



Molecular phylogenies support taxonomic revision of three species of *Laurencia* (Rhodomelaceae, Rhodophyta), with the description of a new genus

Florence ROUSSEAU^{1,*}, Delphine GEY², Akira KURIHARA³,
Christine A. MAGGS⁴, Julie MARTIN-LESCANNE⁵, Claude PAYRI⁶,
Bruno de REVIERS⁷, Alison R. SHERWOOD⁸ & Line LE GALL⁹

^{1,7,9}Institut de Systématique, Evolution, Biodiversité (ISyEB), UMR 7205 CNRS, EPHE, MNHN,
UPMC, Sorbonne Universités, Equipe Exploration, Espèces, Evolution, Muséum National
d'Histoire Naturelle, case postale N° 39, 57 rue Cuvier, 75231 Cedex 05 Paris, France

^{2,5}Outils et Méthodes de la Systématique Intégrative, UMS 2700 MNHN, CNRS,
Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle,
case postale N° 26, 57 rue Cuvier, 75231 Cedex 05 Paris, France

^{3,8}Department of Botany, 3190 Maile Way, University of Hawaii, Honolulu, Hawaii, 96822 U.S.A.

⁴Faculty of Science and Technology, Bournemouth University, Poole House,
Talbot Campus, Poole, Dorset BH12 5BB, U.K.

⁶Institut de Recherche pour le Développement (IRD), UMR ENTROPIE-IRD,
UR, CNRS, BP A5, 98848 Noumea cedex, Noumea, New Caledonia

*Corresponding author E-mail: rousseau@mnhn.fr

²Email: delphine.gey@mnhn.fr

^{3,8}Email: asherwoo@hawaii.edu

⁴Email: cmaggs@bournemouth.ac.uk

⁵Email: Lescanne@mnhn.fr

⁶Email: claud.payri@ird.fr

⁷Email: reviers@mnhn.fr

⁹Email: legall@mnhn.fr

Abstract. The systematics of the *Laurencia* complex was investigated using a taxon-rich data set including the chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene only and a character-rich data set combining mitochondrial cytochrome oxidase 1 (COI-5P), the *rbcL* marker, and the nuclear large subunit of the ribosomal operon (LSU). Bayesian and ML analyses of these data sets showed that three species hitherto placed in the genus *Laurencia* J.V.Lamour. were not closely related to *Laurencia* s. str. *Laurencia caspica* Zinova & Zaberzhinskaya was the sister group of the remaining *Osmundea* Stackh. species, *L. crustiformans* McDermid joined *Palisada* and *L. flexilis* Setch. consisted of an independent lineage. In light of these results a new genus, *Ohelopapa* F.Rousseau, Martin-Lescanne, Payri & L.Le Gall gen. nov., is proposed to accommodate *L. flexilis*. This new genus is morphologically characterized by four pericentral cells in each vegetative axial segment; however, it lacks ‘corps en cerise’ in cortical cells and secondary pit connections between cortical cells, which are characteristic of *Laurencia*. Three novel combinations are proposed to render the classification

closer to a natural system: *Ohelopapa flexilis* (Setch.) F.Rousseau, Martin-Lescanne, Payri & L.Le Gall comb. nov., *Osmundea caspica* (Zinova & Zaberzhinskaya) Maggs & L.M.McIvor comb. nov. and *Palisada crustiformans* (McDermid) A.R.Sherwood, A.Kurihara & K.W.Nam comb. nov.

Keywords. *Laurencia caspica*, *Laurencia crustiformans*, *Laurencia flexilis*, *Ohelopapa*, *Osmundea*.

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Introduction

The *Laurencia* complex consists of species which historically belonged to the genus *Laurencia* J.V.Lamour. (Rhodomelaceae, Ceramiales). Although it is easy to assign specimens to the *Laurencia* complex, it is far more difficult to delimit species and genera within the complex due to extensive morphological plasticity and the significance of inconspicuous anatomical characters.

Over the last two decades, the diversity within the genus *Laurencia* has been revealed by thorough morphological and molecular studies, leading to the recognition of distinctive anatomical features, as well as genetic divergences usually diagnostic at the generic level. The *Laurencia* complex has been split, leading to the resurrection or proposal of new genus names (Table 1). Currently, the *Laurencia* complex consists of seven genera: *Chondrophyucus* (Tokida & Y.Saito) Garbary & J.T.Harper (Garbary & Harper 1998), *Coronaphycus* Metti (Metti *et al.* 2015), *Laurencia* (Lamouroux 1813), *Laurenciella* V.Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C.Oliveira & M.T.Fujii (Cassano *et al.* 2012), *Osmundea* Stackh. (Nam *et al.* 1994), *Palisada* K.W.Nam (Nam 2007) and *Yuzurua* (K.W.Nam) Martin-Lescanne (Martin-Lescanne *et al.* 2010). Despite the recognition of genera within the *Laurencia* complex, a limited number of species have been assigned to the four resurrected genera and the three recently described genera only include one (*Laurenciella* and *Yuzurua*) or two species (*Coronaphycus*) each (Table 1). Therefore, *Laurencia* s. str. is still by far the most speciose genus within the complex, and according to AlgaeBase (Guiry & Guiry 2016) it currently accommodates 145 recognized species and 27 infraspecific taxa among no fewer than 421 taxa (Guiry & Guiry 2016), strongly suggesting that steps toward taxonomic revision are necessary. Presently, most species of the genus *Laurencia* s. str. share the characters of four pericentral cells, trichoblastic spermatangia, tetrasporangial development from pericentral cells (Nam *et al.* 1994; Nam 1999), ‘corps en cerise’ (globular, hyaline bodies) in cortical cells and secondary pit connections between cortical cells. Reproductive characters are not always available, while the other anatomical features are difficult to observe, particularly in herbarium specimens. Consequently, the systematic revision of the *Laurencia* complex relies mostly on molecular databases to complete comprehensive phylogenetic analyses.

In the course of recent floristic surveys we have collected many specimens of the *Laurencia* complex from their type localities, including *Laurencia flexilis* Setch., *L. caspica* Zinova & Zaberzhinskaya and *L. crustiformans* McDermid (e.g., Sherwood *et al.* 2010a, 2010b). These three species have character combinations that do not exactly match the description of *Laurencia* s. str. *Laurencia flexilis* has four pericentral cells, but unlike the type species of *Laurencia*, *L. obtusa* (Huds.) J.V.Lamour., it lacks secondary pit connections and ‘corps en cerise’, (Saito 1967, Masuda *et al.* 1999, 2006). Abe *et al.* (2006) resolved this taxon with moderate support as the earliest diverging lineage within the *Laurencia* complex in an *rbcL* phylogeny. Regrettably, Abe’s data are not publicly available and in the present study we generate novel sequences to address the question of the affinity of this taxon. *Laurencia*

Table 1. Number of specific and infraspecific taxa currently recognized/total number of taxa, according to AlgaeBase (Guiry & Guiry 2016), for each genus of the *Laurencia* complex.

| Genus | Authorship and publication | Current/total |
|--------------------------|---|----------------|
| <i>Chondrophycus</i> | (Tokida & Y.Saito) Garbary & J.T.Harper (Garbary & Harper 1998) | 17/41 |
| <i>Coronaphycus</i> | Metti (Metti <i>et al.</i> 2015) | 2/2 |
| <i>Laurencia</i> | J.V.Lamour., nom. cons. (Lamouroux 1813) | 172/421 |
| <i>Laurenciella</i> | Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C.Oliveira & M.T.Fujii (Cassano <i>et al.</i> 2012) | 1/1 |
| <i>Osmundea</i> | Stackh. (Stackhouse 1809) | 21/24 |
| <i>Palisada</i> | K.W.Nam (Nam 2007) | 24/29 |
| <i>Yuzurua</i> | (K.W.Nam) Martin-Lescanne (Martin-Lescanne <i>et al.</i> 2010) | 1/1 |
| <i>Laurencia</i> complex | | 238/519 |

caspica, described from the Caspian Sea, likewise lacks secondary pit connections and ‘corps en cerise’ and has open spermatangial cups that resemble those of *Osmundea hybrida* (DC.) K.W.Nam from Atlantic Europe and *O. maggsiana* Serio, Cormaci & G.Furnari from the Mediterranean Sea. *Laurencia crustiformans*, an unusual species that consists of a basal crust and nearly unbranched upright axes, was described from the Hawaiian Islands (McDermid 1989, Abbott 1999). It shares with *Laurencia* the presence of secondary pit connections between cortical cells and ‘corps en cerise’, but it differs by bearing tetrasporangia in a right-angle arrangement, as observed in species of *Palisada* and *Yuzurua*.

