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LAURENCIA MARILZAE SP. NOV. (CERAMIALES, RHODOPHYTA) FROM THE CANARY ISLANDS, SPAIN, BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE[1]

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***Laurencia marilzae* Gil-Rodríguez, Sentíes et M.T. Fujii sp. nov. is described based on specimens that have been collected from the Canary Islands. This new species is characterized by distinctive yellow–orange as its natural habitat color, a terete thallus, four pericentral cells per vegetative axial segment, presence of secondary pit-connections between adjacent cortical cells, markedly projecting cortical cells, and also by the presence of *corps en cerise* (one per cell) present in all cells of the thallus (cortical, medullary, including pericentral and axial cells, and trichoblasts). It also has a procarp-bearing segment with five pericentral cells and tetrasporangia that are produced from the third and fourth pericentral cells, which are arranged in a parallel manner in relation to fertile branchlets. The phylogenetic position of this taxon was inferred based on chloroplast-encoded *rbc*L gene sequence analyses. Within the *Laurencia* assemblage, *L. marilzae* formed a distinctive lineage sister to all other *Laurencia* species analyzed. Previously, a large number of unique diterpenes dactylomelane derivatives were isolated and identified from this taxon. *L. marilzae* is morphologically, genetically, and chemically distinct from all other related species of the *Laurencia* complex described.**

Key index words: **Canary Islands; *Laurencia marilzae*; molecular phylogeny; Rhodomelaceae; taxonomy**

Abbreviations: **GTR+I+G, general-time-reversible model of nucleotide proportion of invariable sites and shape parameter of the gamma distribution; MP, maximum parsimony; *rbc*L, LSU of the RUBISCO gene**

The taxonomy of the *Laurencia* J. V. Lamour. complex has undergone several major changes, and currently four genera are assigned to this complex. These changes have led to the resurrection of the genus *Osmundea* (Nam et al. 1994), the elevation of subgenus *Chondrophycus* (in Saito 1967) to generic rank (Garbary and Harper 1998) with generic features as defined by Nam (1999), and recently the genus *Palisada* based on Yamada's (1931) section *Palisadae* (Nam 2006, 2007). Eighteen species of the *Laurencia* complex have been recorded from the Canary Islands (Gil-Rodríguez and Haroun 1993, Haroun et al. 2002), representing 72% of the species described from the east Atlantic Ocean coast (Lawson and John 1982, Maggs and Hommersand 1993, Nam et al. 2000, Gil-Rodríguez and Haroun 2002, Neto et al. 2005).

The *Laurencia* complex in the Canary Islands has been studied taxonomically since the beginning of the 19th century (Bory de Saint-Vincent 1803, Montagne 1840, Børgesen 1930, Gil-Rodríguez and Haroun 1992, 1993, Hernández-González and Gil-Rodríguez 1994, Masuda et al. 1998, Schnetter et al. 2000, Gil-Rodríguez et al. 2003). Ecological and biogeographical aspects of this complex were studied by a number of authors (Haroun and Prud'homme van Reine 1993, Haroun and Gil-Rodríguez 1995, Gil-Rodríguez and Haroun 2002).

The members of *Laurencia* are well known as they synthesize structurally elaborate halogenated natural products (Martín and Darias 1978, Erickson 1983, Norte et al. 1994, 1996, 1997, 1998, Fernández et al. 2000, 2005, Manríquez et al. 2001, Teixeira 2002). These metabolites play a major role in mediating ecological interactions, such as herbivory (Hay and Fenical 1988, Hay and Steinberg 1992), in which these compounds act as defenses against grazing or

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as deterrents against epibiota, due to antifouling activity (Da Gama et al. 2002).

Recently, a species of *Laurencia* from the Canary Islands drew our attention by its distinctive yellow–orange color, different from any other *Laurencia* species in the area. From this species (as *Laurencia* sp. TFC Phyc. 9860), several new diterpene metabolites, which possess a carbon skeleton named dactylomelane, were isolated and structurally determined (Fernández et al. 2005). Anatomical observations on this material revealed the uncommon occurrence of refractile inclusions (*corps en cerise*) in all cells of the thallus. *Corps en cerise* are believed to be the sites of production and/or accumulation of halogenated metabolites (Howard et al. 1980, Young et al. 1980). Recently, Salgado et al. (2008), by studying the localization of bromo-containing products in *Laurencia obtusa* from Brazil, confirmed that *corps en cerise* are the main bromine location site and, consequently, of the halogenated substances (for taxonomic authors, see Table S1 in the supplementary material).

Based on its new chemical compounds allied with detailed morphological studies and chloroplast-encoded *rbc*L gene sequences, a new species of *Laurencia*, *L. marilzae*, is here proposed.

MATERIALS AND METHODS

Samples of *L. marilzae* were collected intertidally from Punta del Hidalgo (28°34′29″ N, 16°19′50″ W) and Paraiso Floral (28°07′39″ N, 16°46′21″ W), Tenerife, Canary Islands, Spain. Previous collections deposited in TFC (Herbario Departamento de Botánica, Universidad de La Laguna, Canary Islands, Spain) were reexamined and included here (Appendix S1 in the supplementary material).

Morphological studies. Anatomical studies were carried out on both fresh specimens and plants fixed in 4% formalin seawater. Sections for microscopic observations were made by hand using a stainless steel razor blade under a Leica MZ12.5 stereoscopic dissection microscope (Wetzlar, Germany) and stained with 0.5% aqueous aniline blue solutions acidified with 1 N HCl (Tsuda and Abbott 1985). Slide preparations were mounted in 80% corn syrup (Karo) (Best Foods, Englewood Cliffs, NJ, USA) after staining for 5–10 min, according to Womersley (1984). Photomicrographs were taken with a Sony W5 digital camera (Tokyo, Japan) coupled to Zeiss Axioshop 2 microscope (Göettingen, Germany). Voucher specimens were deposited in the herbaria TFC, UAMIZ, SP, HRJ. Herbarium abbreviations follow the online *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

DNA analysis. Samples used for molecular analysis were dried in silica gel. The specimens are shown in Table S1, including their GenBank access numbers.

