



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGÍA

BIOLOGÍA EVOLUTIVA

Caracterización genética de poblaciones de *Crocodylus moreletii* y *Crocodylus acutus* en México: variación genética, filogeografía e hibridación

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

Gualberto Jesus Pacheco Sierra

TUTORA PRINCIPAL DE TESIS: DRA. ELLA VÁZQUEZ DOMÍNGUEZ

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CIUDAD DE MÉXICO.

Abril 2017



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Lic. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted, que el Subcomité de Biología Experimental y Biomedicina, en su sesión ordinaria del día 13 de febrero de 2017, aprobó el jurado para la presentación de su examen para obtener el grado de **DOCTOR EN CIENCIAS**, del Posgrado en Ciencias Biológicas, del alumno **PACHECO SIERRA GUALBERTO JESÚS** con número de cuenta **511021369**, con la tesis titulada: "**CARACTERIZACIÓN GENÉTICA DE POBLACIONES DE *Crocodylus moreletii* Y *Crocodylus acutus* EN MÉXICO: VARIACIÓN GENÉTICA, FILOGEOGRAFÍA E HIBRIDACIÓN**", bajo la dirección de la **DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ**:

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 27 de marzo de 2017

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA



Agradecimientos Institucionales

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México por aceptarme en el Programa de Doctorado en Ciencias Biológicas, así como por el apoyo proporcionado durante la realización de esta tesis.

Al Consejo Nacional de Ciencia y Tecnología por la beca de Doctorado otorgada para realizar mis estudios de Posgrado (CONACyT CVU/Becario: 286325/256144).

Al programa de Beca Mixta (CONACyT) y PAEP-UNAM por el apoyo otorgado para realizar estancia de investigación y presentar productos de esta tesis en congresos en el extranjero.

Al Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (Papiit IN202713) y a la Comisión Nacional de Áreas Naturales Protegidas (CONANP/PROCER 2014), por el financiamiento para la realización del presente estudio.

A la Dra. Ella Vázquez Domínguez, quien fue la directora de esta tesis y tutora principal durante mis estudios de posgrado.

Al Dr. Luis Eguiarte Fruns y al Dr. Andrés García Aguayo, integrantes de mi comité tutor, por su constante asesoría e invaluable comentarios durante la realización de mi tesis doctoral.

Agradecimientos Personales

Quiero agradecer de manera especial a dos personas que fueron los responsables directos del éxito de este trabajo: a la Dra. Ella Vázquez, quien me dio la oportunidad de embarcarme en este viaje, me ha dirigido académicamente y con quien crecí enormemente, además de que creyó desde el inicio en mi trabajo, a pesar de las “malas vibras que nos lanzaron”... y a Jerónimo Domínguez Laso, quien compartió desde el inicio la visión que tenía y que gracias a su importante pasión y labor con los cocodrilos hicieron posible el éxito en el muestreo que ahora se ven reflejados en este trabajo. Gracias hermano! Y seguimos...

A los miembros del jurado para la presentación de mi examen: al Dr. Gustavo Casas, Dr. Juan Fornoni, Dr. Luis Eguiarte, Dr. Pierre Charruau y a la Dra. Gabriela Parra por los comentarios, observaciones y consejos realizados a este trabajo.

Al PhD. Zach Gompert de la Utah State University por sus invaluable comentarios y asesorías y por permitirme realizar una estancia en su laboratorio, la cual fue realmente importante en mi formación académica y personal.

A la banda “cocodrilera” que me apoyo con algunas muestras, con apoyo en las salidas de campo y con la buena vibra en mi proyecto: Berenice García “la amigui”, Jose “Lupillo”, Manuel Aeyon, Helios Hernández, Pablo Hernández, Isabel Cardenas, Angel “Judo”, Marco López, Gabriel Barrios, Armando Escobedo, Gabriel Cruz, Roberto “Toby” Ramos, Mauricio Jáuregui, Blanca Rueda y Mariana González.

A toda la gente de la cooperativa de “la Ventanilla” y a la gente de la UMA “Wotoch Aayin” quienes siempre nos han abierto las puertas de sus casas y cuyos proyectos y pasión hacia la conservación de cocodrilos y recursos naturales es ejemplar.

A Jessica Cortés y Kikumi Noda con quienes compartí grandes momentos en el departamento y quienes me brindaron su amistad y apoyo, además de soportar mis largas charlas sobre los cocodrilos.

A grandes amigos que hicieron muy feliz mi estadía en la ciudad de México, a Alana Pacheco, Hugo Siliceo, Laura Ruiz, Mariana Zarazúa, Adrian Caballero y Tania Gutiérrez.

A las personas que me apoyaron en el laboratorio y me respondían o aumentaban mis dudas: Marco Suarez, Susette Castañeda y Tania Garrido.

Fueron muchas las personas que de algún modo se encontraron involucrados en mi trabajo, y que con trabajo en campo, cursos, apoyo moral continuo, etc etc etc, han hecho de este viaje un gran viaje! Por favor, si omití algún nombre, no es porque no fueron importantes, sino porque la memoria ya empieza a fallar... A todos gracias!!

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RESUMEN

La evolución es el resultado de procesos como la selección natural, deriva, mutación, migración y el sistema de apareamiento, cuyas interacciones han forjado la diversidad biológica. Recientemente, el proceso de hibridación ha sido reconocido como un fenómeno biológico asociado con la generación de diversidad a través de la especiación. Dicho proceso, dependiendo de las fuerzas de selección prevalentes entre las especies que hibridizan, puede tener como resultado la extinción de especies o favorecer la especiación. El objetivo principal del presente trabajo fue caracterizar genéticamente las poblaciones de *Crocodylus moreletii* y *C. acutus* en su distribución completa en México, bajo la hipótesis de que estas especies presentarían un complejo proceso de hibridación más allá de la zona histórica de solapamiento de su distribución. Usé dos tipos de marcadores moleculares, microsatélites nucleares y secuencias de ADN mitocondrial, así como datos morfológicos y geográficos. La zona de hibridación entre *C. acutus* y *C. moreletii* se extiende de norte a sur, abarcando prácticamente todo el Golfo de México y el Caribe. Se sugiere que la hibridación se fue promovida a partir de contacto secundario, donde las últimas poblaciones parentales están aisladas en dos islas del Caribe para *C. acutus* (aguas salobres) y en islas al interior del continente (cuerpos de agua dulce como lagunas) en el noroeste de México para *C. moreletii*. Los caracteres morfológicos mostraron un complejo mosaico que no permite identificar a individuos híbridos de las especies parentales en vida libre sin un análisis genético. Asimismo, para la zona del Pacífico también se identificaron individuos híbridos, a pesar de que esta no es zona de distribución natural de *C. moreletii*; los resultados sugieren que estas poblaciones con individuos híbridos son remanentes de flujo genético ancestral entre ambos lados del continente. En cuanto a la antigüedad del proceso de hibridación se determinó que éste es muy antiguo, el cual probablemente inició hace aproximadamente 2.5 millones de años (1.06 – 4.46 ma, 95% HDP) en la región cercana a Campeche, a partir de la combinación de linajes de *C. acutus* del Pacífico y *C. moreletii* del Golfo de México, desplazándose al norte por el Golfo y hacia el sur al Caribe. Se identificaron cinco grupos genéticos diferentes en correspondencia a las clinas geográficas: *C. moreletii* en el noroeste del Golfo de México, híbridos en prácticamente todo el Golfo de México y el Caribe, *C. acutus* de las islas del Caribe, *C. acutus* de la zona noroeste y central del Pacífico y *C. acutus* del sur del Pacífico. Determinar límites entre especies en un sistema donde existe un proceso de hibridación tan complejo como el de estos cocodrilos, es difícil de resolver bajo un modelo estricto de bifurcación entre especies. Las poblaciones que consideramos como *C. moreletii* “puros” o ancestrales, se encuentran en

un potencial proceso de extinción, aunque desde otra perspectiva, la especie puede estar evolucionando bajo un proceso de hibridación. Finalmente, los resultados identificaron que algunos loci presentan señal de selección a favor de los híbridos, lo que sugiere que la hibridación entre estas dos especies de cocodrilos esté bajo un proceso de especiación vía hibridación homoploide.

ABSTRACT

The evolution is the result of processes such as natural selection, genetic drift, mutation, migration and mating system, whose interactions have forged biological diversity. Recently, hybridization has been recognized as a biological phenomenon associated with the generation of diversity through speciation. Such a process, depending on the selection forces prevalent among the species that hybridize, may result in the extinction of species or on the contrary favor speciation. The main goal of the present work was to genetically characterize the populations of *Crocodylus moreletii* and *C. acutus* along their entire distribution in Mexico, under the hypothesis that these species would present a complex hybridization process beyond their historical zone of sympatry. Two types of molecular markers were used, nuclear microsatellites and mitochondrial DNA sequences, as well as morphological and geographic data. Results show that the hybrid zone between *C. acutus* and *C. moreletii* extends from north to south, encompassing practically the entire Gulf of Mexico and the Caribbean. Hybridization was promoted from secondary contact, where the last parental populations are isolated in two Caribbean islands for *C. acutus* (brackish waters) and on islands on the continent (freshwater bodies such as lagoons) in northwestern Mexico for *C. moreletii*. The morphological characters showed a complex mosaic that does not allow to identify hybrid individuals or parental species without a genetic analysis. For the Pacific zone, hybrid individuals were also identified, although this is not a natural area of distribution for *C. moreletii*; results suggest that these populations with hybrid individuals are remnants of ancestral genetic flow between both sides of the continent. Five different genetic groups were identified in correspondence to the geographic clines: *C. moreletii* in the northwest of the Gulf of Mexico, hybrids in practically all the Gulf of Mexico and the Caribbean, *C. acutus* of the Caribbean islands, *C. acutus* from the Pacific Northwest and *C. acutus* from the South Pacific. Divergence results indicate that the hybridization process is very old, which began about 2.5 million years ago (1.06 – 4.47 my, 95% HDP) along a region near Campeche, from the combination of lineages from Pacific *C. acutus* and Gulf of Mexico *C. moreletii*, dispersing to the north by the Gulf and to the south into the Caribbean. Establishing species boundaries is rather difficult in a system where such a complex hybridization process exists, hindering its evaluation under a strict bifurcation model between species. *C. moreletii* might be potentially facing extinction, although from another perspective, it may actually be evolving under a hybridization process. Finally, results showed that some loci have evidence of selection in favor of the hybrids, suggesting that these two crocodiles species are going through a process of homoploid hybrid speciation.

INTRODUCCIÓN GENERAL

La evolución es resultado de procesos como la selección natural, deriva génica, mutación, migración y el sistema de apareamiento. La interacción de estos procesos simultáneamente han determinado (y continúan haciéndolo), en diferentes escalas temporales y espaciales, lo que conocemos como la diversidad biológica (Darwin 1859, Lewontin 1974). La biodiversidad comprende la diversidad genética, la cual hace referencia a la variación heredable de los genes dentro y entre poblaciones de organismos. El estudio de los genes ha cobrado relevancia para el entendimiento de la biodiversidad y para entender los procesos evolutivos que han llevado a las especies a adaptarse y evolucionar. Las especies son una de las unidades fundamentales de la biología, haciéndolas comparables en importancia a los genes, células y organismos (De Queiroz 2007). De manera general, se puede definir a las especies como poblaciones que son diagnosticablemente diferentes, reproductivamente aisladas y cohesivas (ej. *El concepto de Especie Biológica* de Mayr y Dobzhansky; Dobzhansky 1937; Mayr 1942). No obstante, los organismos no son inmutables y los límites entre especies pueden variar según la capacidad de los organismos a adaptarse y evolucionar en relación con su medio ambiente y los factores que los afectan (Wu & Ting 2004; De Queiroz 2007; Harrison 2012).

La Ecología Molecular es una rama de la Ecología, la cual se basa en el uso de diferentes marcadores y herramientas moleculares para responder preguntas planteadas en diferentes niveles de estudio (microevolución, macroevolución y filogeografía), a partir de la caracterización genética de individuos, poblaciones y especies (Eguiarte *et al.* 2007). Esta rama integradora busca describir cualitativa y cuantitativamente la diversidad genética presente en los organismos, mediante la identificación de los diferentes procesos ecológicos y evolutivos asociados con ésta. Estos procesos son fundamentales para tratar de responder diferentes preguntas como: ¿Cuál es la variación genética existente en la población y que procesos evolutivos determinan su distribución? (microevolución); ¿Cuántas especies existen y cuál es su origen? (macroevolución); y ¿Qué procesos geográficos determinan la distribución de los organismos, linajes o especies? (filogeografía) (Eguiarte *et al.* 2007; Beebe & Rowe 2008). Estas preguntas han sido tradicionalmente resueltas con base en el concepto de especie, sin embargo cuando se trata de poblaciones/especies que presentan procesos de hibridación y donde el intercambio genético es continuo, el concepto de especie y los límites entre especies no son tan claros, por lo que dichos procesos deben evaluarse de otra forma (Wu & Ting 2004; De Queiroz 2007; Harrison 2012; Harrison & Larson 2014; Edwards *et al.* 2016; Gompert & Buerkle 2016).

Hibridación

La hibridación se define como la producción de descendencia a partir del entrecruzamiento de individuos pertenecientes a diferentes poblaciones, o entre especies que tengan una poza genética diferente, independientemente de su estatus taxonómico (Barton & Hewitt 1985; Barton 2001; Abbott *et al.* 2013). La hibridación ha sido vista como una fuerza destructiva debido a que, si las barreras de flujo genético son débiles, ésta puede resultar en sumideros genéticos que pueden erosionar la información genética establecida, afectando su origen y mantenimiento, con la consecuente pérdida de biodiversidad (Arnold & Martin 2009; Nolte & Tautz 2010; Gompert *et al.* 2010; Harrison & Larson 2014). Por otro lado, también ha sido vista como una potente fuerza creativa en la evolución, ya que la hibridación puede resultar en la mezcla de genotipos novedosos, con el potencial de adaptarse rápidamente a nuevas condiciones ambientales y, además, dar origen a nuevas entidades taxonómicas (Barton & Hewitt 1985; Seehausen 2004; Arnold & Martin 2009; Nolte & Tautz 2010; Gompert *et al.* 2010; Soltis 2013; Harrison & Larson 2014).

En el contexto geográfico, las zonas de hibridación son áreas donde poblaciones genéticamente distintas se mezclan y producen descendencia. Estas zonas se pueden considerar como laboratorios naturales para el estudio de procesos evolutivos (Barton & Hewitt 1985). En algunos casos, donde la formación de dos especies es resultado de divergencia primaria, éstas pueden presentar una separación incompleta de linajes y, en consecuencia, los patrones genéticos son muy similares a los que se observan en zonas de hibridación mediadas por contacto secundario (Schumer *et al.* 2014). Por otro lado, si la zona de hibridación es producto de un contacto secundario, las especies pueden mantenerse por selección endógena en contra de los híbridos y por migración dentro de la zona de hibridación (zonas de tensión) (Barton & Hewitt 1985), o por selección exógena (ambiental) a lo largo de un ecotono (Endler 1979; Nolte *et al.* 2009; Gompert *et al.* 2010). Las zonas híbridas pueden también presentar un traslape ambiental, donde los híbridos no están aislados espacialmente; en este caso, las características genéticas y fenotípicas pueden traslaparse, manteniendo una combinación de caracteres, lo que origina un gran mosaico donde es difícil determinar cuáles son los límites de la zona de hibridación (Gompert *et al.* 2006, 2010).

Los estudios genéticos a nivel microevolutivo se basan tradicionalmente en la evaluación de las frecuencias alélicas dentro y entre poblaciones y especies, para determinar patrones de variación genética, estructura poblacional, flujo génico y endogamia, entre otros (Hansen & Martins 1996; Diniz-Filho *et al.* 2008)). La estructuración se relaciona principalmente con el flujo

génico, mientras que los niveles de variación genética se relacionan comúnmente con el potencial de adaptación de las especies (Hansen & Martins 1996; Hamilton & Miller 2016). El flujo génico está mediado por la dispersión y migración de los individuos entre poblaciones, que tiene resultados diferentes si se trata de poblaciones distribuidas geográficamente de forma continua o fragmentada, separadas o no por barreras que impidan dicho flujo, o conectadas por corredores que lo faciliten (Hey & Nielsen 2004). Sin embargo en zonas donde existe hibridación, las cuales pueden ser semipermeables, los límites entre poblaciones y especies no son claros y, por lo tanto, los patrones de flujo génico pueden resultar engañosos; es decir, la diversidad y estructuración genética son afectadas directamente por la hibridación interespecífica (Rand & Harrison 1989; Mallet 2005; Dowling & Secor 2011; Harrison 2012; Larson *et al.* 2013; Harrison & Larson 2014; Kenney & Sweigart 2016; Payseur & Rieseberg 2016).

El reconocimiento de la existencia de hibridación interespecífica actualmente ya no es controversial (aunque en el pasado reciente si lo era; Mallet 2005; Baack & Rieseberg 2007; Nolte & Tautz 2010; Harrison 2012; Arnold *et al.* 2012; Harrison & Larson 2014). Sin embargo, aún es complicada la correcta identificación de los individuos que se encuentran bajo un proceso de hibridación, principalmente porque en algunos casos, pueden mostrar patrones similares e incluso idénticos de variación genética y fenotípica a los encontrados en poblaciones de una especie bien definida (Gompert & Buerkle 2016). Recientemente, gracias al uso de diferentes marcadores moleculares y a novedosos modelos y programas estadísticos, es posible identificar diferentes patrones de hibridación y establecer los límites reales de especies en poblaciones donde se ha encontrado hibridación interespecífica (Harrison & Larson 2014; Edwards *et al.* 2016; Pacheco-Sierra *et al.* 2016).

Los estudios macroevolutivos han usado tradicionalmente filogenias moleculares para describir las relaciones entre taxones, ya sea entre especies cercanas o lejanamente relacionadas (Brown *et al.* 2011). Sin embargo, cuando se incluyen híbridos, las reconstrucciones filogenéticas pueden llevar a falsas inferencias acerca de las relaciones entre linajes, debido a una divergencia incompleta de linajes, evolución convergente o reticulación (Edwards *et al.* 2016; Gompert & Buerkle 2016; Abbott *et al.* 2016).

Todos los procesos evolutivos y ecológicos ocurren necesariamente en un contexto geográfico y en múltiples niveles jerárquicos, incluyendo las zonas de hibridación. En este sentido, existe un área de investigación que sirve de nexo entre los estudios microevolutivos y los macroevolutivos: la filogeografía (Avice 2000; Vázquez-Domínguez 2007, Diniz-Filho *et al.* 2008; Hewitt 2011).

La filogeografía permite evaluar y explicar procesos ecológicos y evolutivos, considerando el contexto geográfico, a diferentes niveles temporales y espaciales, utilizando diferentes herramientas moleculares. Las aproximaciones de la filogeografía han facilitado el estudio de los procesos evolutivos que se dan en las zonas de hibridación. De tal manera que con la combinación de diferentes marcadores moleculares con diferente resolución y tasas de divergencia (microsatélites, secuencias de ADN nuclear y mitocondrial y genomas), se han podido describir de manera más precisa los factores exógenos (ambientales y geológicos) y las fuerzas evolutivas (endógenas) asociadas con los procesos de hibridación y, eventualmente, especiación híbrida (Diniz-Filho *et al.* 2008; Edwards *et al.* 2016; Gompert & Buerkle 2016).

La especiación híbrida es la formación de una nueva especie como resultado de la hibridación, sobre lo cual se reconocen dos modelos:

i) especiación por hibridación aloploide (especiación instantánea), basada en la aparición de dos o más cromosomas homólogos derivados de la combinación cromosómica, es decir, la ploidía de los individuos varía; y

ii) especiación por hibridación homoploide, en la que no existe cambios en la ploidía (Buerkle *et al.* 2000; Seehausen 2004; Mallet 2005; Chapman & Burke 2007; Gross & Rieseberg 2008; Mavárez & Linares 2008; Schumer *et al.* 2014).

En el primer caso, la especiación es casi instantánea y dado que los individuos que poseen una nueva ploidía y aunque pueden entrecruzarse con otros individuos que no tenga el mismo número cromosómico, los productos pueden ser rechazados o su descendencia resulta infértil, resultando en un aislamiento reproductivo inmediato (Mallet 2005; Gross & Rieseberg 2008; Abbott *et al.* 2013). Por otro lado, la especiación por hibridación homoploide es más compleja, la cual conlleva procesos de retrocruzamiento e introgresión y donde las tasas de estabilización del genoma son del orden de cientos o miles de generaciones. Cabe mencionar que en ese caso no existe una forma de conocer exactamente cuál es el tiempo necesario para la separación o los componentes críticos de aislamiento que preceden a la fijación y especiación (Buerkle & Rieseberg 2008).

Para responder a la pregunta base: ¿existe hibridación?, la mayoría de los estudios a la fecha se han basado en análisis bayesianos para cuantificar la contribución genética de las especies que están hibridando hacia individuos de ancestría desconocida (Pritchard *et al.* 2000; Anderson & Thompson 2002; Corander *et al.* 2003; Corander & Marttinen 2006). Asimismo, han estimado índices de hibridación con base en la proporción de alelos que se asume fueron heredados por las especies parentales, las cuales se identifican por medio de una función de máxima verosimilitud (Buerkle 2005; Gompert & Buerkle 2010). Sin embargo, la

perspectiva analítica es limitada si sólo se estiman estos índices, en particular si no se toman en cuenta los procesos que pueden estar operando al interior de las poblaciones, los cuales pueden generar valores similares a los que se encuentran en una zona de hibridación (Gompert & Buerkle 2016). Para poder validar correctamente la existencia de un proceso de hibridación interespecífica, es necesario tener un planteamiento correcto de la zona de hibridación; un muestreo que incluya la extensión completa de distribución de las especies de estudio y; que este incluya a las especies parentales. Asimismo, es necesario contar con marcadores moleculares que puedan demostrar mezcla genética, para validar la presencia de híbridos en un gradiente geográfico (Gompert y Buerkle 2016, Pacheco-Sierra *et al.* 2016).

Los primeros estudios que mostraban evidencia de hibridación se basaron en la discordancia filogenética entre marcadores de organelos, marcadores nucleares y morfología (ver Arnold *et al.* 1988, Dowling & secor 1997, Cedeño-Vazquez *et al.* 2008). Recientemente, la mayoría de estudios de este tipo se centran en el análisis de marcadores nucleares que demuestran ancestría híbrida en el genoma y dan evidencia de introgresión, aislamiento geográfico y genómico (Abbott *et al.* 2010, 2013; Brennan *et al.* 2012; Harrison & Larson 2014; Gompert & Buerkle 2016). Para ello se han desarrollado diferentes métodos que permiten determinar la extensión de la introgresión, los cuales se centran en dos tipos de análisis de clinas: los basados en clinas geográficas para determinar qué alelos y frecuencias genotípicas cambian en el espacio; y los que emplean clinas genómicas para examinar los cambios en las frecuencias genotípicas para loci individuales en un gradiente de mezcla genómica (Gompert & Buerkle 2009, 2010, 2011, 2012, 2013; Fitzpatrick *et al.* 2009; Gompert 2016; Payseur & Rieseberg 2016).

Crocodylus acutus* y *Crocodylus moreletii

Los cocodrilos incluyen 24 especies en todo el mundo; entre éstos, el género *Crocodylus* se originó en el Mioceno tardío en la zona tropical del Indo-Pacífico, a partir de donde radiaron rápidamente y se dispersaron globalmente, hasta formar las 12 especies que actualmente componen la familia (Crocodylidae). El cocodrilo americano, *Crocodylus acutus* y el cocodrilo de pantano, *C. moreletii*, divergieron aproximadamente hace 5 millones de años en el Continente Americano (Oaks 2011). Los estudios filogenéticos más recientes muestran que estas dos especies no se encuentran directamente relacionadas filogenéticamente: *C. acutus* es especie hermana de *C. intermedius* (Colombia y Venezuela) y *C. moreletii* de *C. rhombifer* (endémica de Cuba) (Meganathan *et al.* 2010; Meredith *et al.* 2011; Milián-García *et al.* 2011, 2015; Oaks

2011). Estas especies presentan el mismo número de cromosomas ($2N=32$); excepto *C. rhombifer* ($2N=30$; Cohen & Gans 1970).

En México, la distribución geográfica reconocida de *C. acutus* es a lo largo de la franja costera del Océano Pacífico, desde Sinaloa hasta Chiapas y en el Golfo de México en la península de Yucatán, aunque su distribución completa abarca también, Guatemala, Colombia y Venezuela. Por otro lado, *C. moreletii* se distribuye en el Golfo de México y el Caribe a lo largo de las zonas aledañas a la franja costera, desde Tamaulipas hasta Quintana Roo (Álvarez del Toro 1974; Sánchez Herrera *et al.* 2011). La zona de simpatria entre *C. acutus* y *C. moreletii* se ha definido en una franja a lo largo del norte de Yucatán y la región del Caribe mexicano, donde diferentes autores han documentado la existencia de procesos de hibridación (Ray *et al.* 2004; Cedeño-Vázquez *et al.* 2008; Rodríguez *et al.* 2008; Machkour-M'Rabet *et al.* 2009; Pacheco 2010).

Las características morfológicas que distinguen a *C. acutus* y *C. moreletii* se basan principalmente en la coloración, los patrones de escutelación y de escamas caudales, la longitud total en adultos y, principalmente, en la proporción del largo y ancho del hocico (Álvarez del Toro 1974; Platt & Rainwater 2005). No obstante, en la zona donde estas especies son simpátricas, se han encontrado diferentes caracteres mezclados, los cuales hacen difícil su identificación, que posiblemente son resultado precisamente de su hibridación (Cedeño-Vázquez *et al.* 2008; Pacheco 2010). La principal diferencia ecológica entre ambas especies es la asociación de *C. acutus* a cuerpos de agua salada, mientras que *C. moreletii* habita preponderantemente más en cuerpos de agua dulce, ya que aunque ambas especies pueden tolerar agua salada, dado que las dos cuentan con glándulas especializadas que permiten la secreción del exceso de sal en el organismo, en *C. moreletii* no se encuentran tan desarrolladas como *C. acutus* (Taplin *et al.* 1982; Taplin 1988; Mazzotti & Dunson 1989). Dicha característica permite que las dos puedan desplazarse a lo largo de diferentes cuerpos de agua y sin barreras geográficas que limiten su dispersión; ello facilita el flujo génico continuo a lo largo de su distribución.

Durante la década de los años 50 se desarrolló una cacería indiscriminada de las especies de cocodrilos mexicanos, principalmente de *C. moreletii*, debido al alto valor de sus pieles. Ello causó una disminución drástica en sus tamaños poblacionales, por lo que en 1970 se impuso una veda total para la cacería del cocodrilo en México (Álvarez del Toro 1974; Sánchez Herrera *et al.* 2011). Actualmente, ambas especies tienen estatus de 'sujetos a protección especial' en la legislación mexicana (NOM-059-2010; Semarnat 2010). Además, *C. acutus* se encuentra enlistada en el Apéndice I del CITES en el cual se incluyen a todas las

especies en peligro de extinción y *C. moreletii* fue recientemente traspasada del Apéndice I al II en el cual se incluyen especies que no se encuentran necesariamente en peligro de extinción, pero cuyo comercio debe controlarse a fin de evitar una utilización incompatible con su supervivencia, dado que tiene un alto valor comercial (ver CoP 15 Inf. 34). Este cambio se basó en los resultados de un monitoreo de poblaciones de *C. moreletii* a lo largo de su distribución, incluyendo la zona de simpatria, cuyos resultados indicaron que las poblaciones se encuentran estables (Sánchez-Herrera *et al.* 2012). No obstante, la identificación de los individuos en el estudio se basó en la información de la distribución histórica de la especie, sin considerar el potencial de hibridación y la existencia de individuos híbridos, lo cual claramente puede tener repercusiones contrarias en el estatus de conservación de las dos especies.

Así, considerando que las consecuencias de la hibridación son variables y dependen de numerosos factores, incluyendo la adaptación de la descendencia híbrida, la amplitud y naturaleza de la selección, los patrones de dispersión y el contexto ecológico en el cual ésta ocurre (Gompert *et al.* 2010). Asimismo, tomando en cuenta que cuando se realizan estudios genéticos en las zonas de hibridación, sin conocer si este proceso está presente, las inferencias de la información analizada pueden llevar a conclusiones incorrectas, el objetivo principal del presente trabajo fue: Caracterizar morfológica y genéticamente las poblaciones de *Crocodylus acutus* y *Crocodylus moreletii* en México para determinar sus niveles de variación genética, estructura filogeográfica e hibridación.

El presente trabajo está dividido en dos Capítulos, además de una Introducción y una Discusión Generales. En el Capítulo 1, publicado en *Molecular Ecology* con el título de “Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *Crocodylus moreletii*” (Pacheco-Sierra *et al.* 2016), nos planteamos dos preguntas principales:

- i) ¿Está la hibridación e introgresión entre *C. acutus* y *C. moreletii* restringida a la zona de simpatria o esta es geográficamente más amplia? y;
- ii) ¿Pueden identificarse los individuos híbridos de los parentales a partir de las características morfológicas?

Para responder estas preguntas nos propusimos evaluar rigurosamente el nivel y extensión de la hibridación entre *C. acutus* y *C. moreletii* a lo largo del Golfo de México y el Caribe, con ayuda de novedosos análisis estadísticos a partir del uso de microsatélites, aunado a la evaluación de información morfológica de todos los individuos capturados.

A partir de los resultados derivados del Capítulo 1, en donde determinamos que existen muy pocas poblaciones de *C. acutus* y de *C. moreletii* que no tengan algún nivel de admixia (“no-mixturadas”) en vida silvestre en México, y que la zona de hibridación es geográficamente extensa y antigua (miles de generaciones), la cual se formó por contacto secundario, nos planteamos tres preguntas principales en el Capítulo 2:

- i) ¿Cuándo y cómo inicio el proceso de hibridación entre *C. acutus* y *C. moreletii*?
- ii) ¿Pueden distinguirse diferentes linajes en términos de especiación por hibridación homoploide aún cuando el proceso de aislamiento reproductivo es improbable? y;
- iii) ¿Cuáles son las principales fuerzas evolutivas asociadas con la hibridación entre estas especies de cocodrilos?

En este capítulo utilizamos microsatélites y secuencias de ADN mitocondrial a partir de muestras que incluyeron la distribución completa de *Crocodylus* en México. Nuestro objetivo focal fue determinar los límites de especies en una zona de hibridación tipo mosaico y muy extensa mediante el uso de métodos comparativos e integradores para determinar variación genética (microevolución), relaciones filogenéticas (macroevolución) y los componentes geográficos y geológicos asociados (filogeografía).

Capítulo 1

Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *C. moreletii*.

Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *Crocodylus moreletii*

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Abstract

Hybrid zones represent natural laboratories to study gene flow, divergence and the nature of species boundaries between closely related taxa. We evaluated the level and extent of hybridization between *Crocodylus moreletii* and *Crocodylus acutus* using genetic and morphological data on 300 crocodiles from 65 localities. To our knowledge, this is the first genetic study that includes the entire historic range and sympatric zone of the two species. Contrary to expectations, Bayesian admixture proportions and maximum-likelihood estimates of hybrid indexes revealed that most sampled crocodiles were admixed and that the hybrid zone is geographically extensive, extending well beyond their historical region of sympatry. We identified a few geographically isolated, nonadmixed populations of both parental species. Hybrids do not appear to be F₁s or recent backcrosses, but rather are more likely later-generation hybrids, suggesting that hybridization has been going on for several to many generations and is mostly the result of natural processes. *Crocodylus moreletii* is not the sister species of *C. acutus*, suggesting that the hybrid zone formed from secondary contact rather than primary divergence. Nonadmixed individuals from the two species were distinguishable based on morphological characters, whereas hybrids had a complex mosaic of morphological characters that hinders identification in the wild. Very few nonadmixed *C. acutus* and *C. moreletii* populations exist in the wild. Consequently, the last nonadmixed *C. moreletii* populations have become critically endangered. Indeed, not only the parental species but also the naturally occurring hybrids should be considered for their potential conservation value.

Keywords: crocodylia, hybridization, introgression, Mexico, microsatellites, reptiles

Received 4 September 2015; revision received 7 April 2016; accepted 2 May 2016

Introduction

Hybrid zones, which form in geographic areas where genetically distinct populations meet and produce offspring, represent natural laboratories to study evolutionary processes (Endler 1977; Barton & Hewitt 1985; Hewitt 1988; Arnold *et al.* 1999; Nolte *et al.* 2009;

Gompert *et al.* 2010, 2012; Harrison 2012; Abbott *et al.* 2013). Hybrid zones are often narrow and can be stably maintained by endogenous selection against hybrids and by migration into the hybrid zone ('tension zones'; Barton & Hewitt 1985), or by exogenous selection along an ecotone (Endler 1977; Carling & Thomassen 2011). Hybrid zones can exhibit coincident geographic clines in trait values or allele frequencies. Some hybrid zones exhibit sharp clines over short geographic distances (greenshell mussel, *Perna canaliculus*; Gardner &

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Wei 2015), whereas clines in other systems extend throughout large geographic regions (marine mussels; Fraïsse *et al.* 2014). More complex patterns, such as mosaic hybrid zones that show steep clines between habitat patch boundaries, have also been documented (e.g. *Gryllus* spp.; Harrison *et al.* 1987; Ross & Harrison 2002). Indeed, the geographic location of a hybrid zone may reflect differences in the environmental preferences of the species, resulting in a mosaic or an environmental gradient (Confitti *et al.* 2015; Gardner & Wei 2015), like the complex mosaic hybrid zone along Europe observed for the marine mussels *Mytilus edulis* and *Mytilus galloprovincialis* (Fraïsse *et al.* 2014). The long-term evolutionary outcome of hybridization is less clear in cases where no sharp transitions are involved and hybrid zones are naturally diffuse.

Hybridization not only provides information about the nature of species and species boundaries, but can also be an important evolutionary process (Abbott *et al.* 2013). When inherent barriers to gene flow are weak, hybridization can erode species differences and result in hybrid swarms (i.e. all individuals of a population have some level of hybridization) (Barton 2001; Taylor *et al.* 2006; Bossu & Near 2013). Alternatively, hybridization can spread beneficial alleles among differentiated lineages or create novel combinations of genotypes that facilitate rapid adaptation to new environmental conditions (Ellstrand 2003; Martin *et al.* 2006; Nolte *et al.* 2009; Gompert *et al.* 2013). In some instances, hybridization can even lead to the formation of species (Mallet 2005, 2007; Abbott *et al.* 2013). For example, hybridization can generate novel genotypic combinations that allow for adaptation to marginal habitats not occupied by parental species (Rieseberg *et al.* 2003; Gompert *et al.* 2006; Nice *et al.* 2013).

The conservation of hybrid individuals, especially those originating from natural hybridization, is a controversial ongoing debate (Jackiw *et al.* 2015). This is mainly due to the fact that hybrids can have greater fitness allowing for adaptation to novel environments (Allendorf *et al.* 2001; Edmands 2007), whereas on the other hand, hybridization and introgression have contributed to the extinction of species, for instance when rare or endemic species come into contact with more abundant or non-native species (Rhymer & Simberloff 1996; Allendorf *et al.* 2001).

Crocodiles are a group of high conservation concern, in which all 23 species worldwide are in some protected category (IUCN red list). *Crocodylus* originated in a region along the tropics of the Late Miocene Indo-Pacific and rapidly radiated and dispersed around the globe (Oaks 2011). Species in the American continent include *Crocodylus moreletii* and *Crocodylus acutus*, which diverged from each other about 5 million years ago

(Oaks 2011). These species are not each other's closest relatives, rather *C. moreletii* is most closely related to *Crocodylus rhombifer* from Cuba and *C. acutus* is most closely related to *Crocodylus intermedius*, which occurs in Colombia and Venezuela (Meganathan *et al.* 2010; Meredith *et al.* 2011; Milián-García *et al.* 2011, 2015; Oaks 2011). The recognized geographic distribution for *C. moreletii* in Mexico encompasses along the coast of the Gulf of Mexico, from Tamaulipas on the north to Quintana Roo on the Caribbean, while *C. acutus* is distributed mainly on the Pacific coast (from Sinaloa to Chiapas) and the Caribbean (Álvarez del Toro 1974; Sánchez-Herrera *et al.* 2011). The main ecological difference between the species is habitat: *C. acutus* has salt glands and is more commonly associated with salt water (Álvarez del Toro 1974), while *C. moreletii* favours freshwater. However, both species can tolerate salt water (Taplin *et al.* 1982; Taplin 1988; Mazzotti & Dunson 1989), which is a feature directly associated with their high dispersal ability (Sánchez-Herrera *et al.* 2011). In Mexico, these crocodiles do not have substantial geographic or environmental barriers preventing dispersal (Sánchez-Herrera *et al.* 2011).

A sympatric region has been recognized for the two species along the northern Yucatan peninsula and the Caribbean (Cedeño-Vázquez *et al.* 2008), where hybridization has been documented (Ray *et al.* 2004; Cedeño-Vázquez *et al.* 2008; Rodríguez *et al.* 2008; Machkour-M'Rabet *et al.* 2009; Pacheco-Sierra 2010). Although these species have certain morphological features that clearly differentiate them (e.g. head morphology, caudal scalation), they are difficult to identify in the sympatric zones probably due to hybridization (Cedeño-Vázquez *et al.* 2008; Pacheco-Sierra 2010). Hybrid individuals can exhibit a mosaic of parental phenotypes creating uncertainty about the correct identification when based on morphological characters (Allendorf *et al.* 2001). Also, hybridization is not always apparent from morphological observations alone, particularly after several generations of backcrossing (Rhymer & Simberloff 1996; Medina *et al.* 2013); therefore, detection of hybrids is enhanced when assessed both genetically and morphologically.

Recent surveys of hybridization between *C. moreletii* and *C. acutus* (Cedeño-Vázquez *et al.* 2008; Rodríguez *et al.* 2008; Machkour-M'Rabet *et al.* 2009; Pacheco-Sierra 2010) concluded that hybridization and introgression are restricted to the area of sympatry on the Yucatan peninsula and the Caribbean, and González-Trujillo *et al.* (2012) found no evidence of hybridization in central Veracruz populations. However, in those studies the distributional limits of the parental populations of the two species are not clear; hence, the hybrid zone could be larger, especially considering the dispersal

potential of these crocodiles in both salt and freshwater and the lack of major geographic barriers between them. In this study, we aimed to rigorously evaluate the level and extent of hybridization between *C. moreletii* and *C. acutus*. This represents an interesting system to evaluate the outcome of natural hybridization when no sharp boundaries delimit the hybrid zone and limits between species in nature are difficult to establish. This is also an interesting case because crocodiles are long-lived and capable of dispersing large distances (unlike most organisms usually studied in hybrid zones), and thus, we expect a more widespread hybrid zone, perhaps coupled with fuzzier species boundaries. Considering that *C. acutus* and *C. moreletii* are protected species by Mexican and international laws, our results are discussed both in terms of hybridization theory and for their conservation significance. We addressed two specific questions: (i) Is hybridization and introgression between *C. moreletii* and *C. acutus* indeed restricted to the known area of sympatry or is it geographically more widespread? (ii) Can hybrid individuals be differentiated from parental based on morphological characteristics alone? (the answer to this question has practical implications for conservation efforts). We include the most complete sampling regarding number of individuals and extent of distributional cover of the two species in the Gulf of Mexico and the Caribbean.

Materials and methods

Sample collection and microsatellite genotyping

We captured crocodiles from throughout the known distribution of *Crocodylus moreletii* (Gulf of Mexico and the Caribbean) including the reported sympatric zone between *C. moreletii* and *Crocodylus acutus* (the Caribbean) and an isolated *C. acutus* population from Oaxaca (Mexican Pacific Ocean) as an allopatric site for comparison. The sampling scheme was designed to include populations in different environments (coastal areas, inland water bodies and islands) and to maximize the number of individuals collected, to ensure the presence of nonadmixed parental populations from both species. We sampled 65 localities (Fig. 1) and captured an average of seven individuals per locality; from these, we selected a total of 300 individuals for genetic analyses, including all individuals from the putative parental populations and those for which we had morphometric information from the rest of localities. Preliminary identification of all captured individuals was based on characters thought to be diagnostic of the two species (Platt & Rainwater 2005; Platt *et al.* 2009). Crocodiles were measured (see details below) and marked following a unique pattern by clipping tail scutes, following a

numbered code, and liberated at the capture site. Tail scutes were stored in Eppendorf tubes with 99% ethanol, while blood samples were taken from some individuals and stored in K2 EDTA (BD Vacutainer® Blood Collection Tubes; BD Diagnostics).

We isolated and purified DNA from each crocodile sample, ~20 mg of tail scute tissue or 20 µL of blood using Qiagen's DNeasy 250 Blood and Tissue Kit (Cat. No. 69506; Qiagen, Inc.), following the manufacturer's protocol. We genotyped the individuals sampled using 12 polymorphic microsatellite loci using fluorescently labelled microsatellite primers developed for these species (Dever & Densmore 2001; FitzSimmons *et al.* 2001). Microsatellite loci (Table S1, Supporting information) were amplified by polymerase chain reaction PCR in a 7 µL reaction volume containing 2–5 ng of DNA, 2.0 mM MgCl₂, 0.25 µM of dNTP Mix, 0.5 µM of each primer and 0.35 units of Taq polymerase (Vivantis). PCR conditions consisted of an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 1 min (except Cj16, Cj20 and Cj109, 62 °C), 72 °C for 45 s and a final extension at 72 °C for 5 min. Microsatellite products were multiplexed and run on an ABI Prism3730xl and 3100 Genetic Analyser (Applied Biosystems), with ROX-500 as internal size standard and allele size determined with the software GENEMARKER v.1.97 (SoftGenetics). Negative controls were included in all runs, and multiple samples were sized at least twice for reproducibility and correct readings.

We tested for null alleles because their presence could confound some analyses. Inference of null alleles relies on the assumption that the studied population is in Hardy–Weinberg equilibrium, so that the frequency of null alleles can be estimated from a deficit of heterozygotes. Inference of null alleles in admixed populations would be confounded by the reduction in observed heterozygosity (relative to expected heterozygosity) caused by hybridization (Nolte *et al.* 2009). Accordingly, we tested for null alleles only in geographically isolated populations that were unlikely to have experienced admixture (*C. moreletii* from San Luis and *C. acutus* from Ventanilla, Cozumel and Chinchorro); a lack of admixture at these sites was confirmed with downstream analyses (see Results). Null alleles' frequencies were estimated with MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004).

