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Eur. J. Phycol. (2010) **45(3)**: 308–326

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A revised classification of the Sphacelariales (Phaeophyceae) inferred from a *psb*C and *rbc*L based phylogeny

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(Received 19 April 2010; revised 19 April 2010; accepted 1 May 2010)

Phylogenetic relationships within the brown algal order Sphacelariales and with its sister group were investigated using chloroplast-encoded *psb*C and *rbc*L DNA sequences. A pilot study with 21 non-sphacelarialeans, representing nine orders (and some *incertae sedis* taxa), showed a strongly supported monophyly of the Sphacelariales with its sister taxa *Phaeostrophion irregulare*, *Bodanella lauterborni* and *Heribaudiella fluviatilis*. These three taxa were selected as outgroup for further analyses including DNA sequences of 30 sphacelarialean specimens representing all but two of the recognized genera (*Phloiocaulon* and *Ptilopogon* were not sampled). Bayesian Inference and Maximum Likelihood trees showed some incongruence with Maximum Parsimony trees. Trees based on *rbc*L showed some incongruence with trees based on *psb*C and combined alignments. Phylogenetic results were used as the basis for a newly proposed classification of the Sphacelariales that reflects evolutionary history. The Sphacelariales is subdivided into four families: Cladostephaceae (monotypic), Sphacelariaceae, Stypocaulaceae, and a newly created monotypic family Sphacelodermaceae to incorporate *Sphaceloderma caespitula*, comb. nov. (former *Sphacelaria caespitula*). *Sphacelaria radicans* is transferred to a newly created genus *Protohalopteris* and classified in the Stypocaulaceae, which also contains the two unsampled genera *Phloiocaulon* and *Ptilopogon* as well as the genus *Halopteris*. The genera *Stypocaulon* and monotypic *Alethocladus* were merged with *Halopteris*. The Sphacelariaceae were subdivided into six genera including *Sphacelaria* (consisting only of the former subgenus *Propagulifera*) and the monotypic *Sphacella*. *Herpodiscus durvillaeae*, *Sphacelaria pulvinata* and the *Sphacelaria* subgenera *Bracteata* and *Reinkea* were merged in an emended *Herpodiscus*. A new genus *Sphacelorbus* was created for *Sphacelaria nana*. *Battersia* was reinstated for *Sphacelaria mirabilis* and the subgenus *Pseudochaetopteris*, except for *Sphacelaria plumosa* for which *Chaetopteris* was reinstated.

Key words: *Battersia*, *Chaetopteris*, Cladostephaceae, *Herpodiscus*, *Protohalopteris* gen. nov., *psb*C, *rbc*L, Sphacelariales, Sphacelodermaceae fam. nov., *Sphacelorbus* gen. nov.

Introduction

Members of the brown algal order Sphacelariales Migula occur from the Arctic to the tropics, but the main centres of distribution are along the coasts of Europe, southern Australia and New Zealand (Prud'homme van Reine, 1993). The tropical species are all representatives of the *Sphacelaria* subgenus *Propagulifera* Prud'homme which are characterized by bearing propagules, i.e. specialized branchlets for vegetative propagation. The Sphacelariales in general are characterized by a polystichous thallus (although the monotypic genus *Sphacella* Reinke is haplostichous) growing from conspicuous apical cells, and by a temporary blackening of the cell wall when treated with an aqueous solution of sodium hypochlorite bleach (Prud'homme van Reine, 1993; de Reviers & Rousseau, 1999). The family Choristocarpaceae Kjellman was originally included in the order, but its members are haplostichous and do not show the characteristic response to bleach. When DNA sequence data became available, these taxa were removed from the Sphacelariales. The new family Onslowiaceae Draisma et Prud'homme (Draisma & Prud'homme van Reine, 2001) and new order Onslowiales Draisma et Prud'homme (in Phillips *et al.*, 2008) were created for the two oligostichous genera *Onslowia* Searles and *Verosphacela* E.C. Henry. Kawai *et al.* (2007) reinstated the family Discosporangiaceae O.C. Schmidt and the order

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ISSN 0967-0262 print/ISSN 1469-4433 online/10/03000308–326 © 2010 British Phycological Society
DOI: 10.1080/09670262.2010.490959

Discosporangiales O.C. Schmidt for the genus *Discosporangium* Falkenberg and also included the Choristocarpaceae (with the monotypic genus *Choristocarpus* Zanardini) in this order. The Sphacelariales *sensu stricto* (*s.s.*) currently consists of two families, i.e. the Sphacelariaceae Decaisne *emend.* Oltmanns and the Stypocaulaceae Oltmanns, after Draisma *et al.* (2002) concluded that the monotypic Cladostephaceae Oltmanns should be merged with the Sphacelariaceae based on DNA sequence data of the chloroplast-encoded gene for the large subunit of RuBisCO (*rbc*L). Draisma *et al.*'s (2002) molecular phylogenetic analysis of the Sphacelariales *s.s.* included representatives of both families and six out of eight recognized genera: *Cladostephus* C. Agardh, *Sphacelaria* Lyngbye, *Sphacella* Reinke (Sphacelariaceae), *Alethocladus* Sauvageau, *Halopteris* Kützing, and *Stypocaulon* Kützing (Stypocaulaceae). The Stypocaulaceae genera *Phloiocaulon* M. Geyler and *Ptilopogon* Reinke were not represented. Four main clades were resolved. One main clade was formed by the Stypocaulaceae. The Sphacelariaceae (including *Cladostephus*) formed another main clade with the exception of *Sphacelaria radicans* (Dillwyn) C. Agardh and *Sphacelaria caespitula* Lyngbye, which each formed a monotypic clade. No support was found for relationships between any of the four main clades. This may in part be explained by the fact that only half of the *rbc*L sequence was determined for *Sphacelaria radicans*. Southern hemisphere representatives were conspicuously missing in the study by Draisma *et al.* (2002), with the exception of Antarctic *Alethocladus corymbosus* (Dickie) Sauvageau. Draisma *et al.* (2002) discussed the possibilities for a new classification of the Sphacelariales, but (considering their limited taxon sampling and incompletely resolved phylogeny) refrained from proposing any formal change. Meanwhile, Heesch *et al.* (2008) demonstrated that *Herpodiscus durvillaeae* (Lindauer) South should also be transferred to the Sphacelariales. *Herpodiscus durvillaeae*, the only species in its genus, forms a branch on its own within the Sphacelariaceae in a *rbc*L phylogeny.

The availability of additional Sphacelariales collections from the southern hemisphere, including representatives of previously unsampled *Sphacelaria* subgenera, provided an opportunity to re-investigate the phylogeny of this order. The DNA samples used in Draisma *et al.* (2002) were still available and for these an additional DNA marker was sequenced, i.e. the chloroplast-encoded photosystem II CP43 chlorophyll Apo protein gene (*psb*C). Moreover, a closer outgroup for the Sphacelariales is now known: the monotypic Phaeostrophiaceae H. Kawai *et al.*, *Pseudolithoderma roscoffense* Loiseaux, and/or the freshwater genera *Bodanella* W. Zimmermann and *Heribaudiella* Gomont (currently *incertae sedis*) form the sister clade of the Sphacelariales (Kawai *et al.*, 2005; de Reviers *et al.*, 2007; McCauley & Wehr, 2007; Bittner *et al.*, 2008; Phillips *et al.*, 2008).

The aim of this study was to reconstruct the phylogeny of the Sphacelariales on the basis of a combined analysis of *psb*C and *rbc*L DNA sequences and an improved taxon sampling with more representatives of the southern hemisphere than in Draisma *et al.* (2002) and more closely related outgroup taxa (*Bodanella*, *Heribaudiella* and *Phaeostrophion* Setchell et N.L. Gardner) to improve resolution within the Sphacelariales. The length of the alignment was twice that in Draisma *et al.* (2002) and a complete *rbc*L sequence of *Sphacelaria radicans* (Dillwyn) C. Agardh was now included in the analysis. Draisma *et al.* (2002) inferred phylogenies using Maximum Parsimony and Maximum Likelihood methods. In the present study Bayesian Inference was also applied. The inferred phylogeny was used as the basis for a revision of the subdivision of the order.

Materials and methods

Taxon sampling

Taxa used in the phylogenetic analyses are listed in Table 1. Taxa from the Kobe University Macroalgal Culture Collection in Japan (vouchers starting with 'KU') were provided as DNA extracts and in addition Dr Takeaki Hanyuda (Kobe) provided *rbc*L sequences of the specimens KU-508 and KU-1118. Additional cultures were ordered from the Culture Collection of Algae at Göttingen in Germany (vouchers starting with 'SAG'). The Sphacelariales species in Draisma *et al.* (2002) are represented in this study by the same individual specimens and DNA extracts with the exception of *Sphacelaria radicans*. Therefore, a new *rbc*L sequence was determined for *S. radicans* in order to confirm conspecificity. *Pseudolithoderma roscoffense* was not available for this study and therefore its *psb*C sequence could not be determined. *Herpodiscus durvillaeae* specimens were provided by Dr Wendy Nelson (Wellington), but all amplification attempts led to sequences of its host *Durvillaea antarctica* (Cham.) Harvey. New amplification primers were designed (not shown) that did not anneal to *D. antarctica*, but amplification of *H. durvillaeae* remained unsuccessful. Additional specimens of Sphacelariales from New Zealand collected and silica dried in 1998 by Dr W.F. Prud'homme van Reine included specimens of *Ptilopogon botryocladus* (Hooker f. et Harvey) Reinke and *Sphacelaria cirrosa* (Roth) C. Agardh. However, PCR amplifications of these two specimens failed despite repeated efforts. In 2009 two *Cladostephus spongiosus* (Hudson) C. Agardh specimens from Australia were added to the collection.

Table 1. Specimens used in the present study listed by ordinal classification. The Sphacelariales are listed by the traditional subdivision into three families and the subgeneric classification of the genus *Sphacelaria*. EMBL accession numbers for new sequences (this study) are shown in **bold**. More collection details can be retrieved from EMBL.

