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Phylogenetic relationships within the Fucales (Phaeophyceae) assessed by the photosystem I coding *psa*A sequences

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Fucalean brown algae are ecologically important for maintaining intertidal to subtidal ecosystems and are currently a subject for DNA phylogenies. We analyzed the photosystem I coding *psa*A gene (1488 base pairs) from 26 taxa in all families of the Fucales including *Nemoderma tingitanum* and *Microzonia velutina* as outgroup species. A total of 41 taxa, including published sequences from three fucalean and 13 brown algae, were used for phylogenetic analyses. The *psa*A phylogenies confirmed previous ideas, based on previous studies using morphology and nuclear ribosomal genes, suggesting that the fucalean algae are monophyletic, including the Durvillaeaceae and Notheiaceae. However, in all analyses of the *psa*A data, the Notheiaceae, endemic to Australasia, occupied a basal position within the order. All families except the Cystoseiraceae are monophyletic. The *psa*A data together with previous nuclear ribosomal DNA data and autapomorphic morphological characters strongly support that the genera *Bifurcariopsis* and *Xiphophora* should be separated from the Cystoseiraceae and the Fucaceae, respectively. Two new families, Bifurcariopsidaceae and Xiphophoraceae, are proposed to accommodate each of the genera. The *psa*A data concur with the morphological and biogeographical hypotheses that fucalean algae might have originated from Australasian waters and have become established in the northern hemisphere. The present results indicate that the *psa*A region is a new tool for better understanding phylogenetic relationships within fucalean algae.

KEY WORDS: *Bifurcariopsis,* Bifurcariopsidaceae fam. nov., Fucales, Phaeophyceae, Phylogeny, *psa*A, Taxonomy, *Xiphophora,* Xiphophoraceae fam. nov.

INTRODUCTION

The order Fucales, comprising eight families with approximately 41 genera of about 485 species (for species number, see Guiry *et al.* 2005), is one of the largest and most diversified orders within the class Phaeophyceae. The fucalean algae are ecologically important as a major, often dominant component of tropical to temperate marine forests and intertidal communities (Nizzamudin 1962). Some fucoids are economically important sources of food, alginates, and pharmacials (Sun *et al.* 1980), and have served as a useful tool for many developmental researches (e.g. Motomura 1994). The Fucales are distributed worldwide, but those of the northern hemisphere differ considerably from those of the southern hemisphere (Clayton 1984; Reviers & Rousseau 1999).

The order Fucales was described by Bory de Saint-Vincent [1827 (1826–1829), p. 62] (see Silva & de Reviers 2000) and the characters used to define the order together with other phaeophycean orders are primarily based on Kylin's classification system (1933). The taxonomic history, nomenclature, and description of the order were reviewed by Nizzamudin (1962), who recognized eight families: the Ascoseiraceae, Cystoseiraceae, Durvillaeaceae, Fucaceae, Hormosiraceae, Himanthaliaceae, Sargassaceae, and Seirococcaceae. However, Petrov (1963, 1965) excluded the Ascoseiraceae and Durvillaeaceae from the order, which were accommodated, respectively, in the orders Ascoseirales and Durvillaeales. Clayton (1984) reviewed phylogenetic relationships of the major six families on the basis of morphology, anatomy, and cytology, proposing that some fucoids such as the Hormosiraceae from southern Australia could be forerunners in the evolutionary history of the Fucales. Gibson & Clayton (1987) suggested that the Notheiaceae, sometimes excluded from the Fucales (see review by de Reviers & Rousseau 1999) or even placed in a separate order, Notheiales (Womersley 1987), should be classified within the Fucales and be regarded as a primitive lineage because of its haplostichous and anisogamous nature vs parenchymatous and oogamous nature of the other fucalean algae. The inclusion of the Notheiaceae, consisting of a monotypic genus, *Notheia,* in the Fucales has been later supported by Saunders & Kraft (1995), on the basis of nuclear small subunit (SSU) ribosomal DNA (rDNA) sequence data analysis. In a series of studies using the SSU and large subunit (LSU), Rousseau *et al.* (1997, 2001) and Rousseau & de Reviers (1999) redefined the concept of the Fucales, reincluding the Durvillaeaceae and the Notheiaceae within the order.

Horiguchi & Yoshida (1998) transferred the genus *Myagropsis,* formerly classified in the Cystoseiraceae, to the Sargassaceae. Rousseau & de Reviers (1999) included the paraphyletic Cystoseiraceae in the Sargassaceae *sensu lato* (*sensu* De Toni 1895) and excluded *Bifurcariopsis* from the family. Despite its paraphyletic nature, the Cystoseiraceae is still used by some authors (e.g. Gómez & Ribera 2005): eight families are thus currently recognized in the Fucales.