Detection of hybrids

We performed two different analyses to detect nonadmixed and hybrid individuals using Bayesian and maximum-likelihood methods. Bayesian inference of admixture proportions, that is the proportion of each individual's genome derived from each source

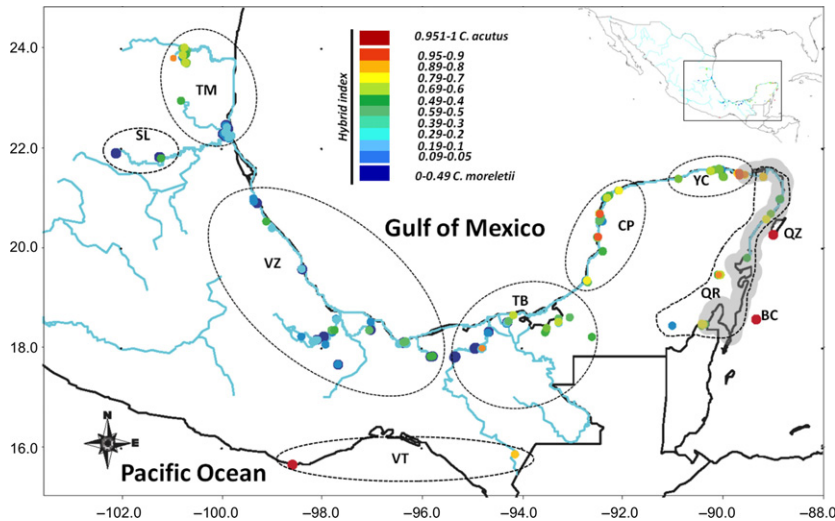


Fig. 1 Map of sampling localities for *Crocodylus acutus* and *Crocodylus moreletii* throughout Mexico and the Caribbean. Circles indicate sampling localities, the blue lines represent the river system and possible routes of migration, while the grey shadow is the historical sympatric zone between the two species. Red and dark blue dots indicate the localities where nonadmixed individuals of *C. acutus* ($h = 0.95-1$, lower bound of the 95% CI > 0.8) and *C. moreletii* ($h = 0-0.05$, upper bound of the 95% CI < 0.2) are present, respectively. Admixed individuals are represented by circles, which are coloured to reflect the gradient in hybrid indexes with a colour gradient (shown in the insert scale from blue to orange, with the corresponding hybrid index value). In order to place the sampling localities at the level of state in the map and in association with their description in Table S2 (Supporting information), we grouped them (dotted circles) and codes refer to: TM: Tamaulipas, SL: San Luis Potosí, VZ: Veracruz, TB: Tabasco, CP: Chetumal, YC: Yucatán, QR: Quintana Roo, QZ: Cozumel, BC: Banco Chinchorro, VT: Ventanilla.

population i (q_i), was conducted using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). We used the admixture model with correlated allelic frequencies, with a value of $K = 2$ source populations, because *C. moreletii* and *C. acutus* represent differentiated entities. Model parameter estimation was conducted based on 1 000 000 Markov chain Monte Carlo (MCMC) iterations following a 500 000 burn-in; 10 replicate MCMC runs were performed. STRUCTURE output files from the multiple runs were modified with STRUCTUREHARVESTER (Earl & vonHoldt 2012), and results were summarized and averaged using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007).

Maximum-likelihood estimation of hybrid indexes was performed using the R package INTROGRESS (Gompert & Buerkle 2010). First, admixture proportions from STRUCTURE were used to identify nonadmixed individuals, which were then used to define the source populations to polarize the hybrid index. A value of $h = 0$ corresponds to nonadmixed *C. moreletii* and $h = 1$ corresponds to nonadmixed *C. acutus* individuals. Individuals selected as parental had point estimates of $q_1 > 0.95$ with the lower bound of the 95% credible intervals (CI) for $q_1 > 0.8$ for *C. acutus* and $q_2 > 0.95$ with the lower bound of the 95% CI for $q_2 > 0.8$ for *C. moreletii*. The remaining crocodiles were analysed as possible hybrid individuals (see Table S2, Supporting information). Most of the selected nonadmixed individuals were from

isolated localities, that is inland lagoons and oceanic islands, for example San Luis (SL), Laguna Las Ilusiones (TB) and Cozumel (QZ) and Chinchorro (BC) islands (Fig. 1). We also calculated the correlation between the admixture proportions (q) and the hybrid indexes (h).

In order to test for the presence of F_1 s, we used a Bayesian method implemented in the NEWHYBRIDS software to assign hybrids to classes based on the genetic data (Anderson & Thompson 2002). Posterior probabilities of assignment to different hybrid classes were inferred from the genetic composition of individuals using a MCMC algorithm. The presence of F_1 s would indicate recent and ongoing hybridization between the parental species. Hence, we estimated the posterior probability that each individual belonged to one of six possible genealogical classes, namely first- and second-generation products of all possible matings between the two species and hybrids: nonadmixed *C. moreletii*, nonadmixed *C. acutus*, F_1 , F_2 , backcrosses to *C. moreletii* and backcrosses to *C. acutus*. Note that later-generation hybrids will often be classified as F_2 s or possibly backcrosses, as they will have a genome composition more similar to these classes. We ran 5 MCMC independent replicates with 1 000 000 sampling iterations and 100 000 burn-in.

Consequently, we used a complementary approach to summarize hybrid genome composition based on the

combination of hybrid index and interspecies ancestry (as in Gompert *et al.* 2014). Interspecific ancestry measures the proportion of an individual's genome where one gene copy was inherited from each parental species, and can be used to distinguish between early- vs. late-generation hybrids. For example, both F_1 s and later-generation hybrids (e.g. F_5 , F_{10} , F_{20} , etc.) have an expected hybrid index of 0.5 (with more variance in later-generation hybrids), but F_1 s should be heterozygous for ancestry (not allelic state) across the entire genome, whereas later-generation hybrids will have reduced interspecific ancestry. We estimated interspecific ancestry by combining microsatellite alleles into allelic classes with frequency differentials between the parental species equal to those when each allele is considered separately (Zhu *et al.* 2005; Gompert & Buerkle 2009). This reduces the complexity of multi-allelic data without a loss of information or distortion of the genetic similarity between reference populations. We then calculated hybrid indexes and interspecific ancestry coefficients based on these allelic classes using *INTROGRESS*. We simulated F_1 , F_2 and backcross individuals by randomly sampling individuals from the parental populations, and calculated hybrid index and interspecific ancestry for these simulated hybrids to compare to the observed data.

Spatial analyses of genetic variation

Next, we analysed spatial patterns of genetic variation in the two crocodylian species and their hybrids. We explored and visualized the genetic relationships among individuals in a multidimensional scaling with principal coordinates analysis (PCoA) using *GENALEX* 6.5 (Peakall & Smouse 2012). We used a matrix of individual pairwise genetic distances (Nei's D_A ; Nei *et al.* 1983) obtained with the software *POPULATION* 1.2.32 (Langella 2002). PCoA aims to find the main axes through a matrix by an eigenanalysis (sometimes referred to as 'singular value decomposition'), which estimates a series of eigenvalues and eigenvectors. Each eigenvalue has an eigenvector, with as many eigenvectors and eigenvalues as there are rows in the initial matrix. Based on the eigenvectors one can visualize the main axes through the initial distance matrix. The result is a rotation of the data matrix in which positions of points relative to each other are not changed. Next, we took each individual on the PCoA and assigned it to a category according to hybrid index (h) values and confidence intervals; that is, if an individual had a $h > 0.95$ and the lower bound of the CI > 0.8 , it was classified as *C. acutus*, and likewise if an individual had $h < 0.05$ and the upper bound of the CI < 0.2 , it was classified as *C. moreletii*. The other individuals

with intermediate hybrid indexes were classified as hybrids.

We tested for isolation by distance based on a matrix of individual genetic distances (Nei's D_A), estimated with *POPULATION*, and a matrix of geographic distances. For the latter, we did not use Euclidean distances estimated from the geographic coordinates because crocodiles disperse mainly via water bodies (coastline, mangroves, lagoons, cenotes, flood zones, rivers). Instead and more accurately, we traced the possible dispersal routes for every individual and estimated the distance between all crocodile pairs sampled. To perform this, we used information from hydrologic, urban, road and agricultural shape files from INEGI (<http://inegifacil.com/maps>) and sampling coordinates for each individual, which were overlapped using *GVSIG* (<http://www.gvsig.org>) to construct a final distribution map. Geographic distances were estimated taking into account the individuals' potential dispersal routes, that is, not linearly but along rivers, coastlines, etc. (Fig. 1) with *GVSIG*. We estimated the geographic distance between every pair of individuals; in nine cases, where individuals were sampled within areas of $< 15 \text{ km}^2$ (e.g. small lagoons, the two islands), we calculated the centroid value for the individuals in that locality and used that distance value for comparisons. The latter was based on the crocodile's known home range (Campbell *et al.* 2013). Next, we performed a Mantel test with the genetic and geographic distance matrices using *GENALEX* (Table S4, Supporting information). This was performed based on individuals (not populations), because we did not identify populations composed of nonadmixed individuals, except the geographically isolated ones chosen as parental.

To formally quantify the spatial extent of the hybrid zone, we fit two-parameter geographic clines models for the microsatellite data (Barton & Hewitt 1985; Szymura & Barton 1991), which also allowed us to place lower bounds on the time since secondary contact. Prior to fitting cline models, we converted latitude and longitude coordinates for the sampled crocodiles into locations along an approximate one-dimensional transect. We conducted this analysis by pooling samples from nearby sites into the ten populations indicated in Fig. 1. One population, Ventanilla, was removed as it was off the main axis of sampling (thus, only nine populations were analysed). We first calculated the distance between each pair of localities using the spherical law of cosines, and next used multidimensional scaling to reduce this matrix of pairwise distances to a single dimension that retained the overall structure (i.e. pairwise distances) of the distance matrix (as in Gompert *et al.* 2010). We used this ordinated dimension as the location along a pseudo-linear transect, which was

strongly correlated with longitude ($r = -0.999$, $P < 0.0001$). We then fit sigmoidal clines with two parameters, cline centre and cline width using a Bayesian framework as implemented in the R package HZAR (Derryberry *et al.* 2014). Clines were fit for each microsatellite locus based on allelic classes (as calculated using INTROGRESS, see above for details). Parameter estimates were based on posterior samples from two MCMC runs with 500 000 iterations and a 10 000 iteration burn-in.

Morphological analyses

We obtained 23 different morphological measures from every captured individual used in this study and selected the seven variables that are useful to distinguish both species (Platt & Rainwater 2005; Pacheco-Sierra 2010): cranial proportion, total length/cranial length and snout–cloacal length/cranial length, tail simple scutes, tail double scutes, subcaudal scalation, and total nuchal and cervical scutellation; these measures were normalized and used for further analyses. We first performed an exploratory principal component analysis (PCA) to examine patterns of variation and covariation for these characters. We next performed a canonical discriminant analysis (CDA; Blackith & Reyment 1971) using Fisher's linear discriminant analysis (Fraleay & Raftery 2002), with SPSS 21.0. We used the same classification criteria, based on the hybrid index (h) as in the PCA, in order to explore whether the morphological measures could discriminate the parental species (*C. moreletii*, *C. acutus*) and the hybrid individuals.

Results

We amplified 12 microsatellite loci for 300 crocodile individuals from 65 different localities throughout their entire geographic range along the Gulf of Mexico and the Caribbean. Individuals genotyped more than once had concordant results. We found no evidence of null alleles in either of the two nonadmixed reference populations. Bayesian admixture proportions for $K = 2$ (qi) indicated that a large proportion of individuals were admixed, but also allowed us to identify nonadmixed individuals and populations (Fig. 2C). The nonadmixed crocodiles were mostly from the geographically isolated populations: *Crocodylus moreletii* from San Luis Potosi (SL; Presa San Diego and Cienega de Cabezas) on the northwest and some individuals from Tabasco from an isolated lagoon population (TB; Laguna Las Ilusiones) and *Crocodylus acutus* from islands in southern Mexico, Cozumel (QZ) and Banco Chinchorro (BC), and the population from Oaxaca on the Pacific coast (VT; La Ventanilla) that we included as an

allopatric population (Fig. 1). These individuals were treated as parental individuals for the hybrid index estimations (h).

Hybrid index estimates also indicated that a large proportion of the crocodiles were admixed (Fig. 2A), and were highly correlated with the Bayesian admixture proportions ($r^2 = 0.84$; $P < 0.001$). Hybrids were common throughout much of the Gulf of Mexico and the Caribbean and exhibited clinal variation with more *C. acutus* like individuals in the known sympatric area and more *C. moreletii* like individuals in the northwest; also, some *C. acutus* individuals were found in the northwest (Tamaulipas, sampling locality Vicente Guerrero; Table S2, Supporting information, Fig. 1). Also, the lower and upper bounds of the confidence intervals were higher overall in the admixed individuals (0.003–0.38 to 0.24–0.90, range of lower and upper bounds, respectively), while lower and upper bounds were negligible in the nonadmixed ones (0–0.13). The latter suggests more uncertainty in the exact genomic composition of hybrids than parentals (Fig. 2A, Table S2, Supporting information).

Posterior probabilities for crocodiles being best-classified as F_1 or recent backcross individuals were uniformly low (Fig. 2B). Instead, most putatively admixed individuals had the greatest posterior probability of being F_2 (these could in fact be F_2 or later-generation hybrids). Similarly, while hybrids exhibited a range of hybrid index values and interspecific ancestry proportions, most hybrids had reduced interspecific ancestry relative to simulated F_{1S} , F_{2S} and backcrosses (Fig. 3). These results suggest that multiple generations have passed since hybridization started and that contemporary hybridization involving nonadmixed parents (that is to say, the production of F_{1S}) is uncommon.

Given these results, we performed a new STRUCTURE analysis assuming $K = 3$ putative source populations, to assess to what degree the admixed individuals are drawn from a third gene pool; we used the same model parameters as with $K = 2$. The same genetic clusters for *C. acutus* and *C. moreletii* were found, but a gradient of admixture proportions for the hybrid individuals was also evident (from north to south; see Fig. 2D).

The PCoA of the genetic distance matrix and the test for isolation by distance demonstrated strong geographic structuring of the genetic variation across the hybrid zone. In particular, the 2-first PCoA axes explained 53% of the variation among individuals, and ordinated the crocodiles in a manner that largely reflected geography (Fig. S1, Supporting information). Moreover, when considering both species and the hybrids, genetic distances were significantly correlated with the geographic distances between samples ($r = 0.34$, $P < 0.01$) (Fig. S2, Supporting information).

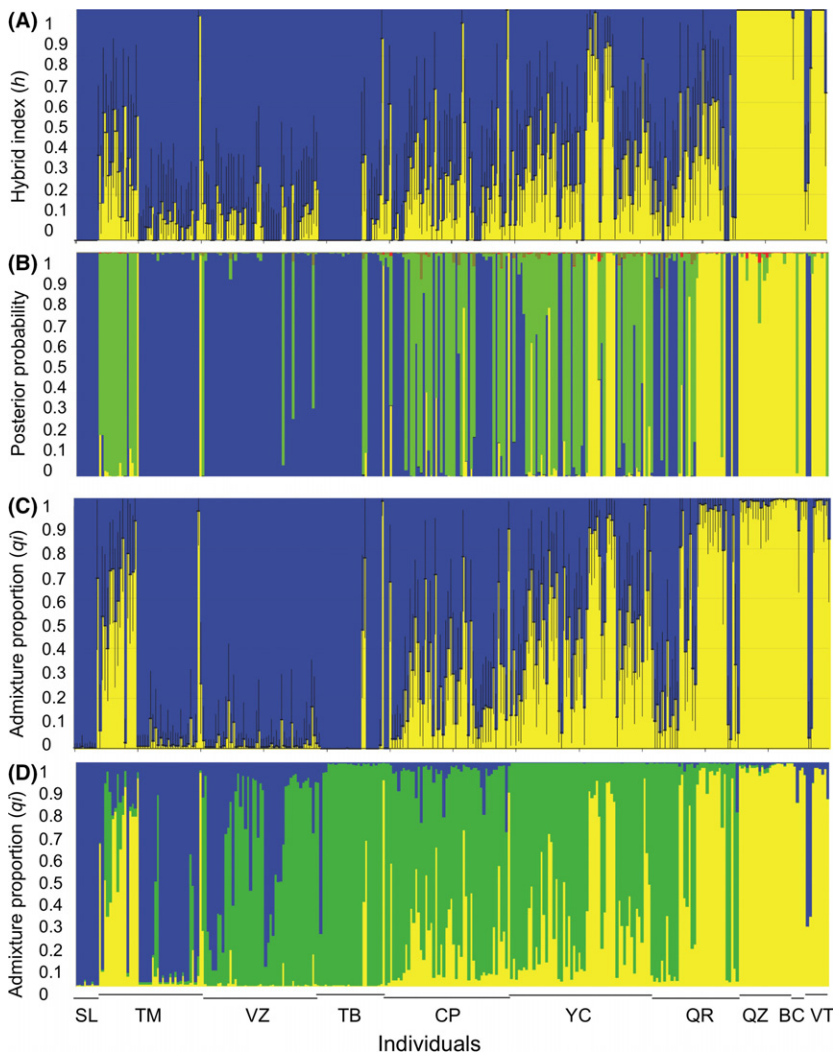


Fig. 2 Bar plots of (A) Maximum-likelihood estimates of hybrid indexes (Buerkle 2005), where solid lines are 95% confidence intervals and blue and yellow bars represent the statistical proportion of the genome inherited from *Crocodylus moreletii* ($1-h$) and *Crocodylus acutus* (h), respectively. (B) Bayesian posterior probability of assignment to different hybrid classes from the software NEWHYBRIDS (Anderson & Thompson 2002), where hybrid classes are indicated as coloured bars as follows: nonadmixed *C. moreletii* (blue), nonadmixed *C. acutus* (yellow), F_1 hybrids (black), F_2 hybrids (green), recent backcrosses to *C. acutus* (red) and to *C. moreletii* (grey). (C) Admixture proportions with $K = 2$ from STRUCTURE (Pritchard *et al.* 2000) where solid lines are 95% credible intervals and different colours represent each admixture proportion: *C. acutus* ancestry (yellow), *C. moreletii* ancestry (blue). (D) Admixture proportions with $K = 3$ from STRUCTURE, where different colours indicate *C. acutus* ancestry (yellow), *C. moreletii* ancestry (blue) and admixed ancestry (green). Admixture proportions are consistent with the hybrid index results. Each vertical bar corresponds to one individual ordered from northwest to southeast populations (left to right, where sampling localities abbreviations are as listed in Table S2, Supporting information and Fig. 1).

Geographic clines were wide for all 12 microsatellite loci with posterior medians ranging from 870 to 27 486 km; the widest clines were essentially flat, and thus, these widths should be interpreted with caution. Samples were pooled into populations by state for this analysis; thus, nine localities were considered (Figs 4 and S3, Supporting information). Little direct information is available about dispersal in *C. acutus* and *C. moreletii*; however, we used what was known to place rough lower bounds on the time since secondary contact. In particular, under a neutral diffusion model, cline width is equal to $2.51 \sigma \sqrt{t}$, where σ is the average dispersal distance between parents and offspring and t is the time since secondary contact in generations (Barton & Gale 1993). Radio telemetry data suggest that *C. moreletii* disperses about 1.2 km per year (Rueda 2015), begin reproducing at 7–10 years of age and continue having offspring until about age 60 (NEPA 2009). Thus, using our narrowest cline (and thus giving the lowest bound for time since secondary contact), our

results suggest secondary contact occurred at least 142.9 generations ago and hence thousands of years ago (here, we assume that the net dispersal away from its birth site is 1 km per year and that crocodiles have had half of their offspring at age 29). In order for secondary contact to have occurred recently, for example, within the past 10 generations, dispersal distances (σ) would need to be 109.6 km per generation. These estimates should of course be interpreted with caution given the limited data available on lifetime dispersal in this crocodiles and the potential for dispersal to vary with habitat.

Finally, the PCA for the morphological measures showed that the first component explained 29.5% of the variation and included the simple and double tail scutes, whereas 29.7% was explained by the second component, grouping the proportion between total length/cranial length and snout–cloacal length/cranial length. The CDA results were significant (Wilks's $\lambda = 0.553$; $P < 0.001$), showing that the first function

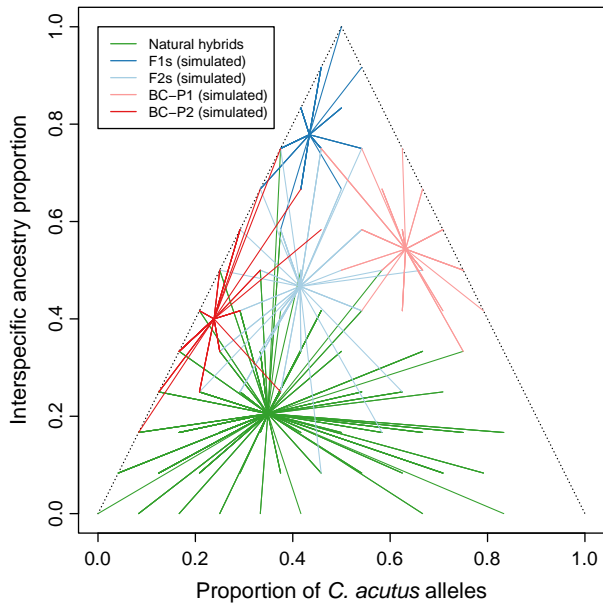


Fig. 3 Triangle plot summarizing patterns of genetic ancestry in hybrids, based on hybrid index values (proportion of *Crocodylus acutus* alleles) and interspecific ancestry (proportion of loci where crocodiles inherited one allele copy from each parental species). Results are shown from natural hybrids and simulated hybrids. In each case, the lines depict the genetic ancestry for individuals and are extended out from the group mean. Dotted lines (forming the triangle) indicate the maximum possible interspecific ancestry for a given hybrid index.

explained 79% of the variance, in which the main morphological features identified were the subcaudal scalation and the total nuchal and cervical scutellation (Fig. 5, Table S3, Supporting information). Also, results showed that 58% of all individuals were correctly classified (either to *C. acutus*, *C. moreletii* or hybrids). Moreover, the CDA was able to distinguish between parental species: considering only the nonadmixed individuals, 100% of *C. acutus* were correctly classified and 81% of the *C. moreletii* (Table S3, Supporting information). Conversely, hybrid individuals were not clearly separated from the nonadmixed ones, where 7% and 45% of individuals were classified as more similar to *C. acutus* and *C. moreletii*, respectively, and 48% as hybrids.

Discussion

This is the first genetic study to include the entire historic distribution for *Crocodylus moreletii* and the sympatric zone where it co-occurs with *Crocodylus acutus* (Fig. 1). In stark contrast to previous studies with more limited geographic sampling (Cedeño-Vázquez *et al.* 2008; Rodríguez *et al.* 2008; Pacheco-Sierra 2010; González-Trujillo *et al.* 2012), our results indicate that a geographically widespread hybrid zone exists between

C. acutus and *C. moreletii* in the Gulf of Mexico and the Caribbean. This hybrid zone includes remote areas on the mainland, some up to 450 km from the coastline in isolated water bodies (Fig. 1). Our results also show that nonadmixed populations of the parental species occur in isolated areas and are morphologically distinguishable, while hybrids are widely dispersed and encompass a mosaic of mixed genetic and morphological characters. Moreover, our results indicate that the hybridization between these two crocodylian species is an ancient process, where secondary contact occurred thousands of years ago.

Geography of hybridization

Bayesian admixture proportions and maximum-likelihood hybrid indexes revealed a widespread hybrid zone, where admixed individuals were found all throughout the Gulf of Mexico and the Caribbean (Figs 1 and 2). The current distribution of the hybrid zone ranges from the southeast of Mexico, including the known area of sympatry in the Yucatan peninsula, all the way up to the north-northwest of Mexico, where the parental populations of *C. moreletii* are distributed. The fact that *C. moreletii* is a sister species of *Crocodylus rhombifer* from Cuba and *C. acutus* of *Crocodylus intermedius* from Colombia and Venezuela (Meganathan *et al.* 2010; Meredith *et al.* 2011; Milián-García *et al.* 2011, 2015; Oaks 2011) supports this hybridization process as an instance of secondary contact and not of primary divergence. We also found significant isolation by distance for individuals across the entire hybrid zone, including admixed and parental crocodiles. In the case of *C. acutus*, we identified only two nonadmixed populations, from Cozumel and Banco Chinchorro, two islands in the Caribbean separated from the mainland 35 and 45 km, respectively. This is consistent with the results of Machkour-M'Rabet *et al.* (2009), whom studied three continental and two island (also Cozumel and Chinchorro) populations of this species in southern Mexico, and concluded that 'islands are the last refuge with genetically pure American crocodiles in the Mexican Caribbean'. Unexpectedly, we only found three nonadmixed *C. moreletii* populations distributed on continental lagoons (Presa San Diego and C. de Cabezas, SL; Lag. Las Ilusiones, TB; Fig. 1, Table S2, Supporting information) in the northern limit of their distribution. These populations represent 'islands' on the mainland, as they have low connection with other water bodies (i.e. there is a geographic barrier to gene flow), decreasing opportunities for gene flow between these *C. moreletii* populations.

In agreement, we documented an isolation by distance pattern and a cline in genetic composition across

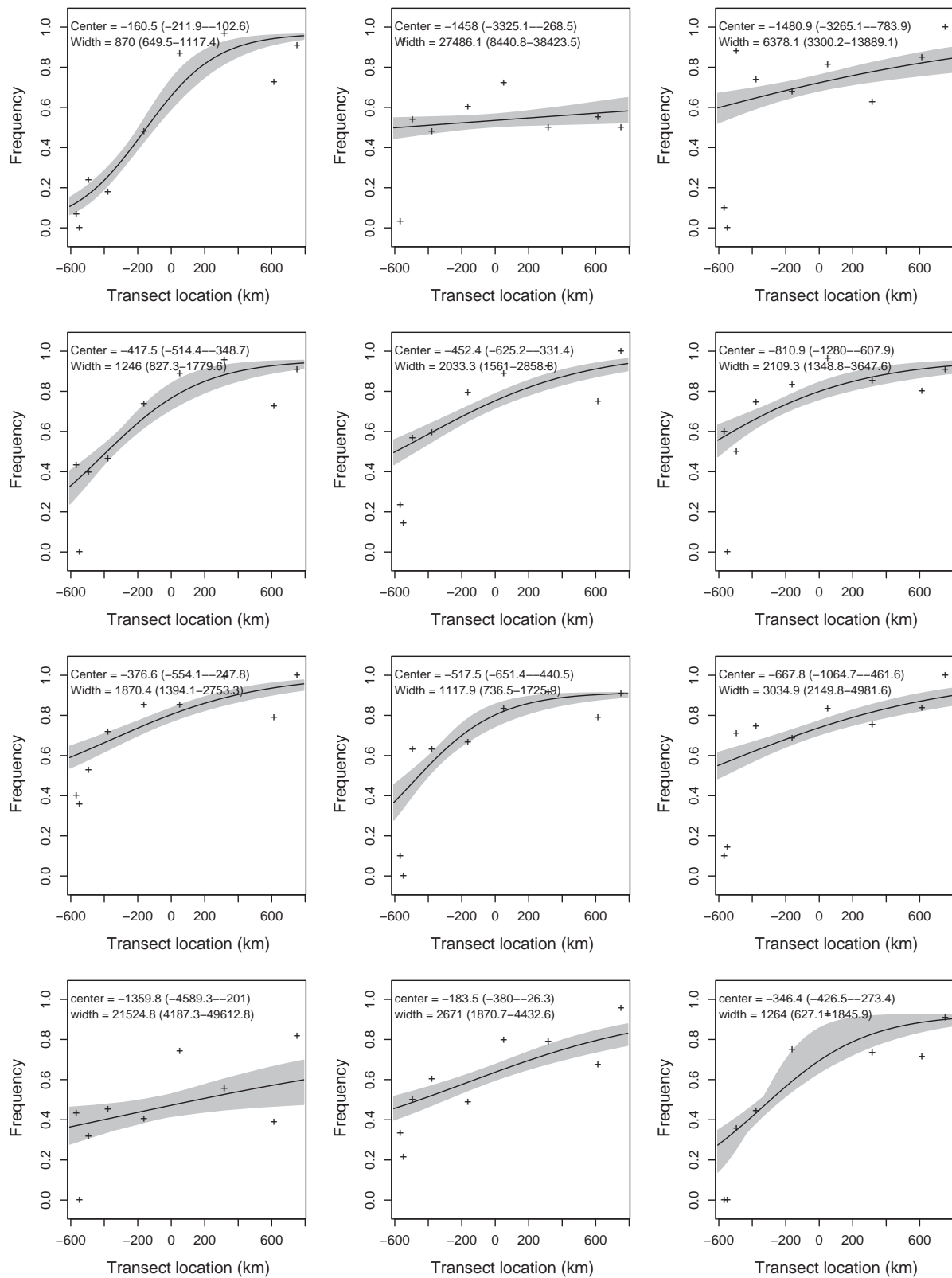


Fig. 4 Plots depict geographic clines for each microsatellite locus. Symbols denote allelic class frequencies for individual populations. Lines and shaded regions denote the point estimate and 95% credible intervals for each cline. Point estimates and credible intervals for cline centre and width are given in each panel.

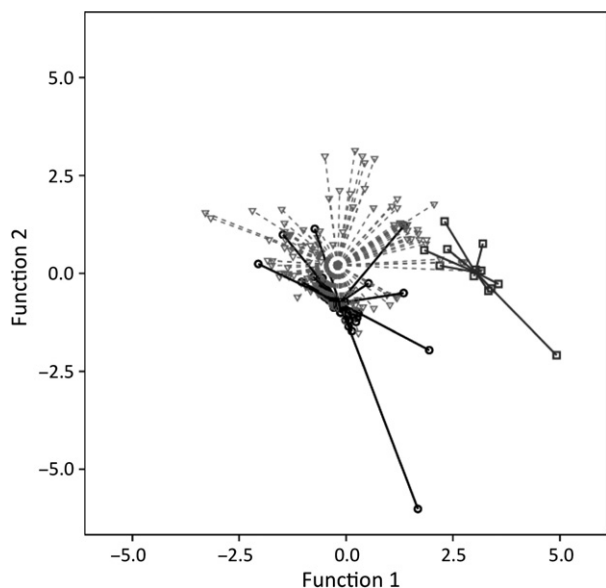


Fig. 5 Canonical discriminant analysis based on seven morphological parameters and labelled with the hybrid index values for all individuals analysed on this study. The intermediate grey squares and lines are nonadmixed *Crocodylus acutus*, the dark grey circles are nonadmixed *Crocodylus moreletii* and the light grey triangles and dashed lines are hybrid individuals, each group with their respective centroid.

the range of the two species and their hybrids, both indicative of neutral diffusion between adjacent populations (Figs 4, S2 and S3, Supporting information). This gradient is evident from the southeast where hybrid individuals are genetically more similar to *C. acutus*, towards the north where hybrids are genetically more similar to *C. moreletii*. This cline reflects the history, geography and demography of secondary contact, which likely occurred thousands of years ago in this system. Specifically, in the absence of strong selection, the genetic composition of hybrids should, at least initially, depend on the magnitude and direction of genetic exchange. As a hybrid zone expands, hybrid individuals at the edges should tend to be genetically more similar to the contiguous parental species because the probability of interbreeding with an individual of the parental species is higher.

The genetic composition of hybrid individuals throughout the hybrid zone is also probably associated with the ecology and habitat preferences of these species. The ability of *C. acutus* individuals to migrate has been related to the lingual salt glands they possess, which confer this species a high tolerance for salt water (Taplin *et al.* 1982; Taplin 1988; Mazzotti & Dunson 1989). Little is known about the osmoregulatory capabilities of *C. moreletii*; however, it can also be found along brackish waters (Cedeño-Vázquez *et al.* 2006). Potential

for dispersal and gene flow in *C. acutus* and *C. moreletii* populations is enhanced by the coastal ecosystems they inhabit and the large hydraulic network connecting populations across rivers, lagoons, swamps, mangroves, cenotes and floodplains characteristic along their distributional area and hybrid zone.

Moreover, our morphological analysis also allowed us to discriminate parental species (with 100% certainty in the case of the *C. acutus*) from each other, but hybrids, which exhibited a wide mosaic of mixed morphological characters, were often not correctly classified. Sánchez-Herrera *et al.* (2011) published a graphic guide for morphologically identifying *C. moreletii* individuals and possible hybrids with *C. acutus*. We could not follow their identification tables because we obtained nearly all possible combinations of states for the morphological characters we analysed (e.g. Rand & Harrison 1989; Dodd & Afzal-Rafii 2004; Eaton *et al.* 2009; Nolte *et al.* 2009; Warren *et al.* 2012; Zigler *et al.* 2012). The latter illustrates that hybrids may resemble one of the parental species phenotypically but not genotypically (cryptic individuals) (Barton 2001; De Queiroz 2007; Fitzpatrick *et al.* 2009; Arnold *et al.* 2012; Abbott *et al.* 2013; Sousa-Neves *et al.* 2013); it also highlights the problem of morphologically identifying hybrid individuals in the field, as their phenotypes depend on the level of admixture or introgression (Hermansen *et al.* 2011; Schumer *et al.* 2014). In fact, such discordance between morphology and genotype (at few loci) is expected for old hybrid zones when hybrids are not uniformly unfit (Abbott *et al.* 2013).

It is important to highlight that, considering our intensive and extensive fieldwork, together with the robust statistical and modelling results we obtained, we are confident that we sampled the parental species to the extent that they exist. This is an essential fact in order to reduce the statistical error in analyses directed to identify hybrid and parental individuals in a study evaluating hybridization (Anderson & Thompson 2002; Gompert & Buerkle 2010). For instance, González-Trujillo *et al.* (2012) report that they did not find hybridization between *C. moreletii* and *C. acutus* in central Veracruz. On the contrary, we identified hybrid individuals in most of the Veracruz state, including one population sampled in both studies (La Mancha). A likely explanation is that González-Trujillo *et al.* (2012) did not include nonadmixed crocodiles from the parental species in their analyses. When aiming to evaluate hybridization between two species, samples from both parental species are required in order to minimize statistical error. If one of the parental populations is small, extinct or poorly sampled, estimates of hybrid indexes or admixture proportions will be shifted, which could give false evidence of the presence or absence of

hybrids. Moreover, when samples of hybrid individuals are analysed as a parental species, the proportion of alleles that define each (nonadmixed) species is lost and, consequently, the hybrids most genetically similar to each of the parental species will be classified as nonadmixed, yielding erroneous results and no hybridization signal between the species being analysed (Anderson & Thompson 2002; Buerkle 2005; Gompert & Buerkle 2009, 2010).

History and evolutionary dynamics of hybridization

Our results support a scenario where hybridization began thousands of years ago, resulting in character and genetic clines that have since begun to decay because of weak or limited selection against hybrids. In other words, in contrast to tension zone models where narrow hybrid zones are maintained by selection against hybrids, hybrids in this system are widespread and are not obviously at a selective disadvantage. Several lines of evidence support this conclusion. The Bayesian admixture proportions inferred when three source populations were assumed clearly identified the nonadmixed parental populations and assigned most hybrids a non-negligible proportion of ancestry from a third source population, but with a wide range of admixture proportions. Likewise, the majority of hybrids were genetically most like a F_2 or late-generation hybrid, with almost no probability of being F_1 or recent backcrosses (Fig. 2B). These results indicate that the hybridization between *C. acutus* and *C. moreletii* has been occurring for at least several generations (Anderson & Thompson 2002), which was corroborated by the cline analysis. If we consider the time needed for the expansion of the hybrid zone along more than 2000 km, in generation times, even for a long-lived species like crocodiles, our results suggest that this hybridization process has been going on over many generations (at least 143 generations ago). Estimation of the historical onset of the hybridization process requires the use of DNA sequence data, which will help to solve this intriguing question (G. Pacheco-Sierra, M. Suárez-Atilano, J. Domínguez-Laso, Z. Gompert & E. Vázquez Domínguez unpublished data). This is further supported by the pattern of isolation by distance indicating gene flow between neighbouring populations, and where nonadmixed populations are geographically isolated in inland lakes or islands.

Conservation implications

Our findings of both an extended hybridization between the two crocodylian species and of only a few isolated parental populations have conservation

implications. Indeed, not only the parental species but also the naturally occurring hybrids must be considered for their conservation value (Jackiw *et al.* 2015). *Crocodylus moreletii* is a species protected by the Mexican law as endangered (Semarnat 2010), and internationally by IUCN and CITES (Cedeño-Vázquez *et al.* 2012; www.cites.org/eng/app/appendices.php). A monitoring programme was established over 5 years ago aiming to ascertain the conservation status of this species; it was focused solely on estimation of population numbers and abundance (Sánchez-Herrera *et al.* 2011). The results obtained in that monitoring programme suggested that *C. moreletii* was not in danger of extinction, based on which it was recently removed from Appendix I to Appendix II on CITES, and also removed from the Endangered Species Act (ESA) for the trade of skins to North America (<http://www.fws.gov/ endangered/laws-policies/>). Our results allow for two conservation considerations: first, it must be taken into account that individuals and populations routinely identified as *C. moreletii* are most likely admixed; hence, changes in the conservation status of *C. moreletii* should consider that many of their populations could include mostly hybrid individuals. Furthermore, our results show that very few populations of parental (nonadmixed) *C. moreletii* remain in the wild, supporting its original status as endangered. Indeed, *C. rhombifer* in Cuba, a critically endangered island endemic, is in the verge of extinction as a result of hybridization with *C. acutus*, also enhanced by anthropogenic threats (Milián-García *et al.* 2015). Hybridization can also significantly decrease reproductive success and fitness, as in the case of the westslope cutthroat trout *Oncorhynchus clarkii lewisi* that shows genetic introgression with the rainbow trout (*Oncorhynchus mykiss*) (Muhlfeld *et al.* 2009). Hence, we advise about *C. moreletii*'s conservation status, which should be reconsidered by national and international authorities, and that new conservation strategies are needed for this species, especially in nonadmixed populations. Second, considering that hybridization between *C. moreletii* and *C. acutus* is an ancient, naturally occurring process, and mostly not determined by anthropogenic factors (e.g. the species overexploitation on the 1950s) as commonly thought (Machkour-M'Rabet *et al.* 2009; Sánchez-Herrera *et al.* 2011), hybridization could be driving evolution of these species, with the potential outcome of the extinction of one (*C. moreletii*) or both parental species, or the formation of a new, hybrid one (Mallet 2005; Abbott *et al.* 2013; Keller *et al.* 2013). This would certainly require an entire new discussion regarding the conservation of these crocodile species. In the meantime, our efforts are next directed to evaluate when the hybridization process started, what historical dispersal routes were

followed and how many different genetic lineages have actually diverged, information that will help to better support conservation actions. It is also key to emphasize the need to perform systematic studies in order to assess whether more isolated *C. acutus* and *C. moreletii* populations exist in the wild, to learn about their population numbers, reproductive success, behaviour and health, aspects that ultimately impact their survival and conservation.

Acknowledgements

Our gratitude to Gabriel Barrios, Armando Escobedo, Gustavo Casas, Marco Lopez and Pierre Charruau that provided tissue loans. We are grateful with Berenice Garcia, Angel Trujillo, Jose Guadalupe Ruiz, the authorities of protected areas (CONANP), all the people from 'La Ventanilla', UMA 'Lucertas' and Parks and Museums Foundation from Cozumel, especially to Hector González, for their invaluable fieldwork support and facilities provided. We deeply thank all the people from the Gompert laboratory at the Utah State University for their assistance and comments to this work during GPS's scientific visit. Susette Casteñeda and Tania Gutiérrez provided molecular and analytical advise. Comments from three anonymous reviewers greatly helped improving the manuscript. Gualberto Pacheco Sierra acknowledges that this work was a part of his doctoral thesis in the Posgrado de Ciencias Biológicas de la Universidad Nacional Autónoma de México. EVD received financial support from Papiit (IN202713) and GPS a scholarship and financial support provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT CVU 286325/Reg. becario 256144), Programa for Postgraduate Studies (PAEP) and UNAM. Scientific collector permit to EVD: Semarnat-FAUT-0168.

References

- Abbott R, Albach D, Ansell S *et al.* (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, **26**, 229–246.
- Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution*, **16**, 613–622.
- Álvarez del Toro M (1974) *Los Crocodylia de México: estudio comparativo*. Instituto Mexicano de Recursos Naturales Renovables, México.
- Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.
- Arnold ML, Bulger MR, Burke JM, Hempel AL, Joseph JH (1999) Natural hybridization: how low can you go and still be important? *Ecology*, **80**, 371–381.
- Arnold ML, Ballerini ES, Brothers AN (2012) Hybrid fitness, adaptation and evolutionary diversification: lessons learned from Louisiana Irises. *Heredity*, **108**, 159–166.
- Barton NH (2001) The role of hybridization in evolution. *Molecular Ecology*, **10**, 551–568.
- Barton NH, Gale KS (1993) Genetic analysis of hybrid zones. In: *Hybrid Zones and the Evolutionary Process* (ed. Harrison RG), pp. 13–45. Oxford University Press, New York, New York.
- Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology, Evolution, and Systematics*, **16**, 113–148.
- Blackith RE, Reyment RA (1971) *Multivariate Morphometrics*. Academic Press, London, UK, New York, New York.
- Bossu CM, Near TJ (2013) Characterization of a contemporaneous hybrid zone between two darter species (*Etheostoma bison* and *E. caeruleum*) in the Buffalo River System. *Genetica*, **141**, 75–88.
- Buerkle CA (2005) Maximum-likelihood estimation of a hybrid index based on molecular markers. *Molecular Ecology Notes*, **5**, 684–687.
- Campbell HA, Dwyer RG, Irwin TR, Franklin CE (2013) Home range utilisation and long-range movement of estuarine crocodiles during the breeding and nesting season. *PLoS ONE*, **8**, e62127.
- Carling MD, Thomassen HA (2011) The role of environmental heterogeneity in maintaining reproductive isolation between hybridizing Passerina (Aves: Cardinalidae) buntings. *International Journal of Ecology*, **2012**, Article ID 295463.
- Cedeño-Vázquez JR, Ross JP, Calmé S (2006) Population status and distribution of *Crocodylus acutus* and *C. moreletii* in southeastern Quintana Roo, Mexico. *Herpetological Natural History*, **10**, 53–66.
- Cedeño-Vázquez JR, Rodriguez D, Calmé S, Ross JP, Densmore LD, Thorbjarnarson JB (2008) Hybridization between *Crocodylus acutus* and *Crocodylus moreletii* in the Yucatan Peninsula. I. Evidence from mitochondrial DNA and morphology. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **309**, 661–673.
- Cedeño-Vázquez JR, Platt SG, Thorbjarnarson JB (IUCN Crocodile Specialist Group) (2012) *Crocodylus moreletii*. *The IUCN red list of threatened species*. Version 2015.2. Available from www.iucnredlist.org. Downloaded on 2 July 2015.
- Confitti IM, Shields GF, Murphy RW, Currie DC (2015) Genetic panmixia within a narrow contact zone between chromosomally and ecologically distinct black fly sibling species (Diptera: Simuliidae). *Journal of Evolutionary Biology*, **28**, 1625–1640.
- De Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology*, **56**, 879–886.
- Derryberry EP, Derryberry GE, Maley JM, Brumfield RT (2014) HZAR: hybrid zone analysis using an R software package. *Molecular Ecology Resources*, **14**, 652–663.
- Dever JA, Densmore LD (2001) Microsatellites in Morelet's crocodile (*Crocodylus moreletii*) and their utility in addressing crocodylian population genetics questions. *Journal of Herpetology*, **35**, 541–544.
- Dodd RS, Afzal-Rafii Z (2004) Selection and dispersal in a multispecies oak hybrid zone. *Evolution*, **58**, 261–269.
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.
- Eaton MJ, Martin A, Thorbjarnarson J, Amato G (2009) Species-level diversification of African dwarf crocodiles (Genus *Osteolaemus*): a geographic and phylogenetic perspective. *Molecular Phylogenetics and Evolution*, **50**, 496–506.
- Edmunds S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, **16**, 463–475.

