UNIVERSIDAD NACIONAL AUTÓNOMA DE MEXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS
BIOLOGÍA EVOLUTIVA

ORIGEN Y EVOLUCIÓN DE ESPECIES DE REPTILES ENDÉMICAS AL VALLE DE CUATRO CIÉNEGAS, COAHUILA, MÉXICO.

TESIS

QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS

PRESENTA:

URI OMAR GARCÍA VÁZQUEZ

TUTOR PRINCIPAL DE TESIS:	**DR. ADRIAN NIETO MONTES DE OCA** FACULTAD DE CIENCIAS, UNAM
COMITÉ TUTOR:	**DRA. SUSANA AURORA MAGALLÓN PUEBLA** INSTITUTO DE BIOLOGÍA **DR. ALEJANDRO ZALDIVAR RIVERÓN** INSTITUTO DE BIOLOGÍA

MÉXICO, CD. MX. **ABRIL 2020**

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POSGRADO
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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE CIENCIAS
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ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Subcomité de Ecología y Manejo Integral de Ecosistemas y Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día **20 de enero de 2020** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **GARCÍA VÁZQUEZ URI OMAR** con número de cuenta **504008230** con la tesis titulada: **"ORIGEN Y EVOLUCIÓN DE ESPECIES DE REPTILES ENDÉMICAS AL VALLE DE CUATROCIÉNEGAS, COAHUILA, MÉXICO"**, realizada bajo la dirección del **DR. ADRIÁN NIETO MONTES DE OCA:**

Presidente:	**DRA. VALERIA FRANCISCA EUGENIA LEOPOLDINA DE MARÍA DE GUADALUPE SOUZA SALDÍVAR**
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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Ciudad Universitaria, Cd. Mx., a 12 de marzo de 2020

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

COORDINACIÓN

AGNS/VMVA/ASR/ipp

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ÍNDICE

Resumen

El Valle de Cuatro Ciénegas (VCC) se encuentra ubicado en el Desierto Chiuahuense, cuya topografía se caracteriza por la alternancia de tierras altas y cuencas de elevación intermedia. En particular, el Valle de Cuatro Ciénegas alberga una de las faunas más ricas en endemismos de Norteamérica. Este endemismo ha sido analizado infiriendo las filogenias de algunas especies acuáticas. Sin embargo, el origen y las relaciones filogenéticas de los vertebrados terrestres de la cuenca son menos conocidas, y únicamente se tienen estudios preliminares que han puesto de manifiesto un origen relictual. En este trabajo se investigan los eventos históricos y procesos ecológicos que dieron origen al alto nivel de endemismo presente en el valle tomando como modelo a los reptiles, uno de los grupos con más especies endémicas y con ayuda de herramientas modernas para evaluar la sincronía en el origen de estas especies desde un punto de vista filogeografico, así como la influencia de los factores ecológicos sobre la distribución actual de las especies del valle y sus especies hermanas. Para ello se estimaron las relaciones filogenéticas de *Terrapene coahuila*, *Gerrhonotus mccoyi*, *Scincella kikaapoa* y una especie no descrita de *Storeria* (con base en datos genéticos multilocus), taxones que ocupan ambientes similares en el VCC, asociados con hábitats acuáticos o semiacuáticos, pero difieren en características como el tamaño del cuerpo y las capacidades de dispersión. Para evaluar la sincronía en el origen de estas especies, se estimó la antigüedad de los eventos de divergencia en los árboles filogenéticos correspondientes calibrando dichos eventos en el tiempo utilizando fósiles, así como las tasas de flujo de genes para probar si estas especies comparten las mismas rupturas filogeográficas. Además, se utilizó un método jerárquico de aproximación bayesiana (hABC, por sus siglas en inglés) implementado en el programa msBayes para probar las hipótesis de divergencia simultánea versus no simultánea.

La mayoría de las filogenias estimadas son similares a las encontradas en estudios previos y revelaron una estrecha relación entre las poblaciones del VCC y las que se encuentran en el este de los Estados

Unidos o la Sierra Madre Oriental de México, patrones observados en otras especies endémicas del Valle. La prueba con el método hABC y los tiempos de divergencia estimados indican un origen asincrónico para las especies estudiadas, con dos eventos de divergencia no simultáneos, y sugieren que la divergencia de los linajes endémicos del VCC estudiados ocurrió durante el Neógeno, lo que indica que los ciclos glaciales del Pleistoceno no jugaron un papel central en su origen. La diversificación de los linajes del VCC estudiados parece estar relacionada con la aparición del Desierto Chihuahuense, asociado con la aridificación neógena del hemisferio norte, y probablemente fue el resultado de una variedad de factores asociados con la formación de nuevos hábitats, como los cambios climáticos importantes, eventos de orogenia durante el Mioceno y cambios globales en la vegetación durante el Plioceno. Las estimaciones del flujo de genes entre las poblaciones endémicas del VCC y sus respectivos grupos hermanos fueron altas en todos los casos, lo que sugiere subdivisiones genéticas profundas borradas por contacto secundario en el Pleistoceno.

Adicionalmente, durante el desarrollo de este trabajo se observó un fuerte impacto sobre la conservación de los grupos estudiados, específicamente en la disminución de los ambientes donde habitan, por lo que a menos que se implementen estrategias de conservación urgentes para regular la extracción de agua en el VCC, estas especies podrían desaparecer en corto plazo.

Abstract

The Cuatro Ciénegas Basin (CCB) is located in the Chihuahuan Desert, whose topography is characterized by alternating high- and medium-elevation basins. The Cuatro Ciénegas Basin (CCB) is home to one of the most endemism-rich faunal communities in North America. This remarkable endemism has been analyzed through the phylogenies of some aquatic species. However, the origin and phylogenetic relationships of the terrestrial vertebrates in the basin are poorly understood, and only known from preliminary studies revealing a relictual origin. This study proposes diverse historic events and ecological processes as the drivers behind the high levels of endemism in the CCB, and it was performed with representatives of the group with the most endemic species and with the help of modern tools that allow the evaluation of the synchrony of species origin from a phylogeographic point of view, as well as the influence of ecological factors on the current distribution of the species in the valley and their sister species. The phylogenetic relationships of *Terrapene coahuila*, *Gerrhonotus mccoyi*, *Scincella kikaapoa*, and *Storeria* sp. were estimated from genetic, multilocus data. These species occupy similar environments in the CCB and are associated with aquatic and semi-aquatic habitats but differ in body size and dispersion capabilities. To evaluate the timing of the origin of these species, we calibrated their respective phylogenetic trees using fossils and estimated their rates of gene flow to test if they share the same phylogeographic breaks. Additionally, a hierarchical Approximate Bayesian Computation (hABC) method was used in the program msBayes to test for synchronous vs asynchronous divergence.

The estimated phylogenies for the majority of species are similar to those found in previous studies of their phylogenetic relationships and revealed a close relationship between populations in the CCB and populations found in eastern United States and the Sierra Madre Oriental in Mexico, patterns observed

in other species endemic to the valley. The HABC model and the estimated divergence times indicate an asynchronous origin for these species, with two non-simultaneous divergence events, and suggest that the divergence of the lineages endemic to the CCB occurred during the Neogene, which indicates that the glacial cycles of the Pleistocene did not play a major role in their origin. The diversification of the lineages in the CCB appears to be related to the appearance of the Chihuahuan Desert, associated with the neogenic aridification of the northern hemisphere, and probably was the result of a variety of factors associated with the formation of new habitats, such as important climate change, orogeny during the Miocene, and global changes in vegetation during the Pliocene. Gene flow estimations between the populations endemic to the CCB and their respective sister groups were high in all cases, which suggests deep genetic divisions blurred by secondary contact during the Pleistocene. Additionally, during this study, conservation of the studied groups was severely impacted, specifically through reduction of their habitat. Thus, unless urgent conservation strategies to regulate water extraction in the CCB are implemented, these species may soon disappear.

Introducción general

La comprensión de los eventos geográficos y procesos ecológicos que conforman los patrones de riqueza de especies es un problema central en la ecología evolutiva (Wiens, 2011). Los estudios sobre el origen y diversificación de diversos taxones proveen ejemplos de cómo los eventos geológicos (Bryson et al., 2012), los procesos ecológicos (Weir y Schluter, 2004) y climáticos (Wiens y Graham, 2005), y la colonización de regiones nuevas (Simpson, 1980) influyen en la acumulación de linajes (Byson et al., 2014). Estos eventos pueden actuar solos o en conjunto para promover la especiación (Wiens et al., 2006).

El estudio comparado de los patrones filogeográficos de varias especies co-distribuidas permite plantear hipótesis sobre su posible origen común; por ejemplo, a través de vicarianza o dispersión, e identificar las causas geológicas, ecológicas o etológicas que pudieron haber influido en dicho origen (Domínguez-Domínguez y Vázquez-Domínguez, 2009). Sin embargo, los estudios filogeográficos comparativos a menudo revelan diferentes edades de divergencia para linajes hermanos de distintos grupos separados por una barrera común, lo que lleva a la conclusión de que el aislamiento de dichos linajes por dicha barrera no fue sincrónico. Se han reportado estudios, donde los tiempos de divergencia no se comparten, sino que los patrones filogeográficos observados son el resultado de varios procesos que ocurrieron en diferentes momentos (Myers et al., 2016). Por el contrario, si un mismo evento histórico afectó de manera similar a diferentes linajes, se esperaría que éstos tengan edades de divergencia similares en función de este evento (Hickerson et al., 2006). Sin embargo, la incongruencia en los tiempos de divergencia entre distintos pares de taxones hermanos no descarta la posibilidad de divergencia simultánea, ya que diferencias en los tiempos de divergencia también pueden deberse a la variación en el proceso de coalescencia. En otras palabras, la diferencia en los tiempos de divergencia estimados podría representar una discrepancia entre el tiempo de divergencia de los genes y el tiempo de divergencia de las poblaciones, que está relacionada con la demografía de cada especie particular (Ornelas et al., 2013). Por otro lado, si los eventos de divergencia fueron sincrónicos, podría esperarse que existan patrones filogeográficos concertados entre las especies co-distribuidas; estos patrones concordantes también podrían corresponder a un solo pulso de diversificación de múltiples pares de poblaciones afectadas por la misma barrera para el flujo de genes (Myers et al., 2017).

La evolución en los desiertos de América del Norte ha interesado a los biogeógrafos y biólogos evolutivos debido a la topografía diversa de la región y su biota igualmente diversa (Wilson y Pitts, 2010). Entre los eventos que pudieron haber tenido una gran influencia en la expansión de los desiertos y los procesos de especiación que en ellos se desarrollaron, están las glaciaciones del Pleistoceno (Haffter, 1969). Esta hipótesis se basa en que diversas zonas han estado sujetas a cambios paleoclimáticos y tectónicos en el pasado reciente (Escalante y Llorente, 1985), y en que cada sucesión de eventos de avance y retroceso de glaciares pudo haber provocado la contracción, aislamiento, y expansión de las áreas de distribución de las especies, con la consecuente diferenciación–especiación e incremento de la riqueza. Así, la extensión de los periodos pluviales durante el Pleistoceno incrementó la humedad de zonas desérticas permitiendo la invasión de hábitats templados. A pesar de que los efectos de estos eventos sobre la flora y fauna han sido sujetos de debate, es posible que diversos periodos de gran precipitación pluvial durante o inmediatamente después del Pleistoceno provocaran una retirada gradual del desierto y la expansión de hábitats más templados y de las áreas de distribución de las especies adaptadas a los mismos. De acuerdo con Milstead (1960), en el desierto Chihuahuense la mayoría de las especies de filiación templada desaparecieron tras el retorno de las condiciones desérticas, pero algunas de ellas se mantuvieron como relictos en torno a diversos oasis, señalando como uno de ellos al Valle de Cuatro Ciénegas.

A pesar de la influencia de las glaciaciones Pleistocénicas en la diversidad actual, algunos estudios sugieren que la diversificación de especies precedió al Pleistoceno (Klicka y Zink, 1997; Zink et al., 2004; Myers et al., 2017; García-Vázquez et al., 2018a). Por lo tanto, en contra de la visión tradicional de que las biotas del desierto regional de América del Norte han divergido recientemente (durante el Pleistoceno), varios investigadores han sugerido que hubo divisiones históricas más profundas (neógenas) en estos desiertos regionales (Hafner y Riddle, 1997; Morafka, 1977; Riddle et al., 2000; Scheinvar et al., 2017). Ante esta evidencia, se han propuestos diversos eventos para explicar estas divergencias. La evidencia paleobiológica, por ejemplo, sugiere que los eventos de elevación del Neógeno que dieron origen a las grandes Sierras de México crearon un efecto de sombra de lluvia sobre la mayor parte de América del Norte y, por lo tanto, condujeron a la formación de las diferentes regiones desérticas (Wilson y Pitts, 2010; Bryson et al., 2011c). Sin embargo, estos procesos no fueron únicos, y algunos autores han propuesto elevaciones secundarias que pudieron haber producido puentes que conectaron la biota montana a través del Altiplano Mexicano (Bryson y Riddle, 2012). De acuerdo con Riddle y Hafner (2006), después de su formación durante el levantamiento piramidal, los semidesiertos alcanzaron su área máxima durante el Plioceno temprano y se redujeron en área durante el Plioceno húmedo tardío y durante los intervalos pluviales del Pleistoceno. Wilson y Pitts (2010) identificaron más de 40 eventos de orogenia asociados a la composición de los desiertos de Norteamérica y sugieren que los eventos evolutivos que datan de entre 15 y 2 millones de años (Ma) podrían considerarse asociados con la construcción de montañas y/o la actividad de formación del desierto (Wilson y Pitts, 2010). Además de los eventos orogénicos durante el Neógeno, microfósiles de las floras del centro de México muestran que climas más fríos y húmedos en relación con el presente estuvieron presentes en gran parte del desierto de Chihuahua durante el Mioceno tardío (8 Ma) (Graham, 1987).

El Valle de Cuatro Ciénegas (VCC) se encuentra ubicado en el Desierto Chiuahuense, cuya topografía se caracteriza por la alternancia de tierras altas y cuencas de elevación intermedia (Milstead, 1960). En particular, el Valle de Cuatro Ciénegas alberga una de las faunas más ricas en endemismos de Norteamérica (Álvarez y Ojeda, 2019), la cual incluye, entre otros, caracoles acuáticos (Taylor, 1966), crustáceos isópodos (Cole y Minckley, 1966), peces (Miller, 1968), tortugas (Milstead, 1967) y lagartijas y serpientes (García-Vázquez et al., 2019). Una marcada diferenciación morfológica y la lenta diferenciación genética de estos taxones sugieren una especiación rápida debido al aislamiento de la cuenca (García-Vázquez et al., 2019). Este proceso de aislamiento ha sido analizado en esta zona geográfica usando filogenias de algunas especies acuáticas (Hulsey et al., 2004; Hsiu-Ping y Hershler, 2007). Sin embargo, las relaciones filogenéticas de los animales terrestres de la cuenca de Cuatro Ciénegas son menos conocidas, y únicamente se tienen estudios preliminares de escorpiones (Williams, 1968), anfibios y reptiles (McCoy, 1984), que han puesto de manifiesto su origen relictual y diversos eventos de especiación local (Meyer, 1973).

El origen de estas especies endémicas ha sido estudiado de manera independiente, existiendo varias teorías. Milstead (1967) ubica al ancestro de *Terrapene coahuila* en el Pleistoceno, mientras que Miller (1968) propone un origen en el Mioceno para uno de los peces endémicos, y sugiere que las pozas donde habitan estos organismos estuvieron presentes en el Valle durante el Cenozoico. De acuerdo con Meyer (1973), el alto grado de endemismo que exhibe la fauna del Valle puede ser explicado por la presencia de cuerpos acuáticos y además sugiere aislamiento por un tiempo prolongado. Taylor (1966) sugiere que el ancestro común a los caracoles acuáticos endémicos al Valle se aisló a principios del Terciario o en el Mesozoico Tardío.

Se han reportado nueve taxones de anfibios y reptiles endémicos al Valle de Cuatro Ciénegas: tres tortugas (*Apalone spinifera ater*, *Trachemys taylori* y *Terrapene coahuila*), cinco lagartijas (*Aspidoscelis gularis pallida*, *A. inornata cienegae*, *Gerrhonotus lugoi*, *G. mccoyi* y *Scincella kikaapoa*) y una rana (*Craugastor augustii fuscofemora*) (McCoy, 1984; García-Vázquez et al., 2019). Adicionalmente, en los últimos años se han descubierto poblaciones de anfibios y reptiles asociadas a los cuerpos de agua cuya distribución y morfología sugiere que se trata de especies no descritas con distribución exclusiva al VCC (García-Vázquez et al., 2019): una serpiente (*Storeria* sp. VCC) y una rana (*Eleutherodactylus* sp.). A pesar de que el estudio taxonómico de estas poblaciones está todavía en curso, estas especies presentan una distribución similar a la de *S. kikaapoa* y *G. mccoyi*, exclusiva de las inmediaciones de los cuerpos de agua (García-Vázquez et al., 2010; 2018b; 2019). En conjunto, estos cuerpos de agua representan microhábitats con condiciones ecológicas diferentes a las observadas en el resto del Valle (García-Vázquez et al., 2019).

Si bien los eventos históricos y geológicos y los procesos ecológicos que dieron origen y que han afectado al VCC han sido bien documentados (Meyer, 1973; Mincley y Jackson, 2007), a la fecha no se sabe en qué medida estos eventos han influido sobre la diversidad actual del Valle. Asimismo, y a pesar de que algunos autores han intentado explicar el origen de las especies endémicas bajo diferentes escenarios (Milstead, 1967; McCoy, 1984; Mincley y Jackson, 2007), no se tiene un estudio que aborde esta problemática desde los puntos de vista filogenético y biogeográfico. Particularmente, la biogeografía comparada puede elucidar la influencia de eventos históricos sobre los patrones actuales de biodiversidad, y puede identificar patrones de co-vicarianza entre taxones no relacionados que habitan las mismas áreas geográficas (Ornelas et al., 2013).

