UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE ECOLOGÍA

LA INVASIÓN DE *Trachemys scripta elegans* EN MÉXICO: ESTADO GENÉTICO, HIBRIDACIÓN Y ÉXITO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS BIOLÓGICAS

PRESENTA:

SAYRA ROSIO ESPINDOLA BARRIENTOS

TUTORA PRINCIPAL DE TESIS: Dra. ELLA VÁZQUEZ DOMÍNGUEZ
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Ciudad Universitaria, CD. MX., Febrero, 2022

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
ENTIDAD INSTITUTO DE ECOLOGÍA
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Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted, que el Comité Académico del Posgrado en Ciencias Biológicas, en su reunión ordinaria del día 06 de diciembre de 2021, aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante: **ESPINDOLA BARRIENTOS SAYRA ROSIO,** con número de cuenta **406031068,** con la tesis titulada: **"LA INVASIÓN DE *Trachemys scripta elegans EN* MÉXICO: ESTADO GENÉTICO, HIBRIDACIÓN Y ÉXITO*",*** bajo la dirección de la **DRA. ELLA GLORIA VÁZQUEZ DOMÍNGUEZ** Tutor Principal, quedando integrado de la siguiente manera:

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

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 09 de febrero de 2022

COORDINADOR DEL PROGRAMA

COORDINACIÓN

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Con cariño
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A mis padres,
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y
María del Refugio Barrientos
Con cariño y gratitud

ÍNDICE

RESUMEN

Las invasiones biológicas son reconocidas como una de las principales causas de pérdida de la biodiversidad a nivel mundial, con importantes impactos sobre las especies nativas, los procesos ecosistémicos, la salud pública y la economía. Su estudio ha permitido comprender algunos procesos ecológicos y evolutivos fundamentales, tanto a nivel de especie, por ejemplo procesos de adaptación y plasticidad fenotípica; como a nivel de comunidades y ecosistemas, tales como resistencias bióticas y factores que determinan los límites en la distribución de las especies, entre otros. Una de las especies más documentadas debido a su gran éxito como invasora es la tortuga de orejas rojas (*Trachemys scripta elegans*), la cual ha sido introducida en más de 70 países a través del comercio internacional de mascotas. Su distribución natural incluye la costa noreste de Estados Unidos y áreas en el norte de Tamaulipas, en México. Hacia el sur por la costa este del país, presenta un gradiente de distribución con dos especies congéneres: *T. cataspila* y *T. venusta*, además de una tercera especie que se encuentra cercana a esta región, *T. taylori*. A lo largo de dicha distribución, existen pocas zonas de contacto entre especies, pero debido a su liberación intencional, en algunas áreas del gradiente puede encontrarse a *T. scripta elegans* coexistiendo con sus congéneres nativos. Con el fin de describir la dinámica de invasión que ocurre en México por la introducción de esta especie, en el presente proyecto utilizamos datos genéticos y de modelado de nicho para: 1) caracterizar los patrones genéticos actuales de las poblaciones de *T. scripta elegans*, tanto en su distribución nativa como de aquellas fuera de su rango natural, 2) evaluar la relación genética con sus congéneres *T. cataspila, T. venusta* y *T. taylori,* y 3) describir las características ambientales que definen el nicho de la tortuga de orejas rojas y la de sus congéneres, para determinar los patrones de distribución entre estas e identificar sitios climáticamente idóneos para su establecimiento.

Iniciamos con la elaboración de un compendio de información sobre reptiles, con especial énfasis en el grupo de tortugas de agua dulce, sobre las características biológicas, ecológicas, demográficas y de vida que las distinguen. Detallamos las técnicas de captura más comunes para su estudio y concluimos enfatizando los importantes problemas de conservación que enfrentan estas tortugas.

Para las estimaciones genéticas utilizamos los siguientes marcadores: 15 microsatélites, el gen mitocondrial citocromo *b*, el gen nuclear R35 y SNPs (*single nucleotide polymorphisms*). Nuestro conjunto de datos incluyó 261 muestras de las cuatro especies, las cuales comprenden individuos capturados en campo, tortugas comercializadas de origen desconocido y muestras de especímenes de museos. A partir de estos datos, estimamos las relaciones evolutivas de *T.*

scripta elegans y sus congéneres (patrones filogenéticos, tiempos de divergencia, introgresión y migración histórica) y la genética de poblaciones de cada especie y entre especies (diversidad genética, diferenciación entre los individuos de *T. scripta elegans* nativos y no nativos, y diferenciación con sus congéneres).

Las estimaciones de nicho ecológico se dividieron en dos partes. En primer lugar, nos enfocamos en la especie invasora, estimando su nicho realizado tanto en el rango nativo como en el no nativo y comparamos, en espacio climático, las diferencias entre ambos nichos (tamaño, forma y sobrelape) y las condiciones climáticas ocupadas en cada caso. Además, generamos una propuesta para estimar el nicho fundamental a partir de diferentes límites de tolerancia fisiológica de temperatura. De forma novedosa, incluimos la distancia a cuerpos de agua como variable, dado que es un factor determinante para su sobrevivencia. Con base en lo anterior, generamos un modelo de idoneidad geográfica y contrastamos los rangos de temperatura ocupados con los rangos que la tortuga es capaz de tolerar.

Por otra parte, estimamos en el espacio ambiental los nichos ecológicos para las cuatro especies estudiadas, mediante modelos de elipsoides construidos a partir de una distribución normal tridimensional de los tres primeros componentes principales de las 19 variables bioclimáticas de WorldClim. A partir de estos modelos obtuvimos el sobrelape de nichos entre pares de especies y elaboramos mapas de idoneidad a partir de valores pareados de distancias al centroide del nicho de cada especie. Tomando lo anterior como referencia, examinamos el efecto de la interacción de las especies en términos de sus ubicaciones relativas y probabilidades en el espacio de idoneidad. Con el fin de contrastar los resultados obtenidos entre *T. scripta elegans* y sus congéneres, realizamos estos mismos análisis con seis especies de tortugas de otras regiones del mundo que, según reportes en la literatura, han sido afectadas por la presencia de la tortuga de orejas rojas.

Los resultados moleculares revelaron una ancestría común entre las poblaciones nativas e introducidas de *T. scripta elegans* pero, al mismo tiempo, una divergencia y una marcada diferenciación reciente, identificando a los individuos nativos e introducidos como dos grupos genéticos distintos. Además, estos últimos presentaron una mayor diversidad genética. Al analizar las relaciones filogenéticas de las cuatro especies, logramos diferenciar claramente dos clados, uno que separa a *T. scripta elegans* de las otras especies. Los análisis de estructura permitieron diferenciar a cada especie, con una subestructura para *T. venusta*. En comparación con el resto de las especies, *T. taylori* presentó los índices más bajos de diversidad genética. Contrario a lo que esperábamos, encontramos un flujo genético reducido, con pocas señales de mezcla entre especies.

En espacio climático estimamos que el nicho realizado es 28.6% mayor en los rangos invadidos con respecto al rango nativo. Las simulaciones basadas en rangos de tolerancia, tanto para México como para todo el mundo, mostraron que *T. scripta elegans* ocupa condiciones análogas y no análogas entre sus rangos nativo y no nativos, con sitios climáticamente idóneos que aún no han sido invadidos. Las comparaciones entre especies basadas en distancias al centroide del nicho, mostraron que las especies nativas de *Trachemys* tienen una idoneidad de nicho climático mayor en las zonas de contacto, contrario a lo que ocurre con especies de otras partes del mundo, donde ocurre un mayor sobrelape y la especie invasora ocupa óptimos ecológicos más cercanos.

De manera general, el conjunto de nuestros resultados genéticos y de modelado nos permitió explicar los patrones particulares del éxito de invasión de TSE en la costa este de México en comparación con otras partes del mundo. Así, los resultados sugieren que, a pesar de contar con una amplia capacidad invasiva, reflejada en una elevada diversidad genética y una amplia tolerancia ambiental, existen otros factores, como la interacción con otras especies cuyos requerimientos son similares, que pueden limitar significativamente su dispersión. Finalmente, la información que presentamos puede ser útil para quienes trabajan en actividades de manejo y conservación de tortugas o de otras especies que enfrentan la amenaza de las especies invasoras.

ABSTRACT

Human-mediated species invasions are recognized as one of the main causes of worldwide biodiversity loss, with important impacts on native species, ecosystem processes, public health and economy. Their study has allowed to understand some fundamental ecological and evolutionary processes at the species level, for example adaptive changes and phenotypic plasticity; and on communities and ecosystems levels, including biotic resistance and factors that determine range limits. One of the most documented species due to its great success as invasive is the North American red-eared slider turtle (*Trachemys scripta elegans*), which has been introduced to more than 70 countries via the international pet trade. Its native distribution encompasses the northeastern coast of the United States with its southern limit in northern Tamaulipas, Mexico. Southwards along Mexico's east coast, it presents a natural distributional gradient with two congeneric species: *T. cataspila* and *T. venusta*; a third species, close to this region, is *T. taylori*. There are few areas of contact between species along with this distribution. Still, given that *T. scripta elegans* has been deliberately introduced into different areas along this gradient, it can be found coexisting with its native congeners. Hence, we aimed to describe the invasion dynamics occurring in Mexico in relation to the introduction of this species, for which we used genetic and niche modeling data to: 1) characterize the current genetic patterns of *T. scripta elegans* populations, both in its native and no-native distributions, 2) evaluate the genetic relationships with its congeners *T. cataspila, T. venusta* and *T. taylori*, and 3) describe the environmental characteristics that define the niche of the North American red-eared slider turtle and their congeners, to determine their distribution patterns and identify climatically suitable sites for its the establishment.

First, we made a compendium of information about reptiles, focusing on the group of freshwater turtles and biological, ecological, demographic and life history features that so neatly distinguish them. We describe the most common trapping techniques for studying these turtles and conclude emphasizing the significant conservation problems they all face.

We used 15 microsatellites, the mitochondrial cytochrome b and R35 nuclear genes, and SNPs (single nucleotide polymorphisms) for the genetic assessment. Our dataset included 261 samples from all four species, comprising field-caught individuals, traded turtles of unknown origin, and museum tissue samples. We estimated the evolutionary relationships between T. scripta elegans and its congeners (phylogenetic patterns, divergence times, introgression and historical migration) and population genetics of each species and between species (genetic diversity and genetic differentiation between *T. scripta elegans* native and non-native individuals, and in relation with its congeners).

The ecological niche analyses were divided into two parts. We first focused on *T. scripta elegans*, estimating its realized niches in both the native and non-native ranges and comparing, in the climatic space, the differences between niches (size, shape and overlap), as well as the climatic conditions occupied by natives and non-natives. In addition, we proposed a novel approach to build its fundamental niche based on key features of the turtle's temperature physiological tolerance limits. Also we included the distance to water bodies as variable, since it is a determining factor for its survival. Based on the above, we generated a geographic suitability model and contrasted the occupied temperature ranges with the optimal, maximum and minimum tolerance limits gathered from the scientific literature.

On the other hand, we estimated the ecological niches in the environmental space for the four species included in this study using ellipsoid models, based on the three-dimensional normal distribution of the first three principal components of the 19 bioclimatic variables from WorldClim. We calculated the proportion of overlap between pairs of species and we elaborated suitability maps from paired values of distances to the centroid of the niche of each species. Next, we examined the interaction effect among species in terms of their relative locations and probabilities in the suitability space. To contrast the results obtained between *T. scripta elegans* and its congeners, we carried out these same analyses with six turtles species from other regions of the world that, according to reports from the literature, have been affected by the presence of the red-eared slider turtle.

Molecular results revealed a common ancestry between native and introduced *T. scripta elegans* populations; but, at the same time, a divergence and a marked recent differentiation, identifying native and introduced individuals as two distinct genetic groups. In addition, the latter presented a greater genetic diversity. By analyzing the phylogenetic relationships of the four species, we were able to depict two distinct clades, one that separates *T. scripta elegans* from the other species. Structure analysis allowed to differentiate each species as independent genetic groups, with a substructure for *T. venusta*. Compared with the rest of the species, *T. taylori* presented the lowest genetic diversity indices. Contrary to what we expected, we found a reduced gene flow, with few signs of inter-species admixture.

We show that the realized niche increases by 28.6% in the invaded ranges with respect to the native one in climatic space. Simulations based on tolerance limits, for worldwide and across Mexico, showed that T. scripta elegans occupies analogue and non-analogue conditions between its native and invaded ranges, also exhibiting unoccupied suitable climatic conditions. Comparisons between species based on niche-center distances showed that native Trachemys species have higher climatic niche suitability in the contact zones, contrary to species from other parts of the world, where there is a greater overlap, and the invasive species occupies closer ecological optima.

Overall, the information collected allowed us to explain differences in *T. scripta elegans* invasion success on the east coast of Mexico compared to other parts of the world. Our findings suggest that despite having a wide invasive capacity, reflected in a high genetic diversity and a wide environmental tolerance, other factors, such as the interaction with other species with similar requirements can significantly limit its dispersal. Finally, the information we present may be helpful for those working in the management and conservation of turtles or other species that face the threat of invasive species.

Introducción general

Invasiones biológicas

En años recientes el tema de las invasiones biológicas ha cobrado particular interés debido a que éstas son reconocidas como una de las principales causas de pérdida de la biodiversidad a nivel mundial. Además, su estudio representa una oportunidad para comprender ciertos procesos ecológicos y evolutivos fundamentales como, por ejemplo, la adaptación a nuevos hábitats, las interacciones bióticas, la dinámica de las comunidades, los factores que determinan los límites en la distribución de las especies, la respuesta de las especies al cambio climático, entre otros (Sax et al., 2007; Lowry et al., 2013; Mollot et al., 2017). Históricamente, las barreras biogeográficas y los mecanismos naturales de dispersión han dado forma a la distribución de las especies, pero en el último siglo, ya sea de manera intencional o accidental, las actividades humanas han transferido cada vez más organismos a través de estas barreras, alterando la magnitud y el ritmo de dispersión al llevar especies a nuevas regiones, muchas veces a lugares en donde no ocurrirían de otra manera (Simberloff et al., 2013; Lowry et al., 2013; Maĉić et al., 2018).

La atención a este fenómeno comenzó en 1958 cuando Charles S. Elton publicó su libro "*The ecology of invasions by animals and plants*", en donde explica, a través de ejemplos, los efectos que las especies invasoras pueden tener sobre los ecosistemas (Elton, 1958). Desde entonces, el estudio de las invasiones biológicas ha buscado explicar cuáles especies son más propensas a convertirse en invasoras y cuáles ambientes son más susceptibles a la invasión, con la finalidad, entre otras, de proponer acciones de manejo y conservación. Es así que el problema se ha abordado desde tres áreas principales de investigación: 1) establecer los rasgos genéticos, fisiológicos o de comportamiento que caracterizan a las especies invasoras; 2) analizar cómo ocurre el proceso de invasión; y 3) estimar los impactos de las especies invasoras sobre las especies nativas, los procesos ecosistémicos, la salud pública y la economía (Cassini, 2020).

Debido a su complejidad, es necesario abordar el problema de las invasiones biológicas desde diferentes perspectivas (estudios poblacionales, experimentos en laboratorio, modelado, entre otros), integrando varios tipos de información (histórica, genética, ambiental y fenotípica) (Sherpa y Després, 2021). En este proyecto nos propusimos evaluar, desde un enfoque genético y ecológico, el proceso de invasión en México de una de las especies invasoras con mayor éxito alrededor del mundo, la tortuga de orejas rojas *Trachemys scripta elegans* (Wied-Neuwied, 1839).

Especies invasoras

El movimiento de organismos más allá de su rango nativo es un proceso biológico fundamental que ocurre y ha ocurrido a lo largo de la historia de la tierra y que tiene implicaciones en la estructura y dinámica de las poblaciones y comunidades, así como en la persistencia, evolución y distribución geográfica de las especies (Nathan, 2001). “Invadir” nuevas áreas significa que es necesario superar las barreras geográficas y ecológicas que limitan dicho movimiento (Blackburn et al., 2011).

Entre las definiciones más aceptadas para describir a una especie como invasora está la que señala que es una especie introducida por los seres humanos fuera de su distribución natural, la cual se ha establecido y extendido exitosamente (Brackburn et al., 2011). A nivel mundial se han documentado casos de especies invasoras para todos los grupos taxonómicos, e incluso se han identificado puntos críticos globales con especies exóticas establecidas en muy diversos ambientes (Pyšek et al., 2020). Más aún, en las últimas décadas el número de especies que se han establecido fuera de su rango nativo ha ido en aumento, en gran medida por la intensificación de actividades como el comercio, el transporte y el cambio de uso de suelo. De acuerdo con estimaciones a futuro, las invasiones seguirán incrementándose para muchos grupos taxonómicos, tanto en relación a las áreas invadidas como en la aparición de nuevas especies invasoras (Seebens et al., 2021).

Respecto a las características asociadas al éxito de invasión de una especie, pueden diferenciarse en general en tres tipos. Las intrínsecas a la especie invasora, como la carga genética de la población fundadora, la plasticidad fenotípica, la historia de vida y las habilidades para dispersarse y competir, entre otros. Las modalidades de introducción, como el origen de las poblaciones, el número de introducciones independientes y la cantidad y frecuencia con la que son dispersados los organismos. Aunado a los mecanismos que ocurren dentro del ambiente invadido y que modulan el proceso de invasión, tanto bióticos como las interacciones entre especies, como abióticos, tales como las condiciones climáticas y las características de paisaje, por mencionar algunas (Catford et al., 2009; Perkins et.al., 2011).

Genética e invasiones

Los enfoques moleculares han sido ampliamente utilizados para resolver preguntas sobre la historia de las invasiones biológicas (Cristescu, 2015). Ello debido a que las características genéticas de las poblaciones invasoras reflejan procesos neutrales como divergencia ancestral, dinámica demográfico-genética durante el proceso de invasión, es decir, efecto fundador o cuellos de botella durante la introducción o surfeo de alelos durante la expansión. También los patrones de estructura y conectividad de la población, a través de flujo genético y diferenciación, y procesos adaptativos que pudieran ocurrir debido a las presiones de selección en los nuevos ambientes, o bien a eventos de mezcla, hibridación e introgresión (Fitzpatrick et al., 2012; Sherpa y Després, 2021).

Por mucho tiempo se habló de una "paradoja genética" en especies invasoras (Frankham, 2005), ya que su introducción supone un número reducido de ejemplares y por ende alta probabilidad de que ocurran cuellos de botella genéticos, en donde se pierde parte de la diversidad genética original y se compromete seriamente el potencial evolutivo para adaptarse al nuevo ambiente, disminuyendo el éxito de establecimiento (Duglosch y Parker, 2008; Simberloff, 2009). Sin embargo, diversos estudios han permitido determinar que las introducciones exitosas generalmente son resultado de múltiples introducciones, lo que favorece la mezcla de individuos (y por lo tanto de sus genotipos) y genera tamaños poblacionales efectivos grandes (Simberloff, 2009). Lo anterior permite que la población invasora mantenga niveles adecuados de diversidad genética y como resultado genera que se pierdan las señales de cuellos de botella, deriva génica o diferenciación (Catford et al., 2009). Si además las introducciones tienen diferentes orígenes (geográficos), es probable que la nueva población presente mayor variabilidad genética en comparación con la observada en su rango nativo (Alda et al., 2013).

Otra área en donde los estudios moleculares han tenido un impacto significativo en el tema de las invasiones biológicas es la detección y la caracterización del entrecruzamiento entre especies nativas e introducidas (Fitzpatrick et al., 2012). La hibridación, o el flujo de genes entre taxones, que conduce a la introgresión, puede aumentar la diversidad genética y las oportunidades de adaptación a las condiciones ambientales cambiantes (Arnold y Martin, 2010; Taylor et al., 2015), pero también puede aumentar el riesgo de extinción de especies, por ejemplo, debido a la depresión por exogamia (Oakley et al., 2015), la introducción de genes maladaptativos (Kidd et al., 2009), o mezclas genéticas ('genetic swamps') (Todesco et al., 2016). Esto es particularmente importante cuando la hibridación ocurre entre especies cercanas y alguna de ellas se encuentra amenazada o en peligro de extinción (Pacheco-Sierra et al., 2018).

Modelado de nicho ecológico

Los modelos de nicho ecológico han sido una herramienta ampliamente utilizada para generar hipótesis geográficas sobre la distribución de las especies (Peterson et al. 2011). Estos modelos utilizan las relaciones que existen entre las condiciones del ambiente y los sitios de presencia de una especie para generar una aproximación de las condiciones donde la especie puede sobrevivir (su nicho ecológico) y, a partir de este, estimar la probabilidad de que ocurra dicha especie en un lugar determinado en función de qué tan favorables resultan las condiciones ambientales; ello expresado en términos de «idoneidad ambiental» (Drake y Richards, 2018). Estos modelos han sido ampliamente utilizados en el estudio de las invasiones biológicas para determinar áreas probables de invasión, estimar riesgos asociados a las especies invasoras como la dispersión de enfermedades, determinar posibles cambios de distribución asociados al cambio climático y al cambio de uso de suelo, así como dilucidar patrones espaciales de expansión posterior a la invasión inicial (Peterson y Vieglais, 2001; Srivastava et al., 2019).

La interpretación de estos modelos con especies invasoras requiere considerar ciertos supuestos que se asumen durante el proceso. Dado que la mayoría de las técnicas de modelado se basan en correlaciones entre la distribución observada y el clima, se supone que se caracteriza el «nicho realizado», aunque directamente no se involucren las interacciones ecológicas (Jiménez-Valverde et al., 2011). Sin embargo, en especies invasoras se ha observado cierta discrepancia en las condiciones ambientales ocupadas entre regiones, lo que sugiere que dependiendo del área que se caracterice, podrían obtenerse representaciones parciales del nicho (Broennimann et al., 2007). Por otra parte, los modelos correlativos también asumen que el nicho se conserva a través del espacio y del tiempo (Pearman et al., 2008). No obstante, se ha sugerido que las especies invasoras podrían experimentar cambios de nicho rápidos en las nuevas regiones a las que llegan en el proceso de invasión. Ello como resultado de la evolución de sus tolerancias ambientales (cambio en el nicho fundamental), o debido a la eliminación de las barreras de dispersión o de las restricciones bióticas (cambio en el nicho realizado) (Tingley et al., 2014).

En busca de estrategias que permitan el mejor aprovechamiento de los modelos de nicho ecológico bajo las suposiciones antes mencionadas, se ha propuesto realizar la comparación e integración entre áreas nativas e invadidas, y así hacer una mejor descripción del nicho realizado (Early y Sax, 2014; Tingley et al., 2014). Otros estudios han tratado de integrar información sobre tolerancias fisiológicas (que definen el nicho fundamental de la especie), interacciones bióticas y barreras de dispersión (que determinan el nicho realizado), con el fin de estimar de una manera más realista los límites geográficos dentro de los cuales puede establecerse una especie invasora (Kearney y Porter, 2009; Soberón y Arroyo-Peña, 2017; Tingley et al., 2014). Estas consideraciones y una propuesta novedosa de análisis se presentan en Espindola et al. (2019).

Por otra parte, se ha documentado una relación significativa entre los atributos de adecuación de las especies y la distancia al centroide de sus nichos ecológicos, sugiriendo que la sobrevivencia de las especies debería ser mayor en aquellas localidades donde existen las condiciones óptimas de su nicho ecológico (Martínez-Meyer et al., 2013; Lira-Noriega y Manthey, 2014; Osorio-Olvera et al., 2020). Así, los estudios que han puesto a prueba esta hipótesis, conocida como "Hipótesis de centro-abundancia", señalan que la posición de una población en el espacio ambiental determina su abundancia (Siqueira et al., 2009; Martínez-Meyer et al., 2013). Con especies invasoras, este enfoque ha permitido explicar patrones de abundancia con respecto al área de distribución en las zonas en donde invaden (Guo, 2014). En este trabajo proponemos utilizar la distancia al centroide de nicho como factor para el potencial de establecimiento de las especies invasoras. Asimismo, dicha propuesta involucra evaluar cómo impacta a dicho potencial de establecimiento el tomar en cuenta la presencia de otras especies nativas que tienen requerimientos ambientales similares (Espindola et al., 2022).

Modelo de estudio: tortugas de agua dulce

Las tortugas dulceacuícolas componen un grupo taxonómica y ecológicamente diverso, con una distribución geográfica muy amplia. Están representadas por numerosas especies de hábitos tanto totalmente acuáticos como semiacuáticos, y constituyen poco más del 60% de las especies de tortugas en el mundo (Burke et al., 2000; Moll y Moll, 2004). Estas tortugas ocupan una gran variedad de hábitats a lo largo de su vida, desde sitios terrestres para anidar y asolearse, hasta ambientes acuáticos para alimentarse y reproducirse (Cadi et al*.,* 2008). La selección de hábitat está asociada a factores tanto bióticos como abióticos, tales como la disponibilidad de recursos, las características fisicoquímicas y ambientales (por ejemplo, temperatura, profundidad del cuerpo de agua, salinidad, intensidad de la luz), y la presencia de competidores y depredadores. Todos ellos tienen un efecto directo en la sobrevivencia y éxito reproductivo de las especies (Bury et al., 2000; Wariss et al., 2012).

México ostenta el segundo lugar de riqueza en tortugas a nivel mundial, sólo después de Estados Unidos (Legler y Vogt, 2013). Respecto a las tortugas continentales, están presentes siete familias, con 13 géneros y 45 especies; y si se incluye a las subespecies se tiene un total de 61 taxa. De éstas, el 60% están consideradas en alguna categoría de riesgo según la Lista Roja de la IUCN y cerca del 40% carecen de la información necesaria para tener una idea clara de su estado de conservación (Macip-Ríos et al., 2015).

En contraste con la situación de peligro de extinción de un gran número de tortugas, algunas especies han tenido enorme éxito para establecerse en nuevos ambientes, como la tortuga de orejas rojas, *Trachemys scripta elegans*, la cual está incluida en la lista de las 100 especies más invasoras del mundo (Lowe et al., 2000). Esta tortuga tiene una extraordinaria capacidad invasiva, gracias sobre todo a su amplia tolerancia a condiciones ambientales (Espindola et al., 2019; Kikillus et al., 2010; Masin et al., 2014; Rödder et al., 2009).

Trachemys scripta elegans en México

La tortuga de orejas rojas se distribuye de forma nativa a lo largo de la costa noreste de Estados Unidos, al norte de Florida a través del valle del río Mississippi, al oeste incluyendo Texas, Oklahoma y Kansas, alcanzando finalmente su límite más al sur en el norte de Tamaulipas, México (Ernst y Siedel, 2006). De acuerdo con la normatividad mexicana (SEMARNAT, 2010), está catalogada como “especie sujeta a protección especial (Pr)”.

En México están reconocidas otras 10 especies de *Trachemys*, cada una distribuida en rangos geográficos separados definidos por los sistemas acuáticos epicontinentales (Legler y Vogt, 2013). Cabe resaltar que a lo largo de la costa este de México presentan un gradiente de distribución de norte a sur, con tres especies congéneres, incluyendo a *T. scripta elegans* (TSE) en el norte de Tamaulipas, poblaciones nativas de *T. cataspila* del norte de Tamaulipas al norte de Veracruz, y *T. venusta* que ocupa del norte de Veracruz hacia el sur hasta Guatemala, Belice y Honduras (Ernst y Siedel, 2006; Siedel y Ernst, 2017). Además, cercana a esta región se encuentra *Trachemys taylori*, una especie microendémica con distribución restringida al sistema de humedales dentro del valle desértico de Cuatrociénegas, en el desierto de Chihuahua, Coahuila, México (Siedel, 2002; Lazcano et al., 2019) (Figura 1). A lo largo de este gradiente natural, TSE ha sido introducida en diferentes áreas donde coexiste con sus congéneres nativos.

Figura 1. Distribución de las diferentes especies del género *Trachemys* en Norte y Centroamérica. Cada polígono negro representa el área de distribución de una especie (o subespecie) diferente. Las especies estudiadas en este proyecto se señalan con la siguiente numeración: 1. *Trachemys scripta elegans*, 2. *T. cataspila*, 3. *T. venusta*, y 4. *T. taylori*. (Modificado de: Fritz et al., 2012).

En el presente proyecto combinamos datos genéticos y de modelado de nicho con el fin de describir la dinámica de invasión de la introducción de *Trachemys scripta elegans* (TSE) en México. Para ello, se establecieron dos objetivos específicos. En el primero nos propusimos caracterizar los patrones genéticos actuales de las poblaciones de TSE, tanto en su distribución nativa como de aquellas fuera de su rango natural. Asimismo, evaluar la relación genética con sus congéneres *T. cataspila, T. venusta* y *T. taylori,* especies con las que TSE coexiste en zonas donde ha sido introducida. En el segundo incluimos el modelado de nicho ecológico para describir las características ambientales que definen el nicho de TSE y la de sus congéneres, de manera que pudiéramos determinar los patrones de distribución entre éstas e identificar sitios climáticamente idóneos para su establecimiento.

Así, el documento de tesis comprende tres capítulos. En el primer capítulo se incluye un compendio de información con el título "*Tortugas dulceacuícolas: una revisión sobre su origen, biología, ecología y técnicas para su captura*", sometido a la *Revista Latinomericana de Herpetología*, donde presentamos una revisión general sobre los reptiles centrándonos en las tortugas de agua dulce. Detallamos aspectos históricos así como de historia natural y ecología, y las técnicas de captura más utilizadas para estudiarlas. Concluimos enfatizando los problemas significativos de conservación que enfrentan todas las tortugas y lo esencial que es contar con información biológica para su conservación.

En el segundo capítulo presentamos una propuesta de análisis que incorpora información sobre requerimientos ambientales fundamentales de TSE, particularmente límites de tolerancia fisiológica de temperatura así como un factor esencial de sobrevivencia, la presencia de cuerpos de agua. Los resultados fueron publicados en *PeerJ*, con el título "*Fundamental niche unfilling and potential invasion risk of the slider turtle Trachemys scripta*", en donde proponemos que en las áreas de invasión, TSE ocupa un subconjunto distinto de su nicho fundamental. Asimismo, presentamos un modelo potencial para México con las áreas donde TSE podría invadir.

Finalmente, a pesar de contar con información sobre el éxito de invasión de TSE en otras partes del mundo y amplias zonas potenciales para México, el trabajo de campo realizado en el presente trabajo reveló una dinámica diferente, ya que encontramos a la especie limitada a cuerpos de agua artificiales o muy perturbados y coexistiendo con sus congéneres, quienes generalmente se encontraban en mayor abundancia. Para tratar de explicar estas observaciones, integramos información de datos genéticos y una propuesta novedosa de análisis de sobrelape de nichos, con el objetivo de evaluar las relaciones históricas entre los linajes de TSE y sus congéneres, los patrones genéticos poblacionales actuales y los límites de distribución de cada especie. Los resultados se presentan en el capítulo 3, con el artículo que lleva por título "*Complex genetic patterns and distribution limits mediated by native congeners of the worldwide invasive red-eared slider turtle*", publicado en *Molecular Ecology*. En este se destaca la divergencia presente entre las poblaciones invasoras y las nativas de TSE, así como un flujo génico reducido y una clara separación genética entre TSE y sus congéneres, a pesar de tener zonas de contacto. Además, con base en la hipótesis de que la especie invasora desplaza a las especies nativas en sitios que están más cerca del centroide de su nicho (es decir, sitios que son más idóneos), demostramos que las especies nativas de *Trachemys* tienen una mayor idoneidad de nicho climático en las zonas de contacto, lo que probablemente previene el establecimiento de TSE y el desplazamiento de las otras especies.

Capítulo 1

Tortugas dulceacuícolas: una revisión sobre su origen, biología, ecología y técnicas para su captura

En revisión en

Revista Latinoamericana de Herpetología

TORTUGAS DULCEACUÍCOLAS: UNA REVISIÓN SOBRE SU ORIGEN, BIOLOGÍA, ECOLOGÍA Y TÉCNICAS PARA SU CAPTURA

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Resumen.-De ser referidos bajo la idea de “criaturas abominables” en la época de Linneo, los reptiles en la actualidad son considerados un grupo fascinante, que hace cerca de 270 millones de años conquistaron tierra firme. En esta revisión recopilamos información sobre el origen, la biología, la ecología y la conservación de las tortugas dulceacuícolas, con el objetivo de ser una lectura de interés para muy diverso público. Así, describimos la historia y la extraordinaria diversificación de los reptiles, para después abordar el origen y evolución de las tortugas y su taxonomía que incluye una enorme riqueza de 360 especies reconocidas hasta ahora. Finalmente, nos concentramos en las tortugas de agua dulce, que constituyen poco más del 60% de las especies de tortugas en el mundo; sobre las características biológicas, ecológicas, demográficas y de historia de vida que las distinguen. Detallamos las técnicas de captura más utilizadas para estudiar a estas tortugas y concluimos enfatizando los problemas significativos de conservación que enfrentan todas las tortugas, y cómo es absolutamente esencial contar con información biológica e histórica como la recabada en esta revisión, así como genética en particular, para su preservación. Para quienes trabajan en actividades de manejo y conservación de las tortugas, la información que aquí presentamos sin duda puede contribuir a la permanencia a largo plazo de este asombroso grupo de reptiles.

Palabras clave.- características de historia de vida, conducta, evolución, reptiles, taxonomía

Abstract.-From being referred to under the concept of “abominable creatures” during Linneo’s times; currently reptiles are considered a fascinating group which ca. 270 million years ago conquered the land environment. In this revision, we collected information about the origin, biology, ecology, and conservation of reptiles, with particular emphasis on the group of freshwater turtles. It will also be interesting reading for a broad diverse public. We describe a little of the history and extraordinary diversification in reptiles to explain the origin and evolution of turtles and their taxonomy of enormous richness con 360 described species so far.

Finally, we concentrate on the freshwater turtles that encompass a little over 60% of the turtle species in the world, about their biological, ecological, demographic and life history features that so neatly distinguish them. We detail the most common trapping techniques for studying these turtles and conclude emphasizing the significant conservation problems faced by all turtles. It is essential to have biological and historical information like that gathered in this revision, jointly with genetic data for their preservation. For those working with turtles' management and conservation activities, the information that we present here can undoubtedly contribute to the long term presence of this amazing group of reptiles.

Key words.-behaviour, evolution, life-history traits, reptiles, taxonomy

INTRODUCCIÓN

El término reptil proviene de la palabra latina «reptĭlis» para hacer referencia a los animales que reptan, esto es, que se arrastran o se deslizan. El estudio de estos organismos estuvo limitado durante mucho tiempo, quizá en parte por el concepto de "criaturas abominables" formulado por Carlos Linneo en 1735 (Garin & Lobos, 2008). Sin embargo, actualmente existe un largo trabajo científico que ha permitido generar un amplio conocimiento sobre los reptiles. El objetivo general de esta revisión fue recopilar información general sobre origen, biología, ecología y conservación de los reptiles, con particular énfasis en el grupo de las tortugas dulceacuícolas, y con un enfoque de divulgación que lo haga de interés para público tanto especializado como de otras áreas.

REPTILES

Hace unos doscientos setenta millones de años –durante el Carbonífero de la era Paleozoica– aparecieron los primeros vertebrados que lograron la conquista de tierra firme: los reptiles (Fontanillas-Pérez et al., 2000). Los más primitivos que se conocen son los cotilosaurios, derivados a partir de una línea primitiva de anfibios pertenecientes a la Subclase Labyrinthodontia. Éstos fueron los primeros en desligarse por completo de la vida en el agua, mediante la adquisición de dos elementos básicos: la respiración pulmonar y huevos capaces de desarrollarse en un ambiente terrestre (Le Garff, 1992). A partir de entonces, se produce una intensa diversificación, dando origen a varias ramas evolutivas muy distintas entre sí.

Los sinápsidos, el grupo de reptiles más antiguo de todos, asumieron un rol dominante en las comunidades terrestres durante el Pérmico (Vitt & Caldwell, 2009). Su evolución fue progresiva hasta desaparecer en el Triásico, probablemente debido a la aparición de otros linajes como los terápsidos, quienes tuvieron su auge durante el Triásico de la era Mesozoica y se extinguieron durante el mismo periodo, dando lugar finalmente a los mamíferos actuales (Fontanillas-Pérez et al., 2000). De manera paralela ya era posible encontrar reptiles de otros grupos: los anápsidos, reptiles sin fenestras temporales (las aberturas del cráneo situadas detrás de las órbitas de los ojos), y los diápsidos, la clase más diversa y los únicos sobrevivientes actuales.

De los diápsidos se reconocen tres linajes principales: los ictiosaurios, los lepidosaurios (que incluyen a su vez a los sauropterigios, los esfenodontos y los escamosos), y los arcosaurios (divididos en pterosaurios, cocodrilos, dinosaurios, tortugas y aves). Su radiación más intensa, que además dio lugar a una gran diversidad, fue durante el Mesozoico (Le Garff, 1992). En este periodo los reptiles fueron los animales terrestres y aéreos dominantes y los principales depredadores en los ambientes marinos. Los sauropterigios y los ictiosaurios fueron reptiles acuáticos extremadamente exitosos que aparecieron muy temprano en el Triásico y permanecieron de forma abundante hasta el final del Cretácico. Los primeros reptiles voladores aparecieron en el Pérmico tardío. Los dinosaurios dominaron los ecosistemas terrestres del Jurásico, extinguiéndose a finales del Cretácico. A finales del Jurásico se hace evidente una radiación marina de Crocodyliformes quienes, junto con las tortugas, conservan muchas de sus características desde entonces (Vitt & Caldwell, 2009).

Los reptiles actuales

Los reptiles se han reconocido como grupo desde 1768, pero en las últimas décadas se ha acumulado evidencia de que no forman un grupo natural (Flores-Villeda y García Vázquez, 2014). Así, existe un profundo desacuerdo en cómo clasificar a los grupos mayores de reptiles, aunque de manera general se reconocen tres grupos: Testudines o Chelonia (las tortugas), Lepidosauria (tuataras, anfisbenias, lagartos y serpientes), y Archosauria (cocodrilos, aves y otros grupos fósiles (Pough et al., 2004). Con base en descripciones originales publicadas desde Linnaeus en 1758 y hasta 2019, se tiene un estimado de 13,361 taxa de reptiles reconocidos (11,050 especies y 2,311 subespecies). Aunque es probable que el número esté incompleto, por el registro de nuevas especies y otras crípticas (especies no distinguibles morfológicamente), representa un buen acercamiento a la diversidad de estos organismos que habitan y han habitado el planeta desde hace cientos de millones de años (Uetz et al., 2019).

1. **Archosauria, Crocodylia.** Los crocodilianos, como los conocemos actualmente, aparecieron en el Cretácico tardío, aunque existen registros fósiles de hace más de 215 millones de años (Triásico medio) (Vitt & Caldwell, 2009). Poseen muchas características que no comparten con los otros grupos vivientes de reptiles. Se caracterizan por tener un cuerpo lagartiforme elongado y fuertemente blindado, con largos hocicos, dientes tecodontos y colas poderosas.

Su armadura está formada por pesadas placas de hueso llamadas osteodermos que se encuentran dentro de la piel dorsal, debajo de las escamas epidérmicas que cubren el cuerpo; en muchas especies también están presentes ventralmente. Sus extremidades son relativamente cortas y sus pies son palmeados. Todas las especies son acuáticas y sus ojos tienen una membrana transparente que los cubre bajo el agua. Además, su corazón presenta cuatro cámaras y poseen varias características morfológicas que les permiten la respiración en ambientes acuáticos. Todos los crocodilianos son ovíparos y la determinación del sexo es dependiente de la temperatura. Presentan comportamientos sociales complejos y son depredadores muy eficientes. Habitan ríos, lagos, lagunas, pantanos e incluso pueden encontrarse en ambientes marinos (Grigg et al., 2001).

Se reconocen tres principales linajes de crocodilianos: Alligatoridae, Crocodylidae y Gavialidae. En los aligatoridos los dientes de la mandíbula inferior se ajustan dentro de unas fosas que se encuentran en la mandíbula superior, por lo que no se ven cuando la boca se encuentra cerrada. Por el contrario, en los crocodilidos los dientes de la mandíbula inferior se acomodan en unas muescas de la mandíbula superior y son visibles aun cuando la boca se encuentre cerrada. Los gavialidos, probablemente los crocodilianos más acuáticos, tienen hocicos estrechos y alargados y se alimentan principalmente de peces (Vitt & Caldwell, 2009; Álvarez del Toro, 1974).

2. **Lepidosauria**. Las principales características que comparte este grupo, y que los distinguen del resto de los reptiles, son: un corte transversal cloacal, la ocurrencia de mudas regulares y el desarrollo de planos de fracturas en la cola. La historia geológica de las familias actuales comenzó en el Triásico tardío en el caso de las tuátaras, en el Jurásico medio para los lagartos —aunque su mayor diversidad fue evidente durante el Cretácico tardío y los géneros modernos provienen del Plioceno—; mientras que la serpiente más antigua que se conoce es del Cretácico temprano y se cree que sus ancestros debieron tener un origen terrestre/subterráneo. En la actualidad, se reconocen dos órdenes: Rhynchocephalia y Squamata (Vidal & Hedges, 2009; Vitt & Caldwell, 2009).

Del orden Rhynchocephalia sólo existe un género, *Sphenodon*, con una sola especie, las tuátaras (Herrera-Flores et al., 2017). Son reptiles de tamaño medio que habitan en las islas de Nueva Zelanda. Son terrestres, solitarios, de hábitos nocturnos, muy longevos y principalmente insectívoros. Su habilidad para mantenerse activos a bajas temperaturas refleja una ancestría de altas latitudes y probablemente de una distribución amplia (Apesteguía & Jones, 2012).

Los Squamata (lagartos, anfisbenias y serpientes) son el grupo con el mayor número de especies vivientes, y pueden ser diagnosticados por más de 70 caracteres anatómicos compartidos, principalmente relacionados con el sistema musculo-esquelético (Vidal & Hedges, 2009). Una de las principales características de estos organismos son un par de órganos copulatorios bien desarrollados llamados hemipenes. Además, la reducción de las extremidades es una tendencia evolutiva dentro de este orden y múltiples linajes han perdido completamente los miembros, característica que implica otros cambios estructurales asociados a nuevos modos de locomoción. Las escamas que cubren la piel varían en forma y tamaño y, generalmente, no presentan glándulas. Los mecanismos de reproducción son muy diversos, tanto en lagartos como en serpientes puede ocurrir oviparidad y viviparidad, dimorfismo sexual y el tiempo de retención de huevos varía considerablemente entre especies (Vitt & Caldwell, 2009).

Los lagartos (*Lepidosauria, Squamata, Lacertilia*) son el grupo más extenso, con cerca de 7,000 especies descritas hasta ahora. Son un grupo parafilético que incluye a los varanos, las iguanas, los coritofánidos, los camaleones, las lagartijas y los geckos. No son un grupo natural ya que incluyen, de forma anidada, a las serpientes y las anfisbenias (Flores-Villeda y García Vázquez, 2014). Por lo anterior, las formas, tamaños, estilos de vida y distribución son ampliamente variables (Pianka & Vitt, 2003).

