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Morphological and molecular studies on the Brazilian native red seaweed *Laurencia oliveirana* (Rhodomelaceae, Ceramiales)

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Article

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Abstract: Morphological and molecular studies were carried out on *Laurencia oliveirana* from the type locality (Arraial do Cabo, Rio de Janeiro, Brazil). This species is easily recognized by its small size, sub-erect habit forming intricate cushion-like tufts and unilateral pectinate branching. The species displays all the typical characters of the genus *Laurencia*, such as the production of the first pericentral cell underneath the basal cell of the trichoblast, tetrasporangia produced from particular pericentral cells, with the third and fourth pericentral cells becoming fertile, without production of additional pericentral cells, spermatangial branches produced from one of two laterals on the suprabasal cell of trichoblasts, and procarp-bearing segment with five pericentral cells. Details of tetrasporangial plants and development of procarp and male plants are described for the first time for the species. The phylogenetic position of *L. oliveirana* was inferred by analysis of the chloroplast-encoded *rbc*L gene sequences from 57 taxa. In all phylogenetic analyses, *L. oliveirana* grouped with *L. caraibica, L. caduciramulosa, L. venusta* and *L. natalensis*, forming a monophyletic clade within the *Laurencia sensu stricto*. The genetic divergence between *L. oliveirana* and the molecularly closest species, *L. caraiba* collected in Brazil, was 2.3%.

Introduction

Currently, the *Laurencia* complex comprises five genera: *Laurencia* J.V. Lamouroux, *Osmundea* Stackhouse, *Chondrophycus* (Tokida & Saito) Garbary & J.T. Harper, *Palisada* (Yamada) K.W. Nam and *Yuzurua* (K.W. Nam) Martin-Lescanne. *Laurencia oliveirana* Yoneshigue possesses all the characters typical of the genus *Laurencia s.s*., such as: production of the first pericentral cell underneath the basal cell of the trichoblast, production of tetrasporangia from a particular pericentral cell, without production of additional pericentral cells, spermatangial branches produced from one of two laterals on the suprabasal cell of trichoblasts, and the procarp-bearing segment with five pericentral cells.

Laurencia oliveirana was proposed by Yoneshigue (1985) from the upwelling region of Arraial do Cabo, Rio de Janeiro. Later, Fujii (1990) reported this species for Ubatuba, northern coast of São Paulo state, and confirmed, by analysis of herbarium material, that the taxon previously named as *Laurencia* sp. by Joly (1965), collected in Bertioga and Ubatuba, São Paulo and Parati, Rio de Janeiro, corresponded to *L. oliveirana*. According to Fujii & Villaça (2003), the *Laurencia* sp. described by Baptista (1977) for the flora of Torres, Rio Grande do Sul, also corresponds to *L. oliveirana*. On the Rio de Janeiro coast, besides the type locality, the species was reported for Ilha Grande, Angra dos Reis by Gestinari et al. (1998). The geographical distribution of this species was expanded by Nunes (1998), who reported it on the coast of Bahia. Later, Horta (2000) and Amado Filho et al. (2006) reported it for the Laje de Santos Marine State Park and Queimada Grande Island, São Paulo, at a depth of 22 m. Although Wynne (2011) cited a record of *L. oliveirana* from Cuba by Areces et al. (2003), the Note "204" was a typo for 203, namely the account by Fujii & Sentíes (2005) of this species from Brazil (Wynne, pers. comm.). Therefore, so far, the occurrence of *L. oliveirana* can be considered to be restricted to the Brazilian coast.

Within the *Laurencia* complex, the genus *Laurencia s.s*. has been highlighted as an important synthesizer of halogenated secondary metabolites,

especially terpenes (Erickson, 1983; Pereira & Teixeira, 1999), with proven antifouling activity (Da Gama et al., 2002; Cassano et al., 2008) and pharmacological importance, as recently compiled by Fujii et al. (2011) for the Brazilian species. The typical *Laurencia s.s.* species possess refractile inclusions named *corps en cerise*, mainly in cortical cells of the thallus, which are the sites of production and/or accumulation of halogenated metabolites (Howard et al., 1980; Young et al., 1980; Salgado et al., 2008). *Corps en cerise*, in number of 1-2 (3) per cortical cell, are cited in the original description of *L. oliveirana* made by Yoneshigue (1985). The presence of *corps en cerise* in *L. oliveirana* makes it a potential producer of secondary metabolites that still need to be investigated.

This study describes and illustrates, in detail, morphological characters of *Laurencia oliveirana*, comparing it with related species, and also infers its phylogenetic position using the chloroplast-encoded *rbc*L gene, confirming its taxonomical position at the species level. Details of tetrasporangial plants and development of the procarp and male plants are described for the first time for this species.

Materials and Methods

Morphological observations

Samples of *Laurencia oliveirana* were collected from the type locality, Ponta da Cabeça, Arraial do Cabo, Rio de Janeiro, Brazil, in 2008. Voucher specimens and material for morphological study were fixed in 4% formalin/seawater or pressed as herbarium sheets. Transverse and longitudinal hand-sections were made with a stainless-steel razor blade and stained with 0.5% aqueous aniline blue solution, acidified with 1N HCl (Tsuda & Abbott, 1985). Living specimens were also examined to check for the presence of *corps en cerise*. Measurements are given as length x diameter. For comparison purposes, additional specimens provided by the Herbarium of the Department of Botany, University of São Paulo (SPF-Algae), and by the Herbarium of the Botanical Institute (SP) were analyzed. Line-drawings were prepared using a camera lucida mounted on a Nikon Eclipse E200 microscope (Tokyo, Japan) and photomicrographs were taken with a Sony W5 digital camera (Tokyo, Japan) coupled to a Nikon microscope. Vouchers are deposited in the SP and SPF herbaria. Herbarium abbreviations follow the online *Index Herbariorum* (http://www.nybg.org/bsci/ih/ih.html).

