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Palisada flagellifera (Ceramiales, Rhodophyta) from the Canary Islands, Spain: a new record for the eastern Atlantic Ocean based on morphological and molecular evidence

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Abstract

Palisada flagellifera (Ceramiales, Rhodophyta) is recorded for the first time in the eastern Atlantic Ocean off Tenerife, La Gomera, La Palma and Fuerteventura, Canary Islands, Spain. The specimens were collected in 2006–2009 growing from the lower intertidal to subtidal zones to 2 m depth at sites exposed to wave action. The species possesses a palisade-like arrangement of cortical cells in cross section, lacks secondary pit connections between them, and has tetrasporangia produced by three fertile pericentral cells (the third and the fourth additional and the second that becomes fertile), and a right-angled arrangement of tetrasporangia. Gametangia were not observed. The phylogenetic relationships were inferred by analyses of the chloroplast-encoded *rbc*L gene sequences from 46 taxa. The Canarian and Brazilian *P. flagellifera* specimens formed a highly supported clade with a low level of genetic variation in the *rbc*L sequences (0.02–0.04%), confirming that they are the same taxonomic entity. This study expands the geographical distribution of *P. flagellifera* to the eastern Atlantic Ocean.

Keywords: Canary Islands; molecular phylogeny; *Palisada flagellifera*; *rbc*L; taxonomy.

Introduction

The *Laurencia* complex is currently divided into four genera: *Laurencia* J.V. Lamouroux *sensu stricto*, *Osmundea* Stackhouse, *Chondrophycus* (Tokida *et* Saito) Garbary *et* J.T. Harper and *Palisada* K.W. Nam (Nam et al. 1994, Garbary and Harper 1998, Nam 1999, 2006, 2007). The genus *Palisada*, based on Yamada's (1931) section *Palisadae*, is characterized by the presence of two pericentral cells per vegetative axial segment, production of the first pericentral cell beneath the basal cell of the trichoblast, spermatangia of the trichoblast type, production of spermatangial branches from one of two laterals on the suprabasal cell of trichoblasts, procarp-bearing segments with four pericentral cells, except in *P. poiteaui* (J.V. Lamouroux) K.W. Nam var. *poiteuai* and *P. poiteaui* var. *gemmifera* (Harvey) Sentíes, M.T. Fujii *et* Díaz-Larrea [Fujii et al. 1996, as *Laurencia poiteaui* (J.V. Lamouroux) M.A. Howe and *L. gemmifera* Harvey], normal developmental pattern of auxiliary cells in the post fertilization process, tetrasporangial production from particular pericentral cells, and presence of fertility in the second pericentral cell of the tetrasporangial axis (Nam et al. 1994, Nam 2006).

Palisada flagellifera (J. Agardh) K.W. Nam was considered a typical member of the subgenus *Palisadae* (Yamada) K.W. Nam, section *Palisadae* Yamada by Fujii et al. (2006) due to the presence of an outermost cortical cell layer arranged in a palisade, the absence of secondary pit connections between cortical cells, the production of one sterile pericentral cell per tetrasporangial axial segment, and by right-angled tetrasporangial arrangement.

The species is reported for the Pacific Ocean [Australia (Cribb 1958), Hawaii (Saito 1969), Solomon Islands and Philippines (Womersley and Bailey 1970)], for the Indian Ocean [Rodriguez Islands and India (Silva et al. 1996)], and for the western Atlantic Ocean [Mexico (Sentíes and Fujii 2002) and Brazil (Fujii and Sentíes 2005, Fujii et al. 2006)]. This paper reports for the first time the presence of *Palisada flagellifera* off the Canary Islands, Spain, based on morphological characters and chloroplast-encoded *rbc*L gene sequences, and expands its geographic distribution to the eastern Atlantic Ocean.

Materials and methods

Specimens for the present study were collected at La Palma, La Gomera, Tenerife, and Fuerteventura (Canary Islands) in 2006–2009 and fixed in 4% formalin solution. Living specimens were also examined to check for the presence of ''*corps en cerise*''. Longitudinal and transverse hand sections were made with a stainless steel razor blade under a stereoscopic dissection microscope and stained with 0.5%

aqueous aniline blue solution, acidified with 1N HCl (Tsuda and Abbott 1985). Measurements are given as length×diameter. Photomicrographs were taken with a Leica (Wetzlar, Germany) stereomicroscope (Leica MZ 12.5) and a compound microscope (Leica DM 2000) with digital camera (Leica DFC 290). Voucher specimens are deposited in TFC, HRJ, UAMIZ and SP. Herbarium abbreviations follow the on-line *Index Herbariorum* (http://www.nybg.org/bsci/ih/ih.html).

Total DNA was extracted using the Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A total of 1467 base pairs of the *rbc*L gene were amplified in three overlapping parts with the primer pairs FrbcLstart×R753, F492×R1150 and F993×RrbcS (Freshwater and Rueness 1994), using the master mix of the Bioneer (Daedeok-Gu, Daejeon, Korea) Premix. All PCR products were analyzed by electrophoresis in 1% agarose to check product size. The PCR products were purified with the Qiagen QIAquick purification kit (Qiagen) following the manufacturer's instructions.

