El Colegio de la Frontera Sur

Caracterización molecular y conectividad del mangle rojo (*Rhizophora mangle*) en el Sur de Quintana Roo, México

TESIS

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Por

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Las personas firmantes abajo, miembros del consejo examinador de Landy Rubí Chablé Iuit hacemos constar que hemos revisado y aprobado la tesis titulada "Caracterización molecular y conectividad del rojo (*Rhizophora* mangle *mangle*) en el sur de Quintana Roo, México", para obtener el grado de **Maestra en Ciencias en Recursos Naturales y Desarrollo Rural**.

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Índice

Resumen

Los manglares son ecosistemas que poseen una gran importancia tanto ecológica como económica. Debido a perturbaciones como el incremento de la población, de actividades antropogénicas a escasa distancia de los bosques de manglar, la contaminación, y el cambio climático, se ha reducido la cobertura de manglar a lo largo del mundo. Dada la importancia de este ecosistema, las amenazas a las que está sujeto y la existencia de pocos estudios genéticos en nuestro país dirigidos a la especie *Rhizophora mangle,* realizamos este estudio en poblaciones establecidas en cuatro grandes zonas (Bahía de Chetumal, Río Hondo, Costa del Caribe Mexicano y Laguna de Bacalar) distribuidas en el sistema hidrológico denominado Corredor Transversal Costero ubicado en el sur del estado de Quintana Roo, utilizando los Inter Secuencias Simples Repetidas (ISSR) como marcador molecular. Los resultados mostraron una clara estructura genética en la comunidad de *R. mangle* y la separación de los individuos que la integran en dos grupos principales: el grupo la Bahía de Chetumal y el grupo de la Costa del Caribe Mexicano, esto como reflejo de procesos históricos como el aumento en el nivel del mar. También se identificó una estructura genética a escala fina (EGEF) lo que podría estar relacionado con factores intrínsecos (e. g., dificultades en la dispersión de propágulos) y factores extrínsecos (e.g., fragmentación del hábitat). Por otro lado, la diversidad genética a nivel local y a nivel especie fue elevada lo que podría estar reflejado en la alta capacidad de adaptación de la especie a ambientes poco favorables. Finalmente, se analizaron las tipologías chaparra y de franja, llegando a la conclusión de que se trata de la misma especie y que podrían formar linajes locales como respuesta adaptativa ecológica y fisiológica al microambiente.

Palabras clave: ISSR*, Rhizophora mangle*, Corredor Costero Transversal, diversidad genética a escala fina, manglar de franja y chaparro.

Capítulo 1

INTRODUCCIÓN GENERAL

Los ecosistemas de manglar se encuentran a lo largo de todas las costas tropicales y subtropicales del mundo, y se ubican entre los 30° N y 30° S (Kangas y Lugo 1990). Los manglares son fundamentales no sólo por ser ecosistemas de alta productividad en las zonas costeras, sino porque son el área de cría, desarrollo y reproducción de muchas especies de importancia ecológica y comercial (Muñiz-Salazar et al. 2013).

En México, las especies de mangle características son *Rhizophora mangle* L. 1753 (Malpighiales, Rhizophoraceae), el mangle rojo; *Avicennia germinans* (L.) L. (Lamiales, Acanthaceae), el mangle negro; *Laguncularia racemosa* (L.) C.F. Gaertin (Myrtales, Combretaceae), el mangle blanco y *Conocarpus erectus* L. (Myrtales, Combretaceae), el mangle botoncillo (López-Portillo y Ezcurra 2002). También se ha registrado *Rhizophora harrisoni* Leechman 1918 (Malpighiales, Rhizophoraceae) y *Avicennia bicolor* Standley 1923 (Lamiales, Acanthaceae) (CONABIO 2009). La estructura, y zonación de cada especie y la extensión de los bosques de manglar a través de la línea de costa presentan relación respecto a las variables del sustrato, tal como el nivel, frecuencia y tiempo de inundación (hidroperíodo), el drenaje del suelo, la salinidad, y la geomorfología del lugar, así como el clima, la disponibilidad de agua dulce y los nutrientes (Cuatrecasas 1958); también influyen las presiones provenientes de las competencias inter- e intra-específicas (Gómez-Aguilar 2013). Las condiciones imperantes en cada sitio serán las que determinen la densidad y el patrón de distribución de los bosques de manglar.

La especie *Rhizophora mangle* vive predominantemente en ambientes con salinidades altas y terrenos con mayor frecuencia de inundación, presenta una serie de adaptaciones fisiológicas como la presencia de neumatóforos (raíces aéreas que permiten la ventilación de sus raíces cuando éstas se encuentran sumergidas), realizan la excreción o exclusión de la sal durante el balance osmótico por medio de glándulas, lo que les permite ocupar sitios con niveles altos de salinidad y como estrategia reproductiva lleva a cabo la viviparidad (Tomlinson 1994). El mangle rojo, especie a la que está enfocado este estudio, posee una amplia distribución en México (Rabinowitz et al. 1986), encontrándose tanto en las costas del Océano Pacífico, como del Atlántico (Tovilla-Hernández y Orihuela-Belmonte 2002). En la costa del estado de Quintana Roo, nuestra área de estudio, habitan todos los tipos de manglar, según la clasificación de Lugo (1980): de franja (o de borde, marginal), chaparros (o enanos), ribereños, de isla, de cayo y de cuenca. El manglar que se extiende en la línea de

costa formando comunidades densas se denomina manglar de franja. Presenta asociaciones integradas solamente de mangle rojo o negro y asociaciones mixtas con mangle blanco y botoncillo. Estos establecimientos dependen del grado de salinidad que puede tolerar cada especie. El manglar chaparro es una comunidad de *R. mangle* que permanece inundada durante la mayor parte del año, por lo que los árboles no rebasan 2 m de altura. Esta característica fenotípica se mantiene porque los nutrimentos no se absorben eficientemente debido a la abundancia de carbonato de calcio del suelo (Granados-Sánchez et al. 1998). Los bosques de manglar chaparro se encuentran en toda la península de Yucatán y el estado de Quintana Roo (Gutiérrez-Mendoza y Herrera Silveira 2015). Aunque existen números estudios dedicados al ecosistema de manglar, muy pocos se han enfocado en la relación existente entre su genética y su tipología (Lira-Medeiros et al. 2010).

En México, hace más de dos décadas se realizó el primer estudio enfocado a la genética de poblaciones del manglar rojo, en ese trabajo se planteó integrar aspectos ecológicos, de distribución y cobertura de manglar con factores genéticos para tener un conocimiento más amplio y sólido de la especie, que posteriormente permitieran generar planes de conservación (Núñez-Farfán et al. 1997). Sandoval-Castro (2012) realizó una evaluación de la diversidad genética de mangle rojo y negro en los ecosistemas de manglar de todo el país, usando microsatélites. En ese estudio, la mayor diversidad genética registrada de *R. mangle* y *A. germinans* fue en la Península de Yucatán. Los microsatélites también han sido utilizados para determinar el éxito de los individuos de mangle rojo en un vivero con fines de reforestación, en Bahía Magdalena, Baja California Sur (Reyes-Medina 2012). Ese estudio se realizó en un área pequeña, la cual presenta numerosos esteros y canales someros rodeados por bosque de mangle. Las poblaciones estudiadas presentaron una alta estructura genética, un bajo nivel de diversidad genética y una heterocigocidad también elevada, probablemente relacionados con el tamaño y la forma del área. En otras partes de Latinoamérica, como en la costa del Pacífico colombiano, Castillo-Cárdenas et al. (2005) realizaron un estudio de la diversidad genética y estructura poblacional de *Pelliciera rhizophorae* Planch y Triana 1862 (Ericales, Tetrameristaceae) y encontraron que esta especie tiene una alta variabilidad genética a pesar de la reducción de su distribución geográfica. Lira-Medeiros et al. (2015), realizaron un análisis comparativo de diversidad genética, usando dos especies de manglar

(*Avicennia schaueriana* y *Laguncularia racemosa),* que habitan en condiciones extremas (pantanos de sal) y en condiciones favorables (ribera del río Piracão, Brasil). Relacionaron las alteraciones morfológicas (desarrollo deficiente) presentadas por ambas especies con la diversidad genética y las condiciones ambientales adversas en las que habitan. Concluyeron que la diferenciación genética hallada entre las plantas de la ribera del río y los pantanos de sal para la especie *A. schaueriana* podría estar relacionada con las alteraciones morfológicas del manglar. Por su parte, *Laguncularia racemosa*, al no tener estructuración genética entre ambas áreas, sus alteraciones morfológicas podrían ser explicadas por medio de mecanismos epigenéticos (expresión de los genes sin alterar la secuencia de ADN). Sin embargo, Lira-Medeiros et al. (2015) recomiendan realizar más estudios que ayuden a comprender la relación que existe entre la variación genética y las alteraciones morfológicas del manglar. Esto abre una perspectiva amplia para que, además de conocer la diversidad genética de los manglares de Quintana Roo, también se indague sobre qué tan estrecha es la relación entre esta diversidad genética y las diferentes tipologías presentes en las poblaciones de *R. mangle*.