En este contexto, el objetivo principal de esta tesis, dividida en cuatro capítulos, es identificar los eventos históricos y procesos ecológicos que dieron origen al alto grado de endemismo presente en el valle tomando como modelo cuatro especies de reptiles, que es el grupo de vertebrados terrestres con más especies endémicas en el valle (*Terrapene coahuila*, *Gerrhonotus mccoyi*, *Scincella kikaapoa* y la especie no descrita del género *Storeria* y con ayuda de herramientas que permitan evaluar la sincronía en el origen de estas especies desde un punto de vista filogeográfico (Ornelas et al., 2013).

En el primer capítulo se presenta un análisis donde se pone a prueba la hipótesis del origen sincrónico de las cuatro especies arriba mencionadas, para lo cual se obtuvieron hipótesis filogenéticas de cada una (basadas en secuencias de mtDNA y nuDNA) que incluyeron representantes de todas sus especies reconocidas, cubriendo toda su área de distribución. A partir de estas hipótesis filogenéticas se estimaron los tiempos de divergencia en cada grupo (usando un reloj molecular relajado) con el programa BEAST v1.6.1 (Drummond y Rambaut, 2007). En todos los casos, los árboles se calibraron a partir del registro fósil. Para probar las hipótesis de un pulso único (síncrono) contra múltiples pulsos de diversificación se utilizó un método de aproximación bayesiana (HABC) implementado en el programa msBayes (Hickerson et al., 2006). Este programa se basa en un modelo coalescente jerárquico donde se utiliza la estimación de hiperparámetros para discriminar las diferencias en el tiempo de divergencia entre pares de taxones en tiempos coalescentes (Hickerson et al., 2006; Daza et al., 2010); por lo tanto, el método HABC distingue razonablemente el aislamiento simultáneo de la incongruencia temporal en la divergencia, y se ha demostrado que es efectivo en una variedad de condiciones con datos de un solo locus (Hickerson et al., 2006; Ornelas et al., 2013) y con un tamaño de muestra de cinco o menos individuos (Hickerson et al., 2007; Topp et al., 2013). Finalmente se discuten las afinidades biogeográficas de cada especie estudiada y se propone una hipótesis biogeográfica con base en los eventos históricos y los procesos ecológicos que pudieron verse involucrados en el origen de estas especies, hipótesis que, contrario a hipótesis previas, pone en evidencia la poca o nula influencia de las glaciaciones pleistocénicas en el origen de estas especies.

En el capítulo dos, usando un conjunto de datos multilocus se evaluó la influencia de los eventos geológicos del Neógeno y del cambio climático del Pleistoceno en la diversificación del género *Gerrhonotus* usando datación molecular y un análisis de reconstrucción de áreas ancestrales; además, se exploró la estructura filogeográfica del género mediante un análisis coalescente de delimitación de especies. A partir de estos análisis se encontró que los levantamientos orogénicos del Mioceno y el Plioceno, probablemente junto con el cambio climático en el Pleistoceno, parecen haber inducido una divergencia alopátrica en una escala espacial relativamente pequeña en este género. Se discuten los resultados principales y se proponen cambios taxonómicos para que el género sea monofilético.

En el tercer capítulo se realiza la descripción formal de una de las especies endémicas del Valle de Cuatro Ciénegas y se discuten aspectos de su afinidad ecológica, sus diferencias en la distribución y hábitat con respecto a otras especies del género y su estado de conservación.

Finalmente, en el capítulo cuatro se recopila y analiza el conocimiento actual de los anfibios y reptiles del Valle de Cuatro Ciénegas, incluidas sus afinidades biogeográficas y la importancia histórica y ecológica de la cuenca para la diversidad de estos grupos en el desierto Chihuahuense.

Capítulo I: Asynchronous origin of the endemic reptiles in the Cuatro Ciénegas Basin, Coahuila, México (Manuscrito con formato para Journal of Biogeography)

Article type: Original Article

Asynchronous origins of endemic reptiles in the Cuatro Ciénegas Basin, Coahuila, Mexico

Running head: Comparative phylogeography of Cuatro Ciénegas Basin endemic reptiles

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ABSTRACT

Aim: Despite the fact that numerous phylogeographical studies have been conducted at the Cuatro Ciénegas Basin (CCB) in central Chihuahuan Desert, it remains unclear how the combined effects of isolation by distance, environmental heterogeneity, or other barriers to gene flow have promoted the high diversity of endemic species in the CCB. Herein, we investigate the evolutionary history of four microendemic species of reptiles (one snake, one turtle, and two lizards) in the CCB to test for codivergence. The focal taxa occupy similar environments, being associated with aquatic or semiaquatic habitats, but they differ in characteristics such as body size and dispersal capabilities.

Location: Northeastern Mexico and south-central United States.

Methods: Using mitochondrial and nuclear DNA sequence data, we test the hypothesis of simultaneous divergence across four endemic species of the CCB. We time-calibrated phylogenetic trees using fossils and estimated rates of gene flow to test whether these species share the same phylogeographic breaks. We also used the hierarchical Approximate Bayesian Computation (hABC) method implemented in the program msBayes to test simultaneous versus non-simultaneous divergence.

Results: The estimated phylogenies for most of the species are similar to those in previous studies of their phylogenetic relationships and revealed a sister-taxon relationship between populations in the CCB and populations found in eastern USA and Sierra Madre Oriental of Mexico. The hABC model testing showed strong support for asynchronous divergence among the studied taxa, with the estimated divergence times encompassing the Late

Miocene and Pliocene. Estimates of gene flow between the CCB endemic populations and their respective sister groups were high in all cases.

Main Conclusions: The divergence of the studied lineages endemic to the CCB ocurred in at least two non-simultaneous events during the Neogene, indicating that Pleistocene glacial cycles did not play a central role in their origin. The diversification of the CCB lineages seems to have been related with the emergence of the Chihuahuan Desert, associated with the Neogene aridification of the Northern Hemisphere, and was likely the result of a variety of factors associated with the formation of new habitats; namely major climatic changes, orogeny events during the Miocene, and global shifts in vegetation. The high estimates of gene flow between the CCB endemic populations and their respective sister groups suggest deep genetic subdivisions blurred by secondary contact in the Pleistocene.

KEYWORDS

Approximate Bayesian computation, codivergence, comparative phylogeography, Cuatro Ciénegas Basin, divergence time estimation, diversification, North American Desert, Neogene.

1 | INTRODUCTION

Reconstructing the historical processes that gave rise to the current biodiversity is a central goal in evolutionary biology. To this purpose, inferring the evolutionary history of the groups inhabiting a particular region is the first step to elucidate the processes by which the biota of the region originated (Colston et al., 2013; García-Vázquez et al., 2018a). The study of different evolutionary histories in the same region has allowed for the recognition of common patterns that have emerged in several regions of North America, such as Baja California (Riddle et al., 2000) and the North American Pacific Northwest (Cartens & Richards, 2007). However, many regional study systems have demonstrated high variation in geographic patterns (Gámez & Castellanos-Morales, 2019).

Evolution in the North American deserts has long interested biogeographers and evolutionary biologists because of the region's diverse topography and its equally diverse biota (Wilson & Pitts, 2010). Traditionally, some authors have suggested that the present North American biota was strongly influenced by the repeated expansion and contraction cycles of the coniferous forests during the Pleistocene climatic fluctuations, which isolated many populations, leading to speciation (Vanzolini, 1970; Bryson et al., 2011a). However, some studies suggest that species diversification preceded the Pleistocene (Lynch, 1988; Klicka & Zink, 1997; Zink et al., 2004; Myers et al., 2017). Thus, contrary to the traditional view that the North American desert biotas have diverged recently (during the late Pleistocene), several researchers have suggested that there are deeper (Neogene) historical divisions in these regional deserts (Hafner & Riddle, 1997; Morafka, 1977; Riddle et al., 2000). Despite the timing of species diversification, many taxa were displaced from much of their current distributions while tracking suitable habitat as glaciers repeatedly advanced and retreated during the Pleistocene. Regardless the timing of species diversification, many taxa were displaced to much of their current distributions while tracking suitable habitat as glaciers repeatedly advanced and retreated during the Pleistocene, which could have erased or obscured previously acquired signals of historical isolation (Bryson et al., 2011a).

On the other hand, concerted phylogeographical patterns between co-distributed species might be expected. A single pulse of diversification might explain these concordant patterns across multiple population pairs distributed on each side of a barrier to gene flow. However, evidence against this has been reported, where divergence times are not shared but rather the observed phylogeographical patterns are the result of various processes occurring at different times (Soltis et al., 2006)

The existence of deeper historical divisions anterior to the Pleistocene in the biotas of North American deserts could indicate some common responses across taxa to vicariant geological events. The degree to which divergent sister taxa collectively reveal a general history of vicariance rather than idiosyncratic dispersal events is a question that ultimately requires analyses of codivergence across multiple codistributed taxa to be answered (Riddle et al., 2000).

Located in the southeastern Chihuahuan Desert (CD) in the Mexican state of Coahuila, the Cuatro Ciénegas Basin (CCB) is a small (approximately 40 km long by 25 km wide), butterfly-shaped intermontane valley with the basin floor about 740 m above sea level and surrounded by mountains rising higher than 2500 m (Meyer, 1973). This valley is surrounded by steep limestone sierras capped by conifer forest. The CCB is nearly bisected from the south by the outputting tip of Sierra San Marcos. Bordering ranges are Sierra San Vicente and Sierra La Purísima to the east, Sierra La Fragua to the west, and Sierra de la Madera and Sierra Menchaca to the northwest and northeast, respectively (Pinkava, 1984). The CCB is a unique place because of its varied aquatic features, including lagoons, playas and associated gypsum dunes, rivers, subterranean tubes, artesian wells, ponds, and cienegas. The vegetation on the basin floor has been divided into several major zones: basin zacaton grasslands, aquatic and semi-aquatic habitats (sedges and marshes), gypsum dune assemblages, and a transition zone of shrubs and trees between the grasslands and the slopes at the foot of the steep limestone sierras that surround the region (Pinkava, 1979, 1984).

The CCB lies in the southeastern portion of the CD, the largest desert in North America, with an extension of over 450,000km^2, and one of the most diverse deserts in the world (Morafka, 1977; Álvarez & Ojeda, 2019). The degree of endemism exhibited by the fauna of the CCB implies long term isolation of the valley and its drainages (Meyer, 1973). However, the time and conditions in which these species originated is unclear, and some authors suggest that different taxa speciated at different times. For example, Milstead (1967) suggested that the ancestor of the endemic box turtle *Terrapene coahuila* became isolated in Cuatro Ciénegas sometime in the Pleistocene. Later, as the environment became more arid, selection favored mutations leading to the distinct aquatic adaptations of the living species. Taylor (1966) wrote that ancestral stocks common to the endemic snails became isolated in the early Tertiary or late Mesozoic. However, Johnson (2005) estimated a younger age for the endemic snail *Mexipyrgus churinceanus*, with the most recent common ancestor of living populations inferred to be approximately 2.5 million years old. Miller (1968) proposed a Miocene origin for one of the endemic fishes, suggesting that the unique spring and pond habitats those organisms require existed in the basin throughout the Cenozoic. On the other hand, Coghill et al. (2019) and Chaves-Campos et al. (2011) estimated an age of 1–1.75 and 1.9–11.2 million years for the origin of the endemic fish *Lepomis megalotis* and the shrimp *Palaemonetes suttkusi*, respectively. Finally, Gámez &

Castellano-Morales (2018) found robust evidence to consider the mountain areas of the CCB as a Pleistocene refuge for a large number of mammals of the CD.

The origin of the basin is quite remarkable, since it is possible that it has been in a stable landmass with similar climatic conditions for the last 15 millon years (Wilson & Pitts, 2010; Souza et al., 2012). If this is true, then the CCB has possibly served many times as a refuge for animal species as dissimilar as copepods and birds, and harbored speciation events in groups that are remarkably different such as mollusks and reptiles (Álvarez & Ojeda, 2019). The geology of the valley lends independent support to these postulations of environmental stability (Meyer, 1973).

Despite the fact that numerous phylogeographical studies have been conducted within the CCB (Johnson, 2005; Chaves-Campos et al., 2011; Coghill et al., 2019), it remains unclear how the combined effects of isolation by distance, environmental heterogeneity, or other barriers to gene flow have promoted the high diversity of the CCB. Testing for simultaneous divergence (vicariance) across different population-pairs that span the same historical barrier to gene flow provides insights into the effects of historical events, and whether species had similar responses (Hickerson et al., 2007). Herein, we investigate the evolutionary history of four microendemic species of reptiles (one snake, one turtle, and two lizards) to test for codivergence. The focal taxa occupy similar environments in the CCB, being associated with aquatic or semiaquatic habitats. However, they differ in important ecological characteristics such as body size and dispersal capabilities (García-Vázquez et al., 2019). Using mitochondrial and nuclear DNA sequence data, we test the hypothesis of simultaneous divergence across four endemic species. We generated time-calibrated phylogenetic trees using fossils and estimated rates of gene flow to test whether these species share the same phylogeographic breaks. In addition, we performed analyses on the multiple species data set to test for simultaneous versus non-simultaneous divergence.

2 | MATERIALS AND METHODS

2.1 | Study taxa and genetic data

Four species of reptiles endemic to the CC were used as focal species: two lizards (*Scincella kikaapoa* and *Gerrhonotus mccoyi*), a turtle (*Terrapene coahuila*), and a population of snake (genus *Storeria*) that likely represents an undescribed species (García-Vázquez et al., 2019). Herein, we refer to this undescribed species as *Storeria* sp. To determine the sister group of each taxon and estimate divergence times, representatives of all species of each genus, as well as external groups, were included in a first estimation of their phylogeny (Appendix S1).

DNA data were obtained from Genbank and from previous and ongoing projects investigating the evolutionary history and species limits of the different groups. For *Scincella*, the mitochondrial fragment included the genes that code for 16S ribosomal RNA (16S) and tRNA-Met (in part) as well as the full length of the genes coding for the NADH dehydrogenase subunit 1 (ND1) and associated tRNAs (Leu, Ile, and Gln), for a total of 962 base pairs (bp) for all individuals (n = 75). Additionally, two nuclear loci were sequenced for a subset of the specimens (n = 39) representing the main clades inferred from our mtDNA dataset, including 716 bp and 612 bp of the genes coding for the megakaryoblastic leukemia 1 (MKL1) and RNA fingerprint 35 (R35) proteins, respectively. For *Storeria*, we included data from two mitochondrial genes: a segment of 1011 bp of the gene coding for the NADH dehydrogenase subunit 2 (ND2) and 930 pb of the gene coding for the NADH dehydrogenase subunit 4 (ND4) for all individuals and outgroups (n = 16). For *Terrapene*, several previously published phylogenies included *Terrapene coahuila* (Spinks et al., 2009; Wiens et al., 2010; Martin et al., 2013); however, the relationships of this taxon are not consistent across them (see Fritz & Havas, 2014). In order to clarify the phylogenetic position of *Terrapene coahuila*, we performed a new analysis based on sequences from two mitochondrial and two nuclear genes. The mitochondrial fragment included segments of 1081 bp and 611 bp of the genes coding for cytochrome b (Cytb) and cytochrome oxidase I (COI), respectively. The nuclear segment included 905 bp of the gene coding for the intron 1 of RNA fingerprint 35 (R35) and 430 bp of the gene coding for the Glyceraldehyde-3-phosphate dehydrogenase (GAPD). Finally, for *Gerrhonotus mccoyi*, we analyzed the mitochondrial fragment (composed of partial sequences of the gene coding for the ND4, complete sequences of the genes coding for the tRNA-His and tRNA-Ser, and partial sequences of the gene coding for tRNA-Leu) and the nuclear fragment composed of partial sequences of the genes coding for the brain-derived neurotropic factor (BDNF) and the prolactin receptor (PRLR) from the phylogenetic study of García-Vázquez et al. (2018a), as well as the same fragments from seven additional samples. In all cases, the analyzed loci were selected because they have been previously shown to be informative at different levels of divergence within the different groups (Alfaro & Arnold, 2002; Bryson & Riddle, 2012; Martin et al., 2013; García-Vázquez et al., 2018a; Pavón-Vázquez et al., 2018).

In order to evaluate synchrony of divergence across the different taxa, we defined two populations for each taxon based on our phylogenetic reconstructions. The first population included representatives of the CCB endemic species and the second included representatives of its respective sister species/clade. Partial sequences of the mitochondrial gene coding for the NADH dehydrogenase subunit 4 (ND4), complete sequences of the genes coding for the tRNA-His and tRNA-Ser, and partial sequences of the gene coding for the tRNA-Leu, for a total of 980 bp, were obtained from 71 samples representing 15-22 individuals from each pair of populations (Table 1; Appendix S2). Primer sequences for ND4 are given in Arévalo et al. (1994); for *Scincella*, in Pavón-Vázquez et al. (2018); for *Storeria* (ND2), in de Queiroz et al. (2008), and for *Gerrhonotus* in García-Vázquez et al. (2018). We focused this last part of the study on mtDNA loci because they represent rapidly evolving and coalescing genomic regions that often reflect population history (Zink & Barrowclough, 2008; Bagley et al., 2018). The GenBank accession numbers for published and new DNA sequences obtained for this paper are provided in Appendixes S1 and S2. For technical details on DNA sequencing and sequence edition see Appendix S3.

2.2 | Phylogenetic reconstruction

For each focal taxon, the phylogeny was inferred using Bayesian inference methods. Partitioned analyses were carried out to improve phylogenetic accuracy. The best-fitting substitution models and partitioning schemes were selected jointly using the Bayesian Information Criterion in the software PARTITIONFINDER v1.1.1 (Lanfear et al., 2012). Bayesian inference analyses were conducted using MRBAYES v3.2.1 (Ronquist et al., 2012). Four runs were conducted using the ‘nruns = 4’ command, each with three heated and one cold Markov chains with sampling every 1000 generations for 60 million generations. Output parameters were visualized using TRACER v1.4 (Rambaut & Drummond, 2007) to ascertain stationarity and convergence. The first 25% of the generations were discarded as burn-in. Nodes were considered strongly supported if Bayesian posterior probability was ≥ 0.95 (Huelsenbeck & Rannala, 2004).

2.3 | Divergence time estimation

The phylogeny and divergence times were estimated simultaneously for each focal taxon using a relaxed Bayesian molecular clock framework implemented in BEAST v1.6.1 (Drummond & Rambaut, 2007). To estimate divergence times, each dataset was used with a relaxed, uncorrelated lognormal clock and node constraints obtained from the fossil record with lognormal distributions. The same partition strategy implemented in the corresponding phylogenetic analysis (see above) was used. Different fossil calibration points for each focal taxon were used (Appendix S4).

