

Morphological and molecular evidence demonstrates the ampho-Atlantic distribution of *Laurencia catarinensis* (Ceramiales, Rhodophyta)

María Machín-Sánchez^{1*}, Valéria Cassano²,
Jhoana Díaz-Larrea³, Abel Senties³, Mutue T. Fujii⁴
and María Candelaria Gil-Rodríguez¹

¹Departamento de Biología Vegetal (Botánica),
Universidad de La Laguna, 38071 La Laguna, Santa Cruz
de Tenerife, Spain, e-mail: mmachinsanchez@gmail.com

²Departamento de Botânica, Instituto de Biociências,
Universidade de São Paulo, Rua do Matão 277, São Paulo,
SP 05508-900, Brazil

³Departamento de Hidrobiología, Universidad Autónoma
Metropolitana-Iztapalapa, a. p. 55-535, México, D.F.
09340, México

⁴Instituto de Botânica, Av. Miguel Estéfano, 3687, São
Paulo, SP 04301-012, Brazil

* Corresponding author

Abstract

We performed morphological and molecular studies of *Laurencia catarinensis* from the Canary Islands. This species has an entangled habit, cushion-like tuft formation, cortical cell walls, slightly to markedly projecting near the apex, and lacking lenticular thickenings in medullary cells. We inferred its phylogenetic position by analyzing the chloroplast-encoded *rbcL* gene sequences from 41 samples. The results demonstrate that specimens of *L. catarinensis* from the Canary Islands, where it is referred to as *L. intricata*, and those from Brazil (including specimens from the type locality in Santa Catarina) form a monophyletic clade with low genetic divergence (0–0.9%). In contrast, specimens of *L. intricata* from the type locality in Cuba, Mexico, and the USA were clearly distinct from *L. catarinensis* collected in Brazil and the Canary Islands, as shown by high genetic divergence values (4.9–5.7%). The type material of *L. catarinensis* from Brazil allowed us to identify all samples from the Canarian Archipelago as *L. catarinensis*. These findings expand the known geographical distribution of *L. catarinensis* to the eastern Atlantic Ocean and demonstrate an ampho-Atlantic distribution of the species.

Keywords: Canary Islands; *Laurencia catarinensis*;
morphology; phylogeny; *rbcL*.

Introduction

The *Laurencia* complex (Ceramiales, Rhodophyta) is currently divided into five genera: *Laurencia* J.V. Lamouroux,

Osmundea Stackhouse, *Chondrophyucus* (Tokida *et* Saito) Garbary *et* Harper, *Palisada* (Yamada) K.W. Nam, and *Yuzurua* (K.W. Nam) Martin-Lescanne (Nam *et al.* 1994, Garbary and Harper 1998, Nam 1999, 2006, 2007, Martin-Lescanne *et al.* 2010). Several morphoanatomical and reproductive characters used in the taxonomy of the complex are of diagnostic value only at the generic level (Saito 1967, Nam *et al.* 1994, Garbary and Harper 1998, Nam 1999, 2006). Moreover, many species have wide morphological plasticity, which complicates taxonomy. Therefore, the genus *Laurencia* is in fact more diverse than previously thought and is now conventionally referred to as the *Laurencia* complex. In this context, molecular markers have proven useful in delimiting taxa in the *Laurencia* complex and inferring their phylogenetic relationships. Thus, molecular techniques have corroborated the current classification system (Nam *et al.* 2000, McIvor *et al.* 2002, Abe *et al.* 2006, Fujii *et al.* 2006, Díaz-Larrea *et al.* 2007, Cassano *et al.* 2009, Gil-Rodríguez *et al.* 2009, Martin-Lescanne *et al.* 2010, Machín-Sánchez *et al.* 2012).

The taxonomy of the *Laurencia* complex in the Canary Islands has been intensively studied since the 19th century (Gil-Rodríguez *et al.* 2009). Seven species of the genus are recorded in recent floristic reports from the Canary Islands: *Laurencia caduciramulosa* Masuda *et* Kawaguchi (Cassano *et al.* 2008); *L. dendroidea* J. Agardh (Cassano *et al.* 2012) [as *L. majuscula* (Harvey) A.H.S. Lucas (Masuda *et al.* 1998, Gil-Rodríguez *et al.* 2003, Haroun *et al.* 2009)]; *L. intricata* J.V. Lamouroux (Gil-Rodríguez *et al.* 2003); *L. marilzae* Gil-Rodríguez, Senties, Díaz-Larrea, Cassano *et* M.T. Fujii (Gil-Rodríguez *et al.* 2009); *L. microcladia* Kützing (Gil-Rodríguez and Haroun 1993, Gil-Rodríguez *et al.* 2003, Aylagas *et al.* 2011); *L. obtusa* (Hudson) J.V. Lamouroux (Gil-Rodríguez *et al.* 2003); and *L. viridis* Gil-Rodríguez *et* Haroun (Gil-Rodríguez and Haroun 1992). *L. catarinensis* Cordeiro-Marino *et* M.T. Fujii has been found only in the western Atlantic Ocean (Brazil) (Cordeiro-Marino and Fujii 1985). Nonetheless, we report here for the first time that *L. catarinensis* occurs in the Canary Islands, thus increasing the distribution of this species to include the eastern Atlantic Ocean.

We describe and illustrate morphological characters of *L. catarinensis* in comparison with related species, and infer its phylogenetic relations by analyzing *rbcL* sequences.

Materials and methods

Samples of *Laurencia catarinensis* were collected in the Canary Islands (Spain) during the period 2007–2011

as follows: La Palma (two sites: Las Salinas-Cancajos, UTM 28R 0230568/3171968; Bco. La Herradura, UTM 28R 0229635/3191032), El Hierro (two sites: La Caleta, UTM 28R 0215534/3078457; Arenas Blancas, UTM 28R 0783684/3074683), La Gomera (two sites: Punta de La Dama, UTM 28R 0273379/3100532; Charco del Conde, UTM 28R 0270286/3108917), Tenerife (one site: El Pris, UTM 28R 0360860/3154548), and Fuerteventura (three sites: El Cotillo, UTM 28R 0595906/3175286; Isla de Lobos, UTM 28R 2845777/1349091; Veril Blanco, UTM 28R 0607540/3181123) (Figure 1).