			EMBL accession no.[c]	
Taxon	Voucher[a]	Country of origin[b]	*psb*C	*rbc*L
Incertae sedis (order not known)				
Asterocladon lobatum D.G. Müll., E.R. Parodi et A.F. Peters	SAG 27.97	Brazil	**FM957119** (1342)	**FM956109** (1467)
Bodanella lauterborni W.Zimm.	SAG 123.79	Germany	**FM957127** (1313)	**FM956110** (1328)
Heribaudiella fluviatilis (Aresch.) Sved.	SAG 13.90	Germany	**FM957128** (1342)	**FM956111** (1390)
Phaeostrophion irregulare Setch. et N.L. Gardner	KU-307	USA (OR)	**FM957126** (1290)	AB117948 (1395)
Porterinema fluviatile (A. Porter) Waern	SAG 124.79	Germany	**FM957112** (1309)	**FM956108** (1004)
Pseudolithoderma roscoffense Loiseaux		England, UK	n.d (0)	EU579935 (1214)
Desmarestiales				
Desmarestia aculeata (L.) J.V. Lamour.	KU-1141∗	Germany (Helgoland)	**FM957124** (1296)	AJ287847 (1158)
Himantothallus grandifolius (A. Gepp et E. Gepp) Zinova	CCAP 1313/1	Antarctica (King George I.)	**FM957125** (1342)	AJ287850 (1255)
Dictyotales				
Dictyota dichotoma (Huds.) J.V. Lamour.	KU-1070∗	Japan	**FM957113** (1288)	AY748312 (1378)
Discosporangiales				
Choristocarpus tenellus (Kütz.) Zanardini	KU-1152∗	Greece/Italy	**FM957111** (1330)	AJ287862 (1375)
Ectocarpales				
Chordaria flagelliformis (O.F. Müll.) C. Agardh	KU-873∗	Japan	**FM957118** (1298)	AB066086 (1467)
Ectocarpus siliculosus (Dillwyn) Lyngb.	KU-1100∗	Australia/?	**FM957117** (1290)	X52503 (1467)
Pylaiella littoralis (L.) Kjellm.	∗	France?	AY876220 (1013)	X55372 (1467)
Fucales				
Durvillaea antarctica (Cham.) Har.	L WELT1[d]	New Zealand	**FN667658** (1342)	**FN667659** (1388)
Fucus vesiculosus L.	–	Portugal	CAX12499 (1365)	CAX12442 (1467)
Laminariales				
Saccharina japonica (Aresch.) C.E. Lane, C. Mayes, Druehl et G.W. Saunders	KU-508	Japan	**FM957121** (1296)	**FN667660**[e] (1426)
Undaria pinnatifida (Harv.) Suring.	KU-206∗	Japan	**FM957120** (1206)	AY851535 (1391)
Onslowiales				
Onslowia endophytica Searles	L SGAD-143	USA (FL)	**FM957114** (1342)	AJ287864 (1375)
Verosphacela ebrachia E.C. Henry	L SGAD-144	USA (FL)	**FM957115** (1321)	AJ287867 (1255)
Sporochnales				
Carpomitra costata (Stackh.) Batters	KU-1158∗	New Zealand/Japan	**FM957123** (1290)	AB045257 (1409)
Syringodermatales				
Syringoderma abyssicola (Setch. et N.L. Gardner) Levr.	KU-758∗	Japan/?	**FM957116** (1293)	AY157698 (1122)
Sphacelariales				
Cladostephaceae				
Cladostephus spongiosus (Huds.) C. Agardh	L SGAD-111 (66.10.2)	Netherlands	**FM957136** (1342)	AJ287863 (1375)
Cladostephus spongiosus	L 14-202	New Zealand	**FM957146** (1229)	n.d. (0)
Cladostephus spongiosus	L 09-10-011	Australia	**FN667652** (1321)	**FN667651** (1390)
Cladostephus spongiosus	L 09-10-060	Australia	as above (1321)	as above (1390)
Stypocaulaceae				
Alethocladus corymbosus (Dickie) Sauv.	CCMP111	Antarctica (Astrolabe I.)	**FM957133** (1342)	AJ287860 (1255)
Halopteris congesta (Reinke) Sauv.	KU-1120	New Zealand	**FM957144** (1321)	n.d. (0)
Halopteris filicina (Gratel.) Kütz.	KU-690∗	Japan/Korea	**FM957130** (1295)	AJ287894 (1375)
Halopteris filicina	L SGAD-173 (68-33-2)	France (Brittany)	**FM957131** (1342)	AJ287895 (1240)
Halopteris gracilescens (J. Agardh) Womersley	KU-1118	New Zealand	**FM957152** (1321)	**FN667655** (1419)

(*continued*)

Table 1. Continued.

Taxon	Voucher[a]	Country of origin[b]	EMBL accession no.[c]	
			*psb*C	*rbc*L
Halopteris virgata (Hook. f. et Harv.) N.M. Adams	L 2	New Zealand	**FM957148** (1321)	**FM956115** (190)
Stypocaulon durum (Rupr.) Okamura	L SGAD138 (HS7)	Canada (NFL)	**FM957134** (1321)	AJ287897 (1255)
Stypocaulon paniculatum (Suhr) Kütz.	KU-1117	New Zealand	**FM957147** (1321)	n.d. (0)
Stypocaulon scoparium (L.) Kütz.	L SGAD130 (94.1)	Portugal (Madeira)	**FM957135** (1321)	AJ287866 (1375)
Sphacelariaceae				
Herpodiscus durvillaeae (Lindauer) South		New Zealand	n.d. (0)	EF460467 (858)
Sphacella subtilissima Reinke	L SGAD-208	Spain (Canary I.)	**FM957110** (1103)	AJ287869 (1375)
subgenus *Bracteata*				
Sphacelaria bracteata (Reinke) Sauv.	L 270B18	New Zealand	**FM957150** (1321)	n.d. (0)
subgenus *Propagulifera*				
Sphacelaria californica (Sauv.) Setch. et N.L. Gardner	L SGAD-101 (93-6-2)	Japan	**FM993107** (761)	AJ287893 (1255)
Sphacelaria cirrosa (Roth) C. Agardh	L SGAD-38 (97-38)	Scotland, UK	**FM957143** (1342)	AJ287865 (1375)
Sphacelaria didichotoma R.D. Saunders	L SGAD-95 (Kita-30)	Japan	**FM957142** (1342)	AJ287889[f] (1375)
Sphacelaria fusca (Huds.) Gray	L SGAD-77 (66-25-1)	Ireland	**FM957141** (1342)	AJ287883[g] (1375)
Sphacelaria tribuloides Menegh.		Japan	n.d. (0)	AJ287891 (1375)
Sphacelaria yamadae Segawa		Japan	n.d. (0)	AJ287890 (1375)
subgenus *Pseudochaetopteris*				
Sphacelaria arctica Harv.	L SGAD-71 (67-56)	Sweden	**FM957139** (1245)	AJ287881 (1375)
Sphacelaria plumigera Holmes	L SGAD-29 (97.29)	Scotland, UK	**FM957140** (1342)	AJ287878 (1375)
Sphacelaria plumigera	KU-687	Japan	**FN667657** (1321)	**FN667656**[e] (1310)
Sphacelaria plumosa Lyngb.	L SGAD-69 (67-49-2/4)	Norway	**FM957109** (1342)	AJ287879 (1255)
Sphacelaria racemosa Grev.	L SGAD-70 (71.11)	Scotland, UK	**FM957138** (1342)	AJ287880 (1375)
subgenus *Reinkea*				
Sphacelaria spuria Sauv.	L 267A18	New Zealand[h]	**FM957149** (1321)	n.d. (0)
subgenus *Sphacelaria*				
Sphacelaria caespitula Lynb.	L SGAD-56 (67-27)	Norway	**FM957129** (1342)	AJ287870 (1255)
Sphacelaria nana Nägeli *ex* Kütz.	L SGAD-63 (67.57.4)	Norway	**FM957137** (1321)	AJ287875 (1255)
Sphacelaria radicans (Dillwyn) C. Agardh	L 67-75-2	Norway	**FM957132** (1321)	**FM956112** (1392)
incertae sedis (subgenus not known)				
Sphacelaria pulvinata Hook. f. et Harvey	L 1-98	New Zealand	**FM957145** (1321)	**FM956114** (954)

CCAP, Culture Collection of Algae and Protozoa, Oban, Scotland.
CCMP, Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West Boothbay Harbor, MA.
KU, Kobe University Macro-Algal Culture Collection, Japan.
L, Netherlands Centre for Biodiversity Naturalis (section NHN), Leiden, The Netherlands.
n.d., not determined.
SAG, Experimental Phycology and Culture Collection of Algae, Göttingen, Germany.
[a]Only of specimens with new determined sequences; ∗ indicates that *psb*C and *rbc*L are determined from different individual specimens.
[b]Where specimens are collected in different countries the *psb*C sample is given first and the *rbc*L sample second, separated by a slash (/).
[c]Number of unambiguously determined nucleotides in parentheses.
[d]Stored under the name of the endophyte *Herpodiscus durvillaeae*.
[e]Sequence provided by Dr Takeaki Hanyuda (Kobe University).
[f]As *S. divaricata* Montagne (see Keum *et al*., 2005).
[g]As *S. rigidula* Kütz. (see Keum *et al*., 2005).
[h]New record for New Zealand.

Laboratory methods

Total DNA was extracted from fresh (SAG cultures and *Sphacelaria radicans*) or silica dried algal tissue using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA was eluted in two fractions of 100 µl and the second fraction was used in PCR reactions, usually diluted 10–100 times with MilliQ water (Millipore, Bedford, MA, USA).

Double-stranded DNA amplifications were performed in a Biometra T3 Thermal Cycler (Horsham, PA, USA). Amplification and sequence primers are listed in Table 2.

Table 2. PCR amplification and sequencing primers.

Primer	Direction	Sequence (5′–3′)	Annealing position in *rbc*L (1467 nt) or *psb*C (1425 nt[c])	Reference
rbcL33F	F	TAAAAGTGACCGKTATGAATC	*rbc*L 32	This study
rbcL68F	F	TGCCWAAATGGGRWAYTGGGATGC	*rbc*L 69	Draisma *et al.* (2001)
rbcL188F	F	TCTACTGCAACATGGAC	*rbc*L 196	Draisma *et al.* (2001)
rbcL496F	F	AGATTAGAYAMATTTGGWCGT	*rbc*L 493	Draisma *et al.* (2001)
rbcL948F	F	ATTTGTAAATGGATGCGTATG	*rbc*L 949	This study
rbcL978F	F	GATCATATTCATGCWGGTACAG	*rbc*L 979	This study
BirF	F	CAGCTAACCGTGTTGC	*rbc*L 1262	Draisma *et al.* (2002)
rbcL1278R	R	GCTAAAATCATACTTTCTA	*rbc*L 1280	This study
rbcL1380R	R	TATCTTWCCATAAATCTAAMGC	*rbc*L 1402	Draisma *et al.* (2001)
S3Rb	R	AAACATCCTTGTGTAASTCTC	*rbc*S[b] 23	This study
psbCF	F	GTGGAAACGCCCTTTAATA	*psb*C 37	This study
psbCF2	F	CGCCCTTTAATATTGTAGC	*psb*C 44	This study
psbCRA[a]	R	CCACCHGGDCCWACWCCC	*psb*C 299	This study
psbCmidF	F	TGGGCYCCYGGTGGTGGAGATG	*psb*C 568	This study
psbCmidR	R	CCYCCWACDARATCTTCCATATTATC	*psb*C 710	This study
psbCFB[a]	F	ATWTTTGGKGGDGARACAAT	*psb*C 1051	This study
psbCR2	R	AAAGYACAGCWTCTGTTTGTC	*psb*C 1405	This study
psbCR1	R	TTAATCAATTGGACGCATTG	*psb*C 1425	This study

F, forward; R, reverse; nt, nucleotides.
[a]Only used in sequencing reactions.
[b]*rbc*S = small subunit of RuBisCO-gene.
[c]1413 nt in *Choristocarpus tenellus* and *Porterinema fluviatile*, and 1428 nt in *Fucus vesiculosus*.