En el sur del estado de Quintana Roo existe un importante sistema hidrológico y ecológico denominado “Corredor Transversal Costero” (CTC), formado por 1) la Laguna de Bacalar, el segundo lago carstico de agua dulce más grande de México, 2) Bahía de Chetumal, la laguna estuarina costera de mayor tamaño del país, y 3) el complejo Sistema Arrecifal Mesoamericano (Hernández-Arana et al. 2015). Las intrincadas conexiones subterráneas de estos sistemas, tienen un origen geológico que ha dado lugar a entornos costeros únicos en su tipo, los cuales permiten el intercambio constante de materia y energía (Hernández-Arana et al. 2015). Sin embargo, organismos y comunidades de elevada importancia ecológica que habitan en este CTC, como los manglares, presentan amenazas para su integridad debido al desarrollo urbano costero y la contaminación orgánica e inorgánica (Sánchez-Sánchez et al. 2009). Un efecto importante del desarrollo urbano es la deforestación del manglar y la vegetación costera, con una consecuente reducción de los servicios ambientales que ofrecen estos ecosistemas, como la protección para la línea de costa contra huracanes y tormentas, la retención de sedimentos y la prevención de erosión causada por el oleaje (Sánchez-Sánchez et al. 2009). Considerando la importancia de los manglares de esta región, se recomienda la realización de estudios ecológicos y

genéticos de estos organismos para tener un conocimiento amplio que podría ser utilizado en la planeación de futuros programas de conservación. Sin embargo, en todo el país, las investigaciones relacionadas con la genética del manglar son escasos; particularmente, en la zona del CTC no se ha realizado alguno. En este estudio se pretende conocer la diversidad genética y determinar si existe una estructura genética en las poblaciones de *Rizhophora mangle* que habitan en la Bahía Chetumal, Rio Hondo, Laguna de Bacalar y la costa de Xcalak, sitios pertenecientes al Corredor Costero Transversal. También, se analizará la relación entre la genética poblacional y las tipologías chaparra y de franja que presenta el mangle rojo en esta área del estado de Quintana Roo.

Capítulo 2

ARTÍCULO

Artículo sometido a publicación en la revista Molecular Ecology

Genetic structure and connectivity of the red mangle at different geographic scales through the “Transverse Coastal Corridor” in southern Quintana Roo

Running title: Fine-scale genetic structure for red mangle

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Abstract

Mangroves forests are ecological and economical valuable resources composed of trees morphologically and physiologically adapted to thrive across a range of habitats. Under those conditions, fine-scale genetic structure (FSGS) might emerge among mangroves inhabiting complex hydrographic systems. We evaluated genetic diversity and structure of fringe and dwarf *Rhizophora mangle* across a range of hydrological conditions from fresh to marine waters within the Transverse Coastal Corridor in southern Quintana Roo (TCC-SQ) Mexico, using inter-simple sequence repeat. Sampling included four hydrological systems, two localities inside each system, and fringe (n = 15) and dwarf (n = 15) trees at each locality. Genetic differentiation was evaluated at local (<100 km) and fine (<10 km) scales using principal coordinate analysis, AMOVA, Bayesian assignment (STRUCTURE), and pairwise genetic differentiation. We also applied an isolation-by-distance analysis at fine-scale level, and genetic diversity was evaluated at all scales levels and between fringe and dwarf typologies. *Rhizophora mangle* exhibited genetic structure at both scales, with high genetic diversity. The genetic structure observed in the TCC-SQ probably reflects the historical dispersion of propagules, whereas the FSGS reflect contemporary processes like restriction in seed dispersal, habitat fragmentation, and local water flow regimes. A higher genetic diversity for dwarf than for fringe form and differentiation between both typologies at fine-scale were observed. We highlighted the importance of knowing the distribution of genetic variation and populations structure in mangrove forests to assist conservation and management programs.

Key words: *Rhizophora mangle*, spatial genetic structure, dwarf and fringe mangroves, Inter Simple Sequences Repeats, Mexico, hydrochory

Introduction

Mangroves forests are worldwide distributed in intertidal zones of tropical and subtropical areas, and represent a productive ecosystem that contributes to different functional processes (e.g. demineralization, organic matter production), and provides important ecological services such as breeding and feeding areas for fauna, and coastal protection against extreme weather events (Zaldívar-Jiménez et al. 2010; Hernandez-Arana et al. 2015). Mangrove trees possess morphological and physiological adaptations to thrive in highly variable environments of marine and estuarine zones (e.g. variation in salinity, nutrients, temperatures, winds, flooding conditions) (Schaeffer-Novelli et al. 1990; Medina et al. 2010; Zaldívar-Jiménez et al. 2010).

Rhizophora mangle L. 1753 (Malpighiales, Rhizophoraceae), the red mangrove, is widely distributed in Mexico (Valderrama-Landeros et al. 2017), and structural variations in their forests have been reported in the Yucatan Peninsula (Medina et al. 2010; Zaldívar-Jiménez et al. 2010), involving three main typologies: fringe, basin, and dwarf mangroves (Zaldívar-Jiménez et al. 2010). Fringe mangroves are in direct contact with water bodies (edge of lagoon or swamp, front of the sea) with high influence of tidal inundation or river discharge (Zaldívar-Jiménez et al. 2010; Gonçalves-Reis et al. 2017) whereas dwarf mangroves are located behind fringe and basin mangroves, or directly in front of water bodies of inland areas, dealing with deficiency in nutrients (phosphorous) and hydrological stress (Zaldívar-Jiménez et al. 2010). The importance and physiological effects of variation of nutrients availability, sedimentation, and hydric stress on the growth of mangroves trees have been widely

discussed (Lovelock et al. 2007; Medina et al. 2010; Naidoo 2010; Zhang et al. 2013; Cerón-Souza et al. 2014; Gonçalves-Reis et al. 2017); however, studies working on the genetic differentiation between mangroves typologies are scarce (Lira-Medeiros et al. 2010).

Genetic structure and connectivity of mangroves forests depends on seed dispersal, pollinators, availability of suitable substrate, level of fragmentation, anthropogenic pressure and coastal/estuarine geomorphology, among other factors (Sengupta et al. 2005; Sandoval-Castro et al. 2012; Ngeve et al. 2017). Long distance seed dispersal (LDD) of mangrove species could be expected considering their hydrochory (passive dispersal of organism by water) nature, that is a highly efficient mechanism for plant dispersion leading to a high gene flow and low population structure among populations (Nilson et al. 2010). Thus, LDD has been demonstrated using genetic tools (Nettle & Dodd 2007) for *Avicennia germinans* L., as well as mark and recapture experiments for different mangrove species (Cerón-Souza et al. 2012). Nevertheless, other studies have shown limited gene dispersal for mangrove species throughout pollen and seed dispersal leading to a fine-scale genetic structure (FSGS) as demonstrated for *Rhizophora racemosa* G. Mey inside a Cameroon estuarine system (Ngeve et al. 2017), *R. mangle* and *A. germinans* at an estuary in Panama (Cerón-Souza et al. 2012), and *A. germinans* and *A. schaueriana* along the Brazilian coast (Mori et al. 2015), among others.

Genetic population structure for *R. mangle* is well documented. For example, Hodel et al. (2016) showed a high genetic connectivity between Atlantic and Gulf coasts of Florida, while Pil et al. (2011) highlighted a genetic differentiation between populations

of North and South part of the State Rio Grande do North in the Brazilian coast, and Arbeláez-Cortes et al. (2007) detected a low but significant structure among populations along the Colombian Pacific coast. In Mexico, the first study at wide geographical scale showed a genetic difference between populations from its Pacific and the Atlantic coasts (Núñez-Farfán et al. 2002), after-which Sandoval-Castro et al. (2014) showed five clades for *R. mangle* along Mexican coasts, with no genetic differentiation for populations from the Gulf of Mexico and the Caribbean region. At regional scale, Sandoval-Castro et al. (2012) reported genetic differentiation in two groups for populations along the northwestern coast of Mexico, and Reyes Medina et al. (2016) showed a genetic structure for *R. mangle* inside the complex Magdalena Bay in Baja California Sur. Genetic studies at regional or smaller geographical scale have never been done in Quintana Roo, or even at the Yucatan Peninsula (YP) scale. The YP region is particularly important for mangrove species because 1) harbors more than 50% of mangrove cover extension of Mexico (Valderrama-Landeros et al. 2017) and it was declared a hotspot for biodiversity conservation (Myers et al. 2000), 2) its coastline is highly vulnerable to climatic events like hurricanes and storm surges which negatively affect mangrove forests (Adame et al. 2013), and 3) the high development of this region (touristic infrastructures) particularly along the shoreline, has destroyed potential habitats for mangrove (Murray 2007; Hirales-Cota et al. 2017).

The “Transverse Coastal Corridor” (TCC) located in the southern part of Quintana Roo (Mexico) is a complex ecological corridor characterized by connectivity processes among ecosystems through hydrological, biogeochemical, and ecological interactions (Hernandez-Arana et al. 2015). Fine-scale genetic structure of *R. mangle* populations could be expected to occur in the TCC, because mangroves inhabits here in three

contrasting hydrological systems (Hernandez-Arana et al. 2015): 1) Bacalar lagoon, the second largest freshwater karstic lake in Mexico, characterized by low level of nutrients (oligotrophic) particularly nitrogen, 2) Chetumal Bay, the largest estuarine-coastal lagoon in Mexico, showing spatial and seasonal variations from oligotrophic to eutrophic, and brackish waters, and 3) The Caribbean coast of Mexico, with the most complex coral reef ecosystem of the Mesoamerican Barrier Reef System. These hydrological systems are interconnected by aboveground (channels and flood zones) and underground flows that facilitate physical, chemical, and biological transfers, driving to ecological and connectivity processes that determine biodiversity and the function of ecosystems (Hernandez-Arana et al. 2015). Mangroves are one of the main vegetation types in the TCC. The fringe, dwarf, island and basin are the principal mangrove typologies along this corridor (Hernandez-Arana et al. 2015).

Several molecular techniques are available to study genetic populations structure and have been largely used, alone or combined, for mangroves studies as isozymes (Núñez-Farfán et al. 2002), microsatellites (e.g. Cerón-Souza et al. 2012; Sandoval-Castro et al. 2012, 2014; Reyes Medina et al. 2016), chloroplast DNA (Huang et al. 2008; Cerón-Souza et al. 2012; Takayama et al. 2013), AFLP (Maguire et al. 2002), RAPD (Dasgupta et al. 2015), and ISSR (Ge & Sun 2001; Su et al. 2007; Huang et al. 2008; Kader et al. 2012; Dasgupta et al. 2015). Inter-simple sequence repeat (ISSR) is a dominant molecular marker which permit to amplify large number of highly polymorphic loci (Machkour-M'Rabet et al. 2017), and have been largely used to evaluate genetic diversity and structure in many plants species (Tanya et al. 2011; Kumar et al. 2016; Thakur et al. 2016), particularly of mangrove species (see above for examples).