- Ellstrand NC (2003) Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **358**, 1163–1170.
- Endler JA (1977) *Variation, Speciation and Clines*. Princeton University Press, Princeton, New Jersey.
- Fitzpatrick BM, Fordyce JA, Gavrillets S (2009) Pattern, process and geographic modes of speciation. *Journal of Evolutionary Biology*, **22**, 2342–2347.
- FitzSimmons N, Tanksley S, Forstner M *et al.* (2001) Microsatellite markers for *Crocodylus*: new genetic tools for population genetics, mating system studies and forensics. In: *Crocodylian Biology and Evolution* (eds Grigg G, Seebacher F, Franklin CE), pp. 51–57. Surrey Beatty & Sons, Chipping, Norton.
- Fraïsse C, Roux C, Welch JJ, Bierne N (2014) Gene-flow in a mosaic hybrid zone: is local introgression adaptive? *Genetics*, **197**, 939–951.
- Fraley C, Raftery AE (2002) Model-based clustering, discriminant analysis, and density estimation. *Journal of the American Statistical Association*, **97**, 611–631.
- Gardner JPA, Wei K-J (2015) The genetic architecture of hybridisation between two lineages of greenshell mussels. *Heredity*, **114**, 344–355.
- Gompert Z, Buerkle CA (2009) A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. *Molecular Ecology*, **18**, 1207–1224.
- Gompert Z, Buerkle CA (2010) Introgress: a software package for mapping components of isolation in hybrids. *Molecular Ecology Resources*, **10**, 378–384.
- Gompert Z, Fordyce JA, Forister ML, Shapiro AM, Nice CC (2006) Homoploid hybrid speciation in an extreme habitat. *Science*, **314**, 1923–1925.
- Gompert Z, Lucas LK, Fordyce JA, Forister ML, Nice CC (2010) Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone. *Molecular Ecology*, **19**, 3171–3192.
- Gompert Z, Parchman TL, Buerkle CA (2012) Genomics of isolation in hybrids. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **367**, 439–450.
- Gompert Z, Lucas LK, Nice CC, Buerkle CA (2013) Genome divergence and the genetic architecture of barriers to gene flow between *Lycaeides idas* and *L. melissa*. *Evolution*, **67**, 2498–2514.
- Gompert Z, Jahner JP, Scholl CF *et al.* (2014) Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. *Molecular Ecology*, **24**, 2777–2793.
- González-Trujillo R, Rodríguez D, González-Romero A *et al.* (2012) Testing for hybridization and assessing genetic diversity in Morelet's crocodile (*Crocodylus moreletii*) populations from central Veracruz. *Conservation Genetics*, **13**, 1677–1683.
- Harrison RG (2012) The language of speciation. *Evolution*, **66**, 3643–3657.
- Harrison RG, Rand DM, Wheeler WC (1987) Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Molecular Biology and Evolution*, **4**, 144–158.
- Hermansen JS, Saether SA, Elgvin TO, Borge T, Hjelte E, Saetre GP (2011) Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture and barriers to gene flow. *Molecular Ecology*, **20**, 3812–3822.
- Hewitt GM (1988) Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution*, **3**, 158–167.
- Jakiw RN, Mandil G, Hager HA (2015) A framework to guide the conservation of species hybrids based on ethical and ecological considerations. *Conservation Biology*, **29**, 1040–1051.
- Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.
- Keller I, Wagner CE, Greuter L *et al.* (2013) Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Molecular Ecology*, **22**, 2848–2863.
- Langella O (2002) POPULATIONS 1.2.29. Population genetic software (individuals or populations distances, phylogenetic trees). Available from <http://bioinformatics.org/~tryphon/populations>.
- Machkour-M'Rabet S, Hénaut Y, Charruau P, Gevery M, Winterton P, Legal L (2009) Between introgression events and fragmentation, islands are the last refuge for the American crocodile in Caribbean Mexico. *Marine Biology*, **156**, 1321–1333.
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*, **20**, 229–237.
- Mallet J (2007) Hybrid speciation. *Nature*, **446**, 279–283.
- Martin NH, Bouck AC, Arnold ML (2006) Detecting adaptive trait introgression between *Iris fulva* and *I. brevicaulis* in highly selective field conditions. *Genetics*, **172**, 2481–2489.
- Mazzotti FJ, Dunson WA (1989) Osmoregulation in crocodilians. *Integrative and Comparative Biology*, **29**, 903–920.
- Medina I, Wang IJ, Salazar C, Amézquita A (2013) Hybridization promotes color polymorphism in the aposematic harlequin poison frog, *Oophaga histrionica*. *Ecology and Evolution*, **3**, 4388–4400.
- Meganathan PR, Dubey B, Batzer MA, Ray DA, Haque I (2010) Molecular phylogenetic analyses of genus *Crocodylus* (Eusuchia, Crocodylia, Crocodylidae) and the taxonomic position of *Crocodylus porosus*. *Molecular Phylogenetics and Evolution*, **57**, 393–402.
- Meredith RW, Hekkala ER, Amato G, Gatesy J (2011) A phylogenetic hypothesis for *Crocodylus* (Crocodylia) based on mitochondrial DNA: evidence for a trans-Atlantic voyage from Africa to the New World. *Molecular Phylogenetics and Evolution*, **60**, 183–191.
- Milián-García Y, Venegas-Anaya M, Frias-Soler R *et al.* (2011) Evolutionary history of Cuban crocodiles *Crocodylus rhombifer* and *Crocodylus acutus* inferred from multilocus markers. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **315**, 358–375.
- Milián-García Y, Ramos-Targarona R, Pérez-Fleitas E, Espinosa-López G, Russello MA (2015) Genetic evidence of hybridization between the critically endangered Cuban crocodile and the American crocodile: implications for population history and in situ/ex situ conservation. *Heredity*, **114**, 272–280.
- Muhlfeld CC, Kalinowski ST, McMahon TE *et al.* (2009) Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters*, **5**, 328–331.
- National Environment and Planning Agency (NEPA) (2009) *Amfrequencyocodile*. Available from <http://www.nrca.org/>

- yourenv/biodiversity/Species/crocodile.htm. Accessed November 2015.
- Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. *Journal of Molecular Evolution*, **19**, 153–170.
- Nice CC, Gompert Z, Fordyce JA *et al.* (2013) Hybrid speciation and independent evolution in lineages of alpine butterflies. *Evolution*, **67**, 1055–1068.
- Nolte AW, Gompert Z, Buerkle CA (2009) Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. *Molecular Ecology*, **18**, 2615–2627.
- Oaks JR (2011) A time-calibrated species tree of *Crocodylia* reveals a recent radiation of the true crocodiles. *Evolution*, **65**, 3285–3297.
- Pacheco-Sierra G (2010) *Caracterización genética de Crocodylus moreletii en la reserva de la Biosfera Ría Lagartos, Yucatán México: estructura genética poblacional y evidencia de hibridación entre C. moreletii y C. acutus*. Master's Thesis. Universidad Autónoma de Yucatán, Mérida, Yucatán, México.
- Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, **28**, 2537–2539.
- Platt SG, Rainwater TR (2005) A review of morphological characters useful for distinguishing Morelet's crocodile (*Crocodylus moreletii*) and American crocodile (*Crocodylus acutus*) with an emphasis on populations in the coastal zone of Belize. *Bulletin of the Chicago Herpetological Society*, **40**, 25–29.
- Platt SG, Rainwater TR, Thorbjarnarson JB *et al.* (2009) Size estimation, morphometrics, sex ratio, sexual size dimorphism, and biomass of Morelet's crocodile in northern Belize. *Caribbean Journal of Science*, **45**, 80–93.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Rand DM, Harrison RG (1989) Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution*, **43**, 432–449.
- Ray DA, Dever JA, Platt SG *et al.* (2004) Low levels of nucleotide diversity in *Crocodylus moreletii* and evidence of hybridization with *C. acutus*. *Conservation Genetics*, **5**, 449–462.
- Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.
- Rieseberg LH, Raymond O, Rosenthal DM *et al.* (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*, **301**, 1211–1216.
- Rodríguez D, Cedeño-Vázquez JR, Forstner MRJ, Densmore LD (2008) Hybridization between *Crocodylus acutus* and *Crocodylus moreletii* in the Yucatan Peninsula: II. Evidence from microsatellites. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **309**, 674–686.
- Ross CL, Harrison RG (2002) A fine-scale spatial analysis of the mosaic hybrid zone between *Gryllus firmus* and *Gryllus pennsylvanicus*. *Evolution*, **56**, 2296–2312.
- Rueda B (2015) *Ámbito hogareño y movimientos del cocodrilo de pantano Crocodylus moreletii en la laguna de las Ilusiones, Villahermosa, Tabasco*. Master's Thesis. UJAT, Mexico.
- Sánchez-Herrera O, López-Segurajáuregui A, García-Naranjo OHA, Benitez-Díaz H (2011) *Programa de Monitoreo del Cocodrilo de Pantano (Crocodylus moreletii) México-Belize-Guatemala*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.
- Schumer M, Rosenthal GG, Andolfatto P (2014) How common is homoploid hybrid speciation? *Evolution*, **68**, 1553–1560.
- Semarnat (2010) *Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo*. Diario Oficial de la Federación, México.
- Sousa-Neves T, Aleixo A, Sequeira F (2013) Cryptic patterns of diversification of a widespread Amazonian woodcreeper species complex (Aves: Dendrocolaptidae) inferred from multilocus phylogenetic analysis: implications for historical biogeography and taxonomy. *Molecular Phylogenetics and Evolution*, **68**, 410–424.
- Szymura JM, Barton NH (1991) The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. *Evolution*, **45**, 237–261.
- Taplin LE (1988) Osmoregulation in crocodylians. *Biological Reviews*, **63**, 333–377.
- Taplin LE, Grigg GC, Harlow P, Ellis TM, Dunson WA (1982) Lingual salt glands in *Crocodylus acutus* and *C. johnstoni* and their absence from *Alligator mississippiensis* and *Caiman crocodilus*. *Journal of Comparative Physiology B*, **149**, 43–47.
- Taylor BW, Flecker AS, Hall RO (2006) Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. *Science*, **313**, 833–836.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Warren BH, Bermingham E, Bourgeois Y *et al.* (2012) Hybridization and barriers to gene flow in an island bird radiation. *Evolution*, **66**, 1490–1505.
- Zhu X, Luke A, Cooper RS *et al.* (2005) Admixture mapping for hypertension loci with genome-scan markers. *Nature Genetics*, **37**, 177–181.
- Zigler KS, Byrne M, Raff EC, Lessios HA, Raff RA (2012) Natural hybridization in the sea urchin genus *Pseudoboletia* between species without apparent barriers to gamete recognition. *Evolution*, **66**, 1695–1708.

G.P.S. and E.V.D. conceived of and designed the study. G.P.S., Z.G. and E.V.D. analysed and interpreted the data. G.P.S. and J.D.L. performed fieldwork and sampling. G.P.S., Z.G. and E.V.D. wrote the manuscript.

Data accessibility

The data used in the present study were deposited in Dryad repository under doi: (temporary url: <http://datadryad.org/review?doi=doi:10.5061/dryad.3780c>). The

deposit file contains data for each sample with the locality (name of locality), coordinates and microsatellite genotypes for all individuals.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Distribution of crocodile individuals in a multidimensional scaling based on results from a principal coordinates analysis (PCoA).

Fig. S2 Correlation between pairwise genetic (D_A ; Nei *et al.* 1983) and geographic distances along the entire distribution area among the 300 crocodile individuals analysed in this study.

Fig. S3 Plot of geographic clines with all microsatellite loci combined, showing allelic class frequencies along a longitudinal transect.

Table S1 Polymorphic microsatellite loci (FitzSimmons *et al.* 2001) for *Crocodylus moreletii* and *Crocodylus acutus* from the Gulf of Mexico and the Caribbean that amplified in the present study.

Table S2 List of samples of *Crocodylus moreletii* and *Crocodylus acutus* from the Gulf of Mexico and the Caribbean, including sample identification and population and locality names and coordinates.

Table S3 Canonical discriminant analysis (Blackith & Reyment 1971) using Fisher's linear discriminant analysis (Fraley & Raftery 2002) based on seven morphological measures from *Crocodylus moreletii*, *Crocodylus acutus* and hybrid individuals from the Gulf of Mexico and the Caribbean.

Table S4 Geographic and genetic distance values between individuals of *Crocodylus moreletii* and *Crocodylus acutus* from the Gulf of Mexico and the Caribbean, which were used to test for isolation-by-distance (see Materials and methods in main text).

SUPPLEMENTARY INFORMATION

Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *C. moreletii*

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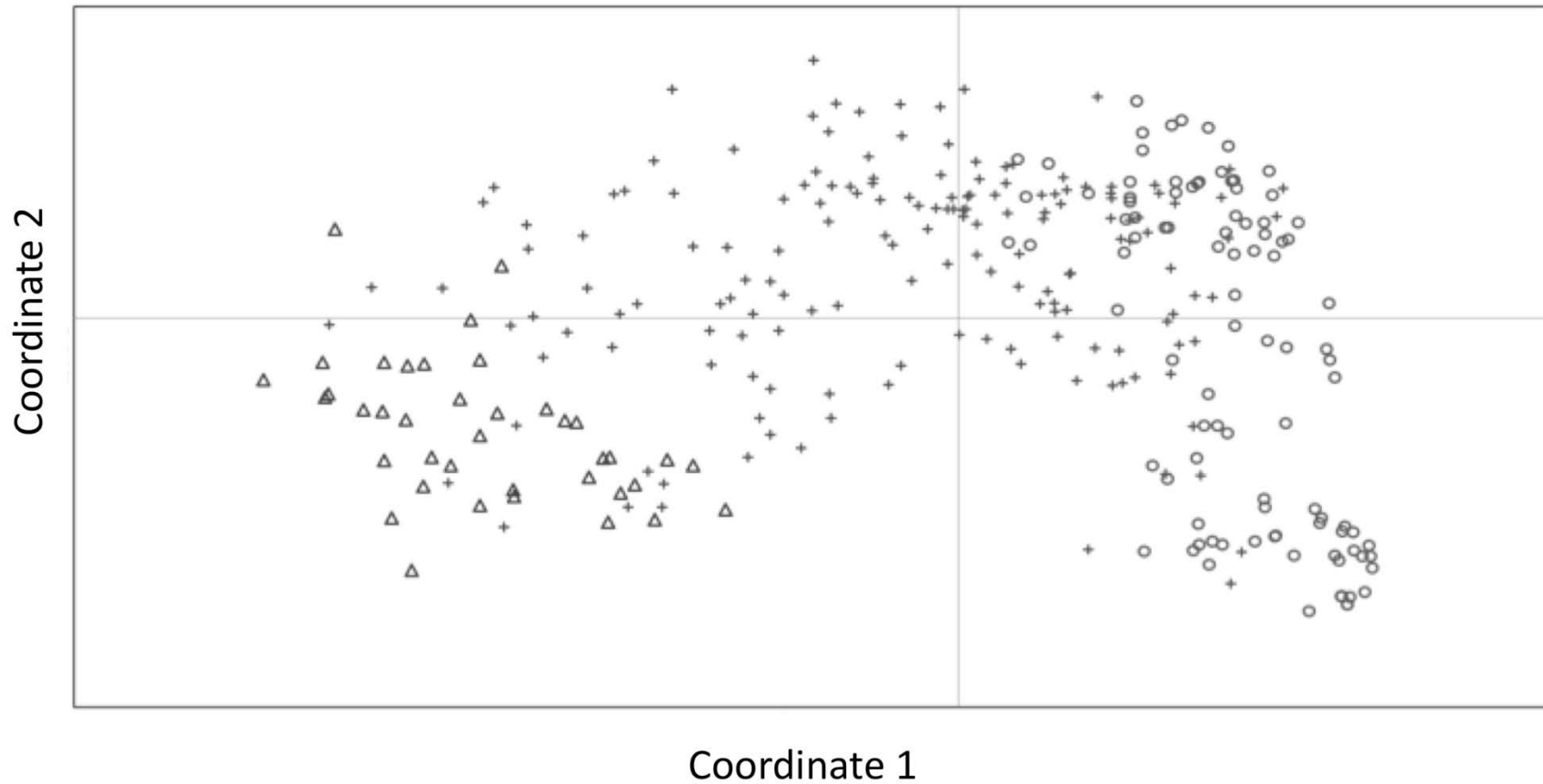


Figure S1. Distribution of crocodile individuals in a multidimensional scaling based on results from a Principal Coordinates Analysis (PCoA). Each individual was assigned, a posteriori, into a class according to hybrid index values (h) and confidence intervals as: triangles are non-admixed individuals of *C. acutus*, circles are non-admixed *C. moreletii* and crosses represent hybrid individuals.

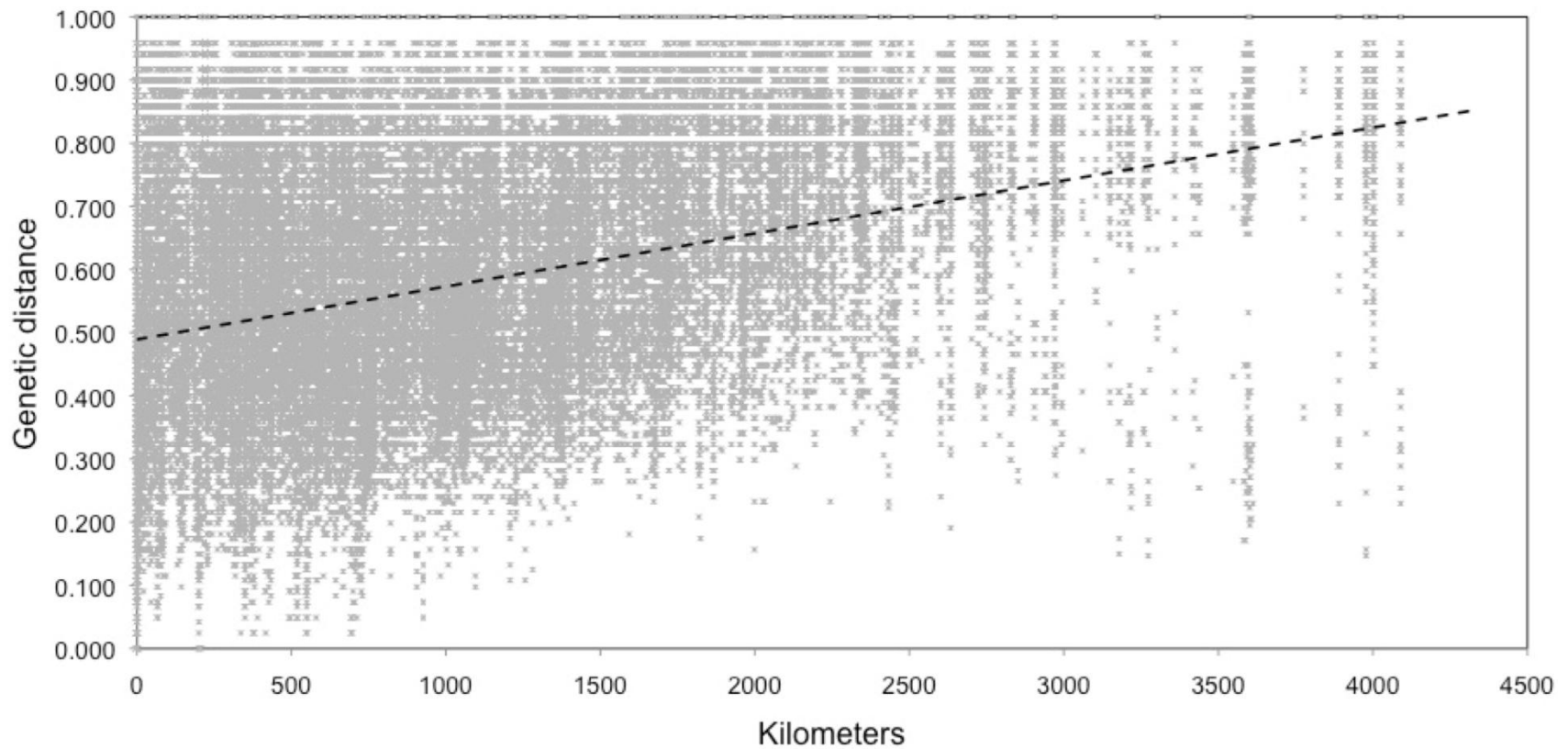


Figure S2. Correlation between pairwise genetic (D_a ; Nei *et al.* 1983) and geographic distances along the entire distribution area among the 300 crocodile individuals analyzed in this study. Gray dots represent pairwise comparisons and the dashed black line is the best-fit regression trend line.

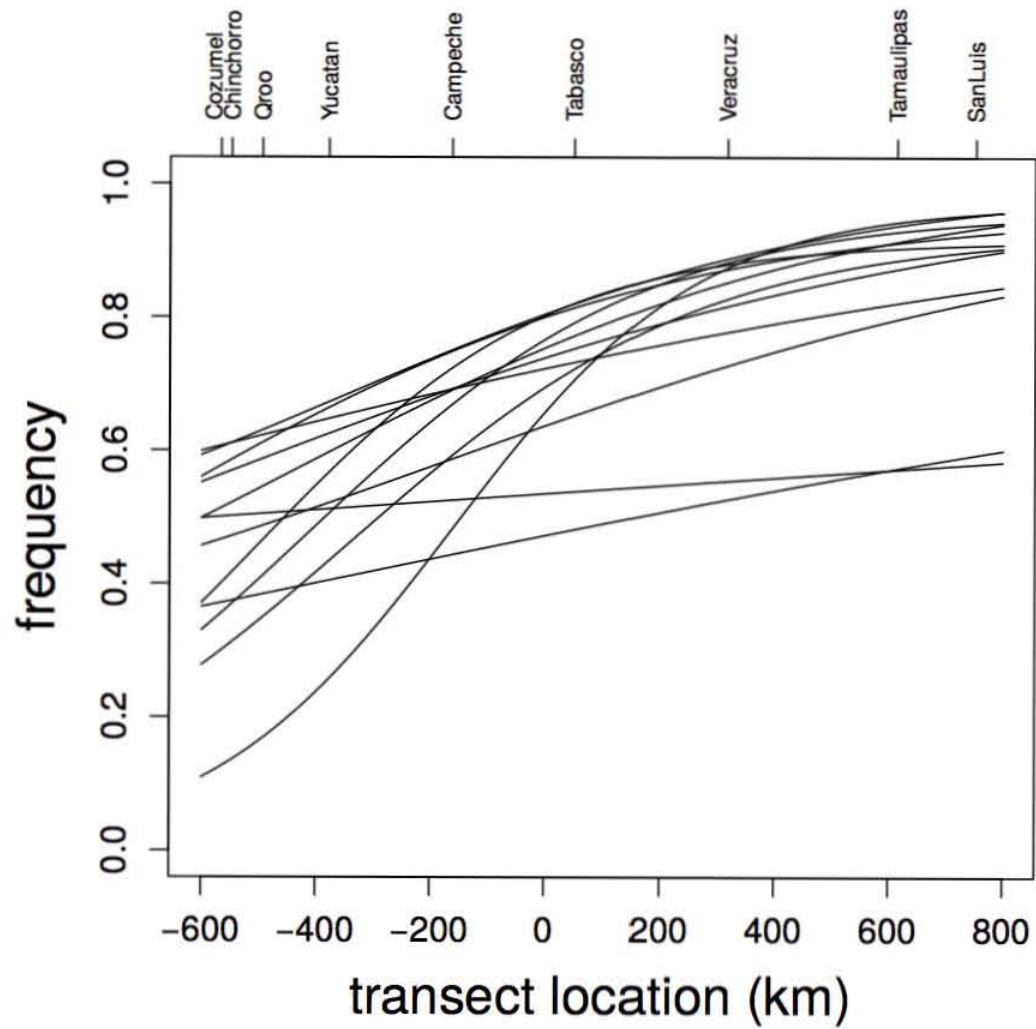


Figure S3. Plot of geographic clines with all microsatellite loci combined, showing allelic class frequencies along a longitudinal transect. Samples were pooled into populations by state for this analysis, thus nine sampling localities are indicated in the figure.

Table S1. Polymorphic microsatellite loci (FitzSimmons *et al.* 2001) for *Crocodylus moreletii* and *C. acutus* from the Gulf of Mexico and the Caribbean that amplified in the present study. Primer sequences (5'-3') and their corresponding dye are given.

Primer name	Sequence 5'-3'	Dye
Cj16	CAT GCA GAT TGT TAT TCC TGA TG TGT CAT GOT GTC AAT TAA ACT C	FAM - F
Cj18	ATCCAAATCCCATGAACCTGAGAG CCG AGT OCT TAC AAG AGG CTG G	HEX - F
Cj20	ACA ATG GGG ATC AGT GCA GAG GTT TCA AAT CCA CAG TCA TAT AGT CC	FAM - F
Cj35	GTTTAGAAGTCTCCAAGCCTCTCAG CTG GGG CAA GGA TTT AAC TCT C	FAM - R
Cj109	GTA TTG TCA ACC CCA CCG TGT C GTT TCC CCT CCA CAG ATT TAC TTG C	FAM - F
Cj119	GTTTGCTGTGGAATGTTTCTAC CGC TAT ATG AAA CGG TGG CTG	HEX - R
Cj127	CCC ATA GTT TCC TGT TAC CTG GTT TCC CTC TCT GAC TTC AGT GTT G	HEX - F
Cj128	ATT GGG GCA GAT AAG TGG ACT C GTT TCT TGC TTC TCT TCC CTA CCT GG	HEX - F
Cj131	GTT TGT CTT CTT CCT CCT GTC CCT C AAA TGC TGA CTC CTA CGG ATG G	FAM - R
CUD68	GCT TCA GCA GGG GCT ACC TGG GGA AAC TGC ACT TTA GG	FAM - R
Cj391	ATG AGT CAG GTG GCA GGT TC CAT AAA TAC ACT TTT GAG CAG CAG	FAM - F
CUJ131	GTC CCT TCC AGC CCA AAT G CGT CTG GCC AGA AAA CCT GT	HEX - R

Table S2. List of samples of *Crocodylus moreletii* and *C. acutus* from the Gulf of Mexico and the Caribbean, including sample identification and population and locality names and coordinates. Includes the summary statistics results for the Bayesian inference of admixture proportions analysis (STRUCTURE 2.3.4; Pritchard *et al.* 2000) and the Maximum-likelihood estimation of hybrid index (h) (R package INTROGRESS; Gompert & Buerkle 2010). Bayesian admixture proportions (q_i) for ($K = 2$; STRUCTURE) and lower and upper credibility intervals (CI) are given.

Sample	Population	Locality	Coordinates WGS84		STRUCTURE				INTROGRESS		h
			Longitude	Latitude	lower CI	upper CI	q_1	$q_2 (1-q_1)$	lower CI	upper CI	
SL01	SanLuis	Presa San Diego	-100.101705	21.906036	0.000	0.019	0.003	0.997	0.000	0.000	0.000
SL02	SanLuis	Presa San Diego	-100.100266	21.907396	0.000	0.019	0.003	0.997	0.000	0.000	0.000
SL03	SanLuis	Presa San Diego	-100.105779	21.908245	0.000	0.019	0.003	0.997	0.000	0.000	0.000
SL04	SanLuis	Presa San Diego	-100.104996	21.912537	0.000	0.053	0.008	0.992	0.000	0.000	0.000
SL05	SanLuis	Presa San Diego	-100.103546	21.907716	0.000	0.019	0.003	0.997	0.000	0.000	0.000
SL06	SanLuis	C. de Cabezas	-99.239420	21.839829	0.000	0.019	0.003	0.997	0.000	0.000	0.000
SL07	SanLuis	C. de Cabezas	-99.236971	21.832713	0.000	0.028	0.005	0.995	0.000	0.000	0.000
SL08	SanLuis	C. de Cabezas	-99.231688	21.835019	0.000	0.022	0.004	0.996	0.000	0.000	0.000
SL09	SanLuis	C. de Cabezas	-99.224737	21.831119	0.000	0.021	0.003	0.997	0.000	0.000	0.000
SL10	SanLuis	C. de Cabezas	-99.204397	21.811128	0.397	0.992	0.681	0.319	0.133	0.673	0.369
SL11	SanLuis	C. de Cabezas	-99.200485	21.807247	0.000	0.254	0.072	0.928	0.029	0.411	0.161
TM01	Tamaulipas	Villa de Casas	-98.709213	23.714266	0.275	0.805	0.528	0.472	0.276	0.836	0.554
TM02	Tamaulipas	Villa de Casas	-98.719231	23.726084	0.232	0.777	0.492	0.508	0.233	0.723	0.468
TM03	Tamaulipas	Villa de Casas	-98.725584	23.731084	0.190	0.643	0.400	0.600	0.078	0.585	0.282
TM04	Tamaulipas	Villa de Casas	-98.709543	23.716330	0.497	0.903	0.711	0.289	0.092	0.691	0.339
TM05	Tamaulipas	Villa de Casas	-98.715306	23.725800	0.501	0.912	0.719	0.281	0.229	0.876	0.568
TM06	Tamaulipas	Villa de Casas	-98.693215	23.723282	0.244	0.798	0.509	0.491	0.190	0.794	0.473
TM07	Tamaulipas	Villa de Casas	-98.695612	23.705908	0.331	0.864	0.589	0.411	0.103	0.570	0.298
TM08	Tamaulipas	Presa V.Guerrero	-98.772430	23.830697	0.485	0.947	0.721	0.279	0.005	0.382	0.100
TM09	Tamaulipas	Presa V.Guerrero	-98.731519	24.026471	0.628	0.996	0.840	0.160	0.312	0.832	0.582
TM10	Tamaulipas	Presa V.Guerrero	-98.695851	23.918332	0.000	0.120	0.023	0.977	0.005	0.316	0.084
TM11	Tamaulipas	Presa V.Guerrero	-98.702248	23.885407	0.521	0.995	0.780	0.220	0.033	0.797	0.357

TM12	Tamaulipas	Presa V.Guerrero	-98.678512	23.963199	0.421	0.976	0.697	0.303	0.017	0.631	0.238
TM13	Tamaulipas	Presa V.Guerrero	-98.772754	24.006591	0.469	0.931	0.707	0.293	0.016	0.595	0.219
TM14	Tamaulipas	Presa V.Guerrero	-98.761735	23.876798	0.717	1.000	0.908	0.092	0.239	0.848	0.540
TM15	Tamaulipas	Estero Lomas	-97.896743	22.474763	0.000	0.052	0.010	0.990	0.000	0.115	0.000
TM16	Tamaulipas	Estero Lomas	-97.905238	22.480032	0.000	0.053	0.010	0.990	0.000	0.102	0.000
TM17	Tamaulipas	Estero Lomas	-97.910862	22.471442	0.000	0.055	0.010	0.990	0.000	0.132	0.000
TM18	Tamaulipas	Estero Lomas	-97.912238	22.466474	0.000	0.054	0.010	0.990	0.003	0.240	0.059
TM19	Tamaulipas	Estero Lomas	-97.916558	22.466356	0.000	0.052	0.010	0.990	0.003	0.229	0.055
TM20	Tamaulipas	Laguna Conejo	-97.880972	22.427639	0.000	0.310	0.121	0.879	0.024	0.398	0.144
TM21	Tamaulipas	Laguna Conejo	-97.885740	22.433786	0.000	0.136	0.031	0.969	0.000	0.112	0.000
TM22	Tamaulipas	Laguna Cañon	-97.868632	22.411096	0.000	0.237	0.084	0.916	0.000	0.106	0.000
TM23	Tamaulipas	Laguna Altamira	-97.895726	22.337848	0.000	0.075	0.014	0.986	0.008	0.371	0.117
TM24	Tamaulipas	Laguna Altamira	-97.892929	22.333736	0.000	0.176	0.044	0.956	0.030	0.424	0.167
TM25	Tamaulipas	Laguna Altamira	-97.903844	22.329862	0.000	0.068	0.013	0.987	0.005	0.330	0.090
TM26	Tamaulipas	Laguna Altamira	-97.916444	22.339376	0.000	0.063	0.012	0.988	0.004	0.286	0.071
TM27	Tamaulipas	Laguna Altamira	-97.920856	22.331406	0.000	0.153	0.038	0.962	0.021	0.350	0.127
TM28	Tamaulipas	Laguna Altamira	-97.907885	22.327407	0.000	0.066	0.012	0.988	0.005	0.313	0.083
TM29	Tamaulipas	Laguna Altamira	-97.916737	22.324821	0.000	0.093	0.017	0.983	0.029	0.418	0.164
TM30	Tamaulipas	Laguna Chairel	-97.898533	22.235110	0.000	0.061	0.011	0.989	0.004	0.267	0.072
TM31	Tamaulipas	Laguna Chairel	-97.910361	22.242865	0.000	0.063	0.012	0.988	0.000	0.132	0.000
TM32	Tamaulipas	Laguna Chairel	-97.958039	22.300669	0.000	0.156	0.043	0.957	0.003	0.236	0.057
TM33	Tamaulipas	Laguna Chairel	-97.970066	22.296811	0.000	0.053	0.010	0.990	0.000	0.112	0.000
TM35	Tamaulipas	Laguna Carpintero	-97.855362	22.235840	0.000	0.054	0.010	0.990	0.003	0.219	0.053
TM36	Tamaulipas	Laguna Carpintero	-97.854593	22.229399	0.000	0.099	0.019	0.981	0.003	0.249	0.062
TM37	Tamaulipas	Laguna Carpintero	-97.855094	22.222319	0.000	0.318	0.123	0.877	0.023	0.343	0.130
TM38	Tamaulipas	Noh Bec	-98.954787	23.812713	0.000	0.053	0.010	0.990	0.000	0.113	0.000
TM39	Tamaulipas	E. tampico	-97.845457	22.199503	0.000	0.136	0.028	0.972	0.005	0.321	0.086
TM40	Tamaulipas	Aldama	-98.954787	23.812713	0.811	1.000	0.948	0.052	0.694	1.000	0.975
TM42	Tamaulipas	Gomez Farias	-98.801751	22.959399	0.060	0.505	0.260	0.740	0.134	0.620	0.348
VZ01	Veracruz	Rio Panuco	-97.795658	22.261961	0.000	0.168	0.032	0.968	0.028	0.409	0.160
VZ02	Veracruz	Tampamachoco	-97.357751	21.005627	0.000	0.037	0.006	0.994	0.004	0.295	0.074

VZ03	Veracruz	Estero Tumulco	-97.351248	20.938211	0.000	0.034	0.006	0.994	0.004	0.284	0.070
VZ04	Veracruz	Estero Chacoaco	-97.290351	20.909730	0.000	0.034	0.006	0.994	0.000	0.132	0.000
VZ05	Veracruz	Estero Chacoaco	-97.290411	20.915244	0.000	0.035	0.006	0.994	0.000	0.132	0.000
VZ06	Veracruz	Estero Lagartos	-97.096046	20.542022	0.000	0.137	0.025	0.975	0.078	0.487	0.239
VZ07	Veracruz	Estro Larios	-96.982713	20.409903	0.000	0.194	0.055	0.945	0.029	0.421	0.162
VZ08	Veracruz	La Mancha	-96.389369	19.593485	0.000	0.045	0.007	0.993	0.007	0.360	0.112
VZ09	Veracruz	La Mancha	-96.386432	19.582741	0.000	0.066	0.011	0.989	0.000	0.202	0.000
VZ10	Veracruz	Papa-Loapan	-95.706286	18.593737	0.000	0.207	0.042	0.958	0.005	0.319	0.084
VZ11	Veracruz	Papa-Loapan	-95.701311	18.576408	0.000	0.419	0.193	0.807	0.008	0.376	0.116
VZ13	Veracruz	Pte. Santa Cruz	-96.121439	18.144412	0.000	0.139	0.025	0.975	0.024	0.376	0.141
VZ14	Veracruz	Pte. Santa Cruz	-96.120240	18.162273	0.000	0.295	0.106	0.894	0.022	0.364	0.133
VZ15	Veracruz	Pte. Santa Cruz	-96.091145	18.173224	0.000	0.041	0.007	0.993	0.000	0.149	0.000
VZ16	Veracruz	Pte. Santa Cruz	-96.080789	18.168074	0.000	0.060	0.010	0.990	0.008	0.375	0.122
VZ12	Veracruz	Temascal	-96.398136	18.240241	0.000	0.062	0.010	0.990	0.004	0.272	0.070
VZ17	Veracruz	Temascal	-96.174993	18.131980	0.000	0.183	0.038	0.962	0.024	0.371	0.139
VZ18	Veracruz	Tlacojalpan	-95.955507	18.241804	0.000	0.057	0.009	0.991	0.000	0.165	0.000
VZ19	Veracruz	Tlacojalpan	-95.951167	18.230109	0.000	0.041	0.007	0.993	0.000	0.189	0.000
VZ20	Veracruz	Tlacojalpan	-95.955305	18.239602	0.000	0.039	0.007	0.993	0.000	0.140	0.000
VZ21	Veracruz	Cosamaloapan	-95.777288	18.319714	0.000	0.038	0.006	0.994	0.004	0.279	0.074
VZ22	Veracruz	Cosamaloapan	-95.792620	18.361949	0.000	0.150	0.025	0.975	0.070	0.516	0.248
VZ23	Veracruz	Cosamaloapan	-95.737561	18.367555	0.000	0.275	0.072	0.928	0.125	0.584	0.320
VZ24	Veracruz	Loma Bonita	-95.919322	18.078873	0.000	0.038	0.006	0.994	0.003	0.241	0.059
VZ25	Veracruz	Playa Vicente	-95.669629	17.675532	0.000	0.040	0.007	0.993	0.000	0.167	0.000
VZ26	Veracruz	Playa Vicente	-95.671033	17.677202	0.000	0.044	0.007	0.993	0.000	0.207	0.000
VZ27	Veracruz	Playa Vicente	-95.668668	17.676002	0.000	0.053	0.009	0.991	0.000	0.246	0.000
VZ28	Veracruz	Catemaco	-95.022300	18.371311	0.000	0.117	0.022	0.978	0.000	0.140	0.000
VZ29	Veracruz	Catemaco	-95.022064	18.370288	0.000	0.045	0.007	0.993	0.000	0.133	0.000
VZ30	Veracruz	Catemaco	-95.020229	18.372091	0.000	0.071	0.012	0.988	0.000	0.112	0.000
VZ31	Veracruz	Catemaco	-95.042143	18.363287	0.000	0.035	0.006	0.994	0.000	0.118	0.000
VZ32	Veracruz	Catemaco	-94.444690	18.085184	0.000	0.298	0.116	0.884	0.052	0.512	0.235
VZ33	Veracruz	Coatzacoalcos	-94.371237	18.114042	0.000	0.066	0.011	0.989	0.027	0.367	0.145

VZ34	Veracruz	Jaguaroundi	-94.374422	18.119283	0.000	0.040	0.007	0.993	0.000	0.144	0.000
VZ35	Veracruz	Jaguaroundi	-94.356508	18.123827	0.000	0.044	0.007	0.993	0.000	0.189	0.000
VZ36	Veracruz	Jaguaroundi	-94.352721	18.123638	0.000	0.317	0.104	0.896	0.073	0.489	0.242
VZ37	Veracruz	Jaguaroundi	-94.360514	18.118786	0.000	0.045	0.008	0.992	0.000	0.160	0.000
VZ38	Veracruz	Jaguaroundi	-94.352928	18.116536	0.000	0.071	0.012	0.988	0.000	0.299	0.002
VZ39	Veracruz	Presa cangrejera	-94.328418	18.112132	0.000	0.043	0.007	0.993	0.005	0.306	0.082
VZ40	Veracruz	Presa cangrejera	-94.324844	18.114700	0.000	0.043	0.007	0.993	0.006	0.345	0.102
VZ41	Veracruz	Presa cangrejera	-94.318018	18.094913	0.000	0.119	0.020	0.980	0.014	0.398	0.150
VZ42	Veracruz	Presa cangrejera	-94.307985	18.088898	0.000	0.185	0.037	0.963	0.029	0.408	0.159
VZ43	Veracruz	Presa cangrejera	-94.298844	18.093411	0.000	0.096	0.016	0.984	0.008	0.362	0.114
VZ44	Veracruz	Presa cangrejera	-94.332372	18.102960	0.007	0.353	0.168	0.832	0.023	0.356	0.134
VZ45	Veracruz	Presa cangrejera	-94.316791	18.137566	0.000	0.286	0.053	0.947	0.072	0.540	0.258
VZ46	Veracruz	Presa cangrejera	-94.323469	18.131620	0.000	0.123	0.021	0.979	0.062	0.469	0.221
VZ47	Veracruz	Sontecomapan	-95.008509	18.526873	0.000	0.061	0.010	0.990	0.000	0.201	0.000
TB01	Tabasco	Lag. Rosario	-93.824848	17.836293	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB02	Tabasco	Lag. Rosario	-93.777913	17.843365	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB08	Tabasco	Lag. Caracol	-93.322473	17.833712	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB09	Tabasco	Lag. Caracol	-93.333310	17.840623	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB10	Tabasco	Lag. Caracol	-93.340849	17.842596	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB11	Tabasco	Lag. Caracol	-93.342444	17.830325	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB12	Tabasco	Lag. Caracol	-93.346783	17.824142	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB13	Tabasco	Lag. Caracol	-93.338316	17.820554	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB14	Tabasco	Lag. Caracol	-93.334700	17.819490	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB15	Tabasco	Lag. Las Ilusiones	-92.939133	17.992474	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB16	Tabasco	Lag. Las Ilusiones	-92.936861	17.996060	0.000	0.001	0.001	0.999	0.000	0.000	0.000
TB17	Tabasco	Lag. Las Ilusiones	-92.931012	17.997234	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB18	Tabasco	Lag. Las Ilusiones	-92.933133	18.003759	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB19	Tabasco	Lag. Las Ilusiones	-92.923273	18.010282	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB20	Tabasco	Lag. Las Ilusiones	-92.924480	18.017085	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB21	Tabasco	Lag. Las Ilusiones	-92.942076	18.013778	0.000	0.001	0.000	1.000	0.000	0.000	0.000
TB22	Tabasco	Paraiso	-93.160863	18.398507	0.202	0.773	0.475	0.525	0.107	0.645	0.336

