Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 94 (2023): e945216

Taxonomy and systematics

Lobophora dispersa (Dictyotaceae: Phaeophyceae), a new record for the coast of Veracruz and insights into *Lobophora* genetic differentiation in the Gulf of Mexico and the Caribbean Sea

Lobophora dispersa *(Dictyotaceae: Phaeophyceae), un registro nuevo para la costa de Veracruz y perspectivas de su diferenciación genética en el golfo de México y el mar Caribe*

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Received: 16 January 2023; accepted: 2 May 2023

Abstract

Species of *Lobophora* (Dictyotales) are distributed throughout the sub-tropical and tropical seas worldwide. Recent analyses have revealed high species diversity in regions previously presumed to host only a single species, such as the Bismarck Sea, Eastern Pacific, Western Atlantic, Mediterranean Sea, and Greater Caribbean. Here, samples from Veracruz and Quintana Roo, Mexico, were collected, and 2 genetic markers (cox3 and psbA) were sequenced. The results confirmed the presence of *L. dispersa* and *L. variegata*. *Lobophora dispersa* is recorded for the first time on the Mexican coast. The distribution of its cox3 haplotypes shows genetic differentiation within the Greater Caribbean and Gulf of Mexico, possibly indicating limited dispersal and isolation by distance. *Lobophora variegata* exhibits lower genetic variability compared to *L. dispersa*, but its haplotypes did not show any obvious pattern. *Lobophora declerckii*, previously reported in the "Anegada de Afuera" reef, Veracruz, was not found, possibly due to its affinity to subtidal depths. Morphologically, *L. dispersa* and *L. variegata* align with previous descriptions, although we observed more variation in thallus cell thickness in *L. dispersa*. However, relying solely on morphological characters is insufficient to confidently identify the species, necessitating further sampling to determine the species diversity in Mexico.

Keywords: Brown algae; Haplotype network; Molecular systematics; *Lobophora variegata*

ISSN versión electrónica: 2007-8706; Universidad Nacional Autónoma de México, Instituto de Biología. https://doi.org/10.22201/ib.20078706e.2023.94.5216

Resumen

Las especies de *Lobophora* (Dictyotales) se distribuyen en mares tropicales y subtropicales del mundo. Estudios recientes revelaron una alta diversidad de especies en regiones en las que se había reportado únicamente una especie, como el mar de Bismarck, Pacífico oriental, Atlántico occidental, mar Mediterráneo y el gran Caribe. En este estudio, se recolectaron ejemplares de Veracruz y Quintana Roo, México, y se secuenciaron 2 genes (cox3 y psbA). Los resultados confirmaron la presencia de *L. dispersa* y *L. variegata*. *Lobophora dispersa* se reporta por primera vez en la costa mexicana. La distribución de sus haplotipos muestra diferenciación genética dentro del gran Caribe y golfo de México, posiblemente indicando dispersión limitada y aislamiento por distancia. *Lobophora variegata* es menos variable y sus haplotipos no muestran un patrón definido. *Lobophora declerckii*, reportada en el arrecife "Anegada de Afuera", Veracruz, no fue encontrada, posiblemente debido a su afinidad por profundidades submareales. Morfológicamente, *L. dispersa* y *L. variegata* concuerdan con descripciones previas, aunque se encontró mayor variación en el grosor del talo en *L. dispersa*. Sin embargo, la morfología es insuficiente para determinar las especies con confianza, requiriendo un mayor muestreo para conocer la diversidad de especies en México.

Palabras clave: Algas pardas; Red de haplotipos; Sistemática molecular; *Lobophora variegata*

Introduction

Lobophora J. Agardh is distributed on tropical and subtropical coasts worldwide (Vieira, Henriques et al., 2020). It is characterized by multilayered fronds growing from a continuous row of apical cells. It has a highly variable morphology, displaying crustose and erect species with fan-shaped, reniform, or dichotomously divided blades, sometimes resembling *Zonaria* C. Agardh (Vieira et al., 2016). *Lobophora* can inhabit several ecological niches such as rocky shores and reefs, from the upper intertidal zone to depths of more than 130 m (Camacho et al., 2019; Puk et al., 2020; Vieira, Henriques et al., 2020).

In recent years, this genus has been the subject of several taxonomic studies, including morphological and molecular data, leading to a re-interpretation of the species limits and distributions (Camacho et al., 2019; Vieira, Henriques et al., 2020). The number of formally described species worldwide increased from 28 (Camacho et al., 2019; Godínez-Ortega et al., 2018; Vieira, Morrow et al., 2020) to 71 (Guiry & Guiry, 2022). Currently, a total of 5 species can be found in the Gulf of Mexico and adjacent regions: *L. declerckii* N.E. Schultz, C.W. Schneider & L. Le Gall; *L. delicata* Camacho & Fredericq; *L. dispersa* Camacho, Freshwater & Fredericq, *L. schneideri* C.W. Vieira; and *L. variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira (Vieira, Morrow et al., 2020). Until 2018, only *L. variegata* was recognized from the Atlantic coast of Mexico (Dreckmann, 1998; Godínez-Ortega et al., 2018; Ortega et al., 2001). This changed with the first record of *L. declerckii* at the "Anegada de Afuera" coral reef near Veracruz (Godínez-Ortega et al., 2018). The occurrence of *L. variegata* in Mexico was confirmed with molecular data for Cancun (Quintana Roo) in the Caribbean (Godínez-Ortega et al., 2018). However, the actual species of *Lobophora* ocurring on Mexican coasts remain uncertain.

The high number of recently discovered species of *Lobophora* around the world, the morphological similarities between species making the identification difficult, and the historical morphology-based records of *Lobophora* in several localities on the Atlantic coast of Mexico (as *L. variegata*; Dreckmann, 1998; García-García et al., 2021; Ortega et al., 2001) prompted the need to re-evaluate the taxonomic identity of *Lobophora* species in Mexico using molecular data. In this study, DNA sequences of the mitochondrial cox3 and plastid psbA were compared from specimens collected from the states of Veracruz and Quintana Roo, Mexico, to investigate the species diversity and the occurrence of genetic differentiation in *Lobophora*.

