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Morphological and molecular characterization of *Lobophora declerckii* and *L. variegata* (Dictyotales, Ochrophyta) on the Atlantic coast of Mexico

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Abstract

The Veracruz Reef System National Park (PNSAV) is located in the central region of Veracruz, off the coast of the municipalities of Veracruz, Boca del Río and Antón Lizardo. It is a complex and important system within the Gulf of Mexico, since it has been declared a biosphere reserve by UNESCO, a Ramsar wetland and an essential component of the southwestern Gulf of Mexico Reef Corridor. *Lobophora* contains 28 currently recognized species and has a pantropical distribution that includes the Atlantic, Pacific, and Indian Oceans in both hemispheres. Recently, some species have been identified from Western Atlantic Ocean, mainly in the Caribbean Sea. However, very little is known about *Lobophora* species diversity on the Mexican coast. In this study, morphological and molecular analyses (MAAT) using cytochrome c oxidase 3 (COX3) sequences as a barcode gene were used to study *Lobophora* spp. The results indicate that there are two species on the Mexican coasts, *Lobophora declerckii* and *L. variegata*. *Lobophora declerckii* represents a new record for the southwest of the Gulf of Mexico and, the presence of *L. variegata* was confirmed for the Mexican Caribbean.

Keywords: Dictyotaceae, *Lobophora*, new records, COX3, Mexico, Caribbean Sea, Gulf of Mexico

Introduction

Lobophora contains 28 currently recognized species (Guiry & Guiry 2018), with the type species *Lobophora nigrescens* J. Agardh (1894: 23). *Lobophora* has a pantropical distribution that includes the Atlantic, Pacific, and Indian Oceans in both hemispheres (Vieira *et al.* 2017). Recently, some species have been identified employing morphological and molecular criteria from several localities in the Western Atlantic Ocean, mainly in the Caribbean Sea. These species include *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira (1977: 217); *L. guadeloupensis* N.E. Schultz, F. Rousseau & L. Le Gall in Schultz *et al.* (2015: 494); *L. declerckii* N.E. Schultz, C.W. Schneider & L. Le Gall in Schultz *et al.* (2015: 493); *L. canariensis* (Sauvageau) C.W. Vieira, De Clerck & Payri in Vieira *et al.* (2016: 699) and *L. littlerorum* C.W. Schneider, N.E. Schultz & L. Le Gall in Schultz *et al.* (2015: 495).

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The distribution of marine algae in the world has been explained through some biogeographic models based on water temperature. For his part, Lüning (1990) explained these similarities in terms of the phenomena associated with plate tectonics, paleoclimates and changes in sea levels from the Cretaceous, and places the Gulf of Mexico in the warm and tropical temperate region. Earle (1972) pointed out that the algal flora of the Gulf of Mexico has very similar phytogeographic affinities with corals. Briggs (1974) accepted as a reference the studies of corals and indicated the extreme north of Veracruz (Cabo Rojo, 21° 30') as the border that divides two provinces: to the north, subtropical or Carolinian Province, and to the south, the tropical or Caribbean Province. Sherman and Duda (1999), however, classified this region into two large marine ecosystems (LME), while Wilkinson *et al.* (2009) also pointed out two marine ecoregions of the world: Gulf of Mexico (North and South) and the Caribbean Sea. Some studies on the geographic distribution of benthic organisms on the Atlantic coast of Mexico suggest a separation of the biological communities in the Caribbean province: the southwest of the Gulf of Mexico and the Caribbean Sea (Jordán-Dahlgren, 2002; Granados-Barba *et al.*, 2003; Ortíz Lozano *et al.*, 2013). This was also observed in the genus *Lobophora*, in which two endemic species from the Caribbean (*L. guadeloupensis* and *L. littlerorum*) were discovered (Vieira *et al.*, 2016). Historically, a single taxon was recognized in the Gulf of Mexico, *L. variegata* (Taylor 1960); however, further study (morphological and molecular) is needed to ascertain the number of *Lobophora* species on the Mexican coasts.

In light of the recent new information on speciation in *Lobophora* from the western Atlantic Ocean, this project analysed new collections of material from subtidal waters of Veracruz and the Caribbean Sea and used morpho-anatomical and molecular analyses (COX3 gene sequence data) to find or confirm the presence of *Lobophora* on the Mexican coasts of the Gulf of Mexico and the Caribbean Sea.

Material and methods

Collected samples

Samples of *Lobophora* were collected from two localities: the first, in the southwestern Gulf of Mexico (Anegada de Afuera, Veracruz); the second, from the Mexican Caribbean (Puerto Morelos, Quintana Roo). The samples from the Gulf of Mexico were collected during February, March and May of the dry season (2014, 2015) and October of the rainy season (2013). In addition, an exsiccata collected in 2011 from the National Herbarium was also analyzed (NI-547, MEXU).

Microscopy

The samples were preserved in a formaldehyde solution neutralized to 4% in seawater, and the others were processed as herbarium specimens. For the morpho-anatomical observations a Mectron brand cryostat was used, coupled to a Reichart Jung 820 microtome brand with disposable double-edged steel razor blades. The cuts were made 20 µm thick, and the sections were stained with a 1% aniline blue solution and mounted with Karo®-Phenol 70/3% (Tsuda & Abbott 1985). Photographs of the studied material were made with a Zeiss optical microscope, equipped with a digital camera Canon PowerShot G6 and stereoscopic microscope Zeiss Axio Zoom.V.16 motorized for photography with extended focus (multifocus). The photographs were digitized with the programs Axio Vision 4.8.2 and Zeiss Efficient Navigation (ZEN), respectively.

The systematic scheme follows de Reviers *et al.* (2015) and Guiry & Guiry (2018). Dry and liquid samples and permanent preparations with its number identifications (NI) were deposited in the National Herbarium of Mexico (MEXU).