Molecular analyses

Samples used for molecular analysis were dried in silica gel. Total DNA was extracted, after grinding in liquid nitrogen, using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A total of 57 *rbc*L sequences were used in this study (Table 1). For each sequence generated, a total of 1467 base pairs of the *rbc*L gene were amplified in three overlapping parts with the primers pairs: FrbcLstart-R753a, F492a-R1150a and F993-RrbcS (Freshwater & Rueness, 1994; Cassano 2009) using PCR master mix (Promega Corp., Madison, WI, USA). All PCR products were analyzed by electrophoresis in 1% agarose to check product size. The PCR products were purified with the MicroSpin™ S-300 HR Columns (GE Healthcare Life Sciences, Piscataway, USA) in accordance with the manufacturer's instructions. Sequencing was carried out with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, NJ, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The primers used for the sequencing were those used for the PCR amplification. The full sequence was obtained from both DNA strands. Multiple alignments for *rbc*L sequences were constructed using the computer program BioEdit 7.0.4.1 (Hall, 1999).

Phylogenetic analyses

Phylogenetic relationships were inferred with PAUP 4.0b10 (Swofford, 2002) and MrBayes v.3.0 beta 4 (Huelsenbeck & Ronquist, 2001). Maximum-parsimony trees (MP) were constructed using the heuristic search option, tree-bisection-reconnection branch swapping, unordered and unweighted characters. Support values for the relationships discovered in each analysis were calculated by performing bootstrap analyses (Felsenstein, 1985), as implemented in PAUP. Ten thousand heuristic search replicates were executed using the TBR branch-swapping algorithm. The model used in the Bayesian analysis was the general-time-reversible model of nucleotide substitution with invariant sites and gamma distributed rates for the variable sites (GTR+I+G). This model was selected based on maximum likelihood ratio tests implemented by the software Modeltest version 3.06 (Posada & Crandall, 1998) with a significance level of 0.01 by Akaike information criterion. For the Bayesian analysis, four chains of the Markov chain Monte Carlo (one hot and three cold) were used, sampling one tree every 10 generations for 1,000,000 generations starting with a random tree. Log-likelihood values were stabilized around 50,000 generations, which were discarded as 'burn in'. A 50% consensus tree (majority rule as implemented by PAUP) was computed after the 'burn in' point. The range of *rbc*L divergence values within and among species was calculated using uncorrected 'p' distances using PAUP.

Table 1. Taxa used in this study for phylogenetic analysis.

Samples	Collection data	GenBank accession numbers
Bostrychia radicans (Montagne) Montagne in Orbigny	USA, Mississippi, St. Louis Bay, 11 Feb. 1998, C.F.D. Gurgel	AF259497
Polysiphonia muelleriana J. Agardh	New Zealand, Deas Cove, Thompson Sound, Fiordland, 03 Oct. 2000, S. Wing and N. Goebel	AY588412
Bryocladia cuspidata (J. Agardh) De Toni	USA, Texas, Port Aransas, 17 May 1998, S. Fredericq and C.F.D. Gurgel	AF259498
Chondria collinsiana M.A. Howe	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano and J.C. De-Paula	GU330225
C. cf *undulatus*	New Caledonia, Loyalty Is., Maré, 22 Mar. 2005, C. Payri	FJ785307
Chondrophycus sp. 1	New Caledonia, Loyalty Is., Lifou, 26 Mar. 2005, C. Payri	FJ785309
Chondrophycus sp. 2	New Caledonia, Loyalty Is., Maré, 21 Mar. 2005, C. Payri	FJ785310
Chondrophycus sp. 3	New Caledonia, Loyalty Is., Beautemps/ Beaupré, 06 Apr. 2005, C. Payri	FJ785311
Laurencia aldingensis Saito & Womersley	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, RJ, 13. Jan. 2005, V. Cassano and J.C. De-Paula	JF810351
L. cf. *brongniartii*	Australia, Tarcoala Beach, 1993, S. Fredericq	EF061654
L. cf. *brongniartii*	Taiwan, Makang Harbour, 11 Jul. 1993, S. Fredericq	AF465814
L. caduciramulosa Masuda & Kawaguchi	Brazil, Rio de Janeiro, Angra dos Reis, Baía da Ribeira, Praia do Velho, 19 Apr. 2006, V. Cassano and J.C. De-Paula	-
L. caraibica P.C. Silva	Mexico, Quintana Roo, Cancún, Isla Mujeres, 23 Feb. 2006, A. Sentíes	EF658642
L. caraibica	Brazil, Rio Grande do Norte, Atol das Rocas, 07 Jul. 2002, R. Villaça	-
L. catarinensis Cordeiro-Marino & M.T. Fujii	Brazil, Espírito Santo, Anchieta, Ponta dos Castelhanos, 05 Oct. 2006, M.T. Fujii and V. Cassano	-
Laurencia dendroidea J. Agardh [as *L. filiformis* (C. Agardh) Montagne]	Brazil, Bahia, Lauro de Freitas, Praia Vilas do Atlântico, 08 Jan. 2008, A. Oliveira	GU330228
L. dendroidea [as *L. majuscula* (Harvey) A.H.S. Lucas]	Brazil, Rio de Janeiro, Angra dos Reis, Praia do Velho, 20 Jul. 2006, V. Cassano and J.C. De-Paula	GU330232
L. flexuosa Kützing	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq	AF465815
L. intricata J.V. Lamouroux	Mexico, Yucatan, Campeche Bay, 14 Feb. 1999, C.F.D. Gurgel	AF465809
L. intricata	USA, Florida, Long Key, Channel 5, 10 Dec. 1998, B. Wysor and T. Frankovich	AY588410
L. intricata	Cuba, Ciego de Ávila, Cayo Coco, 25 Sep. 2005, M.T. Fujii	GU330238
L. cf. *kuetzingii*	New Caledonia, Loyalty Is., Ouvéa, 31 Mar. 2005, C. Payri	FJ785322
L. cf. *mariannensis*	New Caledonia, Lagon Sud-Ouest, Ilot Larégnère, 11 Jul. 2003, C. Payri	FJ785313
L. marilzae Gil-Rodríguez, Sentíes, Cassano, Díaz-Larrea & M.T. Fujii	Spain, Canary Islands, Tenerife, Playa Paraíso, 14 Jul. 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes	EF686001
L. marilzae	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 12 Jul. 2006, M.C. Gil-Rodríguez,	EF686002
L. marilzae	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 Mar. 2007, R. Rocha-Jorge	GU938189
L. cf. *mcdermidiae*	New Caledonia, Ile des Pins, 29 Nov. 2005, C. Payri	FJ785314
L. natalensis Kylin	South Africa, KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq	AF465816
L. cf. *nidifica*	New Caledonia, Ile des Pins, 30 Nov. 2005, C. Payri	FJ785315
L. obtusa (Hudson) J.V. Lamouroux	Ireland, County Donegal, Fanad Head, 06 Jul. 1998, C.A. Maggs	AF281881
L. oliveirana Yoneshigue	Brazil, Rio de Janeiro, Arraial do Cabo, Ponta da Cabeça, 07 Jul. 2008, V. Cassano and J.C. De-Paula	JF810352
L. pyramidalis Bory ex Kützing	France, Brittany, Roscoff, 05 Dec. 2002, F. Rousseau	FJ785316
L. translucida M.T. Fujii & Cordeiro-Marino	Brazil, Espírito Santo, Marataízes, 15 Sep. 2001, M.T. Fujii	AY588408