TB23	Tabasco	Paraiso	-93.168180	18.404753	0.442	1.000	0.763	0.237	0.126	0.707	0.372
TB24	Tabasco	Pantanos de Centla	-92.661778	18.335496	0.000	0.001	0.000	1.000	0.000	0.134	0.000
TB25	Tabasco	Pantanos de Centla	-92.670365	18.309710	0.000	0.001	0.000	1.000	0.000	0.222	0.000
TB26	Tabasco	Pantanos de Centla	-92.648381	18.277524	0.000	0.001	0.000	1.000	0.006	0.333	0.093
TB27	Tabasco	Pantanos de Centla	-92.674069	18.314894	0.000	0.001	0.000	1.000	0.004	0.274	0.070
TB28	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.000	0.001	0.000	1.000	0.006	0.308	0.090
TB29	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.000	0.117	0.014	0.986	0.053	0.435	0.197
TB29-A	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.931	1.000	0.987	0.013	0.598	1.000	0.877
TB30	Tabasco	Yumka	-92.771889	18.013489	0.000	0.001	0.001	0.999	0.028	0.411	0.159
TB31	Tabasco	Yumka	-92.805843	17.991547	0.000	0.001	0.002	0.998	0.031	0.439	0.173
CP01	Campeche	Manguito	-92.161841	18.666062	0.428	0.892	0.667	0.333	0.328	0.847	0.592
CP02	Campeche	Pom Atasta	-92.256715	18.548067	0.000	0.135	0.039	0.961	0.000	0.179	0.000
CP03	Campeche	Pom Atasta	-92.274635	18.538772	0.000	0.136	0.039	0.961	0.003	0.216	0.052
CP04	Campeche	Pom Atasta	-92.284620	18.533553	0.000	0.173	0.050	0.950	0.007	0.375	0.116
CP05	Campeche	Pom Atasta	-92.289700	18.523867	0.000	0.140	0.040	0.960	0.000	0.110	0.000
CP06	Campeche	Pom Atasta	-92.299332	18.495548	0.005	0.235	0.091	0.909	0.000	0.156	0.000
CP07	Campeche	Pom Atasta	-92.291855	18.511583	0.063	0.448	0.235	0.765	0.031	0.423	0.169
CP08	Campeche	Pom Atasta	-92.264917	18.542731	0.002	0.292	0.110	0.890	0.057	0.467	0.213
CP10	Campeche	Rio Chumpan	-91.511359	18.367294	0.163	0.639	0.388	0.612	0.135	0.657	0.362
CP11	Campeche	Rio Chumpan	-91.532363	18.321073	0.127	0.537	0.314	0.686	0.113	0.583	0.313
CP12	Campeche	Rio Chumpan	-91.498598	18.413111	0.292	0.755	0.525	0.475	0.179	0.716	0.428
CP12-A	Campeche	Rio Chumpan	-91.498598	18.413111	0.185	0.675	0.424	0.576	0.178	0.797	0.468
CP13	Campeche	Pital	-91.036970	18.622459	0.050	0.392	0.198	0.802	0.054	0.443	0.202
CP14	Campeche	Sistema Pargo	-91.260245	18.511432	0.010	0.409	0.181	0.819	0.028	0.418	0.161
CP15	Campeche	Sistema Pargo	-91.267360	18.528772	0.435	0.909	0.679	0.321	0.269	0.790	0.525
CP16	Campeche	Sistema Pargo	-91.256343	18.598602	0.103	0.546	0.309	0.691	0.041	0.536	0.221
CP18	Campeche	Rio Caribe	-90.592815	18.227076	0.199	0.671	0.425	0.575	0.107	0.599	0.322
CP19	Campeche	Rio Champoton	-90.717450	19.362779	0.000	0.174	0.052	0.948	0.004	0.244	0.063
CP20	Campeche	Rio Champoton	-90.695545	19.358687	0.458	0.920	0.697	0.303	0.383	0.905	0.657
CP20-A	Campeche	Rio Champoton	-90.685833	19.328080	0.000	0.240	0.078	0.922	0.000	0.331	0.045
CP20-B	Campeche	Rio Champoton	-90.680565	19.319492	0.078	0.456	0.248	0.752	0.091	0.519	0.267

CP21	Campeche	Hampolol	-90.377368	19.942784	0.000	0.185	0.056	0.944	0.005	0.316	0.086
CP22	Campeche	Hampolol	-90.380194	19.940457	0.092	0.513	0.286	0.714	0.091	0.563	0.292
CP23	Campeche	Hampolol	-90.378826	19.941440	0.194	0.629	0.401	0.599	0.096	0.546	0.281
CP24	Campeche	Hampolol	-90.381110	19.939630	0.291	0.757	0.523	0.477	0.122	0.636	0.344
CP25	Campeche	Hampolol	-90.375525	19.944365	0.098	0.543	0.299	0.701	0.053	0.528	0.243
CP26	Campeche	Hampolol	-90.375525	19.944365	0.001	0.313	0.100	0.900	0.005	0.332	0.091
CP28	Campeche	Peten	-90.482520	20.210533	0.098	0.482	0.271	0.729	0.074	0.539	0.262
CP29	Campeche	Peten	-90.485810	20.221311	0.129	0.546	0.320	0.680	0.099	0.548	0.286
CP29-A	Campeche	Peten	-90.475210	20.230127	0.550	0.951	0.767	0.233	0.645	1.000	0.944
CP29-B	Campeche	Peten	-90.478311	20.228308	0.257	0.767	0.507	0.493	0.250	0.786	0.512
CP29-C	Campeche	Peten	-90.481966	20.225753	0.000	0.180	0.053	0.947	0.000	0.209	0.000
CP29-D	Campeche	Peten	-90.487227	20.228616	0.283	0.754	0.511	0.489	0.150	0.618	0.359
CP29-E	Campeche	Peten	-90.487298	20.226673	0.021	0.389	0.176	0.824	0.008	0.384	0.122
CP30	Campeche	El Remate	-90.381173	20.539410	0.001	0.238	0.084	0.916	0.000	0.146	0.000
CP31	Campeche	El Remate	-90.418850	20.542841	0.000	0.145	0.042	0.958	0.000	0.105	0.000
CP32	Campeche	El Remate	-90.421323	20.543251	0.001	0.254	0.091	0.909	0.000	0.143	0.000
CP33	Campeche	El Remate	-90.456290	20.551486	0.026	0.337	0.159	0.841	0.065	0.496	0.235
CP34	Campeche	El Remate	-90.448907	20.560033	0.026	0.367	0.172	0.828	0.004	0.297	0.077
CP35	Campeche	El Remate	-90.417406	20.608397	0.028	0.349	0.165	0.835	0.063	0.485	0.229
CP36	Campeche	El Remate	-90.417843	20.630067	0.028	0.347	0.163	0.837	0.033	0.444	0.179
CP37	Campeche	Isla Arena	-90.440655	20.684636	0.138	0.541	0.323	0.677	0.115	0.608	0.327
CP38	Campeche	Isla Arena	-90.442601	20.708209	0.001	0.217	0.078	0.922	0.081	0.475	0.240
CP39	Campeche	Isla Arena	-90.451098	20.702321	0.440	0.878	0.666	0.334	0.290	0.854	0.575
CP40	Campeche	Isla Arena	-90.448560	20.693314	0.144	0.559	0.338	0.662	0.050	0.442	0.193
CP41	Campeche	Isla Arena	-90.441120	20.692406	0.139	0.556	0.329	0.671	0.003	0.249	0.060
CP42	Campeche	Isla Arena	-90.440091	20.694540	0.003	0.295	0.115	0.885	0.021	0.330	0.121
CP43	Campeche	Isla Arena	-90.439284	20.694611	0.709	0.986	0.877	0.123	0.788	1.000	1.000
YC01	Yucatan	El Palmar	-90.331132	20.999664	0.012	0.311	0.133	0.867	0.004	0.267	0.066
YC02	Yucatan	El Palmar	-90.317733	21.014613	0.177	0.633	0.396	0.604	0.175	0.649	0.389
YC03	Yucatan	El Palmar	-90.298457	21.030374	0.012	0.311	0.133	0.867	0.004	0.267	0.066
YC04	Yucatan	El Palmar	-90.256057	21.060057	0.029	0.458	0.217	0.783	0.086	0.567	0.286

YC05	Yucatan	El Palmar	-90.261774	21.055684	0.022	0.417	0.191	0.809	0.065	0.506	0.237
YC06	Yucatan	El Palmar	-90.250148	21.063386	0.096	0.570	0.316	0.684	0.059	0.481	0.220
YC07	Yucatan	El Palmar	-90.256444	21.058542	0.197	0.681	0.430	0.570	0.124	0.565	0.312
YC07-A	Yucatan	El Palmar	-90.291403	21.018888	0.380	0.877	0.635	0.365	0.250	0.767	0.499
YC07-B	Yucatan	El Palmar	-90.294127	21.014678	0.450	0.953	0.717	0.283	0.295	0.778	0.535
YC07-C	Yucatan	El Palmar	-90.303947	21.006634	0.237	0.777	0.505	0.495	0.074	0.537	0.261
YC08	Yucatan	Sisal	-90.053368	21.159017	0.088	0.604	0.335	0.665	0.081	0.578	0.281
YC09	Yucatan	Sisal	-90.067137	21.158766	0.178	0.713	0.435	0.565	0.165	0.709	0.416
YC09-A	Yucatan	Sisal	-90.054427	21.160256	0.405	0.907	0.660	0.340	0.313	0.822	0.567
YC09-B	Yucatan	Sisal	-90.056372	21.159918	0.280	0.766	0.517	0.483	0.167	0.624	0.373
YC09-C	Yucatan	Sisal	-90.059090	21.159495	0.058	0.504	0.261	0.739	0.036	0.487	0.199
YC09-D	Yucatan	Sisal	-90.049405	21.157391	0.578	0.963	0.793	0.207	0.381	0.866	0.639
YC09-E	Yucatan	Sisal	-90.049405	21.157391	0.430	0.865	0.650	0.350	0.078	0.729	0.356
YC10	Yucatan	San Felipe	-88.227613	21.551921	0.351	0.859	0.602	0.398	0.231	0.730	0.466
YC11	Yucatan	San Felipe	-88.225460	21.552005	0.444	0.945	0.704	0.296	0.258	0.778	0.511
YC12	Yucatan	San Felipe	-88.216059	21.550634	0.026	0.322	0.150	0.850	0.017	0.297	0.104
YC13	Yucatan	San Felipe	-88.208049	21.557335	0.003	0.180	0.063	0.937	0.003	0.222	0.053
YC14	Yucatan	San Felipe	-88.189439	21.560120	0.473	0.952	0.725	0.275	0.182	0.688	0.417
YC15	Yucatan	San Felipe	-88.197914	21.556224	0.209	0.693	0.438	0.562	0.077	0.479	0.235
YC16	Yucatan	San Felipe	-88.179672	21.564551	0.284	0.778	0.525	0.475	0.221	0.666	0.429
YC17	Yucatan	Rio Lagartos	-88.153065	21.578410	0.022	0.359	0.162	0.838	0.050	0.550	0.242
YC18	Yucatan	Rio Lagartos	-88.060133	21.589632	0.147	0.638	0.377	0.623	0.058	0.462	0.212
YC19	Yucatan	Rio Lagartos	-88.056833	21.591748	0.193	0.702	0.436	0.564	0.079	0.492	0.242
YC20	Yucatan	Rio Lagartos	-88.055328	21.592665	0.286	0.841	0.561	0.439	0.204	0.764	0.471
YC21	Yucatan	Rio Lagartos	-88.057922	21.594351	0.216	0.689	0.443	0.557	0.081	0.495	0.245
YC22	Yucatan	Rio Lagartos	-88.005962	21.578107	0.016	0.376	0.164	0.836	0.000	0.158	0.000
YC23	Yucatan	Rio Lagartos	-88.041217	21.577864	0.319	0.801	0.557	0.443	0.250	0.730	0.478
YC24	Yucatan	El Cuyo	-87.644764	21.458028	0.702	0.993	0.882	0.118	0.555	0.983	0.829
YC25	Yucatan	El Cuyo	-87.645685	21.458794	0.656	0.990	0.858	0.142	0.631	1.000	0.921
YC26	Yucatan	El Cuyo	-87.656388	21.461179	0.683	0.992	0.871	0.129	0.514	0.978	0.804
YC27	Yucatan	El Cuyo	-87.656008	21.512701	0.796	0.997	0.927	0.073	0.738	1.000	0.990

YC28	Yucatan	El Cuyo	-87.685641	21.507163	0.554	0.952	0.771	0.229	0.517	0.991	0.790
YC29	Yucatan	El Cuyo	-87.638364	21.506523	0.201	0.721	0.454	0.546	0.004	0.312	0.079
YC30	Yucatan	El Cuyo	-87.638364	21.506523	0.274	0.745	0.506	0.494	0.193	0.722	0.441
YC31	Yucatan	Chipepte	-87.538193	21.484287	0.784	0.997	0.922	0.078	0.604	0.968	0.835
YC32	Yucatan	Chipepte	-87.540051	21.478036	0.816	0.998	0.935	0.065	0.654	0.975	0.862
YC33	Yucatan	Chipepte	-87.542369	21.482892	0.756	0.996	0.911	0.089	0.619	0.971	0.846
YC33-A	Yucatan	Chipepte	-87.544279	21.478861	0.643	0.989	0.849	0.151	0.406	0.896	0.667
YC34	Yucatan	Ranchos	-87.971992	21.445612	0.009	0.313	0.126	0.874	0.006	0.325	0.093
YC35	Yucatan	Ranchos	-87.988679	21.459559	0.272	0.847	0.555	0.445	0.110	0.581	0.310
YC36	Yucatan	Ranchos	-87.980169	21.439976	0.078	0.602	0.319	0.681	0.031	0.471	0.181
YC37	Yucatan	Ranchos	-87.972021	21.445659	0.179	0.674	0.415	0.585	0.127	0.651	0.353
YC38	Yucatan	Ranchos	-87.962846	21.433915	0.284	0.822	0.548	0.452	0.145	0.648	0.372
YC39	Yucatan	Ranchos	-87.969702	21.432999	0.405	0.899	0.655	0.345	0.194	0.713	0.437
YC40	Yucatan	Ranchos	-87.962814	21.433763	0.256	0.771	0.504	0.496	0.037	0.472	0.194
YC41	Yucatan	Ranchos	-87.980206	21.440044	0.100	0.548	0.306	0.694	0.027	0.427	0.158
YC43	Yucatan	Dzilam	-88.866890	21.391827	0.276	0.783	0.519	0.481	0.214	0.688	0.435
YC44	Yucatan	Dzilam	-88.867888	21.391174	0.286	0.794	0.533	0.467	0.128	0.574	0.321
YC45	Yucatan	Dzilam	-88.869090	21.391895	0.130	0.597	0.349	0.651	0.146	0.665	0.379
QR01	Qroo	Rio Yalikin	-87.176357	21.439967	0.875	1.000	0.973	0.027	0.530	0.966	0.789
QR02	Qroo	Rio Yalikin	-87.175245	21.442703	0.353	0.948	0.633	0.367	0.241	0.724	0.471
QR03	Qroo	Rio Yalikin	-87.171652	21.433077	0.490	1.000	0.790	0.210	0.264	0.767	0.515
QR04	Qroo	Rio Yalikin	-87.175688	21.433137	0.121	0.703	0.398	0.602	0.111	0.591	0.317
QR07	Qroo	Moon Palace	-86.833287	20.983244	0.001	0.277	0.110	0.890	0.019	0.320	0.114
QR08	Qroo	Moon Palace	-86.833641	20.991399	0.001	0.423	0.177	0.823	0.091	0.510	0.263
QR09	Qroo	Moon Palace	-86.834583	20.988460	0.000	0.225	0.065	0.935	0.021	0.345	0.124
QR10	Qroo	Moon Palace	-86.838222	20.985260	0.049	0.464	0.229	0.771	0.032	0.428	0.172
QR11	Qroo	Moon Palace	-86.835469	20.982869	0.000	0.252	0.074	0.926	0.000	0.150	0.000
QR12	Qroo	Moon Palace	-86.843447	20.988958	0.207	0.676	0.432	0.568	0.151	0.618	0.361
QR13	Qroo	Moon Palace	-86.846688	20.985790	0.001	0.245	0.089	0.911	0.003	0.258	0.063
QR13-A	Qroo	Moon Palace	-86.849357	20.988862	0.000	0.356	0.134	0.866	0.021	0.344	0.124
QR13-B	Qroo	Moon Palace	-86.845763	20.989830	0.003	0.448	0.197	0.803	0.064	0.520	0.241

QR14	Qroo	Mayacoba	-87.024827	20.690499	0.000	0.268	0.076	0.924	0.062	0.479	0.224
QR15	Qroo	Mayacoba	-87.024827	20.690499	0.561	1.000	0.806	0.194	0.074	0.595	0.279
QR15-A	Qroo	Mayacoba	-87.024827	20.690499	0.809	1.000	0.951	0.049	0.305	0.947	0.643
QR16	Qroo	Xcaret	-87.111819	20.586217	0.152	0.643	0.387	0.613	0.006	0.359	0.101
QR17	Qroo	Xcaret	-87.111819	20.586217	0.187	0.720	0.435	0.565	0.166	0.650	0.388
QR18	Qroo	Xcaret	-87.113466	20.588385	0.597	1.000	0.857	0.143	0.363	0.934	0.665
QR19	Qroo	Xcaret	-87.112978	20.589878	0.112	0.570	0.320	0.680	0.079	0.535	0.267
QR20	Qroo	Punta Allen	-87.497563	19.807935	0.072	0.490	0.257	0.743	0.090	0.666	0.340
QR21	Qroo	Felipe Carrillo Pto.	-88.042901	19.473937	0.648	1.000	0.896	0.104	0.154	0.704	0.408
QR22	Qroo	Felipe Carrillo Pto.	-88.054740	19.475571	0.895	1.000	0.977	0.023	0.302	0.856	0.591
QR23	Qroo	Felipe Carrillo Pto.	-88.081324	19.468733	0.880	1.000	0.974	0.026	0.086	0.732	0.370
QR24	Qroo	Felipe Carrillo Pto.	-88.066687	19.473936	0.903	1.000	0.979	0.021	0.546	1.000	0.829
QR26	Qroo	Felipe Carrillo Pto.	-88.026892	19.471332	0.780	1.000	0.947	0.053	0.330	0.848	0.596
QR28	Qroo	Rio Hondo	-88.337496	18.486960	0.824	1.000	0.958	0.042	0.104	0.690	0.356
QR29	Qroo	Rio Hondo	-88.367995	18.486615	0.809	1.000	0.956	0.044	0.245	0.911	0.588
QR30	Qroo	Rio Hondo	-88.382482	18.480181	0.866	1.000	0.971	0.029	0.331	0.876	0.619
QR31	Qroo	Rio Hondo	-88.386449	18.482179	0.787	1.000	0.948	0.052	0.305	0.870	0.603
QR32	Qroo	Rio Hondo	-88.402319	18.495678	0.882	1.000	0.974	0.026	0.324	0.872	0.610
QR33	Qroo	Rio Hondo	-88.392208	18.487816	0.498	1.000	0.793	0.207	0.041	0.523	0.220
QR34	Qroo	Rio Hondo	-88.402673	18.456735	0.795	1.000	0.951	0.049	0.153	0.841	0.491
QR35	Qroo	Caobas	-88.989324	18.457354	0.000	0.289	0.096	0.904	0.000	0.229	0.000
QR36	Qroo	Caobas	-88.989204	18.457442	0.000	0.151	0.037	0.963	0.000	0.237	0.000
QR38	Qroo	Chajul	-92.132447	15.880450	0.746	1.000	0.933	0.067	0.456	0.935	0.720
QR39	Qroo	Belice	-88.402673	18.456735	0.029	0.658	0.336	0.664	0.006	0.390	0.101
QR40	Qroo	Belice	-88.402673	18.456735	0.000	0.244	0.063	0.937	0.006	0.351	0.098
QZ01	Cozumel	Cozumel	-86.979117	20.281455	0.945	1.000	0.992	0.008	1.000	1.000	1.000
QZ02	Cozumel	Cozumel	-86.979117	20.281455	0.904	1.000	0.986	0.014	1.000	1.000	1.000
QZ03	Cozumel	Cozumel	-86.979117	20.281455	0.940	1.000	0.991	0.009	1.000	1.000	1.000
QZ04	Cozumel	Cozumel	-86.979117	20.281455	0.813	1.000	0.964	0.036	1.000	1.000	1.000
QZ05	Cozumel	Cozumel	-86.979117	20.281455	0.956	1.000	0.993	0.007	1.000	1.000	1.000
QZ06	Cozumel	Cozumel	-86.979117	20.281455	0.917	1.000	0.988	0.012	1.000	1.000	1.000

QZ07	Cozumel	Cozumel	-86.979117	20.281455	0.917	1.000	0.988	0.012	1.000	1.000	1.000
QZ08	Cozumel	Cozumel	-86.979117	20.281455	0.915	1.000	0.988	0.012	1.000	1.000	1.000
QZ09	Cozumel	Cozumel	-86.979117	20.281455	0.802	1.000	0.961	0.039	1.000	1.000	1.000
QZ10	Cozumel	Cozumel	-86.979117	20.281455	0.913	1.000	0.987	0.013	1.000	1.000	1.000
QZ11	Cozumel	Cozumel	-86.979117	20.281455	0.825	1.000	0.974	0.026	1.000	1.000	1.000
QZ12	Cozumel	Cozumel	-86.979117	20.281455	0.834	1.000	0.969	0.031	1.000	1.000	1.000
QZ13	Cozumel	Cozumel	-86.979117	20.281455	0.914	1.000	0.988	0.012	1.000	1.000	1.000
QZ14	Cozumel	Cozumel	-86.979117	20.281455	0.958	1.000	0.993	0.007	1.000	1.000	1.000
QZ15	Cozumel	Cozumel	-86.979117	20.281455	0.971	1.000	0.995	0.005	1.000	1.000	1.000
BC01	Chinchorro	Chinchorro	-87.320031	18.584038	0.992	1.000	0.998	0.002	1.000	1.000	1.000
BC02	Chinchorro	Chinchorro	-87.320031	18.584038	0.992	1.000	0.998	0.002	1.000	1.000	1.000
BC03	Chinchorro	Chinchorro	-87.320031	18.584038	0.984	1.000	0.996	0.004	1.000	1.000	1.000
BC04	Chinchorro	Chinchorro	-87.320031	18.584038	0.962	1.000	0.993	0.007	1.000	1.000	1.000
BC05	Chinchorro	Chinchorro	-87.320031	18.584038	0.992	1.000	0.998	0.002	1.000	1.000	1.000
BC06	Chinchorro	Chinchorro	-87.320031	18.584038	0.993	1.000	0.998	0.002	1.000	1.000	1.000
BC07	Chinchorro	Chinchorro	-87.320031	18.584038	0.994	1.000	0.998	0.002	1.000	1.000	1.000
VT01	Ventanilla	Ventanilla	-96.579730	15.671462	0.952	1.000	0.992	0.008	0.698	1.000	0.966
VT02	Ventanilla	Ventanilla	-96.579730	15.671462	0.626	1.000	0.870	0.130	1.000	1.000	1.000
VT03	Ventanilla	Ventanilla	-96.579730	15.671462	0.912	1.000	0.987	0.013	1.000	1.000	1.000
VT04	Ventanilla	Ventanilla	-96.579730	15.671462	0.928	1.000	0.989	0.011	1.000	1.000	1.000
VT05	Ventanilla	Ventanilla	-96.579730	15.671462	0.915	1.000	0.987	0.013	1.000	1.000	1.000
VT06	Ventanilla	Ventanilla	-96.579730	15.671462	0.000	0.188	0.043	0.957	0.042	0.500	0.214
VT07	Ventanilla	Ventanilla	-96.579730	15.671462	0.000	0.276	0.082	0.918	0.071	0.512	0.248
VT08	Ventanilla	Ventanilla	-96.579730	15.671462	0.785	1.000	0.964	0.036	0.464	0.969	0.748
VT09	Ventanilla	Ventanilla	-96.579730	15.671462	0.934	1.000	0.990	0.010	1.000	1.000	1.000
VT10	Ventanilla	Ventanilla	-96.579730	15.671462	0.952	1.000	0.992	0.008	1.000	1.000	1.000
VT11	Ventanilla	Ventanilla	-96.579730	15.671462	0.948	1.000	0.992	0.008	1.000	1.000	1.000
VT12	Ventanilla	Ventanilla	-96.579730	15.671462	0.826	1.000	0.972	0.028	1.000	1.000	1.000
VT13	Ventanilla	Ventanilla	-96.579730	15.671462	0.929	1.000	0.989	0.011	1.000	1.000	1.000
VT14	Ventanilla	Ventanilla	-96.579730	15.671462	0.585	1.000	0.837	0.163	0.324	0.902	0.641

Table S3. Canonical Discriminant Analysis (Grimm 1973) using Fisher’s linear discriminant analysis (Fraley & Raftery 2002), based on seven morphological measures from *Crocodylus moreletii*, *C. acutus* and hybrid individuals from the Gulf of Mexico and the Caribbean. The species assignment for every individual is based on the genetic data from the hybrid index estimation (see Results in text).

		Predicted group %		
		<i>Crocodylus moreletii</i>	Hybrids	<i>Crocodylus acutus</i>
Original	<i>Crocodylus moreletii</i>	81.4	16.3	2.3
	Hybrids	44.7	48	7.2
	<i>Crocodylus acutus</i>	0	0	100
Cross validation	<i>Crocodylus moreletii</i>	72.1	20.9	7
	Hybrids	45.4	46.1	8.6
	<i>Crocodylus acutus</i>	0	0	100

Capítulo 2

Species boundaries and homoploid hybrids: revisiting hybridization in crocodiles

Species boundaries and homoploid hybrids: revisiting hybridization in crocodiles

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Abstract

Interspecific hybridization plays a key role in the processes of diversification and adaptation, and eventually, if providing an evolutionary advantage, it can lead to speciation. However, species concept leaves out intermediate hybrids because the species boundaries in hybrid zones are genetically and morphologically fuzzy. We combined data from mitochondrial and nuclear molecular markers from *Crocodylus acutus*, *Crocodylus moreletii* and hybrids between these species throughout their entire distribution in Mexico, to evaluate if boundaries between divergent species and hybrids could be distinguished. We also aimed to explain the historical, demographic, and genomic features that characterize this unique hybridization system. Our results showed a complex mosaic of admixed and nonadmixed individuals along the entire distribution of crocodiles in Mexico. The species boundaries were indeed fuzzy and complex to resolve, despite the fact that hybridization is ancestral (our data indicated 2.6 million years). Phylogenetic analyses, phylogeographic patterns and divergence times showed an ancient genetic exchange between individuals from the Gulf of Mexico and the Pacific, and also that hybridization began in the Gulf of Mexico, near Campeche (a historical sympatric zone), which later extended throughout the Gulf to the north and the Caribbean on the south. Five groups were genetically identified, concordant with the geographical cline: non-admixed *C. moreletii*, hybrids, non-admixed *C. acutus* from Caribbean islands, non-admixed *C. acutus* from northwestern and central Pacific coasts, and non-admixed *C. acutus* from southern Pacific localities. The evidence supports that *C. moreletii* might going through a process of extinction as a species or, in our perspective, likely evolving via hybridization. Finally, our results also showed deviation from model of neutral introgression (underdominance and positive selection) in favor of hybrids which, in combination with the phylogenetic relationships observed, suggest a likely ongoing process of homoploid hybrid speciation.

Keywords: crocodylians, divergence, species boundaries, phylogeography, selection, speciation

Introduction

Interspecific hybridization plays a key role in the processes of diversification and adaptation, and eventually in the formation of new species (Gompert *et al.* 2006; Mallet 2007; Abbott *et al.* 2010; Brennan *et al.* 2012; Gante *et al.* 2016). Hybridization can have very distinct outcomes (see Abbott *et al.* 2013), amongst which are cases where a certain balance between selection and hybridization occurs, for example in hybrid zones involving a balance between selection against hybrids and dispersal (tension zones; Barton & Hewitt 1985), in which only some parts of the genome are introgressed between hybridizing populations. In such cases, differentiation might be maintained with no speciation in progress, although with the potential for future divergence between populations (Mallet 2007; Arnold *et al.* 2012).

Hybrid speciation is one key outcome from hybridization, for which two main models are recognized, allopolyploidy and homoploid hybrid speciation. Buerkle & Rieseberg (2008) indicate that in homoploid hybrid speciation, the rate of genome stabilization “is on the order of hundreds of generations; and is no way to know at what time the last significant or critical component of isolation became fixed and speciation is complete”. Although it is still rare to find cases of homoploid hybrid speciation in vertebrates, hybridization and introgression between species is a regular occurrence (Seehausen 2004; Mallet 2007; Schumer *et al.* 2014; Abbott *et al.* 2016). Ultimately, if the hybridization process provides an evolutionary advantage, it can lead to lineages divergence (Gompert & Buerkle 2016). Importantly, introgression via interspecific hybridization is one means for species to acquire beneficial alleles and have the potential to adapt. Adaptive introgression has been detected in nature, evidencing that introgression can be an efficient way to adapt, for instance by enabling populations to respond rapidly to environmental changes, or to cope with different environments via the presence of novel traits in hybrids (Hedrick 2013; Llopart *et al.* 2014).

Mallet (2007) has proposed there is a hyperspace of possible phenotypes and genotypes that can be represented as an adaptive landscape, in which adaptive peaks are scattered about, many of which are unoccupied. Although most early recombinants will be unfit, extreme hybrids can colonize niches unavailable to parents. In the specific case of homoploid hybrids, their progeny can have high genetic variances either via recombination and/or transgressive variation (i.e. phenotypes more extreme than either parent). Such variation may allow hybrid homoploids reach these new adaptive peaks, far from parental optima (Mallet 2007).

It is important to highlight that different characteristics of the hybridization process and the hybrid zone can hinder the identification of hybrid species. Notably, where hybrid homoploidy exists, defining species is complex because species boundaries are semipermeable (by gene

exchange) (Harrison & Larson 2014), genetically and morphologically fuzzy (Pacheco-Sierra *et al.* 2016), and continuous in phylogenetic and phylogeographic terms (Edwards *et al.* 2016). In addition, when hybridization is spread along a wide geographic region encompassing a mosaic hybrid zone formed by secondary contact, identifying reproductive isolation becomes very difficult, especially between parental species and hybrids that occupy the same ecological niche (Pacheco-Sierra *et al.* 2016). Moreover, the prevalent thought is that recent, rapidly radiating and closely related species are most likely to hybridize, as well as those with short generation times (Seehausen 2004; Mallet 2007; Schumer *et al.* 2014; Abbott *et al.* 2016); and that interspecific hybrids are rare at the population level, encompassing less than 0.1% of individuals (Mallet 2005, 2007).

In great contrast to the above expectations and as to which features are expected in populations where hybrids are established, Pacheco-Sierra *et al.* (2016) found evidence of a geographically widespread hybrid zone between two species of crocodiles formed from secondary contact along the Gulf of Mexico and the Caribbean. Using microsatellite loci, they clearly identified the non-admixed parental —and isolated— populations, as well as a third genetic group harboring a gradient of admixture proportions formed by a high number of hybrid individuals (66% of the population of the two species), throughout a wide geographical scale (>2000 km). Hybrids are morphologically intermediate and, although their microsatellite genotypes are characteristic of both parental forms, hybrids form an allele frequency cluster distinguishable from either parent. Also, their results support a scenario in which hybridization began thousands of years ago (142.9 generations, in this long-lived species).

In the present study we expand both the geographic extent and genetic coverage to analyze the entire hybrid zone between *Crocodylus acutus* and *C. moreletii* in Mexico, including the distribution of the parental populations, based on different types of molecular markers (microsatellites, mitochondrial DNA sequences) to explain the historical, demographic, and genomic features that characterize this unique hybridization system. We explore four key questions: (i) When did the hybridization process between *Crocodylus acutus* and *C. moreletii* start? (ii) What are the main types of selection (underdominance, overdominance or directional selection) associated with the hybridization between the two crocodile species? (iii) Can different lineages be distinguished, both in terms of non-admixed and intermediate hybrid individuals? Our ultimate goal was to discern if species boundaries (homoploid hybrids) could be defined in this hybridization system where incomplete reproductive isolation is most common and where the species concept leaves out intermediate hybrid individuals; that is, if this hybridization could be leading to speciation.

Methods

Data collection

We sampled 374 individuals from 92 localities across the distribution of the recognized species *Crocodylus acutus* and *C. moreletii* in Mexico (Fig. 1, Table S1 Supporting Information).

Crocodile individuals were captured and marked following a unique pattern by clipping tail scutes, following a numbered code, and liberated at the capture site (see Pacheco-Sierra *et al.* 2016 for details of the sampling scheme). Samples for genetic analyses included tail scutes and blood.

We extracted genomic DNA from each sample with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. We assessed the quantity and quality of the DNA in a microvolume spectrophotometer NanoDrop 2000 Thermo Scientific™.

mtDNA sequencing

We amplified the mitochondrial cytochrome *b* gene (*cyt b*) of a subset of 271 individuals, with the primers crCYTBF and crCYTBR (Weaver *et al.* 2008), and the gene region encompassing tRNA^{Pro}-tRNA^{Phe}-d-loop with primers L15459 and CR2HA (Glenn *et al.* 2002; Ray & Densmore 2002). Amplification of both fragments was carried out in 25 µL reaction volumes containing: 25-50ng template DNA, 1 unit of Taq DNA polymerase (Vivantis, Selangor, Malaysia), 2 mM MgCl₂, 0.2 µM dNTPs, 10X PCR Buffer, 4X Bovine serum albumin (BioLabs, Ipswich, MA, USA) and 0.5-1.5 µM of each primer. Polymerase chain reaction conditions were performed in a PTC-100 thermocycler (M.J. Research) and consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 1 min, 72°C for 45 sec and a final extension at 72°C for 5 min. Sample purification and sequencing was performed by the UW High Throughput Genomics Center, Seattle, WA. Forward and reverse sequences for each individual were aligned and edited manually using Geneious 9.0.5 (<http://www.geneious.com>; Kearse *et al.* 2012). All chromatograms were reviewed to visually identify heterozygous sites and errors in base calling.

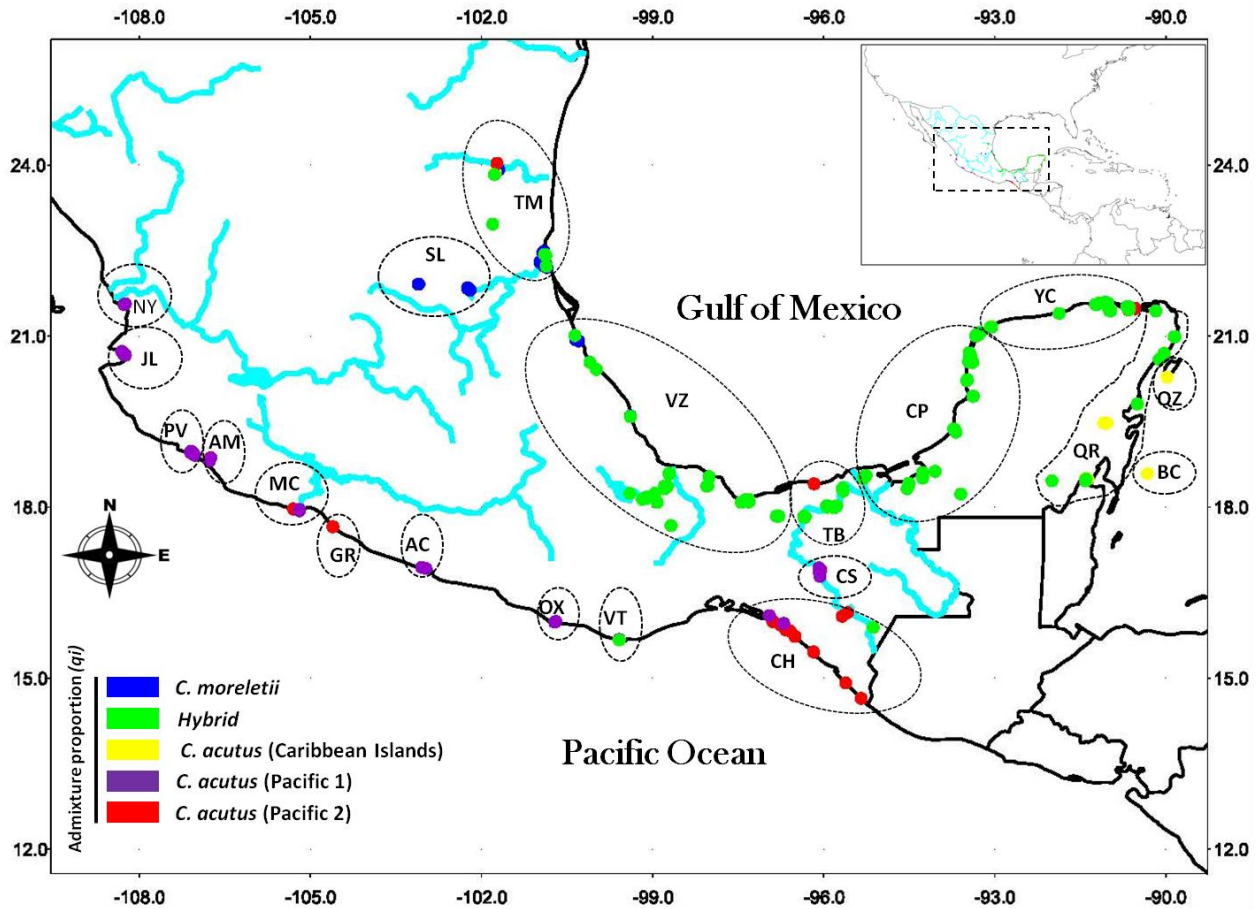


Fig. 1 Map of sampling localities for *Crocodylus acutus* and *C. moreletii* throughout Mexico. Every dot indicates individuals and sampling localities; the blue lines represent the river system. The dots represent Admixture proportions ($K=5$) obtained with Structure (Pritchard *et al.* 2000) and supported for phylogenetic analysis (see below), where different colors indicate ancestry for *C. acutus* from islands (yellow), *C. acutus* from Pacific (group 1; purple), *C. acutus* from Pacific (group 2; red), *C. moreletii* ancestry (blue) and hybrids (green) Samples are ordered following the geographic cline from northeast to southeast along the Gulf of Mexico and Caribbean, following then along the Pacific from southwest to northwest. In order to place the sampling localities at the level of state in the map and in association with their description in Table S1 (Supporting Information), we grouped them (dotted circles) and codes refer to: TM: Tamaulipas, SL: San Luis Potosí, VZ: Veracruz, TB: Tabasco, CP: Campeche, YC: Yucatán, QR: Quintana Roo, QZ: Cozumel, BC: Banco Chinchorro, CS: Cañon del Sumidero, CH: Chiapas, VT: Ventanilla, OX: Chachagua, AC: Acapulco, GR: Guerrero, MC: Michoacan, AM: Amela, PV: Palo Verde, JL: Jalisco and NY: Nayarit.

Genetic structure and differentiation of hybrids

For the present study, we amplified 109 crocodile individuals from the Mexican Pacific coast. Microsatellite genotyping of the new 109 individuals was performed using the same 12 polymorphic microsatellite loci and following the amplification protocols as in Pacheco-Sierra *et al.* (2016). In order to detect non-admixed and hybrid individuals for the species' entire distribution, we considered the new data together with the 265 individuals from Pacheco-Sierra *et al.* (2016). We analyzed the microsatellite data (genotypes per individual) following the same sequence of analyses as in Pacheco-Sierra *et al.* (2016), considering the new complete database (374 samples).

To examine the genetic structure, we first performed a spatially independent analysis based on a Bayesian inference of admixture proportions, that is the proportion of each individual's genome derived from each source population i (q_i), using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). We calculated the probability of individual assignment into population clusters (K) without prior information on the origin of individuals. We conducted several tests using maximum number of populations from $K=2$ to $K=10$, with the admixture model with correlated allelic frequencies, a length value set at 1,000,000 iterations and a burn-in period of 100,000 MCMC repetitions and 10 simulations per K . Each test yielded a log likelihood value of the data (\ln probability for K). We used the Evanno's ΔK test to estimate the maximum number of clusters (Evanno *et al.* 2005); data were processed with STRUCTURE HARVESTER (Earl & vonHoldt 2012) and results were summarized and averaged using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007).

Next, to explore if a potential hybridization pattern was observed considering the entire distribution of the two species in Mexico, we performed a Maximum-likelihood estimation of hybrid indexes with the R package INTROGRESS (Gompert & Buerkle 2010). For this new dataset, non-admixed individuals were identified from admixture proportions based on STRUCTURE results, which showed the same Gulf/Caribbean non-admixed individuals as those identified in Pacheco-Sierra *et al.* (2016), while new non-admixed individuals from Pacific localities were recognized. These individuals were then used to define the source populations to polarize the hybrid index, where a value of $h=0$ corresponds to non-admixed *C. moreletii* and $h=1$ corresponds to non-admixed *C. acutus* individuals (see Pacheco-Sierra *et al.* 2016 for details).

We performed the genomic clines method of Gompert & Buerkle (2009) with the R package INTROGRESS (Gompert & Buerkle 2010), to investigate the genetic architecture of reproductive isolation and adaptative introgression. This method allows to estimate individual-

based clines in genotype frequency as a function of genome-wide admixture (hybrid index) for each molecular locus, and to determine whether these estimated clines are consistent with a null model of neutral introgression (Nolte *et al.* 2009). Specifically, the genomic clines method uses multinomial regression to predict the probability of a given genotype for a locus as a function of genome-wide admixture (e.g., hybrid index between a pair of species or divergent populations; Buerkle 2005). Expected genomic clines can then be generated based on a null model of neutral introgression and then compared with genomic clines from the observed data to identify loci with patterns of introgression inconsistent with neutral expectations (types of selection in hybrids) (Gompert & Buerkle 2009, 2010; Nolte *et al.* 2009). We estimated the genetic diversity (observed and expected heterozygosity) and number of alleles, effective alleles and private alleles using GenAlEx 6.5 (Peakall & Smouse 2012) per sampling locality and regarding the genetic groups identified with STRUCTURE (see Results). To explore the pattern of genetic structure between sampling localities (in a geographic context) and genetic groups, we estimated Nei's genetic distances (Nei *et al.* 1983), migration (Nem), inbreeding (Fis) and differentiation (Fst ; Wright 1951 and Rst ; Slatkin 1991).

The results of Hybrid index (h) and genetic structure (qi) from microsatellites data served as a basis to establish genetic clusters across all the individuals of *Crocodylus* and further analyze hybridization patterns with the mitochondrial data. For that, we performed a Principal Component Analysis (PCA) to examine patterns of variation and covariation between the observed polymorphic sites for the unique haplotypes. We constructed plots based on the first two-principal components and incorporating the hybrid differentiation information: the hybrid index per individual (h) and the assignment proportion (qi) obtained with STRUCTURE (microsatellites). Next, we performed a Canonical Discriminant Analysis (CDA), based on Fisher lineal discriminant analysis (Fralely & Raftery 2002), PCA and CDA was performed in R; (R Development Core Team 2015). We followed the same classification criteria (h and qi as in the PCA), in order to explore if the CDA could distinguish the main groups we had hypothesized (parental and hybrids clusters).

Phylogenetic and diversity analyses

Pacheco-Sierra *et al.* (2016) found that the hybrid zone between *C. acutus* and *C. moreletii* is geographically large, extending well beyond their historical region of sympatry. They also identified a few geographically isolated, non-admixed populations of both parental species, and that hybrids are likely later-generation hybrids, where hybridization has been going on for many generations. Considering that estimation of the historical onset of the hybridization process

requires the use of DNA sequence data, our mitochondrial information can help solve this intriguing question. Accordingly, we performed different analyses to describe the phylogenetic relationships among the two species along their entire distribution in Mexico (both Gulf of Mexico and Pacific coasts), to determine how they are genetically structured, spatially and temporally, and to explore if parentals and hybrids can be differentiated at the more historical, mitochondrial level.

To explore the contribution of each species into the hybridization process between *C. moreletii* and *C. acutus*, we evaluated the genealogical relationships between individuals via phylogenetic analyses both for each gene separately and the concatenated set, considering individuals as operational units (OTU's). We used the Akaike information criterion scores as implemented in the program jModelTest 0.1.1 (Posada 2008) to select the bestfitting models of sequence evolution for our datasets, which were used subsequently for maximum-likelihood (ML) and Bayesian inference (BI) methods.