The aim of this manuscript is to assess the generic affinities of *L. flexilis*, *L. caspica* and *L. crustiformans* within the *Laurencia* complex by generating molecular data for specimens collected at the type localities of these three taxa, and inferring phylogenetic relationships from a character-rich combined dataset (LSU+rbcL+CO1) and a taxon-rich *rbcL* only dataset.

Material and methods

Specimen collection

Newly sequenced specimens were collected in different localities around the world and are listed in Table 2, along with their valid names and GenBank accession numbers (NCBI GenBank) as well as the herbarium where the specimen was deposited. Samples were dried in silica gel or prepared as herbarium specimens and some of them were stored in 5% buffered formalin in seawater. Specimens from the Hawaiian Islands were collected as part of the Hawaiian Rhodophyta Biodiversity Survey (Sherwood *et al.* 2010a, 2010b). During a monitoring project by Environment & Resource Technology Limited (ERT Caspian) for BP Global in Sangachal Bay, Azerbaijan, Caspian Sea, in connection with existing and future operations at the Sangachal oil pipeline terminal (ERT Caspian 2002), the distribution of red algae was surveyed in June 2001 (by grab sampling) and 2002 (snorkeling). *Laurencia caspica* was collected at depths of 4–11 m, mostly more than 1 km from shore, on calcareous hard substrata and barnacle shells. The salinity in this area is 12–13 PSU, typical of the Caspian Sea (Lüning 1990: 123), and macroalgal diversity is very low, with only six red algal species recorded (ERT Caspian 2002) from the 30 species known from the Caspian (Lüning 1990). For molecular investigation, macroalgal samples were collected by van Veen grab at one station in the bay in September 2003. Specimens from New Caledonia were collected as part of the New Caledonia coral reef biodiversity survey in 2003, 2005 (BSM-LOYAUTE Alis Vessel campaign and BIODIP Alis Vessel campaign) and 2008. The specimen of *L. flexilis* was collected at the type locality in Tahiti, French Polynesia, in 2007 and deposited at UPF (Université de Polynésie Française). Acronyms of herbaria follow Thiers (continuously updated).

Table 2. Newly sequenced specimens used in this study with their valid names, their collection data, herbarium where they are deposited and GenBank accession numbers. [continued on next page]

| Taxa | Collection data (Location – Collector(s) – Date – DNA / Voucher collection number) | | Herbarium* | GenBank number | | |
|---|--|------|------------|----------------|----------|-----|
| | | | | <i>rbcL</i> | COI-5P | LSU |
| <i>Chondria scintillans</i> Feldm.-Maz | Roscoff, Brittany, France – F. Rousseau – 5 Dec. 2002 – JML0048 / PC0144059 | PC | KF492775 | KF492717 | KX145612 | |
| <i>Chondrophycus</i> cf. <i>undulatus</i> (Yamada) Garbary & J.T.Harper | Maré, Loyalty Is., New Caledonia – C. Payri – 22 Mar. 2005 – JML0226 / IRD100 | NOU | FJ785307 | KX258814 | KX145614 | |
| <i>Chondrophycus</i> sp. | Lifou, Loyalty Is., New Caledonia – C. Payri – 23 Mar. 2005 – JML0235 / IRD86 | NOU | KX146175 | KX258817 | KX145617 | |
| <i>Chondrophycus</i> sp. | Beautemps/Beaupré, Loyalty Is., New Caledonia – C. Payri – 6 Apr. 2005 – JML0250 / IRD111 | NOU | KX146174 | KX258813 | KX145613 | |
| <i>Chondrophycus</i> sp. | Maré, Loyalty Is., New Caledonia – C. Payri – 21 Mar. 2005 – JML0239/IRD96 | NOU | FJ785310 | KX258816 | KX145616 | |
| <i>Chondrophycus</i> sp. | Beautemps/Beaupré, Loyalty Is., New Caledonia – C. Payri – 6 Apr. 2005 – JML0251 / IRD112 | NOU | FJ785311 | KX258815 | KX145615 | |
| <i>Laurencia</i> cf. <i>brongiartii</i> J.Agardh | Ile des Pins, New Caledonia – C. Payri – 2 Dec. 2005 – JML0150 / IRD124 | NOU | KX146178 | KX258820 | KX145620 | |
| <i>Laurencia</i> cf. <i>calliptera</i> Kütz. | Ile des Pins, New Caledonia – C. Payri – 27 Nov. 2005 – JML0132 / IDP05-223 | NOU | KX146182 | KX258825 | KX145625 | |
| <i>Laurencia</i> cf. <i>majuscula</i> (Harv.) A.H.S.Lucas | Dhofar, Oman – M. Wynne – Sep. 2001 – WYNR154 | MICH | KX146184 | KX258827 | KX145627 | |
| <i>Laurencia</i> cf. <i>mariannensis</i> Yamada | Ilot Larégnère, Lagon Sud-Ouest, New Caledonia – C. Payri – 11 Jul. 2003 – JML0185 / IRD75, NC03-2017 | NOU | FJ785315 | KX258823 | KX145623 | |
| <i>Laurencia</i> cf. <i>natalensis</i> Kylin | Dickwella Village, Sri Lanka – E. Coppejans – 15 Aug. 2006 – HEC15902 | GENT | KX146176 | KX258818 | KX145618 | |
| <i>Laurencia</i> cf. <i>nidifica</i> J.Agardh | Ile des Pins, New Caledonia – C. Payri – 30 Nov. 2005 – JML0141 / IRD122 | NOU | KX146180 | KX258822 | KX145622 | |
| <i>Laurencia</i> cf. <i>obtusa</i> (Huds.) J.V.Lamour. | Banyuls sur Mer, France – L. Bittner – 30 Jul. 2007 – LBC0053 / PC0145178 | PC | KX146185 | KX258828 | KX145628 | |
| <i>Laurencia</i> cf. <i>flexuosa</i> Kütz. | Eastern Cape Province, South Africa – O. De Clerck – 15 Jun. 2003 – ODC1082 | GENT | KX146179 | KX258821 | KX145621 | |
| <i>Laurencia</i> sp. | Odayapiti lagoon, Sri Lanka – E. Coppejans – 8 Nov. 2006 – HEC16009 | GENT | KX146183 | KX258826 | KX145626 | |
| <i>Laurencia</i> sp. | Ilot Larégnère, Lagon Sud-Ouest, New Caledonia – J. Martin-Lescanne – 31 Jan. 2008 – JML0097 / PC0145177 | PC | KX146181 | KX258824 | KX145624 | |
| <i>Laurencia</i> sp. | Maré, Loyalty Is., New Caledonia – C. Payri – 19 Mar. 2005 – JML0216 / IRD97 | NOU | KX146177 | KX258819 | KX145619 | |

