

Molecular support for the establishment of the new genus *Laurenciella* within the *Laurencia* complex (Ceramiales, Rhodophyta)

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Abstract

Currently, five genera are assigned to red seaweeds of the *Laurencia* complex worldwide: *Chondrophyucus*, *Laurencia s.s.*, *Osmundea*, *Palisada* and *Yuzurua*. The genera are segregated on the basis of morphological characters, especially the reproductive traits, and molecular sequences of the plastid-encoded gene *rbcL*. Four of the genera have been resolved as monophyletic, but not *Laurencia s.s.* In this study based on an *rbcL* gene phylogeny we show the presence of a sixth lineage within the *Laurencia* complex, viz., *Laurencia marilzae* plus two unidentified species of *Laurencia* from Brazil. The phylogenetic position of this group, combined with the high genetic divergence from *Laurencia s.s.* (8.2–11%), strongly support the establishment of a sixth genus for the complex, proposed here as *Laurenciella* gen. nov. This new taxon differs from *Laurencia s.s.* and from the other genera of the complex by molecular sequence data, but is indistinguishable from *Laurencia s.s.* by the usual morphological features.

Keywords: *Laurencia* complex; *Laurenciella* gen. nov.; molecular phylogeny; *rbcL*; Rhodomelaceae.

Introduction

The *Laurencia* complex is a large and common group of red seaweeds that are widely distributed around the globe, mainly in the intertidal zone of tropical and subtropical regions (Guiry and Guiry 2012). Species discrimination within this complex is notoriously difficult using traditional morphological

characters because of extensive morphological plasticity combined with the difficulty in defining diacritical characters for the morphology and anatomy. Therefore, molecular markers have been useful in delimiting taxa and inferring their phylogenetic relationships.

Currently, five genera are recognized to the *Laurencia* complex: *Chondrophyucus* (Tokida *et* Saito) Garbary *et* J.T. Harper, *Laurencia* J.V. Lamouroux, *Osmundea* Stackhouse, *Palisada* (Yamada) K.W. Nam and *Yuzurua* (K.W. Nam) Martin-Lescanne. The genera are distinguished by a combination of vegetative and reproductive characters, such as: (1) number of pericentral cells per vegetative axis; (2) position of the first pericentral cell relative to the trichoblast; (3) origin of the tetrasporangia; (4) presence or absence of fertility in the second pericentral cells; (5) number of sterile pericentral cells in the tetrasporangial axis; (6) origin of the spermatangial branches; (7) formation pattern of the spermatangial branches on trichoblasts; (8) number of pericentral cells in procarp-bearing segments of female trichoblasts; and (9) post-fertilization features associated with the timing of auxiliary cell formation (Nam 1999, 2006). Among genera of the complex, *Laurencia* is the only one that possesses four pericentral cells per vegetative axial segment; *Chondrophyucus*, *Osmundea*, *Palisada* and *Yuzurua* have two pericentral cells (Nam *et al.* 1994, Garbary and Harper 1998, Nam 1999, 2006). The genus *Osmundea* is distinguished from the other genera by its tetrasporangial production from random cortical cells rather than from particular pericentral cells, and spermatangial development of the filament-type rather than the trichoblast-type (Nam *et al.* 1994). The genus *Chondrophyucus* is characterized by spermatangial branches produced from two laterals on the suprabasal cell of trichoblasts (one of them remaining partly sterile), and a tetrasporangial axis with second pericentral cells that are never fertile, resulting in an axis with two sterile pericentral cells (Nam 1999). In the genus *Palisada*, the spermatangial branches are produced from one of two laterals on the suprabasal cells of trichoblasts, and the second pericentral cell in the tetrasporangial axis is always fertile; the resulting axis has one sterile pericentral cell (Nam 2006, 2007). The genus *Yuzurua* shares a majority of morphological characters with *Palisada*, from which it was recently segregated, but differs in not having palisade-like cells, in the presence of secondary pit-connections between cortical cells, and in the presence of procarp-bearing segments with five pericentral cells rather than four (Fujii *et al.* 1996).

Most of the molecular approaches have been based on the plastid-encoded *rbcL* gene (Nam *et al.* 2000, McIvor *et al.* 2002, Abe *et al.* 2006, Fujii *et al.* 2006, Cassano *et al.* 2009,

2012, Martin-Lescanne et al. 2010). Although the evolutionary relationships among genera are still not fully understood, the monophyly of *Chondrophyucus*, *Osmundea*, *Palisada* and *Yuzurua* has been strongly confirmed in phylogenetic inferences based on *rbcL* sequences (Nam et al. 2000, McIvor et al. 2002, Fujii et al. 2006, Martin-Lescanne et al. 2010). In contrast, the monophyly of the genus *Laurencia* is not well supported, with *L. marilzae* Gil-Rodríguez, Senties, Díaz-Larrea, Cassano et M.T. Fujii forming a divergent lineage from *Laurencia sensu stricto* (Gil-Rodríguez et al. 2009, Rocha-Jorge et al. 2010, Fujii et al. 2011). Moreover, many interspecific nodes within *Laurencia s.s.* are also not well supported (Garbary and Harper 1998, Abe et al. 2006, Fujii et al. 2006, Martin-Lescanne et al. 2010). According to Martin-Lescanne et al. (2010), the poor resolution at the interspecific nodes within *Laurencia s.s.* may be attributable to either the lack of phylogenetic signals or restricted sampling.

