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A combined barcode and morphological approach to the systematics and biogeography of *Laurencia pyramidalis* and *Laurenciella marilzae* (Rhodophyta)

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In the present study we undertook an integrative approach, using both morphological and molecular data (COI-5P + *rbcL*), to assess the presence of *Laurencia pyramidalis* in Lusitanian Macaronesia. We studied type material of *L. pyramidalis* from the herbarium of the Naturalis Biodiversity Center, the Netherlands, and designated a lectotype and syntypes. Vegetative and reproductive features of *L. pyramidalis* were observed and we included a specimen from the type locality in our molecular analyses. We also investigated the geographical distribution of *Laurenciella marilzae*, a species recently described from the Canary Islands. Barcode sequences (COI-5P and *rbcL*) were generated for *L. pyramidalis* from the type locality (Normandy, France), the Azores, Madeira and the Canary Islands, and for *L. marilzae* from its type locality (Tenerife, Canary Islands), the Azores and Brazil.

Key words: COI-5P, DNA barcoding, *Laurencia* complex, Lusitanian region, Macaronesia, *rbcL*, taxonomy

Introduction

The Macaronesia region consists of five oceanic archipelagos in the north-eastern Atlantic Ocean between 39° N 31° W and 15° N 23° W. From north to south, these are the Azores, the Savage Islands (Ilhas Selvagens) and Madeira (Portugal), the Canary Islands (Spain) and the Cape Verde islands. The Macaronesian islands share many characteristics, although Cape Verde is quite distinct in terms of its climate and biota, having a more tropical climate; it is included within the West African transition province. In the present paper we will focus on the Azores, Madeira and Canary Islands, which belong to the same ecoregion within the Lusitanian province (Spalding *et al.*, 2007). The volcanic islands of the Savage Islands, Madeira and the Canary Islands,

along with related sea mounts, arose from several geological hotspots at various times during the last 60 Ma (Fernández-Palacios *et al.*, 2010), whereas the Azores are much more recent, none emerging more than some 8 Ma ago. The Lusitanian Macaronesian islands have been colonized predominantly by the adjacent North African and European flora and fauna (Juan *et al.*, 2000; Gillespie & Clague, 2009). Similarly, patterns of species richness and composition of algal assemblages across these islands result mainly from the proximity of continental African and European shores, combined with large and meso-scale oceanographic patterns (Tuya & Haroun, 2009).

The marine algal flora of Lusitanian Macaronesia has been investigated sporadically during the past centuries, that of the Canary Islands receiving particular attention (e.g. Bory de Saint-Vincent, 1803; Montagne, 1840; Børgesen, 1930). Since the 1980s, there has been a revival of systematics in this area

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(Gil-Rodríguez & Afonso-Carrillo, 1980a, b; Audiffred & Weisscher, 1984; Audiffred & Prud'homme van Reine, 1985; Prud'homme van Reine, 1988, 1990, 1998; Prud'homme van Reine *et al.*, 1994, 2005; Afonso-Carrillo & Sansón, 1999).

The *Laurencia* complex currently encompasses six genera: *Laurencia*, *Osmundea*, *Chondrophycus*, *Palisada*, *Yuzurua* and the recently described *Laurenciella* (Cassano *et al.*, 2012a). A list of records of the *Laurencia* complex for the Azores, Madeira, Savage Islands and Canary Islands is presented in Table 1. The Canary Islands, the southern most islands in Lusitanian Macaronesia but also the nearest to the continent, have the highest species richness with 26 species recorded (the number varies depending on the authors, see Table 1), whereas only five species have been reported in the Azores, the northernmost, most isolated and most recently formed archipelago. All the species recorded in the Azores, Madeira and the Savage Islands are also encountered in the flora of the Canary Islands, with the exception of two species (*Laurencia nidifica* and *Osmundea osmunda*) recorded only from Madeira. Interestingly, all the members of the *Laurencia* complex found along the Atlantic European coasts have also been reported in Lusitanian Macaronesia, with the sole exception of *Laurencia pyramidalis*. One aim of the present study was therefore to investigate the presence of *Laurencia pyramidalis* in Lusitanian Macaronesia by conducting a floristic survey in the Azores, Madeira and Canary Islands.

Identification of species of the *Laurencia* complex based on anatomical and morphological characters is extremely difficult due to phenotypic plasticity and overlaps in many morphological characters. As a consequence, among the 28 species reported from Lusitanian Macaronesia, 14 species records have been regarded as doubtful (Table 1). DNA barcoding, based on a standardized sequence of the mitochondrial cytochrome c oxidase subunit I gene (COI-5P), has gained recognition as a tool for species delimitation and has proven useful for uncovering new and cryptic species of Rhodophyta, refining species distributions, and detecting invasive and alien species (Saunders, 2005, 2008, 2009; Robba *et al.*, 2006; House *et al.*, 2008; Walker *et al.*, 2009; Clarkston & Saunders, 2010; Le Gall & Saunders, 2010; Manghisi *et al.*, 2010). However, the molecular systematics of the *Laurencia* complex has been based mainly on sequences of the plastid-encoded large subunit of RuBisCO (*rbcL*), which has been used to infer interspecific relationships within the various genera (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, 2012b; Gil-Rodríguez *et al.*, 2009; Martín-Lescanne *et al.*, 2010; Machín-Sánchez *et al.*, 2012a, b).

In the present study we undertook an integrative approach, using both morphological and molecular

data (COI-5P + *rbcL*), to assess whether *L. pyramidalis* is present in Lusitanian Macaronesia. This required us to locate and study the type of *L. pyramidalis* and include a specimen from the type locality in our molecular analyses. In addition, we investigated the distribution of *Laurenciella marilzae*, recently described from the Canary Islands as *Laurencia marilzae* by Gil-Rodríguez *et al.* (2009) and transferred to *Laurenciella* by Cassano *et al.* (2012a).

Materials and methods

Sampling sites

Collection sites in the Azores, Madeira, the Savage Islands and the Canary Islands are indicated in Fig. 1.