* Abbreviations used in accordance with the Index Herbariorum (Thiers continuously updated): BISH: Bishop Museum, Botany Division, Honolulu, Hawaii, USA; BM: Natural History Museum, London, U.K.; GENT: Ghent University, Biology Department, Belgium; MICH: University of Michigan, Ann Arbor, Michigan, USA; NOU: Institut de Recherche pour le Développement, Nouméa, New Caledonia; PC: Museum national d'Histoire naturelle, Non-vascular Plants & Fungi, Paris, France; UPF: Université de Polynésie Française, Faa'a, Tahiti, France.

| Taxa | Collection data (Location – Collector(s) – Date – DNA / Voucher collection number) | Herbarium* | GenBank number | | |
|--|---|------------|----------------|----------|----------|
| | | | <i>rbcL</i> | COI-5P | LSU |
| <i>Laurenciella maritzae</i> (Gil-Rodríguez, Senties, Díaz-Larrea, Cassano & M.T.Fujii) Gil-Rodríguez, Senties, Díaz-Larrea, Cassano & M.T.Fujii | Scedro, Croatia – <i>L. Le Gall</i> – 11 Jun. 2007 – LLG0242 / PC0145179 | PC | KX146186 | KX258829 | KX145629 |
| <i>Ohelopapa flexilis</i> gen. et comb. nov. | Tahiti, French Polynesia – <i>André Pham</i> – 24 Mar. 2007 – 01A-07 / UPF4223 | UPF | KX146187 | KX258830 | KX145630 |
| <i>Osmundea caspica</i> (Zinova & Zaberzhinskaya) comb. nov. | Sangachal Bay, Azerbaijan – <i>ERT Caspian Contractors</i> – Sep. 2003 – CAM1504 / BM001062596 | BM | KX146188 | – | – |
| <i>Osmundea oederi</i> (Gunnerus) G.Furnari | Ile Chausey, Normandy, France – <i>L. Le Gall</i> – LLLG1116 / PC0145180 | PC | KX146189 | KX258833 | KX145633 |
| <i>Osmundea hybrida</i> (DC.) K. W.Nam | St Lunaire, Brittany, France – <i>F. Rousseau</i> – 20 Mar. 1999 – JML0051 / PC0146010 | PC | FJ785317 | KX258831 | KX145631 |
| <i>Osmundea osmunda</i> (S.G.Gmel.) K. W.Nam | Roscoff, Brittany, France – <i>F. Rousseau</i> – 5 Dec. 2002 – JML0049 / PC0146009 | PC | FJ785318 | KX258832 | KX145632 |
| <i>Palisada</i> cf. <i>parvipapillata</i> (C.K.Tseng) K. W.Nam | Maré, Loyalty Is., New Caledonia – <i>C. Payri</i> – 21 Mar. 2005 – JML0221 / IRD98 | NOU | KX146194 | KX258839 | KX145639 |
| <i>Palisada</i> cf. <i>robusta</i> (Yamada) K. W.Nam | Lifou, New Caledonia – <i>C. Payri</i> – 23 Mar. 2005 – JML0228 / IRD92 | NOU | FJ785321 | KX258838 | KX145638 |
| <i>Palisada crustiformans</i> comb. nov. | Makapuu, Oahu, Hawaii, USA – <i>A. Kurihara</i> – 26 May 2007 – BISH 766725 | BISH | KX146196 | HQ422744 | KX145641 |
| <i>Palisada crustiformans</i> comb. nov. | Isaac Hale Beach Park, Hawaii Island, Hawaii, USA – <i>A. Kurihara</i> – 24 Jan. 2008 – BISH 766726 | BISH | KX146195 | HQ422774 | KX145640 |
| <i>Palisada</i> sp. | Polhena Beach, Sri Lanka – <i>E. Coppejans</i> – 16 Aug. 2006 – HEC15931 | GENT | KX146192 | KX258836 | KX145636 |
| <i>Palisada</i> sp. | Makapuu, Oahu, Hawaii, USA – <i>A. Kurihara</i> – 12 Sep. 2006 – BISH 766727 | BISH | KX146190 | KX258834 | KX145634 |
| <i>Palisada</i> sp. | Nouméa, New Caledonia – <i>J. Martin-Lescanne</i> – 4 Apr. 2008 – JML0111 | PC | KX146193 | KX258837 | KX145637 |
| <i>Palisada</i> sp. | Lifou, New Caledonia – <i>C. Payri</i> – 23 Mar. 2005 – JML0231 / IRD85 | NOU | KX146191 | KX258835 | KX145635 |
| <i>Rhodomela confervoides</i> (Huds.) P.C.Silva | Roscoff, Brittany, France – <i>T. Silberfeld</i> – 19 Oct. 2008 – TJS0210 / PC0157429 | PC | KX146197 | KX258842 | KX145642 |
| <i>Yazurua</i> sp. | Guadeloupe, West Indies (16.349028° N, 61.573194° W) – <i>Karubenthos Expedition</i> – 3 May 2012 – FRA1041 / PC0143886 | PC | KX146198 | KX258843 | KX145643 |

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Sample preparation for anatomical studies

Morphology was observed under dissecting and compound microscopes and permanent slides were made from sections stained with acidified aniline blue stain (4% in seawater) and mounted in 50% dilute Karo corn syrup.

Extraction, amplification and sequencing

Total cellular DNA was extracted from tissues preserved in silica gel and dry herbarium specimens using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA or Qiagen GmbH, Hilden, Germany). From 0.5×10^{-3} to 10^{-3} mg of proteinase K was added to the lysis buffer to improve the DNA yield. Except for *L. caspica*, the *rbcL* coding region (1467 bp) was amplified using the following combinations of primers: F-*rbcL* start \times R-753 (Freshwater & Rueness 1994) for the 5' end, *rbcLFC* \times 1011R (Nam & Choi 2000) or F-577 \times R1381 (Freshwater & Rueness 1994) for the middle fragment and F-993 \times R-*rbcS* start (Freshwater & Rueness, 1994) for the 3' end. The protocol used for PCR amplifications was presented in Martin-Lescanne *et al.* (2010).

For *L. caspica*, PCR amplification used a PTC-200 DNA Engine (MJ Research Inc.). All PCR amplifications were carried out using the published primers *rbcLFC* as the forward primer and *rbcLRD* as the reverse primer (Nam *et al.* 2000), and all reactions contained 200 μ M each of dATP, dCTP, dGTP and dTTP, 0.3 μ M of each primer, 2.5 mM MgCl₂ and 1.6 units of Taq polymerase (Bioline). The PCR cycle used was as previously indicated (Nam *et al.* 2000). The mitochondrial marker cytochrome oxidase subunit 1 (CO1) was amplified using the primer pair GAZF1 and GAZR5 with the PCR conditions of Saunders (2005) and Clayden & Saunders (2014). LSU was amplified as three overlapping fragments using primers T01N/T20, T04/T08 and T05/T15, and using the PCR primers and the internal primers T10, T16N, T19N, T22, T24, T25, T30 and T33, following the protocols of Harper & Saunders (2001) and Le Gall & Saunders (2010).

The resulting PCR products were purified and used as templates for cycle sequencing reactions with the same primers as those used for the initial amplifications. These steps were performed by Genoscope (www.genoscope.fr, Evry, France) or Eurofins (France). For *L. caspica*, PCR fragments were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics Ltd., Lewes, UK), according to the manufacturer's instructions. The PCR products were then directly sequenced commercially by MWG-Biotech, Ebersberg, Germany.