Analyses were run for 50 million generations, samples were retained every 1000 generations, and a Yule tree prior was specified. Results were displayed in TRACER v1.5 to confirm acceptable mixing and likelihood stationarity of the Markov chain Monte Carlo (MCMC) analyses, appropriate burn-in, and adequate effective sample sizes (>200 for each estimated parameter). After discarding the first five million generations (10%) as burn-in, parameter values of the samples from the posterior were summarized on the maximum clade credibility tree using TREEANNOTATOR v1.4.8 (Drummond & Rambaut, 2007) with the posterior probability limit set to 0.1 and mean node heights summarized.

2.4 | Gene flow

We used IMa (Hey & Nielsen, 2007) to estimate gene flow between populations from the CCB and their respective sister groups as well as confidence intervals for migration rates; these estimates were used as priors for tests of simultaneous diversification using Approximate Bayesian Computation (ABC) methods. IMa analyses were performed with burn-in periods of 1000000 steps, with 30000000 steps in the chain following burn-in. Convergence was assumed when effective sample sizes (ESSs) were greater than 50 for all parameters (Hey & Nielsen, 2007). We obtained the lower and upper probability density estimates of migration rates between populations (*m1*, *m2*), which were converted to the effective number of migrants per generation by using estimates of theta (Ornelas et al., 2013).

2.5 | Test for synchronous diversification

If the same event had similarly impacted on the diversification of different lineages, it would be expected that these lineages would share similar divergence times across the event. However, incongruence in divergence times between taxon pairs does not rule out the possibility of simultaneous divergence. Even in a speciation event, differences in times of divergence can also be due to variance in the coalescent process; the difference in estimated divergence time may represent a discrepancy between gene divergence time and population divergence time, which is related to the demography of each particular species (Edwars & Berrli, 2000; Ornelas et al., 2013). Here, to address this issue, a hierarchical approximate Bayesian computation (hABC) method implemented in the program msBayes

(Hickerson et al., 2006) was employed to test the hypotheses of a single (synchronous) versus multiple pulses of diversification of four endemic CCB species. MsBayes implements an approximate Bayesian computation approach using a hierarchical coalescent model where hyper-parameter estimation is utilized to discriminate the differences in time of divergence among pairs of taxa in coalescent times (Hickerson et al., 2006; Daza et al., 2010); thus, the hABC method reasonably distinguishes simultaneous isolation from temporal incongruence in the divergence, even with sparse sampling of individuals, and has been shown to be effective over a range of conditions with a single locus (Hickerson et al., 2006; Ornelas et al., 2013) and with population sample size of five or less (Hickerson et al., 2007; Topp et al., 2013).

We allowed the maximum value for Theta to be estimated as part of the analysis. The obtained value was 0.1711312, which is appropriate for mtDNA (Ornelas et al., 2013). The prior for the upper limit of Tau was set to 3 based on the divergence time obtained from the BEAST analysis. The prior for the maximum possible number of divergence events (Ψ) was set to be equal to the number of linage pairs evaluated. Because gene flow after divergence can affect coalescent processes, the mean migration rate (obtained from the IMa analysis) was incorporated as prior information in the simulation stage of msBayes. For the analysis we drew 3×10^6 samples from the hyper-prior and, using the hierarchical approximate Bayesian computation acceptance/rejection algorithm, constructed the hyper-posterior from 1000 samples with a tolerance of 0.0005, because this sampling parameter showed better resolution in the posterior probability density graph. Hickerson et al. (2007) recommends using both Ψ and Ω ($=\mathrm{Var}(\tau)/\mathrm{E}(\tau)$) to evaluate the relative strengths of each hypothesis, where a low value (approximately 0) of Ω and a $\Psi=1$ suggest the data fit a simultaneous model; thus, we used both statistics to evaluate the relative support for each hypothesis (Ornelas et al., 2013).

3 | RESULTS

3.1 | Study taxa and genetic data

Except for *Storeria* sp., our estimated phylogenies revealed a sister-taxon relationship between populations in the CCB and populations mostly found in eastern USA (Fig. 1). In *Storeria*, the CCB population appeared as sister taxon to the populations of *St. dekayi* from the Sierra Madre Oriental of Mexico. The estimated phylogenies for *Gerrhonotus* (García-Vázquez et al., 2018a) and *Scincella* and *Terrapene* (Appendix S5) are similar to those in previous studies of their phylogenetic relationships (Spinks et al., 2009; Linkem et al., 2011; Pyron et al., 2016; Spinks et al., 2016). In the phylogenies estimated herein, *G. mccoyi* was the sister taxon to populations of *G. infernalis* from Texas, USA (García-Vázquez et al., 2018); *S. kikaapoa* was the sister taxon to *S. lateralis*; *Storeria* sp. was the sister taxon to Mexican populations of *St. dekayi* from Puebla and Hidalgo, Mexico; and *Terrapene coahuila* was the sister species to *T. carolina* (including *T. c. carolina* and *T. c. major*; Martin et al., 2013). In all cases, the relationship of the CCB endemic population and its sister taxon was strongly supported (Appendix S5).

In all cases, the sister taxa of the CCB endemic populations have broad geographical distributions. However, in some or most cases this may be due to poorly resolved taxonomy. Because our results corroborate the paraphyly of *T. c. major* with respect to *T. c. carolina* previously documented by Butler et al. (2011) and Martin et al. (2013), here we consider *T. c. carolina* and *T. c. major* as a single lineage (but see Martin et al., 2013). Combined, these taxa are distributed throughout much of the eastern United States, from New York and Kansas south to central Texas and northern Florida (Dodd, 2001). Similarly, *S. lateralis* is found throughout much of eastern USA, from New Jersey, Ohio, and Kansas south to Texas and Florida, as well as in northern Mexico (García-Vázquez et al., 2010; Jackson & Austin, 2010). However, despite this large distribution, it should be noted that only samples from Texas were included in the phylogenetic analysis. In the remaining cases, *Storeria* sp. and *Gerrhonotus mccoyi* were the sister taxa to a clade of *S. dekayi* from Hidalgo and Puebla in the Sierra Madre Oriental, Mexico, and a clade of *G. infernalis* from Texas (Fig. 1), respectively. However, whereas *S. dekayi* and *G. infernalis* (as currently recognized) have both large geographic distributions, they were paraphyletic with regard to the endemic CCB populations of *Storeria* and *Gerrhonotus*, respectively, and it seems likely that they represent composite species rather than single lineages (García-Vázquez et al., 2018a, and personal observation). However, additional study is needed to evaluate their species limits.

3.2 | Divergence time estimates and gene flow

The CCB endemic reptiles included in our study do not appear to share the same temporal pattern of divergence (Fig. 1). The mean divergence times from BEAST ranged from 3.19 Ma (late Pliocene) for *Gerrhonotus* to 8.72 Ma (late Miocene) for *Terrapene*; the additional divergence events represent the split between *Storeria* sp. and the populations of *St. dekayi* from the Sierra Madre Oriental in the mid Pliocene (4.03 Ma) and the split between *Scincella kikaapoa* and *S. lateralis* in the early Pliocene (5.1 Ma) (Table 1). When the 95% highest posterior densities (HPDs) are considered, these results suggest that diversification in the CCB has occurred multiple times between the late Miocene and the late Pliocene. Importantly, the 95% HPDs of the most recently diverged populations do not overlap with those of the more ancient population divergences (Table 1; Fig. 2). Estimates of gene flow between the CCB endemic populations and their respective sister groups were high in all cases (0.30-0.38 migrants per generation; m/g) (Table 2).

3.3 | Test for synchronous diversification

In testing for simultaneous divergence across the four reptile species endemic to the CCB, the msBayes estimates of Omega (Ω) for each of the splits showed no support for simultaneous divergence. The values of Ω (a parameter that measures the incongruence among divergence times along the same phylogeographic barrier) for these four species indicate non-simultaneous divergence (Ω mode = 0.122; Ω mean [95% quantiles] = 0.292 [0.032-0.931]). In fact, we detected two distinct episodes of divergence (Ψ mode = 2) among these taxa (Fig. 3). Mean values for the tMRCA suggest that the turtle *Terrapene coahuila* split in more ancient times, and the lizards *Scincella kikaapoa* and *Gerrhonotus mccoyi* as well as the snake *Storeria* sp. experienced more recent divergence events. Additionally, mean values for the tMRCAs suggest that the splits for most of these endemic reptiles occurred during the Pliocene (Fig. 2).

4 | DISCUSSION

4.1 | Phylogenetic relationships

The phylogenetic relationships recovered for *Terrapene* and *Scincella* were concordant with previous studies (Pyron et al., 2013; Martin et al., 2014); yet, our analyses contained lineages and populations that had not been previously sampled, including those from the CCB. *Terrapene coahuila* was the sister species of *Terrapene carolina*, composed of *T. c. carolina* and *T. c. major* (Martin et al., 2013). As in previous studies of *Scincella*, we recovered a clade composed by *S. assata*, *S. cherriei*, and *S. gemmingeri* to the exclusion of *S. lateralis* (Pyron et al., 2013), with the addition of *S. kikaapoa* as sister to *S. lateralis*. On the other hand, whereas in a previous phylogenomic study Pyron et al. (2016) found strong support for *Storeria storerioides* + (*St. dekayi* + *St. occipitomaculata*) and all species were monophyletic, our analyses recovered *St. dekayi* as sister taxon to *St. occipitomaculata* + *St. storerioides*. Interestingly, whereas Pyron et al.'s (2016) samples of *St. occipitomaculata* were from eastern United States, our samples came from the Sierra Madre Oriental in Mexico. Previous to Pyron et al.'s (2016) work, the latter populations were assigned to *St. hidalgoensis*, in this work the authors synonymized this taxon with *St. occipitomaculata* based on the morphology of a single specimen. However, our results suggest that *St. hidalgoensis* might be a valid species distinct from *St. occipitomaculata*. Also, because in our analyses the Mexican populations of *St. dekayi* were recovered as sister taxon to *Storeria* sp. CCB, *St. dekayi* may be composed of multiple evolutionary independent lineages. However, additional research with a more extensive sampling and including all known populations of *Storeria* is needed to confirm this. Finally, the samples of

Gerrhonotus generated in this study and included in our analysis in addition to those of García-Vázquez et al. (2018a) did not alter the relationships among the lineages found in the latter study.

4.2 | Biogeographic affinities of the endemic reptile species

Our estimated phylogenies for *Gerrhonotus*, *Scincella*, and *Terrapene* revealed a close relationship between populations in the CCB and populations mostly found in eastern USA. On the other hand, *Storeria* sp. CCB was sister to the population of *St. dekayi* from the Sierra Madre Oriental (Fig. 1). Studies that have evaluated the phylogenetic relationships of species endemic to the CCB are limited. However, in congruence with our results, similar patterns have been found in endemic fishes and scorpions. Specifically, a sister relationship between species endemic to the CCB and species from the southeastern USA was recovered for the fish genera *Etheostoma* and *Herichthys* (Hulsey at al., 2004; Near et al., 2011). Furthermore, Ennen et al. (2017) found a strong association between the turtles from the Texas Coast–Southern High Plains Aquatic Province (which includes the Cuatro Cienegas Basin) and the provinces of eastern USA. The province of Texas Coast–Southern High Plains Aquatic Province is considered a remnant of a Late Miocene Great Plains surface that persists via protection from fluvial erosion (Thornbury, 1965; Trimble, 1990). These relationships are apparently related to a complex history of isolation in the Rio Grande Basin (Ennen et al., 2017; García-Vázquez et al., 2018a).

On the other hand, a close relationship between the taxa endemic to the CCB and species from the Sierra Madre Oriental has been observed in numerous taxa, including fish (i.e., *Cyprinella*, Schönhuth et al., 2006; *Cyprynodon*, Haney et al., 2009; *Gambusia*, Lydeard et al., 1995; and *Xiphophorus*, Kang et al., 2013) and scorpions (i.e., *Chihuahuanus*, González-Santillán & Prendini, 2015).While the CCB lies in the southeastern portion of the CD, the mountains that surround the CCB have been considered the northernmost portion of the Sierra Madre Oriental by several authors (Meyer, 1973; Espinosa et al., 2014), given the presence in the former mountains of species associated to the conifer forests of the Sierra Madre Oriental (Pinkava, 1984).

4.3 | Diversification of the endemic species

Determining whether species assemblages experienced shared evolutionary responses to historical/geological and climate-change events is a central but challenging goal of comparative phylogeography (Arbogast & Kenagy, 2001; Bagley et al., 2018). The hABC method for comparative phylogeography used in this study incorporates intrinsic variation in ancestral coalescence and among-taxon demographic histories (Hickerson et al., 2006; 2007; Topp et al., 2013). The approach accounts for potentially confounding stochastic coalescent effects while estimating parameters of phylogeographic datasets in a way that sidesteps the need to calculate explicit likelihood functions (Bagley et al., 2018). In this way, hABC allows testing explicit hypotheses about the timing of genetic divergences that have arisen during the assembly and diversification of regional species assemblages. These methods are effective even with population sample sizes of five or less (Hickerson, et al., 2007; Topp et al., 2013). We used hABC coalescent models and Bayesian estimates of divergence times with a relaxed molecular clock to investigate temporal patterns of diversification of four reptiles endemic to the CCB to test two hypotheses about the origin (synchronic or asynchronic) of these species.

Using hABC, we detected two non-simultaneous divergence events: one exclusive to a single taxon and the other shared by three lineages consistent with our Bayesian divergence dating analyses. We detected a first event for the origin of *Terrapene coahuila* during the late Miocene and a second event for the origin of *Gerrhonotus mccoyi, Scincella kikaapoa*, and *Storeria* sp. in the CCB during the Pliocene (Fig. 1; Table 1). Thus, our results suggest that divergence of the lineages endemic to the CCB occurred during the Neogene, indicating that Pleistocene glacial cycles did not play a central role in their origin, contrary to traditional views for mammals (Findley, 1969), other non-avian reptiles (Morafka, 1977), and birds (Hubbard, 1973) from the Sonoran and Chihuahuan regional deserts (Riddle et al., 2000). The diversification of the CCB lineages was likely the result of a variety of factors associated with the formation of new habitats, namely major climatic changes, orogeny events during the Miocene, and global shifts in vegetation (Zachos et al., 2001; Leavitt et al., 2012).

4.4 | Biogeographical reconstruction

The CCB represents a relict zone, where pluvial periods during or immediately following the Pleistocene apparently caused a gradual withdrawal of the eastern border of the Chihuahuan Desert, which permitted the colonization of more mesic-adapted lineages from the east (Milstead, 1960; Morafka, 1977). Most of these colonists presumably perished with the return of desert conditions, but some lineages remained as relicts clinging to a precarious existence around oases (García-Vázquez et al., 2018b). This is ecologically concordant with the fact that the genera included in this study are mostly mesic-adapted, while the species of the CCB currently occupy habitats exclusively associated with water bodies (Morafka, 1977; García-Vázquez et al., 2019). However, the focal CCB lineages appear to have originated prior to the Pleistocene.

According to our results, the origin of the reptiles endemic to the CCB seems to be related with the emergence of the Chihuahuan desert, associated with the Neogene aridification of the Northern Hemisphere (Morafka, 1977), and with orogenic events during the Miocene (Bryson et al., 2013). While there is ample evidence that the Chihuahuan Desert region was drying during the Miocene, it is unclear how far-reaching this desertification was (Wilson & Pitts, 2010). Microfossil floras, for example, show that cooler, wetter climates were present in much of the Chihuahuan Desert during the late Miocene (8 Ma) relative to the present (Graham, 1987). Based on the various dates given for mountain building and desert formation, Wilson & Pitts (2010) suggest that evolutionary events dating anywhere between 15 and 2 Ma could be considered associated with mountain building and desert formation activity.

An event that could be associated with the origin of some reptiles endemic to the CCB is the expansion of savanna habitats in the late Miocene. Presumably, during the Late Miocene the continuity between the Gulf Coast Fauna and the Plains onto the Mexican Plateau represents an eastward expansion of the savanna corridor that earlier had been largely confined to the Rio Grande Trench (Webb, 1977). The Late Miocene expansion of woodland savanna around much of the Gulf Coast also produced a major disjunction between the mesic forest of the eastern United States and those farther south (Estes, 1970; Martin, 1958; Martin & Harrel, 1957; Webb, 1977). In association with the isolation of the CCB caused by orogeny events in the Early and Middle Miocene (Chavez-Cabello et al., 2005; Wilson & Pitts, 2010), the transformation of an originally more continuous landscape to one of isolated forest patches may have provided opportunities for rapid, early diversification of the ancestor of *Terrrapene coahuila*.

In a biogeographic study of the *Gerrhonotus infernalis* clade, García-Vázquez et al. (2018a) found evidence for a vicariant event between *G. mccoyi* and its sister species associated with the expansion of the southern drainage of the Rio Grande at the end of the Pliocene. This expansion is associated with dispersal and subsequent isolation events between the Mexican Plateau and surrounding sierras (2.7–4.1 Ma), and probably explains the origin of some endemic relict species in the southern portion of the Chihuhuan Desert, including *G. mccoyi* and *Scincella kikaapoa*. Additionally, uplift of the Central Mexican Plateau coupled with the subsequent aridification and late Pliocene development of the Chihuahuan Desert (Jaeger et al., 2005; Bryson et al., 2011c) may have played a role in the final isolation and speciation of some reptiles endemic to the CCB, including *Storeria* sp. However, whereas the origin of *Storeria* sp. may be associated with the uplift and subsequent aridification of the Central Mexican Plateau, unlike *Gerrhonotus* and *Scincella* the ancestor of *Storeria* sp. CCB was probably distributed in the Sierra Madre Oriental.