Las anfisbenias (*Lepidosauria, Squamata, Amphisbaenia*) son un suborden de reptiles adaptados a la vida en el subsuelo cuya mayor característica es que carecen de patas (a excepción del género *Bipes* que aún conserva el par anterior). Están adaptados a la vida en el subsuelo y son capaces de desplazarse tanto en dirección de la cola como de la cabeza. Se reconocen unas 200 especies, distribuidas principalmente en África y Sudamérica (Vidal & Hedges, 2009).

El grupo de las serpientes (*Lepidosauria, Squamata, Serpentes*) poseen además otras características distintivas derivadas principalmente de la reducción de extremidades que presentan. Por ejemplo, su cuerpo tubular requiere que todos los órganos sean alargados y delgados, y en algunas especies pueden encontrarse reducidos (por ejemplo, los pulmones), presentan 120 vertebras precloacales o más y el cráneo es mucho más cinético que el de las lagartijas. Los sentidos del olfato y de la vista se encuentran muy bien desarrollados, logrando imágenes infrarrojas del ambiente. Son cosmopolitas, exceptuando latitudes extremadamente altas. Entre las familias descritas para las serpientes se encuentran: Scolecophidianos, con cabeza y cola roma, escamas suaves y ojos vestigiales; Leptotyphlopidos, el cráneo y la mandíbula superior son inmóviles y sólo presentan dientes en el hueso dentario; Uropeltinos, con mucha especialización para excavar; Boidos, comúnmente conocidas como boas, con los ejemplares más grandes de serpientes; Tropidophiidos, cuando son perturbadas, estas serpientes tienen hemorragias espontáneas en boca y ojos; Bolyeriidos, son las únicas que tienen dividido el hueso maxilar; Acrochordidos, serpientes acuáticas nocturnas con escamas tan aquilladas que su piel da la sensación de lija; Viperidos, con un amplio rango de distribución; Elapidos, todas las serpientes de esta familia son venenosas con una dentición proteroglifa; Atractaspididos, con glándulas venenosas y maxilar notablemente reducido; y Colubridos, la familia más numerosa con aproximadamente el 70% de todas las especies de serpientes (Dereck, 2011; Vitt & Caldwell, 2009).

3. **Testudines**. Las tortugas poseen características inconfundibles que las separan del resto de los reptiles. De hecho, su relación con otros amniotas no se ha determinado completamente. Han existido durante unos 220 millones de años, con éxito en ecosistemas terrestres, de agua dulce y marinos. Hoy en día, más de la mitad de las 360 especies existentes (482 taxones considerando las subespecies) se encuentran en peligro de extinción (Stanford et al., 2020). En las siguientes secciones se presenta un panorama general sobre su origen, su biología, su ecología y los retos de conservación de este excepcional grupo.

ORIGEN Y EVOLUCIÓN DE LAS TORTUGAS

El origen de las tortugas y su peculiar plan corporal todavía no es del todo claro, sobre todo por la dificultad de establecer relaciones con cualquier otro tetrápodo (Carroll, 2013). Debido a la morfología del cráneo y a su armadura dérmica, históricamente se pensaba que las tortugas pertenecían a un grupo de reptiles que divergieron tempranamente, los anápsidos, que son ubicados en un clado monofilético conocido como Parareptilia. Análisis más recientes, que incluyen datos morfológicos y moleculares, han llevado a la hipótesis de que las tortugas evolucionaron a partir de un ancestro común de aves y crocodilianos, ubicándolas dentro de los diápsidos como un grupo hermano de los archosaurios (Crawford et al., 2012; Zardoya & Meyer, 1998). Independientemente de su origen, debido a que el grupo posee muchas características estructurales únicas, siempre se ha considerado monofilético.

El primer fósil que se conoce con afinidades claras con las tortugas actuales es *Odontochelys semitestacea* del Triásico en China, con una antigüedad de 220 millones de años; sólo un poco más viejo que el fósil más antiguo de una tortuga con caparazón completo, *Proganochelys*, del Triásico tardío en Alemania (Reisz & Head, 2008). El aspecto de estos primeros quelonios era básicamente similar a los de hoy en día, cuya mayor diferencia es que éstos aún tenían dientes en ambas mandíbulas y en el paladar (Alderton, 1993; Orenstein, 2012). Durante el Jurásico, las tortugas comenzaron a colonizar los océanos del planeta y se extendieron hacia los continentes actuales de Norteamérica y Asia. Fue durante este periodo que emergieron los antepasados del suborden actual de los Cryptodira. El otro suborden reconocido actualmente, Pleurodira, evolucionó hasta el Cretásico (Vitt & Caldwell, 2009).

Actualmente, gracias a la historia de vida que desarrollaron las tortugas a lo largo de su historia evolutiva, éstas se distribuyen en las regiones tropicales y templadas de todo el mundo en una gran variedad de ecosistemas (Buhlmann et al., 2009; TTWG, 2017). Desafortunadamente, hoy en día las tortugas son el grupo de vertebrados más amenazados. De las 360 especies reconocidas, la Unión Internacional para la Conservación de la Naturaleza (IUCN por sus siglas en inglés) reconoce 187 especies (51.9%) como amenazadas, y 127 especies en peligro de extinción (Stanford et al., 2020).

GENERALIDADES DE LAS TORTUGAS

El caparazón que recubre casi completamente el cuerpo hace de las tortugas los organismos más distinguibles e instantáneamente reconocibles, de hecho, es un carácter exclusivo para este grupo (Nagashima et al., 2013). Este caparazón está compuesto de osificaciones dérmicas que incorporan las costillas, las vértebras y algunas porciones de la cintura escapular. Está dividido en dos secciones, la dorsal conocida propiamente como caparazón y la ventral llamada plastrón. Ambas partes están conectadas por puentes que se encuentran entre las extremidades a cada lado del cuerpo. Observaciones microestructurales han revelado que el caparazón está compuesto por capas exteriores de hueso que cubren una red ósea interior que conforma una capa fibrosa. El plastrón puede ser una estructura sólida o puede ser un grupo de elementos articulados, dependiendo del grupo (Damiens et al., 2012; Lyson et al., 2013).

Todas las tortugas comparten una estructura craneal única, que se caracteriza principalmente por la ausencia de fosas temporales. Además, no presentan dientes, los cuales son reemplazados por un pico queratinoso duro, con un borde córneo cortante llamado tomio. Las extremidades son muy variables y reflejan en gran medida el ambiente en donde se encuentran y el modo de locomoción. Con algunas excepciones, la cola es reducida en comparación con el resto de los reptiles; la cloaca se encuentra en la base de esta, la cual alberga los órganos reproductores. Poseen sentidos del tacto y del olfato bien desarrollados, lo que les permite ubicar zonas para refugiarse, asolearse, detectar presas y aparearse. Todas las tortugas son ovíparas, y generalmente ponen los huevos en nidos excavados en el suelo. No realizan cuidados parentales. Son los vertebrados más longevos que existen actualmente, de crecimiento lento, maduración tardía, y reproducciones repetidas. A excepción de latitudes extremadamente altas, son cosmopolitas, pudiéndose encontrar en hábitats terrestres, dulceacuícolas y marinos (Franklin, 2007).

TAXONOMÍA Y SISTEMÁTICA

La clasificación de las tortugas se ha basado en la estructura del cráneo y del caparazón, caracteres morfológicos y, en algunos casos, datos genéticos. Todas las tortugas existentes pertenecen a uno de dos clados: el Pleurodira y el Cryptodira, los cuales se distinguen básicamente por la forma en la que retraen el cuello; es decir, las tortugas del suborden *Pleurodira* (dividido en tres familias) doblan el cuello hacia un lado, mientras que las del suborden *Cryptodira* (dividido en 11 familias) lo retraen en un plano vertical (Orenstein, 2012; Vitt & Caldwell, 2009). La descripción de las familias que se encuentra a continuación se basa en Cosgrove & Roe (2012), Orenstein (2012), Vitt & Caldwell (2009), Fontanillas-Pérez et al. (2000) y Alderton (1993).

Pleurodira. Las tortugas de este suborden son predominantemente de agua dulce. Se limitan al hemisferio sur, en gran parte de Australia, Sudamérica y África. Hay tres familias representadas:

Chelidae (con 12 géneros): son conocidas comúnmente como tortugas cuello de serpiente, son omnívoras oportunistas.

Pelomedusidae (con dos géneros): con caparazón oblongo y un domo moderadamente alto, habitan principalmente lagos y pantanos; son carnívoras.

Podocnemididae (con tres géneros): son tortugas dulceacuícolas que tienen un caparazón amplio, abovedado y con diseño aerodinámico para el nado activo; son herbívoras, aunque también llegan a comer pequeñas presas y carroña.

Cryptodira. En este suborden se agrupan la mayoría de las tortugas existentes, en 11 familias descritas:

Chelydridae (con dos géneros): estas tortugas poseen una cabeza grande, con el caparazón ancho y aplanado y el plastrón reducido; presentan la cola más larga entre todas las tortugas. Son acuáticas y sólo salen del agua para anidar y dispersarse, sin embargo, no son buenas nadadoras, se desplazan principalmente caminando en el fondo de los cuerpos de agua. Son omnívoras oportunistas y agresivas por naturaleza.

Cheloniidae (con cinco géneros): son tortugas marinas que sólo emergen a tierra para anidar, su principal característica es que sus extremidades anteriores están modificadas en largas aletas. En adultos se ha observado una tendencia a tener dietas especialistas.

Dermochelyidae (monotípica): las tortugas laúd, como se les conoce, están altamente especializadas a zonas marinas pelágicas. Son endotermos inerciales, es decir, que el calor corporal se genera por actividad muscular. Al igual que los quelonidos, sus extremidades anteriores están modificadas como grandes aletas y las posteriores son palmeadas. Su principal presa son los invertebrados gelatinosos, principalmente medusas y salpas. Sin embargo, se sabe que comen otros tipos de alimentos, incluidos pequeños crustáceos y peces (posiblemente simbiontes con medusas), cefalópodos, erizos de mar y caracoles.

Carettochelyidae (monotípica): también conocida como tortuga nariz de cerdo, es una especie que se encuentra al sur de Nueva Guinea y norte de Australia. Su caparazón está cubierto con una suave piel epidérmica. Las extremidades anteriores están modificadas a aletas –como las de las tortugas marinas–, con dos garras bien desarrolladas; su cabeza termina en un hocico ancho, tubular y carnoso. Son tortugas de agua dulce, sigilosas por naturaleza, que viven principalmente en ríos, lagunas y pozos de agua. Son omnívoras.

Trionychidae (con dos subfamilias): son tortugas conocidas como tortugas de caparazón blando, ya que éste es suave, aplanado y reducido y, aunque ha perdido las escamas epidérmicas, está cubierto por una piel gruesa. Las especies de las dos subfamilias habitan en el fondo de los cuerpos de agua o incluso enterradas, esperando a su presa. Sus hocicos son tan largos que les permiten extender la nariz hasta la superficie del agua para respirar. Habitan principalmente ríos y lagos.

Dermatemydidae (monotípica): esta tortuga presenta un caparazón ligeramente abovedado y oblongo, con un gran plastrón y una cabeza moderadamente pequeña. Es acuática, habita áreas de ríos y lagos con poco movimiento del sur de México, Belice y Guatemala. Presenta hábitos nocturnos y es herbívora. Es la tortuga dulceacuícola de mayor tamaño en México y la más amenazada encontrándose en peligro crítico de extinción.

Kinosternidae (con dos subfamilias): también llamadas tortugas de pantano, son una familia propia de América, desde Estados Unidos hasta el norte de Argentina. Su caparazón es moderadamente abovedado y elongado y presentan una cabeza relativamente grande. La subfamilia Kinosteminae presenta un plastrón muy bien desarrollado, generalmente abisagrado y habitan una gran variedad de ambientes dulceacuícolas, en donde generalmente caminan en el fondo al no ser buenas nadadoras. En la subfamilia Staurotypinae el plastrón se encuentra reducido y generalmente sólo son activas durante la época de lluvias.

Emydidae (con dos subfamilias): a esta familia pertenecen tortugas que viven el mayor tiempo en el agua. En general, son tortugas pequeñas con un caparazón abovedado y un plastrón grande, ocasionalmente abisagrado. Este grupo es muy diverso y abundante, incluyendo tortugas acuáticas, semiacuáticas y terrestres. Existe un contraste interesante entre subfamilias, ya que mientras los Emydinos poseen una dieta conservada (omnívoros) y tienen un uso de hábitat diversificado, los Deirochelinos son conservadores en cuanto al uso de hábitat (acuáticos) pero diversos en términos de dieta.

Platysternidae (monotípica): presentan una enorme cabeza que no pueden guardar dentro del caparazón, la cola es larga, los puentes plastrales son flexibles y el techo del cráneo es completo. Es una tortuga pequeña que habita áreas montañosas y rocosas del sureste de Asia. Tiene hábitos nocturnos y durante el día se oculta debajo de las rocas.

Geomydidae (con 23 géneros): es el grupo más diverso de tortugas, con una gran variedad de caparazones y requerimientos de hábitat.

Testudinidae (con 15 géneros): presentan un caparazón muy bien desarrollado, abovedado y todas, sin excepción, presentan miembros fuertes, donde generalmente los posteriores son columnares. Son tortugas completamente terrestres que habitan diversos ambientes (desde desiertos hasta bosques siempre verdes). Son herbívoras, aunque pueden llegar a ser omnívoras oportunistas. Algunas especies pueden alcanzar tamaños gigantescos y ser muy longevas.

TORTUGAS DE AGUA DULCE

Biología

Las tortugas dulceacuícolas componen un grupo taxonómica y ecológicamente diverso, con una distribución geográfica muy amplia. Se encuentran representadas por numerosas especies de hábitos totalmente acuáticos y semiacuáticos, constituyendo poco más del 60% de las especies de tortugas en el mundo (Burke et al., 2000; Moll & Moll, 2004).

En comparación con el resto, las tortugas de agua dulce son de un tamaño modesto, que en pocos casos excede los 300 mm. Pueden presentar dimorfismo sexual generalmente asociado al tamaño, a la coloración o a la forma del caparazón (Legler, 1993). Este dimorfismo sexual puede estar relacionado a aspectos de selección sexual (Berry & Shine, 1980) o como una respuesta a diferencias fisiológicas o ambientales entre sexos (Gibbons & Lovich, 1990). Presentan ojos con una estructura generalizada que funcionan bien bajo una variedad de condiciones. Tienen un oído medio complejo, aunque su sensibilidad auditiva es inferior a la de los cocodrilos y las lagartijas. Su anatomía nasal es primitiva, pero tienen la capacidad suficiente de discriminar señales olfativas (Legler, 1993).

Su locomoción en agua es muy peculiar: debido a su cuerpo rígido y a su cola reducida, los movimientos de propulsión se realizan exclusivamente con las patas, nadando con movimientos alternados de las patas delanteras y las traseras, lo que les proporciona recuperación; en ocasiones sólo usan las extremidades anteriores, mientras que las posteriores actúan sólo como equilibradores o timones. Ello ha generado una gran variedad morfológica de patas como solución a la diversidad de ambientes por donde se desplazan (Pace et al., 2001). Cuando escapan, suelen nadar a toda velocidad hacia el fondo describiendo un camino curveado, se detienen momentáneamente y giran el cuerpo con ayuda del sustrato para nadar nuevamente, pero en dirección opuesta (Legler, 1993).

En cuanto a su comportamiento de reproducción, se sabe que está asociado exclusivamente al ambiente acuático, presentando cortejos de tipo especie-específicos. Si el macho tiene éxito con su cortejo, entonces sujeta fuertemente a la hembra, manteniéndola bajo el agua durante todo el tiempo de copulación. En muchas especies ocurren apareamientos múltiples y la hembra es capaz de retener el esperma por largos periodos (Bowden, 2010).

La determinación del sexo puede ser genética, aunque en la mayoría de las especies depende de la temperatura de incubación. Bajo este último patrón, generalmente a bajas temperaturas de incubación se generan machos y a temperaturas más calientes hembras. De esta forma, las hembras tienen el potencial de controlar la proporción de sexos de su camada y su éxito de desarrollo en el momento de seleccionar la localización del nido (Bowden, 2010). Además, la proporción de sexos puede ser indicador de los procesos ecológicos que ocurren dentro de la población, como por ejemplo, competencia intra-sexual, tiempo invertido en buscar pareja y producción de huevos (Lovich & Gibbons, 1990; Smith & Iverson, 2002).

Aunque con algunas excepciones, la construcción del nido es similar en todas las especies dulceacuícolas. La mayoría de los nidos se ubican en zonas bien drenadas, con suelos arcillosos o arenosos; las hembras excavan con las patas traseras y garras hasta formar un nido en forma de botella (con una cavidad redondeada y una abertura estrecha), donde depositan los huevos y recubren posteriormente con el mismo sustrato, compactándolo con las patas e incluso con el plastrón. Una vez terminado, la hembra se aleja del nido, por lo que no ocurre cuidado parental (Bowden, 2010). Además, se ha visto que las hembras presentan fidelidad a las áreas de anidación, aún incluso cuando han sido transportadas varios kilómetros (Freedberg et al., 2005). Pueden ocurrir comportamientos de hibernación, reduciendo todas sus actividades o incluso enterrándose en el sustrato durante los meses fríos. En general, las tortugas adultas tienen una tolerancia alta a condiciones hipóxicas, por lo que pueden adaptarse fácilmente a periodos con bajos niveles de oxígeno (Bowden, 2010; Jackson, 2000).

Por otra parte, existe cierta evidencia que estas tortugas pueden detectar y procesar una variedad importante de señales químicas, lo que les permite realizar comunicaciones químicas sobre distintos procesos ecológicos: competencia interespecífica, apareamiento y navegación. De esta forma, las hembras pueden regresar a sus sitios de anidación (Bowden, 2010), y se ha visto que tanto hembras como machos pueden ser bastante selectivos respecto a la elección de sitios con la presencia de señales químicas de otros con-específicos (Muñoz, 2004).

Su comportamiento de alimentación es lento, merodeando el fondo y entre la vegetación acuática hasta encontrar a su presa. Generalmente son omnívoras oportunistas, pero se ha visto que los neonatos y los juveniles son más carnívoros que los adultos. Emplean dos técnicas de alimentación: de manera inercial, en donde sujetan a la presa con la mandíbula y la dirigen hacia adentro de la boca con movimientos de cabeza y cuello; y de manera que "chupan" a la presa abriendo la boca lo suficiente para captarla, con un volumen sustancial de agua. El forrajeo terrestre es poco común, aunque si lo hacen, las tortugas regresan al agua para consumir su presa (Bowden, 2010; Legler, 1993). Sus estrategias de alimentación, que además varían en cada etapa de crecimiento, obedecen principalmente a la disponibilidad de alimento según el hábitat, la temporada y la competencia por recursos con otras especies (Moll & Moll, 2004). Como estrategia de osmorregulación, producen urea diluida baja en sodio (Legler, 1993).

Finalmente, uno de los comportamientos más esenciales para sobrevivencia de las tortugas dulceacuícolas es la termorregulación a través del asoleo, que llevan a cabo cuando las temperaturas ambientales superan las temperaturas del agua (asoleo aéreo), o bien, cuando las capas de agua superficiales son más templadas que capas más profundas (asoleo acuático) (Schwarzkopf & Books, 1985). De esta forma, las tortugas pueden mantener una temperatura corporal bastante constante durante todo el día, lo que les permite incrementar el metabolismo, favorecer la digestión, ser más efectivas para alimentarse, reproducirse, crecer, escapar de depredadores, e incluso, cuando están enfermas pueden modificar su termorregulación para provocar fiebres (Bowden, 2010; Dubois et al., 2009; Edwards & Blouin-Demers, 2007). Como una peculiar excepción, la tortuga blanca (*Dematemys mawii*) no necesita asolearse debido a la actividad de las bacterias simbiontes que posee en sus tractos digestivos (Vogt et al., 2011).

Además, estas tortugas incorporan grandes cantidades de energía en forma de biomasa a los flujos de energía y ciclos de nutrientes en cualquier ecosistema que habiten, por lo que representan un componente biológico importante en cada nivel trófico durante todas las etapas de su vida, como depredador y como presa (Moll & Moll, 2004).

Ecología

La dinámica poblacional de las tortugas dulceacuícolas, sus historias de vida y sus interacciones con el medio ambiente se ha descrito con base en el estudio de diferentes especies.

De manera general se sabe que estas tortugas presentan una elevada mortalidad de huevos y juveniles (principalmente por la depredación y explotación comercial a las que se ven expuestas), madurez sexual retrasada y alta sobrevivencia de adultos (ver sección sobre demografía). Sin embargo, estos atributos presentan una gran variación a lo largo del rango geográfico de distribución, asociado a factores geográficos como la latitud y factores ambientales como la estacionalidad, la temperatura y la productividad del hábitat (Judge, 2001). De manera intrínseca, se sabe además que el tamaño corporal es un factor importante en la determinación de caracteres como el tamaño de la camada, la frecuencia de puesta y el tamaño del huevo. Este tamaño corporal está dado por la edad a la que la tortuga madura, es decir, la maduración temprana puede resultar en un tamaño corporal máximo pequeño, pero ofrece la ventaja de una rápida reproducción que asegura el éxito reproductivo, mientras que una maduración tardía contribuye al incremento de la fecundidad y disminuye el costo de la reproducción (Congdon & Gibbons, 1990).

La abundancia de las tortugas está asociada a factores como distancia entre los cuerpos de agua y la disponibilidad de alimento y de sustrato para anidación. Por ejemplo, se ha visto que la abundancia es mayor cuando disminuye la distancia entre charcas, cuando se encuentra una gran cantidad de sustrato orgánico y abundante cobertura vegetal, y cuando aumenta la cantidad de hábitat para anidar cerca de los bordes de las charcas (Marchand & Litvaitis, 2004). Dentro de los cuerpos de agua se ha visto que pueden ocurrir interacciones complejas, como competencia y desplazamiento entre especies de tortugas que coexisten. Por ejemplo, pares de especies que presentan asociaciones negativas son típicamente parapátricas y congéneres, mientras que aquellas simpátricas y filogenéticamente disimiles presentan asociaciones positivas a gran escala y, en ocasiones, segregación a escalas finas (Segurado et al, 2012).

Su desplazamiento puede ser considerado espacialmente en términos intrapoblaciones e interpoblaciones. Los primeros se relacionan con actividades como alimentación, reproducción, termorregulación o refugio, mientras los segundos están relacionados con la migración en hábitats estacionalmente variables, la anidación de las hembras y la exploración del territorio de los machos (Cadi et al., 2008). Se ha visto por ejemplo, que varias especies de tortugas dulceacuícolas pueden desplazarse hasta varios kilómetros en busca de sitios de anidación (Ernst & Lovich, 2009), que pueden abarcar desde tan solo 8 m en el caso del género *Malaclemys* hasta cerca de 1.4 km para *Trachemys*. Además, muchos géneros requieren áreas modestas para anidar (<200 m; *Actinemys, Apalone, Chelydra, Chrysemys, Clemmys, Glyptemys, Graptemys, Macrochelys, Malaclemys, Pseudemys, Sternotherus*), mientras que otros necesitan zonas mayores (*Deirochelys, Emydoidea, Kinosternon, Trachemys*) (Steen et al., 2012). La urbanización, la fragmentación de hábitat y la presencia de depredadores generan impactos importantes en el comportamiento de las tortugas, principalmente en la vagilidad y en las épocas de estivación, sobre todo por la disponibilidad de sitios para realizar sus actividades (Rees et al., 2009).

Las tortugas dulceacuícolas usan una gran variedad de hábitats en el transcurso de sus vidas, desde sitios terrestres para anidar y asolearse, hasta ambientes acuáticos para alimentarse y reproducirse. Su uso varía en respuesta a las condiciones medioambientales, fisiológicas y demográficas (Cadi et al., 2008). La selección de hábitat está asociada a factores tanto bióticos como abióticos, tales como la disponibilidad de recursos, las características físico-químicas ambientales (por ejemplo, temperatura, profundidad del cuerpo de agua, salinidad, intensidad de la luz), y la presencia de competidores y depredadores; los cuales tienen un efecto directo en la sobrevivencia y éxito reproductivo de las especies (Bury et al., 2000; Wariss et al., 2012).

Finalmente, se piensa que estas tortugas cumplen un servicio ecológico importante al actuar como carroñeros en los cuerpos de agua, manteniéndolos libres de contaminación, aunado a sus hábitos alimenticios, que abarcan desde las fitófagas hasta las totalmente carnívoras. Por ello, se piensa que algunas especies pueden ser útiles en el control de poblaciones con base en su alimentación (Cadi et al., 2008).

Demografía

En especies longevas existe poca información sobre aspectos demográficos debido a la complejidad del sistema de estudio, que requiere evaluaciones que abarquen décadas que permitan obtener información suficiente para una estimación confiable de la longevidad, la maduración, las tasas de reproducción, cambios en la proporción de sexos y edades, así como variaciones en la sobrevivencia y la mortalidad (Martins & Souza, 2009; Miller, 2006). Los modelos más utilizados para estimar parámetros demográficos en tortugas son el marcaje-recaptura de hembras neonatales y matrices de crecimiento poblacional basado en el tamaño corporal (Mogollones et al., 2010). De forma general se sabe que las tortugas presentan una elevada mortalidad en huevos y neonatales, maduración sexual retrasada, sobrevivencia elevada en adultos y que pueden llegar a ser bastante longevas (Ernst & Lovich, 2009).

En especies que se han estudiado, se observa comúnmente un patrón reptiliano típico de desarrollo, con un rápido crecimiento de juveniles, seguido de una tasa de crecimiento decreciente hasta llegar a la madurez sexual e indeterminado en el caso de los adultos (Mogollones et al., 2010). La longevidad varía entre especies, con un máximo de 138 años, un mínimo de 20 y un promedio de 43 años (datos estimados a partir de 40 especies, Ernst & Lovich, 2009). La maduración de las hembras se estima a una edad promedio de nueve años, con una puesta de dos hasta 140 huevos dependiendo de la especie y de uno hasta 11 eventos de puesta a lo largo de su vida (Ernst & Lovich, 2009).

En cuanto a la sobrevivencia se han reportado tasas promedio de 0.229 en huevos, 0.672 en juveniles y 0.879 en adultos (Iverson, 1991; Macip-Ríos et al., 2011). Se señala que los tamaños poblacionales son generalmente estables, que se mantienen gracias a la sobrevivencia en adultos, pero que varían enormemente entre especies. Por ejemplo, en el caso de una tortuga endémica de Brasil, *Hydromedusa maximiliani*, se conoce tiene un tamaño poblacional de entre 235 y 318 animales (Martins & Souza, 2009); 38 tortugas en el caso de *Clemmys guttata* (Litzgus & Mousseau, 2004), mientras que para *Trachemys scripta* se han estimado tamaños hasta de 757 individuos por localidad (Miller, 2006).

Algunas correlaciones que se han encontrado entre especies son: 1) en especies de tamaño grande las hembras maduran a una edad más tardía, 2) el tamaño de la puesta y la frecuencia es mayor en especies de mayor tamaño, 3) la longevidad no es necesariamente mayor en especies grandes, y 4) la maduración sexual no está relaciona con la longevidad (Ernst & Lovich, 2009).

Técnicas de captura

Existen dos procedimientos básicos para la captura de tortugas: de manera directa o activa y de manera indirecta o pasiva. La manera directa consiste en que el investigador atrape al animal, con o sin ayuda de herramientas. La manera indirecta involucra el uso de equipos o trampas capaces de atrapar a las tortugas sin que el investigador esté presente (Manzanilla & Péfaur, 2000). La elección del método a utilizar depende en gran medida de la especie de estudio y de los atributos de las poblaciones que se desean evaluar (abundancia, riqueza de especies, densidades, tamaños poblaciones). Ningún método es efectivo en todos los hábitats, ni puede ser aplicado a todas las especies con el mismo éxito (Aguirre-León, 2011; Browne & Hecnar, 2005). A continuación se describen los métodos más utilizados.

1. Técnicas directas. Las capturas directas pueden ser a través de recorridos diurnos o nocturnos, caminando o en vehículos; de forma sistemática o no y puede ejecutarse con o sin

ayuda de instrumentos que aumenten la capacidad de observación y la captura de individuos. Así, es posible utilizar binoculares, telescopios puntuales, lámparas de alto poder para recorridos nocturnos o *goggles* y *snorkel* para buceo, con el fin de localizar a los individuos, que después pueden ser colectados manualmente o con ayuda de una red. En ocasiones se puede remover la vegetación acuática o dar electrochoques al cuerpo de agua con el fin de estresar a los organismos y facilitar su captura (Akre et al., 2012).

Las capturas pueden ser de cuatro formas generalizadas (Aguirre-León, 2011):

a) Colecta oportunista. Cualquier búsqueda no sistemática de organismos corresponde a esta categoría. Generalmente, se realizan caminatas o recorridos en vehículo sin tiempo, área o distancia determinada, a diferentes horas del día o estaciones del año o bajo condiciones climáticas particulares que favorezcan la presencia de la especie de interés.

b) Encuentro visual. Consiste en la observación y colecta de organismos a lo largo de trayectos de distancia o tiempo fijo. Las búsquedas por buceo suelen incluirse en esta categoría al establecerse tiempos fijos de sumersión.

c) Transectos. Son recorridos más o menos rectos de longitud variable en donde se establecen puntos de colecta, ya sea a lo largo del transecto o bien, a un lado y otro del mismo.

d) Cuadrantes. Son áreas completamente delimitadas, de tamaño conocido y estándar para todos los sitos, en donde se realiza la colecta de todos los individuos que se encuentren dentro de dicha área. Los resultados a obtener dependen del tamaño, la forma y el número de cuadrantes utilizados y si el hábitat es homogéneo o heterogéneo.

2. Técnicas indirectas. El trampeo es un método eficiente para capturar a las tortugas dulceacuícolas en diferentes hábitats, sobre todo cuando se encuentran dentro del agua o cuando se trata de tortugas escurridizas que son difíciles de atrapar directamente (Lagler, 1943). Los principales tipos de trampas que se utilizan son:

a) Trampas tipo nasa. Este tipo de trampa es la más comúnmente utilizada para la captura de tortugas dulceacuícolas (ver, por ejemplo, Bluett et al., 2011; Ryan et al., 2008); consisten en tres o cuatro aros de metal de diferentes tamaños cubiertos por una manga de red con una longitud aproximada de 2 m y 15 mm de luz de malla. Los aros conforman pequeños embudos, hechos con el mismo tipo de malla, de tal manera que facilitan la entrada del animal, pero dificultan su salida. Asimismo, para aumentar la eficacia de la trampa, se puede colocar una red de desvío en la entrada de la trampa. El amarre de esta trampa se realiza fuera del agua, dejando así una parte aérea que evita el ahogo de los individuos capturados (Generalitat Valenciana, 2011).

b) Trampas de desvío. Consisten en dos trampas tipo nasa conectadas por una red o trasmallo; comúnmente se coloca cebo (fruta o pescado), que se encuentra suspendido en el centro de la trampa. Regularmente estas trampas se colocan a los márgenes dentro del cuerpo de agua durante dos días (día y noche con revisiones cada cuatro horas) a distancias mínimas de 50 y hasta 200 m (Browne & Hecnar, 2005; SEMARNAT, 2011)

c) Trampas flotantes de asoleo. Se componen de un marco flotante (hecho de tubos de PVC) que sostiene una red de pesca, con una malla de máximo 15 mm de luz, cerrada por abajo. Por la parte superior lo atraviesa una tabla de madera móvil basculante y de sus extremos salen dos tablitas a modo de rampas que flotan hacia el agua por donde suben las tortugas para asolearse (Browne & Hecnar, 2005). Una alternativa a esta técnica es la trampa flotante de cuatro rampas, en la que se instala en todo el exterior del marco de PVC una malla plástica biselada a unos 45° respecto al agua, haciendo accesible a la trampa los cuatro costados (Generalitat Valenciana, 2011). Estas trampas se utilizan en ambientes acuáticos profundos en donde se fijan al fondo con un lastre.

d) Trampas flotantes con cebo. Este tipo de trampas se utiliza para capturar tortugas en aguas abiertas y muy profundas, empleando como atrayente un cebo de pescado. Consisten en una estructura rectangular de hierro cerrada completamente con malla plástica, a excepción de la parte superior en la que se sitúa la tapa, hecha con algún material que flote. En dos caras del cubo se colocan las entradas a modo de embudo y en el interior se coloca suspendido el cebo. La trampa se fija al fondo con un lastre (Generalitat Valenciana, 2011).

e) Trampas sumergidas. Para la captura de tortugas en aguas abiertas también se ha propuesto el uso de redes para cangrejo, cebadas con pescado o fruta. Son trampas que se mantienen bajo el agua, donde el tiempo de sumersión está ligado a los hábitos de forrajeo de la especie que se desea capturar, la profundidad y la claridad del agua. Si bien se ha visto que resulta efectiva para la captura de varias especies tanto carnívoras como omnívoras, es necesario realizar un chequeo constante para evitar que las tortugas mueran ahogadas (Glorioso & Niemiller, 2006).

Existen además un sinfín de modificaciones y alternativas que se han propuesto para la captura de tortugas dulceacuícolas (incluyendo trampas terrestres para mamíferos en donde se han capturado especies que tienen actividad en tierra), que en gran medida están asociadas a requerimientos particulares de estudio (de Lathouder, 2007; Kuchling, 2003; Sharath & Hegde, 2003).

En relación a la efectividad, se ha visto que las trampas con cebo generalmente son útiles para la captura de tortugas de agua dulce omnívoras y que además tienen un forrajeo activo. Las trampas de asoleo se utilizan para capturar especies que no son atraídas tan fácilmente por el cebo, en ambientes con pocas áreas de asoleo naturales y/o donde se encuentre una gran densidad de tortugas. Las capturas a mano, ya sea a través de monitoreos visuales al aire libre o por buceo, son efectivas para complementar otras técnicas de captura y sobre todo en cuerpos de agua claros y de poca profundidad (Sterrett et al., 2010). Los monitoreos nocturnos con lámparas de alta potencia también son útiles para detectar y capturar especies, tanto diurnas como nocturnas, sobre todo en temporadas donde los organismos se mantienen en reposo (Akre et al., 2012). En cualquier caso, el manejo de los organismos debe realizarse con extremo cuidado, de preferencia usando guantes gruesos, manteniendo las manos alejadas de la cabeza y con atención en las garras de las patas (Manzanilla & Péfaur, 2000).

Marcado de organismos.

Existen varias técnicas de marcaje que permiten identificar a los ejemplares y evaluar características de la población, tales como patrones de movimiento, tasas de crecimiento o densidad. Un sistema efectivo de marcaje debe permitir etiquetar a todos los individuos de cualquier tamaño o edad de forma permanente, minimizando la posibilidad de confundir códigos entre individuos y sin que afecte las actividades normales del organismo (Gibbons, 1988).

El método más elemental, y al alcance de todos, es el marcado mediante etiquetas o marcas con pintura (barniz de uñas, pintura indeleble, marcadores de tinta permanente) en el caparazón de la tortuga. Se utiliza para grupos pequeños y tortugas juveniles. Es sencillo aunque no se asegura su permanencia a largo plazo. Para resolver este problema, se han propuesto etiquetas de acero o plásticas que se fijan al borde del caparazón mediante una perforación, aunque generalmente sólo se usan para tortugas de gran tamaño y pueden llegar a ocasionar molestias al ejemplar (SEMARNAT, 2011).

El sistema de Cagle (1939) es el método más antiguo estandarizado que se basa en el marcado mediante la creación de muescas en las escamas marginales del caparazón por medio de pequeñas incisiones o perforaciones, que siguen un patrón de clave único e identificable a largo plazo. Este marcaje es permanente y, al ser generalizado, cualquier persona puede interpretar la codificación de cada individuo (Cagle, 1939). Esta práctica se ha realizado frecuentemente en estudios demográficos, ecológicos, para inventarios y monitoreos de conservación (algunos ejemplos: Beesley et al., 2010; Cook et al., 2007; Limpus, 2008;

Trembath, 2005). Es necesario llevar a cabo esta práctica con extrema precaución, ya que se pueden producir lesiones de tal magnitud que pueden conducir a deformaciones, infecciones e incluso, la muerte del animal (SEMARNAT, 2011).

En algunas especies también se utilizan pequeños anillos numerados que se colocan en las membranas interdigitales de las patas traseras (Beesley et al., 2010; Cook et al., 2007; Limpus, 2008), aunque esta técnica es más común para tortugas marinas (NMFSSFSC, 2008).

Otras propuestas que han surgido actualmente para el marcaje de individuos involucran tecnologías como microchips, implantados de por vida al animal y con el que se pueden realizar seguimientos continuos (Rowe & Kelly, 2005); fotografías, que permiten la identificación individual de organismos adultos (Plummer & Ferner, 2012); y, finalmente, identificación genética, que no sólo permite la identificación exacta de la especie, sino que es posible identificar a cada individuo (Pearse et al., 2001).

CONSERVACIÓN

Las tortugas enfrentan serias amenazas en la actualidad para su sobrevivencia, tales como pérdida y fragmentación de hábitat, contaminación, cambio climático, comercio ilegal de ejemplares y huevos e introducción de especies invasoras (Böhm et al., 2013; Standford et al., 2020). El grupo de trabajo de Genética de la Conservación en Tortugas (Alacs et al., 2007) y diferentes grupos científicos (p.ej. Cortés-Rodríguez et al., 2021) han enfatizado la importancia de la información genética en el entendimiento de la biología de las tortugas, y cómo este conocimiento puede ser aplicado a objetivos para su conservación.

Con base en datos moleculares se ha logrado reconstruir, en buena medida, las relaciones filogenéticas y los tiempo de divergencia entre tortugas (Spinks et al., 2016; Thomson & Shaffer, 2010; Guillon et al., 2012). Más específicamente, se han estudiado patrones de diversificación, con el fin de comprender la relación entre la diversidad existente y las oportunidades ecológicas y los cambios de hábitat (Rodrigues & Diniz-Filho, 2016; Thomson et al., 2021).

Estudios genéticos en tortugas también se han enfocado a resolver problemas de endogamia, identificar flujo génico y unidades de manejo, aclarar situaciones taxonómicas, entender aspectos de comportamiento y relaciones ecológicas (Alacs et al., 2007).

En tortugas dulceacuícolas algunos estudios genéticos han mostrado baja diversidad genética, pérdida de conectividad genética y alta diferenciación, sobre todo en especies con una limitada vagilidad o con escenarios que involucran fragmentación de hábitat, aislamiento o distribución restringida y decremento poblacional (Cortés-Rodríguez et al., 2021; González-

Porter et al., 2011; Reid & Peery, 2014; Shoemaker & Gibbs, 2013; Todd et al., 2010; Vargas-Ramírez et al., 2012).

En contraste con la situación de peligro de extinción de un gran número de tortugas, algunas especies, como la tortuga de orejas rojas (*Trachemys scripta elegans*), ha tenido enorme éxito para establecerse en nuevos ambientes, llegando al grado de ser incluida en la lista de las 100 especies más invasoras del mundo (Lowe et al., 2000). Diversos estudios han puesto en evidencia la amplia capacidad invasiva que tiene esta especie gracias a su amplia tolerancia a condiciones ambientales (Espindola et al., 2019; Kikillus et al., 2010; Masin et al., 2014; Rödder et al., 2009).

CONSIDERACIONES FINALES

Stanford et al. (2020) listan las principales amenazas que han puesto en riesgo crítico de extinción a numerosas especies de tortugas. Si esas amenazas continúan, es altamente probable que en un futuro cercano se pierda este elemento clave de la biodiversidad mundial. La información aquí recabada sobre las características biológicas de este grupo, único en su tipo, concentra una descripción detallada sobre aspectos de historia de vida, conducta, alimentación, entre otros, que sirve de base y consulta para quienes trabajan o desarrollan actividades de manejo y conservación, que abonen a la permanencia a largo plazo de las tortugas.

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Capítulo 2

Fundamental niche unfilling and potential invasion risk of the slider turtle *Trachemys scripta*

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Fundamental niche unfilling and potential invasion risk of the slider turtle *Trachemys scripta*

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ABSTRACT

Background. How species colonize new environments is still a fundamental question in ecology and evolution, assessable by evaluating range characteristics of invasive species. Here we propose a model approach to evaluate environmental conditions and species features to predict niche changes in non-equilibrium contexts. It incorporates potentially range-limiting processes (fundamental niche), hence allowing for better predictions of range shifts, differentiation of analog and non-analog conditions between the native and non-native (invaded) ranges, and identification of environmental conditions not currently available but likely in the future. We apply our approach with the worldwide invasive slider-turtle *Trachemys scripta*.

Methods. We estimated the native and non-native realized niches of *T. scripta* and built its fundamental niche based on key features of the turtle's temperature physiological tolerance limits and survival-associated factors. We next estimated response functions adjusted to the physiological predictor variables and estimated habitat suitability values, followed by a comprehensive set of analyses and simulations to compare the environmental conditions occupied by *T. scripta* (at its native and non-native ranges).

Results. Climatic space analysis showed that the *T. scripta*'s non-native realized niche is 28.6% greater than the native one. Response curves showed that it does not use its entire range of temperature tolerances (density curves for native: 5.3–23.7 °C and non-native: 1.7–28.4 °C ranges). Whether considering the mean temperature of the warmest or the coldest quarter, it occupies a wider range of temperatures along its non-native distribution. Results of the response curves for worldwide (global) and across Mexico (regional) comparisons showed it occupies analog and non-analog conditions between its native and invaded ranges, exhibiting also unoccupied suitable climatic conditions.

Discussion. We demonstrate that *T. scripta* occupies a wider subset of its fundamental niche along its non-native range (within its physiological tolerances), revealing that the species observed niche shift corresponds to a different subset of its fundamental niche (niche unfilling). We also identified suitable environmental conditions, globally and regionally, where the slider turtle could potentially invade. Our approach allows to accurately predict niche changes in novel or non-equilibrium contexts, which can improve our understanding about ecological aspects and geographic range boundaries in current and potential invasions.

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INTRODUCTION

A basic aim of ecology and biogeography is to understand the distribution and abundance of organisms. Additionally, how species colonize new environments is still a fundamental question in ecology and evolution (*Hastings et al., 2005*; *Kueffer, Pyšek & Richardson, 2013*). Biological invasions offer a unique opportunity to explore the patterns and processes of species colonization into novel environments, also a key aspect for monitoring biotic exchange (*Rodríguez-Labajos, Binimelis & Monterroso, 2009*; *Banks et al., 2014*). Indeed, detecting areas where exotic species are likely to establish is a critical challenge for invasion ecology and biodiversity conservation, which in turn can aid in the evaluation and prevention of invasion risk (*Broennimann & Guisan, 2008*; *Higgins & Richardson, 2014*).

