CHONDROPHYCUS ANABELIAE AND *LAURENCIA DIGITATA* (CERAMIALES, RHODOPHYTA) ARE RECORDED FOR THE FIRST TIME FOR VENEZUELA EXPANDING THEIR GEOGRAPHIC DISTRIBUTIONS BEYOND THE TYPE LOCALITIES

CHONDROPHYCUS ANABELIAE Y *LAURENCIA DIGITATA* (CERAMIALES, RHODOPHYTA) SE REGISTRAN POR PRIMERA VEZ PARA VENEZUELA EXPANDIENDO SUS DISTRIBUCIONES GEOGRÁFICAS MÁS ALLÁ DE LAS LOCALIDADES TIPO

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Abstract

Background: Over the course of approximately 12 years, the species of the *Laurencia* complex have been systematically studied in the tropical and subtropical Atlantic Ocean, showing high diversity (48 species), which has been underestimated for the coast of Venezuela.

Questions: What is the species diversity of the *Laurencia* complex in Venezuela?

Studied species: *Chondrophycus anabeliae*, *Laurencia digitata*.

Study site and dates: Cayo Muerto, Parque Nacional Morrocoy, Estado Falcón, Venezuela, 2015.

Methods: For molecular studies, the plastid *rbcL* gene and the mitochondrial DNA barcode marker COI-5P were used, combined with the study of current morpho-anatomical characters used for the identification of the species of the complex.

Results: The occurrence of *Chondrophycus*, as currently circumscribed, was confirmed for the first time for Venezuela. *Chondrophycus anabeliae* and *Laurencia digitata* are reported for the first time beyond the type localities. Tetrasporophytes are described for the first time for *L. digitata*.

Conclusions: Our findings expand the geographic distribution of *Ch. anabeliae* and *L. digitata* for the Venezuelan Caribbean and the Atlantic Ocean, respectively.

Keywords: COI-5P, phylogeny, *rbcL*, Rhodomelaceae, taxonomy.

Resumen

Antecedentes: A lo largo de aproximadamente 12 años se han estudiado de manera sistemática a las especies del complejo *Laurencia* en el Océano Atlántico tropical y subtropical, evidenciando una alta diversidad (48 especies), misma que se ha subestimado para la costa de Venezuela.

Preguntas: ¿Cuál es la diversidad de especies del complejo *Laurencia* en Venezuela?

Especies de estudio: *Chondrophycus anabeliae*, *Laurencia digitata*.

Sitio y años de estudio: Cayo Muerto, Parque Nacional Morrocoy, Estado Falcón, Venezuela, 2015.

Métodos: Para los estudios moleculares, se utilizaron el gen del plástido *rbcL* y el marcador mitocondrial del código de barras de ADN, COI-5P, combinado con el estudio de los caracteres morfoanatómicos actuales utilizados para la identificación de las especies del complejo.

Resultados: La presencia del género *Chondrophycus*, como se circunscribe actualmente, se confirmó por primera vez para Venezuela. *Chondrophycus anabeliae* y *Laurencia digitata* se registran por primera vez fuera de sus localidades tipo. Los tetrasporofitos se describen por primera vez para *L. digitata*.

Conclusiones: Nuestros hallazgos amplían la distribución geográfica de *Ch. anabeliae* y *L. digitata* para el Caribe venezolano y el Océano Atlántico, respectivamente.

Palabras clave: COI-5P, filogenia, *rbcL*, Rhodomelaceae, taxonomía.

The *Laurencia* complex encompasses an extremely diverse group of marine red macroalgae in which new species and genera have been defined in the last three decades (Nam *et al.* 1994, Garbary & Harper 1998, Nam 2007, Martin-Lescanne *et al.* 2010, Cassano *et al.* 2012b, 2019, Metti *et al.* 2015, Machin-Sánchez *et al.* 2016, Senties *et al.* 2016, 2019, Francis *et al.* 2017, Rousseau *et al.* 2017, Collado-Vides *et al.* 2018, Bibi *et al.* 2019). The complex is comprised of 394 species, of these, 211 have been taxonomically accepted at this time (Guiry & Guiry 2020), distributed in eight genera: *Laurencia sensu stricto* J.V.Lamouroux, *Osmundea* Stackhouse, *Chondrophycus* (Tokida & Y.Saito) Garbary & J.T.Harper, *Palisada* K.W.Nam, *Yuzurua* (K.W.Nam) Martin-Lescanne, *Laurenciella* Cassano, Gil-Rodríguez, Senties, Díaz-Larrea, M.C.Oliveira & M.T.Fujii, *Corynecladia* J.Agardh, and *Ohelopapa* F.Rousseau, Martin-Lescanne, Payri & L.Le Gall. *Laurencia s.s.* is the most speciose genus of the complex, with 138 species accepted taxonomically around the world (Guiry & Guiry 2020), whereas *Chondrophycus* is much less representative, with 16 species accepted taxonomically, mostly cited for the Indo-Pacific Ocean. *Chondrophycus anabeliae* Senties, M.T.Fujii, Cassano & Dreckmann is the only species of the genus reported for the western Atlantic Ocean (Senties *et al.* 2016, Wynne 2017).

Venezuela is an important area of occurrence of species of the *Laurencia* complex in the Atlantic. To date, 16 species and one variety have been reported: nine of *Laurencia*, four of *Palisada*, two of *Osmundea* and one of *Yuzurua*. However, the genera *Chondrophycus* (as currently circumscribed), *Corynecladia*, *Laurenciella* and *Ohelopapa* were not mentioned in the region (Rodríguez de Ríos 1979, Ganesan 1989, Gómez *et al.* 2020). Of the species cited for Venezuela, some are rare or endemic. *Laurencia foldatsii* N.Rodríguez Ríos is endemic to Venezuela (Rodríguez de Ríos 1981, Hernández *et al.* 2017); *Laurencia gracilis* J.D. Hooker & Harvey [as *Laurencia filiformis* J.D.Hooker & Harvey *nom. illeg.*] is rare in the Atlantic; apart from Venezuela it is quoted only for the Indo-Pacific; and *Osmundea pinnatifida* (Hudson) Stackhouse and *Osmundea oederi* (Gunnerus) G.Furnari (= *Laurencia bolivarii* N.Rodríguez Ríos) are rare in the western Atlantic. The citations of the *Laurencia* complex species in Venezuela are mostly based on morpho-anatomical characters; only *Laurencia natalensis* Kylin was studied based on molecular data (García-Soto & Lopéz-Bautista 2019).

During our study of the *Laurencia* complex in the tropical and subtropical Atlantic, we identified two species not yet reported for the Venezuelan coast using molecular markers, *rbcl* and COI-5P, combined with morphological data. Our findings confirm the occurrence of the genus *Chondrophycus* for Venezuela, expanding the geographic distribution of *Ch. anabeliae* beyond the type locality, and of *Laurencia digitata* Francis, Bolton, Mattio & R.J. Anderson to the Atlantic Ocean.

Materials and methods

Samples of *Chondrophycus anabeliae* and *Laurencia digitata* were collected in Cayo Muerto, Parque Nacional Morrocoy, Estado Falcón, Venezuela (10° 55' 48.08" N, 68° 15' 31.73" W) in 2015. For each sample, small fragments of the thallus were dried in silica gel for molecular analyses, and the remaining material was preserved in 4 % formalin-seawater or pressed as herbarium vouchers for morphological studies. For morphological examination, transverse and longitudinal hand sections were stained with 0.5 % aqueous aniline blue and acidified with 1N HCl. For each specimen studied, a minimum of 20 measurements of each morphometric character were made. Measurements are given as length × diameter. Images of whole specimens were taken with a Sony W5 digital camera (Sony, Tokyo, Japan), and details of branches and branchlets were captured with Sony W5 coupled to a Stemi SV 6-Zeiss stereomicroscope (Zeiss, Göttingen, Germany). Microscopic diagnostic features were taken with the Sony W5 coupled to a Nikon Eclipse E-200 optical microscope (Nikon, Tokyo, Japan). Voucher specimens were deposited in the herbaria of University of São Paulo (SPF) and Botanical Institute, São Paulo (SP), University of Carabobo, Venezuela (LUC), and Metropolitan Autonomous University (UAMIZ). Abbreviations follow Index Herbariorum (Thiers 2020).