A 50 µL reaction volume contained 1 µl diluted genomic DNA, 5 µl 10× PCR Buffer (Qiagen, Hilden, Germany), 4 µl Qiagen dNTP mix (containing 10 mM each of dNTP), 0.5 µg (in 1 µl) bovine serum albumin (BSA; Promega, Madison, WI, USA), 2 µL 25 mM $MgCl_2$ (Qiagen), 0.25 µl Taq DNA polymerase (Qiagen), 1 µl of each Forward and Reverse primer (25 pmol/µl), and was adjusted to 50 µl with MilliQ water. An initial denaturation step of 94°C for 2 min was followed by 9 cycles: 30 s at 94°C, 1 min at 45°C, and 2 min at 72°C; and then 29 cycles: 30 s at 94°C, 30 s at 50°C, and 2 min at 72°C. The amplification was terminated in a final step of 72°C for 8 min. For shorter fragments the extension time was reduced to 1 min. PCR reactions were first attempted with extreme primer combinations (i.e. rbcL33F/S3Rb and psbCF/psbCR1) and when this failed, smaller overlapping fragments were targeted, e.g. rbcL33F/rbcL1380R + rbcL496F/S3Rb and psbCF2/psbCR2. PCR products were screened for correct length by electrophoresis on a 2% agarose gel and purified using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) following the manufacturer's instructions. Cleaned PCR products were sent to Macrogen (Korea) for sequencing using the PCR primers and internal primers. For the Sphacelariales 1229–1350 out of a total 1425 nucleotides (nt) were determined for *psb*C and 1375–1419 out of 1467 nt for *rbc*L.

Data analysis

The raw data were processed using Sequencher v.4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). If a nucleotide could not be unambiguously determined from the chromatograms, the site was coded with IUPAC ambiguity codes (IUPAC-IUB, 1968) and was treated as uncertainty in the analyses. Sequences were aligned in BioEdit v.7.0.5.3 (Hall, 1999) and checked by eye and codon positions were assigned in MacClade v.4.08 (Maddison & Maddison, 2001). The 3′-end of *psb*C and the 5′-end of *rbc*L were pruned from the alignment, because they contained a number of undetermined sequences for several taxa. A short sequence at the 5′-end of *psb*C was also discarded, because a 9 nt insert was revealed in *Sphacella subtilissima* Reinke, whereas *Sphacelaria plumosa* Lyngbye showed an insert that partly resembled the sequence of the psbCF primer directly after the primer site. *psb*C was represented by nt positions 64–1384 (out of 1425) and *rbc*L by positions 93–1467 (out of 1467).

Pilot analyses were conducted with all taxa from Table 1 using *Choristocarpus tenellus* (Kützing) Zanardini as outgroup in order to confirm the sister group relationship of *Bodanella lauterborni*, *Heribaudiella fluviatilis*, *Phaeostrophion irregulare* and *Pseudolithoderma roscoffense* with the Sphacelariales. The former three taxa were sister to the Sphacelariales in all analyses with strong support, but *P. roscoffense* sometimes branched off earlier. Inclusion of *P. roscoffense* reduced the support for the sister group relationship of the Sphacelariales. Moreover, for *P. roscoffense* only the *rbc*L sequence was available and it was therefore not included in further analyses. In order to avoid outgroup rooting artifacts as demonstrated in Phillips *et al.* (2008), all further analyses included only the Sphacelariales and the sister taxa *Bodanella*, *Heribaudiella* and *Phaeostrophion* as outgroup, but the southern hemisphere *Cladostephus spongiosus* was only represented by specimen L 09-10-011. Three types of phylogenetic inference were conducted: Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). Each of the three methods was carried out with four combinations of

taxon and data sampling. Analyses were made for each gene separately, and for the two genes combined. Combined analyses included 30 Sphacelariales plus three outgroup taxa or only the 25 taxa for which the sequences of both genes were determined. This 25 taxa alignment included *Sphacelaria californica* (Sauvageau) Setchell & N.L. Gardner and *Sphacelaria pulvinata* Hooker f. & Harvey, for which many nucleotides of *psb*C and *rbc*L respectively were not determined (see Table 1), but excluded *Halopteris virgata* (Hooker f. & Harvey) N.M. Adams, for which 190 nt of *rbc*L were determined. Analyses of *psb*C alone included 30 taxa and of *rbc*L alone 28 taxa (both including the three outgroup taxa). The resulting phylogenies were screened for incongruences, i.e. mutually supported conflicting phylogenies with MP bootstrap percentages (BP) > 50%, ML BP > 50% and BI posterior probabilities (PP) > 0.90.

BI was conducted with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) to determine the simplest model of sequence evolution that best fits the data for the combined *psb*C and *rbc*L matrix. MrModeltest v.2.2 (Nylander, 2004) was used to find the best-fitting substitution model. The models of molecular evolution were selected using the Akaike Information Criterion (AIC). The chosen model was GTR + G + I (nst = 6, rate = invgamma). The default priors of MrBayes were used. The data matrix was partitioned by gene (2) and by codon position (3), thus six partitions in total. For each analysis two simultaneous runs were done starting from random trees for 10 000 000 generations, having three 'heated' and one 'cold' chain. Markov chains were sampled every 100th generation. The average standard deviation of split frequencies decreased below 0.01 (indicating that the two runs had reached convergence) within 102 000–367 000 generations. The plot of generation versus log probability was inspected after the run to ensure that stationarity was reached and to determine a suitable burn-in value. Twenty per cent of the trees were discarded as burn-in. The majority-rule consensus tree containing posterior probabilities was built from the remaining sampled trees.

ML phylogenies were estimated using RAxML v.7.0.3 on the CIPRES cluster at the San Diego Supercomputer Center (Stamatakis *et al.*, 2008) with the same model and data partitioning as for the BI analyses and 1000 bootstrapping runs. The GTR + G model was selected in jModelTest v.0.1 (Posada, 2008) for the combined data sets, but for *psb*C alone submodel TVM + G was selected and for *rbc*L alone TIM1 + I + G. However, the −lnL values for the selected models in jModelTest differed only slightly from the −lnL values for the GTR model and RAxML only implements the GTR model.

MP analyses were performed in PAUP* v.4.0b1 (Swofford, 2003). All characters were treated as unordered and equally weighted. MP analyses were conducted using heuristic search methods with 1000 replicates of random taxon addition combined with tree-bisection-reconnection branch swapping (TBR) and the MulTrees option active. To assess support for each clade, MP bootstrap analyses (Felsenstein, 1985) were performed with 10 000 bootstrap pseudoreplicates, TBR swapping of all replicates consisting each of ten random taxon additions.

BI PP are described as strong (1.00), good (0.95–0.99), and weak (0.90–0.94).

MP BP and ML BP are described as strong (90–100%), good (80–89%), moderate (70–79%), weak (50–69%) or no (<50%) support.

Results

Alignment properties

For some taxa either *psb*C or *rbc*L could not be determined (Table 1). A gap spanning 12 nucleotide (nt) positions was needed in the *psb*C sequence of the basal taxa *Choristocarpus* and *Porterinema* in order to restore alignment. The *rbc*L of *Fucus vesiculosus* L. showed a 3 nt insert (duplication). The length of the combined alignment used in the analyses was 2696 nt (*psb*C 1321 nt and *rbc*L 1375 nt). EMBL accession numbers of *psb*C and *rbc*L sequences are listed in Table 1. Average base composition was A 0.28, C 0.16, G 0.22, T 0.34 and did not differ much between the two genes and between the Sphacelariales and the other brown algae. The 2696 nt alignment of 33 taxa contained 935 variable positions of which 637 were potentially informative. In the *psb*C and *rbc*L alignments respectively 38% and 31% of the nt positions were variable (and 27% and 20% were potentially phylogenetically informative). In the *psb*C alignment the ratio of variable positions at third vs. first + second codon positions was 350:150 = 2.3 and in the *rbc*L alignment this ratio was 338:105 = 3.2. More data properties are summarized in Table 3.

Phylogenetic analysis

All analysis results reported here were based on data sets including the Sphacelariales and the outgroup taxa *Bodanella*, *Heribaudiella* and *Phaeostrophion*. All analyses showed maximum support for the monophyly of the Sphacelariales. The results of the BI, ML and MP analyses of the four different alignments (genes combined or separate, and all taxa included or a subset) are summarized in Fig. 1 and a BI phylogeny with PP values based on the complete 2696 nt alignment and 33 taxa is presented in Fig. 2, including MP and ML BPs.

Two striking incongruences were noted between the trees in Fig. 1. The first one was the position of *Sphacelaria caespitula*. This species branched off before all other sphacelarialean taxa in the BI and ML trees, whereas in the MP trees it was sister to a clade that included *Sphacelaria radicans*

Table 3. Summary of data properties of the four alignments. Numbers in parentheses are for the first and second codon positions only.

	*psb*C + *rbc*L		*psb*C	*rbc*L
Number of taxa	33	25	30	28
Length of alignment	2696 (1797)	2696 (1797)	1321 (881)	1375 (916)
Proportion of undetermined nucleotides	15.3%	3.7%	2.5%	5.2%
Average base composition (A/C/G/T)	0.28/0.16/0.22/0.34	0.28/0.16/0.22/0.34	0.26/0.16/0.22/0.36	0.29/0.16/0.22/0.33
Number of constant positions	1761 (1541)	1802 (1569)	821 (713)	942 (811)
Number of variable uninformative positions	298 (115)	300 (97)	147 (66)	149 (48)
Number of potentially informative positions	637 (141)	594 (131)	353 (84)	284 (57)
Number of Most Parsimonious Trees (MPTs)	20 (147)	2 (1)	1 (10)	2 (604)
Number of steps of MPTs	2629 (535)	2376 (468)	1516 (314)	1102 (207)
Selected model in MrModeltest	GTR + G + I	GTR + G + I	GTR + G + I	GTR + G + I
Selected model in jModelTest	GTR + G	GTR + G	TVM + G	TIM1 + I + G
Final ML Optimization Likelihood	−15488.573538	−14142.602181	−8511.303193	−6932.132079

and the Stypocaulaceae. However, *S. caespitula* also branched off first in the MP trees (not shown) if the four data sets were analysed without the third codon positions, although without bootstrap support. The second incongruence concerned the position of *Cladostephus spongiosus*. In the BI and ML trees *Cladostephus* was sister to the clade Sphacelariaceae (1) (i.e. Sphacelariaceae excluding *S. caespitula* and *S. radicans*, see Fig. 2), except in the trees based on *rbc*L alone. In the *rbc*L trees and all MP trees *Cladostephus* grouped with *Sphacelaria nana* and the members of the *Sphacelaria* subgenus *Pseudochaetopteris* Prud'homme, but at best moderately supported. However, *Cladostephus* was sister to the clade Sphacelariaceae (1) in the MP trees if the four data sets were analysed without the third codon position (not shown), but without bootstrap support. For the rest the results of the different analyses were congruent. After the first split (*S. caespitula* from the rest) the Sphacelariales split into two strongly supported clades. One clade comprised *S. radicans* sister to a strongly supported monophyletic Stypocaulaceae clade, but within the Stypocaulaceae the sampled genera were not monophyletic. The other clade consisted of *Cladostephus* sister to the clade Sphacelariaceae (1), but the monophyly of the latter clade was in most trees only weakly supported. The three species of the *Sphacelaria* subgenus *Sphacelaria* were clearly polyphyletic. The species of the *Sphacelaria* subgenus *Pseudochaetopteris* formed a monophyletic clade, except for *S. plumosa* Lyngbye which was sister to a strongly supported clade comprised of three strongly supported subclades: (1) *Sphacella*, (2) *Sphacelaria* subgenus *Propagulifera* and (3) *Herpodiscus* + the *Sphacelaria* samples from New Zealand representing the subgenera *Reinkea* Prud'homme and *Bracteata* Prud'homme and one species not classified in one of the *Sphacelaria* subgenera.