Sequencing was performed using the BigDye terminator cycle sequencing reaction kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Princeton, NJ, USA). The primers used for sequencing were those used for amplification. The full sequence was obtained from both DNA strands. Multiple alignments for *rbc*L sequences were constructed using BioEdit 7.0.4.1 software (Hall 1999). The specimens used in phylogenetic analysis are shown in Table 1, including their GenBank accession numbers (NCBI GENBANK).

Phylogenetic relationships were inferred with PAUP* 4.0b10 (Swofford 2002) and MrBayes v.3.0 beta 4 (Huelsenbeck and Ronquist 2001). Maximum parsimony (MP) trees were constructed using the heuristic search option, treebisection-reconnection branch swapping, unordered and unweighted characters, and gaps in the GenBank sequences were treated as missing data. Support for individual internal branches was determined by bootstrap analysis (Felsenstein 1985), as implemented in PAUP*. For bootstrap analysis, 1000 bootstrap data sets were generated from re-sampled data for the MP analysis. The range of *rbc*L divergence values within and among species was calculated using uncorrected ''p'' distances using PAUP*. The model used in the Bayesian analysis was the general time-reversible model of nucleotide substitution with Invariant sites and gamma-distributed rates for the variable sites (GTR+I+G). This model was selected based on maximum likelihood (ML) ratio test implemented by the software Modeltest version 3.06 (Posada and Crandall 1998) with a significance level of 0.01. For the Bayesian analysis, we ran five chains of the Markov chain Monte Carlo (one hot and four cold), sampling one tree every 1000 generations for 4,000,000 generations starting with a random tree. Stationarity was reached at generation 17,000. Therefore, trees saved until generation 16,500 were the ''burn in'' of the chain, and inferences about the phylogeny were based on those trees sampled after generation 16,500. A 50% consensus tree (majority rule as implemented by PAUP*) was computed after the ''burn in'' point.

Results

Morphological analyses

***Palisada flagellifera* (J. Agardh) K.W. Nam (2007: 54) (Figures 1–13)**

Basionym *Laurencia flagellifera* J. Agardh (1852: 747–748).

Synonym *Chondrophycus flagelliferus* (J. Agardh) K.W. Nam (1999: 463).

Holotype LD 36604–36606!

Type locality ''ad oras Indiae orientalis'' (Indonesia).

Thalli terete, cartilaginous, rigid in texture, forming brown, violet-brown or dark brown tufts up to 9 cm high, with main axes 0.5 (-1) 1.5 mm in diameter, attached to substratum by a discoid holdfast, descending basal branches, and occasionally stolon-like branches (Figures 1 and 2). Branching alternately spiral to irregular, dense in the upper thallus portions and scant or naked in the lower parts. In surface view, cortical cells are isodiametric-polygonal in the middle portions, 15–40×10–37.5 μm, without secondary pit connections (Figure 3). *Corps en cerise* absent. In cross section, thallus with one or two layers of pigmented cortical cells (Figure 4); surface cortical cells radially elongated and arranged as palisade, 23–50×13–25 μm in the middle portions of the main axes (Figure 5). Medullary region with four or five layers cells, 52–60×41–45 μm, without lenticular thickenings. Each vegetative axial segment cutting off two pericentral cells (Figure 6); the first pericentral cell is produced underneath the basal cell of the trichoblast (Figures 7 and 12). Tetrasporangial branches short and compound or long and isolated (Figure 8). Tetrasporangia produced by the second, third and fourth (the two latter additionally produced) pericentral cells (Figures 9 and 13). The fertile pericentral cells cut off two presporangial cover cells distally, the tetrasporangial initial subdistally in abaxial position and one postsporangial cover cell (Figures 10 and 13). Tetrasporangia with a right-angled arrangement, 50–60 μm in diameter (Figures 8 and 11). Gametangia were not observed.

The epilithic specimens were collected from the lower intertidal to subtidal zones to 2 m depth, associated with articulated Corallinaceae and *Cystoseira abies-marina* (S.C. Gmelin) C. Agardh at exposed sites.