Total DNA was extracted, after grinding in liquid nitrogen, by using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A total of 1,467 base pairs of the *rbc*L gene was amplified in three parts with the primer pairs: FrbcLstart-R753, F577-R1150, and F753-RrbcS (Freshwater and Rueness 1994) by 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 in accordance with the manufacturer's instructions. Sequencing was carried out with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Princeton, NJ, USA) on an ''ABI PRISM 3100 Genetic Analyzer'' (Applied Biosystems). The primers used for the sequencing were the same as the ones used for the amplification. The analysis of the sequences was achieved by using the computer program Sequence Navigator (Applied Biosystems).

Phylogenetic analysis. Phylogenetic relationships were inferred with PAUP* 4.0b10 (Swofford 2002) and MrBayes v.3.0 beta 4 (Huelsenbeck and Ronquist 2001). Maximum-parsimony trees (MP) were constructed by using the heuristic search option, tree-bisection-reconnection (TBR) branch swapping, unordered and unweighted characters. Branch length was optimized by using delayed transformation (DELTRAN), which favors parallelisms over reversals. Support values for the relationships discovered in this analysis were calculated by performing bootstrap (Felsenstein 1985) analysis, as implemented in PAUP*. Ten thousand heuristic search replicates were executed by using the TBR branch-swapping algorithm.

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 four chains of the Markov chain Monte Carlo (one hot and three cold), sampling 1 tree every 1,000 generations for 3,000,000 generations starting with a random tree. Stationarity was reached at generation 15,800. Therefore, trees saved until generation 15,000 were the ''burn in'' of the chain, and inferences about the phylogeny were based on those trees sampled after generation 15,000. A 50% consensus tree (majority rule as implemented by PAUP*) was computed after the ''burn in'' point.

The range of *rbc*L divergence values within and among species was calculated using uncorrected ''p'' distances using PAUP*.

RESULTS

Laurencia marilzae sp. nov.

Diagnosis: Alga, colore flavo aurantiaco, in naturali habitatione caespites 7 cm altos forman. Axes cylindrici usque ad 1.5 mm in diametro affixi ad substratum basali disco, quamquam etiam adsint parvae auxiliares adhaerentiae, quae oriuntur ex ramis basalibus; thalli cartilaginea structura atque irregulariter pyramidali ambitu; ramificatio irregulatim alterna et spiraliter disposita, generatim usque ad 2–3 (4) ordines ramificationis; ultimis rami cylindrici ad clavatos; unum ''corps en cerise'' in omnibus thalli cellulis, et in cellulis axialis segmenti et in medullosis cellulis; corticalium cellularum parietes valde projectae sunt; in transversali sectione corticales cellulae numquam radiatim elongatae nec paliformes nec cum secundariis conexionibus; medullosae cellulae cum incrassatis uniformiter parietibus, sed sine lenticularibus crassitudinibus; quattuor pericentrales cellulae per vegetativum segmentum axiale; prima cellula pericentralis sub cellula basali trichoblastorum exoriens; segmentum, ferens procarpium, quinque pericentralibus cellulis, quarum quinta sustinens est; cystocarpium maturum forma subconicali cum prominente ostiolo; tetraspo-

rangia in tertia quartaque pericentrali cellul facta, dispositione parallela ramorum fertilium axe. Habitatio: frequens in locis valde expositis fluctibus.

Plants yellow–orange color in natural habitat forming tufts up to 7 cm high; terete axes up to 1.5 mm in diameter, arising from a discoid holdfast; smaller auxiliary holdfasts present formed from descending basal branches; thalli cartilaginous in texture, irregularly pyramidal in outline; branching irregularly alternate and spirally arranged, usually with 2–3 (4) orders of branches; ultimate branchlets are cylindrical-clavate; *corps en cerise*, one per cell, present in almost all cells of the thalli, including axial segment and other medullary cells; cortical cell walls markedly projecting; in transverse section, cortical cells neither elongated radially nor arranged as a palisade with secondary pit connections; medullary cells with walls uniformly thickened, but lenticular thickenings are absent; four pericentral cells per vegetative axial segment; procarp-bearing segment with five pericentral cells, the fifth becoming the supporting cell; mature cystocarps subconical without protuberant ostiole; tetrasporangia are produced from third and fourth pericentral cells, in parallel arrangement in relation to fertile branchlets.

Morphology: Plants forming yellow–orange tufts up to 7 cm high (Fig. 1a), with terete axes, cartilaginous in texture, irregularly pyramidal in outline, not adhering to herbarium paper when dried. Thalli attached to the substratum by a discoid holdfast and from descending branches formed from the lower portion of axes, which may attach the thalli secondarily by smaller holdfasts (Fig. 1b). Erect

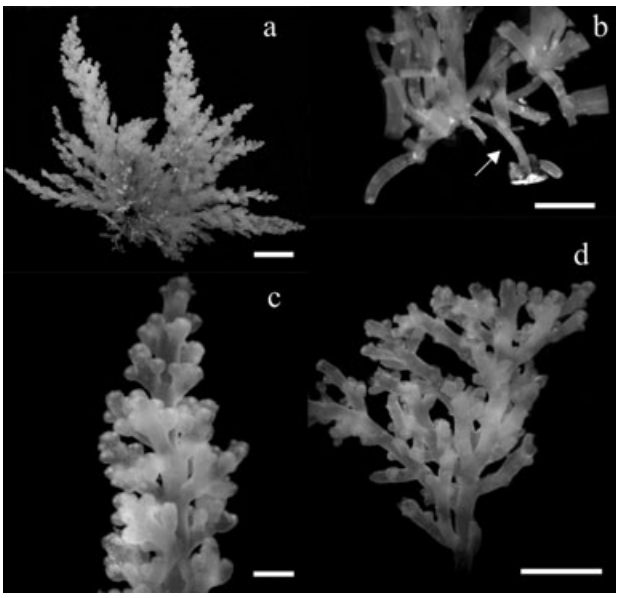

Fig. 1. *Laurencia marilzae* sp. nov. (a) Habit of a plant. Scale bar, 1 cm. (b) Detail of basal portion of the thallus (arrow). Scale bar, 1 mm. (c) Tetrasporangial branches. Scale bar, 2 mm. (d) Female branches. Scale bar, 2 mm.