L. venusta Yamada	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004, J. Díaz-Larrea and A. Sentíes	EF061655
L. viridis Gil-Rodríguez & Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 06 Oct. 2005, M.C. Gil-Rodríguez	EF685999
Laurencia sp.	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano and J.C. De-Paula	-
Osmundea blinksii (Hollenberg & Abbott) K.W. Nam	USA, California, San Mateo Co., Año Nuevo, Greyhound Rock, 17 Jul. 1996, M.H. Hommersand	AY172575
O. oederi (Gunnerus) G. Furnari [as *O. ramosissima* (Oeder) Athanasiadis]	Ireland, Co. Donegal, St. John´s Point, 12 Oct. 1999, C.A. Maggs	AF281880
O. osmunda (S.G. Gmelin) K.W. Nam	Ireland, County Donegal, St. John´s Point, 12 Oct. 1999, C.A. Maggs	AF281877
O. pinnatifida (Hudson) Stackhouse	France, Brittany, Penmarch	AF259495
O. sinicola (Setchell & Gardner) K.W. Nam	USA, California, Orange Co., Crescent Beach, 28 May 2002, S. Murray	AY588407
O. spectabilis (Postels & Ruprecht) K.W. Nam var. *spectabilis*	Mexico, Baja California, Punta Santo Thomas, 2 Jul. 1996, M.H. Hommersand	AY172574
O. splendens (Hollenberg) K.W. Nam	Mexico, Baja California, Bahia Colnett, Drift, 2 Jul. 1996, M.H. Hommersand and J. Hughey	AY172576
O. truncata (Kützing) K.W. Nam & Maggs	Ireland, Lough Hyne, Co. Cork, 11 Nov. 1999, C.A. Maggs	AF281879
Palisada corallopsis (Montagne) Sentíes, M.T. Fujii & Díaz-Larrea	Mexico, Quintana Roo, Cancún, Chaac-Mol Beach, 21 Aug. 2005, J. Díaz-Larrea and A. Sentíes	EF061646
P. flagellifera (J. Agardh) K.W. Nam	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, 03 Aug. 2005, V. Cassano and M.B. Barros-Barreto	GU330221
P. flagellifera	Brazil, São Paulo, Ubatuba, Praia Brava, 25 May 2001, S.M.B. Guimarães and J. Domingos	AF465804
Palisada furcata (Cordeiro-Marino & M.T. Fujii) Cassano & M.T. Fujii	Brazil, Paraíba, Praia de Tambaú, 24 Feb. 2004, M.T. Fujii	GU330226
P. patentiramea (Montagne) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii	Philippines	AF489862
P. perforata (Bory) K.W. Nam	Spain, Canary Islands, Tenerife, Pto. de La Cruz, San Telmo, 14 Jul. 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes	EU256329
P. perforata	Mexico, Quintana Roo, Cancún, Isla Mujeres, 2 Mar. 2007, A. Sentíes and M.C. Gil-Rodríguez	EF658641
P. perforata	Brazil, Rio de Janeiro, Parati, Praia Vermelha, 30 Dec. 2005, V. Cassano	EU256331
Palisada. cf. *robusta*	New Caledonia, Lifou, 23 Mar. 2005, C. Payri	J785321
Palisada cf. *cruciata*	New Caledonia, Ils des Pins, 04 Dec. 2005, C. Payri	FJ785319
P. thuyoides (Kützing) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii	Philippines	AF489863
Yuzurua poiteaui (J.V. Lamouroux) Martin-Lescanne var. *poiteaui*	USA, Florida, Long Key, Ocean Side, 1998, S. Fredericq	EF061652
Y. poiteaui var. *poiteaui*	Mexico, Quintana Roo, Playa del Carmen, 15 Mar 2005, J. Díaz-Larrea and A. Sentíes	EF061653

Results

Morphological study

Laurencia oliveirana Yoneshigue, Taxonomie et ecologie des algues marines dans la region de Cabo Frio (Rio de Janeiro) Brésil, Université d'Aix-Marseille. 1985: 329-330 (Figures 1-23).

Holotype: YY 4016 in Phycological Herbarium, Instituto Nacional de Estudos do Mar, Arraial do Cabo, Rio de Janeiro, Brazil.

Isotype: SPF 24767!

Type locality: Ponta da Cabeça, Arraial do Cabo, Rio de Janeiro, Brazil

Plants with sub-erect habit forming brown-reddish to brown-greenish intricate cushion-like tufts, up to 1.5 cm high (Figures 1-4). The thalli are terete, fleshy in texture, adhering to herbarium paper when dried. Main sub-erect axes with indeterminate growth

175-300 µm in diameter, attached to the substratum by small discoid holdfasts (Figure 4), from which arise erect axes with determinate growth 270-580 µm in diameter in the middle portions of the thalli. The terete axes are sparsely branched. Branching is unilateral pectinate to alternate-distichous with one order of branches. Ultimate branchlets are cylindrical to clavate, simple, long or short with (160) 250-700 (1000) x 130-350 (575) µm.

In surface view, cortical cells are regularly arranged throughout the thalli in longitudinal rows and connected to each other by longitudinally oriented secondary pit connections (Figure 5), with only one *corp en cerise* per cell (Figure 6) and one per trichoblast cell. Cortical cells in surface view are isodiametric-polygonal in the lower and in the upper portions of the thalli and elongate-polygonal in the middle portions, (22.5) 52.5-77.5 (87.5) x 22.5-40 µm. In median longitudinal section through a branchlet, outer cortical cell walls near apices are not projected beyond the surface (Figure 7).