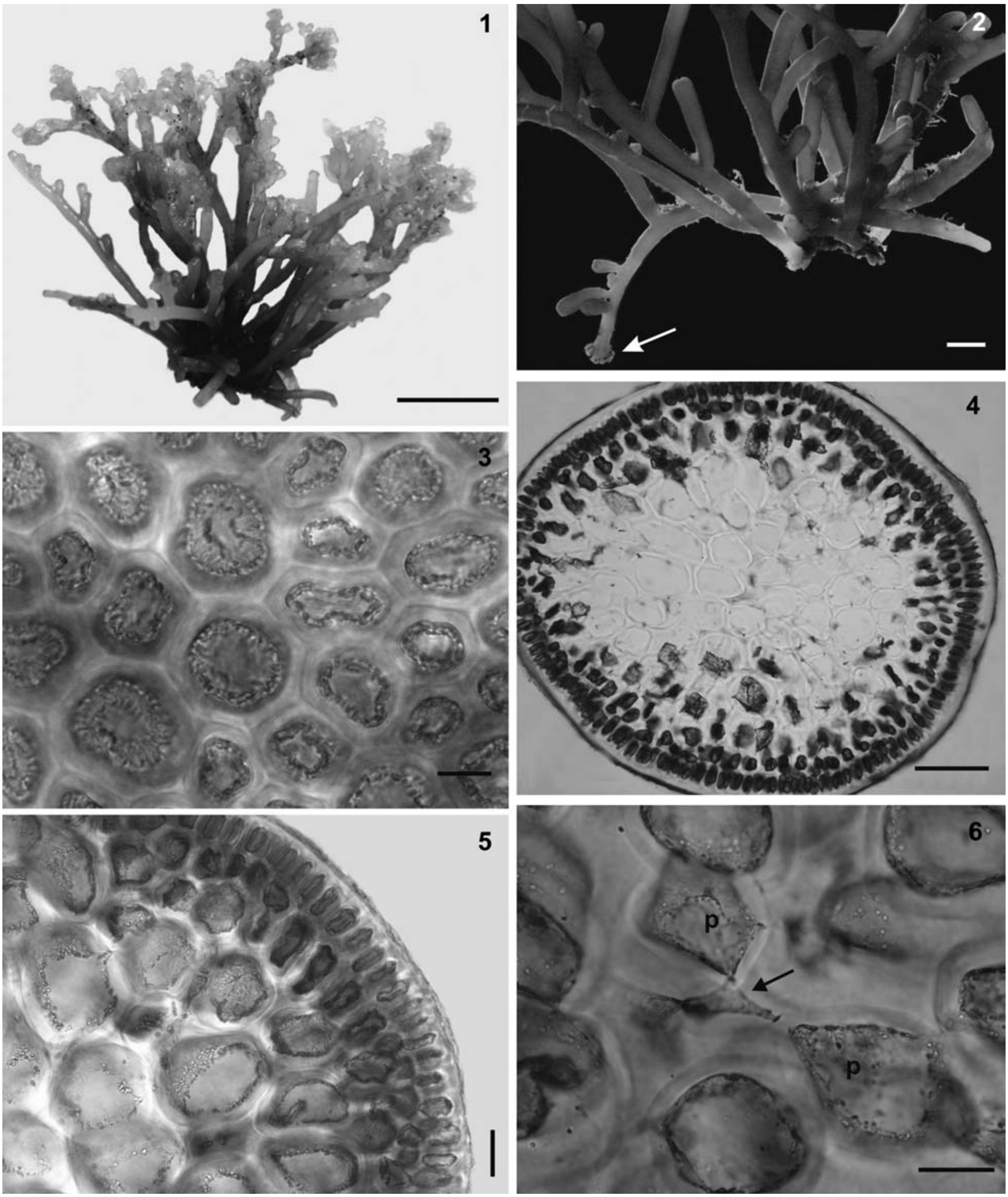
Specimens examined (Spain, Canary Islands) La Palma: Fajana de Barlovento, 24 January 2008, leg. M.C. Gil-Rodríguez, A. Losada and J. Leal Pérez (TFC Phyc. 14138); Bco de la Herradura, 27 September 2008, leg. M.C. Gil-Rodríguez and J. Leal Pérez (TFC Phyc. 14252). La Gomera: Valle Gran Rey, 15 May 2008, leg. M.C. Gil-Rodríguez and A. Cruz (TFC Phyc. 14203); Pta Majona, 16 May 2008, leg. M.C. Gil-Rodríguez and A. Cruz (TFC Phyc. 14215). Ten-

Table 1 Taxa used in this study for phylogenetic analysis.