Different approaches have been applied to understand the features that facilitate the establishment of invasive species into new environments (*Kueffer, Pyšek & Richardson, 2013*), emphasizing the suitability of the abiotic features of the colonized habitat as a key prerequisite for their success (*Kueffer, Pyšek & Richardson, 2013*; *Colangelo et al., 2017*). Correlative ecological niche models (ENMs) have become a common tool to characterize the environmental conditions suitable for invasive species (*Peterson, 2003*; *Jeschke & Strayer, 2008*). ENMs are based on correlations between the species occurrence distribution and environmental data—the realized niche—(see Glossary S1). However, whether species retain their realized niche when introduced elsewhere is an unresolved ecological query, for which findings are largely diverse and sometimes controversial (*Early & Sax, 2014*; *Guisan et al., 2014*), while evidence supports both niche conservatism and niche shifts during invasions (*Petitpierre et al., 2012*; *Guisan et al., 2014*). In fact, *Early & Sax (2014)* contrasted the climatic conditions occupied by 51 plant species in Europe (native) and USA (non-native) distributions and found that a large proportion of the latter distributions occurred outside the climatic conditions occupied in their native ranges. Also, more than half of the invaded ranges of 71 reptiles and amphibians showed niche shifts (*Li et al., 2014*), while *Strubbe, Beauchard & Matthysen (2015)* showed, for an evaluation combining 29 vertebrate species, that niche overlap between native and non-native populations was generally low because of a large degree of niche unfilling (see Glossary S1) in the non-native range.

Evaluating these shifts under the framework proposed by *Guisan et al. (2014)*, which takes into account occurrence data of the realized climatic niche at the native and non-native ranges, is a first step in understanding whether the species' niche is conserved or if it can undergo shifts in the novel environment. It also aids in the assessment of whether the changes detected are likely caused by native niche unfilling in the non-native range, or by expansion into novel environments (*Broennimann & Guisan, 2008*; *Tingley et al., 2014*). However, it is desirable to explicitly incorporate potentially range-limiting processes, namely physiological tolerances and constraints of organisms. Incorporating links between the functional traits of organisms and their environments provides a mechanistic view of Hutchinson's fundamental niche (*Hutchinson, 1957*; *Hutchinson, 1978*) (see Glossary S1), which can then be mapped to the landscape to infer range shifts (*Kearney et al., 2008*; *Kearney & Porter, 2009*; *Rodrigues, Coelho & Ribeiro, 2018*). Furthermore, in order to model a species' niche mechanistically and infer its potential range, the organism's data must be considered in the model not as a point on a map but rather as a set of traits (e.g., morphological, behavioural, physiological; see a detailed explanation in *Kearney & Porter, 2009* and their Fig. 1). Also, adding range-limiting processes to ENMs allows to differentiate analog and non-analog conditions (Glossary S1) between the native and invaded ranges, and to identify environmental conditions that are not currently available but could be in the future (*Jackson & Overpeck, 2000*). However, studies contrasting fundamental and realized niche models in shaping the geographic range limits and niche changes are scarce (but see *Soberón & Arroyo-Peña, 2017* for a meta-analysis; *Rodrigues, Coelho & Ribeiro, 2018* and *Allen-Ankins & Stoffels, 2017* for analyses of thermal niches). Much less has been investigated regarding invasive species (*Rödder et al., 2009*; *Tingley et al., 2014*), likely as a result of limited information on physiological responses, thus attempts to estimate niche changes could render erroneous conclusions.

Trachemys scripta elegans is considered one of the worst invasive species in the world (*Lowe et al., 2000*), which has been studied mainly about its extraordinary potential for impacting indigenous habitats and species (e.g., *Cadi & Joly, 2004*; *Cadi et al., 2004*; *Ficetola, Thuiller & Padoa-Schioppa, 2009*). Previous studies using species distribution models to explore the invasion patterns of *T.s. elegans* have shown that reproductive populations (in central and northern Italy) are associated to warmer climates compared with its native range (*Ficetola, Thuiller & Padoa-Schioppa, 2009*), while *Rödder et al. (2009)* suggested that climatic requirements during egg incubation could be a major driver for the species' native geographic distribution. Recent studies showed that the invasion process of this turtle species has involved niche shifts (*Li et al., 2014*; *Rodrigues et al., 2016*), and conclude that a dynamic method to properly predict its potential invasion risk is still lacking (*Rodrigues et al., 2016*). Moreover, it is still unclear whether *T.s. elegans* is filling its fundamental niche or if, alternatively, there are unoccupied areas that can potentially be invaded.

We here present a model approach for characterizing potential environmental conditions and species features in novel or non-equilibrium contexts, like species invasions (see Fig. 1). It further advances what *Jackson & Overpeck (2000)* and *Guisan et al. (2014)* have proposed, namely to represent the differences among realized and fundamental niches, as well as the different niche changes that can be observed based on the occupied ranges and within analog conditions. Models based on physiological tolerances data or other fundamental traits (i.e., mechanistic models) are independent of the species' current distribution, hence providing a more accurate prediction of where a species can survive and reproduce in the absence of biotic interactions and dispersal limitations (*Soberón, 2007*; *Kearney & Porter, 2009*; *Tingley et al., 2014*). We hereafter refer to the fundamental niche as that of *Soberón & Arroyo-Peña (2017)*: "the fundamental niche (NF) of a species is determined by its physiological range of tolerance to environmental factors in the absence of biotic interactions, whereas the regions of the planet with environments in NF would represent

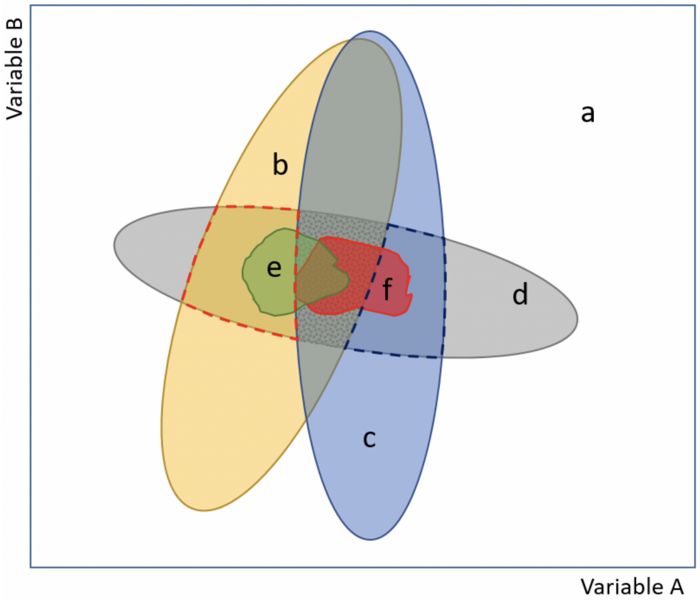

Figure 1 Scheme of our proposed model approach for evaluating potential environmental conditions and niche changes for species invasions. The model explicitly incorporates information about the realized and the fundamental niches (see Glossary S1), and also encompasses both analog and non-analog climate (basic figure based on (*Jackson & Overpeck, 2000*; *Guisan et al., 2014*). The combination of two variables (axes A and B) depicts the environmental space (a), (b) and (c) correspond to the current environment available at the native and the invaded (non-native) ranges, respectively, while (d) delimits the fundamental niche (i.e., areas based on range-limiting processes, like physiological tolerance limits). Importantly, notice that there are areas in (d) that are not currently available, but are areas of potential distribution if an environmental change occurs. The realized (occupied) niche is represented by (e) at the native range and (f) at the invaded range (i.e., areas invaded but outside of the native distribution). Indices of niche shift (unfilling, stability and expansion; see Glossary S1), as described in *Guisan et al. (2014)*, occur inside the central dotted grey area, which corresponds to the analog conditions between the native and invaded ranges. Non-analog conditions but comprising a potential range to be invaded, because it is within the fundamental niche, are indicated with red (native range) and blue (invaded range) broken lines.

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some sort of potential area of distribution for the species”. Accordingly, we describe how to combine into one approach the realized and fundamental niches, encompassing both analog and non-analog conditions, to clearly evaluate if the invaded range represents a niche change but also to adequately define what kind of change it refers to (unfilling, expansion, stability; see Glossary S1), and to identify environmental conditions that are not currently available but could be in the future (*Jackson & Overpeck, 2000*). Importantly, we incorporate a means to identify non-analog regions (mostly absent in invasive species distribution models), which ultimately comprise a potential area of distribution that can be invaded because it is within the species’ fundamental niche range (Fig. 1).

Hence, here we follow our proposed model to assess the current worldwide environmental conditions and distribution ranges of the slider turtle. To this end, we performed a comprehensive set of analyses to compare native and non-native realized and fundamental niches, jointly with estimations of response functions adjusted to the physiological predictor variables, including simulations and response curves, to obtain habitat suitability values. We also test our model regionally, by estimating the potential distribution (invasion risk) for Mexico. Our premise was that we would be able to describe this turtle's niche change patterns throughout its invasion, and resolve whether it is filling its fundamental niche or can invade new environments. Based on our results, we also aimed to exemplify that a more realistic model to define potential areas of invasion risk can be performed based on indirect (i.e., not experimentally based) fundamental traits.

MATERIALS & METHODS

Occurrence and climatic data

Considering the overlapped distribution of *Trachemys scripta* subspecies (*T.s. scripta*. *T.s. elegans* and *T.s. troostii*), and the lack of formal discrimination of the turtle's invasive introductions on the basis of subspecies (*Van Dijk, Harding & Hammerson, 2011*), we obtained georeferenced records for the three subspecies from the Global Biodiversity Information Facility (*GBIF, 2017*) and the *VertNet (2016)* databases. To complement the latter, we also included our own collecting records and those from a thorough revision from the available published literature (data available at https://figshare.com/articles/_/8175158). In order to define *T. scripta*'s native range that encompasses from eastern and central United States of America to extreme north-eastern Mexico, we used as a basis the range maps proposed by *Seidel (2002)* about taxonomic observations on extant species and subspecies of *Trachemys,* updated in *Rhodin et al. (2017)*; next, we used the Freshwater Ecoregions of the World (*WWF/TNC, 2013*) to define range limits based on a global freshwater regionalization. Data points outside these limits were considered as non-native records; that is where the species has been introduced and is considered invasive worldwide (including North America where it has also been introduced outside its native range). We applied a geographic filter to our full set of occurrence data, considering records further than 5 km distance as independent points (multiple points within a cell indicate abundance is higher on those sites in comparison to cells with fewer occurrences). To that end, we used the Ecospat package in R (*Di Cola et al., 2017*) to remove duplicated points within a 5 km buffer, thus reducing overfitting and improving predictions (*De Oliveira et al., 2014*). As a result, we have the most comprehensive data set for the slider turtle, with 2,552 occurrence records, 1,395 from the native distribution range and 1,157 from non-native occurrences around the globe, more than four times the number of records from previous studies.

Climatic data were obtained from the global climate layers of WorldClim (*Fick & Hijmans, 2017*), which represents a statistical summary of temperature, precipitation and radiation at 5′spatial resolution. We included the six following bioclimatic variables: annual mean temperature (BIO1), mean temperature of the warmest quarter (BIO10), mean temperature of the coldest quarter (BIO11), annual precipitation (BIO12), precipitation of driest month (BIO14), and annual mean radiation (RAD), which were selected on the basis of the slider turtle's natural history (i.e., physiology), and also to reduced correlated variables (Pearson's correlation coefficients <0.85). *Trachemys* slider turtles are found in shallow, slow-moving water that has diverse vegetation and nearby places to bask. During seasonal dryness turtles may wander far seeking water sources, rapidly colonizing any newly available habitat; hence, waterbodies availability is a key feature for the activity, dispersal, distribution, and overall life history and survival of this species (*Cox et al., 1998*; *Minton, 2001*; *Buhlmann, Tuberville & Gibbons, 2008*). Accordingly, we constructed a layer depicting the Euclidian distances to the nearest freshwater body (NearDist) (data available at https://figshare.com/articles/_/8175158). For this, we used the Global Lakes and Wetlands Database (GLWD; *WWF, 2017*), a combination of best available sources for lakes and wetlands on a global (world) scale. This raster map has a resolution of 30-second that includes polygons of great lakes (area $\geq$ 50 km^2), great reservoirs (capacity $\geq$ 0.5 km^3) and permanent water bodies (area $\geq$ 0.1 km^2). This is the best resolution available and, although smaller water bodies might not be directly detected, nonetheless polygons encompass the maximum extension of different kinds of water bodies (e.g., marshes, flooding areas). Importantly, this distance to the nearest freshwater body is an indirect measure of water availability—not a measure of dispersal—hence it defines a characteristic of the fundamental niche (*Anderson, Gutierrez & Romano, 2002*; *Braun & Phelps, 2016*).

We tested our proposed model at a regional scale, for Mexico, based on the same variables described above but with a higher resolution (30 arc sec). We used climatic surfaces for the average monthly climate period 1910–2009 (*Cuervo-Robayo et al., 2014*), jointly with a layer we built depicting the Euclidian distances to the nearest freshwater body for Mexico (NearDistMex) using the National Hydrographic System catalogue (*SGM, 2019*).

Comparisons between native and non-native realized niches

We used different approaches to assess similarities and differences between the climatic conditions occupied by *T. scripta* in its native and non-native ranges, all performed in R 3.0.1 (*R Core Team, 2015*). First, in order to characterize the environments available worldwide, we used as reference the occurrence records of all freshwater turtles (not only *Trachemys*) from *GBIF (2017)*, which represent the environments that turtles could occupy. However, because some regions are better sampled for turtles than others and we cannot know if the lack of data is because the environment is not suitable or because they have not been sampled, we constructed a probability layer based on sampling effort by means of assigning a higher probability value where higher occurrences are recorded. We then used the DISMO package to create 100,000 random points worldwide weighted by our probability value. Next, based on the bioclimatic variables of those random points and of the 2,556 *T. scripta* occurrence records (data available at https://figshare.com/articles/_/8175158), we performed a weighted principal components analysis (PCA). Finally, using the first two axes of this PCA we: (*i*) draw contours estimated with a kernel density function (*Broennimann et al., 2012*) to delimit the climatic conditions available on the native range, the non-native ranges (in America, Europe, Asia and Australia) and around the world (areas that have not been invaded); and (*ii*) draw points depicting the estimated realized niche of the native and non-native ranges. This procedure randomizes the position of the kernel density surface of one of the two species within the environmental space available for it, allowing to evaluate whether the realized niches (kernel density surface) occupied in native and non-native ranges are more or less similar to the distribution of similarities under a null model.

As a complementary test, we compared climatic covariance matrices (native versus non-native ranges) with a Common Principal Components Analysis (CPCA; *Phillips & Arnold, 1999*), which compares two or more matrices considering their eigenvectors and eigenvalues in a hierarchical fashion, to describe their structure in relation to the size, shape and orientation of the matrices. We used the latter to establish principal components that were common between matrices (i.e., progressive differences in shape, orientation and size) and to test hypotheses about equality (identical eigenvectors and eigenvalues, i.e., identical size, shape and orientation), proportionality (equal eigenvectors, but eigenvalues differing in a scalar amount, i.e., same shape and orientation, different but proportional size), and unrelated structure (matrices have dissimilar eigenvectors and eigenvalues). The best solution under the model-building approach of the CPCA of the comparison of climatic covariance matrices is indicated by the minimum value of Akaike information criterion (AIC; *Phillips & Arnold, 1999*).

The statistical framework proposed by *Broennimann et al. (2012)* was used to evaluate the assumption of niche conservatism in biological invasions by quantifying the similarity between the native and the non-native realized niches. These comparisons involve a multivariate analysis to calibrate the niche and the occurrence density, performed with ECOSPAT, and the estimation of niche overlap using Schoener's D metric (*Schoener, 1970*), an index ranging from 0 (no overlap) to 1 (total overlap) (*Warren, Glor & Turelli, 2008*; *Broennimann et al., 2012*), with statistical significance assessed on the basis of 100 randomizations ($\alpha = 0.05$). Furthermore, the density of occurrences in environmental space was used to estimate niche expansion (new environmental conditions found in the non-native range), stability (proportion of the native niche conditions found in the non-native one), and unfilling (proportion of the native niche not occupied in the non-native) (*Guisan et al., 2014*), with ECOSPAT. Finally, since the previous method only allows the use of two principal components, for a high-dimensional niche overlap analysis we used a technique based on a multivariate kernel density estimation, implemented in the HYPERVOLUME in R (*Blonder et al., 2014*), that estimates densities based on a Monte Carlo importance sampling approach. To define the six-dimensional hypervolumes (i.e., the six bioclimatic variables described above) for the native and non-native ranges, we chose a threshold that included 100% of the total probability density. We then estimated the overlap (intersection) between the two hypervolumes (native and non-native), and the hypervolume unique to each native or non-native range; results are depicted as pair-plots.

Environmental tolerances, fundamental niche and potential distribution

We performed a literature review (Table S1) to obtain information about physiological optimums and tolerance limits for *T. scripta*. The environmental variable reported more often and consistently was temperature for the following five features: during eggs incubation, hatching, growth, basking, and activity periods. The proximity to freshwater bodies is another critical limit, a fundamental factor for the turtles' survival which they depend upon to complete their life cycles, feeding and reproduction (*Anderson, Gutierrez & Romano, 2002*; *Braun & Phelps, 2016*); thus, we used the Euclidian distance to the nearest freshwater body (NearDist) as an indirect measure of water availability, as explained above. We defined a 10 km buffer for distance to water availability, considering the general 5–10 km average distance reported where turtles can explore (*Cagle, 1944*) and, therefore, reach freshwater bodies. Hence, we considered the combined use of these different temperature tolerance limits and freshwater availability as an accurate approximation of the fundamental niche, as bivariate environmental space, for the slider turtle. Accordingly, we draw response curves in R by using beta and logistic functions adjusted to the optimal, maximum and minimum tolerance limits of *T. scripta*, with VIRTUALSPECIES in R (*Leroy et al., 2015*), which were then used to simulate its potential geographic distribution (see Glossary S1). The tolerance limits used, based on our literature review, were: optimum temperature for eggs incubation (29.5 °C) and daily activity (25.6 °C); ranges for feeding (16.8–32.1 °C), normal activity (10–37 °C), and basking (26.7–31.2 °C); and minimum and maximum tolerances before death (−12.6–42.3 °C). VIRTUALSPECIES allows to define response functions for different predictor variables and to combine the responses to obtain a habitat suitability value. This approach increases the ecological realism for generating environmental suitability of virtual species and to create more adequate species distribution models.

Next, to compare the range of the fundamental niche that is actually occupied by *T. scripta* with the simulated results, kernel density curves based on the temperature variables from all occurrence data (taking into account native and non-native records from which we extracted bioclimatic data) were overlapped with the simulated response curves. Contours and points were also drawn as described above (*Broennimann et al., 2012*), but specifically considering temperature data from the annual mean temperature (BIO1), the mean temperature of the warmest quarter (BIO10), and the mean temperature of the coldest quarter (BIO11), to encompass both the temperature ranges and the extreme tolerance limits of the five described activities; the variable NearDist was also included. The 100,000 generated worldwide random points were used to delimit the bivariate environmental space available, while the occurrence points were used to characterize the bivariate environmental space occupied (data available at https://figshare.com/articles/_/8175158). This data set was compared with the available conditions worldwide, in native and non-native ranges, as well as with the species' physiological tolerance limits. In order to evaluate if combining information about these physiological tolerance limits could improve the prediction of *T. scripta*'s potential invasiveness, we simulated again its potential distribution with VIRTUALSPECIES, this time based on the response curves previously estimated and the higher resolution surfaces built for Mexico.

RESULTS

In accordance with the climatic conditions occupied by *T. scripta* along the two PCA axes (Fig. 2), Schoener's D similarity measure suggests that the niche (the native environmental conditions) has been, at least, partially retained ($D = 0.301$) in the invaded range. This observed niche overlap between the native and non-native niches did not deviate from random expectations (*niche similarity test*, Native to Non-native: $P = 0.029$; Non-native to Native: $P = 0.030$).

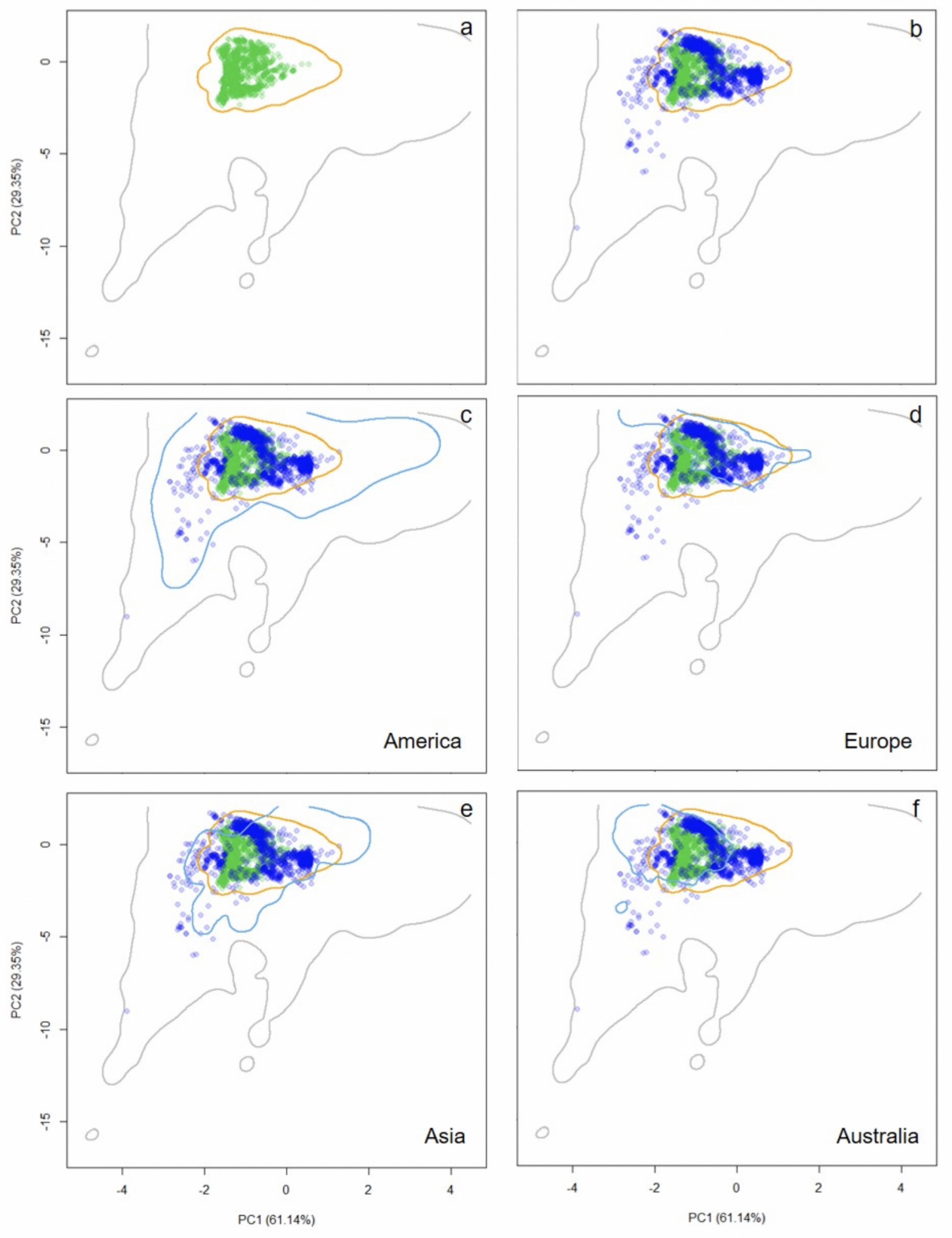

Figure 2 **Distribution of occurrence records of *Trachemys scripta* along the ecological space.** The distribution is represented by the first two principal components of the Principal Components Analysis. The first two principal components accounted for 90.49% of the variation in the data, including mean annual temperature, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of driest month, and mean annual radiation. Contours delimit the range of climatic conditions available around the world (grey line) and at the native range (orange line). Green dots are *T. scripta* occurrence records at its native range (A) and blue dots outside its native range (B); occurrence densities are depicted by the colour darkness of the dots. Blue contours delimit the range of climatic conditions available in America (C), Europe (D), Asia (E), and Australia (F), where *T. scripta* has successfully invaded (graphics were performed in R).

Full-size DOI: 10.7717/peerj.7923/fig-2

The CPCA results showed that the best model that explained the differences between the structure of the climatic matrices of the native and non-native realized niches was the one with dissimilar eigenvectors and eigenvalues (AIC = 42.0; Table S2); that is, matrices have different shape, orientation and size, indicating that even when there are similar climatic conditions at both the native and non-native ranges, non-native individuals of *T. scripta* occupy the available environment in a different fashion. In fact, comparing *T. scripta*'s native realized niche with its non-native one revealed incomplete niche stability (70%), while there was evidence of expansion (30%) into climates available worldwide (Fig. 2).

Results of the six-dimensional climatic space analysis showed that the total hypervolume overlaps by 29% and that the non-native realized niche is 28.5% greater than the native one (Fig. S1). The contribution of the climatic variables to the hypervolume differed between range distributions: regarding the native, mean temperature of warmest quarter was the most important variable, whereas for the non-native it was annual precipitation (Table S3).

The characterization of the fundamental niche (temperature tolerance limits for the different behaviours) of *T. scripta* are depicted in Fig. 3A. Notably, our results comparing temperature density curves between native and non-native realized niche ranges (e and f in Fig. 1, respectively) revealed that *T. scripta* does not use its entire range of temperature physiological tolerances (density curves for native: 5.3–23.7 °C and non-native: 1.7–28.4 °C realized niche ranges; Fig. 3B), namely there are unoccupied areas of higher temperatures within its optimal tolerance limits, while occurrence records are biased towards lower temperatures. Moreover, whether considering the mean temperature of the warmest quarter (Fig. 3C) or the coldest quarter (Fig. 3D), it occupies a wider range of temperatures along its non-native distribution. Results of the response curves based on the fundamental niche and distance to freshwater bodies for worldwide comparisons showed that *T. scripta* occupies analog and non-analog conditions between its native and invaded ranges, both within its tolerance limits, although a few occurrence points fall outside of the limits of its fundamental variables (Figs. 4 and 5). Results also showed suitable climatic conditions, but with no exotic occurrence records, in America, Europe, Asia and, to a lesser extent, Australia (Figs. 4 and 5). Finally, the climatic suitability obtained for Mexico exhibits widespread areas with high potential risk of invasion by *T. scripta* (Fig. 6).

DISCUSSION

Based on our model to evaluate environmental conditions and species features, we show how taking into account both the realized and the fundamental niches (i.e., physiological tolerance limits) for predicting niche changes in novel or non-equilibrium contexts, such as invasions, can improve our understanding about ecological aspects and geographic range boundaries in current and potential invasions (Fig. 1). By considering the temperature physiological tolerances evaluated, we were able to identify that *Trachemys scripta* at its invaded ranges occupies a wider subset of its fundamental niche, which enabled us to ascertain that the species observed niche shift corresponded not to a real change but to a different subset of its fundamental niche (niche unfilling). Our results also show unoccupied areas that have suitable climatic conditions both around the world (Figs. 4 and 5) and regionally within Mexico (Fig. 6) where the slider turtle can potentially invade.

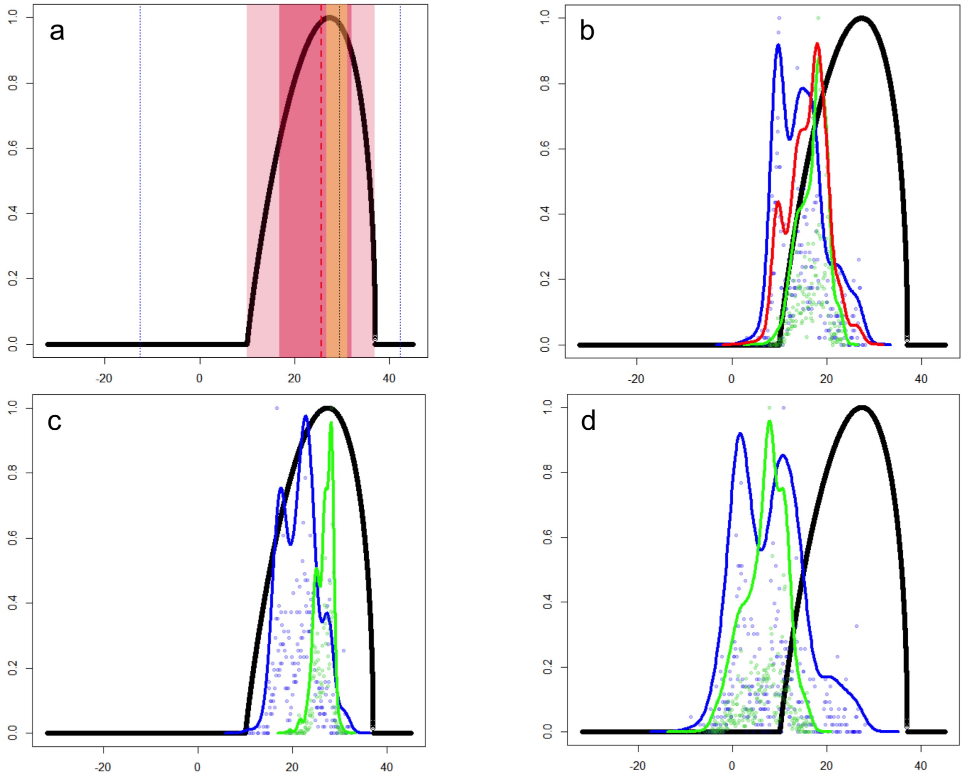

Figure 3 **Temperature tolerance curves of *Trachemys scripta*'s fundamental niche.** (A) Temperature tolerance limits for different behaviors based on literature reports are depicted: optimum temperature for eggs incubation (29.5 °C) and daily activity (25.6 °C) are represented by black and red dotted lines, respectively; ranges for feeding (16.8–32.1 °C), normal activity (10–37 °C), and basking (26.7–31.2 °C) are shaded in dark-pink, light-pink, and yellow, respectively. Minimum and maximum tolerances before death (−12.6–42.3 °C) are represented with blue dotted lines. The thick black line represents the curve encompassing the temperature tolerances estimated in laboratory trials for all the behaviors considered. (B) Comparison between the optimal temperature tolerances (thick black line) and the mean annual temperature (BIO01) ranges actually occupied by the slider turtles at their native (green line) and non-native (blue line) ranges. The red line represents the mean of the total temperature range occupied by *T. scripta* around the World (i.e., native + non-native ranges). Occurrence probabilities are shown by dots for native (green) and non-native (blue) records. (C) and (D) depict the comparison between the optimal temperature tolerances and the occupied ranges based on the mean temperature of the warmest quarter (BIO10) and the coldest quarter (BIO11), respectively. x-axis: temperature (degrees Celsius); y-axis: occurrence likelihood (scale 0 to 1) based on the cumulative frequency of records occurring at a particular temperature (graphics were performed in R).

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Trachemys scripta unfilled realized and fundamental niches

The approach we followed to define *T. scripta*'s fundamental niche, based on temperature physiological tolerances and availability of freshwater bodies, both conditions essential for the persistence of its populations, revealed it has a wide environmental range with favourable climatic conditions (within its tolerance limits). This result is consistent with earlier works suggesting that *T. scripta* has a broad environmental tolerance, particularly regarding temperature ranges (*Rödder et al., 2009*; *Kikillus, Hare & Hartley, 2010*; *Masin et al., 2014*); remarkably, what had not yet been demonstrated is that such broad tolerance does

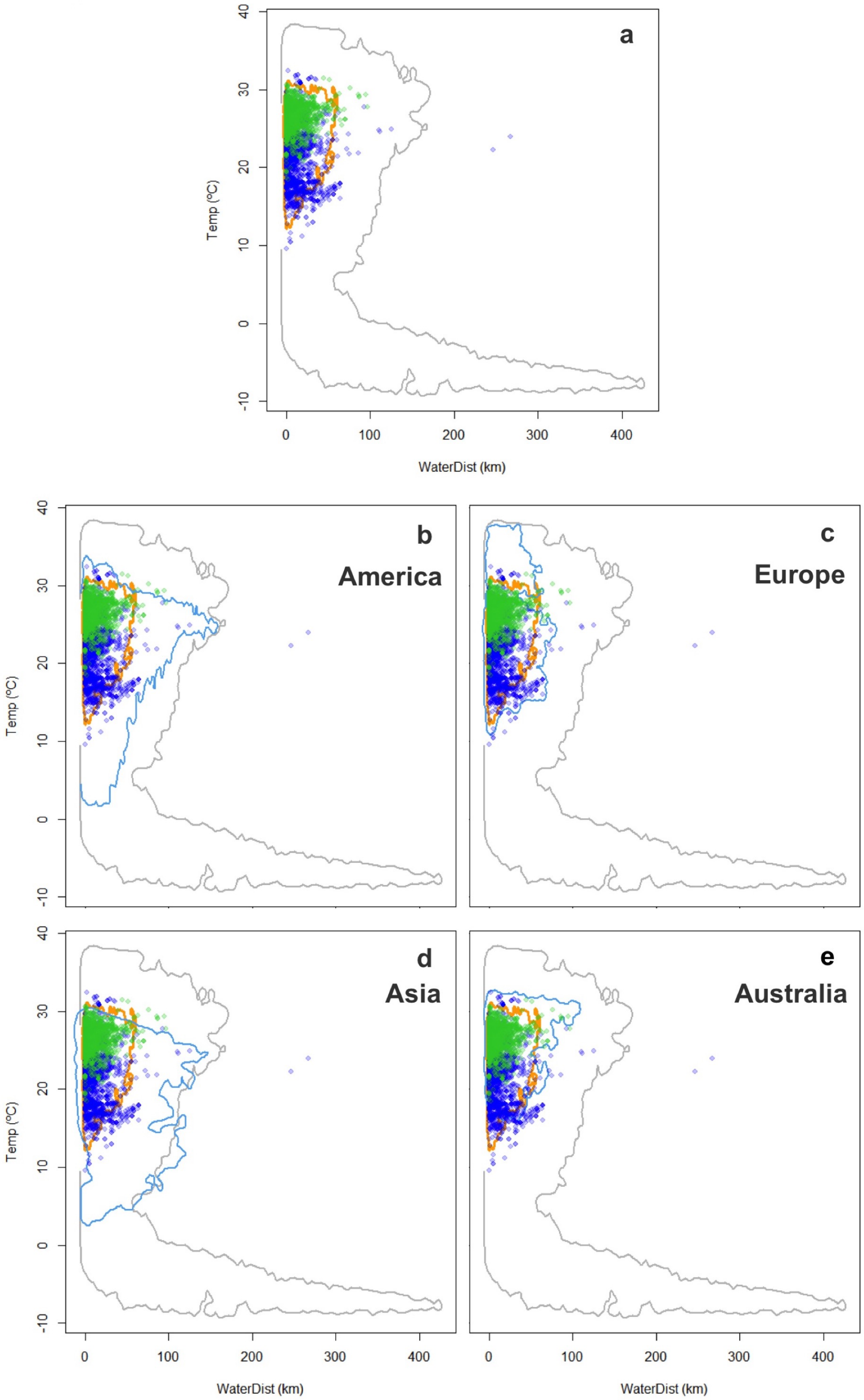

Figure 4 Worldwide distribution of *Trachemys scripta* along the ecological space I. The ecological space is represented by the mean temperature of the warmest quarter (BIO10) and the Euclidian distance to the nearest freshwater body. (continued on next page…)

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Figure 4 (…continued)
Contours delimit the range of the environmental conditions available at the native range (orange line) and around the world (grey line). Green dots are *T. scripta* occurrence records at its native range and blue dots outside its native range (A); occurrence densities are depicted by the colour darkness of the dots. Blue contours delimit the range of climatic conditions available in America (B), Europe (C), Asia (D) and Australia (E), where *T. scripta* has successfully invaded (graphics were performed in R).

encompass a fragment of the range of temperature optimums it requires for performing key behaviors (egg incubation, hatching, growth, basking and activity periods). Accordingly, we consider that the climatic data we used to obtain the ranges of the fundamental niche actually occupied by *T. scripta* allowed us to build a robust model encompassing the environmental conditions required for all these behaviors to occur (i.e., the species' fundamental niche).

Indeed, when compared with the range that *T. scripta* actually occupies, we evidence that it is not experiencing the entire range of optimal conditions along its native distribution; specifically when compared based on the warmest conditions, where suitable climate is unoccupied along its higher spectrum of tolerances (Fig. 3). The latter might reflect biotic features (absent from the climatic models), namely the fact that the distribution of its congener species, *Trachemys cataspila*, starts at the southern limit of the slider turtle's, occupying warmer environmental conditions; these two species do not occur sympatrically, likely due to competition (*Seidel, 2002*). Interestingly, we also found that although non-native areas include similar temperature ranges comparatively with the native ones, non-native individuals have invaded environments that are both warmer and colder than its native range, as documented by *Ficetola, Thuiller & Padoa-Schioppa (2009)* and *Rodrigues, Coelho & Ribeiro (2018)*; however, we show that this has happened without deviating from the turtle's tolerance ranges. This could result from elimination of dispersal barriers and of potential competitors, as shown by studies with different taxa where niche shifts (unfilling and expansion) have been associated with biotic interactions and dispersal limitations, which prevent the species from colonizing the full extent of the available (suitable) conditions (*Hargreaves, Samis & Eckert, 2014*; *Tingley et al., 2014*; *Tingley et al., 2016*; *Strubbe, Beauchard & Matthysen, 2015*); or, conversely, as factors that facilitate the establishment of invasive species, exhibiting where they can potentially be found (*Heikkinen et al., 2007*; *Tingley, Phillips & Shine, 2011*; *Giannini et al., 2013*). Our finding that *T. scripta* is also reaching lower temperatures along its invaded distribution could be associated with the trade of the species as a pet (*Burger, 2009*), where repeated introductions might be forcing the species to occupy areas that are not as optimal (*Ficetola, Thuiller & Padoa-Schioppa, 2009*; *Zhu, Li & Zhao, 2017*). For instance, *Ficetola, Thuiller & Padoa-Schioppa (2009)* recorded *T. scripta* individuals in northern Europe, but where conditions are likely too harsh for successful breeding.

The evaluation of native and non-native distributions provides key information in order to decipher if observed shifts might simply indicate different portions of the fundamental niche occupied by the species (*Araújo & Peterson, 2012*), or suggest an adaptation process to new environmental conditions (*Hill, Chown & Hoffmann, 2013*). When we contrasted *T. scripta*'s native and non-native realized niches along the environmental space, we

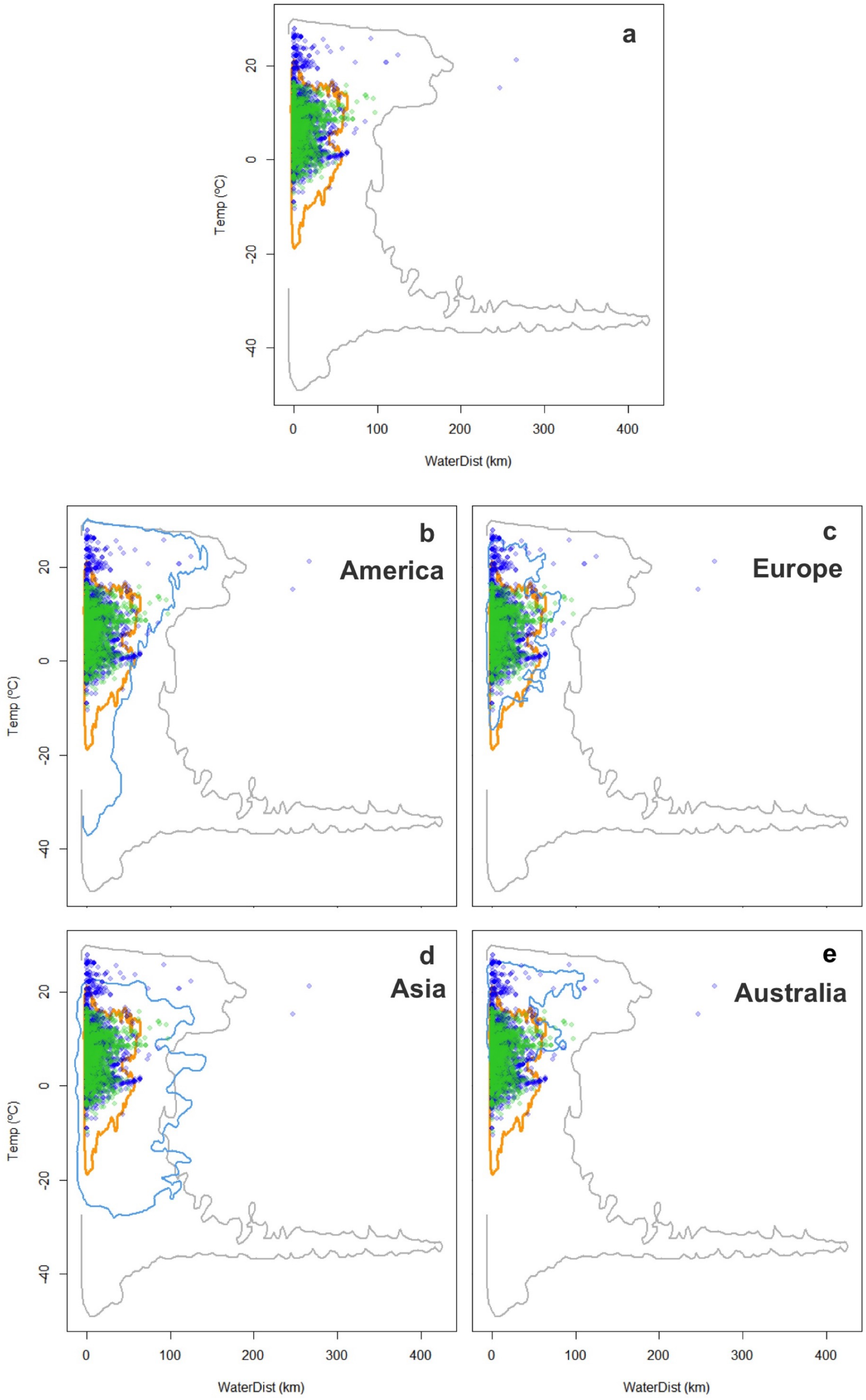

Figure 5 **Worldwide distribution of *Trachemys scripta* along the ecological space II.** The ecological space is represented by the mean temperature of the coldest quarter (BIO11) and the Euclidian distance to the nearest freshwater body. (continued on next page...)

Full-size DOI: 10.7717/peerj.7923/fig-5

Figure 5 (...continued)
Contours delimit the range of the environmental conditions available at the native range (orange line) and around the world (grey line). Green dots are *T. scripta* occurrence records at its native range and blue dots outside its native range (A); occurrence densities are depicted by the colour darkness of the dots. Blue contours delimit the range of climatic conditions available in America (B), Europe (C), Asia (D) and Australia (E), where *T. scripta* has successfully invaded (graphics were performed in R).

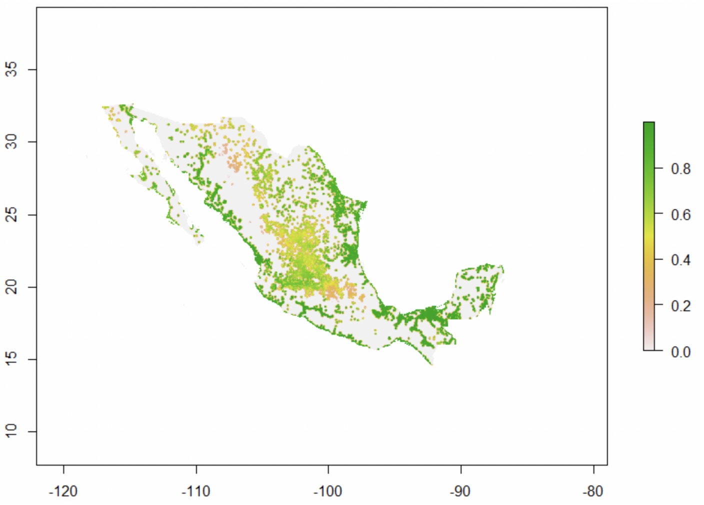

Figure 6 ***Trachemys scripta*'s potential environmental suitability across Mexico.** Map for Mexico drawn with Virtualspecies (*Leroy et al., 2015*), showing the areas of climatic suitability for *T. scripta* based on temperature tolerance curves for different behaviors (eggs incubation, feeding, basking, daily activity) and Euclidian distances to the nearest freshwater body (the species' fundamental niche).