Sequence alignments and phylogenetic analyses

Sequences were obtained for both DNA strands, assembled and corrected using Sequencher™ v. 4.1 (Gene Codes Corporation, Ann Arbor, Michigan) or Codoncode (Dedham, Massachusetts). Alignments of LSU, *rbcL* and CO1 were performed with MEGA v. 6 (Tamura *et al.* 2013). The LSU alignment was adjusted by eye with the objective of minimizing gaps, and four ambiguous regions corresponding to 109 nt were excluded before phylogenetic analyses.

Two datasets were analysed. The first one included 32 taxa for which COI-5P, *rbcL* and LSU (3 genes) were generated (giving 4801 nt alignment length) and a second one was based on only *rbcL* sequences (1395 nt) for 117 taxa. For the *rbcL* dataset, a global alignment (198 taxa) was generated with all GenBank sequences belonging to the *Laurencia* complex and with our newly produced sequences. We then screened the 198 sequences, of which we finally selected 117. We excluded sequences that were too short (less than 900 nt) and conserved only sequences that diverged by more than 2 nt (collapsetypes.pl available at <http://sourceforge.net/projects/collapsetypes/>). Finally, rapid NJ analyses were performed separately on sub-alignments corresponding to typical PCR primer amplifications (0-527, 528-701, 702-1099 and 1100-1395) to reveal a putative conflict signal. The selection of sequences is explained in the [Supplementary file](#).

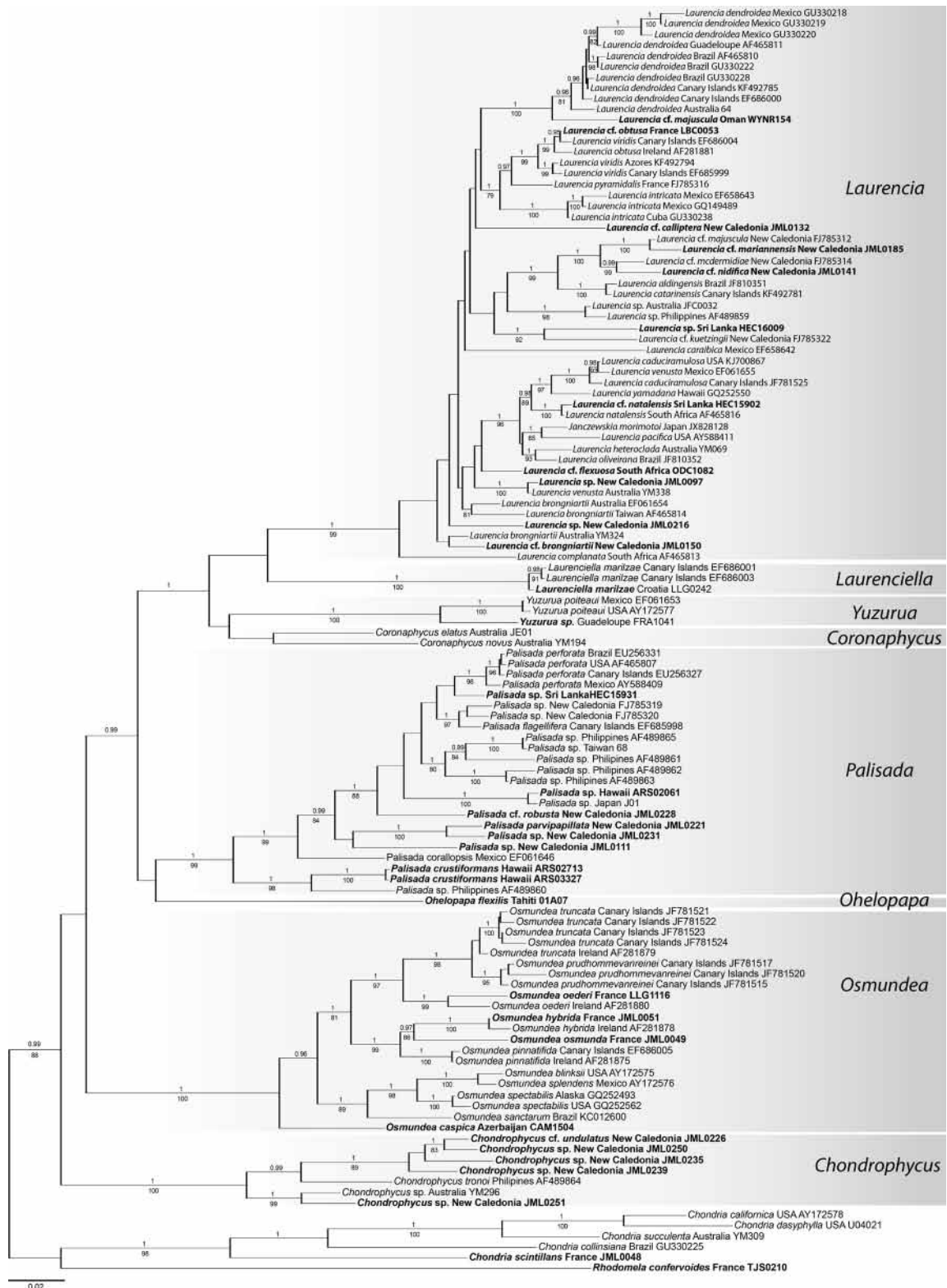


Fig. 1. Tree inferred from *rbcL* using Bayesian analysis (BI) and including 111 specimens of members of the *Laurencia* complex and six outgroup taxa. The numbers above branches indicate Bayesian posterior probabilities (pp) and below branches indicate bootstrap values (bp) inferred from 1000 ML bootstrap replicates (ML); pp < 0.95 and bp < 75% are not shown. Taxa for which new sequences were produced are indicated in bold.

Phylogenetic analyses

Bayesian and ML analyses were performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) and RAxML v. 8.2.0 (Stamatakis 2014) on the cipes portal. For both BI and ML analyses, the *rbcL* data set was partitioned by codon position (1+2 vs 3) while the three-gene data set was analysed in five partitions by gene and codon positions (codon position 1 and 2 merged into one partition). BI analyses were run with GTR+I+G model parameters estimated independently for each partition, with four heated Monte Carlo Markov Chains for 10^7 generations, with sampling intervals of 1000 generations, to produce 10000 trees. Nodal support was assessed by calculating posterior probability (PP) values at each node of the resulting consensus tree after a burn-in of 10% of the trees. The ML analyses were conducted with a GTRGAMMA model with ML estimates of base frequencies. The best-scoring ML tree and 1000 bootstrap trees were obtained using the rapid hill-climbing algorithm (-f a option).

Results

The *rbcL* only dataset (Fig. 1) and the combined dataset including CO1, *rbcL*, and LSU (Fig. 2) were analysed using ML and Bayesian approaches to delimit genera within the *Laurencia* complex and to assess phylogenetic relationships among genera. The two trees (Figs 1–2) were congruent in delimiting strongly supported lineages which overall correspond to the genera currently recognized in the *Laurencia* complex, with a few exceptions. *Laurencia caspica* was resolved as a sister taxon to all the species of *Osmundea* included in these analyses. *Laurencia crustiformans* allied with species of *Palisada* with strong (Fig. 1) to full support (Figs 1–2). *Laurencia flexilis* did not join the remaining species of *Laurencia* and its position varied in the tree depending on the analyses. It was resolved as sister to either *Yuzurua* (Fig. 2) or *Palisada* (Fig. 1). Moreover, our analyses revealed that a specimen collected in the Mediterranean Sea grouped with the recently described genus *Laurenciella* with a divergence from the generitype from the Canary Islands of only 0.5%. In addition, the monotypic genus *Yuzurua* encompassed two lineages with a divergence of 4.7%: one corresponded to a group of specimens from the Caribbean region and the second to a specimen from Guadeloupe. Intergeneric relationships were not supported by either reconstruction method in our taxon-rich dataset (Fig. 1) or in our character-rich dataset (Fig. 2).