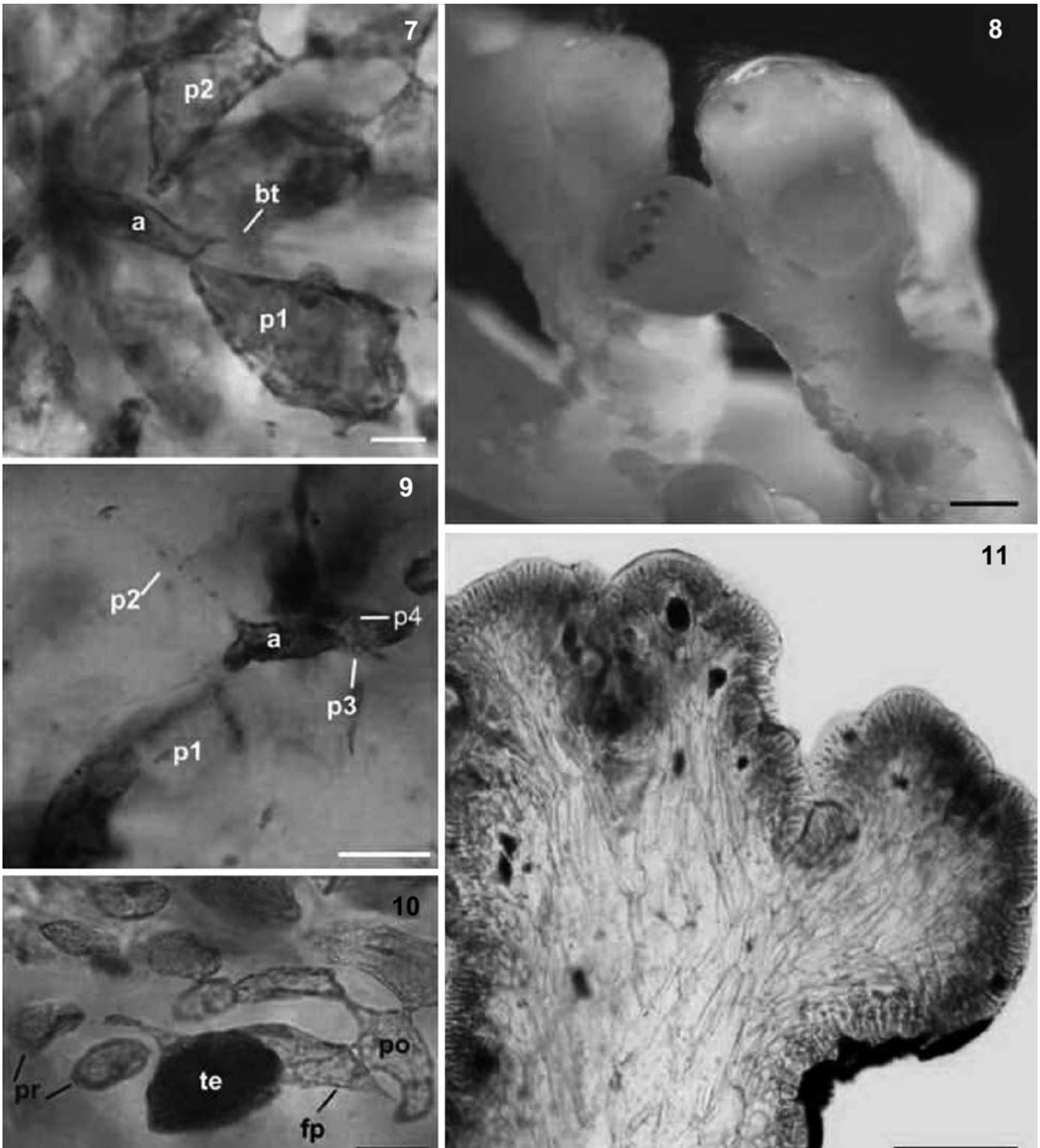
Samples	Collection data including reference	Collectors	GenBank accession nos.
Bostrychia radicans (Mont.) Mont. *in* Orbigny	USA, Mississippi, St. Louis Bay, 11 Feb. 1998 (Lin et al. 2001)	C.F.D. Gurgel	AF259497
Centroceras sp. [as *C. clavulatum* (C. Agardh in Kunth) Mont. in Durieu de Maisonnneuve]	USA, Texas, Port Aransas, Redfish Bay, 18 May 1998 (Lin et al. 2001)	S. Fredericq and C.F.D. Gurgel	AF259490
Ceramium brevizonatum H.E. Petersen	Mexico, Campeche Bay, Laguna de Yulcapeten, 12 Feb. 1998 (Lin et al. 2001)	C.F.D. Gurgel	AF259491
C. dasyphylla (Woodwar) C. Agardh	USA, North Carolina, New Hanover Co., Wrightsville Beach (Freshwater et al. 1994)	–	U04021
Laurencia dendroidea J. Agardh	Brazil, São Paulo, Ubatuba, Praia do Felix, 31 Aug. 2000 (Fujii et al. 2006)	M.T. Fujii	AF465810
L. dendroidea J. Agardh	Brazil, São Paulo, Ubatuba, Ilha Rapada, 19 Jan. 2001 (Fujii et al. 2006)	M.T. Fujii	AF465808
L. cf. *brongniartii*	Taiwan, Makang Harbour, 11 Jul. 1993 (Fujii et al. 2006)	S. Fredericq	AF465814
L. cf. *brongniartii*	Australia, Tarcoala Beach, 1993 (this study)	S. Fredericq	EF061654
L. caraibica P.C. Silva	Mexico, Quintana Roo, Cancún, Isla Mujeres, 23 Feb. 2006 (Gil-Rodríguez et al. 2009)	A. Sentíes	EF658642
L. complanata (Suhr) Kützing	South Africa, KwaZulu-Natal, Port Edward, 8 Feb. 2001 (Fujii et al. 2006)	S. Fredericq and O. De Clerck	AF465813
L. flexuosa Kützing	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001 (Fujii et al. 2006)	S. Fredericq	AF465815
L. intricata J.V. Lamouroux	Mexico, Yucatan, Campeche Bay, 14 Feb. 1999 (Fujii et al. 2006)	C.F.D. Gurgel	AF465809
L. intricata	USA, Florida, Long Key, Channel 5, 10 Dec. 1998 (Fujii et al. 2006)	B. Wysor and T. Frankovich	AY588410
L. intricata	Cuba, Cayo Coco, 25 Sep. 2005 (Cassano et al. unpublished)	M.T. Fujii	–
L. intricata	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 16 Apr. 2004 (Cassano et al. 2009)	J. Díaz-Larrea and A. Sentíes	EF658644
L. intricata	Mexico, Quintana Roo, Playa del Carmen, 2002 (this study)	J. Díaz-Larrea and A. Sentíes	GQ149489
L. intricata	Mexico, Quintana Roo, Tulum, 2004 (this study)	J. Díaz-Larrea and A. Sentíes	GQ149490
L. natalensis Kylin	South Africa, KwaZulu-Natal, Palm Beach, 7 Feb. 2001 (Fujii et al. 2006)	S. Fredericq	AF465816
L. obtusa (Huds.) J.V. Lamour	Ireland, County Donegal, Fanad Head (McIvor et al. 2002)	–	AF281881
L. rigida J. Agardh	Australia (unpublished)	G.C. Zuccarello and J.A. West	AY920852
L. translucida M.T. Fujii *et* Cordeiro-Marino	Brazil, Espírito Santo, Marataízes, 15 Sep. 2001 (Fujii et al. 2006)	M.T. Fujii	AY588408
L. venusta Yamada	México, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004 (Díaz-Larrea et al. 2007)	J. Díaz-Larrea and A. Sentíes	EF061655
L. viridis Gil-Rodríguez *et* Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 6 Oct. 2005 (Gil-Rodríguez et al. 2009)	M.C. Gil-Rodríguez	EF685999
Laurencia sp.	Spain, Canary Islands, Tenerife, Playa Paraíso, 14 Jul. 2006 (Gil-Rodríguez et al. 2009)	M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes	EF686004
Palisada corallopsis (Mont.) Sentíes, M.T. Fujii *et* Díaz-Larrea	Mexico, Quintana Roo, Cancún, Chaac-Mol Beach, 21 Aug. 2005 (Díaz-Larrea et al. 2007)	J. Díaz-Larrea and A. Sentíes	EF061646
P. flagellifera (J. Agardh) K.W. Nam	Brasil, São Paulo, Ubatuba, Praia Brava, 25 May 2001 (Fujii et al. 2006)	S.M.B Gumarães and J. Domingos	AF465804