DNA extraction and PCR amplification

Three specimens of *Lobophora* (1-3) were analyzed. The samples were collected from the 'Ana Elena' wreck located in SAV in the Gulf of Mexico. The samples collected in the field were dehydrated in silica-gel, and one fragment of *L. variegata* collected in the State of Quintana Roo (Mexico) was obtained from National Herbarium of Mexico (NI-547, MEXU). DNA extractions were performed using cetyltrimethylammonium bromide, CTAB (Doyle & Doyle 1987) modified by the addition of 2% (w/v) polyvinylpyrridoline (PVP) to the extraction buffer. DNA was purified and precipitated using isopropanol and then washed in 70% ethanol. DNA quantity and quality was evaluated using a Thermo Scientific NanoDrop ND 2000c spectrophotometer (Thermo Fisher Scientific, U.S.A.). Amplification of the COX3 gene was performed using the primers from Vieira *et al.* (2014): COX3-44F (CATCGCCACCCATTTCAT) and COX3-739R (CATCGACAAAATGCCAATACCA) both from 5′to 3′. Amplification was carried out using the Taq PCR Core Kit (Qiagen, Valencia, California), adding 0.5 µL of serum bovine albumin aqueous solution at 0.4% with

the aim of neutralizing inhibitors potential (Kreader 1996). The mix was prepared with the following volumes: 9.39 μL of H_2O, 1.25 μL of Buffer 10 ×, 0.25 μL of dNTP, 0.125 μL of COX3-44F, 0.125 μL of COX3-739R, 0.5 μL of $MgCl_2$, 0.0625 μL of Taq polymerase, and 0.3 μL of DNA. The PCR was cycled in a PCR System 9700 (Applied Biosystems; Foster City, California 94404, USA) under the following conditions: pre-denaturalization at 94°C for 3 minutes, then 35 cycles at 94°C for one minute, 48°C for one minute, 72°C for one minute, and a final extension at 72°C for 5 minutes as previously reported by Vieira *et al.* (2014). PCR products were visualized by electrophoresis on agarose gel at 1% with 1X TBE using GelRed Nucleic Acid Gel Stain (Biotium). The products were sent to the Laboratorio de Secuenciación de la Biodiversidad y la Salud (México City, México) where they were purified and sequenced by a 3100 Genetic Analyzer (Applied Biosystems Inc.) using the same primers employed for PCR.

Phylogenetic analysis

Following the methods in Schultz *et al.* (2015) and Vieira *et al.* (2016), we assembled a data matrix for twenty-nine species, including twenty representatives of *Lobophora*, eight representatives of *Padina*, and one species of *Microzonia* as the outgroup (Table 1). A total of 33 sequences, four sequences newly produced, were aligned in MEGA7 (Kumar *et al.* 2016) and visually inspected and corrected in Mesquite (Maddison & Maddison 2018).

We performed a phylogenetic analysis with maximum likelihood in RAxML 8.2.9 (Stamatakis 2014). A heuristic search was executed with 100 replicates; two data partitions were set following codon positions, one for first and second positions and another for the third position; the GTRCAT model was used on each partition.

Character support values were estimated with a non-parametric bootstrap (Felsenstein 1985) in RAxML 8.2.9 with 500 replicates. Posterior probabilities were approximated in MrBayes 3.2.6 (Ronquist *et al.* 2012) using the CIPRES platform (Miller *et al.* 2015); the nucleotide model for each partition was selected using a reversible jump MCMC method (Huelsenbeck *et al.* 2004). Character rate variation was modeled using the gamma distribution with four categories. We then ran a MCMC for 10 million generations in two independent sets of four chains each, sampling every 1000 steps, with the burn-in set to 25%, and monitoring convergence, mixing and effective sample size for the parameters with in Tracer 1.7 (Rambaut *et al.* 2018). Bootstrap values and posterior probabilities were drawn on the best tree found in the heuristic search using the maximum likelihood algorithm.

Results

Table 2 presents the morphological and anatomical characteristics of the species in this study and compares them with related taxa from the western coast of the Atlantic and the Pacific. The results identified two species of *Lobophora*: *L. declerckii* (Fig. 1) and *L. variegata* (Fig. 2) in the southwestern Gulf of Mexico and Caribbean Sea, respectively. The first represents a new record for Mexico and the southwestern Gulf of Mexico, and the second species is confirmed for the Mexican Caribbean (Fig. 3).

BLAST

The four COX3 sequences produced, 539 bp in length, were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov) and the following results were found: 1 (NI-1792), 2 (NI-1793) and 3 (NI-1793) were found to be identical to *Lobophora declerckii* (KR260317); sequence 4 (NI-547A) from Quintana Roo was identical to *L. variegata* from Guadeloupe (GenBank accession KX061443). The sequences of *Lobophora declerckii* (1, 2 and 3) were deposited in GenBank under the following accession numbers (MH587570, 587571 and 587572, respectively). In addition, the accession number for *Lobophora variegata* (4) was MH587573.

Phylogenetic analysis

The final alignment included 540 characters for 33 representatives. The best tree found after heuristic searches using maximum likelihood had a score of lnL-5377.7544. The Bayesian inference analysis reached convergence after 10 million generations, where the standard deviation between chains was 0.004. Convergence and mixing were satisfactory, and all the parameters had effective sample size of 6902 or above.

Sequences from Mexican specimens of *Lobophora declerckii* and *L. variegata* were recovered in monophyletic clades with reference sequences (KR260317 and KX061443 from Guadeloupe, respectively, Vieira *et al.* 2016) with very high support (Fig. 4) demonstrating the presence of these species in the southwestern Gulf of Mexico and Caribbean Sea. The first represents a new record for Mexico and the southwestern Gulf of Mexico, and the second is confirmed for the Mexican Caribbean (Fig. 3).

TABLE 1. List of specimens used in this study with collection data and GenBank accession numbers.