For molecular studies, DNA extraction followed manufacturer's instructions of the DNeasy Plant Mini Kit (Qiagen, Valencia, USA). PCR protocols for both markers followed Cassano *et al.* (2019), using for *rbcl* the following pairs of primers: *FrbcLstart*-R492, F492-R1150 and F993-RrbcS (Freshwater & Rueness 1994), and for COI-5P the primer pair GAZF1-GAZR1 (Saunders 2005). For PCR amplification was used the PCR Master Mix (Promega, Madison, Wisconsin USA) in a final volume of 25 µl. The reactions were performed in a Techne TC-4000 thermocycler (Bibby Scientific, Staffordshire, UK). All PCR products were analyzed by electrophoresis in 1 % agarose to check product size and were purified with MicroSpinTMS-300 HR Columns (GE Healthcare Life Sciences, Piscataway, New Jersey, USA) as per manufacturer's instructions.

Sequencing reactions were made using the same PCR primers mentioned above, and the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, California, USA) on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Consensus sequences and multiple sequence alignments for both *rbcl* and COI-5P were generated using BioEdit 7.0.4.1 software (Hall 1999). Multiple sequence alignment for *rbcl* consisted of 93 sequences, including three newly generated sequences from Cayo Muerto, Venezuela; the remaining

sequences were downloaded from GenBank ([Appendix 1](#)). Three Rhodomelacean species were used as outgroups, *Chondria acrorhizophora* Setchell & N.L.Gardner, *Chondria collinsiana* M.Howe, and *Chondria dasyphylla* (Woodward) C.Agardh ([Appendix 1](#)). Neighbor-joining (NJ) analysis was conducted in PAUP v4.0 beta10 ([Swofford 2002](#)) with 2,000 bootstrap replicates. The most appropriate model of sequence evolution for maximum likelihood (ML) and Bayesian inference (BI) was selected using jModeltest v2.1.10 ([Darriba et al. 2012](#)) under the Akaike information criterion (AIC) as implemented on the online server CIPRES Science Gateway v3.3 ([Miller et al. 2010](#)). The model selected was the general-time-reversible model of nucleotide substitution with invariant sites and gamma-distributed rates for the variable sites (GTR+I+G). Maximum likelihood (ML) analysis was performed using IQ-Tree v1.4.3 ([Nguyen et al. 2015](#)) with 1,000 bootstrap replicates on the IQ-Tree web portal. BI analysis was performed using MrBayes v3.2.2 ([Ronquist et al. 2012](#)). For BI analysis, two runs with four MCMC chains (one hot and three cold) were conducted with 4,000,000 generations and sampling every 1,000 generations, starting with a random tree. The first 100,000 generations in both runs were discarded as burn-in to build the consensus tree.

Multiple sequence alignment for COI-5P consisted of 69 sequences, including three newly generated sequences; the remaining were downloaded from GenBank ([Appendix 1](#)). One Rhodomelacean species was used as outgroup, *Chondria baileyana* (Montagne) Harvey ([Appendix 1](#)). The neighbor-joining (NJ) analysis was conducted in PAUP with 2,000 bootstrap replicates. Intra- and interspecific divergence values of *rbcL* and COI-5P were calculated using uncorrected “p” distances in PAUP.

Results

Molecular study. The *rbcL* final alignment included 93 sequences of 1,448 bp in length.

The *Chondrophycus* clade had full support ([Figure 1](#)). The two *rbcL* sequences of *Chondrophycus anabeliae* from Venezuela were identical and formed a subclade with *Ch. anabeliae* from Mexico, its type locality, with high to moderate supports ([Figure 1](#)), diverging by 1.34 %. This subclade was sister to *Chondrophycus* sp. 3 from New Caledonia plus *Chondrophycus* sp. from Australia from which it diverged by 5.17-5.9 %. The subclade formed by *Chondrophycus* cf. *undulatus* (Yamada) Garbary & J.T.Harper, *Ch.* sp.1, *Ch.* sp. 2 from New Caledonia, *Chondrophycus doyti* (Y.Saito) K.W.Nam from Hawaii, and *Chondrophycus tronoi* (E.Ganzon-Fortes) K.W.Nam from Philippines diverged by 1.86 % (*Ch.* sp.1 vs *Ch.* cf. *undulatus*) to 6.4 % (*Ch. doyti* vs *Ch. tronoi*). The interspecific divergence within *Chondrophycus* genus

ranging from 1.86 % to 7.8 % (*Ch. anabeliae* from Mexico vs *Ch.* sp.1 from New Caledonia).

Laurencia s.s. clade was highly supported ([Figure 1](#)). The *rbcL* sequence of *L. digitata* from Venezuela joined with *L. digitata* from South Africa, its type locality, with high to moderate support, diverging by 0.85 %. *Laurencia digitata* is sister to *Laurencia* cf. *kuetzingii* A.J.K.Millar from New Caledonia, diverging by 2.14-2.19 %. This subclade was resolved as sister to *Laurencia pumila* (Grunow) Papenfuss plus *L. pumila* var. *dehoopiensis* Francis, Bolton, Mattio & R.J.Anderson from South Africa plus *Laurencia karachiana* Bibi, Cassano & Rasheed from Pakistan with high to moderate supports. The interspecific divergence between sequences of *L. digitata* and *L. pumila* plus *L. pumila* var. *dehoopiensis* was 4.36-4.89 %, whereas *L. digitata* diverged from *L. karachiana* by 4.7-4.9 %.

The COI-5P final alignment included 69 sequences of 644 bp in length ([Figure 2](#)). We were unable to obtain COI-5P sequence for *L. digitata* from Venezuela due to contamination, even after several amplification attempts. The two identical COI-5P sequences of *Ch. anabeliae* from Venezuela joined to the sequence of *Ch. anabeliae* from the type locality generated in this study (MN597440), diverging only by 0.3 % ([Figure 2](#)). This subgroup joined to *Chondrophycus succisus* (A.B.Cribb) K.W.Nam (as *Laurencia succisa* A.B.Cribb) from Molokai (Hawaii, USA) with 5.4-5.7 % of divergence. The subgroup formed by one sequence of *Ch. doyti* and four of *Ch.* cf. *undulatus*, all from the Hawaiian Islands, showed low genetic divergence (0.16-0.48 %) suggesting that these samples represent the same taxonomic entity. The divergence between these two subgroups (*Ch. anabeliae*-*Ch. succisus* and *Ch. doyti*-*Ch.* cf. *undulatus*) ranging from 8.13 % to 8.76 %.

Morphological study. *Chondrophycus anabeliae* [Senties, M.T.Fujii, Cassano & Dreckmann in Senties et al. 2016: 261, figures 1-18.](#) ([Figures 3A, 4A-E, 5A-D, 6A-D](#))

Type locality. Mexico, Quintana Roo, Isla Mujeres, Garrafón de Castilla; holotype UAMIZ 1240!

Description. Plants forming erect tufts up to 5 cm high, reddish-brown to yellowish-brown, terete to partially compressed axes ([Figures 3A, 4A](#)), cartilaginous in texture, not adhering to herbarium paper when dried. Thalli attached to the substratum by a discoid holdfast. Erect branches irregularly alternate and spirally arranged, usually with 2-3 (4) orders of branches. The main axes are terete and first-order branches partially compressed mainly in the upper third portion of the thalli, up to 2,000 µm in diameter, and slightly narrowing towards the terete apices. Ultimate branchlets cylindrical to clavate and truncate at the apices, 500-2,800 µm long and 525-1,000 µm in diameter.

Chondrophycus anabeliae and *Laurencia digitata* from Venezuela

Figure 1. Consensus tree derived from Maximum likelihood (ML) analyses of *rbcL* sequences. Bootstrap supports for NJ (2000 replicates)/ML (1000 replicates)/posterior probabilities, PP < 0.95 are given at the nodes. Sequence generated in this study in bold; - indicates lack of bootstrap support or values under 70; * indicates full support. Outgroups were removed from the figure only for better ingroups viewing.

Figure 2. NJ analysis for COI-5P sequences. Bootstrap values (2000 replicates) are shown at nodes; values under 70 were not considered. Sequence generated in this study in bold.

Figure 3. A) Habit of *Chondrophyucus anabeliae*. B) Habit of *Laurencia digitata*. Living materials. Scale bar: A and B, 1 cm.