DNA analysis

Specimens for which new sequences were generated in the present study are listed in Supplementary Table S1. Samples for molecular analysis were cleaned, dried and preserved in silica gel. Total DNA was extracted, using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. A total of 64 COI-5P and 55 *rbcL* sequences were included in this study, 57 and 24 sequences being newly generated for COI-5P and *rbcL* respectively. For COI-5P, 670 bp were amplified using the forward primers GazF1 (Saunders, 2005) or GWSFn (Le Gall & Saunders, 2010) in combination with the reverse primers GazR1 (Saunders, 2005) or GWSRx (Saunders, 2009). A total of 1647 bp of the *rbcL* gene and *rbcL-rbcS* spacer was amplified in three fragments with the primer pairs F-*rbcL*start and R-753 (Freshwater & Rueness, 1994) for the 5' end, *rbcL*FC and 1011R (Nam *et al.*, 2000) or F-577 and R1381 (Freshwater & Rueness, 1994) for the central fragment, and F-993 and R-*rbcS* start (Freshwater & Rueness, 1994) for the 3' end. Sequencing reactions were performed by Genoscope (www.genoscope.fr, Evry, France) and Macrogen (dna.macrogen.com, Amsterdam, the Netherlands). Forward and reverse electropherograms were edited and assembled with the software Codoncode (Dedham, Massachusetts, USA) and multiple sequence alignments were constructed using ClustalX 2.1 (Larkin *et al.*, 2007). The COI-5P alignment included 58 newly sequenced taxa (1 from the outgroup + 57 from the ingroup) and 10 taxa (3 outgroup + 7 ingroup) from GenBank. The *rbcL* dataset included 75 taxa from GenBank (3 outgroup + 72 ingroup), for which names were updated according to AlgaeBase (Guiry & Guiry, 2013), and 25 newly generated sequences (1 outgroup + 24 ingroup taxa).

Species assignment was performed (using MEGA version 5.1: Tamura *et al.*, 2011) by cluster analysis of uncorrected *p* distances using the neighbour-joining (NJ) algorithm, with 10 000 replicates for bootstrapping. Phylogenetic analyses used Bayesian Inference (BI). jModelTest v. 0.1.1 (Posada, 2008) was used to select the most appropriate model of sequence evolution for BI analysis of the two datasets, under the Akaike Information Criterion (AIC). The General Time-Reversible model of nucleotide substitution with Gamma-distributed rates for the variable sites (GTR+G) was selected. BI analyses were performed with MrBayes v.

Table 1. *Laurencia* complex species reported from Lusitanian Macaronesia, with sources of information.

Species	Azores	Salvagens	Madeira	Canary Islands
<i>Chondrophycus glandulifer</i> (Kützing) Lipkin & P.C. Silva		John <i>et al.</i> (2004)	Neto <i>et al.</i> (2001) (as <i>L. glandulifera</i>); John <i>et al.</i> (2004)	Haroun <i>et al.</i> (2002) (as <i>Laurencia glandulifera</i>), John <i>et al.</i> (2004)
<i>Laurencia brongniartii</i> J. Agardh				Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia chondrioides</i> Borgesen				Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia cadciramulosa</i> Masuda & Kawaguchi				Cassano <i>et al.</i> (2008)
<i>Laurencia canariensis</i> Montagne ex Kützing				Afonso-Carrillo & Sansón (1999); John <i>et al.</i> (2004); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia catarinensis</i> Cordeiro-Marino & M.T. Fujii				Haroun <i>et al.</i> (2002) (as <i>L. intricata</i>); Machin-Sánchez <i>et al.</i> (2012a)
<i>Laurencia dendroidea</i> J. Agardh				Haroun <i>et al.</i> (2002) (as <i>L. majuscula</i>); Cassano <i>et al.</i> (2012b)
<i>Laurencia flexilis</i> Seichell		Parente <i>et al.</i> (2000)	Neto <i>et al.</i> (2001)	Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia glandulifera</i> (Kützing) Kützing		Parente <i>et al.</i> (2000)	Neto <i>et al.</i> (2001)	Haroun <i>et al.</i> (2002)
<i>Laurencia intricata</i> J.V. Lamouroux				Afonso-Carrillo & Sansón (1999); Machin-Sánchez <i>et al.</i> (2012b) ¹
<i>Laurencia majuscula</i> (Harvey) A.H.S. Lucas		John <i>et al.</i> (2004)	Neto <i>et al.</i> (2001)	Haroun <i>et al.</i> (2002); ^a Cassano <i>et al.</i> (2012a) ¹
<i>Laurencia microcladia</i> Kützing	Tittley <i>et al.</i> (2009)	Parente <i>et al.</i> (2000)	Neto <i>et al.</i> (2001)	Haroun <i>et al.</i> (2002)
<i>Laurencia minuta</i> Vandermeulen, Garbary & Guiry				Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia nidifica</i> J. Agardh			John <i>et al.</i> (2004)	Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux	Neto (1994)	John <i>et al.</i> (2004)	Neto <i>et al.</i> (2001)	Haroun <i>et al.</i> (2002); Gil-Rodríguez <i>et al.</i> (2012) ¹
<i>Laurencia tenera</i> C.K. Tseng				Gil-Rodríguez & Haroun (1992); Haroun <i>et al.</i> (2002)
<i>Laurencia viridis</i> Gil-Rodríguez & Haroun	Gil-Rodríguez & Haroun (1992)	Gil-Rodríguez & Haroun (1992); Parente <i>et al.</i> (2000)	Gil-Rodríguez & Haroun (1992); Neto <i>et al.</i> (2001)	Gil-Rodríguez & Haroun (1992); Haroun <i>et al.</i> (2002)
<i>Laurenciella marilzae</i> (Gil-Rodríguez, Senties, Diaz-Larrea, Cassano & M.T. Fujii)				Gil-Rodríguez <i>et al.</i> (2009) (as <i>Laurencia marilzae</i>)
<i>Osmundea hybrida</i> (De Candolle) K.W. Nam & M.T. Fujii	Neto (1994) (as <i>Laurencia hybrida</i>)	Audiffred & Weisscher (1984) (as <i>L. hybrida</i>); John <i>et al.</i> (2004)	Levring (1974) (as <i>L. hybrida</i>); Neto <i>et al.</i> (2001)	Gil-Rodríguez & Afonso-Carrillo (1980a) (as <i>L. hybrida</i>); Haroun <i>et al.</i> (2002); Machin-Sánchez <i>et al.</i> (2012b) ¹
<i>Osmundea osmunda</i> (S. Gmelin) K.W. Nam & Maggs			Neto <i>et al.</i> (2001); John <i>et al.</i> (2004)	

(continued)

Table 1. Continued.