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Lobophora declerckii N.E. Schultz, C.W. Schneider & L. Le Gall in Schultz *et al.* 2015: 493	Tombant de Port-Louis, Guadeloupe, Caribbean Sea		VERACRUZ: Anegada de Afuera coral reef, Wreck of the 'Ana Elena'	12	19°10'7.20"N, 95°51'39.0"W	J.L. Godínez Ortega	October 4th, 2013	NI-1184B, 1184C, 1350, 1351, 1357, 1203	On a ship hull	Bermudas, Florida Keys, Veracruz, Guadeloupe and Curaçao	This study
							October 5th, 2013	NI-1184A			This study
							March 18, 2014	NI 1487			
							March 19, 2014	NI-1523			
							March 21, 2014	NI-1482, 1497, 1510			
							May 27, 2014	NI-1468, 1469, 1471, 1664			
							May 26, 2014	NI-1668, 1758, 1765, 1776			
							May 27, 2014	NI-1673			
		MH587570 MH587571 MH587572					February 21, 2015	NI-1792-1795, 2449			This study
		KR260317	SuddeRocroy, Vieux-Habitants, Guadeloupe	27		L. LeGall,Y. Buske *et al.*	May 9, 2012	FRA1426			1
Lobophora variegata (J.V. Lamouroux) Womersley ex E.C. Oliveira, 1977: 217	'Antilles', Caribbean Sea		QUINTANA ROO :Cancún		21° 09'38 "N, 86° 50'51"W	E. Jordán	October 2, 1971	NI-2714	Epiphytic on *Avrainvillea* sp.	Bahamas, Florida Keys, St. Kitts and Nevis, Grand Cayman, Jamaica and Guadeloupe	This study
		MH587573	In front of the Academic Unit of Puerto Morelos Reef Systems, ICMyL, UNAM	2	20°52'3.00"N, 86°52'0.62"W	J.L. Godínez Ortega	7 May 2011	NI-547A			This study
		KX061443	Guadeloupe			C. Payri		IRD11140 (NOU)			3

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TABLE 1. (Continued)

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Lobophora canariensis (Sauvageau) C.W. Vieira, De Clerck & Payri	Puerto Orotava [Puerto de la Cruz], Tenerife, Islas Canarias	KR260354	Pointe Grigri, Port-Louis, Guadeloupe	20	32°18'72"N, 65°02'11.76"W	L. Le Gall, Y. Buske *et al.*	May 15, 2012	FRA1777		Bermuda, Guadeloupe, Martinique	1
Lobophora littlerorum C.W. Schneider, N.E. Schultz & L. Le Gall	Petit-Havre, Le Gosier, Guadeloupe, West Indies, Caribbean Sea	KR260347	Petit-Havre, LeGosier, Guadeloupe	1	16°12'32"N, 61°25'32.998"W	L. Charles, R. Penisson *et al.*	May 9, 2012	FRA1404		Caribbean Islands: Guadeloupe	1
Lobophora pachyventera Z. Sun, P.-E. Lim, Ji. Tanaka & H. Kawai	Sunayama Beach, Miyakojima Island, Okinawa, Japan	KM487802	Bovis (fringingreef), Noumea, SouthProvince, New Caledonia			C. Vieira	Jan. 15, 2013	CV3095		China, Japan, Taiwan, Malaysia, New Caledonia	1
Lobophora abscondita C.W. Vieira, Payri & De Clerck	Bovis, Noumea, South Province, New Caledonia	KU353162	Papua New Guinea			C. Payri		PAP509 (NOU)		New Caledonia	3
Lobophora ceylanica (Harvey ex E.S. Barton) C.W. Vieira, De Clerck & Payri	Maldives, Baa Atoll	KM487799	New Caledonia	50		C. Payri	July 20, 2008	IRD7885	on dead coral	Laccadive Islands, India, Pakistan, Sri Lanka, Maldives, New Caledonia	3, 4
Lobophora gibbera C.W. Vieira, Payri & De Clerck	Les Quatres Freres, Touho, North Province, New Caledonia	KU353164	Europa Island			M. Zubia		EUR262 (BOL)		New Caledonia	3
Lobophora obscura (Dickie) C.W. Vieira, De Clerck & Payri	Island of Mangaia	KM487779	New Caledonia			Leg. C. Payri	March 4, 2012	IRD7884 (NOU)		Central Polynesia, Madagascar, Oman, China, Japan, Vietnam, Hawaiian Islands, New Caledonia.	3, 4

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TABLE 1. (Continued)

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Lobophora abaculusa C.W. Vieira, Payri & De Clerck	Beautemps-Beaupre, Ouvea, Loyalty Islands, New Caledonia	KM487893	New Caledonia			Leg. C. Payri	March 21, 2005	IRD277 (NOU)	Growing among Distromium sp.	New Caledonia	3, 4
Lobophora dimorpha C.W. Vieira, Payri & De Clerck	Senez,, Noumea, South Province, New Caledonia	KM487864	New Caledonia			leg. C.W. Vieira	March 13, 2012	IRD10217		New Caledonia	4
Lobophora monticola C.W. Vieira, Payri & De Clerck	Baie de Canala, Canala, North Province, New Caledonia	KM487812	New Caledonia			leg. C. Payri	April 4, 2005	IRD7640 (NOU)		New Caledonia	3, 4
Lobophora hederacea C.W. Vieira, Payri & De Clerck	Abore, Noumea, South Province, New Caledonia	KM487819	New Caledonia			C. Payri	March 14, 2009	IRD7621 (NOU)		New Caledonia	3, 4
Lobophora pacifica (Setchell) C.W. Vieira, De Clerck & Payri	French Polynesia, Fa'aa: Vicinity of Papeete, Moorea	KX581361	French Polynesia			M. Zubia		UPF026 (PC)		Tahiti	3
Lobophora undulata C.W. Vieira, Payri & De Clerck	Laregnere, Noumea, South Province, New Caledonia	KU364220	Chile			M.E. Ramirez		LAF6885 (LAF)		New Caledonia	3
Lobophora rickeri Kraft	Wistari Reef, southern Great Barrier Reef, Queensland, Australia	KU353232	Australia			G.W. Saunders		GWS023108 (UNB)		Réunion, Queensland	3
Lobophora nigrescens J.Agardh	Dromana Bay, VIC, Australia	KU353377	Australia			H. Verbruggen & L. Tyberghein		HV2431 (GENT)		South China Sea, New South Wales, South Australia, New Caledonia	3
Lobophora dichotoma (R.H. Simons) P.C. Silva	Ingwavuma, Kosi Bay, Natal, South Africa	KU353394	South Africa			J.J. Bolton *et al.*		LMD1006 (BOL)		South Africa	3

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TABLE 1. (Continued)

