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Characterization and phylogenetic affinities of the red alga *Chondrophycus flagelliferus* (Rhodomelaceae, Ceramiales) from Brazil on the basis of morphological and molecular evidence

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A detailed study of the vegetative and reproductive morphology of *Chondrophycus flagelliferus* from Brazil is provided. The species possesses axial segments, each bearing two periaxial cells, a situation characteristic for the genus *Chondrophycus*. Within *Chondrophycus*, *C. flagelliferus* belongs to the subgenus *Palisadi*, section *Palisadi*, on the basis of the presence of a palisade-like outer cortical cell layer as seen in transverse sections of branchlets; the absence of secondary pit connections between cortical cells; the fertile periaxial cell with two pre-sporangial cover cells, the tetrasporangium initial and the post-sporangial cover cell that will develop into the corticating system; and the right-angled tetrasporangial arrangement. The phylogenetic position of this species within *Laurencia sensu lato* is inferred from parsimony and Bayesian analyses of chloroplast-encoded *rbc*L sequences from 39 Rhodomelaceae using two Ceramiaceae as the outgroup. This study corroborates the taxonomic decision to split *Laurencia sensu lato* in the genera *Laurencia*, *Chondrophycus* and *Osmundea*, and indicates that *rbc*L provides sufficient phylogenetic signal to infer species-level relationships within the *Laurencia sensu lato* complex. Synapomorphic morphological characters uniting *Laurencia* and *Chondrophycus* include the same origin of the spermatangial filaments and tetrasporangia. The principal character separating both genera is the number of periaxial cells per vegetative axial segment. We hypothesize that the ancestor of the *Laurencia sensu lato* complex most likely possessed two periaxial cells per axial segment. The molecular data indicate that *C. flagelliferus* is closely related to the *C. papillosus* complex, and that, as originally described, *C. translucidus* belongs in the genus *Laurencia*.

KEY WORDS: Algae, Brazil, Ceramiales, *Chondrophycus flagelliferus*, Molecular systematics, Phylogeny, *rbc*L, Rhodomelaceae, Rhodophyta, Taxonomy

INTRODUCTION

The genus *Laurencia* J.V. Lamouroux (1813) has previously been split up in three distinct genera on the basis of a combination of tetrasporangial and vegetative characters: *Laurencia*, *Chondrophycus* (Tokida & Saito) Garbary & J. Harper, and *Osmundea* Stackhouse. The subgenus *Chondrophycus* Tokida & Saito in Saito (1967) was elevated to generic rank as *Chondrophycus* (Tokida & Saito) Garbary & J. Harper (1998) on the basis of the presence of vegetative axial segments, each bearing two rather than the four periaxial cells that characterize *Laurencia*, and by the production of additional tetrasporangial periaxial cells that are absent in the latter. Both genera are distinguished from *Osmundea* by the production of 'trichoblast-type' spermatangial branches and by tetrasporangia originating from a particular periaxial cell (Nam *et al.* 1994). In *Osmundea*, the spermatangial branches originate directly from apical and cortical cells – i.e. are of the 'filament-type' and the tetrasporangia are produced from random cortical cells.

About 23 species of *Laurencia sensu lato* (including *Laurencia*, *Chondrophycus* and *Osmundea*) have been described for Brazil (Taylor 1960; Oliveira Filho 1969, 1977; Cordeiro-Marino *et al.* 1983, 1994; Cordeiro-Marino & Fujii 1985; Yoneshigue 1985; Fujii & Cordeiro-Marino 1996; Wynne 1998; Fujii & Villaça 2003; Yoneshigue-Valentin *et al.* 2003). However, only a few species besides *Chondrophycus translucidus* (Fujii & Cordeiro-Marino) Garbary & J. Harper have been adequately characterized and illustrated (Fujii & Cordeiro-Marino 1996, as *Laurencia translucida*).

The combination *C. flagelliferus* (J. Agardh) K.W. Nam was provided by Nam (1999), but details of this species' morphology were not included. In this paper, we provide new information on the vegetative and reproductive morphology of *C. flagelliferus*, discuss its geographic distribution, and assess its phylogenetic position as inferred from *rbc*L sequence analysis. *rbc*L in the red algae is a 1467 base-pair-long, chloroplast-encoded gene that codes for the large subunit of ribulose 1,5-bisphosphate carboxylase-oxygenase; *rbc*L sequences have been successfully and widely used to generate reliable phylogenetic hypotheses in red algae at different levels of biological organization (e.g. Gelidiales: Freshwater *et al.* 1995; Ceramiales/Delesseriaceae: Lin *et al.* 2001; Hildenbrandiales: Sherwood & Sheath 2003; Gracilariales: Gurgel & Fredericq 2004).

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MATERIAL AND METHODS

Morphological analyses

Voucher specimens and materials for morphological studies were fixed in 4% formalin/seawater and stored in 4% formalin/seawater or pressed as herbarium sheets. Specimens of *C. flagelliferus* were collected along the Brazilian coast encompassing the state of Ceará (3°16′S and 39°10′W) to Santa Catarina (27°14′S and 48°30′W). Living specimens were also examined to check for the presence of '*corps en cerise*'. Longitudinal and transverse hand sections were made with a stainless steel razor blade under a stereoscopic dissection microscope and stained with 0.5% aqueous aniline blue solution acidified with 1 N HCl (Tsuda & Abbott 1985). For critical developmental observations, specimens were stained with a modified Wittmann's aceto-iron-hematoxylin-chloral hydrate solution (Fujii & Guerra 1997). Measurements are given as length × diameter. Photomicrographs were taken with an Olympus BH-2 microscope. Drawings were made with the aid of a Zeiss camera lucida. Voucher specimens are deposited in the herbarium of the Instituto de Botânica, São Paulo, Brazil (SP). The spelling of the generic and subgeneric names within the *Laurencia sensu lato* complex follows that of Furnari *et al.* (2001, 2002).

