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# Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the *rbc*L and 28S nrDNA sequences [1]

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**Abstract** — The position of the brown algal genus *Microzonia*, currently placed in the Cutleriales, was investigated using two different genes: the plastid-encoded *rbc*L gene and the nuclear-encoded rDNA 28S gene. All our analyses show that *Microzonia* is the sister taxon of *Syringoderma* and is not related to the cutlerialean genera *Cutleria* and *Zanardinia*. This result is consistent with morphological characters. It is thus proposed to exclude *Microzonia* from the Cutleriales and to place it in the Syringodermatales. Within the Phaeophyceae, the *Syringoderma-Microzonia* branch diverges after *Choristocarpus* and members of the Dictyotales and the Sphacelariales, as the sister taxon of a clade comprising all other orders. The extreme basal position of *Choristocarpus* is confirmed with both *rbc*L and 28S genes, contrary to previous reports by other authors.

**Cutleriales / marine brown algae / *Microzonia* / molecular phylogeny 28S ribosomal and *rbc*L DNA sequences / Syringodermatales / taxonomy**

**Résumé — Placement du genre *Microzonia* (Phaeophyceae) dans les Syringodermatales, sur la base de l'analyse des séquences des gènes codant pour la *rbc*L et l'ARNr 28S.** La position systématique du genre d'algue brune *Microzonia*, actuellement placé dans les Cutleriales, a été étudiée à l'aide de deux gènes différents : le gène plastidial *rbc*L et le gène nucléaire codant pour l'ARNr 28S. Toutes nos analyses montrent que *Microzonia* est taxon frère de *Syringoderma* et n'est pas associé aux deux genres composant les Cutleriales : *Cutleria* et *Zanardinia*. Ce résultat est soutenu par les caractères morphologiques. Nous proposons donc que *Microzonia* soit exclu des Cutleriales et soit placé dans les Syringodermatales. La branche comprenant *Syringoderma* et *Microzonia* diverge, au sein des Phaeophyceae, après celle de *Choristocarpus*, des Dictyotales et des Sphacelariales, en tant que groupe frère de l'ensemble des représentants des autres ordres. La position basale extrême de *Choristocarpus* est confirmée par l'analyse des deux gènes et non de la seule *rbc*L, contrairement à ce que d'autres auteurs avaient obtenu.

**Algues brunes marines / Cutleriales / *Microzonia* / phylogénie moléculaire / séquences de l'ADN ribosomal 28S et de la *rbc*L / Syringodermatales / taxinomie**

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1. This paper is dedicated with pleasure to Susan Loiseaux-de Goër, on the occasion of her retirement. Susan was the first in France to introduce molecular techniques to algal phylogenetics.

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## INTRODUCTION

*Microzonia velutina* is a fan-shaped brown alga, only known in the southern hemisphere: from New Zealand (Harvey, 1855; Lindauer *et al.*, 1961), Patagonia (Asensi, 1966 & 1971), Stewart island (Adams *et al.*, 1974) and Macquarie island, which is the highest latitude record for this genus (Ricker, 1987).

The species was first described from New Zealand by Harvey (1855) under the name *Zonaria velutina* because of its fan-shaped thallus and "the dense velvety coating of the under surface". In 1859, Kützing (p. 21 and tab. 51, fig. II, a, b) removed this species from *Zonaria* and placed it in the genus *Spatoglossum*. Unilocular reproductive organs were observed by J. Agardh (1894: 18), who showed that they are not on the surface of the thallus as in *Zonaria* but are on uniseriate branched filaments produced by the cortical cells on the ventral surface. On this basis, he created the monotypic genus *Microzonia* for *Zonaria velutina* and made the new combination: *Microzonia velutina* (Harvey) J. Agardh. Agardh (1894) pointed out its resemblance to the Cutleriales, particularly in the sporangial filaments, but maintained the new genus in the Dictyotaceae. In 1954, O'Donnell showed the presence of evanescent marginal hairs and consequently interpreted the growth as trichothallic. This feature and the position of the reproductive organs were considered by O'Donnell (1954) to justify removing *Microzonia* from the Dictyotales. Since she interpreted the growth as trichothallic, she transferred *Microzonia* to the Cutleriales, adding it to the two genera, *Cutleria* and *Zanardinia*. Later, Asensi (1971) and Ricker (1987) showed that, despite marginal hairs, which are not consistently present, growth in *Microzonia* is not trichothallic as in *Cutleria*, but apical. Furthermore, the hairs appear on adult thalli, not on growing plantlets (Asensi, pers. comm.). Ricker (1987) pointed out some resemblance between *Microzonia* and *Syringoderma* by comparing their growth, thallus construction, and the uniseriate filaments bearing unilocular sporangia, but could not decide on its taxonomic position because of the lack of information concerning its life cycle, which remains unknown to date.

