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Phylogenetic analyses of the *Laurencia* complex (Rhodomelaceae, Ceramiales) support recognition of five genera: *Chondrophycus*, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua* stat. nov.

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Molecular phylogenies inferred from *rbc*L sequences including 39 representative members of the *Laurencia* complex confirm the four genera currently recognised within the complex: *Laurencia* sensu stricto, *Osmundea*, *Chondrophycus* and the recently described genus *Palisada*. Furthermore, *Palisada poiteaui* was resolved as a fifth independent lineage suggesting that the complex is actually composed of five rather than four genera. *Palisada poiteaui* is the type species of the subgenus *Yuzurua*, and elevation of this subgenus to generic rank is proposed. This new genus allied strongly with *Laurencia* s.s. However, the other intergeneric relationships were not well supported, suggesting that *rbc*L sequences may not have sufficient signal to clarify infrageneric relationships fully within the *Laurencia* complex.

Key words: *Chondrophycus*, *Laurencia* complex, molecular phylogeny, *Osmundea*, *Palisada*, *rbc*L, *Yuzurua poiteaui*, *Yuzurua* stat. nov

Introduction

The genus *Laurencia* was erected by Lamouroux in 1813; thereafter its taxonomic history has been convoluted, and here we discuss only the major changes that have occurred; the reader is invited to refer to Saito (1967), McDermid (1988), Furnari & Serio (1995) and Furnari *et al.* (2001) for a more comprehensive history. Lamouroux included eight species in the original description of *Laurencia*. Subsequently, Schmitz (1889) recognised *Laurencia obtusa* (Hudson) J.V. Lamouroux as the 'typische Species' for the genus and this species is currently considered the generitype. Thorough anatomical studies during the last four decades (e.g. Saito, 1967; Nam *et al.*, 1994; Garbary & Harper, 1998; Nam, 1999, 2006) have revealed that *Laurencia* is a highly diverse genus, encompassing species that display distinctive features usually diagnostic at the generic level. The genus has therefore been referred to the *Laurencia* complex and, three additional genera, *Osmundea* Stackhouse, *Chondrophycus* (Tokida & Y. Saito) Garbary & Harper, and *Palisada* (Yamada) Nam have been proposed successively to reflect its morphological diversity. Saito (1967) was the first to divide *Laurencia* into two subgenera, *Laurencia* and *Chondrophycus*, based on the occurrence of secondary pit connections between epidermal cells, and the type of tetrasporangial arrangement. The genus *Osmundea* (Stackhouse, 1809), which had been placed in synonymy with *Laurencia* (nom. cons., see Papenfuss, 1947), was resurrected by Nam *et al.* (1994) to accommodate taxa that exhibit a filament-type spermatangial development rather than a trichoblastic-type, and tetrasporangial initials arising from a random epidermal cell rather than a particular pericentral cell. *Osmundea* currently includes 18 species mostly reported from temperate waters

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(Nam *et al*., 2000; McIvor *et al*., 2002; Guiry & Guiry, 2008).

Nam & Saito (1995) showed that the number of pericentral cells in *Laurencia* subgenera (four in subgenus *Laurencia* and two in subgenus *Chondrophycus*) were different, and they also noted other distinctive characters, such as the presence/absence of additional terasporangium-bearing pericentral cells, the position of pericentral cells bearing tetrasporangia, and the number of pericentral cells of the procarp-bearing segment. The taxonomic status of *Chondrophycus* had been a matter of debate for more than 20 years (e.g. Furnari & Serio, 1993) and the subgenus was elevated to generic rank by Garbary & Harper in 1998. Nam (1999) highlighted further diversity within this genus, in both reproductive and vegetative features, and proposed an infrageneric classification including four subgenera: *Chondrophycus*, *Kangjaewonia*, *Palisada* and *Yuzurua*.

Finally, Nam (2006) proposed elevating *Palisada* to generic rank to accommodate members of *Chondrophycus* that have, among other features, the first pericentral cell located underneath the trichoblast rather than on the side, and tetrasporangial axes with one sterile pericentral cell rather than two. Nineteen species were transferred to the genus *Palisada* (Nam, 2006), leaving *Chondrophycus* with 17 species. However, the generic name *Palisada* was only validated the following year with the publication of the Latin diagnosis of this genus (Nam, 2007). Recently, Senties & Díaz-Larrea (2008) transferred *Chondrophycus corallopsis* Montagne to *Palisada*, so the two genera currently include 16 and 20 species, respectively.

Contrasting with Lamouroux's initial concept of the genus *Laurencia*, *Laurencia* s.s. (i.e. *Laurencia* sensu Garbary & Harper, 1998) presently includes more than 140 species and the *Laurencia* complex encompasses almost 200 species (Guiry & Guiry, 2008), which are distributed from temperate to tropical waters (McDermid, 1988).