Morphological observations

We made anatomical studies on fresh specimens of *L. catarinensis* and on plants fixed in 4% formalin seawater. In addition, live specimens were examined for the presence of *corps en cerise*, refractile inclusions that are mainly found in cortical cells of the thallus and constitute the sites of production and/or accumulation of halogenated metabolites (Feldmann and Feldmann 1950; Salgado et al. 2008). A minimum of 20 live and fixed specimens was studied. Transverse and longitudinal hand sections were made under a Leica MZ 12.5 stereoscopic dissection microscope (Leica, Wetzlar, Germany) using a stainless steel razor blade, and then stained with 0.5% aqueous aniline blue solution, acidified with 1 N HCl (Tsuda and Abbott 1985). Measurements are given as length×diameter. Photomicrographs were taken with a Leica DFC290 digital camera coupled to a Leica DM 2000

microscope. Voucher specimens were deposited in the herbarium of the University of La Laguna (TFC). The holotype of *L. catarinensis*, housed in the Herbarium of the Institute of Botany of São Paulo (SP164254), was examined for comparison with material from the Canary Islands. In addition, we reviewed specimens from the Canary Islands deposited in TFC, the herbarium of the Faculty of Biology of Marine Sciences, University of Las Palmas de Gran Canaria (BCM), and the herbarium of the Netherlands Center for Biodiversity Naturalis, section Nationaal Herbarium Nederland, Leiden Rijksherbarium (L). Herbarium abbreviations follow the online Index Herbariorum: <http://www.nybg.org/bsci/ih/ih.html> (Thiers 2011, continuously updated). Specimens we examined morphologically are listed in Table 1.

DNA analysis

Samples for molecular analyses were dried in silica gel. The specimens studied and their GenBank accession numbers are given in Table 1. Total DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. A total of 1467 base pairs of the *rbcL* gene were amplified in three fragments with the primer pairs FrbcLstart-R753, F577-R1150, and F753-RrbcLS (Freshwater and Rueness 1994) using the Taq polymerase chain reaction (PCR) Core Kit (QIAGEN). All PCR products were analyzed by electrophoresis in 1% agarose to confirm product size. The PCR products were purified with the QIAquick Purification Kit (QIAGEN) according

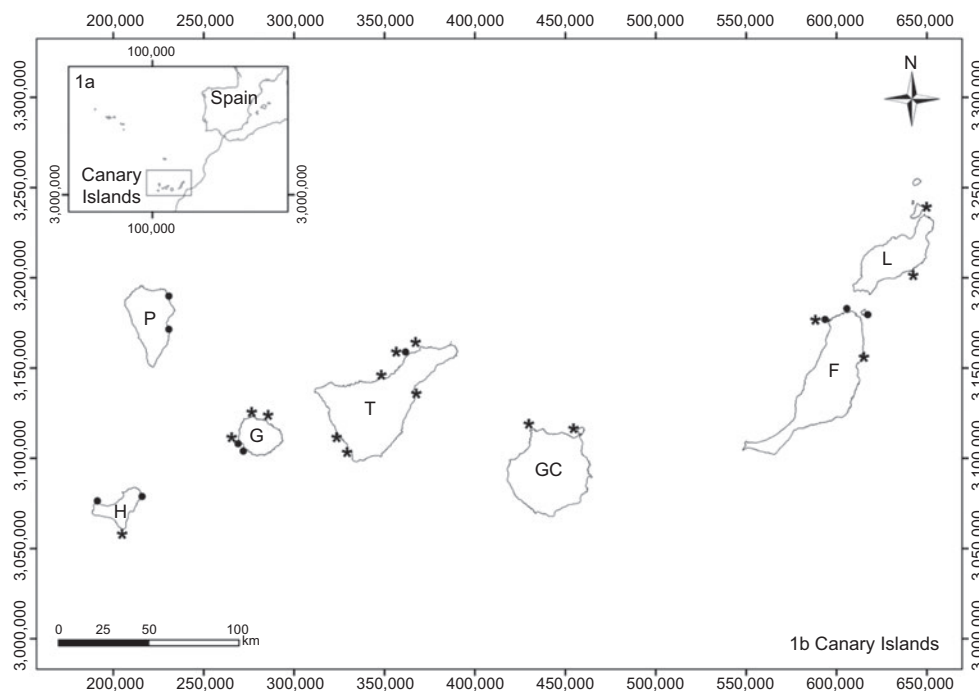


Figure 1 Location of the Canary Archipelago (Spain) in the Atlantic Ocean. Spatial reference WGS_1984_UTM_Zone_28N; units expressed in meters.

(a) Map of the Canary Islands. (1) Map indicating sampling sites of *Laurencia catarinensis* (•) and localities where *L. catarinensis* had been found previously, with vouchers deposited in the herbaria TFC, BCM, and L (*). Islands: La Palma (P), El Hierro (H), La Gomera (G), Tenerife (T), Gran Canaria (GC), Fuerteventura (F), and Lanzarote (L).

Table 1 Specimens subjected to morphological and molecular analysis.