Representative specimens of *C. flagelliferus* from Brazil were examined for morphological study: Ceará State, Trairi, Guajiru beach (F.P. Joventino, August 1981, SP 295121); Rio Grande do Norte State, Natal, Meio beach (M.T. Fujii, 25 May 1986, SP 295027); Pernambuco State, Ipojuca, Serrambi beach (A.L.M. Cocentino, 1992, SP 295111); Alagoas State, Maceió, Pratagi beach (E.C.A. Guedes, August 1989, SP 295124); Bahia State, Camaçari, Arembepe beach, (J.M.C. Nunes, 04 April 1996, SP 317279); Espírito Santo State, Guarapari, Peracanga beach (M.T. Fujii & S.M.P.B. Guimarães, 26 October 1996, SP 295049), Anchieta, Parati beach (M.T. Fujii & S.M.P.B. Guimarães, 30 April 1991, SP 295054); São Paulo State, Ubatuba, Itaguá beach, (M.T. Fujii, 15 October 1997, SP 295114), São Sebastião, Cigarras beach, (M.T. Fujii, 14 November 1986, SP 295045), Itanhaém, Peruíbe beach, (M.T. Fujii, 26 March 1986, SP 295044); Santa Catarina State, Porto Belo, Zimbros beach, (L.C. Ouriques, 27 April 1994, SP 294979).

Molecular analyses

Samples used in the molecular studies were desiccated in silica gel in the field. Silica gel–dried specimens and extracted DNA samples of species worldwide are deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at −20°C. Taxa used in the molecular study are listed in the Appendix with their current names and *rbc*L GenBank accession number.

DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). Protocols for gene amplification, automated sequencing and alignment are identical to those given in Lin *et al.* (2001). Polymerase chain reaction and sequencing primers used in this study were *Frbc*L start, F7, F57, F492, F577, F753, F993, R753, R1381 and *Rrbc*S start, as listed in Freshwater and Rueness (1994).

Phylogenetic analyses of the *rbc*L sequence data set of 41 DNA sequences were performed using the maximum parsimony and Bayesian methods available in the computer programs PAUP* v.4.0 beta 10 (Swofford 2000) and MrBayes v.3.0 beta 4 (Huelsenbeck & Ronquist 2001), respectively. The first 39 nucleotides of all *rbc*L sequences were removed from the data set because many sequences were incomplete at the 3′ end. Parsimony trees obtained under the Fitch criterion of equal weights for all substitutions (Fitch 1971) were inferred in a two-part search scheme, excluding uninformative characters. An initial heuristic search designed to increase the likelihood of swapping within the 'island' of trees leading to the most parsimonious solution (Maddison 1991) consisted of 5000 random sequence additions holding 25 trees at each step, MULPARS and Tree-Bisection-Reconnection algorithms with MULTREES (saving multiple trees) options in effect. Most parsimonious trees found in this first heuristic search were then used as starting points for further searches with MULPARS, MULTREES and STEEPEST DESCENT options in effect until swapping was complete. Support for nodes of parsimony trees was determined by calculating bootstrap proportion values (Felsenstein 1985) on the basis of 1000 resamplings done with the MP settings described above but using the 'simple' stepwise addition option. Outgroup species were selected on the basis of close phylogenetic relationship with the ingroup.

The model used in the Bayesian analysis was the general-time-reversible model (GTR) of nucleotide substitution with gamma-distributed rates for the variable sites, with a percentage of the sites considered invariable (GTR+gamma+inv). This model was selected in both the maximum likelihood ratio test and the Akaike information criterion as implemented by the software Modeltest version 3.7 (Posada & Crandall 1998) with a significance level of 0.01. For the Bayesian analysis, we ran four chains of the Markov chain Monte Carlo (one hot and three cold), sampling 1 tree every 100 generations for 1,000,000 generations starting with a random tree. Stationarity was reached at generation 25,000; therefore trees saved until this generation were discarded as the 'burn in' of the chain. Inferences about the phylogeny were based on those trees sampled after generation 25,000. A 50% consensus tree (majority rule as implemented by PAUP*) was computed from the 9750 + 1 trees saved after the 'burn in' point.

When presented, the range of *rbc*L divergence values within and among species was calculated using uncorrected percentages including all characters (= total number of pairwise substitutions divided by the total number of base pairs sequenced in both sequences).

RESULTS

Chondrophycus flagelliferus (Tokida & Saito) K.W. Nam (1999, p. 463)

