# UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
## División Académica de Ciencias Biológicas

# “ESTABLECIMIENTO DE UN PROGRAMA DE MANEJO GENÉTICO PARA LA TORTUGA BLANCA *(Dermatemys mawii)* EN TRES UNIDADES DE MANEJO DE VIDA SILVESTRE (UMA) DE TABASCO, MÉXICO”

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**Presenta:**

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Dra. Claudia Elena Zenteno Ruiz

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## Dedicatoria

*A DIOS*

*A MIS ABUELOS*

*A MIS PADRES*

*A MI HERMANA*

*A MI HIJA*

## Agradecimientos

A mis padres Isabel Álvarez Estrella y Felipe de Jesús Gallardo Cortez, a mis abuelos Sergio Álvarez Valencia (Q.E.P.D) y Manuela Estrella Aguilar, a mi hermana Dolores Isabel Gallardo Alvarez, a todos mis familiares por haberme tolerado, aconsejado y brindado todo su apoyo y cariño para que pudiera concluir una etapa más en mi formación académica. A mi hija Luz Ariadna Gallardo Recino, por haberme brindado su amor, tiempo, y cariño. Al Dr. Oscar Manuel Cortázar Calacich, (Q.E.P.D por brindarme su amistad, consejo y apoyo durante mi desarrollo académico.

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## Resumen

Las tortugas dulceacuícolas son uno de los grupos de reptiles con mayor riesgo de extinción, debido a diversas actividades antropogénicas, que van desde la destrucción y contaminación de sus hábitats, hasta ser utilizadas de forma excesiva como fuente importante de alimento. Por lo tanto, para la conservación y uso sustentable de las tortugas de agua dulce, se han creado en México programas de reproducción *ex situ*, en sitios denominados como, Unidades de Manejo para la Conservación de la Vida Silvestre (UMAs). En el sureste de México se han creado varias UMAs dedicadas a la crianza de estas especies, siendo la tortuga blanca (*Dermatemys mawii*) una de las más reproducidas. La importancia de la crianza de *D. mawii* en las UMAs, se debe a que es una especie críticamente amenazada debido a la degradación de su hábitat y la caza excesiva para su consumo. Se tiene registro de que, las UMAs dedicadas a la reproducción de *D. mawii*, han tenido éxito en la obtención de crías, así como la comercialización de la especie a nivel nacional e internacional. Estos éxitos se deben a los diferentes planes de manejo que se han establecido en estos sitios y al conocimiento aportado por los diferentes especialistas en quelonios, lo cual ha ayudado a un cuidado adecuado de la especie bajo un manejo *ex situ*. Sin embargo, en las UMAs aún no se ha establecido un programa de manejo genético de los individuos que funcionan como reproductores. El programa de manejo genético es importante, porque permite asegurar la salud genética de la colonia cautiva, así como mantener su variabilidad y viabilidad genética. Por esta razón, el presente estudio propone el establecimiento de un programa de manejo genético para *D. mawii* en tres UMAs en el estado de Tabasco, México. Los objetivos específicos para establecer el programa de manejo genético fueron: 1) Determinar la variabilidad genética y la relación de parentesco entres los individuos fundadores y 2) Establecer las cruzas a partir de las relaciones genéticas entre los individuos fundadores. Para cumplir con los objetivos, se analizaron un total de 185 individuos, de los cuales 117 corresponden a fundadores de las UMAs (90 hembras y 27 machos) y 68 individuos de vida libre. Para el estudio se utilizaron 10 marcadores de microsatélites específicos para *Dermatemys mawii*. Las UMAs

incluidas en la presente investigación fueron: la UMA “La Encantada”, la UMA del gobierno del estado de Tabasco y la UMA de la Universidad Juárez Autónoma de Tabasco. Los sitios silvestres incluidos en el presente trabajo fueron: Río Tabasquillo en el municipio de Centla, Río Mezcalapa en el municipio de Huimanguillo y Laguna “el Chochal” en el municipio de Jonuta. Los resultados mostraron que los individuos fundadores resguardan una diversidad genética ligeramente mayor que las poblaciones silvestres. En otro sentido, los análisis bayesianos en conjunto con el análisis discriminante de componentes principales (DAPC) muestran una evidente separación genética entre las UMAs y las poblaciones silvestres. Una discusión amplia de los resultados de la diversidad genética encontrada en *D. mawii* se aborda en el Capítulo 2. Con referencia a la relación de parentesco, solo se consideraron a los individuos fundadores. Los resultados mostraron que se pueden formar un total 2,430 cruzas, de las cuales, la relación de parentesco indica que 2,220 parejas no se encuentran relacionadas, 145 parejas son medios hermanos, 62 parejas son hermanos completos y 3 parejas tienen una relación de padre-hijo. Además, se propone que, para realizar un programa de manejo genético en las UMAs, se creen 3 grupos reproductores con diferentes objetivos: 1) un grupo dedicado a la conservación de la especie; 2) un grupo dedicado a la investigación de la especie y 3) un grupo dedicado al manejo sustentable de la especie. Una discusión más amplia de esta propuesta se realiza en el capítulo 3. Finalmente, se concluye que esta propuesta brindará información que permita planear las acciones de conservación y uso sustentable de *D. mawii* a nivel nacional e internacional, específicamente dentro del corredor Mesoamericano.

## Abstract

Freshwater turtles are one of the groups of reptiles with the highest risk of extinction, due to various anthropogenic activities, ranging from the destruction and contamination of their habitats, to being used excessively as an important source of food in different parts of the world, by different societies. Therefore, for the conservation, reintroduction and sustainable use of freshwater turtles, different captive breeding programs have been created, in Mexico this activity is carried out in sites called Wildlife Conservation Management Units (UMAs, from its name in Spanish), which promote the sustainable use of wildlife. Especially in the southeast of Mexico, several UMAs dedicated to the breeding of freshwater turtles have been created and the Central American River Turtle (*Dermatemys mawii)* is one of the most reproduced. The importance of the reproduction of *D. mawii* in UMAs is because it is a critically endangered species due to the degradation of its habitat and excessive human hunting for its consumption.

There is a record in which the UMAs dedicated to the reproduction of *D. mawii*, have been successful in obtaining offspring in captivity as well as the commercialization of the species at a national and international level. These successes are due to the different management plans that have been established in these sites and the knowledge contributed by the different chelonian specialists, which has helped to properly care for the species in captivity. But a point that has not yet been established within the UMAs is a program for the genetic management of the founders' individuals. The genetic management program is important because it allows ensuring the genetic health of the captive colony, as well as maintaining its genetic variability and viability. For this reason, the aim of this study is the establishment of a genetic management program for *D. mawii* in three UMAs in the state of Tabasco, Mexico.

The specific goals for establishing the genetic management program are: 1) Determine the genetic variability and the kinship relationship between the founders' individuals. 2) 2) Establish the mating according to their level of relationship and

homozygosity level between the founding breeders. To achieve the objectives, ten specific microsatellite markers were used for Dermatemys mawii in a total of 117 founder individuals (90 females and 27 males). Additionally, 68 individuals from wildlife were included to determine the genetic diversity of the species in wildlife and compare with the founding breeders. The UMAs included in the present investigation were: The UMA “La Encantada”, the Tabasco state government UMA and the Tabasco State Juarez University UMA. The wild sites were: Tabasquillo river, located in Centla, Tabasco, Mezcalapa River located in Huimanguillo, Tabasco and Chochal Lagoon located in Jonuta, Tabasco. Results showed that genetic diversity in wild populations could be considered as medium and are less than values observed for UMAs, and genetic structure analysis highlighted an evident separation between UMAs and wild populations. A broad discussion of the genetic diversity of *D. mawii* is given in Chapter 2. For the kinship relationship only the founding breeders were considered. A total of female-male dyads has been obtained from all genotypes analyzed. Kinship analysis indicated that 2,220 female-male pairs were unrelated, 145 female-male pairs were half-siblings, 62 female-male pairs were full-siblings, and 3 female-male pairs were parents-offspring. A broader discussion of this proposal is explained in Chapter 3. Finally, it is concluded that this proposal can enrich the conservation actions and sustainable use for *D. mawii* at a national and international level, specifically within the Mesoamerican corridor.

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## Capítulo1.- Introducción

La pérdida acelerada de la biodiversidad a nivel global ha ido en aumento desde la era industrial, y en la actualidad persiste (Finderup-Nielsen et al., 2019). Diversos factores han contribuido a la pérdida de la biodiversidad, pero, son las actividades humanas, como la contaminación, la introducción de especies invasoras, la caza excesiva, así como la fragmentación del hábitat, las que mayor impacto han tenido en la perdida de ecosistemas, la extinción de especies y el declive de las poblaciones silvestres. En el mismo sentido, estas actividades antropogénicas han provocado que en los últimos 97 años se haya perdido entre un 5.4 % y un 6.4% de la variabilidad genética a nivel global (Mimura et al., 2017; Leigh et al., 2019). Por tal motivo, con el fin de recuperar la biodiversidad, se han propuesto diferentes estrategias de conservación que involucran a los biólogos, las políticas públicas y a la sociedad. Una de estas estrategias es la conservación *ex situ* (Brooks et al., 2006, Conde et al., 2011; McGowan et al., 2017).

La crianza *ex situ* es considerada como un último recurso para recuperar las especies amenazadas o en peligro de extinción. Sin embargo, esta estrategia de conservación es crucial para evitar exponer a las especies en recuperación de las amenazas antropogénicas, cuando estas son difíciles o imposibles de controlar en el medio silvestre (Spencer et al., 2017). A la vez, la cría en cautividad proporciona a las especies un entorno estable para la reproducción, su objetivo es el de aumentar el tamaño de la población para la reintroducción y el establecimiento de poblaciones silvestres autosustentables (Miller et al., 2018). Los programas de reproducción *ex situ* para la reintroducción, son exitosos, si dan como resultado poblaciones autosuficientes. Sin embargo, el éxito es difícil de evaluar en la mayoría de los casos, porque se requieren datos a largo plazo. Aunado a esto, los biólogos de la conservación no han definido de manera uniforme, los criterios para considerar exitoso un programa con manejo *ex situ* (Robert, 2009).

Existe varios programas de reproducción *ex situ* que han sido exitosos en la recuperación de diferentes especies en peligro de extinción. Alguno de estos

programas exitosos de aves y de mamíferos son: el lince Ibérico (*Lynx pardinus,* Carnivora, Felidae, Temminck, 1827) el cóndor de California (Gymnogyps californianus, Cathartiformes, Cathartidae, Shaw, 1797) así como también el hurón de patas negras (*Mustela nigripes*, Carnivora, Mustelidae, Audubon y Bachman, 1851) (Loercher et al., 2013; Brichieri-Colombi et al., 2019; Kleinman-Ruiz et al., 2019). Adicionalmente, en el orden de las tortugas, la reproducción *ex situ* se ha implementado como una estrategia de conservación, puesto que es uno de los ordenes más amenazados por factores antropogénicos (por ejemplo: la colecta ilegal de individuos silvestres o la destrucción de sus hábitats), que han ocasionado una disminución drástica en sus poblaciones silvestres (López et al., 2010; Masés-García et al., 2016; Tobón et al., 2016; Kanwal y Khan, 2018).

Desde los 70´s se han desarrollado un número importante de investigaciones, monitoreo y programas de crianza *ex situ* de las especies de tortugas marinas como *Chelonia mydas* (Testudines, Cheloniidae, Linnaeus, 1758) o *Lepidochelys olivacea* (Testudines, Cheloniidae, Eschscholtz, 1829) (Silas y Rajagopalan, 1984; Ross, 1999; Barbanti et al., 2019). En cuanto a las especies de tortugas dulceacuícolas, existen proyectos de manejo *ex situ* con diferentes grados de éxito, como el caso de las especies *Batagur baska* (Testudines, Geoemydidae, Gray, 1830), *Batagur borneoensis* (Testudines, Geoemydidae, Schlegel and Müller, 1844), *Chitra chitra* (Testudines, Trionychidae, Nutphand, 1986) y *Platysternon megacephalum* (Testudines, Platysternidae, Gray, 1831) en Tailandia (Van Dijk y Palasuwan, 2000).

Para poder implementar un programa *ex situ* en tortugas, se necesita conocer adecuadamente varios aspectos biológicos y ecológicos de la especie de interés, con el fin de mimetizar el hábitat natural, para que la colonia fundadora tenga un sitio adecuado de reproducción (Kischinovsky, 2017). De igual manera, otros aspectos cruciales son implementar un manejo demográfico que evite la sobrepoblación, así como aplicar un plan de manejo genético que prevenga o disminuya la endogamia, la fijación de alelos deletéreos y que procure mantener el

90% de la heterocigocidad original por al menos 100 generaciones (Ebenhard, 1995; Witzenberger y Hochkirch, 2011, Russello et al., 2018).

De igual manera, es necesario establecer la relación de parentesco de los individuos que servirán como grupo fundador, con el fin de mantener la variabilidad genética durante el desarrollo del programa de crianza *ex situ* (Phillippart, 1995, FitzSimmons y Sumner, 2016). Por tal motivo, para cumplir con este objetivo, los marcadores moleculares como los microsatélites, han sido de gran utilidad. Además, estos análisis genéticos permiten obtener la variación genética y el origen geográfico de los individuos. Y a partir de esos datos, se pueden establecer el tamaño inicial de una colonia fundadora y el parentesco genético entre los individuos (Pereira et al., 2017; Miller et al., 2018).

México, posee el segundo lugar en diversidad de tortugas (Van Dijk et al., 2012). En el país, la crianza *ex situ*, se realiza bajo un marco legal denominado Unidades de Manejo para la Conservación de la Vida Silvestre (UMAs). En 1997, el concepto de UMAs se creó con el objetivo de conservar la vida silvestre en México y forma parte del Sistema Nacional de Unidades de Manejo para la Conservación de la Vida Silvestre (SUMA) (CONABIO, 2012). Las UMAs pueden funcionar como centros de exhibición, productores de pies de cría, bancos de germoplasma y/o centros de investigación. Estas modalidades las convierten en alternativas viables y sustentables que permiten tanto el aprovechamiento como la conservación, reproducción y propagación de las tortugas dulceacuícolas (Ley General de Vida Silvestre, 2000). De esta manera, las UMAs, promueven esquemas alternativos para un uso racional, ordenado y planificado de la vida silvestre y además permite realizar un manejo para la conservación.

Hasta 2017 existían 11,722 UMAs que en conjunto abarcan 38.5 millones de hectáreas, las cuales equivalen al 19% del territorio mexicano (SEMARNAT, 2018). Los beneficios económicos estimados obtenidos de las UMAs son de 5 mil millones de pesos por año. Por otro lado, las UMAs dedicadas a la caza de especies cinegéticas son las más exitosas y se encuentran ubicadas en el norte del país (de

Benito, 2009; Gallina-Tessaro et al., 2009). Sin embargo, las UMAs establecidas en el centro y sureste del país, no han tenido los mismos resultados. Es de resaltar que las UMAs que reproducen las tortugas dulceacuícolas se ubican en la zona sur de México (Weber et al., 2006; de Benito, 2009; CONABIO, 2012).

Del total de las UMAs establecidas en México, 94 se dedican a la reproducción de tortugas de agua dulce, reproduciendo con éxito algunas especies de quelonios como *Trachemys spp* (Testudines, Emydidae, Agassiz, 1857), *Claudius angustatus* (Testudines, Kinosternidae, Cope 1865), *Staurotypus triporcatus* (Testudines, Kinosternidae, Wiegmann 1828) (Reynoso et al., 2016) y *Dermatemys mawii* (Testudines, Dermatemydidae. Gray 1847) (SEMARNAT, 2010; SEMARNAT, 2014; Dirección General de Vida Silvestre y SEMARNAT, 2018). Este éxito de reproducción *ex situ* de quelonios se debe a que las UMAs poseen un desarrollo tecnológico adecuado. Sin embargo, las UMAs carecen de un programa de manejo genético para mantener la variabilidad genética de su colonia cautiva. Estos datos son muy importantes de considerar, puesto que permiten establecer si los organismos reproducidos *ex situ* tienen pérdida de la variabilidad genética. La pérdida de diversidad genética se puede deber a eventos como son el efecto fundador, el cuello de botella, la deriva genética, endogamia y la depresión por endogamia. Se ha reportado que en las granjas donde existen poco o nulo manejo genético, se presentan problemas relacionados a la fecundidad y de una reducción en el tamaño de las crías. La principal causa de estos problemas se considera que es la fijación de alelos deletéreos por una elevada consanguinidad (Williams y Osentoski 2007; Frankham, 2008). Por consiguiente, si no se considera el manejo genético en las UMAs, esto puede ocasionar que solo se tenga un elevado número de individuos, pero que no cuenten con una adecuada variabilidad genética para su liberación e integración de las poblaciones silvestres (Zarza et al., 2016).

La presente investigación propone realizar un plan de manejo genético en tres UMAs que se dedican a la reproducción de la tortuga dulceacuícola *Dermatemys mawii* en el estado de Tabasco. En México se tienen registradas 37 UMAs que

reproducen *D. mawii* de las cuales 17 se encuentran en el estado de Tabasco. Estas UMAs han tenido éxito en reproducir a la especie, lo que le ha permitido la producción de un gran número de individuos. El principal propósito de la reproducción de la tortuga en las UMAs ha sido proteger a la especie de la sobre explotación y poder reforzar sus poblaciones silvestres. Sin embargo, aún no se han llevado a cabo reintroducciones, debido a la falta de información sobre la diversidad genética y la relación de parentesco de los individuos fundadores. Esta información genética es muy importante tenerla debido a que forma parte de los protocolos de reintroducción establecidos por la Comisión de la Supervivencia de Especies de la Unión Internacional para la Conservación de la Naturaleza (*International Union for Conservation of Nature Species Survival Commission* IUCN/SSC, 2013). Estas consideraciones son muy importantes debido a que *D. mawii* tiene poblaciones reducidas y aisladas (Todd et al., 2010; Refsnider y Janzen, 2016).

La importancia de *D. mawii* se debe a que esta especie es la última representante viviente de la familia Dermatemydidae (Vogt et al., 2006), por lo que su pérdida significaría no sólo la extinción de una especie, sino también la desaparición de una familia taxonómica. Además, la tortuga blanca, es importante desde el punto de vista de aprovechamiento, debido a que esta especie es muy apreciada para el consumo humano, alcanzando elevados precios por su carne, lo que paradójicamente ha llevado a la reducción de sus poblaciones (Álvarez del Toro et al., 1979). De igual manera, el conservar a la tortuga blanca permitiría conservar de forma directa su hábitat, el cual ha sufrido reducciones drásticas en el último medio siglo (Tudela, 1989; Zavala y Castillo 2003; Sánchez y Barba, 2005).

Para establecer el programa de manejo genético en las UMAs dedicadas a la crianza de la tortuga blanca, en el presente trabajo se planteó, primero, caracterizar la diversidad genética de los individuos fundadores en tres UMAs del Estado de Tabasco y comparar la diversidad genética obtenida con individuos procedentes de vida libre. Los resultados de esta primera investigación se abordan de manera extensa en el capítulo 2. Como segundo punto, se determinó la relación de

parentesco entre los individuos fundadores y, por último, se estableció el esquema de cruzamiento para la formación de los mejores grupos de reproductores con base a la relación genética que tenían los individuos fundadores. Ambos objetivos se abordan en el capítulo 3.

Por último, este trabajo servirá como antecedente para que en otras especies bajo el esquema de UMAs o crianza *ex situ*, se contemple el manejo genético de los organismos reproducidos; lo que ayudaría a crear mejores estrategias de conservación en las especies en peligro de extinción.

## 1.2.- Antecedentes

### *1.2.1. Descripción de la Tortuga Blanca (Dermatemys mawii)*

La tortuga blanca (*D. mawii*) es la tortuga de agua dulce más grande de Mesoamérica, siendo la única representante viviente de la familia Dermatemydidae. Los organismos de esta especie llegan a medir hasta 60 cm de longitud del caparazón y a pesar como máximo 22 kg (Zenteno-Ruíz et al., 2001).

El caparazón es muy ancho y aplanado, compuesto por cinco escudos vertebrales, cuatro pares costales, 12 pares marginales y un escudo nucal, con dimensiones de un largo total del caparazón que van de 342-480 mm para hembras y 342-450 mm para machos. El color del caparazón puede variar de gris claro a gris olivo, sus extremidades son fuertes y aplanadas con amplias membranas interdigitales, su color de piel es gris oscuro. Los machos, en etapa adulta, tienen a los lados de la cabeza manchas amarillas vermiforme de color amarillo ocre que forman un triángulo de las fosas nasales hasta la zona occipital (Moll, 1989). Las hembras presentan en la parte lateral de la cabeza un color grisáceo con tonalidades pardas (Iverson y Mittermeier, 1980). Las crías y jóvenes presentan una quilla media en el caparazón que usualmente desaparece con la edad (Morales-Álvarez, 2008; Zenteno-Ruíz et al., 2001).

### 1.2.2. Distribución Geográfica

Esta especie se distribuye en el sureste mexicano en los estados de Veracruz, Chiapas, Oaxaca, Tabasco y al sur de la Península de Yucatán llegando a los países de Guatemala y Belice (Fig. 1) (Calderón-Mandujano, 2008).