The phylogeny of the *Laurencia* complex has been studied mainly from anatomical and developmental perspectives, and Garbary & Harper (1998), Nam *et al*. (2000), and Nam (2006), inferred the interspecific relationships among members of the *Laurencia* complex based on cladistic analyses. Molecular studies were initiated with analyses of sequences of the plastid-encoded, large subunit of RuBisCO (*rbc*L) to infer interspecific relationships within *Osmundea* (Nam *et al*., 2000; McIvor *et al*., 2002). Fujii *et al*. (2006) have published a molecular phylogeny including sequences from *Osmundea*, *Laurencia* and *Palisada* (as *Chondrophycus*) species, and confirmed the monophyly of the three genera previously inferred from morphological characters, although with restricted sampling. Abe *et al*. (2006) inferred a molecular phylogeny of the *Laurencia* complex from *rbc*L sequences and confirmed the monophyly of *Osmundea*, however *Palisada* (as *Chondrophycus*) and *Laurencia* were both resolved as non-monophyletic. Finally, Díaz-Larrea *et al*. (2007) published a molecular phylogeny inferred from a data set built to assess species boundaries between *Palisada poiteaui* (J.V. Lamouroux) Nam (as *Chondrophycus poiteaui* (J.V. Lamouroux) Nam) and *Palisada gemmifera* (Harvey) Sentíes, Fujii & Díaz (as *Chondrophycus gemmiferus* (Harvey) Garbary & Harper) and concluded that they were conspecific. To the best of our knowledge, the delimitation of *Palisada* and *Chondrophycus* has not been tested using phylogenies inferred from molecular data. The aim of the present study was therefore to assess the generic boundaries of the four genera currently recognised within the *Laurencia* complex using *rbc*L sequences.

Materials and methods

Specimen collection

Specimens included in molecular analyses are listed in Table 1, along with their valid names and GenBank accession numbers (NCBI GenBank). Newly sequenced specimens were collected by SCUBA in the Western Pacific in the vicinity of New Caledonia (158–169°E and 18–23°S) except for *Laurencia pyramidalis*, *Osmundea osmunda* and *O. hybrida*, which were collected at low tide along the French Atlantic coast of Brittany. A part of each sample was stored in 5% buffered formalin in seawater, and the rest was dried as herbarium specimens and deposited in the herbaria of NOU-IRD (Phycological Herbarium, Institut de Recherche pour le Développement, Nouméa, New Caledonia) and PC Herbarium (abbreviations are in accordance with the Index Herbariorum (Holmgren *et al*., 1990), sciweb.nybg.org/science2.IndexHerbariorum.asp).

Morphological characters for specimen identification were observed using an Olympus BH2 compound microscope (Olympus Optical Co. Ltd., Tokyo, Japan). Most of our specimens were from New Caledonia rather than type localities and given the putative cryptic diversity (including sibling species) within the *Laurencia* complex, we treated the species identification cautiously and used the Latin-derived word 'confer' before the specific epithet of these specimens.

Extraction, amplification and sequencing

Total cellular DNA was extracted from herbarium specimens using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). Between 0.5×10^{-3} to 10^{-3} mg of proteinase K was added to the lysis buffer to improve the DNA yield (Hughey *et al*., 2001). Both the *rbc*L coding region (1,647 base pairs [bp]) and *rbc*L–*rbc*S spacer (~200 bp) were amplified using

Table 1. List of species used in this study for phylogenetic analyses, with their collection data and GenBank accession numbers.