In this study we employed the *rbcL* gene to assess specific and generic discrimination among taxa within the *Laurencia* complex, proposing the establishment of a sixth genus for the complex named here as *Laurenciella* Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C. Oliveira et M.T. Fujii gen. nov.

Materials and methods

Sample collection

The samples were collected in Brazil, Mexico and Canary Islands (Spain). The 85 specimens used in the molecular study are presented in Table 1. A detailed morphological study was carried out on living and 4% formalin/seawater-fixed specimens for all samples collected using a Nikon Eclipse E200 microscope (Tokyo, Japan). Voucher specimens were deposited in HRJ, SP, SPF, UAMIZ, TFC (herbarium abbreviations follow the online *Index Herbariorum* <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Molecular study

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 20 newly generated *rbcL* sequences were included in this study; the rest were obtained from GenBank (Table 1). For each sequence generated, a total of 1467 bp of the *rbcL* gene were amplified in three overlapping parts with the primers pairs: FrbcLstart – R753, F492 – R1150 and F993 – RrbcS (Freshwater and Rueness 1994) using a polymerase chain reaction (PCR) master mix (Promega Corporation, Madison, WI, USA). All PCR products were analyzed by electrophoresis in 1% agarose to check product size. The PCR products were purified with MicroSpinTMS-300 HR Columns (GE Healthcare Life Sciences, Piscataway, NJ, USA) in accordance with the manufacturer's instructions. Sequencing was carried out with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied

Biosystems, Branchburg, 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 and consensus sequences as well as multiple alignments were constructed manually using BioEdit 7.0.4.1 software (Ibis Biosciences, Carlsbad, CA, USA) (Hall 1999).

Phylogenetic analysis

Phylogenetic relationships were inferred with PAUP* 4.0b10 (Sinauer Associates, Inc., Publishers, Sunderland, MA, USA) (Swofford 2002) and MrBayes v.3.0.4 (San Diego, CA, USA) (Huelsenbeck and 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 (GTR+I+G) was selected based on maximum likelihood ratio tests implemented in Modeltest version 3.06 (Posada and Crandall 1998) with a significance level of 0.01 by the 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. The first 100,000 generations 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 *rbcL* divergence values within and among species was calculated using uncorrected 'p' distances using PAUP.

Results

Phylogeny

We sequenced the *rbcL* gene for 21 specimens of the *Laurencia* complex: 13 specimens of *Laurencia s.s.*, five of '*L. marilzae* – *L. sp. 2* – *L. sp. 3* group', one of *Osmundea* and two of *Palisada*. Eighty-five sequences of 1210 bp were analyzed in this study (Table 1). The dataset for *rbcL* sequences consisted of 713 constant characters, 417 parsimony-informative sites and 87 parsimony non-informative sites.

The *Laurencia* complex was separated into six clades represented by the currently recognized genera *Chondrophyucus*, *Laurencia s.s.*, *Osmundea*, *Palisada*, *Yuzurua*, and by a sixth clade, the '*L. marilzae*, *L. sp. 2* and *L. sp. 3* group' (Figure 1). The evolutionary relationships among the genera of the *Laurencia* complex were well-supported only in Bayesian analysis, with posterior probability of 0.98 for *Osmundea* and *Chondrophyucus* clades, and 1.00 for *Laurencia s.s.*, *Yuzurua* and '*L. marilzae* – *L. sp. 2* – *L. sp. 3* group' clades. Although '*L. marilzae*, *L. sp. 2* and *L. sp. 3* group' has been resolved as sister to *Laurencia s.s.* clade, only in Bayesian analysis with moderate support (0.88),

Table 1 Taxa used in phylogenetic analyses.