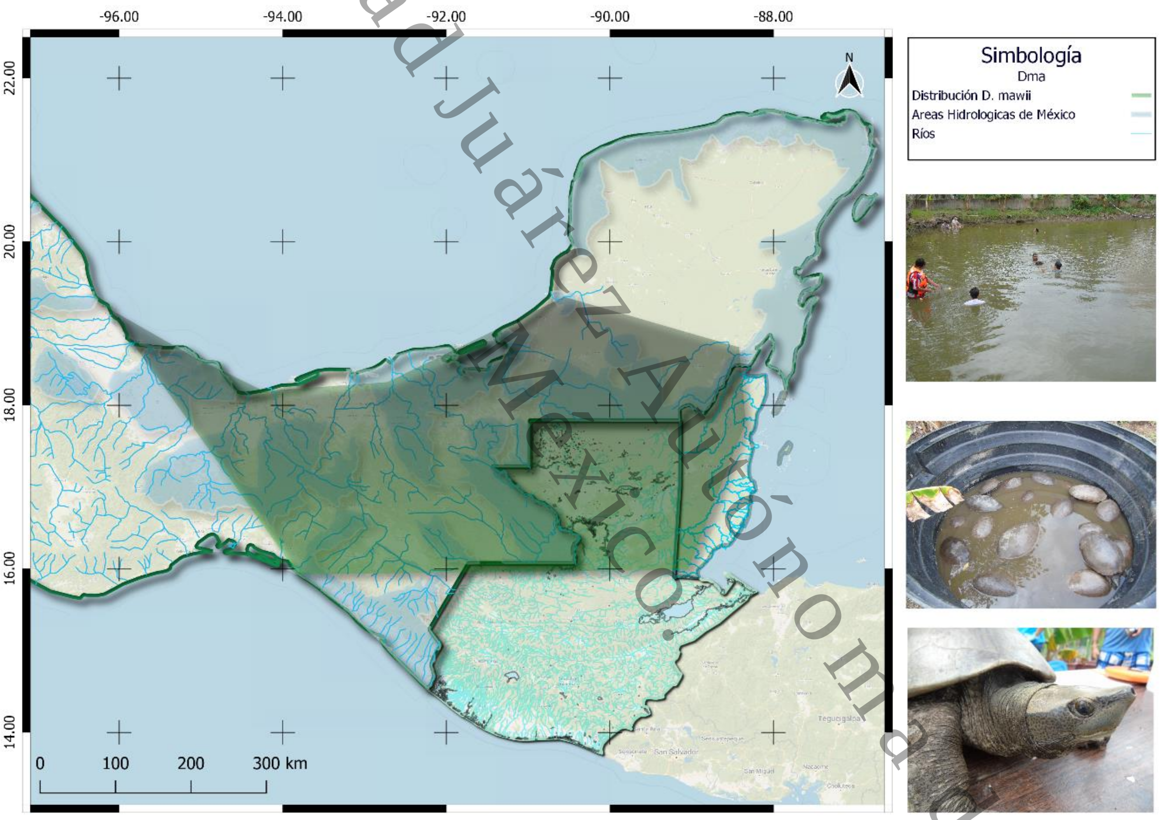

Figura 1.- Distribución geográfica de *Dermatemys mawii* en el sureste de México y Centro América (Iverson y Mittermeier, 1980).

### 1.2.3. Hábitat

La tortuga blanca es de hábitos exclusivamente acuáticos y se ha reportado que puede vivir en diferentes cuerpos de agua como son: ríos caudalosos, lagos con aguas que van desde transparentes hasta poco transparentes, así como en zonas pantanosas. En el mismo sentido, el hábitat de *D. mawii* se caracteriza por poseer una abundante vegetación acuática tanto sumergida como flotante. También, se ha reportado la presencia de la especie en selvas inundables. Durante la época de lluvia, se les encuentra con frecuencia, cerca de árboles sumergidos, los cuales utiliza como refugio (Álvarez del Toro et al., 1979).

### 1.2.4. Alimentación y Reproducción

La tortuga blanca en vida libre se alimenta principalmente de material vegetal acuático, hojas y frutos que caen al agua (Ureña, 2007). Sin embargo, eventualmente pueden consumir proteína animal (Lee,1969; Moll, 1989; Lee, 1996; Kölher, 2003).

Estudios etológicos realizados la tortuga blanca bajo manejo *ex situ*, indican que el cortejo y el apareamiento de esta especie ocurren en los meses de junio y agosto. Estos meses en estado de Tabasco coinciden con la temporada de lluvias. En México, el periodo de anidación de *D. mawii* es de septiembre a marzo (Vogt y Flores-Villela, 1992a). La cópula se realiza inmediatamente después del cortejo y permite la monta, durante la cual, la hembra realiza muy poco movimiento. Una vez que las cloacas entran en contacto y el acoplamiento se realiza, la pareja flota suavemente en aguas poco profundas, la duración de la cópula es aproximadamente de 120 a 240 minutos (SEMARNAT, 2014).

Se ha reportado que *D. mawii*, emerge a tierra únicamente para ovipositar y no se asolea sobre troncos como otras tortugas de agua dulce (Lee, 1969; Lee, 1996; Kölher, 2003). Cada hembra deposita de 6 a 39 huevos por nidada, pudiendo depositar hasta 3 nidadas por temporada (Lee, 1969; Vogt y Flores-Villela, 1992a; Polisar, 1995; Lee, 1996; Kölher, 2003). Los huevos de esta especie son de

cascarón blanco liso, de forma ovalada y duro, este último aspecto permite darle una larga viabilidad al huevo, el cual es menos permeable que el de otras especies.

Los nidos son puestos a una distancia de cero a tres metros a partir de la orilla del río. Es por esta cercanía al río que los nidos pueden inundarse, al subir el nivel del río por las lluvias. Cuando esto ocurre, los embriones de la tortuga blanca presentan diapausa embrionaria. Este proceso biológico consiste en que el embrión detiene en una etapa inicial su desarrollo embrionario, hasta que el nido este seco. De esta manera los embriones requieren poco oxígeno y pueden sobrevivir bajo el agua por semanas. Dependiendo de la diapausa embrionaria, el período de incubación varía de 115 a 223 días (Guichard, 2006).

El sexo de las crías está determinado por la temperatura de incubación (Vogt y Flores-Villela, 1992b). Se cree que *D. mawii*, utiliza las zonas arbóreas, debido a que brindan un microclima que ayuda a conservar los niveles de humedad del sustrato utilizado para la construcción de los nidos, regulando la temperatura de incubación, lo que podría ayudar a mantener un equilibrio natural de la proporción de sexo en las poblaciones de esta especie (Vogt y Flores-Villela, 1986).

### *1.2.5. Investigaciones Realizadas en Dermatemys mawii*

Muchos de los trabajos realizados en la tortuga blanca se han enfocado en conocer la biología y ecología de la especie. Por ejemplo, Ureña-Aranda (2007) menciona que los sistemas lóticos en la cuenca baja del río Papaloapan, en Veracruz, son los más importantes para la conservación del hábitat de las diferentes especies de tortugas dulceacuícolas incluida *D. mawii*.

Otro trabajo donde se evaluó el hábitat de *D. mawii* fue el realizado por Zenteno-Ruíz et al. (2010) quienes hicieron una caracterización espacio-temporal del hábitat y determinaron la presencia-ausencia de la tortuga blanca *D. mawii* en la cuenca del río Grijalva-Usumacinta. El estudio reporta la presencia de la especie en tres localidades, siendo más abundante en Tabasquillo. Además, los autores recalcan que el hábitat, el gradiente y la profundidad son las variables que se relacionan con

la presencia de la especie, evidenciando la importancia que tiene el ambiente ribereño como hábitat para *D. mawii.* Los autores proponen que es posible hacer un plan de acción para la protección de la especie y su hábitat en esta reserva.

De igual forma, otro estudio interesante fue el realizado por Morales-Álvarez et al., (2008) quien evaluó diferencias morfométricas de la concha entre poblaciones de la tortuga blanca *D. mawii* en tres macro cuencas hidrográficas: cuenca del Río Usumacinta, cuenca del Río Azul y cuenca del Río Mopán. El autor menciona que encontró evidencia de un proceso de especiación alopátrica en la tortuga blanca, la cual se ve reflejada en la forma de la disposición de sus escamas en el plastrón. En sus resultados, indica que existe una mayor similitud en las tortugas provenientes de las macrocuencas de los Ríos Usumacinta y Mopán comparadas con la macrocuenca del Río Azul, siendo esta última población más parecida a la macrocuenca del Río Usumacinta. El autor sugiere que la separación entre las macrocuencas del Río Usumacinta y del Río Mopán es más reciente que la separación de la cuenca de Río Azul.

Otra investigación relevante es la realizada por Rangel et al. (2009) quienes analizaron la hematología y bioquímica sérica en *D. mawii*, durante las temporadas de seca y lluviosa del 2006. Los autores analizaron individuos silvestres que provenían de la reserva de la Biosfera Pantanos de Centla e individuos que procedían de una UMA ubicada en el estado de Tabasco. Los resultados mostraron que las tortugas bajo un manejo *ex situ,* durante la estación de seca, presentan mayores niveles de ácido úrico y urea, así como menores niveles de glucosa. Además, detectaron la presencia de *Haemogregarina* sp. en el 100% de los individuos silvestres y no en los procedentes de la UMA.

Con relación a los estudios poblacionales de *D. mawii*, se ha determinado la estructura poblacional y la abundancia relativa de la especie, en la laguna El Perú, ubicada en el Petén, Guatemala (Barahona y López, 2015). Los resultados del estudio indican que capturaron 121 individuos, obteniendo una proporción de sexos 1.37 machos por 1 hembra. El 62% de esos individuos fueron subadultos y 38% de

adultos, no registraron individuos juveniles. Los autores concluyeron que el área de estudio posee una población importante de tortuga blanca, además de ser un sitio importante para la conservación de la especie debido a que posee sitios que pueden servir como anidación, refugio y alimentación.

### *1.2.6. La crianza ex situ de D. mawii en Tabasco*

En 1978 se estableció la primera granja de quelonios dulceacuícolas, en el estado de Tabasco (León, 1989). La creación de esta UMA fue gestionada por la entonces Secretaría de Pesca (Sepesca) en coordinación con el gobierno del estado de Tabasco. De acuerdo con León (1989), su creación fue con el fin de mitigar la grave disminución que ya presentaban las poblaciones de tortugas dulceacuícolas en aquellos años. Esta UMA, conocida como “Granja de Tortugas” del estado de Tabasco, inició sus operaciones manejando cinco especies de tortugas: *Trachemys scripta ornata, Kinosternon leucostomum, Staurotypus triporcatus, Chelydra serpentina y Dermatemys mawii* (León, 1989). Actualmente, la UMA “Granja de Tortugas del estado de Tabasco”, es considerada una granja líder en la reproducción de quelonios dulceacuícolas en México y Latinoamérica, debido a que fue la primera en implementar la incubación semiartificial en un manejo *ex situ*, en las especies hicotea (*Trachemys venusta*) y guao (*Staurotypus triporcatus*).

Después de la UMA “Granja de Tortugas del estado de Tabasco”, cuatro UMAs de tortugas son las que tienen relevancia en el manejo y reproducción de *D. mawii* en el estado de Tabasco: UMA “La Encantada”, UMA “CICEA”; UMA “Arca de Noe” y UMA “Arroyo Tabasquillo”.

La UMA “La Encantada” se localiza en el municipio de Jalpa de Méndez, y se creó en 2002. Esta UMA tiene una colonia cautiva de *D. mawii* adultos importante, con un total de 67 individuos. En el 2003, recibió 60 donaciones de *D. mawii* por parte de la Granja del Estado de Tabasco y en el periodo del 2009-2018, tuvo un aprovechamiento de 69 individuos (CONABIO, 2009, Dirección General de Vida Silvestre (DGVS), SEMARNAT, 2018). Esta UMA se caracteriza por ser manejada

de forma particular por una comunidad, y el objetivo que tienen con *D. mawii* es principalmente para obtener beneficios económicos. El estanque donde se mantiene a los reproductores es de tipo rústico, y se encuentra cercado. Pero, a pesar del número importante de reproductores, no se tiene registro publicado que esta UMA haya tenido nacimientos de *D. mawii.*

La UMA "CICEA", pertenece a la Universidad Juárez Autónoma de Tabasco (UJAT), y se encuentra dentro de las instalaciones de la División Académica de Ciencias Biológicas (DacBiol), en el municipio de Centro. Está UMA fue fundada en 2011, y el principal objetivo es la investigación y la reproducción de tortugas dulceacuícolas con fines de conservación. La UMA "CICEA", cuenta con un número importante de reproductores, pero su colonia es más pequeña comparada con la UMA "La Encantada" y la "Granja de Tortugas". Inició con 22 reproductores adultos, y ha tenido nacimiento *D. mawii* (Gallardo-Álvarez, observación personal). Sus instalaciones son estanques rústicos cercados y cuentan con un mejor control en cuanto a la incubación de los huevos recolectados. El manejo de la UMA está a cargo de investigadores y estudiantes de la misma Universidad.

Las UMAs, "Arca de Noe" y "Arroyo Tabasquillo", son manejadas de forma particular y tienen objetivos de aprovechamiento sustentable de la especie. La UMA "Arca de Noe", se ubica en el municipio de Nacajuca. En el 2006, recibió una donación de 41 individuos de *D. mawii*. Cuenta con un solo estanque rústico donde almacena a los ejemplares de *D. mawii* (Rangel-Mendoza y Weber, 2015). La literatura no menciona si esta UMA ha producido crías, pero es posible que, si lo haya logrado, debido a que, en el periodo 2009-2018, la DGVS reportó que esta UMA tuvo un aprovechamiento de 1048 ejemplares de *D. mawii*, de los cuales, 200 tenían permiso para exportación (Dirección General de Vida Silvestre (DGVS), SEMARNAT, 2018). En otro sentido, la UMA "Arroyo Tabasquillo", se ubica en el municipio de Centla, recibió en 2007, 23 individuos por parte de UMA "Granja de Tortugas", y todos sus individuos se encuentran en un solo estanque rústico (CONABIO, 2009; Rangel-Mendoza y Weber, 2015). Es posible que la población en

la UMA “Arroyo Tabasquillo”, sea de 30 individuos o un poco más de acuerdo con el trabajo realizado por Rangel-Mendoza en 2015. De igual forma, aunque no se menciona con precisión la fecha de fundación de estas UMAs, de acuerdo con las donaciones realizadas por parte de la UMA “Granja de Tortuga”; se puede estimar que al menos estos sitios tienen unos 14 años en operación.

En relación con las demás UMAs de *D. mawii* en Tabasco, sucede la misma situación que con el resto de las UMAs de tortuga blanca en México: es difícil obtener información sobre su manejo y datos de reproducción. Por ejemplo, existe una UMA llamada Granja de tortugas: “Los Guayacanes”, ubicada en el municipio de Huimanguillo. Se tiene reporte que esta UMA ha tenido un aprovechamiento de 269 de tortugas de *D. mawii* entre 2009-2019, pero no se especifica cual es el aprovechamiento que se les ha dado a esos organismos y de donde se obtuvieron las tortugas fundadoras (Dirección General de Vida Silvestre (DGVS), SEMARNAT, 2018). Por la cantidad de individuos, se puede especular que son individuos nacidos dentro de la UMA. De igual forma, por la información compartida por parte de la dependencia de Turismo de Huimanguillo, en la red social Facebook, se puede observar que esta UMA maneja estanques rústicos y cuenta con instalaciones alrededor en buen estado (Turismo Huimanguillo, 2020).

Finalmente, por los datos recabados, se puede estimar que la crianza *ex situ* de *D. mawii* en México y Tabasco, inició hace 42 años. No obstante, el aprovechamiento comercial de la especie se realiza desde hace 21 años en México y aproximadamente 11 año en el estado de Tabasco. De igual manera, a pesar de que existen reportes de individuos nacidos en las UMAs, hasta el momento, no se ha consolidado algún programa de translocación que permita revertir la situación de extinción en la que está inmersa *D. mawii*.

### *1.2.7. Estudios Genéticos en Dermatemys mawii.*

Carr y Bickham (1981) realizaron el estudio del cariotipo de *D. mawii,* obteniendo 28 pares de cromosomas (2n=56) al compararse con el cariotipo de *Chelonia mydas* no encontraron diferencias significativas entre ambos cariotipos.

En investigaciones relacionadas con la ecología molecular de la especie, tenemos el estudio de González-Porter et al. (2011), quienes analizaron la filogeografía de *D. mawii* utilizando fragmentos de la secuencia del ADNmt Cyt b, y los genes ND4. Los autores identificaron 16 haplotipos diferentes y sugieren que la baja diversidad observada de haplotipos en algunas poblaciones y la ausencia de un patrón contemporáneo filogeográfico es causada por una combinación de factores: 1) la expansión de las poblaciones silvestres de *D. mawii*, 2) el flujo de genes entre las poblaciones silvestres y 3) Movimientos de individuos de *D. mawii* por parte de las actividades humanas. Concluyen igual que los recientes cuellos de botella son por la sobreexplotación de la especie.

Un antecedente directo de estudios realizados en poblaciones con manejo *ex situ* es el de Zapata (2012) quien realizó un estudio de caracterización molecular en cuatro UMAs de *D. mawii*. Con base en sus resultados, la autora concluye que las UMAs de *D. mawii* en Tabasco presentan una pérdida de diversidad genética que podría afectar a los individuos en la reproducción, en la malformación de sus crías y podría reducir la capacidad de adaptación de la especie.

González-Porter et al. (2013) utilizando ADN mitocondrial y marcadores Microsatélites realizaron una evaluación de los 3 linajes obtenidos para *D. mawii* en un estudio previo. Los resultados mostraron que, en la cuenca del Grijalva-Usumacinta, las poblaciones separadas por más de 300 kilómetros tienen un alto flujo de genes. También, los autores encontraron que las poblaciones no muestran un patrón significativo de aislamiento por distancia, pero, resaltan que la población de la cuenca del Papaloapan es genéticamente distinta a las poblaciones de la cuenca del Grijalva-Usumacinta.

En otro estudio, Marín (2015) calculó la diversidad y estructura genética de *D. mawii* en tres poblaciones de dos ríos (Tabasquillo y Mezcalapa) y una laguna (el Chochal) del Estado de Tabasco utilizando microsatélites. Los resultados mostraron que la población ubicada en la laguna el Chochal, posee una alta diversidad genética y se encuentra genéticamente más distante de las poblaciones de los ríos Tabasquillo y Mezcalapa.

Por último, Recino (2016) realizó una comparación de la variabilidad genética entre poblaciones silvestres y poblaciones con manejo *ex situ* del estado de Tabasco. Los resultados mostraron una alta heterocigocidad observada (0.586) y elevados valores negativos en el índice de fijación (-0.341) en las UMAs. En las poblaciones silvestres, los resultados de la heterocigocidad observada fueron menores (0.224) al igual que los valores en el índice de fijación (-0.243). La autora concluye que esos valores pueden indicar eventos de cuellos de botella y la pérdida de alelos raros en ambas poblaciones.

### *1.2.8. Manejo Genético en Poblaciones con Manejo Ex situ.*

Cuando las especies se encuentran en peligro de extinción, los investigadores implementan estrategias de conservación, en donde consideran importante mantener la variabilidad genética de la especia a recuperar. De tal modo, los planes de conservación buscan como objetivo, que las poblaciones no disminuyan del mínimo de individuos requeridos para mantener la probabilidad de supervivencia. Además, como las poblaciones que se manejan parten de un número reducido de reproductores, los planes de manejo procuran que los reproductores persistan en la naturaleza o que tengan un manejo *ex situ*, con el fin de mantener la diversidad genética de la especie y la estabilidad demográfica.

El genetista, Sir Otto Frankel, fue uno de los grandes responsables en advertir la importancia de la genética para la conservación biológica (Simberloff, 1988). Sin embargo, es a partir de la década de los 80 que se empezó a ver a la biología de la conservación como una ciencia multidisciplinaria que incluía investigaciones de las

áreas de ecología, biología, genética, etología, entre otras. Gracias a estos enfoques, se logró llegar al consenso de que al menos son siete los principales problemas que se deben evitar cuando se implementa un plan de manejo genético para la conservación biológica: 1) depresión por endogamia, 2) acumulación de alelos deletéreos, 3) perdida de la variación genética en pequeñas poblaciones, 4) adaptación genética al manejo *ex situ*, 5) depresión por exogamia, 6) fragmentación de las poblaciones y reducción de las poblaciones y 7) incertidumbres taxonómicas e introgresión (Frankham, 1995).

De tal manera, el manejo genético se ha implementado en diferentes taxones de vertebrados bajo un manejo *ex situ*, como sería el salmón rojo (*Oncorhynchus nerka*) (Kalinowski et al., 2012), trucha arcoíris (*Oncorhynchus mykiss*) (Christie et. al., 2012), león (*Panthera leo*) (Bruche et. al., 2013), el tigre del sudeste de china (*Panthera tigris amoyensis*) (Fu et. al., 2012), lobo rojo (*Canis rufus*) (Brzeski et al., 2014), Chimpancé (*Pan troglodytes)* (Hvilsom et al., 2013)*,* gorila occidental de llanura (*Gorilla gorilla gorilla*) (Simons et al., 2013), Venado nippon (*Cervus nippon hortulorum*) (Krojerová-Prokešová et al. 2013), panda gigante (*Ailuropoda melanoleuca*) (Yang et al., 2013; Shan et al. 2014), el cocodrilo enano (*Osteolaemus tetraspis*) (Franke et al., 2013), rinoceronte blanco sureño (*Ceratotherium simum simum*) (Guerier et al., 2012), tortuga galápago (*Chelonoidis hoodensis*) (Milinkovitch et al., 2013).

Muchos de estos estudios, para realizar su manejo genético, se basan primero en genotipificar y comparar la variabilidad genética de las poblaciones bajo un manejo *ex situ* con poblaciones silvestres. Como ejemplo tenemos el trabajo realizado en la rana sevosa (*Lithobates sevosus*); en donde los autores encontraron que los individuos fundadores tenían una variabilidad genética similar a las poblaciones silvestres. Además, los resultados mostraron que los individuos fundadores compartían los mismos alelos, aunque en proporciones diferentes. Los autores recomiendan evaluar los planes de manejo genético, específicamente, determinar la variabilidad genética de las parejas seleccionadas para reproducción a fin de

evitar la endogamia y mantener el equilibrio de la representación genética fundadora. Igualmente aconsejan que la liberación de individuos se debería realizar en sitios aislados y restaurados, para que puedan ser monitoreados y medir la eficiencia de la reintroducción (Hinkson et al., 2016).