A new genus, *Silvetia,* consisting of three species previ-

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Table 1. Collection information of samples used in this study.

Family or 'family level' genus	Species	Collection site, reference; voucher no.; GenBank no.
Cystoseiraceae	*Bifurcaria bifurcata* R. Ross	Roscoff, France; PF229; DQ092448
Cystoseiraceae	*B. brassicaeformis* (Kützing) Barton	Simon's Town, Cape Town, South Africa; PF008; DQ092449
Cystoseiraceae	*Coccophora langsdorfii* (Turner) Greville	Nakhodka, Russia; PF232; DQ092450
Cystoseiraceae	*Cystophora* sp.	Brighton Beach, Dunedin, New Zealand; PF137; DQ092451
Cystoseiraceae	*Cystoseira hakodatensis* (Yendo) Fensholt	Hakodate, Hokkaido, Japan; PF025; DQ092452
Durvillaeaceae	*Durvillaea antarctica* Hariot	Brighton Beach, Dunedin, New Zealand; PF189; DQ092453
Durvillaeaceae	*D. willana* Lindauer	Brighton Beach, Dunedin, New Zealand; PF190; DQ092454
Fucaceae	*Ascophyllum nodosum* (L.) Le Jolis	Cho *et al.* (2004)
Fucaceae	*Fucus vesiculosus* Linnaeus	Cho *et al.* (2004)
Fucaceae	*Hesperophycus californicus* P. C. Silva	Laguna Beach, California, USA; PF233; DQ092455
Fucaceae	*Pelvetia canaliculata* Decaisne *et* Thuret	Port Erin Bay, Isle of Man, UK; PF235; DQ092456
Fucaceae	*Pelvetiopsis limitata* (Setchell) Gardner	Cape Beal, Vancouver Island, Canada; PF243; DQ092457
Fucaceae	*Silvetia siliquosa* (Tseng *et* Chang) Serrão *et al.*	Rushan, Shandong, China; PF183; DQ092458
Himanthaliaceae	*Himanthalia elongata* S.F. Gray	Roscoff, France; PF248; DQ092459
Hormosiraceae	*Hormosira banksii* (Turner) Descaisne	Scorching Bay, Wellington, New Zealand; PF254; DQ092460
Nothelaceae	*Notheia anomala* Harvey & Bailey	Melbourne, Australia; PC12/1996; DQ094837
Sargassaceae	*Hizikia fusiformis* (Harvey) Okamura	Seosang, Namhaedo, Korea; PF072; DQ092461
Sargassaceae	*Myagropsis myagroides* (Mertens *ex* Turner) Fensholt	Sangjokam, Goseong, Korea; PF059; DQ092462
Sargassaceae	*Sargassum horneri* (Turner) C. Agardh	Cho *et al.* (2004)
Sargassaceae	*S. muticum* (Yendo) Fensholt	Suryeomri, Gyeongju, Korea; PF020; DQ092463
Sargassaceae	*Turbinaria ornata* (Turner) J. Agardh	Kualoa, Hawaii, USA; PF005; DQ092464
Seirococeaceae	*Marginariella urvilliana* (A. Richard) Tandy	Wellington, New Zealand; PF253; DQ092465
Seirococeaceae	*Seirococcus axillaris* (R. Brown *ex* Turner) Greville	Beachport, Australia; PC02/1997; DQ094836
Bifurcariopsis	*Bifurcariopsis capensis* Papenfuss	Buffesbaii, Cape Town, South Africa; PF006; DQ092466
Xiphophora	*Xiphophora chondrophylla* (R. Brown *ex* Turner) Montagne *ex* Harvey	Cable Bay, New Zealand; ASF326; DQ314586
Xiphophora	*X. gladiata* (Labillardière) Montagne *ex* Kjellman	Brighton Beach, Dunedin, New Zealand; PF188; DQ092467
Xiphophora	*X. gladiata* (Labillardière) Montagne *ex* Kjellman	Southeast Otago, Papotowai, New Zealand; A22676; DQ094833
Ralfsiales	*Nemoderma tingitanum* Schousboe *ex* Bornet	Banyuls, France; PC08/1997; DQ094835
Syringodermatales	*Microzonia velutina* (Harvey) J. Agardh	Culture from New Zealand (D.G. Müller); Müller 1228; DQ094834