The aim of this article is to clarify the taxonomic position of *Microzonia* and that of the Syringodermatales, this order having been proposed by Draisma *et al.* (2001) and Rousseau *et al.* (2001) to be either the sister group of the Sphacelariales or diverging after this order.

Until now, the genes traditionally used at the interordinal level in phylogenetic analyses within the Phaeophyceae were the 18S nrDNA, the partial 28S nrDNA and the *rbc*L. The 18S was soon determined not to be sufficiently informative to infer relationships between orders within the brown algae (reviewed by Reviers & Rousseau, 1999). Draisma *et al.* (2001) showed that partial 28S, including the two divergent domains D1 and D2, and *rbc*L sequences were useful for investigating these phylogenetic relationships. Therefore, we combined partial 28S and *rbc*L sequences to clarify the taxonomic position of *Microzonia*. Because these genes were not suitable for resolving the taxonomic position of the Syringodermatales (Draisma *et al.*, 2001) and because the complete 28S gene appeared to be more suitable to resolve the first divergences of the Phaeophyceae (Rousseau *et al.*, 2001), we explored whether this could resolve the position of the Syringodermatales.

## MATERIAL AND METHODS

### Algal sampling, DNA extraction, PCR and sequencing

The sequences of the examined species, 30 brown algal species for *rbc*L and partial 28S genes, and 11 species for the complete 28S gene, were obtained from GenBank or newly determined for the present study, as specified in Tabs 1 and 2. Field-collected algae were either immediately desiccated in silica gel (Chromatic silica media-grade 12, mesh size 28-200, Merck) according to Chase & Hillis (1991), air-dried as herbarium specimens, frozen at -80°C, or freeze dried (see Tab. 2 for details).

DNA extractions were performed with the DNeasy plant kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. PCR amplifications of fragments were performed as described in Rousseau *et al.* (1997). PCR products were cleaned with QIAquick columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Eleven primer-pairs were used to amplify the complete 28S rDNA gene as described in Rousseau *et al.* (2001) and six primers were used to amplify the complete *rbc*L gene (see Tabs 3 and 4 for further information). For all but one sequence, both strands of the PCR products were directly sequenced with the PCR primers using the CEQ Cycle Sequencing Kit (Beckman, Fullerton, U.S.A.) in the CEQ-2000 DNA Analysis System (Beckman, Fullerton, U.S.A.). The partial 28S sequence of the genus *Zanardinia* was manually sequenced using the radiolabelled primer method of Amersham's thermo Sequenase cycle sequencing kit, following the manufacturer's instructions as described in Rousseau *et al.* (1997). For each species, the final consensus sequences were obtained with Sequencher 4.0.5 software (Gene Codes Corporation, Ann Arbor, U.S.A.).

### Sequence alignment and phylogenetic analyses

Non coding 28S nrDNA sequences were manually aligned taking into account the secondary structure, using the software DCSE v.2.60 (Dedicated Comparative Sequence Editor, De Rijk & De Watcher, 1993). Coding *rbc*L sequences were aligned using the Bioedit sequence Alignment Editor (Hall, 1999). Some regions of the 28S alignment, corresponding to ambiguous sites, were removed prior to all analyses. These regions are indicated in the alignment (available on request from the first author).