Historically, the mountains that surround the CCB have been considered part of the Sierra Madre Oriental (Meyer, 1973; Espinosa et al., 2014). However, the Sierra Madre Oriental appears to be divisible into some unique regions (Salinas-Moreno et al., 2004; Espinosa et al., 2014). Several mesic taxa display distinct genetic breaks across lowlands devoid of coniferous forest (Farjon & Styles, 1997; Bryson et al., 2011a; McCormack et al., 2008; Bryson et al., 2012b). Lowlands with these characteristics appear to be dividing the distributions of *Storeria* sp. CCB and the populations of *S. dekayi* from the Sierra Madre Oriental. Climate change in the area that was punctuated by a dramatic shift in atmospheric conditions during the late Pliocene (Retallack, 1997; Bryson et al., 2013), or perhaps the appearance of filter barriers such as major river drainages (Bryson et al., 2011a), could have promoted the dispersion and subsequent separation of the ancestor of *Storeria* sp. CCB, a pattern observed in several mesic species (Anducho-Reyes et al., 2008; McCormack et al., 2008; Moreno-Letelier & Piñero, 2009; Bryson et al., 2011a).

Finally, the high estimates of gene flow between the CCB endemic populations and their respective sister groups suggest recent divergence (Ornelas et al., 2013). However, expansions of pine-oak woodlands during Pleistocene glacial cycles (Bryson et al., 2011b; Gugger et al., 2011) may have promoted dispersal and periodic bouts of gene flow that could have erased or obscured previously acquired signals of historical isolation (Bryson et al., 2011a). Similar patterns of deep genetic subdivisions blurred by secondary contact have been observed in other mesic North American taxa, like Pines, Aves and Reptiles (Moreno-Letelier & Piñero, 2009; Ornelas, et al., 2010; Bryson et al., 2011b).

5 | CONCLUSIONS

Our comparative data suggests that although some lineages appear to have spatially congruent distributions and genetic breaks, there are differences in the species' geographic origin, and divergences across spatial boundaries are asynchronous among some lineages. The reptile species endemic to the CCB show specialized ecological requirements for humid habitats, and therefore are closely associated with geological features, like the isolation of the CCB. These lineages show signatures of pre-Pleistocenic divergence, in contrast with the diversification times of other taxa that also inhabit the North American deserts. These endemic species may thus offer novel insights into the deeper biogeographic history of the highland landscapes of southeastern North America.

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Table 1. Total numbers of sequenced base pairs and sampled individuals for each taxon in the BEAST analysis. Also presented are the divergence time estimates.

Taxon	Locus (bp)	Number of samples (phylogeny/divergence times)	Divergence time estimates (95% HDP)	Period
Gerrhonotus	ND4 (980), BDNF (720), PRLR (627)	86/46	3.19 Ma (1.86 Ma-5.17 Ma)	Late Pliocene

Scincella	ND1 (962), BDNF (716), R35 (612)	57/40	5.1 Ma (2.51 Ma-6.09 Ma)	Early Pliocene
Storeria	ND2 (1011), ND4 (930)	17/17	4.03 Ma (2.07 Ma-6.03 Ma)	Middle Pliocene
Terrapene	cytb (1081), COI (611), GAPD (430), R35 (905)	24/24	8.72 Ma (6.18 Ma-10.67 Ma)	Late Miocene

Table 2. IMa **e**stimates of gene flow between populations of each focal taxon included in this study.

Taxon	Sample size	m_1			m_2		
		mean	HPD95%Lo	HPD95%Hi	mean	HPD95%Lo	HPD95%Hi
Gerrhonotus	10/10	0.3712	0.0125	0.8835	0.4539	0.0365	0.9235
Scincella	9/13	0.3024	0.0085	0.8125	0.2608	0.0065	0.7555
Storeria	9/7	0.3185	0.0095	0.8355	0.3065	0.0085	0.8245
Terrapene	9/6	0.3385	0.0105	0.8595	0.3464	0.0105	0.8665

Biosketches. The authors share a general interest in the systematics and biogeography of amphibians and reptiles from Mexico. Author contributions: U.O.G.V. and A.N.M.O. conceived the project, analyzed the data, collected specimens and data, and shared in the writing.

Figure 1. Left: BEAST maximum clade credibility (MCC) time tree for all four focal species/lineage splits across the Cuatro Cienegas Basin (CCB). Tip labels are sequence codes used in Appendix S3, colored according to biogeographical position (i.e., in CCB versus outside CBB for sister taxa). Horizontal bars are 95% highest posterior densities (HDPs) for node ages. Scale bars: 10 million years. Rigth: Distribution of samples used in the test for synchronous diversification.

Figure 2. Posterior density plot of divergence times for endemic focal species from the Cuatro Cienegas Basin (CCB).

Figure 3. Result of the ABC analyses using msBayes. Posterior probability densities for Ω (left) and the approximate joint posterior estimates of Var(τ)/E(τ)) against (E(τ) (right) between lineage pairs of lizards, snakes, and turtles. Prior (dashed red lines) and posterior (solid blue lines) distributions.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1: List of samples used in this study and GenBank accession numbers.

Appendix S2: List of samples used in the test for synchronous diversification in this study and GenBank accession numbers.

Appendix S3: Technical details on DNA sequencing and sequence edition.

Appendix S4: Technical details on fossils utilized for the divergence time estimates.

Appendix S5: Genetic structure within *Scincella*, *Storeria*, and *Terrapene* inferred from Bayesian analyses of their respective DNA sequence datasets.

Fig. 1

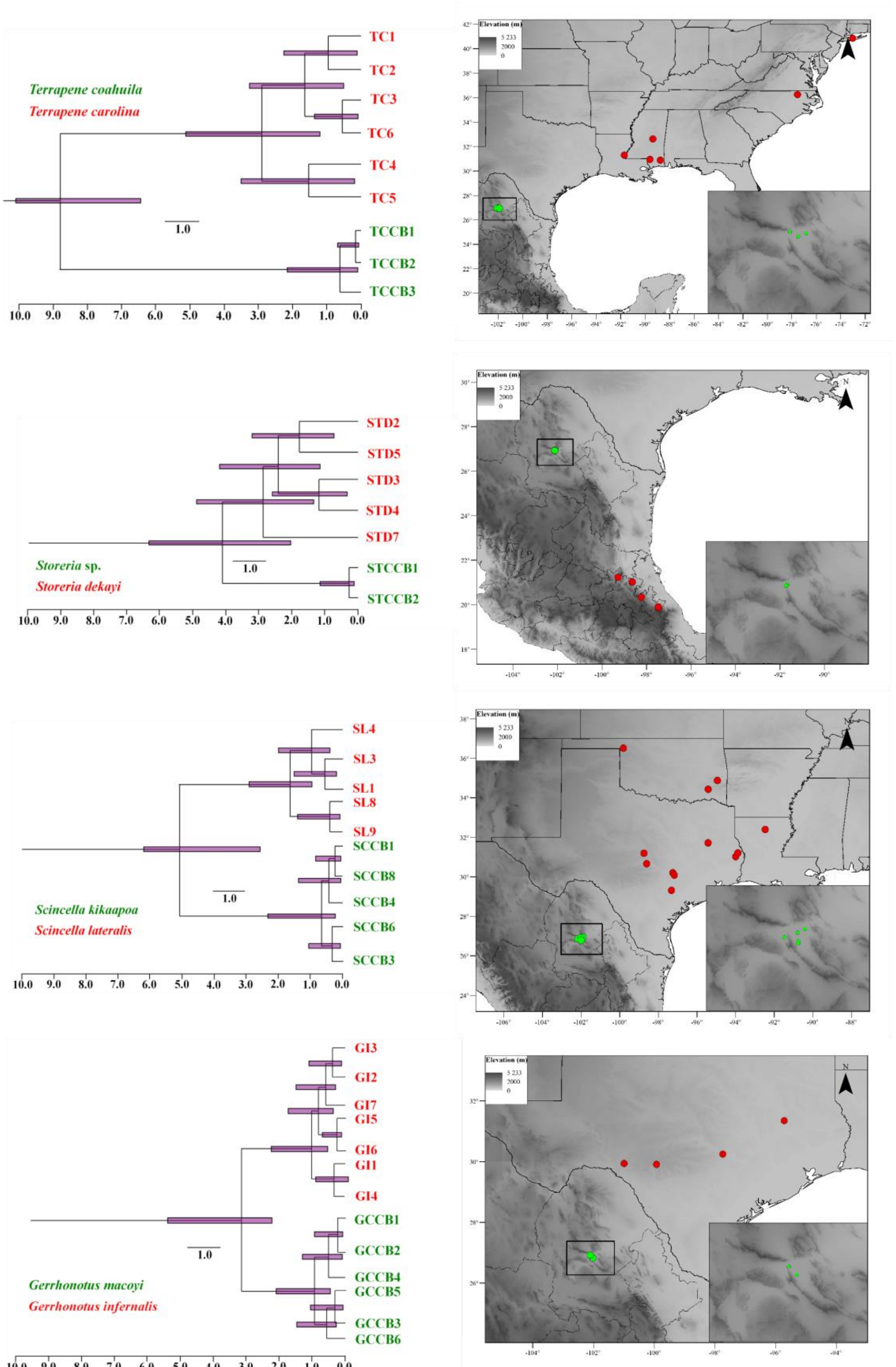
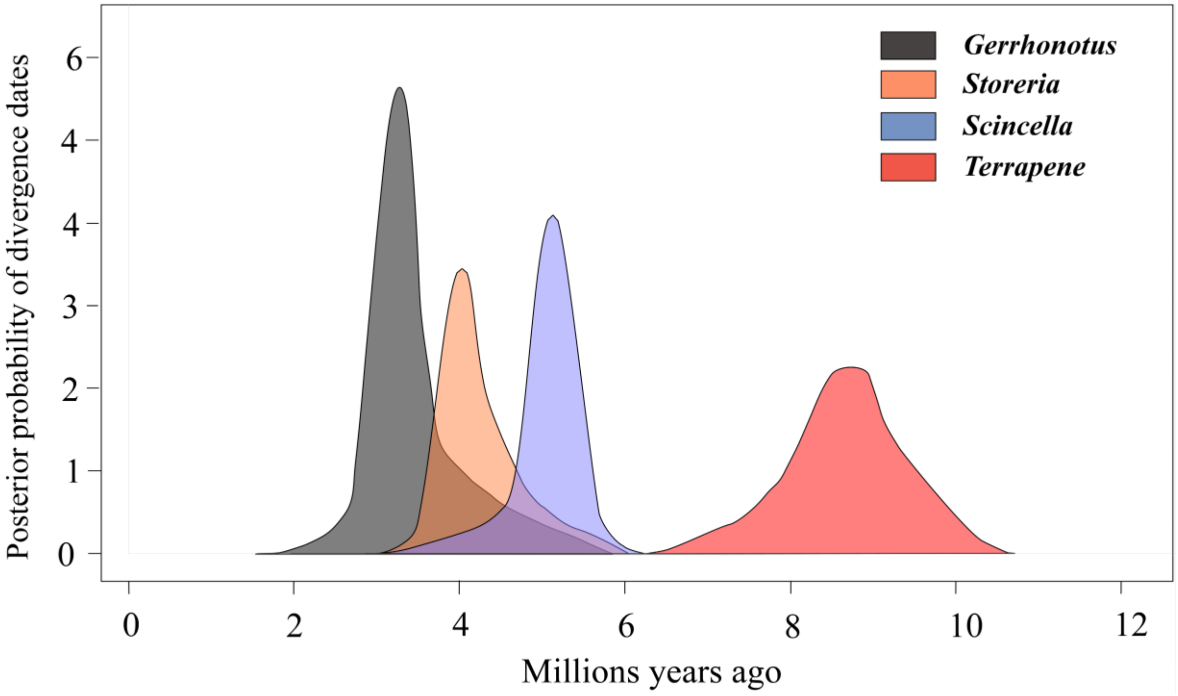
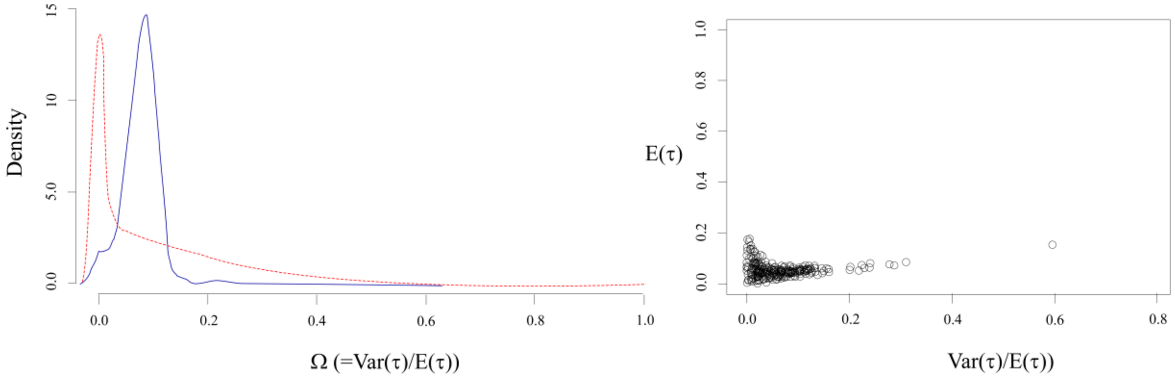

Terrapene coahuila
Terrapene carolina
TC1
TC2
TC3
TC6
TC4
TC5
TCCB1
TCCB2
TCCB3
1.0
10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
Elevation (m)
5 233
2000
0
STD2
STD5
STD3
STD4
STD7
STCCB1
STCCB2
Storeria sp.
Storeria dekayi
SL4
SL3
SL1
SL8
SL9
SCCB1
SCCB8
SCCB4
SCCB6
SCCB3
Scincella kikaapoa
Scincella lateralis
GI3
GI2
GI7
GI5
GI6
GI1
GI4
GCCB1
GCCB2
GCCB4
GCCB5
GCCB3
GCCB6
Gerrhonotus macoyi
Gerrhonotus infernalis
N

Fig. 2



Gerrhonotus
Storeria
Scincella
Terrapene
Posterior probability of divergence dates
6
4
4
3
2
1
0
0
2
4
6
8
10
12
Millions years ago

Fig. 3


Density
15
10
5.0
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Ω (=Var(τ)/E(τ))
E(τ)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
Var(τ)/E(τ))

Appendix S1. Taxon, locality and museum voucher data, and gene sampling for genetic samples used in this study. Institutional codes for museum and collections follow Sabaj-Pérez (2016). ANMO, UOGV, RLR, LCM, IDF, ENS, AMH, JAC, CVS, JJW, FHH and TJL are field identifiers for uncatalogued specimens being deposited in the MZFC. * = Used as outgroup in the phylogenetic reconstruction in MrBayes; ** = used as outgroup for divergence time estimation (fossil calibration) in Beast; *** used as outgroup in both phylogenetic reconstruction and divergence time estimation.

Scincella

Scincid lizard genus *Scincella* is composed of eight species in America: *Scincella assata* with two subspecies (*S. a. assata* and *S. a. taylori*), *S. cherriei* with three subspecies (*S. c. cherriei*, *S. c. ixbaac* and *S. c. stuarti*), *S. gemmingeri* with two subspecies (*S. g. forbesorum* and *S. g. gemmingeri*), *S. incerta*, *S. kikaapoa*, *S. lateralis*, *S. rara* (not included in this study) and *S. silvicola* with two subspecies (*S. s. caudaequinae* and *S. s. silvicola*) (García-Vázquez et al., 2010; Linkem et al., 2011).

Taxon	Country	State	Locality	Voucher	References	Genbank access			Key
						R35	ND1	MKL1	
Aspidoscelis tigris **	USA	California		MVZ179799	Brandley et al., 2011	HM161145	HM160767	-	Sc1
*Cordylus sp.***	Africa		"Africa"	No voucher	Schmitz et al., 2005	-	AY315566	-	Sc2
*Gerrhosaurus major***	Somalia	Awdal		MVZ241366	Brandley et al., 2011	HM161062	HM160779	HM161157	Sc3
Lepidophyma flavimaculatum **				NUM-Az372	Kumazawa, 2007	-	AB162908	-	Sc4
Marisora unimarginata *	México	Guerrero	Chilpancingo	UOGV-628	This study	xxxxx	xxxxx	xxxxx	Sc5
Plestiodon brevirostris **	México	Tlaxcala		IDLH-16	Brandley et al., 2011	HM161075	HM160701	HM160701	Sc6
Plestiodon lynxe **	México	San Luis Potosí		LSUMZ-H14823	Brandley et al., 2011	HM161112	HM160829	HM161207	Sc7
Scincella assata assata	México	Chiapas	Pijijiapan	RLR-1094	This study	-	xxxxx	-	Sc9
Scincella a. taylori	México	Colima	Comala	UOGV-718	This study	xxxxx	xxxxx	xxxxx	Sc8
Scincella a. taylori	México	Guerrero	Chilpancingo	UOGV-629	This study	xxxxx	xxxxx	xxxxx	Sc71
Scincella cherriei cherriei	México	Oaxaca	La gringa	LCM-260	This study	xxxxx	xxxxx	xxxxx	Sc10
Scincella c. cherriei	Honduras			ENS-10902	This study	xxxxx	xxxxx	xxxxx	Sc11