(Table 1 continued)

Samples	Collection data including reference	Collectors	GenBank accession nos.
Palisada flagellifera	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, 03 Aug. 2005 (Cassano et al. unpublished)	V. Cassano and M.B. Barros-Barreto	–
P. flagellifera	Spain, Canary Islands, Tenerife, Playa Paraíso, 12 Jul. 2006	M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes	EF685998
P. patentiramea (Mont.) Cassano, Sentíes, Gil-Rodríguez *et* M.T. Fujii	Philippines (A.O. Lluisma unpublished)	–	AF489862
P. papillosa (C. Agardh) K.W. Nam	Mexico, Baja California, Todos Santos, 24 Oct. 1999 (Fujii et al. 2006)	S. Fredericq	AY588409
Palisada sp. [as *P. papillosa* (C. Agardh) K.W. Nam]	Philippines (A.O. Lluisma unpublished)	–	AF489861
P. perforata (Bory) K.W. Nam (as *P. papillosa*)	USA, Florida, Old Dan's Reef, 8 Apr. 1988 (Fujii et al. 2006)	S. Fredericq	AF465807
P. perforata	Mexico, Quintano Roo, Isla Mujeres, 02 Mar. 2007 (Cassano et al. 2009)	A. Sentíes and M.C. Gil-Rodríguez	EF658641
P. perforata	Spain, Canary Islands, Tenerife, San Telmo, 14 Jul. 2006 (Cassano et al. 2009)	M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes	EU256329
P. perforata (as *P. papillosa*)	Brazil, Espírito Santo, Marataízes, 19 Jun. 1997 (Fujii et al. 2006)	M.T. Fujii	AF465806
P. thuyoides (Kützing) Cassano, Sentíes, Gil-Rodríguez *et* M.T. Fujii	Philippines (A.O. Lluisma unpublished)	–	AF489863
Osmundea blinksii (Hollenberg *et* Abbott) K.W. Nam	USA, California, San Mateo Co., Año Nuevo, Greyhound Rock, 17 Jul. 1996 (McIvor et al. 2002)	M. Hommersand	AY172575
O. oederi (Gunnerus) G. Furnari [as *O. ramosissima* (Oeder) Athanasiadis]	Ireland, Co. Donegal, St. John's Point, 12 Oct. 1999 (McIvor et al. 2002)	C. Maggs	AF281880
O. osmunda (S.G. Gmelin) Nam	Ireland, County Donegal, St. John's Point (McIvor et al. 2002)	–	AF281877
O. pinnatifida (Hudson) Stackhouse	Ireland, County Donegal, St. John's Point (McIvor et al. 2002)	–	AF281875
O. pinnatifida	France, Brittany, Penmarch (Lin et al. 2001)	–	AF259495
O. pinnatifida	Spain, Canary Islands, Tenerife, San Telmo, Puerto de la Cruz, 7 Oct. 2005 (Cassano et al. 2009)	M.C. Gil-Rodríguez	EF686005
O. sinicola (Setchell *et* Gardner) K.W. Nam	USA, California, Orange Co., Crescent Beach, 28 May 2002 (Fujii et al. 2006)	S. Murray	AY588407
O. spectabilis (Postels *et* Ruprecht) K.W. Nam var. *spectabilis*	Mexico, Baja California, Punta Santo Thomas (McIvor et al. 2002)	–	AY172574
O. splendens (Hollenberg) K.W. Nam	México, Baja California, Bahia Colnett, Drift, 02 Jul. 1996 (McIvor et al. 2002)	M. Hommersand and J. Hughey	AY172576
O. truncata (Kützing) K.W. Nam *et* Maggs	Ireland, Lough Hyne, Co. Cork (McIvor et al. 2002)	–	AF281879