Considering the limited information about genetic diversity and fine-scale genetic structure for mangrove species, and the difference between mangrove typologies in the southern part of Mexico, we took the advantage of the occurrence of *Rhizophora mangle* in relatively close distances but in contrasting hydrological systems to focus our study, using ISSR molecular markers, in the following questions: i) do dwarf and fringe mangrove trees express a genetic differentiation pattern at local geographic scale (< 100 km)?, ii) do different patches of *R. mangle* forest constitute a panmictic unit inside the TCC system?, and iii) do *R. mangle* display a genetic structure at fine-scale (< 10 km)?

Materials and Methods

Sampling

Our sampling design considered the diversity of hydrological systems that typologies *of R. mangle* inhabit in the TCC system and their potential connectivity, by including three factors: 1) hydrological regimes at local scale (10 km – <100 km; Ngeve et al. 2017), which could affect the connectivity of *R. mangle* populations inhabiting those regimes, 2) genetic connectivity inside each hydrological system at fine-scale (100 m – <10 km) among *R. mangle* populations at two very close localities per system, and 3) fringe and dwarf typologies of *R. mangle*. Four areas were selected to sample *R. mangle* including the three principal TCC hydrological systems (Bacalar lagoon, Chetumal Bay, and Caribbean coast) and one important connecting (channel) area (Hondo river) (Fig. 1 and Table 1). At each area, two localities were chosen to sample

R. mangle at the fine-scale, due to the presence of fringe and dwarf typologies. At each locality, two transects (one per typology mangrove trees) of 450 m wide and 200 large were delimited. In each transect, five young leaves with petiole were collected from 15 trees of each typology, ensuring a minimal distance of 10 m between samples to avoid sampling the same tree. Immediately after cutting the leaves, PVP-40 (SIGMA-ALDRICH) was added at the cutting petiole zone for preservation. Leaves from each tree were placed and labelled in a botanical leaf press. In the laboratory, leaves of each tree were removed from the leaf press, then repeating the PVP-40 addition at the cutting leaf zone. The leaves from each tree were individually wrapped in aluminum paper and placed in plastic labeled bag, later stored in an ultra-freezer (-70°C) until DNA extraction was performed. Samples of *Rhizophora mangle* were collected under permit SGPA/DGVS/05702/17.

DNA extraction and ISSR-PCR amplification

Total genomic DNA for each sample tree was isolated from a small part of one leaf (~ 0.2 mg). Leaf tissue was grinded to powder using a porcelain mortar and liquid nitrogen, afterwards the powder sample was re-suspended in a lysis solution of CTAB 2% protocol and extraction method was applied (Martínez-Castillo et al. 2014). DNA concentration and quality were determined respectively using a Qubit 2.0 fluorometer (INVITROGEN) and electrophoresis in agarose gel 1%/TAE (1x) with GelRed (BIOTUM) as post-staining.

A total of 23 ISSR primers were screened from which 19 displayed band patterns, nine showed clear and unambiguous scoring, and four [$(GAA)_6$, $(AG)_8C$, $(CA)_8AC$, $(AG)_8YC$]

were selected for further analysis because they exhibited high polymorphism (Table 2). PCR amplifications were performed in a 15 µl reaction volume containing ~2 µl of template DNA, 1.5 µl of 5x buffer (PROMEGA), 0.3 µl of dNTP mix (PROMEGA), 1.8 µl of MgCl2 (PROMEGA), 0.3 µl of primer (1 µM) (INTEGRATE DNA TECHNOLOGY), 0.25 µl Taq Polymerase (GoTaq Flexi, PROMEGA), and 8.85 µl of ultrapure water. All amplifications were processed in a gradient thermocycler (T100, BIO-RAD) under the following conditions: initial denaturation at 94 °C for 5 min, 45 cycles of denaturation at 94 °C for 45 s, primer annealing temperature 52–60 °C depending on the ISSR primer (Table 2) for 45 s, an extension at 72 °C for 90 s, and final extension at 72 °C for 7 min. PCR products were separated by electrophoresis on agarose gel (2% / 1x TAE) using GelRed (BIOTUM) as post-staining method at 110 V for 3 h. A 100 bp DNA ladder (PROMEGA) was used to evaluate the PCR product length. Band patterns were visualized and digitized on an imaging system (Photodoc-IT 65, UVP). The presence (1) or absence (0) of ISSR bands pattern was scored. This information permitted to generate the binary matrix used for analysis, where only individuals that present genetic information for all primers were included. This explains the discrepancy between the number of individuals collected and processed, and the number of individuals used in analysis (Table 1).

Data analysis

First, we verified if fringe and dwarf mangrove typologies presented some level of genetic differentiation and thus if they could be pooled for further analysis. For this, different and complementary analyses were achieved: the number of private bands (loci), a principal cooordinate analysis (PC_oA), and an analysis of molecular variance

(AMOVA with 999 permutations; Excoffier et al. 1992). These analyses were performed using GENALEX 6.5 (Peakall and Smouse 2006, 2012).

Genetic diversity of *R. mangle* was determined using the percentage of polymorphism loci (*PPL*) and Nei's gene diversity (*h*) for the whole dataset, for each hydrological system (including two localities and two mangrove typologies) considered in this study, and for fringe and dwarf mangroves typologies at each locality, using POPGENE 1.31 (Yeh et al. 1999).

Genetic differentiation was approached at different geographic scales. At local-scale, we evaluated the genetic differentiation among hydrological systems by applying Φ_{ST}, an analogue of Wright's F_{ST}, determined for all pairs of hydrological systems (9999 permutations in GENALEX 6.5). In order to confirm these results, we applied two different methods to identify the most probable genetic structure among the four hydrological systems inside the TCC system. First, a Bayesian clustering analysis implemented in STRUCTURE v2.3.3 (basic algorithm: Pritchard et al. 2000, with an extension to the method: Falush et al. 2007; Hubisz et al. 2009) was performed to infer the potential number of populations (or clusters; defined as *K*). This method was designed to identify the optimal number of clusters (*K*) and assigned each individual (sample) with a probability (q_i) to one cluster or more (admixed genotypes). The admixture (considering Locprior option) and allele correlated frequency models were performed. The program was run 10 times for different values of *K* (from 1 to 11) to determine the optimal number of clusters with the Markov chain Monte Carlo algorithm using a burn-in period of 10,000 steps followed by 10,000 steps. To identify the most probable number of *K* that best fitted our data, we used two methods. First, we

identified the maximal value of Ln *P(D)*, the value that indicates the start of "more-or-less plateaus" for larger *K* (Pritchard et al. 2010). Second, we used the Δ*K* method (Evanno et al. 2005) that can be found in the STRUCTURE HARVESTER website (Earl & vonHoldt 2012). When the optimal *K* was obtained, the homogeneity of results for this *K* was confirmed by applying 10 runs under the same conditions but with a burn-in period of 100,000 steps followed by 100,000 steps. Second, the graphical method of the PC_oA was performed with GENALEX 6.5 to show the relationships among samples from all hydrological systems.

The fine-scale genetic structure was approached zooming inside each hydrological system. The genetic differentiation between both localities inside each area was evaluated by AMOVA (9999 permutations) and the graphical method of the PC_oA. Furthermore, we determined the Φ_{ST} parameter between both localities inside each hydrological system (9999 permutations). To test an isolation-by-distance pattern (IBD) we applied a Mantel test which identifies any significant relationships between two data matrices (genetic and geographic). These analyses were performed using GENALEX 6.5.

Finally, to evaluate the genetic relation between fringe and dwarf mangroves, an AMOVA (9999 permutations) was performed considering the two typologies at each locality using GENALEX 6.5.

Results

The selection of four primers allowed to obtain a total of 81 clear and unambiguous fragments of ISSR markers (loci) for 198 sample of *R. mangle* trees from eight localities of the TCC system. The identification of only four private bands (two per mangrove typology; Table 3) confirmed that all samples belonged to the same species.

Do dwarf and fringe mangrove trees possess a genetic differentiation pattern at local geographical scale (< 100 km)?

Genetic analysis of 100 fringe and 98 dwarf forms of *R. mangle* did not support genetic differentiation. AMOVA revealed an extremely low (1%) level of differentiation (Table SM1), and the two first axis of PC_oA did not allow to distinguish groups (Fig. SM1). Accordingly, samples of fringe and dwarf mangrove forms were pooled for the genetic structure and diversity analysis.

Local-scale genetic diversity and structure of Rhizophora mangle

Considering all *R. mangle* individuals throughout the TCC system, POPGENE analysis revealed very high polymorphism (100%) and Nei's gene diversity (h = 0.317 ± 0.172 SD). At the hydrological system level (Table 3) all genetic diversity parameters kept high, with the highest level of diversity for Hondo river (*PPL* = 89%, h = 0.292) and Bacalar lagoon (*PPL* = 81%, h = 0.296), and lowest diversity for Chetumal Bay (*PPL* = 79%, h = 0.271).

The pairwise genetic differentiation coefficients showed a greater differentiation between Chetumal Bay and the Caribbean coast (Φ_{ST} = 0.192), some differentiation between Hondo river and the Caribbean coast (Φ_{ST} = 0.164), and lower differentiation among other paired areas (Table 4). The method of Ln *P*(*D*), to identify the appropriate number of clusters (*K*), suggested a *K* = 3 (Fig. SM2-A), while the Delta *K* method applied in STRUCTURE HARVESTER website showed a peak at *K* = 2, with a very high and close value for *K* = 3 (Fig. SM2-B). Consequently, both genetic structures (*K* = 2 and *K* = 3) are presented. When probability assignment estimated by STRUCTURE was used, all sample trees were partitioned into two clusters showing a gathering of initial localities evaluated by the *Q* probability values (Table 5). Each of the new cluster was clearly defined by Chetumal Bay samples (cluster 1 in red on Fig. 2A), and Caribbean coast (cluster 2 in blue on Fig. 2A) both with very good membership (mean for both localities: 97% and 97% respectively). Both other hydrological system (Bacalar lagoon and Hondo river) displayed a mix of clusters (1 and 2) with different membership proportions (Table 5). Bacalar lagoon showed higher similarity with Chetumal Bay at two localities (membership coefficients of Pedro Santos and Hub Sak to cluster 1; Table 5) while the entrance locality of Hondo river showed high similarity with Chetumal Bay and inward locality of the river was more similar to Caribbean coast (membership coefficients in Table 5).