Taxa	Collection data/References	GenBank accession number
<i>Centroceras</i> sp. [as <i>Centroceras clavulatum</i> (C. Agardh) Montagne]	USA, Texas, Port Aransas, Redfish Bay, 18 May 1998 (Lin et al. 2001)	AF259490
<i>Chondrophycus</i> cf. <i>undulatus</i>	New Caledonia, Loyalty, Maré, 22 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785307
<i>Chondrophycus</i> cf. <i>undulatus</i>	New Caledonia, Loyalty, Maré, 22 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785308
<i>Chondrophycus</i> sp.1	New Caledonia, Loyalty, Lifou, 26 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785309
<i>Chondrophycus</i> sp.2	New Caledonia, Loyalty, Maré, 21 Mar. 2005 (Martin-Lescanne et al. 2010)	FJ785310
<i>Chondrophycus</i> sp.3	New Caledonia, Loyalty, Beautemps/Beaupré, 6 Apr. 2005 (Martin-Lescanne et al. 2010)	FJ785311
<i>Laurencia caduciramulosa</i> Masuda et Kawaguchi	Brazil, Rio de Janeiro, Angra dos Reis, Ilha Grande, Lagoa Azul, 27 Jul. 2005 (V. Cassano, unpublished results)	–
<i>Laurencia caraibica</i> P.C. Silva	Mexico, Quintana Roo, Cancún, Isla Mujeres, 23 Feb. 2006 (Gil-Rodríguez et al. 2009)	EF658642
<i>Laurencia catarinensis</i> Cordeiro-Marino et M.T. Fujii	Brazil, Santa Catarina, Florianópolis, Praia da Barra da Lagoa, 16 Jul. 2008 (V. Cassano, unpublished results)	–
<i>Laurencia catarinensis</i>	Brazil, Espírito Santo, Anchieta, Ponta dos Castelhanos, 05 Oct. 2006 (V. Cassano, unpublished results)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Palma, Las Salinas-Cancajos, 25 Sep. 2008 (TFC Phyc. 14651)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Palma, Las Salinas-Cancajos, 25 Sep. 2008 (TFC Phyc. 14652)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Palma, Bco. De La Herradura, 27 Sep. 2008 (TFC Phyc. 14653)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, El Hierro, La Caleta, 16 Sep. 2009 (TFC Phyc. 14493)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, El Hierro, La Caleta, 16 Sep. 2009 (TFC Phyc. 14498)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, El Hierro, Arenas Blancas, 17 Sep. 2009 (TFC Phyc. 14483)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Gomera, Punta de La Dama, 21 Sep. 2009 (TFC Phyc. 14486)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Gomera, Punta de La Dama, 21 Sep. 2009 (TFC Phyc. 14487)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, La Gomera, Charco del Conde, 23 Sep. 2009 (TFC Phyc. 14503)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, Tenerife, El Prís, 03 Feb. 2011 (TFC Phyc. 14612)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, Fuerteventura, El Cotillo, 24 Jun. 2011 (TFC Phyc. 14403)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, Fuerteventura, Isla de Lobos, 25 Jun. 2011 (TFC Phyc. 14411)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, Fuerteventura, Isla de Lobos, 25 Jun. 2011 (TFC Phyc. 14416)	–
<i>Laurencia catarinensis</i> ^a	Spain, Canary Islands, Fuerteventura, Veril Blanco, 26 Jun. 2011 (TFC Phyc. 14425)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i> J.V. Lamouroux)	Brazil, Rio de Janeiro, Angra dos Reis, Ilha do Bonfim, 13 Dec. 2006 (V. Cassano, unpublished results)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Brazil, Rio de Janeiro, Angra dos Reis, Ponta da Fortaleza, 14 Dec. 2006 (V. Cassano, unpublished results)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Brazil, Rio Grande do Norte, Maracajau, 24 Jun. 2006 (V. Cassano, unpublished results)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Spain, Canary Islands, La Gomera, Charco de Las Condesas, 23 Feb. 2011 (This study)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Spain, Canary Islands, La Gomera, Charco de Las Condesas, 23 Feb. 2011 (This study)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Spain, Canary Islands, Tenerife, El Prís, 03 Feb. 2011 (This study)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>)	Spain, Canary Islands, Tenerife, El Prís, 04 May 2008 (This study)	–

(Table 1 continued)

Taxa	Collection data/References	GenBank accession number
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, La Gomera, North coast, 16 May 2008 (TFC Phyc. 14197)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, La Gomera, Northeast coast, 16 May 2008 (TFC Phyc. 14214)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, La Gomera, Valle Gran Rey, 15 May 2008 (TFC Phyc. 14206)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, El Prís, 04 May 2008 (TFC Phyc. 14103)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, El Prís, 04 May 2008 (TFC Phyc. 14104)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Los Cristianos, 09 Sep. 1999 (TFC Phyc. 14369)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Playa Paraiso, 22 Nov. 2007 (TFC Phyc. 14050)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Playa Paraiso, 22 Nov. 2007 (TFC Phyc. 14056)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Playa Paraiso, 05 May 2008 (TFC Phyc. 14073)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Playa Paraiso, 05 May 2008 (TFC Phyc. 14076)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 05 Apr. 2007 (TFC Phyc. 13164)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Tenerife, Puerto de La Cruz, 03 May 2008 (TFC Phyc. 14114)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia intricata</i>) ^a	Spain, Canary Islands, Lanzarote, Isote del Francés Arrecife, 09 Jun. 2008 (TFC Phyc. 14187)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia cf. intricata</i>) ^a	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 08 Feb. 2007 (TFC Phyc. 13146)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia flexilis</i> Setchell) ^a	Spain, Canary Islands, Fuerteventura, Las Lajas, 01 Dec. 1997 (BCM 5560)	–
<i>Laurencia catarinensis</i> [as <i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux] ^a	Spain, Canary Islands, El Hierro, Punta de La Restinga, 10 Sep. 1977 (L 569)	–
<i>Laurencia catarinensis</i> [as <i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux] ^a	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 23 Mar. 1991 (TFC Phyc. 7009)	–
<i>Laurencia catarinensis</i> [as <i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux] ^a	Spain, Canary Islands, Gran Canaria, Las Canteras, 30 Aug. 1984 (TFC Phyc. 1986)	–
<i>Laurencia catarinensis</i> [as <i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux] ^a	Spain, Canary Islands, Gran Canaria, Las Canteras, 14 Sep. 1986 (TFC Phyc. 5609)	–
<i>Laurencia catarinensis</i> [as <i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux] ^a	Spain, Canary Islands, Lanzarote, Arrecife, 21 May 1980 (L 3135)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Tenerife, El Prís, 26 Nov. 1996 (TFC Phyc. 14065)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Tenerife, El Socorro, 01 Sep. 1980 (TFC Phyc. 2151)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Tenerife, Playa Paraiso, 22 Nov. 2007 (TFC Phyc. 15056)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 30 Oct. 1978 (L 1409)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Tenerife, El Puerto de La Cruz, 02 Nov. 2006 (TFC Phyc. 13117)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Fuerteventura, El Cotillo, 2006 (TFC Phyc. 14161)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia sp.</i>) ^a	Spain, Canary Islands, Lanzarote, La Graciosa, 29 Sep. 1982 (TFC Phyc. 647)	–
<i>Laurencia catarinensis</i> (as <i>Laurencia viridis</i> Gil-Rodríguez et Haroun) ^a	Spain, Canary Islands, Gran Canaria, Sardina Del Norte, 08 Oct. 1991 (BCM 487)	–
<i>Laurencia dendroidea</i> J. Agardh [as <i>L. majuscula</i> (Harvey) A.H.S. Lucas]	Spain, Canary Islands, Tenerife, Puerto de la Cruz, 13 Jul. 2006 (Gil-Rodríguez et al. 2009)	EF686000
<i>Laurencia intricata</i> J.V. Lamouroux	USA, Florida, Long Key, Channel 5, 10 Dec. 1998 (Fujii et al. 2006)	AY588410
<i>Laurencia intricata</i>	Mexico, Yucatan, Campeche Bay, 14 Feb. 1999 (Fujii et al. 2006)	AF465809
<i>Laurencia intricata</i>	Cuba, Ciego de Ávila, Cayo Coco, 25 Sep. 2005 (Cassano et al. 2012)	GU330238
<i>Laurencia intricata</i>	Mexico, Ojo Agua, Puerto Morelos, Quintana Roo, 2004 (Cassano et al. 2009)	EF658644
<i>Laurencia marilzae</i> Gil-Rodríguez, Sentés, Díaz-Larrea, Cassano et M.T. Fujii	Spain, Canary Islands, Tenerife, Playa Paraiso, 14 Jul. 2006 (Gil-Rodríguez et al. 2009)	EF686001
<i>Laurencia marilzae</i>	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 Mar. 2007 (Rocha-Jorge et al. 2010)	GU938189