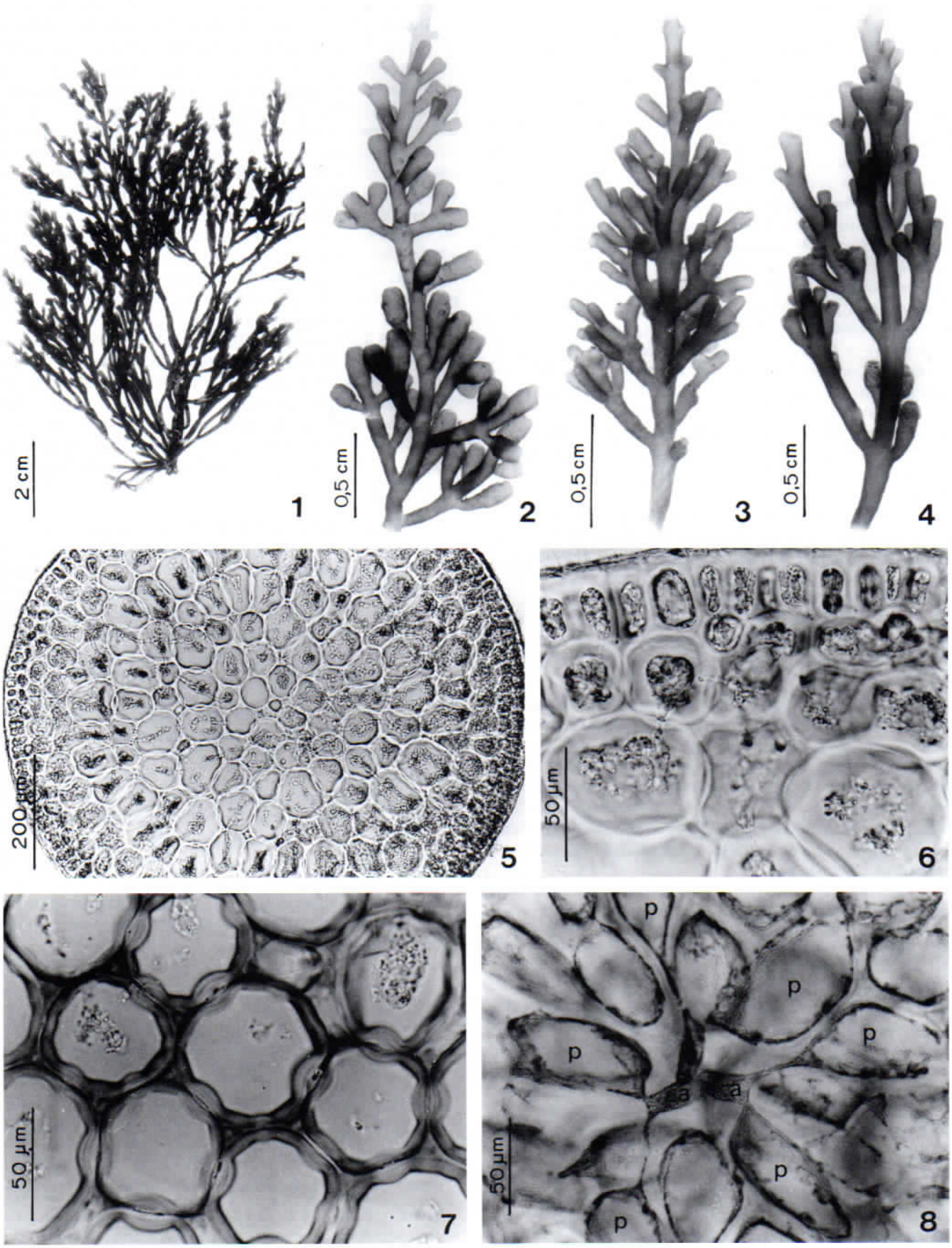
Figs 1–19

BASIONYM: *Laurencia flagellifera* J. Agardh (1852, p. 747).

TYPE: LD 36604–36606!

TYPE LOCALITY: 'ad oras Indiae orientalis' (Indonesia)

HABITAT: Thalli up to 13 cm high, terete, cartilaginous, rigid, not adhering to herbarium paper when dried. Color brown, greenish brown, violet-brown or dark brown. Plants forming


2 cm
1
0,5 cm
2
0,5 cm
3
0,5 cm
4
200 µm
5
50 µm
6
50 µm
7
p
p
p
p
a
a
50µm
p
8

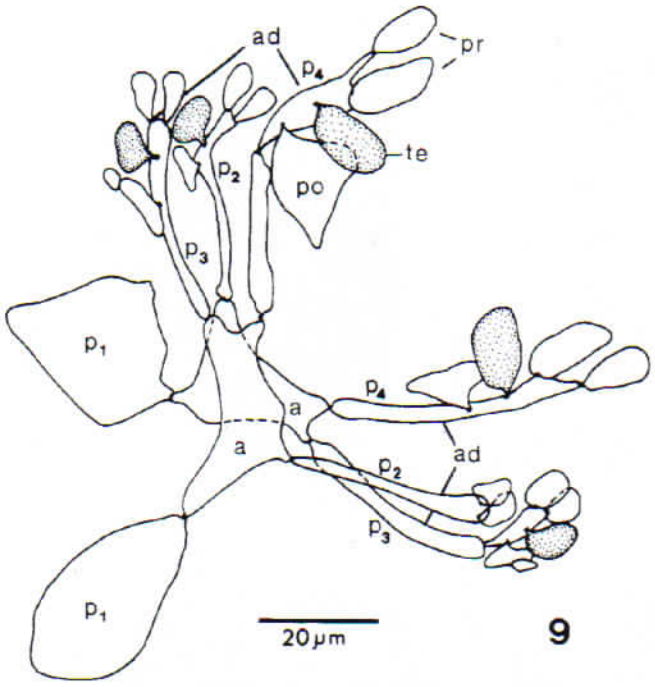


Fig. 9. *Chondrophycus flagelliferus* (SP 295114). Transverse section near the apex of tetrasporangial branchlet, showing each axial segment (a) with one existing (p2) and two (p3-p4) additional fertile periaxial cells (ad). Each fertile periaxial cell with two pre-sporangial cover cells (pr), tetrasporangium initial (te), and post-sporangial cover cell (po).

dense tufts with axes measuring 1–1.5 mm in diameter. Ultimate branchlets short and compound or long and isolated, cylindrical-clavate, 1–6 mm long and up to 800 µm in diameter. Upright axes attached to substratum by two to three descending basal branches terminating into small discoid holdfast and by stolon-like branches that function as secondary attachment organs. Branching alternately spiral to irregular, dense in the upper thallus portions and scant or naked in the lower parts (Figs 1–4). Apical cell sunk in apical pit of branchlet.

VEGETATIVE STRUCTURES: Cortical cells in the middle thallus region isodiametric-polygonal as seen in surface view, 18–40 × 17–30 µm. '*Corps en cerise*' absent. In cross-section, thallus with one or two layers of pigmented cortical cells; surface cortical cells radially elongated and arranged as palisade, 34–56 × 16–24 µm, with inner cells larger and rounded, 30–56 × 28–34 µm (Figs 5–7). Secondary pit connections among cortical cells absent (Fig. 6). Additional cortical cell layers occasionally formed in old thalli. Medullary region with four or five layers of rounded colorless cells becoming smaller toward the thallus center, 100–136 × 80–110 µm. Medullary cells walls thickened (Fig. 7). Each vegetative axial segment cutting off two periaxial cells (Fig. 8); periaxial cells colourless, round, 80–90 × 64–82 µm, slightly smaller than cells in the surrounding layers. Intercellular spaces and lenticular thickenings absent. In median longitudinal sections through a branchlet, outer cortical cell walls near apex not projecting beyond the surface.