ML was conducted with PhyML 3.0 (Guindon *et al.* 2010), using NNI+SPR for branch length and topology optimization. Clade support was assessed with 1,000 non-parametric bootstrap replicates. For the Bayesian inference, implemented in MrBayes 3.1 (Huelsenbeck & Ronquist 2001), we used default settings and four chains sampled every 1000 generations for 50 million generations. Convergence and stationarity within chains were visualized with Tracer 1.4 (Drummond & Rambaut 2007); 25% of generations were discarded as burn-in. We estimated the 50% majority-rule consensus topology and posterior probabilities for each node with the remaining trees. We included sequences from *C. intermedius* (NC015648) and *C. rhombifer* (NC024513), the sister species of *C. acutus* and *C. moreletii*, respectively, and chose the African *C. niloticus* (AJ810452) as outgroup, which is the ancestor of the American crocodylians (Oaks 2011).

We estimated standard genetic diversity estimates within species with DnaSP 5.10 (Librado & Rozas 2009) for each gene separately: nucleotide diversity (p), haplotype diversity (h) and average number of differences among sequences (k). To evaluate if sequences conformed to a neutral model of evolution we also estimated Tajima's D (Tajima 1989), Fu and Li's F (Fu & Li 1993) and Fu's F_S (Fu 1997); statistical significance was determined using the coalescent simulator in DnaSP.

To further investigate the relationships among haplotypes, we constructed unrooted networks among unique haplotypes, using PopArt (available at: <http://popart.otago.ac.nz>), based on the median-joining method (Bandelt *et al.* 1999).

Divergence time estimation

In order to establish the time frame of the hybridization process between *C. acutus* and *C. moreletii* along their distribution area, we estimated divergence times (time to the most recent common ancestor, TMRCA). The analysis was done using a Coalescent Constant Size tree prior and a relaxed-clock dating implemented in BEAST 1.8.2 (Drummond *et al.* 2012), which allows estimation of divergence times simultaneously incorporating rate heterogeneity and uncertainty in the substitution parameters, tree topology, and calibration ages considering a Bayesian framework (Drummond *et al.* 2006; Brandley *et al.* 2010). We considered only unique haplotypes from a modified concatenated dataset discarding tRNA's and including only 1,576 bp of cyt *b+d*-loop mtDNA; the latter with the purpose of incorporating sequences from Oaks (2011) and the following calibration points: the *Mecitops-Crocodylus* split, the split between *C. niloticus* and American *Crocodylus*, the divergence within the *C. intemedius-C. acutus*, and within *C. rhombifer-C. moreletii* clades (Table S1). Final estimation included the GTR+I+G model of evolution across all gene and codon positions, 100,000,000 generations sampled every 5,000th and 25% of initial generations discarded as burnin.

Results

Genetic structure and differentiation of hybrids

Bayesian assignment results (STRUCTURE) show a sequential admixture proportion (q_i), where $K=2$ to $K=5$ (Fig. S1 in Supporting information) gradually separate the non-admixed from the hybrid individuals, where the Gulf of Mexico and the Pacific populations form five distinguishable separate clusters (Fig. 2C). Indeed, this pattern is highly concordant with the hybrid index results (h): the hybrids distribution for the Gulf of Mexico and Caribbean individuals is the same as the one in Pacheco-Sierra *et al.* (2016), showing few non-admixed and isolated individuals for both *C. acutus* and *C. moreletii*, whereas with the new data also admixed and non-admixed individuals from Pacific localities were identified (Fig. 2A, B). Hence, a range of hybrids throughout the entire distribution was observed (Fig. 2), in which five genetic groups were identified (Fig. 2C): two different groups of non-admixed *C. acutus* from the Pacific (group 1, purple; group 2, red), non-admixed *C. acutus* from the Caribbean islands (yellow), non-admixed *C. moreletii* from the Gulf of Mexico (blue), and hybrids predominantly from the Gulf of Mexico but also within some Pacific localities (green).

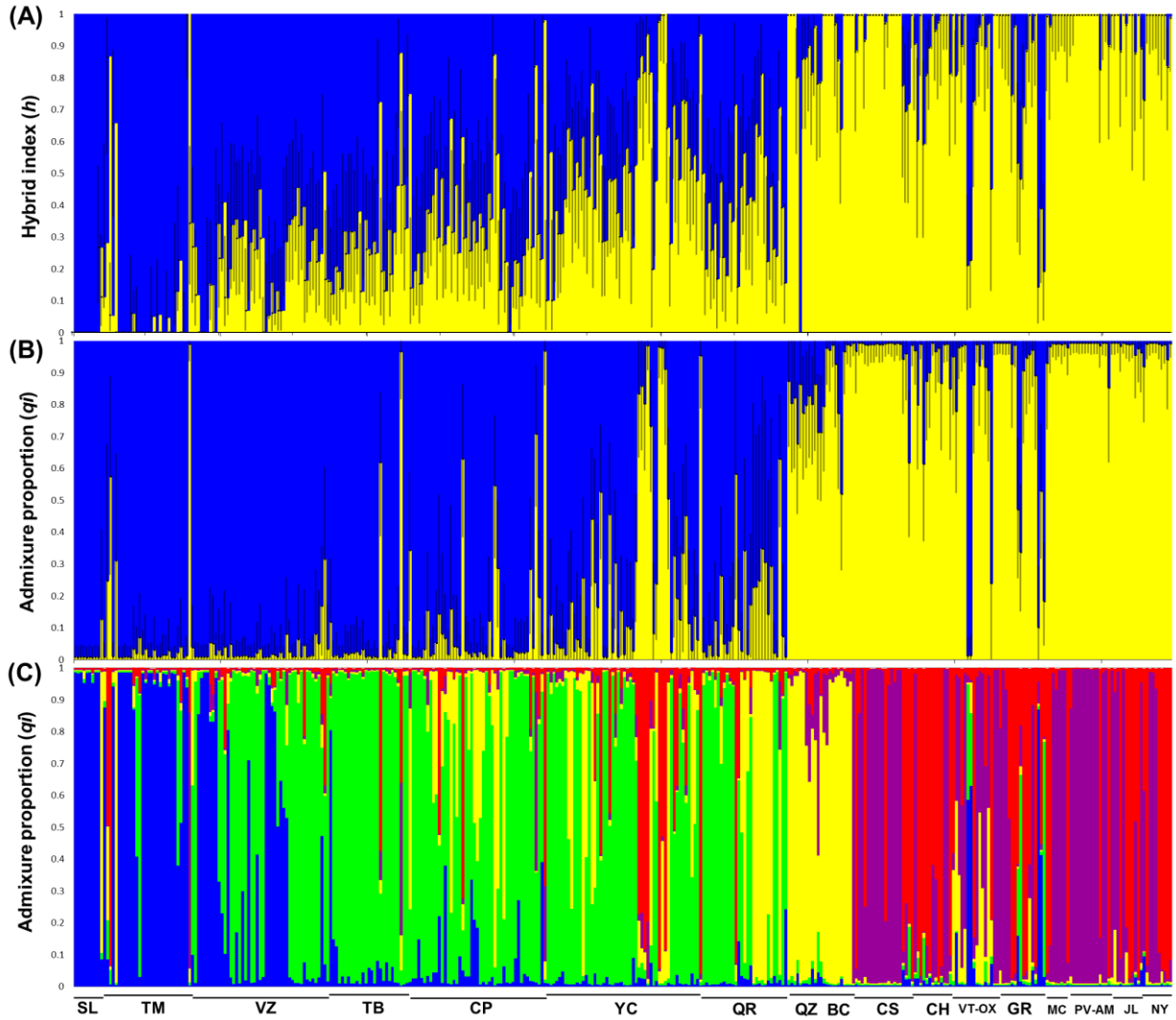


Fig. 2 Bar plots of (A) Maximum likelihood estimates of hybrid indexes (Buerkle 2005), obtained with nuclear markers (microsatellites), where solid lines are 95% confidence intervals and blue and yellow bars represent the statistical proportion of the genome inherited from *C. moreletii* ($1-h$) and *C. acutus* (h), respectively. Admixture proportions with $K=2$ (B), and $K=5$ (C) obtained with STRUCTURE (Pritchard *et al.* 2000), where different colors indicate ancestry for *C. acutus* from islands (yellow), *C. acutus* from Pacific (group 1; purple), *C. acutus* from Pacific (group 2; red), *C. moreletii* ancestry (blue) and hybrids (green) Samples are ordered following the geographic cline from northeast to southeast along the Gulf of Mexico and Caribbean, following then along the Pacific from southwest to northwest (see Table S1 Supporting Information).

The estimates of genomic clines showed that all loci deviate from a model of neutral introgression ($P \leq 0.005$, Fig. 3): five loci were consistent with a model of underdominance (disruptive selection): locus1 (Cj109), locus3 (Cj391), locus6 (Cj127), locus7 (Cj20) and locus9 (CUJ131), and four of these with negative selection (directional selection against P_2/P_2 ; locus 1, 3, 6 and 9). Three loci were consistent with a model of overdominance (balancing selection) in locus2 (Cj128), locus5 (Cj18) and locus11 (Cj119). Finally four loci were consistent with a model of positive selection (directional selection in favor P_1/P_1): locus4 (Cj16), locus8 (CUD68), Locus10 (Cj35) and locus12 (Cj131). The pattern of introgression and proportion of ancestry (Fig. 4) showed that introgression is bidirectional, where near half of the individuals have higher proportion to *C. acutus* ($h=1$) and another half to *C. moreletii* ($h=0$).

The PCA of polymorphic sites from the mitochondrial haplotypes showed that the two first components explained 63% of the variation. When organized based on the classification of the hybrid index and the admixture proportions ($K=5$), the PCA did not show a clear differentiation between clusters (Fig. S3, Supporting information). Nonetheless, the different groups can be readily observed with the CDA (Fig. 5), when using both $K=3$ (Wilk's $\lambda = 0.148$ $P < 0.0001$) and $K=5$ (Wilk's $\lambda = 0.025$, $P < 0.001$), where the group of hybrid individuals is significantly separated and 84.1% and 80.1% respectively, were classified correctly (Table S2, Supporting information). Genetic diversity was low ($H_o=0.121$; $H_e=0.238$) in one of the parental *C. moreletii* populations (lagoon in SLP), with high inbreeding ($F_{is}=0.682$) and no private alleles; contrastingly, *C. acutus* from the Caribbean islands (Cozumel and Banco Chinchorro) showed $H_o=0.689$, $H_e=0.654$; $H_o=0.333$, $H_e=0.389$; $F_{is}=-0.49$, $F_{is}=0.122$ respectively (see Table S3 and S4, Supporting information). Differentiation results (F_{st} and R_{st}) were highly significant between genetic groups and between populations ($P < 0.001$), except in adjacent populations, like San Luis and Tamaulipas (see Table S5 and S7, Supporting information). Accordingly, the highest number of migrants and lower genetic distance were between adjacent populations along the geographic gradient (see details in Table S6 and S8, Supporting information).

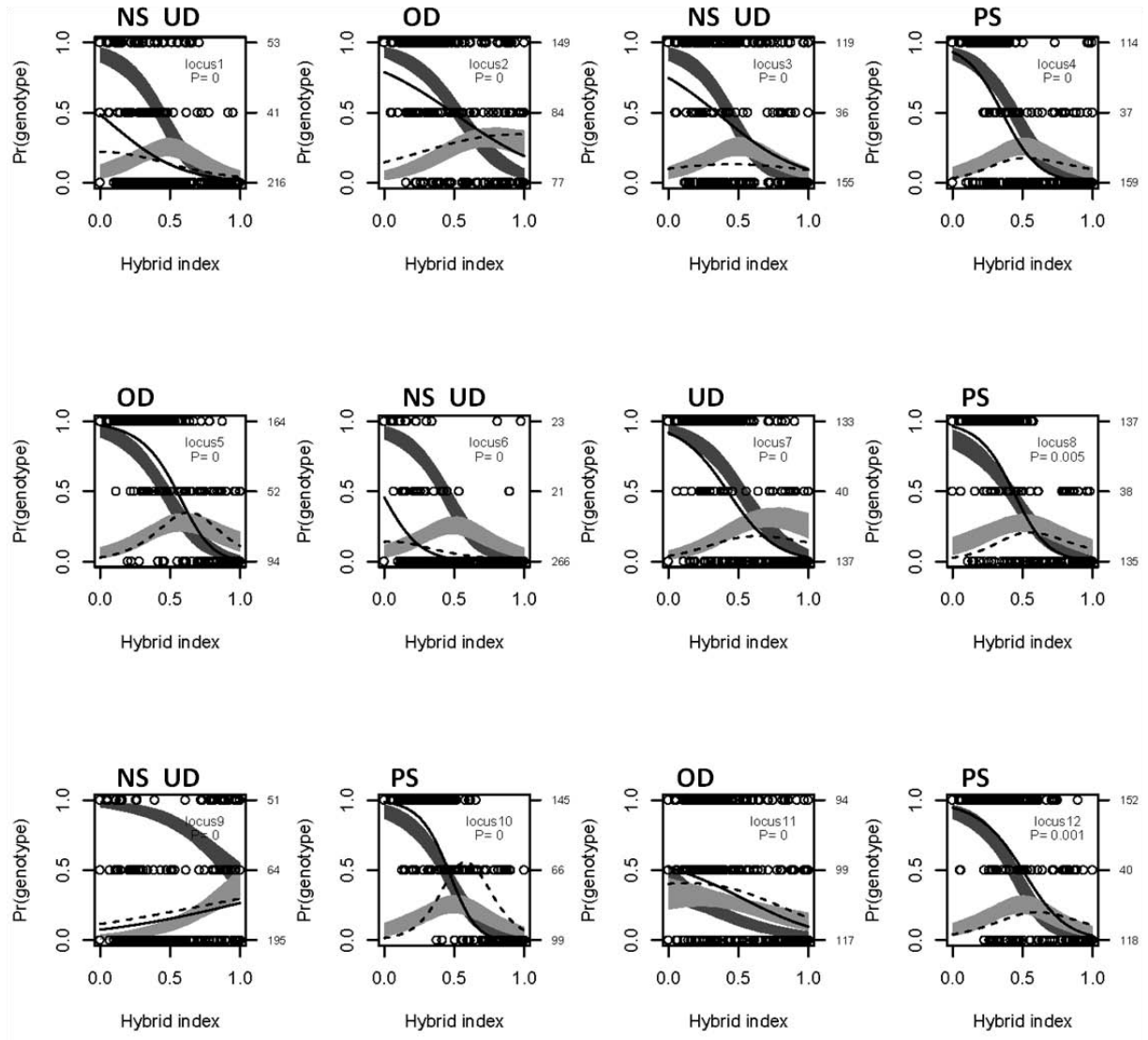


Fig. 3 Genomic cline plots for the 12 nuclear microsatellites used. The vertical axis shows the probability of observing a particular genotype at a locus. Locus number and P -value of the observed genotypic data under a model of neutral admixture are given in each plot. Solid coloured regions represent the 95% confidence intervals for the homozygous (P_1/P_1 ; dark gray) and heterozygous genotype (P_1/P_2 ; light gray), based on 1000 neutral simulations for each locus. The solid and dashed lines denote the genomic clines for the homozygous (P_1/P_1) and heterozygous genotypes (P_1/P_2), respectively, for the observed data. Open circles indicate the observed data and illustrate the relationship between hybrid index and the genotypic classes: homozygous genotype (P_1/P_1 on top and P_2/P_2 bottom) and heterozygous genotype (P_1/P_2 in the middle). Numbers to the right are number of individuals for the respective genotype categories. The following abbreviations denote the classification of loci based on the genomic clines method and accessory summary statistics, NS: consistent with a model of negative selection, PS: consistent with a model of positive selection, UD: consistent with a model of underdominance, OD: consistent with a model of overdominance.

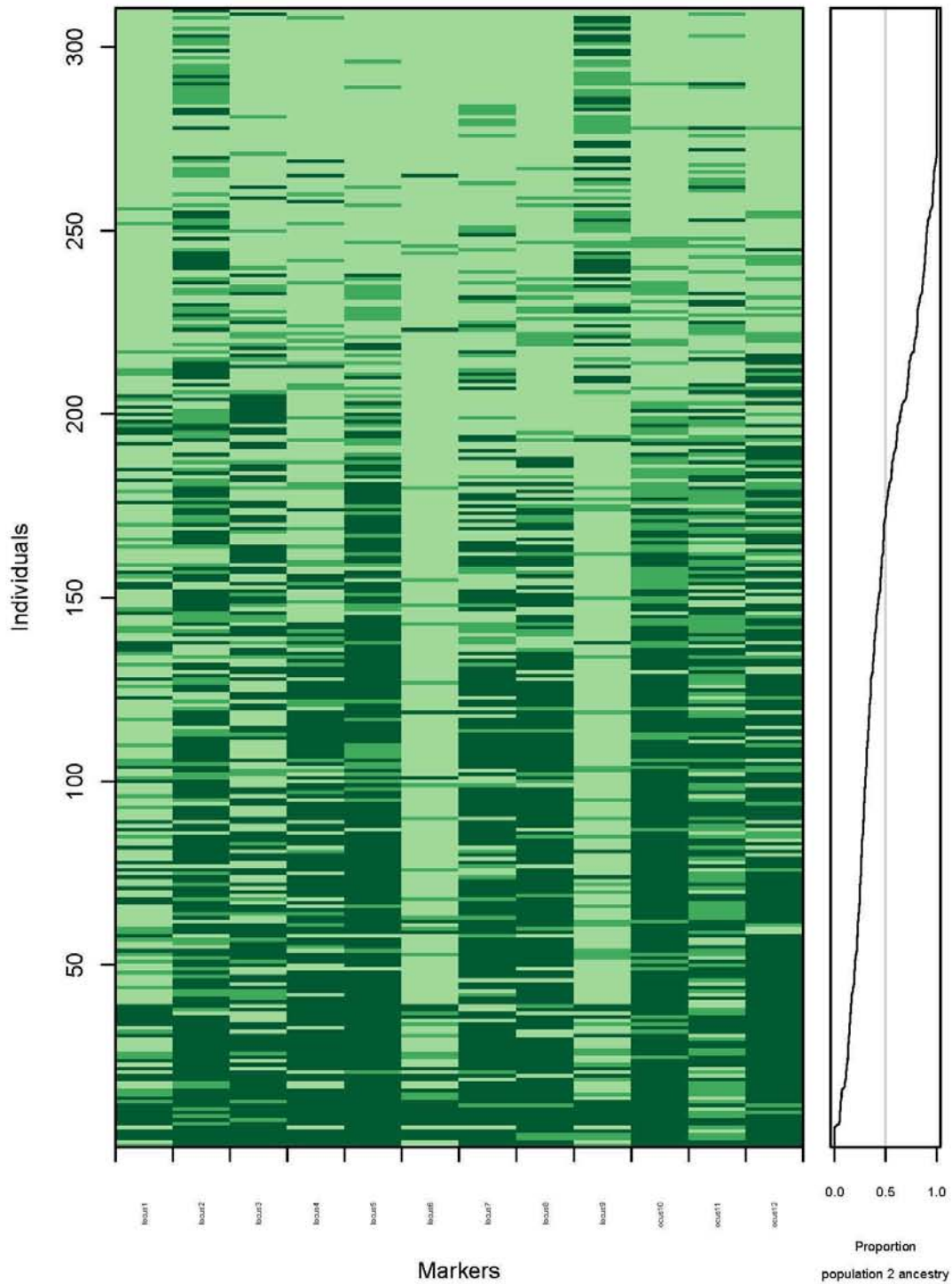


Fig. 4 Plot of introgression patterns for all loci and tested individuals. The individuals are sorted by geographical populations like in Figure 2. Each rectangle represent an individual's genotype at a given locus: dark green (P_1/P_1), green (P_1/P_2) and light green (P_2/P_2). On the right is shown the plot of the fraction of the genome inherited from population 2 for each individual (hybrid index).

Phylogenetic inference

We successfully amplified 1,009 bp and 721 bp of *cyt b* and of tRNA^{Pro}-tRNA^{Phe}-d-loop regions from mtDNA, respectively, across 271 individuals. The concatenated dataset included 123 polymorphic sites, 112 parsimony informative sites and 86 unique haplotypes, with high levels of haplotype diversity ($Hd=0.949$) and low nucleotide diversity ($\pi=0.023$). Average number of nucleotide differences between haplotypes (k) was 40.76. Significant departures from neutrality in molecular variation were found (Tajima's $D=3.05$; Fu and Li's $F=1.08$; Fu's $F_S=-0.51$; all $P<0.05$). The estimated Tr/Tv ratio was 3.74, with mean nucleotide frequencies as 29.13% A, 30.08% C, 13.95% G and 26.84% T. The best-fit model selected for our data was TIM3+I+G (pinv=0.66; $\alpha=0.67$), and the closest equivalent, GTR+I+G was used in subsequent analyses.

Phylogenetic inference methods (BI and ML) showed the same topology, both with each gene separately (not shown) and the concatenated dataset (Fig. S2 in Supporting information). Our phylogeny is similar to that of Oaks (2011) and (Meredith *et al.* 2011), where *C. acutus* appears as a sister species of *C. intermedius* and *C. moreletii* is the closest relative to *C. rhombifer*. Within each species group, a pectinate-like pattern and a continuous diversification were recovered (Fig. 6). There is no clear intraspecific phylogeographic pattern distinguishable, however the topology is highly concordant with the STRUCTURE clustering results and hybridization tests (see below).

We integrated the information about the admixture proportion (qi) from STRUCTURE ($K=5$) with the haplotype network. Results showed a highly concordant pattern with the phylogenetic topology: *C. moreletii* with a star-like configuration and several abundant haplotypes at the center of the network, and a group encompassing hybrid individuals with higher probabilities to *C. moreletii*; *C. acutus* exhibited a similar pattern, although here the dominant haplotypes are at the center (Pacific haplotypes) and the haplotypes from the Caribbean islands are on the edge, showing some unique and some widespread haplotypes with few mutations among them (Fig. 7). The networks for each species are deeply separated (i.e., connected by 66 mutational steps), while the haplotypes in the middle along the connection are hybrids from the Yucatan peninsula (mainly from YC and QR).

By combining the mitochondrial and nuclear genetic information (see Fig. 6), a degree of geographical concordance is evident, in which the phylogenetic tree (Bayesian inference; Fig. 6A), the hybrid index (Fig. 6B) and the admixture assignment ($K=2$ and $K=5$; Fig. 6C and D, respectively) show that the genetic groups are indeed separated by the microsatellites genetic ancestry information: non-admixed *C. acutus* from the Caribbean islands (QZ and BC) are grouped in one clade with the non-admixed *C. acutus* (8 of 11 individuals) from the Pacific

population Ventanilla (VT). This clade is embedded within the *C. acutus* entire clade, encompassing the non-admixed individuals from the Pacific (purple and red groups) and hybrid individuals from the Yucatan peninsula and the Pacific. A separate clade encompasses the non-admixed *C. moreletii* individuals, where all haplotypes are from the Gulf of Mexico and the Yucatan peninsula, and only six haplotypes (GR) from the Pacific. This clade includes one clearly differentiated group with all hybrids from TM, TB, VZ, YC, CP, QR and GR, sister to an unresolved remaining set of haplotypes, but where non-admixed *C. moreletii* from TM and SL are each grouped separately, among the other hybrid individuals from the Gulf of Mexico (YC, VZ, TB, CP, SL and VT).

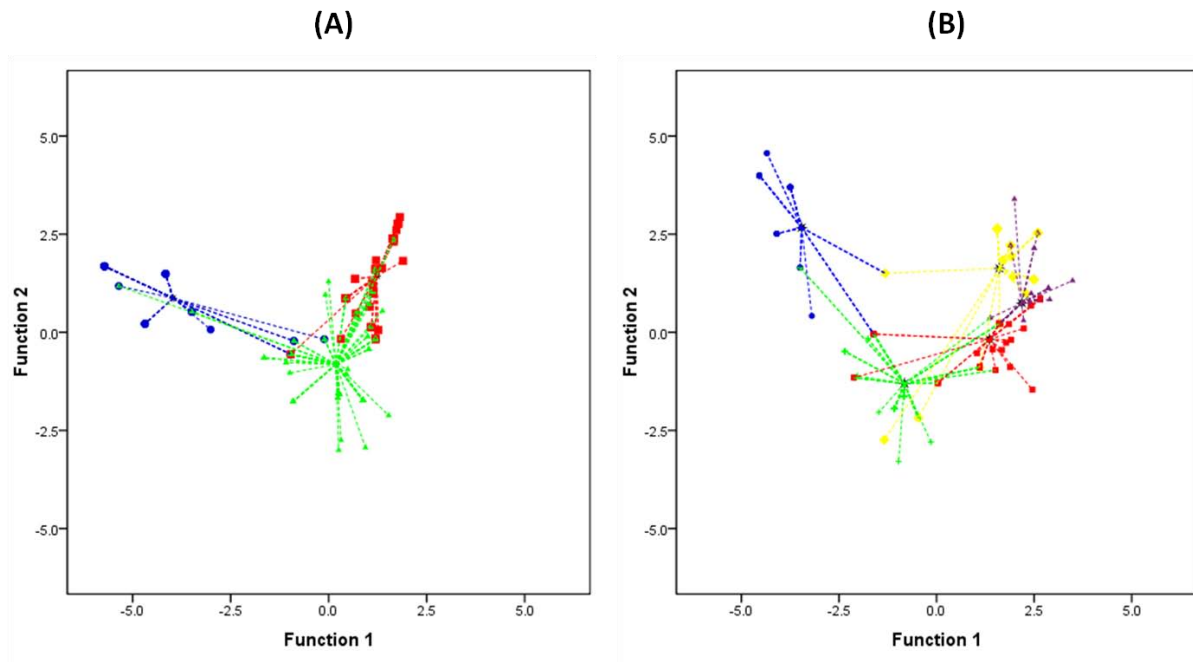


Fig. 5 Multivariate analyses: Canonical Discriminant Analysis based on 124 mtDNA polymorphic sites. (A) Dot colors are based on the statistical proportion of the hybrid index: non-admixed *C. moreletii* (blue), non admixed *C. acutus* (red) and admixed (green). (B) Dot colors are based on the admixture proportions (q_i) from the STRUCTURE (Pritchard *et al.* 2000) results ($K=5$): *C. acutus* ancestry (Caribbean islands: yellow, Pacific group 1: purple, Pacific group 2: red), *C. moreletii* ancestry (blue) and hybrids (green).

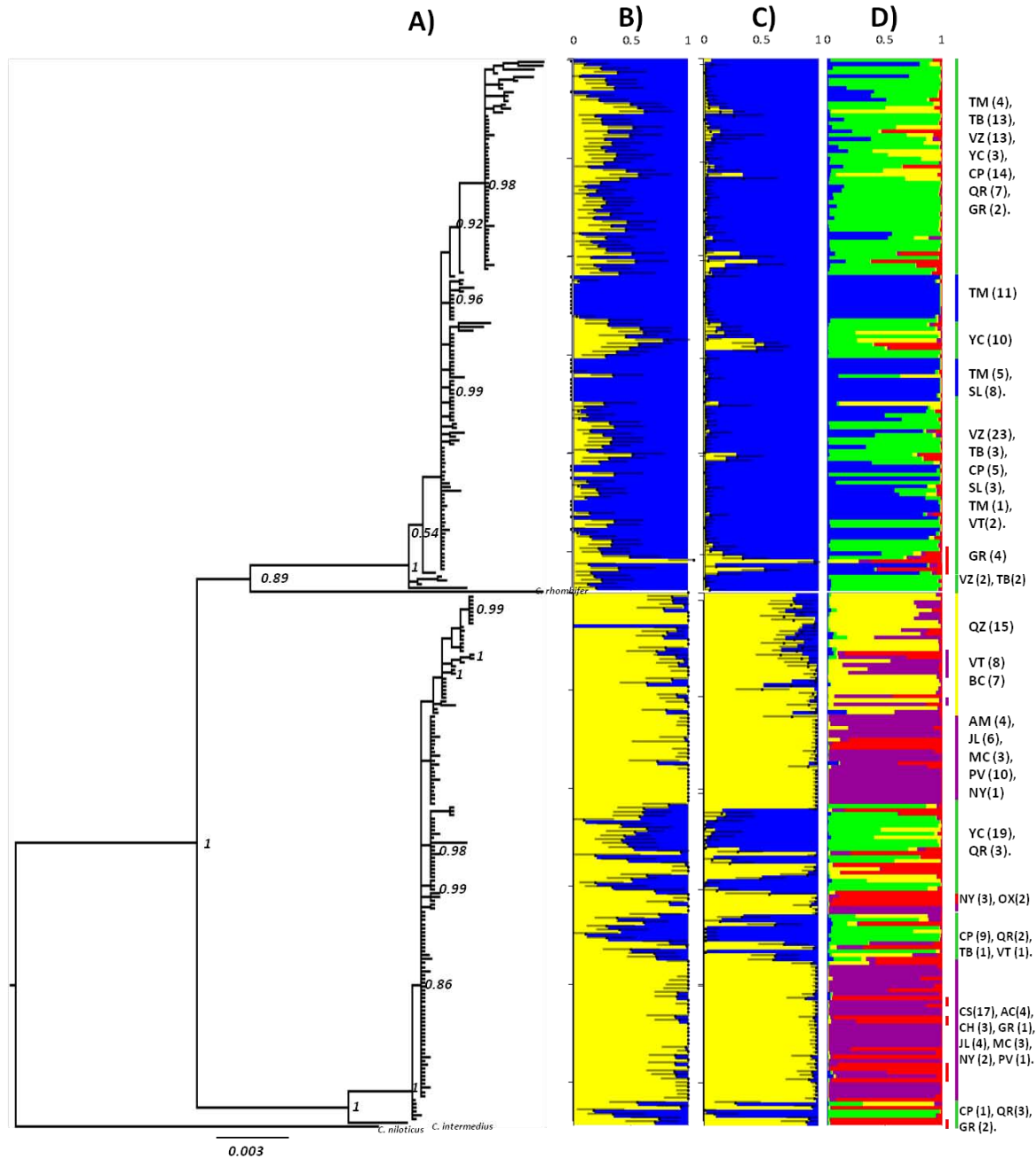


Fig. 6 (A) Individual-based Bayesian phylogenetic analysis based on concatenated cytb-tRNAPro-tRNAPhe-d-loop for *Crocodylus acutus* and *C. moreletii* from Mexico. (B) Maximum likelihood estimates of hybrid indexes (Buerkle 2005) obtained with nuclear markers (microsatellites), where solid lines are 95% confidence intervals and blue and yellow bars represent the statistical proportion of the hybrid index (h) for *C. moreletii* and *C. acutus*, respectively. (C) and (D) Admixture proportions with $K=2$ and $K=5$ respectively, from STRUCTURE, where different colours indicate the ancestry of the different clusters: cluster 1 (blue; *C. moreletii*), cluster 2 (green; hybrids), cluster 3 (yellow; *C. acutus-1* from the Caribbean islands), cluster 4 (purple; *C. acutus-2* from the Pacific) and cluster 5 (red; *C. acutus-3* from the

Pacific). Each horizontal bar in B, C and D corresponds to the individual (haplotype) as depicted from the Bayesian phylogenetic analysis (mtDNA).

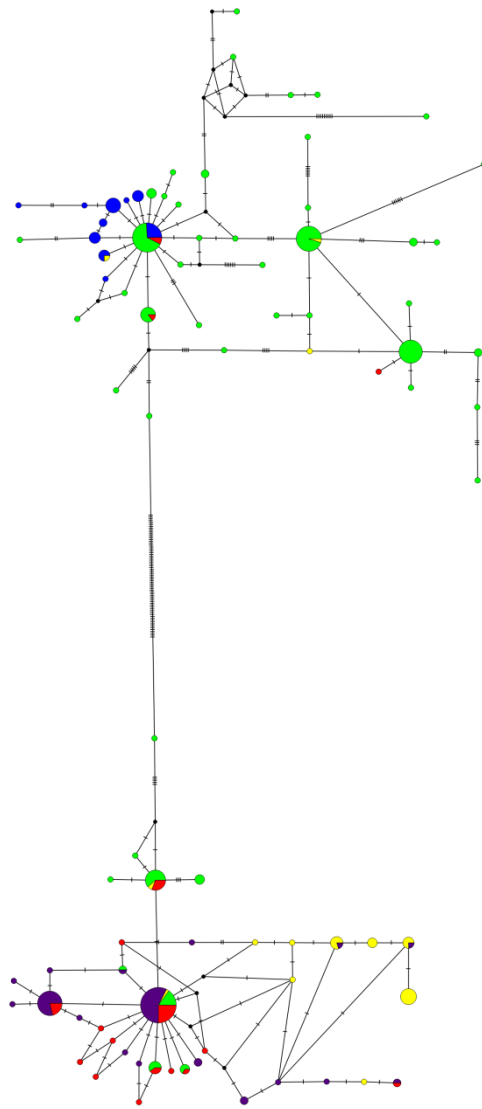


Fig. 7 Minimum network based on the mtDNA haplotypes for *Crocodylus acutus* and *C. moreletii* from Mexico. Circles represent haplotypes and circle size is proportional to haplotype frequency. Colour of circles represents the proportion of individuals classified as *C. acutus* from Caribbean islands (yellow), *C. acutus* from Pacific (group 1; purple), *C. acutus* from Pacific (group 2; red), *C. moreletii* (blue) and hybrids (green) based on the STRUCTURE (Pritchard *et al.* 2000), ($K=5$) and phylogenetic results.

Divergence time estimation

The dataset used for divergence time estimation showed 89 unique haplotypes, 51 of which were assigned to *C. moreletii* and 38 to *C. acutus*. The topology obtained with BEAST evidenced three *C. moreletti* and three *C. acutus* main lineages (Fig. 7). In accordance with our estimations, the divergence between *C. rhombifer* and the Mexican *C. moreletii* occurred 5.93 million years ago (My) (95% HPD: 4.51-7.44), while that between *C. intermedius* and *C. acutus* happened 5.08 My (95% HPD: 3.46-6.8). The TMRCA for *C. moreletii* was calculated at 4.51 My (95% HPD: 3.17-6.01) and for *C. acutus* was dated about 3.74 My (95% HPD: 2.32-5.35) (Table 1). Based on these mitochondrial results that depict the history of females, the hybridization between the two species can be traced back starting approximately 2.6 million years (HPD: 1.06-4.46) to as early as 600,000 years ago.

Table 1. Divergence time-estimation (million years; My) estimated with Beast (see Fig. 6) for *Crocodylus moreletii* and *Crocodylus acutus* from Mexico, based on mtDNA (cytochrome b). The estimated time and highest posterior density (HPD) are indicated for some nodes

Node	Estimated time (My)	95% HPD
A	24.39	19.61-29.94
B	23.13	19.04-27.48
C	7.3	6.09-8.79
D	6.2	5.21-7.33
E	5.52	4.02-7.23
F	4.8	3.91-5.76
G	4.35	3.24-5.29
H	4.21	2.77-5.73
I	2.47	1.06-4.46
J	0.28	0.01-1.78
K	0.23	0.01-0.76

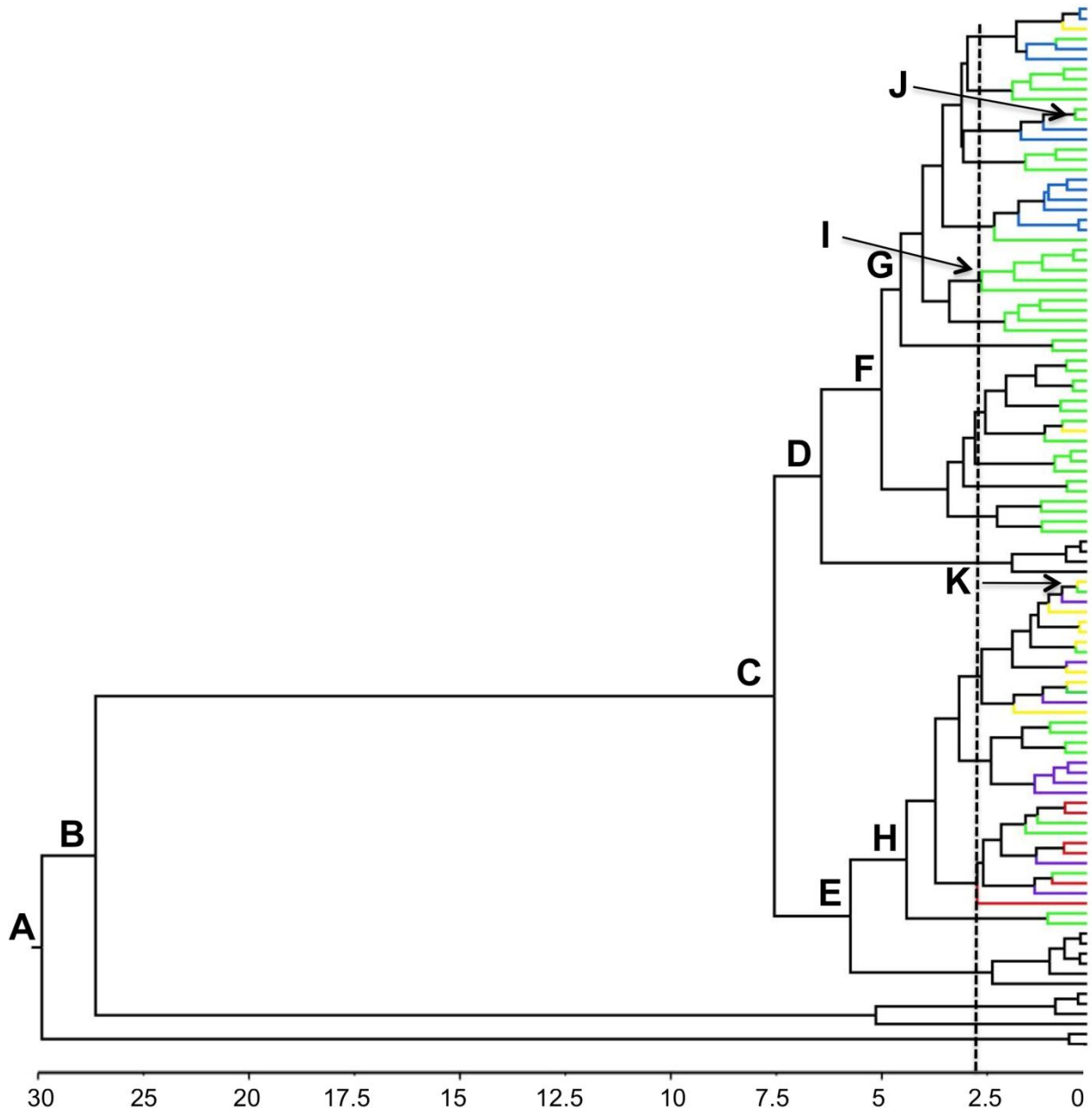


Fig. 8 Divergence times in million years (My; scale bar on the bottom) for *C. moreletii* and *C. acutus* from Mexico, estimated with BEAST and based on concatenated cytb-tRNAPro-tRNAPhe-d-loop. *Mecistops* sp was used as outgroup (GenBank H21719, H21720). The colored bars on the right represent the different genetic groups identified with STRUCTURE (Pritchard *et al.* 2000) (K=5): *C. acutus* from Caribbean islands (yellow), *C. acutus* from Pacific (group 1; purple), *C. acutus* from Pacific (group 2; red), *C. moreletii* (blue) and hybrids (green). The letters at internal nodes refer to divergence times (see Table 1).

Discussion

We here present the first study to evaluate the hybridization between *C. moreletii* and *C. acutus* encompassing the widest distribution on both the Mexican Atlantic and Pacific coasts. The combined use of nuclear and mitochondrial molecular markers to analyze the hybridization between both species along their entire distribution in Mexico revealed a complex mosaic of admixed and non-admixed individuals. Also, our genetic information showed fuzzy boundaries between the non-admixed (parental) species and the hybrids despite the fact that the hybridization process is ancestral (approximately 2.5 million years).

Crocodiles beyond borders: hybridization history between Crocodylus moreletii and C. acutus Pacheco-Sierra *et al.* (2016) found evidence of an extensive hybridization between *C. moreletii* and *C. acutus* in the Gulf of Mexico and Caribbean. They suggested that geographic barriers would likely prevent the genetic exchange between populations from the Gulf of Mexico and those along the Pacific (where only *C. acutus* is naturally distributed). However, our present evidence from the admixture proportions (STRUCTURE $K=5$, Fig. 1, 2), phylogenetic analysis (Fig. 6) and haplotype networks (Fig. 7) show an ancient genetic exchange between individuals from both sides of the continent: hybrids have admixture proportions from *C. acutus* (Caribbean Islands) and *C. acutus* group 2 (southern Pacific localities). The latter is supported by another key finding, the presence of specific *C. acutus* alleles (Fig. 2D) and haplotypes (Fig. 6) from the Caribbean Islands on individuals in a population from Oaxaca (VT; southern Pacific), together with evidence of *C. moreletii* haplotypes on individuals from two Pacific localities in Guerrero and Chiapas (GR y VT). Serrano-Gómez *et al.* (2016) found molecular evidence of the presence of *C. moreletii* in *C. acutus* populations from Oaxaca and Guerrero, the origin of which is now resolved with our study.

Although no overall clear phylogeographic pattern is observed, the mitochondrial and nuclear information reveal a congruent genetic and geographic configuration (Fig. 6), illustrating that hybridization began along the species' historical sympatric zone on the Gulf of Mexico (near Campeche; CP), which later extended throughout the Gulf to the north and the Caribbean on the south. The time of divergence observed is consistent with the hypothesis that the process of hybridization is ancient, as suggested by Pacheco-Sierra *et al.* (2016), indicating that it started around 2.6 million years ago (My), and continued throughout the overlapped historical distribution of both species along the Gulf of Mexico and the Caribbean. This is concordant with the geological history of the Yucatan peninsula, which slowly emerged from south to north, coinciding with historical connectivity of rivers across the southern narrowest part of Mexico,

near the Isthmus of Tehuantepec (Padilla y Sanchez 2007; Vázquez-Domínguez & Arita 2010). Along this region is that we found hybrids and haplotypes from the Caribbean islands. Moreover, the Quaternary climatic oscillations of the last 3 million years that triggered major warm and cold periods, including change of sea levels and floods (Hewitt 2011), could have facilitated dispersal of crocodile individuals between Atlantic and Pacific coasts, as observed for other aquatic species (Razo-Mendivil *et al.* 2013). Indeed, our diversification time results indicate that hybridization between the two species started around 2.6 My, coinciding with these climatic events. In addition, it is important to highlight that our results evidence that the hybridization between *C. moreletii* and *C. acutus* is not the result of human mediated movement as it has been suggested by González-Trujillo *et al.* (2012), since the admixture proportions and haplotypes are ancient, encompassing an extended, mosaic hybridization pattern, with no hybrids distributed in specific patches.

Revisiting the admixture pattern between Crocodylus acutus and C. moreletii

The phylogenetic results showed a clear divergence between the ancestral species, *C. acutus* and *C. moreletii*, in accordance with previous phylogenies (Meganathan *et al.* 2010; Meredith *et al.* 2011; Milián-García *et al.* 2011, 2015; Oaks 2011). Interestingly, five groups were genetically identified that are concordant with the extended geographic cline observed along the species entire distribution (Figs. 1, 2, 8): non-admixed *C. moreletii* (SL and TM), hybrids (almost all Gulf of Mexico and some Pacific individuals), non-admixed *C. acutus* (Caribbean islands), non-admixed *C. acutus* (group 1 from northwestern and central Pacific coast localities) and non-admixed *C. acutus* (group 2 from southern Pacific localities).