Figures 1–6 *Palisada flagellifera* from the Canary Islands.
(1) Habit of a plant. (2) Detail of basal portion. Note descending basal branch terminating in smaller discoid holdfast (arrow). (3) Cortical cells in surface view of the middle portion of a branch without secondary pit connections. (4) Cross section of the middle portion of the thallus. (5) Cross section of ultimate branchlet showing palisade-like arrangement of cortical cells. (6) Detail of the upper portion of a branch with an axial cell (arrow) with two pericentral cells (p). Scale bars: 1 cm in Figure 1; 1 mm in Figure 2; 10 μm in Figure 3; 100 μm in Figure 4; 30 μm in Figure 5; 25 μm in Figure 6.



Figures 7–11 *Palisada flagellifera* from the Canary Islands.
(7) Cross section near the apex of branchlet showing a vegetative axial segment with an axial cell (a) and two pericentral cells (p1, p2). Note the first pericentral cell (p1) produced underneath the basal cell of the trichoblast (bt). (8) Tetrasporangial branches. (9) Cross section of tetrasporangial axial segments showing an axial cell (a) and three fertile pericentral cells (p2–p4). The second pericentral cell (p2) becomes fertile; the third and fourth additional fertile pericentral cells (p3–p4) are formed in the opposite position and the first pericentral cell remains vegetative (p1). (10) Detail of a fertile pericentral cell (fp) with two presporangial cover cells (pr), tetrahedrically divided tetrasporangium (te) and one postsporangial cover cell (po). (11) Longitudinal section through tetrasporangial branchlet showing right-angle arrangement of the tetrasporangia. Scale bars: 10 μm in Figure 7; 200 μm in Figures 8 and 11; 25 μm in Figures 9 and 10.

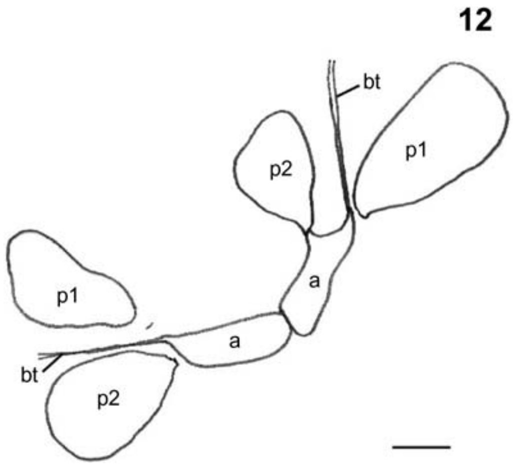


Figure 12 *Palisada flagellifera* from the Canary Islands. (Diagrammatic representation.) Cross section near the apex of branchlet showing two superimposed vegetative axial segments with an axial cell (a) and two pericentral cells (p1, p2). The first pericentral cell (p1) produced underneath the basal cell of the trichoblast (bt). Scale bar: 25 μm.

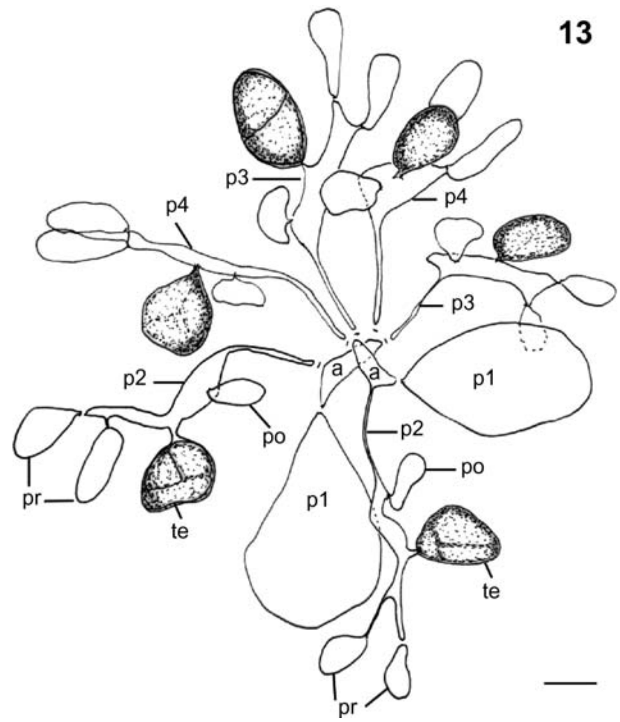


Figure 13 *Palisada flagellifera* from the Canary Islands. (Diagrammatic representation.) Cross section near the apex of a branchlet showing two superimposed tetrasporangial axial segments. Each axial segment (a) with one vegetative pericentral cell (p1) and three fertile pericentral cells, the existing (p2) and the additional (p3 and p4). Presporangial cover cells (pr), tetrasporangial initial (te); postsporangial cover cell (po). Scale bar: 25 μm.