Phylum Rhodophyta Wettst. (von Wettstein 1901)

Subphylum Eurhodophytina G.W.Saunders & Hommers. (Saunders & Hommersand 2004)

Class Florideophyceae Cronquist (Cronquist 1960)

Subclass Rhodymeniophycidae G.W.Saunders & Hommers. (Saunders & Hommersand 2004)

Order Ceramiales Oltm. (Oltmanns 1904)

Family Rhodomelaceae Aresch. (Areschoug 1847)

Genus *Ohelopapa* F.Rousseau, Martin-Lescanne, Payri & L.Le Gall gen. nov.

Fig. 3; Table 2

English diagnosis

With the characters of the genus *Laurencia* such as four pericentral cells, but lacking secondary pit connections between cortical cells and ‘corps en cerise’ inclusions in cortical cells.

Etymology

Ohelopapa means “strawberry” in Tahitian, an allusion to the Tahitian origin, the red color, and the many stolons developed by *Ohelopapa flexilis*.

Type species

Ohelopapa flexilis (Setch.) F.Rousseau, Martin-Lescanne, Payri & L.Le Gall comb. nov. Sole species presently included in the genus.

Ohelopapa flexilis (Setch.) F.Rousseau, Martin-Lescanne, Payri & L.Le Gall comb. nov.

Fig. 3; Table 2

Basionym

Laurencia flexilis Setch., *University of California Publications in Botany* 12: 101, pl. 19, figs 1–6 (Setchell 1926) (in Tahitian algae collected by Setchell W.A., Setchell C.B. & Parks H.E., 1926).

Type material

Holotype

FRANCE: French Polynesia, Tahiti, UC261333 deposited at the Jepson Herbaria (JEPS, University of California, Berkeley).

Original material is also preserved in SAP *vide* Masuda *et al.* (1999, 2006).

Type locality

Reef at Tahara Mountain, Tahiti.

Other material examined

FRANCE: French Polynesia, Tahiti, reef at Tahara Mountain [type locality], 24. Mar. 2007, *André Pham* 01A-07/UPF4223 (UPF).

Distribution

Tropical regions in the Pacific (Setchell 1926; Cribb 1983; Masuda *et al.* 1999), in the Indian Ocean (Silva *et al.* 1996) and on coasts of Japan (Masuda *et al.* 2006).

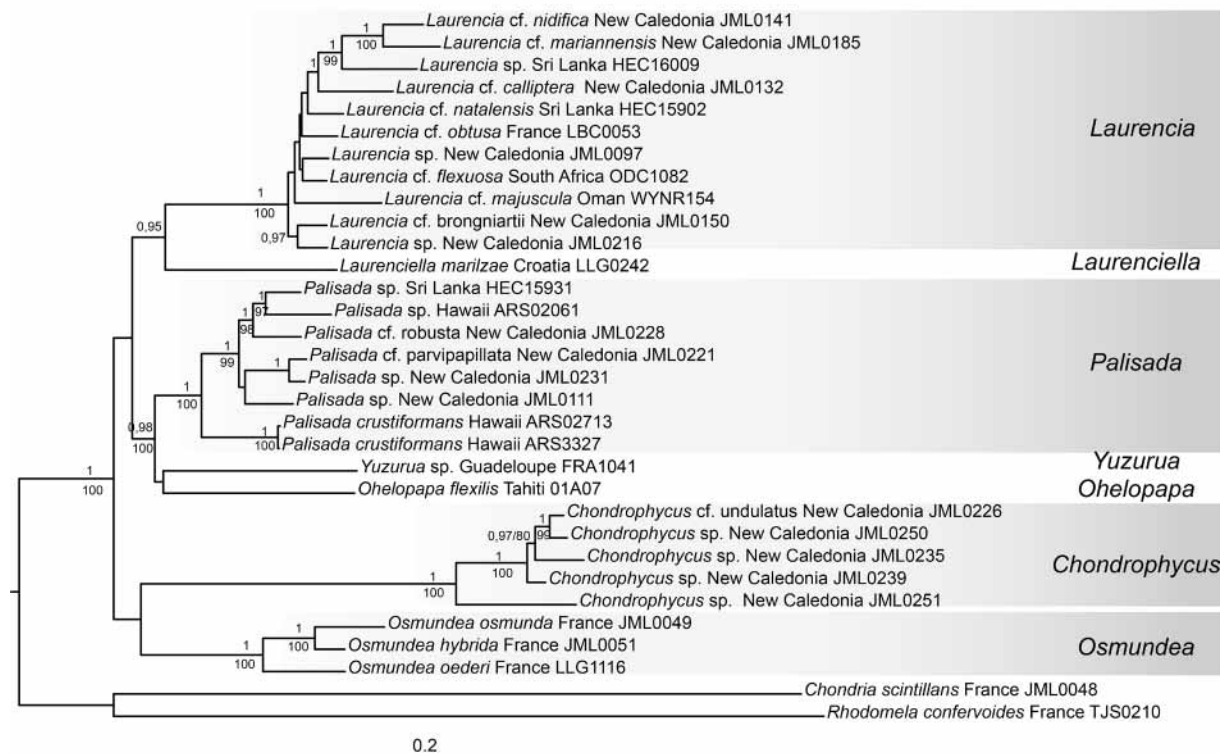


Fig. 2. Tree inferred from COI-5P+*rbcl*+LSU using Bayesian analysis (BI) and including 30 specimens of the *Laurencia* complex and two outgroup taxa. The numbers above branches indicate Bayesian posterior probabilities (pp) and below branches indicate bootstrap values (bp) inferred from 1 000 ML bootstrap replicates (ML); pp < 0.95 and bp < 75% are not shown.

Remarks

Ohelopapa flexilis (Fig. 3A) specimens included in this study matched previous descriptions where anatomical features were thoroughly illustrated (Setchell 1926; Masuda *et al.* 1999, 2006). Notably, we observed, in the outermost cortical layer, translucent cells without secondary pit connections, whereas the inner cortical layer (just below this) consisted of pigmented cells with secondary pit connections (Fig. 3B). This anatomical character was highlighted by Fujii & Cordeiro-Marino (1996) for *C. translucidus* (M.T.Fujii & Cord.-Mar.) Garbary & J.T.Harper. Based on molecular data, Fujii *et al.* (2006) showed that *C. translucidus* pertains to genus *Laurencia* rather than to *Chondrophyucus*. Interestingly, Masuda *et al.* (2006) used the absence of secondary pit connections between superficial cortical cells as a new argument to support the distinction between *L. flexilis* and another morphologically similar species, *L. tropica* Yamada. It would be interesting in the future to test with molecular characters whether *L. tropica* belongs to the genus *Ohelopapa* and also to analyse the taxonomic significance of the presence of an outermost cortical layer formed of translucent cells without secondary pit connections.

Genus *Palisada* K.W.Nam (Nam 2007)

Palisada crustiformans (McDermid) A.R.Sherwood, A.Kurihara & K.W.Nam comb. nov.
Fig. 4; Table 2

Basionym

Laurencia crustiformans McDermid, *Phycologia* 28: 352, figs 2–8 (McDermid 1989).

Type material

Holotype

USA: Hawaii, KM 2050 (BISH) (Abbott 1999: 384).

Type locality

Lualualei Beach Park, Oahu, Hawaii.

Other material examined

USA: Hawaii, Isaac Hale Beach Park, Hawaii Island, 24 Jan. 2008, A. Kurihara ARS03327/BISH 766726 (BISH).