In surface view, the outermost cortical cells are translucent, isodiametric-polygonal in the middle portions, $17.5\text{--}32.5 \times 17.2\text{--}37.5 \mu\text{m}$ without secondary pit connections (Figure 4B). Subcortical cells are pigmented, larger and connected to each other by secondary pit connections (Figure 4C). Outermost cortical cells translucent and subcortical cells present 1 (-2) crystals per cell (Figure 4C, D). In transverse section, thalli formed by two cortical cell layers, and four or five layers of medullary cells (Figure 4E). The cortical cells of translucent outer layer are quadratic, cuneiform to rectangular, smaller than the inner layer cells, measuring $17.5\text{--}30 \times 22.5\text{--}30 \mu\text{m}$ in the ultimate branchlets (Figure 5A), and elongated, $47.5\text{--}55 \times 27.5\text{--}35 \mu\text{m}$ in the middle portions of main axes. The inner layer of cortical cells is composed of pigmented and elongated cells, measuring $40\text{--}65 \times 35\text{--}50 \mu\text{m}$ (Figure 5B) in the middle portions of main axes. Medullary cells are rounded or slightly radially elongated, measuring $65\text{--}145 \times 47.5\text{--}107.5 \mu\text{m}$ in the middle portions of the main axes. Medullary cell walls uniformly thickened, but lenticular wall thickenings are absent. Each vegetative axial segment cuts off two pericentral cells (Figure 5C) that are slightly smaller than the medullary cells of the surrounding layer. In median longitudinal sections through a branchlet, the outer cortical cell walls near the apices are markedly projecting beyond the surface (Figure 5D).

Tetrasporangial branchlets are cylindrical or slightly compressed, simple or compound, $500\text{--}1,500 \times 575\text{--}825 \mu\text{m}$ (Figure 6A). At the apex of fertile branches, each axial segment produces one fertile additional pericentral cell situated oppositely to the pre-existing two pericentral cells which remain vegetative (Figure 6B). The additional cell cuts off two pre-sporangial cover cells distally abaxially positioned in relation to the tetrasporangial initial (Figure 6C). Subsequently, one post-sporangial cover cell is produced and continues to divide, contributing to cortication around the tetrasporangia. Tetrasporangia are

arranged in a right-angled pattern in relation to fertile branchlets (Figure 6D). Mature tetrasporangia are tetrahedrally divided, $50\text{--}100 \mu\text{m}$ in diameter. Gametangia were not observed.

Examined material. Venezuela. Estado Falcón: Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, tetrasporophyte, S. Ardito, M.T. Fujii, A. Senties, V. Cassano (SPF58487, SP470468, LUC7611, UAMIZ 1405). GenBank accession number for *rbcL* (MN597441, MN597442) and for COI-5P (MN597438, MN597439).

Distribution and habitat. This species is currently recorded only for Mexico (Senties et al. 2016), and Venezuela (this study). Epilithic specimens were collected growing in shallow waters on rocky coastline, northeast of Cayo Muerto. The environment is considered as an intertidal zone with medium-sized rocks and moderately strong waves.

Laurencia digitata Francis, Bolton, Mattio & R.J. Anderson in Francis et al. 2017: 812, Figure 5. (Figures 3B, 7A-F, 8A-D)

Type locality. South Africa, KwaZulu-Natal, Cape Vidal; holotype BOL150572.

Description. Plants forming small, very intricate cushion-like tufts, up to 5 cm high, yellowish-brown, terete, cartilaginous in texture, adhering to herbarium paper when dried (Figure 3B). Thallus attached to the substratum by a discoid holdfast, and basal descending branches. Erect branches irregularly alternate and spirally arranged, with up to 3 orders of branches (Figure 7A). Main axes $275\text{--}525 \mu\text{m}$ in diameter in middle portion of the thallus. Ultimate branchlets are cylindrical to clavate with truncated tips, $452\text{--}950 \times 225\text{--}325 \mu\text{m}$ in diameter.

Figure 4. *Chondrophycus anabeliae*. A) Part of branches of a sterile plant. B) Surface view of translucent cortical cells. C) Surface view of subcortical cells. Note secondary pit connections between subcortical cells (arrow) and crystals (arrowhead). D) Surface view of translucent cortical cells showing one crystal per cell. E) Transverse section of the thallus. Scale bar: A, 1 cm; B and D, 25 μ m; C, 50 μ m; E, 100 μ m.

Figure 5. *Chondrophycus anabeliae*. A) Detail of the two-layered cortical cells of ultimate branchlets. Note small translucent outer cortical cells and large pigmented inner cortical cells with secondary pit connections between them. B) Detail of the two-layered cortical cells of main axes in the middle portions of the thallus. Note elongated translucent outer cortical cells. C) Transverse section of upper portion of branch showing an axial cell (a) with two pericentral cells (p). D) Longitudinal section through to a branchlet showing projecting cortical cells. Scale bar: A, D, 50 μ m; B and C, 25 μ m.

In surface view, cortical cells have 1 (-2) *corps en cerise* per cell (Figure 7B). Cortical cells are arranged regularly in longitudinal rows and connected to each other by longitudinally oriented secondary pit-connections (Figure 7C). Cortical cells are rounded to polygonal and slightly longitudinally elongated in middle portions of main axes, 37.5-57.5 $\times$ 27.5-50 μ m. In transverse section, the thallus has 1-2 layers of pigmented cortical cells and 3-4 layers of hyaline medullary cells (Figure 7D). Cortical cells are quadrate, cuneiform to rectangular, not arranged as a palisade, and 25-35 $\times$ 22.5-37.5 μ m in the middle portions of thalli. Medullary cells are rounded to slightly radially elongated, and 55-90 $\times$ 42.5-52.5 μ m, gradually increasing in size toward the center of the thallus. Each vegetative axial segment cut off four pericentral cells slightly larger than the other surrounding cells (Figure 7E). In median longitudinal sections through a branchlet, the outer cortical cell walls near the apices projecting beyond the surface (Figure 7F). Lenticular thickening absent.

Tetrasporangial branchlets are cylindrical, simple or compound, 575-2,125 $\times$ 225-400 μ m (Figure 8A). At the apex of fertile branches, each axial segment produces one fertile pericentral cell, the fourth ones (Figure 8B), the other

pericentral cells remain sterile. Fertile pericentral cell cuts off two pre-sporangial cover cells distally abaxially positioned in relation to the tetrasporangial initial (Figure 8D). Subsequently, one post-sporangial cover cell is produced and continues to divide, contributing to cortication around the tetrasporangia. Tetrasporangia are arranged in a parallel pattern in relation to fertile branchlets (Figure 8A, C). Mature tetrasporangia are tetrahedrally divided, 47.5-75 μ m in diameter. Gametangia were not observed.

Material examined. Venezuela. Estado Falc3n: Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, tetrasporophyte, S. Ardito, M.T. Fujii, A. Senties, V. Cassano (SPF58488, SP470469, UAMIZ 1406). GenBank accession number for *rbcL* (MN597443).

Distribution and habitat. This species is currently recorded only for South Africa (Francis *et al.* 2017) and Venezuela (this study). *Laurencia digitata* was collected as drift specimens in shallow waters on rocky coastline, northeast of Cayo Muerto. The environment is considered as an intertidal zone with medium-sized rocks and moderately strong waves.

Figure 6. *Chondrophycus anabeliae*. A) Detail of tetrasporangial branches. B) Transverse section of tetrasporangial axial segment showing an axial cell (arrow) and two vegetative pericentral cells (p); an additional third fertile pericentral cell is formed in the opposite position (arrowhead). C) Detail of fertile pericentral cell (fp) with two pre-sporangial cover cells (pr), tetrasporangium (te, out of focus); post-sporangial cover cell not shown. D) Longitudinal section through tetrasporangial branchlet showing right-angle arrangement of the tetrasporangia. Scale bar: A, 3 mm; B and C, 25 µm; D, 100 µm.

Discussion

Comparison of *rbcL* and COI-5P sequences of *Ch. anabeliae* from the type locality (Mexico) with our material confirmed the occurrence of this species on the Venezuelan coast, whereas *L. digitata* was confirmed only by comparison with *rbcL* sequence from the type locality (South Africa), since we were unable to generate COI-5P sequences and there are also no sequences available of this marker in databases.