Materials and methods

Specimens of *Lobophora* from Veracruz and Quintana Roo, Mexico (Fig. 1), were collected in the intertidal zone and by snorkeling up to 2 m depth. Specimens were prepared as herbarium vouchers, and a small fragment from the apex of the thallus was brushed and rinsed with distilled water to remove contaminants. The fragment was preserved in silica gel for DNA extraction. Vouchers were deposited in the FEZA herbarium (Thiers, 2022) at the Facultad de Estudios Superiores Zaragoza, UNAM. Other collected vouchers preserved in 4% formalin were morphologically examined.

The CTAB (cetyltrimethylammonium bromide; Doyle & Doyle, 1987) method was used for DNA extractions with the addition of 2% (w/v) polyvinylpyrridoline (PVP). The cytochrome c oxidase subunit 3 (cox3) and D1 protein of Photosystem II (psbA) genes were used following previous

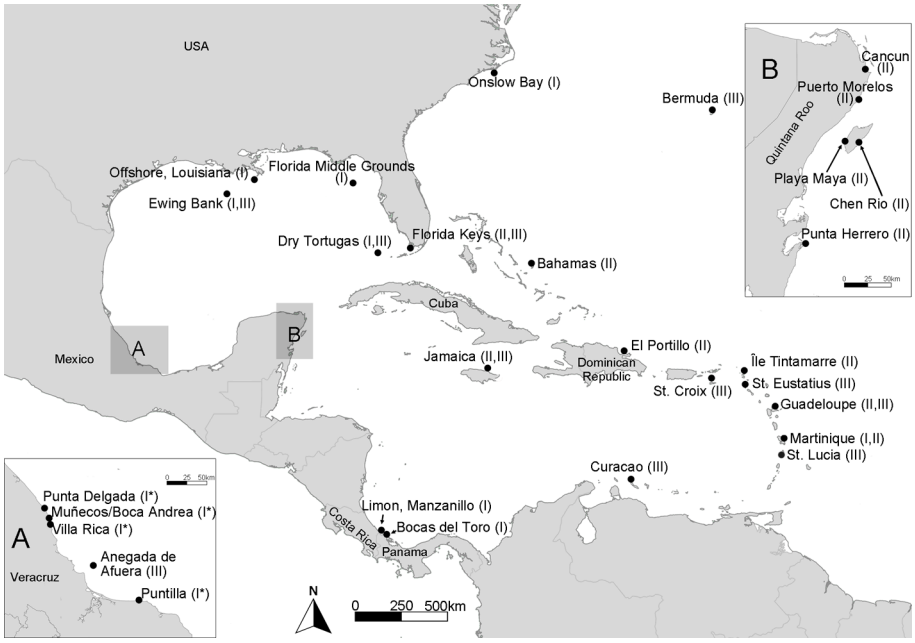

Figure 1. Known distribution of *L. dispersa*, *L. variegata*, and *L. declerckii* (Schultz et al., 2015, Vieira et al., 2016, Godínez-Ortega et al., 2018, Camacho et al., 2019, Vieira, Morrow et al., 2020) in the wider Caribbean. Roman number in brackets indicates the species found at each locality: *L. dispersa* = I, *L. variegata* = II, and *L. declerckii* = III. Insets show details of locations at Veracruz (A) and Quintana Roo (B) states. * indicates new records for *L. dispersa*. The coordinates of the Mexican localities are in Supplementary material: Table S1.

studies within *Lobophora* (Camacho et al., 2019; Vieira et al., 2016; Vieira, De Clerckm et al., 2019). The DNA was amplified with the MyTaq Polymerase Kit (Bioline, Meridian Bioscience Inc., USA) with the following primers: cox3-44F/cox3-739R for cox3 (Silberfeld et al., 2013) and psbA-F/psbA-R1 for psbA (Yoon et al., 2002). The amplification profile consisted of 3 minutes at 94 °C for initial denaturing, followed by 30 cycles of 1 minute at 94 °C, 46 °C for 1 minute and 72 °C for 1 minute, and 7 minutes at 72 °C for final extension. Amplification success was evaluated visually by electrophoresis on 1% agarose. Amplicons were sent to Macrogen (Seoul, Korea) for Sanger sequencing using the amplification primers. A total of 14 specimens of *Lobophora* were sequenced: 9 from the coast of Veracruz and 5 from Quintana Roo (Supplementary material: Table S1). The chromatograms were assembled and edited using Geneious 6 (Biomatters Ltd. available from http://www.geneious.com/).

Independent data matrices were created and aligned in Mega X ver. 10.2.4. using default settings for each gene due to the uneven sequences available (Kumar et al., 2018). Additional sequences of *Lobophora* were downloaded from GenBank (Supplementary material: Table S2). The selected sequences were mainly based on the study of Vieira, Morrow et al. (2020) for the Greater Caribbean region and complemented with others from previous publications (Camacho et al., 2019; Schultz et al., 2015; Vieira et al., 2014, 2016; Vieira, Rasoamanendrika et al., 2021; Vieira, Steen et al., 2021) as well as sequences of Mexican *L. declerckii* and *L. variegata* (Godínez-Ortega et al., 2018). *Padina gymnospora* (Kützing) Sonder was used as an outgroup. Both matrices were analyzed using maximum likelihood (ML) and Bayesian inference (BI). The best molecular model and partition scheme for each codon and data matrix were calculated with PartitionFinder v1.1.0 (Lanfear et al., 2012) using the BIC value model selection option. The selected model for both markers was GTR+I+G in a single partition. In addition, uncorrected pairwise distances ("*p*" distance) were calculated in Mega X.

For ML, IQ-TREE ver. 2.2.0 (Nguyen et al., 2015) was employed, and branch support was calculated by 500 nonparametric bootstrap (BS) replicates. For BI, MrBayes ver. 3.2 (Huelsenbeck & Ronquist, 2001) was executed in CIPRES Science Gateway V. 3.3. (http://www.phylo.org/sub_sections/portal/). Two parallel analyses were performed running for 10,000,000 generations, sampling every 1,000 generations, with unlinked partitions.