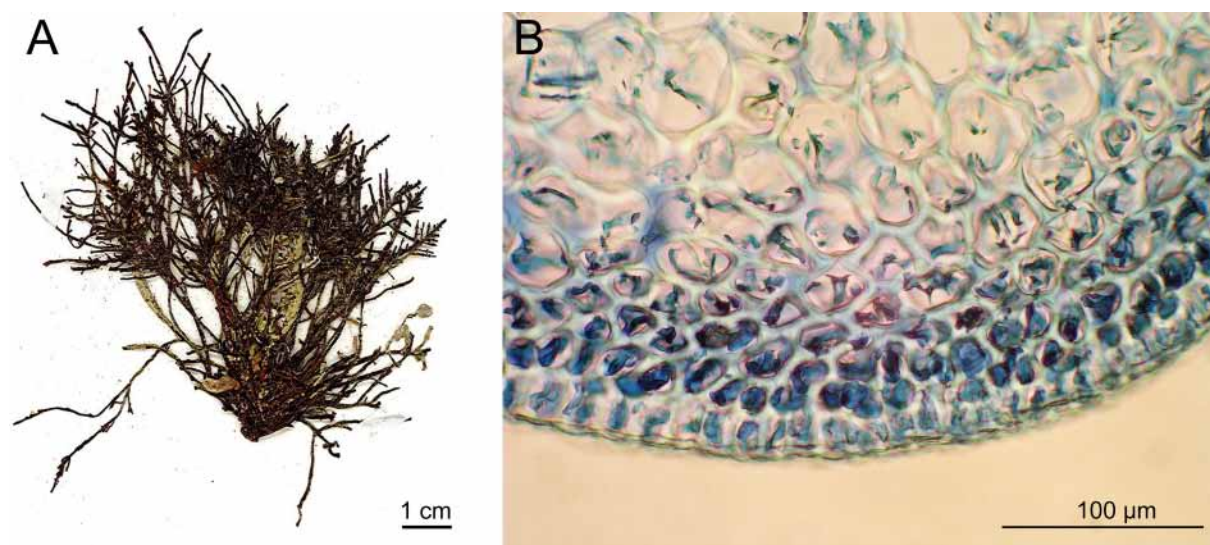


Fig. 3. *Ohelopapa flexilis* (Setch.). **A.** Voucher specimen (01A07/UPF4223) collected at Tahiti (type locality). **B.** Transversal section through outer cortex showing a translucent outermost cortical layer lacking secondary pit connections between cells.

Distribution

Recorded from Hawaii and Tahiti.

Remarks

The *Palisada crustiformans* (Fig. 4) specimen included in this study was sterile and matched previous descriptions by McDermid (1989) concerning vegetative characters. In particular, secondary pit connections between cortical cells were observed (Fig. 4C). Small structures that may or may not be ‘corps en cerise’, were also observed in cortical cells (Fig. 4B–C).

Genus *Osmundea* Stackh. (Stackhouse 1809)

Osmundea caspica (Zinova & Zaberzhinskaya) Maggs & L.M.McIvor comb. nov.
Fig. 5; Table 2

Basionym

Laurencia caspica Zinova & Zaberzhinskaya, *Novosti Sistematiki Nizshikh Rastenii* 1968: 30–32 (Zinova & Zaberzhinskaja 1968).

Type material

AZERBAIJAN: Jun. 1962, *K.M. Petrov*, V.L. Komarov Botanical Institute (LE), Saint-Petersburg.

Type locality

Svinoy, Sangi-Mugan Island, Baku Archipelago, Caspian Sea, Azerbaijan.

Other material examined

AZERBAIJAN: Sangachal Bay, Sep. 2003, *ERT Caspian Contractors BM001062596* (BM).

Distribution

Recorded from the Black Sea (Bulgaria and Romania) and Caspian Sea (Guiry & Guiry 2016), both of which are low salinity bodies of water.

Description

The description of *L. caspica* is published in Russian in a book that is not widely available, so here we provide the following description.

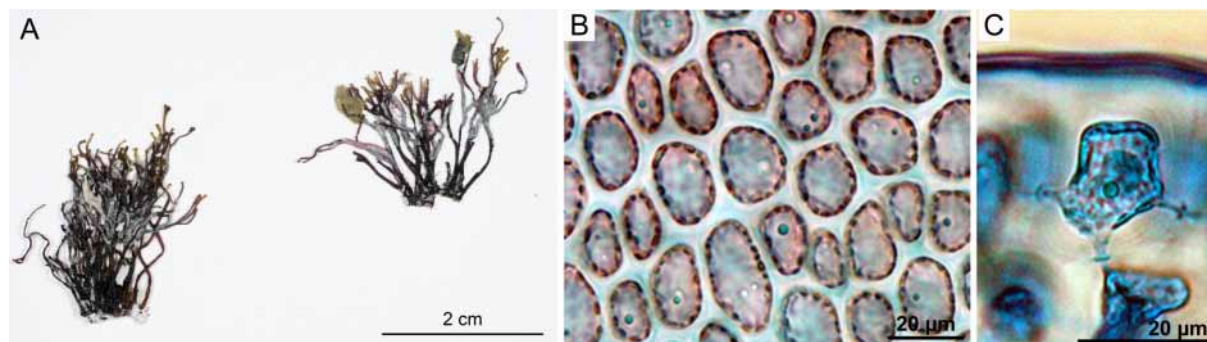


Fig. 4. *Palisada crustiformans* (McDermid). **A.** Voucher specimen of *P. crustiformans* (ARS03327/BISH 766726) collected at Hawaii Island (type locality). **B.** Outer cortical cells in surface view. **C.** Detail of outermost cortical cell showing small spherical structure similar to ‘corps en cerise’ and secondary pit connections with adjacent cells.

Thalli were 5–11 cm high, growing from a solid discoid basal holdfast; erect axes terete, about 1 mm in diameter, irregularly branched to three orders, with blunt apices, similar in general habit to *Laurencia obtusa* with the exception of the holdfast (Fig. 5A). In surface view of the cortex of live thalli, there were no ‘corps en cerise’; in surface view of preserved and stained cortical preparations, secondary pit connections were absent (Fig. 5B). In transverse section of axes, pericentral cells were not distinguishable, and the cortical cells were comparatively large and slightly radially elongated (Fig. 5C). Lenticular thickenings were absent in medullary cells.

Mature non-reproductive thalli, tetrasporophytes and males were collected but females are unknown. Tetrasporangia 80–110 μm in diameter occurred in bands below the apices of lateral branches. They were produced adaxially from random epidermal cells, cut off laterally from the mother cells. Spermatangial receptacles were terminal and open cup-shaped. Spermatangial structures were of the filament type (Nam *et al.* 2000); spermatangial filaments were unbranched, bearing numerous elongate spermatangia, and terminating in single large round to ovoid cells up to 40 μm in diameter (Fig. 5D–E).

Discussion

In the present study we focused on assessing the generic affinities of some species of *Laurencia* which displayed atypical characters for the genus *Laurencia* s. str.