Two data sets were analysed: the first data set included partial 28S, *i.e.* the 5'end of the gene, and *rbc*L sequences for 30 species. In the alignment of 1886 sites, 61 ambiguous sites were excluded and 1825 sites were finally analysed of which 769 were variable and 570 parsimony informative. The second data set included the complete 28S gene and *rbc*L for 11 species. After removing 205 ambiguous sites, this second data set consisted of 4443 sites of which 1143 were variable and 598 parsimony informative. In both analyses, *Tribonema* (Xanthophyceae = Tribophyceae), the closest available taxon outside the Phaeophyceae, was chosen as an outgroup.

The Partition Homogeneity Test (PHT) (Farris *et al.*, 1995) was used to confirm the congruence between the two genes, analysing the first data set, with the following options: 100 replicates, random 50. The P value ($P = 0.19 > 0.05$) indicated a congruence between the genes.

For both data sets, maximum parsimony (MP), distance (Neighbor-joining, NJ), and maximum likelihood (ML) analyses were done. The nucleotide substitution model used for NJ and ML was first determined using ModelTest

Tab. 1. Algal species included in the phylogenetic study (by alphabetical order within the orders), with GenBank accession numbers for their 28S nrDNA and *rbc*L sequences.
* = present work; a = Xanthophyceae (or Tribophyceae); (C) = Complete sequence.

| *Order* | *Species and authors* | *28S gene GenBank accession number* | *rbcL gene GenBank accession number* |
|---|---|---|---|
| Cutleriales | *Cutleria multifida* (J.E. Smith) Greville | [AY157699] * (C) | [AY157692] * |
| | *Zanardinia prototypus* (Nardo) Nardo | [AY157700] * | [AY157693] * |
| Desmarestiales | *Desmarestia aculeata* (Linnaeus) Lamouroux | [AJ229143] | [AJ287847] |
| | *Desmarestia ligulata* (Lightfoot) Lamouroux | [AJ 287434] | [AJ287848] |
| | *Himanthothalus grandifolius* (A. & E.S. Gepp) Zinova | [AJ287433] | [AJ287850] |
| Dictyotales | *Dictyota cervicornis* Kützing | [AJ287436] | [AJ287851] |
| | *Dictyota dichotoma* (Hudson) Lamouroux | [AF130715] (C) | [AJ287852] |
| Ectocarpales | *Myriotrichia clavaeformis* Harvey | [AJ229138] | [AF055408] |
| | *Pylaiella littoralis* (Linnaeus) Kjellman | [AF071782] | [X55372] |
| | *Scytosiphon lomentaria* (Lyngbye) Link | [D16558] (C) | [AB022238] |
| | *Streblonema maculans* (Hamel) South & Tittley | [AF071784] (C) | [AY157694] |
| | *Striaria attenuata* (Greville) Greville | [Z99478] (C) | [AF055415] |
| Fucales | *Ascophyllum nodosum* (Linnaeus) Le Jolis | [AF053106] | [AJ287853] |
| | *Fucus vesiculosus* Linnaeus | [AF053105] (C) | [AY157695]* |
| | *Sargassum muticum* (Yendo) Fensholt | [AF053109] | [AJ287854] |
| *Incertae sedis* (Onslowiaceae) | *Verosphacela ebrachia* Henry | [AJ287445] | [AJ287867] |
| | *Onslowia endophytica* Searles | [AJ287444] | [AJ287864] |
| Laminariales | *Alaria esculenta* (Linnaeus) Greville | [AF071151] | [AF064745] |
| | *Laminaria digitata* (Linnaeus) Lamouroux | [AF331153] (C) | [AY157696] * |
| | *Macrocystis pyrifera* (Linnaeus) C. Agardh | [AF053116] | [AJ287856] |
| Scytothamnales | *Splachnidium rugosum* (Linnaeus) Greville | [AF331154] (C) | [AJ295834] |
| Sphacelariales | *Alethocladus corymbosus* (Dickie) Sauvageau | [AJ287440] | [AJ287860] |
| | *Choristocarpus tenellus* (Kützing) Zanardini | [AY157701]* (C) | [AJ287962] |
| | *Cladostephus spongiosus* (Hudson) C. Agardh | [AF053115] | [AJ287863] |
| | *Sphacella subtilissima* Reinke | [AJ287447] | [AJ287931] |
| | *Sphacelaria cirrosa* (Roth) C. Agardh | [AF071150] (C) | [AJ287865] |
| | *Stypocaulon scoparium* (Linnaeus) Kützing | [AF091285] | [AJ287866] |
| Syringodermatales | *Microzonia velutina* (Harvey) J. Agardh | [AY157702 ] * (C) | [AY157697] * |
| | *Syringoderma abyssicola* Setchell & Gardner | [AY157703 ] * | [AY157698 ] * |
| | *Syringoderma phinneyi* Henry & Müller | [AY157704 ] * (C) | [AJ287868] |
| Tribonematales [a] | *Tribonema aequale* Pascher | [Y07979] (C) | [AF084611] |