The stationarity of the likelihood curve was examined visually, and convergence was analyzed with Tracer 1.7 (Rambaut et al., 2018). The first 2,500,000 generations were discarded as burn-in, and a summary tree including average branch lengths and posterior probability (PP) values was calculated from the remaining trees.

Haplotype networks of the cox3 sequences that grouped with our samples of *L. dispersa* and *L. variegata* were calculated to explore insights of genetic differentiation in PopART ver. 1.7 (Leigh & Bryant, 2015) using the TCS method (Clement et al., 2000), which implements the statistical parsimony algorithm. The haplotypes found were placed on a map to explore potential geographic patterns.

Morphological examination. Thirty-four specimens (23 from Veracruz and 11 from Quintana Roo; Supplementary material: Table S1) were examined morphologically using a Nikon SMZ660 stereoscope. Cross-sections were made by hand with a single-edged razor blade in longitudinal and transverse orientations. Measurements of the medullar, subcortical, and cortical cells were made following the anatomical description used by Schultz et al. (2015), using a Nikon Eclipse 50i microscope. Maximum and minimum values were obtained for each trait and compared with previous descriptions (Camacho et al., 2019; Godínez-Ortega et al., 2018; Torres-Conde et al., 2021; Vieira, Henriques et al., 2020; Vieira, Morrow et al., 2020).

Results

Molecular data from both markers allow the recognition of *Lobophora dispersa* from the coast of Veracruz (Gulf of Mexico) and *L. variegata* from Quintana Roo (Caribbean Sea).

The cox3 data matrix consisted of 697 base pairs (bp) and 107 sequences (13 newly sequenced). BI (Fig. 2) and ML resulted in similar topologies differing only in poorly supported branches. *Lobophora dispersa* from Mexico grouped with the holotype (WNC 33550; USA: North Carolina, Onslow Bay; sequence in Supplementary material: Table S2), while the Mexican samples of *L. variegata* grouped with sequences of *L. variegata sensu* Vieira et al. (2016; see Supplementary material: Table S2). The *Lobophora dispersa* clade was well supported (BS = 90%, PP = 1.0) with low intraspecific genetic variation (0.9 % pairwise difference within the clade). The Mexican samples of *L. dispersa* were found only in Veracruz and grouped in a subclade (BS = 87%, PP = 1.0) different from samples from other localities (e.g., North Carolina, upper Gulf of Mexico, Dry Tortugas). The sister species of *L. dispersa* was *L. rosacea* C.W. Vieira, Payri & De Clerck, as shown in previous studies (BS = 95%, PP = 1.0; Vieira, Morrow et al., 2020) with a genetic distance of 2.9%. *Lobophora variegata* was also well supported (BS = 95%; PP = 0.98). The Mexican samples of *L. variegata* were collected only from Quintana Roo and are highly similar to sequences from other localities (e.g., Bahamas, Dominican Republic, Florida Keys, Guadeloupe, Jamaica; distance within the clade of 0.2%). Its sister species was *L. richardii* C.W. Vieira & Payri (BS = 98%, PP = 1.0; Vieira, Morrow et al., 2020) with a genetic distance of 6.7%.

The psbA data matrix consisted of 956 bp and 76 sequences (6 sequenced here). As in the cox3 dataset, the BI tree is used for discussion (Fig. 3). The psbA phylogeny was congruent with the cox3 topology. *Lobophora dispersa* was recovered in a well-supported clade (BS = 92%, PP = 1.0; distance within the clade of 0.4%) with *L. rosacea* as sister species (BS = 95%, PP = 1.0) with a genetic distance of 1.3%. Mexican samples of *L. dispersa* were grouped in a subclade as well (BS = 81%, PP = 0.97). *Lobophora variegata* was recovered in a well-supported clade with no genetic differentiation (BS = 100%, PP = 1.0; distance within the clade of 0.0%), sister to *L. richardii* (genetic distance of 3.4%) as in the cox3 topology.

The cox3 haplotype networks built for *L. dispersa* (n = 18) recovered a total of 7 haplotypes (Ld1-7; Fig. 4) and 4 subgroups. Group 1 included samples from Veracruz, Mexico (Ld1-3); group 2 included samples from the northern Gulf of Mexico, Florida, and North Carolina, USA (Ld4); group 3 contained samples from Costa Rica and Panama in the southern Caribbean (Ld5); and group 4 included samples from Martinique in the eastern Caribbean region (Ld6, 7). For *L. variegata* (n = 13), a total of 5 haplotypes were found (Lv1-5; Fig. 4). Two haplotypes appear to be widely distributed in the Greater Caribbean: Lv1 occurred along the Mexican Caribbean coast and in Saint Martin, and Lv2 occurred in Mexico, the Dominican Republic, and Florida, USA. Haplotypes Lv3 and Lv4 were found only in Mexico, and Lv5 solely in Jamaica.

Morphological results. The individuals of *Lobophora dispersa* display lobed procumbent blades, either entire or slightly divided (Fig. 5A) to highly divided close to the base of the thallus (Fig. 5B). The color ranged from light brown to yellowish-green but turning darker towards the stipe. Examined thalli were up to 3.2 cm in height and 3.7 cm wide. Anatomically, the thalli consisted of 1 layer of medullary cells, with multiple layers of subcortical and cortical cells on both sides of the medulla (Fig. 5C-F). The basal section of the thallus ranged from 9-10 layers of cells (Fig. 5C, D) to 6-7 in the middle sections (Fig. 5E, F). The ventral side consists of 2-4 layers (1 cortical layer), and the dorsal side of 3-5 layers (1 cortical) depending on the part of the thallus examined and the age of the individual.