Taxon	Country	State	Locality	Voucher	References	Genbank access			Key
						R35	ND1	MKL1	
Scincella c. cherriei	Guatemala			ENS-7113	This study	xxxxx	xxxxx	xxxxx	Sc12
Scincella c. cherriei	Costa Rica			MF-6067	This study	xxxxx	xxxxx	xxxxx	Sc13
Scincella c. ixbaac	México	Campeche		ISZ-211	This study	xxxxx	xxxxx	xxxxx	Sc14
Scincella c. stuarti	México	Veracruz	Las Choapas	JLAL	This study	xxxxx	xxxxx	xxxxx	Sc15
Scincella c. stuarti	México	Veracruz	Orizaba	UOGV-342	This study	xxxxx	xxxxx	xxxxx	Sc16
Scincella cf. gemmingeri	México	Oaxaca	Cerro Baul	JAC-23168	This study	-	xxxxx	-	Sc17
Scincella cf. gemmingeri	México	Oaxaca		JAC-23175	This study	-	xxxxx	-	Sc18
Scincella cf. gemmingeri	México	Chiapas	Tuxtla Gutierrez	IDF-163	This study	xxxxx	xxxxx	xxxxx	Sc19
Scincella cf. gemmingeri	México	Oaxaca	2.1 mi NE Totontepec	JAC-21543	This study	-	xxxxx	-	Sc20
Scincella cf. gemmingeri	México	Oaxaca	Santa Maria Guienagati	JAC-22952	This study	xxxxx	xxxxx	xxxxx	Sc21
Scincella cf. gemmingeri	México	Oaxaca	Santa Maria Guienagati	JAC-23139	This study	-	xxxxx	-	Sc22
Scincella cf. gemmingeri	México	Oaxaca	Pluma Hidalgo	ICS-25	This study	-	xxxxx	-	Sc23
Scincella cf. gemmingeri	México	Oaxaca	Pluma Hidalgo	UOGV-1761	This study	-	xxxxx	-	Sc24
Scincella cf. gemmingeri	México	Oaxaca	Pluma Hidalgo	UOGV-1763	This study	xxxxx	xxxxx	xxxxx	Sc25
Scincella cf. gemmingeri	México	Veracruz	Los Tuxtlas	No voucher	This study	-	xxxxx	-	Sc26
Scincella cf. gemmingeri	México	Veracruz	Los Tuxtlas	UOGV-378	This study	xxxxx	xxxxx	xxxxx	Sc27
Scincella gemmingeri forbesorum	México	Hidalgo	Jacala	AMH-687	This study	-	xxxxx	-	Sc28
Scincella g. forbesorum	México	Hidalgo	Jacala	UOGV-1373	This study	xxxxx	xxxxx	xxxxx	Sc29
Scincella g. gemmingeri	México	Hidalgo		DMA-31	This study	-	xxxxx	-	Sc30
Scincella g. gemmingeri	México	Veracruz	La Selva	UOGV-500	This study	-	xxxxx	-	Sc31
Scincella g. gemmingeri	México	Hidalgo		MZFC-14215	This study	-	xxxxx	-	Sc32
Scincella g. gemmingeri	México	Puebla	Zacatlán	FHH-122	This study	-	xxxxx	-	Sc33
Scincella g. gemmingeri	México	Puebla	Tlatlauquitepec	ISZ-137	This study	-	xxxxx	-	Sc34
Scincella g. gemmingeri	México	Puebla	Zacapoaxtla	UOGV-115	This study	-	xxxxx	-	Sc35
Scincella g. gemmingeri	México	Puebla	Zacapoaxtla	UOGV-112	This study	xxxxx	xxxxx	xxxxx	Sc36
Scincella g. gemmingeri	México	Oaxaca	Totontepec	ANMO-2295	This study	xxxxx	xxxxx	xxxxx	Sc37
Scincella g. gemmingeri	México	Oaxaca	Totontepec	ANMO-2296	This study	-	xxxxx	-	Sc38
Scincella g. gemmingeri	México	Oaxaca	Vista Hermosa	JAC-21632	This study	-	xxxxx	-	Sc39
Scincella g. gemmingeri	México	Oaxaca	Peña del Aguila	CVS-3	This study	-	xxxxx	-	Sc40
Scincella g. gemmingeri	México	Oaxaca	Vista Hermosa	JJW-794	This study	-	xxxxx	-	Sc41
Scincella g. gemmingeri	México	Veracruz	La Perla	JAC-22567	This study	-	xxxxx	-	Sc42
Scincella g. gemmingeri	México	Veracruz	La Perla	UOGV-223	This study	xxxxx	xxxxx	xxxxx	Sc43
Scincella g. gemmingeri	México	Veracruz	Xico	JAC-24979	This study	-	xxxxx	-	Sc44
Scincella g. gemmingeri	México	Oaxaca		UOGV-242	This study	xxxxx	xxxxx	xxxxx	Sc45

Taxon	Country	State	Locality	Voucher	References	Genbank access			Key
						R35	ND1	MKL1	
Scincella incerta	Guatemala			JAC-19573	This study	-	xxxxx	-	Sc46
Scincella kikaapoa	México	Coahuila	Cuatro Ciénegas	UOGV-2123	This study	xxxxx	xxxxx	xxxxx	Sc47
Scincella kikaapoa	México	Coahuila	Cuatro Ciénegas	UOGV-566	This study	-	xxxxx	-	Sc48
Scincella kikaapoa	México	Coahuila	Cuatro Ciénegas	UOGV-569	This study	xxxxx	xxxxx	xxxxx	Sc49
Scincella kikaapoa	México	Coahuila	Cuatro Ciénegas	UOGV-567	This study	-	xxxxx	-	Sc50
Scincella lateralis	USA	Texas		TJL-2296	This study	xxxxx	xxxxx	xxxxx	Sc51
Scincella lateralis	USA	Texas		MF-4740	This study	-	xxxxx	-	Sc52
Scincella lateralis	USA	Texas		TJL-2223	This study	-	xxxxx	-	Sc53
Scincella lateralis	USA	Texas		TJL-2276	This study	-	xxxxx	-	Sc54
Scincella lateralis	USA	Texas		WGS	This study	-	xxxxx	-	Sc55
Scincella lateralis	USA	Louisiana		KU-289470	This study	xxxxx	xxxxx	xxxxx	Sc56
*Scincella reevesi***	China			KU-291934	This study	xxxxx	xxxxx	xxxxx	Sc57
Scincella silvicola caudaequinae	México	Nuevo León	Santiago	JLAL-247	This study	-	xxxxx	-	Sc58
Scincella s. caudaequinae	México	Nuevo León	Santiago	UOGV-561	This study	xxxxx	xxxxx	xxxxx	Sc59
Scincella s. caudaequinae	México	Queretaro	Landa de Matamoros	JAC-24531	This study	xxxxx	xxxxx	xxxxx	Sc60
Scincella s. caudaequinae	México	Queretaro	Landa de Matamoros	JAC-24532	This study	-	xxxxx	-	Sc61
Scincella s. caudaequinae	México	San Luis Potosí		JAC-25991	This study	-	xxxxx	-	Sc62
Scincella s. caudaequinae	México	Tamaulipas		JAC-24547	This study	-	xxxxx	-	Sc63
Scincella s. caudaequinae	México	Tamaulipas		MZFC-647	This study	-	xxxxx	-	Sc64
Scincella s. silvicola	México	Oaxaca	Coyula	LCM-1181	This study	-	xxxxx	-	Sc65
Scincella s. silvicola	México	Oaxaca	Buenos Aires	AMH-191	This study	xxxxx	xxxxx	xxxxx	Sc66
Scincella s. silvicola	México	Oaxaca	Monteflor	CVS-6	This study	-	xxxxx	-	Sc67
Scincella s. silvicola	México	Puebla	Eloxochitlan	UOGV-1232	This study	xxxxx	xxxxx	xxxxx	Sc68
Scincella s. silvicola	México	Puebla	Eloxochitlan	UOGV-910	This study	-	xxxxx	-	Sc69
Scincella s. silvicola	México	Puebla	Cuetzalan	JAC-22499	This study	xxxxx	xxxxx	xxxxx	Sc70
*Sphenomorphus indicus***	China			KU-291935	This study	xxxxx	xxxxx	xxxxx	Sc72
*Sphenomorphus maculatus***	Myanmar			CAS-204863	This study	xxxxx	xxxxx	xxxxx	Sc73
*Xantusia vigilis***	USA	California		MVZ249144	Brandley et al., 2011	HM161146	HM160768	HM160768	Sc74
*Zonosaurus sp***			"Pet trade"	TNHC 55947	Schmitz et al., 2005	-	AY315567	-	Sc75

Storeria

Colubrid snake lizard genus *Storeria* is composed of five species in America, *St. dekayi*, *St. occipitomaculata*, *St. storerioides*, *St. victa* (not included in this study), and an undescribed species from CCB with no subspecies recognized (Pyron at al., 2016; García-Vázquez et al., 2019)

Taxa	Country	State	Locality	Voucher	References	Genbank access		Key
						ND4	ND2	
Storeria dekayi	México	Hidalgo	Tlanchinol	ANMO-1655	This study	xxxxx	xxxxx	St1
Storeria dekayi	México	Puebla	Tlatlauquitepec	ISZ-187	This study	xxxxx	xxxxx	St2
Storeria dekayi	México	Puebla	Tenango de Doria	AMH-122	This study	xxxxx	xxxxx	St3
Storeria dekayi	México	Hidalgo	Tlanchinol	JAC-26039	This study	xxxxx	xxxxx	St4
Storeria dekayi	México	Puebla	Tlatlauquitepec	ISZ-24	This study	xxxxx	xxxxx	St5
Storeria dekayi	USA	Kansas		KU-290676	This study	xxxxx	xxxxx	St6
Storeria dekayi	USA	Texas		TNHC-66469	This study	xxxxx	xxxxx	St7
Storeria occipitomaculata	México	Tamaulipas		JAC-29645	This study	xxxxx	xxxxx	St8
Storeria occipitomaculata	México	Tamaulipas		JAC-29763	This study	xxxxx	xxxxx	St9
Storeria occipitomaculata	México	Nuevo León	Pablillo	UOGV-526	This study	xxxxx	xxxxx	St10
Storeria sp. nov.	México	Coahuila	Cuatro Ciénegas	MZFC-29652	This study	xxxxx	xxxxx	St11
Storeria sp. nov.	México	Coahuila	Cuatro Ciénegas	UOGV-1998	This study	xxxxx	xxxxx	St12
Storeria storerioides	México	Chihuahua		JAC-29229	This study	xxxxx	xxxxx	St14
Storeria storerioides	México	Ciudad de México	Magdalena Contreras	UOGV-737	This study	xxxxx	xxxxx	St15
Storeria storerioides	México	Ciudad de México	Magdalena Contreras	UOGV-1902	This study	xxxxx	xxxxx	St16
*Virginia striata****						AF384852		
*Virginia striata****						AF384852		

Terrapene

Box turtle genus *Terrapene* is composed of six species in America: *Terrapene carolina* with four subspecies (*T. c. bauri*, *T. c. carolina*, *T. c. major* and *T. c. triunguis*), *T. coahuila*, *T. mexicana* with two subspecies (*T. m. mexicana* and *T. m. yucatana*), *T. nelsoni* with two subspecies (*T. n. nelsoni* and *T. n. klauberi*) and *T. ornata* with two subspecies (*T. o. ornata* and *T. o. luteola*) (Butler et al., 2011; Martin et al., 2013; Spinks et al., 2016).

Taxa	Country	State	Locality	Voucher	References	Genbank acces				Key
						Cytb	COI	GAPD	R35	
*Clemmys marmorata****										
*Clemmys marmorata****										
*Emys orbicularis****	Hungary	Mike		HBS-108692	Spinks & Shaffer, 2009	EU787075	-	-	EU787216	Te1
*Emys orbicularis****	France	Ramatuelle		IPMB-4597	Spinks & Shaffer, 2009	EU787065	-	-	EU787185	Te2
*Glyptemys insculpta****	USA	New York	Hofstra University	No voucher	Feldman & Parham, 2002; Wiens et al., 2010	AF258876	-	GQ896117	DQ661020	Te3
Terrapene carolina bauri	USA	Florida	Hernando	No voucher	Martin et al., 2013	KF059126	KF059156	KF059230	-	Te4
Terrapene c. carolina	USA	North Carolina	Jackson Co.	MVZ-137441	Feldman & Parham, 2002; Angielczyk & Feldman, 2013	AF258871	KC181202	-	KC181178	Te5
Terrapene c. carolina	USA	New York	Suffolk Co.	JJW-1852	Wiens et al., 2010	GQ896202	-	GQ896138	GQ896249	Te6
Terrapene c. major	USA	Alabama	Mobile	No voucher	Martin et al., 2013	KF059122	KF059171	KF059229	-	Te7
Terrapene c. major	USA	Mississippi	Forrets	No voucher	Martin et al., 2013	KF059119	KF059170	KF059218	-	Te8
Terrapene c. major	USA	Mississippi	Perry	No voucher	Martin et al., 2013	KF059120	KF059159	KF059217	-	Te9
Terrapene c. major	USA	Louisiana	Concordia	No voucher	Martin et al., 2013	KF059118	KF059158	KF059214	-	Te10
Terrapene c. triunguis	USA	Missouri	Jefferson	No voucher	Martin et al., 2013	KF059142	KF059173	KF059233	FJ770702	Te11
Terrapene c. triunguis	USA	Louisiana	Vernon	No voucher	Martin et al., 2013	KF059133	KF059172	KF059232	FJ770703	Te12
Terrapene coahuila	México	Cuatro Ciénegas	Cuatro Ciénegas	AO-233	Wiens et al., 2010	AF258872	-	GQ896140	-	Te13
Terrapene coahuila	México	Cuatro Ciénegas	Cuatro Ciénegas	No voucher	Martin et al., 2013	KF059124	KF059160	-	FJ770700	Te14
Terrapene coahuila	México	Coahuila	Cuatro Ciénegas	No voucher	Martin et al., 2013	KF059124	KF059161	KF059213	FJ770699	Te15
Terrapene nelsoni	Sonora			No voucher	Martin et al., 2013	KF059080	KF059167	KF059234	KC181180	Te16
Terrapene ornata luteola	USA	New Mexico	Socorro	No voucher	Martin et al., 2013	KF059070	KF059164	KF059178	FJ770701	Te17
Terrapene o. luteola	USA	New Mexico	Socorro	No voucher	Martin et al., 2013	KF059067	KF059165	KF059181	EU787170	Te18
Terrapene o. ornata	USA	Wisconsin	Columbia	No voucher	Martin et al., 2013	KF059079	KF059166	KF059183	DQ649464	Te19
Terrapene o. ornata	USA	Wisconsin	Columbia	No voucher	Martin et al., 2013	KF059076	KF059162	KF059182	HQ266664	Te20
Terrapene mexicana yucatana	México	Yucatán		No voucher	Martin et al., 2013	KF059154	KF059177	KF059215	-	Te21
Terrapene m. mexicana	México	Tamaulipas		No voucher	Martin et al., 2013	KF059153	KF059175	KF059233	-	Te22

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Appendix S2. Taxon, locality and voucher data, and GenBank Accession number for genetic samples used in the test for synchronous diversification in this study. Institutional codes for museum and collections follow Sabaj-Pérez (2016). UOGV, JAC, and TJL are field identifiers for uncatalogued specimens being deposited in the MZFC.

Key	Group	Taxon	Country	State	Locality	Voucher	LAT	LONG	GenBank Access
TC1	Terrapene	*Terrapene carolina carolina*	USA	North Carolina	Jackson Co.	MVZ-137441	36.240676	-77.53812	AF258859
TC2	Terrapene	*Terrapene carolina carolina*	USA	New York	Suffolk Co.	JJW-1852	40.848627	-73.033244	GQ895902
TC3	Terrapene	*Terrapene carolina major*	USA	Alabama	Mobile	No voucher	30.874623	-88.755918	xxxxx
TC4	Terrapene	*Terrapene carolina major*	USA	Mississippi	Forrets	No voucher	32.613907	-89.367412	xxxxx
TC5	Terrapene	*Terrapene carolina major*	USA	Mississippi	Perry	No voucher	30.940269	-89.605395	xxxxx
TC6	Terrapene	*Terrapene carolina major*	USA	Louisiana	Concordia	No voucher	31.290994	-91.699379	xxxxx
TCCB1	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	AO-233	26.911334	-102.016545	AY673523
TCCB2	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.96466	-102.109694	AY673522
TCCB3	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.947969	-101.926091	AY67352
TCCB4	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.911334	-102.016545	xxxxx
TCCB5	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.96466	-102.109694	xxxxx
TCCB6	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.947969	-101.926091	xxxxx
TCCB7	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.911334	-102.016545	xxxxx
TCCB8	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.96466	-102.109694	xxxxx
TCCB9	Terrapene	*Terrapene coahuila*	México	Coahuila	Cuatro Cienegas	No voucher	26.947969	-101.926091	xxxxx
SCCB1	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-2123	26.947969	-101.926091	xxxxx
SCCB2	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-566	26.908996	-102.009192	xxxxx
SCCB3	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-569	26.812757	-102.000538	xxxxx
SCCB4	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-567	26.853752	-102.160133	xxxxx
SCCB5	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	MZFC-17664	26.947969	-101.926091	xxxxx
SCCB6	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	MZFC-17668	26.908996	-102.009192	xxxxx
SCCB7	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-2114	26.78708333	-102.0007222	xxxxx

SCCB8	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-2119	26.78708333	-102.0007222	xxxxx
SCCB9	Scincella	*Scincella kikaapoa*	México	Coahuila	Cuatro Cienegas	UOGV-2120	26.78708333	-102.0007222	xxxxx
SL1	Scincella	*Scincella lateralis*	USA	Texas	Bastrop	MF-4740	30.202059	-97.248885	xxxxx
SL2	Scincella	*Scincella lateralis*	USA	Texas	Bastrop	TJL-2223	30.080427	-97.172751	xxxxx
SL3	Scincella	*Scincella lateralis*	USA	Texas	Jarvis	TJL-2276	31.712434	-95.428837	xxxxx
SL4	Scincella	*Scincella lateralis*	USA	Texas	El llano	WGS	30.659715	-98.608731	xxxxx
SL5	Scincella	*Scincella lateralis*	USA	Louisiana	Ouachita	KU-289470	32.390438	-92.467276	xxxxx
SL6	Scincella	*Scincella lateralis*	USA	Texas	Smith	TJL-2296	29.305786	-97.322684	xxxxx
SL7	Scincella	*Scincella lateralis*	USA	Texas	Smith	KU-289536	31.017518	-93.993279	xxxxx
SL8	Scincella	*Scincella lateralis*	USA	Oklahoma	LeFlore	OMNH-6818	34.871048	-94.942268	xxxxx
SL9	Scincella	*Scincella lateralis*	USA	Oklahoma	Ellis	OMNH-7080	36.512331	-99.806797	xxxxx
SL10	Scincella	*Scincella lateralis*	USA	Oklahoma	Pushmataha	OMNH-9315	34.43306	-95.417355	xxxxx
SL11	Scincella	*Scincella lateralis*	USA	Texas	Jarvis	TJL-2275	31.712434	-95.428837	xxxxx
SL12	Scincella	*Scincella lateralis*	USA	Texas	San Saba	TNHC-53249	31.188524	-98.745455	xxxxx
SL13	Scincella	*Scincella lateralis*	USA	Texas	Sabine	TNHC-65406	31.199809	-93.883562	xxxxx
STD1	Storeria	*Storeria dekayi*	México	Puebla	Tenango de Doria	AMH-122	20.330363	-98.232415	xxxxx
STD2	Storeria	*Storeria dekayi*	México	Hidalgo	Tlanchinol	ANMO-1655	21.00177778	-98.65286111	xxxxx
STD3	Storeria	*Storeria dekayi*	México	Puebla	Tlatlauquitepec	ISZ-187	19.85302778	-97.47269444	xxxxx
STD4	Storeria	*Storeria dekayi*	México	Puebla	Tlatlauquitepec	ISZ-24	19.89663889	-97.47880556	xxxxx
STD5	Storeria	*Storeria dekayi*	México	Hidalgo	Tlanchinol	JAC-26035	21.02103	-98.64006	xxxxx
STD6	Storeria	*Storeria dekayi*	México	Hidalgo	Tlanchinol	JAC-26039	21.02103	-98.64006	xxxxx
STD7	Storeria	*Storeria dekayi*	México	Querétaro	Landa de Matamoros	JAC-24631	21.225507	-99.283121	xxxxx
STCCB1	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	UOGV-2005	26.939243	-102.119264	xxxxx
STCCB2	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	UOGV-1998	26.939243	-102.119264	xxxxx
STCCB3	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC-29652	26.927534	-102.130223	xxxxx
STCCB4	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC-29651	26.927534	-102.130223	xxxxx
STCCB5	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	UOGV-1999	26.919308	-102.134744	xxxxx
STCCB6	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC29645	26.927534	-102.130223	xxxxx
STCCB7	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC-29641	26.927534	-102.130223	xxxxx
STCCB8	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC-29646	26.939243	-102.119264	xxxxx
STCCB9	Storeria	*Storeria sp*	México	Coahuila	Cuatro Cienegas	MZFC-29649	26.927534	-102.130223	xxxxx