ously named under *Pelvetia* from the North Pacific, was described based on rDNA internal transcribed spacer (Serrão *et al.* 1999), and the genus has been supported by plastid RuBisCO sequences (Lee *et al.* 1999) and morphological studies (Cho *et al.* 2001). Later on, Stiger *et al.* (2003) reported that the genus *Sargassum* was divided into three subgenera, *Phyllotrichia, Sargassum,* and *Bactrophycus,* based on internal transcribed spacer data. Recently, Phillips *et al.* (2005), using the protein-coding *rbc*LS operon, found a deep branch for the subgenus *Sargassum* with rapid speciation in closely related taxa. Therefore, although molecular systematics has resulted in a major change in evolutionary concept and classification of the Fucales, and rDNA SSU and LSU data are to date the primary sources for understanding the phylogenetic relationships of the order Fucales (Rousseau *et al.* 1997, 2001; Rousseau & de Reviers 1999).

Plastid *psa*A gene encodes photosystem I P700 apoprotein A1 in algae to vascular plants and is more variable and informative than the *rbc*L in brown algae (Cho *et al.* 2004). Despite its variability, the *psa*A sequences are easily aligned because of the absence of gaps, even among the entire brown algal group. In the present study, we introduced novel *psa*A sequences from 23 species of the Fucales, representing all eight families and two outgroup species (*Nemoderma tingitanum* and *Microzonia velutina*). We believe that the *psa*A gene is a suitable marker for the unravelling of the phylogenetic patterns in the Fucales.

MATERIAL AND METHODS

Taxon sampling

Twenty-six taxa, representing all of the described families in the Fucales and including three previously studied species, were collected in the present study. Fifteen brown algae were used as outgroup taxa, of which the *psa*A sequences from 13 species were downloaded from GenBank (Cho *et al.* 2004) and those of *Nemoderma tingitanum* and *Microzonia velutina* were analyzed in the present study. A total of 42 brown algal taxa were included in our study. The specimens and their corresponding GenBank accession numbers newly determined in the present study are listed in Table 1. Voucher specimens are deposited in the herbarium of Chungnam National University, Daejeon, Korea (CNUK), and at Paris Cryptogamy, France.

Analyses of the *psa*A region

Total DNA was extracted from approximately 0.01 g of dried thalli ground in liquid nitrogen using a DNeasy Plant Mini Kit (Qiagen Gmbh, Hilden, Germany), according to the manufacturer's instruction, and then dissolved in 150 litres of distilled water. Extracted DNA was stored at −20°C and used to amplify the *psa*A region.

The *psa*A region was amplified and sequenced using primers psaA130F, psaA870F, psaA970R, and psaA1760R (Yoon *et al.* 2002). The polymerase chain reaction (PCR) products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instruction. The sequences of the forward and reverse strands were determined for all taxa using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at the Center for Research, CNUK. The electropherogram output for each sample was edited using the program Sequence Navigator v. 1.0.1 (Applied Biosystems).

All the sequences of the *psa*A gene from 42 taxa were collated using the multisequence editing program SeqPup (Gilbert 1995), and aligned by eye to compare our sequences with those published previously (Cho *et al.* 2004).

Phylogenetic analyses

The 42 *psa*A sequences were used for the phylogenetic analyses. For maximum likelihood (ML) and Bayesian analyses, we performed the likelihood ratio test using the Modeltest 3.06 version (Posada & Crandall 1998) to determine the best available model for the *psa*A data set. For each of both analyses, the best model was a general time reversible model with shape parameter of the gamma distribution (Γ) and portion of invariable sites (I). The ML tree for the *psa*A data was constructed under the following conditions by heuristic search methods (10 random sequence-addition) – substitution rate matrix: $A \leftrightarrow C = 4.631659$, $A \leftrightarrow G = 5.737771$, $A \leftrightarrow T = 0.233261$, $C \leftrightarrow G = 4.663257$, $C \leftrightarrow T = 20.285354$, $\Gamma = 0.505334$, $I = 0.459532$, and empirical base frequencies: $A = 0.29853$, $C = 0.15151$, $G = 0.18898$, $T = 0.36098$. Bootstrap (BS) values for the ML tree were evaluated with 100 BS replicates by heuristic search (single random sequence-addition).

Maximum parsimony (MP) trees were constructed for the *psa*A data set with PAUP* (Swofford 2002) using a heuristic search algorithm with the following settings: 100 random sequence-addition replicates, tree bisection–reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies. The BS values for the resulting nodes were assessed using bootstrapping with 1000 pseudoreplicates (100 random sequence-addition).