Tab. 2. Algal species (in alphabetical order) used for DNA extraction, preservation method, collecting localities and persons having supplied the samples.

| *Species* | *Preservation method* | *Locality and collecting date* | *Supplied by* |
|---|---|---|---|
| *Choristocarpus tenellus* | Silica gel | Mediterranean sea culture (28 xii 1999) | D.G. Müller |
| *Cutleria multifida* | Silica gel | Pleubian (France) (03 ix 1996) | A. Asensi |
| *Desmarestia aculeata* | Herbarium | Coutainville (France) (17 viii 1999) | F. Rousseau |
| *Fucus vesiculosus* | – 80 °C | Roscoff (France) (6 x 1994) | B. de Reviers |
| *Laminaria digitata* | Silica gel | Roscoff (France) (4 xii 1996) | A. Asensi |
| *Microzonia velutina* | Silica gel | Culture from New Zealand (28 xii 1999) | D.G. Müller |
| *Streblonema maculans* | Freeze drying | Culture from Roscoff (France) (10 iii 1993) | A. Asensi |
| *Syringoderma abyssicola* | Silica gel | Culture from Japan (06 iv 2000) | D.G. Müller |
| *Syringoderma phinneyi* | Silica gel | Culture from British Columbia (Canada) (06 iv 2000) | D.G. Müller |
| *Zanardinia prototypus* | Silica gel | Aigua Freda (Spain) (02 viii 1998) | C. Rodriguez |

Tab. 3. Primers used to amplify and sequence the 28S gene.

| *28S* | *Forward (F) or Reverse (R)* | *Base composition (5'-3')* | *Position / Scytosiphon 28S sequence [D16558]* |
|---|---|---|---|
| C2' | F | GAA AAG AAC TTT GRA RAG AGA GT | 767 |
| D3' | F | CCG YGG CGC AAT GAA AGT GA | 1191 |
| C4' | F | TAG TAG CTG GTT CCC TCC GA | 1299 |
| C4 | R | TCG GAG GGA ACC AGC TAC TA | 1318 |
| C6 | R | TGC GTT GTT ACA CAC TCC TTA GC | 1613 |
| C6' | F | TCA CCT GCC GAA TCA ACT AGC | 1638 |
| C7 | R | ACT ACC ACCA AGA TCT GCA C | 1803 |
| D7' | F | CCG GCG AGA GTT NTC TTT TCT | 2016 |
| C8' | F | AAC TTC GGG ATA AGG ATT GGC TC | 2257 |
| C8 | R | CCT CAG AGC CAA TCC TTT TC | 2284 |
| D8 | R | ATT CCC CTT GCC GCT CCA GTT | 2452 |
| C9' | F | AAC GGC GGG AGT AAC TAT GA | 2568 |
| C9 | R | GTC TTCT TTC CCC GCT GAT T | 2721 |
| D10' | F | GGG CGGC ACA TCT GTT AA | 2935 |
| D10 | R | AGG ACAC CTG TGT TAT CGT TT | 2971 |
| C11' | F | CGA TGTC GGC TCT CCT ATC | 3180 |
| C11 | R | AAC CTGT CTC ACG ACG GTC T | 3284 |
| D12 | R | ACA AAG CCT ACT CTC ATG CTT AC | 3612 |