Taxa	Collection data (location; collector(s); date; collection number[a])	GenBank no.	Ref.
Out groups			
Bostrychia radicans (Montagne) Montagne	St Louis Bay, MS, USA; C.F.D. Gurgel; 11.ii.1998	AF 259497	Lin *et al.*, 2001
Bryocladia cuspidata J. Agardh	Port Aransas, TX, USA; S. Fredericq & C.F.D. Gurgel; 17.v.1998	AF 259498	Lin *et al.*, 2001
Chondria californica (Collins) Kylin	La Jolla, CA, USA; M. Volovsek; 01.vi.1996	AY 172578	McIvor *et al.*, 2002
Chondria dasyphylla (Woodward) C. Agardh	Wrightsville Beach, NC, USA; D.W. Freshwater; Date not available	U 04021	Freshwater *et al.*, 1994
Halopithys incurva (Hudson) Batters	Jersey, Channel Islands; F. Bunker; 12.vi.1998	AF 281882	Nam *et al.*, 2000
Polysiphonia muelleriana J. Agardh	Thompson Sound, Fiordland, New Zealand; S. Wing & N. Goebel; 03.vii.2000	AY 588412	Fujii *et al.*, 2006
Chondrophycus			
C. cf. *undulatus* (Yamada) Garbary & Harper	Maré, Loyalty Is. New Caledonia; C. Payri; 22.iii.2005; IRD100	FJ 785307	
C. cf. *undulatus* (Yamada) Garbary & Harper	Maré, Loyalty Is. New Caledonia; C. Payri; 22.iii.2005; IRD82	FJ 785308	
C. sp1[a]	Lifou, Loyalty Is. New Caledonia; C. Payri; 26.iii.2005; IRD80	FJ 785309	
C. sp2[a]	Maré, Loyalty Is. New Caledonia; C. Payri; 21.iii.2005; IRD96	FJ 785310	
C. sp3[a]	Beautemps/Beaupré, Loyalty Is. New Caledonia; C. Payri; 06.iv.2005; IRD112	FJ 785311	
Laurencia			
L. arbuscula Sonder	Ubatuba, São Paulo, Brazil; M.T. Fujii; 19.i.2001	AF 465810	Fujii *et al.*, 2006
L. brongniartii J. Agardh	Makang Harbour, Taiwan; S. Fredericq & S.M. Lin; 11.vii.1993	AF 465814	Fujii *et al.*, 2006
L. catarinensis Cordeiro-Marino & Fujii	Ilhabella, São Paulo, Brazil; M.T. Fujii; 19.i.2001	AF 465808	Fujii *et al.*, 2006
L. flexuosa Kützing	Palm beach, Kwa-Zulu Natal, South Africa; S. Fredericq; 07.ii.2001	AF 465815	Zuccarello & West, 2006
L. intricata J.V. Lamouroux	Long Key, FL, USA; B. Wysor & T. Frankovich; 10.xii.1998	AY 588410	Fujii *et al.*, 2006
L. cf. *kuetzingii* A. Millar	Ouvéa, Loyalty Is. New Caledonia; C. Payri; 31.iii.2005; IRD104	FJ 785322	
L. cf. *majuscula*[a] (Harvey) A.H.S. Lucas	Ile des Pins, New Caledonia; C. Payri; 02.xii.2005; IRD132	FJ 785312	
L. cf. *mariannensis*[a] Yamada	Ilot Larégnère, Lagon Sud-Ouest, New Caledonia; C. Payri; 11.vii.2003; IRD75	FJ 785313	
L. cf. *mcdermidiae*[a] I.A. Abbott	Ile des Pins, New Caledonia; C. Payri; 29.xi.2005; IRD119	FJ 785314	
L. natalensis Kylin	Palm beach, Kwa-Zulu Natal, South Africa; S. Fredericq; 07.ii.2001	AF 465816	Fujii *et al.*, 2006
L. cf. *nidifica*[a] J. Agardh	Ile des Pins, New Caledonia; C. Payri; 30.xi.2005; IRD122	FJ 785315	
L. obtusa (Hudson) J.V. Lamouroux	Fanad Head, Donegal, Ireland; C. Maggs; 06.xii.1998	AF 281881	Nam *et al.*, 2000
L. pyramidalis[a] Bory de Saint-Vincent ex Kützing	Roscoff, Brittany, France; F. Rousseau; 05.xii.2002; PC0146011 (JML0042)	FJ 785316	
L. rigida J. Agardh	Botany Bay, NSW, Australia; G. Zuccarello & J. West; 11.v.2000	AY 920852	Zuccarello & West, 2006
L. venusta Yamada	Puerto Moroles, Quintana Rhoo, Mexico; J. Diaz & A. Senties; Date not available	EF 061655	Diaz-Larrea *et al.*, 2007
Osmundea			
O. blinksii (Hollenberg & I.A. Abbott) Nam	Año Nuevo, CA, USA; M. Hommersand; 17.vii.1996	AY 172575	McIvor *et al.*, 2002
O. hybrida[a] (A.P. de Candolle) Nam	St Lunaire, Brittany, France; F. Rousseau; 20.iii.1999; PC0146010 (JML0051)	FJ 785317	
O. osmunda[a] (S.G. Gmelin) Nam	Roscoff, Brittany, France; F. Rousseau; 05.xii.2002; PC0146009 (JML0049)	FJ 785318	
O. pinnatifida (Hudson) Stackhouse	St John's Point, Donegal, Ireland; C. Maggs; 12.x.1999	AF 281875	Nam *et al.*, 2000
O. ramosissima (Oeder) Athanasiadis	St John's Point, Donegal, Ireland; C. Maggs; 12.x.1999	AF 281880	Nam *et al.*, 2000
O. sinicola (Setchell & N.L. Gardner) Nam	Crescent Beach, CA, USA; S. Murray; 20.v.2002	AY 588407	Fujii *et al.*, 2006
O. spectabilis var. *diegoensis* (E.Y. Dawson) Nam	Point Loma, CA, USA; M. Hommersand; 07.vii.1996	AY 172573	McIvor *et al.*, 2002
O. spectabilis var. *spectabilis* (Postels & Ruprecht) Nam	Cambria, CA, USA; M. Hommersand; 10.xii.1996	AY 172572	McIvor *et al.*, 2002
O. splendens (Hollenberg) Nam	Bahia Colnett, Baja California, Mexico; M. Hommersand & J. Hughey; 02.vii.1996	AY 172576	McIvor *et al.*, 2002
O. truncata (Kützing) Nam & Maggs	Lough Hyne, Cork, Ireland; C. Maggs; 13.xi.1999	AF 281879	Nam *et al.*, 2000

(*continued*)

Table 1. Continued.