Species	Azores	Salvagens	Madeira	Canary Islands
<i>Osmundea pinnatifida</i> (Hudson) Stackhouse	Neto (1994) (as <i>Laurencia pinnatifida</i>)	John et al. (2004)	Neto et al. (2001)	Haroun et al. (2002); Machin-Sánchez et al. (2012b)
<i>Osmundea truncata</i> (Kützing) K.W. Nam & Maggs		Parente et al. (2000); John et al. (2004)	Neto et al. (2001)	Haroun et al. (2002); Machin-Sánchez et al. (2012b)
<i>Palisada corallopsis</i> (Montagne) Senties, M.T. Fujii & Diaz-Larrea		Parente et al. (2000) (as <i>Chondrophycus corallopsis</i>); John et al. (2004) (as <i>C. corallopsis</i>)	Neto et al. (2001) (as <i>C. corallopsis</i>)	Haroun et al. (2002) (as <i>C. corallopsis</i>); Gil-Rodríguez et al. (2012) ¹
<i>Palisada flagellifera</i> (J. Agardh) K.W. Nam		Parente et al. (2000) (as <i>Chondrophycus patentirameus</i>)		Gil-Rodríguez et al. (2010)
<i>Palisada patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii		Parente et al. (2000) (as <i>Chondrophycus perforatus</i>); John et al. (2004) (as <i>C. perforatus</i>)	Neto et al. (2001) (as <i>C. perforatus</i>)	Audiffred & Prud'homme van Reine (1985) (as <i>Laurencia paniculata</i>); Gil-Rodríguez et al. (2012) ¹
<i>Palisada perforata</i> (Bory de Saint-Vincent) K.W. Nam				Haroun et al. (2002) (as <i>Chondrophycus papillosus</i> and as <i>C. perforatus</i>); Cassano et al. (2009)
<i>Palisada thuyoides</i> (Kützing) Cassano, Senties, Gil-Rodríguez & M.T. Fujii				Gil-Rodríguez & Haroun (1993) (as <i>Laurencia paniculata</i>); Gil-Rodríguez et al. (2012) ¹
<i>Yizurina poiteaui</i> (J.V. Lamouroux) Martin-Lescanne		Parente et al. (2000) (as <i>Chondrophycus poiteaui</i>); John et al. (2004) (as <i>C. poiteaui</i>)		Haroun et al. (2002) (as <i>C. poiteaui</i>); Gil-Rodríguez et al. (2012) ¹

¹These papers made changes to the taxonomy of the species. Record regarded as doubtful.

3.2 (Ronquist *et al.*, 2012). The analyses were run with four heated Monte-Carlo Markov Chains for 1×10^6 generations, with sampling intervals of 100 generations, to produce 10 000 trees. After verifying that stationary stage had been reached by plotting $-\ln L$ against generation time, the first 2500 trees were discarded and majority rule consensus trees generated from the remaining (post-‘burn-in’) trees.

Morphological observations

Anatomical studies were performed on fresh specimens of *Laurencia pyramidalis* and *Laurenciella marilzae* fixed in 4% formalin seawater. Additionally, freshly collected specimens were examined to check for the presence of *corps en cerise* (Feldmann & Feldmann, 1950; Paradas *et al.*, 2010). 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 & Abbott, 1985). 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). Additionally, we examined specimens of the *Laurencia* complex deposited in the following herbaria: TFC, the herbarium of the Faculty of Biology of Marine Sciences, University of Las Palmas de Gran Canaria (BCM); the herbarium Ruy Telles Palhinga of the University of Azores (AZB); the herbarium of the Naturalis Biodiversity Center, the Netherlands (L); the herbarium of the Muséum National d’Histoire Naturelle, Paris, France (PC). Furthermore, we studied type material of *L. marilzae* in TFC. Herbarium abbreviations follow the on-line Index Herbariorum: <http://sciweb.nybg.org/science2/IndexHerbariorum.asp> (Thiers, 2013, continuously updated).

Results

Sampling and typification of Laurencia pyramidalis

In the course of our survey we collected specimens with a gross morphology similar to *Laurencia pyramidalis* in the Canary Islands, Madeira and the Azores, and we found *Laurenciella marilzae* in the Azores as well as in the Canary Islands (its type locality). Specimens were deposited in the TFC Herbarium (Supplementary material, Table S2).

Laurencia pyramidalis was described by Kützing (1849, p. 854) based on a herbarium specimen from Lenormand collected in France (‘ad oras Galliae’), named as *L. pyramidalis* by Bory de Saint Vincent. Harvey (1849), unaware of the description published earlier by Kützing (1849), described a specimen from the Indian Ocean coast of South Africa as a variety of *Laurencia obtusa* (Hudson) J.V. Lamouroux, referring to it as *L. obtusa* var. *pyramidalis* Bory; however, since the name was never published by Bory, the correct attribution of the variety is to Bory *ex* Harvey. The name *Laurencia obtusa* var. *pyramidalis*