Samples	Collection data/reference	GenBank accession number
<i>Bostrychia radicans</i> (Montagne) Montagne in Orbigny	USA, Mississippi, St Louis Bay, 11 February, 1998, C.F.D. Gurgel (Lin et al. 2001)	AF259497
<i>Bryocladia cuspidata</i> (J. Agardh) De Toni	USA, Texas, Port Aransas, 17 May, 1998, S. Fredericq and C.F.D. Gurgel (Lin et al. 2001)	AF259498
<i>Chondria collinsiana</i> M.A. Howe	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 January, 2005, V. Cassano and J.C. De-Paula (Cassano et al. 2012)	GU330225
<i>C. dasyphylla</i> (Woodward) C. Agardh	USA, North Carolina, New Hanover Co., Wrightsville Beach (Freshwater et al. 1994)	U04021
<i>Gracilaria tenuistipitata</i> C.F. Chang et B.M. Xia var. <i>liui</i> Zhang et B.M. Xia	China, Haikou, 5 June, 1990, E.C. Oliveira (Hagopian et al. 2004)	AY673996
<i>Polysiphonia muelleriana</i> J. Agardh	New Zealand, Deas Cove, Thompson Sound, Fiordland, 3 October, 2000, S. Wing and N. Goebel (Fujii et al. 2006)	AY588412
<i>Chondrophycus</i> cf. <i>undulatus</i>	New Caledonia, Loyalty Island, Maré, 22 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785307
<i>Chondrophycus</i> sp. 1	New Caledonia, Loyalty Island, Lifou, 26 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785309
<i>Chondrophycus</i> sp. 2	New Caledonia, Loyalty Island, Maré, 21 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785310
<i>Chondrophycus</i> sp. 3	New Caledonia, Loyalty Island, Beautemps/Beaupré, 6 April 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785311
<i>Laurencia aldingensis</i> Saito et Womersley	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 January, 2005, V. Cassano and J.C. De-Paula (this study)	JF810351
<i>L. aldingensis</i>	Brazil, Espírito Santo, Anchieta, Ilhote de Ubu, 30 June, 2007, E.M. Stein (this study)	–
<i>L. cf. brongniartii</i>	Australia, Tarcoala Beach, S. Fredericq, 1993 (Gil-Rodríguez et al. 2010)	EF061654
<i>L. cf. brongniartii</i>	Taiwan, Makang Harbour, S. Fredericq, 11 July, 1993 (Fujii et al. 2006)	AF465814
<i>L. caduciramulosa</i> Masuda et Kawaguchi	Brazil, Rio de Janeiro, Angra dos Reis, Baía da Ribeira, Praia do Velho, 19 April, 2006, V. Cassano and J.C. De-Paula (this study)	–
<i>L. caduciramulosa</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 6 May, 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea (Machín-Sánchez et al. 2012a)	JF781525
<i>L. caraibica</i> P.C. Silva	Mexico, Quintana Roo, Cancún, Isla Mujeres, 23 February, 2006, A. Senties (Gil-Rodríguez et al. 2009)	EF658642
<i>L. catarinensis</i> Cordeiro-Marino et M.T. Fujii	Brazil, Santa Catarina, Florianópolis, Prainha da Barra da Lagoa, 16 July, 2008, P.A. Horta (this study)	–
<i>L. catarinensis</i> (as <i>L. intricata</i>)	Brazil, Rio de Janeiro, Angra dos Reis, Ponta da Fortaleza, 14 December, 2006, V. Cassano, M.T. Széchy and M.T. Fujii (this study)	–
<i>L. catarinensis</i>	Brazil, Espírito Santo, Anchieta, Ponta dos Castelhanos, 5 October, 2006, M.T. Fujii and V. Cassano (this study)	–
<i>L. catarinensis</i> (as <i>L. intricata</i>)	Brazil, São Paulo, Santos, Laje de Santos Marine State Park, 18 August, 2008, E.C. Oliveira (this study)	–
<i>L. catarinensis</i> (as <i>L. intricata</i>)	Brazil, Rio Grande do Norte, Maracajaú, 24 June, 2006, M.T. Fujii and I.B. Silva (this study)	–
<i>L. catarinensis</i> (as <i>L. intricata</i>)	Spain, Canary Islands, Tenerife, El Pris, 4 May, 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea (Machín-Sánchez et al. 2012a)	–
<i>L. catarinensis</i> (as <i>L. intricata</i>)	Spain, Canary Islands, Tenerife, Playa Paraíso, 5 May, 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea (this study)	–
<i>Laurencia dendroidea</i> J. Agardh [as <i>L. filiformis</i> (C. Agardh) Montagne]	Brazil, Bahia, Lauro de Freitas, Praia Vilas do Atlântico, 8 January, 2008, A. Oliveira (Cassano et al. 2012)	GU330228
<i>L. dendroidea</i> [as <i>L. majuscula</i> (Harvey) A.H.S. Lucas]	Brazil, Rio de Janeiro, Angra dos Reis, Praia do Velho, 20 July, 2006, V. Cassano and J.C. De-Paula (Cassano et al. 2012)	GU330232
<i>L. dendroidea</i> (as <i>L. arbuscula</i>)	Brazil, São Paulo, Ubatuba, Praia do Felix, 31 August, 2000, M.T. Fujii (Fujii et al. 2006)	AF465810
<i>L. dendroidea</i> (as <i>L. filiformis</i>)	Brazil, São Paulo, Santos, Laje de Santos Marine State Park, 18 August, 2008, E.C. Oliveira (this study)	–