REPRODUCTIVE STRUCTURES: Tetrasporangial branches short and compound or long and isolated, 2.0–6.0 mm long × 810–1270 µm in diameter (Fig. 4). At the apex of fertile branches, each axial segment producing two additional periaxial cells in opposite position to the existing two normal periaxial cells. Each of the additional periaxial cells and the second normal periaxial cell becoming fertile and cutting off two pre-sporangial cover cells distally and the tetrasporangial initial subdistally in abaxial position. Subsequently, one post-sporangial cover cell is produced and by continuous dividing it develops in the cortical cell system (Figs 9, 10). The pre-sporangial cover cells do not divide and display a transverse-type alignment in relation to the fertile axis in surface view. Tetrasporangial maturation is in a clockwise spiral and the final arrangement is in right-angle type in relation to fertile branchlets (Figs 11, 12). Mature tetrasporangia are tetrahedral, 55–65 × 38–45 µm.

In female thalli, each procarp-bearing segment produces five pericentral cells, the fifth of which becomes the supporting cell of a four-celled carpogonial branch (Figs 13, 14). Details of the post-fertilization stages (Figs 14, 15) are basically identical to those described in *Chondrophycus translucidus* (M.T. Fujii & Cordeiro-Marino) Garbary & J. Harper (Fujii & Cordeiro-Marino 1996). Carposporangia are clavate, 123–170 × 31–44 µm. Fully developed cystocarps are subconical with protuberant ostiole, 975–1170 µm in diameter, with the lower half immersed within the parent branch (Figs 3, 16, 17).

Fertile male branches are characteristically swollen, 1.5–3.0 mm in diameter (Fig. 2). In longitudinal sections through a fertile branchlet, the spermatangial pits are cup-shaped and an axial cell row is discernible at the base (Fig. 18). Spermatangia are produced by fertile trichoblasts terminating in a vesicular sterile cell, 16–46 × 19–35 µm; spermatia ovoid, 9.8–12.5 × 5.7–6.4 µm with a large nucleus at their tips (Fig. 19).

*rbc*L ANALYSIS: The entire *rbc*L data set (i.e. including outgroup sequences) contained 1428 characters of which 543 varied, and 424 were parsimony informative. Tree lengths of 100,000 randomly generated trees had a skewed distribution ($g = -0.4892$, $P < 0.01$), indicating the presence of non-random structure (Hillis & Huelsenbeck 1992). MP analyses

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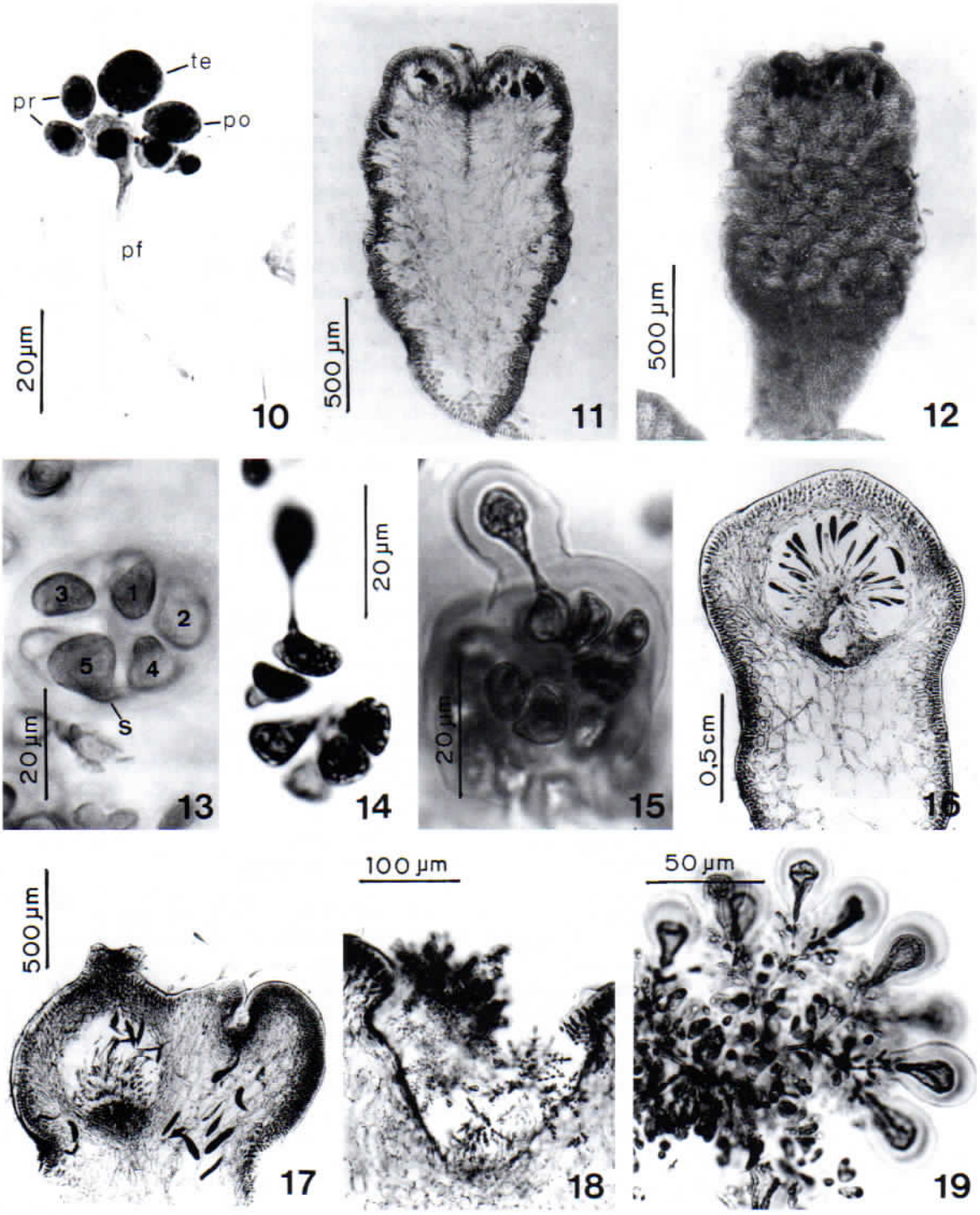
Figs 1–4. *Chondrophycus flagelliferus* (SP 295045).
Fig. 1. Habit.
Fig. 2. Male branches.
Fig. 3. Female branches.
Fig. 4. Tetrasporangial branches.
Figs 5–8. *Chondrophycus flagelliferus* (SP 295114).
Fig. 5. Cross-section through thallus.
Fig. 6. Longitudinal section through thallus showing cortex.
Fig. 7. Detail showing medullary cells with thickened cell walls.
Fig. 8. Three superimposed axial cells (a), each of them bearing two periaxial cells (p).