Considering our results for *rbcL*, intraspecific value between the Venezuelan and South African *L. digitata* (0.85 %) is within the range observed in previous works for *Laurencia* s.s. (0-1.35 %) reported by Cassano *et al.* (2012a, b), Metti *et al.* (2013), and Collado-Vides *et al.* (2018). Interspecific divergences for the *rbcL* gene between *L. digitata* and *Laurencia* species closest molecularly (i.e., *L. cf. kuetzingii*, *L. pumila*, *L. pumila* var. *dehoopiensis*, and *L. karachiana*, 2.14-4.9 %) were within the variation observed for *Laurencia* s.s. by Cassano *et al.* (2012b, 1.0-6.8 %) and Cassano *et al.* (2019, 2.4-3.7 %).

There are no data available in the literature for the *rbcL* gene to compare the intraspecific divergence for *Chondrophycus*. However, the intraspecific value obtained for *Ch. anabeliae* (1.34 %) is below the interspecific variation range described for the genus by Cassano *et al.* (2012b, 1.8-6.9 %), by Senties *et al.* (2016, 3.4-7.8 %), and that found in this study (1.86-7.8 %).

For COI-5P gene, the intraspecific divergence values reported for the *Laurencia* complex are low, not exceeding 1 %. The lowest range of intraspecific divergence was observed in this study (0-0.3 %) for samples of *Ch. anabeliae* from the Caribbean Sea (Venezuela and Mexico), whereas divergences up to 0.52 % were reported by Machín-Sánchez *et al.* (2014) for *Laurencia*, up to 0.67 % by Machín-Sánchez *et al.* (2016) for *Osmundea*, and up to 0.7 % by Cassano *et al.* (2012b), Machín-Sánchez *et al.* (2014), and Collado-Vides *et al.* (2018) for *Laurenciella*. The interspecific divergence obtained in this study for COI-5P (5.4-8.76 %) is within the range found for other genera of the complex, i.e. for *Laurenciella* species (7.4-9.2 %, Collado-Vides *et al.* 2018), and for *Laurencia* species (2.6-10.2 %, Cassano *et al.* 2019).

Figure 7. *Laurencia digitata*. A) Part of branches of a sterile plant. B) Surface view of cortical cells showing *corpus en cerise* in living material. C) Surface view of cortical cells showing secondary pit-connections (arrow). D) Transverse section of the thallus. E) Transverse section of upper portion of branch showing an axial cell (a) with four pericentral cells (p). F) Longitudinal section through a branchlet showing projecting cortical cells. Scale bar: A, 3 mm; B and C, 25 µm; D, 100 µm; E and F, 50 µm.

Figure 8. *Laurencia digitata* A) Detail of tetrasporangial branches. B) Transverse section of two superimposed tetrasporangial segments showing an axial cell (a) and one fertile pericentral cell, the fourth (arrow); the other pericentral cells (p) remain sterile (pit connections between axial and pericentral cells, out of focus). C) Longitudinal section through a tetrasporangial branchlet showing parallel arrangement of tetrasporangia. D) Detail of fertile pericentral cell (fp) with two pre-sporangial cover cells (pr, only one visualized), tetrasporangium (te); post-sporangial cover cell not shown. Scale bar: A, 500 µm; B and D, 25 µm; C, 40 µm.

Morphologically, Venezuelan *Ch. anabeliae* shares all diagnostic characters described for the species by [Senties *et al.* \(2016\)](#), such as: (i) thallus slightly compressed; (ii) two cortical cell layers, the outmost layer formed by translucent cells with conspicuous cell wall projections near the apices and absence of secondary pit connections, and the inner layer formed by pigmented and pit connected cells; and (iii) tetrasporangia produced from one additional fertile pericentral cell. The presence of 1 (-2) crystals per cortical and subcortical cells is a characteristic unique for *Ch. anabeliae* from Venezuela; they were not found in Mexican *Ch. anabeliae* and were not described for other *Chondrophyucus* species.

Venezuelan and South African *Laurencia digitata* share the cushion-like tufted habit and absence of lenticular thickenings. However, the South African material differed from ours by color (reddish-brown), branching pattern

(alternate but subopposite and/or subverticillate at the tips of some branches), and absence of cortical cell walls projections near the apices ([Francis *et al.* 2017](#)). The tetrasporophyte has typical features of the genus *Laurencia* and is described for the first time for this species.

Chondrophyucus anabeliae seems to be restricted to the Caribbean Sea so far. The range of distribution of *Ch. anabeliae* represents the limits from the western to the southeast Caribbean, whereas *L. digitata*, previously considered endemic to South Africa by [Francis *et al.* \(2017\)](#), presents a disjunct distribution in the Indian and Atlantic Oceans. The disjunct distribution of *L. digitata* is similar to that of *L. natalensis* whose occurrence was recorded only for the Indian Ocean, nevertheless with wider distribution [South Africa, Mozambique, Kenya, Mauritius, Sri Lanka and Vietnam ([Guiry & Guiry 2020](#))], and it was cited for Venezuela by [García-Soto & López-Bautista](#)

(2019). However, more studies of biogeography and phylogeography are needed in the area, which will allow us to explain this further.

Although macroalgal floristic studies have been carried out for the Venezuelan coast (e.g., Gómez *et al.* 2017), the diversity for this region is still underestimated. Similar underestimations have already been reported for other groups of red algae (e.g., Adey *et al.* 2015, Basso *et al.* 2015, Hind *et al.* 2015, Ardito *et al.* 2017, Núñez-Resendiz *et al.* 2018). For this reason, it is necessary to continue performing floristic surveys that incorporate molecular-assisted alpha taxonomy to accurately identify all and potential new species from this region, especially for ecologically and economically important taxonomic groups such as those included in the *Laurencia* complex.

The use of *rbcL* gene for phylogenetic inference, and the COI-5P barcode marker for species delimitation allied to morphological study revealed the presence of two species of the *Laurencia* complex for Venezuela, *Chondrophycus anabeliae* and *Laurencia digitata*. Both constitute new records for the region and their first occurrence outside the type localities. Our findings expand the geographic distribution of *Ch. anabeliae* to the southeast of the Caribbean Sea, where the species seems to be restricted so far, whereas the first report of *L. digitata* for the Atlantic Ocean established a disjunct distribution of this species in the Atlantic and Indian Oceans.

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Literature cited

- Adey WH, Hernandez-Kantun JJ, Johnson G, Gabrielson, PW. 2015. DNA sequencing, anatomy, and calcification patterns support a monophyletic, subarctic, carbonate reef-forming *Clathromorphum* (Hapalidiaceae, Corallinales, Rhodophyta). *Journal of Phycology* **51**: 189-203. DOI: <https://doi.org/10.1111/jpy.12266>
- Ardito S, Núñez-Resendiz ML, Dreckmann KM, Senties A. 2017. *Gracilaria falconii* sp. nov. (Gracilariales, Rhodophyta): a new species with flat axes from Venezuela. *Phytotaxa* **292**: 271-278. DOI: <https://doi.org/10.11646/phytotaxa.292.3.7>
- Basso D, Caragnano A., Le Gall L, Rodondi G. 2015. The genus *Lithophyllum* in the north-western Indian Ocean, with description of *L. yemenense* sp. nov., *L. socotraense* sp. nov., *L. subplicatum* comb. et stat. nov., and the resumed *L. affine*, *L. kaiseri*, and *L. subreduncum* (Rhodophyta, Corallinales). *Phytotaxa* **208**: 183-200. DOI: <http://dx.doi.org/10.11646/phytotaxa.208.3.1>
- Bibi R, Cassano V, Medeiros RD, Rashid S, Rasheed M. 2019. Morphological and molecular systematic investigation of *Laurencia karachiana* sp. nov. (Ceramiales, Rhodophyta) from Karachi, Pakistan. *Phytotaxa* **404**: 23-40. DOI: <http://dx.doi.org/10.11646/phytotaxa.404.1.3>
- Cassano V, Metti Y, Millar AJK, Gil-Rodríguez MC, Senties A, Díaz-Larrea J, Oliveira MC, Fujii MT. 2012a. Redefining the taxonomic status of *Laurencia dendroidea* (Ceramiales, Rhodophyta) from Brazil and the Canary Islands. *European Journal of Phycology* **47**: 67-81. DOI: <https://doi.org/10.1080/09670262.2011.647334>
- Cassano V, Oliveira MC, Gil-Rodríguez MC, Senties A, Díaz-Larrea J, Fujii MT. 2012b. Molecular support for the establishment of the new genus *Laurenciella* within the *Laurencia* complex (Ceramiales, Rhodophyta). *Botanica Marina* **55**: 349-357. DOI: <https://doi.org/10.1515/bot-2012-0133>
- Cassano V, Santos GN, Santos EM, Nunes JMC, Oliveira MC, Fujii MT. 2019. *Laurencia longiramea* sp. nov. for Brazil and an emendation of the generic delineation of *Corynecladia* (Ceramiales, Rhodophyta). *Phycologia* **58**: 115-127. DOI: <https://doi.org/10.1080/00318884.2018.1523519>
- Collado-Vides L, Cassano V, Santos GN, Senties A, Fujii MT. 2018. Molecular and morphological characterization of *Laurencia intricata* and *Laurenciella mayaimii* sp. nov. (Ceramiales, Rhodophyta) in South Florida, USA. *Phycologia* **57**: 287-297. DOI: <https://doi.org/10.2216/17-80.1>
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModeltest2: more models, new heuristics and parallel computing. *Nature Methods*. **9**: 772. DOI: <https://doi.org/10.1038/nmeth.2109>
- Francis C, Bolton JJ, Mattio L, Mandiwana-Neudani TG, Anderson RJ. 2017. Molecular systematic reveals increased diversity within the South Africa *Laurencia* complex (Rhodomelaceae, Rhodophyta). *Journal of Phycology*. **53**: 804-819. DOI: <https://doi.org/10.1111/jpy.12543>