(Table 1 continued)

Samples	Collection data/reference	GenBank accession number
<i>L. dendroidea</i> (as <i>L. majuscula</i>)	Spain, Canary Islands, Tenerife, Puerto de la Cruz, 13 July, 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Sentfés (Gil-Rodríguez et al. 2009)	EF686000
<i>L. flexuosa</i> Kützing	South Africa, S. KwaZulu-Natal, Palm Beach, 7 February, 2001, S. Fredericq (Fujii et al. 2006)	AF465815
<i>L. intricata</i> J.V. Lamouroux	Mexico, Campeche, Campeche Bay, 14 February 1999, C.F.D. Gurgel (Fujii et al. 2006)	AF465809
<i>L. intricata</i>	USA, Florida, Long Key, Channel 5, 10 December, 1998, B. Wysor and T. Frankovich (Fujii et al. 2006)	AY588410
<i>L. intricata</i>	Cuba, Ciego de Ávila, Cayo Coco, 25 September, 2005, M.T. Fujii (Cassano et al. 2012)	GU330238
<i>L. cf. kuetzingii</i>	New Caledonia, Loyalty Is., Ouvéa, 31 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785322
<i>L. cf. majuscula</i>	New Caledonia, Ile des Pins, 2 December, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785312
<i>L. cf. mariannensis</i>	New Caledonia, Lagon Sud-Ouest, Ilot Larégnère, 11 July, 2003, C. Payri (Martin-Lescanne et al. 2010)	FJ785313
<i>L. marilzae</i> Gil-Rodríguez, Sentfés, Díaz-Larrea, Cassano et M.T. Fujii	Spain, Canary Islands, Tenerife, Playa Paraíso, 14 July 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Sentfés (Gil-Rodríguez et al. 2009)	EF686001
<i>L. marilzae</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, M.C. Gil-Rodríguez, 12 July, 2006 (Gil-Rodríguez et al. 2009)	EF686002
<i>L. marilzae</i>	Mexico, Isla Mujeres, Quintana Roo, 2008, A. Sentfés and M.T. Fujii (Sentfés et al. 2011)	HQ115065
<i>L. marilzae</i>	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 March, 2007, R. Rocha-Jorge (Rocha-Jorge et al. 2010)	GU938189
<i>L. cf. mcdermidiae</i>	New Caledonia, Ile des Pins, 29 November 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785314
<i>L. natalensis</i> Kylin	South Africa, KwaZulu-Natal, Palm Beach, 7 February 2001, S. Fredericq (Fujii et al. 2006)	AF465816
<i>L. cf. nidifica</i>	New Caledonia, Ile des Pins, 30 November, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785315
<i>L. obtusa</i> (Hudson) J.V. Lamouroux	Ireland, County Donegal, Fanad Head, 6 July, 1998, C.A. Maggs (McIvor et al. 2002)	AF281881
<i>L. oliveirana</i> Yoneshigue	Brazil, Rio de Janeiro, Arraial do Cabo, Ponta da Cabeça, 7 July, 2008, V. Cassano and J.C. De-Paula (this study)	JF810352
<i>L. pyramidalis</i> Bory ex Kützing	France, Brittany, Roscoff, 5 December, 2002, F. Rousseau (Martin-Lescanne et al. 2010)	FJ785316
<i>L. translucida</i> M.T. Fujii et Cordeiro-Marino	Brazil, Espírito Santo, Marataízes, 15 September, 2001, M.T. Fujii (Fujii et al. 2006)	AY588408
<i>L. venusta</i> Yamada	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, J.D. Larrea & A. Sentfés, 18 April, 2004 (Díaz-Larrea et al. 2007)	EF061655
<i>L. viridis</i> Gil-Rodríguez et Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 6 October, 2005, M.C. Gil-Rodríguez (Gil-Rodríguez et al. 2009)	EF685999
<i>Laurencia</i> sp. 1	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 January, 2005, V. Cassano and J.C. De-Paula (this study)	–
<i>Laurencia</i> sp. 1	Brazil, Espírito Santo, Anchieta, Ilhote de Ubu, 30 June, 2007, E. Stein (this study)	–
<i>Laurencia</i> sp. 2	Brazil, Espírito Santo, Anchieta, Ilhote de Ubu, 30 June, 2007, E. Stein (this study)	–
<i>Laurencia</i> sp. 3	Brazil, São Paulo, Ilha Vitória, 19 May, 2008, M.T. Fujii (this study)	–
<i>Laurencia</i> sp. 3	Brazil, São Paulo, Santos, Laje de Santos Marine State Park, 18 August, 2008, E.C. Oliveira (this study)	–
<i>Laurencia</i> sp. 3	Brazil, São Paulo, Santos, Laje de Santos Marine State Park, 18 August, 2008, E.C. Oliveira (this study)	–
<i>Laurencia</i> sp. 3	Brazil, São Paulo, Santos, Laje de Santos Marine State Park, 18 August 2008, E.C. Oliveira (this study)	–
<i>Osmundea blinksii</i> (Hollenberg et Abbott) K.W. Nam	USA, California, San Mateo County, Año Nuevo, Greyhound Rock, 17 July, 1996, M.H. Hommersand (McIvor et al. 2002)	AY172575