Full-size DOI: 10.7717/peerj.7923/fig-6

found a shift in shape, orientation and size (climatic matrices). In fact, the hypervolume analysis results suggest that the introduction of *T. scripta* into new areas has facilitated the use of available climates in a different way. For instance, while our findings show that temperature is the most significant variable determining the areas that are occupied by the turtle in its native range, it is precipitation at its non-native range. We acknowledge that the hypervolume method's assumptions have been criticized (*Qiao et al., 2017*), nonetheless our results are consistent with the fact that the species always occupies a set of environmental conditions within its physiological tolerance limits and encompassing both analog and non-analog conditions. Furthermore, our results hence support the hypothesis about invasive species not in equilibrium with the environment at their native range (*Elith, Kearney & Phillips, 2010*; *Owens et al., 2013*), and ratify the importance of fully considering the species' capacity to use a wider climate gradient on both space and time (*Broennimann & Guisan, 2008*; *Václavík & Meentemeyer, 2012*; *Tingley et al., 2014*). Accordingly, we show that the slider turtle's documented niche expansion (*Li et al., 2014*; *Rodrigues et al., 2016*) in fact represents a different subset of the species' fundamental niche.

More complex ways when defining potential invasion risk

Notably, our results show both worldwide suitable conditions (in America, Europe, Asia, Australia) as well as regional areas (Mexico) that have not yet been invaded despite encompassing the range of climatic conditions where *T. scripta* has successfully invaded (Figs. 4–6). In addition to the relationship between the distribution of organisms and climate availability, there are more complex approaches to define potential invasion risks (*Gallien et al., 2010*; *Qiao et al., 2017*), which could also explain why *T. scripta* does not occupy the total area with suitable environment in its non-native (and neither native) distribution. *Václavík & Meentemeyer (2012)* have pointed out the need to consider the stage of the invasion to avoid underestimation of habitats at risk of invasion. The history of invasiveness of *T. scripta* is relatively recent: it has been transported globally since the 1950s as one of the most popular turtle pets (*Burger, 2009*), facilitating its introduction into new habitats. Considering the long generation times of these turtles, it is highly likely that *T. scripta* can continue spreading and colonizing new portions of its fundamental niche, which is currently not being fully occupied, allowing it to respond favourably under a climate change scenario (*Hellmann et al., 2008*; *Cosner, 2014*; *Early & Sax, 2014*).

Ecological niche models need to be taken with caution when used to forecast species invasions and their response to environmental change, as it has frequently been shown they may underestimate the potential spread of invasive species (see *Parravicini et al., 2015*; *Merow et al., 2017*). Occurrence data from within and outside of a species native range have been amply used in studies evaluating invasion risk and niche change in exotic species (*Guisan et al., 2014*; *Li et al., 2014*). Studies have, nonetheless, shown that geographic predictions should be used carefully, especially when species are experiencing niche shifts, because it can lead to underestimation of the potential invasion risk. We believe one crucial condition to evaluate invasion risk is having the most accurate fundamental niche possible of the species in question. Biotic interactions also play an important role during the invasion process and, therefore, are key in defining the range that an invasive species can or cannot occupy. Indeed, although the slider turtle has been able to establish widely, its success has not been ubiquitously identical. One likely reason for the latter is that invasions are species- and case-specific, mainly because it involves complex biotic interactions (i.e., with competitors or predators) not easily detected by environmental modeling approaches. Thus, although still unresolved (see *Wisz et al., 2013*; *Cunningham et al., 2016*), it is essential to find means to include biotic interactions when ENM predictions are used to evaluate invasion risk. Also, for an accurate invasiveness assessment, the distinction between colonizing areas where the species can establish and reproduce from those where it can only survive needs be considered; for instance, by comparing predictions with and without those records. In our case, we consider that *T. scripta* ‘s observed non-native range is an adequate proxy of its invasive potential, given that it can survive for decades in areas outside its breeding requirements (*Ficetola, Thuiller & Padoa-Schioppa, 2009*) and that the observed non-native ranges do not deviate from its fundamental niche. Finally, spatial heterogeneity in environmental conditions is another challenge to consider when searching for better invasion risk models. Incorporating landscape genetics methods (*Balkenhol et al., 2013*) could aid in detecting barriers or corridors embedded in the geographic space, ultimately helping improve predictions associated with the expansion range of invasive species (*Tingley et al., 2013*).

CONCLUSIONS

Our approach (Fig. 1) emphasizes the importance of considering key information regarding the survival of species to detect areas that have suitable conditions, areas that could therefore be potential for invasion. It highlights the need to incorporate links between fundamental niche information, namely functional traits and species constraints that influence the survival and spread of organisms to new habitats, into ENM, to improve our current ability to predict potential invasions. It can be applied with different species for which data pertaining their fundamental niche exists. As we show, even if information about a species' functional traits is not based on biophysical models or experimental trials (sensu *Kearney & Porter, 2009*), combining temperature physiological limits (or other physiological constraint) for different vital activities can provide accurate approximations to derive a mechanistic formulation of its fundamental niche. Furthermore, we show that incorporating response functions analyses and comparing empirical and simulated potential distributions provide a key step to understand the processes limiting species' ranges, as well as to predict and accurately describe niche changes and range shifts. Although our results did not exhibit differences in analog and non-analog conditions between the native and invaded ranges, our approach permits to detect such differences where they exist. Undoubtedly, this allows for more robust predictions for invasive species that, as has been evidenced more and more often, are not in environmental equilibrium (*Kearney & Porter, 2009*; *Elith, Kearney & Phillips, 2010*). Indeed, our findings about climatic suitability regions for *T. scripta*, as in our example for Mexico, exhibit a more precise identification of those areas that have the highest potential for a successful introduction of this turtle. Moreover, considering the continuous movement of the slider turtle into new environments via human introductions, and the wide range of environmental conditions suitable and not yet occupied by this turtle, the risk of invasion is rather significant. Integrating other approaches, such as stages of invasion, future climate change, environmental heterogeneity, and biotic interactions to evaluate this and other species invasion processes is a major challenge, yet necessary in order to achieve more reliable models for the management and control of invasive species.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Sayra Espindola conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Juan L. Parra conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Ella Vázquez-Domínguez conceived and designed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

Data is available at Figshare: Vázquez-Domínguez, Ella; Espindola, Sayra; Parra, Juan (2019): DataTrachemysScripta.csv. figshare. Dataset. https://doi.org/10.6084/m9.figshare.8175158.v1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7923#supplemental-information.

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ANEXOS

Fundamental niche unfilling and potential invasion risk of the slider turtle *Trachemys scripta*

Sayra Espindola, Juan L. Parra, Ella Vázquez-Domínguez

Supplemental Figure S1

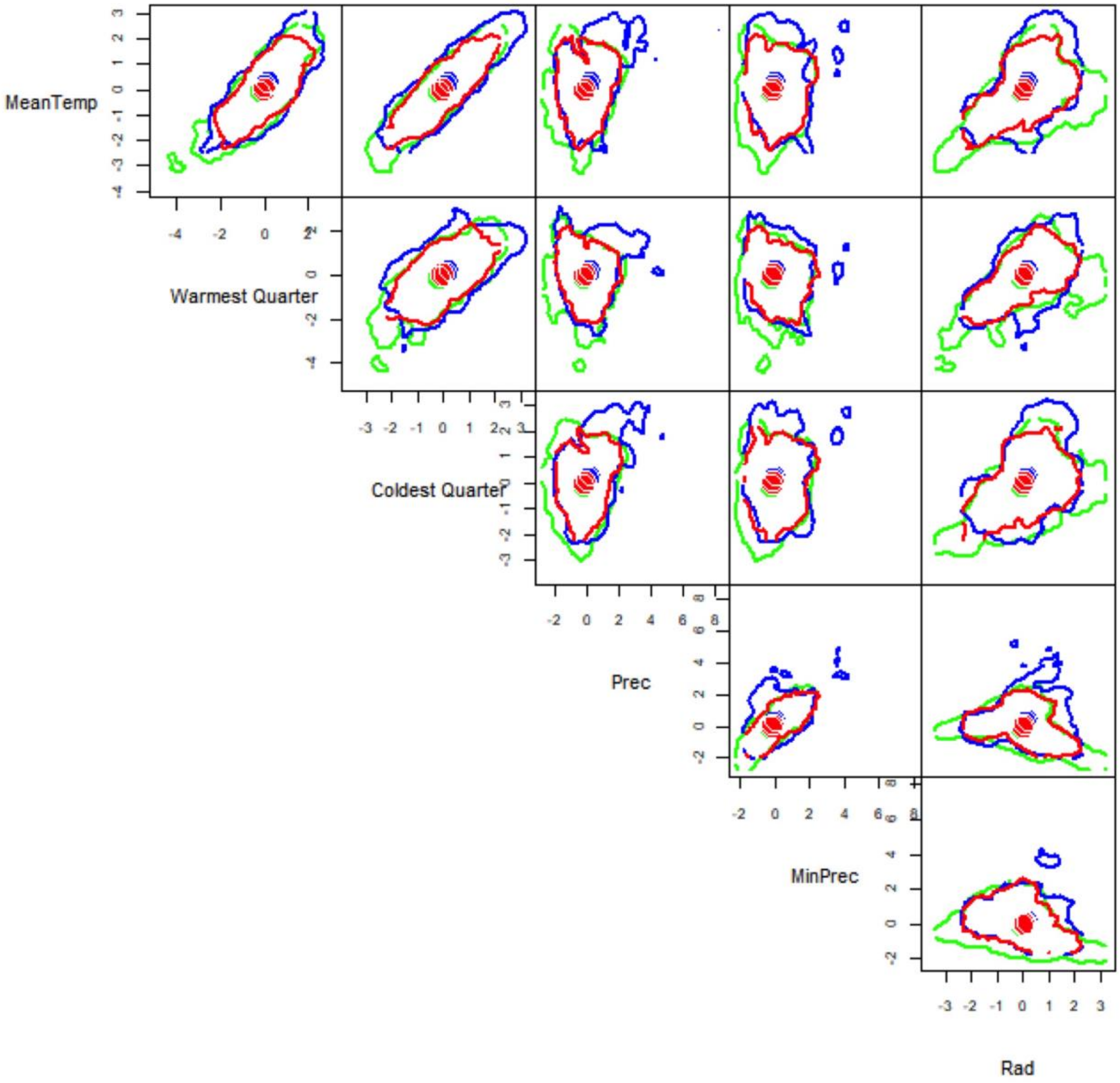

Figure S1. Six-dimensional hypervolumes for native and non-native *Trachemys scripta*. Estimated six-dimensional hypervolumes for native (green line), non-native (blue line), and intersection (red line) between *Trachemys scripta* native and non-native niches along the climatic space (HYPERVOLUME package in R; Blonder *et al.* 2014). Variables were selected on the basis of the slider turtle's natural history, and z-transformed for the analysis. Contours include 95% of simulated data.

Supplemental Tables S1 – S3

Table S1. Literature review from where information about occurrence records, physiological optimums and tolerance limits for Trachemys scripta was obtained. Complete references are listed at the bottom.

Title	Year	Ref.
The growth of the Slider turtle, *Pseudemys scripta elegans*.	1946	1
Acute and chronic temperature effects on cardiovascular regulation in the red-eared slider (*Trachemys scripta*).	2015	2
Varying Hydric Conditions during Incubation Influence Egg Water Exchange and Hatchling Phenotype in the Red-Eared Slider Turtle.	2008	3
Anoxia tolerance and freeze tolerance in hatchling turtles.	2005	4
Trachemys scripta (Slider terrapin).	2012	5
An experimental study of the influence of embryonic water availability, body size, and clutch on survivorship of neonatal red-eared sliders, *Trachemys scripta elegans*.	2002	6
Basking Behavior of the Turtle *Pseudemys scripta*: Effects of Digestive State, Acclimation Temperature, Sex, and Season.	1988	7
Dietary and Habitat Shift with Size of Red-Eared Turtles (*Pseudemys scripta*) in a Southern Louisiana Population.	1983	8
Critical Thermal Maxima in Turtles.	1966	9
Experimental Test of the Effects of Fluctuating Incubation Temperatures on Hatchling Phenotype.	2007	10
Living at Extremes: Development at the Edges of Viable Temperature under Constant and Fluctuating Conditions.	2009	11
Acute and persistent effects of pre- and posthatching thermal environments on growth and metabolism in the red-eared slider turtle, *Trachemys scripta elegans*.	2012	12
Preferred body temperatures in five neartic freshwater turtles: a preliminary study.	1993	13
Cold Tolerance in Hatchling Slider Turtles (*Trachemys scripta*).	1997	14
Temperature, phenotype, and the evolution of temperature-dependent sex determination: how do natural incubations compare to laboratory incubations?	2010	15
Immigration and Dispersal of Slider Turtles *Pseudemys scripta* in Mississippi Farm Ponds.	1984	16
Basking Behavior of Emydid Turtles (*Chysemys picta, Graptemys geographica*, and *Trachemys scripta*) in an Urban Landscape.	2009	17
Effects of body temperature on righting performance of native and invasive freshwater turtles: Consequences for competition.	2012	18
Feeding status and basking requirements of freshwater turtles in an invasion context.	2012	19
Translating natural history into geographic space: a macroecological perspective on the North American Slider, *Trachemys scripta* (Reptilia, Cryptodira, Emydidae).	2009	20

Sexual and Seasonal Differences in Behavior of *Trachemys scripta* (Testudines: Emydidae).	1999	21
Response of Red-Eared Slider, *Trachemys scripta elegans*, Eggs to Slightly Differing Water Potentials.	1998	22
Year-to-Year Variation in Growth in the Red-Eared Turtle, *Trachemys scripta elegans*.	1995	23
Natural history notes on nesting, nests, and hatchling emergence in the red-eared slider turtle, *Trachemys scripta elegans*, in west central Illinois.	1997	24
Annual and Local Variation in Reproduction in the Red-Eared Slider, *Trachemys scripta elegans*.	1998	25
Temperature-Dependent Sex Determination in the Red-Eared Slider Turtle, *Trachemys scripta*.	1998	26
Linking climate and physiology at the population level for a key life-history stage of turtles.	2005	27
Temperature, Genes, and Sex: a Comparative View of Sex Determination in *Trachemys scripta* and *Mus musculus*.	2005	28

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Table S2. Comparison between climatic covariance matrices for *Trachemys scripta*, showing their corresponding Akaike information criterion (AIC) values. (Step-up and model building approaches).

Model		**AIC**
higher	**lower**	
Equality	Proportional	6634.758
Proportional	CPC	2443.042
CPC	CPC(4)	1710.512
CPC(4)	CPC(3)	1587.103
CPC(3)	CPC(2)	1509.213
CPC(2)	CPC(1)	1205.078
CPC(1)	Unrelated	732.403
Unrelated	---	42.000

Table S3. Contribution of the evaluated variables to the total hypervolume differences between *Trachemys scripta* native and non-native niches. The importance score reported is the ratio of the n-dimensional hypervolume relative to each of the n-1 dimensional hypervolumes, where larger values indicate that a variable contributes proportionally more to the overall volume.

Variable	**Native occupied niche**	**Non-native occupied niche**
bio01	0.9686791	0.9576779
bio10	1.1684712	1.1302481
bio11	1.0670855	0.992571
bio12	1.1333105	1.2999334
bio14	1.152466	1.1045099
radAnual	1.1497316	1.110255

Supplemental Glossary S1

Glossary

Analog climate: the combination of climatic conditions present in one area (or time) that is within the envelope of climatic conditions found in a different area (or time) and used for comparison. The contrary is referred to as **non-analog climate**.

Environmental space: all the possible combinations between two environmental variables (or more in a n-multidimensional space), which can be represented –or not– on the geographic space; also referred to as “environmental range”. In Figure 1, the environmental space is delimited by the blue square box (“a”).

Fundamental niche: of a species consisting of the suite of environmental (abiotic) conditions in a n-dimensional environmental space that permit survival and reproduction of individuals (Hutchinson, 1978). It is determined by an organism’s physiological range of tolerance to environmental factors where a species can survive in the absence of biotic interactions (Soberón & Arroyo-Peña, 2017). Hence, the physiological requirements of a species (i.e. optimum, minimum and maximum physiological tolerance limits) can be measured to delimit the dimensions of its fundamental niche. In Figure 1, “d” depicts the fundamental niche; notice that it also refers to areas of the fundamental niche that are not currently available on the geographic space.

Native range: the complete geographic area where a species is naturally distributed (where an exotic species is native), whereas **Non-native range** is the geographic area where an exotic (non-native) species is distributed outside its native range. In Figure 1 “b” and “c” depict the environment available at the native and non-native ranges, respectively.

Niche expansion: proportion of the exotic niche that does not overlap with the native niche.

Niche overlap: the intersection of two niches in the n-dimensional environmental space.

Niche shift: a change in the limits of the niche envelope in environmental space.

Niche stability: proportion of the non-native niche overlapping with the native niche.

Niche unfilling: proportion of the native niche that does not overlap with the non-native niche.

Potential native distribution: suitable areas inside the native geographic range (occupied or not); also referred to as “potential native range”.

Potential non-native distribution: suitable areas outside the native geographic range (occupied or not); also referred to as “potential non-native range”.

Realized niche: it is the actual area occupied by a species (quantified from field observations and occurrence records); also referred to as “ecological niche”; usually a more restricted region of the environmental (abiotic) conditions obtained after accounting for biotic interactions, populations dynamics and dispersal limitations. It comprises a subset of the fundamental niche, which is constrained by biotic factors and may prevent individuals of a species from occupying part of its fundamental niche. The geographic projection of the realized niche is based on the correlation between occurrence records and bioclimatic variables. In Figure 1, “e” and “f” depict the realized (occupied) niche at the native and non-native ranges, respectively.

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Capítulo 3

Complex genetic patterns and distribution limits mediated by native congeners of the worldwide invasive red-eared slider turtle

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Complex genetic patterns and distribution limits mediated by native congeners of the worldwide invasive red-eared slider turtle

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Abstract

Non-native (invasive) species offer a unique opportunity to study the geographical distribution and range limits of species, wherein the evolutionary change driven by interspecific interactions between native and non-native closely related species is a key component. The red-eared slider turtle, *Trachemys scripta elegans* (TSE), has been introduced and successfully established worldwide. It can coexist with its native congeners *T. cataspila*, *T. venusta* and *T. taylori* in Mexico. We performed comprehensive fieldwork, executed a battery of genetic analyses and applied a novel species distribution modelling approach to evaluate their historical lineage relationships and contemporary population genetic patterns. Our findings support the historical common ancestry between native TSE and non-native ($\mathrm{TSE}_{\mathrm{alien}}$), while also highlighting the genetic differentiation of the exotic lineage. Genetic patterns are associated with their range size/endemism gradient; the microendemic *T. taylori* showed significant reduced genetic diversity and high differentiation, whereas $\mathrm{TSE}_{\mathrm{alien}}$ showed the highest diversity and signals of population size expansion. Counter to our expectations, lower naturally occurring distribution overlap and little admixture patterns were found between TSE and its congeners, exhibiting reduced gene flow and clear genetic separation across neighbouring species despite having zones of contact. We demonstrate

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Sayra Espindola and Ella Vázquez-Domínguez contributed equally to this work.

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that these native *Trachemys* species have distinct climatic niche suitability, probably preventing establishment of and displacement by the $\text{TSE}_{\text{alien}}$. Moreover, we found major niche overlap between $\text{TSE}_{\text{alien}}$ and native species worldwide, supporting our prediction that sites with closer ecological optima to the invasive species have higher establishment risk than those that are closer to the niche-centre of the native species.

KEYWORDS
niche-centrality, population genomics, species distribution models, species invasions, *Trachemys*

1 | INTRODUCTION

How the combined effects of evolutionary, biogeographical and ecological forces have driven the distribution of natural species, coupled with how interacting factors (e.g., physical and climatic environment, species interactions, resources availability) define the limits of species' geographical ranges, remains a fundamental question in ecology and evolution. Non-native (invasive) species offer a unique opportunity to explore patterns and processes governing the geographical distribution and range limits of species (Espindola et al., 2019; Goldberg & Lande, 2007; Grayson & Johnson, 2018). For instance, Tingley et al. (2014) demonstrated that the rapid and extreme success of the cane toad (*Rhinella marina*) invasion of Australia is related to a shift in the species' realized niche, as opposed to evolutionary shifts in the range-limiting traits that define its fundamental niche. An assessment of terrestrial gastropods found that native ranges reflect natural dispersal limitation and biogeographical realms, while their distribution in non-native regions is mostly explained by prevailing climate (Capinha et al., 2015).

Reconstructing human-mediated species invasion histories has been possible with the use of genetic approaches that isolate ecological and evolutionary forces acting on non-native species during the invasion process and shaping their distribution limits (Cristescu, 2015; Suárez-Atilano et al., 2019). Likewise, genetic diversity within populations of non-native species depends on a variety of factors, including the frequency of bottlenecks and founder effects, the possibility and frequency of multiple introductions and admixture, and demographic characteristics (Brennan et al., 2014; Suárez-Atilano et al., 2019). Native species also experience novel pressures when a potential invader arrives, including increased competition, range displacement and decreases in population size, introduction of maladapted alleles and potential decrease in fitness by hybridization and introgression, and even alteration of their evolutionary trajectory in response to selective pressures and rapid adaptive changes (Stuart et al., 2014; Suarez & Tsutsui, 2008; Vyšniauskienė et al., 2018). Notably, these processes have rarely been evaluated along the distribution of non-native species with their congeners, or in scenarios where they co-occur. An example illustrating how interspecific interactions between native and non-native closely related species can drive evolutionary change is shown by the lizards *Anolis carolinensis* and *A. sagrei* on islands in Florida, where the native *A. carolinensis* moved to high perches and adaptively evolved larger toepads after the *A. sagrei* invasion (Stuart et al., 2014).

Species invasions have been studied under different approaches; among these, species distribution models (SDMs) have been used to assess probable risk areas based on the species' niche and its climatic suitability (e.g., Srivastava et al., 2019). A key concept in SDMs is that fitness is expected to be highest in areas with environments closest to the centre of the fundamental niche (Maguire, 1973; Martínez-Meyer et al., 2013; Osorio-Olvera et al., 2019), which is defined as the physiological range of tolerance to environmental factors where a species can survive in the absence of biotic interactions (Espindola et al., 2019; Hutchinson, 1957; Soberón & Arroyo-Peña, 2017). In fact, a significant relationship between species' fitness attributes (e.g., genetic diversity and abundance) with the distance to the centre of the niche has been documented in a diverse array of taxa (Lira-Noriega & Manthey, 2014; Osorio-Olvera et al., 2020). Contrasting niche-centre distances between native and invasive species can be a useful approach to estimate the potential for establishment of the latter in sites that are suitable for both species. This idea is based on the hypothesis that the invading species would displace the native one in those sites that are closer to its niche centre.

Among vertebrates, turtles represent an exceptional case for exploring genetic and distributional invasion processes, and studies have revealed complex patterns of genetic variation regarding both historical lineage diversification (Fritz et al., 2012; Kraus, 2015) and contemporary changes resulting directly from human-mediated invasions (Fong & Chen, 2010; Parham et al., 2013, 2020). The slider turtle genus *Trachemys* is one of the most widely distributed American reptile groups (Siedel & Ernst, 2017). The North American red-eared slider turtle, *Trachemys scripta elegans* (hereafter TSE), is well known globally mainly because it has been introduced and successfully established worldwide, in at least 70 countries (CABI, 2019), due to its popularity as a reptile pet (GISD 2018). It is classified as one of the 100 worst invasive species worldwide (Lowe et al., 2000), displaying an extraordinary potential for impacting native species and habitats (Cadi et al., 2004; Ficetola et al., 2009; Parham et al., 2013, 2020; Pearson et al., 2015).

Trachemys scripta elegans is distributed natively throughout the northeastern coast of the USA, to northern Florida, through the Mississippi River valley, west through Texas, Oklahoma and Kansas, finally reaching its southernmost limit at northern Tamaulipas, Mexico. Other congeneric species distributed in Mexico have an interesting natural distribution that follows that of TSE in a southwards gradient along Mexico's east coast, including native populations of *T. cataspila* (northern Tamaulipas to northern Veracruz) and *T. venusta* (northern Veracruz southwards to Guatemala, Belize and Honduras; Ernst & Siedel, 2006; Siedel & Ernst, 2017). *Trachemys taylori*, a microendemic species with a markedly restricted distribution, inhabits the wetlands system within the Cuatrociénegas desert valley, in the Chihuahua desert, Coahuila, México (Lazcano et al., 2019; Seidel, 2002a). Along this natural gradient, TSE has been introduced in different areas where it can coexist with its native congeners. The physiology and patterns of invasion of TSE have been well studied (see Cadi et al., 2004; Ryan et al., 2014; Willmore & Storey, 1997), and with regard to phylogeny, ecology and niche characterization (e.g., Espindola et al., 2019; Fritz et al., 2012; Jo et al., 2017; Tucker, 2001). However, historical and contemporary patterns of genetic diversity and structure remain little known in this invasive taxon, particularly comparing native and non-native populations within TSE, and among closely related species co-occurring along their distribution (but see Parham et al., 2013, 2020; Vamberger et al., 2020).

Hence, we aimed to evaluate the historical lineage relationships and contemporary population genetic patterns of these four *Trachemys* species. We specifically targeted the following questions: (i) What are the phylogenetic relationships among native and non-native TSE and in relation to its *Trachemys* congeners? (ii) Do the genetic diversity and structure patterns within and among the four species reflect their range size/endemism gradient from microendemic (*T. taylori*) to widespread invasive (TSE)? (iii) Do native turtles show signals of genetic admixture and introgression among them and with the invasive TSE in Mexico? (iv) Do these *Trachemys* species exhibit distinct climatic niche suitability, enough to prevent the establishment of and displacement by the non-native TSE? (v) Is the establishment of invasive TSE and the displacement of native turtles worldwide associated with the distance from their niches' centre?

We performed comprehensive fieldwork in Mexico and executed a battery of genetic and genomic analyses and a novel species distribution modelling approach to address these questions. We used three sets of molecular markers, microsatellite loci, mitochondrial and nuclear sequences, and single nucleotide polymorphisms (SNPs). Considering the historical dispersal and vicariance events of slider turtles (Fritz et al., 2012), as well as the history of invasion of TSE (Espindola et al., 2019; Ficetola et al., 2009; Pearson et al., 2015) and evidence of hybridization in other regions where TSE has been introduced (Parham et al., 2013, 2020), we anticipated complex genetic patterns in the *Trachemys* species studied here. Namely, we predicted disjunct population structure among the four species, historical and contemporary genetic differentiation between native and non-native TSE, and reduced genetic diversity and high structure in the microendemic *T. taylori*. We also expected to find high introgression and admixture between TSE and its congeners, which will also be supported by markedly shared suitability of their ecological niches. Finally, given that TSE has invaded and displaced native turtle species worldwide, we predicted that sites that are closer to TSE' niche-centre would have higher establishment risk than those that are closer to the niche-centre of the native species.

2 | MATERIALS AND METHODS

2.1 | Field sampling, museum samples and traded individuals

We performed several field trips in 2014 and 2015 at 11 localities throughout the natural distribution of *Trachemys venusta* and *T. cataspila* along the Mexican east coast (Gulf of Mexico and the Caribbean) and *T. taylori* in the Cuatrociénegas valley. Notably, we did not find *T. scripta elegans* (TSE hereafter) at any of the naturally distributed populations with its congeneric species, where we expected it to occur given its invasive nature. Thus, we obtained TSE samples (outside its native range) only in targeted sites at artificial waterbodies (botanical gardens, parks, urban lakes), where it was undoubtedly transported intentionally. Turtles were trapped with baited hoop traps and hand nets, with a consecutive effort of 1–3 days per site. All captured individuals were measured (length and width of carapace and plastron) and weighed; tissue samples (~3 mm^2) were obtained from interdigital tissue. All animals were released at the sampling site; fieldwork was carried out in strict adherence to the guidelines for work with amphibians and reptiles (Beaupre et al., 2004), and with the respective scientific collection permit from Secretaría del Medio Ambiente y Recursos Naturales (Semarnat-FAUT-0168). In order to have samples that covered most of TSE's native distribution along the western USA (Seidel, 2002b), 39 samples (muscle and skin) were obtained from museum specimens (the Herpetology Collections at the Field Museum of Natural History, the American Museum of Natural History, and the Smithsonian National Museum of Natural History) (Figure 1a; Table S1). Finally, in order to gather data about traded turtles of unknown origin, 23 samples were obtained from the Herpetology Laboratory "Vivario" (FES-Iztacala, UNAM). Both fresh and museum tissue samples were stored at −20°C in 90% ethanol until later use.

2.2 | DNA extraction and amplification (microsatellite loci, mitochondrial and nuclear genes)

DNA extraction and amplification protocols are available in Appendix S1. Briefly, we extracted total DNA from fresh tissue with the DNeasy Blood and Tissue Extraction Kit (Qiagen), while for tissue museum samples, many of which were preserved in formaldehyde and later stored in ethanol, DNA was isolated using a modification of the QIAamp DNA FFPE Tissue Kit (Qiagen).

Microsatellite loci were characterized with 15 fluorescently labelled primer pairs developed for TSE (list of primers in Appendix S1,

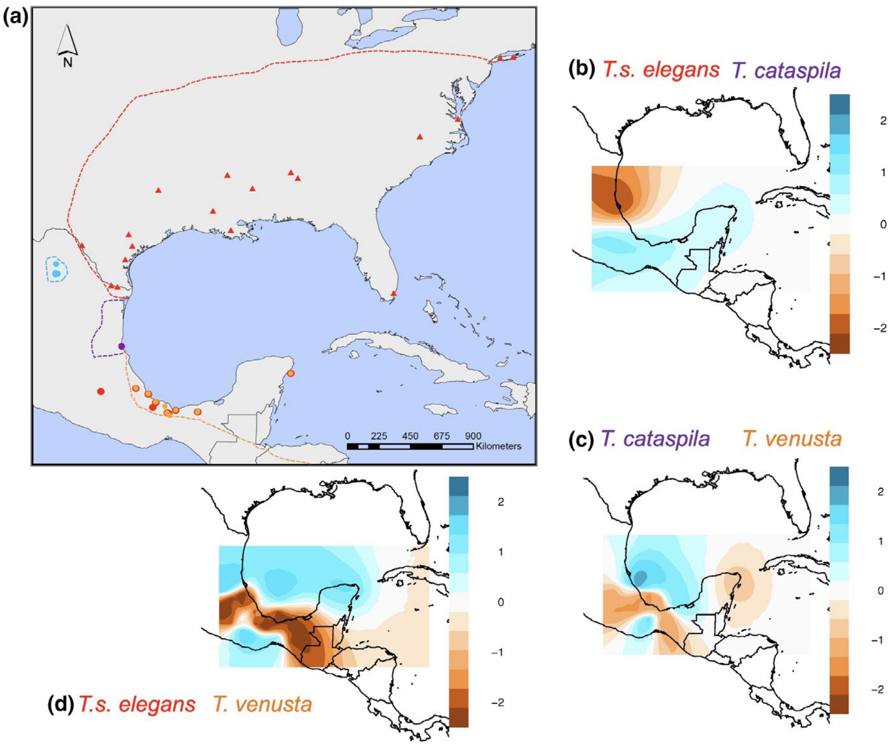

FIGURE 1 (a) Map of sample localities (from field work, museum collections, and the Herpetology Laboratory; Table S1) for four *Trachemys* species: *T. s. elegans* in its native (museum samples; red triangles) and non-native (red dots) distribution, *T. cataspila* (purple), *T. venusta* (orange) and *T. taylori* (blue). Dashed lines depict the approximate distribution range for each species. (b–d) Historical migration patterns (EEMS results) depicting regions of significantly reduced gene flow between the neighbouring species, *T. s. elegans* and *T. cataspila* (b) and *T. cataspila* and *T. venusta* (c), and between the non-neighbouring *T. s. elegans* and *T. venusta* (d). Colour figure available in the online version

Table S2). We included negative controls in all runs and genotyped two or more randomly chosen samples to ensure correct readings and data consistency. Potential null alleles and scoring errors were assessed with MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004), using 1000 Monte Carlo simulations. The mitochondrial cytochrome *b* (cyt *b*; 57 samples) gene and the nuclear genomic intron 1 of the RNA fingerprint protein 35 (R35; 41 samples) gene were amplified for a subset of fresh tissue samples from fieldwork; no museum sample could be successfully amplified despite multiple trials, probably due to the rather fragmented DNA we were able to obtain (Appendix S1 for PCR protocols).

2.3 | Genotyping by sequencing (GBS) and SNP calling

A subset of 178 samples was sent to the Genomic Diversity Facility (GDF) at Cornell University for GBS library construction and sequencing services, on a single lane on an Illumina Hi-Seq 2500 (Illumina) with single-end 100-bp reads. Raw data were processed with *ipyrad*, a simple and reproducible RADseq assembly and analysis framework that is computationally efficient and suitable for both population genetic and phylogenetic scale data sets. The assembly workflow in *ipyrad* is fully self-contained, capable of taking raw Illumina data and producing assembled output files without the need for pre- or post-processing by other software (Eaton & Overcast, 2020). Our workflow was based on a *de novo* assembly method and with the filtering protocol described in Appendix S1. We obtained a final data set of 160 individuals after filtering (see Results).

2.4 | Population genetics and evolutionary relationships

To assess our questions regarding evolutionary relationships and population genetics, we performed a hierarchical set of analyses, for which we distinguished between *T. scripta elegans* individuals from its native (TSE) and non-native ($\text{TSE}_{\text{alien}}$) distribution. Based on the mitochondrial and nuclear sequences data sets, we first determined the genetic differentiation among TSE samples from its native distribution and the non-native ones from our sampling in Mexico, as well as its divergence in relation with the *Trachemys* congeners. Thus, we performed phylogenetic inferences and estimated times of divergence. Second, focusing on a more contemporary evaluation, we estimated genetic diversity values and population genetic structure (differentiation) patterns, based on microsatellite loci, per species and for both TSE and $\text{TSE}_{\text{alien}}$. Finally, based on the SNPs data set, we further evaluated phylogenetic and differentiation patterns, and estimated introgression and historical migration.

2.5 | Mitochondrial and nuclear phylogenetic analyses and divergence time estimation

For the phylogenetic analysis and in order to have a more complete data set for TSE along its native distribution, we incorporated five R35 and eight cyt *b* TSE sequences from GenBank. In addition, we included four cyt *b* sequences for *T. scripta*, one *T. venusta* and one *T. cataspila* (Table S3), as well as sequences for *Chrysemys picta*, *Malaclemys terrapin* and *Trachemys decussata* as outgroups, the first for both genes (cyt *b* and R35) while the other two were available only for cyt *b* (GenBank; Table S3; detailed description in Appendix S1).

Phylogenetic relationships were estimated with maximum likelihood (ML) for both genes with PHYML 3.0 (Guindon et al., 2010), using an NNI+SPR search for branch length optimization; clade support was assessed with 1000 bootstrap replicates. Posterior probabilities and a 50% majority-rule consensus trees were obtained and the resulting trees visualized with FIGTREE version 1.4.2 (Rambaut, 2014). To evaluate the relationship at the level of mitochondrial haplotypes specifically within *T. scripta elegans*/*T. scripta*, we constructed an unrooted network among unique cyt *b* haplotypes with POPART (Leigh & Bryant, 2015), based on the minimum-spanning method (Bandelt et al., 1999).

Lineage divergence was estimated with the cyt *b* data set and a Bayesian framework in BEAST 1.8.3 (Suchard et al., 2018). We used the GTR model across all gene and codon positions, with empirical base frequencies and four gamma categories. We used the same outgroup species as in the phylogenetic analysis, based on which we provided calibration points and error estimates derived from a lognormal distribution, as follows: split of *Chrysemys picta* and the ingroup at 22.5 million years ago (Ma), *Malaclemys terrapin* and the ingroup 13.0 Ma (95% highest posterior density [HPD]: 9.5–17.2), *T. decussata*/*T. scripta*–*T. venusta* split at 9.4 Ma (95% HPD: 7.9–11.2) and *T. decussata*–*T. scripta* at 8.6 Ma (95% HPD: 6.9–10.5) (Fritz et al., 2012; Spinks et al., 2016). The time to the most recent common ancestor (TMRCA) was estimated with an uncorrelated lognormal tree prior and a constant population size prior, running two Markov chain Monte Carlo (MCMC) chains for 10×10^7 generations, sampling every 1000th iteration. TRACER version 1.7.0 was used to monitor convergence and adequate mixing (effective sample size, ESS > 200; Rambaut et al., 2018) and a maximum clade credibility tree (burn-in 10%) was built with TREEANNOTATOR version 2.4.6 (Drummond et al., 2012) and visualized with FIGTREE. Additionally, we conducted a skyline plot analysis using BEAST to infer potential population fluctuations specifically for the non-native TSE. Model parameters were sampled each 5000 generations, with a total of 50 million generations, under a relaxed lognormal molecular clock model, with assumption of uniform distributions, root time set based on the lineage divergence results, and 10% discharged as burn-in. Convergence of results was visualized with TRACER.

2.6 | Diversity, differentiation and demographic analyses (microsatellite loci)

We tested for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between all pairs of loci using GENEPOP version 6 (Rousset, 2008) per species. We estimated genetic diversity indices using GENALEX version 6 (Peakall & Smouse, 2012), including allelic richness (A_r), a rarefied measure that considers the different sampling numbers based on a standard sample size of the smallest sample with complete genotypes at all loci, estimated with *adegenet* in R (Jombart, 2008). Genetic diversity was analysed with the microsatellites because only for this marker did we have TSE native samples and the greatest number of individuals for all species. Complementarily, the SNP data were directed for phylogenetic, historical migration and introgression assessments.

Population structure between and within species was inferred with two methods: a model-free multivariate method, discriminant analysis of principal components (DAPC) with *adegenet*; and with STRUCTURE version 2.3.4 (Pritchard et al., 2000), a Bayesian approach to infer the most likely number of genetic clusters (*K*). Based on the complete database (four *Trachemys* species), we tested for $K = 1–8$ genetically distinct clusters, and further examined structure within some of the identified clusters at the species level.

To evaluate genetic differentiation between species and genetic clusters (identified with STRUCTURE; see Results), we estimated genetic divergence between populations (F_{ST}) using FSTAT (Goudet, 1995) and 10,000 randomizations, and estimated Nei's genetic distance (D_{NEI}) with GENALEX. Further description of all analyses is given in Appendix S1.

2.7 | Phylogenetic relationships with SNPs

We obtained an unrooted species tree topology, based on the 160 individuals data set, with a coalescent method using SVDQUARTETS (Chifman & Kubatko, 2014), a program designed for SNP data, with the default parameters. To obtain a phylogenetic tree incorporating an outgroup, we built an SNP-based outgroup from the painted turtle *Chrysemys picta* genome (Badenhorst et al., 2015) with SAMTOOLS 1.6 (Li, 2011), and used FASTTREE (Price et al., 2009) to infer an approximately ML phylogenetic tree, using the GTR model and the gamma option to rescale the branch lengths. See Appendix S1 for details.

2.8 | Individual ancestry, migration and potential introgression (SNPs)

With the aim of evaluating genetic structure at a fine scale based on SNPs, we performed a DAPC analysis with *adegenet* and secondly used the sparse non-negative matrix factorization method (sNMF; Frichot et al., 2014), which computes regularized least-square estimates of admixture proportion to estimate individual ancestry coefficients. We ran sNMF with the command-line version, *K* from 2 to 7, six series and default parameters. The optimum *K* was calculated by minimal cross-entropy and results plotted as a barplot. Individuals are considered admixed if they show >75% ancestry. We also estimated nucleotide diversity per species, mean F_{ST} and weighted F_{ST} between species using VCFTOOLS (removing all sites with missing data with the --max-missing 1.0 flag).

We evaluated historical migration patterns with the method of estimated effective migration surfaces (EEMS; Petkova et al., 2016). Genetic differentiation is represented as a function of migration rates, correlating genetic variation with geography. If genetic similarity decreases rapidly for the observed values, in comparison with expected values, this indicates a low effective migration (i.e., a barrier to gene flow; Petkova et al., 2016). We built a pairwise dissimilarity matrix and a geographical distance matrix for all individuals to perform paired comparisons (TSE–TC; TC–TV; TSE–TV). With these matrices we ran EEMS with runeems_snps, using a deme size of 1200, with three independent starting chains for 5×10^6 MCMC iterations, 5×10^4 of burn-in, thinning of 5000 and different starting seeds. The three runs were combined and visualized with *reemsplots2* in R (https://github.com/dipetkov/reemsplots2).

To test for introgression among species we used the ABBA/BABA test (also called D-statistic), which is a powerful test for a deviation from a strict bifurcating evolutionary history, namely testing for an excess of shared derived variants between nonsister taxa (Durand et al., 2011; Zheng & Janke, 2018). We built three hypotheses ([TC,TV],TSE; [TT,TC],TSE; [TV,TT],TSE) plus a null model, using two randomly chosen individuals per species (to test each hypothesis twice); *Chrysemys picta* was used as the outgroup. To run ABBA/BABA we used *evobiR* in R (http://www.uta.edu/karyodb/evobiR/) with the *CalcD* function and a jackknife (1000) significance test (see Appendix S1 for full details). A test is significant if the resulting Z score (the D-statistic value divided by its standard error) is above 3 (Zheng & Janke, 2018). Finally, to have a measure of admixture fraction, we used the DSUITE program (Malinsky et al., 2021) to estimate the f4-ratio for the significant comparisons obtained with ABBA/BABA.

2.9 | Niche overlap and niche suitability between TSE and native species

To evaluate our last questions about the establishment of invasive TSE and the displacement of native turtles, we explored niche suitability and niche overlap between TSE and its congeners *T. venusta*, *T. cataspila* and *T. taylori*; for worldwide comparisons, we selected a set of native turtle species from different parts of the world where invasive TSE has been documented and negative interactions have been observed: *Actinemys marmorata* (Western USA; Silbernagel et al., 2013; Spinks et al., 2003), *Mauremys leprosa* (south Europe–north Africa; Meyer et al., 2015; Polo-Cavia et al., 2014), *Emys orbicularis* (Europe; Cadi and Joly, 2004; Polo-Cavia et al., 2014), *Mauremys reevesii* (Asia; Geng et al., 2018; Jo et al., 2017), and *Emydura macquarii* and *Chelodina longicollis* (Australia; Burgin, 2006; Mo, 2019). A data set of available occurrence records per species was gathered from the Global Biodiversity Information Facility (GBIF.org., 2015); our own collecting records in Mexico were also included. To clean this data set, we removed fossil records, doubtful occurrences, wrongly georeferenced localities and duplicate records. We thinned the occurrences to avoid spatial autocorrelation problems, using a distance of 5 km, based on an average movement distance. The final data set included the following records per species: *T. s. elegans* $n = 4270$, *T. venusta* $n = 104$, *T. cataspila* $n = 26$, *T. taylori* $n = 22$, *A. marmorata* $n = 648$, *E. orbicularis* $n = 752$, *M. leprosa* $n = 1535$, *M. reevesii* $n = 219$, *E. macquarii* $n = 642$ and *C. longicollis* $n = 1559$.