Tab. 4. Primers used to amplify and sequence the *rbc*L gene.

| *Primer name* | *Sequence (5'-3')* | *Forward (F) or reverse (R)* | *Approximate annealing position* |
|---|---|---|---|
| A32 | TAA AAA GTG ACC GTT ATG AAT CTG | F | 32 |
| F68 | GCN AAA ATG GGN WAY TGG GAT GC | F | 68 |
| A630 | TAA CTC WCA ACC ATT CAT GCG | F | 630 |
| R641 | TTC TCT CCA ACG CAT GAA TG | R | 641 |
| R1015 | TTT AAC CAT TAA AGG ATC TCC TTC | R | 1015 |
| R1381 | ATA TCT TTC CAT ARR TCT AAW GC | R | 1381 |

version 3.06 (Posada & Crandall, 1998), which suggested the general time reversible model of DNA substitution (Rodriguez *et al.*, 1990), following a gamma distribution and invariable sites. In all analyses, gaps were considered as missing characters. For MP and ML analyses, a general heuristic search was done using PAUP version 4.0b3a (Swofford, 1998) using TBR branch swapping. For MP analyses one hundred random taxon addition replicates were performed in each heuristic search. For distance analyses, the Neighbor-joining algorithm (Saitou & Nei, 1987) was used to reconstruct the tree.

The robustness of trees was assessed for all analyses using bootstrap proportions (Felsenstein, 1985). For MP and NJ methods, 1000 bootstrap replications were performed and for ML method, only 100 bootstrap proportions were performed due to computing time. Decay indices were obtained for the MP tree using the software TreeRot (Sorenson, 1999).

## RESULTS

For the first data set, four most parsimonious trees were obtained with a consistency index (CI) of 0.441, a retention index (RI) of 0.546 and a length of 2698 steps. The strict consensus of these trees was calculated. For the second data set, a single most parsimonious tree of 2295 steps was obtained with a CI of 0.6771 and a RI of 0.4429. For each data set, the same topology was obtained regardless of the method used (MP, NJ, ML), and therefore only the MP consensus trees are shown (Figs 1, 2).

After sequencing the complete 28S gene for *Choristocarpus tenellus* we noted that the 5' end of our 28S sequence appeared very different from that determined by Draisma *et al.* (2001). This ambiguity may have been caused by contamination and may explain why Draisma *et al.* (2001) found the two genes to be incongruent and the taxonomic position of *Choristocarpus* unclear: *i.e.* this taxon diverged either first at the base of the Phaeophyceae using the *rbc*L gene, or within the Sphacelariales clade using ribosomal DNA. Consequently, to compare the *rbc*L sequence with the available one used by Draisma *et al.* (2001) [GenBank accession number: AJ287861], we partially sequenced the *rbc*L gene of *Choristocarpus tenellus* and, this time, both sequences were similar. Therefore we used our 28S sequence and the *rbc*L sequence available on GenBank.

Our results show that the genus *Choristocarpus* always diverges first, at the base of the phaeophycean clade, followed by the Dictyotales, the Sphacelariales, and then by a clade formed by *Microzonia* and *Syringoderma* species. Theses first divergences were supported by high bootstrap and decay indices values (see Figs 1 and 2).

In the present study, *Microzonia* appears as a sister taxon of the genus *Syringoderma*. This node is supported by a bootstrap value of 100 in all analyses, and a decay index of 31-32 according to the considered data set. For the data set including the type of the genus, *Syringoderma abyssicola* (Setchell & Gardner) Levring, the two species of *Syringoderma* appear as a monophyletic group, and as the sister taxon of *Microzonia*. Nevertheless, the position of the clade, which contains *Syringoderma* and *Microzonia*, is better supported by high bootstrap and decay index values in the second data set than in the first one. This may be due to the higher information contained in the complete 28S nrDNA or it may be caused by the lower number of species in the second data set.