In transverse section, thalli present one or two layers of pigmented cortical cells and three or four layers of colorless medullary cells (Figure 8). Cortical cells in transverse section are quadratic to cuneiform, neither radially elongated nor arranged as a palisade, 20-32.5 x 25-40 µm in the middle portions of the thalli. Medullary cells are rounded or slightly radially elongated, becoming larger toward the thallus center, 40-105 µm x 37.5-82.5 µm in the middle portions of the thalli. Lenticular thickenings are occasionally present. When present, they occur in medullary cell walls of main axes or lateral branches and especially at the bases of cystocarps (Figures 9 and 19). Each vegetative axial segment cuts off four pericentral cells (Figure 10). The first pericentral cell is produced underneath the basal cell of the trichoblast.

Male plants with clavate tip branches, characteristically swollen, simple, 780-1125 µm in diameter (Figure 3). In longitudinal section through a fertile branchlet, the spermatangial pits are cup-shaped, and an axial cell row is discernible at the base (Figure 11). Spermatangial trichoblasts arise from the axial cell, consisting of fertile and sterile branches (Figure 12); the fertile branches produce many ovoid spermatangia, 7.5-10 x 2.5-5 µm, and terminate in vesicular sterile cells, 17.5-27.5 µm x 12.5-20 µm (Figure 13); each spermatium possesses an apical nucleus (Figure 12).

In the female plants, each procarp-bearing segment produces five pericentral cells (Figure 14), the fifth of which becomes the supporting cell of a four-celled carpogonial branch with two groups of sterile cells (basal and lateral), which develop from the supporting cell of the carpogonial branch (Figures 15-17). After fertilization, the supporting cell cut off its distal end, producing the auxiliary cell. Gonimoblast filaments produce many carposporangia terminally, which are clavate to cuneiform-elongate, 90-147.5 x 30-52.5 µm. Fully developed cystocarps are pyriform, prominent and without a protuberant ostiole, arranged along the upper third to subapical portions of the thallus, 580-750 µm in diameter (Figures 2 and 18).

Tetrasporangial plants with cylindrical and simple branchlets, 1425-8000 x 325-550 µm (Figures 4 and 20). At the apex of fertile branches, each axial segment produces two fertile pericentral cells, the third and the fourth ones (Figure 21); the first and the second pericentral cells remain sterile. Each fertile pericentral cell cuts off two pre-sporangial cover cells distally and abaxially the tetrasporangial initial. Subsequently, one post-sporangial cover cell is produced (Figure 22) and continues dividing and contributes to produce cortication around the tetrasporangium. 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 a parallel pattern in relation to fertile branchlets (Figure 23). Mature tetrasporangia are tetrahedrally divided, 50-85 µm in diameter.

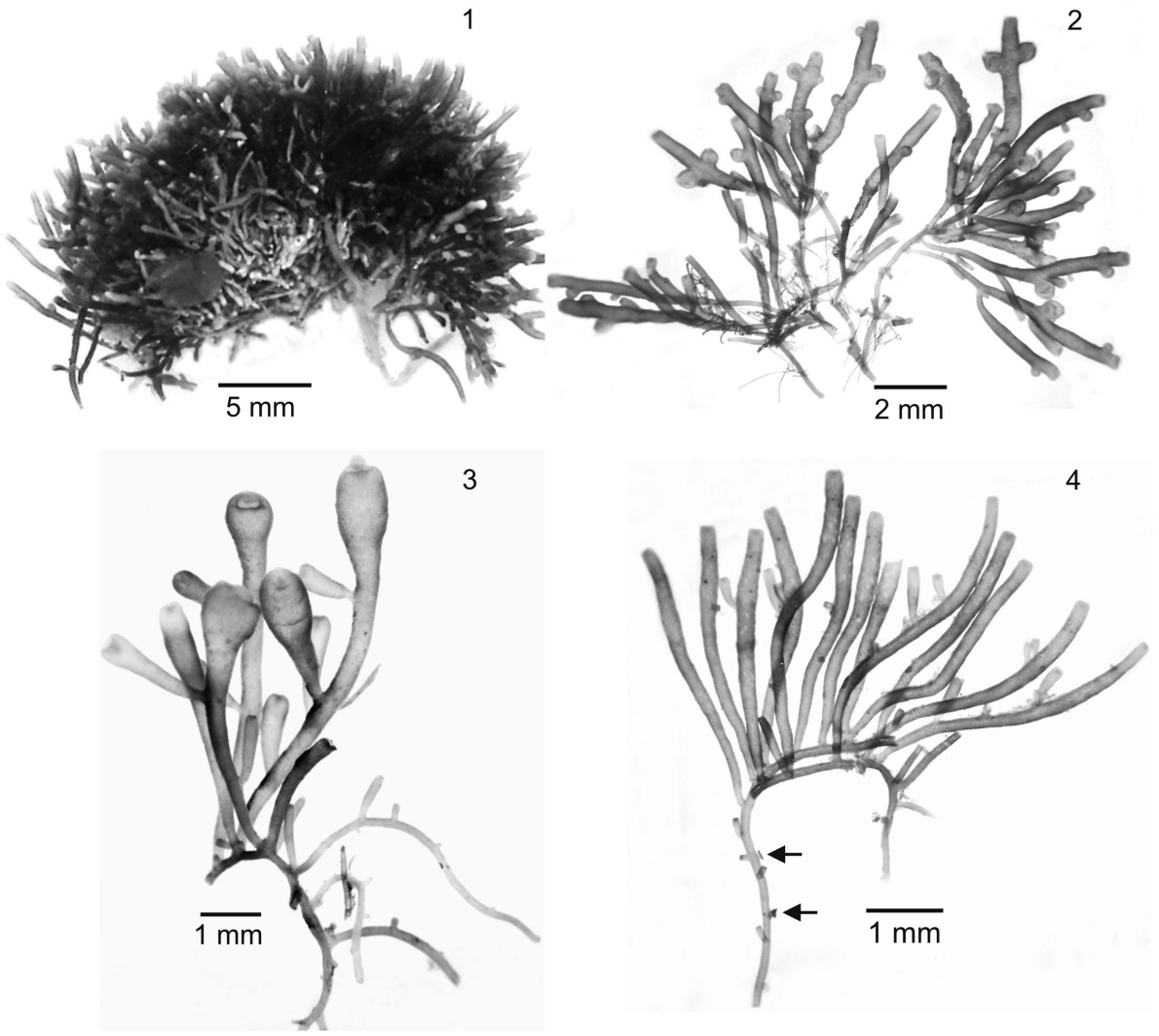
The epilithic specimens were collected from the intertidal zone at exposed wave-action sites, associated with turfs of articulated Corallinaceae. In this study, *L. oliveirana* was found in the type locality only in winter.