Taxa	Collection data (location; collector(s); date; collection number[a])	GenBank no.	Ref.
Palisada			
P. corallopsis (Montagne) Senties, Fujii & Diaz	Cancun, Quintana Rhoo, Mexico; J. Diaz & A. Senties; Date not available	EF 061646	Diaz-Larrea *et al.*, 2007
P. cf. *cruciata*[a] (Harvey) Nam	Ile des Pins, New Caledonia; C. Payri; 04.xii.2005; IRD 127	FJ 785319	
P. flagellifera (J. Agardh) Nam	Ubatuba, São Paulo, Brazil; S.M.P.B. Guimaraes & J. Domingos; 25.v.2001	AF 465804	Fujii *et al.*, 2006
P. papillosa-1 (C. Agardh) Garbary & Harper	Puerto Morelos, Quintana Rhoo, Mexico; J. Diaz & A. Senties; Date not available	EF 061651	Diaz-Larrea *et al.*, 2007
P. papillosa-2 (C. Agardh) Garbary & Harper	Todos Santos, Baja California, Mexico; S. Fredericq; 24.vii.1999	AY 588409	Fujii *et al.*, 2006
P. papillosa-3 (C. Agardh) Garbary & Harper	Content Key, FL, USA; M. Hommersand; 12.iii.1997	AY 172577	McIvor *et al.*, 2002
P. cf. *perforata*[a] (Bory de Saint-Vincent) Nam	Lifou, New Caledonia; C. Payri; 23.iii.2005; IRD93	FJ 785320	
P. poiteaui (J.V. Lamouroux) Nam	Playa del Carmen, Quintana Rhoo, Mexico; J. Diaz & A. Senties; Date not available	EF 061653	Diaz-Larrea *et al.*, 2007
P. cf. *robusta*[a] (Yamada) Nam	Lifou, New Caledonia; C. Payri; 23.iii.2005; IRD92	FJ 785321	

[a]Samples for which we obtained *rbc*L sequences.

the following combinations of primers (Table 2): F-rbcLstart × R-753 (Freshwater & Rueness, 1994) for the 5′ end, rbcLFC × 1011R (Nam *et al.*, 2000) or F-577 × R1381 (Freshwater & Rueness, 1994) for the middle fragment, and F-993 × R-rbcS start (Freshwater & Rueness, 1994) for the 3′ end. The protocol used for PCR amplifications was modified from Nam *et al.* (2000) and Lin *et al.* (2001). Sequence-amplifications were performed by PCR in a final 30 µl volume. For the primer pair rbcLFC × 1011R the cycle was 5 min of initial denaturation at 94°C followed by 40 cycles of 60 s at 94°C, 60 s at 52°C and 60°C at 72°C and a final extension at 72°C during 5 min. Conditions for amplifications with other primer pairs were: 4 min at 96°C for denaturation, followed by 35 cycles of 60 s at 94°C, 60 s at temperatures varying from 42°C to 50°C, and 90 s at 72°C, with a final 8 min extension at 72°C. The resulting PCR products were purified and used as templates for cycle sequencing reaction with the same primers as for the initial amplifications. These steps were performed by Genoscope (www.genoscope.fr, Evry, France).

Sequence alignments and phylogenetic analyses

Sequences were obtained for both DNA strands and assembled and corrected using Sequencher™ 4.1 (Gene Codes Corporation, Ann Arbor, Michigan). Twenty-six sequences from GenBank were included to broaden the taxonomic range of the phylogeny, but only after carefully checking that they (i) belonged to the tribe Laurenciae, (ii) covered at least 70% of the full length of the *rbc*L gene and (iii) were associated with a published manuscript. Three of these selected sequences displayed suspicious phylogenetic affinities: *Chondrophycus translucidus* (Fujii & Cordeiro-Marino) Garbary & Harper (AY585408), *Laurencia complanata* (Suhr) Kützing (AF465813) and *Palisada flagellifera* (J. Agardh) Nam (EF061647). We therefore performed distance analyses partitioning the *rbc*L gene into two equivalent-sized fragments (~702 bp) or three fragments (coinciding with the regions bordered by the universal red algal primers F-rbcLstart × R-753, F-577 × R1381 and F-993 × R-rbcS start). It appeared that the position of the three taxa changed considerably depending on the partition used, suggesting that they were likely to be chimeric, and therefore we excluded them from our analyses. Out-group species (see Table 1) were chosen from the Rhodomelacean sequences available in GenBank. Alignments were performed with MEGA version 3.1 (Kumar *et al.*, 2004) using the CLUSTAL algorithm (Thompson *et al.*, 1994).

Phylogenetic analyses of rbc*L*

Maximum parsimony (MP) analyses were performed using PAUP* version 4.0b10 (Swofford, 2003) and used a heuristic search with 1000 random additions, unordered and unweighted characters, with tree bisection-reconnection branch swapping in effect. The program Modeltest version 3.7 (Posada & Crandall, 1998) was used to determine the model that best fit

Table 2. Primers used for amplification and sequencing in this study.

*rbc*L	Sequence (5′–3′)	References
Forward primers		
F-rbcL start	TGTGTTGTCGACATGTCTAACTCTGTAGAAG	Freshwater & Rueness (1994)
rbcLFC	ACTCCTCAACCAGGAGTAGATCCAG	Nam *et al.* (2001)
F-577	GTATATGAAGGTCTAAAAGGTGG	Freshwater & Rueness (1994)
F-993	GGTACTGTTGTAGGTAAATTAGAAGG	Freshwater & Rueness (1994)
Reverse primers		
R-rbcS start	TGTGTTGCGGCCGCCCTTGTGTTAGTCTCAC	Freshwater & Rueness (1994)
R-753	GCTCTTTCATACATATCTTCC	Freshwater & Rueness (1994)
1011R	TGACCACAATGAATACCACCTGAAGC	Nam *et al.* (2001)
R-1150	GCATTTGTCCGCAGTGAATACC	Freshwater & Rueness (1994)
R-1381	ATCTTTCCATAGATCTAAAGC	Freshwater & Rueness (1994)