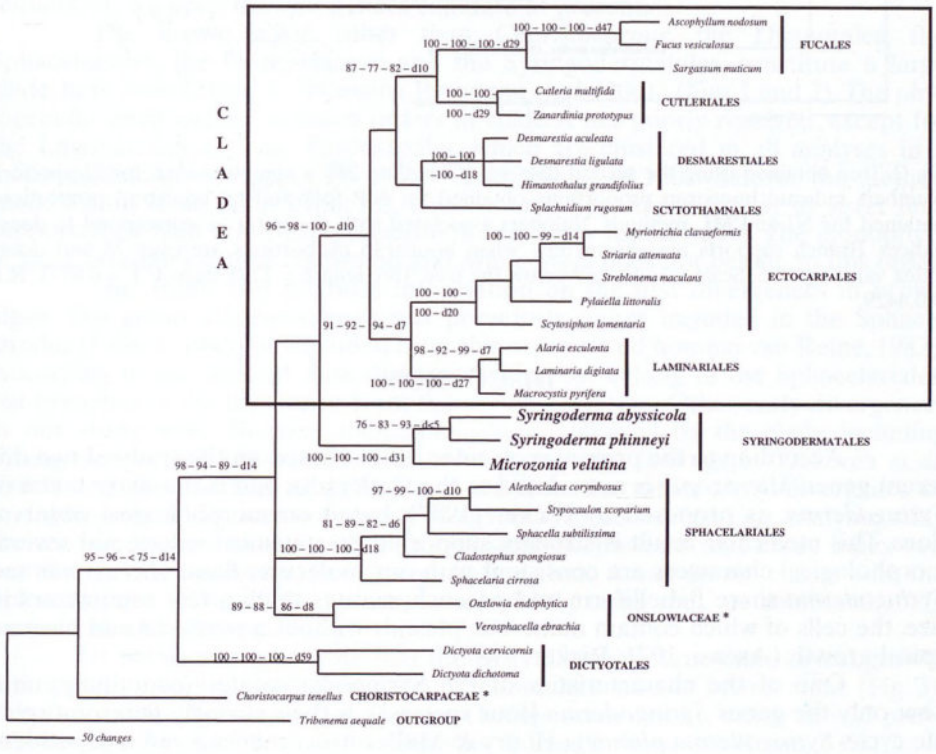


Fig. 1. Strict consensus tree of four equally parsimonious trees obtained using the first data set (partial 28S + complete *rbc*L for 30 species). Numbers indicate bootstrap proportions obtained for MP, followed by bootstrap proportions obtained for NJ and ML methods. Numbers associated with the letter 'd' correspond to decay indices. Branch supports are shown only when bootstrap proportions are over 75 and decay index values over 5. Scale is indicated below the tree. Tree length = 2698 steps, C.I. = 0.441, R.I. = 0.546. (*) = no order name available yet.