An increase to three clusters continued to show the cluster 1 and cluster 2 well defined for Chetumal Bay and Caribbean coast respectively (membership coefficient in Table 5) while the other two areas exhibited a more complex situation (Fig. 2B). In the third cluster, the inward locality of Hondo river was well defined (membership coefficient of 95%; cluster 3 in yellow in Fig. 2B), while the entrance of this river was influenced by

Chetumal Bay and inward Hondo river locality (membership coefficient in Table 5). Each locality of Bacalar lagoon presented different situation. The Pedro Santos locality showed an admixture of cluster 1 (Chetumal Bay; red in Fig. 2B) and cluster 2 (Caribbean coast; blue in Fig. 2B) with low contribution of cluster 3 (yellow in Fig. 2B), while Huub' Sak locality showed contribution mainly from both Chetumal Bay (cluster 1 in red in Fig. 2B) and Hondo river inward (cluster 3 in yellow in Fig. 2B) with very low contribution of Caribbean coast (cluster 2 in blue in Fig. 2B) (Table 5 for membership proportions). When principal coordinate analysis (PC_oA) was performed, a clear separation of Chetumal Bay and Caribbean coast (red and blue respectively on Fig. 3) occurred, as found with Bayesian analysis of STRUCTURE. In the positive side of *y*-axis, a large number of points of the inward locality of Hondo river (yellow triangle in Fig. 3) formed the third cluster as proposed by STRUCTURE analysis. Other localities (entrance of Hondo river and the two localities of Bacalar lagoon; Fig. 3) had different contribution level from the three principal groups (Chetumal Bay, Caribbean coast, and inward locality of Hondo river) in agreement with STRUCTURE analysis for $K = 3$.

Fine-scale genetic structure of Rhizophora mangle

Both localities of each hydrological system showed some genetic structuration throughout AMOVA (Table 6) and PCoA (Fig. 4), with high Φ_{ST} coefficients between them: Chetumal bay ($\Phi_{ST} = 0.117$, $P = 0.0001$), Bacalar lagoon ($\Phi_{ST} = 0.188$, $P = 0.0001$), Hondo river ($\Phi_{ST} = 0.178$, $P = 0.0001$), and Caribbean coast ($\Phi_{ST} = 0.138$, $P = 0.0001$). Furthermore, Mantel test indicated a significant IBD pattern inside each hydrological system: Chetumal bay ($r^2 = 0.06$, $P = 0.001$), Bacalar lagoon ($r^2 = 0.238$,

P = 0.001), Hondo river (r^2 = 0.217, P = 0.001), and Caribbean coast (r^2 = 0.099, P = 0.001).

Genetic characterization of fringe and dwarf mangrove typologies

Generally, dwarf mangroves exhibited slightly higher genetic diversity parameters (*PPL* and *h*; Table 7) than fringe mangroves at each locality. Interestingly, although both typologies of mangroves did not distinguish genetic differentiation in the analysis integrating all samples, a fine-scale genetic differentiation was evident through AMOVA (Table 8). At all localities (except for Hondo river inward), AMOVA showed a high and significant genetic differentiation.

Discussion

This study represents an important step in the genetic knowledge of *Rhizophora mangle* in Mexico considering that is the first one at a fine geographic scale and in a complex transversal hydrographic system, including the dwarf and fringe typologies of this species. Results highlights a strong genetic structure for *Rhizophora mangle* community across the Transverse Coastal Corridor, a complex hydrographic system in the south of Quintana Roo, Mexico. Our results (Φ_{ST} coefficients, STRUCTURE and PC_oA analysis) showed a clear separation between Chetumal Bay (including the entrance of Hondo river) and the Mexican Caribbean coast, which was unexpected considering the well-established interconnection between Chetumal Bay and the Caribbean Sea through its wide and long mouth (Hernández-Arana et al. 2009) and other minor interconnections through narrow and short natural (Bacalar Chico) and

artificial (Zaragoza) channels (Hernández-Arana & Ameneyro-Angeles 2011). Hondo river and Bacalar lagoon mangroves trees consisted of a mix of both principal clusters (Chetumal Bay and Caribbean coast) with *K* = 2; however, with *K* = 3 Hondo river (mainly its inward part) emerged as the third cluster (yellow in Fig. 2), isolated from both other clusters; the south part of Bacalar (Huub' Sak) was similarly related with Hondo river and Chetumal Bay clusters but much less related with the Caribbean coast. Those results contrast with studies showing high connectivity for mangrove species as *R. mangle* and *Avicennia germinans* in the Florida coasts (Hodel et al. 2016) and *R. mangle* forming genetic homogeneous groups inside wide geographical areas along Mexican coasts (Núñez-Farfán et al. 2002; Sandoval-Castro et al. 2014). Nonetheless, our results are in accordance with other studies showing fine-scale genetic structure (FSCG) for mangrove species as *R. racemosa* which presented high genetic structure inside estuaries along the coast of Cameroon, reflecting the historical sea level rise due to climate change but also intrinsic and extrinsic contemporary processes (Ngeve et al. 2016, 2017). Also, Cerón-Souza et al. (2012) showed genetic differentiation for *A. germinans* and *R. mangle* in estuaries on the Caribbean and Pacific coasts of Panama, highlighting the importance of coastal geomorphology and limitation of seed dispersal to explain that level of structure. In this sense, when considering three clusters, the north of Bacalar (Pedro Santos locality) was more related with the Caribbean coast while the south of Bacalar and Hondo river showed more relation with the Chetumal Bay.

Genetic structure of *R. mangle* observed with a contemporary genetic marker could reflect the consequences of the historical sea level rise occurring between 4600 and 4000 B.P. (Torrescano & Islebe 2006) and the variation of mangrove coverage due to

successive episodes of dry periods in this region beginning around 3400 B.P. to ~2000 B.P. when climatic conditions became favorable for tropical forests and mangroves (Aragón-Moreno et al. 2018). Those historical events could have isolated forest mangroves which were maintained by sea current movement along the Mesoamerica Reef System, the recent increase in mean sea level (Ruiz-Ramírez et al. 2014), and the geomorphology and hydrological characteristics of the TCC systems. Loss of connectivity among hydrographic systems of the TCC is reinforced by the dispersal limitation of *R. mangle* seed (see below). The unique source of connectivity between Chetumal Bay and the south part of Mexican Caribbean coast is the wide mouth (~ 15 km) of Chetumal Bay; however, this does not allow a genetic homogenization among both systems. This is not surprising, because runoff coming from Honduras (Caribbean Current) is transported towards Chinchorro Banks flowing south-to-north through the Yucatan Channel (Yucatan Current), but another part flows north-to-south along the coast of southern Quintana Roo (more or less around Majahual) and Belize and re-circulating into the Gulf of Honduras (offshore gyre rotting counterclockwise), finally a part taken toward the outside of the Mesoamerican Barrier Reef System (Ezer et al. 2005; Chérubin et al. 2008). Furthermore, water movement from Chetumal Bay flows north-to-south towards coast of Belize and Gulf of Honduras (Ezer et al. 2005). Natural and artificial channels (Bacalar Chico and Zaragoza respectively) which connect Chetumal Bay and Caribbean coast of Quintana Roo didn't represent a source of exchange for *R. mangle* trees probably because the density of mangrove trees increases the retention of propagules as suggested in other studies (Ngeve et al. 2017), and also because the size (2,600 km^2) of the Chetumal Bay and its complex hydrodynamic (Avalos-Cuevas et al. 2017) would limit the connection between the Bay and the coastal area. All of those particular movement of water in this region could

explain the lack of connectivity between Chetumal Bay and Caribbean coast. A wider sampling around the coasts of Chetumal Bay, and along the Caribbean coast, will be necessary to have a better view about the connectivity of mangrove populations inside the Bay and the connectivity level along the Mesoamerican Reef System. We are aware that our hypothesis of mangrove populations distribution is speculative because is based on only one nuclear genetic marker, and that the use of different molecular markers as cpDNA or genomic data (e.g., RADseq) need to be investigated.

At the species level, the genetic diversity observed for *R. mangle* in the TCC was high and consistent with other studies using ISSR molecular markers. For example, *Lumnitzera littorea* (Combretaceae) a non-viviparous mangrove from tropical Asia and north Australia showed high genetic diversity (*PPL* = 76,5%, H_e = 0.240) (Su et al. 2007), and *Kandelia obovate* (Rhizophoraceae), a tropical and subtropical mangrove from China, also showed high values of genetic diversity (*PPL* = 83%, *H* = 0.363) (Chen et al. 2010), while a cumulative study using AFLP + ISSR on *Merope angulata* (Rutaceae) showed a medium polymorphism (43%) and high heterozygosity (H_e = 0.393) (Jena et al. 2015). In contrast, other studies have shown extremely low genetic diversity (*Nypa fruticans*, *P* = 3% and H_e = 0.0113, Jian et al. 2010; *Bruguiera gymnorrhiza*, *P* = 24.36 % and *h* = 0.0785, and *Heritiera fomes*, *P* = 12.76 % and *h* = 0.0592, Dasgupta et al. 2015). The genetic diversity at species level, reflecting long-term evolution, is a prerequisite for its survival and development (Shen et al. 2005). At the population level, the genetic diversity reflects the adaptability and evolution of the specie, showing the genotype richness in specific environments (Chen et al. 2010), and provides valuable information about the status of a species and an assessment of its conservation value (Dasgupta et al. 2015). All local communities of *R. mangle* in the

TCC system exhibited higher genetic diversity suggesting greater ability to adapt and evolve (Li & Chen 2004). Probably, the conspicuousness inside each hydrographic system in the TCC of *R. mangle*, the most common mangrove species in this region (Diaz-Gallegos & Acosta-Velázquez 2009, Adame et al. 2013), permitted to obtain the high genetic variation.