Figs 10–19. *Chondrophycus flagelliferus* (SP 295049).
Fig. 10. Fertile periaxial cell (pf) with two pre-sporangial cover cells (pr), tetrasporangium initial (te), and post-sporangial cover cell (po) that will develop into the corticating system.
Fig. 11. Longitudinal section through tetrasporangial branchlet, showing right-angle arrangement of the tetrasporangia.
Fig. 12. Tetrasporangial branchlet in surface view.
Fig. 13. Procarp-bearing segment with five pericentral cells, the fifth becoming the supporting cell.
Figs 14–15. Stages of carpogonial branch development.
Fig. 16. Longitudinal section through cystocarp.
Fig. 17. Detail of female branch with partly immersed cystocarp.
Fig. 18. Male branch with spermatangial branches in cup-shaped apical pit.
Fig. 19. Detail of terminal part of mature spermatangial branches.

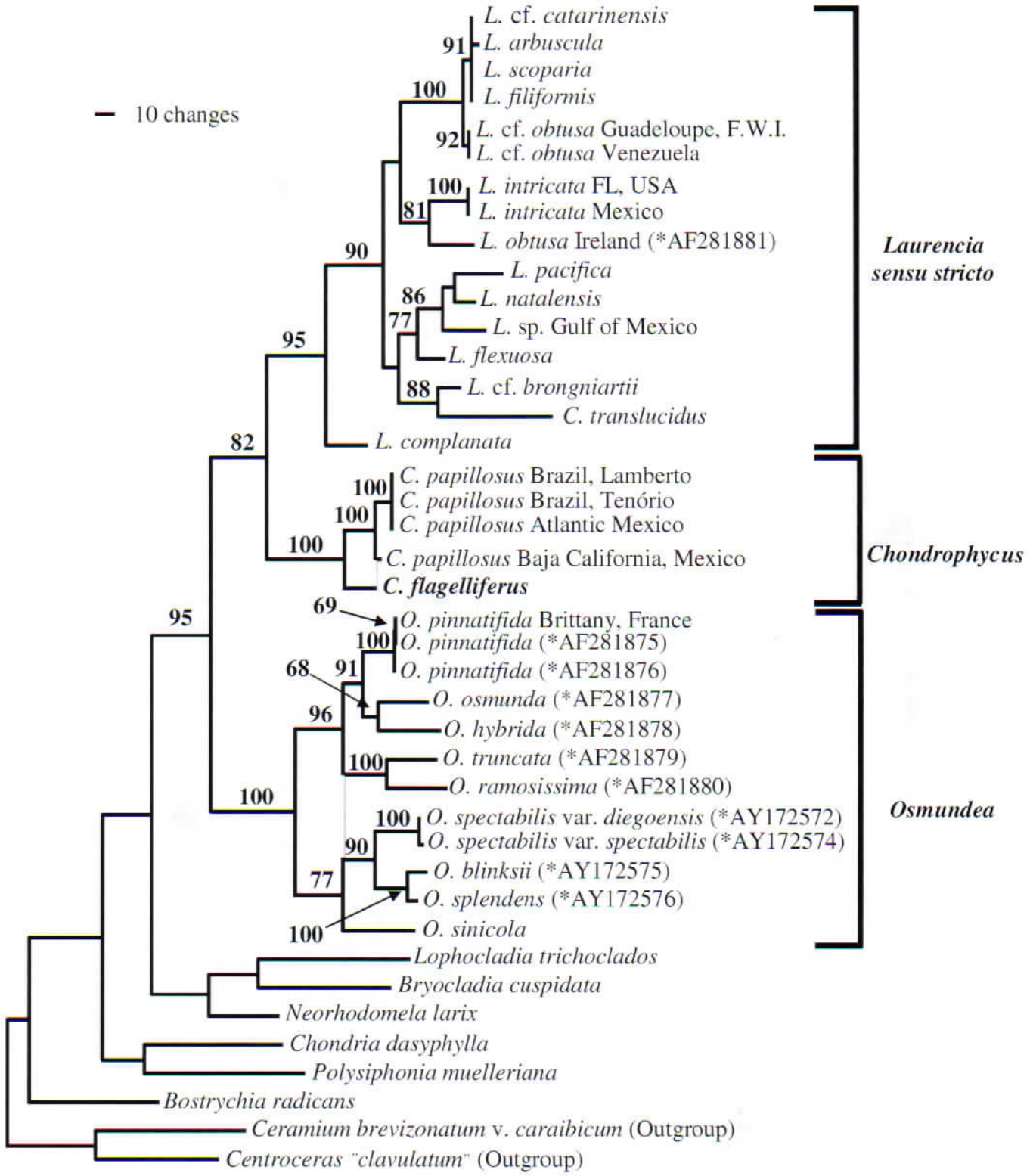


Fig. 20. Phylogenetic position of *Chondrophycus flagelliferus* within the *Laurencia sensu lato* complex. One of 78 most-parsimonious trees based on the analysis of 41 Ceramiales *rbc*L DNA sequences, 39 of which are Rhodomelaceae. Tree length = 1697 evolutionary steps, consistency index = 0.393, retention index = 0.654, number of parsimony-informative characters included = 443 (with outgroups). Sequences with GenBank numbers marked with an * are from McIvor *et al.* (2002).