Discussion

Phylogeny

The 12 nucleotide (nt) gap in the *psb*C sequence of *Choristocarpus tenellus* and *Porterinema fluviatile* (A. Porter) Waern may be an indicator of early divergence within the Phaeophyceae. Therefore, this gap can also be expected in other basal brown algal taxa like *Ishige* Yendo, *Diplura* Hollenberg and *Petroderma* Kuckuck, and in the sister class of the Phaeophyceae, i.e. Schizocladiophyceae (Kawai *et al.*, 2003; de Reviers *et al.*, 2007; Lim *et al.*, 2007; Bittner *et al.*, 2008).

The *rbc*L BI and ML trees were incongruent with the *psb*C and combined BI and ML trees, but there was no strong support for this incongruence. Statistical tests for incongruence (e.g. incongruence length difference test; Farris *et al.*, 1994) between the two markers were not conducted, because these tests have shown to be unreliable under certain conditions (Hipp *et al.*, 2004 and references therein). The combined analysis of incongruent data sets can in some cases lead to a more robust phylogeny, but if the parts of the resulting tree are in strongly supported conflict between data sets this should be interpreted with caution (Wiens, 1998). Congruence of *psb*C trees with the combined trees may be attributed to the higher contribution of variable characters by *psb*C. This was explored by performing a BI analysis on the 25 taxon *psb*C + *rbc*L data set with the first half (660 nt) of the *psb*C gene pruned from the alignment so that *psb*C was represented by 220 variable characters and *rbc*L by 433. The resulting BI tree (not shown) had the same topology as the BI tree of

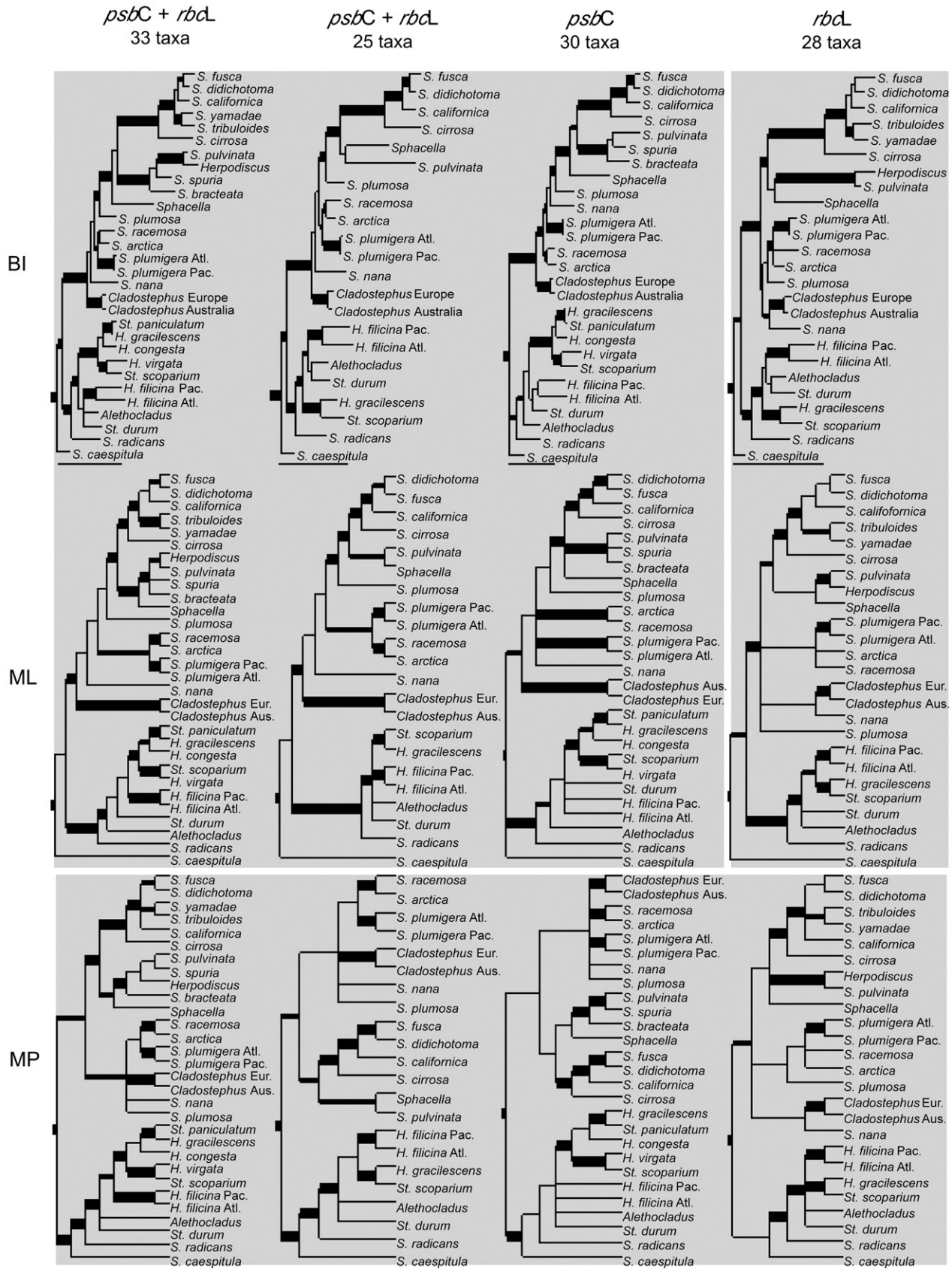

Fig. 1. Phylogenetic trees ordered by inference method (in rows) and data set (in columns). The three outgroup taxa have been pruned from the trees (after analysis). Monotypic genera are indicated by genus name. *H.* = *Halopteris*, *S.* = *Sphacelaria*, *St.* = *Stypocaulon*, Atl. = Atlantic, Aus. = Australia, Eur. = Europe, Pac. = Pacific. BI = Bayesian Inference, ML = Maximum Likelihood, MP = Maximum Parsimony. Scale bars under BI trees represent 0.1 expected changes per site. The ML and MP trees are represented by the 50% majority rule consensus of the bootstrap trees. Thickest branches have 1.00 posterior probability (PP) (BI trees) or 80–100% bootstrap support (BS) (ML and MP trees). Intermediate branches have 0.95–0.99 BI PP or 70–79% BS. Thinnest branches have 0.50–0.94 BI PP or 50–69% BS. Trees within the same grey block have similar topologies. The upper left tree is also shown in Fig. 2.

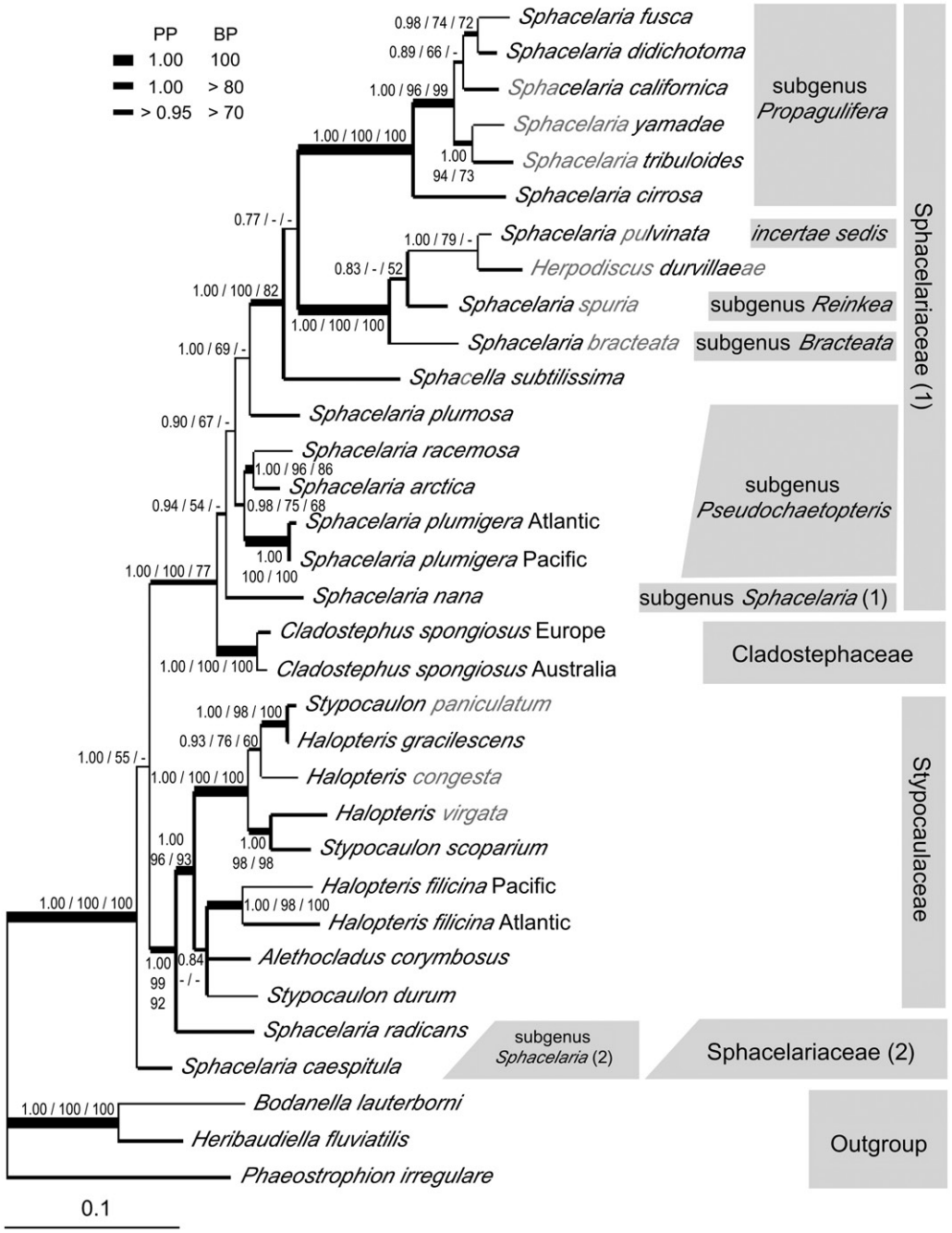

Fig. 2. Bayesian Inference (BI) phylogeny for the Sphacelariales based on a combined analysis of *psb*C and *rbc*L DNA sequence data. Traditional family classification and *Sphacelaria* subgenera are indicated in grey boxes. BI Posterior Probabilities (PP) and Maximum Likelihood (ML) Bootstrap Percentages (BP) and Maximum Parsimony (MP) BP are given near nodes (BI PP/ML BP/MP BP). Dashes (-) indicate percentages <50% (or that the node did not occur in the MP or ML tree). Branch support is also indicated by branch thickness (see inset upper left). In cases where a taxon was represented by a substantial amount of undetermined nucleotides in the alignment, then the genus name (for undetermined *psb*C) or the species epithet (for undetermined *rbc*L) is indicated (partially) in grey (see Table 1 for exact numbers of determined nucleotides).

the 2696 nt alignment, except that *S. nana* Nägeli ex Kützing was sister to *Cladostephus* (not supported, BI PP 0.87) and the *Alethocladus* + *Stypocaulon durum* (Ruprecht) Okamura clade became paraphyletic (not supported, BI PP 0.55). All other branches had similar BI PP values in both trees, except that the *Sphacella* + *S. pulvinata* clade was supported in the tree of the pruned alignment (BI PP 0.97). This outcome suggests that the *psb*C tree is a more accurate reflection of phylogenetic relationships than the *rbc*L tree. Moreover, the position of *Cladostephus* on a separate branch in the *psb*C and combined BI and ML trees is in agreement with the traditional classification in a separate family based on morphological grounds (Draisma *et al.*, 2002).