Geographical distribution

Atlantic Ocean, Brazil, Northeastern: Bahia (Nunes, 1998); Southeastern: Rio de Janeiro (Yoneshigue, 1985; Gestinari et al., 1998; Cassano 2009), São Paulo (Joly, 1965, as *Laurencia* sp.; Horta, 2000; Fujii and Sentíes, 2005; Amado Filho et al., 2006); South: Rio Grande do Sul (Baptista, 1977, as *Laurencia* sp.).

Examined material

Isotype: *Laurencia oliveirana* Yoneshigue [´*Laurencia oliverae*`]: BRAZIL: Rio de Janeiro: Arraial do Cabo, Ponta da Cabeça, tetrasporangial plant, 25.viii.1983, Y. Yoneshigue YY4016 (SPF24767), 07.vii.2008, female, male and tetrasporangial plants, V. Cassano and J.C. De-Paula (SP399.857), Parati, Praia de Panema, 12.v.1963, tetrasporangial plant, A.B. Joly, E.C. Oliveira, M. Cordeiro-Marino, N. Yamaguishi and Y. Ugadim (SPF55393), São Paulo: Picinguaba, Praia Brava, 20.vii.1989, M.T.Fujii and M.N. Fujii, Bertioga, between Praia Preta and Prainha, 08.vi.1963, E.C. Oliveira, M. Cordeiro-Marino, N. Yamaguishi and Y. Ugadim (SPF028230), Ubatuba, Praia de Fora, 21.vii.1963, A.B. Joly, E.C. Oliveira, M. Cordeiro-



Figures 1-4. Habit of *Laurencia oliveirana.* 1. Aspect of an intricate tuft associated with articulated Corallinaceae. 2. Female plant. 3. Male plant. 4. Tetrasporangial plant. Note basal portion with discoid holdfasts (arrows).

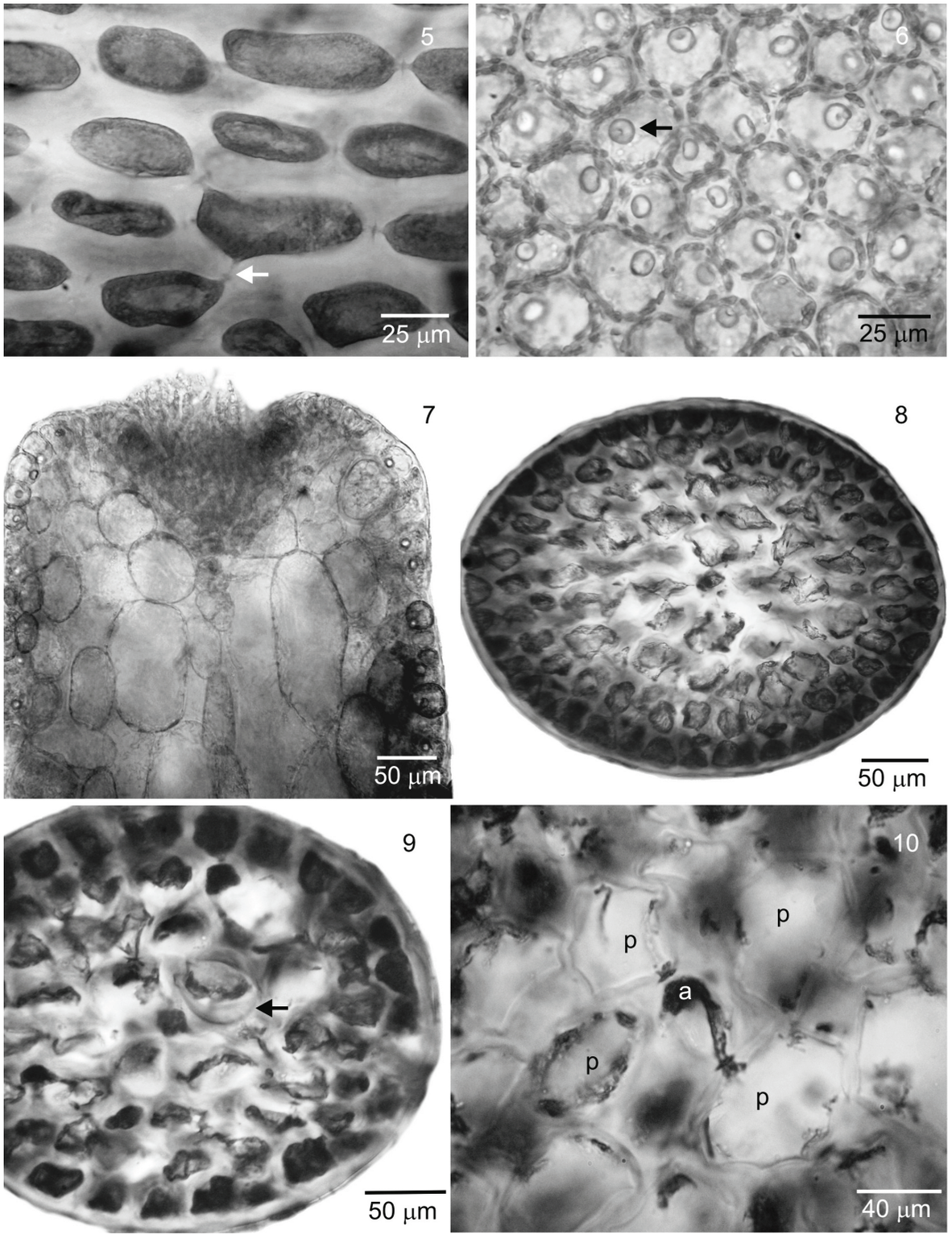
Marino, N. Yamaguishi and Y. Ugadim (SPF24756), Ilha das Couves, 22.vii.1963, tetrasporangial plant, A.B. Joly, E.C. Oliveira, M. Cordeiro-Marino, N. Yamaguishi and Y. Ugadim (SPF24757), Praia do Bonete, 19.viii.1963, tetrasporangial plant, A.B. Joly, E.C. Oliveira, M. Cordeiro-Marino, N. Yamaguishi and Y. Ugadim (SPF026565), Ilha Anchieta, Praia do Sul, 18.viii.1963, A.B. Joly, E.C. Oliveira, M. Cordeiro-Marino, N. Yamaguishi and Y. Ugadim (SPF24760).