(Table 1 continued)

Taxa	Collection data/References	GenBank accession number
<i>Laurencia maritzae</i>	Mexico, Isla Mujeres, Quintana Roo, 2008 (Sentíes et al. 2011)	HQ115065
<i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux	Ireland, County Donegal, Fanad Head, 06 Jul. 1998 (Nam et al. 2000)	AF281881
<i>Laurencia venusta</i> Yamada	México, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004 (Díaz-Larrea et al. 2007)	EF061655
<i>Laurencia viridis</i> Gil-Rodríguez et Haroun	Spain, Canary Island, Tenerife, Punta del Hidalgo, Roca Negra, 06 Oct. 2005 (Gil-Rodríguez et al. 2009)	EF685999
<i>Osmundea hybrida</i> (A.P. de Candolle) K.W. Nam	Ireland, County Donegal, Fanad Head, 07 Nov. 1999 (Nam et al. 2000)	AF281878
<i>Osmundea oederi</i> (Gunnerus) G. Furnari	Ireland, County Donegal, St John's Point, 12 Oct. 1999 (Nam et al. 2000)	AF 281880
<i>Osmundea osmunda</i> (S.G. Gmelin) K.W. Nam et Maggs	Ireland, County Donegal, St. John's Point (McIvor et al. 2002)	AF281877
<i>Osmundea pinnatifida</i> (Hudson) Stackhouse	Spain, Canary Island, Tenerife, San Telmo, Puerto de la Cruz, 07 Oct. 2005 (Cassano et al. 2009)	EF686005
<i>Osmundea spectabilis</i> (Postels et Ruprecht) K.W. Nam var. <i>spectabilis</i>	Mexico, Baja California, Punta Santo Thomas (McIvor et al. 2002)	AY172574
<i>Osmundea truncata</i> (Kützinger) K.W. Nam et Maggs	Spain, Canary Island, Tenerife, Barranquera, 05 May 2008 (Machín-Sánchez et al. 2012)	JF781523
<i>Palisada corallopsis</i> (Montagne) Sentíes, M.T. Fujii et Díaz-Larrea	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004 (Díaz-Larrea et al. 2007)	EF061646
<i>Palisada flagellifera</i> (J. Agardh) K.W. Nam	Spain, Canary Island, Tenerife, Playa Paraiso, 16 Jul. 2006 (Gil-Rodríguez et al. 2010)	EF685998
<i>Palisada flagellifera</i>	Spain, Canary Island, Tenerife, Playa Paraiso, 14 Jul. 2006 (Gil-Rodríguez et al. 2009)	EF685998
<i>Palisada perforata</i> (Bory) K.W. Nam	Spain, Canary Island, Tenerife, San Telmo, 14 Jul. 2006 (Cassano et al. 2009)	EU256329
<i>Yuzurnia poiteaui</i> (J.V. Lamouroux) Martin-Lescanne var. <i>gemmifera</i> (Harvey) M.J. Wynne	Mexico, Quintana Roo, Playa del Carmen, 17 Apr. 2004 (Díaz-Larrea et al. 2007)	EF061649
<i>Yuzurnia poiteaui</i> (J.V. Lamouroux) Martin-Lescanne	USA, Florida, Content Key, 12 Mar. 1997 (McIvor et al. 2002)	AY172577

^aTaxa not included in the phylogenetic analysis.

Taxa marked in bold were examined morphologically.

to the manufacturer's recommendations. Cycle sequencing was carried out on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA), using the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems). Primers were used for PCR amplification as well as cycle sequencing. Sequences were analyzed with Sequence Navigator software, version 1.0.1 (Applied Biosystems).

Phylogenetic analyses

Phylogenetic relationships were inferred with PAUP* version 4.0b10 (Swofford 2002) and MrBayes v.3.0 beta 4 (Huelsenbeck and Ronquist 2001). Maximum parsimony (MP) trees were constructed by applying the heuristic search option, tree-bisection-reconnection (TBR) branch swapping, with unordered and unweighted characters. Branch length was optimized by using delayed transformation (DELTRAN), which favors parallelisms over reversals. Modeltest software, version 3.7 (Posada and Crandall 1998), was used to find the model of sequence evolution least rejected in each data set by a hierarchical likelihood ratio test. Once the evolution model had been determined, maximum likelihood (ML) searches were performed by applying the estimated parameters (substitution model, gamma distribution, proportion of invariant sites, and frequencies of the bases). Maximum likelihood analysis was then employed to construct the most likely tree from the data set.