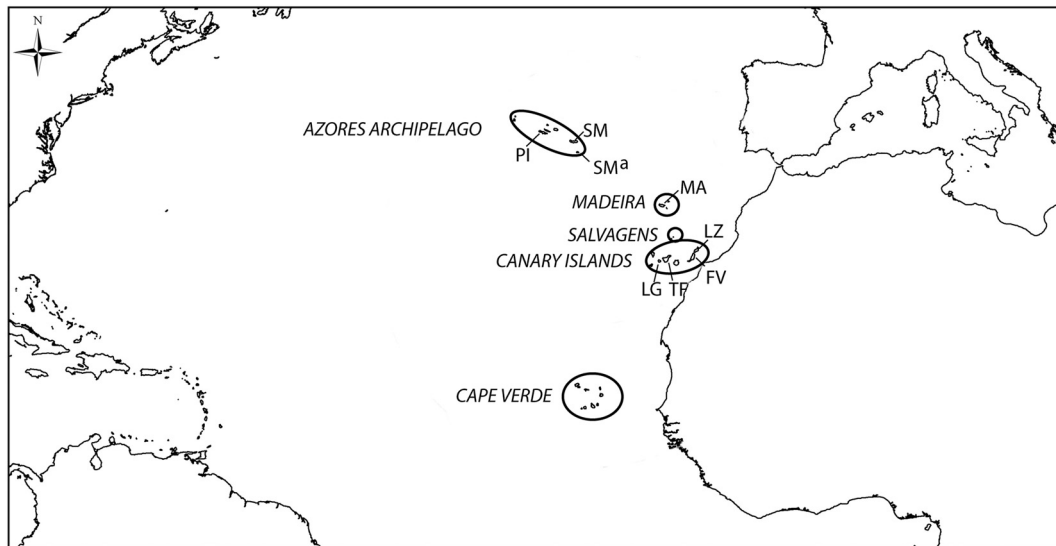


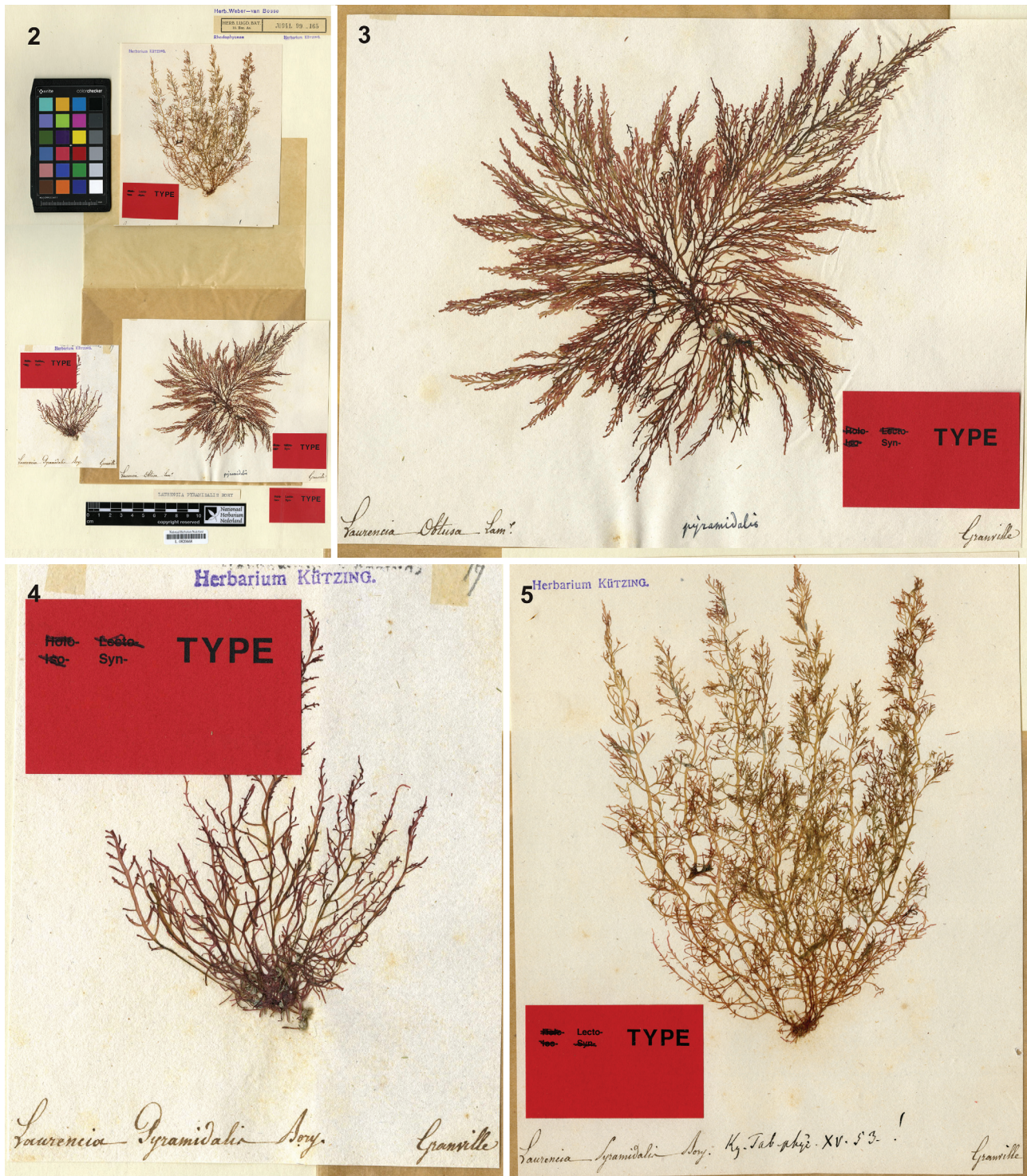
Fig. 1. Map of Atlantic Ocean showing the Macaronesian archipelagos. Sites where Macaronesian specimens were collected in the present study are in the **Azores**: Pico (**PI**: Pocinho-Monte Candelaria 38.49686466° N/ 28.53992863° W; Barca-Madalena 38.53988167° N/ 28.52058390° W; Prainha do Norte 38.47720139° N/ 28.20443794° W; Lajes do Pico-Poça de Baleia 38.38998195° N/ 28.25144459° W; Lajes do Pico-Fábrica de Baleia 38.38862491° N/ 29. 19430601° W; Santa Cruz Ribeiras 38.404° N/ 28.1872° W), São Miguel (**SM**: Cerco da Caloura-Baía 37.7570843° N/ 25.81716330° W; Ferraria 37.8579856° N/ 25.85265462° W; Mosteiros 37.8992112° N/ 25.82103420° W), Santa Maria (**SM^a**: Boca de Ribeira Seca 36.94337327° N/ 25.16456403° W; Emissores 36.99720678° N/ 25.17678807° W; Anjos-Este 37.00542551° N/ 25.16444382° W; Anjos-Ponta dos Frades 37.00788999° N/ 25.15019092° W; Anjos-Piscinas 37.00458430° N/ 25.15727202° W); in **Madeira** (**MA**: Seixal-Praia da Laje 32.82554110° N/ 17.11529253° W; Porto Moniz-Piscinas 32.86802811° N/ 17.17135116° W; Ponta de Sao Jorge-Casi 32.8357° N/ 16.9053° W); in the **Canary Islands**: Fuerteventura (**FV**: Garcey 28.345492° N/ 14.178111° W; El Cotillo 28.7013° N/ 14.0182° W), Lanzarote (**LZ**: Arrecife 28.957972° N/ 13.544525° W; Pechigueras 28.855217° N/ 13.872631° W), La Gomera (**LG**: El Charco de las Condesas 28.0839772° N/ 17.33672469° W; El Charco del Conde 28.05150° N/ 17.20269° W; Punta de la Dama 28.031° N/ 17.183° W), Tenerife (**TF**: El Pris 28.50981317° N/ 16.42174616° W; Puerto de la Cruz 28.4175° N/ 16.5462° W; Punta del Hidalgo 28.5739° N/ 16.5462° W).

had in fact been used previously by Zanardini (1847) but without providing any description; therefore Zanardini's name was not validly published. J. Agardh (1852, p. 752) claimed that *L. obtusa* var. *pyramidalis* Bory ex Harvey was a misapplied name for *Laurencia virgata* (C. Agardh) J. Agardh and he also listed *L. obtusa* var. *pyramidata* Bory ex J. Agardh with *L. pyramidalis* Kützing as a synonym. In contrast, Maggs & Hommersand (1993, p. 405) considered *L. obtusa* var. *pyramidata* Bory ex J. Agardh as a synonym of *L. pyramidalis* Bory ex Kützing and mentioned three probable syntypes from France (Cherbourg) in L, LD and BM. They claimed that 'no specimens have been found in L that were obviously examined by Kützing'. We reviewed the material in L and found three samples from Granville (Normandy, France) in Kützing's herbarium (Fig. 2). One of these (Fig. 5) was clearly the one that Kützing used to prepare an illustration in his 'Tabulae Phycologicae' (1865, 15: p. 19, Tab. 53 a), in which he shows the habit of *L. pyramidalis*; we therefore here designate this specimen as the lectotype of *Laurencia pyramidalis*. This sample was stamped 'Herbarium Kützing', and on it was written '*Laurencia pyramidalis* Bory' (in Lenormand's handwriting), 'Kg. Tab.