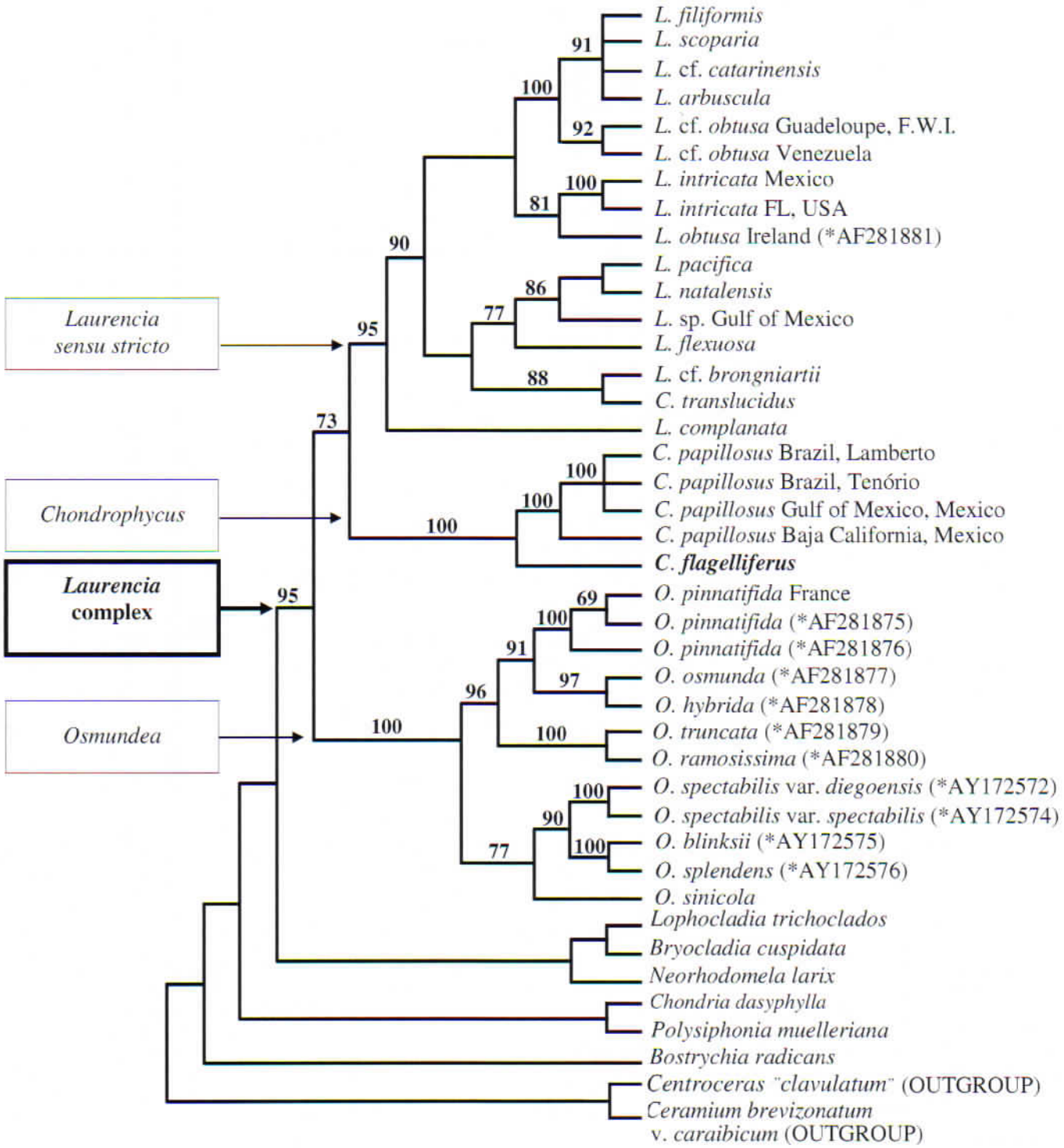


Fig. 21. Phylogenetic position of *Chondrophycus flagelliferus* within the *Laurencia sensu lato* complex based on a Bayesian analysis of *rbc*L DNA sequence. Fifty percent majority-rule consensus of 98,317 + 1 trees sampled after the run reached stationarity at generation 16,830 (total number of generations run = 1.0×10^6). Evolutionary model used in the Bayesian analysis was the GRT+Γ, selected by a maximum likelihood ratio test. Sequences with GenBank numbers marked with an * are from McIvor *et al.* (2002).

resulted in 39 equally MPT of 1637 steps (tree length), consistency index = 0.384, and retention index = 0.658.

In both analyses the *Laurencia sensu lato* assemblage contained three distinct clades, with high support, corresponding to the genera *Chondrophycus, Laurencia sensu stricto* and *Osmundea* (bootstrap proportion values: 100, 90, 100; Bayesian posterior possibilities: 100, 100, 100, respectively). In all phylogenetic trees, the genus *Chondrophycus* is sister to the genus *Laurencia, C. translucidus* pertains to genus *Laurencia* instead of to *Chondrophycus*, and *C. flagelliferus* is sister to the *C. papillosus* complex. The taxon known as *C. papillosus* from the Baja California, Mexico, is a species distinct from

the Atlantic entity (Figs 20, 21). The relationships among the *C. papillosus* sequences from the Atlantic and the taxa within the *L. arbuscula* clade are not resolved in either analysis because of the absence or lower levels of genetic variation among these sequences.