Contrary to other *Laurencia* species, *L. flexilis* has neither secondary pit connections between cortical cells nor ‘corps en cerise’ but axial cells of its sterile branches have four pericentral cells like species of

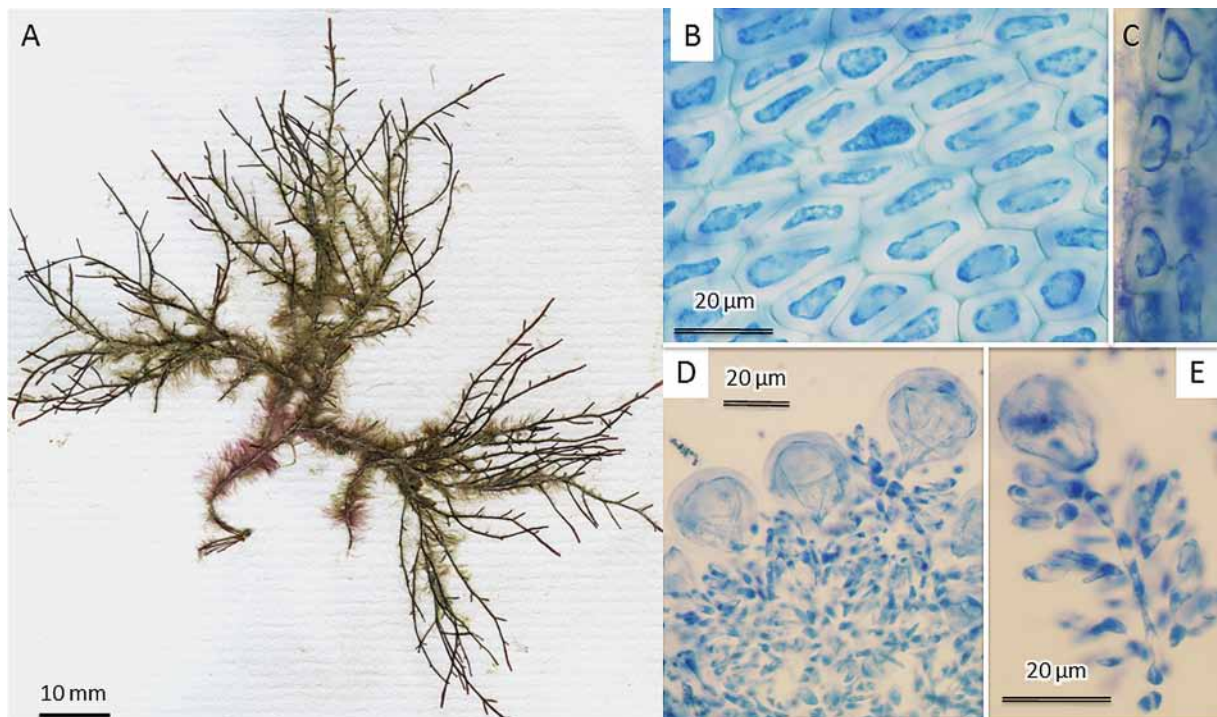


Fig. 5. *Osmundea caspica* (A.D.Zinova & Zaberzhinskaya). Sangachal Bay, Azerbaijan, September 2003, dredged. **A.** Habit of herbarium voucher specimen with epiphytic acrochaetioid red algae. **B.** Outer cortical cells in surface view, elongated along the thallus axis, lacking secondary pit connections. **C.** Longitudinal section through outer cortex (scale bar as in B). **D.** Part of mature spermatangial receptacle, showing spermatangial filaments each terminating in a large rounded sterile cell. **E.** Spermatangial filament bearing mature spermatia and terminating in a rounded sterile cell.

Laurencia s. str. (Masuda *et al.* 1999, observed both in specimens from Malaysia and Setchell's original material from Tahiti preserved in SAP; Masuda *et al.* 2006, based on both Setchell's original material and Japanese specimens). In the molecular analyses of Abe *et al.* (2006), *L. flexilis* was resolved as an independent divergent lineage of *Laurencia* s. str. with no supported relationships with any other genera. In our analyses, we confirm that *L. flexilis* does not have strong affinities with any of the currently described genera of the *Laurencia* complex and we therefore propose here to accommodate *L. flexilis* in *Ohelopapa* gen. nov.

When Nam (2007) elevated *Palisada* to generic rank, he included a cladistic analysis based on the anatomical characters of *Laurencia crustiformans* and resolved it within a species group for which he proposed novel combinations to be accommodated in *Palisada*. Surprisingly, he did not propose the transfer of *Laurencia crustiformans* to *Palisada*. Given that our molecular phylogeny is congruent with his cladistic analysis, we here propose the new combination *Palisada crustiformans*.

Osmundea caspica was described from the Caspian Sea in 1968 as *Laurencia caspica*, previously identified as *L. hybrida* (DC.) T.Lestib. (now *O. hybrida*; see Zinova 1967). It lacks 'corps en cerise' and secondary pit connections between cortical cells, a couple of features shared by almost all the *Laurencia* s. str. species. *Osmundea caspica* is very similar to *Osmundea maggsiana* D.Serio, Cormaci & G.Furnari from the Mediterranean in the anatomical features considered important in this genus, sharing a solid discoid holdfast, terete axes, lacking 'corps en cerise' and secondary pit connections in the cortex, lacking lenticular thickenings in the medulla, and having cup-shaped spermatangial receptacles with individual spermatangial filaments terminating in 1–2 large cells (Fig. 5; Serio *et al.* 1999; Furnari 2001). The main difference is that whereas *O. caspica* is up to 11 cm high, and the axes are less than 1 mm in diameter, *O. maggsiana* is smaller (up to 5.5 cm) but more robust, up to 3 mm in diameter. Also, in *O. maggsiana* cortical cells are markedly radially elongated in longitudinal section ("palisade-like", Serio *et al.* 1999; Fig. 5), whereas in *O. caspica* cortical cells are isodiametric in longitudinal section (Fig. 5C). *Osmundea maggsiana* is apparently known only from a single collection from Pantelleria Island, Straits of Sicily (Serio *et al.* 1999), consisting of males and tetrasporophytes but no cystocarpic thalli. *Osmundea caspica* also resembles *O. hybrida* from the Atlantic, but the spermatangial filaments terminate in single cells rather than in groups of up to 20 large cells as in *O. hybrida* (Maggs & Hommersand 1993). The spermatangial development on filaments terminated by large sterile cells and the origin of tetrasporangia from outer cortical cells observed in *O. caspica* are distinctive characters of the genus *Osmundea*.

Despite its evident morphological similarities with *Osmundea*, *L. caspica* has never been transferred to *Osmundea*. It is widespread and common in the Caspian Sea (Zinova 1967), and it has also been reported from the Black Sea (Gómez Garreta *et al.* 2001). It is the only species of *Osmundea* that occurs in low-salinity conditions, occurring in the Caspian at 12–13 PSU.

The disjunct distribution of *Osmundea* was considered by McIvor *et al.* (2002) to correspond to a late Tethyan distribution pattern: "the genus would likely have originated in the western Tethyan Ocean after effective closure of the Tethyan Seaway between 60 Ma and 20 Ma during Oligocene or Miocene." McIvor *et al.* (2002) noted the apparent absence of species of *Osmundea* in the tropical western Atlantic and Indo-West Pacific Oceans. The discovery of *Osmundea* in the Caspian Sea strengthens the hypothesis of a Tethyan origin. In the late Eocene (ca 37 Ma), the tropical Tethys Ocean vanished due to the northward movement of India (Scotese 2016). The western relic Tethys connected the Atlantic Ocean to the Indo-Pacific, and the Turgai Strait opened to the Polar Sea (Rögl 1998). The Caspian Sea is a remnant of the epicontinental Paratethys, a gulf of the original Tethys Ocean (van Baak *et al.* 2013; Scotese 2016), which was connected to the North Sea during the early Oligocene (ca 32 Ma) but then ca 30 Ma the closure of open seaways isolated Paratethys, leaving its only marine connections to the