our data for maximum likelihood (ML) and Bayesian inference (BI) analysis, using the Akaike Information Criterion (AIC). ML analysis was carried out with PhyML 2.4.4 (Guindon & Gascuel, 2003) using a BioNJ starting tree, and parameter values estimated during the run. ML and MP analyses were subjected to bootstrap resampling (1000 replicates and 1000 replicates with 10 random additions, respectively) to estimate robustness (Felsenstein, 1985). MrBayes version 3.1 (Huelsenbeck & Ronquist, 2001) was used to complete BI, with the sequence data analysed as a single partition (an analysis using codon partitioning was also run, but topologies were unaffected therefore the simplest partition scheme was selected). Analyses were run with four heated Monte-Carlo Markov Chains for 2,000,000 generations. Output trees and data were sampled every 100 generations. Appropriate burn-in for each run was determined by plotting the overall likelihood against generations prior to estimating the posterior probability distribution. In all analyses, likelihood values were stable after the first 200,000 generations, and the final results were based on the pooled samples from the stationary phase of the two independent runs.

Results

Sampling and analyses of rbc*L sequences*

Complete *rbc*L (1467 bp) and *rbc*L–*rbc*S spacer sequences were successfully generated for 16 specimens (Table 1). In order to minimize missing data and, when combined with sequences available on GenBank, 59 bp at the 5′ end of the *rbc*L and the spacer region at the 3′ end were removed leading to a final alignment of 1404 characters. This alignment showed no gap and no stop codon (except for the final one), 545 (38.8%) sites were variable, and 138 were parsimony-informative. One hundred and one sites were variable and 67 (66%) were parsimony-informative at first codon positions; 35 sites were variable and 11 (31%) parsimony-informative at second codon positions; 409 sites were variable and 360 (88%) were parsimony-informative at third codon positions.

Phylogenetic analyses

Parsimony analysis of our dataset resulted in nine equally most parsimonious trees, which were 1954 steps long, with a consistency index of 0.4012 and a retention index of 0.6562. The best model selected under an Akaike Information Criterion was the general time reversible model (GTR) of nucleotide substitution, with the percentage of sites considered invariable and a gamma distribution of rates for variable sites (GTR + I + G).

All our phylogenetic analyses (Fig. 1) strongly supported the monophyly of the *Laurencia* complex and resolved five, fully supported lineages. *Laurencia* s.s., *Chondrophycus* and *Osmundea*, were resolved as monophyletic genera. The two remaining lineages accommodated taxa currently assigned to the genus *Palisada*. Supra-generic relationships were not supported except for the relationship between the *P. poiteaui* group and *Laurencia* s.s. recovered in all our analyses (Fig. 1).

The genus *Laurencia* s.s. was resolved as a monophyletic lineage including the generitype *L. obtusa*, plus 14 other species. Infrageneric relationships within *Laurencia* s.s. lacked resolution, however two lineages appeared strongly supported. The first included specimens from New Caledonia (*L.* cf. *mcdermidiae*, *L.* cf. *nidifica*, *L.* cf. *forsteri* and *L.* cf. *mariannensis*), and the second, fully supported, encompassed *L. arbuscula* and *L. catarinensis*, two specimens from South America. Two other lineages were moderately supported; the first contained cosmopolitan species (*L. pyramidalis*, *L. obtusa* and *L. intricata*), and the second included taxa mainly from the Indian and Pacific oceans (*L. flexuosa*, *L. venusta*, *L. natalensis* and *L. rigida*).

One of the lineages in our analysis contained five specimens from New Caledonia. Two specimens were identified as *Chondrophycus* cf. *undulatas*, based on descriptions by Yamada (1931) and Nam (1999). The other three were difficult to identify to species due to the lack of reproductive structures and problematic tissue rehydration.