DISCUSSION

Chondrophycus flagelliferus was originally described by J. Agardh (1852, as *Laurencia*) on the basis of a specimen from Indonesia ('ad oras Indiae orientalis'). The species presents all generic features as defined by Nam (1999) and it is easily recognized in the field by its cartilaginous and loosely branched thalli. Anatomically the species can be characterized by a set of features, such as lacking pit connections between epidermal cells, two pericentral cells per each vegetative axial segment, right-angle arrangement of tetrasporangia, and the presence of radially elongated outer cortical cells arranged in palisade as seen in cross-section. On the basis of this taxonomical evidence, *C. flagelliferus* clearly belongs to the subgenus *Palisadi*, section *Palisadi* (Nam 1999). Additional members of the section *Palisadi* includes *C. palisadus* (Yamada) K.W. Nam, *C. intermedius* (Yamada) Garbary & J. Harper, *C. capituliformis* (Yamada) Garbary & J. Harper, *C. dinhii* (Masuda & Kogame) K.W. Nam, *C. concretus* (Cribb) K.W. Nam, and *C. tronoi* (Ganz.-Fort.) K.W. Nam.

Among the species of the section *Palisadi*, *C. intermedius* is taxonomically the closest related to *C. flagelliferus* on the basis of comparative morphology (pers. obs.). The latter species has been occasionally cited as occurring in the Pacific Ocean, such as southeast Queensland, Australia (Cribb 1958), Hawaii (Saito 1969), and in the Indian Ocean (Rodriguez Island) (Silva *et al.* 1996). On the other hand, *C. intermedius* is the most widespread species in these regions, with a distribution ranging from Japan (Yamada 1931, Saito 1967, Nam & Saito 1995), Korea (Kang 1966, Nam & Saito 1995), the Philippines (Cordero 1977) and Tanzania (Jaasund 1970) to Reunion Island (Silva *et al.* 1996). Whereas *C. flagelliferus* is the most common species of the *Laurencia* complex in Brazil, it seems that *C. intermedius* occupies the same niche in the other regions of the world.

Chondrophycus intermedius can be differentiated from *C. flagelliferus* by the presence of short papilliform branchlets in the former, whereas they are elongate and non-papilliform in the latter. Besides, procarp structure in *C. flagelliferus* is different from that in the majority of species belonging to Nam's (1999) section *Palisadi*. *Chondrophycus flagelliferus*, like many species of *Chondrophycus*, *Laurencia* and *Osmundea*, possesses five pericentral cells in the procarp-bearing segment of the trichoblast (Nam & Sohn 1994, Nam *et al.* 1991, 1994, Nam & Saito 1990, 1995, Fujii & Cordeiro-Marino 1996, Fujii *et al.* 1996) rather than four as found in *C. intermedius* and other species of the section *Palisadi* (Nam & Saito 1995, Masuda & Kogame 1998, Masuda *et al.* 1998). Although the tetrasporangia are produced from the second, third, and fourth pericentral cells in both species, a fifth fertile tetrasporangial branch can be occasionally formed in *C. intermedius* (Nam 1999) but not in *C. flagelliferus*.

*rbc*L sequence analysis of a small data set of members of *Laurencia sensu lato* clearly corroborates the taxonomic distinction of the three genera *Laurencia*, *Chondrophycus* and *Osmundea*. Synapomorphic morphological characters uniting *Laurencia* and *Chondrophycus* include the same origin of the spermatangial filaments and tetrasporangia. The principal character separating both genera is the number of periaxial cells per vegetative axial segment. Consideration regarding the evolution of periaxial cell number does not conform to that proposed by Garbary & Harper (1998); instead, we are of the opinion that the ancestor of the *Laurencia sensu lato* complex possessed two periaxial cells per vegetative axial segment, the plesiomorphic condition. In our view this interpretation is more parsimonious than that advanced by Garbary & Harper (1998).

More studies are needed to elucidate the taxonomy of species of the *Laurencia sensu lato* complex in Brazil and the western Atlantic, but our data demonstrate that the species referred to as *Laurencia obtusa* in the western Atlantic is distinct from eastern Atlantic *L. obtusa*, a species described from England (Hudson 1778), and that *C. flagelliferus* is closely related to the *C. papillosus* complex. However, the entity going under the name *C. papillosus* from Baja California, Mexico, needs to be critically reexamined. The *rbc*L sequences from the taxon going under the name *L. scoparia*, *L. arbuscula*, and *L. filiformis* from Brazil do not indicate the degree of genetic variation shown in other *Laurencia* species, suggesting that these taxa encompass a single morphologically diverse species in Brazil. *Laurencia filiformis* (C. Agardh) Montagne (1845:125), a species cited for many different parts of the world, is known for having many synonyms. The molecular data indicate that, as originally described, *C. translucidus* belongs in the genus *Laurencia*.

All species currently placed in the genus *Osmundea* formed a monophyletic clade with robust support. However, two clades representing different geographical areas were observed (North Pacific and Atlantic Europe), with bootstrap above 80% corroborating the distinct biogeographic pattern within the genus as observed by McIvor *et al.* (2002).

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APPENDIX

Taxonomic identification, collection data, GenBank accession numbers, % of the *rbc*L gene sequenced, and authorship of the *rbc*L sequences used in this study:

Bostrychia radicans (Montagne) Montagne, St. Louis Bay, Mississippi, USA; coll. C.F.D. Gurgel, 11 Feb. 1998, (AF259497, 90%).

Bryocladia cuspidata (J. Ag.) De Toni, Port Aransas, Texas, USA; coll. S. Fredericq & C.F.D. Gurgel, 17 May 1998, (AF259498, 98%).

Centroceras "clavulatum" (C. Agardh) Montagne; Redfish Bay, Port Aransas, Texas, USA, coll. S. Fredericq & C.F.D. Gurgel, 18 May 1998, (AF259490, 97%).

Ceramium brevizonatum v. *caraibicum* H. Petersen *et* Børgesen in Børgesen; Laguna de Yulcapeten, Campeche Bay, Mexico, coll. C.F.D. Gurgel, 13 Feb. 1998 (AF259490, 97%).