ML and MP branch supports were calculated by nonparametric bootstrapping analysis (Felsenstein 1985), as implemented in PAUP*.

Ten thousand heuristic search replicates were executed with the TBR branch-swapping algorithm. The general time-reversible model of nucleotide substitution with invariant sites and gamma-distributed rates for the variable sites (GTR+I+G) were used for the Bayesian analysis. This model was selected based on an ML ratio test implemented by the software Modeltest, version 3.06 (Posada and Crandall 1998), with a significance level of 0.01. For Bayesian analysis, we ran four chains of the Markov chain Monte Carlo, sampling one tree every 1000 generations for 4,000,000 generations, starting with a random tree. A 50% consensus tree-majority rule (as implemented by PAUP*) was computed after the burn-in point. The range of *rbcL* divergence values within and between species was calculated using uncorrected "p" distances obtained from PAUP*.

Results

Morphological analyses

Laurencia catarinensis Cordeiro-Marino *et* M.T. Fujii, *Revista Brasileira de Botânica* 8: 47–53, Figures 1–18. 1985.

Holotype Institute of Botany of São Paulo (SP164254)!

Type locality Praia Mole, Santa Catarina, Brazil.

Distribution Brazil and Canary Islands (Spain).

Morphology Plants form intricate, cushion-like tufts, which are up to 5 cm high (Figure 2A) and attach to the substratum by a small holdfast (Figure 2B). Numerous branches

develop from the prostrate axes. Thalli are pink-purple to greenish, usually pink tipped, terete, delicate, soft in texture, and adhere to the herbarium paper when dried. The main axes are usually prostrate with indeterminate growth; from these, erect branches arise, 600–840 µm in diameter. Branching is sparse, alternate-spiral to irregular, and includes up to three orders of branches (Figure 2C); anastomoses occur between the branches.

Vegetative structures In surface view, cortical cells contain 1 or 2 (-3) *corps en cerise* (Figure 2D); trichoblast cells in living specimens contain one. Cortical cells are regularly arranged along the thalli in a longitudinal row and connected to each other by longitudinally oriented secondary pit connections (Figure 2E). Cortical cells of the upper portion of the thallus are polygonal (Figure 2D,E), longitudinally elongate in the middle, 70–136×32–56.5 µm in size, and polygonal to elongate in the lower portion, measuring 45–89×30–51.5 µm. In longitudinal sections, cortical cells project slightly to markedly outward (Figure 2F). Each vegetative axial segment cuts off four pericentral cells. There are no lenticular thickenings in medullary cells.

Reproductive structures Tetrasporangial plants with cylindrical and simple or compound branchlets, 910–3400×440–650 µm in size. Mature tetrasporangia tetrahedrally divided, 80–110 µm, and arranged in a parallel pattern in relation to the fertile branchlets (Figure 3A). All axial segments at the apices of fertile branches produce two fertile pericentral cells (the third and the fourth); the first and the second pericentral cell remain vegetative (Figure 3B). Each fertile pericentral cell cuts off two presporangial cover cells with abaxial alignment, distally to the initial tetrasporangium. Subsequently, one postsporangial cover cell is formed, which continues dividing and contributes to the cortication around the tetrasporangium (Figure 3C,D).

The species is frequently found in lower intertidal to subtidal zones at depths of 1–2 m at sites of moderate wave action, usually associated with articulated calcareous algae.

Previously misapplied names for *L. catarinensis* in the Canary Islands, Spain *L. intricata* [Gil-Rodríguez and Haroun 1993 (pp. 114–115), Masuda *et al.* 1998 (p. 274), Haroun *et al.* 2002 (p. 152), 2003 (p. 156), 2008 (p. 170), 2009 (p. 164), Gil-Rodríguez *et al.* 2003 (p. 30)].

Molecular analyses

Variation in the *rbcL* sequences We obtained the *rbcL* gene sequences from four specimens of *L. catarinensis* (as *L. intricata*) from the Canary Islands. A total of 41 sequences were analyzed; *Centroceras* sp. [as *C. clavatum* (C. Agardh in Kunth) Montagne in Durieu de Maisonneuve] was included as the out group (Table 1).

Genetic divergences in the *rbcL* gene sequence are given in Table 2. The clade that included samples of *L. catarinensis* (as *L. intricata*) had lowest levels of genetic variation. Intraspecific divergences obtained for *L. catarinensis* varied