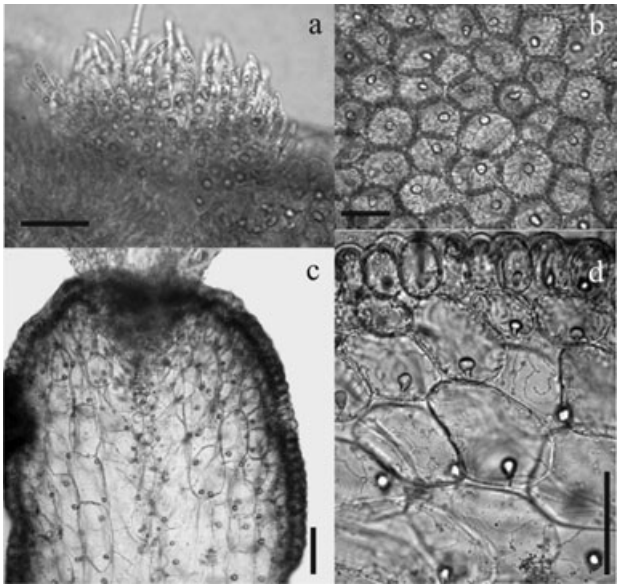

Fig. 2. *Laurencia marilzae* sp. nov. showing a single *corp en cerise* present in all cells of the thallus. (a) Apical region of the branch with abundant trichoblasts; each cell of trichoblast bearing a *corp en cerise*. Scale bar, 20 μm. (b) Cortical cells in superficial view. Scale bar, 50 μm. (c) Longitudinal section of a branch showing *corps en cerise* in cortical and medullary cells. Scale bar, 100 μm. (d) Longitudinal section in detail showing *corps en cerise* in medullary cells. Scale bar, 50 μm.

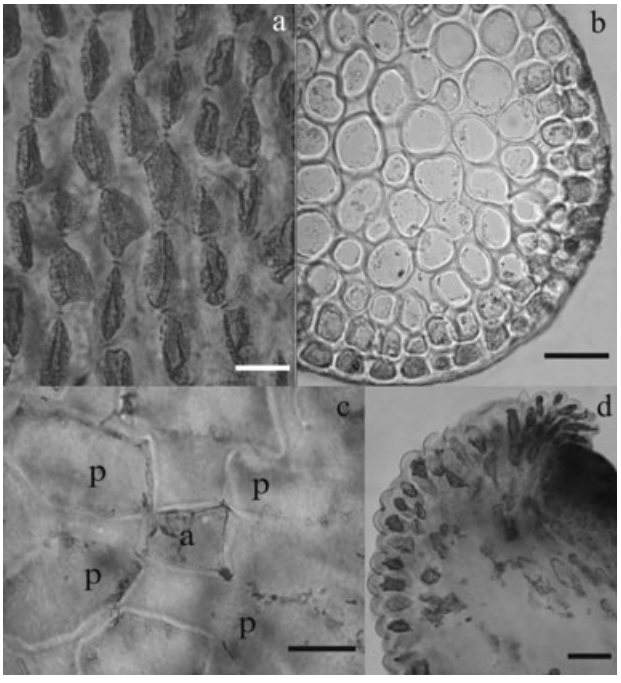

Fig. 3. *Laurencia marilzae* sp. nov. (a) Cortical cells of the middle portion of the thallus in surface view showing secondary pit connections. Scale bar, 20 μm. (b) Transverse section of the thallus. Scale bar, 50 μm. (c) Transverse section of the upper portion of a branch showing an axial cell (a) with four pericentral cells (p). Scale bar, 25 μm. (d) Longitudinal section of a branch showing projecting cortical cells. Scale bar, 40 μm.

branches irregularly alternate and spirally arranged (Fig. 1, c and d), usually with 2–3 (4) orders of branches. The main axes are 740–1,100 μm in diameter in the lower portions of the thalli, 992–1,440 μm in diameter in the middle portions, and 720–1,060 μm in diameter at the apices. First-order branches up to 3 cm long and 800–1,152 μm in diameter bearing simple or compound second-order branches up to 0.4 cm long and 640–850 μm in diameter. Ultimate branchlets cylindrical-clavate, 800–3,680 μm long and 540–820 μm in diameter (Fig. 1, c and d).

Vegetative structures: Trichoblasts are subdichotomously branched to 3 or 4 orders at the apex of each branch. A single *corp en cerise* presents in each cell of the thallus (trichoblasts, cortical and medullary cells, including axial and pericentral cells) (Fig. 2, a–d). In surface view, cortical cells are regularly arranged in longitudinal rows and connected to each other by secondary pit connections (Fig. 3a). Cortical cells are isodiametric-polygonal in the upper portions of the thalli, 22–55 μm long and 28–45 μm wide; longitudinally elongated in the middle portions, 45–90 μm long and 28–53 μm wide; and elongate-polygonal in the lower portions of the thalli, 30–88 μm long and 25–50 μm wide. In transverse section, thalli with one layer of pigmented cortical cells and four or five layers of colorless medullary cells (Fig. 3b). Cortical cells measuring 30–55 μm long and 28–48 μm wide in the middle portions of main branches. Medullary cells are rounded or slightly radially elongated, measuring 75–148 μm long and 40–100 μm wide in the middle portions of the main axes. Medullary cell walls lack lenticular thickenings. Intercellular spaces and secondary cortication are present in the older portions of thalli. Each vegetative axial segment cuts off four pericentral cells (Fig. 3c), which are slightly larger than surrounding medullary cells (Fig. 3b). In median longitudinal section, cortical cells near apices of branchlet project markedly (Fig. 3d).