GI1	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Crockett	TJL-2350	29.911378	-99.927052	MH181257
GI2	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Real	TJL-2351	31.345397	-95.718372	MH181258
GI3	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Devils	No voucher	29.933717	-100.995241	JN547348
GI4	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Devils	No voucher	29.933717	-100.995241	JN547346
GI5	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Austin	Austin_1	30.242617	-97.743784	MH181242
GI6	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Austin	Austin_2	30.242617	-97.743784	MH181243
GI7	Gerrhonotus	*Gerrhonotus infernalis*	USA	Texas	Crockett	TJL-2352	29.911378	-99.927052	xxxxx
GCCB1	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29667	26.82580556	-102.0224722	MH181314
GCCB2	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29668	26.82580556	-102.0224722	xxxxx
GCCB3	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	UOGV-1440	26.91997222	-102.1147778	MH18131
GCCB4	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	UOGV-1438	26.91997222	-102.1147778	MH181311
GCCB5	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	UOGV-1439	26.91997222	-102.1147778	MH181312
GCCB6	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29655	26.91997222	-102.1147778	xxxxx
GCCB7	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29656	26.91997222	-102.1147778	xxxxx
GCCB8	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29657	26.91997222	-102.1147778	xxxxx
GCCB9	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29658	26.91997222	-102.1147778	xxxxx
GCCB10	Gerrhonotus	*Gerrhonotus mccoyi*	México	Coahuila	Cuatro Cienegas	MZFC-29659	26.91997222	-102.1147778	xxxxx

Appendix S3. Technical details on DNA sequencing and sequence edition

DNA extraction, amplification, and sequencing and sequence edition. Total genomic DNA was extracted from liver, muscle, or shed skins using proteinase K (25 mg/mL in 10 mM Tris-HCl, pH 7.5) in lysis buffer (100 mM Tris, 5 mM Na_2EDTA, 200 mM NaCl, 0.2% SDS) and incubated at 52 °C. Shed skins often required 2–3 days to fully digest, and an additional 12.5 µl of proteinase K were added every 24 h. Samples were cleansed with two rinses of phenol:chloroform:isoamyl alcohol (25:24:1) followed by a final rinse of chloroform:isoamyl alcohol (24:1). Additionally, we used the standard protocol from the commercial extraction kit DNeasy Blood & Tissue Kit (QUIAGEN). All gene regions were amplified via the polymerase chain reaction (PCR) in a 25 µl reaction volume containing 0.5–1.0 µl deoxynucleoside triphosphates (dNTPs; 10 mM), 18–19.25 µl double-distilled water, 0.2–0.5 µl each primer (10 mM), 2.5 µl 1 X PCR buffer, 1.2 mM $MgCl_2$ (Fisherbrand, Pittsburgh, PA, USA), 0.15-0.20 µl Taq DNA polymerase (Fisherbrand), and 1.0–1.5 µl template DNA. For mtDNA, DNA was denatured at 94 °C for 2 min, followed by 38–40 cycles of: 94 °C for 30 s, 48–50 °C for 45 s, and 72 °C for 45 s. A final extension phase of 72 °C for 7 min terminated the protocol. For nuclear genes, DNA was denatured at 94 °C for 2.5 min, followed by 38 cycles of: 94 °C for 15 s, 51* °C for 20 s (* where temperature is reduced by 0.3 degrees each cycle), and 72 °C for 1 min. A final extension phase of 72 °C for 7 min terminated the protocol. Double-stranded amplified products were checked by electrophoresis on a 1% agarose gel. PCR products were purified with polyethylene glycol precipitation (Lis, 1980). DNA templates were sequenced in both directions with the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Inc.) and an ABI 3100 automated DNA sequencer (Applied Biosystems, Inc.) using the amplification primers (Table S3). Sequences were assembled and edited in the Staden Package v 1.6.0 (Whitwham & Bonfield, 2005).

Table S3. Primers used in this study.

Primer name	Sequence 5' → 3'	Gene	Group	Reference
ND4	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC	ND4	All	Arévalo et al., 1994
LEU	CAT TAC TTT TAC TTG GAT TTG CAC CA	ND4	All	Arévalo et al., 1994
L5238	ACM TGA CAA AAA ATY GC	ND2	*Storeria*	De Queiroz et al., 2004
H5382	GTG TGG GCR ATT GAT GA	ND2	*Storeria*	De Queiroz et al., 2005
16AR2	CCC GMC TGT TTA CCA AAA ACA	ND1	*Scincella*	Reeder, 2003
16DR	CTA CGT GAT CTG AGT TCA GAC CGG AG	ND1	*Scincella*	Leaché & Reeder, 2002
ND1-INTR2a	GGY TCT TTR RTR ADA GTT THA CNC	ND1	*Scincella*	This study
ND1-INTF4a	GTR GCM CAA ACM HAT YTC MTA YGA	ND1	*Scincella*	This study
ND1-INTR2	CRA AKG GGC CDG CTG CRT AYT CTA C	ND1	*Scincella*	Schmitz et al., 2005
TMET	TCG GGG TAT GGG CCC RAR AGC TT	ND1	*Scincella*	Leaché & Reeder (2002)
MKL1_f1	GTG GCA GAG CTG AAG CAR GAR CTG AA	MKL1	*Scincella*	Townsend et al., 2008
MKL1_r2	GCR CTC TKR TTG GTC ACR GTG AGG	MKL1	*Scincella*	Townsend et al., 2008
R35-F	GAC TGT GGA YGA YCT GAT CAG TGT GGT GCC	R35	*Scincella*	Brandley et al., 2011
R35-R	GCC AAA ATG AGS GAG AAR CGC TTC TGA GC	R35	*Scincella*	Brandley et al., 2011
BDNF-F	GAC CAT CCT TTT CCT KAC TAT GGT TAT TTC ATA CTT	BDNF	*Gerrhonotus*	Brandley et al., 2011
BDNF-R	CTA TCT TCC CCT TTT AAT GGT CAG TGT ACA AAC	BDNF	*Gerrhonotus*	Brandley et al., 2011
PRLR_f1	GAC ARY GAR GAC CAG CAA CTR ATG CC	PRLR	*Gerrhonotus*	Townsend et al., 2008
PRLR_r3	GAC YTT GTG RAC TTC YAC RTA ATC CAT	PRLR	*Gerrhonotus*	Townsend et al., 2008

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Appendix S4. Fossils utilized for the divergence time estimates

Gerrhonotus (García-Vázquez et al., 2018)**.** (1) 99 Ma (Albian-Cenomanian boundary) for the most recent common ancestor (MRCA) of Anguidae, Aniellidae, and Helodermatidae based on the fossil helodermatid *Primaderma* (Nydman, 2000); (2) 70 Ma, latest date for Upper Cretaceous MRCA of Anguidae and Aniellidae based on the fossil anguid *Odaxosaurus* (Gilmore, 1928); (3) early Pliocene (Clarendonian) for the MRCA of Gerrhonotinae based on the fossils "*Gerrhonotus*" *mungerorum* (Holman, 1975; Good, 1988) and *Paragerrhonotus ricardensis* (Estes, 1963; Good, 1988).

***Scincella*.** (1) 70 Ma, latest date for Upper Cretaceous MRCA of Scincidae based on the fossils *Contogenys* and *Sauriscus* (Estes, 1983; Wiens et al., 2006; Mulcahy et al., 2012); (2) 65 Ma (Upper Cretaceous) for the MRCA of Xantusidae, Cordylidae and Gerrhosauridae based on the fossil *Konkasaurus* (Mulcahy et al., 2012); (3) 151 Ma, inferior Cretaceous (Berriasiense), for the MRCA of Scincomorpha based on the fossil *Sakurasaurus* (Brandley et al., 2011).

***Storeria*.** (1) 12-17 Ma (middle Miocene) for the MRCA of *Storeria* based on the fossil *Storeria* sp (Holman, 2000); (2) 93 Ma, Upper Cretaceous (Turoniense), for the MRCA of Boidae and Colubridae based on the fossil *Coniophis* (Mulcahy et al., 2012); (3) 130 Ma (early Cretaceous) for the MRCA of Serpentes based on the fossil *Laparentophis defrennei* (Pyron, 2010).

***Terrapene*.** (1) 29 Ma, latest date for Upper Oligocene MRCA of Emydinae based on the fossil *Chrysemis antiqua* (Crawford et al., 2015); (2) 15 Ma (early Miocene) for the MRCA of *Graptemys* based on the fossil *Pseudemys idahoensis* (Gilmore, 1933); (3) 12.5 Ma, middle Miocene

(Serravalliense), for the MRCA of *Terrapene* based on the fossil *Terrapene ornata* (Spinks & Shaffer, 2009; Martin et al., 2013).

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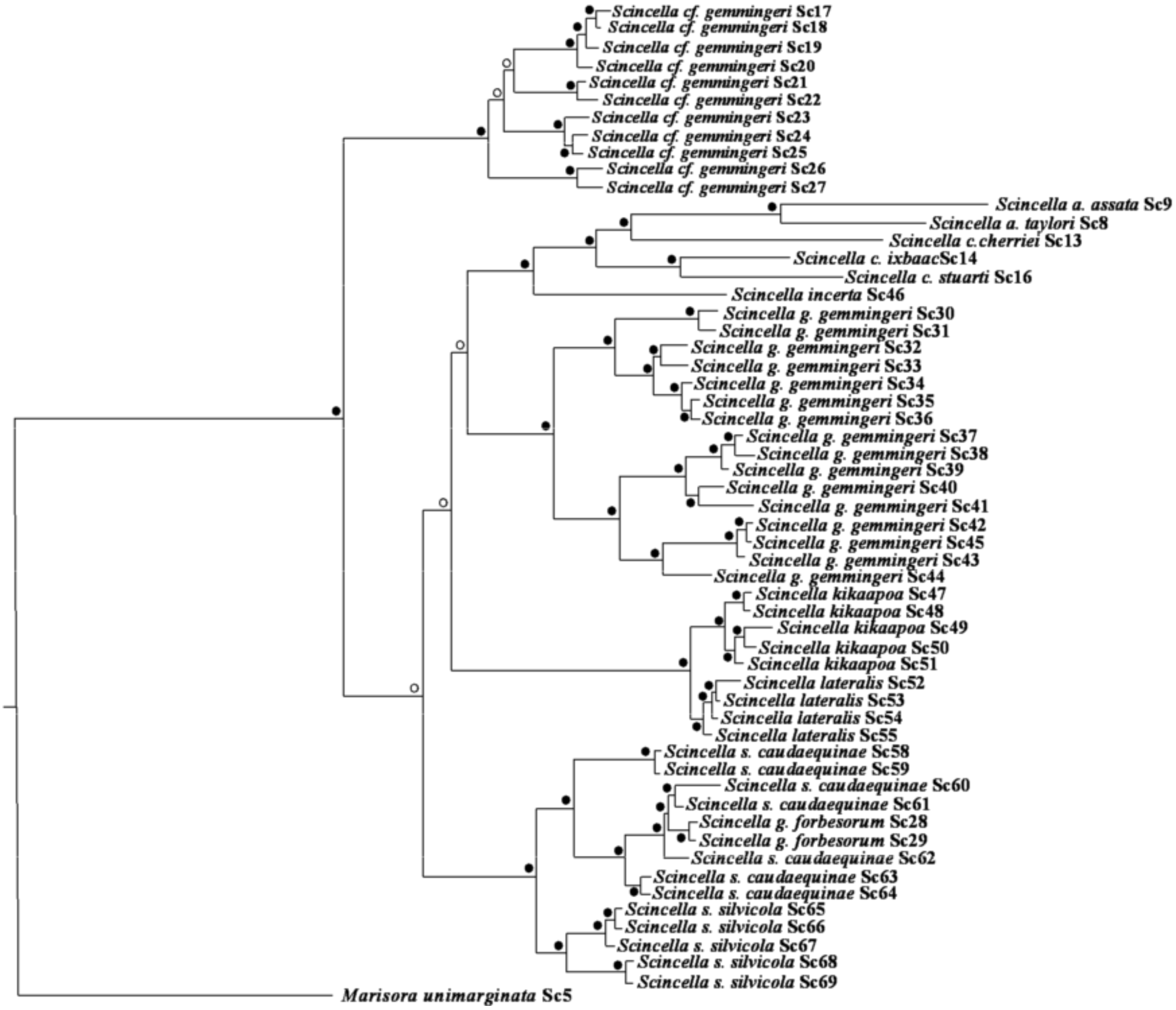
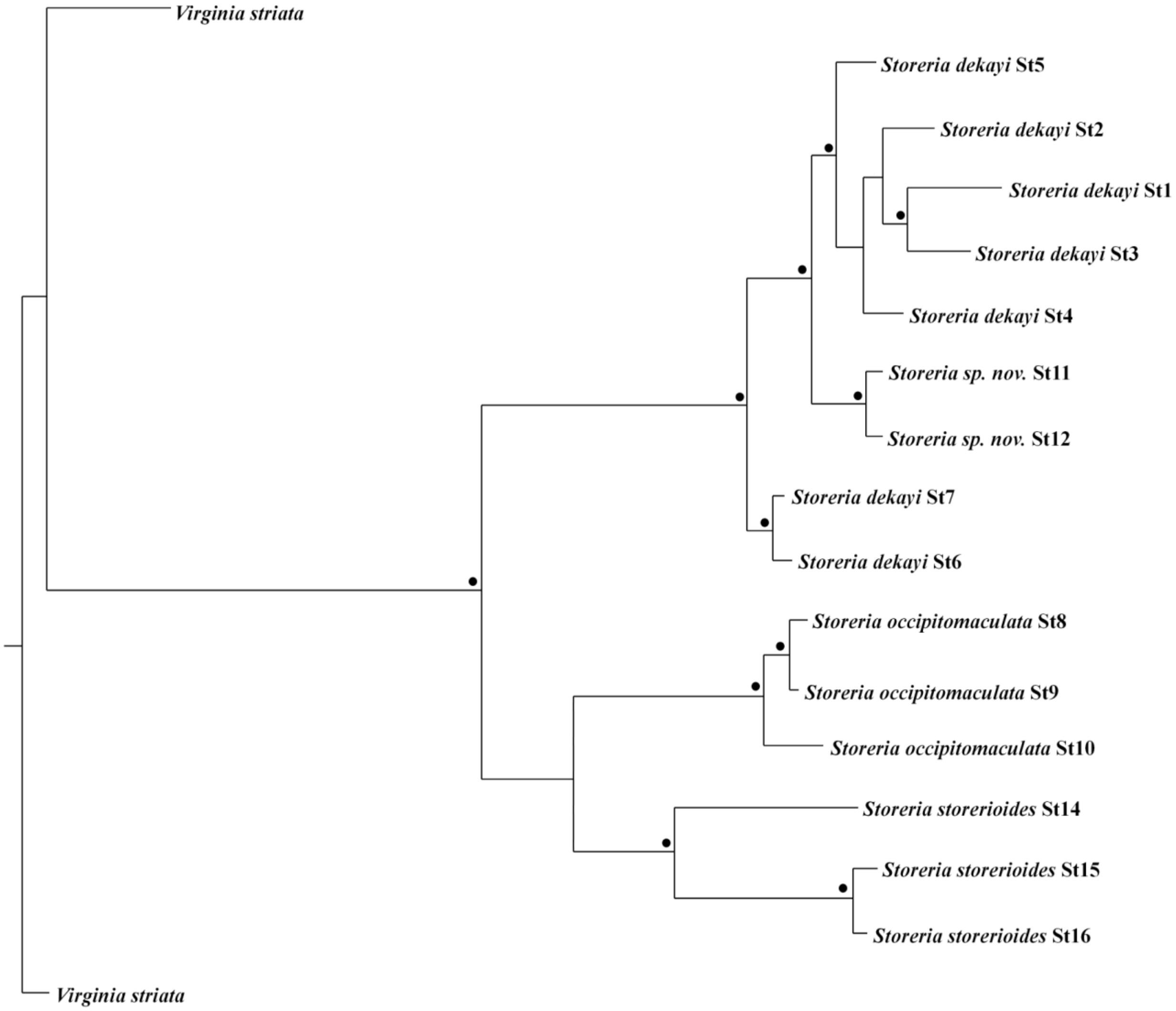
Appendix S5. Genetic structure within *Scincella*, *Storeria*, and *Terrapene* inferred from Bayesian analyses of their respective DNA sequence datasets. Black dots represent strongly supported nodes (Bayesian posterior probability value ≥0.95).