The inadequacy of *rbc*L to resolve the correct phylogeny may be a consequence of the saturation in the third codon position that has been reported in several brown algal studies (Draisma *et al*., 2001; Bittner *et al*., 2008; Phillips *et al*., 2008). The relative number of variable positions in third codon positions compared to first and second codon positions is much higher in *rbc*L (ratio 3.2) than in *psb*C (ratio 2.3), although the proportion of third codon positions that are variable is higher in *psb*C (80%) than in *rbc*L (74%).

There was also incongruence between the results of different phylogenetic inference methods. There has been a long-standing debate whether parsimony or model based methods (ML and BI) perform better without general agreement (Sober, 2004; Yang, 2006; Mc Manus, 2009). The fact that MP analysis of data sets including only the more conservative first and second codon positions resulted in the same topology as the BI and ML trees supports this being the correct topology. The morphological argument for the position of *Cladostephus* can also be put forward as well as the weak or no bootstrap support for its placement in the trees based on all codon positions. However, the basal paraphyly in the clade Sphacelariaceae (1) in the BI and ML trees is also weakly or not supported and branch internode lengths are short. This is probably a consequence of lack of informative data and can only be resolved with a much larger set of characters.

Another point of caution is the implication of missing data (Lemmon *et al*., 2009). Analyses with highly incomplete taxa may have limited ability to avoid long-branch attraction in parsimony, but can be very beneficial when using model-based methods (Wiens, 2005). Wiens (2004) found that the reduced accuracy associated with including incomplete taxa is caused by these taxa bearing too few complete characters rather than too many missing data. Wiens (2009) showed that adding taxa with missing data to a data set can be highly beneficial and improve phylogenetic accuracy; accuracy decreased only in a few cases. In the present study analyses were done with and without missing data (Fig. 1). Missing data are indicated by grey taxon names (Fig. 2). Taxa with missing data were represented in the *Sphacelaria* subgenus *Propagulifera*, in the family Stypocaulaceae, and in the clade composed of *Herpodiscus durvillaeae* and the three *Sphacelaria* species from New Zealand. These three clades were resolved in all analyses with strong support, suggesting sufficient complete characters. There is no reason to doubt the validity of the *Propagulifera* and the Stypocaulaceae as they are well-defined morphological clades that were also resolved in an analysis based on morphological characters alone (Draisma *et al*., 2002). The clade with Australasian taxa was not included in that study. The three *Sphacelaria* species of that clade were represented by complete *psb*C sequences and formed a strongly supported monophyletic clade in all analyses. This clade at the end of a long branch is unambiguous. *Herpodiscus durvillaeae* was only represented by *rbc*L (3′-end missing), not by *psb*C. *Sphacelaria pulvinata* was also represented by *rbc*L (5′-end missing). The *rbc*L sequences of *H. durvillaeae* and *S. pulvinata* overlapped by 557 nt in which they differed by 9 nt (1.64%). This sequence divergence is lower than for any other pairwise species comparison in the present study. European and Australian *Cladostephus spongiosus* differed by 1.46% in these same 557 nt positions and Atlantic and Pacific *Sphacelaria plumigera* Holmes differed by 1.63%. The MP analysis of only these 557 nt (28 taxa, not shown) resolved the monophyly of *H. durvillaeae* + *S. pulvinata* together at the end of a long branch with 100% bootstrap support. The *Propagulifera* were also supported 100% and together with *Sphacella* these three clades formed a monophyletic clade (73% support) as they do in all analyses (Fig. 1). Taken together it can be concluded that the three Australasian *Sphacelaria* species and *Herpodiscus durvillaeae* together form a monophyletic clade. Additional support for the position of *H. durvillaeae* comes from an ML and BI analysis of partial *rbc*L and LSU rDNA by Heesch *et al*. (2008). In their trees *H. durvillaeae* is sister to *Sphacella* (ML BP 66%, BI PP 0.72), but the only other Sphacelariales sampled were *Cladostephus spongiosus*, *Sphacelaria cirrosa* and two Stypocaulaceae.

A 0.95 BI PP consensus tree of the tree in Fig. 2 is shown in Fig. 3, with the exception of clade Sphacelariaceae (1) which had 0.94 BI PP. This consensus tree will be the basis for the discussion of a new classification. The aim was to strive for a classification that reflects shared evolutionary history and probably can withstand future improved phylogenies.

Revised classification (Fig. 3)

The evolutionary model-based analyses strongly indicate that *Sphacelaria caespitula* branches off first within the Sphacelariales. Therefore, a new family needs to be created for this species in order to keep the three traditional families. The alternative would be to merge all sphacelarialean families into a single family, but considering the degree of genetic variation within the order compared with that in other brown algal orders it is justifiable to recognize more than one family in the Sphacelariales (Draisma *et al*., 2003).

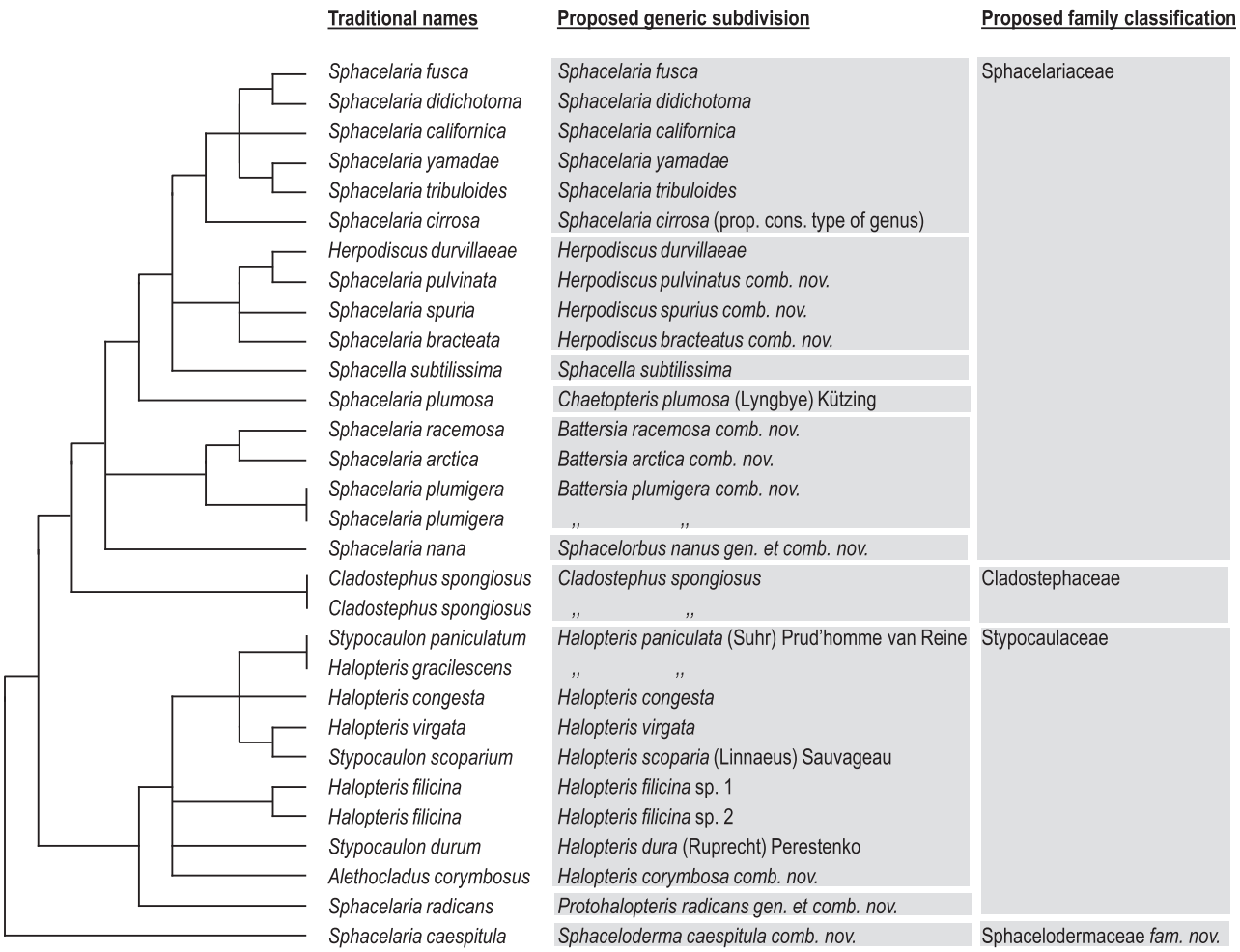

Fig. 3. Summarized phylogeny of the Sphacelariales including the taxa of the present study. Recent species names are given on left, and the newly proposed classification on right.

For *S. caespitula* the genus name *Sphaceloderma* Kuckuck can be reinstated. *Sphaceloderma helgolandica* Kuckuck is the type of this monotypic genus and currently considered a synonym of *Sphacelaria caespitula*. The new family Sphacelodermaceae is proposed here to accommodate only the genus *Sphaceloderma*.

It is proposed to include *Sphacelaria radicans* in the Stypocaulaceae. Prud'homme van Reine (1982) already discussed the fact that *Sphacelaria radicans* (and perhaps *Sphacelaria reticulata* Lyngbye) possesses characters that fit incorporation in more than one family. Sauvageau (1903) suggested an intermediate position for *S. radicans* (and related species *S. caespitula* and *S. nana* [as *S. olivacea* (Dillwyn) Greville] between the Hemiblastées (*Sphacelaria* and related genera) and the Holoblastées (*Halopteris* Kützing and *Stypocaulon* Kützing). In 1914 Sauvageau suggested that *S. radicans* and related species ought to be classified in a new genus, but did not suggest a name. In the new classification proposed here *S. radicans* will not be included in the genus *Halopteris*, because it is not known where the unsampled genera *Phloiocaulon* and *Ptilopogon* split off in the tree. Moreover, the morphology of *S. radicans* differs substantially. Therefore, a new genus *Protohalopteris* is proposed to accommodate *S. radicans* and it is classified in the Stypocaulaceae. *Protohalopteris*, gen. nov. (Gk. πρωτο- [proto-] = first) differs from the other Stypocaulaceae in having a radial pattern of cell division in transverse section (vs. periclinal in the other genera), zoidangia not formed in axils, and no differentiation into a medulla and cortex (for terminology see Sauvageau, 1900–1914; Prud'homme van Reine, 1982; Draisma *et al.*, 2002). *Protohalopteris* shows a combination of hypacroblastic and acroblastic branching. It has the latter branching mode in common with the other Stypocaulaceae while none of the other Sphacelariales shows acroblastic branching. Another common feature of the Stypocaulaceae including *Protohalopteris* is that phaeophycean hairs, if present, are formed in clusters.