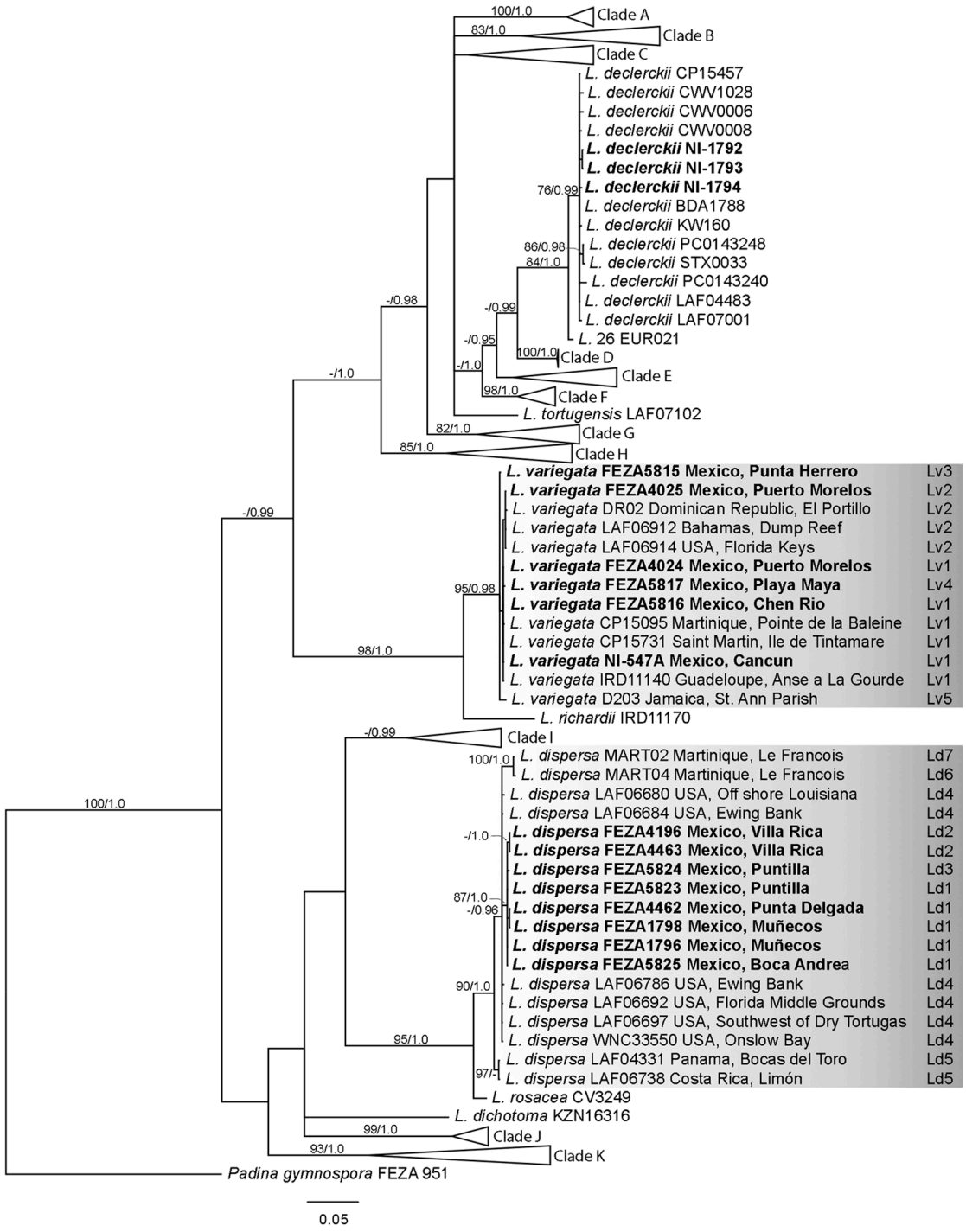

Figure 2. Bayesian topology of *Lobophora* based on the *cox*3 dataset. Numbers on branches indicate the bootstrap percentages (BS)/ posterior probabilities (PP); only values above 75% or 0.95, respectively, are shown. Bold indicates individuals from the coasts of Mexico. The haplotype number used in the haplotype network is at the right of each sequence. The complete tree can be found in Supplementary material: Fig. S1.