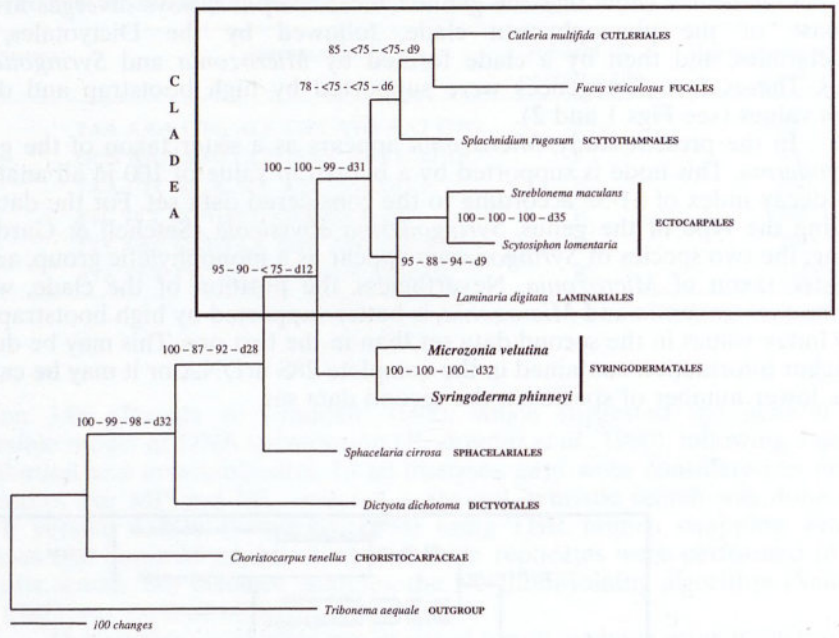


Fig. 2. Tree obtained using the second data set (Complete 28S + complete *rbc*L for 11 species). Numbers indicate bootstrap proportions obtained for MP, followed by bootstrap proportions obtained for NJ and ML methods. Numbers associated with the letter 'd' correspond to decay indices. Branch supports are shown only when bootstrap proportions are over 75 and decay index values over 5. Scale is indicated below the tree. Tree length = 2295 steps, C.I. = 0.6771, R.I. = 0.4429.

## DISCUSSION

According to the present molecular results, based on the study of two different genes, *Microzonia* is not related to the Cutleriales and is the sister taxon of *Syringoderma*, as proposed by Ricker (1987), based on morphological observations. This molecular result is strongly supported by statistical values and several morphological characters are consistent with our molecular data. *Microzonia* and *Syringoderma* share flabelliform and parenchymatous thalli a few centimeters in size, the cells of which contain numerous plastids without a pyrenoid and have an apical growth (Asensi, 1971; Ricker, 1987).

One of the characteristics of the Syringodermatales (containing, until now, only the genus *Syringoderma* [four species]), is their strongly heteromorphic life cycle. *Syringoderma phinneyi* Henry & Müller has a reduced and independent gametophyte; *Syringoderma abyssicola* and *Syringoderma floridana* Henry have a non-independent and short-lived gametophyte, and the poorly known *Syringoderma australe* Levring apparently has a direct life cycle (Henry, 1984). The life cycle of *Microzonia* remains unknown; this may reflect the fact that either the gametophytic phase is extremely reduced as in *Syringoderma abyssicola* and *Syringoderma floridana*, or sexuality is missing as in *Syringoderma australe* (Henry, 1984). Further, *Syringoderma abyssicola* has sessile unilocular organs and

numerous filaments perpendicular to the dorsal face of the thallus (Walker & Henry, 1978) and *Syringoderma australe* bears unilocular organs on such filaments (Levring, 1940), while *Syringoderma phinneyi* (Henry & Müller, 1983) and *Syringoderma floridana* (Henry, 1984) have unilocular organs directly on the thallus surface. *Microzonia* has unilocular sporangia on branched filaments (Agardh, 1894; O'Donnell, 1954), which are probably homologous structures of those in *Syringoderma*. We noted a high diversity in the number of spores within the Syringodermatales: the sporangia of *Syringoderma abyssicola* contain 8 to 16 flagellated spores (Kawaï & Yamada, 1990), those of *Syringoderma floridana* probably more than 16 (Henry, 1984), and those of *Syringoderma phinneyi* more than 100 (Henry & Müller, 1983). The number of spores of *Syringoderma australe* is presently unknown. The sporangia of *Microzonia* contain 16 to 32 spores and perhaps as many as 64 (O'Donnell, 1954).

The molecular and morphological characters are congruent and we therefore propose to include the genus *Microzonia* in the Syringodermatales. Because the life cycle of *Microzonia* is unknown despite culture studies (Asensi and Müller, com. pers.), because of the presence of apical hairs in *Microzonia*, which were not observed in *Syringoderma*, and because *Syringoderma australe* was not sequenced, we keep the two genera separate at present.

The brown algae other than *Choristocarpus*, the Dictyotales, the Sphacelariales, the Onslowiaceae and the Syringodermatales, constitute a large clade, here called clade A, following Rousseau *et al.* (2001) (Figs 1 and 2). The phylogenetic relationships between orders in clade A are poorly resolved, except for the Laminariales and the Ectocarpales, which are clustered in all analyses in a monophyletic group supported by bootstrap values of 100 whatever the method used. Within clade A, as expected, *Cutleria* and *Zanardinia* appear as sister taxa supported by high bootstrap proportions and decay index values. The monophyly of the Cutleriales is thus confirmed and *Microzonia* does not belong to this order.