According to Prud'homme van Reine (1991, 1993) and Kawai & Prud'homme van Reine (1998) the genera *Stypocaulon* and *Halopteris* can

be separated on the basis of differences in reproduction and branching pattern. The present results, however, clearly indicate that the genus *Stypocaulon* cannot be maintained and should be merged with the genus *Halopteris*. This was already suggested by Sauvageau (1903) and Womersley (1987) included *Stypocaulon* in *Halopteris*. This means that names within the genus *Halopteris* have already been proposed and therefore new combinations need not be proposed here. The results of the present study also indicate that *Alethocladus* should be included in *Halopteris* and a new combination for its single species is proposed in the present paper. *Alethocladus* was distinguished from the other genera by the occurrence of exclusively, strictly acrohomoblastic branching. The genus *Halopteris sensu* the present authors (i.e. including *Stypocaulon* and *Alethocladus*) is characterized by leptocaulous growth, whereas *Phloiocaulon* and *Ptilopogon* show auxocaulous growth. However, Sauvageau (1904) reported slightly auxocaulous growth for *Stypocaulon paniculatum* (Suhr) Kützing (as *Halopteris hordacea* [Harvey] Sauvageau).

Stypocaulon paniculatum and *Halopteris gracilescens* (J. Agardh) Womersley can be considered conspecific based on the present *psb*C sequence data, allowing some intraspecific sequence divergence. Their *psb*C sequences differ at 7 out of 1321 nt positions, which represents 0.53%. Womersley (1987) already treated *H. gracilescens* as a synonym of *S. paniculatum* (as *Halopteris paniculata* [Suhr] Prud'homme). The two *Halopteris filicina* (Grateloup) Kützing specimens in the present study differ by 97 out of 1294 nt in *psb*C and 64 out of 1240 nt in *rbc*L, which represent respectively 7.50% and 5.16%. These percentages exceed the level of expected intraspecific variation. Atlantic and Pacific *H. filicina* should therefore be considered as two cryptic species for which currently no distinguishing diagnostic characters are known. Keum *et al.* (1995), however, reported the occurrence of propagule-like structures in Korean plants, which have never been reported for Atlantic *H. filicina*. Nevertheless, it is premature to conclude that one entity only occurs in the Atlantic and the other only in the Pacific, based on only one or two isolates per region. Atlantic and Pacific *Sphacelaria plumigera* specimens differed by 0.15% in *psb*C and by 0.79% in *rbc*L and are considered conspecific. European and Australasian *Cladostephus spongiosus* specimens differed by 1.29–1.51% in *psb*C and by 1.0% in *rbc*L and are also considered conspecific despite their disjunct bipolar temperate distribution. In accordance with the sequence divergence it can be assumed that the disjunct distribution is not the result of recent introduction. There are no multiple *psb*C sequences of other brown algal species available to compare with apart from *S. plumigera* in the present study. The smallest known *psb*C sequence divergence between two brown algal species is 2.79% (between *Stypocaulon*/*Halopteris paniculatum* and *Halopteris congesta* [Rinke) Sauvageau) and outside the Sphacelariales between the kelps *Undaria pinnatifida* (Harvey) Suringar and *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl et G.W. Saunders (3.64%) (data from present study). The present data indicate that sequence divergence in *psb*C is somewhat higher than in *rbc*L (Table 3). The smallest reported *rbc*L sequence divergence between two brown algal species is 1.59% between *Desmarestia viridis* (O.F. Müller) J.V. Lamouroux (Genbank AF207799) and *D. tabacoides* (Genbank AB037140) (Kawai *et al.*, 2007). Kawai *et al.* (2007) concluded that a 3.3% *rbc*L sequence divergence between Mediterranean and Australian specimens of *Discosporangium mesarthrocarpum* was enough to consider them to be different species. There are multiple *rbc*L sequences available in EMBL/Genbank for a few brown algal species: *Chordaria chordaeformis* (O.F. Müller) C. Agardh ($n = 8$), *Chordaria flagelliformis* (O.F. Müller) C. Agardh ($n = 15$), *Colpomenia peregrina* Sauvageau ($n = 33$), *Colpomenia sinuosa* (Mertens *ex* Roth) Derbès et Solier ($n = 7$), *Desmarestia viridis* ($n = 2$), *Petrospongium rugosum* (Okamura) Setchell et N.L. Gardner ($n = 4$), *Sphacelaria caespitula* ($n = 3$), and *Stypocaulon scoparium* ($n = 2$). These species showed up to 2% intraspecific *rbc*L variation, but interspecific variation within the same genus was always higher. The paraphyletic species *Chorda filum* (L.) Stackhouse shows 3% intraspecific sequence divergence ($n = 8$) and may represent multiple cryptic species (Sasaki & Kawai, 2007). Pacific and Atlantic *Scytosiphon lomentaria* (Lyngbye) Link ($n = 5$) were considered to be different species based on 1.8–2.3% *rbc*L sequence divergence (Cho *et al.*, 2007).

The present study supports maintaining the monotypic family Cladostephaceae as sister family of the Sphacelariaceae in contrast to Draisma *et al.* (2002) who concluded this family should be merged with Sphacelariaceae based on their *rbc*L MP tree. Cladostephaceae is characterized by auxocaulous growth and simultaneous occurrence of several different branching modes, whereas Sphacelariaceae has strictly leptocaulous growth and hemiblastic formation of laterals. The circumscription of Sphacelariaceae should, in addition to a (semi-) isomorphic life history (in all but one species for which the life history is known), also include a heteromorphic life history with a minute gametophyte transformed completely

into a four-celled plurilocular zoidangium (in *Herpodiscus durvillaeae*).

Treating the Sphacelariaceae as a monogeneric family would require the least number of name changes. *Sphacelaria* currently represents about 60 species and of all brown algal genera only *Sargassum* C. Agardh and *Dictyota* J.V. Lamouroux contain more species (Guiry & Guiry, 2009). *Sphacella* and *Herpodiscus* are both monotypic. However, it is proposed here to recognize the six subclades in Sphacelariaceae (1) as separate genera. The genus *Sphacelaria* is probably mostly associated with the 24 propagule-bearing species and it seems therefore desirable to link the name *Sphacelaria* to the subgenus *Propagulifera*, which shows a widespread distribution and of which *S. cirrosa* is the type. However, *Sphacelaria reticulata* Lyngbye is the nomenclatural type of the genus, but it is not a member of *Propagulifera*. This species was placed by Prud'homme van Reine (1982) in the subgenus *Sphacelaria* together with *S. caespitula*, *S. radicans* and *S. nana*. However, it is a matter of speculation where *S. reticulata* would fit in the phylogeny as it is the only *Sphacelaria* species that shows strictly dichoblastic branching and the subgenus *Sphacelaria* is clearly polyphyletic (Fig. 2). Prud'homme van Reine (1982) placed *S. reticulata* in the vicinity of *S. radicans* on the basis of the resemblance in morphology of its filaments and the occasional occurrence of dichoblastic branching in *S. radicans*. Live material of *S. reticulata* was never studied and it is unlikely to be forthcoming. The species is only known from one locality in Fyn (Denmark) where it was found washed ashore mixed with other *Sphacelaria* species on the beaches of Hofmansgave together with large amounts of *Zostera marina* L. in 1816, in 1846 and for the last time in 1867 (Prud'homme van Reine, 1982). Prud'homme van Reine (1982) speculated that *S. reticulata* disappeared together with *Zostera marina* as a result of the seagrass disease occurring after 1930 and *S. reticulata* has not returned since. Although it was probably not the intention of Lyngbye, *S. reticulata* was published one year before the publication of nine other species in his new genus (Lyngbye, 1818, 1819). Considering the problems with *S. reticulata* it seems justifiable to propose to conserve the name *Sphacelaria* with another generitype. A formal proposal to conserve the name *Sphacelaria* with *S. cirrosa* as the generitype will be published elsewhere (Draisma & Prud'homme van Reine, in press). *Sphacelaria cirrosa* (as *S. pennata*) was one of the species described by Lynbye in 1819. *Sphacelaria cirrosa* as the new lectotype of the genus would automatically become the type of the subgenus *Sphacelaria* *emend.* making the name *Propagulifera* superfluous.

Prud'homme van Reine (1982) questioned the placement of *Sphacelaria nana* in the subgenus *Sphacelaria* and separated it from the other three species in the subgenus at an early stage in a phylogenetic diagram. It is proposed to create a new genus, *Sphacelorbus* (Latin *orbus* = orphan), for *Sphacelaria nana*. It is proposed to reinstate the genus *Battersia* Reinke with an emended description (*Battersia mirabilis* Reinke *ex* Batters is the type) and to transfer the species of the *Sphacelaria* subgenus *Pseudochaetopteris* to *Battersia*, except for *Sphacelaria plumosa* for which it is proposed to reinstate the monotypic genus *Chaetopteris* Kützing (*Chaetopteris plumosa* [Lyngbye] Kützing is the type). Draisma *et al.* (2002) concluded that *Battersia mirabilis* (as *Sphacelaria mirabilis* [Reinke *ex* Batters] Prud'homme) grouped with the members of *Pseudochaetopteris* based on 190 nt of the *rbc*L 3'-end (Genbank AJ287882) and the complete 272 nt RuBisCO spacer (RBS) (AJ287930). RBS sequences are extremely variable in the Sphacelariales and the complete spacer can only be aligned among the subgenus *Propagulifera* (Draisma *et al.*, 2002). However, the 3'-end of the RBS is best conserved and a NCBI BLAST search (Altschul *et al.*, 1997) revealed that 87% of the *B. mirabilis* RBS is alignable with *S. arctica* Harvey (AJ287929), 66% with *S. plumigera* (AJ287926) and 59% with *S. racemosa* Greville (AJ287928). Only 48% is alignable with *Cladostephus spongiosus* and 37% or less with all other taxa.

Sphacella is retained as a separate monotypic genus and it is proposed to emend the description of the genus *Herpodiscus* and to transfer *Sphacelaria pulvinata* and the members of the *Sphacelaria* subgenera *Bracteata* and *Reinkea* to *Herpodiscus*. From the low *rbc*L sequence divergence between *Herpodiscus durvillaeae* and *S. pulvinata* it may be speculated that they are conspecific as they are both parasitic endemics to New Zealand. However, there are conspicuous differences. *Herpodiscus durvillaeae* is haplostichous, has terminal unilocular sporangia and grows on *Durvillaea antarctica*, whereas *S. pulvinata* is oligostichous, has lateral unilocs and grows on *Carpophyllum maschalocarpum* (Turner) Greville (Harvey, 1855; South, 1974; Adams, 1994; Heesch *et al.*, 2008). It is clear, however, that the two species are closely related. The taxonomic affinity of *S. pulvinata* was unknown before, but Prud'homme van Reine (1993) suspected that this species and *Sphacelaria affinis* Dickie, which both have solitary unilocular zoidangia with plurilocs being unknown, were related to the subgenera

Reinkea and *Bracteata* respectively. The subgenera *Bracteata* and *Reinkea* are both characterized by sessile unilocular zoidangia, arranged in short, adaxial, lateral sympodia (Prud'homme van Reine, 1993). They are epiphytes on Fucales, but the only species from the northern hemisphere, i.e. *Sphacelaria sympodiocarpa* Sauvageau (endemic in Gulf of Biscay), can also grow epilithically. Prud'homme van Reine (1993) considered these two subgenera to be closely related and even suggested possible paraphyly of *Bracteata*. The subgenera differ by the common presence (*Reinkea*) or practical absence (*Bracteata*) of secondary transverse cell walls and pericysts in the secondary segments, with the exception of the pinnately branched *Sphacelaria spuria* Sauvageau (subgenus *Reinkea*) in which pericysts are unknown. *Sphacelaria spuria* was previously only known from the type specimen collected in Brighton, Port Phillip, Victoria (Australia). The specimen in the present study is a new record for New Zealand and no pericysts were observed. Prud'homme van Reine (1993) suggested that pericysts in pinnately branched specimens may be less functional and thus may disappear again.