De igual forma, el monitoreo genético y los planes de manejo genético surgen de evaluaciones de planes de reproducción y reintroducción creados en décadas pasadas, cuando las consideraciones genéticas dentro de la conservación biológica eran pocas veces consideradas. Como ejemplo, podemos mencionar el trabajo realizado en la pava yacutinga (*Aburria jacutinga*), un ave que se distribuye en Sudamérica (Brasil, Argentina y Paraguay). Esta especie debido a que se encuentra en peligro de extinción, desde la década de los 80´s fue reproducida en tres aviarios, sin embargo, las consideraciones genéticas no fueron tomadas en cuenta cuando se empezaron a reproducir. Por tal motivo, uno de los primeros aspectos genéticos a evaluar fueron la variabilidad genética dentro y entre los aviarios, donde se encontró la existencia de dos linajes, así como la presencia de un evento de cuello de botella en uno de los aviaros por exceso de heterocigotos. Dentro de las recomendaciones que se realizaron a los manejadores de los aviaros fueron: 1) reintroducir a los organismos en zonas donde se encuentre extinta la especie en lugar de introducir a los individuos en zonas donde ya se encuentren poblaciones establecidas, esto con el fin de evitar interrumpir los complejos adaptativos locales, 2) evitar la endogamia, manejando los grupos cautivos por separado para minimizar la adaptación al manejo *ex situ*, 3) entrecruzar las generaciones previas antes de la liberación para mejorar la reintroducción, y 4) implementar un libro genealógico (Oliveira-Jr. et al., 2016). Evaluaciones parecidas se han realizado en otras especies de aves como son el faisán de Wallich (*Catreus wallichii*) y el tragopán occidental (*Tragopan melanocephalus*) donde evaluaron a los individuos fundadores y detectaron endogamia en las poblaciones cautivas (Mukesh et al., 2016).

Por último, se ha propuesto que para tener un mejor aprovechamiento de los datos genéticos para un manejo genético eficiente, se debe de realizar un marco de

referencia donde se incluyan de manera holística la composición genética de la población en el pasado (componente pasado del marco de referencia), la composición genética actual de las poblaciones bajo un manejo *ex situ* (componente presente del marco de referencia) y composición genética a futuro (componente futuro) después de los eventos de reproducción y liberación. Esta propuesta ha sido aplicada en dos especies de peces pequeños *Nannopeca osbscura* y *N. australis.* Estas especies son endémicas de la cuenca baja Murray-Darling en Australia y sus poblaciones fueron amenazadas por una sequía prolongada. El modelo fue aplicado a dos poblaciones que fueron rescatadas, reproducidas bajo un manejo *ex situ* y subsecuentemente reintroducidas. El modelo holístico reveló similitudes del impacto de la sequía en la genética de las poblaciones rescatadas. Igualmente, los datos revelaron el mantenimiento exitoso de la diversidad genética bajo un manejo *ex situ*, así como la supervivencia y el reclutamiento de crías obtenidas en el manejo *ex situ* y liberadas en el medio silvestre (Attard et al., 2016).

### *1.3.- Justificación*

A nivel ecológico, las tortugas desempeñan un papel importante debido a que participan en el ciclo de nutrientes, permitiendo servir como alimento a otros organismos, así como en el proceso de aporte de nutrientes en los cuerpos de agua (bioturbación). Otro papel importante de las tortugas es que algunas especies, como *D. mawii*, al ser herbívoras, pueden llegar a desempeñar un rol esencial en la reproducción de las especies de plantas que consumen a través de la dispersión de sus semillas. Además, un rol en donde participan activamente las tortugas es en el flujo de energía a través de los ecosistemas, debido a que pueden funcionar como depredadores y controlar las poblaciones de algunos invertebrados (Cortés-Gómez et al., 2015). Por otra parte, para el ser humano, las tortugas dulceacuícolas son muy importantes porque representan una fuente de alimento, así como un ingreso económico para las comunidades locales.

Por tal motivo, para conservar y aprovechar de manera sustentable a las especies de tortugas amenazadas, en México se han creado las Unidades de Manejo para la Conservación de Vida Silvestre (UMAs). Este esquema ha ayudado a incrementar el número de organismos de la tortuga blanca y a que la especie sea utilizada de manera sustentable. De igual manera, muchas UMAs, al encontrarse cerca de área naturales protegidas, han permitido que exista una interconexión con estas áreas, fomentando la creación de corredores biológicos y potenciando la conservación biológica de las especies reproducidas.

Pero, a pesar de que las UMAs son importantes para la conservación de especies amenazadas, uno de los aspectos menos atendidos cuando se establece una UMA, es el manejo de la variabilidad genética y la relación de parentesco de los individuos fundadores. Un desconocimiento sobre esta información genética puede afectar la viabilidad de estas poblaciones criadas bajo un esquema *ex situ*, ocasionando que se incumplan los objetivos de conservación, especialmente al fin último que es la reintroducción. Esto se debe a que individuos con una variabilidad genética muy homocigota pueden causar perdida de variabilidad genéticas en las poblaciones

naturales. Además, las UMAs no han implementado un programa de manejo genético, lo que aumenta el riesgo de provocar depresión endogámica y perdida de alelos en las poblaciones con manejo *ex situ*, y que estas tengan una mayor distancia genética con las poblaciones silvestres.

Por estos motivos, el presente trabajo propone caracterizar la genética de los organismos fundadores de tortuga blanca en tres UMAs del estado de Tabasco, lo que permitirá identificar el pool genético de *D. mawii* con el que cuentan estas instituciones para su reproducción. La importancia de conocer esta información es que permite establecer planes de cruzas más eficientes para mantener la variabilidad genética adecuada en las generaciones producidas en estas UMAs y asegurar que los organismos liberados al medio silvestre no disminuyan la diversidad genética en las poblaciones naturales de la tortuga blanca.

## 1.4.- Hipótesis

Ho1: La estructura genética de los individuos fundadores de las UMAs y los individuos silvestres será similar, debido a que ambos provienen de vida libre.

Ho2: Se espera que los individuos fundadores no tengan una relación de parentesco entre ellos, debido a que las UMAs se manejan de forma independiente.

## 1.5.- Objetivos

### *1.5.1. Objetivo General*

Establecer un programa de manejo genético en *Dermatemys mawii* en tres UMA del estado de Tabasco.

### *1.5.2. Objetivos específicos*

- Determinar la variabilidad genética de los individuos fundadores en tres UMAs dedicadas a la reproducción de *Dermatemys mawii.*
- Comparar la variabilidad genética de los individuos fundadores entre tres UMAs dedicadas a la reproducción de *Dermatemys mawii* y con individuos de *D. mawii* procedentes de vida libre.
- Evaluar las relaciones de parentesco de los individuos fundadores en cada UMA y entre las 3 UMAs dedicadas a la reproducción de *Dermatemys mawii.*
- Establecer el esquema de cruzamiento a partir de las relaciones genéticas entre los individuos fundadores de tres UMAs dedicadas a la reproducción de *Dermatemys mawii.*

## Capítulo 2.- Genetic diversity and population structure of founders from wildlife conservation management units and wild populations of critically endangered *Dermatemys mawii*

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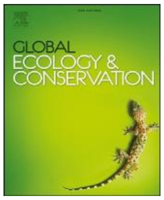

Original Research Article

# Genetic diversity and population structure of founders from wildlife conservation management units and wild populations *Dermatemys mawii*

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## ABSTRACT

Many endangered freshwater turtle species are born in captivity for conservation and future reintroduction into the wild. However, in order to improve breeding programs, an assessment of the genetic diversity of the founder individuals is required to avoid genetic problems such as inbreeding, fixation of deleterious alleles, or loss of allelic diversity due to genetic drift. In this research, we assessed the genetic diversity of the founder individuals from three Wildlife Management Units (UMA) dedicated to the reproduction of Dermatemys mawii in southeast Mexico, and from three wild populations using ten microsatellite markers. Dermatemys mawii is a freshwater turtle that is critically endangered due principally to fragmentation, loss, degradation, and contamination of its habitat, in addition to hunting for human consumption. Furthermore, genetic relationships among UMAs and wild populations, as well as within each kind of group, were investigated by means of Bayesian analysis (STRUCTURE software) and discriminant analysis of principal component (DAPC). Genetic diversity in wild populations could be considered as medium and are less than values observed for UMAs. Genetic diversity for UMAs and wild populations were discussed considering origin of individuals, translocation between UMAs, habitat quality among other factors. Genetic structure analysis highlighted an evident separation between UMAs and wild populations (Bayesian and DAPC analyses), and the hierarchical analysis of structure among UMAs reflected the origin and relationship among them, whereas geographical situation of wild populations is the best explanation for its hierarchical structure. In light of our results, some conservation and management recommendations are provided for this endangered freshwater turtle.

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## 1. Introduction

Turtles have played a significant role in Mesoamerican populations, with a diverse range of uses such as food or to making musical instruments. There is evidence that the Mayans used turtle shells in ceremonies imitate the sounds of thunder (Zender, 2006), and the earth was represented by a turtle by this ethnic group and others in southeast Mexico (Taube, 2009). The turtle has also been an important part of gastronomy in the state of Tabasco for all the ethnic groups that settled in the region (Guevara-Chumacero et al., 2017) with several chronicles describing the importance of these animals as a source of food, taking advantage of the meat, bones, and shell; they were also utilized as utensils and musical instruments (Both, 2008).

The Central American River Turtle, Dermatemys mawii Gray 1847 (Testudines, Dermatemydidae), is an endemic freshwater turtle of Mesoamerica and the last representative of the primitive family Dermatemydidae (Polisar and Horwich, 1994).

It is a species exclusive of aquatic environments, inhabiting rivers, clear bodies of water, lakes, and marshy areas with abundant aquatic vegetation (Alvarez Del Toro et al., 1979). Its diet is mainly herbivorous, although it can occasionally consume animal protein (Lee, 1969; Moll, 1989; Lee, 1996; Campbell, 1998; Kohler, 2008). Generally, courtship and mating occur in the months of June and August, and the nesting period takes place from September to March (Vogt &Flores-Villela, 1992). The species undergo embryonic diapause, and on average, females lay 2.6 clutches per year, with around 11 to 18 eggs per clutch. The incubation period varies from 115 to 223 days, depending on embryonic diapause. Dermatemys mawii is a sex-dependent temperature species (Legler and Vogt, 2013; Vogt et al., 2011). Little research has been carried out on life history in free-living conditions (Lee, 1969; Campbell, 1972; Moll, 1989; Polisar, 1996); however, the longevity record in captivity belongs to an adult male from the Jacksonville Zoo and Gardens (Florida) which lived 35.7 years in captivity, with an estimated age of around 40 years (Platt et al., 2016). The same zoo has a wild-caught Guatemalan female that has been in captivity for 22.7 years, unfortunately its age or size class at the time of acquisition is unknown. These two individuals represent the oldest wild-caught *D. mawii* recorded to date. The longest-living captive-hatched *D. mawii* is a 22.3-year-old female of Guatemalan descendence that was hatched at the Philadelphia Zoo in January 1994 and currently resides at Jacksonville Zoo and Gardens (Platt et al., 2016).

Although, this species was abundant until the mid-20th century throughout its distribution range which extended from the southeast of Mexico to Belize and Guatemala, at the present time, this large freshwater turtle is at risk of extinction due to hunting (consumption of meat), illegal trade, and habitat fragmentation (Vogt et al., 2011; SEMARNAT, 2014). Dermatemys mawii is considered as one of the 25 most endangered chelonian species in the world, therefore it is classified as “critically endangered” by the IUCN Red List and included in the Appendix II of CITES (Macip-Ríos et al., 2015; Turtle Conservation Coalition, 2018). In Mexico, *D. mawii* is considered as priority species for conservation by CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), and the NOM-059-SEMARNAT-2010 established by SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales) classify *D. mawii* as endangered (meaning “in danger of extinction”) (SEMARNAT, 2010a, b; Macip-Ríos et al., 2015). Therefore, in Mexico, *D. mawii* is protected from capture for which it is obligatory to obtain permits (Oficial de la Federación, 2000; 2015). Wildlife Management and Use Units (UMA, from its name in Spanish; Weber et al., 2006) are viable alternatives to allow conservation, reproduction, and reintroduction of this species. Official data in Mexico report 37 breeding places (UMA) for *D. mawii*; the principal aims of these UMA are reproduction, conservation, and sustainable use (CONABIO, 2008). However, it is important to take into account genetic considerations to maintain genetic variability within the UMA (CEC, 2017).

To improve the conservation of the species, captive breeding programs have been implemented and carried out in Mexico through the development of the UMAs. UMAs are sites where wild species are managed for their conservation and sustainable use and can work as exhibition centers, germplasm banks, and centers of research. This action plan aims to preserve biodiversity and generate opportunities for economic diversification of the rural sector. UMAs experienced an exponential growth in the number of registrations between 2012 and 2017 with 322 new UMAs totaling 11,722 UMAs covering more than 38.5 million hectares which is equivalent to 19% of the Mexican territory (SEMERNAT, 2018). Correspondingly, indigenous communities and small owners have been able to direct their efforts and their economy to the management and use of wildlife with a financial movement of more than 5 billion Mexican pesos (around 263 million US$) a year and a double purpose: contributing to repopulate wildlife in Mexico and improve its economy. Mexican UMAs are enjoying good results in term of economic gains and recovery of wild populations of the managed species (de Benito, 2009; Gallina-Tessaro et al., 2009). Most of the successful cases in the management of UMAs are associated with the hunting ranches of white-tailed deer (*Odocoileus virginianus*, Zimmermann, 1780, Artiodactyla, Cervidae), the red brocket (*Mazama americana*, Erxleben, 1777, Artiodactyla, Cervidae), and the grey brocket (*Mazama gouazoubira*, Fischer, 1814, Artiodactyla, Cervidae) which are located in the northern states of Mexico (Tamaulipas, Coahuila, Chihuahua, Sinaloa, Sonora, Baja California Norte, Baja California Sur, Zacatecas, Durango and Nuevo Leon). However, in central and southern states of the country the scope of the projects undertaken have not had the same results (de Benito, 2009). Particularly, in southeast Mexico, many UMAs have bred freshwater turtles for commercial purposes (e.g., for human consumption, or pets) or for reintroduction in wild populations (Rangel et al., 2018).

Unfortunately, husbandry techniques in UMAs may be based on traditional knowledge of rural consumers and the experience of local producers (Rangel-Mendoza et al., 2014) and, consequently, this could affect the initial objectives of the UMA. Effective management of the UMA should integrate knowledge of several aspects of the species biology and other factors such as optimal site conditions, quality management of the organisms, excellent sanitary care, and adequate knowledge on the genetic diversity of the founder individuals (Huntley and Langton, 1994; Syed et al., 2007). Particularly, genetic diversity of founder individuals of UMAs used for our study is unknown. Indeed, at the time of the creation of UMAs, the use of molecular tools was limited, furthermore, the integration of genetic study in UMAs (or farms) is relatively recent and requires a financial technical cost and technical support not always available. There is a lack of genetic information associated with UMAs for freshwater turtles, as genetic diversity, and fitness conservation; those information are important to avoid low genetic variability, fixation of deleterious alleles, loss of genetic diversity due to drift and inbreeding and outbreeding problems (Alacs et al., 2007; Williams and Osentoski, 2007). Therefore, genetic tools could help evaluate the genetic structure of individuals among UMAs, and considerably enhance the exchange of breeding individuals, thus improving genetic management.

An important problem in UMAs is that the geographic origin of the founder turtles is uncertain; this could cause problems when different UMAs exchange or donate individuals to increase the number of breeding individuals (Syed et al., 2007). This problem may be due to the presence of technical problems such as lack of training for staff that manages the organism, insufficient staff to carry out all the activities, and a lack of organization of UMA records and documents (Gallina-Tessaro et al., 2009).

Genetic diversity in both captive and wild populations has been determined by different neutral polymorphic markers such as RAPDs, AFLPs, RFLPs, ISSRs, microsatellites, or SNPs (Alcantara, 2007; Shirley et al., 2015; Hurt et al., 2017; Atkinson et al., 2018). In particular, microsatellites have been successfully used in genetic characterization in captive breeding programs of endangered species (Aranguren-Mendez et al., 2005 ) as for example for *Alligator sinensis* Fauvel, 1879 (Crocodilia, Alligatoridae) (Xu et al., 2005), and other species (e.g., Capra ibex L., 1758, Artiodactyla, Bovidae; Maudet et al., 2002, and *Ctenosaura pectinata* Wiegmann, 1834, Squamata, Iguanidae; Zarza, Reynoso and Emerson, 2016), to identify loss of genetic diversity in captive populations (e.g., Mauritius kestrel Temminck, 1821, Falconiformes, Falconidae; Ewing et al., 2008, and *Gorilla gorilla gorilla* Savage, 1847, Primates, Hominidae; Simons et al., 2013), and to determine the effective population size (e.g., *Gypaetus barbatus* Linneus, 1758, Accipitriformes, Accipitridae; Gautschi et al., 2003, and for Galapagos tortoise, Testudines, Testudinidae; Milinkovitch et al., 2013).

In Mexico, 94 UMAs have been established to reproduce native freshwater turtles ( Dirección General de Vida Silvestre (DGVS) & SEMERNAT, 2018) and they have achieved successful breeding of some freshwater turtle species such as Trachemys spp (Testudines, Emydidae, Agassiz, 1857) (SEMARNAT, 2011), Claudius angustatus (Testudines, Kinosternidae, Cope 1865), Staurotypus triporcatus (Testudines, Kinosternidae, Wiegmann 1828) (Reynoso et al., 2016), and *D. mawii* (SEMARNAT, 2010a,b) among others. In the case of *D. mawii*, Mexico registered 37 UMAs (17 in Tabasco state) that have achieved successful reproduction as a result of ex situ management and allowed the production of a large number of individuals. Successful captive breeding identified the biological requirements for survival in captivity (e.g., adequate infrastructure to achieve successful mating, nesting viability, incubation temperature, survival of the hatchlings, diet) (SEMARNAT, 2009). The main purpose of the reproduction has been to protect turtles from overexploitation in the wild; Nevertheless, the reintroduction of individuals from UMAs to their natural environment to reinforce wild populations has not been achieved due to the lack of genetic information of individuals and their genetic compatibility with wild populations, which is part of the reintroduction protocol established by the IUCN/SSC (2013). Genetic monitoring in UMAs is important in order to control and detect inbreeding and the loss of genetic diversity, which could result in loss of adaptation capacity and generate problems such as outbreeding at the moment of reintroduction; this is particularly important because *D. mawii* is highly threatened and has small and isolated populations (Todd et al., 2010; Refsnider and Janzen, 2016).

The aim of this research was to determine the level of genetic diversity and structure of founder individuals in three UMAs in southeast Mexico dedicated to the reproduction and reintroduction of *D. mawii*. Particularly, we focused on the following objectives: (i) to determine the genetic diversity of founder individuals in three UMAs, (ii) to determine the genetic structure inside and between UMAs, (iii) to compare genetic information (diversity and structure) from UMAs with wild populations in order to evaluate the feasibility of reintroduction. Finally, we will discuss the usefulness of this type of genetic study for the management of Mexican UMAs.

## 2. Methods

### *2.1. Sites description and samples collection*

### *2.1.1. UMAs*

Skin samples were collected from three different freshwater turtle UMAs: (1) “La Encantada” (LE$_{\text{UMA}}$) located in Jalpa de Mendez, Tabasco (18 20$_0$09$_{00}$N-9302$_0$53$_{00}$W). This was created in 2002 and represents the second largest freshwater turtle UMA in Tabasco. Its aim is the reproduction of two freshwater turtle's species *D. mawii* and T. venusta, in addition to trade and conservation. The founder individual of *D. mawii* for this UMA came from wild environments in Tabasco where turtles from the Tabasco State Government UMA (TSG$_{\text{UMA}}$) were also sourced. This privately managed UMA was selected for our study because it has the second highest rate of production for *D. mawii* individuals and access was facilitated; (2) Tabasco State Government UMA (TSG$_{\text{UMA}}$), located in the town of Nacajuca in Tabasco (1811$_0$23$_{00}$N-9259$_0$37$_{00}$W) was created in 1978, with the aims of reproduction, research, education, and conservation of seven freshwater turtle species (including *D. mawii*). Another important aim is breeding stocks that are donated to other new UMAs. This UMA has the largest captive population of *D. mawii* in Mexico (>800 individuals in 2006) (Rangel-Mendoza et al., 2009). Founder individuals of *D. mawii* came from natural river habitat near the border with Guatemala (Vogt, Personal communication). This UMA was selected for our study because it was the first to reproduce a *D. mawii* population in Tabasco state and in the southeast of Mexico; (3) Tabasco State Juárez University UMA (TSJU$_{\text{UMA}}$) located in Villahermosa, Tabasco (1759$_0$26$_{00}$N-9258$_0$16$_{00}$W) was created in 2011 with the aim of research, reproduction, and conservation of native freshwater turtles. This UMA is dedicated to the breeding of two species of freshwater turtles *T. venusta* and *D. mawii*. The TSJU$_{\text{UMA}}$ founders of *D. mawii* came from TSG$_{\text{UMA}}$ and wild individuals.

This UMA was selected for our study because their principal activities are focused on environmental education, reproduction, and conservation of *D. mawii*, and access was facilitated by the University. Additional information related to the UMAs is presented in Table 1 and Fig. 1.

It should be clarified that even though the management plan of many UMAs establish the releasement of individuals as part of these objectives, none have reintroduced *D. mawii* into the wild. The principal purposes of the UMAs where we collected individuals for this research are reproduction, environmental education, research, and conservation which are some of the specific purposes of UMAs included in article 39 of the General Law of Wildlife (Oficial de la Federacion, 2000).

### *2.1.2. Wild populations*

Samples of wild individuals were collected at three places: (1) Tabasquillo River (TR) located in Centla, Tabasco (1824$_0$0$_{00}$N9239$_0$0$_{00}$W); it is a lotic and calm body water with tributaries of the Grijalva-Usumacinta delta rivers system. This site was selected because the presence of *D. mawii* is reported and represents an adequate habitat for the reproduction of *D. mawii* (Zenteno et al., 2010);

(2) Chochal Lagoon (CL) is a freshwater lagoon located in Jonuta, Tabasco (1826$_{0}$44$_{00}$N-9216$_{0}$30$_{00}$W) permanently flooded by rains and connected to the Usumacinta River drainage system. Like TR, this place is a preserved area where *D. mawii* is still abundant. Both localities (TR and CL) are within the buffer zone of the Pantanos de Centla Biosphere Reserve; (3) Mezcalapan River (MR) located in Huimanguillo, Tabasco (1836$_{0}$0$_{00}$N-9239$_{0}$0$_{00}$W) which is lotic and a large body of water that is part of the Grijalva River drainage system. This site was chosen because it is one of the most disturbed areas in the state of Tabasco due to agriculture, activities related to the oil industry, and modifications to the riverbed due to the construction of dams in the neighboring state of Chiapas which impact rivers and potential habitats for freshwater turtles. In this locality, although the sampling effort was similar at all study sites, the number of individuals captured was lower, certainly reflecting the reality of a low population density due to the previously mentioned inadequate habitat conditions.