The results of hybridization and admixture proportions for the populations from the Gulf of Mexico and the Caribbean are mostly concordant with the patterns found by Pacheco-Sierra *et al.* (2016), although with a few differences that, because we now included new individuals from the Mexican Pacific, are the result of the parental ancestry being from those populations. Specifically, in Pacheco-Sierra *et al.* (2016) the Laguna Las Ilusiones sampling locality –in Tabasco (TB)– was identified as a non-admixed *C. moreletii* population, whereas we now found that it is composed of hybrid individuals. The latter is because their *C. acutus* ancestry in fact comes from Pacific localities. This corroborates what Pacheco-Sierra *et al.* (2016) discussed regarding potential false positives: “If one of the parental populations is small, extinct or poorly sampled, estimates of hybrid indexes or admixture proportions will be shifted, which could give false evidence of the presence or absence of hybrids”.

We detected evidence of introgression on both directions along the two species entire distribution in Mexico (Fig. 4), genetically and geographically, from the parental species on the extremes of the hybrid mosaic (northern and southern populations, *C. moreletii* and *C. acutus* respectively) to hybrids and vice versa. Our results also showed different types of selection in favor of hybrids which, in combination with the phylogenetic relationships observed, suggest a likely ongoing process of homoploid hybrid speciation. This is in agreement with the divergence times topology (node F, Fig. 8) that shows one monophyletic, divergent clade encompassing hybrid individuals from central Gulf of Mexico, separated from non-admixed *C. moreletii* and *C. acutus* populations. Genetic analyses (mtDNA and nDNA) and morphological analyses (Pacheco-Sierra *et al.* 2016) clearly identify a differentiated group of hybrids, again in agreement with a pattern of homoploid hybrid speciation. Importantly, this group is not geographically isolated, thus allowing “promiscuity” among groups, at least with those bounding this region, resulting in incomplete reproductive isolation. In addition, our genetic evidence suggests that hybridization between *C. moreletii* and *C. acutus* can be a source of genetic novelty, expressed as different allele combinations between non-admixed and hybrid individuals (i.e., adaptive introgression). However, species boundaries between non-admixed and admixed groups remain fuzzy, both despite and because of the ancient –and still ongoing– diversification and hybridization processes between the two crocodile species.

Types of selection and symmetry of introgression

The estimates of genomic clines show five microsatellite loci consistent with a model of underdominance, namely selection against hybrids resulting in disruptive selection (Gompert & Buerkle 2009, 2011; Larson *et al.* 2013). Such a process would suggest barriers to admixture, which is unlikely because hybridization in the present case is ancient, while reproductive isolation between the parental and hybrid groups is incomplete. Disruptive selection could be the result of independent environmental fitness or genetic incompatibility (Gompert & Buerkle 2009, 2010; Nolte *et al.* 2009; Larson *et al.* 2013), however four of the five markers under dominance exhibited directional selection against P_2/P_2 (*C. moreletii*), indicating a disadvantage to *C. moreletii*. The latter is a likely result based on the fact that the non-admixed *C. moreletii* populations are isolated, a result found both in Pacheco-Sierra *et al.* (2016) and in the present study. These populations have very low population numbers, which accordingly showed high homozygosity levels (low gene flow and low genetic diversity). Individual loci show little introgression towards any of the parental genotypes (Fig. 4), consistent with high rates of gene flow and admixture along the hybrid zone. This asymmetrical pattern of introgression is most

likely due to the lack of non-admixed *C. moreletii* populations. The fact that non-admixed *C. moreletii* individuals are so scarce along its historical natural distribution highlights that *C. moreletii* might be in the process of disappearing as a species or, in our perspective, likely evolving via hybridization.

On the other hand, three loci are consistent with a model of overdominance (balancing selection) or selection in favor of hybrids, suggesting an adaptive role for the hybrid genotypes, which may result in allele frequencies being balanced among populations (Rieseberg *et al.* 1999; Baack & Rieseberg 2007; Nolte *et al.* 2009; Meirmans 2012; Christe *et al.* 2017). Finally, four loci are consistent with a model of positive selection favouring the *C. acutus* genotype (Arnold & Martin 2009; Teeter *et al.* 2010; Soltis 2013; Owens *et al.* 2016; Christe *et al.* 2017). Accordingly, the types of selection observed, in favor of hybrids and non-admixed *C. acutus* alleles, suggest a process of adaptive introgression that could potentially lead to speciation (Arnold & Martin 2009; Gompert & Buerkle 2009; Sweigart 2009; Abbott *et al.* 2013; Soltis 2013; Hamilton & Miller 2016). Non-admixed populations, whether measured by sampling locality or by the genetic group identified (Structure), showed lower genetic diversity and higher inbreeding in comparison with hybrids, further supporting the isolation and reduced population sizes of the parentals. The results regarding genetic distance, migration rates and population structure were concordant with the genomic clines along the geographic gradient, however these must be taken with caution because, in hybridization systems like this one, consequences of gene flow do not depend on taxonomy or a specific definition of species, but rather on the nature of differences between groups (i.e. hybrids, non-admixed) (Gompert & Buerkle 2016). Estimates of genomic clones were based on twelve microsatellites only, and the results should be taken as suggestive of selection in favour of hybrids, however these are few markers to provide sufficient evidence, and more accurate analyzes are recommended that could provide solid evidence of evolutive process and selection forces operating in this hybridization process, based on more informative molecular markers (ie, SNPs).

Final thoughts

Our ultimate goal was to discern if species boundaries (homoploid hybrids) could be defined in this hybridization system where incomplete reproductive isolation is most common and where the species concept leaves out intermediate hybrid individuals; that is, if natural selection could be promoting selection in favor of hybrids, leading to speciation. The haplotype networks (Fig. 7) show that hybrid individuals from Campeche (CP), Yucatan (YC) and Quintana Roo (QR) correspond to the haplotype connections between the highly divergent *C. acutus* and *C.*

moreletii genetic groups, highlighting that species boundaries in this hybrid system are fuzzy and complex to resolve, particularly under a strictly bifurcating evolutionary model (Wu 2001; Mallet 2007; Harrison & Larson 2014, 2016; Gompert & Buerkle 2016). Nonetheless, hybrids can be regarded as differentiated genetic groups; as such, the monophyletic, divergent clade encompassing hybrid individuals from central Gulf of Mexico we found could represent incipient species boundaries (homoploid hybrids).

We found a rather interesting pattern for *C. acutus*: this species is differentiated into two Pacific and one Caribbean island non-admixed groups, both by mitochondrial (Figs. 6, 7, 8) and nuclear (Figs. 2, 6, 8) genes. We believe *C. acutus* from the islands can be considered as a distinct lineage (monophyletic mtDNA clade and microsatellite genetic cluster), whereas those from the Pacific suggest a complex “intraspecific” hybridization process that needs to be evaluated in much more detail (Choleva *et al.* 2014).

To distinguish the evolutionary and ecological processes that have shaped this hybridization system remains a difficult task, mainly because crocodiles have consistently mixed on different time and spatial scales (Mastrantonio *et al.* 2016). Also, we are aware that our study has important limitations because we resolve genetic patterns based on mitochondrial markers and a few nuclear loci, which limit the potential to accurately determine species boundaries based on traditional models in which hybrids need be represented by a strictly bifurcating evolution. In accordance, we are working to further explore this extraordinary complex hybridization system between *Crocodylus moreletii* and *C. acutus* with next generation sequencing, with which we will be able to more accurately evaluate introgression and selection processes.

Acknowledgements

We deeply thank Luis Eguiarte and Andrés García for discussion throughout the entire project, which significantly helped to improve it. Gualberto Pacheco Sierra acknowledges that this paper was a part of his doctoral thesis in the Doctorado de Ciencias Biológicas de la Universidad Nacional Autónoma de México. EVD received financial support from Papiit (IN202713) and GPS a scholarship and financial support provided by the National Council of Science (CONACyT CVU 286325/Reg. becario 256144), Programa for Postgraduate Studies (PAEP) and UNAM. Scientific collector permit to EVD: Semarnat-FAUT-0168.

References

- Abbott R, Albach D, Ansell S *et al.* (2013) Hybridization and speciation. *Journal of evolutionary biology*, **26**, 229-246.
- Abbott RJ, Barton NH, Good JM (2016) Genomics of hybridization and its evolutionary consequences. *Molecular Ecology*, **25**, 2325–2332.
- Abbott RJ, Hegarty MJ, Hiscock SJ, Brennan AC (2010) Homoploid hybrid speciation in action. *TAXON*, **59**, 1375–1386.
- Arnold ML, Ballerini ES, Brothers a N (2012) Hybrid fitness, adaptation and evolutionary diversification: lessons learned from Louisiana Irises. *Heredity*, **108**, 159-166.
- Arnold ML, Martin NH (2009) Adaptation by introgression. *Journal of biology*, **8**, 82.
- Baack EJ, Rieseberg LH (2007) A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development*, **17**, 513–518.
- Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.
- Barton NH, Hewitt GM (1985) Analysis of Hybrid Zones. *Annual Review of Ecology, Evolution, and Systematics*, **16**, 113–148.
- Brandley MC, Wang Y, Guo X *et al.* (2010) Bermuda as an evolutionary life raft for an ancient lineage of endangered lizards. *PLoS ONE*, **5**, 3–6.
- Brennan AC, Barker D, Hiscock SJ, Abbott RJ (2012) Molecular genetic and quantitative trait divergence associated with recent homoploid hybrid speciation: a study of *Senecio squalidus* (Asteraceae). *Heredity*, **108**, 87–95.
- Buerkle CA (2005) Maximum-likelihood estimation of a hybrid index based on molecular markers. *Molecular Ecology Notes*, **5**, 684–687.
- Buerkle CA, Rieseberg LH (2008) The rate of genome stabilization in homoploid hybrid species. *Evolution*, **62**, 266–275.
- Choleva L, Musilova Z, Kohoutova-Sediva A *et al.* (2014) Distinguishing between Incomplete Lineage Sorting and Genomic Introgressions: Complete Fixation of Allospecific Mitochondrial DNA in a Sexually Reproducing Fish (Cobitis; Teleostei), despite Clonal Reproduction of Hybrids (N Johnson, Ed.). *PLoS ONE*, **9**, e80641.
- Christe C, Stölting KN, Paris M *et al.* (2017) Adaptive evolution and segregating load contribute to the genomic landscape of divergence in two tree species connected by episodic gene flow. *Molecular Ecology*, **26**, 59–76.
- Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, 699–710.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology*, **7**, 214.
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.
- Edwards S V., Potter S, Schmitt CJ, Bragg JG, Moritz C (2016) Reticulation, divergence, and the phylogeography–phylogenetics continuum. *Proceedings of the National Academy of Sciences*, **113**, 8025–8032.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.
- Fraley C, Raftery AE (2002) Model-Based Clustering, Discriminant Analysis, and Density Estimation. *Journal of the American Statistical Association*, **97**, 611–631.
- Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.
- Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.
- Gante HF, Matschiner M, Malmstrøm M *et al.* (2016) Genomics of speciation and introgression

- in Princess cichlid fishes from Lake Tanganyika. *Molecular Ecology*, **25**, 6143–6161.
- Glenn TC, Staton JL, Vu AT *et al.* (2002) Low mitochondrial DNA variation among American alligators and a novel non-coding region in crocodylians. *The Journal of experimental zoology*, **294**, 312–324.
- Gompert Z, Buerkle CA (2009) A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. *Molecular ecology*, **18**, 1207–1224.
- Gompert Z, Buerkle CA (2010) Introgress: a Software Package for Mapping Components of Isolation in Hybrids. *Molecular ecology resources*, **10**, 378–384.
- Gompert Z, Buerkle CA (2011) Bayesian estimation of genomic clines. *Molecular ecology*, **20**, 2111–2127.
- Gompert Z, Buerkle CA (2016) What, if anything, are hybrids: enduring truths and challenges associated with population structure and gene flow. *Evolutionary Applications*, **9**, 909–923.
- Gompert Z, Fordyce J a, Forister ML, Shapiro AM, Nice CC (2006) Homoploid Hybrid Speciation in an Extreme Habitat. *Science*, **314**, 1923–1925.
- González-Trujillo R, Rodríguez D, González-Romero A *et al.* (2012) Testing for hybridization and assessing genetic diversity in Morelet's crocodile (*Crocodylus moreletii*) populations from central Veracruz. *Conservation Genetics*, **13**, 1677–1683.
- Guindon S, Dufayard JF, Lefort V *et al.* (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, **59**, 307–321.
- Hamilton JA, Miller JM (2016) Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology*, **30**, 33–41.
- Harrison RG, Larson EL (2014) Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, **105**, 795–809.
- Harrison RG, Larson EL (2016) Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. *Molecular Ecology*, **25**, 2454–2466.
- Hedrick PW (2013) Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*, **22**, 4606–4618.
- Hewitt GM (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica*, **139**, 617–638.
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.
- Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.
- Kearse M, Moir R, Wilson A *et al.* (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.
- Larson EL, Andrés J a., Bogdanowicz SM, Harrison RG (2013) Differential introgression in a mosaic hybrid zone reveals candidate barrier genes. *Evolution*, **67**, 3653–3661.
- Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.
- Llopart A, Herrig D, Brud E, Stecklein Z (2014) Sequential adaptive introgression of the mitochondrial genome in *Drosophila yakuba* and *Drosophila santomea*. *Molecular Ecology*, **23**, 1124–1136.
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends in ecology & evolution*, **20**, 229–237.
- Mallet J (2007) Hybrid speciation. *Nature*, **446**, 279–283.
- Mastrantonio V, Porretta D, Urbanelli S, Crasta G, Nascetti G (2016) Dynamics of mtDNA introgression during species range expansion: insights from an experimental longitudinal

- study. *Scientific Reports*, **6**, 30355.
- Meganathan PR, Dubey B, Batzer M a, Ray D a, Haque I (2010) Molecular phylogenetic analyses of genus *Crocodylus* (Eusuchia, Crocodylia, Crocodylidae) and the taxonomic position of *Crocodylus porosus*. *Molecular phylogenetics and Evolution*, **57**, 393–402.
- Meirmans PG (2012) The trouble with isolation by distance. *Molecular Ecology*, **21**, 2839–2846.
- Meredith RW, Hekkala ER, Amato G, Gatesy J (2011) A phylogenetic hypothesis for *Crocodylus* (Crocodylia) based on mitochondrial DNA: evidence for a trans-Atlantic voyage from Africa to the New World. *Molecular phylogenetics and evolution*, **60**, 183–191.
- Milián-García Y, Ramos-Targarona R, Pérez-Fleitas E *et al.* (2015) Genetic evidence of hybridization between the critically endangered Cuban crocodile and the American crocodile: implications for population history and in situ/ex situ conservation. *Heredity*, **114**, 272–280.
- Milián-García Y, Venegas-Anaya M, Frias-Soler R *et al.* (2011) Evolutionary history of Cuban crocodiles *Crocodylus rhombifer* and *Crocodylus acutus* inferred from multilocus markers. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **315 A**, 358–375.
- Nolte AW, Gompert Z, Buerkle CA (2009) Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. *Molecular Ecology*, **18**, 2615–2627.
- Oaks JR (2011) A time-calibrated species tree of crocodylia reveals a recent radiation of the true crocodiles. *Evolution*, **65**, 3285–3297.
- Owens GL, Baute GJ, Rieseberg LH (2016) Revisiting a classic case of introgression: hybridization and gene flow in Californian sunflowers. *Molecular Ecology*, **25**, 2630–2643.
- Pacheco-Sierra G, Gompert Z, Domínguez-Laso J, Vázquez-Domínguez E (2016) Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *Crocodylus moreletii*. *Molecular Ecology*, **25**, 3484–3498.
- Padilla y Sánchez RJ (2007) Evolución geológica del sureste mexicano desde el Mesozoico al presente en el contexto regional del Golfo de México. *Boletín de la Sociedad Geológica Mexicana*, **LIX**, 19–42.
- Peakall R, Smouse PE (2012) GenAEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics (Oxford, England)*, **28**, 2537–9.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Ray DA, Densmore L (2002) The crocodylian mitochondrial control region: general structure, conserved sequences, and evolutionary implications. *The Journal of experimental zoology*, **294**, 334–345.
- Razo-Mendivil U, Vázquez-Domínguez E, Pérez-Ponce de León G (2013) Discordant Genetic Diversity and Geographic Patterns Between *Crassicutis cichlasomae* (Digenea: Apocreadiidae) and its Cichlid Host, “*Cichlasoma*” *urophthalmus* (Osteichthyes: Cichlidae), in Middle-America. *Journal of Parasitology*, **99**, 978–988.
- Rieseberg LH, Archer M a, Wayne RK (1999) Transgressive segregation, adaptation and speciation. *Heredity*, **83**, 363–372.
- Schumer M, Rosenthal GG, Andolfatto P (2014) How common is homoploid hybrid speciation? *Evolution; international journal of organic evolution*, **68**, 1553–1560.
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends in ecology & evolution*, **19**, 198–207.
- Serrano-Gómez SS, Guevara-Chumacero LM, Barriga-Sosa IDLA *et al.* (2016) Low levels of genetic diversity in *Crocodylus acutus* in Oaxaca and Guerrero, Mexico, and molecular-

- morphological evidence of the presence of *C. moreletii*. *Biochemical Systematics and Ecology*, **69**, 51–59.
- Soltis PS (2013) Hybridization, speciation and novelty. *Journal of Evolutionary Biology*, **26**, 291–293.
- Sweigart A (2009) Sculpin hybrid zones: natural laboratories for the early stages of speciation. *Molecular Ecology*, **18**, 2547–2548.
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.
- Teeter KC, Thibodeau LM, Gompert Z *et al.* (2010) The variable genomic architecture of isolation between hybridizing species of house mice. *Evolution*, **64**, 472–485.
- Vázquez-Domínguez E, Arita HT (2010) The Yucatan peninsula: Biogeographical history 65 million years in the making. *Ecography*, **33**, 212–219.
- Weaver JP, Rodriguez D, Venegas-Anaya M *et al.* (2008) Genetic characterization of captive Cuban crocodiles (*Crocodylus rhombifer*) and evidence of hybridization with the American crocodile (*Crocodylus acutus*). *Journal of experimental zoology. Part A, Ecological genetics and physiology*, **309**, 649–660.
- Wu C (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**, 851–865.

SUPPLEMENTARY INFORMATION

Species boundaries and homoploid hybrids: revisiting hybridization in crocodiles

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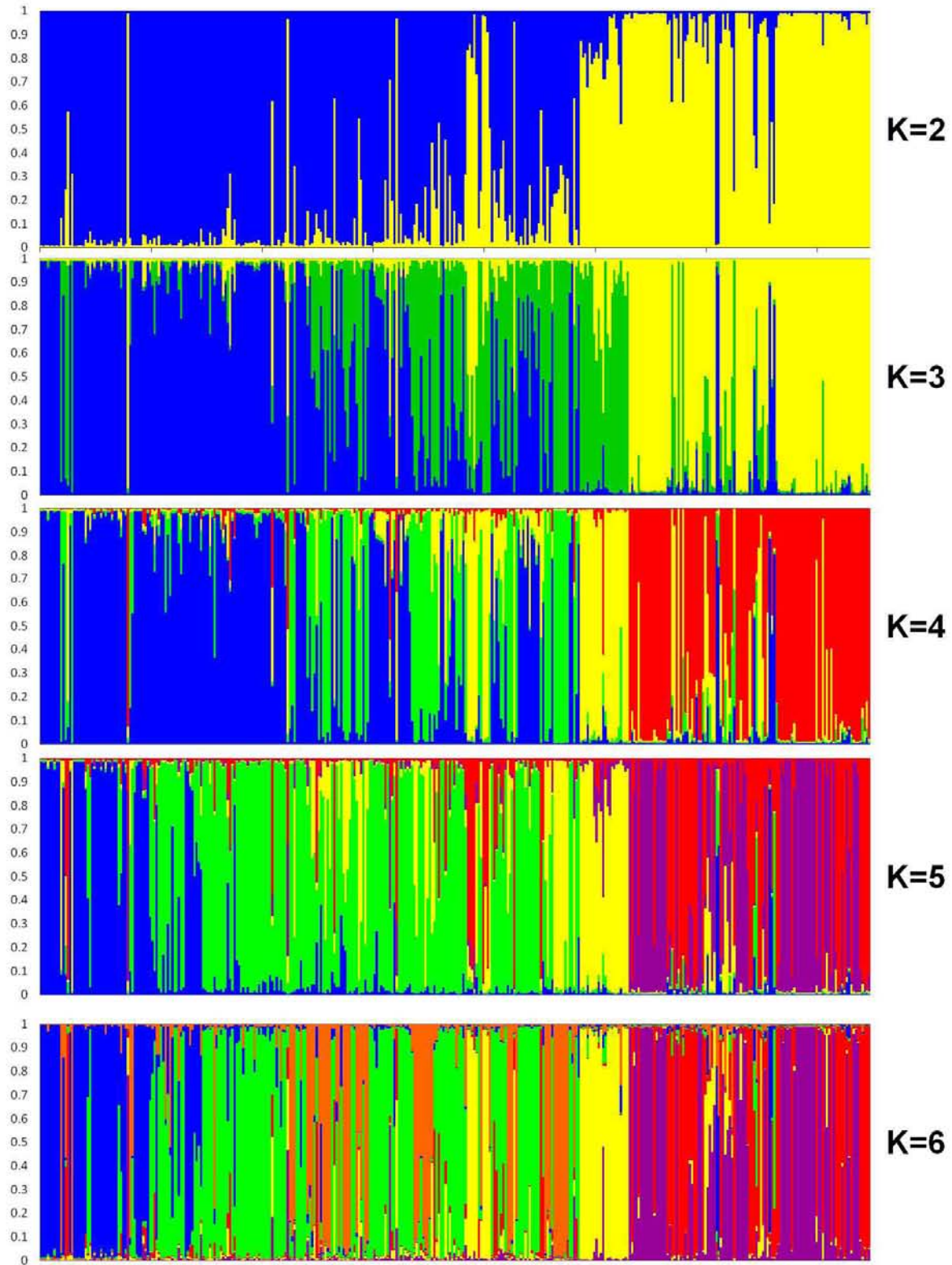


Figure S1. Admixture proportions ($K=2$ to $K=6$) obtained with STRUCTURE (Pritchard *et al.* 2000) for *Crocodylus acutus* and *C. moreletii* from Mexico, based on microsatellite loci.

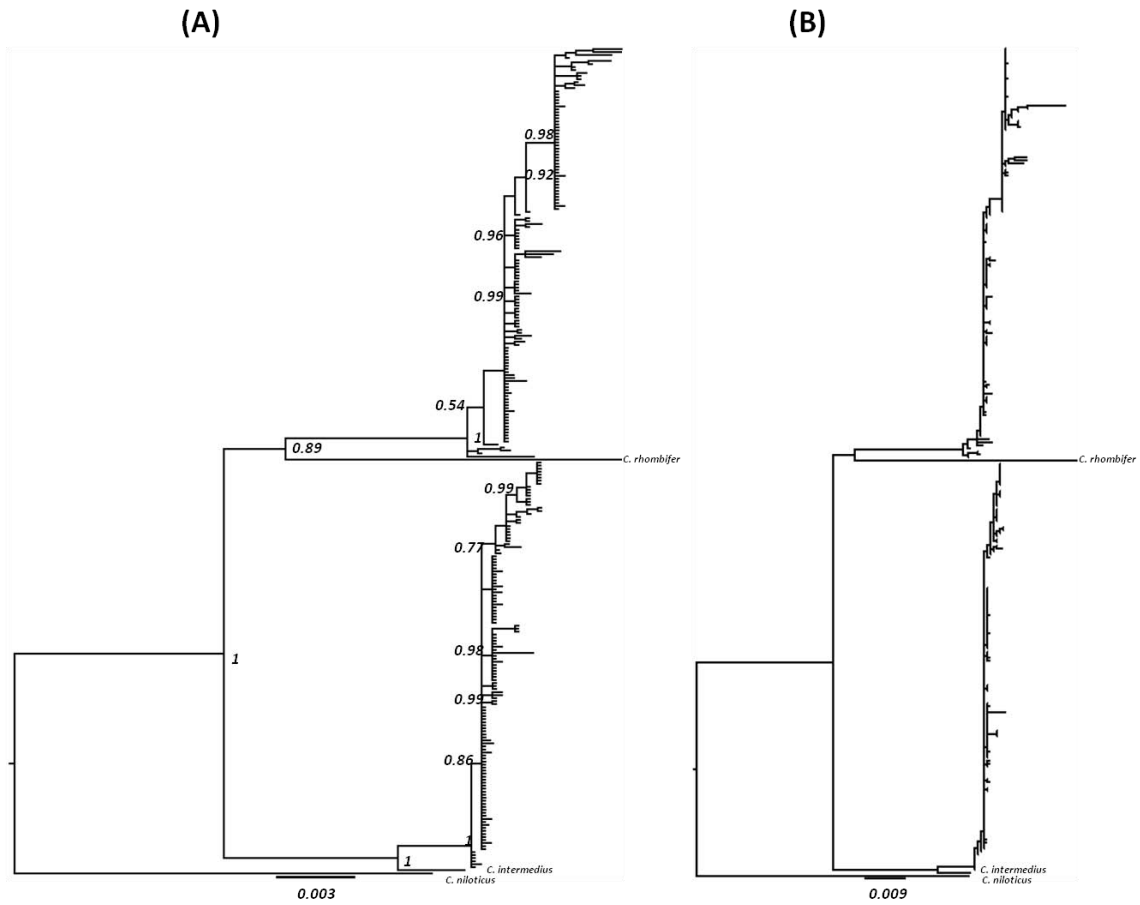


Figure S2. A) Bayesian and B) Maximum Likelihood phylogenetic trees based on mtDNA sequences for *Crocodylus acutus* and *C. moreletii* from Mexico.

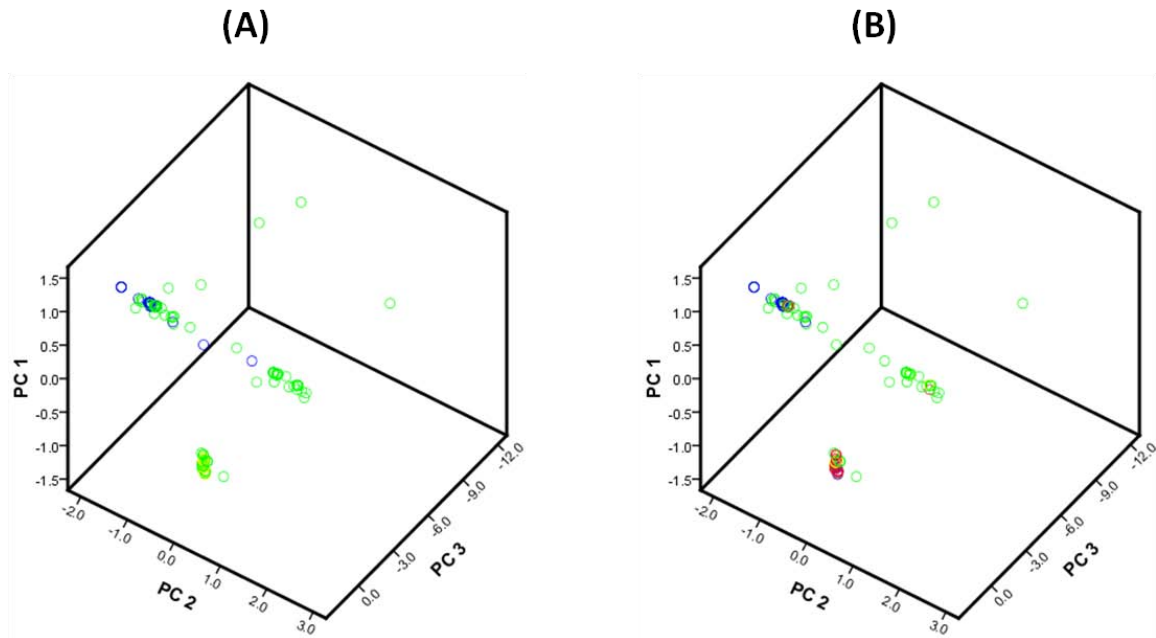


Figure S3. Principal component analysis from polymorphic sites of mtDNA sequences and integer factors based on STRUCTURE results (Pritchard et al. 2000) for $K=3$ (A) and $K=5$ (B), for *Crocodylus acutus* and *C. moreletii* from Mexico.

Table S1. List of samples of *Crocodylus* from the Gulf of Mexico and Pacific, including sample identification, population, locality names, and coordinates. Includes the summary statistics results for the Bayesian inference of admixture proportions analyses (STRUCTURE 2.3.4, $K=5$; Pritchard *et al.* 2000) and the Maximum-likelihood estimation of hybrid index (h) (R package INTROGRESS; Gompert & Buerkle 2010). Lower and upper credibility intervals (CI) are given.

Sample no.	Population	Locality	Coordinates WGS84		INTROGRESS (Gompert & Buerkle 2010) Hybrid index results				STRUCTURE (Pritchard <i>et al.</i> 2000) results ($K = 5$)				
			Longitude	Latitude	Lower IC	Upper IC	C. a. - h	C. m. - h	C. moreletii	Hybrid	C.acutus (islands)	C. acutus (Northwestern Pacific)	C. acutus (Southern Pacific)
SL01	SanLuis	Presa San Diego	100.101705	21.906036	0	0	0	1	0.985	0.006	0.003	0.003	0.003
SL02	SanLuis	Presa San Diego	100.100266	21.907396	0	0	0	1	0.982	0.009	0.003	0.003	0.003
SL03	SanLuis	Presa San Diego	100.105779	21.908245	0	0	0	1	0.985	0.006	0.003	0.003	0.003
SL04	SanLuis	Presa San Diego	100.104996	21.912537	0	0	0	1	0.953	0.013	0.026	0.003	0.005
SL05	SanLuis	Presa San Diego	100.103546	21.907716	0	0	0	1	0.984	0.007	0.003	0.003	0.003
SL06	SanLuis	C. de Cabezas	-99.239420	21.839829	0	0	0	1	0.985	0.006	0.003	0.003	0.003
SL07	SanLuis	C. de Cabezas	-99.236971	21.832713	0	0	0	1	0.952	0.027	0.004	0.003	0.014
SL08	SanLuis	C. de Cabezas	-99.231688	21.835019	0	0	0	1	0.985	0.006	0.003	0.003	0.003
SL09	SanLuis	C. de Cabezas	-99.224737	21.831119	0	0	0	1	0.985	0.006	0.003	0.003	0.003
SL10	SanLuis	C. de Cabezas	-99.204397	21.811128	0.0890894	0.526695	0.2674157	0.7325843	0.086	0.019	0.883	0.003	0.009
SL11	SanLuis	C. de Cabezas	-99.200485	21.807247	0.0184714	0.3082599	0.1106241	0.8893759	0.875	0.021	0.093	0.004	0.006
TM08	Tamaulipas	Presa V.Guerrero	-98.772430	23.830697	0.0748554	0.5957476	0.2805817	0.7194183	0.086	0.122	0.295	0.003	0.495
TM09	Tamaulipas	Presa V.Guerrero	-98.731519	24.026471	0.5614227	0.992084	0.8674793	0.1325207	0.006	0.047	0.01	0.003	0.934
TM10	Tamaulipas	Presa V.Guerrero	-98.695851	23.918332	0.0029041	0.2197983	0.0533139	0.9466861	0.942	0.012	0.034	0.005	0.007
TM14	Tamaulipas	Presa V.Guerrero	-98.761735	23.876798	0.3639909	0.8905567	0.6568014	0.3431986	0.005	0.006	0.981	0.003	0.005
TM15	Tamaulipas	Estero Lomas	-97.896743	22.474763	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM16	Tamaulipas	Estero Lomas	-97.905238	22.480032	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM17	Tamaulipas	Estero Lomas	-97.910862	22.471442	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM18	Tamaulipas	Estero Lomas	-97.912238	22.466474	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM19	Tamaulipas	Estero Lomas	-97.916558	22.466356	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM20	Tamaulipas	Laguna Conejo	-97.880972	22.427639	0.0031702	0.2429885	0.0592667	0.9407333	0.869	0.007	0.068	0.006	0.051

TM21	Tamaulipas	Laguna Conejo	-97.885740	22.433786	0	0.1331887	0	1	0.408	0.56	0.005	0.003	0.024
TM22	Tamaulipas	Laguna Cañon	-97.868632	22.411096	0	0.1448917	0	1	0.03	0.885	0.004	0.007	0.075
TM23	Tamaulipas	Laguna Altamira	-97.895726	22.337848	0	0	0	1	0.982	0.006	0.004	0.005	0.004
TM24	Tamaulipas	Laguna Altamira	-97.892929	22.333736	0	0	0	1	0.953	0.006	0.005	0.03	0.006
TM25	Tamaulipas	Laguna Altamira	-97.903844	22.329862	0	0	0	1	0.984	0.006	0.004	0.003	0.003
TM26	Tamaulipas	Laguna Altamira	-97.916444	22.339376	0	0	0	1	0.984	0.006	0.003	0.003	0.003
TM27	Tamaulipas	Laguna Altamira	-97.920856	22.331406	0.002698	0.2074357	0.0497536	0.9502464	0.948	0.009	0.005	0.02	0.017
TM28	Tamaulipas	Laguna Altamira	-97.907885	22.327407	0	0	0	1	0.984	0.006	0.004	0.003	0.003
TM29	Tamaulipas	Laguna Altamira	-97.916737	22.324821	0.0030176	0.2291319	0.0562299	0.9437701	0.94	0.016	0.029	0.004	0.011
TM30	Tamaulipas	Laguna Chairel	-97.898533	22.235110	0	0	0	1	0.983	0.006	0.003	0.004	0.004
TM31	Tamaulipas	Laguna Chairel	-97.910361	22.242865	0	0	0	1	0.98	0.009	0.003	0.004	0.004
TM32	Tamaulipas	Laguna Chairel	-97.958039	22.300669	0.002515	0.1935897	0.0463764	0.9536236	0.941	0.01	0.015	0.029	0.005
TM33	Tamaulipas	Laguna Chairel	-97.970066	22.296811	0	0	0	1	0.984	0.007	0.003	0.003	0.003
TM35	Tamaulipas	Laguna Carpintero	-97.855362	22.235840	0	0	0	1	0.981	0.01	0.003	0.003	0.003
TM36	Tamaulipas	Laguna Carpintero	-97.854593	22.229399	0.0222333	0.3444999	0.1292068	0.8707932	0.379	0.581	0.017	0.003	0.02
TM37	Tamaulipas	Laguna Carpintero	-97.855094	22.222319	0.0667077	0.463787	0.2268348	0.7731652	0.514	0.352	0.024	0.006	0.105
TM38	Tamaulipas	Noh Bec	-98.954787	23.812713	0	0	0	1	0.985	0.006	0.003	0.003	0.003
TM39	Tamaulipas	E. tampico	-97.845457	22.199503	0	0.1269753	0	1	0.938	0.027	0.007	0.004	0.024
TM40	Tamaulipas	Aldama	-98.954787	23.812713	0.7990513	1	1	0	0.004	0.009	0.044	0.803	0.141
TM42	Tamaulipas	Gomez Farias	-98.801751	22.959399	0.1504774	0.5873794	0.3437691	0.6562309	0.099	0.533	0.36	0.003	0.005
VZ01	Veracruz	Rio Panuco	-97.795658	22.261961	0.091854	0.5225657	0.2692739	0.7307261	0.011	0.946	0.036	0.003	0.004
VZ02	Veracruz	Tampamachoco	-97.357751	21.005627	0.0195096	0.3243147	0.1167146	0.8832854	0.855	0.132	0.005	0.004	0.004
VZ03	Veracruz	Estero Tumilco	-97.351248	20.938211	0	0	0	1	0.968	0.021	0.004	0.003	0.004
VZ04	Veracruz	Estero Chacoaco	-97.290351	20.909730	0	0	0	1	0.985	0.006	0.003	0.003	0.003
VZ05	Veracruz	Estero Chacoaco	-97.290411	20.915244	0	0	0	1	0.985	0.006	0.003	0.003	0.003
VZ06	Veracruz	Estero Lagartos	-97.096046	20.542022	0.0388839	0.3623538	0.1536374	0.8463626	0.832	0.017	0.012	0.087	0.052
VZ07	Veracruz	Estro Larios	-96.982713	20.409903	0.0375808	0.3510089	0.1485997	0.8514003	0.832	0.029	0.007	0.048	0.084
VZ08	Veracruz	La Mancha	-96.389369	19.593485	0	0.1537582	0	1	0.968	0.017	0.005	0.006	0.004
VZ09	Veracruz	La Mancha	-96.386432	19.582741	0.1501357	0.5806438	0.3413232	0.6586768	0.329	0.628	0.004	0.034	0.005

VZ10	Veracruz	Papa-Loapan	-95.706286	18.593737	0.076563	0.467757	0.2326268	0.7673732	0.23	0.732	0.01	0.004	0.025
VZ11	Veracruz	Papa-Loapan	-95.701311	18.576408	0.1903469	0.6608248	0.4090661	0.5909339	0.102	0.627	0.012	0.007	0.253
VZ13	Veracruz	Pte. Santa Cruz	-96.121439	18.144412	0.0182068	0.3029993	0.1087754	0.8912246	0.805	0.083	0.094	0.003	0.013
VZ14	Veracruz	Pte. Santa Cruz	-96.120240	18.162273	0.0533888	0.4390717	0.2000341	0.7999659	0.028	0.93	0.011	0.019	0.012
VZ15	Veracruz	Pte. Santa Cruz	-96.091145	18.173224	0.1357998	0.5958021	0.3384711	0.6615289	0.008	0.982	0.003	0.003	0.004
VZ16	Veracruz	Pte. Santa Cruz	-96.080789	18.168074	0.1600787	0.5928484	0.3558633	0.6441367	0.17	0.812	0.009	0.005	0.005
VZ12	Veracruz	Temascal	-96.398136	18.240241	0.1171363	0.5370051	0.2964794	0.7035206	0.01	0.977	0.005	0.003	0.004
VZ17	Veracruz	Temascal	-96.174993	18.131980	0.1189603	0.545254	0.3023216	0.6976784	0.3	0.675	0.01	0.004	0.012
VZ18	Veracruz	Tlacojalpan	-95.955507	18.241804	0.1583593	0.588798	0.3525817	0.6474183	0.019	0.957	0.006	0.005	0.013
VZ19	Veracruz	Tlacojalpan	-95.951167	18.230109	0.0037827	0.263213	0.0683142	0.9316858	0.711	0.262	0.013	0.009	0.005
VZ20	Veracruz	Tlacojalpan	-95.955305	18.239602	0.0978419	0.5363054	0.2820006	0.7179994	0.011	0.979	0.004	0.004	0.003
VZ21	Veracruz	Cosamaloapan	-95.777288	18.319714	0.1300116	0.5740659	0.3243194	0.6756806	0.013	0.975	0.003	0.004	0.004
VZ22	Veracruz	Cosamaloapan	-95.792620	18.361949	0.0901741	0.498319	0.2605066	0.7394934	0.415	0.505	0.023	0.052	0.006
VZ23	Veracruz	Cosamaloapan	-95.737561	18.367555	0.2286695	0.6826261	0.4492092	0.5507908	0.02	0.944	0.016	0.012	0.009
VZ24	Veracruz	Loma Bonita	-95.919322	18.078873	0.1046568	0.5529153	0.2963006	0.7036994	0.03	0.96	0.004	0.003	0.003
VZ25	Veracruz	Playa Vicente	-95.669629	17.675532	0	0.1103711	0	1	0.983	0.007	0.004	0.003	0.003
VZ26	Veracruz	Playa Vicente	-95.671033	17.677202	0.0028677	0.2171802	0.0526507	0.9473493	0.947	0.025	0.012	0.01	0.006
VZ27	Veracruz	Playa Vicente	-95.668668	17.676002	0.0399066	0.3643864	0.1564793	0.8435207	0.881	0.011	0.043	0.006	0.06
VZ28	Veracruz	Catemaco	-95.022300	18.371311	0.0032294	0.2459359	0.0602183	0.9397817	0.863	0.067	0.008	0.006	0.056
VZ29	Veracruz	Catemaco	-95.022064	18.370288	0.0220966	0.3508991	0.1298808	0.8701192	0.502	0.485	0.004	0.003	0.005
VZ30	Veracruz	Catemaco	-95.020229	18.372091	0.0037394	0.2624964	0.0677282	0.9322718	0.646	0.313	0.006	0.004	0.031
VZ31	Veracruz	Catemaco	-95.042143	18.363287	0.0037391	0.2687902	0.068281	0.931719	0.561	0.428	0.004	0.003	0.004
VZ32	Veracruz	Catemaco	-94.444690	18.085184	0.1112764	0.5204319	0.2848037	0.7151963	0.529	0.228	0.126	0.105	0.011
VZ33	Veracruz	Coatzacoalcos	-94.371237	18.114042	0.1591531	0.5562266	0.3371949	0.6628051	0.028	0.951	0.005	0.008	0.008
VZ34	Veracruz	Jaguarondi	-94.374422	18.119283	0.1576126	0.596163	0.3544416	0.6455584	0.02	0.968	0.004	0.003	0.004
VZ35	Veracruz	Jaguarondi	-94.356508	18.123827	0.1647983	0.6076071	0.3656404	0.6343596	0.012	0.977	0.004	0.004	0.003
VZ36	Veracruz	Jaguarondi	-94.352721	18.123638	0.2542654	0.6723741	0.4550445	0.5449555	0.011	0.879	0.089	0.004	0.017
VZ37	Veracruz	Jaguarondi	-94.360514	18.118786	0.1487882	0.5713533	0.3363477	0.6636523	0.008	0.981	0.004	0.004	0.003
VZ38	Veracruz	Jaguarondi	-94.352928	18.116536	0.19547	0.6257719	0.394653	0.605347	0.076	0.685	0.016	0.011	0.213