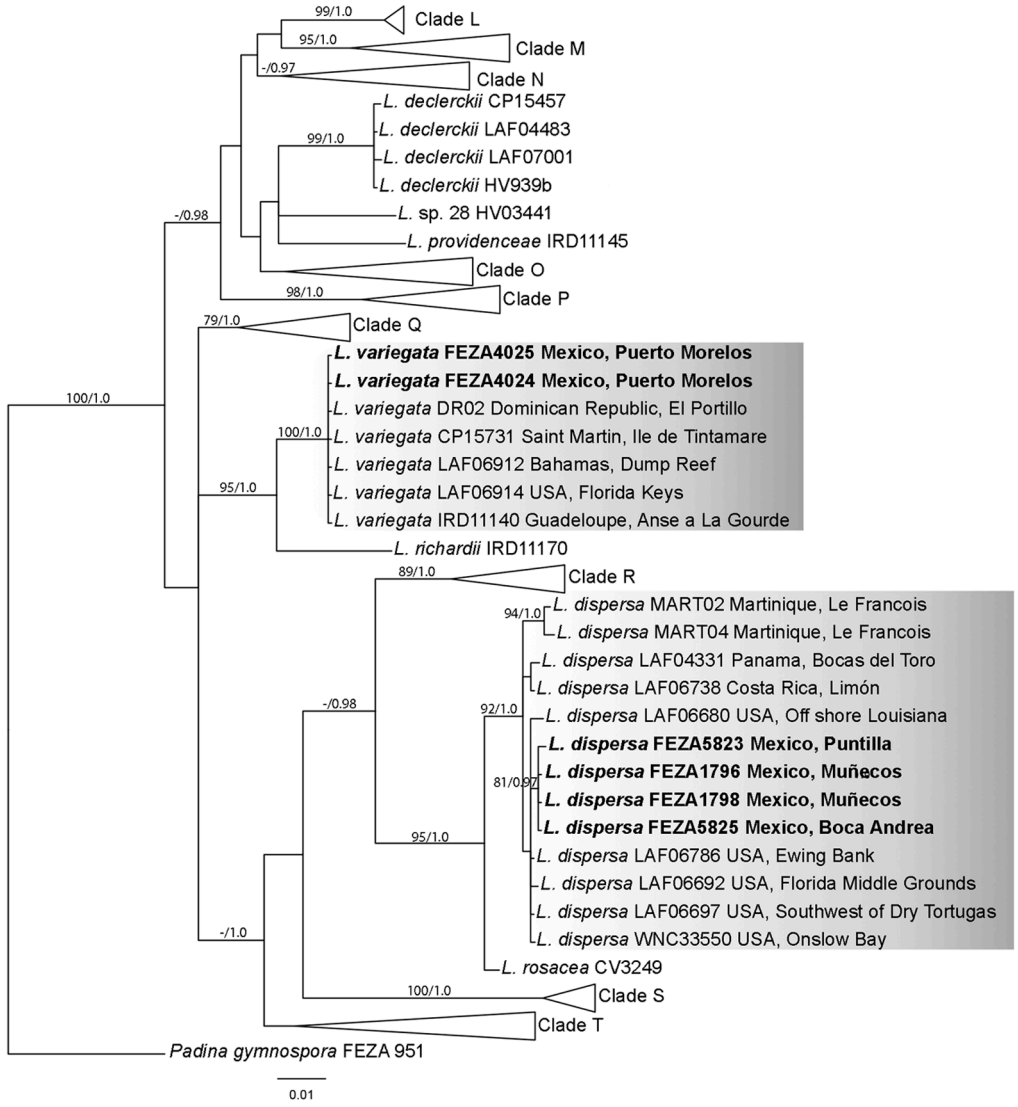

Figure 3. Bayesian topology of *Lobophora* from the psbA matrix. Numbers on branches indicate the bootstrap percentages (BS)/ posterior probabilities (PP); only values above 75% or 0.95, respectively, are shown. Bold indicates individuals from the coasts of Mexico. The complete tree can be found in Supplementary material: Fig. S2.

Transverse sections occasionally reveal the division of the medullary cells (Fig. 5C, E). Measurements of cells can be found in Table 1.

The analyzed individuals of *L. variegata* formed lobed erect blades (Fig. 6A), stipitate to decumbent, and are sometimes divided (Fig. 6B). The color ranged from brown to light brown and yellow-brown, turning darker toward the stipe. The analyzed thalli were up to 7.5 cm in height and 6.1 cm wide. Anatomically, the thalli consisted of 1 layer of medullary cells, plus 3 ventral cell layers (1 cortical layer) and 3 layers on the dorsal side (1 cortical) (Fig. 6C, D). Other measurements are shown in Table 1.

Discussion

The molecular data show the occurrence of *L. dispersa* on the coast of Veracruz because the Mexican sequences are highly similar to the type material (Camacho et al., 2019;

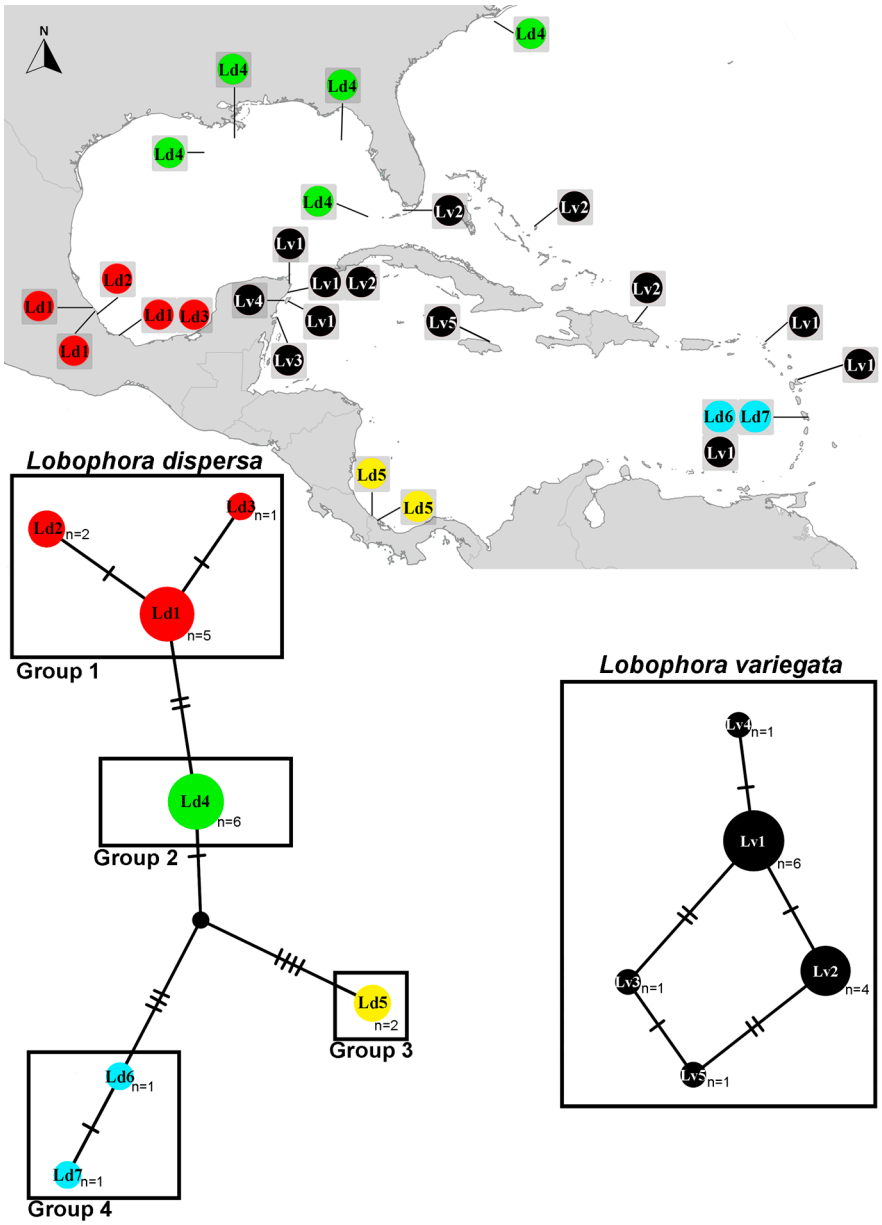

Figure 4. Cox3 haplotype network of *L. dispersa* (lower left) and *L. variegata* (lower right) and their distribution in the Greater Caribbean (upper). Circles indicate the location of each haplotype. Colors indicate haplotype groups found in the analysis.