Reproductive structures: Tetrasporangial branchlets are cylindrical-clavate, simple or compound, measuring 576–3,984 μm long and 528–864 μm in diameter

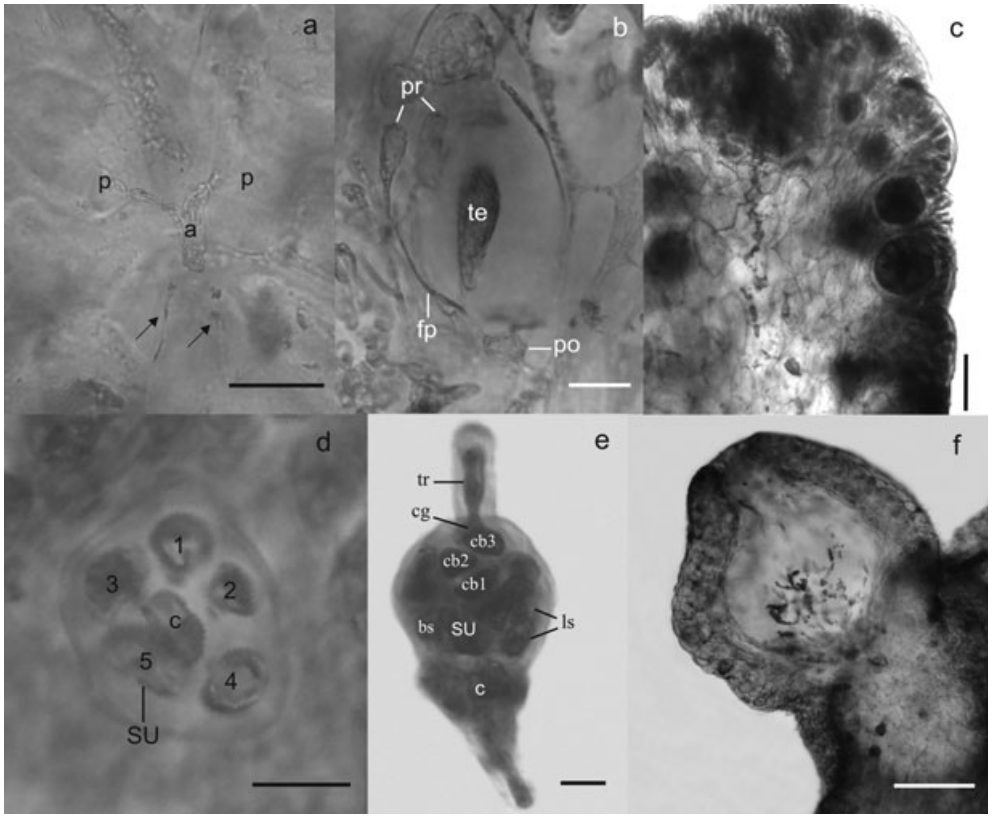

FIG. 4. *Laurencia marilzae* sp. nov. (a) Transverse section near the apex of branchlet showing tetrasporangial axial segment; axial cell (a) with two vegetative pericentral cells (p) and two fertile pericentral cells (arrows). Scale bar, 25 μm. (b) Detail of a fertile pericentral cell (fp) with two presporangial cover cells (pr), the tetrasporangial initial (te), and one postsporangial cover cell (po). Scale bar, 25 μm. (c) Longitudinal section through a tetrasporangial branchlet showing parallel arrangement of the tetrasporangia. Scale bar, 100 μm. (d) Procarp-bearing segment with five pericentral cells, the fourth becoming the supporting cell (su); central cell of procarp-bearing segment (c). Scale, 10 μm. (e) Procarp before fertilization with four-celled carpogonial branch (cb), lateral sterile group (ls), and basal sterile group (bs); carpogonium (cg), trichogyne (tr), supporting cell (su), central cell of procarp-bearing segment (c). Scale bar, 10 μm. (f) Longitudinal section through cystocarp. Scale bar, 200 μm.

FIG. 5. *Laurencia marilzae* sp. nov. thalli in natural habitat growing in the lower intertidal zone, intermingled with other macroalgae.

(Fig. 1c). At the apex of fertile branches, each axial segment produces tetrasporangia from the third and fourth pericentral cells (Fig. 4a). The fertile pericentral cells cutting off two presporangial cover cells distally and the tetrasporangial initial subdistally in abaxial position, and subsequently, one postsporangial cover cell is produced that continues dividing and contributes to produce the corticating system around the tetrasporangium (Fig. 4b). The presporangial cover cells do not divide and display a transverse-type alignment in relation to the fertile axis in surface view. Tetrasporangia in parallel arrangement in relation to fertile branchlets (Fig. 4c). Mature tetrasporangia are tetrahedrally divided, measuring 65–125 μm in diameter.

In female thalli, the procarp forms with five pericentral cells (Fig. 4d), the fifth becoming the supporting cell of a four-celled carpogonial branch, a trichogyne and two groups of sterile cells (Fig. 4e); it undergoes the typical postfertilization process of *Laurencia*. Carposporangia are clavate, 62–160 μm long and 18–30 μm in diameter. Fully developed cystocarps are subconical without protuberant ostiole, 760–1,060 μm in diameter (Fig. 4f).

Habitat: L. marilzae is an annual plant that grows rapidly during winter-spring months and decays in late summer, near lower intertidal zone intermingled with other macroalgae. Plants always occur on overhanging rocks subject to strong wave-action (Fig. 5).