The environmental data used to model niches and estimate the proportion of overlap was derived from a principal component analysis on the 19 bioclimatic variables from WorldClim version 1.4, at 2.5′ spatial resolution (~5-km^2 cell; Hijmans et al., 2005). We adopted the first three principal components (PCs), which summarized ~85% of the total variance of the original WorldClim data set (Figure S1) to describe ecological niches. Niches were determined in environmental space by means of ellipsoid models based on the three-dimensional normal distribution (Osorio-Olvera, Lira-Noriega, et al., 2020; Osorio-Olvera, Yañez-Arenas, et al., 2020) applied to the first three PCs. This calls for estimating a niche centre and a positive definite covariance matrix to represent the niche, based on the occurrence points of each species. The modelled niches are each 95% highest density regions (HDRs) in three dimensions, which are in fact ellipsoids because of the multivariate normal assumption (see Appendices S2-Glossary and S3-HDR). Let us denote by A the ellipsoid corresponding to TSE having centre μ and covariance Σ, and by B_k the ellipsoids of each of the other turtles, having centres μ_k and covariances Σ_k. The R package *ntbox* (Osorio-Olvera, Lira-Noriega, et al., 2020) provides functions for estimating these niche centres and covariance matrices.

Quantifications of niche overlap are the volumes of the intersections $A \cap B_k$, which were approximated numerically by Monte Carlo integration as follows: by first examining the range of each PC dimension (PC_j, $j = 1, 2, 3$), a hyperrectangle was constructed that contained the union $A \cup B_k$, and a large number of points (5×10^6) was uniformly generated within the hyperrectangle. The proportion of points that randomly lie in $A \cap B_k$ multiplied by the volume of the hyperrectangle is an approximation to the volume of $A \cap B_k$. We used *ntbox* for this numerical purpose, as functions are provided to easily check if a given random point is within a given ellipsoidal niche or not.

Once niche centres and covariances were determined for each species, we addressed relative appropriateness of occurrences that lie in intersections $A \cap B_k$ by computing suitability indexes. Consider $M(p, \mu, \Sigma)$, the Mahalanobis distance between point p and the multivariate normal distribution with mean μ and covariance matrix Σ. That is, if the coordinates of p are (x, y, z), $M(p, \mu, \Sigma) = \left[(x, y, z)^T - \mu\right]^T \Sigma^{-1} \left[(x, y, z)^T - \mu\right]$. For every occurrence point $p_i \in A \cap B_K$ of TSE let $M_{1i} = M(p_i, \mu, \Sigma)$ and $M_{2i} = M(p_i, \mu_k, \Sigma_k)$. For every occurrence point $q_j \in A \cap B_k$ of species k, let $M_{1j} = M(q_j, \mu, \Sigma)$ and $M_{2j} = M(q_j, \mu_k, \Sigma_k)$. Suitability values at a given occurrence point are defined as $S = e^{-M/2}$, which rescales Mahalanobis distances to $(0, 1)$, where $M = 0$ (maximum suitability) corresponds to $S = 1$, and suitability decreases the further away a point is located from the corresponding niche centre. Pairs of values $\left(S, S_{2i}\right)$ and $\left(S_{1j}, S_{2j}\right)$ can thus be obtained and plotted on the unit square, the suitability space. We refer to these plots as suitability maps for each species.

A main point of interest is to study the observed bivariate distributions of $\left(S_{1i}, S_{2i}\right)$ and $\left(S_{1j}, S_{2j}\right)$ and compare them with what these distributions would have been under the hypothesis of independence, namely no association of the species' distributions when the invasive species is present. If TSE and species k are statistically independent of each other, then the multivariate normals that determine niche location for each species are independent. This in turn induces a specific but unspecified behaviour or density for $\left(S_{1i}, S_{2i}\right)$ and $\left(S_{1j}, S_{2j}\right)$ in the intersection $A \cap B_k$. This density is analytically unknown but can be studied by Monte Carlo simulation.

We employed three descriptive statistics to help discern differences between the observed behaviour of $\left(S_{1i}, S_{2i}\right)$ and $\left(S_{1j}, S_{2j}\right)$ relative to the behaviour that would be expected under independence. First, if $f\left(S_1, S_2\right)$ is the (bivariate) density of $\left(S_{1i}, S_{2i}\right)$ and $f_k\left(S_1, S_2\right)$ is the density of $\left(S_{1j}, S_{2j}\right)$, we define a distance between these two densities as:

$$D^* = \frac{1}{2} \iint \left| f(u,v) - f_k(u,v) \right| du dv.$$

We note that this distance is related to the familiar Shoener D statistic to measure overlap between two density functions (Warren, Glor & Turelli, 2008), by means of $D = 1 - D^*$. This enabled us to examine what the effect of species interactions is in terms of their relative locations and probabilities in suitability space. Second, we consider the sizes (areas) of the 95% HDRs for f and f_k. These are denoted by H_1 and H_2 and are useful to examine how the interaction individually altered the way in which species distribute with high probability in suitability space. Of course, f and f_k are unknown, but since we have data observed over $A \cap B_k$, we can use them to compute density estimates $\hat{f}$ and $\hat{f}_k$ using kernel density estimation (see Appendix S3). The plots of these density estimates are useful descriptors, but in turn they also allowed us to obtain estimates $\hat{D}*$, $\hat{H}_1$ and $\hat{H}_2$ for each instance of k.

The statistical significance of $\hat{D}*$, $\hat{H}_1$ and $\hat{H}_2$ is determined by Monte Carlo simulation under the hypothesis of independence. This means independently simulating pairs of three-variate normal samples (of the same size as observed) within $A \cap B_k$, converting sampled points to suitabilities, computing kernel density estimates $\hat{f}$ and $\hat{f}_k$, and thereby collecting a large number of sample values (5000 in our study) of $\hat{D}*$, $\hat{H}_1$ and $\hat{H}_2$. The distributions of these simulated values under independence were used to compute p-values by comparing with the corresponding observed values. Both upper- and lower-tailed p-values were considered in order to determine if observed values are significant in a single-tail sense.

3 | RESULTS

Our data set included 261 samples, comprising individuals from the field, traded turtles of unknown origin and museum samples. We could not classify five individuals to species in the field due to a markedly mixed external morphology (TSX01–TSX05; Figure S2); these were later identified with the genomic data (see below). Thus, we worked with a final data set of 69 *Trachemys venusta*, 13 *T. cataspila*, 12 *T. taylori*, 128 TSE$_{\text{alien}}$ and 39 TSE (museum) turtle individuals (Table S1).

3.1 | Sequence-based phylogeny and times of divergence

We successfully amplified 57 cyt *b* sequences (782 bp) and 41 R35 (800 bp), encompassing samples from all sampling localities in Mexico and the four *Trachemys* species studied. The best nucleotide substitution models were GTR and HKY85 for cyt *b* and R35, respectively. The ML topology with both genes consistently showed two well-differentiated clades, one for TSE (native and non-native) and the second encompassing the other three species (Figure S3). Based on the nuclear R35 gene (Figure S3a), TSE included eight haplotypes (two and four exclusive for TSE and TSE$_{\text{alien}}$ respectively, and two shared); *T. venusta* formed a separate group (four haplotypes), while *T. cataspila* and *T. taylori* were not well resolved, each having two exclusive haplotypes while sharing one. Cyt *b* included seven haplotypes, two (TSE) and three (TSE$_{\text{alien}}$) exclusive and two shared; it had low resolution for the other three species, with no clear separation among them (Figure S3b). Results from the minimum spanning haplotype network for TSE showed one central, most abundant haplotype, which includes both native and non-native samples. A few unique terminal haplotypes, one widespread, encompass non-native haplotypes separated by one mutation step, while the others separated by >3–4 mutations are native (Illinois) or lead to *T. s. scripta* (Figure S4a).

The topology obtained with BEAST for the estimation of the times of divergence is concordant with the ML phylogenetic relationships depicting two distinct clades, one for TSE (native and non-native) and the other where only *T. venusta* is more clearly separated (Figure S5). The divergence of the *Trachemys* haplotypes (node A in Figure S5) occurred 12.9 Ma (95% HPD: 12.6–13.3). The TMRCA leading to *T. venusta* and to *T. taylori*/*T. cataspila* was dated at 9.5 Ma (9.3–9.7, node B) and 9.4 Ma (9.2–9.7, node C), respectively; diversification times for each clade (TV and TT/TC) started ~1.7 and ~2.2 Ma respectively. The TMRCA between *T. s. scripta* and *T. s. elegans* (node D) was 8.4 Ma (8.1–8.6), followed by a wide range of diverging times within the TSE clade, which diverged at 3.2 Ma (node E) into two monophyletic groups, one including native and non-native haplotypes (1.9 Ma) and a younger one, exclusively of non-native TSE, from 1.6 Ma to as recent as a few thousand years ago. The TSE$_{\text{alien}}$ skyline plot showed a pattern of population size expansion towards the present (Figure S5). Both analyses had convergence with ESS >200.

3.2 | Genetic diversity, structure and bottlenecks (microsatellite loci)

We successfully amplified all 15 polymorphic microsatellite loci for 256 individuals. Null alleles were detected for two loci (TSE06 and TSE02) but based on the Brookfield 1 index results (<15% null alleles), all loci were retained for further analyses. After Bonferroni correction, deviations from HWE were observed at multiple loci and for each species, in all cases showing significant heterozygote deficiency ($p < .001$). No linked loci were found. Regarding genetic variability results, allelic richness (A_r) ranged from 3.1 (*T. taylori*) to 10.9 (TSE_{alien}) (Table 1). With the exception of *T. taylori* ($H_O = 0.255$; $H_{NEI} = 0.424$), moderate to high observed and expected heterozygosity values were observed for all species, ranging from $H_O = 0.580$ (TSE) to $H_O = 0.792$ (TSE_{alien}) and $H_{NEI} = 0.767$ (*T. cataspila*) and $H_E = 0.890$ (TSE_{alien}). Lower genetic diversity indices in *T. taylori* were statistically significant in comparison with the other species (Mann–Whitney *U* tests, $p = .003$).

Consistent results were provided by both approaches used to evaluate genetic structuring. First, the four *Trachemys* species were separated as independent genetic groups with the DAPC analysis (Figure 2a). Similarly, STRUCTURE results clustered each species separately, although interestingly it identified five clusters (LnPr($K = 5$) = −1780.4), clearly separating the native (TSE) and non-native individuals (TSE_{alien}) as two different clusters (Figure 2b). In a second hierarchical STRUCTURE (per species), five clusters were found within *T. venusta* (LnPr($K = 5$) = −3571.5; Figure 2c). In agreement, strong allelic and genetic differentiation was found (G exact test; $p < .05$), where the highest pairwise differentiation was found between *T. taylori* and the other species/genetic clusters ($F_{ST} = 0.163$–0.184, $D_{NEI} = 0.739$–1.012), and the lowest between TSE and TSE_{alien} with both indices (Table 2).

3.3 | Species relationships, genomic ancestry, migration and introgression

Results from GBS rendered a total of 483×10^6 raw reads, an average number of raw reads per sample of 3.02×10^6, and 210,809 raw SNPs. The final data set after filtering included 160 samples (none for the TSE museum samples), with an average number of loci/sample =55,982, coverage = 16× and 63,385 SNPs. Based on this SNPs data set, both the coalescent unrooted species (SVDQUARTETS; Figure S4b) and the approximately-ML (FASTTREE) phylogenetic trees (Figure 3a) exhibited a better resolved topology (in comparison with that from cyt *b* and R35 sequences), clearly separating the four species. This was further supported by the DAPC and sNMF results that showed four clusters as the optimum genetic structure (Figure 2d,e), concordant with the four species. The SVDQUARTETS tree showed some individuals with potential admixture (*T. venusta* TV56 and TV64 with *T. cataspila* TC), which was confirmed by sNMF where TV56 and TV64 showed high admixture with TC (>75% TC ancestry). FASTTREE and sNMF results also allowed us to correctly identify the five individuals we were not able to classify on the field (Figure S2): TSX01 = TC; TSX03, TSX04 = TV; TSX02, TSX05 = TSE. We performed an sNMF analysis for each TV and TSE separately, obtaining $K = 3$ and $K = 2$ respectively, where the former separated three TV samples from Cozumel Island (TV58, TV60, TV61) (Figure S6a); the two TSE genetic clusters had no geographical pattern (Figure S6b). The SNP nucleotide diversity per species, which correlates with effective population size at migration/drift equilibrium, was TC =0.0046, TT =0.0050, TSE =0.0034 and TV =0.0027. Results of the weighted F_{ST} showed the highest values between TSE and the other species (Table S4), consistent with the FASTTREE and SVDQUARTETS relationships. Given that the TSE samples are biased towards non-native individuals, it could have an impact on these analyses (i.e., lower nucleotide diversity), due to reduced genome-wide variation as a result of an invasion bottleneck (which is also apparent in the "star-like" formation of TSE in the SVDQUARTETS tree).

Regarding historical migration patterns, the EEMS results exhibited regions of lower than expected migration across the geographical distributions of the species. Specifically, regions of significantly reduced gene flow are indicated between neighbouring species, TSE and *T. cataspila* (Figure 1b) and between *T. cataspila* and *T. venusta* (Figure 1c); while also between TSE and *T. venusta* (Figure 1d). Introgression results based on the *D* statistics (ABBA/BABA test) showed signal of historical introgression between *T. cataspila* and TSE, which share a natural contact zone, and in one case between *T. venusta* and TSE (Figure 3b; Table S5), while not between other congeners. The admixture fraction values were concordant, where f4-ratio = 0.025–0.026 (TSE–TC) and f4-ratio = 0.003 (TSE–TV).

3.4 | Niche overlap and geographical range structure

Based on niche simulations, distinct patterns were observed with overlapping climatic niches between TSE and native turtle species from different parts of the world. Results showed an overlap (with respect to the native species) from 100% with *Mauremys leprosa* (i.e., the climatic niche of the native species is completely within

	N	n_o	A_r	H_O	H_E	H_{NEI}	F_{IS}
TSE	39	13.40	9.71	0.580	0.861	0.884	0.327
TSE_{alien}	128	20.87	10.89	0.792	0.890	0.893	0.107
T. venusta	64	16.27	8.92	0.620	0.788	0.794	0.215
T. cataspila	13	8.02	7.10	0.626	0.737	0.767	0.151
T. taylori	12	3.33	3.15	0.255	0.406	0.424	0.331

Note: TSE and TSE_{alien}: native and non-native *Trachemys scripta elegans*, respectively. Number of individual samples (N), observed (n_o) number of alleles, allelic richness (A_r), observed (H_O) and expected (H_E) heterozygosity, Nei's unbiased expected heterozygosity (H_{NEI}), and fixation index (F_{IS}).

TABLE 1 Genetic diversity for *Trachemys* species analysed in the study (five genetic clusters identified; see STRUCTURE results), based on 15 microsatellite loci

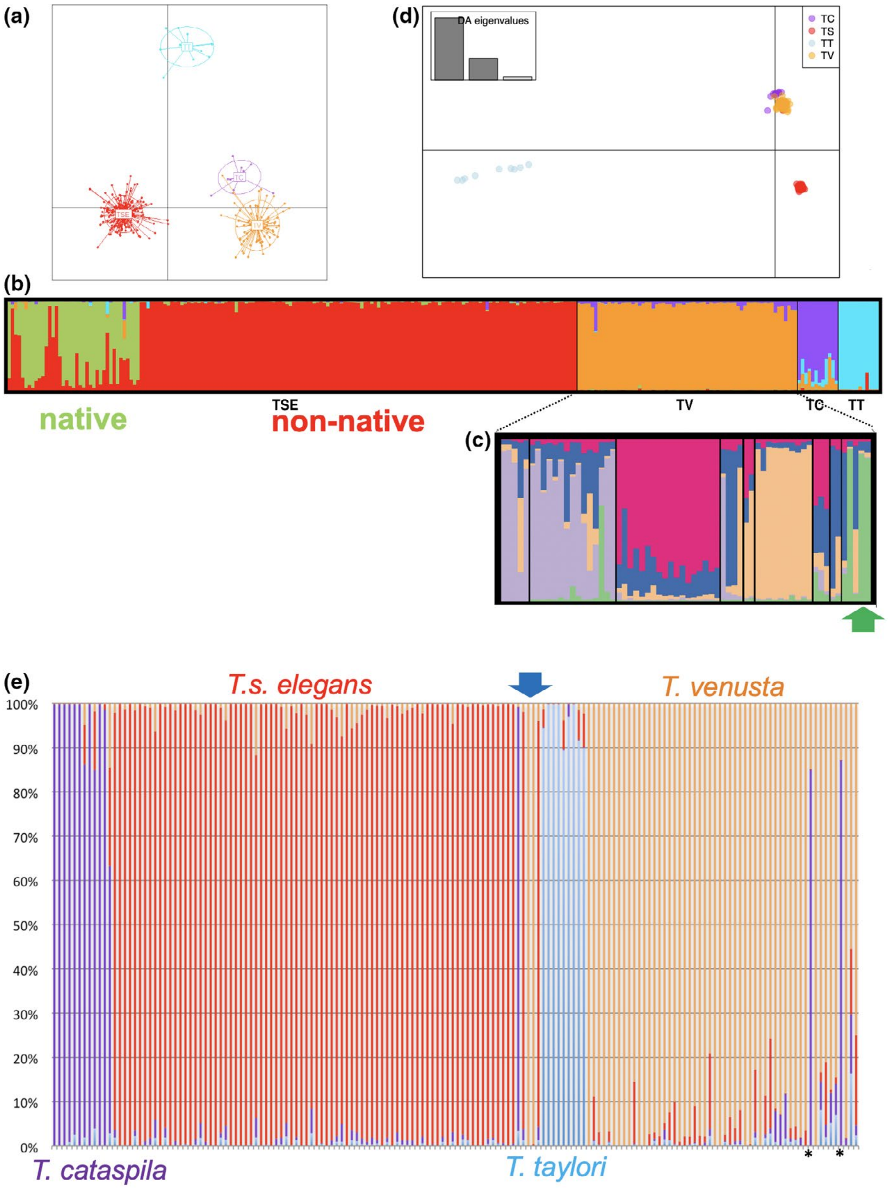

FIGURE 2 Genetic differentiation based on (a) DAPC analysis where the four *Trachemys* species were separated as independent genetic groups, and (b) STRUCTURE ($K = 5$), where the native (TSE) and non-native individuals ($\text{TSE}_{\text{alien}}$), and also (c) *Trachemys venusta*, were separated into two and five different clusters, respectively (based on 15 microsatellite loci); green arrow indicates individuals from Cozumel Island. (d) DAPC and (e) ancestry proportions results based on individual ancestry coefficients (sNMF) for the four *Trachemys* species (based on 63,385 SNPs). Blue arrow indicates the five individuals not identified in the field (TSX01 = TC; TSX03, TSX04 = TV; TSX02, TSX05 = TSE) and the asterisks depict highly admixed individuals (*T. venusta* TV56 and TV64 with *T. cataspila* TC). Colour figure available in the online version

TABLE 2 Genetic differentiation between *Trachemys* species analysed in the study (five genetic clusters identified; see STRUCTURE results), measured as F_{ST} (above diagonal) and Nei's genetic distance (below diagonal), based on 15 microsatellite loci

	TSE	$\text{TSE}_{\text{alien}}$	*T. venusta*	*T. cataspila*	*T. taylori*
TSE	—	0.030	0.050	0.070	0.180
$\text{TSE}_{\text{alien}}$	0.547	—	0.057	0.076	0.167
T. venusta	0.626	0.853	—	0.055	0.163
T. cataspila	0.692	0.871	0.395	—	0.184
T. taylori	1.012	0.887	0.739	0.783	—

Note: TSE and $\text{TSE}_{\text{alien}}$: native and non-native *Trachemys scripta elegans*, respectively.

the climatic niche of the non-native species) to 45% with *Mauremys reevesii*, which has a niche larger than TSE (Figure S7). In geographical space, niche suitability (understood as maps depicting the proximity to the niches' centres) showed larger suitability areas for the non-native species, except in *Mauremys reevesii*, where it was noticeably higher for the native species ($p < 2.2\text{e-}16$) (Figure S7[5a–5b]). When we examined the effect of species interactions, a significant change was observed both in their relative location and in the size of their HDRs. With the exception of *Actinemys marmorata*, the change generally included a greater distance (D^*) and a smaller area but conserved a marked overlap between the invasive and the native species (Figure S8; Table S6).

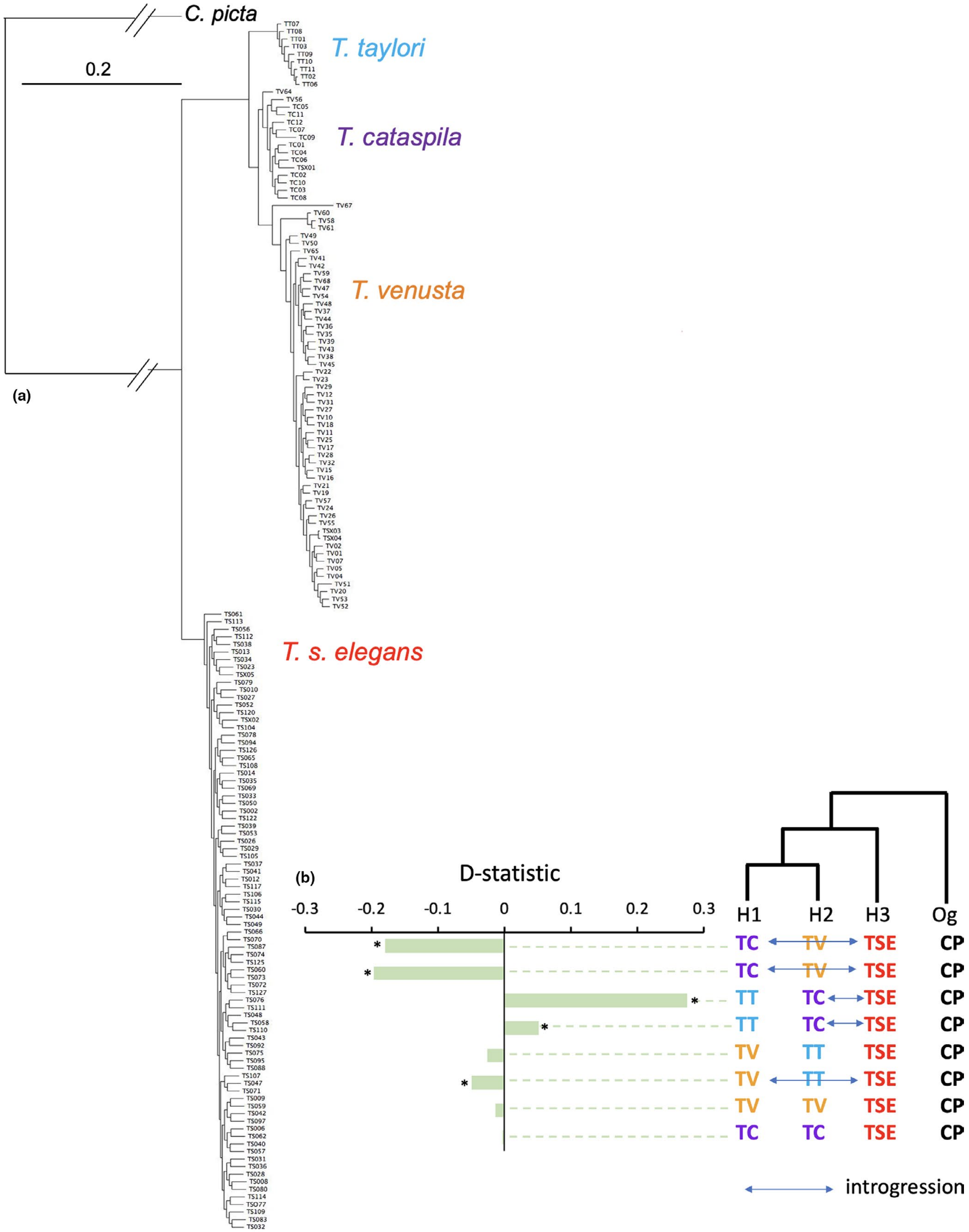

FIGURE 3 (a) Approximately-maximum-likelihood phylogenetic tree (FASTTREE) based on 63,385 SNPs of *Trachemys* species (*T. scripta elegans*, *T. cataspila*, *T. venusta*, *T. taylori*); *Chrysemys picta* was used as the outgroup. (b) Diagram of the ABBA-BABA results and the direction of introgression between species, where a negative value of the D-statistic indicates excess gene flow from H3 to H1, and a positive value from H3 to H2. **Z* score >3. Colour figure available in the online version

Results for *Trachemys* species from Mexico were different. The niche overlap (with respect to the native congeneric species) was 100% for *T. taylori*, 66% for *T. cataspila* and 23% for *T. venusta* (Figure 4a). In contrast to the results obtained regarding native turtles worldwide, a larger suitable area was observed for native congeners in Mexico ($p < 2.2e\text{-}16$), coinciding with the natural distributional area occupied by each species (Figure 4b). Additionally, a significant change was observed when bivariate distributions were compared, with a greater distance and a smaller area, but this time the overlap zones decreased considerably (Figure 5).

4 | DISCUSSION

Understanding how niche differences affect biological invasions and their genetic consequences is crucial for conserving native biodiversity. We present the first assessment of the genetic and ecological niche patterns of *Trachemys scripta elegans*, a widely known invasive species, and three of its native congeneric *Trachemys* species. As predicted, our findings showed historical and contemporary divergence between native and non-native TSE and reduced genetic diversity and high differentiation in the microendemic *T. taylori*. Notably, contrary to our expectation, they did not show shared suitability of their ecological niches (neither high introgression nor admixture).

4.1 | Complex genetic patterns and disjunct population structure

Our first question aimed to evaluate the evolutionary relationships among native and non-native TSE and with lineages of its *Trachemys* congeners. Fritz et al. (2012) and Parham et al. (2013) had each assessed three of the four species we studied; the phylogenetic relationships we evaluated for these four *Trachemys* showed that TSE is a distinct lineage from the other species; notably, native (TSE) and non-native ($\text{TSE}_{\text{alien}}$) include both exclusive and shared cyt *b* and R35 haplotypes, indicative of their historical common ancestry while also showing divergence between them. This is further supported by the more contemporary differentiation (microsatellites), where the native and non-native individuals are clearly separated as two distinct genetic clusters.

Our divergence results agree with the historical biogeography described by Fritz et al. (2012), which showed that *Trachemys* dispersed from North America to Central America (>10 Ma). The two species groups diverged within Central America around 8 Ma, generating *T. scripta* (>5.5 Ma), *T. venusta* and *T. cataspila*, the last two diverging much later (2–1 Ma). We identified two lineages within the TSE clade, one with native and no-native haplotypes and the other with exclusively non-native ones, again highlighting the genetic differentiation of the exotic lineage, which exhibits the highest diversification (different haplotypes) mostly spanning contemporary times.

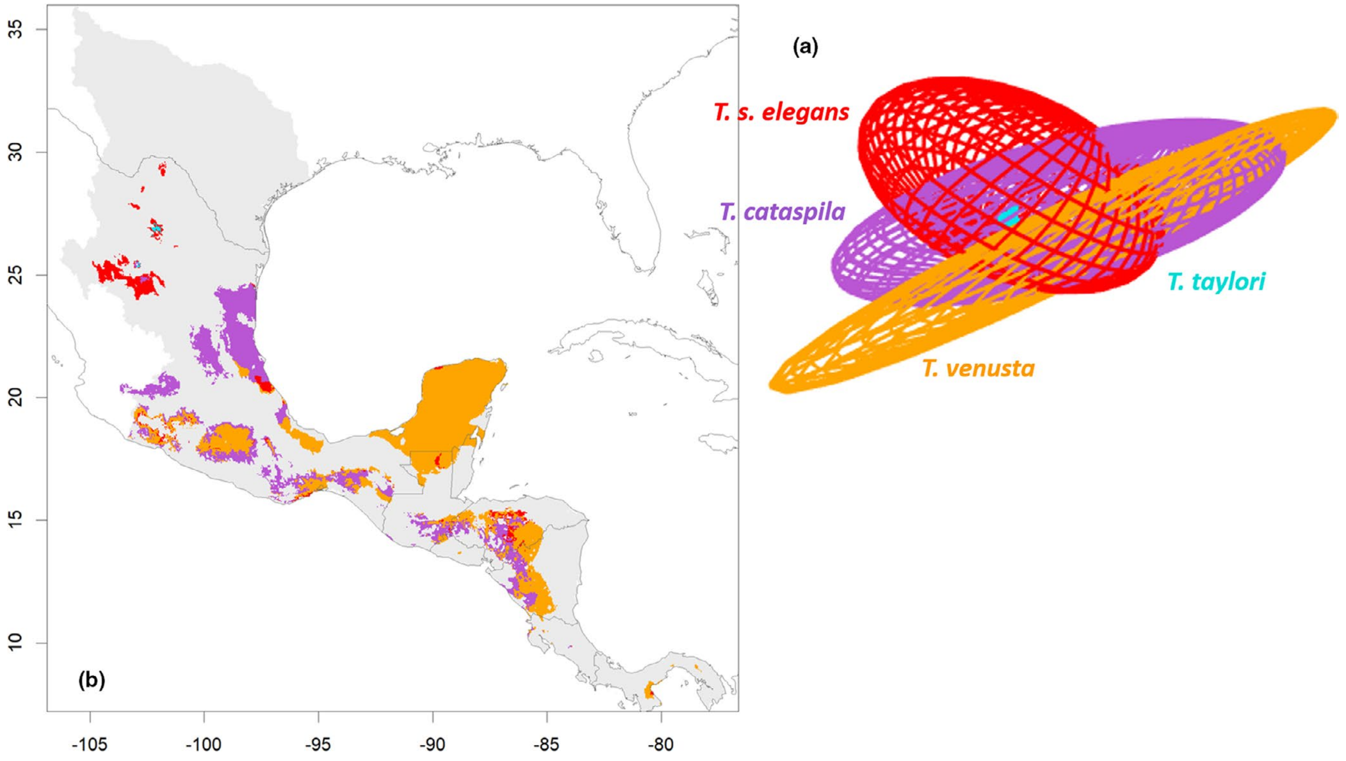

FIGURE 4 Niche overlap between the four *Trachemys* species. (a) Graphic representation of ecological niche overlap, based on ellipsoid models, depicting *T. scripta elegans* (red) and each native species from Mexico (*T. taylori*, blue; *T. cataspila*, purple; *T. venusta*, orange). (b) Map showing the sites along the distribution of each native species that are closer to the niche-centre of *T. scripta elegans*. If the site has a greater suitability (understood as the proximity to the niche-centre) for *T. scripta elegans* it is coloured red, otherwise, it is coloured according to that of each native species (as in a). Grey colour depicts areas where no overlap occurs. Colour figure available in the online version

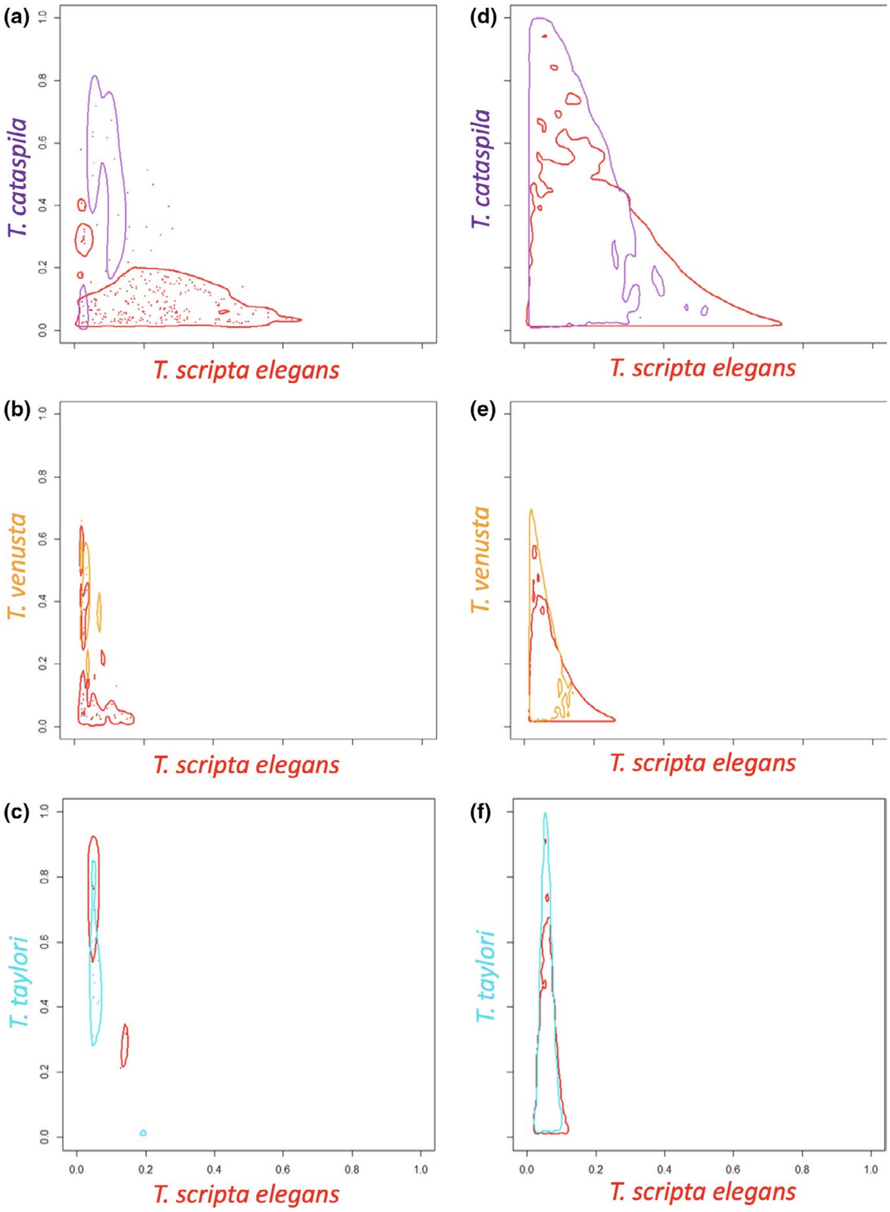

FIGURE 5 Suitability maps for *Trachemys scripta elegans* (in red) and its congeners *T. cataspila* (purple), *T. venusta* (orange) and *T. taylori* (blue). The panel on the right (d–f) shows what the highest density regions (HDRs) would be like for densities f and f_k if species are distributed independently of each other. This is obtained by Monte Carlo simulations, generating a large number of independent samples and applying kernel density estimates. By contrast, the left panel (a–c) is the suitability map based on observed data over $A \cap B_k$. Colour figure available in the online version

Moreover, the relationships among TSE haplotypes are in agreement with this pattern of more recent, unique non-native haplotypes.

We next asked if the genetic diversity and structure patterns within and among species would reflect their range size/endemism gradient from microendemic (*T. taylori*) to the widespread invasive $\text{TSE}_{\text{alien}}$ and predicted that *T. taylori* will show reduced genetic diversity and high differentiation. Indeed, clear genetic structure of *T. taylori* is exhibited by DAPC using both microsatellite loci and SNPs which depict it as a unique genetic cluster. In fact, the four *Trachemys* species were separated as independent genetic groups. *Trachemys taylori*, microendemic to the desert-spring ecosystem of the Cuatrociénegas Basin (CCB), Coahuila, Mexico, is only found in permanent and seasonal wetlands across the CCB valley (Seidel, 2002a). The CCB wetland has shrunk 90% in the last 60 years, mainly as a result of water extraction and channelling through a complex system of artificial courses and canals, significantly reducing the natural distribution of many species, including other endemic turtles (*Terrapene coahuila*, *Apalone atra*; Cortés-Rodríguez et al., 2021). Very little is known about the biology and ecology of *T. taylori*, but there is no doubt that they have rather low population sizes, probably due to their endemism but also to the contemporary habitat loss and fragmentation across the CCB, as recently described for *T. coahuila* (Cortés-Rodríguez et al., 2021). Accordingly, *T. taylori* are highly diverged from other *Trachemys* species and they present the lowest genetic diversity.

In contrast, but not unexpectedly, the highest genetic diversity was shown by the $\text{TSE}_{\text{alien}}$ individuals, jointly with a signal of population size expansion. This may be associated with diverse factors, namely that they originated historically from distinct geographical locations and/or from multiple sources or lineages, and probably also from multiple introductions (Kolbe et al., 2007; Suárez-Atilano et al., 2019).

4.2 | Distribution limits mediated by native congeners

We aimed to determine the invasion scenario of TSE along its distribution gradient in Mexico in relation to its congeners. We expected that they would show high genetic admixture and introgression, which would also be evidenced by markedly shared suitability of their ecological niches. Counter to our expectations, we found lower than anticipated naturally occurring distribution overlap between $\text{TSE}_{\text{alien}}$ and its congeners along the Gulf of Mexico coastal region, a pattern supported by different results. During our extensive fieldwork, we did not find $\text{TSE}_{\text{alien}}$ individuals at the naturally distributed areas of its congeneric species. Also, we show that the four species are clearly genetically differentiated, while non-native TSE individuals comprise a unique genetic cluster. In addition, we identified little admixture between $\text{TSE}_{\text{alien}}$ and any of its congeners. Parham et al. (2020) found evidence of admixture between the subspecies *T. scripta elegans* and *T. scripta*, and between the former and *T. gaigeae*, which in addition share morphological features. We found two *T. venusta* individuals having high admixture with *T. cataspila*; these are also recognized as subspecies, *T. venusta* and *T. venusta cataspila* (Fritz et al., 2012; Parham et al., 2013), probably due to their potential to hybridize, a process that would need to be further evaluated but is beyond the scope of our study. Interestingly, *T. venusta* is genetically differentiated as five clusters (with the microsatellite data) that are roughly associated with their geographical distribution, from northern Veracruz to southern Tabasco and a population from the Caribbean island Cozumel as another cluster (some of the Cozumel individuals were also differentiated by SNPs; Figure 2; Figure S6a).

Our findings show regions of reduced gene flow precisely across neighbouring species, that is between northern TSE and *T. cataspila*, and between the latter and *T. venusta*, in agreement with their natural gradient distribution southwards along the Gulf of Mexico and the Yucatan peninsula in Mexico (Figure 1), exhibiting clear genetic separation despite having zones of contact. Gene flow is probably ancient rather than recent, given the geographical and genetic structuring among them, with introgression occurring in the past. Indeed, we found that the most likely past introgression was between *T. cataspila* and TSE, while *T. cataspila* and *T. venusta*, which are sister species and neighbours geographically (contact zone in southern Tamaulipas), exhibit significant low migration between them.

4.3 | Suitability, environmental centrality and establishment risk

Comparative studies between native and invasive vertebrates that explicitly include biological interactions or functional features are quite limited (Espindola et al., 2019; Polo-Cavia et al., 2014). Thus, contrasting niche suitability could be a useful tool to include, indirectly, the interaction that can occur when a species is introduced to habitats occupied by other species, based on the environmental centrality hypothesis, where fitness is expected to be highest in those sites with environments closest to the centre of the fundamental niche (Martínez-Meyer et al., 2013; Osorio-Olvera et al., 2019). To further assess the observed distribution limits and low admixture patterns mediated by TSE's congeners, we used our novel distribution modelling approach to determine if the congeneric *Trachemys* species exhibited distinct climatic niche suitability, probably preventing establishment of and displacement by the non-native TSE. Our results suggest strongly that this is the case; we demonstrate that the niche overlap between non-native TSE and four out of the six worldwide native species we analysed is >90%. Specifically, the climatic niche of *Mauremys leprosa* and *Emys orbicularis* (native to Europe), *Actinemys marmorata* (western coast of the USA and Mexico), and *Chelodina longicollis* (Australia) are completely (or mostly) within the climatic niche of the non-native species (TSE). Thus, the available climatic niches that TSE has encountered in those regions probably facilitated its successful establishment. The latter, jointly with specific biological and life history traits, potentially enabled it to displace the native species (Cadi & Joly, 2004; Meyer et al., 2015). In fact, Lambert et al. (2019) provide evidence that TSE can compete with native *Actinemys marmorata* in the wild. What we found for *Trachemys* species from Mexico is markedly different. Niche overlap was 66% with *T. cataspila* and 23% with *T. venusta*, which follows the natural geographical climatic cline along their distribution. Surprisingly, niche overlap with *T. taylori* was 100%, despite the rather unique ecosystem of the Cuatrociénegas wetlands it inhabits. This is a crucial result, because it shows that non-native TSE, at least climatically, could easily adapt to the CCB habitat, and become a threat to the micro-endemic *T. taylori*. During our fieldwork we did not find TSE individuals, but they have been nonetheless documented (McGaugh, 2012).

Because TSE has invaded and displaced native turtle species worldwide, we predicted that sites that are closer to its niche-centre would have higher establishment risk than those that are closer to the niche-centre of the native species. Our findings fully support our prediction; niche suitability, understood as the proximity to the centre of the niche, was greater for the non-native species in all our comparisons with the exception of *Mauremys reevesii* (native to Asia), which also showed the lowest niche overlap. This turtle is known for its ability to hybridize with other Geoemydidae and is also one of the most commonly raised species on China's modern-day turtle farms (Jo et al., 2017; da Nóbrega Alves et al., 2008). By contrast, statistically significantly greater suitability was observed for the three native *Trachemys*, coinciding with the natural distribution area occupied by each species, again corroborating the much lower establishment risk of TSE regarding the congeners we evaluated.

4.4 | Future directions

Our findings show that diverse historical, biogeographical and ecological processes have defined the current natural and invasive distribution of TSE. In this process, its ecological niche has played a key role, showing that in non-native areas it will successfully establish and outcompete a potential competitor if local environmental conditions are closer to its ecological optimum (niche centre) than to the ecological optima of the native species. Moreover, the historical and contemporary patterns of genetic diversity and structure of these closely related *Trachemys* turtles are key to understanding the distribution limits of TSE. Our genetic evaluation was enhanced by the novel distribution modelling approach applied, contrasting niche suitability based on niche-centre distances between congeners/native and non-native species to explore distribution patterns of their shared suitability space. To complement our findings regarding the niche centrality hypothesis, future comparisons between relative niche-centre distances and genetic features (genetic diversity and genetic differentiation within and between species) could potentially improve predictions about sites with higher establishment risk. First to corroborate whether populations closer to the niche centre have higher abundance and are more genetically diverse (Cruz-Nicolás et al., 2020), and second to elucidate the relationship between genetic distances and niche separation between closely related species (Tromas et al., 2018). Finally, our results set the basis for future work, where using whole genome or gene-targeted sequencing and with the inclusion of a higher number of field-sampled individuals would allow directly assessment of hybridization and specific gene introgression and connection between loci and traits.