see above). However, the Veracruz samples correspond to newly discovered haplotypes. The genetic variation and haplotype network of cox3 for this species suggests genetic differentiation based on the collection site. Such genetic differences could be attributed to reproductive strategies or isolation by distance (Couceiro et al., 2011; Krueger-Hadfield et al., 2011; Li et al., 2016). Interestingly, no reproductive structures have been reported so far for *L. dispersa* (Camacho et al., 2019; Vieira, Aharonov et al., 2019; Vieira, Morrow et al., 2020) raising questions regarding its dispersal capabilities, phenology, and life cycle. Some of these questions may be answered with further sampling at different times and scale, and finer population genetic analyses. Similarly, patterns of genetic differentiation through the Gulf of Mexico can be found in *Padina gymnospora* and *P. boergesenii* Allender & Kraft,

Table 1

Comparison of morphological and anatomical characters of *Lobophora dispersa*, *L. variegata*, and *L. declerckii*. Measurements are in μm, except for thallus height and width (cm). '-' = Not available.

	Lobophora dispersa		*Lobophora variegata*				*Lobophora declerckii*		
Color	Light brown to dark green	Light to dark brown	Light to dark brown, olive green	Dark orange, brown to dark green	Light brown	Yellow- green	Light green	Light brown	Brown
Growth form	Flabellate, fan-shaped; blade entire to highly divided; procumbent	Erect, procumbent; fan-shaped, stipitate	Flabellate, fan-shaped; blade entire to highly divided; decumbent	Decumbent, fasciculate, ruffled	Erect; simple or lobed	Simple or lobed to lacerate	Conk-like/ decumbent	Decumbent, simple or lobed	Simple or lobed
Thallus height	1.8-3.7	-	2.3-6.1	-	1-3	2.2-4.4	-	1-5	1.3-4.9
Thallus width	1.9-3.2	-	1.2-7.6	-	7-13	3.1-5.5	-	2-7	65-83
Blade thick	110-167.5	78-164	140-180	124-197	135-145	128-190	55-85	70-110	65-83
Total number of cells	6-10	5-8	7	5-7	5-7	5-7	3-5	5	3-5
Number of dorsal cells	3-5	2-4	3	2-3	3	2-3	1-2	2	1-2
Number of cortical cells	1	1	1	1	1	1	1	1	1
Number of ventral cells	2-4	2-3	3	2-3	3	2-3	1-2	1-2	1-2
Dorsal side cells									
Cortical cells height	4.3-14.5	-	8.9-13.5	-	5-12	10-13	15-22	17-16	8-12
Cortical cells length	10.8-50.9	-	20.8-37.9	-	24-50	-	-	24-42	-
Cortical cells width	7.14-14.8	-	11.1-11.7	-	12-19	-	-	8-29	-
Subcortical cells height	9.1 -15.2	-	8.8-16.5	-	10-24	6-12	-	7-15	6-10
Subcortical cells length	27.4-74.9	-	45.0-65.9	-	54-107	-	-	44-104	-
Subcortical cells width	14.3-25.1	-	15.5-21.4	-	20-39	-	-	8-41	-
Medullar cells									
Cells height	20.1-45.3	23-60	42.1 -54.2	50-94	35-73	53-87	30-50	27-75	27-48
Cells length	59.3-107.7	48-125	46.5-65.2	68-94	53-91	78-90	62-100	53-103	67-98
Cells width	13.9-23.6	18-28	18.8-22.4	23-43	24-40	28-40	25-45	18-41	23-40
Ventral side cells									
Subcortical cells height	8.7-16.4	-	9.7-13.1	-	11-27	8-10	-	7-15	6-10
Subcortical cells length	28.8 -68.2		46.3-69.5		60-100			44-104	
Subcortical cells width	9.82-23.3		18.6-21.1		23-37			8-41	
Cortical cells height	7.8-16.4		7.6-14.2		7-18	6-11	14-20	7-17	8-11
Cortical cells length	17.9-47.0		23.3-55.6		34-64			15-79	
Cortical cells width	8.7-24.0		7.8-19.0		11-17			9-35	
Sporangia									
Diameter	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	90-105	Not observed	Not observed
Height							125-150		
References	This study	Camacho et al., 2019, Vieira, Aharonov et al., 2019; Vieira, Henriques et al., 2020; Vieira, Morrow et al., 2020	This study	Vieira et al., 2016	Godínez-Ortega et al., 2018	Torres-Conde et al., 2021	Vieira, Morrow et al., 2020	Godínez-Ortega et al., 2018	Torres-Conde et al., 2021