Chondria dasyphylla (Woodwar) C. Agardh; Morehead City, North Carolina, USA, coll. D.W. Freshwater, (U04021, 96.5%).

Chondrophycus flagelliferus (J. Agardh) K.W. Nam; Praia Brava, Ubatuba, São Paulo, Brazil, coll. S.M.P.B. Guimarães & J. Domingos, 25 May 2001, (SP356238, LAF#: L41) (AF465804, 99%).

Chondrophycus papillosus (C. Agardh) Garbary & J. Harper; basaltic rocky shores in front of the lighthouse, Santana City, Vera Cruz State, Mexico, 19°51.62'N and 96°27.67'W, coll. C.F.D. Gurgel, 09 Feb. 1999, (LAF#: L42) (AF465807, 96.7%).

Chondrophycus papillosus (C. Agardh) Garbary & J. Harper; Praia do Lamberto, Ubatuba, São Paulo, Brazil, coll. M.T. Fujii, 31 Aug. 2000, (SP356239, LAF#: L23) (AF465806, 99.1%).

Chondrophycus papillosus (C. Agardh) Garbary & J. Harper; Praia do Tenório, Ubatuba, São Paulo, Brazil, coll. S.M.P.B. Guimarães & J. Domingos, 26 May 2001, (SP356245, LAF#L58) (AY593972, 58.4%).

Chondrophycus papillosus (C. Agardh) Garbary & J. Harper; Todos Santos, Baja California, Mexico, coll. S. Fredericq, 24 Oct. 1999, (LAF#: L36) (AY588409, 99.1%).

Chondrophycus translucidus (M.T. Fujii & Cordeiro-Marino) Garbary & J. Harper; Marataizes, Espírito Santo State, Brazil, coll. M.T. Fujii, 15 Sep. 2001, (SP356242, LAF#: 377) (AY588408, 98.9%).

Laurencia arbuscula Sonder; Ilha das Couves, Ubatuba, São Paulo, Brazil, coll. M.T. Fujii, 19 Jan. 2001, (SP356240, LAF#: L25) (AF465810, 95.6%).

Laurencia cf. *brongniartii* J. Agardh; Makang Harbor, Taiwan, coll. S. Fredericq & S.M. Lin, 11 Jul. 1993, (AF465814, 95.6%).

Laurencia cf. *catarinensis* Cordeiro-Marino & Fujii; Saco do Eustáquio, Ilhabela, São Paulo, Brazil, coll. M.T. Fujii, 19 Jan. 2001, (SP356241, LAF#: L15) (AF465808, 97.7%).

Laurencia cf. *obtusa* (Hudson) Lamouroux; Pointe de la Verdure, Guadeloupe, F.W.I.; coll. A. Renoux, 20 Mar. 1994, (LAF#:L38) (AF465811, 84.6%).

Laurencia cf. *obtusa*; Isla Pelona, Venezuela, coll. C.F.D. Gurgel, 26 Jun. 1999, (LAF#:L22) (AF465812, 84.1%).

Laurencia complanata (Suhr) Kützing; Port Edward, Kwa-Zulu-Natal, South Africa; coll. A. Millar, 08 Feb. 2001, (LAF#:8.2.01.2.12, L32) (AF465813, 97.7%).

Laurencia filiformis (C. Agardh) Montagne; Praia Brava, Ubatuba, São Paulo, Brazil, coll. S.M.P.B. Guimarães & J. Domingos, 25 May 2001, (LAF#: L40) (AF465818, 54.4%).

Laurencia flexuosa Kützing; Palm Beach, Kwa-Zulu Natal, South Africa, coll. S. Fredericq, 07 Feb. 2001, (LAF#: 7.2.01.1.19, L44) (AF465815, 96.6%).

Laurencia intricata Lamouroux; Campeche Bay, Champoton, Mexico, 19° 17.67' N and 90° 46.26'W, coll. C.F.D. Gurgel, 14 Feb. 1999, (LAF#:L45) (AF465809, 96.9%).

Laurencia intricata Lamouroux; Channel 5 (ocean side), Long Key, Florida, USA, coll. B. Wysor & T. Frankovich, 10 Dec. 1998, (LAF#:L55) (AY588410, 100%).

Laurencia natalensis Kylin; Palm Beach, Kwa-Zulu Natal, South Africa, coll. S. Fredericq, 07 Feb. 2001, (LAF#: 7.2.01.1.20, L49) (AF465816, 100%).

Laurencia pacifica Kylin; Central beach, Moss Beach, CA, USA, coll. S. Fredericq, 17 Feb. 1992, (LAF#:L37) (AY588411, 68.7%).

Laurencia scoparia J. Agardh; Marataizes, Espírito Santo, Brazil, coll. M.T. Fujii, 15 Sep. 2001, (SP356244, LAF#: 27) (AY593971, 56%).

Laurencia sp.; Flower Garden Banks National Marine Sanctuary, Gulf of Mexico, USA, coll. S. Fredericq, 14 Apr. 2000, (LAF#:L46, AF465819, 95%).

Lophocladia trichoclados (C. Agardh) Schmitz; Old Dan's Reef, Keys Marine Lab, Florida, USA, coll. S. Fredericq, 08 Apr. 1998, (AF465819, 95%).

Neorhodomela larix (Turner) Masuda; Pigeon Point, CA, USA, coll. M.H. Hommersand, (AF259499, 70%).

Osmundea pinnatifida (Hudson) Stackhouse; Penmarch, Brittany, France, coll. M.H. Hommersand, Jul. 1993, (AF259495, 97%)

Osmundea sinicola (Setchell & N.L. Gardner) K.W. Nam; Crescent Beach, Orange Co., California, USA, coll. S. Murray, 28 May 2002, (LAF#:680) (AY588407, 96.8%).

Polysiphonia muelleriana J. Agardh; Deas Cove, Thompson Sound, Fiordland, New Zealand, coll. S. Wing & N. Goebel, 03 Oct. 2000, (WELT#:ASA356; LAF#:223) (AY588412, 96.7%).