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CONFLICT OF INTEREST

The authors declare no commercial or financial conflicts of interest.

AUTHOR CONTRIBUTIONS

S.E., E.V.D. and E.M.M. conceptualized and designed different parts of the research. S.E. and E.V.D. performed fieldwork and laboratory work. S.E., E.V.D., E.A.M., I.O. and B.N.R. conducted genetic and genomic data analyses. S.E., M.N., L.O.O. and E.M.M. performed species niche modelling analyses. S.E., E.V.D., M.N., L.O.O. and F.T.B. wrote the paper, and all authors reviewed the manuscript and agreed on the submission.

BENEFIT-SHARING STATEMENT

Benefits generated: a research collaboration was developed with scientists from different backgrouds and institutions; also, the research addresses a priority concern, in this case invasive species and conservation of endemics.

DATA AVAILABILITY STATEMENT

The data used in this study are available as: Sequences are deposited in GenBank (accession nos.: cytochrome *b* OL869975–OL870030; R35 OL870031–OL870071). The microsatellite genotypes and the assembled GBS data (vcf file) are available in Dryad at doi: https://doi.org/10.5061/dryad.gf1vhhmrh. The *Trachemys* genomic data are part of an ongoing study, and we are performing further analyses and a corresponding scientific manuscript is in preparation, and therefore the corresponding author should be contacted regarding the raw fastqs files. Sampling localities, genetic protocols and other data are available in the Supporting Information.

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SUPPORTING INFORMATION

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ANEXOS

a) Appendices S1-S3

Appendix S1. Extended description of materials and methods used in the study

Molecular genetics protocols

DNA extraction

We extracted total DNA from fresh tissue with the DNeasy Blood and Tissue Extraction Kit (QIAGEN), following the manufacturer's protocols. For tissue museum samples, many of which were preserved in formaldehyde and later stored in ethanol, DNA was isolated using a modification of the QIAamp DNA FFPE Tissue Kit (QIAGEN). Before DNA extraction, samples were gradually dehydrated with ethanol, followed by a rehydration in an alkali buffer and incubation at high temperature, to eliminate formaldehyde and recover DNA fragments (Fang et al., 2002; Campos & Gilbert, 2012). DNA extractions were quantified with the Qubit fluorometer and quality was assessed with 1% agarose gels stained with GelRed (Biotium) and visualized with UV light.

Microsatellites loci

Microsatellite loci were characterized with 15 fluorescently labeled primers developed for TSE (Xin et al., 2012; Simison et al., 2013). List of primers used and PCR amplification protocol are available in Table S2 and Appendix S1. TSE02-TSE80 primers were amplified as in Xin et al. (2012), in a 5 µl final volume. For Tsc108-330 primers developed by Simison et al. (2013), we designed a multiplex PCR protocol containing □25ng template DNA, 0.5 units of Taq DNA polymerase, 3mM $MgCl_2$, 0.2 mM dNTPs, 0.45 µM of each primer and 10x reaction PCR buffer (200 mM Tris-HCl pH=8.4, 500 mM KCl). Primer combinations were as follows: 1) Tsc108 & Tsc243; 2) Tsc260 & Tsc328; and 3) Tsc263, Tsc330 & Tsc299. For all primer pairs, cycling conditions were: initial 5 min denaturation at 95°C; 30 cycles consisting of 94°C for 30 seg, primer-specific annealing temperature for 30 seg and 72°C for 40 seg, and 10 min final extension at 72°C. Annealing temperatures (AT) are in Table S2.

Microsatellites products were run on an ABI Prism3730xl and 3100 Genetic Analyzer (Applied Biosystems), with ROX-500 as internal size standard. Allele size was determined with GeneMarker 1.97 (SoftGenetics) and rounded to integers using TANDEM 1.8 (Matschiner & Salzburger, 2009). We included negative controls in all runs and genotyped two or more randomly chosen samples to ensure correct readings and data consistency. Potential null alleles and scoring errors were assessed with MicroChecker 2.2.3 (Van Oosterhout et al., 2004), using 1,000 Monte Carlo simulations.

Mitochondrial and nuclear sequences

Cytochrome *b* (Fritz et al. 2011). The cyt *b* gene was amplified in a 25 µl final volume containing □25ng template DNA, 0.5 units of Taq DNA polymerase, 2.5mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer and 10x reaction PCR buffer (200 mM Tris-HCl pH=8.4, 500 mM KCl). PCR conditions were as follows: initial 5 min denaturation at 94°C; 30 cycles consisting of 94°C for 30 seg, 58°C for 30 seg and 72°C for 1 min, and 10 min final extension at 72°C.

Genomic intron 1 of the RNA fingerprint protein 35 gene (Fujita et al. 2004). The R35 gene was amplified in a final volume of 25 µl containing □25ng template DNA, 0.5 units of Taq DNA polymerase, 3mM MgCl2, 0.2mM dNTPs, 0.5µM of each primer and 10x reaction PCR buffer (200 mM Tris-HCl pH=8.4, 500 mM KCl). PCR conditions were as follows: initial 5 min denaturation at 94°C; 35 cycles consisting of 94°C for 30 seg, 60°C for 1 min 30 seg and 72°C for 2 min, and 10 min final extension at 72°C. Mitochondrial and nuclear PCR products were sent to Macrogen-Korea for both forward and reverse sequencing.

Genotyping by Sequencing (GBS) and SNPs calling with *ipyrad*

A subset of 178 samples was sent to the Genomic Diversity Facility (GDF) at Cornell University for Genotyping by Sequencing library construction (GBS; Elshire et al., 2011) and sequencing services. Libraries were prepared using the EcoT221 enzyme for genome complexity reduction, with recognition sequence 5'-ATGCA/T-3' and 3'-T/ACGTA-5'. The resulting fragments were tagged with individual barcodes, samples were pooled and PCR-amplified, multiplexed, purified and sequenced on a single lane on an Illumina Hi-Seq 2500 (Illumina Inc, USA) with single-end 100 bp reads. Considering that the parameters used in de novo locus identification and genotyping may affect downstream analyses and resulting inference, we examined a range of values of parameters to optimize assembly in the ipyrad pipeline (Eaton & Overcast, 2020); the final processing and filtering protocol used is:

Demultiplex
Assembly method: *denovo*
Restriction overhang (cut1): TGCA
Max low quality base calls (Q<20) in a read: 5
phred Q score offset: 33
Min depth for statistical base calling: 8
Min depth for majority-rule base calling: 8
Max cluster depth within samples: 10000
Clustering threshold for de novo assembly: 0.85

Max number of allowable mismatches in barcodes: 0
Filter for adapters/primers: 1
Min length of reads after adapter trim: 35
Max alleles per site in consensus sequences: 2
Max N's (uncalled bases) in consensus: 5
Max Hs (heterozygotes) in consensus: 8
Min # samples per locus for output: 87
Max # SNPs per locus: 0.2
Max # of indels per locus: 5
Max # heterozygous sites per locus: 0.5

Mitochondrial and nuclear phylogenetic analyses

For the phylogenetic analysis among the four *Trachemys* species, in addition to the sequences we amplified, we incorporated from GenBank five R35 and eight cyt *b* sequences in order to have a more complete dataset for TSE along its native distribution, plus four cyt *b* sequences for *T. scripta scripta*, one *T. venusta* and one *T. cataspila* (Table S3); we also included sequences for *Chrysemys picta*, *Malaclemys terrapin* and *Trachemys decussata* as outgroups, the former for both genes (cyt *b* and R35) while the other two were available only for cyt *b* (GenBank; Table S3). Sequence datasets were assembled and reviewed by eye with Bioedit 7.0.5.2 (Hall, 1999), while Geneious v.9.1.8 was used for alignment, blasting and edition.

We chose the best nucleotide substitution model for both cyt *b* and R35 datasets based on the corrected Akaike Information Criterion (AIC) in PHYML 3.0 (Guindon et al., 2010), using the default options and optimized NNI search.

Diversity, differentiation, and demographic analyses (microsatellites loci)

We tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between all pairs of loci using GENEPOP v.6 (Rousset, 2008) per species, with an exact probability test (Markov chain parameters: 10,000 dememorizations, 100 batches, 10,000 iterations per batch), and sequential Bonferroni corrections. In cases of deviation from HWE, we also tested whether this was attributed to heterozygosity deficit or excess. We estimated the following genetic diversity indices: observed number of alleles (n_o), observed (H_O) and expected (H_E) heterozygosity, and Nei's unbiased expected heterozygosity (H_{NEi}; Nei, 1978), using GENALEX v.6 (Peakall & Smouse, 2012). Allelic richness (A_r), a rarefied measure that considers the different sampling numbers based on a standard sample size of the smallest sample with complete genotypes at all loci, was estimated with *adegenet* in R (Jombart, 2008).

We inferred population structure between and within species with two methods: a model-free multivariate method, Discriminant Analysis of Principal Components (DAPC) with *adegenet*; we also used STRUCTURE v.2.3.4 (Pritchard et al., 2000), a Bayesian approach to infer the most likely number of genetic clusters (*K*). Based on the complete database (four *Trachemys* species), we tested for *K*=1-8 genetically distinct clusters with 20 replicates for each *K*, with 500,000 iterations and a burn-in of 100,000, including *a priori* locality data, and an admixture model with correlated allele frequencies. We further examined structure within some of the identified clusters at the species level, performing new runs using the same parameters described. For all tests, the optimal *K* was calculated according to Evanno et al. (2005) with the STRUCTUREHarvester online web server (Earl & Von Holdt, 2012), while Distruct 1.1 (Rosenberg, 2004) was used to graphically display the genetic structure results.

Phylogenetic relationships with SNPs

We obtained an unrooted species tree topology with a coalescent method using SVDquartets (Chifman & Kubatko, 2014), a program designed for SNP data that computes a score based on singular value decomposition of a matrix of site pattern frequencies, corresponding to a split on a phylogenetic tree. These quartet scores are used to select the best-supported topology for quartets of taxa, which in turn can be used to infer the species phylogeny using quartet methods. We ran the program with the final dataset using 160 individuals and with the default parameters.

To obtain a phylogenetic tree incorporating an outgroup, we built a SNP-based outgroup from the Painted turtle *Chrysemys picta* genome (Badenhorst et al. 2015; (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF_000241765.3_Chrysemys_picta_bellii-3.0.3_genomic.fna.gz) with Samtools 1.x (Li 2011). We next used FastTree (Price et al., 2009) to infer an approximately-maximum-likelihood phylogenetic tree, using the GTR model and the gamma option to rescale the branch lengths.

Individual ancestry, migration, and potential introgression (SNPs)

The historical migration patterns were evaluated with the method of Estimated Effective Migration Surfaces (EEMS; Petkova et al., 2016). Migration patterns are adjusted by the similarity between the observed genetic differences and those expected under a stepping-stone model; the obtained estimations are interpolated across a uniformly spaced grid (the landscape) to create the migration surfaces. Genetic differentiation is represented as a function of migration rates, correlating genetic variation with geography; portions of a species range where population divergence deviates from patterns expected under isolation-by-distance are highlighted. If genetic similarity decreases rapidly for the observed, in comparison with expected values, indicates a low effective migration (i.e. a barrier to gene flow; Petkova et al., 2016). We built a pairwise dissimilarity matrix and a geographic distance matrix for all individuals to perform paired comparisons (TSE-TC; TC-TV; TSE-TV). With these matrices we ran EEMS with runeems_snps, using a deme size of 1200, with three independent starting chains for 5x10^6 MCMC iterations, 5x10^4 of burn-in, thinning of 5000 and different starting seeds. The three runs were combined and visualized with reemsplots2 in R (https://github.com/dipetkov/reemsplots2).

To test for introgression among species we used the ABBA/BABA test (also called D statistics), which is a powerful test for a deviation from a strict bifurcating evolutionary history, namely testing for an excess of shared derived variants between non-sister taxa (Durand et al., 2011; Zheng & Janke, 2018). In brief, consider three ingroup populations (or species) and an outgroup with the relationship (((P1,P2),P3),O), where P1 and P2 are more related to one another than either to P3; given a haploid genome sequence for each population (i.e. H1, H2 and H3), ABBA sites are those at which H2 and H3 share a derived allele 'B', whereas H1 has the ancestral state 'A', as defined by the outgroup sample. Similarly, BABA represents sites at which H1 and H3 share the derived state. We built three hypotheses [(TC,TV),TSE; (TT,TC),TSE; (TV,TT),TSE)] plus a null model, using two randomly chosen individuals per species (to test each hypothesis twice); *Chrysemys picta* was used as outgroup. To run ABBA/BABA we used *evobiR* in R (http://www.uta.edu/karyodb/evobiR/) with the CalcD function and a jackknife (1000) significance test.

Appendix S2. Glossary of mathematical symbols used in niche analysis

A	Niche ellipsoid for TSE
μ, Σ	Mean vector and covariance matrix for TSE niche
B_k	Niche ellipsoid for alternative species k
μ_k, Σ_k	Mean vector and covariance matrix for alternative species k
$M(p, \mu, \Sigma)$	Generic Mahalanobis distance between a point p and the mean of multivariate normal distribution with mean μ and covariance matrix Σ
S	Generic suitability index defined by $S = e^{-M/2}$, where M is a Mahalanobis distance
S_{1i}	Suitability of TSE at the i-th presence point of TSE in $A \cap B_k$
S_{2i}	Suitability of species k at the i-th presence point of TSE in $A \cap B_k$
S_{1j}	Suitability of TSE at the j-th presence point of species k in $A \cap B_k$
S_{2j}	Suitability of species k at the j-th presence point of species k in $A \cap B_k$
$f(u,v)$	Bivariate density function of (S_{1i}, S_{2i}) at (u,v)
$f_k(u,v)$	Bivariate density function of (S_{1i}, S_{2i}) at (u,v)
D	(Quadratic) Distance between densities $f(u,v)$ and $f_k(u,v)$
$\hat{f}(u,v)$	Kernel density estimate of $f(u,v)$
$\hat{f}_k(u,v)$	Kernel density estimate of $f_k(u,v)$
$\widehat{D}$	(Quadratic) Distance between densities $\hat{f}(u,v)$ and $\hat{f}_k(u,v)$
H_1	Area of highest density region (HDR) corresponding to $f(u, v)$
H_2	Area of highest density region (HDR) corresponding to $f_k(u,v)$
$\widehat{H}_1$	Area of highest density region (HDR) corresponding to $\hat{f}(u, v)$
$\widehat{H}_2$	Area of highest density region (HDR) corresponding to $\hat{f}_k(u,v)$

Appendix S3. Kernel density estimation and highest density regions

Suppose $(X_1, Y_1), \ldots, (X_n, Y_n)$ are sample points of a bivariate random vector, assumed to originate from a two-dimensional density function given by $f(x,y)$. Kernel Density estimation (Scott, 1992; Simonoff, 1996) is a method for inferring the density function, which may be unknown. It is based on the notion of a kernel function, K, that is nonnegative and integrates to 1. The *kernel estimate* of f is defined as

$$\hat{f}(x,y) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{h^2}K\left(\frac{X_i - x}{h}\right)K\left(\frac{Y_i - y}{h}\right),$$

where h is a positive constant called the *bandwidth* that controls the degree of smoothing of the ensuing estimate. A common choice for K is the standard normal density function. Because we are interested in studying bivariate densities restricted to the $(0,1) \times (0,1)$ unit square (what we called the suitability space in the text), a special provision called edge correction must be taken into account. Function bivariate.density in R package sparr implements kernel density estimation with edge correction to confine estimated density functions to the unit square. In addition, a data-driven bandwidth selection algorithm, that is, a value of h based on data values and an optimality criterion is also provided in that package (Davies et al. 2018).

Next consider an arbitrary bivariate density function, $g(x,y)$. The *highest density region* (HDR) is a useful device for summarizing and describing g. It is a subset of the plane that represents location of points of high density spanning a specified probability and is particularly useful for dealing with uncharacteristic and multimodal densities. The $100 \times (1-\alpha)\%$ HDR (Hyndman, 1996) is the subset $R(c) = \{(x,y) | g(x,y) \geq c\}$, where c is the largest constant such that

$$\iint_{R(c)} f(x,y)\, dx\, dy \geq 1 - \alpha.$$

If $g(x,y,z)$ is a three-dimensional density, the HDR is likewise defined to be the subset $R(c) = \{(x,y,z) | g(x,y,z) \geq c\}$, where c is the largest constant such that

$$\iiint_{R(c)} f(x,y,z)\, dx\, dy\, dz \geq 1 - \alpha.$$

In any case, the HDR is a small set that encompasses high probability. The HDR is a neighborhood of the mode (or modes) of the density having a specific area in two dimensions, or a volume in three dimensions. R library ks provides convenient functions for plotting boundaries of HDRs of given α in two dimensions, as opposed to plotting multiple contour levels of a kernel density estimate, which is a different graphical concept. Since any kernel density estimate is a legitimate density function, then the HDR notion can be directly applied to the estimate as well, as was done in our analysis.

b) Tables S1-S5

Table S1. List of *Trachemys* samples used in the study, from fieldwork individuals and from museum specimens, including all distribution and field data.

(a) Samples of *Trachemys scripta elegans*, *T. venusta, T. catraspilla* and *T. taylori* obtained from the field: traded samples from unknown origin obtained from FES-Iztacala. Sample ID, date, sampling locality, sex, weight, measures of carapace length/width/height and plastron length/width are indicated. Samples amplified with microsatellites loci (SSR), mitochondrial cytochrome b (cyt b) and nuclear genomic intron 1 of the RNA fingerprint protein 35 (R35) genes, and single nucleotide polymorphisms (SNP) are highlighted.

	Species	Sample ID	Date	Locality	Sex	Carapace			Plastron		weight (g)	Amplified genes			
						length (cm)	width (cm)	height (cm)	length (cm)	width (cm)		SSR	cyt *b*	R35	SNP
1	*Trachemys scripta elegans*	001-TSE	31/01/2014	CDMX	F	-	-	-	-	-	-	X	X		
2	*Trachemys scripta elegans*	002-TSE	31/01/2014	CDMX	F	-	-	-	-	-	-	X	X	X	X
3	*Trachemys scripta elegans*	003-TSE	31/01/2014	CDMX	F	-	-	-	-	-	-	X	X		
4	*Trachemys scripta elegans*	004-TSE	31/01/2014	CDMX	Y	-	-	-	-	-	-	X	X		
5	*Trachemys scripta elegans*	005-TSE	31/01/2014	CDMX	F	-	-	-	-	-	-	X			X
6	*Trachemys scripta elegans*	006-TSE	31/01/2014	CDMX	J	-	-	-	-	-	-	X	X	X	X
7	*Trachemys scripta elegans*	007-TSE	22/03/2014	Boca del Río, Ver	M	15	11.6	-	12.7	-	460	X	X	X	
8	*Trachemys scripta elegans*	008-TSE	23/03/2014	Boca del Río, Ver	F	21.2	15.7	-	18.6	-	1520	X			X
9	*Trachemys scripta elegans*	009-TSE	23/03/2014	Boca del Río, Ver	F	22.4	16.8	-	21.1	-	1930	X			X
10	*Trachemys scripta elegans*	010-TSE	23/03/2014	Boca del Río, Ver	M	16.5	12.4	-	14.5	-	630	X			X
11	*Trachemys scripta elegans*	011-TSE	23/03/2014	Boca del Río, Ver	M	12.6	9.7	-	11.2	-	300	X	X		
12	*Trachemys scripta elegans*	012-TSE	25/03/2014	Tlacotalpan, Ver	F	19.8	14.2	-	18.4	-	1230	X	X		X
13	*Trachemys scripta elegans*	013-TSE	26/03/2014	Tlacotalpan, Ver	F	23.5	17.1	-	19.8	-	1520	X	X		X
14	*Trachemys scripta elegans*	014-TSE	26/03/2014	Tlacotalpan, Ver	F	20.1	16.1	-	18.7	-	1110	X	X	X	X
15	*Trachemys scripta elegans*	015-TSE	26/03/2014	Tlacotalpan, Ver	M	14.2	11	-	12.1	-	320	X			
16	*Trachemys scripta elegans*	016-TSE	05/09/2014	Boca del Río, Ver	F	11.78	9.62	4.89	10.86	7.77	290	X			
17	*Trachemys scripta elegans*	017-TSE	05/09/2014	Boca del Río, Ver	F	11.67	8.41	4.95	10.94	7.27	290	X	X		
18	*Trachemys scripta elegans*	018-TSE	05/09/2014	Boca del Río, Ver	F	15.2	12	5.18	13.77	9.91	560	X			
19	*Trachemys scripta elegans*	019-TSE	05/09/2014	Boca del Río, Ver	F	13.66	10.94	5.91	12.93	8.65	400	X			
20	*Trachemys scripta elegans*	020-TSE	05/09/2014	Boca del Río, Ver	M	16.3	16.12	5.7	10.71	8.03	360	X			
21	*Trachemys scripta elegans*	021-TSE	05/09/2014	Boca del Río, Ver	M	12.81	10.05	4.66	11.55	8.3	300	X			
22	*Trachemys scripta elegans*	022-TSE	05/09/2014	Boca del Río, Ver	-	16.99	11.86	5.8	14.5	9.87	610	X			

23	*Trachemys scripta elegans*	023-TSE	05/09/2014	Boca del Río, Ver	J	9.34	8.51	4.09	8.43	6.5	166	X	X	X	X
24	*Trachemys scripta elegans*	024-TSE	09/09/2014	Tlacotalpan, Ver	M	17.68	12.82	7.75	15.65	9.74	690	X			
25	*Trachemys scripta elegans*	025-TSE	09/09/2014	Tlacotalpan, Ver	Y	5.13	4.9	2.33	4.71	3.98		X			
26	*Trachemys scripta elegans*	026-TSE	10/09/2014	Chacaltianguis, Ver	M	11.28	9.21	4.59	10.18	7.3	240	X	X	X	X
27	*Trachemys scripta elegans*	027-TSE	12/09/2014	Texistepec, Ver	M	17.23	12.12	6.86	14.38	9.7	630	X	X	X	X
28	*Trachemys scripta elegans*	028-TSE	12/09/2014	Acayucan, Ver	M	18.84	14.66	7.85	14.33	10.37	970	X	X	X	X
29	*Trachemys scripta elegans*	029-TSE	12/09/2014	Acayucan, Ver	F	12.35	9.03	5.15	10.07	7.54	270	X			X
30	*Trachemys scripta elegans*	030-TSE	12/09/2014	Acayucan, Ver	F	26.31	22.31	-	17.81	12.32	1520	X			X
31	*Trachemys scripta elegans*	031-TSE	12/09/2014	Acayucan, Ver	F	21.01	15.61	-	13.67	16.08	1260	X			X
32	*Trachemys scripta elegans*	032-TSE	12/09/2014	Acayucan, Ver	F	21.49	26.94	-	27.3	17	2000	X			X
33	*Trachemys scripta elegans*	033-TSE	12/09/2014	Acayucan, Ver	-	-	-	-	-	-	-	X			X
34	*Trachemys scripta elegans*	034-TSE	12/09/2014	Acayucan, Ver	-	-	-	-	-	-	-	X	X	X	X
35	*Trachemys scripta elegans*	035-TSE	12/09/2014	Acayucan, Ver	-	-	-	-	-	-	-	X			X
36	*Trachemys scripta elegans*	036-TSE	12/09/2014	Acayucan, Ver	-	-	-	-	-	-	-	X			X
37	*Trachemys scripta elegans*	037-TSE	14/09/2014	Acayucan, Ver	-	-	-	-	-	-	-	X			X
38	*Trachemys scripta elegans*	038-TSE	17/09/2014	Coatzacoalcos, Ver	F	15.3	11.2	7	14.3	9.13	570	X			X
39	*Trachemys scripta elegans*	039-TSE	17/09/2014	Coatzacoalcos, Ver	F	19.8	15.93	9.6	19.3	11.41	-	X	X	X	X
40	*Trachemys scripta elegans*	040-TSE	17/09/2014	Coatzacoalcos, Ver	-	13.6	11.21	5.5	12.81	8.9	450	X	X	X	X
41	*Trachemys scripta elegans*	041-TSE	17/09/2014	Coatzacoalcos, Ver	-	9.08	7.94	3.68	8.46	6.22	120	X			X
42	*Trachemys scripta elegans*	042-TSE	17/09/2014	Coatzacoalcos, Ver	-	15.3	12.21	7.23	13.44	9.24	520	X			X
43	*Trachemys scripta elegans*	043-TSE	17/09/2014	Coatzacoalcos, Ver	-	19.02	14.62		18.11	11.5	1210	X			X
44	*Trachemys scripta elegans*	044-TSE	17/09/2014	Coatzacoalcos, Ver	F	16.31	11.2	6.9	14.6	9.1	610	X			X
45	*Trachemys scripta elegans*	046-TSE	18/09/2014	Villahermosa, Tab	F	19.3	13.7	7.8	16	11.11	930	X			
46	*Trachemys scripta elegans*	047-TSE	18/09/2014	Villahermosa, Tab	F	20.6	14.9	9.3	12.4	12.6	1170	X			X
47	*Trachemys scripta elegans*	048-TSE	18/09/2014	Villahermosa, Tab	F	17.2	14.31	8.1	16.7	11.3	1040	X			X
48	*Trachemys scripta elegans*	049-TSE	18/09/2014	Villahermosa, Tab	F	16.7	12.9	7.6	15.3	10.5	660	X			X
49	*Trachemys scripta elegans*	050-TSE	18/09/2014	Villahermosa, Tab	F	17.33	12.7	7.4	13	10.12	790	X			X
50	*Trachemys scripta elegans*	051-TSE	18/09/2014	Villahermosa, Tab	M	14.9	12.2	6.9	13.6	9.4	620	X			
51	*Trachemys scripta elegans*	052-TSE	18/09/2014	Villahermosa, Tab	M	14.1	10.5	6.8	12.6	8.21	410	X			X
52	*Trachemys scripta elegans*	053-TSE	18/09/2014	Villahermosa, Tab	M	14.7	11.11	6.54	14.33	8.51	450	X			X
53	*Trachemys scripta elegans*	054-TSE	18/09/2014	Villahermosa, Tab	M	12.81	10.53	5.9	11.61	8	410	X			
54	*Trachemys scripta elegans*	055-TSE	18/09/2014	Villahermosa, Tab	M	15	10.82	7.1	13.9	8.81	510	X			
55	*Trachemys scripta elegans*	056-TSE	18/09/2014	Villahermosa, Tab	J	-	-	-	-	-	-	X	X	X	X
56	*Trachemys scripta elegans*	057-TSE	19/09/2014	Villahermosa, Tab	F	17.9	12.9	8.4	17.3	10.6	950	X			X
57	*Trachemys scripta elegans*	058-TSE	19/09/2014	Villahermosa, Tab	F	22.8	20.1		20.7	14.8	2120	X			X
58	*Trachemys scripta elegans*	059-TSE	19/09/2014	Villahermosa, Tab	M	28	23.3	7.5	23.4	16	2790	X			X

59	*Trachemys scripta elegans*	060-TSE	19/09/2014	Villahermosa, Tab	F	21.2	14.9	9.8	17.7	12.2	1520	X			X
60	*Trachemys scripta elegans*	061-TSE	19/09/2014	Villahermosa, Tab	J	13.8	10.9	6	12.6	8.1	360	X	X		X
61	*Trachemys scripta elegans*	062-TSE	19/09/2014	Villahermosa, Tab	J	15.5	12.4	7.1	14.3	9.8	640	X			X
62	*Trachemys scripta elegans*	063-TSE	19/09/2014	Villahermosa, Tab	M	14.6	11	6.2	12.4	8.5	440	X			
63	*Trachemys scripta elegans*	064-TSE	19/09/2014	Villahermosa, Tab	F	19	12.9	8.4	16.2	10.2	990	X			
64	*Trachemys scripta elegans*	065-TSE	19/09/2014	Villahermosa, Tab	M	13.2	11	6.3	12.6	8.9	450	X	X	X	X
65	*Trachemys scripta elegans*	066-TSE	06/09/2015	CDMX	-	-	-	-	-	-	-	X			X
66	*Trachemys scripta elegans*	067-TSE	06/09/2015	CDMX	-	-	-	-	-	-	-	X			
67	*Trachemys scripta elegans*	068-TSE	06/09/2015	CDMX	-	-	-	-	-	-	-	X			
68	*Trachemys scripta elegans*	069-TSE	10/09/2015	Xalapa, Ver	F	15	13	6	14	10.3	300	X			X
69	*Trachemys scripta elegans*	070-TSE	10/09/2015	Xalapa, Ver	F	18.7	14.6	6.4	18	12.3	900	X			X
70	*Trachemys scripta elegans*	071-TSE	10/09/2015	Xalapa, Ver	F	16.4	13.1	5.7	17	10.5	700	X	X	X	X
71	*Trachemys scripta elegans*	072-TSE	10/09/2015	Xalapa, Ver	F	14.5	10.9	5.1	13.4	8.4	500	X	X	X	X
72	*Trachemys scripta elegans*	073-TSE	10/09/2015	Xalapa, Ver	F	16.7	13.4	5.4	14.9	11.5	700	X			X
73	*Trachemys scripta elegans*	074-TSE	10/09/2015	Xalapa, Ver	M	14	11.1	5	12.9	9.6	500	X			X
74	*Trachemys scripta elegans*	075-TSE	10/09/2015	Xalapa, Ver	F	16.6	14.4	5.7	15.7	11.4	400	X			X
75	*Trachemys scripta elegans*	076-TSE	10/09/2015	Xalapa, Ver	M	10.8	9	4.4	10	7.3	200	X			X
76	*Trachemys scripta elegans*	077-TSE	10/09/2015	Xalapa, Ver	J	11.5	10	4	11	8.2	300	X			X
77	*Trachemys scripta elegans*	078-TSE	10/09/2015	Xalapa, Ver	M	15	11.6	5.3	13.8	9.5	600	X			X
78	*Trachemys scripta elegans*	079-TSE	10/09/2015	Xalapa, Ver	F	11	10.5	4.2	12.2	9.1	300	X			X
79	*Trachemys scripta elegans*	080-TSE	10/09/2015	Xalapa, Ver	M	13	17.2	6.5	16.5	10.1	800	X			X
80	*Trachemys scripta elegans*	081-TSE	26/08/2015	Cozumel, Q.Roo	M	12.63	9.84	5.69	11.63	7.89	200	X			
81	*Trachemys scripta elegans*	082-TSE	26/08/2015	Cozumel, Q.Roo	F	17	11	6	15.5	10	600	X			
82	*Trachemys scripta elegans*	083-TSE	26/08/2015	Cozumel, Q.Roo	M	9.91	7.86	3.84	8.81	6.21	70	X			X
83	*Trachemys scripta elegans*	084-TSE	26/08/2015	Cozumel, Q.Roo	J	12.45	9.75	4.6	12.14	7.76	100	X			
84	*Trachemys scripta elegans*	085-TSE	26/08/2015	Cozumel, Q.Roo	J	11.36	8.65	4.32	9.99	7.14	80	X			
85	*Trachemys scripta elegans*	086-TSE	26/08/2015	Cozumel, Q.Roo	F	21	15.5	7.7	28.3	12.3	1700	X			
86	*Trachemys scripta elegans*	087-TSE	26/08/2015	Cozumel, Q.Roo	M	15.3	13.14	6.17	14.37	10.11	400	X			X
87	*Trachemys scripta elegans*	088-TSE	26/08/2015	Cozumel, Q.Roo	M	13.37	9.85	5.29	11.43	6.89	120	X			X
88	*Trachemys scripta elegans*	089-TSE	26/08/2015	Cozumel, Q.Roo	M	14.23	10.86	5.29	12.82	8.56	300	X			
89	*Trachemys scripta elegans*	090-TSE	26/08/2015	Cozumel, Q.Roo	F	19	14	7.3	17	11.2	900	X			
90	*Trachemys scripta elegans*	091-TSE	26/08/2015	Cozumel, Q.Roo	F	19.7	14.5	7	17.2	12.13	800	X			
91	*Trachemys scripta elegans*	092-TSE	26/08/2015	Cozumel, Q.Roo	F	18.9	15	6.5	18	12.2	700	X	X		X
92	*Trachemys scripta elegans*	093-TSE	26/08/2015	Cozumel, Q.Roo	J	12.06	9.88	5	11.4	7.69	100	X			
93	*Trachemys scripta elegans*	094-TSE	26/08/2015	Cozumel, Q.Roo	J	8.89	7.8	3.98	7.96	5.89		X			X
94	*Trachemys scripta elegans*	095-TSE	26/08/2015	Cozumel, Q.Roo	M	11.54	9.56	4.914	10.27	6.83	200	X			X

95	*Trachemys scripta elegans*	096-TSE	26/08/2015	Cozumel, Q.Roo	M	16.5	12.5	5.6	15	10.8	700	X			
96	*Trachemys scripta elegans*	097-TSE	26/08/2015	Cozumel, Q.Roo	M	11.47	9.24	4.33	10.32	7.19	100	X	X		X
97	*Trachemys scripta elegans*	098-TSE	26/08/2015	Cozumel, Q.Roo	F	16.5	13.5	6.1	15.6	10.2	700	X			
98	*Trachemys scripta elegans*	099-TSE	26/08/2015	Cozumel, Q.Roo	F	18.5	14	6	16	10.8	900	X			
99	*Trachemys scripta elegans*	100-TSE	26/08/2015	Cozumel, Q.Roo	M	13.08	10.9	5.62	11.98	9.74	200	X			
100	*Trachemys scripta elegans*	101-TSE	26/08/2015	Cozumel, Q.Roo	M	10.71	7.73	4.21	9.05	6.1		X			
101	*Trachemys scripta elegans*	102-TSE	26/08/2015	Cozumel, Q.Roo	M	12.99	9.73	5.07	11.29	7.09	200	X			
102	*Trachemys scripta elegans*	103-TSE	26/08/2015	Cozumel, Q.Roo	F	20.3	13.6	7	18.6	12	1200	X			
103	*Trachemys scripta elegans*	104-TSE	26/08/2015	Cozumel, Q.Roo	F	23.5	18	7.5	21.5	13.8	1600	X			X
104	*Trachemys scripta elegans*	105-TSE	31/08/2015	Cozumel, Q.Roo	M	9	7.2	3.5	7.5	5.6	100	X			
105	*Trachemys scripta elegans*	106-TSE	31/08/2015	Cozumel, Q.Roo	M	13.6	10.5	5	12.5	9.3	400	X			X
106	*Trachemys scripta elegans*	107-TSE	31/08/2015	Cozumel, Q.Roo	F	12.5	10.5	4.2	11.9	7.8	30	X	X	X	X
107	*Trachemys scripta elegans*	108-TSE	31/08/2015	Cozumel, Q.Roo	M	12	8.8	4	9.8	7.1	200	X			X
108	*Trachemys scripta elegans*	109-TSE	31/08/2015	Cozumel, Q.Roo	M	13.6	9.3	4.5	12.2	7.8	390	X			X
1	*Trachemys scripta elegans*	110-TSE	01/03/1994	unknown origin	F	22	18	9.5	20	13.2	4200	X			X
2	*Trachemys scripta elegans*	111-TSE	02/02/1995	unknown origin	F	22.4	18	9	21.5	13.5	3400	X			X
3	*Trachemys scripta elegans*	112-TSE	03/02/1995	unknown origin	F	18.2	14	6.3	15.5	10.5	2400	X			X
4	*Trachemys scripta elegans*	113-TSE	24/02/1995	unknown origin	F	21	17	7.5	18.7	13.6	3600	X			X
5	*Trachemys scripta elegans*	114-TSE	24/02/1995	unknown origin	M	13.1	11.6	4.3	12.3	8.2	400	X			X
6	*Trachemys scripta elegans*	115-TSE	10/06/1996	unknown origin	M	13	10.8	4.6	12.2	8.4	800	X			X
7	*Trachemys scripta elegans*	116-TSE	26/08/1996	unknown origin	F	17	14	6.4	16.2	10.3	1800	X			
8	*Trachemys scripta elegans*	117-TSE	25/08/1998	unknown origin	F	19.5	15	6.5	18.6	11.8	2600	X			X
9	*Trachemys scripta elegans*	118-TSE	-	unknown origin	F	19.5	15	7	15.5	11.3	2800	X			
10	*Trachemys scripta elegans*	119-TSE	07/11/2000	unknown origin	F	21	17	8	20	12.8	3400	X			
11	*Trachemys scripta elegans*	120-TSE	04/06/2003	unknown origin	F	15.6	12.6	5	14.4	9.5	1200	X			X
12	*Trachemys scripta elegans*	121-TSE	06/06/2003	unknown origin	-	-	-	-	-	-	-	X			
13	*Trachemys scripta elegans*	122-TSE	10/08/1995	unknown origin	F	19.3	14.5	7	15.8	10.8	2000	X			X
14	*Trachemys scripta elegans*	123-TSE	15/12/1993	unknown origin	M	17.1	14	6.2	15	9.4	1400	X			
15	*Trachemys scripta elegans*	124-TSE	27/02/1996	unknown origin	F	21	15.8	8.8	19	12.5	3000	X			
16	*Trachemys scripta elegans*	125-TSE	28/06/1996	unknown origin	F	19.2	15.2	8	17.7	11.7	2400	X			X
17	*Trachemys scripta elegans*	126-TSE	20/03/2003	unknown origin	F	16.1	12.8	6.5	15.2	9.8	1600	X			X
18	*Trachemys scripta elegans*	127-TSE	20/03/2003	unknown origin	F	16	12.2	6.5	15.6	10	1400	X	X		X
1	*Trachemys venusta*	001-TV	22/03/2014	Boca del Río, Ver	F	-	-	-	-	-	-	X			X
2	*Trachemys venusta*	002-TV	22/03/2014	Boca del Río, Ver	F	-	-	-	-	-	-	X			X
3	*Trachemys venusta*	003-TV	22/03/2014	Boca del Río, Ver	M	-	-	-	-	-	-	X			
4	*Trachemys venusta*	004-TV	22/03/2014	Boca del Río, Ver	F	-	-	-	-	-	-	X		X	X

5	*Trachemys venusta*	005-TV	22/03/2014	Boca del Río, Ver	F	15.7	12.2	-	14	-	590	X			X
6	*Trachemys venusta*	006-TV	23/03/2014	Boca del Río, Ver	F	11.5	9	-	10.8	-	230	X			
7	*Trachemys venusta*	007-TV	23/03/2014	Boca del Río, Ver	M	9.8	8.1	-	8.8	-	150	X			X
8	*Trachemys venusta*	008-TV	23/03/2014	Boca del Río, Ver	F	10.3	7.9	-	8.6	-	150	X			
9	*Trachemys venusta*	009-TV	23/03/2014	Boca del Río, Ver	F	10.4	8.3	-	9	-	150	X			
10	*Trachemys venusta*	010-TV	25/03/2014	Tlacotalpan, Ver	F	18.4	13.6	-	16.9	-	750	X			X
11	*Trachemys venusta*	011-TV	25/03/2014	Tlacotalpan, Ver	F	19.1	13.7	-	17.9	-	930	X			X
12	*Trachemys venusta*	012-TV	26/03/2014	Tlacotalpan, Ver	F	18.3	9.5	-	15	-	610	X		X	X
13	*Trachemys venusta*	013-TV	26/03/2014	Tlacotalpan, Ver	F	15.5	11.2	-	14	-	520	X		X	
14	*Trachemys venusta*	014-TV	26/03/2014	Tlacotalpan, Ver	M	14.7	10.3	-	12.6	-	390	X			
15	*Trachemys venusta*	015-TV	26/03/2014	Tlacotalpan, Ver	M	14.9	10.9	-	13.2	-	430	X			X
16	*Trachemys venusta*	016-TV	26/03/2014	Tlacotalpan, Ver	F	15.8	11.4	-	12.9	-	430	X			X
17	*Trachemys venusta*	017-TV	26/03/2014	Tlacotalpan, Ver	F	19	15.5	-	19.8	-	1350	X			X
18	*Trachemys venusta*	018-TV	26/03/2014	Tlacotalpan, Ver	F	23.5	17.5	-	21.8	-	1740	X			X
19	*Trachemys venusta*	019-TV	26/03/2014	Tlacotalpan, Ver	F	20.6	14.7	-	18.6	-	1080	X			X
20	*Trachemys venusta*	020-TV	27/03/2014	Catemaco, Ver	F	27.9	20.8	-	24.8	-	3000	X	X		X
21	*Trachemys venusta*	021-TV	27/03/2014	Catemaco, Ver	F	29.8	20.3	-	24.5	-	3210	X			X
22	*Trachemys venusta*	022-TV	27/03/2014	Catemaco, Ver	J	31.6	21.6	-	27.3	-	2890	X			X
23	*Trachemys venusta*	023-TV	27/03/2014	Catemaco, Ver	M	14.3	11.2	-	13.4	-	420	X			X
24	*Trachemys venusta*	024-TV	05/09/2014	Boca del Río, Ver	F	16.86	11.56	6.7	14.36	9.74	560	X	X	X	X
25	*Trachemys venusta*	025-TV	05/09/2014	Boca del Río, Ver	J	8.68	6.96	3.35	7.36	5.7	70	X			X
26	*Trachemys venusta*	026-TV	05/09/2014	Boca del Río, Ver	J	9.02	7.99	4.01	9.26	6.32	160	X			X
27	*Trachemys venusta*	027-TV	07/09/2014	Tlacotalpan, Ver	F	19.9	13.03	7.86	17	10.87	790	X			X
28	*Trachemys venusta*	028-TV	07/09/2014	Tlacotalpan, Ver	F	19.18	12.69	7.69	17.44	10.51	790	X			X
29	*Trachemys venusta*	029-TV	07/09/2014	Tlacotalpan, Ver	M	19.24	11.79	7.04	15.77	10.11	630	X			X
30	*Trachemys venusta*	030-TV	08/09/2014	Tlacotalpan, Ver	F	22.57	14.35	9.43	20.63	12.06	1300	X			
31	*Trachemys venusta*	031-TV	08/09/2014	Tlacotalpan, Ver	F	20.79	13.48	8.73	18.48	11.08	1040	X			X
32	*Trachemys venusta*	032-TV	08/09/2014	Tlacotalpan, Ver	F	14.51	10.18	6.11	13.26	8.07	410	X	X	X	X
33	*Trachemys venusta*	033-TV	08/09/2014	Tlacotalpan, Ver	M	140.37	9.94	6.13	12.61	8.35	370	X			
34	*Trachemys venusta*	034-TV	08/09/2014	Tlacotalpan, Ver	F	13.58	9.58	5.5	12.17	77.73	330	X			
35	*Trachemys venusta*	035-TV	12/09/2014	Texistepec, Ver	M	12.16	8.8	4.95	10.32	7.03	250	X			X
36	*Trachemys venusta*	036-TV	12/09/2014	Texistepec, Ver	-	-	-	-	-	-	-	X	X		X
37	*Trachemys venusta*	037-TV	12/09/2014	Texistepec, Ver	-	-	-	-	-	-	-	X			X
38	*Trachemys venusta*	038-TV	12/09/2014	Texistepec, Ver	-	-	-	-	-	-	-	X			X
39	*Trachemys venusta*	039-TV	12/09/2014	Texistepec, Ver	-	-	-	-	-	-	-	X			X
40	*Trachemys venusta*	040-TV	12/09/2014	Texistepec, Ver	-	-	-	-	-	-	-	X			

