979. Genetic affinity of Ectocarpus siliculosus (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia. *Phycologia* 18: 312–318.

Müller D.G., Parodi E.R. & Peters A.F. 1998. *Asterocladon lobatum* gen. et sp. nov., a new brown alga with stellate chloroplast arrangement, and its systematic position judged from nuclear rDNA sequences. *Phycologia* 37: 425–432.

Müller K.M., Sheath R.G., Vis M.L., Crease T.J. & Cole K.M. 1998. Biogeography and systematics of *Bangia* (Bangiales, Rhodophyta) based on the RUBISCO spacer, *rbc*L gene and 18S rRNA gene sequences and morphometric analyses. I. North America. *Phycologia* 37: 95–207.

Peters A.F. & Ramírez M.E. 2001. Molecular phylogeny of small brown algae, with special reference to the systematic position of

Caepidium antarcticum (Adenocystaceae, Ectocarpales). *Cryptogamie Algologie* 22: 187–200.

Posada D. & Crandall K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Pringsheim E.G. 1946. *Pure cultures of algae.* Cambridge University Press, Cambridge, UK, 119 pp.

Pueschel C.M. & Stein J.R. 1983. Ultrastructure of a freshwater brown alga from western Canada. *Journal of Phycology* 19: 209–215.

Rousseau F. & De Reviers B. 1999. Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. *Cryptogamie Algologie* 20: 5–18.

Rousseau F., Burrowes R., Peters A.F., Kuhlenkamp R. & de Reviers B. 2001. A comprehensive phylogeny of the Phaeophyceae based on nrDNA sequences resolves the earliest divergences. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie* 324: 305–319.

Saunders G.W., Potter D. & Andersen R.A. 1997. Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales (Heterokonta) based on analysis of molecular and combined data. *Journal of Phycology* 33: 310–318.

Schloesser R.E. & Blum J.L. 1980. *Sphacelaria lacustris* sp. nov., a freshwater brown alga from Lake Michigan. *Journal of Phycology* 16: 201–207.

Siemer B.L., Stam W.T., Olsen J.L. & Pedersen P.M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and spacer sequences. *Journal of Phycology* 34: 1038–1048.

Starmach K. 1977. *Phaeophyta-brunatnice. Rhodophyta – krasnorosty*. Flora slodkowodna Polski, Vol. 14. Panstwowe Wydawnictwo Naukowe, Warsaw/Krakow. 445 pp.

Starr R.C. & Zeikus J.A. 1987. UTEX – the culture collection of algae at the University of Texas at Austin. *Journal of Phycology Supplement* 23: 1–47.

Swofford D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony and Other Methods* (software). Version 4.0 Beta 10, Sinauer Associates, Sunderland, MA.

Szymanska H. & Zakrys B. 1990. New phycological records from Poland. *Archiv für Hydrobiologie Supplement* 87: 25–32.

Tan I.H. & Druehl L.D. 1994. A molecular analysis of *Analipus* and *Ralfsia* (Phaeophyceae) suggests the order Ectocarpales is polyphyletic. *Journal of Phycology* 30: 721–729.

Van den Hoek C., Mann D.G. & Jahns H.M. 1995. *Algae: an introduction to phycology* Cambridge University Press, Cambridge, UK, 623 pp.

Waern M. 1952. Rocky-shore algae in the Oregund Archipelago. *Acta Phytogeographica Suecica* 30: 1–298.

Wehr J.D. 2003. Brown algae. In: *Freshwater algae of North America. Ecology and classification* (Ed. by J.D. Wehr & R.G. Sheath), pp. 757–773. Academic Press, San Diego, CA.

Wehr J.D. & Perrone A.A. 2003. A new record of *Heribaudiella fluviatilis*, a freshwater brown alga (Phaeophyceae) from Oregon. *Western North American Naturalist* 63: 517–523.

Wehr J.D. & Stein J.R. 1985. Studies on the biogeography and ecology of the freshwater phaeophycean alga *Heribaudiella fluviatilis*. *Journal of Phycology* 21: 81–93.

West J.A. & Kraft G.T. 1996. *Ectocarpus siliculosus* (Dillwyn) Lyngb. from Hopkins River Falls, Victoria – the first record of a freshwater brown alga in Australia. *Muelleria* 9: 29–33.

Wilce R.T. 1966. *Pleurocladia lacustris* in Arctic America. *Journal of Phycology* 2: 57–66.

Wilce R.T., Webber E.E. & Sears J.R. 1970. *Petroderma* and *Porterinema* in the New World. *Marine Biology* 5: 119–135.

Zimmermann W. 1928. Über algenbestände aus der Tiefenzone des Bodensees. Zur Ökologie und Soziologie der Tiefseepflanzen. *Zeitschrift für Botanik* 20: 1–28 + 2 pl.

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