Fine-scale genetic structure (FSGS) was found inside each hydrographic system suggesting a limitation in dispersal capacity (pollen and seed) (Cerón-Souza et al. 2012; Ngeve et al. 2017) or environmental barriers limiting the development of propagules. Several factors have been proposed to understand FSGS in mangrove species. The most obvious intrinsic factor is the limitation of dispersion of mangrove propagules caused by the high density of the root network, characteristic of *Rhizophora* spp., making new mangrove trees to get stranded very close of their parent trees (Van der Stocken et al. 2015; Ngeve et al. 2017). Extrinsic factors could be responsible of the limitation of dispersion of mangrove propagules explaining the FSGS, as geomorphology of the shore, habitat fragmentation, and water flow regimes inside each system (Cerón-Souza et al. 2012; Ngeve et al. 2017). For example, the rapid urban development around Bacalar lagoon, the Chetumal Bay, and in the Xcalak community of the Caribbean coast could cause mangrove forest fragmentation, which together with the presence of the artificial Zaragoza Channel and the Chile Verde river both with flows into the Chetumal Bay could contribute to explain the FSGS. The study of *R. mangle* communities in the complex hydrographic systems of the TCC put in evidence the role of intrinsic and extrinsic factors to explain the FSGS as suggested before by other studies (Cerón-Souza et al. 2012; Mori et al. 2015; Ngeve et al. 2017).

Nevertheless, to refine our understanding of causes linked to the FSGS in the TCC, further investigations would be needed but they are beyond the purpose of this study.

Genetic studies that evaluate the level of genetic variation between different typologies of nearby mangrove trees are scare. Lira-Medeiros et al. (2010) using the methyl-sensitive amplified polymorphism method highlighted the importance of the epigenetic variation in the mangrove *Laguncularia racemosa*, being more important than genetic variation to explain the typology differences (smaller trees near a salt marsh than in riverside habitats). Description differences in size development of *R. mangle* trees is largely documented (Lopez-Portillo et al. 1989; Granados-Sánchez et al. 1998; Medina et al. 2010; Zaldívar-Jiménez et al. 2010), being dwarf forms generally associated to limited resources and hydrological stress (Lira-Medeiros et al. 2010; Zaldívar-Jiménez et al. 2010). The need to know the responses to those constrains by dwarf typology would also help to explain the slightly higher values of genetic diversity for dwarf typology than that found in fringe typology trees. However, to confirm those results, a wide sampling trees and a powerful genetic method capable to screen high number of loci (as RADseq genomic data) should be applied. Interestingly, we observed a genetic differentiation (AMOVA) between dwarf and fringe mangrove trees within and between almost all localities, separated from 200 m to 30 km. Those differences could reflect the formation of local lineages (dwarf *vs.* fringe) as a response to some ecological and physiological adaptations to microenvironment (Schwarzbach & Ricklefs 2001). Although our study suggest that some genetic variation exist between fringe and dwarf mangrove typologies, more genomic, ecologic, and physiologic studies are needed to understand the complex relation among fringe and dwarf *R. mangle* trees.

Mangrove forest is one of the most important ecosystem for the aquatic and terrestrial biodiversity in tropical and subtropical regions, they represent high valuable ecological and economical resources and yet, they suffer a high loss rate generally correlated with human density and anthropogenic activities, as urban development, aquaculture, pollution and fragmentation, among others (Alongi 2002; Cerón-Souza et al. 2012). The understanding about the distribution of genetic diversity and the organization of populations in a mangrove community would assist conservation and management programs (Schwarzbach & Ricklefs 2001) particularly in economic growing regions as in the case of the southern Quintana Roo. Our study permitted to bring to light the presence of some genetic structure in the southern Quintana Roo that probably reflect historical processes and contemporary events. We also showed that Bacalar, even that is presenting anthropogenic pressure, exhibit the higher genetic diversity for *R. mangle* trees which make it a good candidate for a conservation priority area. Genetic structure observed in the TCC suggests that mangrove areas could constitute different management units for which management and conservation programs could be adapted depending on intrinsic and extrinsic factors that limit the connectivity and the genetic diversity of mangrove trees.

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Data Accessibility Statement

All dataset used in this study will be accessible on FigShare when the manuscript will be accepted for publication.

Authors Contribution

Designed research: MMS, EAJ, HAHA, LAH, and HY; Performed research: CIL; Resources: MMS and HAHA; Analysis: CIL and MMS; Project administration: MMS; Wrote the paper: CIL, MMS, EAJ, HY, HAHA.

Table 1. Sampling information of *Rhizophora mangle* collected through the Transverse Coastal Corridor of southern Quintana Roo, Mexico.

Hydrological system	Locality	GC	Mangle typology	*N*1	*N*2
Chetumal Bay	Tamalcab	18°36'N-88°12'W	Fringe	15	15
			Dwarf	15	13
	Playa Cayo Venado	18°46'N-88°06'W	Fringe	15	15
			Dwarf	15	13
Bacalar lagoon	Pedro Santos	18°56'N-88°09'W	Fringe	15	11
			Dwarf	15	12
	Huub' Sak	18°48'N-88°16'W	Fringe	15	6
			Dwarf	15	9
Caribbean coast	Xcalak	18°16'N-87°50'W	Fringe	15	12
			Dwarf	15	12
	Bacalar Chico	18°08'N-87°51'W	Fringe	15	15
			Dwarf	15	15
Hondo river	Entrance	18°29'N-88°18'W	Fringe	15	13
			Dwarf	15	12
	Inward	18°29'N-88°19'W	Fringe	15	13
			Dwarf	15	12

GC: geographic coordinates, *N*1: number of collected samples, *N*2: number of samples with genetic information used for analysis.

Table 2. ISSR primers information used for *Rhizophora mangle*.

Primer code	Primer sequence (5'→3')	T_a (°C)	*N* bands	Size (bp)
$(GAA)_6$	GAA GAA GAA GAA GAA GAA	52	19	100-1500
$(AG)_8C$	AG AG AG AG AG AG AG AG C	57	17	200-1500
$(CA)_8AC$	CA CA CA CA CA CA CA CA AC	60	19	200-1500
$(AG)_8YC$	AG AG AG AG AG AG AG AG YC	58	26	300-1500

T_a: annealing temperature, *N* bands: number of bands over all localities, size (bp): range size of the DNA fragments. YC: degenerated sites.

Table 3. Genetic diversity parameters estimated with POPGENE for *Rhizophora mangle* from four hydrological systems of the Transverse Coastal Corridor in the southern Quintana Roo.

Area	*n*	*N* Loci	*N* PoL	*N* PrL	*PPL* (%)	*h* (SD)
Chetumal Bay	56	71	64	1	79.01	0.271 (0.196)
Bacalar lagoon	38	71	66	1	81.48	0.296 (0.182)
Hondo river	50	78	72	2	88.89	0.292 (0.190)
Caribbean Coast	54	73	66	1	81.48	0.291 (0.192)

n: number of samples, *N* Loci: total number of bands per area, *N* PoL: total number of polymorphic bands per area, *N* PrL: total number of private bands per area, *PPL* (%): percentage of polymorphic loci, *h* (SD): Nei's gene diversity per area (standard deviation).

Table 4. Pairwise genetic differentiation values (Φ_{PT}; below diagonal) determined for *Rhizophora mangle* among four hydrological systems of the Transverse Coastal Corridor system.

	Chetumal Bay	Bacalar lagoon	Hondo river	Caribbean
Chetumal Bay		0,0001	0,0001	0,0001
Bacalar lagoon	0,119		0,0001	0,0001
Hondo river	0,132	0,113		0,0001
Caribbean	**0,192**	0,143	**0,164**	

Above diagonal: probability for Φ_{PT} based on 9999 permutations. Highest values are in bold.

Table 5. Membership probabilities (*Q*) of each pre-defined sample localities in each of the new clusters for *K* = 2 and *K* = 3.

Hydrological system	Locality	Clusters for *K* = 2		Clusters for *K* = 3		
		1	2	1	2	3
Chetumal Bay	Tamalcab (*n* = 28)	**0.993**	0.007	**0.996**	0.000	0.004
	Playa Cayo Venado (*n* = 28)	**0.951**	0.049	**0.928**	0.064	0.008
Bacalar lagoon	Pedro Santos (*n* = 23)	**0.596**	0.404	**0.510**	0.439	0.051
	Huub' Sak (*n* = 15)	**0.822**	0.178	**0.469**	0.074	**0.457**
Caribbean coast	Xcalak (*n* = 24)	0.010	**0.990**	0.005	**0.993**	0.002
	Bacalar Chico (*n* = 30)	0.046	**0.954**	0.049	**0.931**	0.020
Hondo river	Entrance (*n* = 25)	**0.895**	0.105	**0.554**	0.016	0.430
	Inward (*n* = 25)	0.469	**0.531**	0.011	0.036	**0.952**

The highest value assigned of a sample to one of the new clusters is indicated in bold. *n*: number of individual of *Rhizophora mangle* trees.

Table 6. Analysis of molecular variance (AMOVA) based on nuclear ISSR markers for *Rhizophora mangle* inside each hydrological system of the Transverse Coastal Corridor system.