Additional examined materials for other taxa: *Laurencia caraibica:* Atol das Rocas, 07.vii.2002, R. Villaça (SP399940).

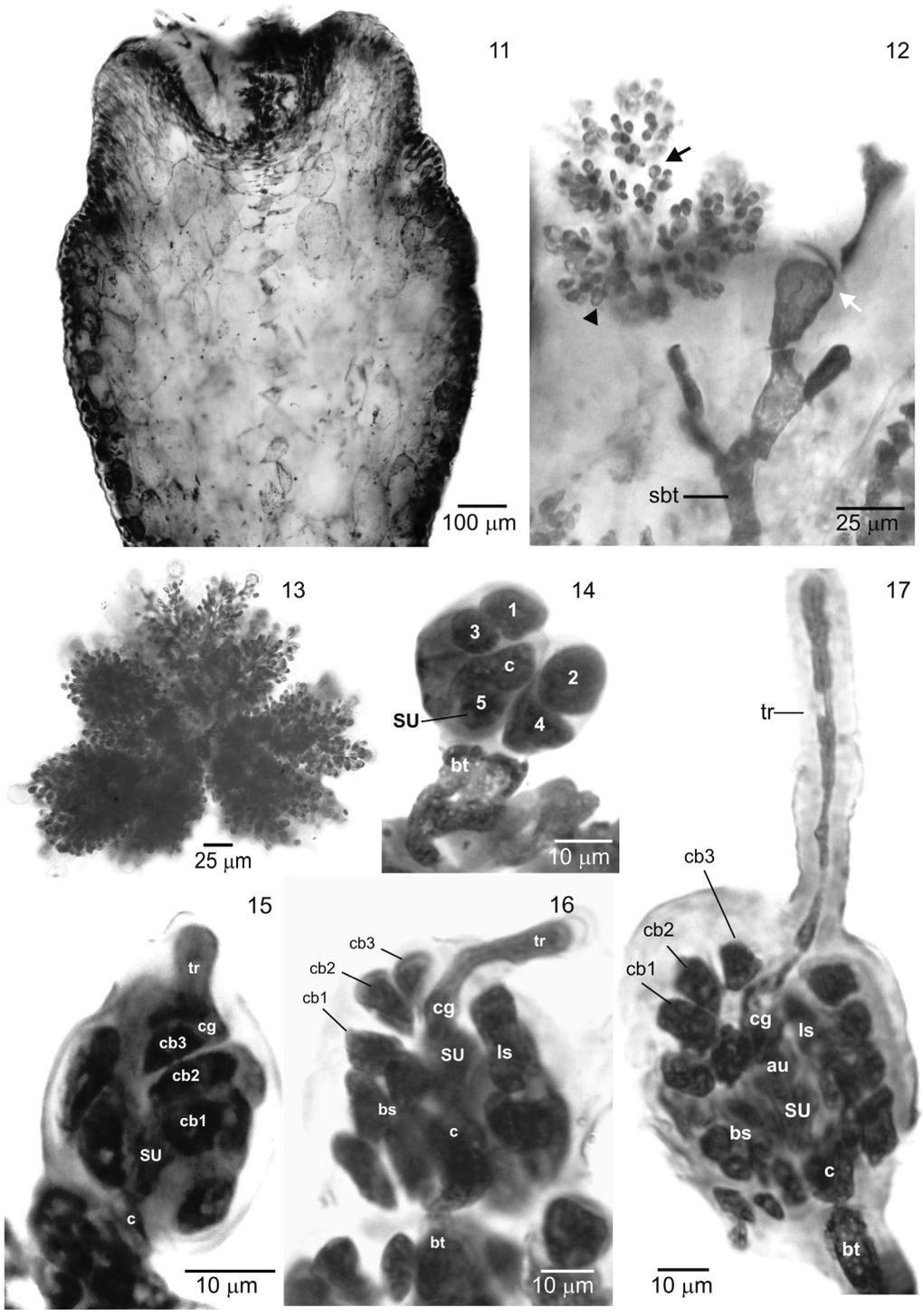
Molecular study

In this study, a total of 57 sequences were analyzed, including members of the Ceramiales *Bostrychia radicans* (Montagne) Montagne, *Bryocladia cuspidata* (J. Agardh) De Toni, *Chondria collinsiana* M. A. Howe and *Polysiphonia muelleriana* J. Agardh as outgroups (Table 1). In all *rbc*L sequences, a total of 250 nucleotides were removed from the beginning and/or end of the sequences since many sequences from the GenBank were incomplete, producing a data set of 1217 base pairs. The data set consisted of 738 constant characters, 399 parsimony-informative sites and 80 parsimony non-informative sites. The analyses show that the *Laurencia* complex is monophyletic and it was separated into the five clades currently accepted as different genera (Figure 24).