Phyc. XV. 53. !' (in Kützing's handwriting) and 'Granville' (again in Lenormand's handwriting). One of the remaining syntypes (Fig. 3) was labelled '*Laurencia obtusa* Lamx.' (handwriting of Lenormand), '*pyramidalis*' (handwriting of Kützing) and 'Granville' (Lenormand's handwriting), but had no 'Herbarium Kützing' stamp, while the second syntype (Fig. 4) had a 'Herbarium Kützing' stamp and the inscriptions (in Lenormand's handwriting) '*Laurencia pyramidalis* Bory' and 'Granville', and the number 17. Thus Kützing's comment that the species was 'ad oras Galliae' does not refer to Cherbourg but to Granville, which is a town along the western coast of Normandy. The three type samples are displayed in Figs 2–5.

Molecular identification and phylogenetic analyses

On the basis of the lectotypification of *Laurencia pyramidalis* on a specimen from Granville, we included in our molecular analysis a specimen recently collected at Chausey, a small archipelago which is under the jurisdiction of the town of Granville. The COI-5P sequence generated for this specimen (LLG1855) was identical to sequences



Figs 2–5. *Laurencia pyramidalis*: type specimens from the herbarium of the Naturalis Biodiversity Center (section NHN), Leiden (L). 2. Herbarium sheet L 0820668 bearing lectotypes and syntypes (Herbarium Kützing). 3. Syntype, enlarged from Fig. 2. 4. Syntype, enlarged from Fig. 2. 5. Lectotype, enlarged from Fig. 2.

obtained from 16 specimens collected in Macaronesia (Fig. 6A). Moreover, these specimens belonged to a fully supported clade containing 11 additional specimens collected in Madeira, the Azores and the Canary Islands, whose sequences were very similar to LLG1855, though not identical. Altogether, three haplotypes were assigned to *L. pyramidalis*, displaying divergences of less than 0.52%. Eight *rbcL* sequences

of *L. pyramidalis* were generated from Macaronesia and were clearly conspecific with a sequence from Brittany, France. Furthermore, two haplotypes were detected and were congruent with the COI-5P data.

A specimen of *Laurenciella marilzae* collected from the type locality (Punta del Hidalgo Tenerife, Canary Islands) belonged to a fully supported clade also containing other specimens from the Canary

Islands, and specimens from the Azores and Brazil (Fig. 6A). The ten sequences belonged to three haplotypes with divergences ranging from 0.0% to 0.70%. Sequences of *rbcL* generated for three specimens from Canary Islands and one specimen from the Azores were identical to most (five out of six) sequences of *L. marilzae* available in GenBank. No sequence variation was observed in *rbcL*.

Other representatives of the *Laurencia* complex collected in Macaronesia and included in our analyses were resolved as distinct lineages (Fig. 6A), confirming that they were distinct from *L. pyramidalis* and *Laurenciella marilzae*. The results include the first COI-5P for *Palisada flagellifera* (from the Canary Islands); *Palisada perforata* (a sample from the type locality – Tenerife, Canary Islands); *Osmundea pinnatifida* (from the Azores); *Laurencia viridis* (from the Azores, Madeira and the Canary Islands, including a sequence from the type locality, Punta del Hidalgo); and *L. catarinensis* and *L. dendroidea* (both from the Canary Islands).

All our phylogenetic analyses of *rbcL* sequences (NJ and BI) resolved the genus *Laurencia sensu stricto* as a monophyletic lineage (Fig. 6B). However, relationships among *Laurencia* species were only moderately or poorly supported. Furthermore, although all the specimens of *Laurenciella marilzae* grouped together in a fully supported lineage, the phylogenetic affinities of this genus were not resolved. Interestingly, *Laurencia catarinensis* from Macaronesia was resolved with full support as sister species to a lineage encompassing specimens from the South Pacific.

Morphological observations

Supplementary Table S2 lists the specimens from TFC Herbarium for which we obtained morphological data. The type specimens and samples of *Laurencia pyramidalis* (from Normandy and Macaronesia) included in this study exhibited a similar habit, with spirally arranged branches around the main axes and densely ramified branchlets conferring a pyramidal shape to the plant. The Macaronesian specimens of *L. pyramidalis* (Figs 7–22) were in accordance with the descriptions given by Kützing (1849), Maggs & Hommersand (1993) and Serio *et al.* (2004): the thalli were terete, 10–18 cm high, brownish red to purple in colour, soft in texture, attached to the substratum by a stoloniferous holdfast, and pyramidal in outline, with three to four orders of branches arranged in three whorls. In surface view, cortical cells usually contained one *corps en cerise* in living specimens and were connected to each other by secondary pit connections; the medullary cells lacked lenticular wall thickenings. The main difference between

Macaronesian specimens and those from continental Europe was the presence in the former of two pericentral fertile cells in tetrasporangial segments (Fig. 21), instead of one as described by Serio *et al.* (2004) for Mediterranean Sea samples. Furthermore, Macaronesian specimens were green to yellow-greenish and tended to be smaller than specimens from the continent. All specimens of *Laurenciella marilzae* observed in this study (e.g. Figs 23–25) fitted the descriptions given by Gil-Rodríguez *et al.* (2009), Rocha-Jorge *et al.* (2010) and Senties *et al.* (2011). *Corps en cerise* structures were present in all cells of the thallus (Figs 24, 25), a unique and very distinctive feature of this species.

Discussion

To the best of our knowledge, this study constitutes the first report of *Laurencia pyramidalis* for Macaronesia. This species has a broad distribution in the Atlantic, with records for France (Bouxin & Dizerbo, 1971, as *L. obtusa* var. *pyramidata*), Britain and Ireland (Maggs & Hommersand, 1993), Italy (Furnari *et al.*, 1999; Serio *et al.*, 2004), Portugal (Araújo *et al.*, 2009), Spain (Conde *et al.*, 1996; Gorostiaga *et al.*, 2004; Bárbara *et al.*, 2005; Cires Rodríguez & Cuesta Moliner, 2010) and Morocco (Dangeard, 1949). The presence of this species in the Azores, Madeira, the Savage Islands and the Canary Islands extends its distribution range westwards.