VZ39	Veracruz	Presa cangrejera	-94.328418	18.112132	0.0292056	0.4128834	0.1635772	0.8364228	0.022	0.967	0.004	0.004	0.004
VZ40	Veracruz	Presa cangrejera	-94.324844	18.114700	0.0612493	0.4685927	0.2221793	0.7778207	0.016	0.971	0.004	0.005	0.004
VZ41	Veracruz	Presa cangrejera	-94.318018	18.094913	0.1148536	0.5188328	0.2889185	0.7110815	0.025	0.945	0.012	0.005	0.013
VZ42	Veracruz	Presa cangrejera	-94.307985	18.088898	0.132852	0.5729573	0.3271048	0.6728952	0.012	0.946	0.007	0.022	0.014
VZ43	Veracruz	Presa cangrejera	-94.298844	18.093411	0.0612493	0.4685927	0.2221793	0.7778207	0.028	0.945	0.006	0.01	0.011
VZ44	Veracruz	Presa cangrejera	-94.332372	18.102960	0.0836888	0.4812851	0.2464758	0.7535242	0.473	0.276	0.073	0.033	0.144
VZ45	Veracruz	Presa cangrejera	-94.316791	18.137566	0.2668664	0.7508125	0.5043	0.4957	0.069	0.529	0.008	0.026	0.368
VZ46	Veracruz	Presa cangrejera	-94.323469	18.131620	0.0423516	0.3847046	0.1658949	0.8341051	0.016	0.954	0.006	0.006	0.019
VZ47	Veracruz	Sontecomapan	-95.008509	18.526873	0.0303864	0.3965514	0.160656	0.839344	0.804	0.102	0.006	0.039	0.049
TB01	Tabasco	Lag. Rosario	-93.824848	17.836293	0.0202114	0.3301706	0.120028	0.879972	0.148	0.836	0.006	0.006	0.004
TB02	Tabasco	Lag. Rosario	-93.777913	17.843365	0.0552105	0.4512837	0.2067934	0.7932066	0.126	0.849	0.007	0.014	0.005
TB08	Tabasco	Lag. Caracol	-93.322473	17.833712	0.0350933	0.4668015	0.1913185	0.8086815	0.01	0.979	0.004	0.003	0.003
TB09	Tabasco	Lag. Caracol	-93.333310	17.840623	0.0235594	0.362032	0.1364543	0.8635457	0.031	0.956	0.006	0.003	0.004
TB10	Tabasco	Lag. Caracol	-93.340849	17.842596	0.1268745	0.5628751	0.3167535	0.6832465	0.009	0.973	0.005	0.006	0.006
TB11	Tabasco	Lag. Caracol	-93.342444	17.830325	0.0719882	0.507731	0.2469234	0.7530766	0.032	0.93	0.005	0.019	0.015
TB12	Tabasco	Lag. Caracol	-93.346783	17.824142	0.1268745	0.5628751	0.3167535	0.6832465	0.01	0.972	0.006	0.006	0.006
TB13	Tabasco	Lag. Caracol	-93.338316	17.820554	0.1268745	0.5628751	0.3167535	0.6832465	0.009	0.972	0.006	0.006	0.006
TB14	Tabasco	Lag. Caracol	-93.334700	17.819490	0.089994	0.5128609	0.2640599	0.7359401	0.018	0.962	0.006	0.008	0.006
TB15	Tabasco	Lag. Las Ilusiones	-92.939133	17.992474	0.1821798	0.6149579	0.3804842	0.6195158	0.008	0.978	0.004	0.004	0.006
TB16	Tabasco	Lag. Las Ilusiones	-92.936861	17.996060	0.0220795	0.3425946	0.12838	0.87162	0.045	0.921	0.005	0.013	0.015
TB17	Tabasco	Lag. Las Ilusiones	-92.931012	17.997234	0.1674408	0.5805926	0.3536981	0.6463019	0.009	0.981	0.003	0.003	0.004
TB18	Tabasco	Lag. Las Ilusiones	-92.933133	18.003759	0.0905537	0.5000162	0.2615082	0.7384918	0.058	0.929	0.004	0.005	0.004
TB19	Tabasco	Lag. Las Ilusiones	-92.923273	18.010282	0.0833748	0.4767311	0.2447305	0.7552695	0.051	0.939	0.004	0.003	0.003
TB20	Tabasco	Lag. Las Ilusiones	-92.924480	18.017085	0.1112219	0.5247016	0.2859554	0.7140446	0.01	0.979	0.003	0.004	0.004
TB21	Tabasco	Lag. Las Ilusiones	-92.942076	18.013778	0.0849234	0.4845311	0.2490167	0.7509833	0.081	0.909	0.004	0.003	0.003
TB23	Tabasco	Paraiso	-93.168180	18.404753	0.4658696	0.925627	0.723176	0.276824	0.057	0.235	0.042	0.091	0.575
TB24	Tabasco	Pantanos de Centla	-92.661778	18.335496	0.0512372	0.4244098	0.1925474	0.8074526	0.017	0.972	0.004	0.003	0.004
TB25	Tabasco	Pantanos de Centla	-92.670365	18.309710	0.022231	0.3530169	0.1306617	0.8693383	0.076	0.906	0.007	0.007	0.005
TB26	Tabasco	Pantanos de Centla	-92.648381	18.277524	0.1290027	0.5723816	0.3220123	0.6779877	0.017	0.959	0.005	0.013	0.006

TB27	Tabasco	Pantanos de Centla	-92.674069	18.314894	0.0479894	0.4074303	0.1821043	0.8178957	0.062	0.926	0.006	0.003	0.003
TB28	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.1257199	0.5589668	0.3141443	0.6858557	0.013	0.959	0.014	0.007	0.006
TB29	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.2439461	0.6909304	0.4602834	0.5397166	0.011	0.931	0.011	0.009	0.037
TB29-A	Tabasco	Pantanos de Centla	-92.785959	18.003803	0.6420626	0.9887982	0.8785078	0.1214922	0.003	0.047	0.111	0.479	0.358
TB30	Tabasco	Yumka	-92.771889	18.013489	0.2457576	0.6981822	0.4641835	0.5358165	0.007	0.969	0.013	0.005	0.006
TB31	Tabasco	Yumka	-92.805843	17.991547	0.1307934	0.5804584	0.3264391	0.6735609	0.011	0.965	0.014	0.005	0.005
CP01	Campeche	Manguito	-92.161841	18.666062	0.4886532	0.9393587	0.7480991	0.2519009	0.014	0.228	0.63	0.114	0.013
CP02	Campeche	Pom Atasta	-92.256715	18.548067	0.0242098	0.3625153	0.1387846	0.8612154	0.025	0.965	0.004	0.003	0.003
CP03	Campeche	Pom Atasta	-92.274635	18.538772	0.0515276	0.4266733	0.1935989	0.8064011	0.025	0.965	0.004	0.003	0.003
CP04	Campeche	Pom Atasta	-92.284620	18.533553	0.0835178	0.4880689	0.2480983	0.7519017	0.148	0.832	0.008	0.008	0.004
CP05	Campeche	Pom Atasta	-92.289700	18.523867	0.0267105	0.3961387	0.152607	0.847393	0.027	0.964	0.004	0.003	0.003
CP06	Campeche	Pom Atasta	-92.299332	18.495548	0.0832727	0.5045886	0.2523129	0.7476871	0.012	0.954	0.008	0.004	0.022
CP07	Campeche	Pom Atasta	-92.291855	18.511583	0.1872474	0.6187969	0.3861997	0.6138003	0.034	0.766	0.083	0.105	0.012
CP08	Campeche	Pom Atasta	-92.264917	18.542731	0.1691919	0.6228243	0.3750484	0.6249516	0.015	0.947	0.029	0.004	0.005
CP10	Campeche	Rio Chumpan	-91.511359	18.367294	0.2086188	0.6932157	0.4330169	0.5669831	0.045	0.532	0.412	0.005	0.005
CP11	Campeche	Rio Chumpan	-91.532363	18.321073	0.2885378	0.7448917	0.5157896	0.4842104	0.044	0.559	0.387	0.005	0.005
CP12	Campeche	Rio Chumpan	-91.498598	18.413111	0.1010066	0.5730696	0.2985341	0.7014659	0.221	0.221	0.033	0.004	0.522
CP12-A	Campeche	Rio Chumpan	-91.498598	18.413111	0.2515552	0.7308523	0.483661	0.516339	0.011	0.681	0.236	0.007	0.065
CP13	Campeche	Pital	-91.036970	18.622459	0.107627	0.5069493	0.2762042	0.7237958	0.38	0.482	0.056	0.057	0.024
CP14	Campeche	Sistema Pargo	-91.260245	18.511432	0.1356762	0.5882928	0.3349688	0.6650312	0.01	0.828	0.151	0.004	0.008
CP15	Campeche	Sistema Pargo	-91.267360	18.528772	0.4400007	0.8668311	0.6712742	0.3287258	0.007	0.225	0.755	0.006	0.008
CP16	Campeche	Sistema Pargo	-91.256343	18.598602	0.1226368	0.5735027	0.3152083	0.6847917	0.021	0.804	0.138	0.006	0.031
CP18	Campeche	Rio Caribe	-90.592815	18.227076	0.2453407	0.6988611	0.4644479	0.5355521	0.008	0.524	0.456	0.008	0.004
CP19	Campeche	Rio Champoton	-90.717450	19.362779	0.0963177	0.4671281	0.2503904	0.7496096	0.095	0.881	0.008	0.009	0.007
CP20	Campeche	Rio Champoton	-90.695545	19.358687	0.3710589	0.8283839	0.6137711	0.3862289	0.05	0.126	0.082	0.019	0.723
CP20-A	Campeche	Rio Champoton	-90.685833	19.328080	0.1186485	0.5313838	0.2970117	0.7029883	0.032	0.939	0.012	0.012	0.005
CP20-B	Campeche	Rio Champoton	-90.680565	19.319492	0.2006715	0.6438218	0.4079517	0.5920483	0.032	0.712	0.248	0.003	0.005
CP21	Campeche	Hampolol	-90.377368	19.942784	0.0979442	0.480894	0.2564847	0.7435153	0.336	0.646	0.007	0.006	0.005
CP22	Campeche	Hampolol	-90.380194	19.940457	0.147702	0.5979554	0.3523923	0.6476077	0.209	0.305	0.479	0.003	0.003

CP23	Campeche	Hampolol	-90.378826	19.941440	0.0979744	0.5416699	0.2837362	0.7162638	0.185	0.209	0.6	0.003	0.003
CP24	Campeche	Hampolol	-90.381110	19.939630	0.1357315	0.6708181	0.3741684	0.6258316	0.007	0.522	0.46	0.003	0.008
CP25	Campeche	Hampolol	-90.375525	19.944365	0.1426052	0.572214	0.330704	0.669296	0.015	0.725	0.246	0.003	0.011
CP26	Campeche	Hampolol	-90.375525	19.944365	0.0730092	0.5347633	0.2605191	0.7394809	0.007	0.98	0.004	0.003	0.006
CP28	Campeche	Peten	-90.482520	20.210533	0.2165471	0.6788763	0.4368368	0.5631632	0.016	0.895	0.012	0.025	0.052
CP29	Campeche	Peten	-90.485810	20.221311	0.1580408	0.5993823	0.3562898	0.6437102	0.03	0.791	0.172	0.003	0.004
CP29-A	Campeche	Peten	-90.475210	20.230127	0.6046297	1	0.8737146	0.1262854	0.003	0.057	0.923	0.006	0.011
CP29-B	Campeche	Peten	-90.478311	20.228308	0.3105176	0.7990118	0.560961	0.439039	0.01	0.297	0.625	0.061	0.007
CP29-C	Campeche	Peten	-90.481966	20.225753	0.0228652	0.3530431	0.1326841	0.8673159	0.01	0.978	0.005	0.003	0.004
CP29-D	Campeche	Peten	-90.487227	20.228616	0.1881579	0.6225336	0.3892981	0.6107019	0.064	0.148	0.779	0.003	0.005
CP29-E	Campeche	Peten	-90.487298	20.226673	0.060683	0.4722918	0.2219891	0.7780109	0.013	0.771	0.209	0.003	0.004
CP30	Campeche	El Remate	-90.381173	20.539410	0	0.2088374	0	1	0.01	0.98	0.004	0.003	0.004
CP31	Campeche	El Remate	-90.418850	20.542841	0.0251093	0.3769711	0.1441578	0.8558422	0.015	0.975	0.004	0.003	0.004
CP32	Campeche	El Remate	-90.421323	20.543251	0.07516	0.4566231	0.2272468	0.7727532	0.089	0.876	0.005	0.021	0.01
CP33	Campeche	El Remate	-90.456290	20.551486	0.0716408	0.445677	0.219166	0.780834	0.271	0.702	0.012	0.009	0.007
CP34	Campeche	El Remate	-90.448907	20.560033	0.006743	0.3909842	0.1143935	0.8856065	0.011	0.973	0.005	0.005	0.007
CP35	Campeche	El Remate	-90.417406	20.608397	0.0798669	0.483806	0.241953	0.758047	0.04	0.934	0.007	0.011	0.007
CP36	Campeche	El Remate	-90.417843	20.630067	0.1146937	0.5377745	0.2950129	0.7049871	0.031	0.943	0.007	0.013	0.007
CP37	Campeche	Isla Arena	-90.440655	20.684636	0.257764	0.7536997	0.504506	0.495494	0.017	0.721	0.046	0.024	0.192
CP38	Campeche	Isla Arena	-90.442601	20.708209	0.1051477	0.4888166	0.2677686	0.7322314	0.016	0.956	0.004	0.01	0.013
CP39	Campeche	Isla Arena	-90.451098	20.702321	0.5952514	0.9876899	0.8380586	0.1619414	0.013	0.344	0.005	0.562	0.077
CP40	Campeche	Isla Arena	-90.448560	20.693314	0.0896374	0.6071843	0.3090127	0.6909873	0.007	0.817	0.015	0.01	0.151
CP42	Campeche	Isla Arena	-90.440091	20.694540	0.0768274	0.4575881	0.2303096	0.7696904	0.39	0.536	0.055	0.007	0.011
CP43	Campeche	Isla Arena	-90.439284	20.694611	0.7214568	1	0.9805964	0.0194036	0.012	0.011	0.056	0.236	0.685
YC01	Yucatan	El Palmar	-90.331132	20.999664	0.0057332	0.3541063	0.09953	0.90047	0.01	0.973	0.005	0.007	0.005
YC02	Yucatan	El Palmar	-90.317733	21.014613	0.3260528	0.7928632	0.5664628	0.4335372	0.005	0.913	0.037	0.032	0.013
YC03	Yucatan	El Palmar	-90.298457	21.030374	0.0057332	0.3541063	0.09953	0.90047	0.01	0.973	0.005	0.007	0.005
YC04	Yucatan	El Palmar	-90.256057	21.060057	0.1569761	0.6535509	0.3826553	0.6173447	0.018	0.922	0.014	0.033	0.012
YC05	Yucatan	El Palmar	-90.261774	21.055684	0.1076692	0.5825846	0.3102574	0.6897426	0.03	0.923	0.013	0.022	0.012

YC06	Yucatan	El Palmar	-90.250148	21.063386	0.1226075	0.5594927	0.3110278	0.6889722	0.048	0.896	0.013	0.036	0.007
YC07	Yucatan	El Palmar	-90.256444	21.058542	0.2042449	0.6646487	0.4200431	0.5799569	0.008	0.961	0.023	0.004	0.004
YC07-A	Yucatan	El Palmar	-90.291403	21.018888	0.3914361	0.8590307	0.6417971	0.3582029	0.006	0.457	0.524	0.004	0.009
YC07-B	Yucatan	El Palmar	-90.294127	21.014678	0.3765435	0.8121324	0.6057007	0.3942993	0.007	0.238	0.746	0.004	0.005
YC07-C	Yucatan	El Palmar	-90.303947	21.006634	0.2180662	0.7060758	0.4476025	0.5523975	0.006	0.962	0.019	0.008	0.005
YC08	Yucatan	Sisal	-90.053368	21.159017	0.2815397	0.7829929	0.5353361	0.4646639	0.013	0.919	0.028	0.012	0.029
YC09	Yucatan	Sisal	-90.067137	21.158766	0.2619431	0.728314	0.491012	0.508988	0.008	0.92	0.056	0.007	0.01
YC09-A	Yucatan	Sisal	-90.054427	21.160256	0.3821225	0.8235943	0.6145123	0.3854877	0.017	0.016	0.948	0.003	0.016
YC09-B	Yucatan	Sisal	-90.056372	21.159918	0.2370772	0.6771569	0.4493707	0.5506293	0.036	0.371	0.585	0.003	0.006
YC09-C	Yucatan	Sisal	-90.059090	21.159495	0.1834654	0.7063068	0.428093	0.571907	0.008	0.951	0.032	0.004	0.005
YC09-D	Yucatan	Sisal	-90.049405	21.157391	0.5181777	0.9621542	0.7813314	0.2186686	0.005	0.254	0.694	0.035	0.011
YC09-E	Yucatan	Sisal	-90.049405	21.157391	0.13832	0.6927134	0.3894204	0.6105796	0.041	0.269	0.334	0.003	0.354
YC10	Yucatan	San Felipe	-88.227613	21.551921	0.3836677	0.8250315	0.6179185	0.3820815	0.006	0.851	0.096	0.023	0.024
YC11	Yucatan	San Felipe	-88.225460	21.552005	0.2831092	0.8259262	0.5603678	0.4396322	0.005	0.39	0.015	0.005	0.586
YC12	Yucatan	San Felipe	-88.216059	21.550634	0.1112219	0.5247016	0.2859554	0.7140446	0.011	0.977	0.005	0.003	0.004
YC13	Yucatan	San Felipe	-88.208049	21.557335	0.1139328	0.5237768	0.2887443	0.7112557	0.03	0.96	0.004	0.003	0.003
YC14	Yucatan	San Felipe	-88.189439	21.560120	0.2366893	0.7426205	0.4821681	0.5178319	0.007	0.497	0.019	0.021	0.455
YC15	Yucatan	San Felipe	-88.197914	21.556224	0.2484476	0.7205568	0.477425	0.522575	0.005	0.971	0.012	0.004	0.008
YC16	Yucatan	San Felipe	-88.179672	21.564551	0.2658161	0.7123926	0.483898	0.516102	0.012	0.741	0.028	0.075	0.145
YC17	Yucatan	Rio Lagartos	-88.153065	21.578410	0.1667038	0.6274769	0.3752832	0.6247168	0.011	0.977	0.005	0.003	0.003
YC18	Yucatan	Rio Lagartos	-88.060133	21.589632	0.1053886	0.5609808	0.3003423	0.6996577	0.015	0.856	0.016	0.048	0.065
YC19	Yucatan	Rio Lagartos	-88.056833	21.591748	0.281546	0.7675799	0.5245687	0.4754313	0.005	0.967	0.017	0.006	0.005
YC20	Yucatan	Rio Lagartos	-88.055328	21.592665	0.3193801	0.8187782	0.5791647	0.4208353	0.004	0.934	0.019	0.025	0.017
YC21	Yucatan	Rio Lagartos	-88.057922	21.594351	0.2857294	0.7291516	0.5039233	0.4960767	0.005	0.959	0.008	0.013	0.015
YC22	Yucatan	Rio Lagartos	-88.005962	21.578107	0.0876133	0.5283412	0.2654812	0.7345188	0.09	0.858	0.006	0.008	0.039
YC23	Yucatan	Rio Lagartos	-88.041217	21.577864	0.3039501	0.7455107	0.5250379	0.4749621	0.011	0.697	0.075	0.069	0.148
YC24	Yucatan	El Cuyo	-87.644764	21.458028	0.5325472	0.9761413	0.7986291	0.2013709	0.028	0.032	0.147	0.02	0.773
YC25	Yucatan	El Cuyo	-87.645685	21.458794	0.6413862	0.996479	0.8691112	0.1308888	0.025	0.05	0.045	0.111	0.769
YC26	Yucatan	El Cuyo	-87.656388	21.461179	0.5413514	0.9859313	0.8179218	0.1820782	0.024	0.028	0.056	0.009	0.884

YC27	Yucatan	El Cuyo	-87.656008	21.512701	0.7215287	1	0.9361219	0.0638781	0.004	0.005	0.015	0.181	0.795
YC28	Yucatan	El Cuyo	-87.685641	21.507163	0.5938688	0.9596631	0.8159071	0.1840929	0.075	0.055	0.679	0.089	0.102
YC29	Yucatan	El Cuyo	-87.638364	21.506523	0.0130654	0.5800695	0.1983106	0.8016894	0.004	0.797	0.009	0.005	0.186
YC30	Yucatan	El Cuyo	-87.638364	21.506523	0.2398128	0.7267973	0.4759351	0.5240649	0.017	0.564	0.404	0.011	0.004
YC31	Yucatan	Chipepte	-87.538193	21.484287	0.7568926	1	0.9787062	0.0212938	0.003	0.006	0.038	0.01	0.943
YC32	Yucatan	Chipepte	-87.540051	21.478036	0.8544413	1	1	0	0.003	0.006	0.448	0.014	0.529
YC33	Yucatan	Chipepte	-87.542369	21.482892	0.8456409	1	1	0	0.003	0.014	0.093	0.006	0.884
YC33-A	Yucatan	Chipepte	-87.544279	21.478861	0.4062942	0.8485752	0.6425772	0.3574228	0.012	0.013	0.93	0.003	0.042
YC34	Yucatan	Ranchos	-87.971992	21.445612	0.0808221	0.5535954	0.2788724	0.7211276	0.006	0.963	0.009	0.016	0.006
YC35	Yucatan	Ranchos	-87.988679	21.459559	0.4827743	0.8932117	0.7143734	0.2856266	0.005	0.474	0.007	0.005	0.509
YC36	Yucatan	Ranchos	-87.980169	21.439976	0.3447245	0.8500262	0.6093818	0.3906182	0.036	0.575	0.005	0.014	0.371
YC37	Yucatan	Ranchos	-87.972021	21.445659	0.2434502	0.7282046	0.4808464	0.5191536	0.009	0.913	0.014	0.047	0.016
YC38	Yucatan	Ranchos	-87.962846	21.433915	0.4859866	0.9159445	0.7320601	0.2679399	0.006	0.759	0.042	0.066	0.127
YC39	Yucatan	Ranchos	-87.969702	21.432999	0.4755682	0.9126488	0.7243464	0.2756536	0.008	0.568	0.024	0.031	0.37
YC41	Yucatan	Ranchos	-87.980206	21.440044	0.339039	0.8005028	0.5790674	0.4209326	0.005	0.942	0.005	0.021	0.026
YC43	Yucatan	Dzilam	-88.866890	21.391827	0.2878044	0.7364196	0.5110458	0.4889542	0.041	0.303	0.647	0.005	0.005
YC44	Yucatan	Dzilam	-88.867888	21.391174	0.3387563	0.7657976	0.5565016	0.4434984	0.007	0.504	0.419	0.004	0.067
YC45	Yucatan	Dzilam	-88.869090	21.391895	0.2436591	0.721401	0.4767691	0.5232309	0.024	0.844	0.049	0.014	0.069
QR01	Qroo	Rio Yalikin	-87.176357	21.439967	0.7215287	1	0.9361219	0.0638781	0.004	0.01	0.009	0.017	0.96
QR04	Qroo	Rio Yalikin	-87.175688	21.433137	0.2563914	0.7500418	0.4999244	0.5000756	0.005	0.941	0.038	0.01	0.006
QR07	Qroo	Moon Palace	-86.833287	20.983244	0.0528406	0.4389807	0.1994042	0.8005958	0.013	0.974	0.005	0.003	0.005
QR08	Qroo	Moon Palace	-86.833641	20.991399	0.1742184	0.6774258	0.4093805	0.5906195	0.005	0.955	0.015	0.014	0.011
QR09	Qroo	Moon Palace	-86.834583	20.988460	0.0727949	0.5291321	0.258449	0.741551	0.007	0.98	0.006	0.004	0.004
QR10	Qroo	Moon Palace	-86.838222	20.985260	0.1044256	0.6443638	0.3428038	0.6571962	0.007	0.868	0.087	0.011	0.027
QR11	Qroo	Moon Palace	-86.835469	20.982869	0.0109264	0.5006509	0.1677728	0.8322272	0.006	0.98	0.004	0.004	0.007
QR12	Qroo	Moon Palace	-86.843447	20.988958	0.2329986	0.7286634	0.4737751	0.5262249	0.011	0.689	0.18	0.027	0.094
QR13	Qroo	Moon Palace	-86.846688	20.985790	0.0648203	0.4980861	0.2360879	0.7639121	0.016	0.921	0.018	0.005	0.04
QR13-A	Qroo	Moon Palace	-86.849357	20.988862	0.0324341	0.4426264	0.1792279	0.8207721	0.008	0.972	0.006	0.005	0.009
QR13-B	Qroo	Moon Palace	-86.845763	20.989830	0.1742184	0.6774258	0.4093805	0.5906195	0.01	0.939	0.009	0.011	0.031

QR14	Qroo	Mayacoba	-87.024827	20.690499	0.1263084	0.6289871	0.3486965	0.6513035	0.007	0.936	0.004	0.009	0.044
QR15-A	Qroo	Mayacoba	-87.024827	20.690499	0.3570825	0.9763463	0.7150376	0.2849624	0.004	0.012	0.052	0.004	0.928
QR16	Qroo	Xcaret	-87.111819	20.586217	0.024083	0.3919436	0.1434561	0.8565439	0.142	0.505	0.007	0.005	0.342
QR17	Qroo	Xcaret	-87.111819	20.586217	0.251086	0.6797646	0.45838	0.54162	0.034	0.45	0.504	0.006	0.006
QR18	Qroo	Xcaret	-87.113466	20.588385	0.3064287	0.8128734	0.5650552	0.4349448	0.026	0.048	0.888	0.005	0.033
QR19	Qroo	Xcaret	-87.112978	20.589878	0.1953628	0.6378895	0.4017208	0.5982792	0.023	0.652	0.315	0.005	0.005
QR20	Qroo	Punta Allen	-87.497563	19.807935	0.1963524	0.6815126	0.4253226	0.5746774	0.067	0.779	0.009	0.084	0.061
QR23	Qroo	Felipe Carrillo Pto.	-88.081324	19.468733	0.1468645	0.7363448	0.414972	0.585028	0.005	0.004	0.983	0.003	0.005
QR26	Qroo	Felipe Carrillo Pto.	-88.026892	19.471332	0.4058146	0.8683864	0.6552134	0.3447866	0.006	0.024	0.96	0.004	0.006
QR29	Qroo	Rio Hondo	-88.367995	18.486615	0.3029147	0.8910115	0.6172764	0.3827236	0.01	0.008	0.972	0.004	0.006
QR30	Qroo	Rio Hondo	-88.382482	18.480181	0.5605617	0.9722326	0.8139274	0.1860726	0.004	0.017	0.969	0.003	0.007
QR32	Qroo	Rio Hondo	-88.402319	18.495678	0.2788492	0.8195202	0.5533483	0.4466517	0.005	0.004	0.979	0.003	0.009
QR33	Qroo	Rio Hondo	-88.392208	18.487816	0.0586067	0.4915438	0.2235071	0.7764929	0.019	0.208	0.759	0.002	0.012
QR34	Qroo	Rio Hondo	-88.402673	18.456735	0.1633904	0.801142	0.4591129	0.5408871	0.009	0.018	0.931	0.003	0.039
QR35	Qroo	Caobas	-88.989324	18.457354	0.0908918	0.5040164	0.2632732	0.7367268	0.033	0.946	0.006	0.006	0.009
QR36	Qroo	Caobas	-88.989204	18.457442	0.081541	0.4686762	0.2398623	0.7601377	0.012	0.975	0.006	0.004	0.003
QR38	Qroo	Chajul	-92.132447	15.880450	0.4782548	0.8877428	0.7074911	0.2925089	0.02	0.017	0.706	0.214	0.043
QR39	Qroo	Belice	-88.402673	18.456735	0.1473745	0.6821794	0.3934924	0.6065076	0.005	0.899	0.007	0.007	0.082
QR40	Qroo	Belice	-88.402673	18.456735	0.0270745	0.405793	0.1558863	0.8441137	0.242	0.743	0.004	0.004	0.007
QZ01	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.005	0.015	0.947	0.011	0.023
QZ02	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.012	0.022	0.91	0.036	0.02
QZ03	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.005	0.013	0.957	0.016	0.008
QZ04	Cozumel	Cozumel	-86.979117	20.281455	0.5618118	0.9582224	0.8021469	0.1978531	0.041	0.042	0.891	0.014	0.012
QZ05	Cozumel	Cozumel	-86.979117	20.281455	0	0	0	1	0.005	0.006	0.98	0.005	0.004
QZ06	Cozumel	Cozumel	-86.979117	20.281455	0.6413035	0.9926959	0.8611385	0.1388615	0.009	0.01	0.966	0.009	0.005
QZ07	Cozumel	Cozumel	-86.979117	20.281455	0.6399357	0.9935927	0.8629434	0.1370566	0.01	0.01	0.736	0.232	0.012
QZ08	Cozumel	Cozumel	-86.979117	20.281455	0.696157	1	0.9000124	0.0999876	0.007	0.021	0.614	0.243	0.114
QZ09	Cozumel	Cozumel	-86.979117	20.281455	0.5901417	0.959323	0.8143805	0.1856195	0.055	0.026	0.728	0.045	0.146
QZ10	Cozumel	Cozumel	-86.979117	20.281455	0.7454911	1	0.9622069	0.0377931	0.011	0.013	0.749	0.208	0.019

QZ11	Cozumel	Cozumel	-86.979117	20.281455	0.5390994	0.9604509	0.7834607	0.2165393	0.044	0.128	0.239	0.58	0.009
QZ12	Cozumel	Cozumel	-86.979117	20.281455	0.5502976	0.9431008	0.787449	0.212551	0.03	0.049	0.832	0.007	0.081
QZ13	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.009	0.017	0.771	0.187	0.016
QZ14	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.006	0.009	0.742	0.221	0.022
QZ15	Cozumel	Cozumel	-86.979117	20.281455	1	1	1	0	0.005	0.004	0.955	0.026	0.009
BC01	Chinchorro	Chinchorro	-87.320031	18.584038	1	1	1	0	0.004	0.004	0.947	0.027	0.019
BC02	Chinchorro	Chinchorro	-87.320031	18.584038	0.7434984	1	0.9710607	0.0289393	0.003	0.004	0.965	0.024	0.005
BC03	Chinchorro	Chinchorro	-87.320031	18.584038	0.6271083	0.9952769	0.8569043	0.1430957	0.005	0.006	0.966	0.016	0.006
BC04	Chinchorro	Chinchorro	-87.320031	18.584038	0.4045518	0.8469639	0.6399403	0.3600597	0.024	0.007	0.96	0.005	0.005
BC05	Chinchorro	Chinchorro	-87.320031	18.584038	1	1	1	0	0.003	0.004	0.962	0.022	0.008
BC06	Chinchorro	Chinchorro	-87.320031	18.584038	1	1	1	0	0.004	0.004	0.934	0.047	0.011
BC07	Chinchorro	Chinchorro	-87.320031	18.584038	1	1	1	0	0.003	0.003	0.951	0.026	0.017
CS01	Chiapas1	Cañon del Sumidero	-93.058260	16.779395	1	1	1	0	0.003	0.003	0.004	0.986	0.004
CS02	Chiapas1	Cañon del Sumidero	-93.060707	16.783047	0.6328882	1	0.8852511	0.1147489	0.007	0.007	0.011	0.03	0.945
CS03	Chiapas1	Cañon del Sumidero	-93.061130	16.783386	0.8815379	1	1	0	0.003	0.008	0.008	0.839	0.142
CS04	Chiapas1	Cañon del Sumidero	-93.063278	16.785429	1	1	1	0	0.003	0.003	0.004	0.986	0.004
CS05	Chiapas1	Cañon del Sumidero	-93.062843	16.784609	0.7041663	1	0.9182901	0.0817099	0.004	0.004	0.022	0.221	0.749
CS06	Chiapas1	Cañon del Sumidero	-93.064328	16.808328	1	1	1	0	0.004	0.004	0.004	0.98	0.008
CS07	Chiapas1	Cañon del Sumidero	-93.063439	16.814390	1	1	1	0	0.004	0.003	0.003	0.986	0.003
CS08	Chiapas1	Cañon del Sumidero	-93.061748	16.814708	1	1	1	0	0.004	0.003	0.003	0.986	0.003
CS09	Chiapas1	Cañon del Sumidero	-93.068645	16.839708	1	1	1	0	0.003	0.003	0.003	0.987	0.004
CS10	Chiapas1	Cañon del Sumidero	-93.070575	16.840071	1	1	1	0	0.004	0.003	0.004	0.984	0.005
CS11	Chiapas1	Cañon del Sumidero	-93.082606	16.935878	1	1	1	0	0.004	0.004	0.003	0.981	0.007
CS12	Chiapas1	Cañon del Sumidero	-93.085132	16.932499	0.7652139	1	0.974387	0.025613	0.003	0.003	0.005	0.194	0.794
CS13	Chiapas1	Cañon del Sumidero	-93.072566	16.913581	0.8971325	1	1	0	0.003	0.003	0.012	0.928	0.054
CS14	Chiapas1	Cañon del Sumidero	-93.075542	16.910183	1	1	1	0	0.003	0.003	0.003	0.987	0.004
CS15	Chiapas1	Cañon del Sumidero	-93.076416	16.904971	1	1	1	0	0.003	0.006	0.005	0.942	0.044
CS16	Chiapas1	Cañon del Sumidero	-93.069164	16.887721	1	1	1	0	0.003	0.003	0.004	0.984	0.006
CS17	Chiapas1	Cañon del Sumidero	-93.064686	16.893132	1	1	1	0	0.003	0.004	0.004	0.982	0.007

CS18	Chiapas1	Cañon del Sumidero	-93.061236	16.891879	0.4876536	0.9662349	0.7773777	0.2226223	0.053	0.014	0.008	0.035	0.89
CS19	Chiapas1	Cañon del Sumidero	-93.053094	16.893030	0.407585	0.9094872	0.6966444	0.3033556	0.022	0.006	0.007	0.226	0.739
CS20	Chiapas1	Cañon del Sumidero	-93.050880	16.894791	0.4068814	0.9488538	0.7206684	0.2793316	0.024	0.164	0.009	0.004	0.798
CH01	Chiapas2	Costa Playa Linda	-92.339365	14.638934	0.8948421	1	1	0	0.002	0.006	0.008	0.009	0.975
CH02	Chiapas2	Costa Playa Linda	-92.343262	14.642925	0.6990287	0.9936801	0.9082984	0.0917016	0.025	0.015	0.004	0.938	0.017
CH03	Chiapas2	Costa Barra San Jose	-92.614026	14.914012	0.2965037	0.863882	0.6040397	0.3959603	0.026	0.064	0.02	0.003	0.887
CH04	Chiapas2	Costa Angostura	-92.578253	16.150019	0.8088061	1	1	0	0.003	0.004	0.004	0.023	0.966
CH05	Chiapas2	Costa Buenavista	-93.586762	15.817218	0.2977414	0.8572384	0.5927082	0.4072918	0.019	0.134	0.015	0.005	0.826
CH06	Chiapas2	Costa Boca del Cielo	-93.655873	15.841019	0.5906947	0.953243	0.8110101	0.1889899	0.008	0.015	0.004	0.102	0.871
CH07	Chiapas2	Costa Puerto Arista	-93.699914	15.959245	0.7014261	0.9872337	0.8970514	0.1029486	0.027	0.006	0.008	0.007	0.952
CH08	Chiapas2	Costa Puerto Arista	-93.754043	15.931840	0.8915876	1	1	0	0.003	0.004	0.006	0.369	0.618
CH09	Chiapas2	Costa Zapotal	-93.170195	15.456895	0.7929289	0.9972862	0.9500625	0.0499375	0.007	0.01	0.012	0.308	0.663
CH10	Chiapas2	Costa Angostura	-92.669861	16.084840	0.7340802	1	0.9769239	0.0230761	0.005	0.003	0.005	0.004	0.983
CH11	Chiapas2	Costa El Manguito	-93.502225	15.733011	0.776274	1	1	0	0.013	0.016	0.005	0.03	0.936
CH12	Chiapas2	Costa Acapetahua	-92.864426	15.176349	0.8911	1	1	0	0.003	0.005	0.004	0.984	0.004
CH13	Chiapas2	Costa Villa del Mar	-93.947817	16.090308	0.8903601	1	1	0	0.003	0.004	0.003	0.985	0.004
CH14	Chiapas2	Costa Puerto Arista	-93.886706	15.989920	0.5552779	0.9789456	0.8150132	0.1849868	0.007	0.033	0.007	0.051	0.902
VT01	Oaxaca	Ventanilla	-96.579730	15.671462	0.8075096	1	1	0	0.004	0.014	0.347	0.054	0.581
VT02	Oaxaca	Ventanilla	-96.579730	15.671462	0.5912128	0.9519462	0.8090116	0.1909884	0.168	0.006	0.408	0.396	0.022
VT03	Oaxaca	Ventanilla	-96.579730	15.671462	0.8203046	1	1	0	0.006	0.006	0.338	0.619	0.031
VT04	Oaxaca	Ventanilla	-96.579730	15.671462	0.7224449	0.9838383	0.9022895	0.0977105	0.006	0.008	0.044	0.51	0.431
VT05	Oaxaca	Ventanilla	-96.579730	15.671462	0.8416748	1	1	0	0.005	0.005	0.171	0.803	0.016
VT06	Oaxaca	Ventanilla	-96.579730	15.671462	0.0684363	0.4335235	0.2107707	0.7892293	0.586	0.362	0.006	0.021	0.025
VT07	Oaxaca	Ventanilla	-96.579730	15.671462	0.075191	0.4550515	0.2267776	0.7732224	0.63	0.229	0.096	0.007	0.038
VT08	Oaxaca	Ventanilla	-96.579730	15.671462	0.4564893	0.9257697	0.728282	0.271718	0.057	0.021	0.009	0.067	0.846
VT09	Oaxaca	Ventanilla	-96.579730	15.671462	0.7146695	0.993712	0.9109025	0.0890975	0.006	0.007	0.116	0.842	0.029
VT10	Oaxaca	Ventanilla	-96.579730	15.671462	0.7415651	1	0.9643045	0.0356955	0.005	0.005	0.536	0.444	0.011
VT11	Oaxaca	Ventanilla	-96.579730	15.671462	0.9018298	1	1	0	0.003	0.004	0.302	0.673	0.018
VT12	Oaxaca	Ventanilla	-96.579730	15.671462	0.6966787	0.9931652	0.8947821	0.1052179	0.014	0.065	0.014	0.598	0.31

VT13	Oaxaca	Ventanilla	-96.579730	15.671462	0.7670238	1	0.9714234	0.0285766	0.005	0.007	0.549	0.415	0.024
VT14	Oaxaca	Ventanilla	-96.579730	15.671462	0.1299367	0.8386764	0.4506951	0.5493049	0.071	0.023	0.114	0.003	0.789
OX01	Oaxaca	Chacahua	-97.710059	15.981205	1	1	1	0	0.003	0.003	0.004	0.983	0.007
OX02	Oaxaca	Chacahua	-97.690921	15.991723	1	1	1	0	0.003	0.003	0.004	0.985	0.005
AC01	Guerrero	Acapulco	100.047028	16.942642	0.8936671	1	1	0	0.003	0.003	0.044	0.76	0.19
AC02	Guerrero	Acapulco	100.052729	16.942977	0.8297771	1	1	0	0.003	0.003	0.003	0.985	0.006
AC03	Guerrero	Acapulco	-99.973778	16.925188	0.7959594	1	1	0	0.003	0.004	0.004	0.922	0.067
AC04	Guerrero	Acapulco	-99.971287	16.923292	0.7774219	1	1	0	0.004	0.004	0.003	0.516	0.473
GR01	Guerrero	Ixtapa	101.598574	17.653399	0.5025937	0.923083	0.746628	0.253372	0.008	0.007	0.004	0.006	0.975
GR02	Guerrero	Ixtapa	101.598574	17.653399	0.7250061	1	0.9645933	0.0354067	0.004	0.004	0.009	0.025	0.958
GR03	Guerrero	Ixtapa	101.598574	17.653399	0.283476	0.7855787	0.5338299	0.4661701	0.159	0.211	0.007	0.009	0.614
GR04	Guerrero	Ixtapa	101.598574	17.653399	0.2440261	0.7295907	0.4826811	0.5173189	0.016	0.649	0.026	0.128	0.18
GR05	Guerrero	Ixtapa	101.598574	17.653399	0.4483968	0.9078617	0.709103	0.290897	0.008	0.009	0.006	0.01	0.967
GR06	Guerrero	Ixtapa	101.598574	17.653399	0.655883	1	0.8871636	0.1128364	0.005	0.009	0.009	0.026	0.951
GR07	Guerrero	Ixtapa	101.598574	17.653399	0.7877969	1	1	0	0.004	0.009	0.264	0.106	0.616
GR08	Guerrero	Ixtapa	101.598574	17.653399	0.7080766	0.9947373	0.9149221	0.0850779	0.05	0.013	0.013	0.808	0.116
GR09	Guerrero	Ixtapa	101.598574	17.653399	0.776274	1	1	0	0.008	0.015	0.017	0.021	0.94
GR10	Guerrero	Ixtapa	101.598574	17.653399	0.0263384	0.3601026	0.1424445	0.8575555	0.87	0.011	0.007	0.07	0.042
GR11	Guerrero	Ixtapa	101.598574	17.653399	0.1552072	0.6722492	0.3903575	0.6096425	0.412	0.011	0.005	0.019	0.553
GR12	Guerrero	Ixtapa	101.598574	17.653399	0.0350932	0.4696812	0.1922443	0.8077557	0.03	0.738	0.008	0.008	0.216
MC01	Michoacan	Lazaro Cardenas- Barra de Santa Ana	102.294122	17.965836	0.7590095	1	0.9957369	0.0042631	0.004	0.009	0.008	0.021	0.957
MC02	Michoacan	Lazaro Cardenas- Balsas	102.186558	17.942008	0.6697586	1	0.9639639	0.0360361	0.003	0.006	0.006	0.114	0.872
MC03	Michoacan	Lazaro Cardenas- Balsas	102.187289	17.949722	0.8850653	1	1	0	0.003	0.004	0.007	0.97	0.017
MC04	Michoacan	Lazaro Cardenas- Balsas	102.183704	17.936267	0.8575071	1	1	0	0.003	0.005	0.004	0.921	0.066
MC05	Michoacan	Lazaro Cardenas- Balsas	102.187277	17.945275	0.8850653	1	1	0	0.003	0.004	0.003	0.984	0.005
MC06	Michoacan	Lazaro Cardenas- Balsas	102.186866	17.953231	0.9019086	1	1	0	0.003	0.008	0.004	0.977	0.008
MC07	Michoacan	Lazaro Cardenas- Balsas	102.185789	17.960066	0.8850653	1	1	0	0.003	0.004	0.003	0.984	0.006

PV01	Colima	Palo Verde	104.023579	18.896411	0.787834	1	1	0	0.005	0.007	0.006	0.056	0.926
PV02	Colima	Palo Verde	104.032359	18.906599	0.8702184	1	1	0	0.003	0.006	0.022	0.841	0.128
PV03	Colima	Palo Verde	104.040850	18.918316	0.9029803	1	1	0	0.003	0.003	0.003	0.988	0.003
PV04	Colima	Palo Verde	104.040266	18.928851	1	1	1	0	0.003	0.003	0.003	0.987	0.005
PV05	Colima	Palo Verde	104.050556	18.926758	1	1	1	0	0.003	0.003	0.003	0.987	0.004
PV06	Colima	Palo Verde	104.065849	18.970522	1	1	1	0	0.003	0.003	0.003	0.987	0.004
PV07	Colima	Palo Verde	104.047879	18.938857	1	1	1	0	0.003	0.003	0.003	0.987	0.004
PV08	Colima	Palo Verde	104.064473	18.948445	1	1	1	0	0.003	0.003	0.003	0.987	0.004
PV09	Colima	Palo Verde	104.076332	18.964951	1	1	1	0	0.003	0.003	0.003	0.986	0.004
PV10	Colima	Palo Verde	104.089702	18.983885	1	1	1	0	0.003	0.003	0.003	0.986	0.005
PV11	Colima	Palo Verde	104.096713	18.963340	1	1	1	0	0.003	0.003	0.003	0.987	0.004
AM01	Colima	Amela	103.749496	18.860605	0.5964946	0.9799047	0.8280041	0.1719959	0.005	0.004	0.029	0.115	0.846
AM02	Colima	Amela	103.757482	18.854631	0.8573185	1	1	0	0.004	0.003	0.004	0.973	0.016
AM03	Colima	Amela	103.745534	18.849937	0.9149868	1	1	0	0.003	0.004	0.004	0.984	0.005
AM04	Colima	Amela	103.751079	18.845460	0.6076438	1	0.9022789	0.0977211	0.005	0.013	0.007	0.005	0.969
AM05	Colima	Amela	103.768451	18.813351	0.8956387	1	1	0	0.004	0.004	0.02	0.964	0.008
JL01	Jalisco	El salado	105.243053	20.663309	0.8896297	1	1	0	0.003	0.003	0.172	0.747	0.074
JL02	Jalisco	El salado	105.243358	20.663733	1	1	1	0	0.003	0.003	0.008	0.975	0.011
JL03	Jalisco	El salado	105.243719	20.664361	0.6473485	1	0.8850351	0.1149649	0.007	0.004	0.004	0.073	0.912
JL04	Jalisco	Boca de Tomates	105.270053	20.669018	1	1	1	0	0.003	0.003	0.003	0.986	0.005
JL05	Jalisco	Boca de Tomates	105.269588	20.670647	0.7422463	1	0.9683987	0.0316013	0.003	0.003	0.102	0.078	0.813
JL06	Jalisco	Boca de Tomates	105.269178	20.670666	1	1	1	0	0.004	0.004	0.004	0.013	0.975
JL07	Jalisco	El Quelele	105.294339	20.728614	0.5096041	1	1	0	0.005	0.004	0.003	0.036	0.952
JL08	Jalisco	El Quelele	105.292881	20.726582	0.6335429	1	0.8790344	0.1209656	0.029	0.009	0.008	0.473	0.48
JL09	Jalisco	El Quelele	105.291896	20.724910	1	1	1	0	0.006	0.005	0.008	0.009	0.972
JL10	Jalisco	El Quelele	105.299087	20.726260	0.6716907	0.9921871	0.8944333	0.1055667	0.051	0.005	0.018	0.881	0.045
NY01	Nayarit	San Blas	105.258167	21.556328	0.4770143	0.9174207	0.7318032	0.2681968	0.095	0.012	0.004	0.503	0.385

NY02	Nayarit	San Blas	105.254946	21.564244	0.9103521	1	1	0	0.003	0.004	0.037	0.022	0.934
NY03	Nayarit	San Blas	105.245379	21.563766	0.8762001	1	1	0	0.003	0.004	0.008	0.972	0.012
NY04	Nayarit	La Tobará	105.257811	21.548467	0.8896372	1	1	0	0.003	0.003	0.003	0.977	0.014
NY05	Nayarit	La Tobará	105.257811	21.548467	0.8933182	1	1	0	0.003	0.003	0.005	0.924	0.064
NY06	Nayarit	La Tobará	105.257811	21.548467	0.8097805	1	1	0	0.004	0.003	0.004	0.094	0.895
NY07	Nayarit	La Tobará	105.257811	21.548467	0.7311821	1	1	0	0.007	0.006	0.034	0.007	0.945
NY08	Nayarit	La Tobará	105.257811	21.548467	0.8585302	1	1	0	0.003	0.005	0.004	0.035	0.953
NY09	Nayarit	La Tobará	105.257811	21.548467	0.6350892	0.9612698	0.8364333	0.1635667	0.005	0.006	0.005	0.008	0.976
NY10	Nayarit	La Tobará	105.254107	21.550244	0.8205189	1	0.9904207	0.0095793	0.003	0.004	0.007	0.069	0.917

Table S2. Canonical discriminant analysis using Fisher’s linear discriminant analysis (Fralely & Raftery 2002), based on 124 polymorphic sites from sequences of mtDNA of 271 individuals of crocodiles from the Gulf of Mexico and the Pacific. The species assignment for every individual is based on the genetic data from the hybrid index estimation (A) and admixture proportions (B; STRUTURE, K=5) (see Results in main text).