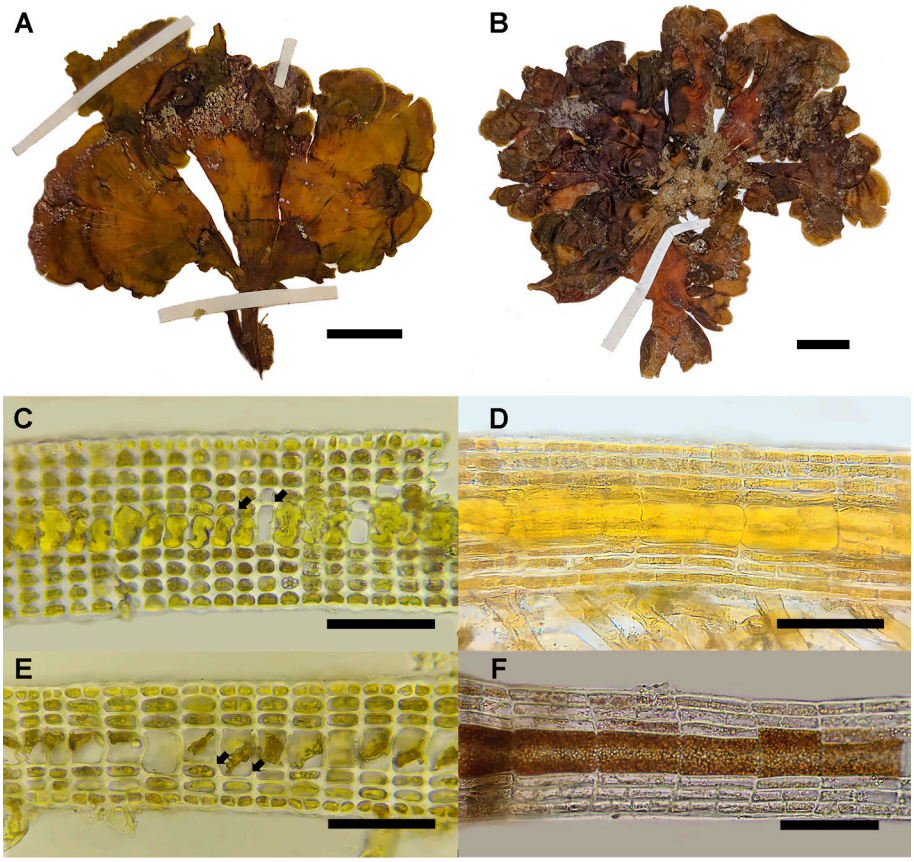

Figure 5. Habit and sections of *L. dispersa*. A, Specimen (FEZA 4463) showing slightly divided lobes; scale bar = 1 cm. B, Specimen (FEZA 1796) showing highly divided lobes; scale bar = 1 cm. C, Transverse section of the basal section of the thalli showing up to 10 cell layers: 1 layer dorsal cortical, 3-4 dorsal subcortical, 1 medullary, 3 ventral subcortical, and 1 ventral cortical layer; arrows indicate the division of medullary cells and increase of cell layers; scale bar = 100 µm. D, Longitudinal section of the basal section of the thalli showing up to 9 cell layers: 1 dorsal cortical, 3 dorsal subcortical, 1 medullary, 3 ventral subcortical, and 1 ventral cortical bearing rhizoids; scale bar = 100 µm. E, Transverse section of the mid portion showing up to 7 cell layers: 1 dorsal cortical, 2 dorsal subcortical, 1 medullary, 1-2 ventral subcortical, and 1 ventral cortical; arrows indicate the division of medullary cells and increase of cell layers; scale bar = 100 µm. F, Longitudinal section of the mid portion showing up to 7 cell layers: 1 dorsal cortical, 2 dorsal subcortical, 1 medullary, 2 ventral subcortical, and 1 ventral cortical; scale bar = 100 µm.

where most individuals from Veracruz are genetically distinct from those in Campeche and the Yucatán Peninsula (Díaz-Martínez et al., 2016). Ocean currents in the Caribbean and the Gulf of Mexico, which originated from the rising of the Yucatán Peninsula and the closure of the Central American isthmus (Pindell & Kennan, 2009), have been invoked to explain the distribution and endemicity of red algae in the region, particularly in relation to the isolation of populations in Campeche, located in the southeastern Gulf of Mexico (Dreckmann et al., 2018; Hernández et al., 2021; Núñez-Resendiz et al., 2017, 2019), either improving or reducing the connectivity between populations. The genetic pattern observed in *L. dispersa* could be influenced by the heterogeneity of habitats in the region (because of the influence of currents and geological history; Dreckmann & Sentíes, 2013) and/or other intrinsic species factors such as reproductive modes (Ardehed et al., 2015; Krueger-Hadfield et al., 2013).

The occurrence of *L. variegata* on the Mexican Caribbean coast is also corroborated as the cox3 sequences are highly similar to the type material (Antilles, West Indies) studied by Viera et al. (2016). For this species, the genetic distances in both markers, cox3 and psbA, was low, even considering specimens from distant regions, contrasting with *L. dispersa*. The haplotype network analysis revealed the presence of at least 3 distinct

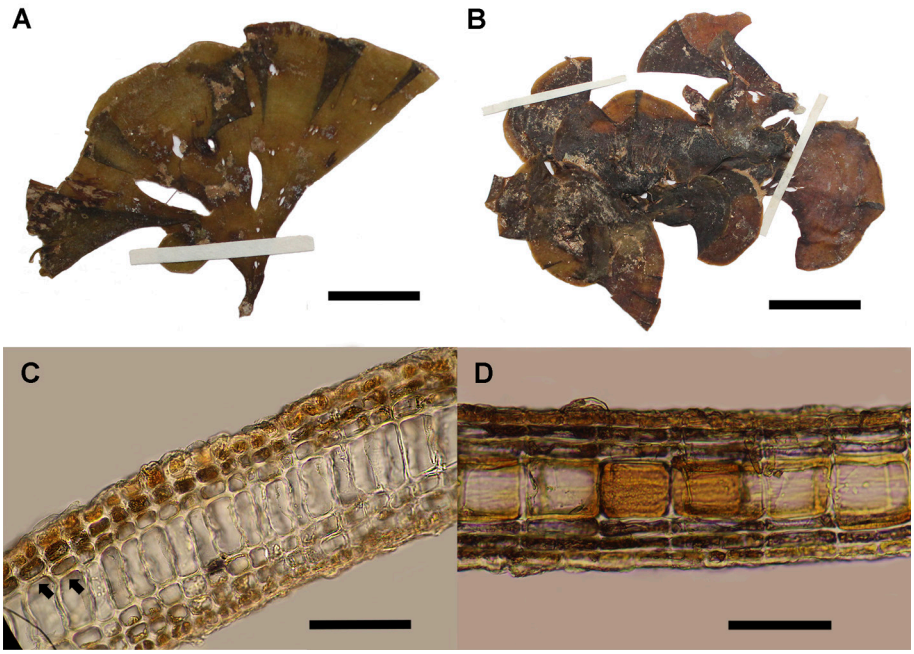

Figure 6. Habit and sections of *L. variegata*. A, Specimen (FEZA 4025) showing slightly divided lobes (almost entire); scale bar = 1 cm. B, Specimen (FEZA 4024) of *L. variegata* showing highly divided lobes; scale bar = 1 cm. C, Transverse section of the mid-portion showing up to 7 cell layers: 1 dorsal cortical, 2 dorsal subcortical, 1 medullary, 2 ventral subcortical, and 1 ventral cortical; arrows indicate the division of medullary cells and increment of cell layers; scale bar = 100 µm. D, Longitudinal section of the mid portion showing up to 7 cell layers: 1 dorsal cortical, 2 dorsal subcortical, 1 medullary, 2 ventral subcortical, and 1 ventral cortical; scale bar = 100 µm.