(Table 1 continued)

Samples	Collection data/reference	GenBank accession number
<i>O. oederi</i> (Gunnerus) G. Furnari [as <i>O. ramosissima</i> (Oeder) Athanasiadis]	Ireland, County Donegal, St John's Point, 12 October, 1999, C.A. Maggs (McIvor et al. 2002)	AF281880
<i>O. osmunda</i> (S.G. Gmelin) K.W. Nam	Ireland, County Donegal, St John's Point, 12 Oct. 1999, C.A. Maggs (McIvor et al. 2002)	AF281877
<i>O. pinnatifida</i> (Hudson) Stackhouse	Spain, Canary Islands, La Palma, La Fajana de Barlovento, 24 January, 2008, M.C. Gil-Rodríguez (this study)	–
<i>O. pinnatifida</i>	France, Brittany, Penmarch (Lin et al. 2001)	AF259495
<i>O. sinicola</i> (Setchell et Gardner) K.W. Nam	USA, California, Orange County, Crescent Beach, 28 May, 2002, S. Murray (Fujii et al. 2006)	AY588407
<i>O. spectabilis</i> (Postels et Ruprecht) K.W. Nam var. <i>spectabilis</i>	Mexico, Baja California, Punta Santo Thomas, 2 July, 1996, M.H. Homersand (McIvor et al. 2002)	AY172574
<i>O. splendens</i> (Hollenberg) K.W. Nam	Mexico, Baja California, Bahía Colnett, Drift, 2 July, 1996, M.H. Homersand and J. Hughey (McIvor et al. 2002)	AY172576
<i>O. truncata</i> (Kützinger) K.W. Nam et Maggs	Ireland, Lough Hyne, County Cork, 11 November, 1999, C.A. Maggs (McIvor et al. 2002)	AF281879
<i>O. truncata</i>	Spain, Canary Islands, Tenerife, La Barranquera, 5 May, 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea (Machín-Sánchez et al. 2012b)	JF781523
<i>Palisada corallopsis</i> (Montagne) Senties, M.T. Fujii et Díaz-Larrea	Mexico, Quintana Roo, Cancún, Chaac-Mol Beach, 21 August, 2005, J. Díaz-Larrea and A. Senties (Díaz-Larrea et al. 2007)	EF061646
<i>P. cf. cruciata</i>	New Caledonia, Ile des Pins, 4 December, 2005, C. Payri (Martin- Lescanne et al. 2010)	FJ785319
<i>P. flagellifera</i> (J. Agardh) K.W. Nam	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, 3 August, 2005, V. Cassano and M.B. Barros-Barreto (Gil-Rodríguez et al. 2010)	GU330221
<i>P. flagellifera</i>	Brazil, São Paulo, Ubatuba, Praia Brava, 25 May, 2001, S.M.B. Guimarães and J. Domingos (Fujii et al. 2006)	AF465804
<i>P. flagellifera</i>	Spain, Canary Islands, Tenerife, Playa Paraíso, 12 July, 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Senties (Gil-Rodríguez et al. 2010)	EF685998
<i>P. furcata</i> (Cordeiro-Marino et M.T. Fujii) Cassano et M.T. Fujii	Brazil, Paraíba, Praia de Tambaú, 24 February, 2004, M.T. Fujii (Cassano et al. 2012)	GU330226
<i>P. patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodríguez et M.T. Fujii	Philippines (A.O. Lluisma, unpublished)	AF489862
<i>P. perforata</i> (Bory) K.W. Nam	Spain, Canary Islands, Tenerife, Puerto de La Cruz, San Telmo, 14 July, 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Senties (Cassano et al. 2009)	EU256329
<i>P. perforata</i>	Brazil, Rio de Janeiro, Parati, Praia da Lula, 9 February, 2005, V. Cassano (this study)	–
<i>P. perforata</i>	Brazil, São Paulo, Ubatuba, Praia Vermelha do Sul, 13 October, 2008, E.C. Oliveira (this study)	–
<i>P. perforata</i>	Mexico, Quintana Roo, Cancún, Isla Mujeres, 2 March, 2007, A. Senties and M.C. Gil-Rodríguez (Cassano et al. 2009)	EF658641
<i>P. cf. perforata</i>	New Caledonia, Lifou, 23 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785320
<i>Palisada</i> sp. [as <i>P. papillosa</i> (C. Agardh) K.W. Nam]	Mexico, Baja California, Todos Santos, 24 October, 1999, S. Fredericq (Fujii et al. 2006)	AY588409
<i>Palisada. cf. robusta</i>	New Caledonia, Lifou, 23 March, 2005, C. Payri (Martin-Lescanne et al. 2010)	FJ785321
<i>P. thuyoides</i> (Kützinger) Cassano, Senties, Gil-Rodríguez et M.T. Fujii	Philippines (A.O. Lluisma, unpublished)	AF489863
<i>Yuzurua poiteaui</i> (J.V. Lamouroux) Martin-Lescanne var. <i>gemmifera</i> (Harvey) M.J. Wynne	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 16 April, 2004, J. Díaz-Larrea and A. Senties (Díaz-Larrea et al. 2007)	EF061648
<i>Y. poiteaui</i> var. <i>gemmifera</i>	Cuba, La Habana, Rincon de Guanabo, 29 July, 2005, J. Díaz-Larrea and A.A. Mallea (Díaz-Larrea et al. 2007)	EF061650
<i>Y. poiteaui</i> var. <i>poiteaui</i>	USA, Florida, Long Key, Ocean Side, 1998, S. Fredericq (Díaz-Larrea et al. 2007)	EF061652
<i>Y. poiteaui</i> var. <i>poiteaui</i>	Mexico, Quintana Roo, Playa del Carmen, 15 March, 2005, J. Díaz- Larrea and A. Senties (Díaz-Larrea et al. 2007)	EF061653