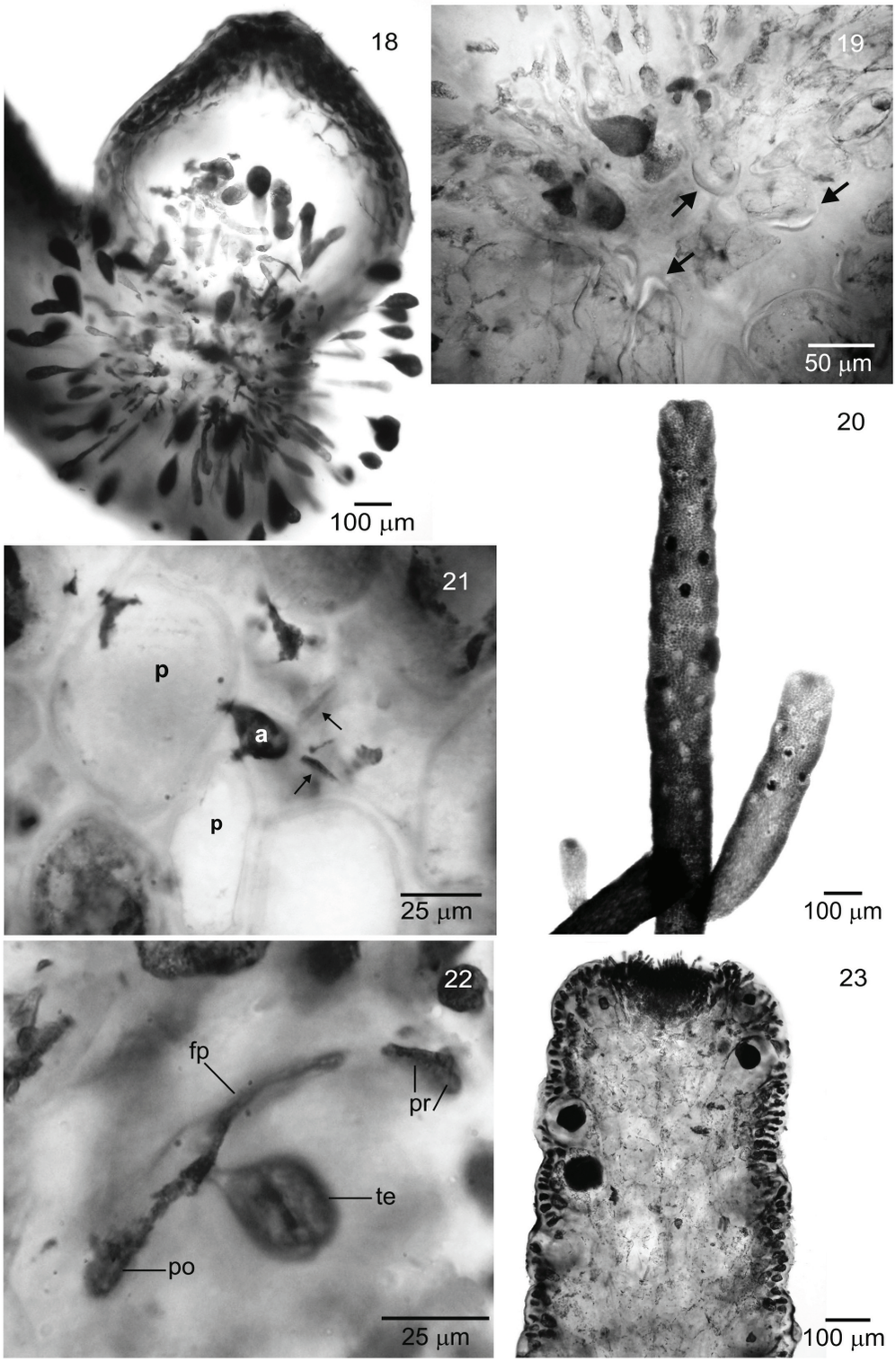
In all phylogenetic analyses, *L. oliveirana* positioned within the *Laurencia sensu stricto* forming a monophyletic clade with *L. caraibica* P.C. Silva, *L. caduciramulosa* Masuda & Kawaguchi, *L. venusta* Yamada and *L. natalensis* Kylin with high support to posterior probability (100%). *Laurencia oliveirana* grouped in all analyses with *L. caraibica* from Brazil,



Figures 5-10. Vegetative characters. 5. Cortical cells in surface view of middle portion of the thallus showing secondary pit-connections (arrow). 6. Cortical cells in surface view, showing one *corp en cerise* per cell in living material (arrow). 7. Longitudinal section through a branchlet showing non-projecting cortical cells. Note *corps en cerise* in cortical cells in living material. 8. Transverse section of the thallus showing two layers of pigmented cortical cells and three layers of colorless medullary cells. 9. Transverse section showing lenticular thickening in pericentral cell (arrow). 10. Transverse section of the upper portion of a branch showing an axial cell (a) with four pericentral cells (p).



Figures 11-17. Reproductive characters. 11-13. Details of male plants. 11. Longitudinal section through a branchlet showing spermatangial branches in cup-shaped tips. 12. Detail of spermatangial branches on trichoblast with two laterals, sterile (white arrow) and spermatangial (arrowhead) branches on its suprabasal cell (sbt). Note spermatangium with an apical nucleus (black arrow). 13. Detail of trichoblast-type spermatangial branches with terminal vesicular sterile cells. 14-17. Details of female plants showing development of procarp. 14. Procarp-bearing segment with five pericentral cells, the fifth becoming the supporting cell (su) of the carpogonial branch, central cell of procarp-bearing segment (c), basal cell of trichoblast (bt). 15. Procarp before fertilization with four-celled carpogonial branch (cb), carpogonium (cg), trichogyne (tr), supporting cell (su), central cell of procarp-bearing segment (c). 16. Carpogonial branch more developed showing lateral sterile group (ls), basal sterile group (bs). 17. Carpogonial branch after fertilization showing auxiliary cell (au) formed from supporting cell (su).



Figures 18-23. Reproductive characters. 18-19. Details of female plants. 18. Longitudinal section through a female branchlet with prominent cystocarp without a protuberant ostiole. 19. Longitudinal section of a cystocarp showing in detail its base with lenticular thickenings (arrows). 20-23. Details of tetrasporangial plants. 20. Detail of tetrasporangial branchlets. 21. Transverse section of tetrasporangial axial segment showing an axial cell (a) and two fertile pericentral cells, the third and the fourth (arrows); the other pericentral cells remain vegetative (p). 22. Detail of a fertile pericentral cell (fp) with two pre-sporangial cover cells (pr), tetrasporangium (te) and one post-sporangial cover cell (po). 23. Longitudinal section through a tetrasporangial branchlet showing parallel arrangement of the tetrasporangia.