Source of variation	df	SS	MS	Est. Variance	% variance	*P*
Chetumal Bay						
Among localities	1	39,911	39,911	1,122	12	0.001
Within localities	54	458,607	8,493	8,493	88	
Total	55	498,518		9,615	100	
Bacalar lagoon						
Among localities	1	48,264	48,264	2,147	19	0.001
Within localities	36	334,104	9,281	9,281	81	
Total	37	382,368		11,428	100	
Hondo river						
Among localities	1	60,480	60,480	2,043	18	0.001
Within localities	48	451,520	9,407	9,407	82	
Total	49	512,000		11,450	100	
Caribbean coast						
Among localities	1	47,225	47,225	1,435	14	0.001
Within localities	52	465,775	8,957	8,957	86	
Total	53	513,000		10,392	100	

Table 7. Genetic diversity parameters estimated with POPGENE for *Rhizophora mangle* between the fringe and dwarf typologies at each locality.

Hydrological system	Locality	Fringe mangrove			Dwarf mangrove		
		n	*PPL*	*h* (SD)	n	*PPL*	*h* (SD)
Chetumal Bay	Tamalcab	15	48.15	0.182 (0.387)	13	55.56	0.208 (0.215)
	Playa Cayo Venado	15	58.02	0.222 (0.215)	13	58.02	0.210 (0.207)
Bacalar lagoon	Pedro Santos	11	54.32	0.197 (0.210)	12	60.49	0.246 (0.212)
	Huub' Sak	6	49.38	0.183 (0.206)	9	56.79	0.223 (0.216)
Caribbean coast	Xcalak	12	46.91	0.187 (0.215)	12	54.32	0.221 (0.217)
	Bacalar Chico	15	66.67	0.240 (0.208)	15	61.73	0.223 (0.201)
Hondo river	Entrance	13	54.32	0.177 (0.204)	12	65.43	0.233 (0.209)
	Inward	13	60.49	0.191 (0.194)	12	61.73	0.231 (0.206)

n: number of samples, *PPL*: percentage of polymorphic loci, *h* (SD): Nei's gene diversity (standard deviation).

Table 8. Analysis of molecular variance (AMOVA) based on nuclear ISSR markers for *Rhizophora mangle* between fringe and dwarf mangroves typologies at each locality.

Source of variation	df	SS	MS	Est. Variance	% variance	*P*
Tamalcab						
Among typology	1	28,738	28,738	1,519	17	0.0001
Within typology	26	197,262	7,587	7,587	83	
Total	27	226,000		9,106	100	
Playa Cayo Venado						
Among typology	1	15,202	15,202	0,491	6	0.019
Within typology	26	217,405	8,362	8,362	94	
Total	27	232,607		8,853	100	
Pedro Santos						
Among typology	1	20,024	20,024	1,017	11	0.002
Within typology	21	175,280	8,347	8,347	89	
Total	22	195,304		9,364	100	
Huub' Sak						
Among typology	1	19,744	19,744	1,470	14	0.0026
Within typology	13	119,056	9,158	9,158	86	
Total	14	138,800		10,628	100	
Xcalak						
Among typology	1	19,125	19,125	0,932	10	0.0023
Within typology	22	174,750	7,943	7,943	90	
Total	23	193,875		8,875	100	
Bacalar Chico						
Among typology	1	26,033	26,033	1,150	12	0.0001
Within typology	28	245,867	8,781	8,781	88	
Total	29	271,900		9,931	100	
Hondo river entrance						
Among typology	1	42,820	42,820	2,750	24	0.0001
Within typology	23	195,500	8,500	8,500	76	
Total	24	238,320		11,250	100	
Hondo river inward						
Among pops	1	12,309	12,309	0,286	3	0.066
Within pops	23	200,891	8,734	8,734	97	
Total	24	213,200		9,021	100	

Figure Legends

Figure 1. Hydrological systems and localities in the Transverse Coastal Corridor in the southern Quintana Roo, Mexico, where *Rhizophora mangle* tree samples were collected.

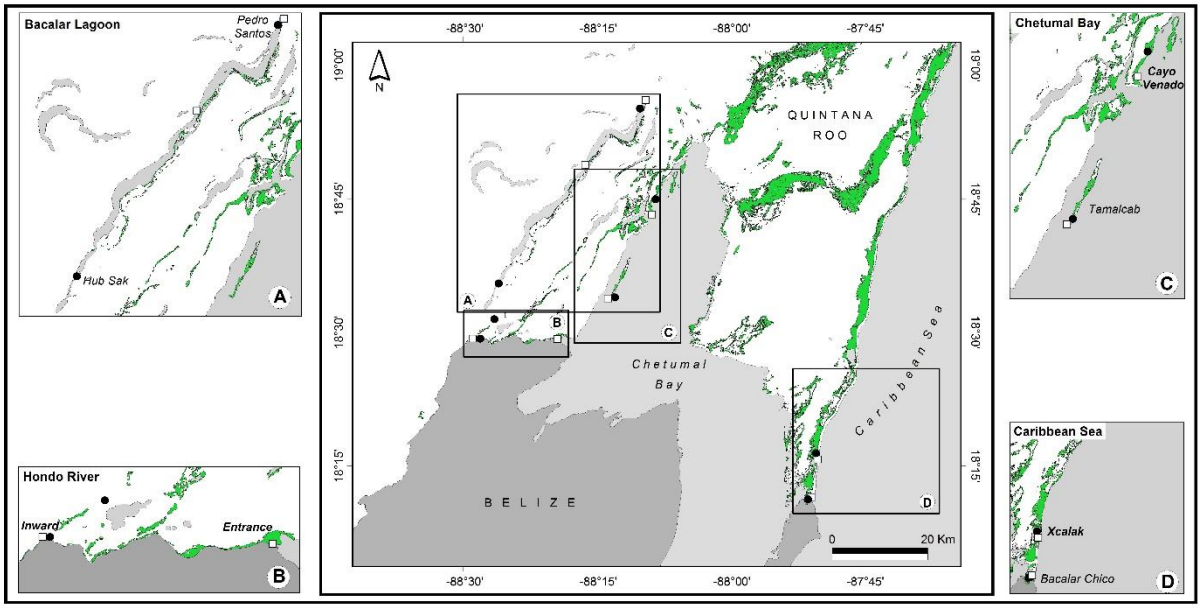
Figure 2. Bayesian analysis of *Rhyzophora mangle* in the Transverse Coastal Corridor, Yucatan Peninsula, Mexico, computed by STRUCTURE 2.3.1 software. (A) results for K = 2, (B) results for K = 3. Each individual is represented by a single vertical line broken into K segments of length proportional to the estimated membership (probability q_i) in the K clusters.

Figure 3. Principal coordinate analysis (PCoA) for the red mangrove, *Rhyzophora mangle*, in the Transverse Coastal Corridor, Yucatan Peninsula, Mexico, using ISSR molecular markers. Chetumal Bay (red color): Tamalcab (square) and Playa Cayo Venado (triangle), Laguna Baccalar (green color): Pedro Santo (square) and Hub Sak (triangle), Rio Hondo (yellow color): entrance (square) and inward (triangle), and Caribbean coast (blue color): Baccalar Chico (square) and Xcalak National Reef Park (triangle).

Figure 4. Principal coordinate analysis (PC_oA) for the red mangrove, *Rhyzophora mangle*, at each hydrological system considered into the Transverse Coastal Corridor, Yucatan Peninsula, Mexico, using ISSR molecular markers. Chetumal Bay (A): Tamalcab (square) and Playa Cayo Venado (triangle), Laguna Baccalar (B): Pedro Santo (square) and Hub

Sak (triangle), Rio Hondo (C): entrance (square) and inward (triangle), and Caribbean coast (D): Baccalar Chico (square) and Xcalak National Reef Park (triangle).

Fig. 1



Hydrological systems: (A) Bacalar lagoon, (B) Hondo river, (C) Chetumal Bay, (D) Carribean Sea; Black circle (dwarf mangrove trees), white square (fringe mangrove trees), light grey (water bodies), green (mangrove forest cover; source: CONABIO 2016).