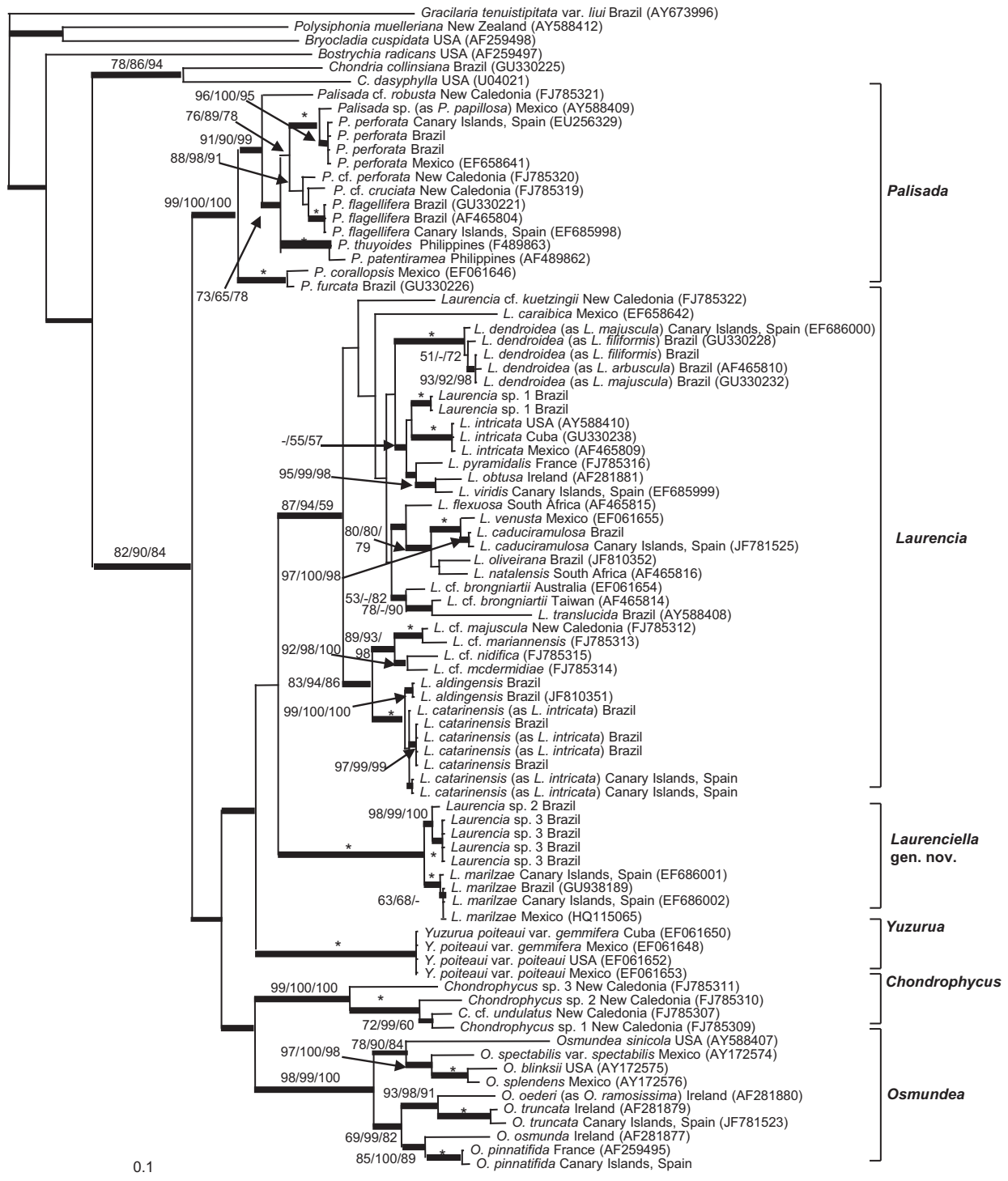


Figure 1 Bayesian analysis inferred from *rbcL* sequences for the *Laurencia* complex. The posterior probabilities (when >95%) are shown as thicker branches. Bootstrap supports for MP/NJ (2000 replicates)/ML (1000 replicates) are shown at the nodes. Asterisks indicate 100% bootstrap support.

the former clearly represents an independent lineage within the *Laurencia* complex with full support in all analyses.

The genetic divergences are summarized in Table 2. Our results clearly showed that the '*L. marilzae* – *L. sp. 2* – *L. sp. 3* group' constitutes a new genus within the *Laurencia* complex, which is here proposed as

***Laurenciella* Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C. Oliveira et M.T. Fujii gen. nov.**

Diagnosis *Cum characteribus generis Laurenciae sensu stricto i.e.: cellula apicali depressa in apicalis ramuli fovea; cortice extens; omnibus segmentis axialibus cum quattuor*

Table 2 Range of genetic divergence of the *rbcL* gene within the *Laurencia* complex.

Genera/marker	<i>rbcL</i>		
		Interspecific divergence (%)	Intraspecific divergence (%)
<i>Laurenciella</i> and <i>Laurencia</i>	8.2–11.0	<i>Laurenciella</i> (0.7) ^a 1.4–1.5	0–0.2
<i>Laurenciella</i> and <i>Palisada</i>	8.7–10.2	<i>Laurencia</i> 1.0–6.8	0–0.62
<i>Laurenciella</i> and <i>Chondrophycus</i>	10.4–11.3	<i>Palisada</i> 1.4–6.4	0–0.2
<i>Laurenciella</i> and <i>Osmundea</i>	10.2–13.4	<i>Chondrophycus</i> 1.8–6.9	–
<i>Laurenciella</i> and <i>Yuzurua</i>	10.2–10.4	<i>Osmundea</i> 1.9–10	0.4–1.0
<i>Laurencia</i> and <i>Palisada</i>	7.2–11.5	<i>Yuzurua</i> –	0–0.1
<i>Laurencia</i> and <i>Chondrophycus</i>	8.8–12.2		
<i>Laurencia</i> and <i>Osmundea</i>	9.2–13.7		
<i>Laurencia</i> and <i>Yuzurua</i>	9.0–10.7		