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Lobophora guadeloupensis N.E. Schultz, F. Rousseau & L. Le Gall	Ilet Gosier, Guadeloupe, Antilles, Caribbean Sea	KR260344	Guadeloupe			F. Rousseau *et al.*		0143243 (PC)		Guadeloupe, Martinique	3
Lobophora rosacea C.W. Vieira, Payri & De Clerck	Ricaudy, Noumea, South Province, New Caledonia	KU353411	Kenya			O. De Clerck		ODC1571 (GENT)		New Caledonia	3
Padina ramonribae Avila-Ortíz, Pedroche & Díaz-Martínez	Cayaquitos, Guerrero, Mexico	HG974411	Guerrero (Mexico)		17°18'4"N, 101°3'6.3"W			FEZA 1781		Mexico (Pacific)	5
Padina boergesenii Allender & Kraft	U.S. Virgin Islands	JQ363946	Dickwella, Sri Lanka			E. Coppejans		HEC15913		Italy, Bermuda, Mexico (Pacific), Central and South America, Eritrea, Libya, Madagascar, Mozambique, South Africa, Mauritius, Rodrigues Island, Arabian Gulf, India, Israel, Lebanon, Levant states, Oman, Pakistan, Sri Lanka, South China Sea, Indonesia, Houtman Abrolhos, Lord Howe Island, Queensland, Federated States of Micronesia, Fiji, Guam, Hawaiian Islands, Northwestern Hawaiian Islands.	6

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TABLE 1. (Continued)

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Padina antillarum (Kützing) Piccone	Trinidad	JQ363930	Weligama, Sri Lanka			E. Coppejans		HEC154511		Salvage Islands, Florida, Mexico, Brazil, Angola, Côte d'Ivoire, Gambia, Ghana, Kenya, Liberia, Madagascar, Mauritania, Senegal, Sierra Leone, Togo Goa, Pakistan, Sri Lanka, China, Indonesia, Myanmar, Singapore, Vietnam.	6
Padina crispata Thivy	Golfo Dulce, Costa Rica	HG974413	Mexican Pacific Coast					FEZA-1792		Mexico, Baja California, Costa Rica, Panama, Galápagos Islands.	5
Padina durvillei Bory	Concepcion: D'Urville	JQ363961	Los Cerritos, Sinaloa, Mexico			C. Rodríguez		AM716		Baja California, Costa Rica, Islas Revillagigedo, Mexico (Pacific), Chile, Colombia, Ecuador, Galápagos Islands, Peru, Temperate South America, Ghana, Liberia, Sierra Leone, South Africa, Togo, Taiwan.	6
Padina glabra Gaillard	Point de Fann, Dakar, Senegal	JQ363965	Isla Espíritu Santo, Baja California Sur, Mexico			H. Verbruggen, C. Ortuno, J. Hernand		HV1751		Florida, Texas, Senegal, India, Oman	6

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TABLE 1. (Continued)

Taxa	Type locality	GenBank accession no.	Collection Site	Deep (m)	Coordinates	Collector	Date	Voucher no.(MEXU)	Habitat	Distribution in western Atlantic	Ref.
Padina gymnospora (Kützing) Sonder	St Thomas, Virgin Islands	AB820959	Kenting, Taiwan			May 31, 2007		KU-d3601		Spain, Atlantic Islands, Florida, Georgia, Mexico (Atlantic), Mexico (Pacific), North Carolina, Texas, Baja California, Belize, Costa Rica, Panama, Caribbean Islands, South America, Africa, Indian Ocean Islands, Asia, Australia and New Zealand, Pacific Islands	7
Padina mexicana E.Y. Dawson	Turner's Island reef, off Tiburon Island	HG974402	Mexican Pacific Coast					FEZA-1761		Mexico (Pacific), Ghana	5
Microzonia phinneyi (E.C.Henry & D.G.Müller) Camacho & Fredericq	Victoria, British Columbia, Canada	EU681467	Cultured strain			A. Peters		FRA0140		British Columbia, California, Washington	6

1) Schultz *et al.* 2015.

2) Vázquez-Machorro *et al.* 2016 (as *L. variegata*).

3) Vieira *et al.* 2016.

4) Vieira et al (2014).

5) Díaz Martínez *et al.* (2016).

6) Silberfeld *et al.* (2013).

7) Ni-Ni-Win *et al.* (2013).

The largest clade included 20 species of *Lobophora* (Table 1). *Padina* was recovered in a separate clade with very low bootstrap support (58%) and no posterior probability support. This clade included eight species of *Padina* (Table 1). The Bayesian inference analysis recovered *Microzonia phinneyi* (E.C. Henry & D.G. Müller) Camacho & Fredericq in Camacho *et al.* (2018: 420) in a close relationship with species of *Padina*, resulting in a low posterior probability for the genus.