### *2.2. Sample collection*

Skin samples were collected from the interdigital membrane of the legs during 2017 and 2018. A total of 117 samples were obtained from UMAs and 68 from wild populations. The original founder individuals from UMAs were identified thanks to the assigned tags in the shell, and descendants were not included in our study. Turtles were captured using a trammel net while wild individuals were caught using fyke or frame nets (Vogt, 1980). Before taking samples, the skin was disinfected with 70% ethanol, and after an antiseptic (methylthioninium chloride) was applied to the wound to avoid infections. Tissues were preserved in a salt-saturated solution (250 mM EDTA pH 7.5 and 20% DMSO) and stored at 80 C until DNA extraction (Gonzalez-Porter et al., 2011). All the organisms were subject to observation for a period of 2 h, and after having confirmed that they were in good condition, they were released in places close to where they were captured. Capture of individuals, biological sampling, transportation, and ethical procedures used in this study were those recommended by the guide for the management of amphibians and living reptiles of the American Society of Ichthyologists and Herpetologists (American Society of Ichthyologists and Herpetologists, 2004), and by the management plan for Dermatemys (SEMARNAT, 2010a, b). The capture permit SGPA/DGVS/011,085/16 was provided through the Secretariat of Environment and Natural Resources (SERMARNAT) of Mexico.

#### *2.2.1. DNA extraction and microsatellite genotyping*

DNA was extracted using the Qiagen DNeasy Tissue kit (Qiagen, Inc., Valencia, CA), and quality and concentration were verified using a UVeVis spectrophotometer (Thermo ScientificTM NanoDropTM One). Ten specific microsatellite loci were used in our study; seven of them were reported in Andree et al. (2010) and used by Gonzalez-Porter et al. (2013), and three microsatellite primers (Dm4A-11, Dm3A-43, and Dm3A-61) were designed from the sequences reported in GenBank (Gonzalez-Porter et al., unpublished GenBank information). PCR was performed in a total volume of 22 μl containing 100 ng of

**Table 1**

Information on wildlife management unit (UMA) and wild populations of Dermatemys mawii obtained for this study. Geographic coordinates (GC), number of individuals (N) which represent initial founders for UMA.

| | Locality/name | Township | Abbreviation | GC | N |
|---|---|---|---|---|---|
| UMA | La Encantada | Jalpa de Mendez | LE$_{UMA}$ | 182000900 N-930205300 W | 67 |
| | Tabasco State Government | Nacajuca | TSG$_{UMA}$ | 181102300 N-925903700 W | 28 |
| | Tabasco State Juárez University | Centro | TSJU$_{UMA}$ TR | 175902600 N 925801600 W | 22 |
| Wild Populations | Tabasquillo River | Centla | | 18240000N-92390000W | 37 |
| | Mezcalapan River | Huimanguillo | MR | 18360000N-92390000W | 8 |
| | Chochal Lagoon | Jonuta | CL | 182604400N-921603000W | 23 |

**Fig. 1.** Study area in southeast Gulf of Mexico. The points represent the sampling areas. Wildlife management unit (UMA): La Encantada (LE$_{UMA}$), Estatal representing the Tabasco State Government UMA (TSG$_{UMA}$), Cicea representing the Tabasco State Juárez University UMA (TSJU$_{UMA}$), and wild populations: El Chochal representing the Chochal Lagoon (CL), Huimanguillo representing the Mezcalapan River (MR), and finally Tabasquillo representing the Tabasquillo River (TR).

template DNA, 15 ml of Platinum PCR® SuperMix (22 U/ml recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22 mM Tris-HCl pH 8.4, 55 mM KCl, 1.65 mM MgCl2, 220 mM dGTP, 220 mM dATP, 220 mM dTTP, 220 mM dCTP, and stabilizers; Invitrogen), and 200 nM of each primer. DNA was amplified by PCR reactions using the following conditions: for the locus Dm3A-72, an initial denaturation at 94 C for 7 min, 38 cycles of denaturation at 94 C for 40 s, primer annealing temperature at 64 C for 40 s, an extension at 72 C for 45 s, and final extension at 72 C for 7 min. PCR conditions for other primers were the following: initial denaturation at 94 C for 7 min, 45 cycles of denaturation at 92 C for 1 min, primer annealing temperature at 50e65 C for 1 min depending of the primer (Table S1), extension at 72 C for 1 min, and a final extension at 72 C for 7 min. All PCR amplifications were performed in a T100 Thermal Cycler (Biorad). PCR products were analyzed on 3.4% high-resolution agarose gel (UltraPureTM Agarose-1000, Invitrogen) (Roubos et al., 2010), stained with ethidium bromide, running at 70 V for 2 h with a 100-bp DNA ladder (Biorad) as size standard. Gels were photo-documented using Molecular Imager® Gel Doc TM (Biorad), and the amplified fragments were analyzed using the automatic gel documentation and image analyzer Bio-Vison software (Vilber, US). Microsatellites reproducibility was tested considering the allelic dropout (when a heterozygote is typed as a homozygote; Valiere, 2002) and false allele (when homozygote a is typed as a heterozygote; Valiere, 2002) rates using Gimlet 1.3.3 (Valiere, 2002). The criteria used to test the genotyping error were: (1) analyze based on 5% of samples (ten individuals chosen at random) from the total sample size as suggested by Bonin et al. (2004), (2) the same DNA extract was used in the reproducibility test and experiments, and (3) each DNA sample was replicated three times (twice in the same PCR run and once in the other PCR run) (Machkour-M'Rabet et al., 2017).

### *2.3. Data analysis*

#### *2.3.1. Genetic diversity*

Genetic diversity was estimated through allele number (NA), number of effective alleles (NE), observed heterozygosity (Ho), expected heterozygosity (He), percentage of polymorphic loci (PPL), and inbreeding coefficient (F) using Genalex 6.502 (Peakall and Smouse, 2006). The Hardy-Weinberg equilibrium (HWE) for heterozygote deficit was tested with Genepop 4.0.10 (Raymond and Rousset,1995; Rousset, 2008). Presence of null alleles per loci was estimated with FreeNA (Chapuis and Estoup, 2007). All of these parameters were determined for each UMA and each wild population and considering all dataset of UMAs and wild populations respectively as only one group. Bottleneck events were tested for each UMA, each wild population, all UMAs, and all wild populations by comparing levels of He related to excess $H_E$ relative to expected equilibrium heterozygosity (Heq) using Bottleneck 1.2.02 (Cornuet and Luikart, 1996; Piry et al., 1999). Two mutational/drift equilibrium models were considered: stepwise mutational model (SMM) and two-phase model (TPM), with 95% of single-step mutation for the TPM model and a variance among multiple steps of 12 (Piry et al.,1999). Significance was assessed from one-tailed Wilcoxon ranktests, and bottleneck event will be considered only if both models were significant (Harencar et al., 2018). The Mezcalapan River locality had only eight individuals, therefore, considering its sensitivity to sample size, the bottleneck test was not applied (Gonzalez-Porter et al., 2013).

### *2.3.2. Genetic structure*

To determine the level of genetic differentiation, $F_{ST}$ values were estimated with FreeNA which implement the ENA correction method to calculate $F_{ST}$ values excluding null alleles (Chapuis and Estoup, 2007). Pairwise $F_{ST}$, and a global value of $F_{ST}$ were determined considering only UMAs and wild populations respectively. To identify the most probable genetic structure for UMAs and wild populations, we used two different methods: (1) a Bayesian analysis implemented in Structure 2.3.1 (Pritchard et al., 2000). This method allows to determine the optimal number of groups, or clusters (K), assigning each individual to one or more groups indicating that they are admixture. The admixture and correlated allele frequency models were applied. In addition, we used the locprior model which is recommended when the level of structure could be weak (Hubisz et al., 2009). To determine the optimal number of clusters, the program was run ten times for different numbers of K (K from 1 to 10), and for each run the Markov Chain Monte Carlo algorithm was run with a burn-in period of 500,000 steps followed by 500,000 steps, after which the results were implemented in Structure Harvester (Earl and vonHoldt, 2011) that determines the most probable K value (the DK method also known as Evanno method; Evanno et al., 2005); (2) the discriminant analysis of principal components (DAPC), a multivariate method available through the adegenet package (Jombart, 2008) for the R software (R Core Team, 2013). This package and its corresponding analysis where used and described in Jombart et al. (2010), and Jombart and Collins (2017). This relatively recent method has the advantage of not using a hypothesis for the population genetics model, so therefore it is free of assumptions on the Hardy-Weinberg Equilibrium (Jombart et al., 2010). This is salient, considering that all populations of this study present a significant departure from HWE. Firstly, the molecular data were converted into an adequate data frame to be analyzed using the adegenet package, and the first trials have allowed us determine the adequate number of principal components. The function find. cluster was used to determine the optimal number of the principal components and the number of clusters for the six proposed localities based on the Bayesian Information Criteria (BIC) whose result is presented as a graphical representation using the scatter function implemented in the ade4 package (Dray and Dufour, 2007). In this scatterplot, the original “groups” (UMAs or wild populations) were represented as inertia ellipses where the dispersion of individuals around the gravity center is marked by segments linking points (individuals) to the ellipse. The cross validation was checked using the xvalDapc function from adegenet package, this compares the experimental obtained values into an iterative probe to check the robustness of the result.

## **3. Results**

### *3.1. Genetic diversity*

Genotyping error rates could be considered as low (average allelic dropout rate of 2.7% and average false allele rate of 9.1; Table S2 for detail per microsatellite) when compared with other studies (see Table 4 in Valiere et al., 2007); representing good reproducibility of our microsatellite primers. Genetic diversity parameters (Na, Ne, Ho, He) were slightly higher for UMAs than for wild populations (Table 2 for details). The global values of He for UMAs (0.613) was significantly higher (MannWithney U test: U ¼ 416.00 and P ¼ 0.03) than for wild population (0.527). A significant difference in He values among wild populations was found (Kruskal-Wallis test: $H_{2,30}$ ¼ 5.89, P ¼ 0.05) with a post-hoc test showing a difference between TR and MR localities ($P < 0.05$), and a significant difference (One-Way ANOVA: F ¼ 4.23, df ¼ 2, P ¼ 0.025) was observed among UMAs with the

Encantada UMA attaining the highest value (Newman-Keuls post-hoc test: $P < 0.05$). General polymorphism is high (from 80% to 100%) for UMAs as well as for wild populations, with the exception of the Mezcalapan River which presents a very low value (50%), probably a consequence of its low number of individuals. Presence of null alleles was detected at four loci for UMAs and six loci for wild populations (Table S3). Inbreeding coefficient (F) was higher for wild populations (0.451) than for UMAs (0.414), and all HWE tests for heterozygote deficit were significant ($P < 0.001$; Table 2). A bottleneck event was detected for MR population, as well as for $LE_{UMA}$ (Table 2).

### *3.2. Genetic structure*

Genetic differentiation ($F_{ST}$) between pairs of wild populations is higher than between pairs of UMAs (Table 3). The highest $F_{ST}$ value among pairs of UMAs was found between $TSJU_{UMA}$ and $LE_{UMA}$ ($F_{ST}$¼ 0.113) and between CL and MR ($F_{ST}$¼ 0.0380) for wild population pairs. When considering pairwise among UMA groups and wild populations, all UMAs present the highest genetic differentiation with MR wild locality, and the lowest genetic differentiation with TR (Table 3). Using probability assignments estimated by Structure, all individuals (UMAs and wild) were clearly separated into two clusters (Fig. 2A; dark grey for UMAs and light grey for wild populations) as suggested by DK method, both with very high membership probabilities (Table S4). This separation into two groups is confirmed by the DAPC analysis (Fig. 3) showing a clear separation between UMAs and wild populations along the first axis (negative side for UMAs and positive side for wild populations). When only UMAs were analyzed, the DK method suggested K ¼ 3 (Figs. 2Be1) that separates La Encantada UMA ($LE_{UMA}$), which has two well defined clusters (white and light grey in Figs. 2Be1; membership probabilities in Table S5), from both other UMAs (dark grey in Figs. 2Be1; high membership probabilities for the third cluster: Table S5). However, in order to observe more internal structure, we decided to illustrate the genetic structure proposed by the Bayesian analysis when K ¼ 4 and K ¼ 5. For a K ¼ 4, the Tabasco State Juárez University UMA ($TSJU_{UMA}$) begins to show a slight difference from the Tabasco State Government UMA ($TSG_{UMA}$) (very dark grey in Fig. 2Be; Table S5), and with K ¼ 5, a new cluster can be observed well defined into the $LE_{UMA}$ (black in Figs. 2Be3; Table S5). When only wild populations were considered, the DK method suggested K ¼ 2 (Figs. 2Ce1) that clearly separates (high membership probabilities for each new clusters: Table S6) TR and MR populations (light grey with dots), from the CL population (white with dots). However, if we considered a genetic structure with K ¼ 3, in order to identify a finer structure, the MR population differs from TR (Fig. 2Ce) with a very high membership probability (Table S6). The totality of clustering of UMA and wild populations that could be observed through the hierarchical analysis of Structure are observed in a specific way when applying the DAPC analysis. In addition, to indicate a separation between UMAs and wild populations, each “group” (UMA or wild) are relatively well separated, creating a total of six clusters (Fig. 3). Nevertheless, the double genetic profiles observed for $LE_{UMA}$ with Bayesian analysis of Structure (Figs. 2Be1, 2B-2) was not highlighted by the DAPC analysis, as was the more complex genetic composition of the TR wild population (Fig. 2Ce).

## **4. Discussion**

This is the only study that evaluates, through microsatellites, the level of genetic diversity of both captive and wild populations of a critically endangered species of freshwater turtle in Mexico. All populations (wild and UMAs) showed significant deficit in heterozygotes (high value of F), which could suggest inbreeding in natural and captive populations, but also sub-structuring in populations. Although, the inbreeding process and population structuring cannot be rejected, the presence of high number of null alleles in wild and captive populations could probably explain this deviation from HWE, and that given that the founding individuals in the UMAs originate from different places, it is likely that a Wahlund effect will be added to the null alleles presence in the UMAs. Wild populations showed high values of polymorphism (with the exception of the Mezcalapan River) which could suggest open populations (Machkour-M'Rabet et al., 2014); this is not unexpected considering the high mobility of this freshwater turtle (Calderon-Mandujano, 2017). The low value observed for the Mezcalapan River population is probably due to the low number of individuals collected. The high value of polymorphism founded in UMAs is probably the result of the different origin of individuals. As with the results obtained by Gonzalez-Porter et al. (2013), no indications of recent bottlenecks were identified for wild populations, whereas the UMA La Encantada (LE) showed a significant value for the bottleneck test. This result could indicate a founder effect, especially when considering that to develop a new UMA, the breeding stock must come from the UMA of the Tabasco State Government (TSG$_{UMA}$), where a complete single clutch could result in problems of inbreeding due to highly related organisms.

Genetic diversity observed in our study for *D. mawii* is similar to the results from other studies that use microsatellites for highly threatened species. For example, Spitzweg et al. (2018) obtained similar values of genetic diversity (H$_e$ around 0.6) for wild and captive populations of a critically endangered riverine turtle (Batagur baska Gray, 1830; Testudines, Geoemydidae),

**Table 2**

Genetic diversity statistics for wildlife management unit (UMA) and wild populations for Dermatemys mawii in southeast Mexico. Number of individuals (n), percentage of polymorphic loci (PPL), mean number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (F), Hardy-Weinberg equilibrium test for heterozygote deficit (PHWE), results of bottleneck test considering the two-phase model (PTPM) and the stepwise mutational model (PSMM). Not significant (ns), not applicable (na), * P < 0.05, ** P < 0.01, *** P < 0.001. For site abbreviations see Table 1. Global represent analysis when all populations of a particular group are considered as only one population.

| | Wildlife management unit | | | | Wild populations | | | |
|---|---|---|---|---|---|---|---|---|
| | LEUMA | TSG$_{UMA}$ | TSJU$_{UMA}$ | Global | TR | MR | CL | Global |
| N | 67 | 28 | 22 | 117 | 37 | 8 | 23 | 68 |
| PPL (%) | 100 | 80 | 90 | 90 | 100 | 50 | 90 | 80 |
| $N_a$ | 8.0 | 5.1 | 3.8 | 8.2 | 6.4 | 2.0 | 4.0 | 7.5 |
| $N_e$ | 5.245 | 3.052 | 2.565 | 5.138 | 4.083 | 1.673 | 2.806 | 4.926 |
| $H_o$ | 0.557 | 0.358 | 0.304 | 0.406 | 0.496 | 0.158 | 0.346 | 0.387 |
| $H_e$ | 0.786 | 0.551 | 0.501 | 0.613 | 0.746 | 0.310 | 0.545 | 0.527 |
| F | 0.314 | 0.343 | 0.360 | 0.414 | 0.330 | 0.479 | 0.368 | 0.451 |
| PHWE | *** | *** | *** | *** | *** | *** | *** | *** |
| PTPM | ** | ns | ns | ns | ns | na | ns | * |
| PSMM | ** | ns | ns | ns | ns | na | ns | ns |

**Table 3**

Pairwise F$_{ST}$ for all populations pairs considering wildlife management unit (UMA) and wild populations of Dermatemys mawii. Values were determined including null alleles (below diagonal) using GENEALEX and using the ENA correction method implemented in FREENA to exclude null alleles (above diagonal and in italic). Bold values are significant. For abbreviation populations see Table 1.

| | | UMA | | | Wild populations | | |
|---|---|---|---|---|---|---|---|
| | | LEUMA | TSG$_{UMA}$ | TSJU$_{UMA}$ | TR | MR | CL |
| UMA | LEUMA | e | 0.072 | 0.113 | 0.077 | 0.228 | 0.152 |
| | TSG$_{UMA}$ | 0.085 | e | 0.111 | 0.103 | 0.302 | 0.223 |
| | TSJU$_{UMA}$ | 0.127 | 0.124 | e | 0.128 | 0.315 | 0.302 |
| Wild populations | TR | 0.088 | 0.096 | 0.131 | e | 0.247 | 0.162 |
| | MR | 0.218 | 0.289 | 0.319 | 0.229 | e | 0.380 |
| | CL | 0.168 | 0.236 | 0.324 | 0.176 | 0.370 | e |

and Schmidt et al. (2018) showed similar values of genetic diversity ($H_e$ around 0.5) for wild and captive individuals of the endangered Mary River turtle (*Elusor macrurus* Cann and Legler, 1994; Testudines, Chelidae) considering these values as low. The unique genetic study on *D. mawii*, that included populations from a wide geographical range, exhibited heterozygosity values from low to medium ($H_e$ from 0.31 to 0.57) with highly variable number of individuals per locality (from 2 to 30) (Gonzalez-Porter et al., 2013). These values (around 0.5) for threatened freshwater turtle species could probably be regarded as medium, recognizing that fishes and reptiles present a higher level of genetic diversity than other vertebrates, and that threatened species demonstrate a lower degree of genetic diversity than species of lesser conservation concern (Willoughby et al., 2015). Indeed, genetic diversity for other river turtles with less threatened status are higher; for example, Petre et al. (2015) obtained high values of genetic diversity ($H_e$ around 0.75) in all sampled localities of Louisiana for the near threatened terrapin turtles (Malaclemys terrapin Schoepff,1793; Testudines, Emydidae), and Laporte et al. (2013) showed very high values of genetic diversity (around 0.80) for the painted turtle (Chrysemys picta Schneider 1783; Testudines, Emydidae).

Even though a loss of genetic diversity in captive populations was expected (Frankham, 2008), our results showed significantly higher genetic diversity in UMAs than in wild populations, while other studies show no significant difference in genetic diversity between captive and wild turtle populations (Schmidt et al., 2018; Spitzweg et al., 2018). Different explanations for the high values in captive populations can be proposed: (1) individuals collected in the UMA were exclusively founders, thus genetic diversity in the UMA is a reflection of the genetic diversity of wild populations over many years, (2) individuals found in the UMA could come from a variety of localities as the majority come from seizures of trafficked individuals from many areas, carried out by Mexican environmental agency PROFEPA (Procuradoria Federal de Proteción al Ambiente), thus increasing genetic diversity, (3) in recent decades, wild freshwater turtle populations of Tabasco are subject to strong human pressures, probably decreasing genetic diversity, such as fragmentation and contamination of habitat due to high economic development of the region (e.g., livestock, agriculture, petrochemical industry) drastically altering the landscape (see references in Arriaga-Weiss et al., 2008), and traditional human consumption of turtles in this region (see references in Gonzalez-Porter et al., 2013) adds further pressure on turtle populations, and finally (4) we cannot rule out the possible bias due to the low value of $H_e$ obtained for one wild population which could be due to the low number of individuals (N ¼ 8 in Mezcalapan River) collected at this particular point.

Historic origin of the founders is probably the explanation for the difference observed in genetic diversity among UMAs. Certainly, the UMA “La Encantada” (LE) presented the highest value of $H_{e,}$ probably a consequence of the founding individuals having two different origins (personal communication from owner of the UMA): (1) other UMAs (Tabasco State Government UMA, $TSG_{UMA}$) and (2) donations of wild animals confiscated by PROFEPA. This double origin is also observed in the genetic structure among UMA with two genetic groups clearly separated. The founding individuals of both other UMAs ($TSG_{UMA}$ and $TSJU_{UMA}$) have a less variable origin; all founders of the Tabasco State Government UMA ($TSG_{UMA}$) founded in 1978 have been captured from the Tzendales River on the border of Mexico and Guatemala (Dr. RC. Vogt, personal communication), and regarding the founders of the Tabasco State Juárez University UMA ($TSJU_{UMA}$), the most recent (2011), came from PROFEPA and the other UMA ($TSG_{UMA}$). This translocation of individuals between both UMAs ($TSG_{UMA}$ and $TSJU_{UMA}$) is clearly observed in the genetic structure revealed by Bayesian analysis.