A) INTROGRESS hybrid index results

Predicted group membership				
number of individuals (%)				
	<i>C. moreletii</i>	<i>C. acutus</i>	hybrids	total
<i>C. moreletii</i>	25 (80.6)	0	6 (19.4)	31
<i>C. acutus</i>	0	68 (90.7)	7 (9.3)	75
hybrids	3 (1.8)	27 (16.4)	135 (81.8)	165

B) STRUCTURE results (K=5)

Predicted group membership						
number of individuals (%)						
	<i>C. moreletii</i>	hybrids	<i>C. acutus</i> (Caribbean islands)	<i>C. acutus</i> (northwestern and central Pacific)	<i>C. acutus</i> (southern Pacific)	Total
<i>C. moreletii</i>	27 (87.1)	4 (12.9)	0	0	0	31
hybrids	2 (1.6)	102 (82.3)	0	7 (5.6)	13 (10.5)	124
<i>C. acutus</i> (Caribbean islands)	1 (3.7)	2 (7.4)	21 (77.8)	2 (7.4)	1 (3.7)	27
<i>C. acutus</i> (northwestern and central Pacific)	0	0	3 (5.5)	50 (90.9)	2 (3.6)	55
<i>C. acutus</i> (southern Pacific)	0	3 (8.8)	0	14 (41.2)	17 (50)	34

Table S3. Genetic diversity based on 12 microsatellite loci and mtDNA sequences, estimated for *Crocodylus acutus* and *C. moreletii* in Mexico. Population listing follows the geographic gradient.

Population	Microsatellites						mtDNA		
	Na	Ne	Pa	Ho	He	Fis	h	Pi	K
SanLuis	2.250	1.454	0.000	0.121	0.238	0.682	0.727	0.00059	1.018
Tamaulipas	6.500	2.054	0.000	0.286	0.458	0.413	0.874	0.00616	10.649
Veracruz	6.083	2.517	0.167	0.300	0.467	0.279	0.861	0.0032	5.523
Tabasco	5.250	2.169	0.083	0.301	0.458	0.360	0.811	0.00733	12.674
Campeche	8.000	3.275	0.500	0.379	0.612	0.378	0.788	0.02164	37.424
Yucatan	9.083	3.727	0.417	0.436	0.694	0.360	0.794	0.02219	38.375
Qroo	9.167	3.709	0.583	0.372	0.696	0.470	0.886	0.02407	41.61
Cozumel	4.833	3.259	0.250	0.689	0.654	-0.049	0.648	0.00051	0.876
Chinchorro	2.750	2.202	0.083	0.333	0.389	0.122	0.542	0.00044	0.762
Chiapas1	7.083	2.800	0.333	0.367	0.579	0.384	0.065	0.00004	0.065
Chiapas2	7.917	5.478	0.083	0.435	0.794	0.453	0.667	0.00039	0.667
Oaxaca	6.833	3.792	0.250	0.464	0.678	0.328	0.964	0.01571	27.164
Guerrero	8.167	5.274	0.000	0.536	0.774	0.303	0.833	0.02285	39.5
Michoacan	3.917	2.953	0.333	0.500	0.599	0.135	0.733	0.00054	0.933
Colima	5.917	2.886	0.333	0.344	0.563	0.426	0.345	0.00021	0.364
Jalisco	6.583	4.171	0.083	0.458	0.666	0.260	0.711	0.00054	0.933
Nayarit	6.583	4.270	0.250	0.450	0.733	0.376	1	0.00116	2

Number of alleles (Na) effective alleles (Ne) and private alleles (Pa); Ho: observed heterozygosity; He: expected heterozygosity; Fis, fixation index; h: haplotype diversity; Pi: nucleotide diversity; K: number of polymorphic sites.

Table S4. Genetic diversity based on 12 microsatellite loci, estimated for *Crocodylus acutus* and *C. moreletii* in Mexico, considering the genetic groups identified with STRUCTURE (K=5) (Pritchard et al. 2000).

Genetic group	Na	Ne	Pa	Ho	He	Fis
<i>C. moreletii</i>	5.833	1.857	0.000	0.227	0.380	0.436
hybrids	12.417	3.280	1.167	0.364	0.616	0.399
<i>C. acutus</i> (islands)	10.333	4.738	0.750	0.475	0.765	0.377
<i>C. acutus</i> (Pacific 1)	12.583	4.987	0.833	0.418	0.746	0.448
<i>C. acutus</i> (Pacific 2)	12.333	5.023	1.833	0.452	0.735	0.381

Number of alleles (Na) effective alleles (Ne) and private alleles (Pa); Ho: observed heterozygosity; He: expected heterozygosity; Fis, fixation index.

Table S5. Matrix of pairwise *Fst* (above diagonal) and *Rst* (below diagonal) between populations of *Crocodylus sp.* along the geographic gradient in Mexico. Values on red are not significant ($P>0.05$), whereas all the rest are significant ($P<0.001$).

	SanLuis	Tamaulipas	Veracruz	Tabasco	Campeche	Yucatan	Qroo	Cozumel	Chinchorro	Chiapas1	Chiapas2	Oaxaca	Guerrero	Michoacan	Colima	Jalisco	Nayarit
SanLuis	-	0.058	0.193	0.326	0.211	0.242	0.238	0.411	0.642	0.476	0.374	0.386	0.322	0.537	0.509	0.449	0.439
Tamaulipas	0.025	-	0.082	0.175	0.128	0.164	0.160	0.323	0.485	0.402	0.300	0.298	0.233	0.409	0.425	0.358	0.349
Veracruz	0.083	0.004	-	0.045	0.059	0.104	0.113	0.308	0.479	0.405	0.298	0.295	0.233	0.385	0.421	0.368	0.350
Tabasco	0.095	0.019	0.011	-	0.064	0.091	0.119	0.314	0.481	0.407	0.283	0.299	0.229	0.379	0.425	0.377	0.342
Campeche	0.183	0.106	0.092	0.039	-	0.033	0.035	0.213	0.348	0.313	0.199	0.202	0.160	0.282	0.328	0.279	0.248
Yucatan	0.393	0.304	0.291	0.211	0.066	-	0.023	0.153	0.288	0.246	0.130	0.146	0.094	0.201	0.255	0.204	0.172
Qroo	0.189	0.124	0.131	0.066	0.024	0.084	-	0.179	0.302	0.272	0.142	0.166	0.112	0.222	0.276	0.218	0.187
Cozumel	0.525	0.487	0.498	0.411	0.307	0.215	0.281	-	0.278	0.210	0.139	0.133	0.120	0.207	0.220	0.156	0.150
Chinchorro	0.773	0.620	0.657	0.535	0.495	0.533	0.456	0.600	-	0.363	0.249	0.249	0.229	0.368	0.357	0.323	0.287
Chiapas1	0.631	0.532	0.546	0.473	0.373	0.335	0.359	0.142	0.663	-	0.064	0.082	0.118	0.069	0.074	0.083	0.098
Chiapas2	0.531	0.448	0.472	0.382	0.315	0.283	0.291	0.147	0.563	0.059	-	0.057	0.018	0.025	0.089	0.037	0.009
Oaxaca	0.450	0.365	0.376	0.305	0.249	0.238	0.219	0.122	0.588	0.118	0.017	-	0.067	0.088	0.118	0.090	0.076
Guerrero	0.462	0.404	0.433	0.345	0.290	0.263	0.274	0.146	0.497	0.082	0.000	0.038	-	0.061	0.105	0.038	0.025
Michoacan	0.640	0.570	0.592	0.503	0.454	0.435	0.424	0.186	0.672	0.211	0.024	0.091	0.027	-	0.048	0.051	0.009
Colima	0.670	0.626	0.644	0.585	0.548	0.547	0.535	0.349	0.730	0.381	0.229	0.274	0.202	0.060	-	0.054	0.071
Jalisco	0.542	0.479	0.504	0.406	0.341	0.308	0.294	0.081	0.573	0.023	0.004	0.034	0.024	0.076	0.272	-	0.000
Nayarit	0.672	0.611	0.630	0.546	0.479	0.438	0.453	0.141	0.689	0.150	0.066	0.140	0.072	0.000	0.116	0.062	-

Table S6. Matrix of pairwise Nei's genetic distance values (above diagonal) and number of effective migrants per generation (Nem; below diagonal) between populations of *Crocodylus sp.* along the geographic gradient.

	SanLuis	Tamaulipas	Veracruz	Tabasco	Campeche	Yucatan	Qroo	Cozumel	Chinchorro	Chiapas1	Chiapas2	Oaxaca	Guerrero	Michoacan	Colima	Jalisco	Nayarit
SanLuis	-	0.055	0.190	0.387	0.294	0.447	0.409	1.029	2.109	1.467	1.375	0.994	0.808	2.012	1.726	1.354	1.724
Tamaulipas	4.070	-	0.100	0.229	0.210	0.333	0.315	0.927	1.616	1.412	1.181	0.843	0.624	1.602	1.627	1.272	1.475
Veracruz	1.048	2.794	-	0.061	0.084	0.166	0.182	0.801	1.604	1.401	1.052	0.781	0.581	1.281	1.522	1.323	1.378
Tabasco	0.517	1.182	5.331	-	0.101	0.155	0.217	0.894	1.565	1.519	1.068	0.877	0.629	1.272	1.676	1.570	1.448
Campeche	0.936	1.702	3.979	3.659	-	0.090	0.104	0.728	1.204	1.312	0.957	0.712	0.612	1.242	1.462	1.402	1.317
Yucatan	0.782	1.278	2.149	2.497	7.294	-	0.104	0.591	1.067	1.038	0.713	0.590	0.436	0.930	1.111	1.067	0.977
Qroo	0.801	1.312	1.969	1.857	6.939	10.474	-	0.781	1.219	1.352	0.899	0.756	0.588	1.192	1.380	1.321	1.248
Cozumel	0.358	0.524	0.563	0.546	0.921	1.384	1.146	-	0.756	0.681	0.783	0.503	0.585	0.834	0.716	0.636	0.741
Chinchorro	0.139	0.266	0.272	0.270	0.468	0.619	0.577	0.650	-	1.142	1.089	0.718	0.819	1.245	1.025	1.179	1.164
Chiapas1	0.275	0.372	0.367	0.365	0.548	0.767	0.669	0.941	0.439	-	0.225	0.242	0.412	0.232	0.181	0.266	0.344
Chiapas2	0.419	0.584	0.589	0.632	1.003	1.667	1.516	1.551	0.754	3.627	-	0.354	0.282	0.248	0.316	0.314	0.274
Oaxaca	0.398	0.588	0.598	0.586	0.985	1.468	1.257	1.634	0.755	2.816	4.104	-	0.363	0.372	0.348	0.404	0.422
Guerrero	0.526	0.824	0.821	0.843	1.309	2.411	1.986	1.826	0.840	1.865	14.034	3.475	-	0.349	0.356	0.281	0.303
Michoacan	0.215	0.362	0.399	0.410	0.638	0.992	0.874	0.958	0.430	3.400	9.885	2.595	3.867	-	0.191	0.284	0.205
Colima	0.241	0.338	0.344	0.338	0.511	0.730	0.656	0.889	0.451	3.117	2.565	1.870	2.137	4.967	-	0.199	0.263
Jalisco	0.307	0.448	0.429	0.413	0.647	0.974	0.895	1.353	0.524	2.779	6.583	2.537	6.372	4.690	4.345	-	0.157
Nayarit	0.319	0.467	0.465	0.481	0.757	1.200	1.088	1.420	0.621	2.306	28.958	3.034	9.706	28.552	3.247	0.000	-

Table S7. Matrix of pairwise *Fst* (above diagonal) and *Rst* (below diagonal) between the genetic groups identified with STRUCTURE (Pritchard et al. 2000). All values are significant ($P < 0.001$).

	<i>C. moreletii</i>	hybrids	<i>C. acutus</i> (islands)	<i>C. acutus</i> (Pacific 1)	<i>C. acutus</i> (Pacific 2)
<i>C. moreletii</i>	-	0.141	0.208	0.326	0.339
hybrids	0.133	-	0.060	0.213	0.230
<i>C. acutus</i> (islands)	0.212	0.057	-	0.114	0.136
<i>C. acutus</i> (Pacific 1)	0.409	0.257	0.134	-	0.032
<i>C. acutus</i> (Pacific 2)	0.498	0.401	0.259	0.094	-

Table S8. Matrix of pairwise Nei's genetic distance (above diagonal) and number of effective migrants per generation (*Nem*; below diagonal) between the genetic groups identified with STRUCTURE (Pritchard et al. 2000).

	<i>C. moreletii</i>	hybrids	<i>C. acutus</i> (islands)	<i>C. acutus</i> (Pacific 1)	<i>C. acutus</i> (Pacific 2)
<i>C. moreletii</i>	0.000	0.198	0.373	1.126	1.209
hybrids	1.529	0.000	0.141	0.824	0.934
<i>C. acutus</i> (islands)	0.952	3.921	0.000	0.600	0.744
<i>C. acutus</i> (Pacific 1)	0.517	0.922	1.944	0.000	0.137
<i>C. acutus</i> (Pacific 2)	0.488	0.836	1.584	7.680	0.000

DISCUSIÓN Y CONCLUSIONES FINALES

Discusión general

Este es el primer estudio genético que incluye la distribución completa en México para *C. acutus* y *C. moreletii* y con combinación de dos tipos de marcadores moleculares (microsatélites nucleares y secuencias de ADN mitocondrial) y análisis genéticos y estadísticos novedosos, estudiamos a este complejo y extraordinario sistema de hibridación entre las dos especies de cocodrilos mexicanos. En los Capítulos presentados, determinamos los niveles de hibridación y de variación genética en un contexto microevolutivo, además de explorar la ancestría de este proceso y de una posible especiación por medio de la hibridación en un contexto macroevolutivo. En ambos casos, logramos incorporar la información en un gradiente geográfico, bajo este contexto presentamos un modelo filogeográfico con el cual fue posible identificar los procesos que han llevado a ambas especies hasta este punto en su evolución.

En el primer capítulo nos enfocamos en determinar la extensión de la zona de hibridación en el Golfo de México y el Caribe, incluyendo localidades hacia el interior del continente en zonas lagunares y rivereñas. Los resultados determinaron que la zona de hibridación abarca prácticamente todo el Golfo de México y la península de Yucatán, “desplazando” a las especies parentales hacia poblaciones aisladas en dos islas en el Caribe, Cozumel y Banco Chinchorro para el caso de *C. acutus*, y a *C. moreletii* en islas continentales. Este proceso es el resultado de contacto secundario y no de divergencia primaria como ocurre en casos donde puede existir divergencia incompleta (Gompert & Buerkle 2016). Los análisis de clinas genéticas y geográficas son concordantes, mientras que la correlación entre distancias genéticas y geográficas muestran aislamiento por distancia; este análisis no está basado en distancias geográficas euclidianas como comúnmente se hace, sino bajo un escenario de estimación de distancias geográficas entre individuos, considerando un continuo a lo largo de los ríos, lagunas y zonas inundables que son la principal vía de migración en estas especies.

Por otro lado, se identificó un complejo mosaico de mezcla entre caracteres genéticos y morfológicos en la zona de hibridación. Cabe resaltar que los resultados obtenidos soportan un escenario donde la hibridación es ancestral y no un proceso reciente, donde las clinas genéticas muestran que los híbridos no presentan desventajas selectivas, contrario a los modelos de zonas de tensión donde las zonas de hibridación son mantenidas por selección en contra de los híbridos. Sin embargo, con los microsatélites no fue posible evaluar otras preguntas sobre procesos históricos y de divergencia, las cuales fueron abordadas en el

segundo capítulo: i) ¿Cuándo inicio el proceso de hibridación entre *C. acutus* y *C. moreletii*?; ii) ¿Cuáles son los tipos de selección asociados con la hibridación entre estas especies de cocodrilos?; iii) ¿Se pueden distinguir diferentes linajes en términos de no-mixturados (parentales) e híbridos?

Así, el objetivo en el segundo capítulo fue responder a dichas preguntas, para lograr discernir si los límites entre especies podrían resolverse en un sistema donde existe amplia hibridación pero con la particularidad de que el aislamiento reproductivo es improbable dado el intercambio genético continuo, sobre todo porque las especies parentales y los híbridos poseen una compatibilidad cromosómica idéntica (hibridación homoploide). Esto representó un reto, en particular porque el concepto tradicional de especies deja fuera a los híbridos intermedios, los cuales pueden, sin embargo, estar en proceso de especiación.

En el segundo capítulo incluimos muestras de todo México y combinamos análisis basados en microsatélites de ADN nuclear, los cuales son útiles para identificar procesos de hibridación, y ADN mitocondrial que es ideal para identificar linajes y estimar tiempos de divergencia. Los resultados confirmaron que la hibridación es ancestral, iniciando hace aproximadamente 2.5 millones de años (1.06 – 4.46, 95% HDP). Los tiempos de divergencia deberán confirmarse con datos genómicos tipo SNPs, análisis que están en proceso, dado que se estimaron sólo con base en ADN mitocondrial. Las proporciones de ancestría muestran que un linaje del sur del Pacífico fue el promotor de esta hibridación, cuyos tiempos de divergencia coinciden con el Pleistoceno, periodo asociado con ciclos climáticos y diferentes eventos geológicos (Padilla y Sanchez 2007; Vázquez-Domínguez & Arita 2010) que propiciaron el contacto entre ambos lados del continente, a través de continuos en los sistemas hídricos, permitiendo la dispersión de especies como los cocodrilos (Razo-Mendivil *et al.* 2013). Cabe resaltar que se identificó un grupo monofilético compuesto exclusivamente de individuos híbridos, lo cual apoya el potencial de diferenciación genética dentro de este peculiar sistema de hibridación. Asimismo, se determinó que existen diferentes tipos de selección a favor de los híbridos (sobredominancia) y selección en contra de las especies parentales (selección negativa), sin embargo los límites entre especie en este sistema son aun difusos y complejos de resolver bajo un modelo estricto de bifurcación entre especies.

El concepto de especie ha sido debatido por décadas y existen múltiples definiciones (ver De Queiroz 2007). Bajo estos conceptos, una especie es un linaje metapoblacional que evoluciona independientemente, el cual tiene que estar intrínsecamente aislado reproductivamente de otros linajes, tiene que ocupar un nicho diferente y debe ser

fenotípicamente distinguible (De Queiroz 2007). Siguiendo estos preceptos, se pueden definir claramente los límites entre especie, sin embargo en un sistema de hibridación como el que se presenta en este trabajo, estos límites son semipermeables, genética y morfológicamente difusos, y discontinuos en términos filogenéticos y filogeográficos.

Los análisis identificaron un grupo monofilético de híbridos, linaje que está formado por individuos de diferentes zonas en el continuo geográfico dentro de la zona de hibridación a lo largo del Golfo de México. Sin embargo, también se tienen individuos que no están “separados genéticamente”, de los cuales no se tiene evidencia suficiente de aislamiento reproductivo, a pesar del aislamiento geográfico de las especies parentales en zonas insulares y continentales. En este sentido, Buerkle & Rieseberg (2008) soportan la idea de que la tasa de estabilización del genoma en las zonas de hibridación es del orden de cientos o miles de generaciones, y que no es posible saber en qué momento se fija el último componente significativo o crítico del aislamiento, ni cuándo se alcanza la especiación, en particular en especies con tasas generacionales grandes, como es el caso de los cocodrilos, así como en una zona de hibridación tan amplia como la que se determinó en nuestro trabajo (>3000 km).

Recomendaciones de conservación

En el Capítulo 1 incluimos un apartado llamado “*Conservation implications*”, en el cual describimos brevemente el estatus de conservación nacional e internacional con un enfoque hacia *C. moreletii*, dada la importancia que actualmente presenta para el comercio de productos y subproductos en el mercado internacional, así como el potencial estatus de peligro de extinción dado el aislamiento de individuos parentales (“puros”) de esta especie. Los resultados muestran evidencia de proceso de hibridación ancestral, el cual además sugiere que existe un proceso de especiación por hibridación homoploide a lo largo del Golfo de México. Sin embargo, con nuestros resultados no es posible clarificar si es posible determinar una especiación completa (en términos de delimitación de especies), por lo que ante los “ojos” de la legislación, aun es necesario tratarlos como un grupo híbrido.

Como se ha mencionado anteriormente, *C. moreletii* se encuentra actualmente en el apéndice II y *C. acutus* en el apéndice I de la lista de especies en riesgo de CITES. La Resolución Conf. 10.17 (Rev. COP 14) establece que los individuos híbridos adquieren el nivel de protección de la especie parental más protegida, en este caso, los individuos híbridos se agregarían al apéndice I; sin embargo esto es aplicable únicamente a los linajes recientes (hasta 4 generaciones), por lo que los individuos híbridos en realidad se encuentran

desprotegidos *per se*. Por otro lado, se establece también que la fauna y flora híbridos pueden incluirse en los apéndices, pero sólo si forman poblaciones distintivas y estables en vida libre (Resolución Conf. 9.24, Rev. COP16). Los resultados del “Programa para el Monitoreo del cocodrilo de pantano (*Crocodylus moreletii*)” impulsado por la CONABIO, evidencian que las poblaciones de cocodrilos en el Golfo de México se encuentran poblacionalmente estables (~76,000 individuos en la naturaleza), “lo que ubica a la especie en buen estado y con potencial de desarrollar proyectos productivos sustentables” (Sánchez Herrera *et al* 2012, 2015). Sin embargo, si se toma en cuenta que nuestros resultados muestran que las poblaciones que se han manejado como *C. moreletii* en realidad son poblaciones de híbridos, tenemos un nuevo escenario en el que es necesario una discusión más puntual sobre el estado de conservación que debe adquirir esta nueva entidad taxonómica y a *C. moreletii per se*. Recientemente se aprobó realizar un programa de ranqueo de *C. moreletii* en tres puntos focales en México (Campeche, Veracruz y Quintana Roo), es decir, extraer huevos directamente de vida silvestre para criarlos en cautiverio y mantener la línea de producción de algunas granjas de cocodrilo y comunidades humanas asentadas en estos sitios. Lo anterior es muy delicado dado que, aunque no sabemos si hay hibridación en estos sitios, ya que en nuestro trabajo no obtuvimos muestras de estos sitios en particular (Palizada, Campeche; Chacchobben, Quintana Roo y la Mancha, Veracruz), sin embargo, si consideramos los patrones geográficos y genéticos observados, es altamente probable que sean poblaciones híbridas. En consecuencia, es muy preocupante que programas de este tipo se incluyan en una agenda nacional sin los estudios genéticos necesarios.

Nuestros resultados también evidencian que no es posible identificar parentales e híbridos únicamente por características morfológicas, ya que son altamente crípticos. Con nuestros resultados identificamos un complejo mosaico de caracteres morfométricos para la zona de hibridación y, aunque es posible diferenciar a *C. moreletii* de *C. acutus*, los individuos híbridos mantienen caracteres mezclados de ambas especies. Así, la única forma para determinar si son individuos parentales o híbridos es a partir de pruebas genéticas y con base en marcadores nucleares que identifican mezcla genética.

La determinación del nivel taxonómico que debe considerarse en este grupo de híbridos entre *C. acutus* y *C. moreletii* es discutible, ya que es necesario el uso de marcadores más eficaces para determinar los niveles de divergencia y simulaciones a nivel nuclear. En este sentido, nuestros esfuerzos están dirigidos al uso de marcadores tipo SNPs (Single nucleotide polymorphisms), que nos permitan delimitar si el grupo de híbridos ya puede considerarse como

una especie diferente o un grupo híbrido en el que no se ha estabilizado su genoma. Esto claramente nos ayudará a proponer mejores mecanismos de conservación para el grupo de cocodrilos en México, y proporcionar además un modelo que apoye que las entidades no-recientemente híbridas deben ser consideradas como grupos evolutivamente únicos, en proceso de especiación vía hibridación.

Conclusiones

- 1) Se detectó un proceso de hibridación entre *C. acutus* y *C. moreletii* ampliamente extendido a lo largo del Golfo de México y el Caribe.
- 2) Este proceso inició a partir de contacto secundario, aparentemente hace mucho tiempo (ca. 2.6 millones de años).
- 3) Se identificaron haplotipos mitocondriales que unen a los clados de cada especie y sus híbridos en localidades de la región formada por Yucatán, Campeche y Quintana Roo.
- 4) El proceso de hibridación se formó en la región antes mencionada, particularmente en Campeche, a partir de la cual se extendió al norte a lo largo del Golfo de México y al sur hacia la península de Yucatán y el Caribe.
- 5) Las proporciones de ancestría muestran que un linaje de *C. acutus* al sur del Pacífico fue el promotor de la hibridación, cuyos tiempos de divergencia coinciden con el Pleistoceno, periodo asociado con ciclos climáticos y diferentes eventos geológicos que propiciaron el contacto entre ambos lados del continente, a través de continuos en los sistemas hídricos, permitiendo la dispersión.
- 6) Los individuos con linaje de *C. acutus* de las islas del Caribe están asociados con linajes de la región cercana al Istmo de Tehuantepec en el Pacífico, los cuales posteriormente divergieron alopátricamente.
- 7) La combinación de análisis de los marcadores genéticos es compatible con las clinas geográficas, resaltando el aislamiento por distancia, creando un panorama continuo entre ambos datos, genéticos y geográficos.
- 8) *C. moreletii* se encuentra en un proceso crítico de extinción o, alternativamente, está potencialmente en proceso de especiación por medio de hibridación.
- 9) *C. acutus* de las islas del Caribe representan un grupo alopátrico y genéticamente separado de los linajes del Pacífico, el cual, como *C. moreletii*, está aislado y que podría desaparecer por tamaños poblaciones pequeños, deriva y/o endogamia.

10) Finalmente, nuestros resultados muestran diferentes tipos de selección a favor de los híbridos, los cuales, en combinación con los análisis de relaciones filogenéticas, sugieren un probable proceso en curso de especiación por hibridación homoploide.

Literatura citada

- Abbott R, Albach D, Ansell S *et al.* (2013) Hybridization and speciation. *Journal of evolutionary biology*, 26, 229–46.
- Abbott RJ, Barton NH, Good JM (2016) Genomics of hybridization and its evolutionary consequences. *Molecular Ecology*, 25, 2325–2332.
- Abbott RJ, Hegarty MJ, Hiscock SJ, Brennan AC (2010) Homoploid hybrid speciation in action. *TAXON*, 59, 1375–1386.
- Álvarez del Toro M (1974) Los Crocodylia de México: estudio comparativo. Instituto Mexicano de Recursos Naturales Renovables, México.
- Anderson EC, Thompson E a (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, 160, 1217–29.
- Arnold ML, Martin NH (2009) Adaptation by introgression. *Journal of biology*, 8, 82.
- Avise JC (2000) *Phylogeography: The history and formation of species*. Harvard University Press, Cambridge, Massachusetts.
- Arnold ML, Ballerini ES, Brothers A N (2012) Hybrid fitness, adaptation and evolutionary diversification: lessons learned from Louisiana Irises. *Heredity*, 108, 159–66.
- Barton NH (2001) The role of hybridization in evolution. *Molecular ecology*, 10, 551–68.
- Baack EJ, Rieseberg LH (2007) A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development*, 17, 513–518.
- Barton NH, Hewitt GM (1985) Analysis of Hybrid Zones. *Annual Review of Ecology, Evolution, and Systematics*, 16, 113–148.
- Beebee T, Rowe G (2008) *An Introduction to Molecular Ecology*. Oxford University Press.
- Brennan AC, Barker D, Hiscock SJ, Abbott RJ (2012) Molecular genetic and quantitative trait divergence associated with recent homoploid hybrid speciation: a study of *Senecio squalidus* (Asteraceae). *Heredity*, 108, 87–95.
- Brown RM, Jordan WC, Faulkes CG *et al.* (2011) Phylogenetic Relationships in *Pterodroma* Petrels Are Obscured by Recent Secondary Contact and Hybridization (D Ortiz-Barrientos, Ed.). *PLoS ONE*, 6, e20350.

- Buerkle CA (2005) Maximum-likelihood estimation of a hybrid index based on molecular markers. *Molecular Ecology Notes*, 5, 684–687.
- Buerkle CA, Morris RJ, Asmussen MA, Rieseberg LH (2000) The likelihood of homoploid hybrid speciation. *Heredity*, 84, 441–451.
- Buerkle CA, Rieseberg LH (2008) The rate of genome stabilization in homoploid hybrid species. *Evolution; international journal of organic evolution*, 62, 266–75.
- Cedeño-Vázquez JR, Rodríguez D, Calmé S *et al.* (2008) Hybridization between *Crocodylus acutus* and *Crocodylus moreletii* in the Yucatan Peninsula: I. Evidence from mitochondrial DNA and morphology. *Journal of experimental zoology. Part A, Ecological genetics and physiology*, 309, 661–673.
- Chapman M a, Burke JM (2007) Genetic divergence and hybrid speciation. *Evolution; international journal of organic evolution*, 61, 1773–80.
- Cohen M, Gans C (1970) The chromosomes of the order Crocodylia. *Cytogenetics*, 9, 81–105.
- Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, 15, 2833–2843.
- Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, 163, 367–374.
- Darwin C (1859). *On the Origin of Species by Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life*. Prometheus Books: Buffalo.
- de Aguiar MAM, Baranger M, Baptestini EM, Kaufman L, Bar-Yam Y (2009). Global patterns of speciation and diversity. *Nature* 460:384–387.
- De Queiroz K (2007) Species concepts and species delimitation. *Systematic biology*, 56, 879–86.
- Diniz-Filho JAF, De Campos Telles MP, Bonatto SL *et al.* (2008) Mapping the evolutionary twilight zone: Molecular markers, populations and geography. *Journal of Biogeography*, 35, 753–763.
- Diniz-Filho JAF, De Campos Telles MP, Bonatto SL *et al.* (2008) Mapping the evolutionary twilight zone: Molecular markers, populations and geography. *Journal of Biogeography*, 35, 753–763.
- Dobzhansky, T. 1937. *Genetics and the origin of species*. Columbia Univ. Press, New York.
- Dowling, T., & Secor, C. (1997). The Role of Hybridization and Introgression in the Diversification of Animals. *Annual Review of Ecology and Systematics*, 28, 593-619.

- Edwards S V., Potter S, Schmitt CJ, Bragg JG, Moritz C (2016) Reticulation, divergence, and the phylogeography–phylogenetics continuum. *Proceedings of the National Academy of Sciences*, 113, 8025–8032.
- Eguiarte LE, Souza V, Aguirre X (2007) *Ecología molecular*. Instituto Nacional de Ecología, SEMARNAT, México, D.F.
- Endler J a. (1979) Gene Flow and Life History Patterns. *Genetics*, 93, 263–284.
- Fitzpatrick BM, Fordyce J a, Gavrillets S (2009) Pattern, process and geographic modes of speciation. *Journal of evolutionary biology*, 22, 2342–7.
- Gompert Z (2016) Bayesian inference of selection in a heterogeneous environment from genetic time-series data. *Molecular Ecology*, 25, 121–134.
- Gompert Z, Buerkle CA (2009) A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. *Molecular Ecology*, 18, 1207–24.
- Gompert Z, Buerkle CA (2010) Introgress: a Software Package for Mapping Components of Isolation in Hybrids. *Molecular Ecology resources*, 10, 378–84.
- Gompert Z, Buerkle CA (2011) Bayesian estimation of genomic clines. *Molecular Ecology*, 20, 2111–27.
- Gompert Z, Buerkle CA (2012) bgc: Software for Bayesian estimation of genomic clines. *Molecular Ecology resources*, 12, 1168–76.
- Gompert Z, Buerkle CA (2013) Analyses of genetic ancestry enable key insights for molecular ecology. *Molecular Ecology*, 22, 5278–5294.
- Gompert Z, Buerkle CA (2016) What, if anything, are hybrids: enduring truths and challenges associated with population structure and gene flow. *Evolutionary Applications*, 9, 909–923.
- Gompert Z, Fordyce J a, Forister ML, Shapiro AM, Nice CC (2006) Homoploid hybrid speciation in an extreme habitat. *Science (New York, N.Y.)*, 314, 1923–5.
- Gompert Z, Lucas LK, Fordyce J a, Forister ML, Nice CC (2010) Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone. *Molecular ecology*, 19, 3171–92.
- Gross BL, Rieseberg LH (2008) The ecological genetics of homoploid hybrid speciation. *The Journal of heredity*, 96, 241–52.
- Hamilton JA, Miller JM (2016) Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology*, 30, 33–41.

- Hansen, T. F., and E. P. Martins. 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* 50:1404–1417.
- Harrison RG (2012) The language of speciation. *Evolution*, 66, 3643–3657.
- Harrison RG, Larson EL (2014) Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, 105, 795–809.
- Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, 167, 747–760.
- Hewitt GM (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica*, 139, 617–38.
- Kenney AM, Sweigart AL (2016) Reproductive isolation and introgression between sympatric *Mimulus* species. *Molecular Ecology*, 25, 2499–2517.
- Larson EL, Andrés J a., Bogdanowicz SM, Harrison RG (2013) Differential introgression in a mosaic hybrid zone reveals candidate barrier genes. *Evolution*, 67, 3653–3661.
- Lewontin RC (1974) *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.
- Machkour-M'Rabet S, Hénaut Y, Charruau P *et al.* (2009) Between introgression events and fragmentation, islands are the last refuge for the American crocodile in Caribbean Mexico. *Marine Biology*, 156, 1321–1333.
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends in ecology & evolution*, 20, 229–37.
- Mavárez J, Linares M (2008) Homoploid hybrid speciation in animals. *Molecular Ecology*, 17, 4181–4185.
- Mayr E. (1942) *Systematics and the origin of species*. Columbia University Press; New York: 1942.
- Mazzotti FJ, Dunson WA (1989) Osmoregulation in crocodylians. *Integrative and Comparative Biology*, 29, 903–920.
- Meganathan PR, Dubey B, Batzer M a, Ray D a, Haque I (2010) Molecular phylogenetic analyses of genus *Crocodylus* (Eusuchia, Crocodylia, Crocodylidae) and the taxonomic position of *Crocodylus porosus*. *Molecular phylogenetics and evolution*, 57, 393–402.
- Meredith RW, Hekkala ER, Amato G, Gatesy J (2011) A phylogenetic hypothesis for *Crocodylus* (Crocodylia) based on mitochondrial DNA: evidence for a trans-Atlantic voyage from Africa to the New World. *Molecular phylogenetics and evolution*, 60, 183–91.

- Milián-García Y, Ramos-Targarona R, Pérez-Fleitas E *et al.* (2015) Genetic evidence of hybridization between the critically endangered Cuban crocodile and the American crocodile: implications for population history and in situ/ex situ conservation. *Heredity*, 114, 272–280.
- Milián-García Y, Venegas-Anaya M, Frias-Soler R *et al.* (2011) Evolutionary history of Cuban crocodiles *Crocodylus rhombifer* and *Crocodylus acutus* inferred from multilocus markers. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 315 A, 358–375.
- Nolte a W, Gompert Z, Buerkle C a (2009) Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. *Molecular ecology*, 18, 2615–27.
- Nolte AW, Tautz D (2010) Understanding the onset of hybrid speciation. *Trends in genetics : TIG*, 26, 54–8.
- Oaks JR (2011) A time-calibrated species tree of crocodylia reveals a recent radiation of the true crocodiles. *Evolution*, 65, 3285–3297.
- Pacheco G (2010) Caracterización genética de *Crocodylus moreletii* en la reserva de la biosfera Ría Lagartos, Yucatán México: estructura genética poblacional y evidencia de hibridación entre *C. moreletii* y *C. acutus*. Universidad Autónoma de Yucatán.
- Pacheco-Sierra G, Gompert Z, Domínguez-Laso J, Vázquez-Domínguez E (2016) Genetic and morphological evidence of a geographically widespread hybrid zone between two crocodile species, *Crocodylus acutus* and *Crocodylus moreletii*. *Molecular Ecology*, 25, 3484–3498.
- Padilla y Sanchez RJ (2007) Evolución geológica del sureste mexicano desde el Mesozoico al presente en el contexto regional del Golfo de México. *Boletín de la Sociedad Geológica Mexicana*, LIX, 19–42.
- Payseur BA, Rieseberg LH (2016) A genomic perspective on hybridization and speciation. *Molecular Ecology*, 25, 2337–2360.
- Platt SG, Rainwater TR (2005) A review of morphological characters useful for distinguishing morelet's crocodile (*Crocodylus moreletii*) and american crocodile (*Crocodylus acutus*) with an emphasis on populations in the coastal zone of Belize. *Bull. Chicago Herp. Soc.*
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–59.

- De Queiroz K (2007) Species concepts and species delimitation. *Systematic biology*, 56, 879–86.
- Rand DM, Harrison RG (1989) Ecological Genetics of a Mosaic Hybrid Zone: Mitochondrial, Nuclear, and Reproductive Differentiation of Crickets by Soil Type. *Evolution*, 43, 432–449.
- Ray DA, Dever JA, Platt SG *et al.* (2004) Low levels of nucleotide diversity in *Crocodylus moreletii* and evidence of hybridization with *C. acutus*. *Conservation Genetics*, 5, 449–462.
- Razo-Mendivil U, Vázquez-Domínguez E, Pérez-Ponce de León G (2013) Discordant Genetic Diversity and Geographic Patterns Between *Crassicutis cichlasomae* (Digenea: Apocreadiidae) and its Cichlid Host, “Cichlasoma” urophthalmus (Osteichthyes: Cichlidae), in Middle-America. *Journal of Parasitology*, 99, 978-988.
- Rodríguez D, Cedeño-Vázquez JR, Forstner MRJ, Densmore LD (2008) Hybridization between *Crocodylus acutus* and *Crocodylus moreletii* in the Yucatan Peninsula: II. Evidence from microsatellites. *Journal of experimental zoology. Part A, Ecological genetics and physiology*, 309, 674–86.
- Sánchez Herrera O, López Segurajáuregui A, García Naranjo Ortiz de la Huerta A, Benitez Díaz H (2011) Programa de Monitoreo del Cocodrilo de Pantano (*Crocodylus moreletii*) México-Belice-Guatemala. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.
- Sánchez Herrera, O., G. López Segurajáuregui, A. García Naranjo Ortiz de la Huerta y H. Benítez Díaz. 2012. Informe del Programa de Monitoreo del Cocodrilo de Pantano en México Temporada 2011. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México 72.pp
- Sánchez Herrera, O., E. Rivera-Téllez, G. López Segurajáuregui, A. García Naranjo Ortiz de la Huerta, y H. Benítez Díaz. 2015. Informe del Programa de Monitoreo del Cocodrilo de Pantano en México, Temporadas 2011 a 2013. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México 36.pp
- Schumer M, Rosenthal GG, Andolfatto P (2014) How common is homoploid hybrid speciation? *Evolution; international journal of organic evolution*, 68, 1553–60.
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends in ecology & evolution*, 19, 198–207.
- Semarnat (2010) Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federación, 10 diciembre 2010, México.

- Soltis PS (2013) Hybridization, speciation and novelty. *Journal of Evolutionary Biology*, 26, 291–293.
- Taplin LE (1988) Osmoregulation in crocodylians. *Biological Reviews*, 63, 333–377.
- Taplin LE, Grigg GC, Harlow P, Ellis TM, Dunson WA (1982) Lingual salt glands in *Crocodylus acutus* and *C. johnstoni* and their absence from *Alligator mississippiensis* and *Caiman crocodilus*. *Journal of Comparative Physiology B*, 149, 43–47.
- Vázquez-Domínguez E (2007) Filogeografía y vertebrados. In: *Ecología Molecular* (eds Eguiarte L, Souza V, Aguirre X), pp. 441-466. SEMARNAT, CONABIO, INE, UNAM, México.
- Vázquez-Domínguez E, Arita HT (2010) The Yucatan peninsula: Biogeographical history 65 million years in the making. *Ecography*, 33, 212–219.
- Wu C, Ting C (2004) Genes and speciation. *Nature*, 5, 114–122.