Fig. 2

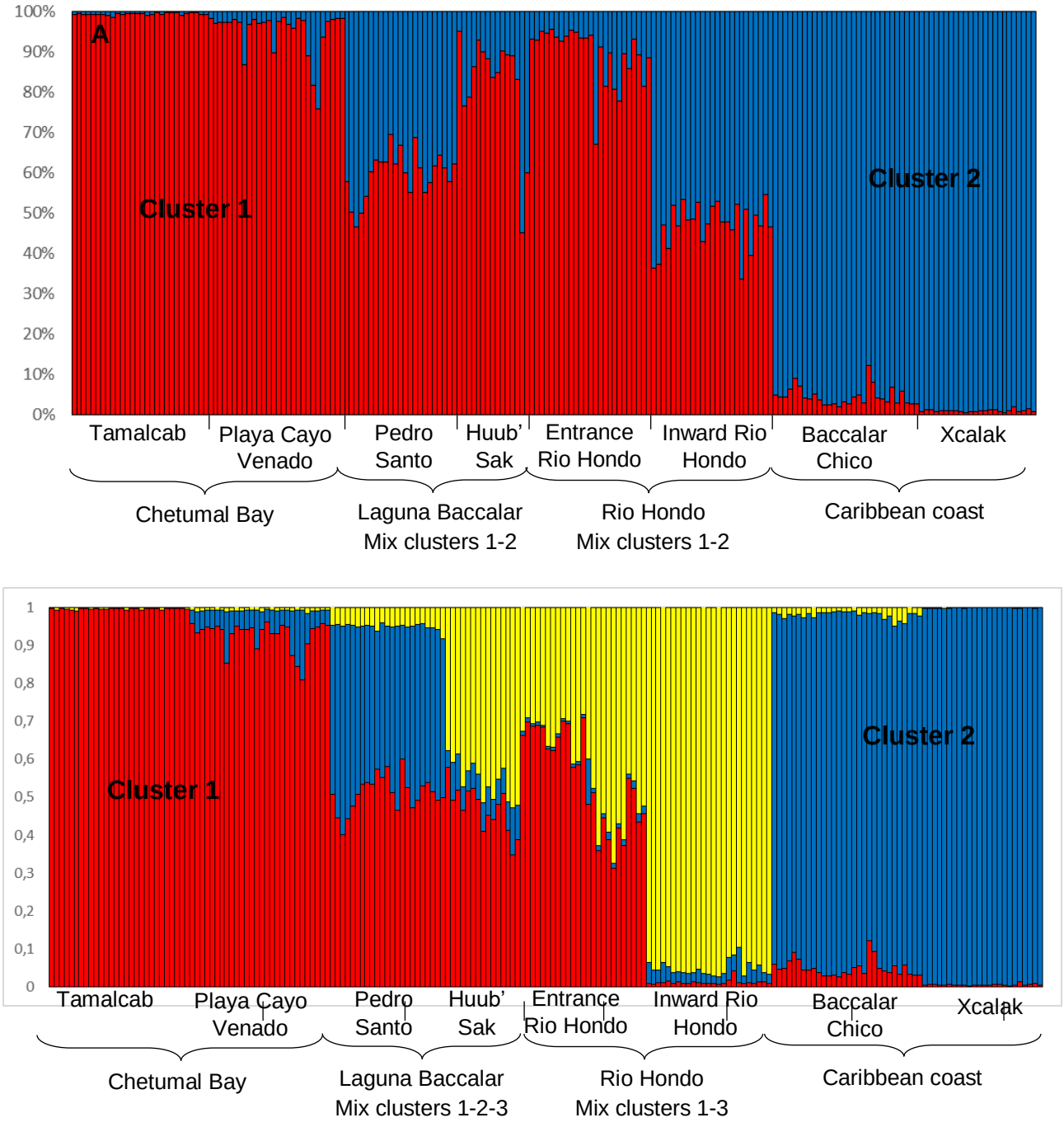

A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cluster 1
Cluster 2
Tamalcab
Playa Cayo
Venado
Pedro
Santo
Huub'
Sak
Entrance
Rio Hondo
Inward Rio
Hondo
Baccalar
Chico
Xcalak
Chetumal Bay
Laguna Baccalar
Mix clusters 1-2
Rio Hondo
Mix clusters 1-2
Caribbean coast
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Cluster 1
Cluster 2
Tamalcab
Playa Cayo
Venado
Pedro
Santo
Huub'
Sak
Entrance
Rio Hondo
Inward Rio
Hondo
Baccalar
Chico
Xcalak
Chetumal Bay
Laguna Baccalar
Mix clusters 1-2-3
Rio Hondo
Mix clusters 1-3
Caribbean coast

Fig. 3

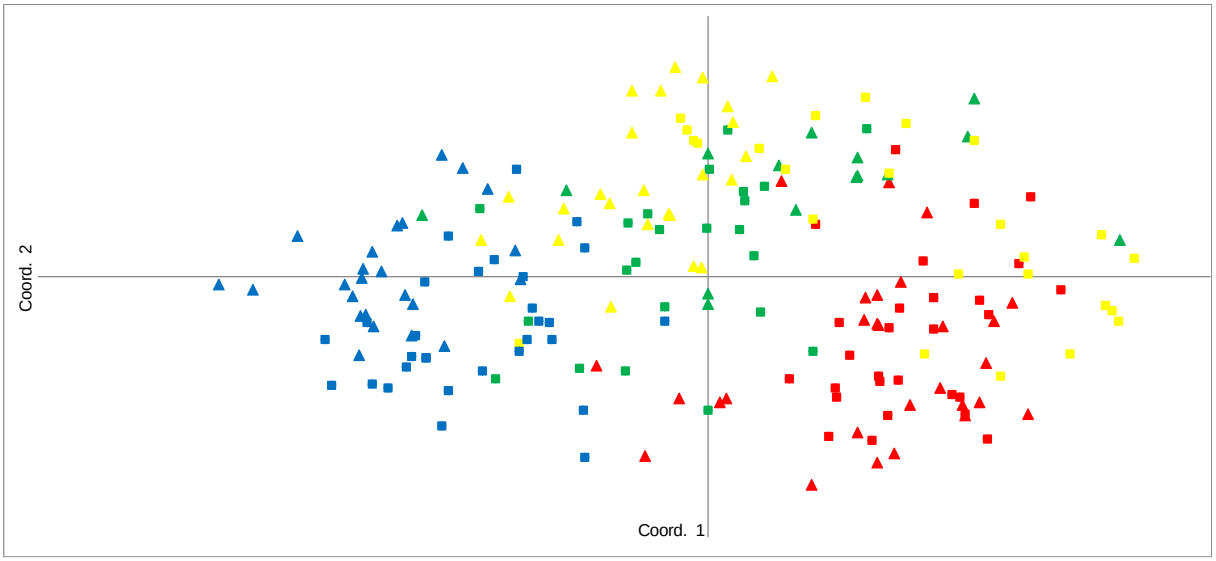
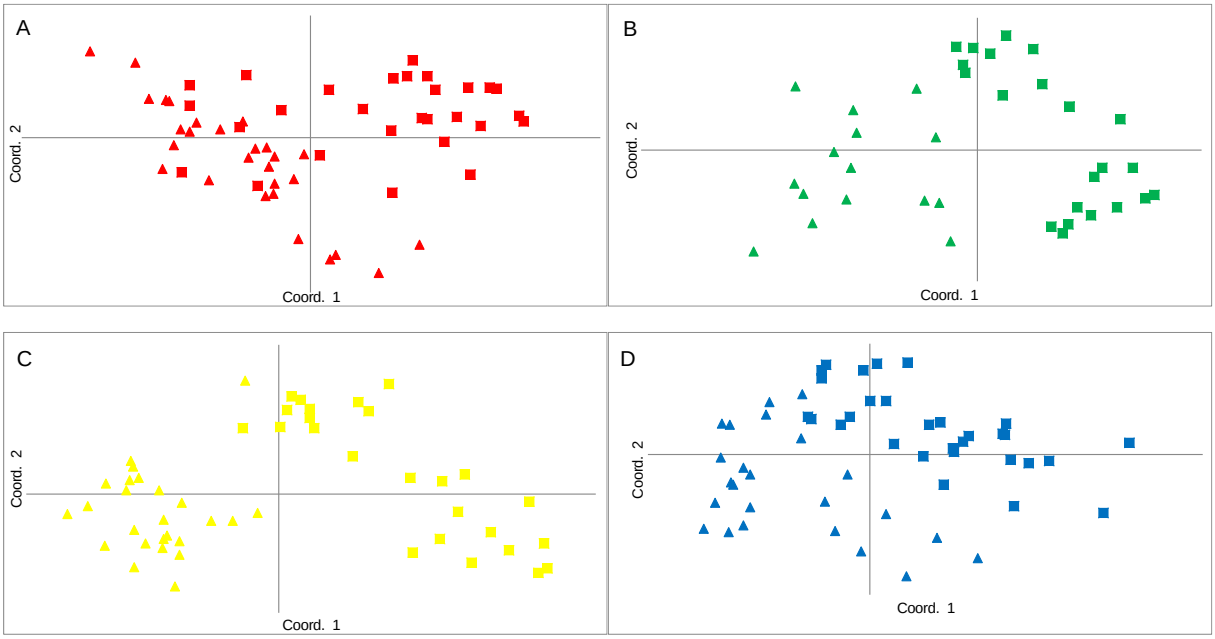


Fig. 4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D
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Coord. 1

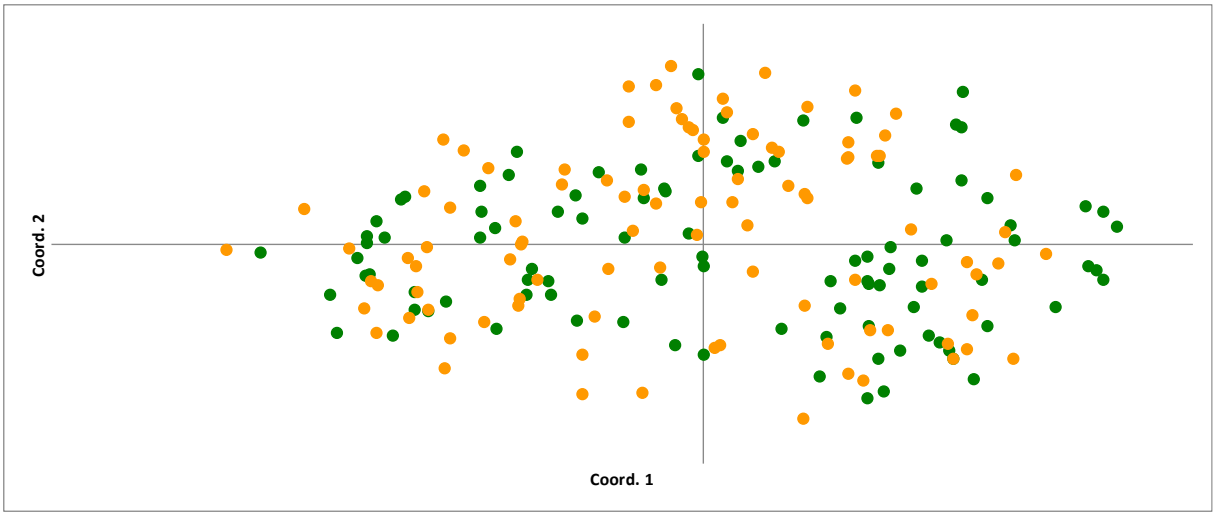

Figure SM1. Two first axis of the principal coordinate analysis (PC_oA) for two typologies of *Rhyzophora mangle* (fringe and dwarf) obtained by ISSR molecular markers. Fringe typology (green), dwarf typology (orange).