Our study also provides information on the first divergences in brown algae. The genus *Choristocarpus* was previously either included in the Sphacelariales (Fritsch, 1945) or excluded from this order (Prud'homme van Reine, 1982). According to our present data this taxon does not belong to the Sphacelariales, but branches as the first taxon from the brown algal tree. Other early divergences in our study were *Dictyota* then *Sphacelaria*, followed by the clade including *Syringoderma* and *Microzonia*. This corroborates the findings by Draisma *et al.* (2001) and Rousseau *et al.* (2001) and confirms the rather early divergence of *Syringoderma*, which was not clearly resolved in those studies. Our analysis, using the second data set, shows with high bootstrap proportions and decay index values that the Syringodermatales diverge before clade A and do not cluster with the Sphacelariales, as found by Draisma *et al.* (2001) using *rbc*L or combined *rbc*L, partial 28S and 18S sequences.

It is interesting to note that the early diverging taxa share apical growth and contain several plastids without a pyrenoid, like most of brown algae (Fig. 3). The Dictyotales, Sphacelariales and the Onslowiaceae family, which should probably be placed in their own order (Draisma & Prud'homme van Reine, 2001), have sexual life histories with isomorphic phases. In *Choristocarpus*, thalli with either uni- or plurilocular organs are known (Fritsch, 1945); these thalli look similar and could also indicate an isomorphic life cycle. This kind of life cycle is thus probably the ancestral condition in brown algae (Fig. 3). It will be interesting to examine other small, rare, and poorly known brown algal taxa such as *Discosporangium* or *Zosterocarpus* for their position relative to the early divergences in the brown algae tree, before establishing the Onslowiales and Choristocarpales.

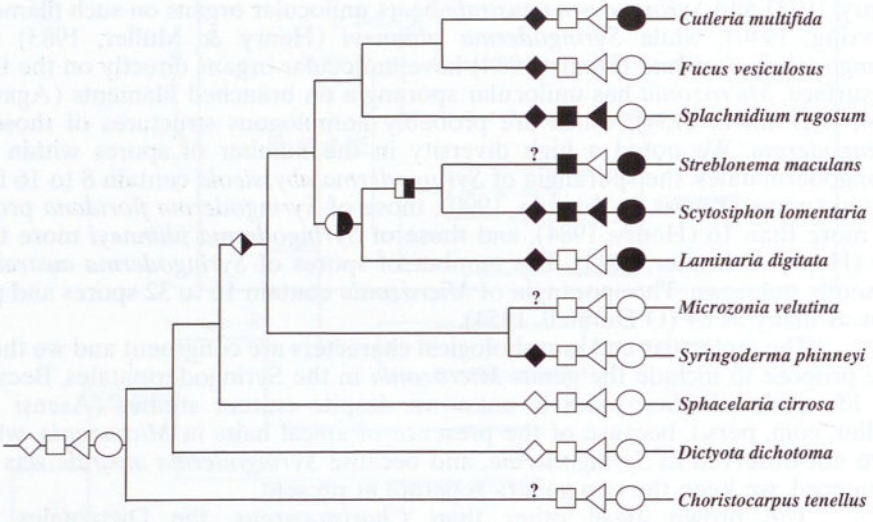


Fig. 3. Phylogenetic tree using the second data set (complete 28S + complete *rbc*L for 11 species) and showing morphological characters. Life cycle isomorphic (◇) or heteromorphic (◆); pyrenoid absent (□) or present (■); plastids several (◁) or only one (◀); growth apical (○) or intercalary (●). ? = life cycle unknown or uncertain: see the text for *Choristocarpus tenellus* and *Microzonia velutina*; for '*Streblonema maculans*', unpublished results show that our strain is related neither to Punctariaceae (of which it might be a phase in the life cycle) nor to other *Streblonema* species for which sequences are known, and therefore its life cycle is considered unknown.

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