Holotype: Punta del Hidalgo, Northern Tenerife, Canary Islands, Spain, collected on 12.vii.2006 by M. C. Gil-Rodríguez, A. Sentíes & M. T. Fujii, tetrasporophyte. Deposited in TFC Phyc 13129.

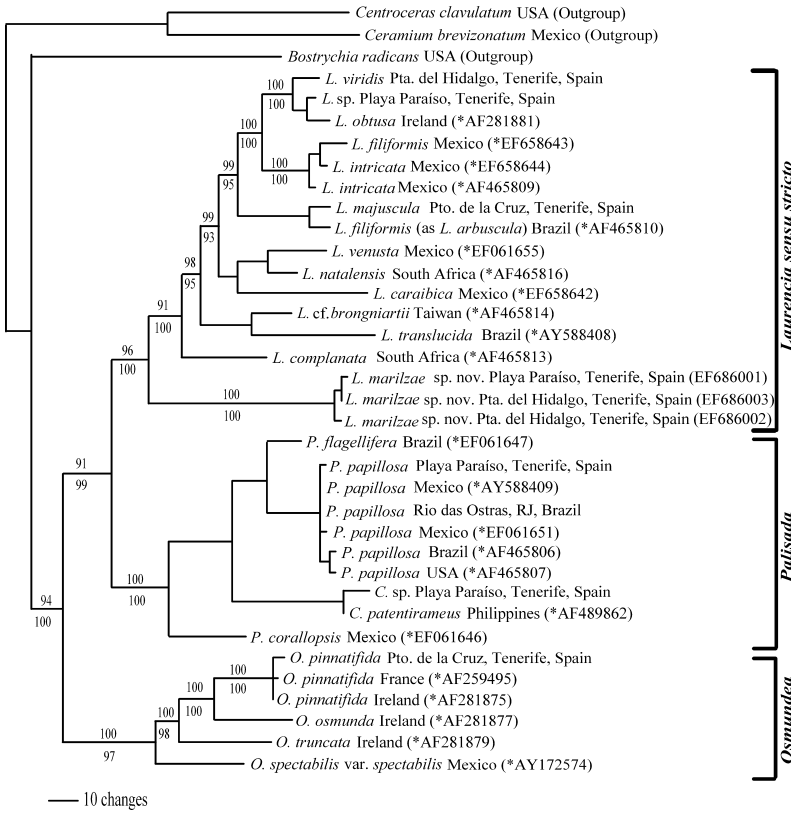

FIG. 6. Phylogenetic relationships of the *Laurencia* complex based on Bayesian analysis of *rbc*L DNA sequences. Fifty percent majority-rule consensus tree sampled after the run reached stationarity at generation 15,800 (total number of generations ran = 3.0×10^6). Evolutionary model used in the Bayesian analysis was the GTR+I+G, selected by a maximum-likelihood ratio test. Bootstrap (above) and Bayesian posterior probabilities (below) values are indicated at the nodes. (*) GenBank sequences.

Isotypes: UAMIZ, SP, HRJ, BM, P, L, BCM, MA.

Etymology: Species named in honor of the late Dr. Marilza Cordeiro-Marino, a Brazilian phycologist of the Instituto de Botânica, for her significant contributions to the knowledge of the *Laurencia* complex.

Molecular analyses: A total of 36 sequences were analyzed including *Ceramium brevizonatum*, *Centroceras clavulatum*, and *Bostrychia radicans* (Montagne) Montagne as outgroups. The topology of majority-rule Bayesian trees is shown in Figure 6.

The analyses show that the *Laurencia* complex is monophyletic with high bootstrap and posterior probabilities values in relation to the members of the outgroups. The *Laurencia* complex was separated into three clades, corresponding to the three genera: *Laurencia, Osmundea,* and *Palisada*. In all the analyses, the earliest diverging clade was *Osmundea,* with *Laurencia* and *Palisada* as sister taxa. The clade that corresponded to *Palisada* included five species: three of *Palisada* (*P. corallopsis*, *P. flagellifera*, and *P. papillosa*) and two species of *Chondrophycus* (*C. patentirameus* and *Chondrophycus* sp.), suggesting that these two later species should be transferred to *Palisada*. The clade that corresponded to the genus *Laurencia* included 13 species. *L. marilzae* forms a strongly supported sister clade to all other *Laurencia* species. There is little genetic variation among the samples of this species analyzed (0.06%–0.2%).

DISCUSSION

The presence of four pericentral cells per axial segment, cortical cells with secondary pit connections, and lack of lenticular thickenings place the new species in the section *Laurencia* (Yamada 1931, Saito 1967, Saito and Womersley 1974).

L. marilzae is morphologically close to *L. majuscula* reported for the Mediterranean Sea (Serio et al. 2000, Furnari et al. 2001) and for Sultanate of Oman (Wynne et al. 2005). Both species share the presence of cortical cells that are projected beyond the surface in apical and subapical portions of the thallus and one *corp en cerise* per cell. However, *L. marilzae* possesses *corps in cerise* in all cells of the thallus, while in *L. majuscula* they occur primarily in cortical cells and in the trichoblasts and rarely in medullary cells. The taxon identified as *L. majuscula* from the Canary Islands by Masuda et al. (1998) clearly differs from the present species by having *corps in cerise* only in superficial cortical cells and trichoblasts, being two, three, or four in cortical cells and one in trichoblasts. The DNA sequence obtained from the sample of *L. majuscula* from the Canary Islands supports the distinction between these species. The morphological and chemical comparison among *Laurencia* species reported for the Canary Islands is shown in Table 1.

The genus *Laurencia* produces principally bromine-containing natural metabolites with sesquiterpene skeletons, although there are several examples of bromine diterpenes as well (Erickson 1983).