Individuals of *L. pyramidalis* from Lusitanian Macaronesia were found growing either in mid-to-lower intertidal rock pools or in turfs covering rocks, especially in the Azores, where the macroalgal turfs are one of the most conspicuous assemblages of the intertidal shores (Wallenstein *et al.*, 2009). The species frequently occurred in non-calcareous turfs, sometimes together with *L. viridis*. The morphology of the specimens studied was in agreement with the type material of *L. pyramidalis* and with modern material from the type locality deposited in PC (PC0157557), which was included in our molecular analysis. It is noteworthy that *L. pyramidalis* specimens from Macaronesia were never taller than 7 cm, whereas mainland specimens are larger (13 cm in the lectotype, 17 cm for PC0157557). We observed the presence and absence of annular thickenings in medullary, pericentral and axial cells, and determined this to be a variable vegetative character. It would be interesting to test whether the size differences observed between *L. pyramidalis* specimens from Atlantic islands and the continent result from adaptive responses to environmental conditions.

Laurenciella marilzae was originally described as *Laurencia marilzae*, based on morphological and molecular studies from specimens collected in the

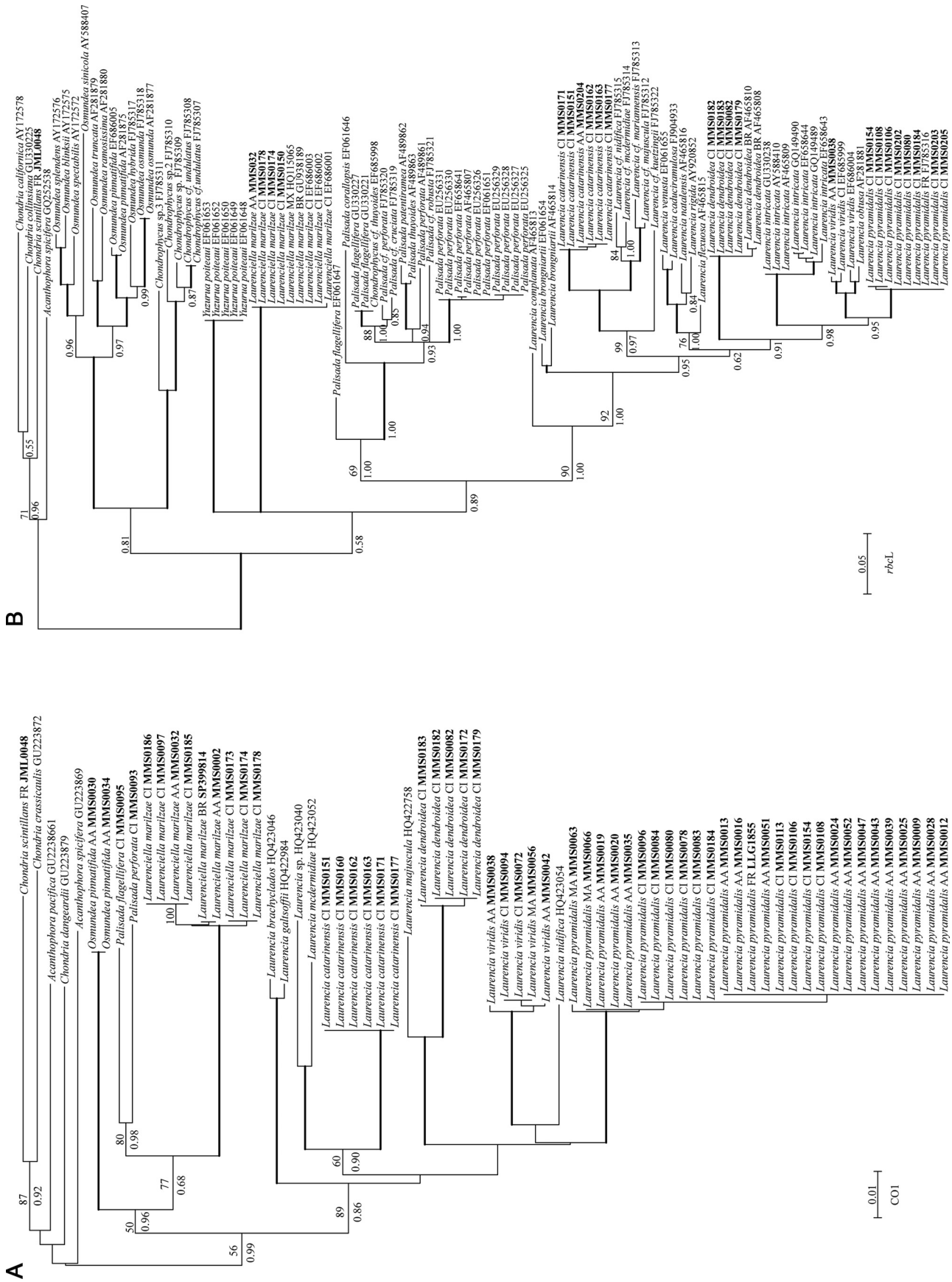
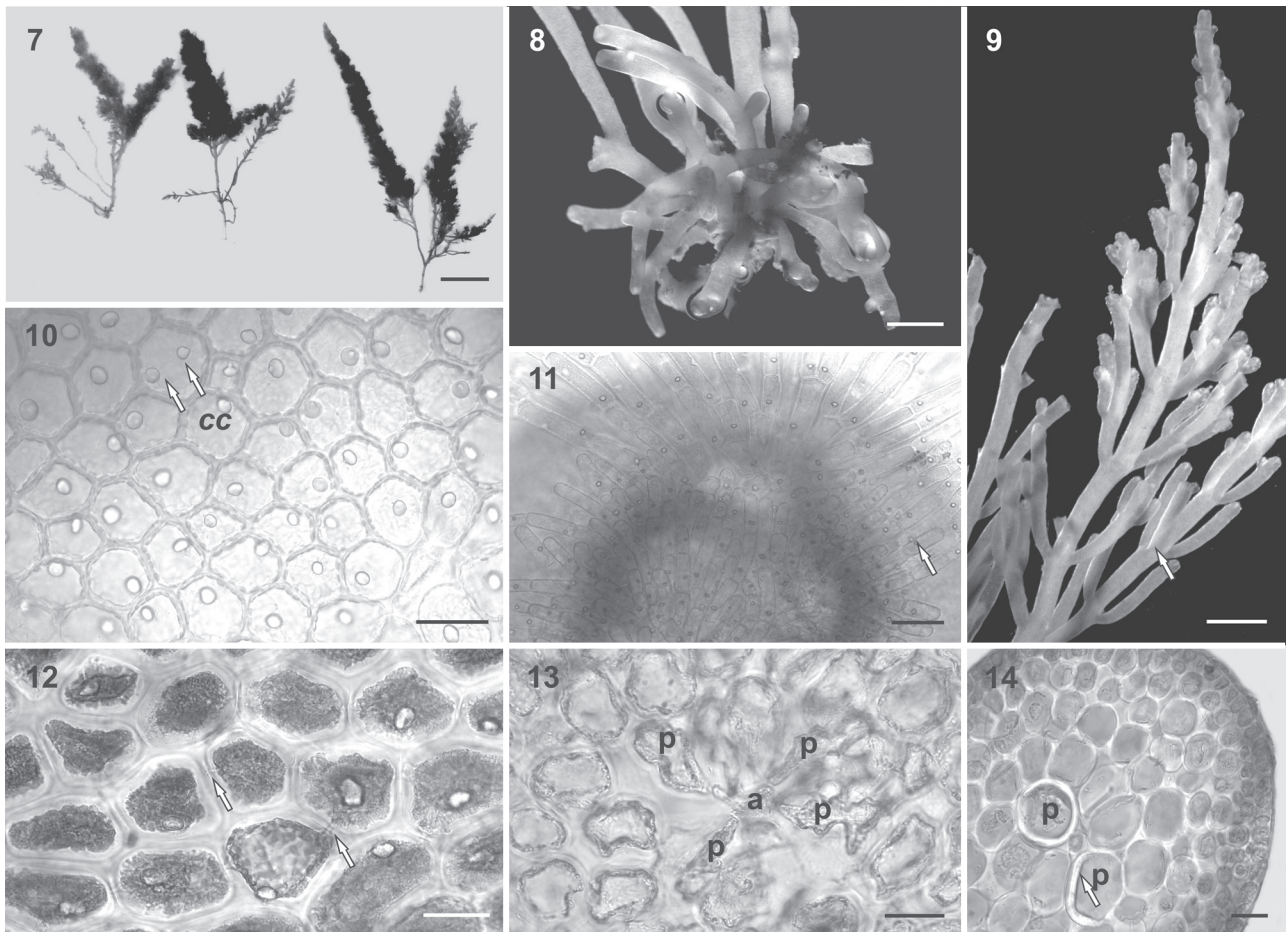