Mediterranean in the west (Rögl 1998). Complete isolation of the Caspian and Black Seas occurred in the Miocene (ca 18 Ma) followed by a complex series of marine transgressions and interconnections during which the Mediterranean–Indian Ocean seaway finally closed ca 15 Ma, but the southern basins of the Caspian (1 km deep) never dried up (Rögl 1998; van Baak *et al.* 2013). The Caspian Sea contains ancient palaeoendemics such as the sturgeons (Osteichthyes: Acipenseridae; Choudhury & Dick 1998). The phylogenetic position of *O. caspica* at the first divergence of the *Osmundea* group (Fig. 1) strongly supports a late Tethyan origin of the genus within the tropical Laurencieae. The phylogeny of *Osmundea* shows a deep divergence between the North East Atlantic [including Atlantic islands]–Mediterranean clade and an American clade (Fig. 1; Machín-Sánchez *et al.* 2016). McIvor *et al.* (2002) noted the apparent absence of species of *Osmundea* from the tropical western Atlantic in contrast to observations on Tethyan distributions (Hommersand 2007) that “More often than not related taxa also occur in the western Atlantic Ocean and Caribbean Sea”. The discovery of two *Osmundea* species in SE Brazil (*Osmundea sanctarum* M.T.Fujii & Rocha-Jorge from offshore islands of the State of São Paulo (Rocha-Jorge *et al.* 2013) and *Osmundea lata* (M.Howe & W.R.Taylor) Yonesh., M.T.Fujii, & Gurgel from a depth of 60 m off Cabo Frio, State of Rio de Janeiro; Yoneshigue-Valentin *et al.* 2003) has now shown that the distribution of *Osmundea* is typically Tethyan. In addition, the known distribution of *Osmundea sinicola* (Setch. & N.L.Gardner) K.W.Nam now includes Pacific Panama as well as the Galapagos Islands (Littler & Littler 2010). The earliest diverging species in the American clade is *O. sanctarum*, which is further evidence that this clade originated by vicariance as the South Atlantic opened up ca 100 Ma in the Cretaceous (Scotese 2016), then spread to the NE Pacific and diversified (Fig. 6).

In addition to clarifying the generic affinities of some species so far known as *Laurencia*, this paper provides new records for the two recently described genera *Laurenciella* and *Yuzurua*. *Laurenciella*

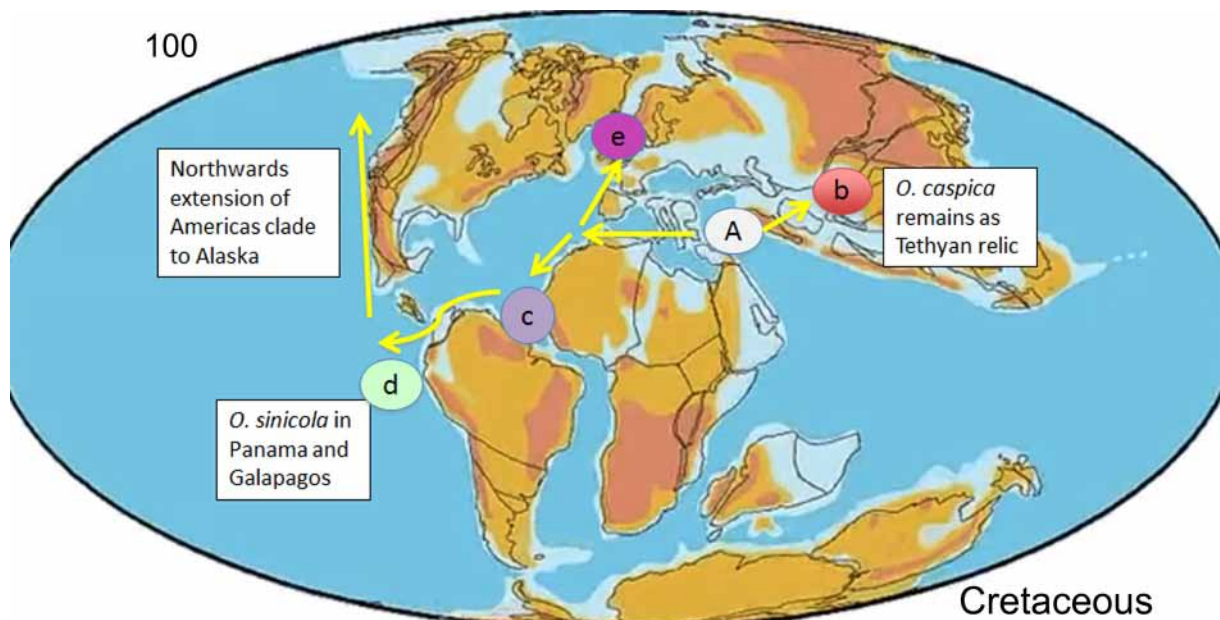


Fig. 6. Scenario of *Osmundea* biogeography using frame from Scotese animation (www.scotese.com/) at 100 Ma. South America and Africa are starting to move apart. Hypothetical ancestors (A) of widespread and diverse *Osmundea* flora along Tethyan shores of which *Osmundea caspica* (A.D.Zinova & Zaberzhinskaya) comb. nov. (b) is almost certainly a relic. Since 100 Ma, *Osmundea* has colonized the Atlantic Ocean (c, e). Brazilian *O. sanctarum* M.T.Fujii & Cord.-Mar. (c) is likely a relic of the lineage at the origin of the clade that entered the Pacific (d) and colonized the Americas.

marilzae (Gil-Rodríguez, Sentfies, Díaz-Larrea, V.Cassano & M.T.Fujii) Gil-Rodríguez, Sentfies, Díaz-Larrea, V.Cassano & M.T.Fujii was initially described based on specimens from the Canary Islands of Macaronesia (Gil-Rodríguez *et al.* 2009) as a new species of *Laurencia*. When the new genus *Laurenciella* was described, *L. marilzae* was reported both in Brazil and in the Canary Islands. In 2014, the species was also reported in Mexico and in many islands of Macaronesia (Machín-Sánchez *et al.* 2014). Here, we further extend the distribution of the genus to the Mediterranean Sea; however, we question whether this new record is conspecific with *L. marilzae* as it falls in the grey zone between intra- and interspecific divergence (0.5% of divergence in *rbcL*). Unfortunately, our specimens were pressed as herbarium vouchers, a technique which does not preserve tissues of members of the *Laurencia* complex well enough for a thorough anatomical study. The genus *Yuzurua* was proposed to accommodate *L. poiteaui* (J.V.Lamour) M.Howe, the type locality of which is Santo Domingo, Dominican Republic, based on molecular data for specimens from Mexico and USA (Martin-Lescanne *et al.* 2010). In the present study we report one specimen of *Yuzurua* from Guadeloupe which displays a divergence that clearly indicates that the two lineages are not conspecific (4.7%). Further anatomical studies are needed to assess whether we need to describe a new species for this Guadeloupean record.

Conclusion and perspectives

This study, which is the first examination of the *Laurencia* complex based on both a taxon-rich dataset (*rbcL*) as well as a character-rich data set (combining COI-5P+*rbcL*+LSU), has once again indicated that the *Laurencia* complex is more diverse than currently recognized at the generic level and has confirmed that molecular-assisted systematics constitutes a helpful tool to assign species of the notoriously difficult *Laurencia* complex to a genus. Despite the fact that the genera of the *Laurencia* complex are currently well delimited using molecular characters, relationships among them are still poorly resolved. More molecular data, as well as broader taxon sampling, are still necessary to further improve our understanding of this taxonomic complex in an evolutionary framework. Advances in next generation sequencing create novel opportunities to develop phylogenomic analyses. The recent publication of a plastid genome of a species of *Laurencia* (Verbruggen & Costa 2015) paves the way toward plastome phylogenetic analyses of the *Laurencia* complex which will likely break the stranglehold of unresolved phylogenetic relationships.

Acknowledgments

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