It is proposed to leave *incertae sedis S. affinis* (endemic in Kerguelen) and all other *Sphacelaria* species, namely the two freshwater species *S. fluviatilis* C.C. Jao (from China and USA) and *S. lacustris* Schloesser et Blum (from USA), *S. kovalamensis* V. Krishnamurty et Baluswami in Krishnamurty (from southern India, no unilocs known). The reported plate-like, lobed and ribbon-like chromatophores in *S. kovalamensis* (Krishnamurty & Baluswami, 1988) are otherwise unprecedented in the Sphacelariales. Five species with a very restricted known distribution are only known from sterile specimens: *Sphacelaria isocnema* Kraft (from Lord Howe Island, Australia), *S. limicola* Lindauer (from Stewart Island, New Zealand), *S. masonii* Setchell et Gardner (from Clarion Island, Pacific Mexico), *S. sauvageaui* Weber Bosse (from Rotti Island, S.W. Timor, Indonesia), and *S. reticulata* = *Disphacella reticulata* (Lyngbye) Sauvageau (from Odense Fjord, Denmark, presumed extinct). The unilocular sporangia recorded by Lyngbye (1818, 1819) in *S. reticulata* were not found again by Sauvageau (1903) or Prud'homme van Reine (1982).

Proposed taxonomic changes

Sphacelodermaceae Draisma, Prud'homme et H. Kawai, fam. nov.

DIAGNOSIS: Thalli e crusta basali crassa polystromatica filamentis angustis sparse ramosis erectis aspectu fruticum humilium aspergilliformium gaudent.

TYPE GENUS: *Sphaceloderma* Kuck. in: *Wiss. Meeresuntersuch. Abt. Helgoland, Neue Folge* 1: 232, fig. 7; 1894.

Description

Plants with thalli forming low brush-like bushes of thin sparsely branched erect filaments arising from a thick polystromatic basic crust.

***Sphaceloderma caespitula* (Lyngbye) Draisma, Prud'homme et H. Kawai, comb. nov.**

BASIONYM: *Sphacelaria caespitula* Lynb. in: *Tent. hydrophytol. dan.* 105; 1819, descr. (as *Sphacelaria caespitula*) in Prud'homme van Reine in: *Tax. Rev. Eur. Sphacel.* 1982: 80–93, figs 118–151; 1982.

MOST RECENT SYNONYM: *Sphaceloderma helgolandica* Kuck. in: *Wiss. Meeresuntersuch. Abt. Helgoland, Neue Folge* 1: 232, fig. 7; 1894. TYPE: probably lost.

TYPE: Lyngbye *s.n.*, Naes, Österö, Faeroes. *ad stipitem Fuci digitali inter Conferva Rothii*, 29/VII/1817 (C: lectotype; LD).

***Protohalopteris* Draisma, Prud'homme et H. Kawai, gen. nov.**

DIAGNOSIS: Filamenta sparse ramosa e numeris discorum basalium minutorum saepe aggregatis cum pericystis turmisque pilorum, ramificatione praecipue hypacroblastica aliquando ramis lateralibus e ramificatione acroheteroblastica dichoblasticave, zoidangia unilocularia sessilia vel stipite unicellulario, zoidangia plurilocularia stipite 1–4-cellulis.

TYPE SPECIES: *Protohalopteris radicans* (Dillwyn) Draisma, Prud'homme et H. Kawai, comb. nov.

Description

Sparsely branched erect filaments arising from a number of often crowded small basal discs and having pericysts and groups of hairs. Branching mainly hypacroblastic, but occasionally some laterals arise by acroheteroblastic or by dichoblastic branching. Unilocular zoidangia are sessile or with unicellular stalks; plurilocular zoidangia have a stalk of 1–4 cells.

***Protohalopteris radicans* (Dillwyn) Draisma, Prud'homme et H. Kawai, comb. nov.**

BASIONYM: *Conferva radicans* Dillwyn in: *Brit. Conferv.* p. 58, t. C; 1809.

MOST RECENT SYNONYM: *Sphacelaria radicans* (Dillwyn) C. Agardh in: *Syst. Alg.* p. 165; 1824. Descr. in Prud'homme van Reine in: *Tax. Rev. Eur. Sphacel.* p. 62–80, figs 60–117, plate 3a. 1982.

TYPE: Mrs. Hutchins *s.n.*, leg. W. Borrer jr. in Ed. Forster's herbarium (BM sh. 142.832, lectotype).

***Sphacelorbus* Draisma, Prud'homme et H. Kawai, gen. nov.**
DIAGNOSIS: Plantae gregariae vel caespitosae tegetes humiles velutinas formantes vel pannos minutos velutinos disco monostromatico vel polystromatico, vel stolonibus reptantibus ramosis aut eramosis. Filamenta erecta lateralibus hypacroblasticis disperses solitariis plerumque eramosis cetera filamentis erectis similibus. Lateralia transformatione rhizoideis non-corticato vel stolonibus facere possunt. Zoidangia unilocularia and plurilocularia solitaria vel in turmis minutis stipitibus paucicellaribus.
TYPE SPECIES: *Sphacelorbus nanus* (Nägeli *ex* Kützing) Draisma, Prud'homme et H. Kawai, comb. nov.

Description
Plants gregarious or caespitose, forming low felty mats or small felty patches with a monostromatic or polystromatic attachment disc or with creeping, branched or unbranched, stolons. Erect filaments with scattered, solitary, usually unbranched, hypacroblastic laterals which do not differ from the erect filaments. Laterals may transform into non-corticating rhizoids or into stolons. Unilocular and plurilocular zoidangia solitary or in small groups on few-celled stalks.

***Sphacelorbus nanus* (Nägeli *ex* Kützing) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria nana* Nägeli *ex* Kützing in: *Tab. phycol.* p. 26, t. 87, fig. 1; 1855, descr. in Prud'homme van Reine: *Tax. Rev. Eur. Sphacel.*: p. 93–115, figs 151–211, plates 3b, 4; 1982.
TYPE: Nägeli 214: Torquay (L. sh. 937.71-710: lectotype).

***Battersia* Reinke *ex* Batters 1889: 59 *emend.* Draisma, Prud'homme et H. Kawai.**
TYPE SPECIES: *Battersia mirabilis* Reinke *ex* Batters.

Description
Plants attached by a polystromatic basal crust. Erect filaments present or absent, fully or partly covered by descending, corticating rhizoids. The polysiphonous secondary segments are divided by longitudinal walls that are of the periclinal type and secondary transverse cell walls are often frequent. Unilocular and plurilocular zoidangia are formed on unicellular or multicellular branched or unbranched thin fertile filaments, which arise from the basal crust or from the erect main axes and their branches.

***Battersia arctica* (Harvey) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria arctica* Harv: in: *Ner. bor.-amer.* Part III, suppl. p. 124; 1858, descr. in Prud'homme van Reine in *Tax. Rev. Eur. Sphacel*: 147–160, figs 318–352; 1982.
TYPE: Lyall *s.n.*, Isle of Disko, E. Greenland, in tide pools VI/1852 (BM: holotype; TCD).

***Battersia plumigera* (Holmes) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria plumigera* Holmes in: *Grevillea* 11: 141; 1883. Descr. in Prud'homme van Reine in *Tax. Rev. Eur. Sphacel.*: 131–146, figs 264–317; 1982.
TYPE: Borrer *s.n.*, Eastbourne, Beachy Head, 2/X/1808 (as *Conferva pennata* Hudson) (BM: lectotype; LD, TCD).

***Battersia racemosa* (Greville) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria racemosa* Grev.in *Scott. crypt. fl.* 2: t. 96 (as *Sphacellaria*). 1824. Descr. in Prud'homme van Reine in *Tax. Rev. Eur. Sphacel.*: 160–168, figs 353–379; 1982.
TYPE: Richardson *s.n.*, Firth of Forth, opposite Caroline Park, 4/II/1819. Growing on rocks near high water mark (BM, GL, LD).

***Herpodiscus* South *emend.* Draisma, Prud'homme et H. Kawai**

Description
Plants forming little tufts on parenchymatic brown algae and on seagrasses, occasionally epililithic. Filaments with apical growth, haplostichous, oligostichous or polystichous, with or without secondary transverse cell walls. Unilocular zoidangia solitary or in adaxial, lateral sympodia, plurilocular zoidangia on branched small laterals. Life cycle isomorphic, strongly heteromorphic or unknown.
TYPE SPECIES: *Herpodiscus durvillaeae* (Lindauer) South in: *J. R. Soc. New Zealand* 4 (4): 455–461, figs 1–6 (as *H. durvilleae*).

***Herpodiscus bracteatus* (Reinke) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria pulvinata* Hooker f. et Harvey var. *bracteata* Reinke in *Biblioth. Bot.* 23: 17, pl. 5, fig. 11; 1891. Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: 156, figs 49A–F (as *Sphacelaria bracteata*), 1987.
MOST RECENT SYNONYM: *Sphacelaria bracteata* (Reinke) Sauvgeau in *J. Bot (Morot)* 14: 250–254, figs 7, 8; 1900 (= *Rem. Sphacélar.* p. 25–29).
TYPE: Jessen *s.n.* 'An einen *Cystophyllum* von Neu Holland' com. Reinbold (KIEL: holotype; PC: isotype). NB: the host is a *Cystophora*.

***Herpodiscus bornetii* (Harvey) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria bornetii* Har. in *J. Bot (Morot)*1: 57–59; 1887,

TYPE: Cape Horn, Tierra del Fuego, Chile, on a shell of a mussel; microslide in herb. Bornet (PC).

***Herpodiscus carpoglossi* (Womersley) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria carpoglossi* Womersley in *Austral. J. Bot.*: 15:197, fig. 2; 1967. Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: 154–155, figs 45 D, 48; 1987.
TYPE: Womersley A. 29527, Victor Harbor, S. Australia, on *Carpoglossum confluens*, drift, 19/XI/1965 (ADU: holotype).