Genetic diversity among wild populations is highly variable from very low to high with the greatest values for Tabasquillo River and Chochal Lagoon, two sites inside the buffer zone of the Pantanos de Centla Biosphere Reserve, while the Mezcalapan River showed the lowest value. The Centla Biosphere Reserve is a large extension of wetland (302 706 ha) with an official management plan and is considered as terrestrial priority area for Mexico (see references in Guerra-Martínez and Ochoa-Gaona, 2008). Even though some activities are authorized inside the Reserve (e.g., livestock, oil industry, fisheries), the conservation status should help to maintain adequate environmental conditions for numerous species limiting the reduction of population size, their isolation, and consequently curbing the erosion of genetic diversity (Keyghobadi, 2007). Furthermore, Tabasquillo River and Chochal Lagoon areas are under the protection of local communities limiting the access to these areas (permission required) which provide some level of protection to animal populations. Very few studies are focused on the population density of *D. mawii* (Barahona and Lopez, 2015; Calderon-Mandujano, 2017), particularly in Tabasco state (Zenteno Ruiz et al., 2010). Zenteno Ruiz et al. (2010) studied abundance of *D. mawii* in the three principal rivers of the Centla Biosphere Reserve, confirming the presence of the species in all rivers, with the highest density of individuals in the Tabasquillo River.

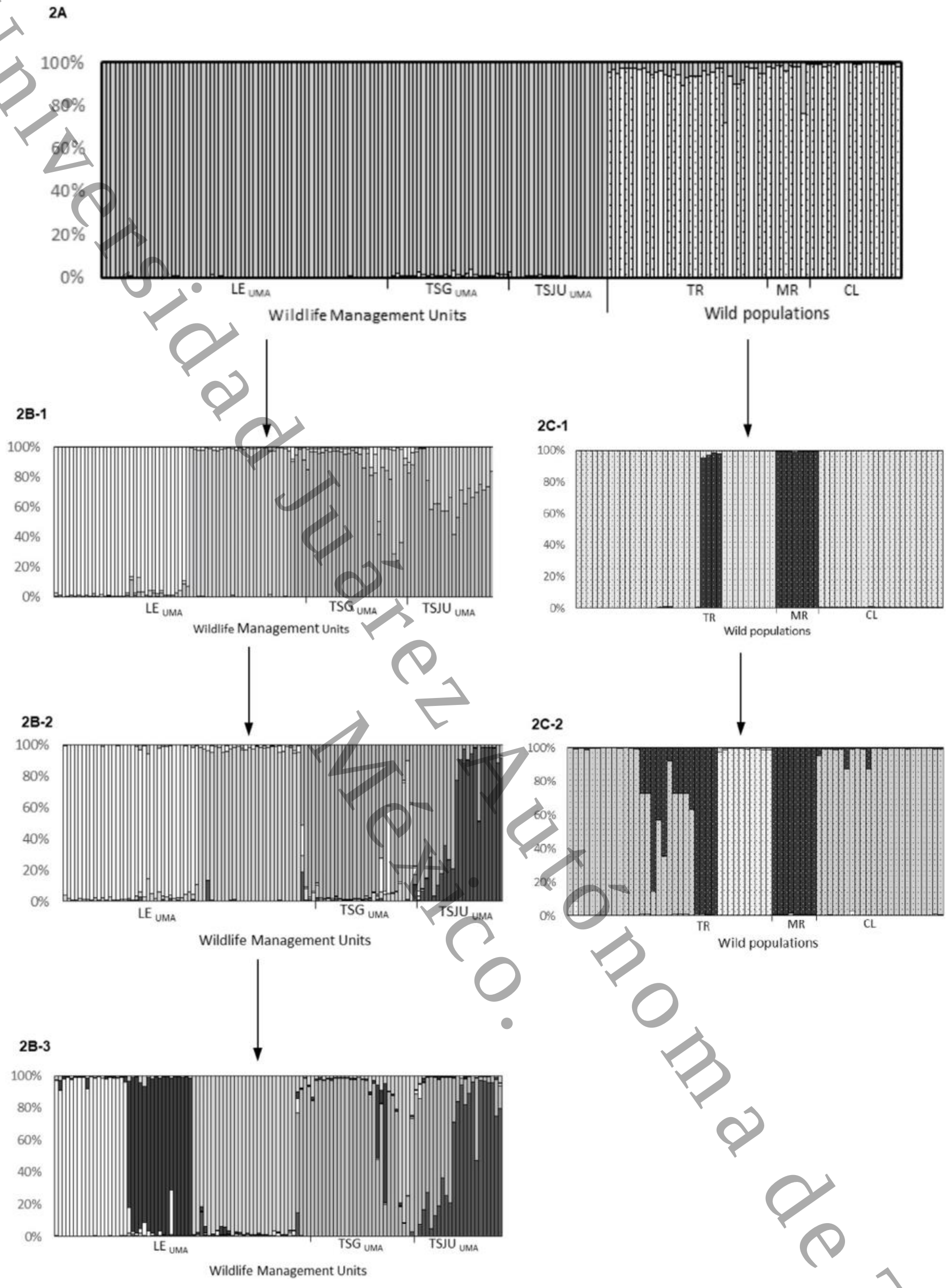

**Fig. 2**. Bayesian analysis for Dermatemys mawii in southeast Mexico computed by STRUCTURE 2.3.1. Each single vertical line represents an individual and its proportional membership probability among the K clusters. (A) results for K ¼ 2 considering all wildlife management unit (UMA) and wild population, (B1-3) results of the hierarchical STRUCTURE analysis for the three wildlife management units considering K from 3 to 5, (C1-2) results of the hierarchical STRUCTURE analysis for the three wild populations considering K ¼ 2 and K ¼ 3. For UMAs and localities abbreviations see Table 1.

They mentioned a higher level of conservation of *D. mawii* habitat in this river which provides better food resources, shelter, and nesting sites. This situation could explain the highest genetic diversity observed in the population of the Tabasquillo River. In contrast, the Mezcalapan River population is located in an area (region of Chontalpa) without particular protection, where habitat is subject to more disturbance (high rate of deforestation and the construction of several dams), contamination (Ramos-Herrera et al., 2012), and hunting for human consumption, all of which at could provoke a decrease in population size, increasing the erosion of genetic diversity and explaining observed low genetic diversity. However, we cannot reject the possible effect of sample size considering the low number of individuals collected in the Mezcalapan River. Nevertheless, Petre et al. (2015) obtained high value for genetic diversity ($H_e$¼ 0.743; Table 1) in the Sabine locality, where only 8 individuals were captured. To confirm the positive effect of the Biosphere Reserve on genetic diversity of *D. mawii*, we suggest an increase in both the size and number of point samples when collecting in different protected areas in southeast Mexico.

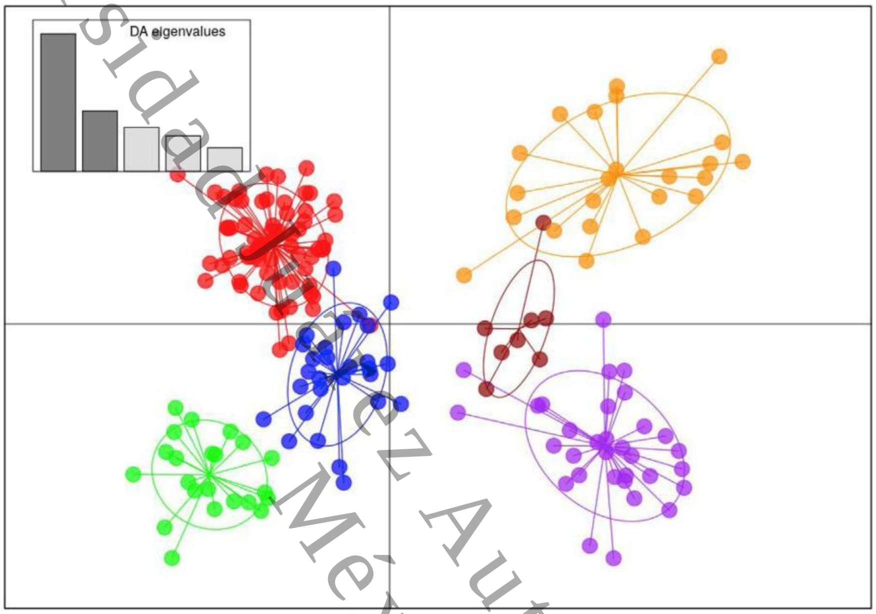

**Fig. 3.** Scatterplot of discriminant analysis of principal components (DAPC) for wildlife management unit (UMA) and wild populations of Dermatemys mawii in southeast Mexico obtained by adegenet package for R software. Colors represent the six clusters: La Encantada UMA (red), Tabasco State Government UMA (blue), Tabasco State Juárez University UMA (green), Tabasquillo River (purple), Mezcalapan River (brown), and Chochal Lagoon (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The global genetic structure analysis showed a clear separation between wild and captive populations of *D. mawii*, suggesting different geographical origins of founding individuals of UMAs (e.g., founders $TSG_{UMA}$ originate from a river close to the border with Guatemala; Dr. RC Vogt, personal communication) when compared to the wild populations used in our study. Also, this clear separation reflected the translocations that took place between UMAs, resulting in the homogenization of the genetic pool among UMAs in this region (Bayesian analysis with K ¼ 2, and DAPC analysis). The lowest values for genetic differentiation (Wright F-Statistic) were observed between the UMAs and the Tabasquillo River (TR) locality. This is not surprising since the Pantanos de Centla Biosphere Reserve is where PROFEPA operations are carried out to confiscate illegally caught individuals (PROFEPA, 2008, 2014, 2015a; 2015b); once confiscated, they are handed over to authorized UMAs. The hierarchical analysis of Structure (Bayesian analysis) and the Wright F-Statistic among UMAs probably reflects the origin of individuals: (1) “La Encantada” UMA showed the two different origin of individuals (white and light grey in Figs. 2Be1), (2) the translocation of individuals from $TSG_{UMA}$ to $TSJU_{UMA}$ UMAs (dark grey in Figs. 2Be1) is evident, and (3) lower values of $F_{ST}$ are between the two more recent UMAs ($LE_{UMA}$ and $TSJU_{UMA}$) and the older one ($TSG_{UMA}$), clearly reflecting that the two newer UMAs received individuals at the time of their establishment from the $TSG_{UMA}$. This assertion was confirmed by the owners of the UMAs and by Dr. Zenteno who participated in the founding of the UMAs. The genetic structure observed among wild populations analyzed in this study explains perfectly the geographic situation of these populations; the Wright F-Statistic are higher between the Mezcalapan River (MR) and the two other wild populations (Tabasquillo River and Chochal Lagoon) reflecting that this population is the most distant from the others. Of course, habitat fragmentation due to strong human disturbances in the Chontalpa region (Tudela,1992; Ramos-Herrera et al., 2012) could probably affect the connectivity of this more isolated population (MR) with others. Genetic isolation due to habitat fragmentation has been reported in other freshwater turtles such as Mesoclemmys dahli Zangerl & Medem 1958 (Testudines, Chelidae) (Gallego-García et al., 2018). Interestingly, Bayesian analysis showed that the Tabasquillo River has three genetic profiles while the other two localities presented only one genetic profile each. This could be explained because the Tabasquillo River area (Centla) is the confluence zone of the River Grijalva and River Usumacinta, where there is a high degree of connectivity with other populations along a vast area of rivers and drainage basins. The unique genetic profile observed in the two other populations could be a result of closed and more isolated populations.

Although *D. mawii* is an exclusive freshwater turtle listed as critically endangered, few studies have been conducted on its ecology, reproduction, abundance, and even less on population genetics. Generally, studies are focused on a specific region, and only Gonzalez-Porter et al. (2011, 2013) evaluated genetic structure over the entire range of distribution (Mexico, Belize, and Guatemala). Our study is the first that considers the genetic characteristic of captive *D. mawii* individuals and compares it with wild populations. Our results, using nuclear markers, permit conclusions about contemporary events (e.g., fragmentation, human consumption, contamination) and have allowed us to establish a data baseline for the development of further studies. Considering our genetic results associated with selected founder individuals, we recommend the progressive development of more captive turtle centers of *D. mawii* throughout its distribution range and integrating a genetic management plan to optimize genetic diversity of individuals and limiting possible inbreeding depression. These centers would have a variety of functions such as education, research, sale for human consumption, among others. Furthermore, we propose the elaboration of potential distribution maps over the entire distribution range in order to plan reintroduction of individuals from captive centers to new potential areas. These reintroductions could improve connectivity among local populations throughout river systems and then restore population size and genetic diversity, therefore limiting the risk of local extinction. Without doubt, although outcrossing offers a solution for genetic rescue, we cannot ignore the risk of the outbreeding depression effect. However, recent work by Frankham (2016) demonstrated the beneficial effect on descendant fitness (to at least F3 generation) after outcrossing among inbreed populations. Furthermore, several studies have confirmed the successful translocation/reintroduction of individuals to rescue populations with low genetic diversity (Xia et al., 2014; Michaelides et al., 2015; Ochoa et al., 2016; Pelletier et al., 2017). However, the possibility of reintroduction from captive to wild populations needs to be analyzed with caution, considering for example the decision tree proposed by Frankham et al. (2011), using a larger number of microsatellites or other more precise genetic markers such as genome-wide markers (He et al., 2016), undertaking trial breeding among captive and wild populations to evaluate the potential effect on reproductive success (Slade et al., 2014), and establishing a genetic monitoring program for reintroduced and wild populations among others. Recently, Malone et al. (2018) proposed an integrative approach to optimize the successful of reintroduction which could serve as a model for future reintroduction programs. Finally, when the management of critical endangered species involves several countries, international collaboration is vital. Furthermore, interdisciplinary programs that study different aspects of *D. mawii* populations (e.g., habitat quality, impact of water contamination on individuals, genetic diversity, among others) over the entire range of its distribution (Mexico, Belize, Guatemala) will be fundamental for the successful implementation of species conservation management.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00616.

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Results: Our results have led to the proposal of three breeding groups: (1) conservation breeding group, made up of 16 females and 7 males that are unrelated and with a lower level of homozygosity (< 0.4); (2) research breeding group consisting of 45 females and 16 males that are unrelated or half-sibling relationship and with a medium level of homozygosity (<0.6); and (3) a sustainable breeding group made up of 29 females and 4 males not necessary unrelated and with a high level of homozygosity (> 0.6).

Conclusion: The UMAs can create 3 breeding groups with different goals: 1) species conservation, 2) species research, and 3) sustainable use of species.

Implications for Conservation: Our proposal can enrich the conservation actions and sustainable use for *D. mawii* at a national and international level, specifically within the Mesoamerican corridor.

# Genetic relationship between founders of a threatened freshwater turtle in Mexican Wildlife Management Unit. A conservation strategy.

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# Abstract

**Background and Research Aims**: For the captive breeding of endangered freshwater turtles, a genetic management plan must be established, to maintain genetic variability. In Mexico, freshwater turtles are reproduced within wildlife management units (UMA for its Spanish acronym) whose function is conservation and sustainable management. Particularly, in southeastern Mexico, *Dermatemys mawii*, a critically endangered turtle has been reproduced in UMAs, however, UMAs do not have a genetic management plan. Consequently, our work proposes to determine the kinship relationship and homozygosity by loci index of the founders in three UMAs dedicated to the reproduction of *D. mawii* for the establishment of breeding groups.

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## Introduction

Captive breeding programs are widely used to recover endangered species and could help to reduce anthropogenic threats which are difficult to address directly (Spencer et al., 2017). These conservation programs aim to increase the population size of the threatened species in captivity, with the final goal of reintroducing individuals and establishing self-sustaining populations in the wild (Miller et al., 2018). Consequently, these programs require a management plan for their captive colonies which should consider all the ecological and biological requirements necessary for its success (Prayaga & Reverter, 2007). Additionally, the genetic management plan must consider and minimize the risks due to breeding in captivity, such as inbreeding depression, fixation of deleterious alleles due to genetic drift, loss of genetic diversity, and adaptation to captivity (Frankham, 2008). These management plans should preserve 90% of the original heterozygosity for at least 100 years under the conservation strategy (Ballou et al., 2010; Frankham et al., 2010). To minimize the risks and optimize genetic variability in captivity, the genetic management program must consider the appropriate reproductive groups through the knowledge of kinship relationships among captive individuals (Miller et al., 2018). Consequently, the use of genetic information (e.g., genetic diversity, origin, kinship) in the management plan could be the key factor for success within (Alacs et al., 2007) Wildlife Management Units.

Today, many species of turtles, especially freshwater ones, are in danger of extinction due to different human activities such as pollution, habitat fragmentation, as well as excessive hunting (Kanwal & Kahn, 2018; Stanford et al., 2020). Therefore, several Management Units have been created as a conservation strategy for this group of vertebrates (Stanford et al., 2020, He et al., 2010). For example, in Thailand different programs were developed for several freshwater turtles with varying degrees of success (Van Dijk & Palasuwan, 2000). Furthermore, it is important that Management Units implement a genetic management program to optimize the chance of success, particularly for breeding selection (Fienieg & Galbusera, 2013) for turtle management (Alacs et al., 2007; Williams & Osentoski, 2007).

In Mexico, freshwater turtles have been bred in captivity since the 70´s; but, since 1997, captivity breeding programs take place at sites called Wildlife Management Units (UMAs, by its Spanish acronym; Recino-Reyes et al., 2020). UMAs constitute a very important tool for conservation in Mexico, and promote alternative schemes for a rational, orderly, and planned use of wildlife and allow conservation management (Pineda-Vazquez et al., 2019). Until 2017, a total of 11,722 UMAs had been recorded in Mexico but only 94 are dedicated to the reproduction of freshwater turtles and several of them are in the southeast region of the country (SEMARNAT, 2018; Dirección General de Vida Silvestre & SEMARNAT, 2018). These UMAs have successfully reproduced some chelonians species such as *Trachemys spp* Agassiz, 1857 (Testudines, Emydidae), *Claudius angustatus* Cope 1865 (Testudines, Kinosternidae), *Staurotypus triporcatus* Wiegmann 1828 (Testudines, Kinosternidae), and *Dermatemys mawii* Gray, 1847 (Testudines, Dermatemydidae) (Reynoso et al., 2016; SEMARNAT, 2014).

The Central American river turtle, *D. mawii* is an important species for conservation. This species is the last representative of the Dermatemydidae and is endemic to Mesoamerica with a distribution range from the southeast of Mexico to Belize and Guatemala (Vogth et al., 2011). The *D. mawii* wild populations have been hunted, illegally trafficked, and their habitat has become so fragmented that their natural populations have greatly decreased during the last few decades (SEMARNAT, 2014; Calderón-Mandunajo et al., 2017; Jennings, 2020). Therefore, this Chelonia species is considered among the 25 most threatened species of turtles in the world (Turtle Conservation Coalition, 2018). It is classified as "critically endangered" by the IUCN Red List, included in the Appendix II of CITES, and it is classified as "Threatened" in Mexico by the Norma Oficial Mexicana (SEMARNAT, 2010; Macip-Ríos et al., 2015). Furthermore, *D. mawii* is an important freshwater turtle for sustainable use as it is used as pet, it represents an important natural economic resource for people who reproduce them in captivity (principally for food, musical instruments, craftwork) and it has played a historical role in culture and gastronomy since Mayan times (Guevara-Chumacero, et al., 2016; Sharpe et al., 2020). Consequently, great efforts have been made to develop the appropriate protocol for their reproduction in UMAs and from the 94 UMAs of Mexico that manage freshwater turtles, 37 are dedicated to reproducing *D. mawii* of which 17 are in Tabasco state. These UMAs have been able to successfully reproduce *D. mawii* in captivity.

The principal goals of *D. mawii* UMAs have been to protect this turtle from over-exploitation and reinforcement of its wild populations (Pineda-Vazquez et al., 2019). UMAs in Mexico are authorized only for native species and for their development; technical assistance and infrastructure are implemented, technicians are trained, acquisition of a breeding colony is facilitated, and scientific studies on the species are supported (Avila-Foucat & Pérez-Campuzano, 2015). However, these UMAs present several problems which are principally related to operational and administrative management (García-Garduño et al., 2017). Other important problems are that UMAs do not have a genetic management plan for reproduction in captivity, and generally they do not know the geographical origin of specimens; consequently, UMAs ignore the genetic diversity, the genotype, and the relationship among their founding breeders. Only one study has been carried out in UMAs of *D. mawii* and the authors report an observed difference in heterozygosity between UMAs; however, these sites have a higher genetic diversity than wild populations (Gallardo-Alvarez et al., 2019). The gap of genetic information in breeding programs could cause inbreeding or outbreeding depression when different UMAs exchange or donate individuals to increase the number of breeders (Williams & Osentoski, 2007; Zarza et al., 2016). These problems can have an impact on the success of the UMAs. We can reasonably believe that the lack of genetic study in UMAs at the time of their establishment is due to the limited and onerous use of molecular tools; the consideration of genetic information in captive management programs is relatively recent and requires technical and financial support that is not always available in México (Torres-Florez et al., 2018).

The development of a genetic management plan in the UMAs of *D. mawii* is fundamental because it is necessary to comply with reintroduction protocols established by the IUCN/SSC (IUCN/SSC; 2014) which indicate that reintroduced individuals must provide adequate genetic diversity and that captive individuals should be from populations with appropriate demographic, genetic, welfare and health management, and behavior. Because *D. mawii* has small and isolated wild populations (Briggs-Gonzalez et al., 2019), it is necessary to ensure that captive individuals of *D. mawii* that will be reintroduced enrich the genetic diversity of the wildlife populations, which will allow them to better adapt to environmental and climatic changes (Refsnider & Janzen, 2016).

Considering the importance of the genetic information within management plans in Mexican UMAs of *D. mawii*, we propose for the first time to establish a genetic management plan for the founder population in three UMAs in the southeast of Mexico. More precisely, the following questions are addressed: (1) Determine homozygosity level in the founder population, (2) Determine the kinship relationship among founders for each UMA, (3) Propose breeding groups for specific purposes as conservation, research, and sustainable use, and finally (4) our results will be discussed in a larger context of conservation and management of the species in its geographical distribution range.