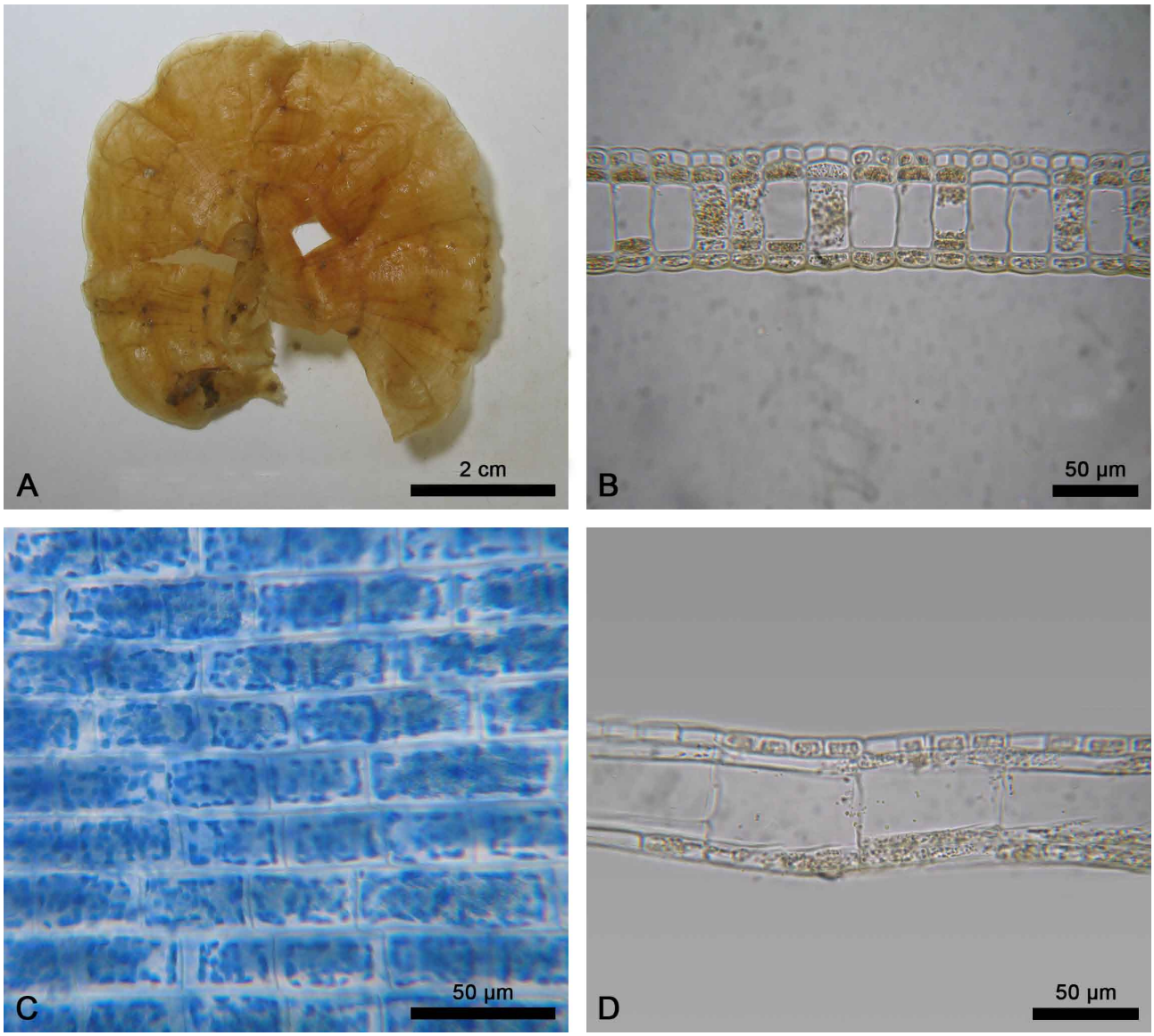

FIGURE 1. *Lobophora declerckii* N. E. Schultz, C. W. Schneider & L. Le Gall. A: Habit of thallus. B: Cross section of a blade. C: Surface view of a blade. D: Longitudinal section of a blade.

Discussion

For the first time, the presence of *Lobophora declerckii* is reported on the Mexican coasts of the western Atlantic and from the southwestern Gulf of Mexico. *Lobophora declerckii* is distinguished from its congeners by its external cortical cells; in transverse view there are 1-2 dorsally positioned cells and 1 ventrally located cells for each medullary cell (Table 2). In addition, the dorsal external cortex in longitudinal view generally has four cells for each medullary cell, sometimes 2 or 3 cells, possessing two cortical layers on each side of the medulla, although sometimes only one layer is present due to the degree of development. *Lobophora canariensis* (Western Atlantic), *L. pachyventera* and *L. dimorpha* (both southwestern Pacific) showed some similarities with *L. declerckii* with respect to the number of cortical layers (2); nevertheless, *L. canariensis* presented more cells in the subcortical dorsal layer (2 cells for each medullary cell) in longitudinal view. Besides that, its aspect is erect rather than decumbent. On the other hand,

TABLE 2. Comparision of morphological and anatomical characters among some species of *Lobophora*.

Characters	*L. declerckii* N.E. Schultz, C.W. Schneider & L. Le Gall	*L. variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira	*L. canariensis* (Sauvageau) C.W. Vieira, De Clerck & Payri[1]	*L. pachyventera* Z. Sun, P.E. Lim, J. Tanaka & H. Kawai[2]	*L. dimorpha* C.W. Vieira, Payri & De Clerck[3]	*L. littlerorum* C.W. Schneider, N.E. Schultz & L. Le Gall[1]	*L. guadeloupensis* N.E. Schultz, F. Rousseau & L. Le Gall[1]
Thallus							
Blades	simple or lobed	simple or lobed	simple or reniform	unbranched	reniform (orbicular lobes)	simple or lobed	simple and tufted
Height	1-5 cm	1-3 cm	2-3.8 cm	2-3 cm	2 cm	2-3.5 cm	2-4 cm
Width	2-7 cm	7-13 cm	1.2-4 cm	3-4 cm	4 cm	3-5 cm	1.5-4 cm
Growth-form	decumbent	erect	erect or decumbent	decumbent	decumbent or erect	crustose	erect
Thickness	70-110 µm	135-145 µm	50-100 µm	99-125.4 µm	80–140 µm	95-140 µm	65-95 µm
Color	light brown	light brown	light brown	yellow brown	-	greenish to brown	brown
Number of layers	5	5–7	5	6-7	5-6	5	
Medullary cells							
Number of cells	1	1	1	1	1	1	1-2
Heigth	27-75 µm	35-73 µm	28-52 µm	-	-	38-40 µm	34-50 µm
Length	53-103 µm	53-91 µm	60-100 µm	-	-	60-80 µm	55-105 µm
Width	18-41 µm	24-40 µm	25-38 µm	-	-	32-46 µm	25-32 µm
Number of cortical layers							
Dorsal layers	2	3	2	2	2-3	2 (-3)	2
Ventral layers	1-2	3	2	(2-)3(-4)	2	2	2
Dorsal cortex							
Number of cells	2-4[a], 1-2[b]	2-3	4[a], 3-2[b]	5-7[a], 3[b]	6-8[a], 2[b]	4-7	2
Heigth	7-16 µm	5-12 µm	8-11 µm	-	-	12-14 µm	8-12 µm
Lenght	24-42 µm	24-50 µm	15-22 µm	-	-	10-16 µm	32-52 µm
Width	8-29 µm	12-19 µm	8-18 µm	-	-	9-15 µm	5-16 µm
Ventral cortex					-		
Number of cells	2-4[a], 1[b]	2-3	2[a], 2-1[b]	2[a], 2[b]	2-3[a], 1[b]	2	3-2[a], 2[b]
Heigth	7-17 µm	7-18 µm	8-12 µm	-	-	12-16 µm	9-13 µm
Length	15-79 µm	34-64 µm	38-40 µm	-	-	24-34 µm	32-52 µm
Width	9-35 µm	11-17 µm	8-12 or 18-25 µm	-	-	12-23 µm	5-16
Dorsal subcortex							
Layers	1	1-2	1	1	1-2	2-1	1
Number of cells	1	1	2-1[a], 1[b]	1	1-2	2, (1)	1
Heigth	7-15 µm	10-24 µm	7-12 µm	9.6-11.2 µm		12-18 µm	10-14 µm
Length	44-104 µm	54-107 µm	30-45 or 74-98 µm	47.7-71.5 µm		26-32, (58-66)	58-105 µm
Width	8-41 µm	20-39 µm	25-38 µm	23.5-33.9 µm		30-35 µm	20-32 µm
Ventral subcortex							
Layers	1	1-2	1	2	1	1	1
Number of cells	1	1	2-1[a], 1[b]	1	1-3	1	1
Heigth	5-17 µm	11-27 µm	7-10 µm	-	-	12-16 µm	11-12 µm
Length	57-116 µm	60-100 µm	32-45 or 74-98 µm	-	-	57-70 µm	58-105
Width	8-41 µm	23-37 µm	25-34 µm	-	-	32-45 µm	22-32
Superficial cells				-	-		
Length	8-36 µm	29-42 µm	-	-	-	-	
Width	18-104 µm	11-17 µm	-	-	-	-	
Sporangia	absent	absent	present	present	absent	-	present
Diameter			65-80 µm	70-80 µm	-	-	90-105 µm
Heigth			70-90 µm	-	-	-	125-150 µm