Bayesian phylogenetic analyses were performed using MrBayes 3.0 (Huelsenbeck & Ronquist 2001). Each analysis was initiated from a random starting tree and the program was set to run four chains of Markov chain Monte Carlo iterations simultaneously for 2,000,000 generations with trees sampled every 100th generation. The first 3001 trees, including the start tree, were burned to stabilize the likelihood scores. The last 17,000 trees were consensed by 50% majority rule to obtain a Bayesian probability (BP) supporting value.

RESULTS

The *psa*A sequences determined in the present study totaled 1488 base pairs. For the 27 aligned sequences from our fucalean taxa, 456 (30.6%) bases were variable and 321 (21.6%) were parsimoniously informative. There were excesses of adenine and thymine at all codon positions (30.06% and 36.28%, respectively). Transitions occurred twice more than transversions over all codon positions (Ti/Tv = 1.88). For the *psa*A alignment of 42 sequences including outgroups, 649 (43.6%) bases were variable and 521 (35.0%) were parsimoniously informative. There were excesses of adenine and thymine at all codon positions (29.85% and 36.1%, respectively). Transitions occurred more frequently than transversions for all codon positions (Ti/Tv = 1.40).

The pairwise divergence of the *psa*A within the Fucales averaged 8.52%. It was 6.43% between the Fucaceae and *Xiphophora,* and 5.79% between *Xiphophora* and *Hormosira. Bifurcariopsis capensis* differed from Cystoseiraceae by 9.57% divergence and from *Himanthalia elongata* by 6.99% divergence.

In the *psa*A phylogenies (Fig. 1), all the fucalean algae investigated here produced a monophyletic clade (84% BS for ML, 69% BS for MP, and BP = 1.0). *Notheia* was the basalmost taxon of the Fucales. The Cystoseiraceae clustered with the Sargassaceae with maximum support. However, *Bifurcaria bifurcata* from France was more related to *Cystophora* sp. from New Zealand (90% BS for ML, 100% BS for MP, and BP = 0.96) than to *B. brassicaeformis* from South Africa. The genus *Bifurcariopsis* did not cluster with the Cystoseiraceae in any of the analyses. The monophyly of *Sargassum, Hizikia, Myagropsis,* and *Turbinaria,* previously classified in the Sargassaceae, was resolved with maximum support. The Cystoseiraceae studied here were not recovered, nor was the genus *Bifurcaria* within the family. The remaining fucalean algae formed a clade (62% BS for ML, 77% BS for MP, and BP = 0.99). The Fucaceae, the Hormosiraceae, and *Xiphophora* clustered with strong support (98% BS for ML, 92% BS for MP, and BP = 1). Fucacean genera such as *Fucus, Hesperophycus, Pelvetiopsis, Pelvetia, Ascophyllum,* and *Silvetia* were perfectly supported in any of the analyses (99% BS for ML, 100% BS for MP, and BP = 1.0). The sister relationship between the Fucaceae and the Hormosiraceae was not supported, and *Xiphophora* occupied a basal position of the two families. Relationships among the Durvillaeaceae, Seirococcaceae, Himanthaliaceae, and *Bifurcariopsis* were not supported in any of the present analyses.

DISCUSSION

The present study, including 26 fucalean taxa, includes all eight families of the order Fucales. The taxon sampling covers approximately 60% of all 41 genera recognized in the Fucales (Clayton 1984; de Reviers & Rousseau 1999; Serrão *et al.* 1999), including all seven genera of the Fucaceae, and the monogeneric Himanthaliaceae, Hormosiraceae, Notheiaceae, and Durvillaeaceae. The three remaining families were less represented with two of five genera for the Seirococcaceae, four of 17 for the Cystoseiraceae, and four of eight for the Sargassaceae.