## Methods

### *Wildlife Management Units (UMAs)*

Skin samples were collected only from founder individuals from three UMAs dedicated to reproduction of *D. mawii* located in Tabasco state, Mexico (Figure 1): **(1)** "La Encantada" (LE) located in town Jalpa de Méndez (18°20'09''N-93°02'53''W; N = 67), **(2)** Tabasco State Government UMA (TSG) located in town Nacajuca (18°11'23''N-92°59'37''W; N = 28), and **(3)** Tabasco State Juárez University UMA (TSJU) located in Villahermosa (17°59'26''N-92°58'16''W; N = 22). The capture permit SGPA/DGVS/011,085/16 was provided through the Secretariat of Environment and Natural Resources (SERMARNAT) of Mexico. Characteristics of UMAs can be consulted at Gallardo et al. (Gallardo-Alvarez et al., 2019). We should clarify that UMAs selected for this study have achieved theirs aims of reproduction, environmental education, and research, but to date, they have not carried out translocation for population restoration of *D. mawii*. The aims of UMA are included in article 39 of the General Law of Wildlife in México (Oficial de la Federación Diario, 2000).

## *Sample collection*

Skin samples were collected during 2017 by cutting 1 cm of tissue from the interdigital membrane of the legs. Before taking samples, cutting skin area was disinfected with 70% alcohol and an antiseptic (methylthioninium chloride) was applied to prevent infection. Founders were identified using the numerical marking assigned in each UMA and captured using a trammel net. After sample collection, all individuals were subject to observation for a period of 2h after that they were released to their respective ponds. Skin samples were preserved in a salt-saturated solution (250 mM EDTA pH 7.5 and 20% DMSO) and stored at -80°C until DNA extraction (Gonzalez-Porter et al., 2011). The procedures for capturing individuals, sampling, transporting and ethical procedures in this study were those recommended by the guide for the management of amphibians and living reptiles of the American Society of Ichthyologists and Herpetologists (American Society of Ichthyologists and Herpetologists, 2004), and by the management plan for *Dermatemys* (SEMARNAT, 2014).

## *DNA extraction and microsatellite genotyping*

DNA was extracted with Qiagen DNeasy Tissue kit (Qiagen, Inc., Valencia, CA). DNA quality and concentration were verified using a UV-Vis spectrophotometer (Thermo ScientificTM NanoDropTM One). We used ten microsatellite' loci for genotyping all founder individuals; seven of them previously used (Andree et al., 2010; Gonzalez-Porter et al., 2013), and three microsatellite primers (Dm4A-11, Dm3A-43 and Dm3A-61) were designed for the *D. mawii* study from the sequences reported in GenBank (González-Porter et al., unpublished). Detailed laboratory procedures and microsatellite information were widely described in Gallardo et al. (Gallardo et al., 2019).

## *Data analyses*

### *Kinship analysis*

The pedigree relationships between founder individuals were evaluated using the ML-RELATE program (Kalinowski et al., 2007) which calculates by default the strongest relationships (highest likelihood). For each pair of individuals, the following kinship relationships were determined: unrelated (U), half-siblings (HS), full-siblings (FS), and parents-offspring (PO). Each relationship category is assigned based on a test (statistical test and simulations) that determines which relations are consistent with the data (confidence set option: 0.05 level of significance and 1000 simulations) (Kalinowski et al., 2007). Simultaneously, ML-RELATE provides a list of several possible relationships (the putative corresponding to the highest likelihood, and the alternatives), therefore, we used the likelihood ratio test (1000 simulations; specific hypotheses test option) proposed by ML-RELATE to obtain a *P* value and confirm the kinship relationships originally suggested. If *P* was small ($P < 0.05$) the alternative hypothesis was rejected, and the highest relationship maintained as proposed by the highest likelihood. If the *P* value was large ($P > 0.05$), this indicated that putative and alternative relationships were consistent with the data, so the lowest relationship was selected (U < HS < FS < PO) (Kalinowski et al., 2007). Furthermore, we calculated homozygosity by loci (*HL*) using STORM PROGRAM (Frasier, 2008) homozygosity index that weighs the contribution of each locus depending on their allelic variability with values that range from 0 (all loci are heterozygous) to 1 (all loci are homozygous) (Aparicio et al., 2006).

*Formation of breeder groups*

To develop the genetic management plan for *D. mawii* in Mexican UMAs, three important considerations have been taken into account: 1) UMAs have all founder individuals in the same ponds, 2) possibility to consider translocation between UMAs if no adequate pairs of breeders are determined, and 3) the polyandry mating system favoring the occurrence of multiple paternity (Fantin et al.; 2018). The design of our genetic management plan was based on the level of kinship between males and females. The level of homozygosity of the reproducing individuals was considered as maintaining a high level of heterozygosity between founders is important to ensure sufficient genetic variation. (Williams & Osentoski, 2007). With this information, we propose the formation of three breeder groups that could meet some specific purposes. **(1)** breeder group for conservation with the objective to conserve, reintroduce, and share individuals with other UMA. For this group, we selected only females and males with an unrelated kinship relationship (U), and individuals that present a low value of homozygosity (*HL* index ≤ 0.4); **(2)** breeder group for scientific research with the objective to maintain genetically valuable individuals with the possibility of transfer to the "conservation breeder group", or translocation to other UMAs to enrich genetic diversity, and to produce individuals for scientific purposes. In this breeder group, we selected females and males with unrelated (U) or half-sibling (HS) kinship relationship and with a *HL* index ≤ 0.6; finally, **(3)** breeder group for sustainable uses with an economical aim that consists of producing individuals for pets or traditional gastronomy, among others (Pineda-Vazquez et al., 2019). In this group, we include all other individuals.

Finally, considering that each UMA has the possibility to host only one breeding group according to its types of facilities, we propose that each UMA only hosted one breeding group. The breeder group for conservation purpose could be hosted in the TSG UMA, the group for scientific research in the TSJU UMA, and the group for sustainable use in the LE UMA.

## Results

Genotyping error rates could be considered as low (average allelic dropout rate of 2.7% and mean false allele rate of 9.1; see 30). A total of 117 founder individuals' genotypes were analyzed from the three UMAs (90 females and 27 males). We can observe that all UMA showed a deficit in females, particularly La Encantada (Figure 2).

### *Kinship analysis*

A total of 2,430 female-male dyads have been obtained from all genotypes analyzed and the mean *HL* index value was 0.46 (min: 0 and max: 1). Kinship analysis indicated that 2,220 female-male pairs were unrelated (Figure 3). Three female-male pairs have a PO relationship when all UMAs were considered. All pedigree relationships female-male pairs and *HL* index values are presented in Table 1.

A detailed analysis per UMA show that the UMA LE has a total of 522 female-male pairs. Their pedigree relationships are the following, 442 U (85% of the dyads), 51 HS (10% of the dyads), and 29 FS (5% of the dyads). The *HL* index values range from 0.00 to 0.76 with a mean value of 0.365. The UMA TSG has a total of 192 female-male pairs. Their pedigree relationships are the following, 148 U (77% of the dyads), 29 HS (15% of the dyads), and 15 FS (8% of the dyads). The *HL* index values range from 0.34 to 1 with a mean value of 0.547. Finally, the UMA TSJU has a total of 96 female-male pairs. Their pedigree relationships are the following, 63 U (66% of the dyads), 21 HS (22% of the dyads), and 12 FS (12% of the dyads). The *HL* index values range from 0.44 to 1 with a mean value of 0.639.

## ***Selection of candidate breeders***

*Selection of candidate breeders for conservation.*

Because of the low number of males in UMAs, we first selected males with the *HL* index < 0.4, then we selected females presenting only unrelated relationship (*U*) with these males and an *HL* index < 0.4. A total of seven males were considered (five from LE, one from TSG, and one from TSJU) and 16 females (14 from LE and two from TSG) (individuals highlighted with the superscript a in Table 1).

*Selection of candidate breeders for* research

We selected all males with a *HL* index < 0.6 leading to a total number of 16 males (four from LE, 10 from TSG, and two from TSJU). Subsequently, we looked for all females with a *HL* index < 0.6 and with a *U* or *HS* relationship with those males. We selected a total of 45 females (36 from LE, three from TSG, and six from TSJU) (individuals highlighted with the superscript b in Table 1).

*Selection of candidate breeders for sustainable uses*

This group consists of principally individuals from TSG and TSJU UMAs which have the most homozygous individuals. In this group, we proposed four males (one from TSG and three from TSJU) and 29 females (eight from LE, 11 from TSG, and 10 from TSJU) (individuals highlighted with the superscript c in Table 1).

## Discussion

Captive breeding programs are widely used and recommended for critically endangered, species and with molecular advances, it is possible to determine the kinship relationships of founders to establish good genetic management in captivity and maximize the genetic pool in the founding colony (IUCN/SSC, 2014; Mahood et al., 2021). In Mexico, this conservation strategy is achieved at sites called Management Units (Zenteno-Ruiz et al., 2021). These programs establish management strategies whose objectives are to obtain demographically self-sufficient and genetically healthy populations (Ivy & Lacy, 2012). It has been shown that the most effective way to obtain healthy genetics in captive breeding programs is to minimize the degree of kinship when proposing breeding pairs (Rudnick & Lacy, 2008) thus, molecular markers are highly useful to ascertain the relationship between individuals for breeding (Ivy & Lacy, 2010; Jiménez-Mena et al., 2016). The use of adequate breeding pairs (mean unrelated) minimizes the risk of inbreeding (Ivy & Lacy, 2010; Miller et al., 2010). In our research, we were able to determine the kinship relationships in *D. mawii* to establish genetic management in captivity, which is a new approach to conservation for UMAs in Mexico. Different captive breeding programs for endangered species of reptiles have used this strategy. For example, for the loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) (Sakaoka et al., 2012), the Siamese and saltwater crocodiles (*Crocodylus siamensis*, Schneider, 1801 and *C. porosus*, Schneider, 1801) (Lapbenjakul et al., 2017), and the Galapagos giant tortoise (*Chelonoidis spp*.) (Miller et al., 2018).

The captive reproduction of freshwater turtles has been implemented for commercial purposes (pet or human consumption) as well as for the recovery of populations of endangered species. For example, the red-eared slider (*Trachemys scripta elegans*, Wied-Neuwied, 1839) is reproduced exclusively in farms for commercial purposes (Mali et al., 2015). The Asian giant softshell turtle (*Pelochelys cantorii*, Gray, 1864), a critically endangered species, has been part of a recovery project since 1989 that considers the monitoring of wild populations within natural reserves in China, as well as in units management (Xiaoyou et al., 2019). Therefore, due to the economic and ecological importance that this group of reptiles represent, it is important to begin to implement genetic management considerations in captive breeding programs so that the objective of healthy genetics is met

More specifically, the captive reproduction of *D. mawii* in southeastern Mexico in UMAs has been successful with births in captivity (Vogt et al., 2011). However, genetic analysis to identify the kinship relationship of reproductive individuals has not been implemented. For this reason, the offspring born in UMAs, have been used only for commercial purposes (Dirección General de Vida Silvestre & SEMARNAT, 2018). Here, we present the first study considering the genetic relationship to conform breeding groups in UMAs.

Our results indicate that most individuals in TSG and TSJU have *HL* values close to 1, so, they present low level of heterozygosity (Aparicio et al., 2006). We can hypothesize that the geographical origin of the founders may explain these results. The owners of the TSG UMA mention that their founding colony probably comes from a single wild population near to Guatemala (Gallardo et al., 2019) increasing the risk of related individuals in founders. This information, together with our results, suggests that the wild population could have been small. The records suggest that *D. mawii* is almost extirpated in Mexico and it is exceedingly rare and difficult to capture (Vogt & Flores-Villela, 1992; Morales & Suarez, 2010). For example, in Rio Hondo, Quintana Roo, it has been reported an average of 1.74 turtles/net into 2009 and 2010 (Calderón-Mandujano et al., 2017). In Tabasco, in the Northeastern portion of Pantanos de Centla Biosphere Reserve (RBPC), an average of 0.312 turtles/net was reported in the Tabasquillo river and 0.041 turtles/net in the Grijalva river during the dry season while during the rainy season, the average was 0.500 turtles/net in the Tabasquillo river, 0.083 turtles/net in the Grijalva river and 0.041 turtles/net in the Usumacinta River (Zenteno-Ruiz et al., 2010). In addition, the kinship relationships observed among the founder individuals in the TSG UMA could be a reflection of the social behavior generally present in the freshwater turtles. Freshwater turtles show a fidelity to the nesting site (nest-site fidelity) and juvenile individuals have less extensive movements than adults over several years (Rowe et al., 2005; Sheridan et al., 2010). For example, it has been reported that some species, such as the European pond turtle (*Emys orbicularis*, Linnaeus, 1758), can remain in the same distribution area for a long period of time (31 years) (Escoriza et al., 2020). Similarly, in a population of Eastern Box Turtles (*Terrapene carolina carolina*, Linnaeus, 1758), several of the captured individuals show a high level of relationship (Moore et al., 2020). Therefore, our results in the TSG UMA suggest that founder individuals came from a small population with common parents and therefore, the founding colony in this UMA have a high proportion of HS and FS relationship. Another reason to explain these results is the translocation of individuals between the UMAs. TSG UMA was the first UMA in Mexico to reproduce *D. mawii* and its offspring have served as a founding batch for other UMAs such as TSJU and LE. Almost all of the founder individuals from TSJU originate from TSG and the individuals donated present a high proportion of FS or HS relationship because they share parents. Furthermore, the owners of LE UMA comment that their founding colony not only comes from individuals donated by the TSG UMA, but also from wildlife individuals; this can explain the lower level of related individuals in this UMA. In contrast, 48 individuals have HL values close to 0, so, they present a higher level of heterozygosity (Aparicio et al., 2006). The identification of these individuals is crucial from a genetic management point of view, as they could constitute the breeder group for conservation and increase the reproductive success in captivity. The theory of the advantage of the heterozygous individual indicates that individuals with higher heterozygosity, in relation to those of the same sex, have greater aptitude (Brown, 1997; Garcia-Navas et al., 2009). Furthermore, it has been proposed that females show preference for less inbred males (Pilakouta & Smiseth, 2017) and that these selected males could pass genes that will increase the survival or reproductive success of her offspring, which could increase offspring genetic diversity and reduce deleterious recessive allele expression (Cutrera et al., 2012; Fan et al., 2021). The presence of "good genes" in males is very important, because in species with polyandry reproduction such as turtles, the sperm is subject to sperm competition, so the female will have selection mechanisms (such as keeping or expelling sperm); so that only seminal material that the female detects as adequate fertilizes the eggs (Firman et al., 2017).

The number and genetics of founder individuals are a key factor for the success of any breeding program (Miller et al., 2010; Willoughby et al., 2017). Many wildlife management officials consider that the number of founder individuals should represent almost 95% of the genetic diversity in the wild and as a rule, it has been proposed that an initial number of 20 or 30 founder individuals will normally ensure that captivity programs preserve 90% of the original genetic diversity for 200 years (Foose et al., 1986; Soulé et al., 1986). However, determining the number of suitable founders can be very difficult and complex, especially if there are no more animals to capture in the wild or the facilities at the farms are too small to increase the captive population (Willis & Willis, 2010). Sometimes, turtle captive breeding programs s start with few individuals as for example with the successful reproductive program of the Hood Island giant tortoise (*Chelonoidis hoodensis*, Van Denburgh, 1907) endemic to the Española Island. This program began with an initial population of 15 individuals (12 females and 3 males), and in 60 years, it has produced 2000 tortoises which have been released on the Española Island of Galápagos (Miller et al., 2018; Gibbs et al., 2014). Another successful program is the Batagur Project which aims to recover the *Batagur baska* (Gray, 1830) a freshwater turtle. This project began with 14 males and 6 females, and the first offspring obtained were in 2012 (Weissenbacher et al., 2015; Spitzweg et al., 2018). Similarly, the project to recover the critically endangered Burmese roofed turtle (*Batagur trivittata*, Duméril & Bibron, 1835), began with 22 adult individuals in 2002 and for the 2014-2015 nesting season yielded 53 hatchling turtles (Çilingir et al., 2017, https://myanmar.wcs.org). Considering these backgrounds, we can consider that a conservation captive breeding program for threatened freshwater turtles can be initiated with a small colony and it can be successful in hatchling production and reintroduction into the wild. Therefore, we propose the captive breeding of *D. mawii*, throughout its distribution range, to help recover their wild populations. Also, we suggest that the captive breeding of *D. mawii* includes the genetic management of the species, based on determining the kinship relationship between the founding individuals. Many captive management programs use a pedigree-based mean kinship (MK) strategy for the breeding of individuals that are least related and maintain long-term genetic diversity (Lacy, 1994). Moreover, if the pedigree is unknown, the UMA managers use molecular markers to estimate the genetic relationship among the founders, to identify or prevent the effect of inbreeding on their descendants (Hogg et al., 2019).

Similarly, this study will be the first to propose genetic management for *D. mawii* in captivity. Today, in Belize, The Hicatee Conservation and Research Center (HCRC; http://hicatee.org/hcrc/), is a project to recover *D. mawii* in captivity which began in 2014 from a colony with 22 individuals. In 2015, the HCRC had their first offspring born in captivity and currently their founding colony is made up of 45 individuals (28 females and 17 males) (Briggs-Gonzalez et al., 2019). However, it is not known if the HCRC project uses genetic analysis to establish mating among breeders and monitoring the offspring genetic diversity. In Guatemala, there is no documented captive conservation program for the Central American river turtle, but it is believed to be home to the country's largest remaining population; it is estimated that over 4,000 turtles exist in the lowland Maya Biosphere Reserve in Guatemala (García-Anleu et al., 2007). Finally, in Mexico, the conservation of *D. mawii* is carried out under the political system called UMA, which also includes objectives for a sustainable use of this freshwater turtle (Zenteno-Ruíz et al., 2019). Therefore, our results could contribute to conservation actions and programs for *D. mawii* since our study addresses the problem from a genetic perspective. In Mexico, the UMA of *D. mawii* have been created without considering the genetics of its founding colony, so our study shows the importance of a molecular approach in the captive program of freshwater turtles and could be a guide to enrich the captive management program already established in UMAs.

In the UMAs where the present research was carried out, we recommend the creation of 3 breeding groups with different goals: 1) species conservation, 2) species research, and 3) sustainable use of species. This proposal is based on the following arguments: **(1)** This strategy can decrease the expression of recessive deleterious alleles due to the increase of less related individuals for reproduction. Our results indicate that each UMA has few unrelated female-male pairs and with adequate heterozygosity. This situation increases the possibility of inbreeding in each UMA leading to some problems such as inbreeding depression in future generations; **(2)** A genetic rescue (GR) is promoted, which is defined as an increase in population fitness (growth) due to the immigration of new alleles (Ingvarsson, 2001). For management and conservation, GR is especially useful because it focuses on restoring genetic diversity and increasing fitness in small populations that are isolated and typically, but not necessarily, suffering from inbreeding effects (Whiteley et al., 2015). GR has been implemented in fragmented or isolated wild populations, leading to beneficial results (increased fitness) (Frankham, 2015). Different programs have demonstrated the importance of GR in captivity or for reintroduction projects (Hasselgren et al., 2021; Hedrick & Fredrickson, 2010). Thus, our recommendation could promote GR, allowing an interaction among unrelated founder individuals, increasing new allelic combinations and offspring that descend from parents with greater genetic diversity; **(3)** Support UMA administration to define their specific goals. In Mexico, UMA management plans propose combining conservation and sustainable use goals at the same time. In Tabasco, turtle UMAs have different degrees of development, and different levels of operational and productive consolidation (Zenteno-Ruiz et al., 2021), so, our proposal could help them define more specific goals according to their interests (commercial, conservation or both); **(4)** Also, we believe that the cooperation between the three UMAs could be advantageous. Therefore, it is essential that the heads of the UMAs reach an agreement which satisfies the needs of these UMAs and allows the exchange of the reproducers.

We suggest the following actions: 1) The LE UMA primary goal is production to obtain economic benefits. We suggest that the owners are linked with the corresponding market, so that they can commercialize their products obtained from the individuals they have destined for use. In the same way, because this UMA has females with the highest level of genetic diversity, we suggest an exchange or donation with TSG and TSJU UMAs, and that the 37 females identified for sustainable purposes may be on this site; 2) The TSG UMA has the main function of donating individuals to serve breeders in other UMAs, therefore, we suggest that the 32 individuals join this site to form a research group. In the same way, this UMA can lend and exchange its males to the TSG UMA, so that these UMAs have a more balanced sex ratio. It is important to note that the research group is made up mostly of individuals who have low heterozygosity but are not related. Therefore, it is possible to produce individuals with high genetics because the advantage of dissimilar mate theory proposes that reproduction between genetically different individuals can maximize heterozygosity and fitness of their offspring (Landry et al., 2001) 3) The TSJU UMA main goal is conservation, therefore, we suggest that the conservation breeding group be managed by this UMA as it has the facilities and scientific specialists in freshwater chelonians. Likewise, TSJU UMA must work collaboratively with TSG UMA to obtain other allelic combinations from the research breeding group.

## Implications for Conservation

Our study proposes, for the first time, a genetic management plan in captivity, for *D. mawii* in Mexico. This plan is pioneer in the region and important because it contemplates establishing in the UMAs, three reproductive groups with specific objectives: conservation breeding group, research breeding group, and sustainable farming group. This genetic management strategy will allow a better reproduction of the species, avoiding inbreeding problems and the fixation of deleterious alleles in the captive colonies. Our proposal to create three breading groups with defined objectives can enrich the conservation actions and sustainable use for *D. mawii* at a national and international level. Furthermore, for optimum protection and management of *D. mawii*, the creation of a conservation program for this species in Mexico is required, which considers comprehensive in situ - ex situ management under the IUCN guidelines (IUCN/SSC, 2014). In the same sense, it has been recognized that there are natural sites in Mexico where *D. mawii* is still distributed, many of them close to UMAs, thus, our proposed genetic management will serve as a guideline to establish a genetic reintroduction and enrichment program between UMAs and the sites where *D. mawii* still inhabits. Countries such as Guatemala and Belize could be interested in joining efforts to undertake regional conservation actions. Therefore, our work also serves to strengthen the conservation efforts of the species within the Mesoamerican corridor (CONABIO, 2009; Zenteno-Ruiz et al., 2016).