[1]Schultz *et al.* (2015). [2]Sun *et al.* (2012). [3]Vieira *et al.* (2014). [a]Longitudinal view. [b]Transverse view.

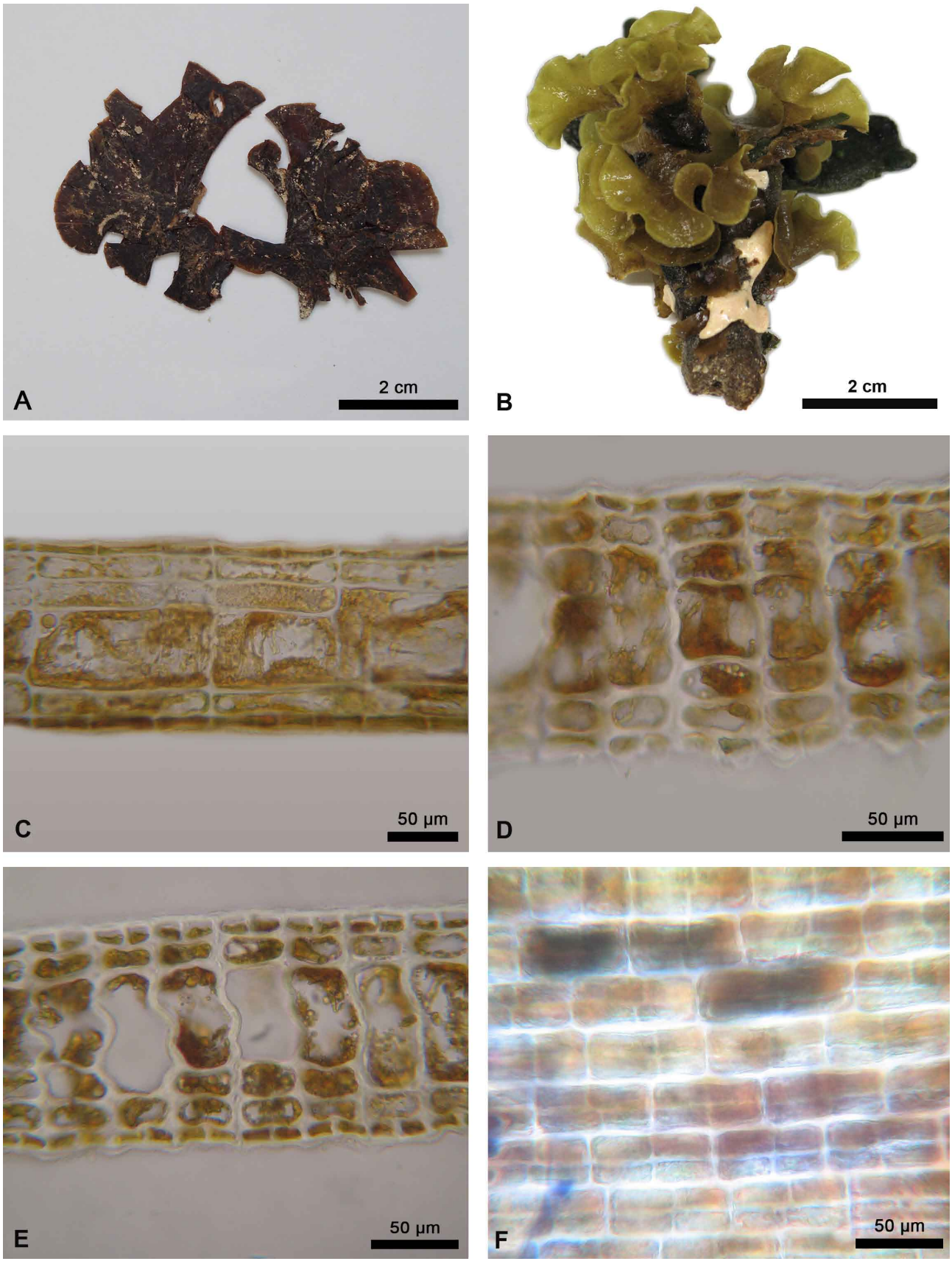

FIGURE 2. *Lobophora variegata* (J. V. Lamouroux) Womersley ex E. C. Oliveira. A: Aspect of a herbarium specimen. B: Habit of a living plant. C: Longitudinal section of a blade. D, E: Cross section of a blade. F: View of the ventral surface of the blade.