***Herpodiscus implicatus* (Sauv.) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria implicata* Sauv. in *J. Bot* (*Morot*) 15: 228–230, fig. 27; 1901 (= Rem. Sphacél. p. 118–121). Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: 152–154, figs 47 D–G, 1987.
TYPE: F. von Mueller *s.n.*, Australia, on *Cystophora scalaris*, and Raoul *s.n.* New Zealand, on *Cystophora (Blossevillea) retorta*, 1843 (PC: syntypes).

***Herpodiscus multiplex* (Womersley) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria multiplex* Womersley in *Mar. Benth. Fl. S. Austral.* II: 152, figs 45 C, 47 A–C; 1987.
TYPE: Woelkerling A. 34204, Sarge Bay, E. side of Cape Leeuwin, W. Australia, on *Plathytalia angustifolia*, drift (ADU: holotype; isotypes distributed in 'Marine Algae of Southern Australia' No. 240).

***Herpodiscus pulvinatus* (Hooker f. et Harvey) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria pulvinata* Hook. f. & Harv. in Harvey in J.D. Hooker: *Bot. antarct. voy.* II, *Fl. nov.-zel.* II, p. 221–222; 1855, pl. 110, publ. 11/VII/1854. descr. in Adams: *Seaw. New Zealand* p. 80, pl. 20; 1994.
TYPE: Colenso *s.n.*, North Island, New Zealand, on *Carpophyllum maschalocarpus*, (TCD: holotype).

***Herpodiscus reinkei* (Sauvageau) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria reinkei* Sauv. in *J. Bot (Morot)* 14: 313–317, fig. 12; 1900 (= Rem. Sphacél. p. 43–47). Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: p. 154, figs 47 H–I; 1987.
TYPE: F. von Mueller *s.n.*, Georgetown, Tasmania, on *Cystophora subfarinacea*, herb. Thuret (PC: holotype).

***Herpodiscus sympodiocarpus* (Sauvgeau) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria sympodiocarpa* Sauvageau Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: p. 154, figs 47 H–I; 1987.
Descr. in Prud'homme van Reine in *Tax. Rev. Eur. Sphacel.* p. 258–260, figs 647–660; 1982.
TYPE: Sauvageau *s.n.*, Guéthary, Basses-Pyrénées, France, 15/IX/1898, dredged on *Cystoseira baccata* (PC: lectotype; L).

***Herpodiscus spurius* (Sauvageau) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria spuria* Sauv. in *J. Bot (Morot)* 14: 318–322, fig. 13; 1900 (= Rem. Sphacél. p. 47–51). Descr. in Womersley in *Mar. Benth. Fl. S. Austral.* II: p. 151–152, figs 46 I–K; 1987.
TYPE: Harvey's Australian Algae 14F: on *Cystophora botryocystis*, Brighton Beach, Port Phillip, Victoria, Australia, in herb. Thuret (PC: holotype).

***Herpodiscus stewartensis* (Lindauer) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria stewartensis* Lindauer in *Pacif. Sci.* 3: 340–341, figs 1a–c; 1949. Descr. in Adams: *Seaw. New Zealand* p. 80, no fig. 1994.
TYPE: E.A. Willa *s.n.*, Port Pegasus, Stewart Island, on *Xiphophora chondrophylla*. 20/III/1948. AKU 000033 = herb. Lindauer 9955 (AKU: holotype; isotypes distributed in Lindauer Algae New Zealand Exsiccatae 13: no. 311).

***Halopteris corymbosa* (Dickie) Draisma, Prud'homme et H. Kawai, comb. nov.**
BASIONYM: *Sphacelaria corymbosa* Dickie in *J. Linn. Soc., Bot.* 15: 199; 1876.
MOST RECENT SYNONYM: *Alethocladus corymbosus* (Dickie) Sauvageau in *J. Bot. (Morot)* 17: 346–353, fig. 54; 1903 (= Rem. Sphacél. p. 280–288).
TYPE: Rev. A.E. Eaton *s.n.*, Swains Bay, Kerguelen, 30/I/1875 (BM: holotype).

The sister clade of the Sphacelariales
The members of the sister clade of the Sphacelariales, i.e. *Bodanella*, *Heribaudiella*, *Phaeostrophion*, and possibly *Pseudolithoderma roscoffense*, are all restricted to the northern hemisphere (Table 4) and so are the basal sphacelarialean genera *Sphaceloderma*, *Protohalopteris* gen. nov., *Sphacelorbus* gen. nov., *Battersia*, and *Chaetopteris*, but not *Cladostephus* which has a bipolar distribution. A northern hemisphere origin of the order can nevertheless be hypothesized. Sphacelarialean species must then have crossed the equator from north to south multiple times within the Stypocaulaceae and within the crown of Sphacelariaceae (1). Other species that show a disjunct bipolar distribution are *Sphacella subtilissima* and *Sphacelaria cirrosa* whereas some members of the (former) *Sphacelaria* subgenus *Propagulifera* have a continuous distribution

Table 4. Analytical comparison of families of the revised Sphacelariales and its sister taxa. Data from Draisma *et al.* (2002), Fletcher (1987), Heesch *et al.* (2008), Kawai *et al.* (2005), Mathieson (1967), McCauley & Wehr (2007), Prud'homme van Reine (1982) and Wehr & Sheath (2003).

Family	Cladostephaceae	Sphacelariaceae	Sphacelodermaceae fam. nov.	Stypocaulaceae	Phaeostrophiaceae	*Incertae sedis*[c]	*Incertae sedis*
Genera included	*Cladostephus*	*Battersia*, *Chaetopteris*, *Sphacelorbus* gen. nov., *Herpodiscus*, *Sphacelaria*[a], *Sphacella*	*Sphaceloderma*	*Halopteris*[b], *Protohalopteris* gen. nov., *Phloiocaulon*, *Ptilopogon*	*Phaeostrophion*	*Pseudolithoderma pro parte*[c]	*Bodanella*, *Heribaudiella*
Distribution	bipolar in temperate waters	worldwide	cold-temperate NE Atlantic	worldwide, except tropics	cold NE Pacific	temperate NE Atlantic	temperate North America, Europe, China, Japan
Habitat	marine (sub- and intertidal)	marine (sub- and intertidal) and brackish[d]	marine (subtidal)	marine (sub- and intertidal), *Protohalopteris* also brackish	marine (intertidal)	marine (intertidal)	freshwater (lakes and streams)
Cell wall blackening	positive	positive	positive	positive	n.d.	n.d.	negative
Thallus structure	polystichous; auxocaulous	polystichous, haplostichous or oligostichous; leptocaulous	polystichous; leptocaulous	polystichous; leptocaulous or auxocalous	parenchymatous, partly haplostichous; leptocaulous	haplostichous (pseudo-parenchymatous); leptocaulous	haplostichous (pseudo-parenchymatous); leptocaulous
Growth	apical	apical	apical	apical	chiefly from a localized basal meristem in parenchymatous part, but apical in haplostichous part	apical	apical
Branching	main axis hypacroblastic, laterals acroheteroblastic	hemiblastic, but acrohomoblastic observed in *H. durvillaeae*	meriblastic	acroheteroblastic or acrohomoblastic, *Protohalopteris* also meriblastic	hypacroblastic	dichoblastic	dichoblastic
Hairs	solitary or in bundles	solitary or absent	absent	in bundles or absent	absent	absent	solitary terminal
Life history	isomorphic	(semi-) isomorphic, but heteromorphic in *H. durvillaeae*	isomorphic	isomorphic	presumed direct	presumed direct	presumed direct
Plurilocular zoidangia	lateral	lateral in isomorphic species[e]	lateral	axillary, but sessile in *Protohalopteris*	embedded in thallus	terminal	terminal (unknown in *Bodanella*)
Unilocular zoidangia	lateral	mostly lateral, sometimes terminal	lateral	axillary, but sessile in *Protohalopteris*	embedded in thallus	unknown	terminal

[a]To be conserved with a new type.
[b]Including former *Alethocladus* and *Stypocaulon*.
[c]*Pseudolithoderma roscoffense* is currently classified in the Lithodermataceae. However, according to Parente *et al.* (2005; unpublished results presented at VIIIth International Phycological Congress in Durban, South Africa) the type of the genus, i.e. *P. extensum* (P.L. Crouan & H.M. Crouan) S. Lund is not closely related to *P. roscoffense* and there is no DNA sequence data available for the type of the family, i.e. *Lithoderma fasticens* Areschoug.
[d]Two freshwater *Sphacelaria* species are considered *incertae sedis*.
[e]In *H. durvillaeae* the gametophyte is transformed into a four-celled plurilocular zoidangium.

from their most northern to their most southern distribution.

The basal Sphacelariales *Sphacelorbus nanus* comb. nov., *Protohalopteris radicans* comb. nov., and *Cladostephus spongiosus* occur in the intertidal, although the latter two are also found in the marine subtidal. *Protohalopteris radicans* comb. nov. and *Sphacelorbus nanus* comb. nov. have also been found in salt-marshes, often near freshwater streams and often on the highest parts of the marshes (Prud'homme van Reine, 1982). In brackish waters they have been found permanently submerged. *Pseudolithoderma roscoffense* and *Phaeostrophion irregulare* both occur in the intertidal and must thus be tolerant of fresh water (Table 4). It can therefore be hypothesized that the ancestor of the freshwater genera *Heribaudiella* and *Bodanella* was an alga that lived in the intertidal or perhaps in brackish water. However, the presumed first species to branch off within the Sphacelariales, i.e. *Sphaceloderma caespitula* comb. nov. is a subtidal, permanently submerged and strictly marine species which does not penetrate far into brackish waters (Prud'homme van Reine, 1982).

The Phaeostrophiaceae, *Pseudolithoderma roscoffense*, *Bodanella* and *Heribaudiella*, are currently not classified in an order. De Reviers *et al*. (2007) speculated that they may be incorporated in the Sphacelariales in the future. However, combining them in a separate order is also an option to consider, if their monophyly and sister-relationship to the Sphacelariales were convincingly demonstrated. *Bodanella* and *Heribaudiella* could be placed in a newly created family and a separate new family could be created for *Pseudolithoderma roscoffense* (see Table 4 footnotes), or all taxa could be classified in the Phaeostrophiaceae. However, before making these taxonomic decisions it is better to await more DNA sequence data of *Pseudolithoderma roscoffense* and other members of the genus. Moreover, McCauley & Wehr (2007) demonstrated that *Heribaudiella* is not monophyletic either. *Heribaudiella fluviatilis* from Canada is not identical with *H. fluviatilis* from Germany but sister to *Bodanella lauterborni* from Germany in their *rbc*L phylogeny.

Acknowledgements

We are grateful to Paul Silva (Berkeley, USA) for useful discussions about nomenclature and we thank Dr Jan-Frits Veldkamp (Leiden, the Netherlands) for providing the Latin diagnoses. Willem Prud'homme van Reine's collection trips to New Zealand and Australia were sponsored by the (Schure-)Beijerinck-Popping Fund of the Royal Dutch Academy of Sciences (KNAW). Wendy Nelson (Wellington, New Zealand) is thanked for sending samples of *Herpodiscus durvillaeae* and Takeaki Hanyuda (Kobe, Japan) for providing the *rbc*L sequences of *Saccharina japonica* and *Halopteris gracilescens*. Two anonymous reviewers are thanked for their valuable comments and suggestions for improving the manuscript. The present research was supported in part by a grant from the US National Science Foundation (DEB 0629564).

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