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## Declaration of Conflicting Interests

The authors declare that they have no conflicting interests.

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## Data Accessibility Statement

Knowledge Network for Biocomplexity site, whit identifier urn:uuid:ca4fbe59-6b8c-46a9-8f52-84da38db1cd8, contains the R-values data file. The datasets analyzed for this study are available from the corresponding author on reasonable request.

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**Table 1.** Selection of *D. mawii* individuals to constitute breeding groups for UMAs in south Mexico.

## Figure legends

**Figure 1.** Study area in UMAs of *Dermatemys mawii* located in Southern Gulf of Mexico. Color points represent the UMA location. Photos credits: Lesher-Gordillo J.M., Zenteno-Ruiz C.E, Gallardo-Alvarez M.A.

**Figure 2.** Number of individuals breeders of Dermatemys mawii analyzed from three UMAs in southeast Gulf of Mexico.

**Figure 3.** Total number of female-male dyads for the different level of relatedness for Dermatemys mawii from three UMAs in southeast Mexico.

Figure 1. Study area in UMAs of Dermatemys mawii located in Southern Gulf of Mexico. Color points represent the UMA location. Photos credits: Lesher-Gordillo J.M., Zenteno-Ruiz C.E, Gallardo-Alvarez M.A.

137x97mm (300 x 300 DPI)

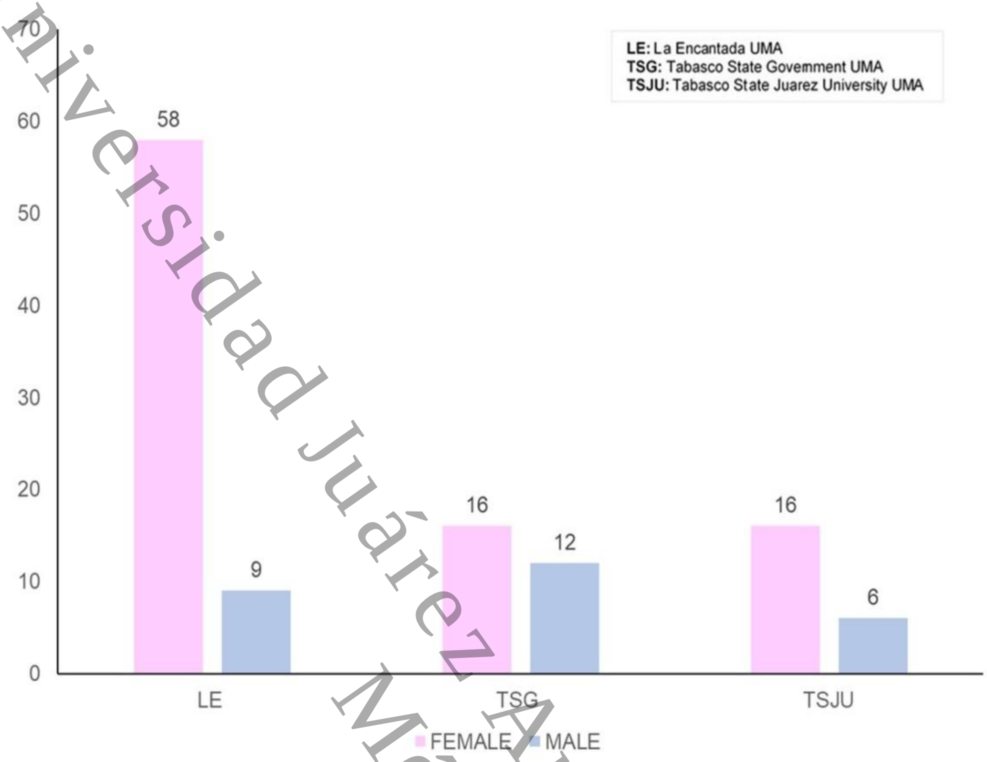

Figure 2. Number of individuals breeders of Dermatemys mawii analyzed from three UMAs in southeast Gulf of Mexico

104x74mm (300 x 300 DPI)

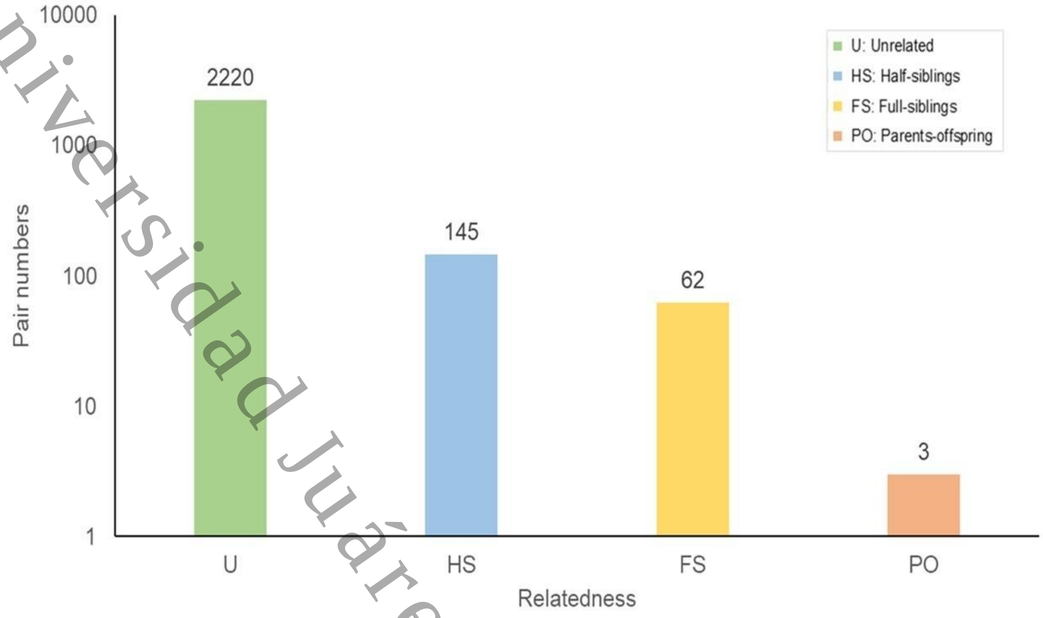

Figure 3. Total number of female-male dyads for the different level of relatedness for Dermatemys mawii from three UMAs in southeast Mexico.

107x61mm (300 x 300

Table 1. Selection of *D. mawii* individuals to constitute breeding groups for UMAs in south Mexico.

| | | | | Male | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **ID UMA** | [b]No ID | [b]205 | [a]207 | [b]149 | [a]No ID | [a]No ID | [b]158 | [a]No ID | [a]204 | [b]28 | [a]111 | [b]115 | [b]116 | [a]118 | [b]119 | [b]124 | 133 | [b]134 | [b]137 | [a]139 | [b]7001 | [a]141 | [a]101 | [b]PROFEPA | 23 | 108 | 10 |
| | | | **UMA** | LE | LE | LE | LE | LE | LE | LE | LE | LE | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSG | TSJ | TSJ | TSJ | TSJ | TSJ | TSJ |
| | **ID UMA** | **UMA** | ***HL*** | 0.58 | 0.42 | **0.31** | 0.47 | **0.20** | **0.28** | 0.52 | **0.30** | **0.28** | 0.57 | 0.55 | 0.49 | 0.49 | **0.38** | 0.56 | 0.58 | 0.68 | 0.48 | 0.50 | 0.57 | 0.43 | 0.44 | **0.20** | 0.48 | 1.00 | 0.73 | 0.78 |
| **Famale** | [b]145 | LE | 0.41 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]170 | LE | 0.42 | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]164 | LE | 0.42 | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]269 | LE | **0.24** | U | FS | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]193 | LE | **0.32** | U | HS | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]6 | LE | **0.37** | HS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]254 | LE | 0.44 | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]5 | LE | 0.65 | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]154 | LE | 0.42 | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]4 | LE | **0.38** | HS | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]185 | LE | **0.28** | FS | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]171 | LE | **0.34** | HS | FS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]152 | LE | **0.24** | HS | HS | FS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]181 | LE | 0.42 | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]No ID | LE | 0.49 | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]No ID | LE | **0.36** | U | U | U | HS | HS | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]No ID | LE | **0.38** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U |
| | [a]201 | LE | **0.38** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U |
| | [d]No ID | LE | **0.38** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]176 | LE | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]244 | LE | 0.41 | U | U | U | U | U | HS | U | HS | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]172 | LE | 0.41 | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]179 | LE | 0.76 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]3 | LE | **0.13** | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]219 | LE | 0.52 | U | U | U | U | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]197 | LE | **0.39** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]266 | LE | **0.32** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]No ID | LE | **0.31** | U | U | HS | FS | HS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [d]No ID | LE | **0.23** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]203 | LE | **0.31** | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]189 | LE | 0.46 | U | U | U | U | U | FS | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]153 | LE | 0.41 | U | U | U | U | U | U | U | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]253 | LE | **0.39** | U | U | U | U | U | HS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]No ID | LE | 0.41 | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | [b]237 | LE | 0.42 | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS | U | U | U |
| | [b]208 | LE | 0.48 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]264 | LE | **0.36** | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]183 | LE | 0.41 | U | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]160 | LE | **0.38** | U | U | U | U | U | FS | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]232 | LE | 0.58 | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [b]222 | LE | **0.33** | U | U | U | U | U | FS | HS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]No ID | LE | **0.34** | U | U | U | U | U | HS | U | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]241 | LE | **0.37** | U | U | U | U | U | U | HS | FS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]191 | LE | **0.28** | U | U | U | U | U | FS | U | FS | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]174 | LE | **0.35** | U | U | U | U | U | FS | HS | HS | HS | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]203 | LE | **0.25** | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [b]212 | LE | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [b]262 | LE | **0.22** | U | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [a]156 | LE | **0.37** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | FS | U | U | U | U | U | U | HS |
| | [b]No ID | LE | **0.37** | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [b]170 | LE | **0.11** | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | [c]No ID | LE | **0.26** | U | U | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | U | HS | U | PO | U | U | U | U | U | U | U |
| | [b]No ID | LE | 0.55 | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | HS | U | HS | U | U | U | U | U | U | U |
| | [c]206 | LE | **0.00** | U | HS | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | PO | U | U | U | U | U | U | U |
| | [a]157 | LE | **0.28** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [a]164 | LE | **0.18** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | U | U | U | U | PO | U | U |
| | [b]175 | LE | **0.22** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | HS | U | U |
| | [a]221 | LE | **0.38** | U | U | U | U | U | U | HS | U | U | U | U | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]24 | TSG | 0.57 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]42 | TSG | 0.41 | U | U | U | U | U | U | U | U | U | HS | HS | U | HS | FS | FS | U | U | HS | U | U | U | U | U | U | U | U | U |
| | [a]100 | TSG | **0.34** | U | U | U | U | U | U | U | U | U | HS | U | HS | HS | U | U | U | U | U | U | U | HS | HS | U | U | U | U | U |
| | [c]105 | TSG | 0.55 | U | U | U | U | U | U | U | U | U | U | FS | U | U | U | U | FS | U | U | U | U | U | U | U | U | U | U | U |
| | [c]112 | TSG | 0.59 | U | U | U | U | U | U | U | U | U | U | FS | U | HS | U | HS | FS | U | U | U | U | U | U | U | U | U | U | U |
| | [c]120 | TSG | 0.42 | U | U | U | U | U | U | HS | U | U | U | FS | U | HS | HS | FS | FS | U | U | U | U | U | U | U | U | U | U | U |
| | [c]122 | TSG | 0.85 | U | U | U | U | U | U | U | U | U | HS | FS | FS | HS | HS | U | HS | U | U | U | U | U | U | U | U | U | U | U |
| | [c]123 | TSG | 0.51 | U | U | U | U | U | U | U | U | U | U | FS | U | HS | U | HS | HS | U | HS | U | U | U | U | U | U | HS | U | U |
| | [c]125 | TSG | 0.56 | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | FS | U | U | U | U | U | U | U | HS | U | U | U |
| | [c]129 | TSG | 0.73 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]130 | TSG | 0.67 | FS | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U |
| | [c]131 | TSG | 1.00 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U |
| | [a]132 | TSG | **0.37** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U |
| | 136 | TSG | 0.46 | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | FS | FS | U | U | U | U | U | U | U | U | U |
| | [b]138 | TSG | 0.52 | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [b]140 | TSG | 0.51 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS | U | U | U | HS | U | HS |
| | [c]15 | TSJ | 0.54 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | HS | U | U | U | U | U |
| | [b]7 | TSJ | **0.32** | U | U | U | HS | FS | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | HS | U | U | U | U |
| | [b]103 | TSJ | **0.32** | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | HS | HS | U | U | U | U | HS | U | U | U | U |
| | [b]52 | TSJ | 0.48 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS | HS | U | U | U |
| | [b]4 | TSJ | **0.36** | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | HS | FS | U | U |
| | [c]PROFEPA | TSJ | 0.62 | U | U | U | U | U | U | U | U | U | FS | U | U | HS | HS | U | U | U | HS | U | U | HS | U | U | U | U | U | U |
| | [c]100 | TSJ | 0.76 | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | HS |
| | [c]1 | TSJ | 0.87 | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | HS | U | U | U | U | U | HS | FS | U | FS |
| | [b]13 | TSJ | 0.51 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | HS | HS |
| | [c]31 | TSJ | 1.00 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | FS | HS | HS |
| | [b]107 | TSJ | 0.85 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | [c]110 | TSJ | 0.86 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | FS | U |
| | [c]114 | TSJ | 0.63 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | FS | HS |
| | [c]128 | TSJ | 0.76 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | FS | FS | U |
| | [c]131 | TSJ | 0.85 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U | FS | HS | U |
| | [c]143 | TSJ | 0.71 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS | HS |

## Capítulo 4. Consideraciones finales

En México, la conservación de los quelonios empezó en la década de los 70, siendo en las especies marinas donde se desarrollaron los mayores esfuerzos de conservación. Estos esfuerzos han consistido en la protección de los nidos, o la translocación de los huevos a criaderos especializados, técnica que se conoce como “head-starting” o crianza controlada (García et al. 2003, Senko et al. 2011, Burke et al. 2015). No obstante; en las tortugas dulceacuícolas, no es viable utilizar estas estrategias debido a los altos costos que implica su implementación, además de que, muchas de las poblaciones de tortugas dulceacuícolas se encuentran desconectadas a nivel paisaje (Spencer et al. 2017). Por esta razón, en México, desde hace 42 años, para la conservación y uso sustentable de las tortugas dulceacuícolas, la crianza *ex situ* ha sido implementada, y hoy en día, esta estrategia se desarrolla en las UMAs. Por otro lado, se tiene registro que, la mayoría de las UMAs de tortugas dulceacuícola, se encuentran en el sureste del país, principalmente en el estado de Tabasco.

De tal manera, durante el desarrollo del presente estudio, se obtuvo información general sobre la situación de las UMAs de tortugas dulceacuícolas, en particular, de aquellas que reproducen a *D. mawii*. Los datos obtenidos permitieron concluir, que las UMAs que reproducen a la tortuga blanca, han tenido éxito en obtener crías bajo un manejo *ex situ*, las cuales han sido comercialidades a nivel nacional e internacional. También se encontró que estas mismas crías han sido utilizadas como pie de cría para fundar otras UMAs. Otro aspecto importante es que México cuenta para las UMAs, un manual de plan de manejo intensivo para *D. mawii,* el cual da las recomendaciones sobre cómo deben ser las instalaciones y el cuidado de la especie durante el *manejo ex situ*.

Sin embargo, una propuesta para un programa de manejo genético en las UMAs que reproducen a *D. mawii*, aun no se ha realizado. Por tal motivo, el presente estudio es relevante, debido a que es el primero en abordar este tema en las tres

UMAs más importantes dedicadas a la reproducción de la tortuga blanca dentro del estado de Tabasco.

Por consiguiente, para realizar nuestra propuesta de manejo genético, uno de los primeros aspectos abordados en el estudio fue determinar la diversidad genética de los individuos fundadores de las UMAs y comprar la información obtenida con individuos de vida silvestre procedentes del estado de Tabasco. Los resultados nos permiten concluir que los individuos fundadores poseen una mayor heterocigosidad que los individuos silvestres, no obstante, para ambos grupos, la heterocigosidad vario de mediana a baja. Además, se encontró que los individuos silvestres de *D. mawii* se encuentran genéticamente separados de los individuos fundadores. Por lo tanto, concluimos que los individuos fundadores en las UMAs de Tabasco posiblemente tienen un origen geográfico distinto y reflejan la diversidad genética de la población de donde proceden. Igualmente, se observó que no existe cuellos de botella recientes para las poblaciones silvestres analizadas. Pero, la disminución en el número de individuos en las poblaciones silvestres de *D. mawii* dentro del estado de Tabasco a causa de presiones humanas, han afectado su heterocigosidad. En el mismo sentido y de manera particular, se concluye que los individuos fundadores de la UMA “La Encantada” poseen una mayor heterocigosidad en comparación de las UMAs “CICEA” y del estado de Tabasco.

En otro punto, el segundo aspecto a evaluar en el presente estudio fue la relación de parentesco entre individuos fundadores. A partir de los datos obtenidos, concluimos que en las UMAs existe una proporción mayor de hembras que de machos. Además, al analizar en conjunto encontramos evidencia que solo en la UMA “La Encantada”, es posible formar grupos reproductores que cumplan con los criterios de tener parejas no estar relacionados y con una baja homocigosidad.

Por tal motivo, para nuestro programa de manejo genético, se propone crear tres grupos reproductores con objetivos específicos considerando a todos los individuos fundadores:

- El primer grupo de reproductores se propone para la conservación de la especie (grupo de conservación) y se encuentra integrado por individuos no relacionados y con valor del índice de HL <0.4. El grupo lo conforman 25 individuos, siete machos (cinco pertenecientes a la UMA “La Encantada”, uno de la UMA del “Estado de Tabasco” y uno de la UMA de la UJAT/Dacbiol “CICEA”) y 16 hembras (14 de la UMA “La Encantada” y dos de la UMA del “Estado de Tabasco”). Se propone a la UMA de la UJAT/Dacbiol “CICEA” para administrar este grupo de conservación.

- El segundo grupo se propone para realizar investigación y recuperación de la diversidad genética de la especie. Este grupo está integrado por individuos no relacionados o con una relación de medio hermanos, y con valor del índice HL <0.6. El grupo lo conforman, 61 individuos: 16 machos (cuatro de la UMA “La Encantada”, 10 de la UMA del “Estado de Tabasco” y dos de la UMA de la UJAT/Dacbiol “CICEA”) y 45 hembras (36 de la UMA “La Encantada”, tres de la UMA del “Estado de Tabasco” y seis de la UMA de la UJAT/Dacbiol “CICEA”). Se propone a la UMA del “Estado de Tabasco” con colaboración de la UMA de la UJAT/Dacbiol “CICEA” para administrar este grupo de conservación.

- El tercer grupo se propone para realizar un manejo sustentable de la especie. Este grupo se encuentra integrado por individuos mayormente homocigotos y emparentados. El grupo lo conforman 33 individuos, cuatro machos (uno de la UMA del “Estado de Tabasco” y tres de la UMA de la UJAT/Dacbiol “CICEA) y 29 hembras (ocho de la UMA “La Encantada”, 11 de la UMA del “Estado de Tabasco” y tres de la UMA de la UJAT/Dacbiol “CICEA”). Se propone a la granja “La Encantada” para administrar este grupo de aprovechamiento.

La estrategia de proponer tres grupos ayudará a las UMAs involucradas a:

1) Disminuir la expresión de alelos deletéreos recesivos, debido a que evita la reproducción de individuos emparentados.

2) Se promueve un rescate genético, debido a que incrementa la posibilidad de un flujo genético entre las UMAs.

3) Se ayuda a las UMAs para tener objetivos más específicos para cumplir.

Consideramos igual que necesario la colaboración de las tres UMAs para que se puedan permitir los intercambios propuestos, con el fin de no afectar los planes de manejo existentes.

De igual manera, a partir de la realización del presente estudio, se pudo identificar otros aspectos y temas de investigación, que pueden enriquecer y mejorar la crianza de la *D. mawii*. Estos temas serían:

- Mejorar las instalaciones para la crianza de los organismos.
- Verificaciones continuas de la composición alélica de los individuos destinados a la liberación.
- Mejorar el conocimiento biológico sobre los requerimientos alimentarios de la especie.
- Aumentar el conocimiento de los requerimientos reproductivos para la obtención de individuos saludables y que conserven una adecuada diversidad genética.
- Establecer mejores normas sobre el cuidado sanitario de *D. mawii* en condiciones *ex situ*.
- Identificación la existencia y la proporción de hembras masculinizadas o machos feminizados.
- Entender la competencia espermática en tortugas, debido a que esta selección significaría que solo ciertos tipos de machos dejen su descendencia lo cual puede sesgar la variabilidad genética de la descendencia sobre un solo macho o par de machos.
- Se necesita incrementar el número de machos en las tres UMAs para tener una proporción de sexos más equilibrada.

- Monitoreos sobre la estructura poblacional de *D. mawii* en vida libre en el estado de Tabasco.
- Integrar individuos procedentes de poblaciones silvestres del estado de Tabasco a las UMAs, para poder contar con este pool genético en los programas de conservación de la especie.
- Caracterizar molecularmente diferentes poblaciones dentro del estado de Tabasco, con el fin de identificar los sitios donde sería más viable los programas de reintroducción. Igualmente, esta caracterización permitiría proponer programas de conservación *in situ*.
- Desarrollar un programa que tenga como objetivo principal la conservación *D. mawii* y que integre estrategas de conservación tanto *ex situ* como *in situ*.

Finalmente, el presente trabajo encontró que las Unidades de Manejo para la Conservación de la Vida Silvestre han sido relevantes para entender la reproducción de *D. mawii* con un manejo ex situ, y son sitios viables para conservar esta especie. En el mismo sentido, el integrar las anteriores propuestas dará la oportunidad de desarrollar un programa de conservación nacional mejor planificado para la tortuga blanca que a su vez pueda servir como ejemplo para la conservación de otros reptiles en peligro de extinción y que son reproducidos en las UMAs.