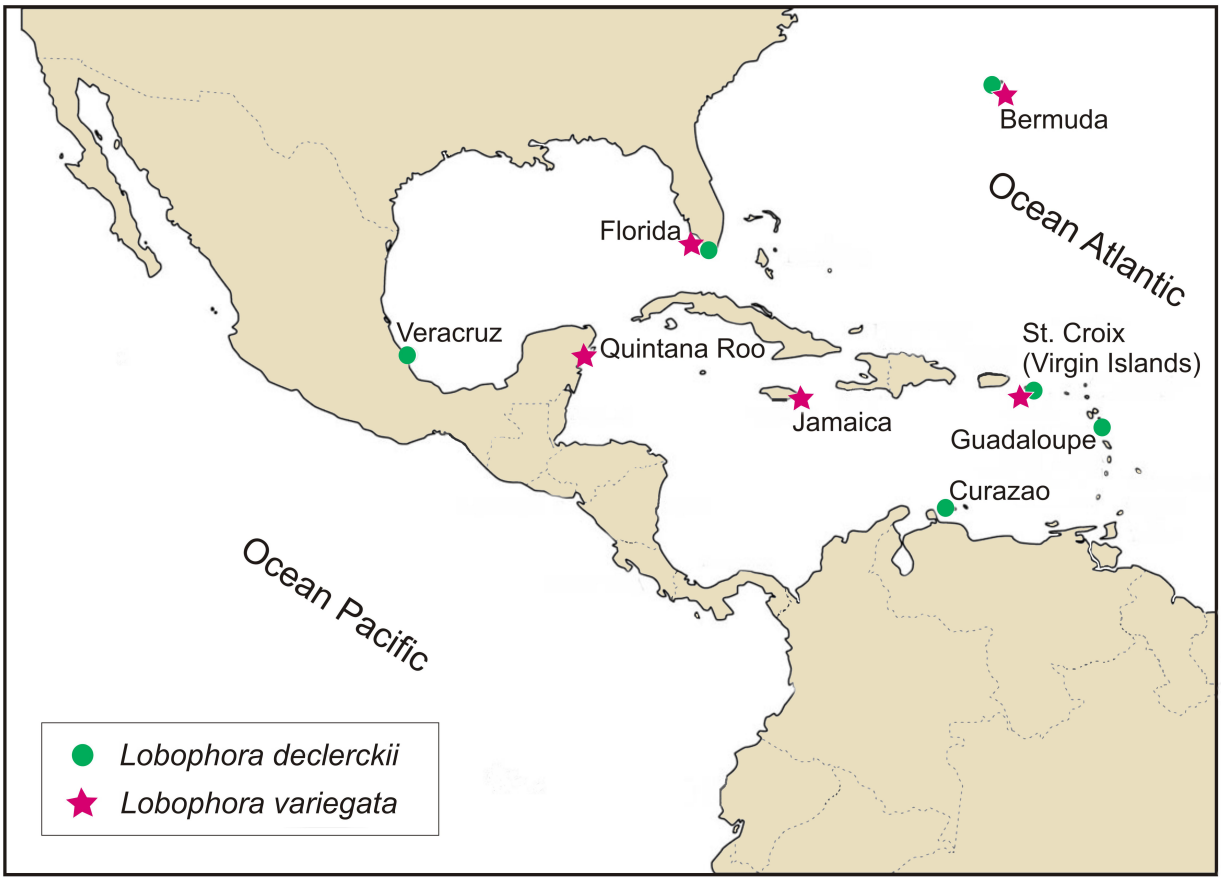

FIGURE 3. Distribution of *Lobophora* from the Atlantic coast of Mexico and surrounding areas (Schultz *et al.* 2015; Vieira *et al.* 2016).

L. pachyventera differs from *L. declerckii* by presenting further cells in the dorsal cortex for each medullary cell (5-7) in longitudinal view; although it shows the same thickness as *L. declerckii* with a decumbent habit and rhizoids present on the ventral side of the thallus (Vieira *et al.*, 2014). *Lobophora dimorpha* has 5 or 6 layers and 2 or 3 on each side of the medulla (Vieira *et al.*, 2014); in contrast, *L. dimorpha* has a different number of subcortical cells (2-3), and in the case of *L. declerckii* has only one, plus displays a distinct anatomy with a fibrous stipe and a reniform thallus. According to Schultz *et al.* (2015), *L. declerckii* is found exclusively at depths greater than 10 m, which is confirmed with the Mexican species here found at 12 m. *Lobophora littlerorum* differed by crustose growth form. In this study four sequences of COX3 were recovered in a clade with a reference sequence of *L. declerckii* (KR260317) from Guadeloupe Island, with very high support (Fig. 4), and next to a larger clade with six species, including *L. littlerorum* (KR260347, Guadeloupe), and *L. pachyventera* (KM487802, New Caledonia).

Lobophora variegata in the Mexican Caribbean is confirmed. The single specimen we collected differs morphologically and anatomically from the other species reported in Table 2. Its erect biological form is rare, except in *L. guadeloupensis* that also is erect. *Lobophora guadeloupensis* differs in having more medullary layers (2) than *L. variegata* which has only one. *Lobophora guadeloupensis* is thinner (65-95 µm) and the number of cortical layers (dorsal and ventral) is fewer (2) than *L. variegata* (135-145 µm, 3 cortical layers).

Lobophora variegata was considered to be a common species widely distributed in the tropics (Guiry & Guiry 2018); recent studies, however, have revealed that its distribution is more restricted based on morphological and molecular results (Vieira *et al.* 2016). Schultz *et al.* (2015) proposed an epitype for this species [MICH, GenBank KR260367] from material collected at St. Croix Island (U.S. Virgin Islands, Lesser Antilles); although the molecular analysis was not possible, a morphological study was implemented to verify the species. However, Vieira *et al.* (2016) later showed that the morphological characteristics were not sufficient for a taxonomic identification of this species. Vieira *et al.* (2016) obtained gene sequences from the holotype specimen of *Dictyota variegata* J.V. Lamouroux (1809: 40) deposited in the Lamouroux Herbarium in France (CN). Using this sequence they established the true identity of *D. variegata* from the Caribbean. Therefore, the lectotypification superseded the epitypification of *Dictyota variegata* sensu Schultz *et al.* (2015), whose St. Croix and Bermuda samples are probably another species (Wynne, 2017).