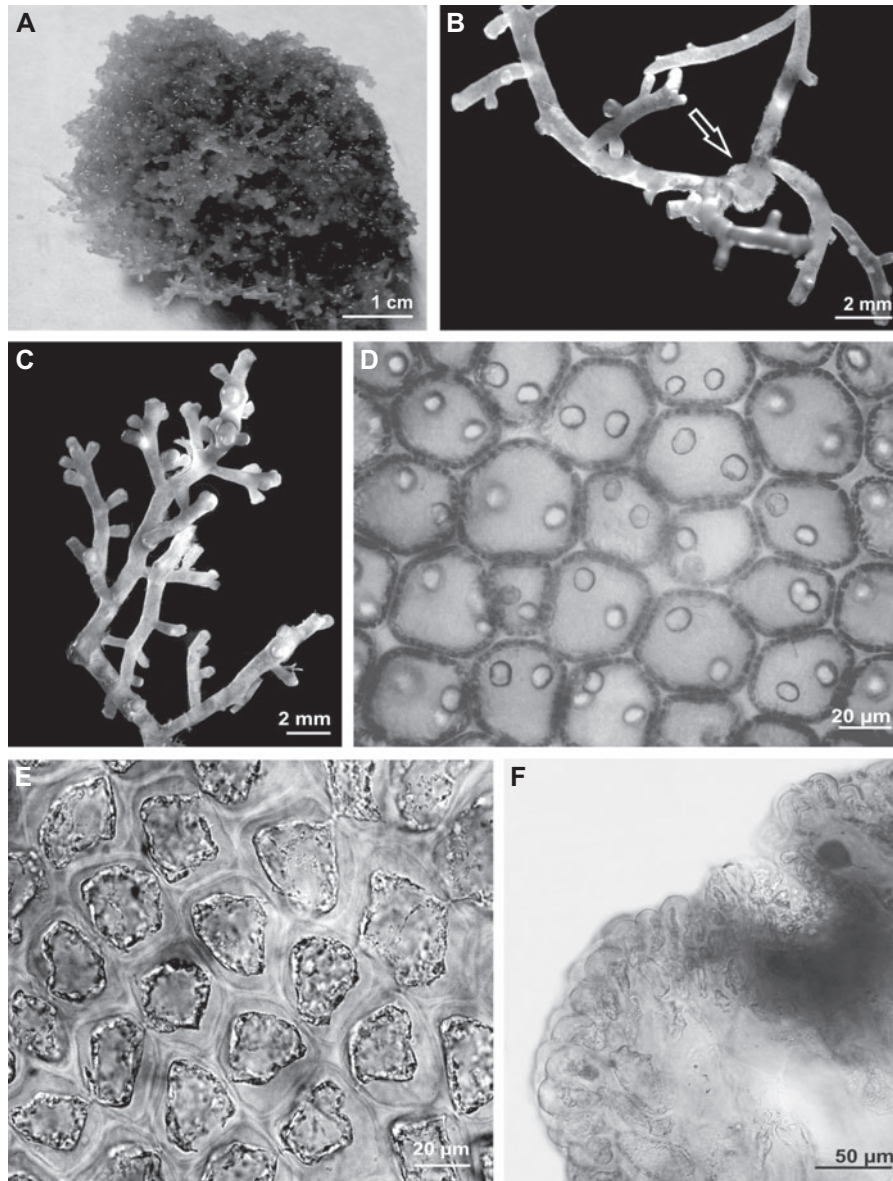


Figure 2 *Laurencia catarinensis* from the Canary Islands: habit and vegetative structures.

(A) Habit of a plant. (B) Detail of discoid holdfast (arrow). (C) Detail of the branches. (D) Cortical cells in surface view showing 1, 2 (-3) *corps en cerise* per cell in live material. (E) Cortical cells in surface view showing secondary pit connections. (F) Longitudinal section through a branchlet showing slightly projecting cortical cells.

from 0% to 0.9% among samples from the Canary Islands and Brazil, and from 0.1% to 0.5% among Canarian samples. Interspecific divergences between *L. catarinensis* and *L. intricata* samples varied from 4.9% to 5.7%.

Phylogeny The data set consisted of 800 constant characters and 80 parsimony noninformative sites. MP produced three trees of 1000 steps (CI=0.85, HI=0.15), and the resulting topology was similar to the Bayesian and ML topologies. Accordingly, we show only the topology of the majority rule Bayesian tree (Figure 4). The analysis revealed that the clade corresponding to the genus *Laurencia sensu stricto* included nine taxa: *L. viridis*, *L. obtusa*, *L. intricata*, *L. dendroidea*,

L. caraibica P.C. Silva, *L. catarinensis*, *L. venusta* Yamada, *L. caduciramulosa*, and *L. marilzae*. Within the assemblage, *L. catarinensis* formed a well-supported monophyletic clade that included the samples from Santa Catarina, Brazil (the type locality) and was clearly separated from the *L. intricata* and the *L. caraibica* sample.

The model used in the Bayesian analysis was the GTR+I+G, and estimated the following parameters: gamma distributions=1.1012; proportion of invariant sites=0.4800; frequency of the bases: A=0.3300, C=0.1539, G=0.2010, and T=0.3200; rate matrix: (A–C)=4.7308, (A–G)=6.1207, (A–T)=4.9034, (C–G)=1.095, (C–T)=33.1801. ML analysis resulted in a topology of an -lnL score of 3280.1850.

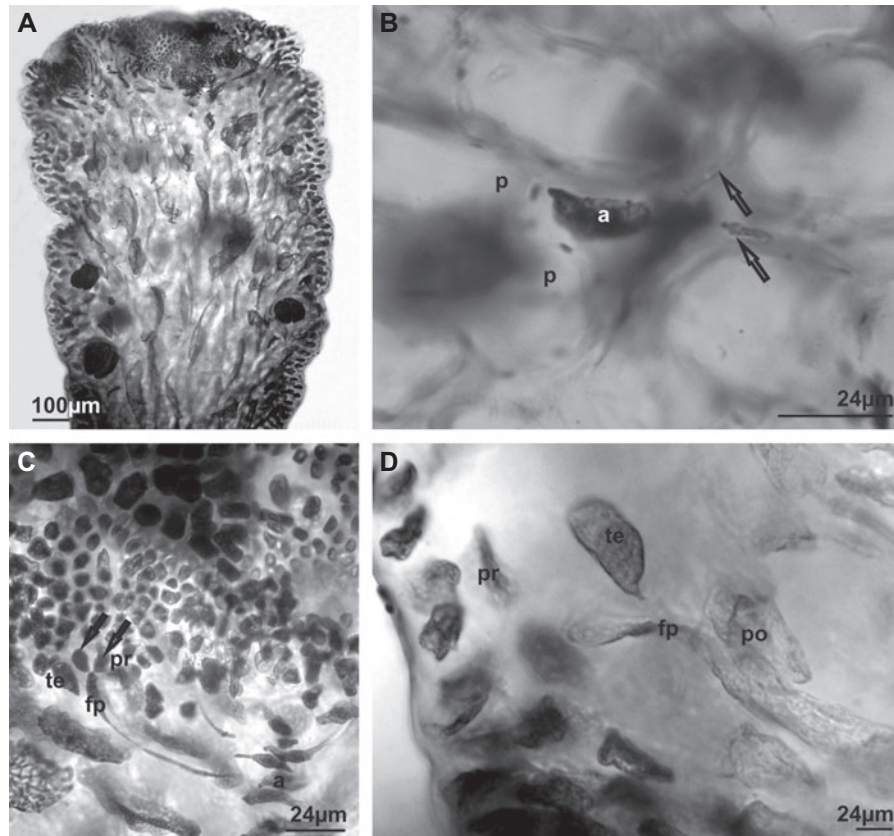


Figure 3 *Laurencia catarinensis* from the Canary Islands: reproductive structures.