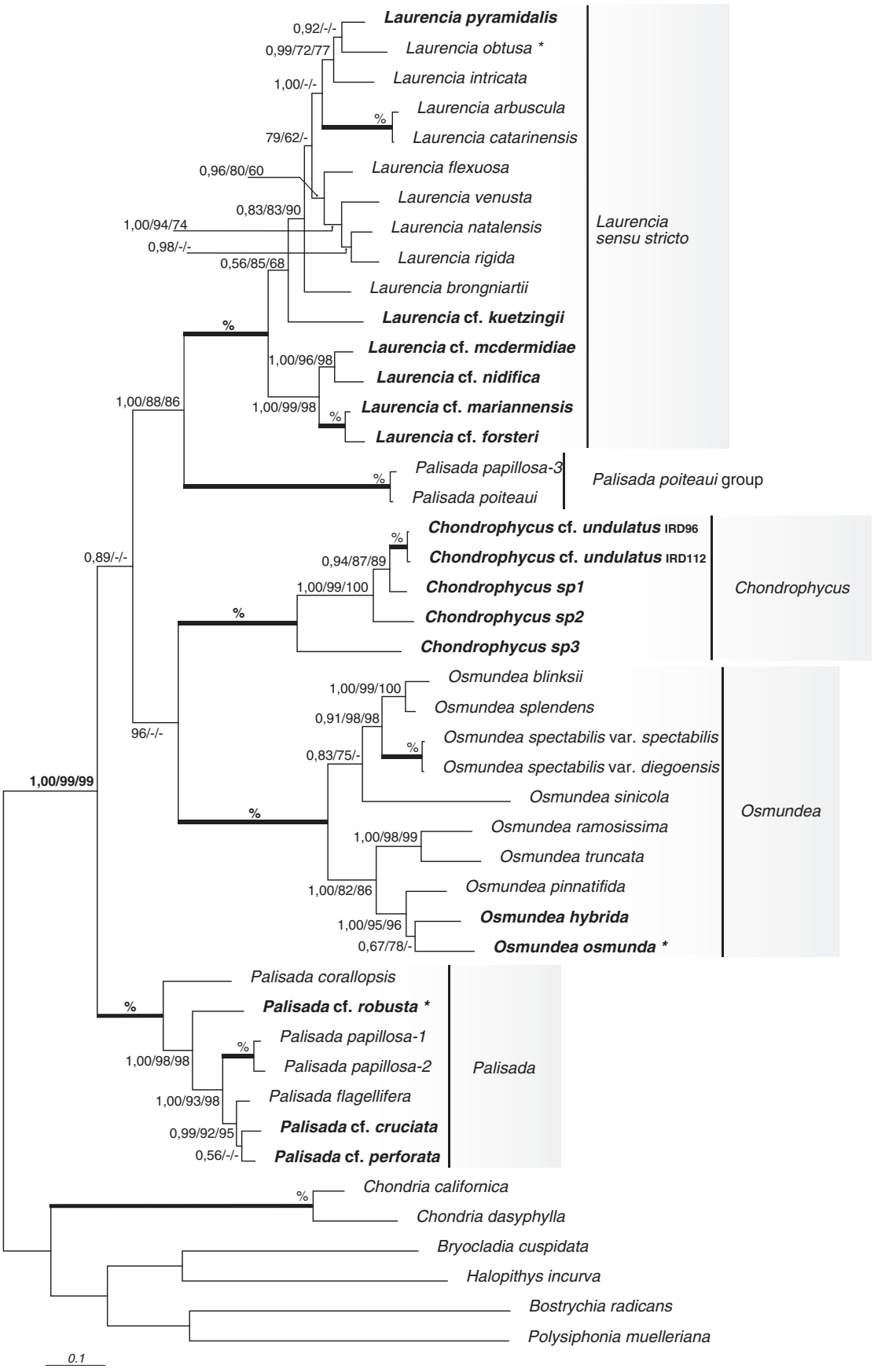

Fig. 1. Bayesian phylogram inferred from analyses of *rbc*L sequences for 39 *Laurencia* complex taxa and six outgroup species. Numbers above branches correspond to support values for Bayesian inference posterior probability/Maximum Likelihood bootstrap/and Maximum Parsimony bootstrap, respectively. Bold lines and % indicate a fully supported node in all the three analyses. Taxa marked in bold indicate newly determined sequences. Asterisks indicate generitypes, and the scale bar refers to the substitutions per site.

Specimens were therefore referred to as sp1, sp2, and sp3. These specimens formed small, dark red clumps, cartilaginous in texture, with compressed fronds, and irregularly ramified axes with short wart-like ultimate branchlets. Epidermal cells were neither palisade-like in transversal section, nor secondarily pit connected, and lenticular thickenings were not observed in medullary cells. In longitudinal section, we observed projecting, domed epidermal cells near the branchlet in *Chondrophycus* sp3, a character mentioned by Nam (1999) for some species of *Chondrophycus*. Based on our observations and molecular results, we assigned this clade to the genus *Chondrophycus*.

Taxa belonging to the genus *Osmundea*, including the type species *O. osmunda*, formed a fully supported and well-resolved lineage. Two lineages within this genus were moderately supported: one containing the North-East Pacific species (*O. blinksii*, *O. splendens*, *O. spectabilis* and *O. sinicola*) and the second containing species from North Atlantic coasts (*O. ramosissima*, *O. truncata*, *O. pinnatifida*, *O. hybrida* and *O. osmunda*). *Osmundea* was resolved as sister to *Chondrophycus* with high support in BI.

Phylogenetic analyses resolved specimens assigned to *Palisada* in two distinct lineages. One, fully supported in all analyses, included a specimen from New Caledonia, which presented the morpho-anatomical features of *P. robusta,* the generitype of *Palisada*; we therefore assigned this lineage to *Palisada*. The second lineage, fully supported in all analyses, included *P. poiteaui* and *P. papillosa-3*, two specimens with only three differences in their sequences (divergence 0.2%).