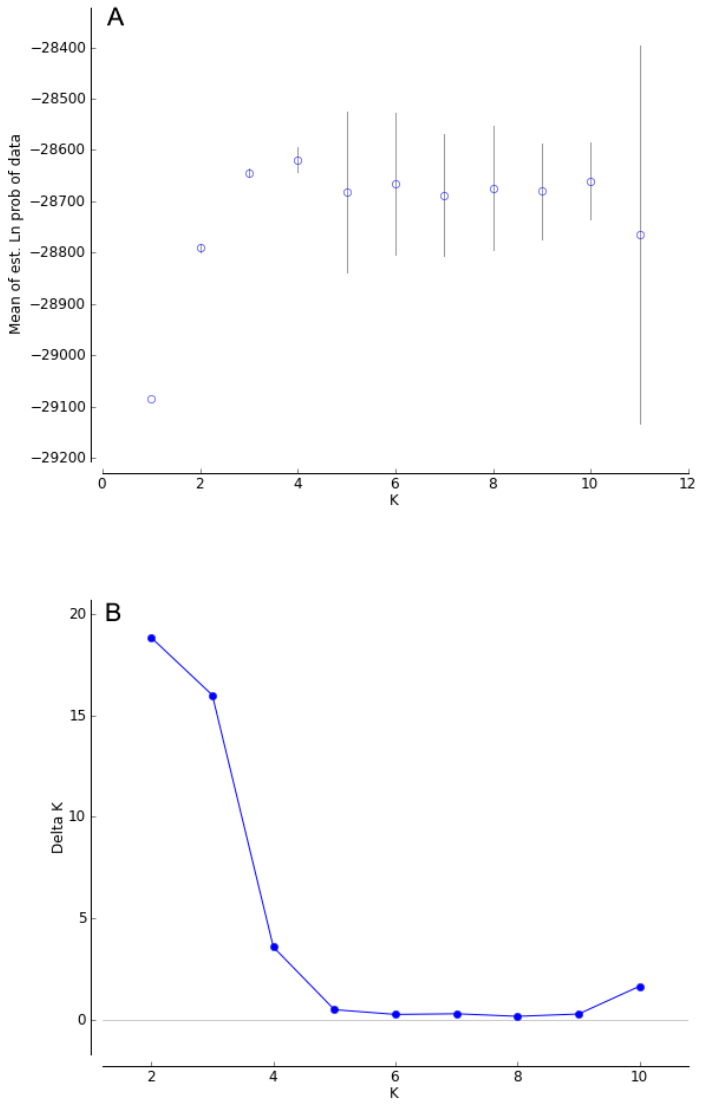

Figure SM2. Determination of the optimal number of *K* from STRUCTURE analysis. Ten replicates for values of *K* from 1 to 11 under the LOCPRIOR model were realized. Classical method based on the mean value of Ln *P(D)* for each value of *K* (A) and, Δ*K* determined using STRUCTURE HARVEST website following Evanno method (B).

Table SM1. Analysis of Molecular Variance (AMOVA) between two *Rhyzophora mangle* typologies (fringe and dwarf) in the Transverse Coastal Corridor, Yucatan Peninsula, Mexico, using ISSR molecular markers.

Source of variation	df	SS	MS	Est. Variance	% variance	*P*
Among typology	1	23,595	23,595	0,127	1%	0.001
Within typology	196	2162,966	11,036	11,036	99%	
Total	197	2186,561		11,162	100%	

Capítulo 3

CONCLUSIONES GENERALES

Este estudio se suma a los pocos realizados en nuestro país enfocado a la genética de poblaciones de *Rhizophora mangle,* y los resultados obtenidos son por demás interesantes porque nos ayudarán a comprender mejor a esta especie. Encontramos que una distancia relativamente pequeña y los flujos de agua a través de canales entre la Bahía de Chetumal y la Costa del Caribe no fueron un impedimento para que entre ambos complejos hidrológicos exista una clara separación genética, formando dos grupos (*K*=2) bien diferenciados. En este caso, los manglares de Río Hondo y Bacalar quedaron mezclados con esos dos grupos principales. Con la formación de tres grupos (*K*=3), la parte interna del Río Hondo emergió como un tercer grupo, mientras que la parte Sur de Bacalar (Hub Sak) se relacionó con la Bahía de Chetumal y Río Hondo, pero mucho menos con la Costa del Caribe. Es destacable haber encontrado una sólida estructura genética en la población de *R. mangle* que habita en un sistema hidrológico de alta complejidad e importancia como lo es el Corredor Transversal Costero de la costa Sur de Quintana Roo (CTC).

La estructura genética de *Rhizophora mangle* encontrada con los ISSR podría ser el reflejo del cambio histórico del nivel del mar que se produjo entre 4600 y 4000 A.P. y la variación de la cobertura del manglar derivado de periodos secos en esta región, que comenzó alrededor de 3400 A.P. a ~ 2000 A.P. Estos eventos históricos pudieron haber aislado los manglares de esta región. La limitada dispersión de la semilla de *R mangle* refuerza la pérdida de conectividad entre los sistemas hidrográficos del CTC. Esto sumado a factores como la compleja hidrodinámica del sitio, posiblemente la elevada densidad de los árboles de mangle que produce un aumento en la retención de propágalos y las pocas fuentes de intercambio para los árboles de *R. mangl*e como en el caso de la Bahía de Chetumal y la parte Sur de la costa del Caribe mexicano que a pesar de tener una amplia desembocadura (~ 15 km) no permite una homogeneización genética entre ambos sistemas.

La diversidad genética observada en *R. mangle* a nivel especie en el CTC fue alta. De igual manera, todas las comunidades locales de *R. mangle* en este estudio y pertenecientes al CTC mostraron una elevada diversidad genética. Esto refleja la evolución a largo plazo, lo que es importante para la supervivencia y adaptabilidad de las

especies. *Rhizophora mangle* es una especie que muestra alto nivel de adaptabilidad incluso desarrolla mecanismos de supervivencia que le permite adaptarse a ambientes extremosos lo que coincide con la alta variabilidad genética encontrada en este estudio.

Una estructura genética a escala fina (EGEF) fue evidente dentro de cada sistema hidrológico. Para explicar esta estructura se han propuesto factores intrínsecos y extrínsecos. El factor intrínseco más obvio es la alta densidad de las raíces de *R. mangle*, lo que podría reducir la capacidad de dispersión de sus propágulos provocando que los nuevos árboles de mangle migren a muy poca distancia de sus árboles de origen. Por otra parte, entre los factores extrínsecos que podrían estar involucrados en la limitación de la dispersión del manglar hemos propuesto la geomorfología de la costa, la fragmentación del hábitat y los regímenes hidrológicos de cada sistema. Por ejemplo, la suma de factores extrínsecos como el desarrollo urbano alrededor de la Laguna de Bacalar, la Bahía de Chetumal y la comunidad de Xcalak en la Costa del Caribe podría causar la fragmentación de los bosques de manglar que aquí habitan, esto junto a la presencia del flujo del canal artificial denominado Canal de Zaragoza y el flujo procedente de la laguna Chile Verde, ambos dirigidos hacia la Bahía de Chetumal, podrían contribuir a la explicación de la presencia de la estructura genética a fina escala aquí encontrada.

En la actualidad existen escasos estudios que exploren las marcadas diferencias entre las tipologías del manglar desde un punto de vista genético, la mayoría de los trabajos relacionan estas diferencias con factores como el estrés hídrico y recursos limitados. Encontramos que los árboles de manglar chaparro y de franja presentaron una diferenciación genética (AMOVA) dentro de y entre casi todas las localidades que forman parte de este estudio. Estas diferencias podrían reflejar la formación de linajes locales (chaparros y de franja) como respuesta a adaptaciones ecológicas y fisiológicas a microambientes. Aunque cierta variación genética fue hallada entre las tipologías de manglar chaparro y de franja, se necesita desarrollar nuevos estudios genómicos, ecológicos y fisiológicos para confirmar lo aquí planteado y para ampliar nuestros conocimientos respecto a las tipologías de manglar, un tema apenas explorado desde la perspectiva genética. Resultados interesantes como los mostrados en este documento dan paso a nuevas interrogantes, lo que puede desembocar en nuevos estudios en el

campo de la genética de poblaciones, el cual cada vez cobra más importancia para la conservación.

Finalmente, queremos destacar la importancia del ecosistema de manglar, tanto para la ecología como para la economía del Sur de Quintana Roo. Los densos bosques de manglar protegen a la línea de costa de los efectos de los huracanes y son el hogar de muchas especies comerciales que brindan sustento a las familias locales, sólo por mencionar algunas de sus valiosas funciones. Por eso es prioritaria la realización de estudios como el presente, que nos permitan comprender la distribución de la diversidad genética y la organización de las poblaciones de la comunidad de manglar rojo del Sur de Quintana Roo, porque a pesar de todos los servicios ambientales y económicos que nos brinda este ecosistema, hoy en día se ha incrementado la pérdida de su cobertura, generalmente correlacionada con la densidad humana y las actividades antropogénicas como el desarrollo urbano, la acuicultura, la contaminación y la fragmentación. Este trabajo de investigación dio a conocer la presencia de una estructura genética en nuestra área de estudio la cual forma parte del complejo sistema hidrológico Corredor Transversal Costero, que es de enorme importancia por sus características únicas. También dimos a conocer que la Laguna de Bacalar, a pesar de presentar presión antropogénica, exhibe la mayor diversidad genética de *R. mangle* lo que la convierte en una buena candidata para ser declarada área prioritaria de conservación. El ampliar nuestros conocimientos en la genética poblacional de *R. mangle* nos permitirá crear programas de conservación y manejo mejor diseñados y ajustados a los factores intrínsecos y extrínsecos que limitan la conectividad y diversidad genética de los manglares.

Capítulo 4

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ANEXOS

22-May-2018

Dear Dr. Machkour-M'Rabet:

Your manuscript entitled "Genetic structure and connectivity of the red mangle at different geographic scales through the “Transverse Coastal Corridor” in southern Quintana Roo" by Chablé Iuit, Landy; Machkour-M'Rabet, Salima; Espinoza-Ávalos, Julio; Hernández-Arana, Hector; López-Adame, Haydée; Hénaut, Yann has been successfully submitted online and is presently being given full consideration for publication in Molecular Ecology. Please read this message carefully as it contains important information about the assessment of your manuscript.

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