haplotypes from the Caribbean coast of Mexico, with no obvious differentiation patterns observed. At first glance, this finding appears to be consistent with other macroalgae in the Western Atlantic such as *Hypnea* sp. 1 (Nauer et al., 2019) and *Gracilaria usneoides* (C. Agardh) J. Agardh (Núñez-Resendiz et al., 2017), where surface ocean currents could be promoting connectivity and genetic exchange between populations. However, the genetic diversity observed in *Hypnea* sp. and *Gracilaria usneoides* is higher compared to *Lobophora variegata*. To further test these hypotheses, additional sampling and molecular studies, such as population genetics, phylogeography, and time-calibrated analyses, will be necessary.

The morphology of *Lobophora dispersa* found in Mexico mostly fits with previous descriptions, although some variations in cortical and subcortical cells are newly reported (Table 1). Remarkably, we have found specimens with up to 10 layers, 2 more than previously reported. In *L. variegata*, the specimens examined are similar to previous reports from Mexico (Godínez-Ortega et al., 2018), Cuba (Torres-Conde et al., 2021), and other Caribbean localities (Camacho et al., 2019; Vieira et al., 2016; Vieira, Henriques et al., 2020; Vieira, Morrow et al., 2020), but the overall measurement ranges were smaller. This could be attributed to where sections were made or the developmental stage of the samples, which can make identification difficult based only on thallus thickness (Vieira et al., 2014).

With the occurrence of *L. dispersa,* the current number of *Lobophora* species reported on the Mexican coast of the Gulf of Mexico and the Caribbean Sea rises to 3. Based only on morphology, it has been proposed that some species can be differentiated using the number of cell layers, thickness, and growth pattern (Vieira et al., 2014). *Lobophora* species in Mexico cannot be confidently identified based only on these traits. The number of cell layers and thickness of thalli between *L. dispersa* (5-10 layers) and *L. variegata* (5-7 layers) overlap, although they can be distinguished by growth form: *L. dispersa* is procumbent while *L. variegata* is erect to decumbent. On the other hand, both can be mistaken with *L. declerckii* which, despite being thinner, its number of cell layers (5) is similar to the slender individuals of *L. dispersa* and *L. variegata*. *Lobophora declerckii* also can share a decumbent growth with *L. variegata*, although a "conk-

like" form similar to a shelf is reported in *L. declerckii* as well (Vieira, 2020; Vieira, Morrow et al., 2020). Having these similar and overlapping features between species, it is clear that molecular tools are necessary to support species identification (Puk et al., 2020; Vieira et al., 2014).

Interestingly, *Lobophora dispersa*, *L. variegata*, and *L. declerckii* were not found at the same sites. In previous studies, *Lobophora* species are reported to co-exist due to microhabitat preferences in the same localities and limited intraspecific and interspecific competition (Vieira, Morrow et al., 2020). However, niche preferences could be influencing the distribution of the species in the region at small and broad scales. In this regard, local studies in Palau (Micronesia) have shown the existence of ecologically generalist and specialist species where wave exposure is an important factor determining the *Lobophora* species assemblages at large scales, while wave exposure, depth, and herbivory are relevant at small scales (Puk et al., 2020). In addition, other studies in red algae such as *Bostrychia intricata* (Bory) Montagne (Muangmai et al., 2015) and *Caloglossa ogasawaraensis* Okamura (Kamiya & West, 2014) have shown a correlation between distinct genetic lineages adapted to specific growth conditions and, therefore, different microhabitats. For *L. declerckii*, it is possible that the only record from "Anegada de Adentro" coral reef is related to an affinity for deeper zones, while *L. dispersa* could be more adapted to the intertidal and shallow shores of Veracruz. Therefore, exploration of the sub-tidal zone is needed to investigate the diversity of *Lobophora* species in the region.

Two other interesting questions are: why do *L. variegata* and *L. dispersa* not occur in the same localities even when both occur in the intertidal zone, and why is there no molecular confirmation of *L. variegata* in the southern and great part of the northern Gulf of Mexico? Although this could be the effect of limited sampling, it could be alternatively related to the water temperature of each marine region delimiting the distribution of both species and a biogeographic factor. In this regard, the existence of a transition zone between the Gulf of Mexico and the Caribbean biotic components on the Yucatán peninsula has been suggested, which is attributed to the influence of seaweed diversity and endemicity of each region (Vilchis et al., 2018).

In conclusion, we have expanded the knowledge of this genus by reporting the occurrence of *L. dispersa* in Veracruz, updating the distribution of *L. variegata* along the coast of Quintana Roo, and pointing out some insights into the genetic diversity of *Lobophora* in the region. However, there is a clear need for a broader and more efficient sampling effort to represent more accurately the genetic diversity occurring throughout the Gulf of Mexico and the Caribbean Sea. It is well known that further sampling in poorly explored areas of species with wide distributions can result in better-supported conclusions (Dijoux et al., 2014; Lee et al., 2013; Zuccarello et al., 2006). As *Lobophora* is reported in Tamaulipas and Campeche (as *L. variegata*; Dreckmann, 1998; García-García et al., 2021) the occurrence of other species, as well as some co-existing in the same localities, could have been overlooked.

Acknowledgements

We thank Uri O. García Vázquez from the Molecular Systematics Laboratory at FES Zaragoza, and the Molecular Systematics Laboratory at Instituto de Biología (UNAM) for facilitating the molecular work. We thank the "Carrera de Biología" from FES Zaragoza for providing transport and facilities, Arturo Ubaldo-Fuentes for his help taking slide photographs, and we also thank the reviewers who assisted in improving this manuscript. This work was supported by "Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica" (PAPIIT) funding IA204921 (Universidad Nacional Autónoma de México). LHA thanks the "Posgrado en Ciencias Biológicas, UNAM" and received fellowship 779816 from Conahcyt.

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