41	*Trachemys venusta*	041-TV	13/09/2014	Acayucan, Ver	J	-	-	-	-	-	-	X	X		X
42	*Trachemys venusta*	042-TV	13/09/2014	Acayucan, Ver	F	25.09	17.84	9.07	21.6	13.64	1810	X			X
43	*Trachemys venusta*	043-TV	13/09/2014	Texistepec, Ver	M	15.44	11	6.03	13.65	9	380	X			X
44	*Trachemys venusta*	044-TV	13/09/2014	Texistepec, Ver	M	15.5	10.74	5.76	12.83	8.32	310	X	X		X
45	*Trachemys venusta*	045-TV	13/09/2014	Texistepec, Ver	F	14.7	10.91	5.68	11.81	8.76	310	X			X
46	*Trachemys venusta*	045b-TV	17/09/2014	Coatzacoalcos, Ver	M	16	11.41	6.9	14.29	9.27	610	X			
47	*Trachemys venusta*	046-TV	13/09/2014	Texistepec, Ver	F	15.84	11.5	6.92	12.12	9.59	540	X			
48	*Trachemys venusta*	047-TV	17/09/2014	Coatzacoalcos, Ver	F	11.41	9.43	4.8	10.03	7.22	230	X			X
49	*Trachemys venusta*	048-TV	17/09/2014	Coatzacoalcos, Ver	J	10.53	8.1	4.3	9.4	6.2	160	X	X		X
50	*Trachemys venusta*	049-TV	18/09/2014	Villahermosa, Tab	J							X			X
51	*Trachemys venusta*	050-TV	19/09/2014	Villahermosa, Tab	F	23.8	20.5	8.2	21	15.4	1860				X
52	*Trachemys venusta*	051-TV	10/09/2015	Xalapa, Ver	M	26	18.8	11.5	26	15.6	3600	X	X	X	X
53	*Trachemys venusta*	052-TV	10/09/2015	Xalapa, Ver	M	16.1	11.9	5.5	14.5	9.8	500	X			X
54	*Trachemys venusta*	053-TV	10/09/2015	Xalapa, Ver	M	19	13.8	6.6	17.1	10.4	1100	X			X
55	*Trachemys venusta*	054-TV	10/09/2015	Xalapa, Ver	F	25	19	8.6	22.4	15	2500	X	X		X
56	*Trachemys venusta*	055-TV	10/09/2015	Xalapa, Ver	M	18	13.5	5.5	15.2	11	800	X			X
57	*Trachemys venusta*	056-TV	10/09/2015	Xalapa, Ver	F	26.5	19.5	9	24	16.3	3000	X		X	X
58	*Trachemys venusta*	057-TV	26/08/2015	Cozumel, Q.Roo	F	21.5	16.5	7.7	19.8	12.6	1300	X			X
59	*Trachemys venusta*	058-TV	26/08/2015	Cozumel, Q.Roo	M	18	13.5	5.5	15.2	10.2	700	X	X	X	X
60	*Trachemys venusta*	059-TV	26/08/2015	Cozumel, Q.Roo	F	21	13	7.3	18.6	11.6	700	X	X	X	X
61	*Trachemys venusta*	060-TV	31/08/2015	Cozumel, Q.Roo	M	17	12.5	10.9	16.5	6.2	800	X			X
62	*Trachemys venusta*	061-TV	31/08/2015	Cozumel, Q.Roo	M	15.2	10	4.9	12.4	8.6	400	X			X
19	*Trachemys venusta*	064-TV	04/10/1994	unknown origin	-	-	-	-	-	-	-				X
20	*Trachemys venusta*	065-TV	01/06/2003	unknown origin	-	-	-	-	-	-	-				X
21	*Trachemys venusta*	067-TV	25/01/1998	unknown origin	F	25	18	10	22.4	14	4200	X			X
22	*Trachemys venusta*	068-TV	-	unknown origin	F	24	19.8	9.9	22.6	17.1	2550				X
1	*Trachemys cataspila*	001-TC	30/10/2014	Tampico, Tam	F	37.5	27	14	31	19	8000	X		X	X
2	*Trachemys cataspila*	002-TC	30/10/2014	Tampico, Tam	J	7.2	7.2	2.5	5.8	4.6		X		X	X
3	*Trachemys cataspila*	003-TC	30/10/2014	Tampico, Tam	M	34	23	10	25	15	6000	X			X
4	*Trachemys cataspila*	004-TC	30/10/2014	Tampico, Tam	F	25.3	17.5	9	20.5	13	4000	X	X		X
5	*Trachemys cataspila*	005-TC	30/10/2014	Tampico, Tam	F	36.5	25	15	30.5	17.5	9000	X	X		X
6	*Trachemys cataspila*	006-TC	31/10/2014	Tampico, Tam	F	34	31	12	27	17.8	5000	X	X	X	X
7	*Trachemys cataspila*	007-TC	31/10/2014	Tampico, Tam	F	34.5	31	17	27.5	17.5	5000	X	X		X
8	*Trachemys cataspila*	008-TC	31/10/2014	Tampico, Tam	M	36	31	16	26.5	16.7	6000	X	X	X	X
9	*Trachemys cataspila*	009-TC	31/10/2014	Tampico, Tam	M	39	36	18	31.5	20	8500	X	X	X	X
10	*Trachemys cataspila*	010-TC	31/10/2014	Tampico, Tam	M	31.5	26.5	11	23.5	15	3000	X			X

11	*Trachemys cataspila*	011-TC	31/10/2014	Tampico, Tam	F	36	32	16	29	20	5000	X	X		X
12	*Trachemys cataspila*	012-TC	31/10/2014	Tampico, Tam	J	8.8	8.8	3.5	7.2	5.8		X	X	X	X
1	*Trachemys taylori*	001-TT	26/05/2015	Cuatrociénegas, Coah	F	17.5	16	6.33	14.8	9.6	600	X	X	X	X
2	*Trachemys taylori*	002-TT	26/05/2015	Cuatrociénegas, Coah	M	16.9	15	7	14	9		X	X	X	X
3	*Trachemys taylori*	003-TT	28/05/2015	Cuatrociénegas, Coah	F	20.3	18	7.044	16.3	10.8	800	X	X		X
4	*Trachemys taylori*	004-TT	29/05/2015	Cuatrociénegas, Coah	J	11.62	8.51	5.076	9.82	6.67		X			
5	*Trachemys taylori*	005-TT	30/05/2015	Cuatrociénegas, Coah	M	19.5	16	6.084	16	10	600	X	X	X	
6	*Trachemys taylori*	006-TT	30/05/2015	Cuatrociénegas, Coah	J	16	14	5.952	13	10	500	X		X	X
7	*Trachemys taylori*	007-TT	09/06/2015	Cuatrociénegas, Coah	M	22.5	19.5	6.862	18.4	10	1100	X	X	X	X
8	*Trachemys taylori*	008-TT	15/06/2015	Cuatrociénegas, Coah	M	24.4	21	6.5	18.5	7.5	970	X		X	X
9	*Trachemys taylori*	009-TT	17/06/2015	Cuatrociénegas, Coah	J	13.5	11.4	4.315	11	5	240	X	X	X	X
10	*Trachemys taylori*	010-TT	17/06/2015	Cuatrociénegas, Coah	-	-	-	-	-	-		X	X	X	X
11	*Trachemys taylori*	011-TT	14/03/2016	Cuatrociénegas, Coah	-	-	-	-	-	-	870	X	X	X	X
12	*Trachemys taylori*	012-TT	29/05/2015	Cuatrociénegas, Coah	-	-	-	-	-	-		X			
1	unclassified on the field	TX01=TC	05/09/2014	Boca del Río, Ver	M	13.95	10.08	5.48	12.39	8.22	430	X			X
2	unclassified on the field	TX02=TSE	06/09/2014	Boca del Río, Ver	F	16.57	12.96	6.02	15.07	9.94	620	X	X		X
3	unclassified on the field	TX03=TV	06/09/2014	Boca del Río, Ver	F	20.78	14.87	7.3	19.79	12.05	1040	X			X
4	unclassified on the field	TX04=TV	17/09/2014	Coatzacoalcos, Ver	M	14.11	10.52	5.6	12.83	8.11	420	X			X
5	unclassified on the field	TX05=TSE	17/09/2014	Coatzacoalcos, Ver	M	13.62	11.1	5.3	12.8	8.3	420	X	X		X
23	*Trachemys* sp	TO01=TV	-	unknown origin	F	19	16.5	7.5	17.5	11.4	2400		X	X	

(b) Samples of *Trachemys scripta elegans* obtained from museum collections, with museum number. AMNH: American Museum of Natural History; FMNH: Field Museum of Natural History; SNMNH: Smithsonian National Museum of Natural History.

	Species	Sample ID	Date	Locality	Sampling locality	Museum	Catalog number	Amplified gene
								SSR
1	*Trachemys scripta elegans*	AM-01	11/05/1962	Lusiana, USA	Lusiana, USA	AMNH	157826	x
2	*Trachemys scripta elegans*	AM-03	26/11/1988	Hidalgo, Texas, USA	Hidalgo, Texas, USA	AMNH	172726	x
3	*Trachemys scripta elegans*	AM-04	27/02/1978	Kenedy, Texas, USA	Kenedy, Texas, USA	AMNH	172668	x
4	*Trachemys scripta elegans*	AM-06	20/09/1988	Nueces, Texas, USA	Nueces, Texas, USA	AMNH	172733	x
5	*Trachemys scripta elegans*	AM-08	13/09/1980	Gonzales, Texas, USA	Gonzales, Texas, USA	AMNH	172655	x
6	*Trachemys scripta elegans*	AM-09	13/10/1985	Tyler, Texas, USA	Tyler, Texas, USA	AMNH	172734	x
7	*Trachemys scripta elegans*	AM-10	18/07/1988	Suffolk, New York, USA	Suffolk, New York, USA	AMNH	134546	x
8	*Trachemys scripta elegans*	AM-11	14 Dec 1975	Shelby, Alabama, USA	Shelby, Alabama, USA	AMNH	114547	x
9	*Trachemys scripta elegans*	AM-12	07/06/1976	Jefferson, Alabama,USA	Jefferson, Alabama,USA	AMNH	117738	x
10	*Trachemys scripta elegans*	AM-13	18/07/1988	Suffolk, New York, USA	Suffolk, New York, USA	AMNH	134545	x
11	*Trachemys scripta elegans*	AM-14	11/07/1963	Tamaulipas, Mex	Tamaulipas, Mex	AMNH	157895	x
12	*Trachemys scripta elegans*	AM-16	03/06/1975	Terrebonne, Luisiana, USA	Terrebonne, Luisiana, USA	AMNH	157892	x
13	*Trachemys scripta elegans*	AM-17	11/07/1963	Tamaulipas, Mex	Tamaulipas, Mex	AMNH	157894	x
14	*Trachemys scripta elegans*	AM-18	29 Apr 1989	Holmes, Mississippi, USA	Holmes, Mississippi, USA	AMNH	157897	x
15	*Trachemys scripta elegans*	AM-19	17 Apr 1983	Terrebonne, Luisiana, USA	Terrebonne, Luisiana, USA	AMNH	157893	x
16	*Trachemys scripta elegans*	AM-20	18/07/1988	Suffolk, New York, USA	Suffolk, New York, USA	AMNH	134547	x
17	*Trachemys scripta elegans*	AM-24	Apr 1990	Nassau, New York, USA	Nassau, New York, USA	AMNH	136216	x
18	*Trachemys scripta elegans*	AM-25	Apr 1990	Nassau, New York, USA	Nassau, New York, USA	AMNH	136217	x
19	*Trachemys scripta elegans*	FM-01	06 Apr 1938	Coahuila, Mex	Coahuila, Mex	FMNH	28843	x
20	*Trachemys scripta elegans*	FM-02	06 Apr 1938	Coahuila, Mex	Coahuila, Mex	FMNH	28844	x
21	*Trachemys scripta elegans*	FM-09	26 Aug 1938	Coahuila, Mex	Coahuila, Mex	FMNH	55630	x
22	*Trachemys scripta elegans*	FM-11	26 Aug 1938	Coahuila, Mex	Coahuila, Mex	FMNH	55632	x
23	*Trachemys scripta elegans*	FM-18	26 Aug 1938	Coahuila, Mex	Coahuila, Mex	FMNH	55639	x
24	*Trachemys scripta elegans*	FM-21	26 Aug 1938	Coahuila, Mex	Coahuila, Mex	FMNH	55642	x
25	*Trachemys scripta elegans*	FM-22	26 Aug 1938	Coahuila, Mex	Coahuila, Mex	FMNH	55643	x
26	*Trachemys scripta elegans*	FM-24	14/07/1963	Mississippi, USA	Mississippi, USA	FMNH	194228	x

27	*Trachemys scripta elegans*	FM-25	30/03/1964	Mississippi, USA	Mississippi, USA	FMNH	194277	X
28	*Trachemys scripta elegans*	SI-01	24/03/1915	North Carolina, USA	North Carolina, USA	SNMNH	60892	X
29	*Trachemys scripta elegans*	SI-02	06/07/1915	North Carolina, USA	North Carolina, USA	SNMNH	60893	X
30	*Trachemys scripta elegans*	SI-03	12/07/1982	Florida, USA	Florida, USA	SNMNH	249559	X
31	*Trachemys scripta elegans*	SI-04	13/07/1982	Florida, USA	Florida, USA	SNMNH	249560	X
32	*Trachemys scripta elegans*	SI-05	14/07/1982	Florida, USA	Florida, USA	SNMNH	249561	X
33	*Trachemys scripta elegans*	SI-06	29/07/1982	Florida, USA	Florida, USA	SNMNH	249562	X
34	*Trachemys scripta elegans*	SI-07	13/07/1982	Florida, USA	Florida, USA	SNMNH	249646	X
35	*Trachemys scripta elegans*	SI-12	30/07/1982	Florida, USA	Florida, USA	SNMNH	249651	X
36	*Trachemys scripta elegans*	SI-15	10/06/1980	Virginia, USA	Virginia, USA	SNMNH	324272	X
37	*Trachemys scripta elegans*	SI-16	09/04/1987	Virginia, USA	Virginia, USA	SNMNH	518289	X
38	*Trachemys scripta elegans*	SI-17	09/04/1987	Virginia, USA	Virginia, USA	SNMNH	518290	X
39	*Trachemys scripta elegans*	SI-18	15/04/1987	Virginia, USA	Virginia, USA	SNMNH	518291	X

Table S2. List of microsatellites primers used for the genetic analyses with *Trachemys* species, with their corresponding sequence, annealing temperature applied and source.

Locus	Primer sequence (5'-3')	AT (°C)	Published source
TSE02	F: TCAGACGTGGCCTTCCTC R: AATCAAACGCTGCTCCCT	66	Xin et al. 2012
TSE06	F: ACCCTGACATCTGCCGACA R: GAGACCTTCCGCTGCTGC	68	Xin et al. 2012
TSE09	F: ACGGAGGACACTGCTTGA R: TTGCTTGGCTAAGGTGGA	64	Xin et al. 2012
TSE10	F: TTTCAAACACCCCTCCAG R: CACCTAGCACCATTTTCC	60	Xin et al. 2012
TSE14	F: CTGTCGGTGTCTTGTCCC R: TGAGCCCAGAAGTAGTGATG	64	Xin et al. 2012
TSE21	F: GGAACCGCAAGGAGGAAA R: GCCATGCAACTGAGCACC	66	Xin et al. 2012
TSE78	F: AAGGCAGCACAAATGGAG R: ACAGAATGTGGCAGGGAC	66	Xin et al. 2012
TSE80	F: AGACAGTTGCTTCCTTGA R: CATCCCCTTGCTTTTAGT	60	Xin et al. 2012
Tsc108	F:CGCAGTCAAAACACCTTCAG R:TTCACCTCCCCAGATCTCAC	55	Simison et al. 2013
Tsc243	F:GCAAAACCTGGAGATTTTCAA R:TTTCGATGGAAAATGGCTTT	55	Simison et al. 2013
Tsc260	F:TGCAAATGGAGTTGCAAGA R: TCCATTTGAACCTGGGAGAA	55	Simison et al. 2013
Tsc328	F:TGGATTGCATTATTAGAAATGGT R:CCCACCAACCACCATAATTC	55	Simison et al. 2013
Tsc263	F:TGTGCACGGGAGTTGTATG R:TTCTATTTGCCAAAAATTGCAT	55	Simison et al. 2013
Tsc330	F:TGGCTTATTTTGCAGCCTGA R:CCAACTTTCACTCCCATTGC	55	Simison et al. 2013
Tsc299	F:CCATGTGCCATCTGTCTACCT R:GATCAAGGGATGAGGGTCAA	55	Simison et al. 2013
cyt *b*	F: GATTTAAGCCGAGACCTGTG R: TCTTTGGTTTACAAGACCAATGC	58	Fritz et al. 2011
R35	F: ACGATTCTCGCTGATTCTTGC R: GCAGAAAACTGAATGTCTCAAAGG	60	Fujita et al. 2004

AT: annealing temperature

Table S3. List of GenBank accession numbers of mitochondrial cytochrome *b* (cyt *b*) and nuclear genomic intron 1 of the RNA fingerprint protein 35 (R35) sequences used for phylogenetic analyses.

Species	cyt *b*	R35
Chrysemys picta	HE590298	HE590495
Malaclemys terrapin	HE590304	
Trachemys venusta	HE590362	
Trachemys cataspila	HE590363	
Trachemys decussata	HE590331	
Trachemys scripta scripta	FJ770618	
	FJ770619	
	HE590356	
	HE590358	
Trachemys scripta elegans	U81351	EU787163
	FJ770617	FJ770705
	FJ770618	JN707490
	FJ770619	JN707528
	EU787024	HE590519
	HE590356	
	HE590358	
	KM216748	

Table S4. Results of the ABBA/BABA test (*D* statistics) (Green et al. 2010; Durand et al. 2011). Three hypotheses plus a null model were tested (H1-H3 and Null), using two randomly chosen individuals per species (to test each hypothesis twice) and *Chrysemys picta* as outgroup. TC: *Trachemys cataspila*; TV: *T. venusta*; TSE: *T. scripta elegans*; TT: *T. taylori*.

Test	**H1(A)**	**H2(B)**	**H3(B)**	***D***	***Z***	***D stat***	***p***
H1a	TC01	TV45	TSE9	-0.1792	20.359	0.0089	0
H1b	TC11	TV10	TSE66	-0.1963	22.757	0.0086	0
H2a	TT02	TC01	TSE9	0.2748	30.64	0.0089	0
H2b	TT07	TC11	TSE66	0.0510	5.335	0.0096	<0.001
H3a	TV45	TT02	TSE9	-0.0255	2.855	0.0090	0.0043
H3b	TV10	TT07	TSE66	-0.0497	5.727	0.0087	<0.001
Null1	TV45	TV10	TV58	-0.0132	2.241	0.0059	0.025
Null2	TC01	TC11	TC06	-0.0032	0.506	0.0063	0.612

Table S5. Niche overlap (%) and observed and HDRs between *Trachemys scripta elegans* and native species analyzed in the study. Arrows in *p*-values denote if observed values were significant (single-tail: upper (↑) and lower-tailed (↓)).

	Overlap (%)	$\hat{D}$	*p-value*	$\widehat{H_1}$	*p-value*	$\widehat{H_2}$	*p-value*
TSE — *T. venusta*	23.1	77.041	0 ↑	0.050	0.0048 ↓	0.045	0.0036 ↓
TSE — *T. cataspila*	66	15.676	0.0006 ↑	0.126	0 ↓	0.189	0.0378 ↓
TSE —*T. taylori*	100	20.809	0.139	0.152	0.091	0.080	0.0022 ↓
TSE — *A. marmorata*	95	0.888	0.0272 ↓	0.628	0 ↑	0.575	0 ↓
TSE — *M. reevesii*	45	9.870	0 ↑	0.292	0 ↓	0.243	0 ↓
TSE — *M. leprosa*	100	4.925	0 ↑	0.432	0 ↓	0.506	0 ↓
TSE — *E. orbicularis*	98.5	3.034	0 ↑	0.404	0 ↓	0.544	0 ↓
TSE — *C. longicollis*	89.6	1.594	0 ↑	0.516	0 ↓	0.728	0 ↑
TSE — *E. macquarii*	70.5	2.186	0 ↑	0.598	0 ↓	0.753	0.474

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c) Figures S1-S7

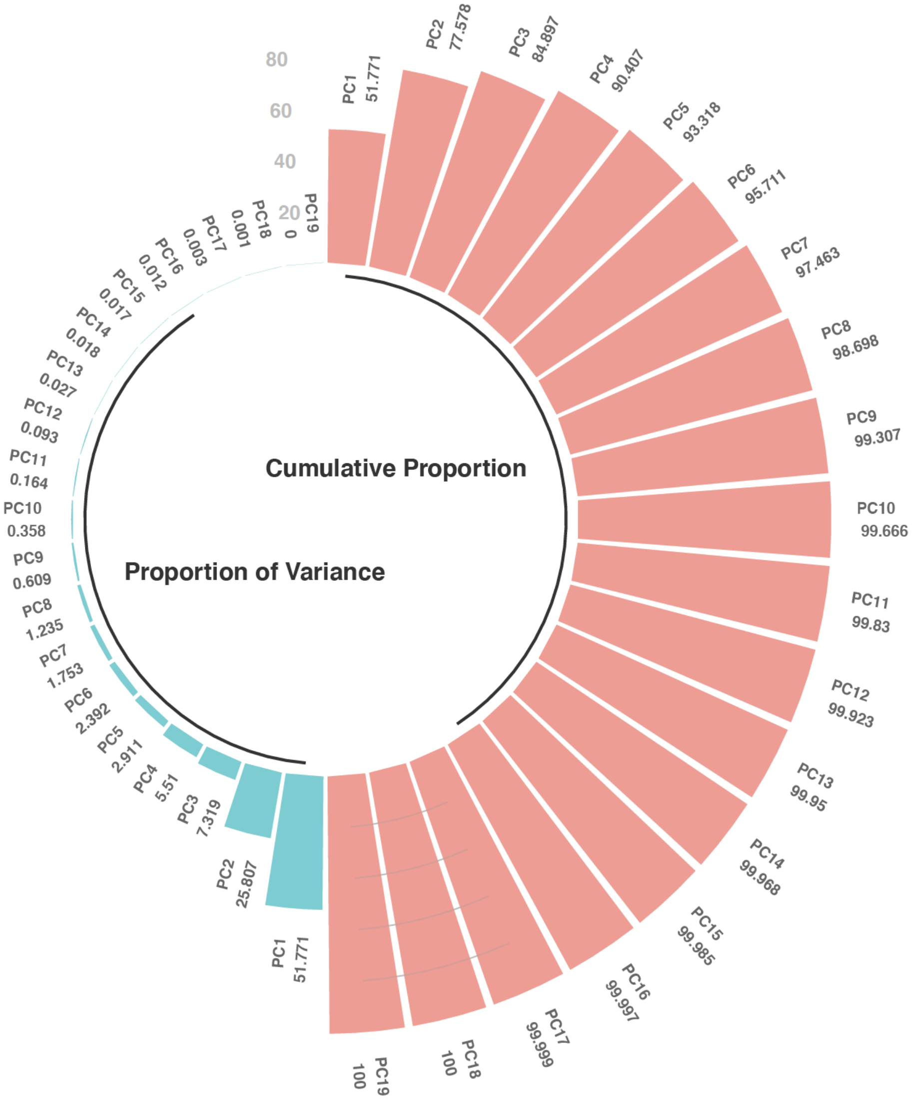


Figure S1. Percentage of variance explained by the principal components of the WorldClim 19 bioclimatic variables. Blue bars depict the proportion of variance accounted by each component to the total variance in all the variables. Red bars show the cumulative proportion of variance explained by the first 19 components.

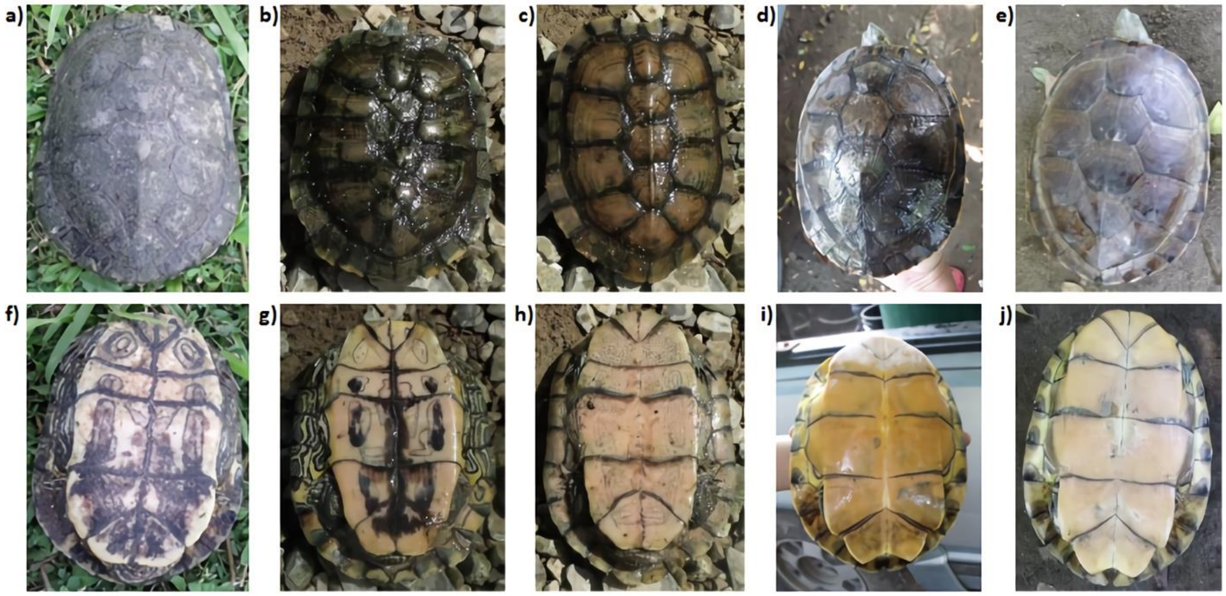


Figure S2. Photos of *Trachemys* individuals that could not be identified on the field given their mixed or unusual morphology (from left to right: TSX01, TSX04, TSX05, TSX02, TSX03; Table S1). Variation in color, patterns, and shapes in both dorsal and ventral parts of each turtle is shown. Genomic data allowed to completely differentiate between *T. scripta elegans* (shell: a-c; plastron: f-h) and *T. venusta* (shell: d-e; plastron: i-j).

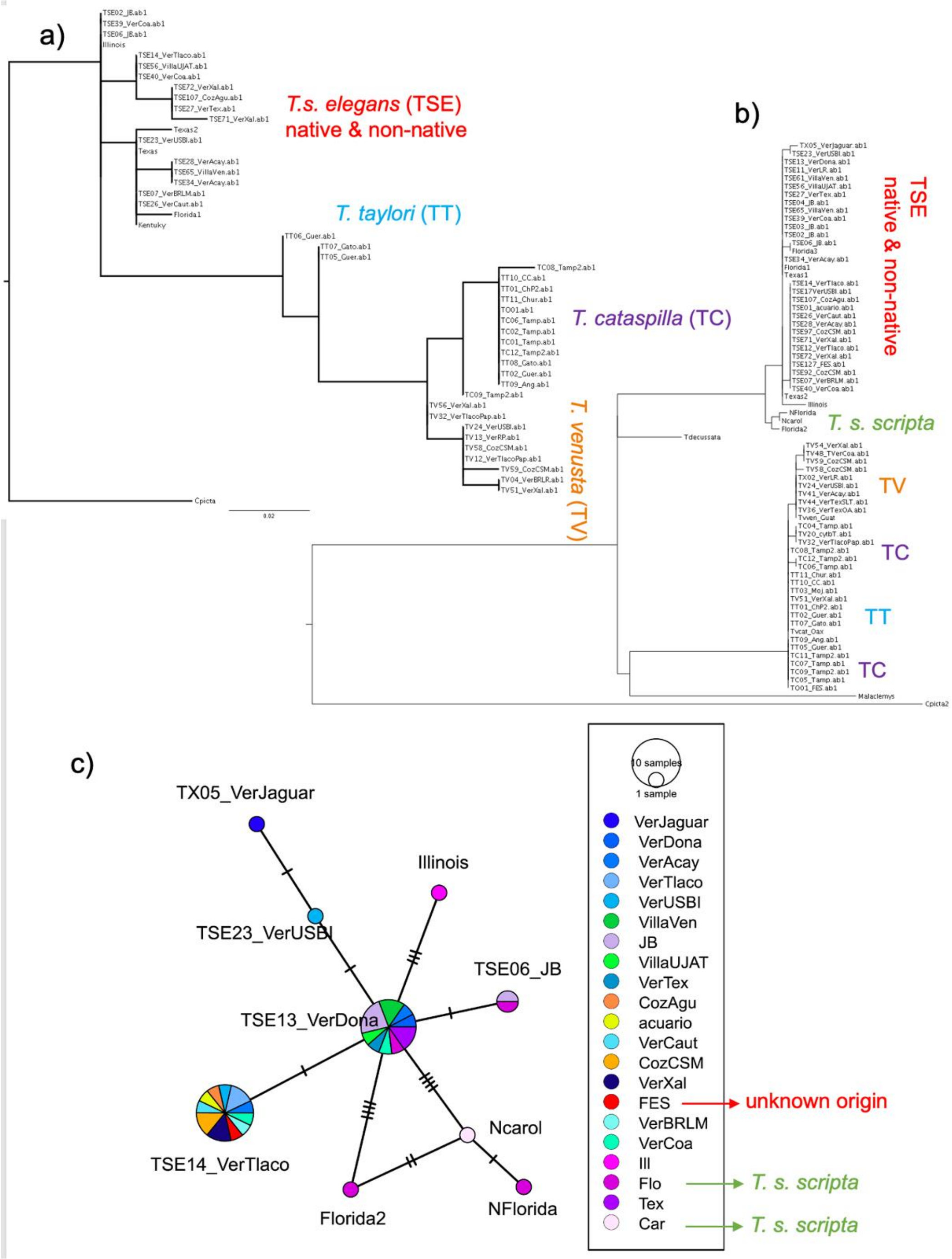


Figure S3. Maximum likelihood phylogenetic tree of *Trachemys* species (*T. scripta elegans*, *T. cataspila*, *T. venusta*, *T. taylori*), based on the (a) R35 and (b) cytochrome *b* genes. *Chrysemys picta* and *Malaclemys terrapin* were used as outgroups. The scale bar represents substitutions per site. (c) Minimum spanning haplotype network for *Trachemys scripta elegans* and *T. scripta scripta*. Circles represent haplotypes and circle size is proportional to haplotype frequency. Color of circles depicts the sampling locality or site of origin of samples (see insert; unknown origin, see Table S1).

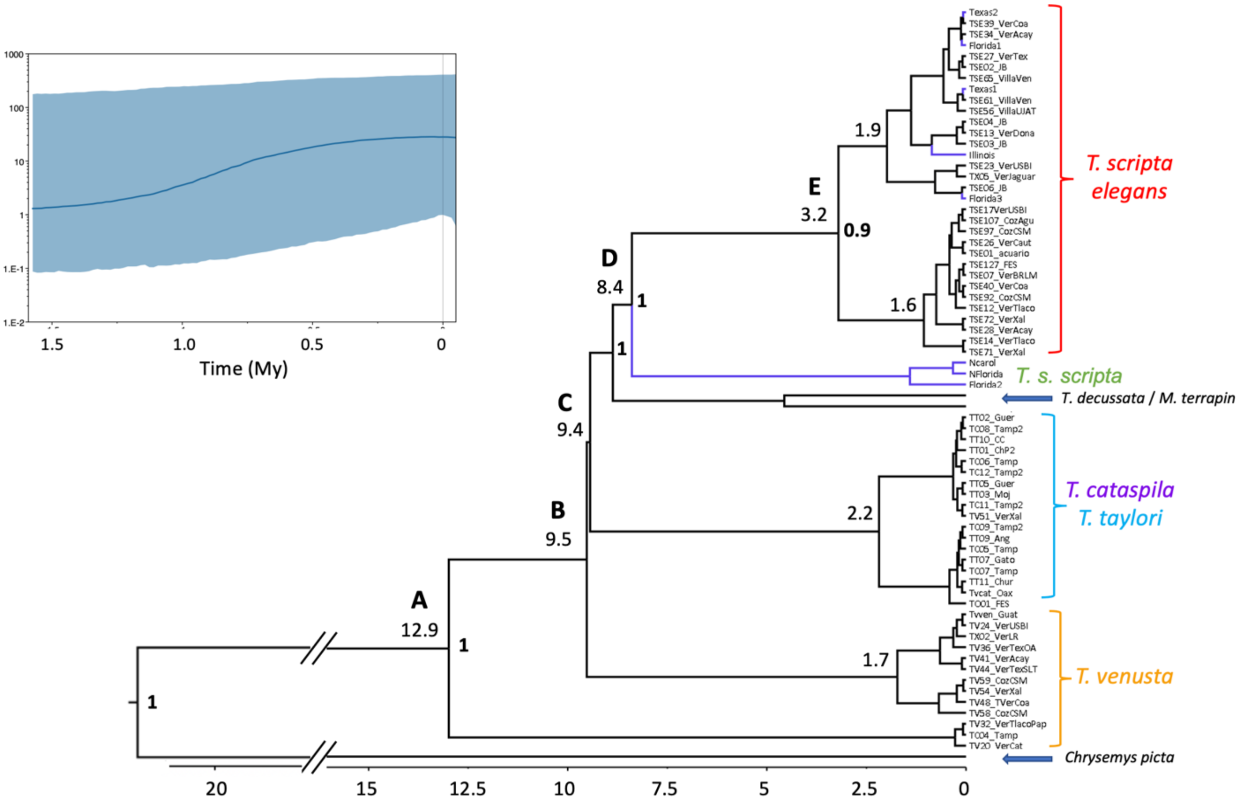
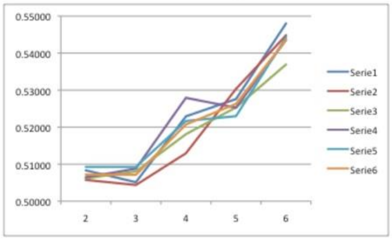


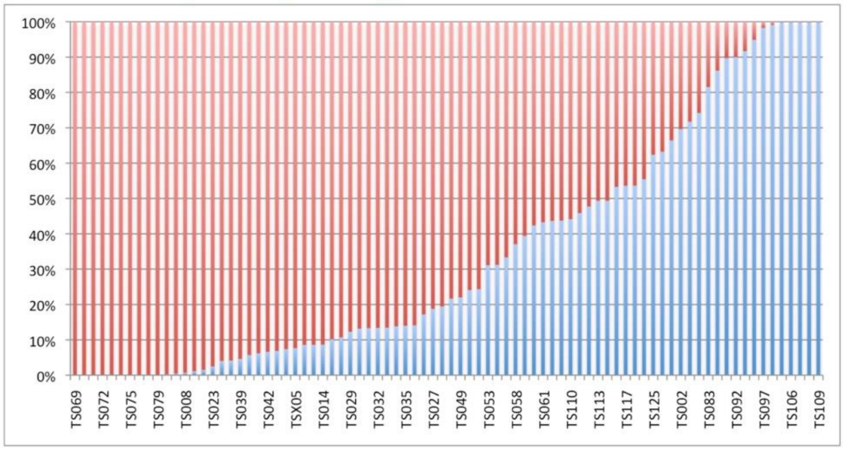
Figure S4. Divergence-time estimation (timescale in millions of years; My) above nodes, based on the cytochrome *b* data, of the *Trachemys* species. *Chrysemys picta*, *Malaclemys terrapin* and *Trachemys decussata* sequences were used as outgroups and for calibration points. Posterior probabilities >0.8 are indicated for some nodes, while capital letters are for reference in the results. Skyline plot for non-native *T. scripta elegans* in upper left corner.

a) *Trachemys venusta* (*K*=3)

TV058, 60, 61: Cozumel; TV56, 64: *T. cataspilla*



b) *Trachemys scripta elegans* (*K*=2)



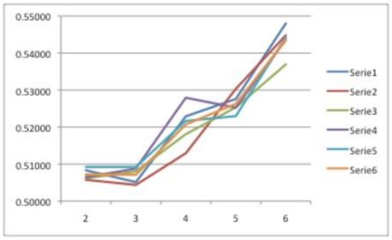
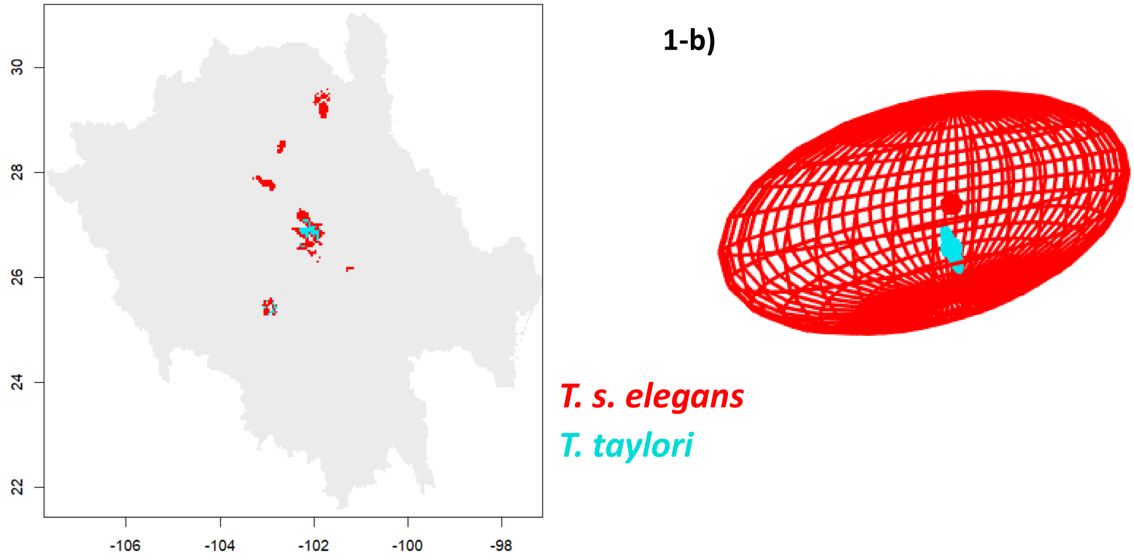
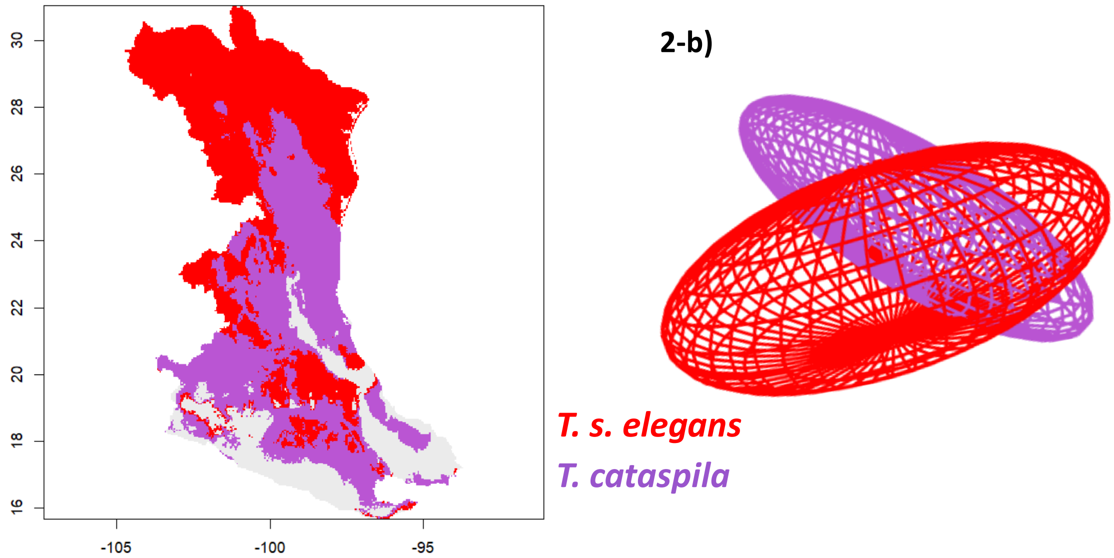


Figure S5. sNMF results for (a) *Trachemys venusta* (*K* = 3) and (b) *T. scripta elegans* (*K* = 2).