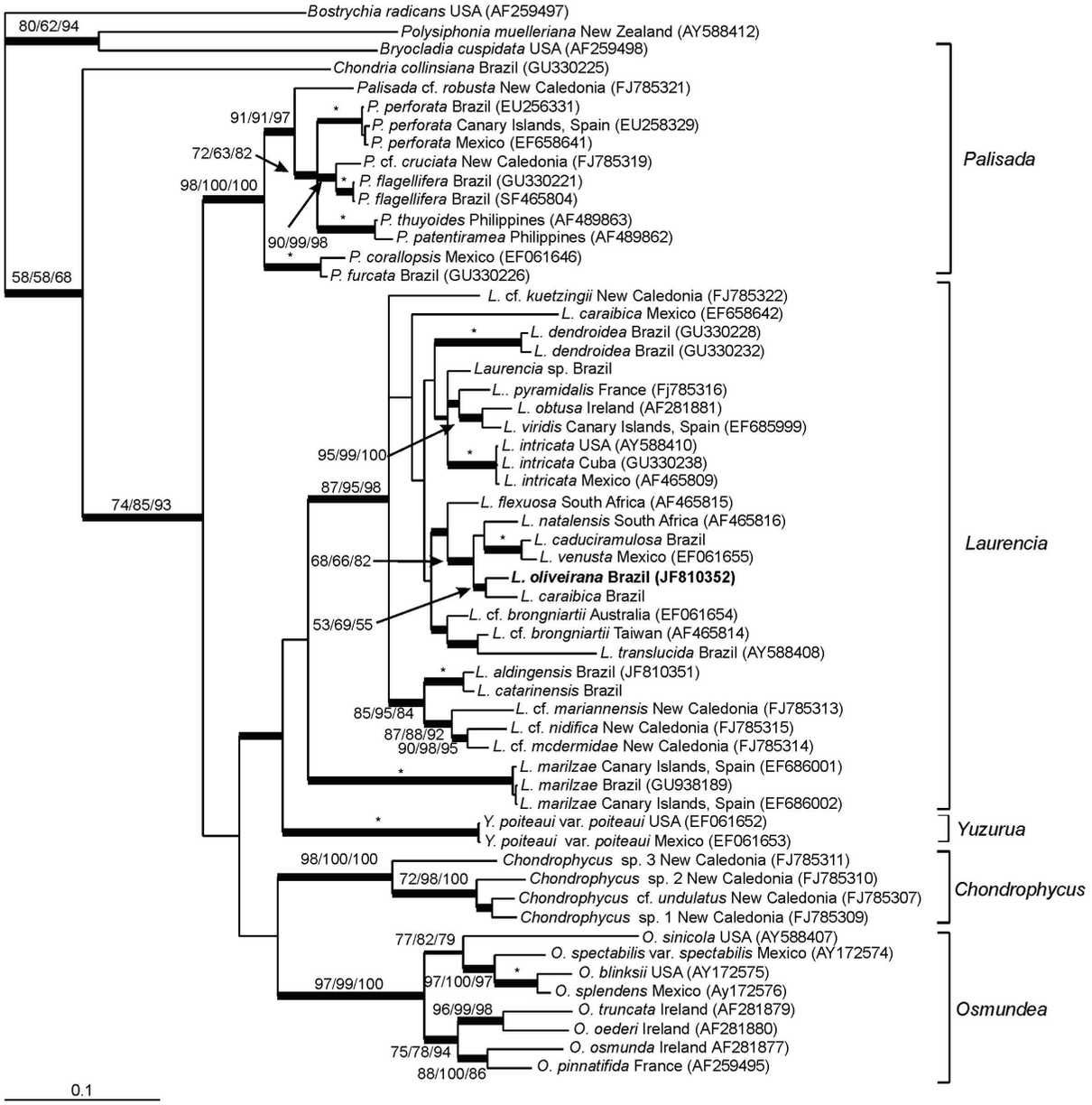


Figure 24. Consensus tree derived from Bayesian analyses of *rbc*L sequences. The posterior probabilities, PP (when >95%) are shown as thicker branches. Bootstrap supports for MP/NJ (2000 replicates)/ML (1000 replicates) are shown at the nodes; *indicates bootstrap supports = 100%.

with a genetic divergence of 2.3% between the two species. On the other hand, *L. caraibica* from Mexico positioned phylogenetically distant from *L. caraibica* from Brazil with a genetic divergence of 7.4%.

Discussion

Laurencia oliveirana is easily recognized by its small size, sub-erect habit forming intricate cushion-like tufts, usually associated with articulated Corallinaceae algae, and predominantly unilateral pectinate branching. Lenticular thickenings, described as absent by Yoneshigue (1985), Fujii (1990) and Fujii & Sentíes (2005), were observed for the first time for this species in tetrasporangial and female plants, mainly at the bases of cystocarps.

Laurencia oliveirana is morphologically similar to *L. caraibica* (type locality: Abraham Bay, Mariguana [Mayaguana], Bahamas), a species that also presents small size and intricate habit forming fastigiate mats. *Laurencia caraibica* was reported for the first time in Brazil from Atol das Rocas as *L. pygmaea* Weber-van Bosse (Oliveira Filho & Ugadim, 1974; 1976). However, Fujii & Villaça (2003), upon

examining of the type material of *L. caraibica* (US 68437) and comparing it with *L. pygmaea* identified by Oliveira Filho & Ugadim (1974, 1976), concluded that the Atol das Rocas material corresponded to *L. caraibica*. Edwards & Lubbock (1983) recorded *L. caraibica* (as L. nana M.A. Howe) for the São Pedro e São Paulo Archipelago, located off the coast of northeastern Brazil.

Laurencia caraibica was considered to be distinct from *L. oliveirana* by the presence of abundant lenticular thickenings in the medullary cell walls and frequent anastomoses between the branches, absent in *L. oliveirana* (Fujii & Villaça, 2003). The observation in this study of lenticular thickenings in *L. oliveirana* refutes this feature as distinguishing between these two species. However, the abundance of lenticular thickening found in *L. caraibica* was not observed in *L. oliveirana*. The examination of *L. caraibica* from the Atol das Rocas showed that it differs from *L. oliveirana* by its more delicate thallus with smaller diameter (160-210 µm), presence of anastomoses between the branches, and decumbent axes, giving the thallus a dorsiventral aspect. Besides, according to Schneider et al. (2010), who reported *L. caraibica* from the Bermudas, this species is a delicate decumbent plant whose erect branches attach through secondary haptera to other branches or the host, a diagnostic character for this species. The analysis of *rbc*L gene sequences obtained in this study confirmed that *L. oliveirana* from the type locality and *L. caraibica* from the Atol das Rocas are distinct species, whose genetic divergence was 2.3%. On the other hand, the molecular data showed that the specimen sequenced as *L. caraibica* of the Mexican Caribbean (Díaz-Larrea, 2008) is a taxonomic entity distinct from *L. caraibica* described for Brazil, showing high genetic divergence (7.4%). Mexican plants of *L. caraibica* present a more robust thallus than those described for Brazil, reaching 5 cm high, with axes ranging from 0.8 to 1 mm in diameter, branches compressed and branching dichotomous below and irregular above, up to 3 orders of branches (Sentíes & Fujii, 2002). A more detailed morphological comparative review of *L. caraibica* from Brazil and from the Caribbean Sea and related species is necessary to clarify the taxonomic position of these taxa, including a broader sampling for molecular analysis.

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