<i>Palisada</i> and <i>Chondrophycus</i>	8.8–10.5		
<i>Palisada</i> and <i>Osmundea</i>	9.1–13.2		
<i>Palisada</i> and <i>Yuzurua</i>	8.9–11.1		
<i>Chondrophycus</i> and <i>Osmundea</i>	9.5–12		
<i>Chondrophycus</i> and <i>Yuzurua</i>	10.5–11.2		
<i>Yuzurua</i> and <i>Osmundea</i>	11.2–12.9		

^aLow divergence between *Laurencia* sp. 2 and *L.* sp. 3 (see Discussion).

cellulis pericentralibus; prima cellula pericentrali sub trichoblastorum basali cellula facta; spermatangiis typi trichoblasti; ramulis spermatangialibus ab una duorum lateralium ramulorum in trichoblasti cellula suprabasica factis; segmentis procarpiaferentibus quinque pericentralibus cellulis; tetrasporangiis e cellula pericentrali peculiari factis. Ab allis generibus Laurenciae sensu lato differt in rbcL molecularibus sequentiis.

With the characters of the genus *Laurencia* s.s. i.e., apical cell always sunk in apical pit of branchlet; central axis recognizable only near apical cell; forming extensive cortex; vegetative axial segments with four pericentral cells; first pericentral cell produced underneath the basal cell of the trichoblast; spermatangial development of the trichoblast type; spermatangial branches produced from one of two laterals on the suprabaasal cell of trichoblasts; procarp-bearing segments with five pericentral cells; tetrasporangial production from particular pericentral cells. Distinct from other genera of the *Laurencia* complex by the *rbcL* molecular sequences.

Etymology The generic name refers to the resemblance with *Laurencia*; *-ella*, diminutive.

Type species *Laurenciella marilzae* (Gil-Rodríguez, Senties, Díaz-Larrea, Cassano et M.T. Fujii) Gil-Rodríguez, Senties, Díaz-Larrea, Cassano et M.T. Fujii comb. nov.

Holotype TFC Phyc 13129; GenBank no.: EF686002 (*rbcL* sequence of the holotype).