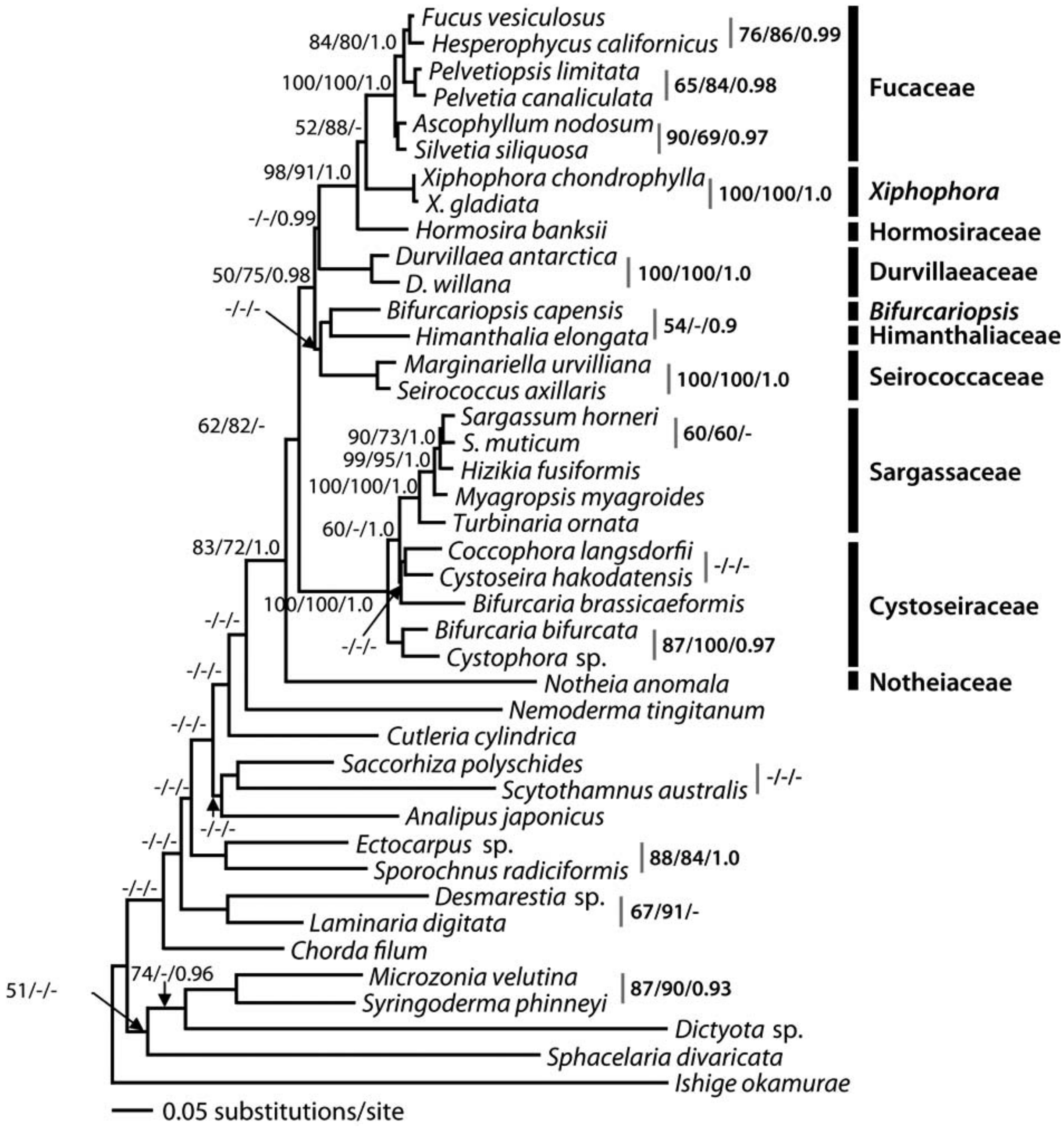


Fig. 1. Maximum likelihood tree for the Fucales estimated from the *psa*A sequence data ($-\ln L = 15040.66494$). The supporting values shown above the branches are from ML BS/MP BS/BP value. Dashes indicate <50% support of bootstrap (or <0.9 for BP).

This is the first report to document protein-coding plastid *psa*A phylogeny in the Fucales and the second report of the psaA data in brown algae since the report by Cho *et al.* (2004). The *psa*A sequence variability (approximately 30%) among fucalean representatives is suitable to infer phylogenetic relationships within the order, bringing supplementary and complementary information to previously published rDNA data. At the scale of the order, our trees are generally congruent with those of nuclear ribosomal DNA (nrDNA) phylogenies (Rousseau & de Reviers 1999; Rousseau *et al.* 1997, 2001). At the family level, our trees are very similar to those inferred from *rbc*L sequences for the Sargassaceae-Cystoseiraceae group (Phillips *et al.* 2005), and are also symmetrical with ITS trees for the Fucaceae (Serrão *et al.* 1999). These results indicate that the *psa*A region is a new molecular marker for a better understanding of phylogenetic relationships among the fucalean algae.

In all *psa*A trees, the order Fucales is monophyletic, having *Nemoderma tingitanum* as its sister. The sister relationship between the Fucales and *N. tingitanum* is not supported, as it is in previously published nrDNA data (Rousseau *et al.* 2001). *Nemoderma tingitanum,* a small crustose brown alga, is currently classified in the Nemodermataceae within the Ralfsiales (Feldmann 1937; Nakamura 1972) and this relationship is surprising. However, *Nemoderma* is different from the ralfsialean algae in having multiple plastids without pyrenoids, intercalary unilocular sporangia, and isomorphic life cycle with anisogamy (Kuckuck 1912) rather than one plate-like plastid without pyrenoid, terminal unilocular sporangia, and a direct life cycle of the Ralfsiaceae. The Fucales have many plastids without pyrenoids, like *Nemoderma;* however, this is a plesiomorphic condition in brown algae (Rousseau *et al.* 2001) and it cannot be interpreted as morphological evidence of a possible relationship. However, phylogeny of the Ralfsiales is beyond the scope of the present study.