(A) Longitudinal section through a tetrasporangial branchlet showing parallel arrangement of tetrasporangia. (B) Transverse section of a tetrasporangial axial segment showing an axial cell (a) with two fertile pericentral cells (arrows) and two sterile pericentral cells (p). (C) Longitudinal section through the apical portion of a tetrasporangial branchlet showing tetrasporangium (te) originating from a fertile pericentral cell (fp), an axial cell (a), and two presporangial cover cells (pr) (arrows). (D) Longitudinal section through a tetrasporangial branchlet showing detail of a fertile pericentral cell (fp) with two presporangial cover cells (pr), a tetrasporangium (te), and a postsporangial cell (po).

Discussion and conclusions

Laurencia catarinensis was originally described by Cordeiro-Marino and Fujii (1985) from Santa Catarina, Brazil. This species is characterized by a markedly entangled thallus formation that produces cushion-like tufts, which are typically associated with articulated Corallinaceae; tufts are often greenish with

Table 2 Divergences in the *rbcL* sequence within the *Laurencia* complex.

Intergeneric divergences (%)		Interspecific divergences (%)	
<i>Chondrophycus-Laurencia</i>	9.0–11.3	<i>Chondrophycus</i>	3.2–6.9
<i>Chondrophycus-Osmundea</i>	9.6–11.8	<i>Laurencia</i>	3.3–10.5
<i>Chondrophycus-Palisada</i>	9.5–10.7	<i>Osmundea</i>	4.9–8.6
<i>Chondrophycus-Yuzurua</i>	10.0–11.0	<i>Palisada</i>	6.2–7.6
<i>Laurencia-Osmundea</i>	9.7–13.7	<i>Yuzurua</i>	0.3
<i>Laurencia-Palisada</i>	6.8–11.3		
<i>Laurencia-Yuzurua</i>	9.1–11.1		
<i>Osmundea-Palisada</i>	10.1–12.7		
<i>Osmundea-Yuzurua</i>	11.3–12.4		
<i>Palisada-Yuzurua</i>	10.0–10.3		

pink apices. Cortical cells project slightly to markedly near the apices of the branches, and lack lenticular thickenings in medullary cell walls. *L. intricata* was proposed by Lamouroux (1813) without description of the specific site of the type locality in the Antilles. Although the species was determined to be invalid by Silva et al. (1987), it was reinstated by Silva et al. (1996), taking the illustration provided by Lamouroux (1813) as sufficient to validate a publication based on the International Code of Botanical Nomenclature, Art. 44.2 (Greuter et al. 1994). However, these illustrations do not show enough details for an informed interpretation of this species. Once established, *L. intricata* was widely reported for tropical as well as temperate regions throughout the world (Masuda et al. 1998). The morphological similarity between *L. catarinensis* and *L. intricata* was recognized by Fujii and Senties (2005), who segregated the species by differences in the color of the thalli and the degree of cortical cell projection.

Our review of *L. catarinensis* from the Canary Islands shows that it shares habit and the majority of the vegetative and reproductive anatomical characteristics with *L. intricata*. Cassano (2009) examined in detail various specimens of *L. intricata* from Cuba, Mexico, Florida, and the Lesser Antilles that are deposited in the herbarium of the University of