erife: Playa Paraíso, 12 July 2006, leg. M.C. Gil-Rodríguez, M.T. Fujii and A. Sentíes (TFC Phyc. 13127); 21 November 2007, leg. M.C. Gil-Rodríguez (TFC Phyc. 14048); 05 May 2008, leg. M.C. Gil-Rodríguez, E. Aylagas, V. Cassano, M.T. Fujii and J. Díaz-Larrea (TFC Phyc. 14083). Pta. del Hidalgo, 08 February 2007, leg. M.C. Gil-Rodríguez (TFC Phyc. 13144; 13145; Caleta del Hueso-Pta. del Hidalgo, 05 April 2007, leg. M.C. Gil-Rodríguez (TFC Phyc. 13162). Fuerteventura: Faro del El Cotillo, 25 June 2009, leg. M.C. Gil-Rodríguez, E. Aylagas and M. Machín (TFC Phyc. 14407); Veril Blanco, 26 June 2009, leg. M.C. Gil-Rodríguez, E. Aylagas and M. Machín (TFC Phyc. 14428). Isla de Lobos, 25 June 2009, leg. M.C. Gil-Rodríguez, E. Aylagas and M. Machín (TFC Phyc. 14415).

Additional material examined (Brazil) Espírito Santo State: Guarapari, Peracanga beach, 26 October 1996, leg. M.T. Fujii and S.M.P.B. Guimarães (SP 295049), Anchieta, Parati beach, 30 April 1991, leg. M.T. Fujii and S.M.P.B. Guimarães (SP 295054). Rio de Janeiro State: Armação dos Búzios, Rasa beach, 17 July 2004, leg. V. Cassano (SP 399860), 13 January 2005, leg. V. Cassano and J.C. De-Paula (SP 399861), Rio das Ostras, Areias Negras, 03 August 2005, leg V. Cassano and M.B. Barros-Barreto (SP 399864), Cemitério's beach, 02 August 2005, leg. V. Cassano and M.B. Barros-Barreto (SP 399941). São Paulo State: Ubatuba, Itaguá beach, 15 October 1997, leg. M.T. Fujii (SP 295114), São Sebastião, Cigarras beach, 14 November 1986, leg. M.T. Fujii (SP 295045), Itanhaém, Peruíbe beach, 26 March 1986, leg. M.T. Fujii (SP 295044).

Molecular analyses

A total of 46 sequences were analyzed including the outgroups *Bostrychia radicans*, *Ceramium brevizonatum*, *Centroceras* sp. and *Chondria dasyphylla* (Table 1). The topology of the majority rule Bayesian tree is shown in Figure 14. The analyses show a monophyletic *Laurencia sensu lato* group with high support values in relation to members of the outgroups. The *Laurencia sensu lato* assemblage separated into three clades with high support values, corresponding to the genera that form the group: *Laurencia, Palisada* and *Osmundea.* The earliest diverging clade was *Osmundea*, and *Laurencia* and *Palisada* were sister groups forming a clade with high support values. The monophyletic clade that corresponded to the genus *Laurencia* included 11 taxa: *L. complanata*, *L. caraibica, L. viridis, L. obtusa, L. intricata, L. flexuosa, L. natalensis, L. venusta, L. rigida, L. dendroidea* and *L. cf. brongniartii.* The monophyletic clade that corresponded to the genus *Osmundea* included eight species: *O. sinicola, O. spectabilis var spectabilis, O. blinksii, O. splendens, O. osmunda, O. pinnatifida, O. truncata* and *O. oederi* (as *O. ramosissima*). Finally, the *Palisada* assemblage included three independent clades: the earliest diverging clade included *P. corallopsis*. The other two sister clades included 11 taxa: the first clade included *Palisada* sp. (as *P. papillosa*), *P. thuyoides* and *P. patentiramea*. The other sister clade included different *P. perforata* and *P. flagellifera* populations. The *P. flagellifera* assemblage included Brazilian and Canary Islands specimens with high support values. The