The Notheiaceae and the Durvillaeaceae are consistently retrieved as members of the Fucales. This result confirms previous nrDNA data (Saunders & Kraft 1995; Rousseau & de Reviers 1999; Rousseau *et al.* 2001). Inclusion of *Notheia* and *Durvillaea* within the Fucales is supported with morphological characters (see review by de Reviers & Rousseau 1999). However, there is some incongruency between plastid *psa*A and nrDNA data concerning the position of *Notheia: psa*A phylogenies showed *Notheia* as the first divergence of the fucalean algae, whereas in the rDNA data, the Seirococcaceae occupied the basal position and instead *Notheia* is then a sister of the Cystoseiraceae-Sargassaceae group.

The early divergence of the Notheiaceae in the Fucales is not well supported by BS values and requires further investigations. However, we point out that *Notheia* is anisogamous, like *Nemoderma,* the sister of the Fucales, whereas all other fucalean algae are oogamous (Gibson & Clayton 1987). It is therefore speculated that anisogamy is ancestral within the Fucales. Also, *Notheia* has a peculiar lipidic composition with diacylglycerylhydroxymethyl-β-alanine (DGTA) and phosphatidylcholine (PC), whereas the other fucalean algae have only DGTA (Eichenberger *et al.* 1993). The presence of altritol in *Notheia,* as in *Himanthalia, Hormosira, Xiphophora,* and *Bifurcariopsis,* can be regarded as symplesiomorphic but this would imply four independent losses of this hexitol in the Cystoseiraceae-Sargassaceae group, Durvillaeaceae, Seirococcaceae, and Fucaceae. The inverse scenario is equiparsimonious, leading to three independent gains and one loss (i.e. the same number of evolutionary events). Regarding the number of eggs in the oogonium, 16 eggs becoming eight per oogonium, which is formed in *Notheia,* might be considered primitive within the Fucales; in this hypothesis, the number of eggs would basically tend to decrease along the evolutionary process. By contrast, Jensen (1974) and Clayton (1984) hypothesized that the production of four eggs in *Bifurcariopsis* and *Durvillaea* is an ancestral type because the four cells resulting from the meiosis were interpreted as homologous of tetraspores, the hypothesis made earlier than the inclusion of the *Notheia* in the Fucales (Gibson & Clayton 1987). Other morphological details of *Notheia* and other families are given in Table 2.

The Sargassaceae and Cystoseiraceae produced a monophyletic clade strongly supported by BS values and BPs. The monophyly of the Sargassaceae, consisting of *Sargassum, Hizikia, Myagropsis,* and *Turbinaria* in the present study, is retrieved in all trees with maximum support. The six cystoseiracean representatives did not form a monophyletic group in any of analyses of the *psa*A data. This phylogenetic result confirms the suggestion, proposed by Rousseau & de Reviers (1999), to merge the Cystoseiraceae within the Sargassaceae. The *psa*A phylogenies also confirm the paraphyly of the genus *Bifurcaria,* shown in previous rDNA data (Rousseau & de Reviers 1999; Rousseau *et al.* 2001). Because the type of the genus is *B. bifurcata* (Ross 1958), a new genus should be established for the intertidally dominant *B. brassicaeformis* from South Africa. Before the taxonomic revision of the genus, the study on *B. galapegensis* (Piccone & Grunow) Womersley is necessary. More taxon sampling will resolve relationships among cystoseiracean algae.

Bifurcariopsis did not cluster with the other members of the Cystoseiraceae included in the present study but formed a sister relationship with *Himanthalia,* both having altritol (Chudek *et al.* 1984). The sister relationship between *Bifurcariopsis* and *Himanthalia,* although poorly supported, is interesting because the former occurs in South Africa, while the latter is distributed along the North Atlantic coast. The results corroborate the views of Rousseau & de Reviers (1999) and also those of Draisma *et al.* (2003), who strongly considered establishing its own family to accommodate *Bifurcariopsis.* Here we describe a new family:

Bifurcariopsidaceae fam. nov.