- Freshwater DW, Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* **33**: 187-194. DOI: <https://doi.org/10.2216/i0031-8884-33-3-187.1>
- Ganesan EK. 1989. *A Catalog of benthic marine algae and seagrasses of Venezuela*. Fondo Editorial CONICIT, Caracas, Venezuela.
- Garbary D, Harper JT. 1998. A phylogenetic analysis of the *Laurencia* complex (Rhodomelaceae) of the red algae. *Cryptogamie Algologie* **19**: 185-200.
- García-Soto G, López-Bautista JM. 2019. *Laurencia natalensis* (Ceramiales, Rhodophyta): a new record for the Atlantic Ocean. *Caribbean Journal of Science* **49**: 201-208. DOI: <https://doi.org/10.18475/cjos.v49i2.a9>
- Gómez S, García M, Carballo Y, Gil N. 2017. *Macroalgas béticas del Parque Nacional Archipiélago Los Roques, Venezuela. Guía ilustrada*. Venezuela, Caracas, Distrito Capital: Sello Editorial Ediciencias, UCV. ISBN: 978-980-00-2859-9
- Gómez S, Carballo YB, García M, Gil N. eds. 2020. Web Ficoflora Venezuela. Catálogo Digital de la Ficoflora de Venezuela. Publicación electrónica. Caracas, Venezuela: Universidad Central de Venezuela, <http://www.ciens.ucv.ve/ficofloravenezuela/> (accessed April 06, 2020).
- Guiry MD, Guiry GM. 2020. *AlgaeBase. World-wide electronic publication*. National University of Ireland, Galway. <http://www.algaebase.org/> (accessed March 07, 2020).
- Hall TA. 1990. BioEdit: a user-friendly biological alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.
- Hernández OE, Senties A, Dreckmann KM, Cassano V, Fujii MT. 2017. Species diversity and biogeographical patterns of *Laurencia sensu stricto* (Rhodophyta) in the Atlantic Ocean. *Hidrobiologica* **27**: 301-314. DOI: <https://doi.org/10.24275/uam/izt/dcbs/hidro/2017v27n3/Hernandez>
- Hind KR, Miller KA, Young M, Jensen C, Gabrielson PW, Martone PT. 2015. Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA. *American Journal of Botany* **102**: 1-19. DOI: <https://doi.org/10.3732/ajb.1500308>
- Machín-Sánchez M, Le Gall L, Neto AI, Rousseau F, Cassano V, Senties A, Fujii MT, Díaz-Larrea J, Prud'homme van Reine W, Bonillo C, Gil-Rodríguez MC. 2014. A combined barcode and morphological approach to the systematics and biogeography of *Laurencia pyramidalis* and *Laurenciella marilzae* (Rhodophyta). *European Journal of Phycology* **49**: 115-127. DOI: <https://doi.org/10.1080/09670262.2014.893017>
- Machín-Sánchez M, Rousseau F, Le Gall L, Cassano V, Neto A, Senties A, Fujii MT, Gil-Rodríguez MC. 2016. Species diversity of the genus *Osmundea* (Rhodophyta, Ceramiales) in the Macaronesian Region. *Journal of Phycology* **52**: 664-681. DOI: <https://doi.org/10.1111/jpy.12431>
- Martin-Lescanne J, Rousseau F, De-Reviers B, Payri C, Couloux A, Cruaud C, Le Gall L. 2010. Phylogenetic analyses of the *Laurencia* complex (Rhodomelaceae, Ceramiales) support recognition of five genera: *Chondrophycus*, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua* stat. nov. *European Journal of Phycology* **45**: 51-61. DOI: <https://doi.org/10.1080/09670260903314292>
- Metti Y, Millar AJK, Cassano V, Fujii MT. 2013. Australian *Laurencia majuscula* (Rhodophyta, Rhodomelaceae) and the Brazilian *Laurencia dendroidea* are conspecific. *Phycological Research* **61**: 98-104. DOI: <https://doi.org/10.1111/pre.12009>
- Metti Y, Millar AJK, Steinberg P. 2015. A new molecular phylogeny of the *Laurencia* complex (Rhodophyta, Rhodomelaceae) and a review of key morphological characters result in a new genus, *Coronaphycus*, and a description of *C. novus*. *Journal of Phycology* **51**: 929-942. DOI: <https://doi.org/10.1111/jpy.12333>
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA: The Institute of Electrical and Electronics Engineers (IEEE), pp. 1-8. DOI: <https://doi.org/10.1109/GCE.2010.5676129>
- Nam KW. 2007. Validation of the generic name *Palisada* (Rhodomelaceae, Rhodophyta). *Algae* **22**: 53-55. DOI: <https://doi.org/10.4490/ALGAE.2007.22.2.053>
- Nam KW, Maggs CA, Garbary DJ. 1994. Resurrection of the genus *Osmundea* with an emendation of the generic delineation of *Laurencia* (Ceramiales, Rhodophyta). *Phycologia* **33**: 384-395. DOI: <https://doi.org/10.2216/i0031-8884-33-5-384.1>
- Nguyen LT, Schmidt HA, Von-Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268-274. DOI: <https://doi.org/10.1093/molbev/msu300>
- Núñez-Resendiz ML, Ardito S, León-Tejera H, Dreckmann KM, Senties A. 2018. *Solieria incurvata* (Solieriaceae, Rhodophyta), a new species from Venezuela based on morpho-anatomical and molecular evidence. *Botanica Marina* **61**: 383-393. DOI: <https://doi.org/10.1515/bot-2018-0010>

- Rodríguez de Ríos N. 1979. El género *Laurencia* Lamouroux en Venezuela. Maracay, Venezuela: Promotional Report, Facultad de Agronomía, Universidad Central de Venezuela.
- Rodríguez de Ríos N. 1981. Dos especies nuevas de *Laurencia* (Rhodophyta, Ceramiales). *Ernstia* **2**: 1-11.
- Ronquist F, Teslenko M, Mark P, Ayres DL, Darling A, Hohna A, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model choice across a large model space. *Systematic Biology* **61**: 539-542. DOI: <https://doi.org/10.1093/sysbio/sys029>
- Rousseau F, Gey D, Kurihara A, Maggs CA, Martin-Lescanne J, Payri C, De Reviers B, Sherwood A, Le Gall L. 2017. Molecular phylogenies support taxonomic revision of three species of *Laurencia* (Rhodomelaceae, Rhodophyta), with the description of a new genus. *European Journal of Taxonomy* **269**: 1-19. DOI: <https://doi.org/10.5852/ejt.2017.269>
- Saunders GW. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of Royal Society of Biological Sciences* **360**: 1879-1888. DOI: <https://doi.org/10.1098/rstb.2005.1719>
- Sentías A, Cassano V, Dreckmann KM, Gil-Rodríguez MC, Stein EM, Fujii MT. 2016. *Chondrophycus anabeliae* (Rhodomelaceae, Ceramiales), a new species in the *Laurencia* complex from the Mexican Caribbean. *Phytotaxa* **283**: 259-270. DOI: <http://dx.doi.org/10.11646/phytotaxa.283.3.2>
- Sentías A, Dreckmann KM, Hernández OE, Núñez-Resendiz ML, Le Gall L, Cassano V. 2019. Diversity and distribution of *Laurencia sensu stricto* (Rhodomelaceae, Rhodophyta) from the Mexican Pacific, including *L. mutuea* sp. nov. *Phycological Research* **67**: 1-12. DOI: <https://doi.org/10.1111/pre.12382>
- Swofford D. 2002. PAUP 4.0 b10: *Phylogenetic analysis using parsimony*. Massachusetts, USA: Sinauer Associates, Sunderland.
- Thiers B. 2020. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://www.nybg.org/bsci/ih/ih.html> (accessed September 16, 2019).
- Wynne MJ. 2017. A checklist of benthic marine algae of the tropical and subtropical Western Atlantic: fourth revision. *Nova Hedwigia Beiheft* **145**: 1-202. ISBN: 978-3-443-51067-1

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Appendix 1. Taxa used in this study for molecular analysis.

Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>Chondria baileyana</i> (Montagne) Harvey	Canada, Nova Scotia, Pomquet (far on Monks Head Road), 16 Aug. 2012, G.W. Saunders, A. Savoie, C. Longtin, K. Dixon, M. Bruce	KU564345	-
<i>C. californica</i> (Collins) Kylin	USA, California, San Diego Co., Beach Club Reef (La Jolla Shores), 1 Jul. 1996, M. Volovsek	-	AY172578
<i>C. collinsiana</i> M.A. Howe	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula	-	GU330225
<i>C. dasyphylla</i> (Woodward) C. Agardh	USA, North Carolina, Carteret Co., Bogue Sound	-	U04021
<i>Chondrophycus anabeliae</i> Senties, M.T.Fujii, Cassano & Dreckmann	Mexico, Quintana Roo, Isla Mujeres, Garrafón de Castilla, 12 Feb. 2007, A. Senties, M.C. Gil-Rodríguez	MN597440	KX815262
<i>Ch. anabeliae</i>	Venezuela, Estado Falcón, Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, S. Ardito, M.T. Fujii, A. Senties, V. Cassano	MN597438 MN597439	MN597441 MN597442
<i>Ch. dotyi</i> (Y. Saito) K.W. Nam	USA, Hawaii	HQ423050	-
<i>Ch. dotyi</i>	USA, Hawaii, Oahu, Sandy Beach, 31 May 2015, E.M. Stein	-	KX815263
<i>Ch. cf. undulatus</i> (Yamada) Garbary & J.T. Harper	New Caledonia, Loyalty Is., Maré, 22 Mar. 2005, C. Payri	-	FJ785307
<i>Ch. cf. undulatus</i>	USA, Maui, 12 Dec. 2007	GU223886	-
<i>Ch. cf. undulatus</i>	USA, Hawaii	HQ422752 HQ423055 HQ422996	-
<i>Ch. succisus</i> (A.B. Cribb) K.W. Nam	USA, Molokai, 11 Feb. 2007	GU223884	-
<i>Ch. tranoi</i> (E. Ganzon-Fortes) K.W. Nam	Philippines, A.O. Lluisma	-	AF489864
<i>Ch. sp.</i>	Australia, Norfolk Island, Collins Head, 21 Mar. 2005, Y. Metti, A. Millar		KY120337
<i>Ch. sp. 1</i>	New Caledonia, Loyalty Is., Lifou, 26 Mar. 2005, C. Payri	-	FJ785309
<i>Ch. sp. 2</i>	New Caledonia, Loyalty Is., Maré, 21 Mar. 2005, C. Payri	-	FJ785310
<i>Ch. sp. 3</i>	New Caledonia, Loyalty Is., Beautemps/Beaupré, 06 Apr. 2005, C. Payri	-	FJ785311
<i>Corynecladia clavata</i> J. Agardh	Australia, Victoria, Walkerville, 20 Jan. 2015, P. Díaz-Tapia, M. Brookes	-	MF094079
<i>C. clavata</i>	Australia, Victoria, The Caves, 21 Jan. 2015, P. Díaz-Tapia, M. Brookes	MH704456	-
<i>C. clavata</i> (as Ceramiales sp.)	Australia, Tasmania, 24 Jan. 2004, G.W. Saunders, R. Withall	HM915955	-
<i>C. elata</i> (C. Agardh) Cassano, M.C. Oliveira & M.T. Fujii	Australia, Western Australia, Rottnest Island, 15 Nov. 2008, J. Eu	-	KY120339
<i>C. nova</i> (Metti) Cassano, M.C. Oliveira & M.T. Fujii	Australia, NSW, Jervis Bay, Plantation Point, 15 Feb. 2005, Y. Metti, A. Millar	-	KY120340
<i>Laurencia aldingensis</i>	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula	-	JF810351
<i>L. alfredensis</i> Francis, Bolton, Mattio & Anderson	South Africa, 04 Jul. 2008, R.J. Anderson, J.J. Bolton	-	KY927749
<i>L. brachyclados</i> Pilger	USA, Hawaii	HQ423046	-

Chondrophycus anabeliae and *Laurencia digitata* from Venezuela

Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>L. cf. brongiartii</i> J. Agardh	Australia, Tarcoola Beach, 1996, S. Fredericq	-	EF061654
<i>L. caduciramulosa</i> Masuda & Kawaguchi	Brazil, Rio de Janeiro, Angra dos Reis, Praia do Velho, 19 Apr. 2006, V. Cassano, J.C. De-Paula	-	KJ700865
<i>L. caraibica</i> P.C. Silva	Mexico, Quitana Roo, Cancún, Isla Mujeres, 2006, A. Senties	-	EF658642
<i>L. caraibica</i>	Venezuela, Falcon, Cabo San Roman, 06 Oct. 2012, G. Garcia-Soto		MH388533
<i>L. catarinensis</i> Cordeiro-Marino & M.T. Fujii	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 02 Jun. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez	-	KF492781
<i>L. catarinensis</i>	Spain, Canary Islands, Lanzarote, Pechiguerras, 15 Jan. 2013, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492718	-
<i>L. complanata</i> (Suhr) Kützing	South Africa, 09 Dec. 2010, R.J. Anderson, J.J. Bolton	-	KY927738
<i>L. corymbosa</i> J. Agardh	South Africa, 19 Aug. 2008, R.J. Anderson, J.J. Bolton	-	KY927760
<i>L. dendroidea</i> J. Agardh	Brazil, Bahia, Lauro de Freitas, Praia Vilas do Atlântico, 08 Jan. 2008, A. Oliveira	-	GU330228
<i>L. dendroidea</i>	Venezuela, Falcon, Playa Buchuacos, 06 Oct. 2012, G. Garcia-Soto	-	MH388528
<i>L. dendroidea</i> (as <i>L. majuscula</i>)	Australia, NSW, Kiama Harbour, North side, 03 Apr. 2004, Y. Metti, D. Williams	-	-
<i>L. dendroidea</i>	Spain, Canary Islands, La Gomera, Punta de La Dama, 21 Sept. 2009, E. Aylagas, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492725	-
<i>L. dendroidea</i>	Spain, Canary Islands, Lanzarote, Pechiguerras, 15 Jan. 2013, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492728	-
<i>L. dendroidea</i> (as <i>L. majuscula</i>)	USA, Molokai, 10 Feb. 2007	GU223887	-
<i>L. dichotoma</i> Francis, Bolton, Mattio & Anderson	South Africa, 22 Mar. 2011, J.J. Bolton	-	KY927786
<i>L. digitata</i> Francis, Bolton, Mattio & Anderson	South Africa, 04 Jul. 2008, R.J. Anderson, J.J. Bolton	-	KY927748
<i>L. digitata</i>	Venezuela, Estado Falcón, Parque Nacional Morrocoy, Cayo Muerto, 19 May 2015, S. Ardito, M.T. Fujii, A. Senties, V. Cassano	-	MN597443
<i>L. flexuosa</i> Kützing	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq	-	AF465815
<i>L.cf. flexuosa</i>	South Africa, Eastern Cape Province, 15 Jun. 2003, O. De Clerck	KX258821	-
<i>L. filiformis</i> (C. Agardh) Montagne	Western Australia, Geraldton, Tarcoola Beach, 21 Sept. 1995, M.H. Hommersand, F.H. Hommersand	-	MH704449
<i>L. galtsoffii</i> M.A. Howe	USA, Hawaii	HQ422984	-
<i>L. glomerata</i> (Kützing) Kützing	South Africa, 03 Mar. 2009, R.J. Anderson, J.J. Bolton	-	KY927763
<i>L. heteroclada</i> Harvey f. <i>decussata</i> Cribb	Australia, NSW, Arrawarra headland, 28 Jul. 2004, Y. Metti	-	KY120344
<i>L. intricata</i> J.V. Lamouroux	Cuba, Ciego de Ávila, Cayo Coco, 25 Sept. 2005, M.T. Fujii	-	GU330238
<i>L. intricata</i>	USA, Florida, Long Key, Channel 5 (ocean side), 10 Dec. 1998, B. Wysor, T. Frankovich	-	AY588410
<i>L. karachiana</i> Bibi, Cassano & Rasheed	Pakistan, Karachi, French Beach (Buleji), 13 Aug. 2018, R. Bibi	MK796229	MK796228
<i>L. cf. kuetzingii</i> A. Millar	New Caledonia, Loyalty Is., Ouvéa, 31 Mar. 2005, C. Payri	-	FJ785322

Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>L. laurahuertana</i> Mateo-Cid, Mendoza-González, Senties & Díaz-Larrea	Mexico, Quintana Roo, Punta Herrero, 12 Apr. 2012, A.C. Mendoza González, L.E. Mateo-Cid	-	KF279401
<i>L. longiramea</i> Cassano, G.N. Santos, J.M.C. Nunes, M.C. Oliveira & M.T. Fujii	Brazil, Espírito Santo, Anchieta, Ilhote de Ubu, 30 Jun. 2007, E.M. Stein	MH704454	-
<i>L. longiramea</i>	Brazil, Rio de Janeiro, Armação dos Búzios, Praia Rasa, 13 Jan. 2005, V. Cassano, J.C. De-Paula	MH704455	MH704451
<i>L. cf. majuscula</i> (Harvey) A.H.S. Lucas	Oman, Dhofar, Sept. 2001, M. Wynne	-	KX146184
<i>L. cf. mariannensis</i> Yamada	New Caledonia, Lagon Sud-Ouest, Ilot Larégnère, 11 Jul. 2003, C. Payri	-	FJ785313
<i>L. mcdermidiae</i> I.A. Abbott	USA, Oahu, 08 Apr. 2007	GU223877	-
<i>L. mcdermidiae</i>	New Caledonia, Ile des Pins, 09 Nov. 2005, C. Payri	-	FJ785314
<i>L. multiclavata</i> Francis, Bolton, Mattio & Anderson	South Africa, 29 Mar. 2010, R.J. Anderson	-	KY927766
<i>L. mutuae</i> Senties, Cassano & Dreckmann	Mexico, Guerrero, Acapulco, Isla la Roqueta, 07 Jun. 2017, A. Senties, K.M. Dreckmann	MK182532	MK159179
<i>L. natalensis</i> Kylin	South Africa, S. KwaZulu-Natal, Palm Beach, 07 Feb. 2001, S. Fredericq	-	AF465816
<i>L. natalensis</i>	Venezuela, Falcon, Cabo San Roman, 06 Oct. 2012, G. Garcia-Soto		MH388523
<i>L. nidifica</i> J. Agardh	USA, Hawaii	HQ422750	-
<i>L. nidifica</i>	USA, Hawaii	HQ422751	
<i>L. nidifica</i>	USA, Hawaii	HQ423054	-
<i>L. cf. nidifica</i>	New Caledonia, Ile des Pins, 30 Nov. 2005, C. Payri	-	FJ785315
<i>L. nipponica</i>	Russia, Sakhalin, 23 Jun. 2003	GU223874	-
<i>L. obtusa</i> (Hudson) J.V. Lamouroux	Ireland, County Donegal, Fanad Head, C.A. Maggs	-	AF281881
<i>L. obtusa</i>	France, Languedoc-Roussillon, Pyrenees-Orientales, Cap Beart, Banyuls-sur-Mer, 11 Jul. 2007, L. Bittner	KX258828	-
<i>L. oliveirana</i> Yoneshigue	Brazil, Rio de Janeiro, Arraial do Cabo, Ponta da Cabeça, Praia Grande, 07 Jul. 2008, V. Cassano, J.C. De-Paula	-	JF810352
<i>L. pacifica</i> Kylin	USA, California, Stillwater Cove, Pebble Beach, 20 May 2010, B. Clarkston, K. Hind, S. Toews	KM254466	-
<i>L. pacifica</i>	USA, California, Moss Beach, Central Beach, 17 Feb. 1992, S. Fredericq	-	AY588411
<i>L. pumila</i> (Grunow) Papenfuss	South Africa, 10 Jun. 2009, R.J. Anderson, J.J. Bolton	-	KY927765
<i>L. pumila</i> var. <i>dehoopiensis</i> Francis, Bolton, Mattio & Anderson	South Africa, 19 Aug. 2008, R.J. Anderson	-	KY927757
<i>L. pyramidalis</i> Bory ex Kützing	France, Brittany, Roscoff, 05 Dec. 2002, F. Rousseau	-	FJ785316
<i>L. pyramidalis</i>	Portugal, Madeira, Seixal, Praia da Laje, 07 Jul. 2011, E. Nogueira, V. Cassano, A. Senties	KF492733	-
<i>L. pyramidalis</i>	Portugal, Madeira, Porto Muniz, 07 Jul. 2011, M.T. Fujii, A. Neto, M. Machín-Sánchez	KF492739	-

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Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>L. pyramidalis</i>	Portugal, Azores, São Miguel, Mosteiros, 27 Jun. 2011, M.T. Fujii, A. Prestes, A. Pacheco, M. Machín-Sánchez	KF492751	-
<i>L. pyramidalis</i>	Spain, Canary Islands, Fuerteventura, Garcey, 10 Sept. 2012, M. Machín-Sánchez	KF492756	-
<i>L. pyramidalis</i>	Spain, Canary Islands, Tenerife, Puerto de La Cruz, 20 May 2011, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492746	-
<i>L. rigida</i> J. Agardh	Australia, NSW, Botany Bay, 11 May 2000, G.C. Zuccarello, J.A. West	-	AY920852
<i>L. saitoi</i> Perestenko	USA, California, Monterey, McAbee Beach, 21 May 2010, B. Clarkston, K. Hind, S. Toews	KM254876	-
<i>L. snackeyi</i> (Weber van Bosse) M. Masuda	Unspecified	MF093985	-
<i>L. sodwaniensis</i> Francis, Bolton, Mattio & Anderson	South Africa, 22 Mar. 2011, C.M. Francis	-	KY927790
<i>L. stegengae</i> (Stegenga, Bolton & Anderson) Francis, Bolton, Mattio & Anderson	South Africa, 18 Mar. 2010, R.J. Anderson	-	KY927743
<i>L. tasmanica</i> J.D. Hooker & Harvey	Australia, Victoria, Tween Reef, between Cape Paterson and Inverloch, P. Díaz-Tapia	-	MF094081
<i>L. venusta</i> Yamada	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 2004, J. Díaz-Larrea, A. Senties	-	EF061655
<i>L. viridis</i> Gil-Rodríguez & Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 06 Oct. 2005, M.C. Gil-Rodríguez	-	EF685999
<i>L. viridis</i>	Spain, Canary Islands, Fuerteventura, El Cutillo, 07 Mar. 2011, M.C. Gil-Rodríguez	KF492757	-
<i>L. viridis</i>	Portugal, Azores, Santa Maria, Boca de Ribeira Seca, 02 Jul. 2011, M.T. Fujii, A. Neto, J. Pombo, M. Machín-Sánchez	KF492760	-
" <i>L. yamadana</i> " M.A. Howe	USA, HI, Maui, Kihei, 05 Apr. 2006, A.L. Carlile, J.R. Waaland	-	GQ252550
<i>Laurencia</i> sp.	New Caledonia, Loyalty Islands, Maré, 21 Mar. 2005, C. Payri	KX258820	KX146182
<i>Laurencia</i> sp.	New Caledonia, Loyalty Islands, Maré, 19 Mar. 2005, C. Payri	KX258819	-
<i>Laurencia</i> sp.	USA, Oahu	GU223889 GU223891 GU223893 GU223894	-
<i>Laurencia</i> sp.	USA, Lanai	GU223892	-
<i>Laurencia</i> sp.	Sri Lanka, Odayapiti lagoon, 08 Nov. 2006, E. Coppejans	KX258826	-
<i>Laurencia</i> sp.1	Australia, Victoria, Mallacoota, H. Verbruggen, K. Dixon	-	MK125344
<i>Laurencia</i> sp.2	Australia, Victoria, Mallacoota, H. Verbruggen, K. Dixon	-	MK125395
<i>Laurenciella marilzae</i> (Gil-Rodríguez <i>et al.</i>) Gil-Rodríguez <i>et al.</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 12 Jul. 2006, M.C. Gil-Rodríguez, M.T. Fujii, A. Senties	-	EF686002
<i>La. marilzae</i>	Spain, Canary Islands, Lanzarote, Pechiguerras, 15 Jan. 2013, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492762	-

Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>La. marilzae</i>	Portugal, Azores, São Miguel, Cerco da Caloura-Baía, E. Nogueira, V. Cassano, A. Senties	KF492765	-
<i>La. marilzae</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492769	-
<i>La. marilzae</i>	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 Mar. 2007, R. Rocha-Jorge	KF270693	GU938189
<i>La. marilzae</i>	Croatia, Scedro, 11 Jun. 2007, J. Utge, L. Le Gall	KX258829	KX146186
<i>La. mayaimii</i> L. Collado-Vides, Cassano & M.T. Fujii	USA, Florida, Biscayne Bay at Deering Estate, 12 Aug. 2013, L. Collado-Vides, V. Cassano, M.T. Fujii	MG004176	-
		MG004177	-
<i>La. mayaimii</i>	USA, Florida, Key Largo, John Pennekamp Park, 14 Aug. 2013, L. Collado-Vides, V. Cassano, M.T. Fujii	MG004178	MG004183
<i>Laurenciella</i> sp.	USA, Florida, Key Biscayne, Crandon Park, 12 Aug. 2013, L. Collado-Vides, V. Cassano, M.T. Fujii	MG004179	MG004184
<i>Ohelopapa flexilis</i> (Setchell) F. Rousseau, Martin-Lescanne, Payri & L. Le Gall	French Polynesia, Tahiti, Tahara reef, 24 Mar. 2007, A. Aphan	KX258830	KX146187
<i>Osmundea blinksii</i> (Hollenberg & I.A. Abbott) K.W. Nam	USA, California, San Mateo Co., Año Nuevo, Greyhound Rock, 17 Jul. 1996, M.H. Hommersand	-	AY172575
<i>O. caspica</i> (Zinova & Zaberzhinskaya) Maggs & L.M. McIvor	Azerbaijan, Sangachal Bay, 01 Sept. 2003	-	KX146188
<i>O. hybrida</i> (A.P. de Candolle) K.W. Nam	France, Brittany, St. Lunaire, 20 Mar. 1999, F. Rousseau	-	FJ785317
<i>O. hybrida</i>	France, Brittany, Roscoff, Finistere, 12 May 2002, F. Rousseau	KX258831	-
<i>O. oederi</i> (Gunnerus) G. Furnari	Ireland, Co. Donegal, St. John's Point, 12 Oct. 1999, C.A. Maggs	-	AF281880
<i>O. osmunda</i> (S.G. Gmelin) K.W. Nam & Maggs	Ireland, Co. Donegal, St. John's Point, 12 Oct. 1999, C.A. Maggs	-	AF281877
<i>O. osmunda</i>	France, Brittany, Le Loup, 19 May 2011, L. Couceiro, M. Robuchon	KJ960875	-
<i>O. pinnatifida</i> (Hudson) Stackhouse	France, Brittany, Penmarch	-	AF259495
<i>O. pinnatifida</i>	France, Brittany, Le Loup, 08 Mar. 2012, L. Couceiro, M. Robuchon	KJ960886	-
<i>O. sanctarum</i> M.T. Fujii & R. Rocha-Jorge	Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 19 Aug. 2012, R. Rocha-Jorge, M.B. Barros-Barreto	-	KC012601
<i>O. sinicola</i> (Setchell & N.L. Gardner) K.W. Nam	USA, California, Orange Co., Crescent Beach, 28 May 2002, S. Murray	-	AY588407
<i>O. spectabilis</i> (Postels & Ruprecht) K.W. Nam var. <i>spectabilis</i>	Mexico, Baja California, Punta Santo Thomas, 02 Jul. 1996, M.H. Hommersand	-	AY172574
<i>O. spectabilis</i>	USA, California, Aquarium Reef, Monterey Bay, 23 May 2010, B. Clarkston, S. Toews	KM254974	-
<i>O. spectabilis</i>	USA, California, Monterey, McAbee Beach, 21 May 2010, B. Clarkston, K. Hind, S. Toews	KM254320	-
<i>O. splendens</i> (Hollenberg) K.W. Nam	Mexico, Baja California, Drift, Bahia Colnett, 02 Jul. 1996, M.H. Hommersand, J. Hughey	-	AY172576
<i>O. splendens</i>	USA, California, Santa Cruz (Four Mile), 19 May 2010, B. Clarkston, K. Hind, S. Toews	KM254377	-

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Samples	Collection data	GenBank accession numbers	
		COI-5P	<i>rbcL</i>
<i>Palisada cervicornis</i> (Harvey) Collado-Vides, Cassano & M.T. Fujii	USA, Florida, Key Largo, Pickles Reef, 14. Aug. 2013, A. Duran	-	MG030375 MG020476
<i>P. corallopsis</i> (Montagne) Senties, M.T. Fujii & Díaz-Larrea	Mexico, Yucatán, Cancún, Chaac Mool Beach, 2005, J. Díaz-Larrea, A. Senties	-	EF061646
<i>P. crustiformans</i> (McDermid) A.R. Sherwood, A. Kurihara & K.W. Nam	USA, Hawaii	HQ422768	-
<i>P. crustiformans</i>	USA, Hawaii, Oahu, Makapuu, 26 May 2007, A. Kurihara	-	KX146196
<i>P. flagellifera</i> (J. Agardh) K.W. Nam	Brazil, Rio de Janeiro, Rio das Ostras, Praia do Cemitério, 03 Aug. 2005, V. Cassano, M.B.B. Barreto	-	GU330227
<i>P. flagellifera</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492772	-
<i>P. furcata</i> (Cordeiro-Marino & M.T. Fujii) Cassano & M.T. Fujii	Brazil, Paraíba, Praia de Tambaú, 24 Feb. 2004, M.T. Fujii	-	GU330226
<i>P. parvipapillata</i> (C.K. Tseng) K.W. Nam	USA, Oahu, 18 Sept. 2007	GU223895	-
<i>P. perforata</i> (Bory) K.W. Nam	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Faro, Bahia Izquierda, 06 Oct. 2005, M. Gil-Rodríguez	-	EU256327
<i>P. perforata</i>	Mexico, Quintana Roo, Isla Mujeres, 2007, A. Senties, M.C. Gil-Rodríguez	-	EF658641
<i>P. perforata</i>	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 13 Jan. 2012, M.C. Gil-Rodríguez, M. Machín-Sánchez	KF492773	-
<i>P. cf. robusta</i> (Yamada) K.W. Nam	New Caledonia, Lifou, 23 Mar. 2005, C. Payri	-	FJ785321
<i>P. yamadana</i> (M.A. Howe) K.W. Nam	USA, Hawaii	HQ422794	-
<i>Palisada</i> sp.	New Caledonia, Ilot Bayes, 01 Jan. 2001, C. Payri	-	KX146194
<i>Yuzurua poiteaui</i> (J.V. Lamouroux) Martin-Lescanne var. <i>gemmifera</i> (Harvey) M.J. Wynne	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 2004, J. Díaz-Larrea, A. Senties	-	EF061648
<i>Y. poiteaui</i> var. <i>gemmifera</i>	Cuba, La Havana, Rincon de Guanabo, 2005, J. Díaz-Larrea, A. Areces	-	EF061650
<i>Y. poiteaui</i> var. <i>poiteaui</i>	USA, Florida, Long Key, Ovan Side, 1998, S. Fredericq	-	EF061652
<i>Y. poiteaui</i> var. <i>poiteaui</i>	Mexico, Quintana Roo, Cancún, Playa del Carmen, 2005, J. Díaz-Larrea, A. Senties	-	EF061653
<i>Yuzurua</i> sp.	West Indies, Guadeloupe, Grand Cul-de-Sac Marin, Chenal Ilet Colas, 03 May 2012, F. Rousseau, Y. Buske, J. Espinosa, M. Snyder, G. Dirberg	KX258843	-