Discussion

Monophyly of the Laurencia *complex*

In our molecular phylogenetic analyses, the four genera forming the *Laurencia* complex are resolved as a strongly supported, monophyletic group, confirming previous studies based on molecular and morpho-anatomical characters (Garbary & Harper, 1998; Nam *et al*., 2000; McIvor *et al*., 2002; Abe *et al*., 2006; Fujii *et al*., 2006 and Díaz-Larrea *et al*., 2007). These results suggest that the main morpho-anatomical features (apical cells sunk in apical pits of branchlets, a recognisable axial cell row only near the apical cell and an extensive cortex (Kylin, 1956; Saito & Womersley, 1974; Womersley, 2003; Nam, 2006) used to distinguish members of the *Laurencia* complex from the remaining Rhodomelaceae are phylogenetically significant. However, using cladistic analyses of morphological characters, Nam & Choi (2001) and Nam (2006) resolved *Laurencia clavata* Sonder (an Australian endemic) as sister to the remaining species of the complex, and suggested that *L. clavata* may link the *Laurencia* complex and the genus *Chondria* C. Agardh. On the one hand, *L. clavata* has basally constricted branches, many refractive discoid starch grains in medullar and sub-cortical cells, which are characters shared with *Chondria*. On the other hand, it lacks specialized plates in its spermatangial structure, which is a characteristic feature of the tribe *Chondriae*, and has four pericentral cells, epidermal cells with secondary pit connections and unrecognizable axial cell rows in mature thalli, characters attributed to the *Laurencia* complex. Based on these observations, Nam (2006) proposed resurrecting the genus *Corynecladia* J. Agardh to accommodate *L. clavata*. Similarly, *Laurencia flexilis* Setchell, which also has basally constricted branches (Masuda *et al*., 2006), was resolved by Abe *et al*. (2006) as an early divergence in the *Laurencia* complex. Because Abe *et al*.'s sequences are not deposited in any public database, it was not possible to include their sequence of *L. flexilis* in the present study. Further analyses including both *C. clavata* and *L. flexilis* are needed to improve circumscription of the *Laurencia* complex, and to determine the evolutionary relationships of these two enigmatic species.

Interspecific relationships within the Laurencia *complex*

The five lineages resolved in our analyses are fully supported and correspond respectively to the genera *Laurencia* s.s., *Chondrophycus*, *Osmundea*, *Palisada*, and to a lineage encompassing *P. poiteaui* and *P. papillosa-3*. *Palisada* is divided into two unrelated lineages and is therefore polyphyletic. One of these two lineages (Fig. 1) corresponds to *Palisada* since it includes a specimen that has the morpho-anatomical features of *P. robusta,* the generitype of *Palisada*. In addition, all the species of this group have epidermal cells with a conspicuous palisade structure (Martin-Lescanne, personal observation; see also Table 3). The second lineage, the '*P. poiteaui* group' (Fig. 1), includes two specimens identified morphologically as *P. papillosa* and *P. poiteaui*, although their *rbc*L sequences only differed by three base pairs (uncorrected p distance value = 0.2%). Two other specimens identified as *P. papillosa* were resolved within the *Palisada* lineage rather than the *P. poiteaui* group. Consequently, specimens assigned to *P. papillosa* did not form a monophyletic taxon. *Palisada poiteaui* and specimens referred to as *P. papillosa-3* were both collected in the Gulf of Mexico (Florida) (McIvor *et al*., 2002; Senties & Diaz-Larrea, 2008), are similar in habit and

Table 3. Diagnostic characters used to identify the different genera of the *Laurencia* complex.

	Vegetative structure					Male reproductive structure Spermatangial branch			Female reproductive structure	
Genus	Axial cells	Secondary pit connections	*Corps en cerise*	Position of the first P relative to the trichoblast	E arrangement	Development	Production	Pit shape	Auxiliary cell timing	Procarp-bearing segment
Osmundea	2P[c,e]	±[a]	–[b,d]	Side[g]	NPa[f]	Filament type[d]	Absente[g]	Pocket/Cup[d]	Normal[e,g]	5/6P[d]
Laurencia	4P[c,e]	+[a]	+[b,e]	Underneath[g]	NPa[f]	Trichoblastic type[d]	SOL[g]	Cup[d]	Normal[e,g]	5P[d]
Chondrophycus	2P[c,e]	–[a]	–[e]	Side[g]	NPa[f]	Trichoblastic type[d]	STL[g]	Cup[d]	Delayed[e,g]	5P[g]
Palisada	2P[f]	–[f]	–[f]	Underneath	Pa[a,h†]	Trichoblastic type[d]	SOL[g]	Cup[g]	Normal[e,g]	4/5P[g]
Yuzurua	2P[f]	+[f]	–[f]	?	NPa[f]	Trichoblastic type[f]	?	Cup[g]	?	5P[g]

	Tetrasporangia							
Genus	Origin	Pericentral position	Orientation	Arrangement of tetrasporangia	Presporangial cover cell arrangement	Tetrasporangia axis	Fertility on the second P	Additional tetrasporangial P
Osmundea	E[d]	Random E[d]	Lateral[d]	Parallel[a]	Parallel[d]	NA[g]	NA[g]	NA[g]
Laurencia	Particular P[d]	3rd, 4th[c]	Abaxial[d]	Parallel[a]	Transverse[d]	2/3 SP	–[g]	–[g]
Chondrophycus	P[d,g]	Additional P[d]	Abaxial[d]	Right-angle[a]	Transverse[d]	2 S[g]	–[g]	+[d]
Palisada	P[g]	Additional P[f]	Abaxial[f]	Right-angle[f]	Transverse[f]	1 S[g]	+[g]	+[d]
Yuzurua	P[g]	Additional P[f]	Abaxial[f]	Right-angle[f]	Transverse[f]	1 S[g]	+[g]	+[g]

Abbreviations: E: epidermal cells; NA: not applicable; NPa: non-palisadic; Pa: palisadic; P: pericentral cells; SP: sterile pericentral cells; STL: development of spermatangial branches from two laterals on suprabasal cell of trichoblast; SOL: development of spermatangial branches from one of the two laterals; +: present; –: absent; ?: unknown.
[a]Saito (1967); [b]McDermid (1988); [c]Nam & Saito (1991); [d]Nam *et al.* (1994); [e]Garbary & Harper (1998); [f]Nam (1999); [g]Nam (2006), [h]Personal observations. [†]Character unclear for *Palisada papillosa*, *Palisada maris-rubri* and *Palisada iridescens*.

difficult to distinguish (Littler & Littler, 2000), especially when they are fertile. We believe that *P. papillosa-3* may have been misidentified and is probably *P. poiteaui*; however, the voucher specimen should be re-examined to confirm this hypothesis.