Bifurcariopsidaceae nova familia. Thallus constatus ex iterum atque iterum bifurcates, teretibus parenchymatis repente haptorone exorientibus. Ex trilatera apicali cellula crescens. Monoecius thallus. Terminales partes extremorum ramorum spissescentes in receptaculos continentes bisexuales conceptaculos cum oogonio producente quatuor ova, et antheria in terminales fasciculos in longis nullis ramosis filamentis. Oogamia. Altritol praesens.

GENUS TYPUS: *Bifurcariopsis* Papenfuss 1940: 212, Notes on South African algae 1. *Botaniska Notiser* 1940: 200–226.

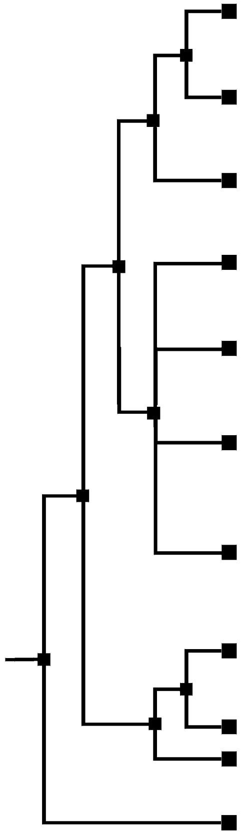
Thallus made of repeatedly bifurcate, terete parenchymatous axis arising from a creeping holdfast. Growth from one three-sided apical cell. Thallus monoecious. Terminal portions of ultimate branches thickening into receptacles containing bisexual conceptacles with four eggs produced by oogonium and antheridia in terminal clusters on long unbranched filaments. Oogamy. Altritol present.

TYPE GENUS: *Bifurcariopsis* Papenfuss, 1940: 212.

The family presently contains only one species, *B. capensis* (Areschoug) Papenfuss, which is endemic to South Africa.

A morphological and biochemical comparison of *Xiphophora* and other fucalean algae is given in Table 2. In the present analysis, the six fucacean genera *Ascophyllum, Fucus, Hesperophycus, Pelvetia, Pelvetiopsis,* and *Silvetia* are recovered as a monophyletic clade with maximum support. This result concurs with previously studied ITS (Serrão *et al.* 1999) and rDNA data (Rousseau & de Reviers 1999; Rousseau *et al.* 2001). These six genera are from the northern hemisphere, and share the anterior flagellum shorter than the posterior, and fucoserratene or finavarene as pheromones. Their spermatozoids also have a proboscis, a structure is absent in *Xiphophora* and *Hormosira* but possibly homologous of a similar structure found in the Seirococcaceae. The genus *Xiphophora,* like *Hormosira,* is from the southern hemisphere and the anterior flagella of the spermatozoids are longer than the posterior. Furthermore, in both genera, the flagella of the spermatozoids are inserted with an acute angle rather than at 180° in the six genera of the Fucaceae and possess a spine on the posterior flagella (a character shared by *Himanthalia*). Thus, despite some affinities with *Xiphophora* and *Hormosira,* the former has been classified in the Fucaceae because it has one four-sided apical cell and receptacles, and because the latter possesses four three-sided apical cells and no receptacles. In

Table 2. Morphological details of the Fucales at the familial level. Synthetic tree modified from Fig. 1 appears on the left side of the table and only supported nodes are resolved in the tree in the table headings. Data from Jensen 1974, Chudek *et al.* 1984, Clayton 1984, 1994, Gibson & Clayton 1987, Eichenberger *et al.* 1993, Pohnert & Boland 2002, and Womersley 1978.



Taxon name	Number of apical cells	Shape of apical cell	Branching	Structure	Receptacles	Type of gamy	Eggs / oogonium	Sperm flagella angle	Sperm flagella spine	altritol synthesis	Lipid	Pheromone	Distribution
Fucaceae	1	4-sided	dichotomous	parenchymatous	apical, marginal	oogamy	1, 2, 4, 8	180°	absent	no	DGTA	fucoserratene, finavarrene	Northern Hemisphere
Hormosiraceae	4	3-sided	di, tricho- tomous	parenchymatous	scattered conceptacles	oogamy	4	acute	present	yes	DGTA	hormosirene	Australasia
Xiphophora	1	4-sided	dichotomous	parenchymatous	apical	oogamy	4	acute	present	yes	DGTA	hormosirene	Australasia
Himanthaliaceae	1	3-sided	Dichotomous	Parenchymatous	Apical	Oogamy	1	Acute	Present	Yes	DGTA	?	North Atlantic
Bifurcariopsis	1	3-sided	monopodial	parenchymatous	apical	oogamy	4	?	?	yes	DGTA	?	South Africa
Durvillaeaceae	0	-	bilateral	haplostichous	scattered conceptacles	oogamy	4	?	?	no	DGTA	hormosirene	Australasia, Chile
Seirococcaceae	<2	4-sided	bilateral	parenchymatous	marginal or axillary	oogamy	1	180°	absent	no	DGTA	?	Southern Hemisphere
Sargassaceae	1	3-sided	radial or bilateral	Parenchymatous	clustered in axils to terminal	Oogamy	1	90°	Absent	No	DGTA	?	Worldwide
Cystoseiraceae	1	3-sided	radial or bilateral	parenchymatous	terminal on laterals	oogamy	1	90°	absent	no	DGTA	cystophorene	Worldwide
Notheiaceae	3	3-sided	Irregular	Haplostichous	scattered conceptacles	Anisogamy	8	?	Absent	Yes	PC, DGTA	?	Australasia