TABLE 1. Comparison of *Laurencia marilzae* sp. nov. with morphologically related species reported from the Canary Islands, Spain.

	Vegetative structure				Tetrasporangia	Female structure		Chemical constituents	
Species	Type of attachment	*Corps en cerise* per cell	Cortical cell walls (projection)	Lenticular thickenings	Tetrasporangia P (position)	Procarp-bearing segment	Cystocarp shape	Major secondary metabolites	References
L. intricata	Stolon-like branches	Two-four	Present	Absent	3rd, 4th	With 5P	Ovoid	caespitol (sesquiterpene)	Nam and Saito (1995), Masuda et al. (1998), Furnari et al. (2001)
L. majuscula	Discoid holfast and stolon-like branches	One-four	Present (slight)	Absent	3rd, (2nd), 4th	Unknown	Urceolate	elatol, isoobtusol (sesquiterpenes)	Masuda et al. (1998), Serio et al. (2000)
L. microcladia	Stolon-like branches	One (rarely two or three)	Present	Present	3rd, 4th	Unknown	Urceolate	not available	Furnari et al. (2001)
L. obtusa	Stolon-like branches	One	Absent	Absent	3rd, 4th	With 5P	Ovoid	elatol, obtusol, obtusano, isoobtusol (sesquiterpenes)	Martín et al. (1989), Nam et al. (1994), Furnari et al. (2001)
L. viridis	Stolon-like branches	One	Absent	Absent	Unknown	Unknown	Urceolate	viridianol (sesquiterpene)	Gil-Rodríguez and Haroun (1992), Norte et al. (1994, 1997)
L. marilzae sp. nov.	Discoid holdfast and auxiliary holdfasts	One (in all cells of the thallus)	Present (markedly)	Absent	3rd, 4th	With 5P	Subconical	dactylomelane (diterpene)	Fernández et al. (2005), This study

P, pericentral cells.

All species show the following characters in common: four pericentral cells per axial segment, cortical cells with secondary pit connections, without palisade-like arrangement, tetrasporangia with parallel arrangement, and spermatangial development of trichoblast-type.

Within *Laurencia* complex, *L. marilzae* is the first example with a large number of diterpenes dactylomelane derivatives (Fernández et al. 2005). In contrast, four sesquiterpenes were previously reported for *L. majuscula* from different localities. The sesquiterpenes pacifenol and dehydrochloroprepacifenol were isolated from *L. majuscula* from the Mediterranean Sea (Caccamese et al. 1986, 1987), whereas isoobtusol and elatol were reported for *L. majuscula* from the Gran Canaria (Masuda et al. 1998), differing from the diterpenes reported for *L. marilzae*.

The interspecific divergence values are comparable to those reported by other authors for the genus *Osmundea*. Nam et al. (2000) estimated divergence percentages which varied from 5% (*O. osmunda* vs. *O. pinnatifida*) to 9% (*O. hybrida* vs. *O. truncata*), and McIvor et al. (2002) recorded values of 2% (*O. blinksii* vs. *O. splendens*) to 9% (*O. blinksii* vs. *O. truncata*). Díaz-Larrea et al. (2007) obtained interspecific divergence values for the species of *Laurencia,* which varied from 6% to 8%, whereas those for *Palisada* (as *Chondrophycus*) varied from 6% to 9%.

L. marilzae formed a distinctive and well-supported clade within the *Laurencia* complex. The high levels of genetic variation between the samples of *L. marilzae* from the rest of *Laurencia* species suggest that this species should be assigned to a new taxon within *Laurencia* sensu stricto. As verified by Nam (2006), our results obtained from the *rbc*L analysis confirm the existence of the *Laurencia* complex as a monophyletic clade including *Palisada, Laurencia,* and *Osmundea* separated into clearly defined clades. *Laurencia* and *Palisada* appear as closely related groups, with the separation of the two genera confirmed in our analysis by the presence of four and two periaxial cells per each axial segment, respectively.

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Børgesen, F. 1930. Marine algae of the Canary Islands especially from Tenerife and Gran Canaria. Part III. Ceramiales. *Kongel. Danske Vidensk. Selsk. Biol. Medd.* 9:1–159.

Bory de Saint-Vincent, J. B. G. M. 1803. *Essais sur les îles Fortunées et l'antique Atlantide, ou precis de l'histoire general de l'archipel des Canaries.* Baudouin, Paris, 522 pp., 4 pls., 3 maps.

Caccamese, S., Compagnini, A. & Toscano, R. M. 1986. Pacifenol from the Mediterranean red alga *Laurencia majuscula*. *J. Nat. Prod.* 49:173–4.

Caccamese, S., Compagnini, A., Toscano, R. M., Nicolo, F. & Chapuis, G. 1987. A new labile bromoterpenoid from the red alga *Laurencia majuscula*: dehydrochloroprepacifenol. *Tetrahedron* 43:5393–9.

Da Gama, B. A. P., Carvalho, A. G. V., Coutinho, R. & Yoneshigue-Valentin, Y. 2002. The effects of seaweeds metabolites on biofouling. *Biofouling* 18:13–20.

Díaz-Larrea, J., Sentíes, A., Fujii, M. T., Pedroche, F. F. & Oliveira, M. C. 2007. Molecular evidence for *Chondrophycus poiteaui* var. *gemmiferus* comb. *et* stat. nov. (Ceramiales, Rhodophyta) from the Mexican Caribbean Sea: implications for the taxonomy of the *Laurencia* complex. *Bot. Mar.* 50:250–6.

Erickson, K. L. 1983. Constituents of *Laurencia*. *In* Scheur, P. J. [Ed.] *Marine Natural Products, vol. 5.* Academic Press, New York, pp. 131–257.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–91.

Fernández, J. J., Souto, M. L., Gil, L. V. & Norte, M. 2005. Isolation of naturally occurring dactylomelane metabolites as *Laurencia* constituents. *Tetrahedron* 61:8910–5.