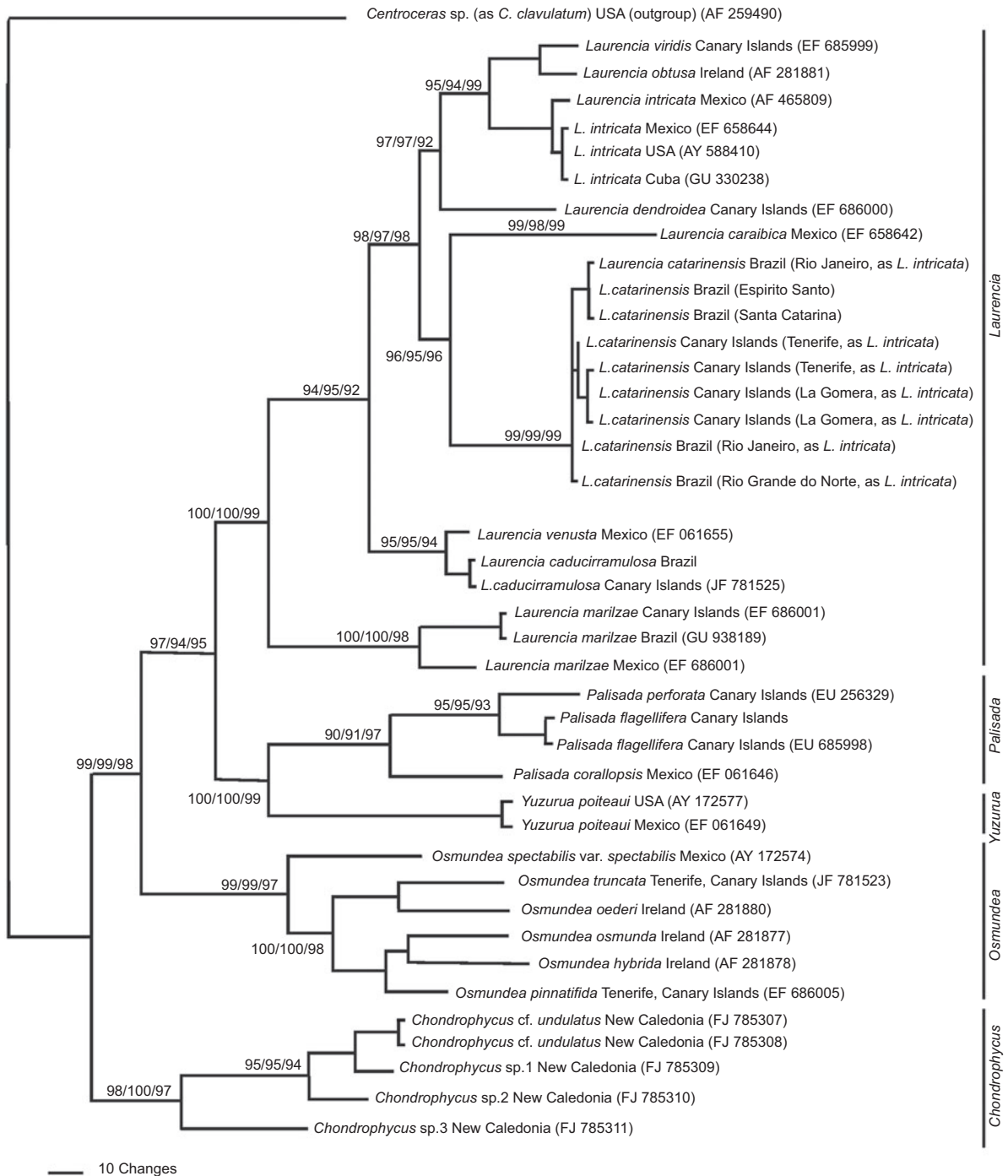


Figure 4 Phylogenetic relationships in the *Laurencia* complex based on Bayesian analysis of *rbcL* DNA sequences. MP bootstrap, ML bootstrap, and Bayesian posterior probability values are indicated at the nodes.

Michigan (MICH). The specimens analyzed were similar, showing an entangled habit and sparse branching arising at wide to right angles, cortical cell walls markedly projecting beyond the surface, and thickened medullary cells with abundant lenticular thickenings, except for the material from Mexico, which had thinner medullary cell walls and fewer lenticular thickenings. Though easily observable in the material examined by Cassano (2009), lenticular thickenings are absent from descriptions of

this species prepared by other authors (Saito 1967, Nam and Saito 1995, Masuda et al. 1998, Furnari et al. 2001, Senties and Fujii 2002, Dawes and Mathieson 2008). We were not able to examine the type specimen of *L. intricata* in our study. However, the discrepancy between the descriptions of *L. intricata* from the literature and our observations indicates the need for a critical review of this species, including a molecular study with broader sampling from different locations.

The morphology of the specimens collected in the Canary Islands was in agreement with the type material of *L. catarinensis*. Previously collected material of *L. intricata* from the Canaries, deposited in TFC, BCM, and L, was also examined. Our analyses revealed that these materials had been misidentified and all corresponded to *L. catarinensis*. Based on our morphological observations, *L. catarinensis* can be distinguished from *L. intricata* from the Caribbean Sea and the Gulf of Mexico by the lack of lenticular thickenings, which have never been observed in specimens from the type locality (Cordeiro-Marino and Fujii 1985), Rio de Janeiro (Cassano 2009), or the Canary Islands [this study, Masuda et al. 1998 (as *L. intricata*)]. Although lenticular thickenings represent a problematic diagnostic character within *Laurencia sensu stricto*, as they may vary between individuals of the same species, we consider them a useful criterion for separating *L. catarinensis* from *L. intricata*.

Our molecular analysis showed that the nine samples of *L. catarinensis* from the Canary Islands and Brazil, together with a sample from the type locality, Santa Catarina, formed a well-supported clade and diverged by percentage values of 0–0.9%. These data demonstrate that all these samples must be identified as *L. catarinensis*, corroborating the results previously obtained by Cassano (2009). On the other hand, these specimens had high genetic divergence from *L. intricata* from Mexico, Florida (USA), and Cuba (4.9–5.7%) and were phylogenetically distant. Cuba may represent the type locality of *L. intricata*, originally assigned by Lamouroux (1813) to the Antilles without specifying the collecting site. Therefore, the phylogenetic interference and genetic divergence found in *rbcL* sequences support the identification of *L. intricata* and *L. catarinensis* as distinct species even though their morphological convergence suggests that these taxa form a species complex. Moreover, these taxa occupy distinct geographic areas, and our results indicate that the current distribution of *L. intricata* in the Atlantic Ocean is restricted to the Caribbean Sea and the Gulf of Mexico.

In summary, our morphological and molecular evidence confirms that the species previously referred to as *L. intricata* on the coasts of the Canary Islands must be recognized as *L. catarinensis*. The phylogenetic analysis of *rbcL* sequences revealed that *L. intricata* from Mexico, Florida (USA), and Cuba (the type locality) is a distinct species, not grouped with any of the *L. catarinensis* samples from the Canary Islands or Brazil. From a phytogeographical perspective, *L. catarinensis* is currently considered as a taxon from subtropical and tropical zones of the western Atlantic Ocean. However, this report increases its distribution to the eastern Atlantic Ocean (Canary Islands) and makes *L. catarinensis* an ampho-Atlantic species.

Acknowledgments

We thank E. Aylagas, A. Cruz, J. Leal Pérez, and A. Losada Lima for help with field collection, V. Garzón for his advice on cartography, and the University Institute of Tropical Diseases (University of La Laguna) and M. Hernández-Ferrer for kindly receiving the first author in their laboratory. We also thank the Center for Biodiversity Naturalis (section NHN), Leiden, for providing the specimens from

the Netherlands, and the curator of the Herbarium of the Faculty of Biology of Marine Sciences, University of Las Palmas de Gran Canaria (BCM) for his support. M. Machín-Sánchez was financed by a grant from the Government of the Canary Islands. This work was partially supported by project MEC (CGL 2007-60635/BOS), MICINN (CGL 2010-14881), and by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), (Proc. 10/52244-2). M.T. Fujii thanks CNPq for the Research Productivity Fellowship (Proc. 301438/2009-9). M.K. Pec critically revised the manuscript and helped improve the English.

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Received 26 August, 2011; accepted 28 February, 2012; online first 29 March, 2012