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# ANEXOS

## 1.- Tablas

| | | | **Machos** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **ID, UM** | 207 | No, ID | No, ID | No, ID | 204 | 118 | 101 |
| | | | **UM** | La encantada | La encantada | La encantada | La encantada | La encantada | Estado de Tabasco | Universidad Juárez |
| **Hembras** | **ID, UM** | **UM** | ***HL*** | 0.31 | 0.20 | 0.28 | 0.30 | 0.28 | 0.38 | 0.20 |
| | 269 | La encantada | 0.24 | U | U | U | U | U | U | U |
| | 193 | La encantada | 0.32 | U | U | U | U | U | U | U |
| | 6 | La encantada | 0.37 | U | U | U | U | U | U | U |
| | No, ID | La encantada | 0.38 | U | U | U | U | U | U | U |
| | 201 | La encantada | 0.38 | U | U | U | U | U | U | U |
| | No, ID | La encantada | 0.38 | U | U | U | U | U | U | U |
| | 197 | La encantada | 0.39 | U | U | U | U | U | U | U |
| | 266 | La encantada | 0.32 | U | U | U | U | U | U | U |
| | No, ID | La encantada | 0.23 | U | U | U | U | U | U | U |
| | 264 | La encantada | 0.36 | U | U | U | U | U | U | U |
| | 156 | La encantada | 0.37 | U | U | U | U | U | U | U |
| | 157 | La encantada | 0.28 | U | U | U | U | U | U | U |
| | 164 | La encantada | 0.18 | U | U | U | U | U | U | U |
| | 221 | La encantada | 0.38 | U | U | U | U | U | U | U |
| | 100 | Estado de Tabasco | 0.34 | U | U | U | U | U | U | U |
| | 132 | Estado de Tabasco | 0.37 | U | U | U | U | U | U | U |

Tabla 1.- Relación de parentesco del grupo de tortugas *D. mawii* destinado para conservación en tres UMAs del Estado de Tabasco. *HL*= índice de Homocigosidad por Loci (Homozygosity by loci).

| | | | Machos | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **ID, UMA** | No, ID | 205 | 149 | 158 | 28 | 111 | 115 | 116 | 119 | 124 | 134 | 137 | 139 | 7001 | 141 | PROFEPA |
| | | | **UMA** | La Encantada | | | | Estado de Tabasco | | | | | | | | | | Universidad Juárez | |
| **Hembras** | **ID, UMA** | **UMA** | ***HL*** | 0.58 | 0.42 | 0.47 | 0.52 | 0.57 | 0.55 | 0.49 | 0.49 | 0.56 | 0.58 | 0.48 | 0.50 | 0.57 | 0.43 | 0.44 | 0.48 |
| | 145 | La Encantada | 0.41 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 170 | | 0.42 | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 164 | | 0.42 | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 254 | | 0.44 | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 154 | | 0.42 | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 4 | | 0.38 | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 152 | | 0.24 | HS | HS | HS | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 181 | | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | No, ID | | 0.36 | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 176 | | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 244 | | 0.41 | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U | U |
| | 172 | | 0.41 | U | U | U | U | U | U | U | U | HS | U | U | U | U | U | U | U |
| | 179 | | 0.76 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 3 | | 0.13 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 219 | | 0.52 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 203 | | 0.31 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 189 | | 0.46 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 153 | | 0.41 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | No, ID | | 0.41 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS |
| | 237 | | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS |
| | 208 | | 0.48 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 183 | | 0.41 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 160 | | 0.38 | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | 232 | | 0.58 | U | U | U | HS | U | U | U | U | U | U | U | U | HS | U | U | U |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 222 | | 0.33 | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | No, ID | | 0.34 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 241 | | 0.37 | U | U | U | HS | U | U | U | U | U | U | U | U | U | U | U | U |
| | 191 | | 0.28 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 174 | | 0.35 | U | U | U | HS | U | U | U | U | HS | U | U | U | U | U | U | U |
| | 203 | | 0.25 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | 212 | | 0.42 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | 262 | | 0.22 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | No, ID | | 0.37 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | 170 | | 0.11 | U | U | U | U | U | U | U | U | U | U | U | U | HS | U | U | U |
| | No, ID | | 0.55 | U | U | U | U | U | U | U | U | U | U | HS | U | HS | U | U | U |
| | 175 | | 0.22 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 24 | Estado de Tabasco | 0.57 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 138 | | 0.52 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 140 | | 0.51 | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS | U | U |
| | 7 | Universidad Juárez | 0.32 | U | U | HS | U | U | U | U | U | U | U | HS | U | U | U | U | U |
| | 103 | | 0.32 | U | U | U | U | U | U | U | U | U | U | HS | U | U | U | U | U |
| | 52 | | 0.48 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS | HS |
| | 4 | | 0.36 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | HS |
| | 13 | | 0.51 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |
| | 107 | | 0.85 | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U | U |

Tabla 2.- Relación de parentesco del grupo destinado para investigación de tortugas *D. mawii* en tres UMAs del estado de Tabasco. *HL*= índice de Homocigosidad por Loci (Homozygosity by loci).

| | | | **ID, UMA** | 133 | 23 | 108 | 10 |
|---|---|---|---|---|---|---|---|
| | | | **UMA** | Estado de Tabasco | Universidad Juárez | Universidad Juárez | Universidad Juárez |
| | **ID, UMA** | **UMA** | ***HL*** | 0.68 | 1.00 | 0.73 | 0.78 |
| **Hembras** | 5 | La Encantada | 0.65 | U | U | U | U |
| | 185 | La Encantada | 0.28 | U | U | U | U |
| | 171 | La Encantada | 0.34 | U | U | U | U |
| | No, ID | La Encantada | 0.49 | U | U | U | U |
| | No, ID | La Encantada | 0.31 | U | U | U | U |
| | 253 | La Encantada | 0.39 | U | U | U | U |
| | No, ID | La Encantada | 0.26 | U | U | U | U |
| | 206 | La Encantada | 0.00 | U | U | U | U |
| | 42 | Estado de Tabasco | 0.41 | U | U | U | U |
| | 105 | Estado de Tabasco | 0.55 | U | U | U | U |
| | 112 | Estado de Tabasco | 0.59 | U | U | U | U |
| | 120 | Estado de Tabasco | 0.42 | U | U | U | U |
| | 122 | Estado de Tabasco | 0.85 | U | U | U | U |
| | 123 | Estado de Tabasco | 0.51 | U | HS | U | U |
| | 125 | Estado de Tabasco | 0.56 | U | U | U | U |
| | 129 | Estado de Tabasco | 0.73 | U | U | U | U |
| | 130 | Estado de Tabasco | 0.67 | U | U | U | U |
| | 131 | Estado de Tabasco | 1.00 | U | U | U | U |
| | 136 | Estado de Tabasco | 0.46 | FS | U | U | U |
| | 15 | Universidad Juárez | 0.54 | U | U | U | U |
| | PROFEPA | Universidad Juárez | 0.62 | U | U | U | U |
| | 100 | Universidad Juárez | 0.76 | U | U | FS | HS |
| | 1 | Universidad Juárez | 0.87 | U | FS | U | FS |
| | 31 | Universidad Juárez | 1.00 | U | FS | HS | HS |
| | 110 | Universidad Juárez | 0.86 | U | HS | FS | U |
| | 114 | Universidad Juárez | 0.63 | U | HS | FS | HS |
| | 128 | Universidad Juárez | 0.76 | U | FS | FS | U |
| | 131 | Universidad Juárez | 0.85 | U | FS | HS | U |
| | 143 | Universidad Juárez | 0.71 | U | HS | HS | HS |

Tabla 3.- Relación de parentesco del grupo de tortugas de *D. mawii* destinado para uso sustentable en tres UMAs del Estado de Tabasco. *HL*= índice de Homocigosidad por Loci (Homozygosity by loci).

## 2.- Figuras.

Figura 2.- Mapa de localización de la UMA del estado de Tabasco.

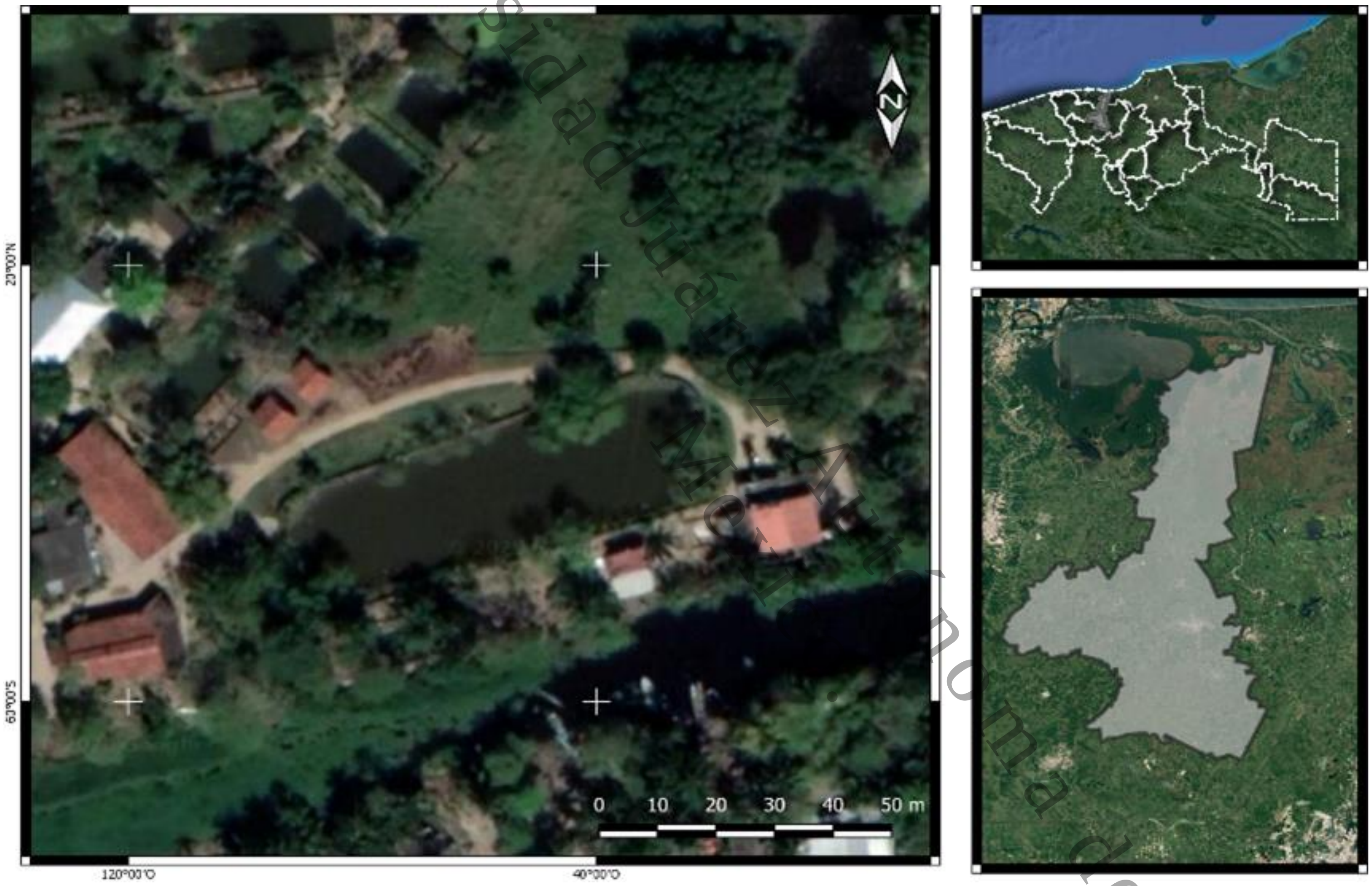

Figura 3.- Mapa de localización de la UMA "La Encantada".

Figura 4.- Mapa de localización de la UMA UJAT/Dacbiol “CICEA”.

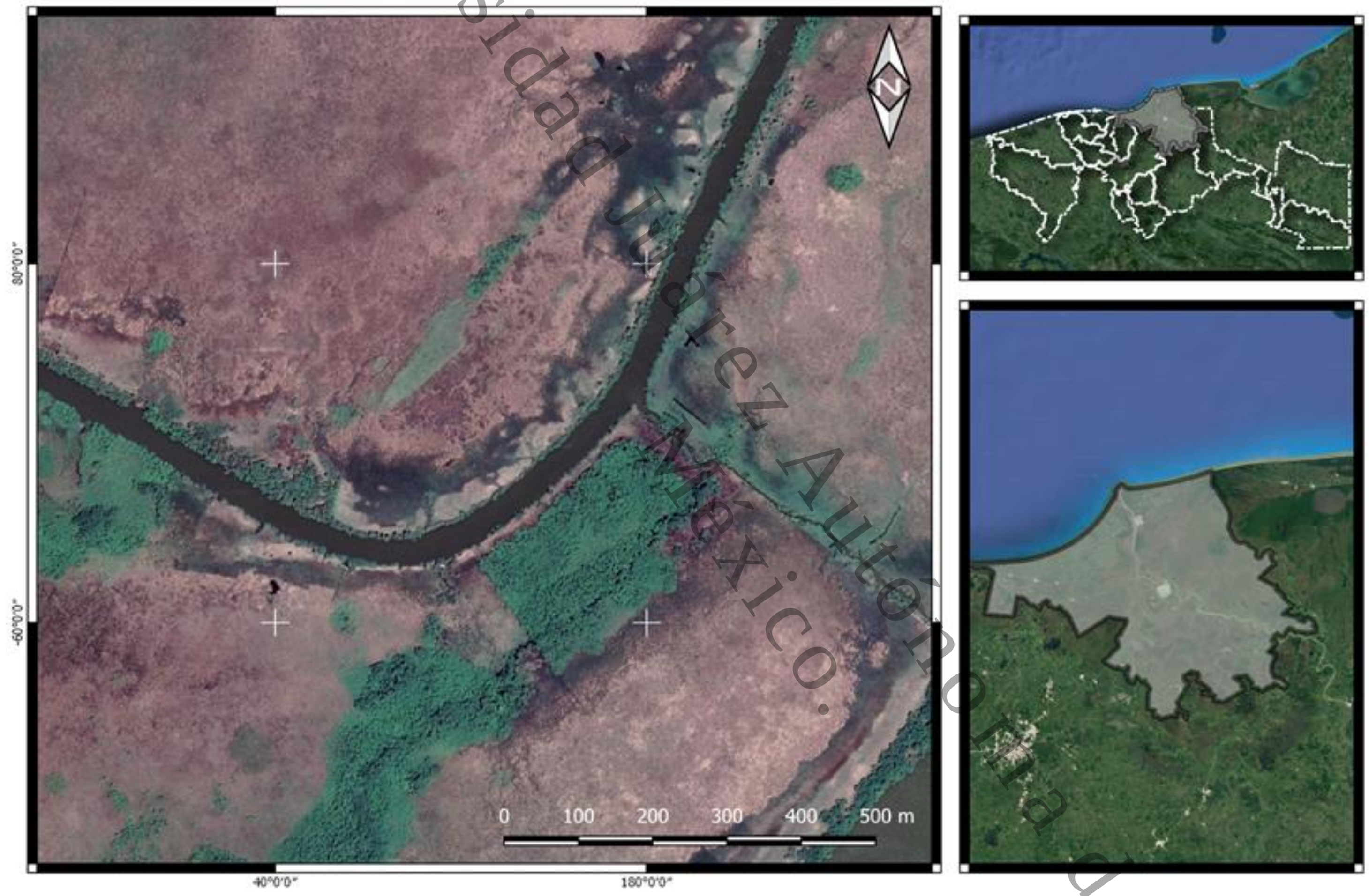

Figura 5.- Mapa de localización del sitio natural Arroyo Tabasquillo, Centla, Tabasco.

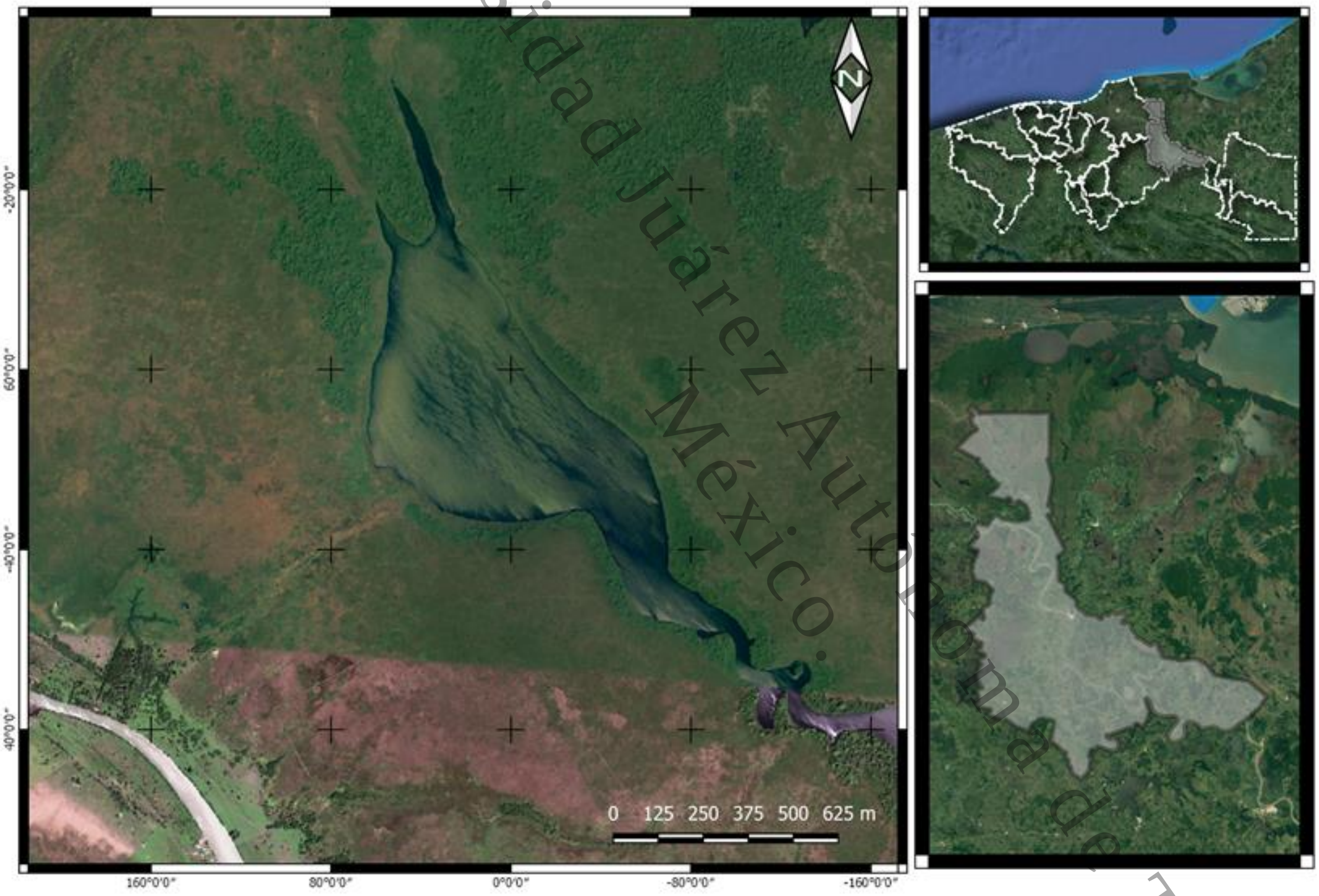

Figura 6.- Mapa de localización del sitio natural: laguna “El Chochal”, Jonuta, Tabasco.

Figura 7.- Mapa de localización del cuerpo de agua conectado al sistema del río Mezcalapa, Huimanguillo, Tabasco

# UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
# DIVISIÓN ACADÉMICA DE CIENCIAS BIOLÓGICAS

Coordinación de Investigación y Posgrado

Doctorado en Ecología y Manejo de Sistemas Tropicales

## ACTA DE EVALUACIÓN DE EXAMEN PREDOCTORAL

El examen predoctoral es uno de los requisitos para obtener el grado de Doctor en Ciencias en Ecología y Manejo de Sistemas Tropicales. Su objetivo es asegurar que el estudiante cuente con la base de conocimientos indispensables y suficientes para pretender el grado de Doctor. El estudiante que haya cubierto los requisitos que su comité tutorial le solicite, tendrá derecho a presentar el examen. Los lineamientos generales y procedimientos para este examen serán determinados por el Comité Académico de Posgrado. La forma y estructura específica para cada examen serán determinadas por los cinco sinodales asignados para llevarlo a cabo. (DACBIOL – UJAT. 2011. Plan de estudios del Doctorado en Ciencias en Ecología y Manejo de Sistemas Tropicales).

Nombre del Alumno (a): Manuel Ignacio Gallardo Álvarez
Matrícula: 162G14001

**Nombre y opinión del Comité Revisor:**

Presidente: Dr. Guillermo Castañón Nájera
Opinión: La defensa realizada al examen escrito y oral por el Doctorando Manuel Ignacio le permiten optar por el grado de Doctor en Ciencias.

Sinodal: _Dra. Patricia Ramos Morales
Opinión: Manuel realizó una excelente defensa del escrito que presentó para la candidatura al Doctorado.

Sinodal: Dra. Judith Andrea Rangel Mendoza
Opinión: Excelente defensa de su temario demostró un abordaje múltiple y crítico para atender la problemática real que rodea a la especie objetivo de su estudio.

Sinodal: Dr. Wilfrido Miguel Contreras Sánchez
Opinión: Manuel realizó una defensa satisfactoria de su examen oral, manifestando en todo momento un amplio dominio de los temas abordados.

Sinodal: Dra. Ena Edith Mata Zayas
Opinión: El estudiante defendió satisfactoriamente las preguntas del examen oral y escrito, mostrando conocimiento y manejo de los temas abordados en la evaluación.

**DICTAMEN FINAL**: Aprobado por Unanimidad

**FIRMAS**

Dra. Patricia Ramos Morales
Sinodal

Dr. Guillermo Castañón Nájera
Presidente

Dr. Wilfrido Sánchez
Sinodal

Judith Rangel M.

Dr. Judith Rangel Mendoza
Sinodal

Dr. Ena Edith Mata Zayas
Sinodal

Villahermosa, Tabasco a __11_ de _Julio de 2020