Fernández, J. J., Souto, M. L. & Norte, M. 2000. Marine polyether triterpenes. *Nat. Prod. Rep.* 17:235–46.

Freshwater, D. W. & Rueness, J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia* 33:187–94.

Fujii, M. T., Guimaraes, S. M. P. B., Gurgel, C. F. & Fredericq, S. 2006. Characterization and phylogenetic affinities of the red alga *Chondrophycus flagelliferus* (Rhodomelaceae, Ceramiales) from Brazil on the basis of morphological and molecular evidence. *Phycologia* 45:432–41.

Furnari, G., Cormaci, M. & Serio, D. 2001. The *Laurencia* complex (Rhodophyta, Rhodomelaceae) in the Mediterranean Sea: an overview. *Cryptogam. Algol.* 22:331–73.

Garbary, D. J. & Harper, J. T. 1998. A phylogenetic analysis of the *Laurencia* complex (Rhodomelaceae) of the red algae. *Cryptogam. Algol.* 19:185–200.

Gil-Rodríguez, M. C. & Haroun, R. 1992. *Laurencia viridis* sp. nov. (Ceramiales, Rhodomelaceae) from the Macaronesian Archipelagos. *Bot. Mar.* 35:227–37.

Gil-Rodríguez, M. C. & Haroun, R. 1993. The genus *Laurencia* (Rhodomelaceae, Rhodophyta) in the Canary Islands. *Cour. Forschungsinst. Senckenb.* 159:113–7.

Gil-Rodríguez, M. C. & Haroun, R. 2002. Vegetative and reproductive phenology of *Chondrophycus perforatus* and *Laurencia viridis* (Rhodomelaceae, Rhodophyta) in Tenerife, Canary Islands. *Constancea 83.20* [online publication of the Jepson Herbarium, U. C., Berkeley], http://ucjeps.berkeley.edu/constancea/83/.

Gil-Rodríguez, M. C., Haroun, R., Ojeda, A., Berecibar, E., Domínguez, P. & Herrera, B. 2003. Protoctistas. *In* Moro, L., Martín, J. L., Garrido, M. J. & Izquierdo, I. [Eds.] *Lista de especies marinas de Canarias (algas, hongos, plantas y animales).* Consejería de Política Territorial y Medio Ambiente del Gobierno de Canarias, Tenerife, Canarias, pp. 3–30.

Haroun, R. & Gil-Rodríguez, M. C. 1995. Reproductive strategies of *Laurencia perforata* (Bory) Montagne, (Ceramiales, Rhodomelaceae) in the Canary Islands. *Nova Hedwigia* 61:269–74.

Haroun, R. J., Gil-Rodríguez, M. C., Díaz de Castro, J. & Prud'Homme van Reine, W. F. 2002. A check-list of the marine plants from the Canary Islands (Central Eastern Atlantic Ocean). *Bot. Mar.* 45:139–69.

Haroun, R. & Prud'homme van Reine, W. F. 1993. A biogeographical study of *Laurencia* and *Hypnea* species of the Macaronsian Region. *Cour. Forschungsinst. Senckenb.* 159:119–25.

Hay, M. E. & Fenical, W. 1988. Marine plant herbivore interaction: the ecology of chemical defense. *Annu. Rev. Ecol. Syst.* 19:111–45.

Hay, M. E. & Steinberg, P. D. 1992. The chemical ecology of plant-herbivore interactions in marine versus terrestrial communities. *In* Rosenthal, J. A. & Berenbaum, M. R. [Eds.] *Herbivores: Their Interaction with Secondary Plant Metabolites, Ecological and Evolutionary Process, vol. 2.* Academic Press, San Diego, pp. 372–408.

Hernández-González, M. C. & Gil-Rodríguez, M. C. 1994. Existence of plants bearing tetrasporangia and spermatangial