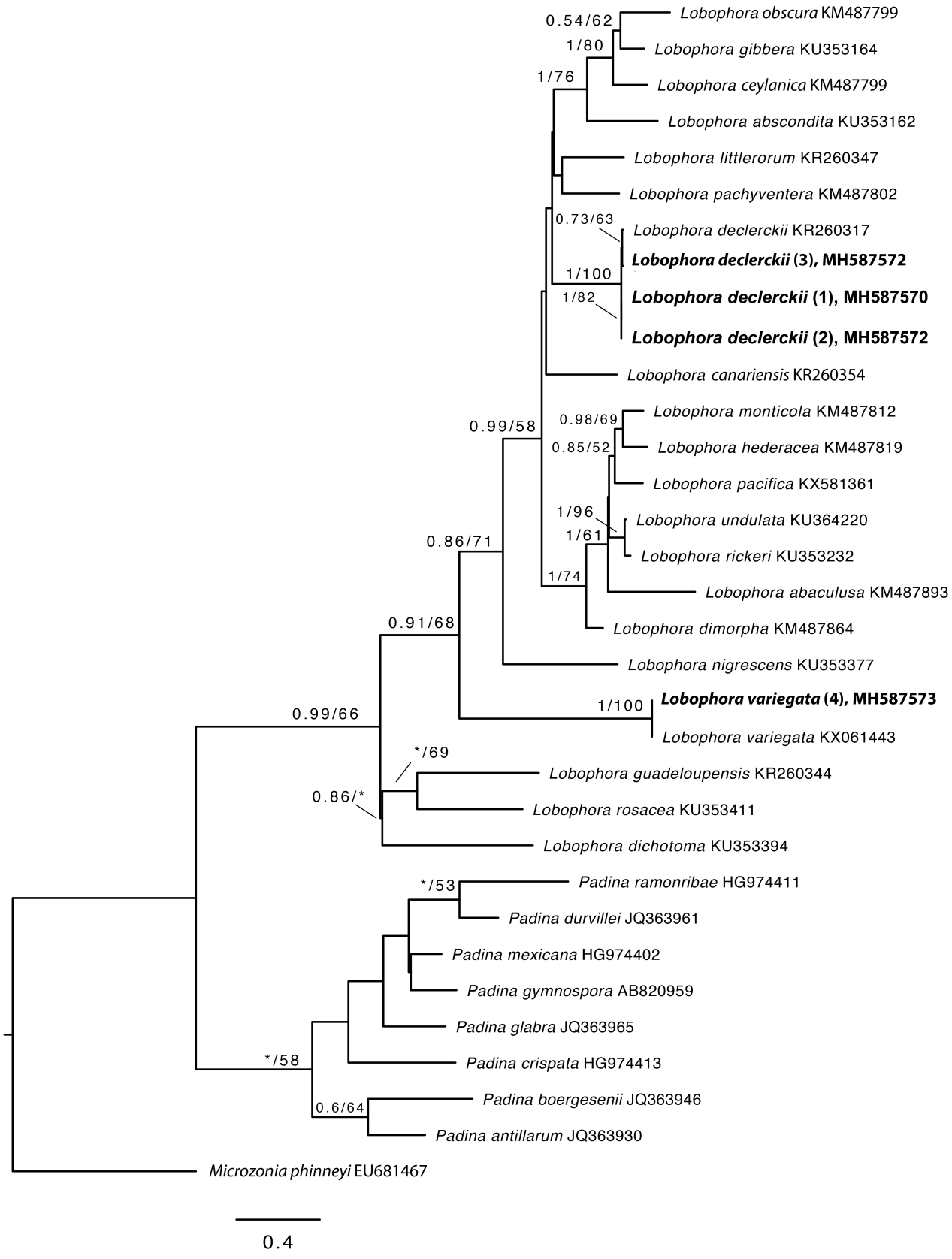

FIGURE 4: Best tree found after maximum likelihood heuristic search. Posterior probabilities and bootstrap frequencies are indicated above branches. Only values above 0.50 or 50% are shown, * represents branches not recovered in the Bayesian majority rule consensus or in the bootstrap consensus tree.

Lobophora was recovered as monophyletic, but with mixed support, low bootstrap support and a high posterior probability (Fig. 4). Posterior probabilities usually are higher than bootstrap frequencies, but even considering that, both statistics measure different aspects of support (García-Sandoval 2014). The discrepancy recovered in the present analysis may be related to the molecular marker and that only one marker was considered.

We generated sequences for COX3 that were recovered in a clade with a sequence of *L. variegata* (KX061443, from Guadeloupe) with very high support (Fig. 4). *Lobophora variegata* was separated from the rest of the *Lobophora* species included in the phylogenetic tree, and this was confirmed by the anatomical evidence (Table 2). Vieira *et al.* (2017) considered this species endemic to the West Indies (Caribbean Sea), which is confirmed with the Mexican record. Schultz *et al.* (2015) cited five species of *Lobophora* for the western Atlantic (*L. variegata*, *L. guadeloupensis*, *L. declerckii*, *L. canariensis* [as *L. payriae*] and *L. littlerorum*), two of which are endemic to Guadeloupe Island, Lesser Antilles (*L. guadeloupensis* and *L. littlerorum*). Of the three remaining species, two of them are present in Mexico (*L. declerckii* and *L. variegata*), but one (*L. canariensis*) remains unknown with a current distribution cited as occurring on both sides of the North Atlantic (Wynne 2017). *Microzonia phinney* (outgroup) is subtended by a relatively large branch, which indicates a large dissimilarity with the *Lobophora-Padina* clade (Fig. 4). As Schultz *et al.* (2015) indicated, there are many specimens of *Lobophora* in North American herbaria with varying morphologies, and for this reason, it is necessary to confirm the actual number of species present in the Gulf of Mexico and in the Caribbean Sea.

Conclusion

For the first time, on the basis of molecular and morphological bases, *L. declerckii* and *L. variegata* are reported for the Mexican coasts of the Atlantic Ocean. This study shows that future investigations are necessary to accurately classify and document the species of *Lobophora* from Mexico.

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