The *P. poiteaui* group was resolved as sister to *Laurencia* s.s. with moderate (ML, MP) to full (BI) support. Interestingly, *P. poiteaui* and *P. gemmifera* (which is now considered as conspecific with *P. poiteaui*, Díaz-Larrea *et al*., 2007) were two species transferred to *Palisada* by Nam (2006), which lack palisadic epidermal cells. Both species also differ from other *Palisada* species by having five rather than four pericentral cells in procarp-bearing segments (Fujii *et al*., 1996). Nam (2006) resolved these taxa as sister to the remaining species of *Palisada* based on morphological characters. The *P. poiteaui* group and *Laurencia* s.s. also have secondary pit connections between epidermal cells, a feature exhibited by most representatives of *Laurencia* s.s. but not of *Palisada*. *Palisada poiteaui* should either be included in *Laurencia* s.s. and this genus emended to accommodate it, or placed in a separate genus. Since *P. poiteaui* has two pericentral cells, whereas *Laurencia* s.s. has four, and lacks *corps en cerise*, in contrast to other *Laurencia* s.s. species, it seems preferable to accommodate it in a distinct genus (Table 3). This is consistent with the sequence divergences obtained in molecular analyses, *P. poiteaui* being as distant from *Laurencia* s.s. species (9–11%) as *Chondrophycus* species are from *Osmundea* (10–11%), and distances between *P. poiteaui* and *Palisada* species also ranged from 10–11%. Nam (1999) proposed the subgenus *Yuzurua* to accommodate *P. poiteaui*, as well as *Palisada parvipapillata* (C.K. Tseng) Nam (as *Chondrophycus parvipapillatus* (C.K. Tseng) Garbary & Harper), *Palisada iridescens* (Wynne & Ballantine) Nam (as *C. iridescens* (Wynne & Ballantine) Garbary & Harper) and *C. gemmiferus* (now included in *P. poiteaui*, see above). Nam (1999) designated *P. poiteaui* (as *C. poiteaui*) as the type species of the subgenus. We propose elevating *Yuzurua* to generic rank. At this time, we formally transfer *P. poiteaui* to *Y. poiteaui*, however we refrain from including *P. parvipapillatus* and *P. iridescens* in the new genus because of the lack of molecular data and the unknown state of the key characters in these species.

Species belonging to the genus *Laurencia* s.s. form a fully supported monophyletic cluster in which all species sequenced in the present study display *Laurencia* s.s. features: four pericentral cells per vegetative axial segment, longitudinally oriented secondary pit connections between contiguous superficial cortical cells and, when fresh material was available, *corps en cerise* within superficial cortical and trichoblast cells (Table 3). As in previous molecular and morpho-anatomical phylogenies (Garbary & Harper, 1998; Abe *et al*., 2006; Fujii *et al*., 2006), general infrageneric relationships were poorly resolved, and only a few interspecific nodes were well supported (Fig. 1). Since interspecific nodes were well supported in other genera, the absence of supported relationships within *Laurencia* s.s. could be due to either a weak phylogenetic signal (maximum divergence 0.05%) or insufficient taxon sampling. As mentioned in our results, some geographic structuring in our trees was observed but wider geographic sampling is needed to discuss any biogeographical scenarios.

Our molecular data largely echo the morpho-anatomical features used to delineate genera and sub-genera within the *Laurencia* complex. This suggests that molecular tools are helpful for assigning unidentified specimens of the *Laurencia* complex to a genus, especially when distinguishing morpho-anatomical characters cannot be observed, as it is often the case for reproductive features.

Despite the fact that the genera of the *Laurencia* complex are molecularly well-defined, relationships among them are currently poorly resolved and more molecular data and a larger taxon sampling are still necessary to further improve our understanding of this taxonomic complex in an evolutionary framework.

Taxonomic conclusion

Genus *Yuzurua* (Nam) Martin-Lescanne stat. nov. with the characters of the subgenus *Yuzurua* Nam (1999, *Eur. J. Phycol.* 34: 467)

TYPE SPECIES: *Yuzurua poiteaui* comb. nov. (J.V. Lamouroux) Martin-Lescanne.

BASIONYM: *Fucus poiteaui* J.V. Lamouroux (1805, *Ann. Mus. Hist. Nat*. Paris 20: 63–64).

SYNONYMS: *Chondrophycus poiteaui* (J.V. Lamouroux) K.W. Nam (1999, *Eur. J. Phycol.* 34: 463); *Palisada poiteaui* (J.V. Lamouroux) K.W. Nam (2007, *Algae* 22: 54).

TYPE LOCALITY: Santo Domingo, Dominican Republic.

TYPE MATERIAL: CN, unnumbered.

SPECIES PRESENTLY INCLUDED IN THE GENUS: *Yuzurua poiteaui*.

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