receptacles (mixed phases) in the genus *Laurencia* Lamouroux (Rhodomelaceae). *Nova Hedwigia* 59:189–94.
Howard, B. M., Nonomura, A. M. & Fenical, W. 1980. Chemotaxonomy in marine algae: secondary metabolite synthesis by *Laurencia* in unialgal culture. *Biochem. Syst. Ecol.* 8:329–36.
Huelsenbeck, J. P. & Ronquist, F. R. 2001. MrBayes. Bayesian inference of phylogeny. *Biometrics* 17:754–5.
Lawson, G. W. & John, D. M. 1982. The marine algae and coastal environment of tropical West Africa. *Nova Hedwigia* 70:1–455.
Lin, S., Fredericq, S. & Hommersand, M. H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *J. Phycol.* 37:881–99.
Maggs, C. A. & Hommersand, M. H. 1993. *Seaweeds of the British Isles. Volume 1. Rhodophyta. Part 3A. Ceramiales.* Natural History Museum, London, 444 pp.
Manríquez, C. P., Souto, M. L., Gavín, J. A., Norte, M. & Fernández, J. J. 2001. Several new squalene-derived triterpenes from *Laurencia. Tetrahedron* 57:3117–23.
Martín, J. D., Caballero, P., Fernández, J. J., Norte, M., Rérez, R. & Rodríguez, M. L. 1989. Metabolites from *Laurencia obtusa. Phytochemistry* 28:3365–7.
Martín, J. D. & Darias, J. 1978. Algal sesquiterpenoids. *In* Scheuer, P. J. [Ed.] *Marine Natural Products, vol. 1.* Academic Press, New York, pp. 125–73.
Masuda, M., Kogame, K., Arisawa, S. & Suzuki, M. 1998. Morphology and halogenated secondary metabolites of three Gran Canarian species of *Laurencia* (Ceramiales, Rhodophyta). *Bot. Mar.* 41:265–77.
McIvor, L., Maggs, C. A., Guiry, M. D. & Hommersand, M. H. 2002. Phylogenetic analysis of the geographically disjunct genus *Osmundea* Stackhouse (Rhodomelaceae, Rhodophyta). *Constancea 83.9* [online publication of the Jepson Herbarium, U.C., Berkeley], http://ucjeps.berkeley.edu/constancea/83/mcivor_etal/osmundea.html.
Montagne, C. 1840. Plantes Cellulaires. *In* Webb, P. B. & Berthelot, S. [Eds.] (1835–1850). *Histoire Naturelle des îles Canaries. Phytographia Canariensis.* Tomo III (Parte 2, Sección 4). Bethune, Paris, 208 pp.
Nam, K. W. 1999. Morphology of *Chondrophycus undulatus* and *C. parvipapillata* and its implications for the taxonomy of the *Laurencia* (Ceramiales, Rhodophyta) complex. *Eur. J. Phycol.* 34:455–68.
Nam, K. W. 2006. Phylogenetic re-evaluation of the *Laurencia* complex (Rhodophyta) with a description of *L. succulenta* sp. nov. from Korea. *J. Appl. Phycol* 18:679–97.
Nam, K. W. 2007. Validation of the generic name *Palisada* (Rhodomelaceae, Rhodophyta). *Algae* 22:53–5.
Nam, K. W., Maggs, C. A. & Garbary, D. J. 1994. Resurrection of the genus *Osmundea* with an emendation of the generic delineation of *Laurencia* (Ceramiales, Rhodophyta). *Phycologia* 33:384–95.
Nam, K. W., Maggs, C. A., McIvor, L. & Stanhope, M. J. 2000. Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *J. Phycol.* 36:759–72.
Nam, K. W. & Saito, Y. 1995. Vegetative and reproductive anatomy of some *Laurencia* (Ceramiales, Rhodophyta) species with a description of *L. maris-rubri* sp. nov. from the Red Sea. *Phycologia* 34:157–65.
Neto, N., Tittley, I. & Raposeiro, P. M. 2005. *Flora marinha do litoral dos Açores.* Secretaria Regional do Ambiente e do Mar. Nova Gráfica, SNESup, San Miguel, Azores, 148 pp.
Norte, M., Fernández, J. J. & Souto, M. L. 1994. Viridianol, a rearranged sesquiterpene with a novel carbon skeleton from *Laurencia viridis. Tetrahedron Lett.* 35:4607–10.
Norte, M., Fernández, J. J. & Souto, M. L. 1996. Viridiols, two new diterpenes from *Laurencia viridis. Nat. Prod. Lett.* 8:263–9.
Norte, M., Fernández, J. J. & Souto, M. L. 1997. New polyether squalene derivatives from *Laurencia. Tetrahedron* 5:4649–54.
Norte, M., Fernández, J. J. & Souto, M. L. 1998. Evaluation of the cytotoxic activity of polyethers isolated from *Laurencia. Bioorg. Med. Chem.* 6:2237–43.
Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.
Saito, Y. 1967. Studies on Japanese species of *Laurencia*, with special reference to their comparative morphology. *Mem. Fac. Fish. Hokkaido Univ.* 15:1–81.
Saito, Y. & Womersley, H. B. S. 1974. The southern Australian species of *Laurencia* (Ceramiales, Rhodophyta). *Aust. J. Bot.* 22:815–74.
Salgado, L. T., Viana, N, Andrade, L. R., Leal, R. N., Da Gama, B., Attias, M., Pereira, R. C. & Amado Filho, G. M. 2008. Intracellular storage, transport and exocytosis of halogenated compounds in marine red alga *Laurencia obtusa. J. Struct. Biol.* 162:345–55.
Schnetter, R., Gil-Rodríguez, M. C., Medina, M., Döring, J., Henne, K.-D. & Hernández-González, M. C. 2000. Distinction of gametophytes and tetrasporophytes by microfluorometry in *Osmundea pinnatifida* (Rhodophyta) from Canary Islands. *Phycologia* 39:147–52.
Serio, D., Cormaci, M. & Furnari, G. 2000. *Laurencia majuscula* (Harvey) Lucas (Rhodophyta, Rhodomelaceae): a little known, but probably widely distributed species in the Mediterranean Sea. *Biol. Mar. Mediterranea* 7:737–40.
Swofford, D. L. 2002. *PAUP. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates, Sunderland, Massachusetts.
Teixeira, V. T. 2002. Produtos naturais marinhos. *In* Pereira, R. C. & Soares-Gomes, A. [Eds.] *Biologia Marinha.* Interciência, Engenho Novo, pp. 249–79.
Tsuda, R. T. & Abbott, I. A. 1985. Collecting, handling, preservation and logistics. *In* Littler, M. M. & Littler, D. S. [Eds.] *Handbook of Phycological Methods, Ecological Field Methods: Macroalgae, vol. IV.* Cambridge University Press, Cambridge, UK, pp. 67–86.
Womersley, H. B. S. 1984. *The Marine Benthic Flora of Southern Australia. Part I.* Government Printer, South Australia, Adelaide, 329 pp., 102 figs., 16 plates.
Wynne, M. J., Serio, D., Cormaci, M. & Furnari, G. 2005. The species of *Chondrophycus* and *Laurencia* (Rhodomelaceae, Ceramiales) occurring in Dhofar, the Sultanate of Oman. *Phycologia* 44:497–509.
Yamada, Y. 1931. Notes on *Laurencia*, with special reference to the Japanese species. *Univ. Calif. Publ. Bot.* 16:185–310.
Young, D. N., Howard, B. M. & Fenical, W. 1980. Subcellular localization of brominated secondary metabolites in the red alga *Laurencia snyderae. J. Phycol.* 16:182–5.

Supplementary Material

The following supplementary material is available for this article:

Table S1. Samples used for the phylogenetic analysis.

Appendix S1. Specimens examined morphologically.

This material is available as part of the online article.