Fig. 6. Phylogram generated using neighbour-joining analyses from COI-5P sequences (A) and phylogenetic tree generated using Bayesian inference from *rbcL* sequences (B). Voucher numbers (in bold) and the geographical origin of each specimen were indicated. FR: France, AA: Azores archipelago, MA: Madeira, CI: Canary Islands, MX: Mexico, BR: Brazil. Bootstrap values > 60% are indicated above nodes and Bayesian posterior probabilities are indicated under nodes. Bold branches indicate strongly supported nodes (bootstrap values > 95% and Bayesian probability > 0.99).

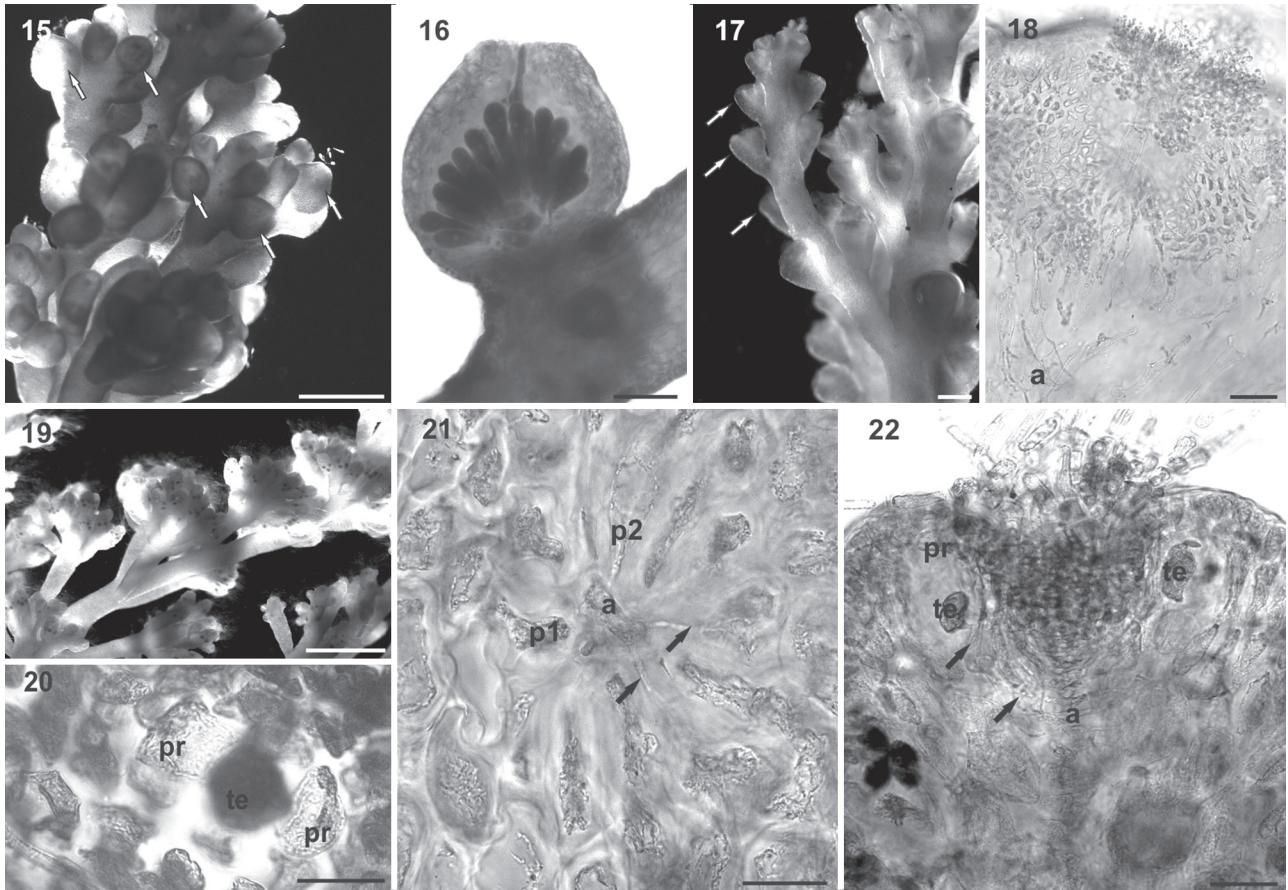


Figs 7–14. *Laurencia pyramidalis* from the Lusitanian Macaronesian region. **7.** Habit. The main axes have sparse branching in the lower portions and first order laterals that decrease in length upwards and bear three further orders of branching in the same arrangement, resulting in a thallus that is pyramidal in outline. Scale bar = 2 cm. **8.** Basal anchorage crust with stoloniferous branches. Scale bar = 1 mm. **9.** Spiral branching with three or four orders of whorled branches. Branches issued from the first-order branches curved markedly towards the main axis, becoming almost parallel to it. Scale bar = 2 mm. **10.** In surface view, cortical cells contain one or two *corps en cerise* (cc) in living specimens (arrows). Scale bar = 30 μ m. **11.** Branches with truncate tips showing dense hyaline trichoblasts with one *corps en cerise* per cell in living specimens (e.g. arrow). Scale bar = 50 μ m. **12.** Surface view showing polygonal cortical cells connected to each other by secondary pit connections (arrows). Scale bar = 30 μ m. **13.** Transverse section of the upper portion of a branch showing an axial cell (a) with four pericentral cells (p). Scale bar = 30 μ m. **14.** Transverse section of a thallus showing medullary cells with thickened walls. Note pericentral cells (p) with annular thickenings (arrow). Scale bar = 50 μ m.

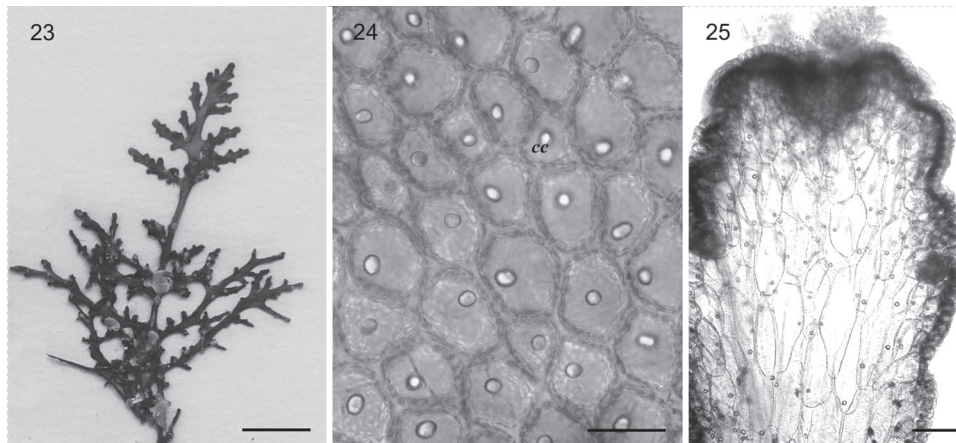
Canary Islands (Gil-Rodríguez *et al.*, 2009). However the distributional range of this species was not assessed in that study. *Laurenciella marilzae* has subsequently been reported from deep waters in southeastern Brazil (Rocha-Jorge *et al.*, 2010) and in the Mexican Caribbean (Senties *et al.*, 2011). It occurs on both sides of the Atlantic and has probably a preference for tropical and warm temperate waters. It is noteworthy that, despite extensive sampling of members of the *Laurencia* complex in Brittany (Le Gall & Rousseau, unpublished data), *Laurenciella marilzae* has never been encountered there. It would be interesting to assess its presence in the warmer waters along the coasts of the Iberian Peninsula. The present report reveals that, within Macaronesia, *L. marilzae* is not restricted to the Canary Islands but also occurs in