Basionym *Laurencia marilzae* Gil-Rodríguez et al. (2009): J. Phycol. 45: 265–269, Figures 1–5).

Discussion

The *Laurencia* complex was resolved as a monophyletic group in all analyses, as in previous molecular studies based on the *rbcL* gene (Abe et al. 2006, Fujii et al. 2006, 2011, Cassano et al. 2009, 2012, Martin-Lescanne et al. 2010). The complex was split into six lineages represented by the well-supported genera *Chondrophycus*, *Laurenciella*, *Osmundea*, *Palisada* and *Yuzurua*, and by the moderate-to-well-supported genus *Laurencia*.

The intergeneric divergence values between *Laurenciella* and *Laurencia* s.s. were in the range of genetic variation observed among the other genera of the complex (Table 2). The interspecific divergence values obtained in this study were similar to those previously reported for the *Laurencia* complex (McIvor et al. 2002, Nam et al. 2000, Díaz-Larrea et al. 2007, Cassano et al. 2009, Martin-Lescanne et al. 2010, Rocha-Jorge et al. 2010).

The new genus established here, *Laurenciella*, includes a species described as *Laurencia marilzae* from the Canary Islands (Gil-Rodríguez et al. 2009), later reported from deep waters in southeastern Brazil (Rocha-Jorge et al. 2010), and from the Mexican Caribbean (Senties et al. 2011). The most distinctive characteristic of this species is the presence of one, rarely two, *corps in cerise* scattered in all cells of the thallus, a feature not shared with *L.* sp. 2 and *L.* sp. 3, which formed a full supported clade with *L. marilzae* and whose *corps in cerise* are restricted to only cortical cells and trichoblasts. Our molecular data indicate that *L. marilzae* is distinct from *L.* sp. 2 and *L.* sp. 3. These latter two taxa may be conspecific since the divergence in the *rbcL* sequences between *L.* sp. 2 and *L.* sp. 3 was low (0.7%, 8 bp in 1210 positions). However, sequences of *cox1* (the 5' region of the mitochondrial cytochrome *c* oxidase I gene, Saunders 2005) obtained for both taxa (data not

shown) had higher genetic divergence between *L. sp. 2* and *L. sp. 3* (4.1%, 27 bp divergent in 644 positions). This variation is within the range of interspecific divergences observed for other red algae (Saunders 2005, Robba et al. 2006, Yang et al. 2007, Clarkston and Saunders 2010). Morphologically, *L. sp. 2* and *L. sp. 3* differ in their habit, but only tetrasporangial plants of the two species have been found; therefore these taxa need further morphological studies and broader molecular data to clarify their taxonomic position.

Laurenciella shares all generic diagnostic characters with *Laurencia s.s.*; it is not possible to distinguish them by traditional morphological characters. These morphological features include: four pericentral cells per vegetative axial segment, first pericentral cell produced underneath the basal cell of the trichoblast, tetrasporangia formed from particular pericentral cells, without formation of additional fertile pericentral cells, spermatangial development of trichoblast-type with spermatangial branches produced from one of two laterals on the suprabasal cells of trichoblasts, and five pericentral cells in procarp-bearing segments of female trichoblasts. When *Laurencia marilzae* was described, Gil-Rodríguez et al. (2009) highlighted the high level of genetic divergence between the samples of this species and the rest of *Laurencia s.s.* The possibility that it represented a new genus was also pointed out by Rocha-Jorge et al. (2010) based on the high genetic distance (8.6–10.7%) from *Laurencia s.s.* However, because of the lack of *rbcL* sequences representing all genera of the complex, this taxon remained assigned to *Laurencia* until more sequences became available for comparison.

Molecular data, especially those obtained by DNA Barcode markers (*cox1* and UPA, the universal plastid amplicon, domain V of the 23S rRNA gene) (Presting 2006) have resolved more genetic groups of species than expected. The genetic groups can be further supported by morphological and anatomical features, as verified by Saunders (2008) for the Dumontiaceae, Clarkston and McDonald (2010) for the Rhodymeniaceae, Clarkston and Saunders (2010) for the Kallymeniaceae and Le Gall and Saunders (2010) for the Phylloporaceae. In contrast, the genetic groups formed for the genus *Batrachospermum* Roth are not supported by morphological characters that formally distinguish them as distinct species (Sherwood et al. 2008). Clarkston and Saunders (2010) have argued that when multiple species-level markers support the same group of genetic species, they must be recognized as different species, even if after extensive examination there are no morphological characters to support a formal separation. Recently, Sutherland et al. (2011) reviewed the Bangiales, establishing new genera based largely on molecular phylogenetic data. In our phylogenetic analyses with broader taxon sampling using *rbcL* sequences and also *cox1* and UPA (data not presented), the *L. marilzae*, *L. sp. 2* and *L. sp. 3* group remained as a separate lineage within the *Laurencia* complex, justifying its separation as a new genus.

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