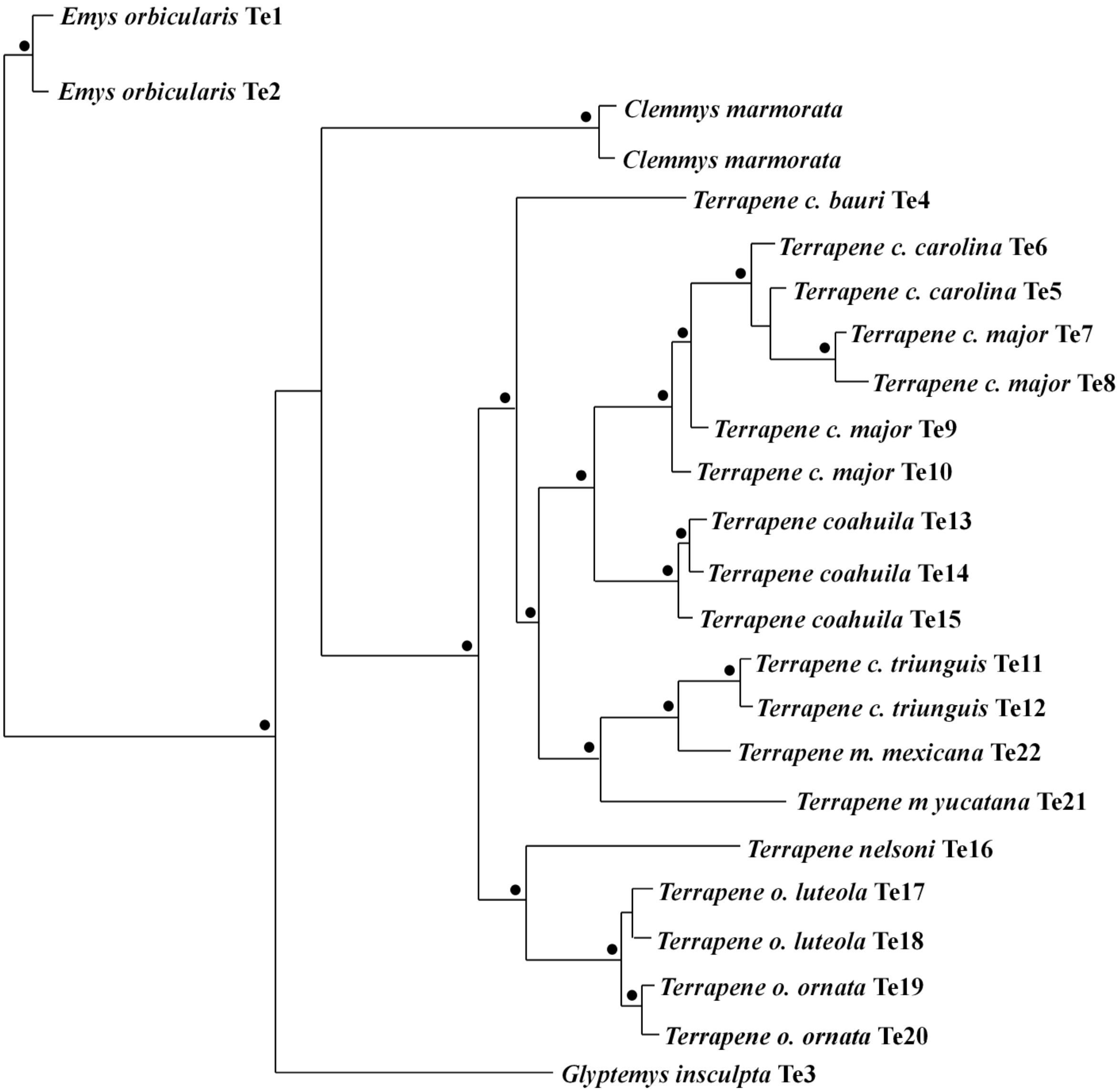
Scincella



Storeria


Virginia striata
Storeria dekayi St5
Storeria dekayi St2
Storeria dekayi St1
Storeria dekayi St3
Storeria dekayi St4
Storeria sp. nov. St11
Storeria sp. nov. St12
Storeria dekayi St7
Storeria dekayi St6
Storeria occipitomaculata St8
Storeria occipitomaculata St9
Storeria occipitomaculata St10
Storeria storerioides St14
Storeria storerioides St15
Storeria storerioides St16
Virginia striata

Terrapene


Emys orbicularis **Te1**
Emys orbicularis **Te2**
Clemmys marmorata
Clemmys marmorata
Terrapene c. bauri **Te4**
Terrapene c. carolina **Te6**
Terrapene c. carolina **Te5**
Terrapene c. major **Te7**
Terrapene c. major **Te8**
Terrapene c. major **Te9**
Terrapene c. major **Te10**
Terrapene coahuila **Te13**
Terrapene coahuila **Te14**
Terrapene coahuila **Te15**
Terrapene c. triunguis **Te11**
Terrapene c. triunguis **Te12**
Terrapene m. mexicana **Te22**
Terrapene m yucatana **Te21**
Terrapene nelsoni **Te16**
Terrapene o. luteola **Te17**
Terrapene o. luteola **Te18**
Terrapene o. ornata **Te19**
Terrapene o. ornata **Te20**
Glyptemys insculpta **Te3**

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RESEARCH PAPER

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Molecular systematics and historical biogeography of the genus *Gerrhonotus* (Squamata: Anguidae)

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Abstract

Aim: Multiple geological and climatic events have created geographical or ecological barriers associated with speciation events, playing a role in biological diversification in Mexico. Here, we evaluate the influence of Neogene geological events and of Pleistocene climate change in the diversification of the genus *Gerrhonotus* using molecular dating and ancestral area reconstruction.

Location: Mexico and south-central United States.

Methods: A multilocus sequence dataset was generated for 86 individuals of *Gerrhonotus* from most Mexican biogeographical provinces and belonging to five of the seven currently recognized species, as well as two putative undescribed species. Phylogeographical structure was explored using Poisson-Tree-Processes molecular species delimitation. Divergence events were estimated based on the fossil record using a relaxed uncorrelated lognormal clock. Ancestral areas were estimated at divergence events across the tree using a probabilistic Bayesian approach.

Results: Extensive geographical structure was evident within three well-supported clades. These clades probably diverged from each other in the early to mid-Miocene, and their divergence was followed by six divergences in the late Miocene and eight divergences in the Pliocene. The ancestral origin of *Gerrhonotus* with keeled dorsal scales (keeled-scale *Gerrhonotus*) was reconstructed to be across the Pacific Coast Province. Our phylogenetic analyses did not support the monophyly of *Gerrhonotus*.

Main conclusions: Miocene and Pliocene geomorphology, perhaps in conjunction with climate change, appears to have induced allopatric divergence on a relatively small spatial scale in this genus. The late Miocene–Pliocene reduction in the highlands along the Tehuantepec fault probably created a large marine embayment that led to an early divergence in a clade of *Gerrhonotus*. Our analysis suggests uplifting of the Trans-Mexican Volcanic Belt during this same time period resulted in additional diversification. This was followed by more recent, independent colonization events in the Pliocene from the Mexican Plateau to the Sierra Madre Oriental, Sierra Madre Occidental, Tamaulipas and Edwards Plateau provinces. A genus *Gerrhonotus* with the keeled-scale species in addition to *Coloptychon rhombifer* (= G.

rhombifer) is strongly supported. Inclusion of the smooth dorsal-scale species in the genus is uncertain and maintained only tentatively.

KEYWORDS

ancestral area reconstruction, *Coloptychon*, divergence dating, diversification, *Gerrhonotus*, Isthmus of Tehuantepec, Mexican Plateau, Trans-Mexican Volcanic Belt

1 | INTRODUCTION

Inferring the evolutionary history of the groups in a particular region is the first step to elucidate the processes by which the fauna of that region originated (Colston et al., 2013). Multiple phylogeographical studies of species with broad distributions in the Mexican Transition Zone (MTZ) provide an illustrative case (e.g. Bryson, Pastorini, Burbrink, & Forstner, 2007; León-Paniagua, Navarro-Sigüenza, Hernández-Banos, & Morales, 2007). The MTZ is a complex area where Neotropical and Nearctic biotas overlap, spanning the region from the deserts of south-western United States and northern Mexico to the dry and humid forests of the Nicaraguan lowlands (Morrone, 2010).

Multiple geological and climatic events have created geographical or ecological barriers associated with speciation events, playing a role in biological diversification in Mexico (Bryson, García-Vázquez, & Riddle, 2012a; León-Paniagua et al., 2007), but four are considered of primary importance and have received the most attention (Bryson, García-Vázquez & Riddle, 2011; Ferrusquía-Villafranca & González-Guzmán, 2005; Vanzolini, 1970). One of these events was the formation over 30 million years ago (Ma) of three of the four major mountain ranges in Mexico (i.e. the Sierra Madre Occidental [SMO], Sierra Madre Oriental [SME] and Sierra Madre del Sur [SMS]; Ferrusquía-Villafranca & González-Guzmán, 2005). The formation of these mountains probably pre-dates the origin of most extant species (Bryson, García-Vázquez, & Riddle, 2012b). Another event was the formation of the Trans-Mexican Volcanic Belt (TVB) during the Neogene, which resulted in major mountain formations that almost completely subdivided Mexico. The uplift of these mountains during two major volcanic episodes, one around 10–19 Ma and another one around 3–7.5 Ma, created new geographical barriers and undoubtedly affected both the timing and tempo of diversification of the biota (Anducho-Reyes, Cognato, Hayes, & Zuniga, 2008; Bryson et al., 2012b). A third event was the faulting and marine seaway development across the Isthmus of Tehuantepec in southern Mexico around 3 Ma. The Isthmus is a narrow lowland region that has been typically invoked as a biogeographical barrier for many upland taxa (Castoe et al., 2009). The last event was the repeated expansion and contraction cycles of the coniferous forests during Pleistocene climatic fluctuations, which isolated many populations of forest-adapted taxa in refugia, leading to speciation (Vanzolini, 1970). A number of studies have identified other major biogeographical barriers across Mexico, such as river drainages within the major sierras (Bryson, Murphy, Graham, Lathrop & Lazcano-Villareal, 2011; Bryson et al., 2007, 2012a, 2012b; León-Paniagua et al., 2007). Yet, the effectiveness of these barriers in isolating lineages throughout the past several million years remains unclear (Bryson et al., 2012b).

The anguid lizard genus *Gerrhonotus* (Gerrhonotinae) is distributed throughout most of the MTZ (Figure 1). Currently, seven species are formally recognized (Banda-Leal, Nevárez-de los Reyes, & Bryson, 2017). Of these, *G. infernalis* is the most widely distributed, occurring from central Texas in the USA south to Hidalgo, Mexico, with isolated populations in several parts of northern Mexico (Good, 1994). *Gerrhonotus liocephalus* is distributed from central Guerrero and central Puebla south and east to south-eastern Chiapas, with an isolated population in the Los Tuxtlas mountain range in southern Veracruz (Good, 1994). *Gerrhonotus ophiurus* occurs from south-eastern Tamaulipas to central Veracruz, Mexico (García-Padilla & Villegas-Ruiz, 2010). The other four species (*G. farri*, *G. lugoi*, *G. lazcanoi* and *G. parvus*) have restricted distributions in northern Mexico. *Gerrhonotus lugoi* is found near the Cuatro Ciénegas Basin (CCB), Coahuila, and into adjacent Nuevo León; *G. parvus* and *G. lazcanoi* range across central Nuevo León, and *G. farri* is known only from a small region of Tamaulipas (Banda-Leal et al., 2017). In addition to these seven species, the existence of two undescribed species has been suggested. Good (1994) tentatively assigned populations from western Mexico (Sinaloa, Colima and Jalisco) to *G. liocephalus*, but suggested that they could represent one or more distinct species. Castiglia, Anessi, Bezerra, García, and Flores-Villela (2010) found support for the specific status of the populations from Jalisco in a phylogenetic analysis based on mitochondrial DNA (mtDNA) data. Similarly, Contreras-Arquieta (1989) suggested that the population of *Gerrhonotus* in the vicinity of the CCB represented a new subspecies of *G. liocephalus*. However, examination of several specimens of *Gerrhonotus* from the CCB and the surrounding mountains suggests that this population actually represents a distinct, undescribed species (García-Vázquez et al., submitted). However, both of these putative species remain undescribed. Good (1994) and Pyron, Burbrink, and Wiens (2013) performed phylogenetic analyses that included some, but not all, of the currently recognized species of *Gerrhonotus*. Also, the latter authors found that *Coloptychon*, an enigmatic alligator lizard from Middle America, is nested within *Gerrhonotus*. Thus, the monophyly of *Gerrhonotus* is uncertain.

In this study, the phylogenetic relationships of *Gerrhonotus* were described in order to evaluate the role of the major orogenic events and Pleistocene climatic fluctuations on lineage diversification. Samples of five of the seven recognized and the two putative undescribed species of *Gerrhonotus* were included. One mitochondrial and

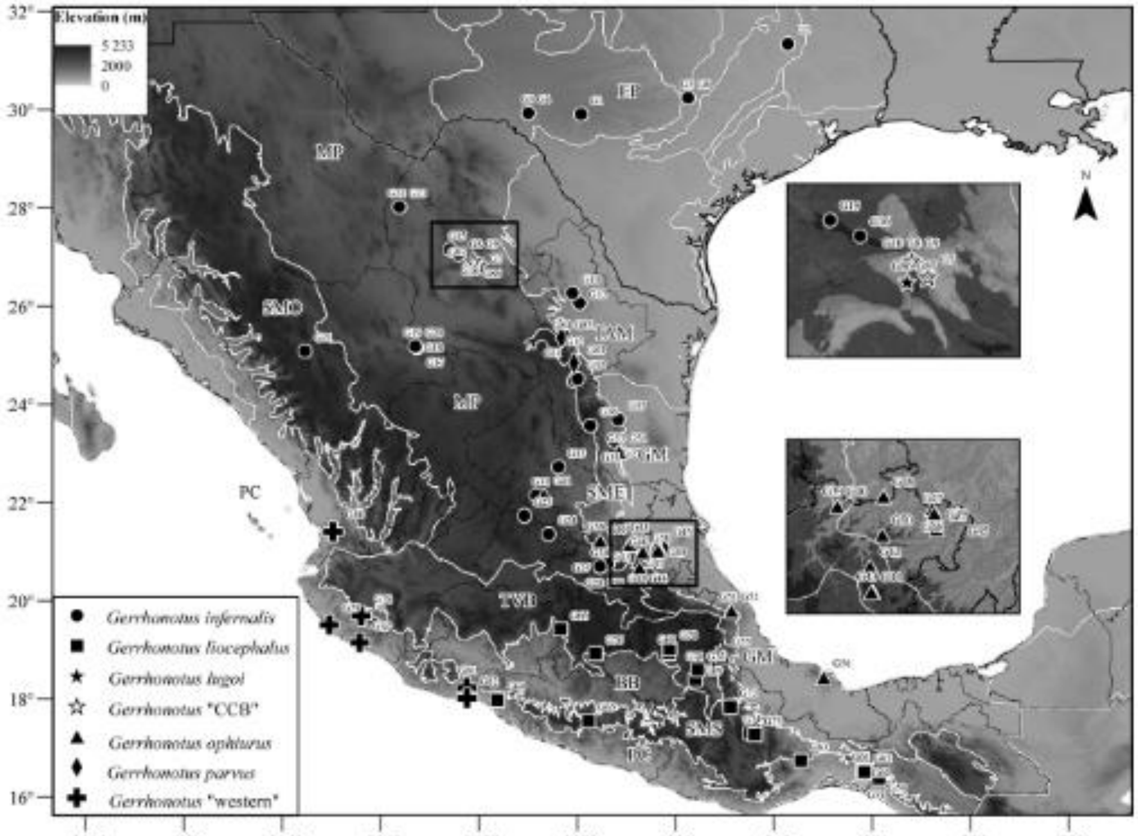

FIGURE 1 Sampling localities for genetic samples of *Gerrhonotus* used in this study (see also Appendix S1). White lines delineate the Biogeographical Provinces and North American Terrestrial Ecoregions (Morrone, 2005; Wiken et al., 2011): Mexican Plateau (MP), Gulf of Mexico (GM), Pacific Coast (PC), Sierra Madre Oriental (SME), Sierra Madre Occidental (SMO), Sierra Madre del Sur (SMS), Balsas Basin (BB), Tamaulipas (TAM), Trans-Mexican Volcanic Belt (TVB) and Edwards Plateau (EP). Continuous black lines represent political borders

two nuclear loci were sequenced. Phylogeographical structure was inferred and a time-calibrated species tree estimated from these data. Finally, ancestral ranges at each divergence event were reconstructed. The resulting patterns of diversification are discussed in the context of mountain building and Pleistocene climatic fluctuations.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and laboratory methods

Eighty six individuals of *Gerrhonotus* were collected from throughout the distribution of the genus (Figure 1, see Appendix S1). Our sampling spans most of the geographical distributions of the currently recognized species with the exception of *G. farri* and *G. lazcanoi*, two species known each only from the holotype. Samples of the two putative undescribed species from western Mexico and CCB also were included. To time calibrate our phylogenetic tree, sequences from seven other anguid genera and their closest relatives within Anguimorpha were included (Pyron et al., 2013; Appendix S1). Partial sequences of the mitochondrial gene coding for the NADH dehydrogenase subunit 4 (*ND4*), complete sequences of the genes coding for the tRNA-His and tRNA-Ser, and partial sequences of the gene coding for tRNA-Leu were obtained for a total of 980 base pairs (bp) for all of the 86 individuals of *Gerrhonotus* and outgroups. Two nuclear loci also were sequenced for a subset of the specimens ($n = 46$) representing the main lineages inferred from our mtDNA dataset (see below), including 720 bp of the gene coding for the brain-derived neurotropic factor (*BDNF*) and 627 bp of the gene coding for the prolactin receptor (*PRLR*). Loci were selected because they have been previously shown to be informative at different levels of divergence within anguid lizards (Bryson & Riddle, 2012; Vidal et al., 2012). Primer sequences for ND4 are given in Arévalo, Davis, and Sites (1994), and for *BDNF* and *PRLR* in Townsend, Alegre, Kelley, Wiens, and Reeder (2008). See Appendix S2 for technical details on DNA sequencing and sequence edition.