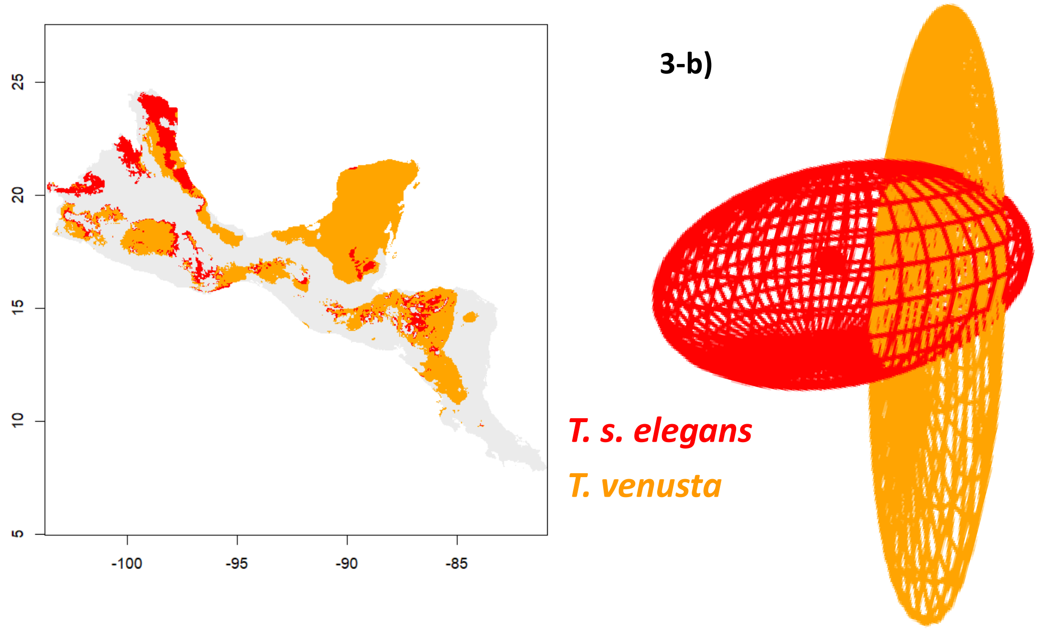
1-b)

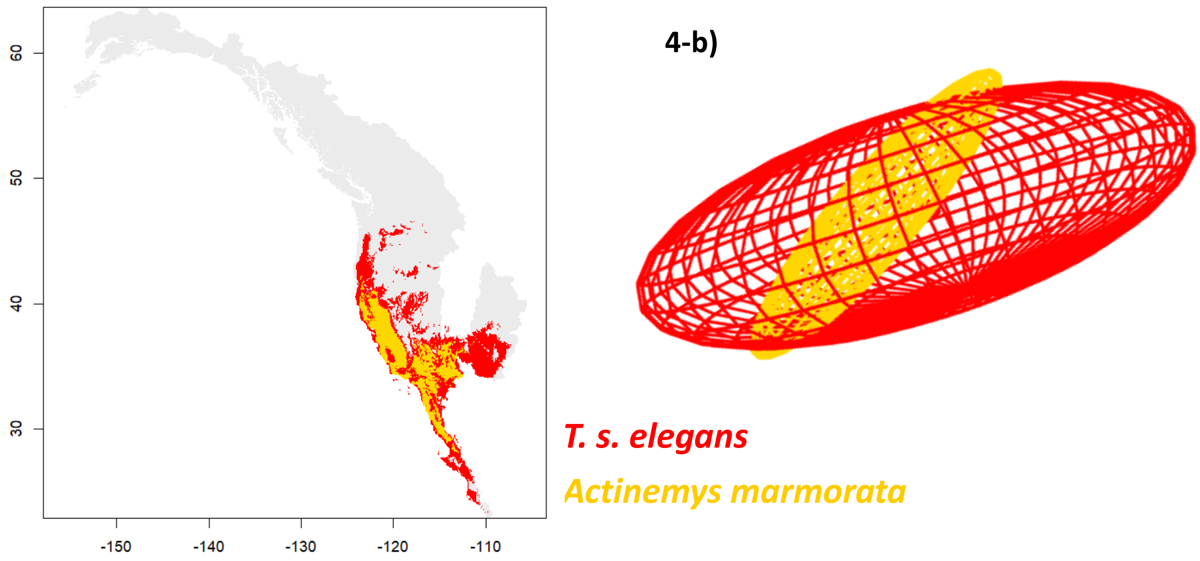

30
28
26
24
22
-106
-104
-102
-100
-98
T. s. elegans
T. taylori

2-b)


30
28
26
24
22
20
18
16
-105
-100
-95
T. s. elegans
T. cataspila

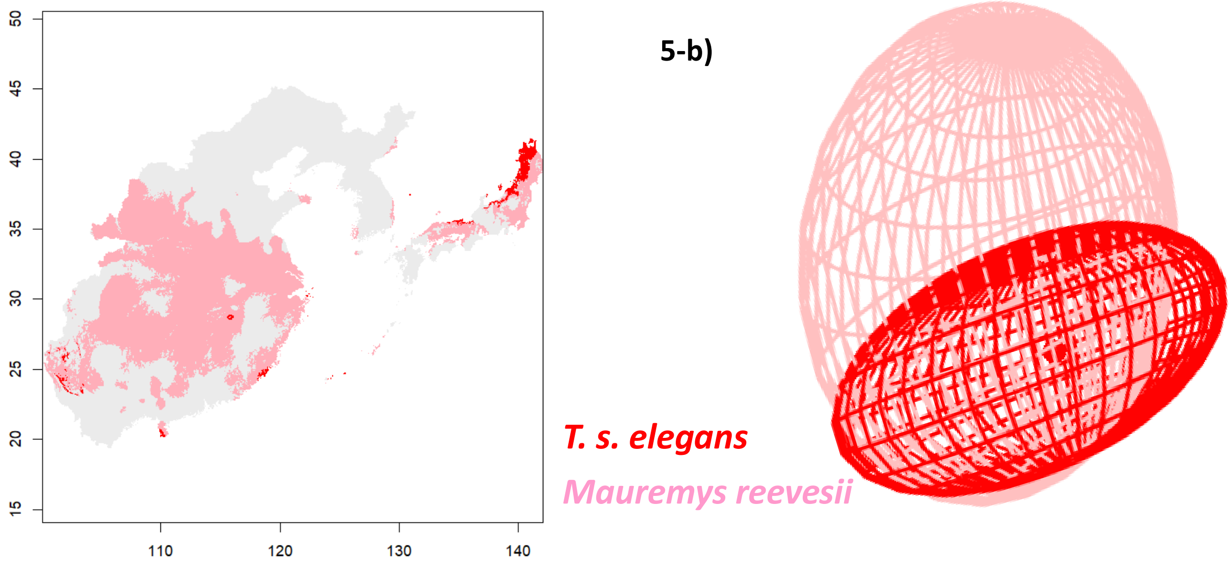
3-b)


25
20
15
10
5
-100
-95
-90
-85
T. s. elegans
T. venusta



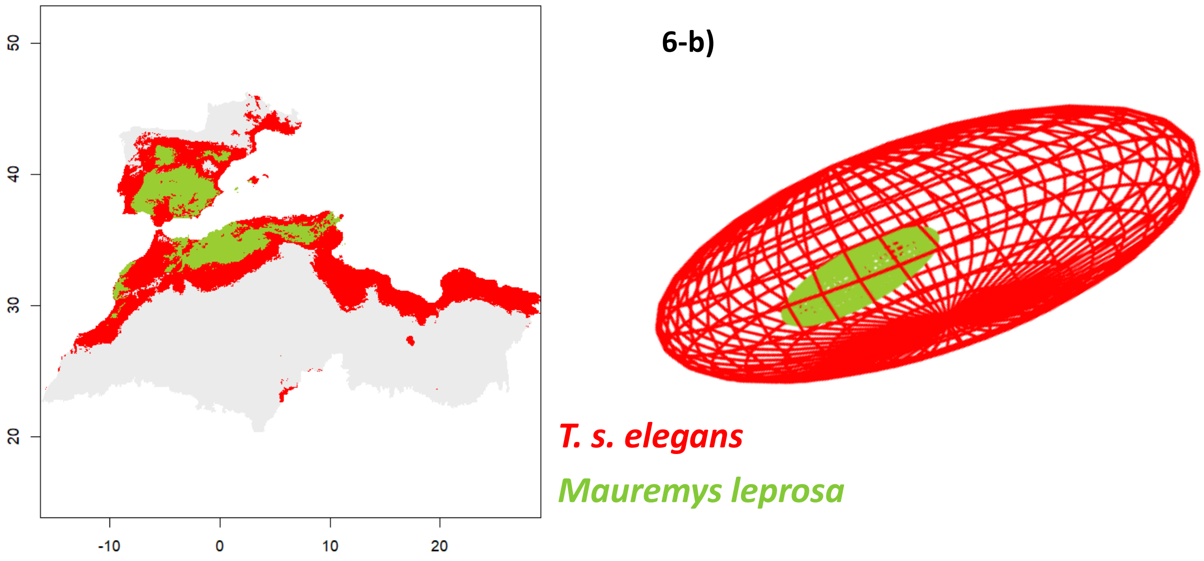
4-b)

60
50
40
30
-150
-140
-130
-120
-110
T. s. elegans
Actinemys marmorata



5-b)

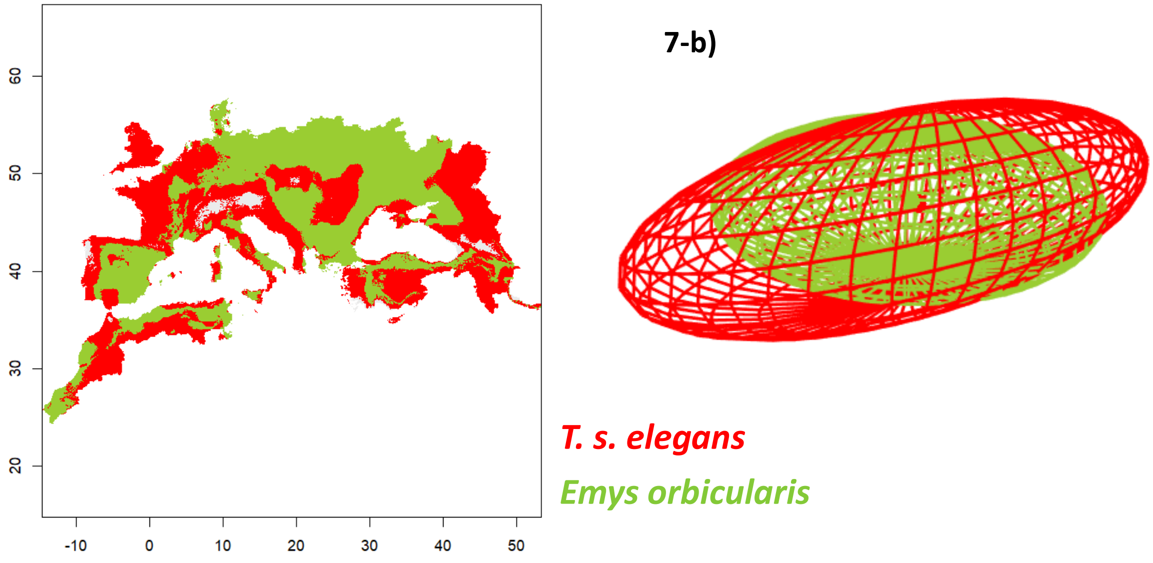
50
45
40
35
30
25
20
15
110
120
130
140
T. s. elegans
Mauremys reevesii



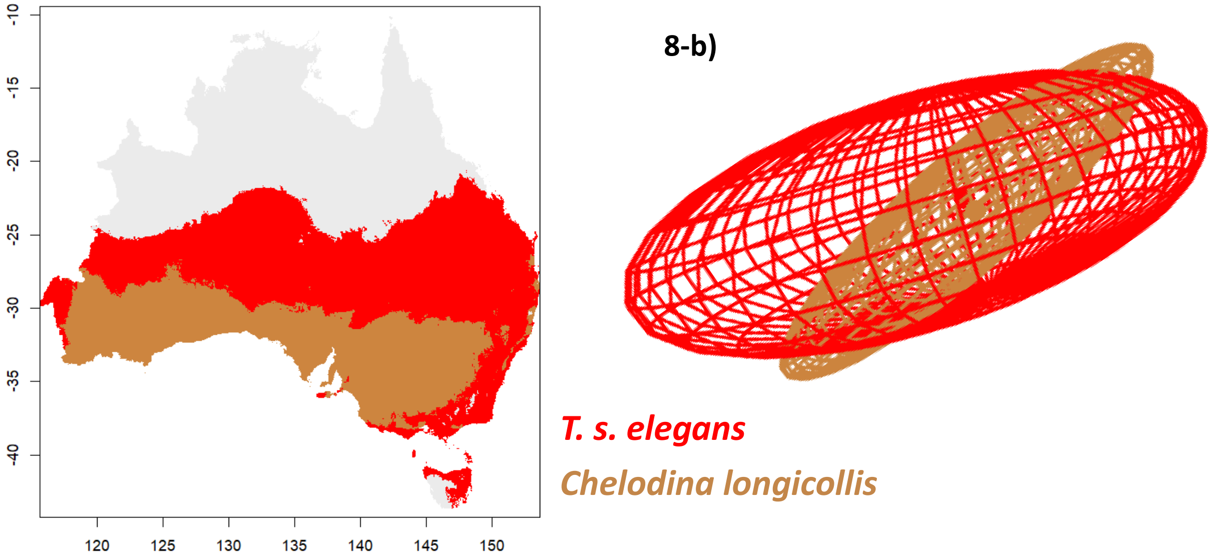
6-b)

50
40
30
20
-10
0
10
20
T. s. elegans
Mauremys leprosa

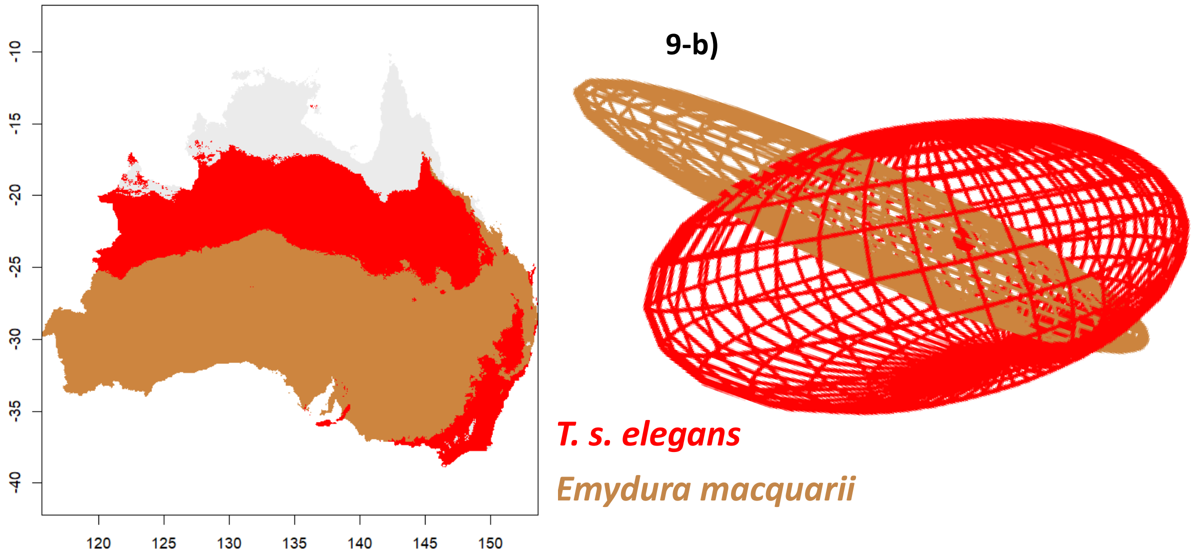
7-b)


60
50
40
30
20
-10
0
10
20
30
40
50
T. s. elegans
Emys orbicularis

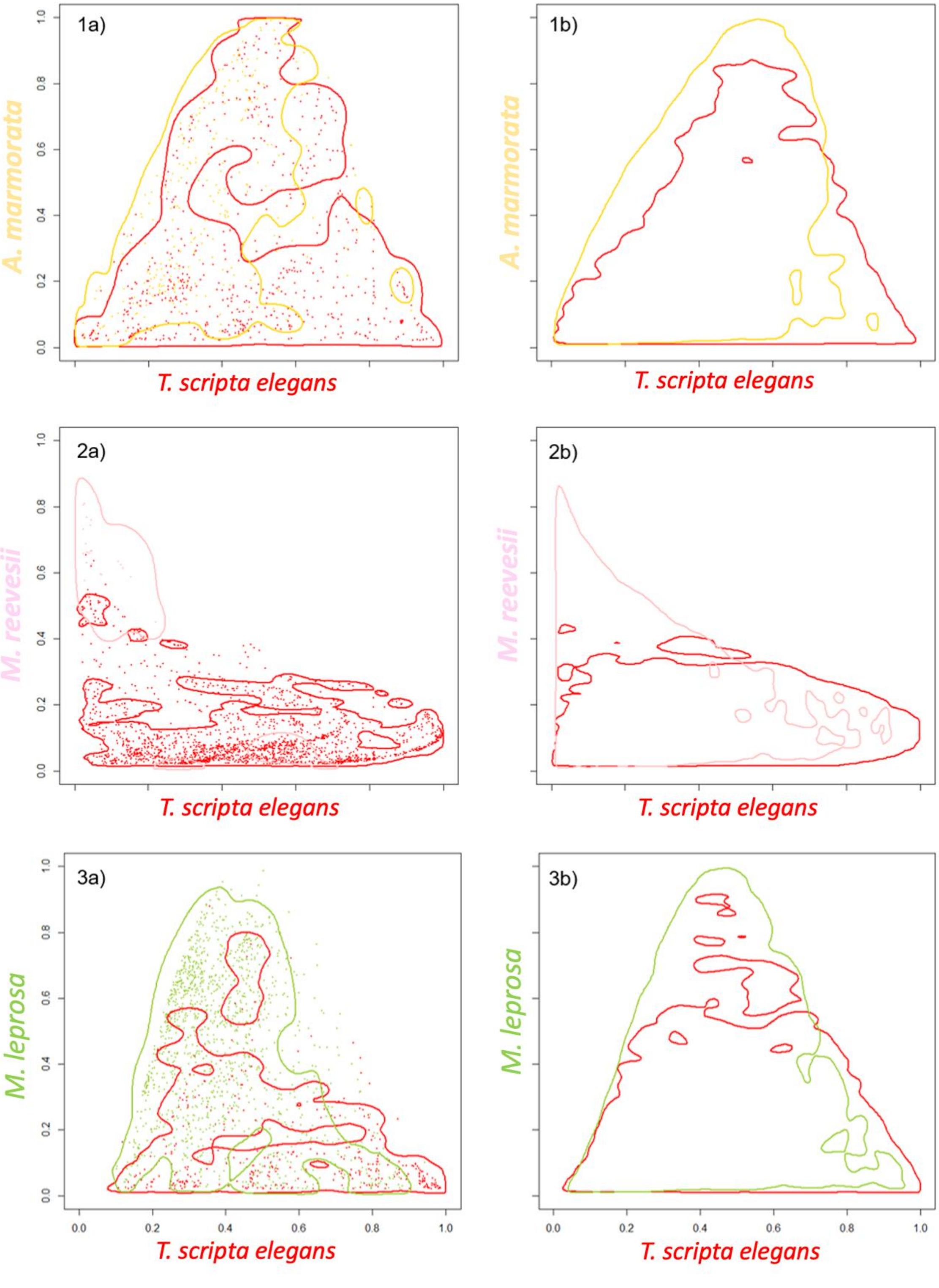
8-b)


-10
-15
-20
-25
-30
-35
-40
120
125
130
135
140
145
150
T. s. elegans
Chelodina longicollis

9-b)


-10
-15
-20
-25
-30
-35
-40
120
125
130
135
140
145
150
T. s. elegans
Emydura macquarii

◂ **Figure S6.** On the right: Graphic representation of ecological niche overlap, based on ellipsoid models, between *T. scripta elegans* (red) and each native species of different parts of the world: Western United States (4-*Actinemys marmorata*; yellow); Asia (5-*Mauremys reevesii*; pink); Europe (6-*Mauremys leprosa* and 7-*Emys orbicularis*; green), and Australia (8-*Chelodina longicollis* and 9-*Emydura macquarii*; brown). On the left: Maps showing the sites along the distribution of each native species that are closer to the niche-center of *T. scripta elegans*. If the site has a greater suitability (understood as the proximity to the niche-center) for *T. scripta elegans* is colored red; otherwise, it is colored according to each native species color (as mentioned above). Gray color depicts areas where no overlap occurs.


1a)
1b)
2a)
2b)
3a)
3b)
A. marmorata
A. marmorata
M. reevesii
M. reevesii
M. leprosa
M. leprosa
T. scripta elegans
T. scripta elegans
T. scripta elegans
T. scripta elegans
T. scripta elegans
T. scripta elegans
0.0
0.2
0.4
0.6
0.8
1.0

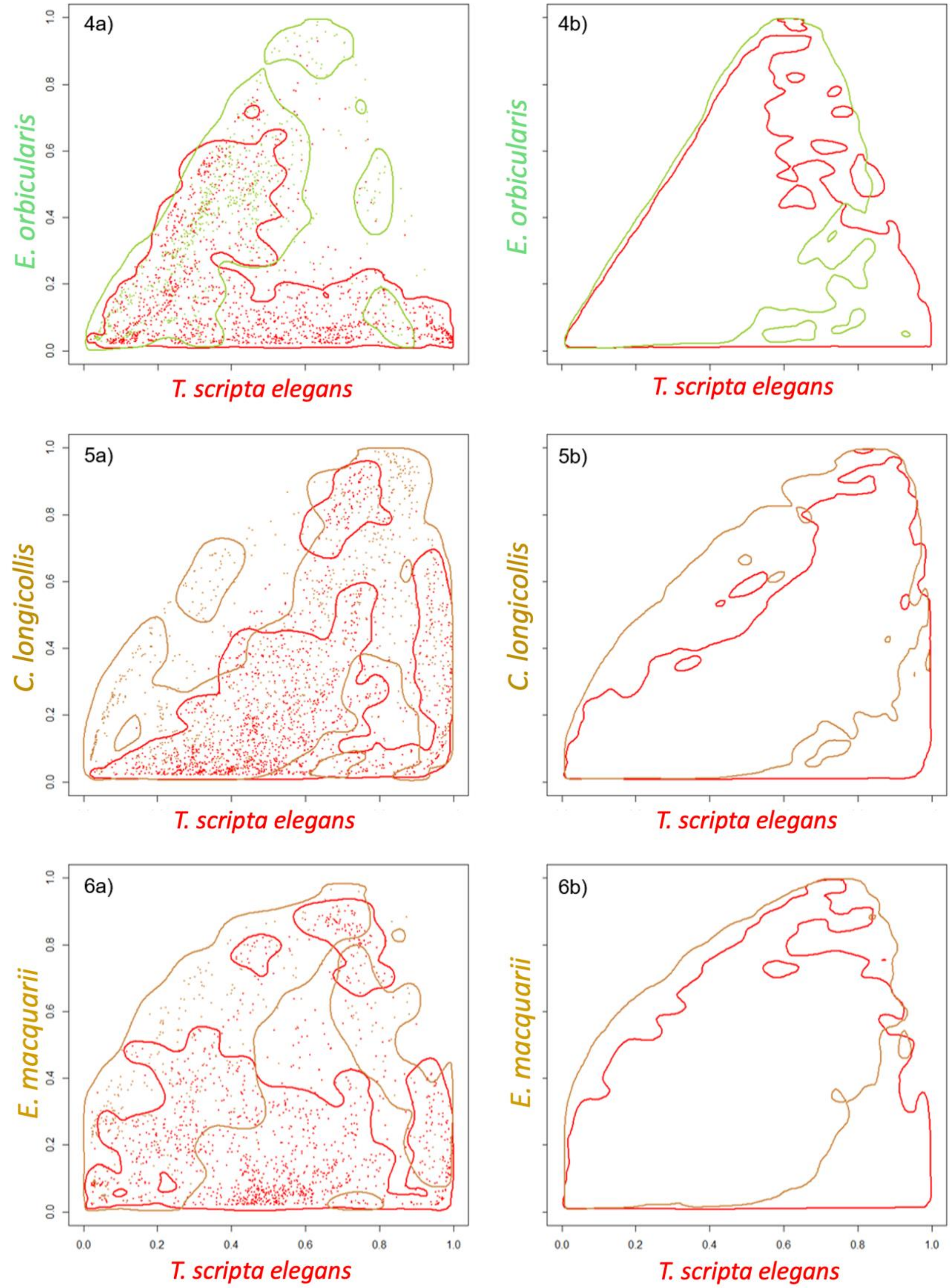


Figure S7. Suitability maps for each k. The panel on the right shows what the HDRs would be like for densities f and f_k if species truly distributed independently of each other. This panel is obtained by Monte Carlo simulation, generating a large number of independent samples, and applying kernel density estimates. In contrast, the left panel is the observed suitability map based on observed data over $A \cap B_k$.

Discusión y conclusiones

Discusión general

Dada la complejidad de las invasiones biológicas, el estudio de las especies invasoras ha tenido –y continúa teniendo– un potencial enorme para explorar diferentes teorías evolutivas, ecológicas y genéticas. Por ejemplo, sobre cambio de nicho, especiación, intercambios bióticos, procesos microevolutivos, efectos sinérgicos con otros procesos ambientales como cambio climático y consecuencias de la homogenización de la biodiversidad a nivel global.

Entre las especies más utilizadas para poner a prueba preguntas sobre estos y otros temas, se encuentra la tortuga de orejas rojas *Trachemys scripta elegans*, que ha sido introducida, a través del comercio internacional de mascotas, en al menos 76 países y todos los continentes (con excepción de la Antártida), por lo que actualmente es una de las especies más extendidas fuera de su área de distribución nativa (TTWG, 2017; CABI, 2019). Este gran éxito como especie invasora la ha puesto en el reflector de numerosos estudios, debido en gran parte a los impactos negativos que ha generado sobre otras tortugas nativas donde ha invadido. Así, existe información acerca de su filogeografía, su historia de vida, sus requerimientos ambientales, su habilidad competitiva, las áreas potenciales de invasión, entre otros (algunos ejemplos; Cadi y Joly, 2004; Cadi et al., 2004; Rödder et al., 2009; Fritz et al., 2012; Polo-Cavia et al., 2012; Crescente et al., 2014; Pearson et al., 2015; Banha et al., 2017; Rodrígues et al., 2018; Nori y Rojas-Soto, 2019; Parham et al., 2020).

Sin embargo, cabe enfatizar que este es el primer estudio que se realiza para México, en donde proponemos novedosas perspectivas de análisis, asociadas al interesante hecho de que aquí (al menos para la costa este) su invasión no ha sido tan exitosa como en otras partes del mundo.

Entre los aspectos relevantes de este trabajo está el uso de distintos tipos de información. Respecto a los datos genéticos, incluimos tres tipos de marcadores: microsatélites, secuencias nucleares y mitocondriales, y SNPs (*single nucleotide polymorphisms*), que en conjunto permitieron evaluar con alta precisión las características genéticas a diferentes resoluciones espaciales y temporales (Roux y Wieczorek, 2009). Así, fue posible evaluar desde migración histórica entre especies con el uso de SNPs, hasta patrones contemporáneos de estructura y diversidad genética basados en los resultados con microsatélites. Por otra parte, para el modelado de nicho ecológico hicimos uso de datos de ocurrencia provenientes de múltiples bases de datos nacionales e internacionales, e incluimos información climática, datos de tolerancias fisiológicas, disponibilidad de cuerpos de agua y presencia de otras especies, infiriendo, desde una perspectiva más integral, no solo las áreas más probables de invasión, sino también algunos factores que determinan los límites de su distribución.

Poblaciones nativas versus poblaciones introducidas de TSE

Conforme a nuestras predicciones, los resultados moleculares mostraron un patrón genético complejo, revelando una ancestría común entre las poblaciones nativas e introducidas de TSE pero, al mismo tiempo, una divergencia entre éstas, reflejado también en una marcada diferenciación genética entre individuos nativos e introducidos, identificados como dos grupos genéticos distintos. Además, el conjunto de individuos no nativos presentó una mayor diversidad genética. Dado que la introducción de esta especie está asociada a su liberación intencional después de su comercio como mascota, los patrones observados podrían estar asociados a que los organismos provienen de distintos orígenes geográficos y a partir de múltiples introducciones, como se ha observado para otras especies invasoras (por ejemplo, lagartijas del género Anolis, Kolbe et al., 2007; y boas, Suárez-Atilano et al., 2019). En este contexto, será necesario realizar estudios futuros en donde se incluyan datos de ejemplares provenientes de los criaderos en donde se reproducen para su venta con el fin de poder definir las rutas de introducción y las fuentes de las cuales provienen los organismos que son liberados, información que facilitaría el diseño de medidas específicas de vigilancia para evitar futuras introducciones.

Adicionalmente, los resultados de los modelos de nicho ecológico que generamos utilizando datos de tolerancia fisiológica revelaron características muy particulares. En primer lugar, tal como se ha sugerido previamente (Rödder et al., 2009; Masin et al., 2014; Rodrígues et al., 2016), encontramos que TSE tiene una amplia gama ambiental con condiciones climáticas favorables, lo que sin duda le ha permitido establecerse en numerosas regiones del mundo. Sin embargo y de manera contrastante, TSE no cubre todo el rango de condiciones climáticas óptimas a lo largo de su distribución nativa. En segundo lugar, al investigar si la especie conserva o no su nicho al ser introducida a nuevos ambientes, demostramos que TSE retiene, al menos en parte, las características que ocupa en su rango nativo, pero además, en las áreas no nativas ocupa un subconjunto más amplio de su nicho fundamental, ocupando zonas que no abarca en su rango nativo pero sin desviarse de sus rangos de tolerancia. Por ejemplo, en concordancia con lo documentado previamente (Ficetola et al., 2009; Rodrigues et al., 2018), observamos que en las áreas no nativas ocupa zonas tanto más frías como más cálidas de las que ocupa en su distribución nativa. Lo anterior podría estar asociado a dos posibles escenarios. Por una parte, en las zonas invadidas se eliminan las barreras y competidores que en su área nativa impiden que colonice la extensión completa de las condiciones ambientales adecuadas (Tingley et al., 2016). Otra posibilidad podría asociarse a la naturaleza de su introducción, en donde repetidas liberaciones podrían 'forzar' a la especie a ocupar áreas que no son óptimas (Ficetola et al., 2009). Los resultados anteriores sugieren que existen otros factores que limitan significativamente su dispersión y su distribución, tanto en las áreas en donde habita naturalmente como donde ha sido liberada intencionalmente (al menos en las áreas de la costa este de México que evaluamos). Al respecto, si bien las características climáticas son relevantes en determinar la oportunidad que tiene una especie invasora para establecerse (Colangelo et al., 2017), se ha reconocido la necesidad de integrar información adicional, tanto fisiológica como del hábitat (rangos nativo y no nativo) para lograr identificar con mayor precisión áreas adecuadas de establecimiento (Gallien et al., 2010; Enders et al., 2020). De hecho, gracias a la integración de información fundamental sobre la sobrevivencia de esta especie, logramos identificar de una manera más precisa aquellas áreas en México con el mayor potencial para una invasión exitosa de TSE.

Así, tanto los resultados genéticos como de modelado de nicho sugieren que TSE cuenta con el potencial para lograr una invasión exitosa en México. Sin embargo, de acuerdo con lo que observamos en campo, al explorar la situación de la especie en la costa este del país, aun cuando es capaz de mantenerse en los cuerpos de agua donde es liberada (cuerpos de agua artificiales o muy perturbados), al parecer no ha logrado dispersarse ni desplazar a otras tortugas nativas. Algunas propuestas que podrían explicar nuestros hallazgos se describen a continuación.

Integrando como variable a otras especies

La presencia de TSE como especie invasora en México provee un modelo excepcional para poner a prueba hipótesis que integran variables bióticas, en particular la interacción con otras especies, a la explicación de la modulación del éxito de una invasión. Ello debido a que la situación de TSE en México es muy singular, ya que otras especies congéneres como *T. cataspila* y *T. venusta* tienen una peculiar distribución natural que sigue a la de TSE en un gradiente hacia el sur a lo largo de la costa este de México; además de *T. taylori*, una especie microendémica cuya distribución se restringe al valle de Cuatrociénegas, en Coahuila (Seidel, 2002). La distribución de estas especies es alopátrica, con una historia biogeográfica compleja con escasas o nulas zonas de contacto salvo la frontera de la distribución entre TSE y *T. cataspila* y, particularmente, aquellos lugares donde TSE ha sido liberada.

Aunque existen estudios sobre las relaciones filogenéticas de tortugas donde se ha incluido al género *Trachemys* (Fritz et al., 2012; Parham et al., 2013), nuestros resultados son los primeros que incluyen las cuatro especies estudiadas. Así, al analizar las relaciones filogenéticas con las especies congéneres, logramos diferenciar claramente dos clados, uno que separa a TSE de las otras especies. Pero es cuando se analiza con otros marcadores (microsatélites) que se detecta la diferenciación genética entre individuos de TSE de origen nativo de aquellos no nativos, separando asimismo a las cuatro especies en grupos genéticos independientes. Respecto a las interacciones entre especies, logramos identificar ciertas señales de mezcla, reducido flujo génico entre especies vecinas y señales de introgresión histórica entre TSE y *T. cataspila*. Estas evidencias muestran que hay una marcada separación entre especies a pesar de que existe potencial de hibridación (Parham et al., 2020), posiblemente asociado a factores ambientales como los que identificamos, que delimitan su distribución geográfica, así como a procesos de exclusión conductuales y de interacciones entre especies, que es necesario evaluar.

Dicha exclusión podría explicar por qué la dinámica de invasión en México ha sido distinta a lo ocurrido en otras partes del mundo. Para probar esta idea, y como una de las propuestas más novedosas de este estudio, fue el abordar la hipótesis de centralidad de nicho bajo el supuesto de interacción entre especies. Para ello distinguimos dos grupos: por una parte, tomamos datos de seis especies de tortugas nativas de otras regiones del mundo que han sido afectadas negativamente por la presencia de TSE. Por otra parte, realizamos análisis con datos de las tres especies estudiadas de *Trachemys* en México y de las cuales no hay evidencia clara de afectaciones. Para las primeras observamos, en el espacio geográfico, una idoneidad de nicho, entendida como la proximidad al centroide de nicho, mayor para TSE que para las especies nativas. Asimismo, el efecto de interacción reveló cambios significativos de tamaño y forma en la densidad de distribución, pero conservando un marcado sobrelape del nicho. En contraste, la idoneidad fue mayor para las especies nativas de México comparado con TSE, coincidiendo con el área geográfica que ocupa naturalmente cada especie. Además, al comparar la densidad de la distribución de las *Trachemys* nativas cuando está presente TSE, observamos una considerable disminución de tamaño y forma respecto a lo esperado, esta vez disminuyendo también el sobrelape que existe entre TSE y sus congéneres.

Aun cuando la interacción de las especies fue estimada de manera indirecta, nuestros resultados son relevantes en términos de manejo y conservación. La mayor diversificación del género *Trachemys* está en México, con varias especies con distribución restringida (Macip-Ríos et al., 2015) y que, aun cuando su historia evolutiva podría conferirles cierta resistencia al establecimiento o dispersión de TSE, la constante introducción de ejemplares de esta especie podría finalmente generar afectaciones y poner en riesgo a las poblaciones nativas, como es el caso de *T. taylori*. Así, la capacidad de respuesta para remover de manera temprana a los ejemplares que son liberados en zonas donde existen congéneres podría resultar en una estrategia efectiva de manejo para evitar su establecimiento. No obstante, dada la gran riqueza de especies de tortugas dulceacuícolas (Legler y Vogt, 2013), otras especies de otros géneros con diferente morfología, ecología y comportamiento pueden ser más vulnerables a la invasión de esta especie, tal como se ha documentado recientemente para tortugas nativas de Sonora (*Kinosternon sonoriense*) (Drost et al., 2021). Adicionalmente, para futuros estudios también será necesario evaluar otros impactos potenciales asociados a la presencia de esta especie; por ejemplo, en el funcionamiento de los ecosistemas de agua dulce donde ha sido liberada, la transmisión de parásitos o patógenos y la depredación de especies vulnerables (Pérez Salerno y van den Burg, 2021).

Otros factores por considerar

En el capítulo 2 propusimos un modelo que integra la disponibilidad de cuerpos de agua como factor esencial para la sobrevivencia de la especie, lo que nos permitió identificar. a partir de un acercamiento más integral, áreas con condiciones ambientales favorables para su potencial de invasión. En el capítulo 3 indagamos la interacción con otras especies, demostrando que considerar dicha variable es determinante en cuanto al éxito de establecimiento. Sin duda existen otros factores no considerados en este estudio, que individualmente o en sinergia con otros, pudieran tener efecto en el éxito o el fracaso de una especie invasora, por lo que será necesario continuar proponiendo modelos alternativos que integren otras variables como: éxito reproductivo, presión de propágulo, estado de conservación y riqueza de especies del hábitat receptor, disponibilidad de recursos, relación con los asentamientos humanos y patrones de comercio y abandono, entre otros (Lowry et al., 2013).

Los patrones de comercio y abandono son particularmente relevantes en reptiles, pues su comercio como mascotas es la principal vía de introducción de este grupo; ello es de tal magnitud que se estima que alrededor de un tercio de los reptiles del mundo así han sido introducidos con éxito (Hulme et al. 2008). Aunque este tipo de información puede ser difícil de determinar, debido en gran medida a que mucho del comercio es ilegal, el usar esta variable en los modelos predictivos podría afinar los resultados al proveer datos relacionados con la presión de propágulo. De hecho, un estudio para predecir el potencial de invasión en reptiles y anfibios demostró que el número de veces que una especie es introducida es la variable más fuertemente asociada con el éxito de su establecimiento (van Wilgen y Richardson, 2012). Particularmente para TSE, estudios previos han demostrado que los patrones de su introducción están influenciados por el número total de tortugas, mientras que el éxito de establecimiento se asocia a la frecuencia de liberación, las zonas en donde están siendo liberadas y el número de especies nativas presentes (Kikillus et al., 2012; García-Díaz et al., 2015). Asimismo, Fonseca y colaboradores (2021) demostraron que integrando variables de actividad humana (densidad poblacional, uso de suelo y accesibilidad) a modelos de distribución de especies fue posible refinar los resultados para identificar las áreas más susceptibles a la invasión de *Trachemys dorbigni*.

Sin embargo, aunque las actividades humanas resultan un buen indicador para dilucidar patrones de introducción, otros estudios resaltan la importancia de incorporar variables asociadas a la dinámica poblacional de la especie introducida, para poder definir su capacidad de persistir y establecer poblaciones reproductivas (Ficelota et al., 2009), un rasgo fundamental para considerar exitosa una invasión (Blakburn et al., 2011). Particularmente para especies longevas como las tortugas, los adultos pueden sobrevivir por largos periodos de tiempo en hábitats sin las condiciones óptimas para reproducirse (por ejemplo, demasiado frías o calurosas), por lo que un primer reto es poder discriminar entre las poblaciones reproductoras de aquellas que no lo son. Por el tipo de muestreo realizado en este estudio no fue posible incluir esta información; no obstante, para futuros estudios será esencial incluir datos al respecto pues se ha demostrado que, en Europa, donde TSE se ha introducido ampliamente, ha logrado establecer poblaciones reproductivas sólo en pocas localidades (Ficelota et al., 2009). Al respecto, cabe resaltar que aún sin ser poblaciones reproductivas, la presencia de altas densidades de organismos, producto de una dinámica poblacional “fuente-sumidero” mediada por los humanos, es altamente probable que genere impactos sobre la vegetación y las comunidades de especies nativas, por lo que es clave que los datos de presencia no sean desestimados. De hecho, se ha sugerido que aun cuando las especies invasoras no hayan completado el proceso de invasión en una región, es crucial considerar su presencia en los planes estratégicos para la atención de especies invasoras (Rouget et al., 2016).

Por otra parte, también es importante tomar en consideración los métodos de análisis, ya que particularmente en los modelos de nicho ecológico, los resultados pueden depender del tipo de datos (presencia, ausencia, *background*), la escala y la forma de calibración (Robinson et al., 2010). Como ya se ha mencionado, los modelos de nicho desarrollados en este trabajo permitieron integrar información clave enriqueciendo nuestros análisis y las conclusiones derivadas de éstos. Sin embargo, existen otras perspectivas de análisis que no incluimos y que podrían considerarse para futuras investigaciones. Por ejemplo, Nori y Rojas-Soto (2019) resaltan la importancia de incorporar restricciones biológicas en los análisis que implican comparaciones entre regiones y transferibilidad de nicho, ya que muchas especies poseen requerimientos ambientales específicos, como es el caso de TSE cuya ocurrencia está fuertemente asociada a hábitats dulceacuícolas. Así, el uso de datos que reflejen dichas restricciones puede producir resultados más confiables. Por su parte, Václavík y Meentemeyer (2011) señalan que la etapa de invasión puede tener una profunda influencia en los resultados de los modelos de nicho, ya que afectan la medida en que las observaciones de ocurrencia coinciden con el nicho ambiental de la especie; ellos proponen un modelo que integra datos en escenarios a lo largo del tiempo. En cualquier caso, dada la complejidad que representa el estudio de especies invasoras, un enfoque prudente podría ser adoptar varios modelos e identificar las ventajas relativas de cada uno, lo que probablemente dependerá de la finalidad de cada estudio (Robinson et al., 2010).

Conclusiones y conservación

El conjunto de información generada en este trabajo, que integró el modelado del nicho (fundamental y realizado) de *Trachemys scripta elegans*, la genética poblacional de ésta, así como la filogenia, la filogeografía y la evaluación de la relación entre idoneidad y sobrelape del nicho ecológico entre TSE y sus congéneres, nos permitió explicar las diferencias del éxito de invasión de TSE en la costa este de México en comparación con otras partes del mundo.

Demostramos que TSE tiene una amplia tolerancia ambiental, particularmente en lo que respecta a los rangos de temperatura. Con relación a sus tolerancias fisiológicas, observamos que a lo largo de su distribución nativa TSE no ocupa todo el rango de condiciones óptimas, mientras que en las áreas de invasión ocupa un subconjunto más amplio. El enfoque utilizado para el análisis de estos resultados enfatizó la importancia de considerar información clave sobre la sobrevivencia de las especies para mejorar tanto la capacidad actual de predecir áreas que potencialmente pueden ser invadidas, como nuestra comprensión sobre los aspectos que determinan los límites geográficos de establecimiento o dispersión en invasiones actuales y potenciales.

Nuestra evaluación genética puso en evidencia que los patrones históricos y contemporáneos de diversidad genética y estructura de TSE y sus congéneres son relevantes para comprender sus límites de distribución y el intercambio genético que pudo haber ocurrido o que ocurre actualmente entre sus poblaciones. Asimismo, nuestros análisis permitieron establecer que el nicho ecológico juega un papel importante en este proceso, mostrando que en áreas no nativas TSE logrará establecerse y desplazar a otras especies si las condiciones ambientales locales están más cerca de su óptimo ecológico (centro de nicho) que de los óptimos ecológicos de las especies nativas. Si bien estos análisis no incorporan información directa sobre interacciones entre especies, representan una excelente aproximación para explicar lo que ocurre con las especies nativas cuando ocurre la introducción de potenciales competidores.

Nuestros resultados sientan las bases para futuras investigaciones; por ejemplo, para deducir rutas de introducción, para evaluar hibridación e introgresión de genes específicos y la conexión entre loci y rasgos fisiológicos, a través del uso del genoma completo o la secuenciación de genes objetivo y la inclusión de un mayor número de individuos muestreados en el campo. Asimismo, para explorar nuevas propuestas de análisis que incluyan explícitamente datos genéticos e interacciones bióticas en el modelado del nicho y de la distribución de las especies. Otro desafío para investigaciones futuras será caracterizar los escenarios que pudieran generar la sinergia entre el proceso de invasión de TSE y otras amenazas. Así, ha sido ampliamente documentado el impacto del cambio climático en la distribución, la reproducción y el comportamiento de las especies, efecto del que no están exentas las especies invasoras. Sin embargo, mientras las especies nativas pueden verse seriamente amenazadas (Markovic et al., 2014), en especies invasoras el cambio climático podría favorecer su distribución al incrementarse las zonas disponibles para reproducirse y propagarse, y por lo tanto incrementar los impactos que conlleva su presencia (Hellmann et al., 2008). De hecho, para TSE se ha sugerido que un incremento en la temperatura podría aumentar las zonas disponibles para anidación y alargar el periodo de puesta, favoreciendo el reclutamiento y la supervivencia de crías (Butler, 2019; Ficetola et al., 2009; Tucker et al., 2008).

Resaltamos además la importancia de integrar otros enfoques, como la etapa de la invasión, la frecuencia y las vías de introducción, así como la heterogeneidad ambiental (incluidos cambios climáticos a largo plazo y restricciones de hábitats específicos), entre otros; para evaluar éste y otros procesos de invasión de especies, ya que, aunque es un desafío importante, es clave para lograr modelos más confiables para el manejo y control de las especies invasoras.

Finalmente, la información que presentamos puede ser útil para quienes trabajan en actividades de manejo y conservación de las tortugas. Por ejemplo, con base en nuestros resultados, podemos señalar que la introducción continua de individuos de TSE en las pozas y humedales de Cuatrociénegas donde habita *T. taylori*, con su condición de microendémica y con poblaciones fragmentadas y aisladas (Cortés-Rodríguez et al., 2020), representa un riesgo significativo para su conservación. Además, los modelos de análisis que propusimos en este trabajo pueden ser aplicados al estudio de otros grupos biológicos que se encuentran en riesgo y que enfrentan el gravísimo problema de las invasiones biológicas.

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