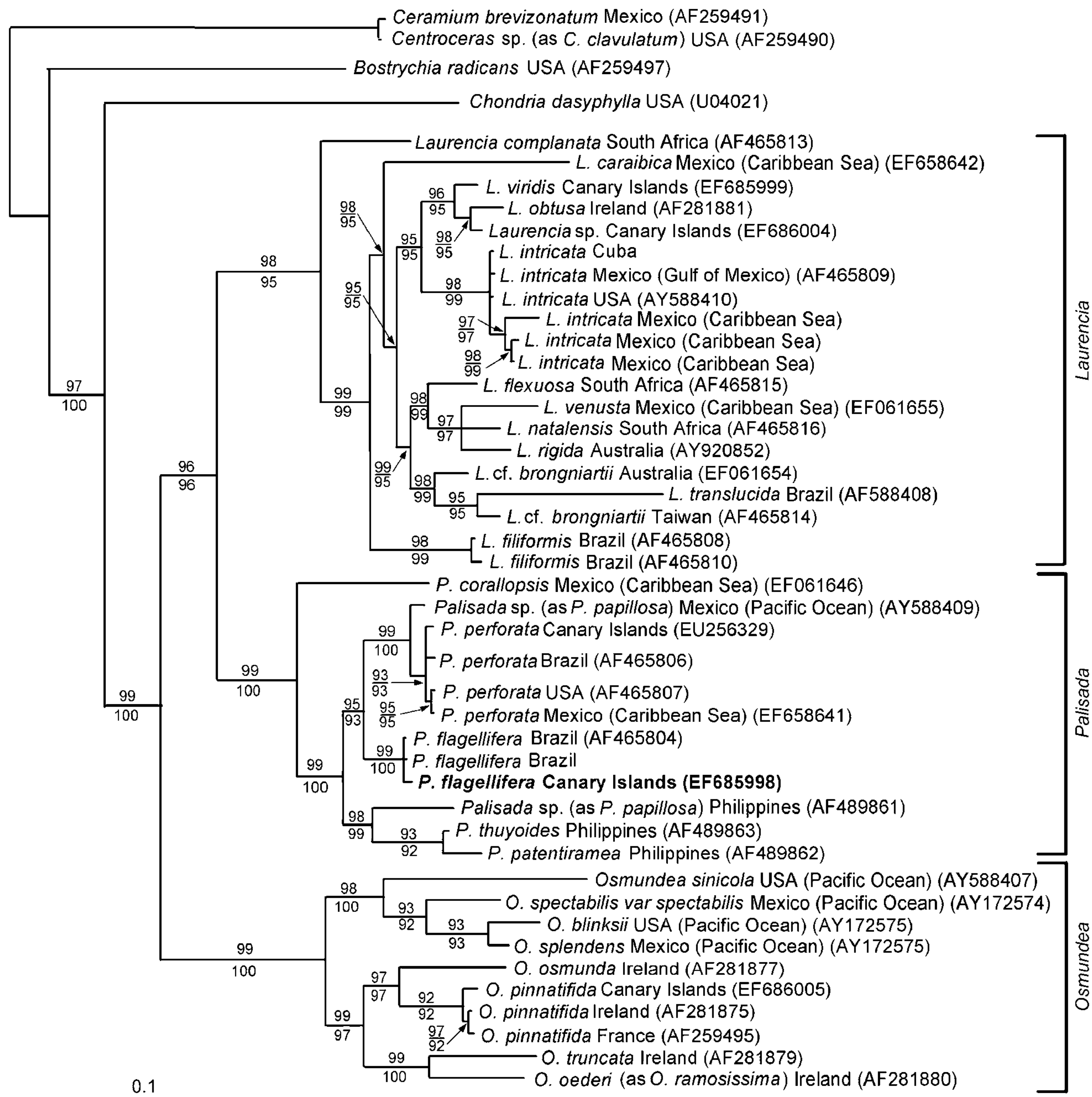


Figure 14 Phylogenetic relationships of the *Laurencia* complex based on Bayesian analysis of *rbc*L DNA sequences.
Evolutionary model used in the Bayesian analysis was GTR+I+G selected by a maximum-likelihood ratio test. Bootstrap (above) and Bayesian *posterior* probability (below) values are indicated at the nodes.

level of genetic variation between their sequences was 0.02–0.04%, confirming that the three populations represent the same taxonomic entity.

Discussion

Palisada flagellifera was originally described from Indonesia (''ad oras Indiae orientalis'' in Hb. Hookeri) as *Laurencia flagellifera* by J. Agardh (1852). The type locality was mistakenly believed to be India by Silva et al. (1996). Morphologically, the Canary Islands and Brazilian specimens are in agreement with the holotype of *P. flagellifera* and share the same habit and cartilaginous and loosely branched thalli, two pericentral cells per vegetative axial segment, radially elongated outer cortical cells arranged in palisades in cross section, lacking pit connections between the cortical cells, and right-angle arrangement of tetrasporangia [Fujii et al. 2006, as *Chondrophycus flagelliferus* (J. Agardh) K.W. Nam]. These specimens are also in agreement with the generic circumscription given by Nam (2006) to *Palisada* by having tetrasporangia produced from particular pericentral cells and the second pericentral cell always fertile resulting in a tetrasporangial axis with only one sterile pericentral cell (the 1st one) and the production of the first pericentral cell underneath the basal cell of the trichoblast. The number of pericentral cells in procarp-bearing segments in *P. flagellifera* was re-examined in the Brazilian specimens (Cassano 2009). This re-examination revealed that the procarp-bearing segment possesses four pericentral cells, instead of five as previously interpreted by Fujii et al. (2006). This feature could not be confirmed in the present study because female plants were never found in the Canary Islands.

The phylogeny results (Figure 14) demonstrated that *Palisada flagellifera* has molecular affinities with *P. perforata,* as previously shown by Fujii et al. (2006), Díaz-Larrea (2008) and Cassano et al. (2009). These species are easily distinguished by their habit and by the ''papiliform'' branches present in *P. perforata* and absent in *P. flagellifera*. The species also have differences in the number of additional pericentral fertile cells produced in the tetrasporangial axis, which are two for *P. flagellifera* and one for *P. perforata* (Nam and Saito 1991, Fujii et al. 2006, Cassano et al. 2009).

Palisada flagellifera represents a new record for the Canary Islands and for the eastern Atlantic Ocean.

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