*psa*A trees, the two *Xiphophora* species made a monophyletic clade, which is positioned as basal of the Fucaceae/Hormosiraceae clade.

The current classification of *Xiphophora* in the Fucaceae appears to be in conflict with the *psa*A phylogenies. By contrast with the strong monophyly of the six core fucacean genera, the sister relationship between *Xiphophora* and the groups of the six core fucacean genera is not well resolved. Furthermore, *Xiphophora* has a combination of characters, which make it quite different from the core fucacean algal group. Because the relationship of *Xiphophora* with *Hormosira* is not supported, *Xiphophora* cannot be included in the Hormosiraceae, as it does not belong to the Fucaceae either. The taxonomic separation of *Xiphophora* from the Fucaceae has been strongly considered by Rousseau & de Reviers (1999) and followed by Draisma *et al.* (2003), based on rDNA data. We finally conclude that the genus *Xiphophora* as an independent fucalean group is best expressed by placement of the genus in a separate family, as follows:

Xiphophoraceae fam. nov.

Xiphophoraceae nova familia. Thallus cum plus minusve dichotomis parenchymatis ramis, divisis vel hapteroideis hapteronibus exorientibus. Nulla vesicula. Ex una quadrilatera apicali cellula in angustum apicalem sulcum crescens. Monoecius thallus. Terminali rami, in receptaculos cum uni-bisexualibusve, dispersis ordinibusve, ostiolis super plana pagina conceptaculis evoluti. Oogonia producentia quatuor ova. Antheridia in ramosis paraphysibus. Oogamia. Altritol praesens.

GENUS TYPUS: *Xiphophora* Montagne, 1842: 12, *Prodromus generum, specierumque phycearum novarum, in itinere ad polum antarcticum regis Ludovici Philippi Jussu ab illustri Dumont d'Urville peracto collectarum, notis diagnosticis tatum huc evulgatarum, descriptionibus vero fusioribus nec non iconibus analyticis jam jamque illustrandarum* auctore C. Montagne D.M., Parisiis, apud Gide editorium, 1842, [1]-16.

Thallus with more or less dichotomous parenchymatous branches arising from a divided or hapteroid holdfast. Vesicles absent. Growth from a single, four-sided apical cell in a narrow apical groove. Thallus monoecious. Terminal branches developing into receptacles with unisexual or bisexual conceptacles scattered or in lines, with ostioles on the flat surface. Oogonia producing four eggs; antheridia on branched paraphyses. Oogamy. Altritol present.

TYPE GENUS: *Xiphophora* Montagne, 1842: 12.

The family is presently monogeneric with two species *X. chondrophylla* (R. Brown *ex* Turner) Montagne *ex* Harvey and *X. gladiata* (Labillardière) Kjellman, both endemic to southern Australia and New Zealand.

The Australasian waters have been suggested for a long time to be a diversity center of the fucalean algae (Clayton 1984), considering that all the families except the Himanthaliaceae from the North Atlantic, Bifurcariopsidaceae from South Africa, and the Fucaceae from the North Pacific occur in Australia and New Zealand. The number of the fucalean algae decreases in Antarctica, Chile, and South Africa (Clayton 1984; de Reviers & Rousseau 1999) and the Fucaceae family is dominant only in the northern hemisphere. When we map our *psa*A phylogenies with the current distribution patterns of the fucalean algae, the Australasian family Notheiaceae is consistently the most basal in the Fucales. The *psa*A trees therefore support Clayton's hypothesis (1984) that the first fucalean algae might have evolved on the southern Australian shores. Although Clayton (1984) inferred that they occurred there before the separation of lands of present Australia and New Zealand at the end of the Cretaceous period (approximately 80 million years ago), there is to date no molecular clock in the Fucales. Considering the above scenario, the Australian forerunners of fucalean algae migrated several times to the northern hemisphere (see review by de Reviers & Rousseau 1999).

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