the Azores. The Azores specimens share similar morphological characters with specimens from the type locality in Tenerife, Canary Islands, i.e. yellow-orange thalli in the natural turf habitat, an irregularly pyramidal outline, discoid holdfast and a single *corps en cerise* in each cell of the thallus (Gil-Rodríguez *et al.*, 2009). Moreover, in the Azores, specimens grow near the lower intertidal zone, generally forming turfs with other macroalgae, similar to *L. marilzae* in the Canary Islands.

The *Laurencia* complex provides an interesting model for understanding the biogeography of the Macaronesian algal flora; however, increased specimen sampling is required in both Macaronesia and along the continental coasts of Europe, Africa and America.



Figs 15–22. *Laurencia pyramidalis* from the Lusitanian Macaronesian region: reproductive structures. **15.** Female gametophyte with cystocarps (arrows) located on the penultimate branches; they are subapical, sessile and prominent. Scale bar = 1 mm. **16.** Longitudinal section showing a slightly pyriform cystocarp with a non-protuberant ostiole and clavate carposporangia. Scale bar = 200 μ m. **17.** Male gametophyte with spermatangial receptacles (arrows) located on the ultimate fertile branchlets. Scale bar = 500 μ m. **18.** Longitudinal section of a cup-shaped spermatangial receptacle. An axial cell (a) is discernible at the base bearing a fertile branch with many ovoid spermatangia. Scale bar = 50 μ m. **19.** Tetrasporangial plants with cylindrical branchlets. Scale bar = 2 mm. **20.** Surface view of a tetrasporangium (te) with two presporangial cover cells (pr). Scale bar = 30 μ m. **21.** Transverse section near the apex of axial tetrasporangial segments with an axial cell (a), two vegetative pericentral cells (p1, p2) and two fertile pericentral cells (arrows). Scale bar = 30 μ m. **22.** Longitudinal section through a tetrasporangial branchlet showing the parallel arrangement of the tetrasporangia. Each fertile pericentral cell (arrows) cuts off presporangial cover cells (pr) distal to the initial tetrasporangium (te). Scale bar = 50 μ m.



Figs 23–25. *Laurenciella marilzae* from the Azores. **23.** Habit. Scale bar = 5 mm. **24.** In surface view, cortical cells contain one *corps en cerise* (cc) in living specimens. Scale bar = 30 μ m. **25.** Longitudinal section through a branch showing *corps en cerise* in cortical and medullary cells. Scale bar = 10 μ m.

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Supplementary information

The following supplementary material is available for this article, accessible via the Supplementary Content tab on the article's online page at

Table S1. Specimens for which barcode (COI-5P) and *rbcL* sequences were generated in the present study, along with their valid names, vouchers, details of collection data, and GenBank accession numbers

Table S2. Collection details of specimens observed in this study along with their herbarium information, also indicating specimens newly collected for this study.

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