2.2 | mtDNA phylogenetic inference

The full mtDNA dataset (n = 100, including outgroups) was analysed to survey range-wide genetic structure and delineate geographically cohesive lineages (see below). The mtDNA phylogeny was inferred using Bayesian inference and maximum likelihood (ML) phylogenetic methods. For both methods, partitioned analyses were carried out to improve phylogenetic accuracy. The best-fitting substitution models and partitioning schemes were selected jointly using the Bayesian Information Criterion in the software PartitionFinder 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012). Bayesian inference analyses were conducted using MrBayes 3.2.1 (Ronquist et al., 2012). Four runs were conducted using the "nruns = 4" command, each with three heated and one cold Markov chains with sampling every 100 generations for 40 million generations. Output parameters were visualized using Tracer 1.4 (Rambaut & Drummond, 2007) to ascertain stationarity and convergence. The first 25% of the generations were discarded as burn-in. Maximum likelihood analyses were conducted using RAxML 7.2.6 (Stamatakis, 2006) under the GTRCAT model, with 1,000 nonparametric bootstrap replicates to assess nodal support. Nodes were considered strongly supported if their Bayesian posterior probability was ≥0.95 and their bootstrap value was ≥70% (Huelsenbeck & Rannala, 2004).

2.3 | Phylogeographical estimation

Poisson-Tree-Processes molecular species delimitation (PTP; Zhang, Kapli, Pavlidis, & Stamatakis, 2013) was used to delineate genetically distinct geographical clusters of samples (henceforth "lineages"). PTP was used because this approach does not require ultrametrization of trees (and its associated biases), showing more robustness to changes in the elected phylogenetic reconstruction method (Tang, Humphreys, Fontaneto, & Barraclough, 2014; Zhang et al., 2013). In the PTP model, speciation or branching events are modelled in terms of the number of substitutions (represented by branch lengths), so it only requires a phylogenetic input tree. Although this method is a useful tool for identifying structured phylogeographical lineages, divergence by itself is not evidence of speciation, and therefore obtaining species hypotheses only with molecular evidence and genetic methods can overestimate the number of species, considering divergent populations as different lineages (Tang et al., 2014). The delimitation of species must be carried using integrative approaches, including bioinformatics, genetic and morphological aspects. The lack of any of these aspects implies biases in the generation of hypotheses of the number of species proposed (Pyron, Hsieh, Lemmon, Lemmon, & Hendry, 2016). Because obtaining other types of evidence for integrative species delimitation in *Gerrhonotus* is beyond the goals of this study, species delimitation was not further pursued. The PTP analysis was conducted on the Web server of the Exelixis Lab (http://species.h-its.org/ptp/). A maximum likelihood tree was used as input. This tree was estimated as above but with a reduced number of terminals (n = 61), as PTP tends to overestimate the number of recognized species when there is an uneven sampling of individuals per species (Zhang et al., 2013). Thus, all identical sequences were first removed and the PTP analysis then ran for 200,000 MCMC generations with a thinning value of 100 and burn-in of 25%. Outgroups were removed to improve species delimitation (Cottontail et al., 2014).

Strongly supported clusters concordant with geography and proposed as distinct species by PTP were each considered as a distinct "lineage." However, when several clusters proposed as distinct species by PTP comprised a strongly supported, morphologically homogeneous clade distributed in the same biogeographical region, the clade was considered as a single lineage. Single divergent samples from unique geographical areas also were referred to as lineages when they were recognized as distinct, evolutionary independent from other samples by the PTP model and represented apparently isolated populations.

2.4 | Divergence time estimates

Testing temporal aspects of biogeographical hypotheses requires a time-calibrated phylogeny (Colston et al., 2013). To this end, divergence times and phylogeny were estimated simultaneously using a relaxed Bayesian molecular clock framework implemented in Beast 1.6.1 (Drummond & Rambaut, 2007). Because of potential problems associated with model parameter variance across heterogeneous datasets (Guiher & Burbrink, 2008), and to capture genetic diversity in other levels but reduce computational burden and sequencing costs, 1–2 individuals were selected from each currently recognized species or phylogeographical lineage inferred from the mtDNA dataset (n = 43) and two nuclear genes sequenced for this reduced dataset (see above).

To estimate divergence times, our multilocus dataset was used with a relaxed, uncorrelated lognormal clock and node constraints obtained from the fossil record with lognormal distributions to estimate divergence dates throughout the tree. The same partition strategy implemented in the phylogenetic analysis (see above) was used, except for the addition of a separate partition for each nuclear gene. Three fossil calibration points of Anguids and relates groups were used. See Appendix S3 for technical details on calibration points.

Analyses were run for 40 million generations, samples were retained every 1,000 generations and a Yule tree prior was specified. Results were displayed in Tracer 1.5 to confirm acceptable mixing and likelihood stationarity of the Markov chain Monte Carlo (MCMC) analyses, appropriate burn-in and adequate effective sample sizes (>200 for each estimated parameter). After discarding the first four million generations (10%) as burn-in, parameter values of the samples from the posterior were summarized on the maximum clade credibility tree using TreeAnnotator 1.4.8 (Drummond & Rambaut, 2007) with the posterior probability limit set to 0.1 and mean node heights summarized.

2.5 | Ancestral area reconstruction

The ancestral range at each divergence event was reconstructed using Bayesian binary Markov chain Monte Carlo analysis (BBM) as implemented in RASP 3.2 (Yu, Harris, Blair, & He, 2015). This program determines the probability of an ancestral range at a node by averaging over a posterior set of trees, thereby accounting for phylogenetic uncertainty (Bryson, Savary, & Prendini, 2013). A total of 36,000 post-burn-in trees were loaded from the divergence time analyses into Rasp. Each sample from the phylogeny was assigned to one of the following nine terminal biogeographical provinces and North American terrestrial ecoregions (Morrone, 2005; Wiken, Jiménez-Nava, & Griffith, 2011): (1) Mexican Plateau (MP); (2) Gulf of Mexico (GM); (3) Pacific Coast (PC); (4) Sierra Madre Oriental (SME); (5) Sierra Madre Occidental (SMO); (6) Sierra Madre del Sur (SMS); (7) Tamaulipas (TAM); (8) Trans-Mexican Volcanic Belt (TVB) and (9) Edwards Plateau (EP). The probabilities for nodes in the phylogeny were estimated. The analyses were conducted for 1 million generations with sampling every 100 using 10 chains. The first 25% of generations were discarded as burn-in.

3 | RESULTS

3.1 | mtDNA phylogenetic inference

The final dataset consisted of 980 aligned nucleotide positions. The partitions and models that best fit the data were as follows: *ND4* first position and tRNAs, TrN+G; *ND4* second position, HKY+I; and *ND4* third position, TrN+G. All sequences were deposited in GenBank (Appendix S1). ML and Bayesian inference analyses resulted in highly congruent phylogenetic trees with most clades strongly supported (Figure 2). The recovered relationships between the genera of Anguinae were in agreement with recently published phylogenies (e.g. Pyron et al., 2013), thereby providing a solid platform for divergence dating analysis.

Our phylogenetic analyses did not support the monophyly of *Gerrhonotus* (Figure 2): *Gerrhonotus lugoi* was the sister taxon to *Barisia* (although this relationship was not strongly supported), and the strongly supported group with the remaining species of *Gerrhonotus* was paraphyletic with respect to *Coloptychon*, while *G. parvus* was strongly supported as sister taxon to the latter species and *Coloptychon*. Except for *G. infernalis*, which was paraphyletic with respect to *G.* "CCB," all of the species of *Gerrhonotus* were monophyletic. Furthermore, *G. infernalis*, *G. ophiurus* and *G. liocephalus* each contained several strongly supported, geographically delimited lineages (including singletons).

3.2 | Phylogeographical estimation

Gerrhonotus lugoi was excluded from the PTP analyses because of its uncertain phylogenetic relationships. The samples of *Coloptychon* and *G. parvus* also were excluded after preliminary analyses including them resulted in evident over-splitting, likely due to their significant divergence and long-branch attraction. Thus, the analysis was focused on the group of keeled-scale *Gerrhonotus* ("core" *Gerrhonotus*). In addition, the samples of *G. liocephalus* from the TVB and SMS, which were estimated to comprise three distinct, evolutionary independent clusters by the PTP model, were considered as a single lineage because these clusters comprised a strongly supported, morphologically homogeneous clade and were distributed in the same biogeographical regions, yet were not segregated by region (i.e. two of the clusters were distributed both in TVB and SMS, and were not sister taxon to each other).

Within the group of keeled-scale *Gerrhonotus*, extensive geographical structure was evident within three well-supported major clades (Figure 2). One clade comprised all populations of *G. infernalis*, *G. ophiurus* and the putative undescribed species from CCB. The second clade corresponded to *G. liocephalus*, and the third clade to the populations of the putative undescribed species from western Mexico. Evolutionary independent clusters estimated by the PTP model were largely concordant with geography (i.e. individuals from regions in close geographical proximity generally formed monophyletic groups) (Figure 3). All of the haplotypes from the PC north from Guerrero comprised a clade (clade I, Figure 2). Although the PTP model recognized three evolutionary independent clusters within this clade, the clade was considered as a single lineage because it was strongly supported, morphologically homogeneous (i.e. all the specimens have 8–9 dorsal crossbands and dark bars on the lateral fold) and restricted to the ecologically homogeneous PC. Thus, on the basis of these samples and consistent with previous research (Good, 1994), the PC lineage was considered to represent a single undescribed species, hereafter referred to as *G.* "western."

Samples of *G. ophiurus* comprised three strongly supported main lineages recognized as distinct species by PTP (Figures 2 & 3). One lineage included the haplotypes from southern SME (II), whereas two lineages contained the haplotypes from the GM: one was composed of haplotypes from extreme south-eastern San Luis Potosí, adjacent Hidalgo and central Veracruz (III), whereas the other one contained haplotypes from central Veracruz and the Los Tuxtlas region in southern Veracruz (IV). Previously, the population from Los Tuxtlas was assigned to *G. liocephalus* by Good (1994) on the basis of external morphology. Although this assignment is in conflict with our results, the phenotype of our specimen is consistent with that of other populations of *G. ophiurus* (e.g. in the possession of a cantholoreal and 10 dorsal crossbands on the body), which supports its assignment to this taxon. The PTP model recognized 12 evolutionary independent lineages within *G. infernalis*. In two cases, however, two or more lineages proposed by the PTP model formed a strongly supported, morphologically homogeneous clade and were distributed in the same biogeographical province with no evident geographical barriers between them. One case involved the haplotypes from southern MP (lineage IX, Figures 2 & 3), which were estimated to represent three distinct lineages in the Sierra Gorda region by the PTP model. The second case involved four samples estimated to represent two distinct lineages by the PTP model (lineage VI, Figures 2 & 3), despite all samples being distributed in the TAM and separated from each other by less than 60 km. Based on this, for the purposes of our biogeographical interpretation only nine lineages within the widespread *G. infernalis* were recognized. These nine distinct,

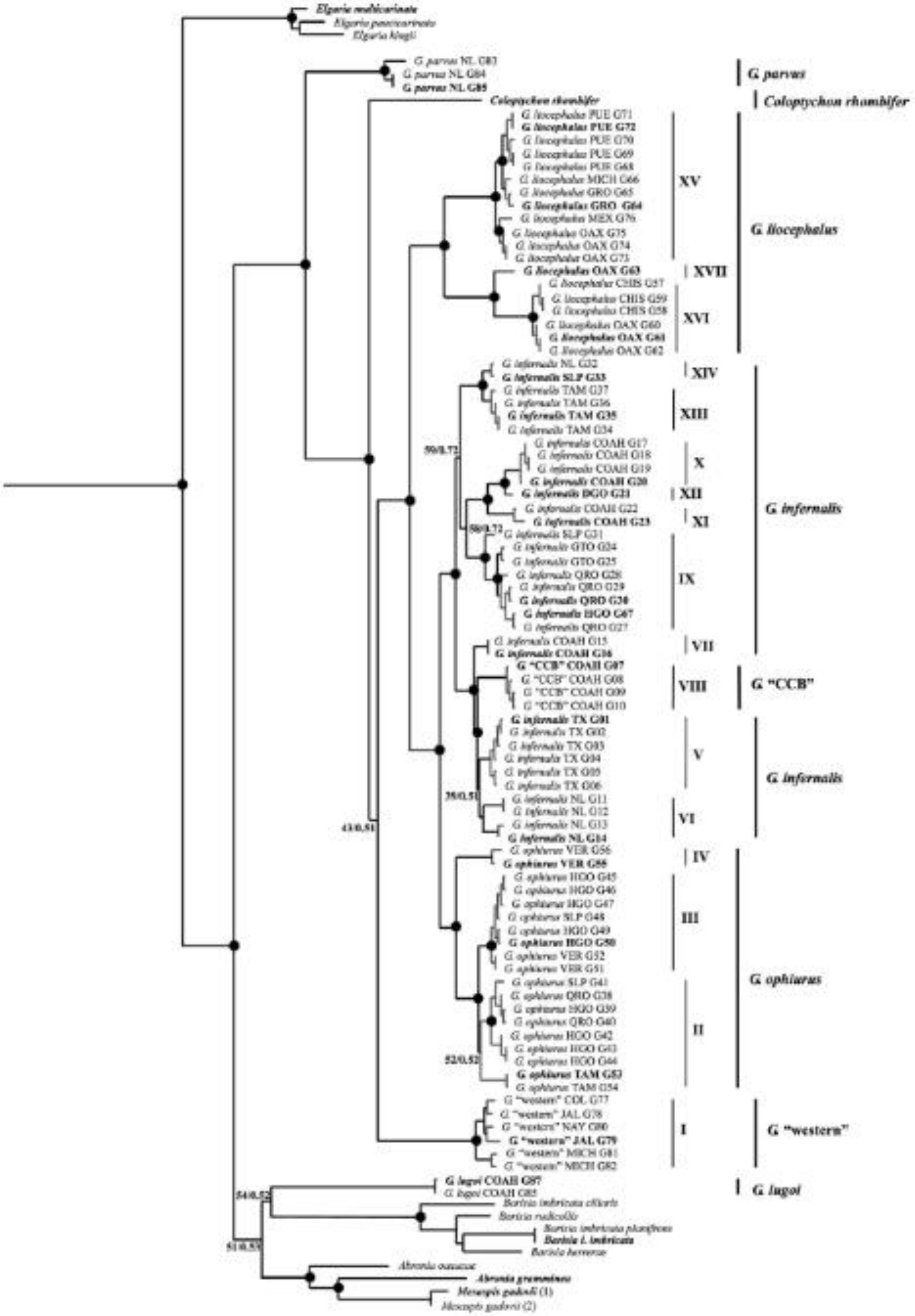
Elgaria multicarinata
Elgaria paucicarinata
Elgaria kingii
G. parvus NL G83
G. parvus NL G84
G. parvus NL G85
G. parvus
Coloptychon rhombifer
Coloptychon rhombifer
G. liocephalus PUE G71
G. liocephalus PUE G72
G. liocephalus PUE G70
G. liocephalus PUE G69
G. liocephalus PUE G68
G. liocephalus MICH G66
G. liocephalus GRO G65
G. liocephalus GRO G64
G. liocephalus MEX G76
G. liocephalus OAX G75
G. liocephalus OAX G74
G. liocephalus OAX G73
XV
G. liocephalus
G. liocephalus OAX G63
XVII
G. liocephalus CHIS G57
G. liocephalus CHIS G59
G. liocephalus CHIS G58
G. liocephalus OAX G60
G. liocephalus OAX G61
G. liocephalus OAX G62
XVI
G. infernalis NL G32
G. infernalis SLP G33
XIV
G. infernalis TAM G37
G. infernalis TAM G36
G. infernalis TAM G35
G. infernalis TAM G34
XIII
59/0.72
G. infernalis COAH G17
G. infernalis COAH G18
G. infernalis COAH G19
G. infernalis COAH G20
X
G. infernalis HGO G21
XII
G. infernalis COAH G22
G. infernalis COAH G23
XI
G. infernalis
58/0.72
G. infernalis SLP G31
G. infernalis GTO G24
G. infernalis GTO G25
G. infernalis QRO G28
G. infernalis QRO G29
G. infernalis QRO G30
G. infernalis HGO G67
G. infernalis QRO G27
IX
G. infernalis COAH G15
G. infernalis COAH G16
VII
G. "CCB" COAH G07
G. "CCB" COAH G08
G. "CCB" COAH G09
G. "CCB" COAH G10
VIII
G. "CCB"
G. infernalis TX G01
G. infernalis TX G02
G. infernalis TX G03
G. infernalis TX G04
G. infernalis TX G05
G. infernalis TX G06
V
G. infernalis
39/0.51
G. infernalis NL G11
G. infernalis NL G12
G. infernalis NL G13
G. infernalis NL G14
VI
43/0.51
G. ophiurus VER G56
G. ophiurus VER G55
IV
G. ophiurus HGO G45
G. ophiurus HGO G46
G. ophiurus HGO G47
G. ophiurus SLP G48
G. ophiurus HGO G49
G. ophiurus HGO G50
G. ophiurus VER G52
G. ophiurus VER G51
III
G. ophiurus
G. ophiurus SLP G41
G. ophiurus QRO G38
G. ophiurus HGO G39
G. ophiurus QRO G40
G. ophiurus HGO G42
G. ophiurus HGO G43
G. ophiurus HGO G44
G. ophiurus TAM G53
G. ophiurus TAM G54
52/0.52
II
G. "western" COL G77
G. "western" JAL G78
G. "western" NAY G80
G. "western" JAL G79
G. "western" MICH G81
G. "western" MICH G82
I
G. "western"
G. lugoi COAH G87
G. lugoi COAH G85
G. lugoi
54/0.52
Barisia imbricata ciliaris
Barisia rudicollis
Barisia imbricata planifrons
Barisia i. imbricata
Barisia herrerae
51/0.53
Abronia oaxacae
Abronia graminea
Mesaspis gadovii (1)
Mesaspis gadovii (2)

FIGURE 2 Genetic structure within *Gerrhonotus* inferred from a maximum likelihood analysis of the mitochondrial DNA dataset. Black dots represent strongly supported nodes (bootstrap value ≥70; Bayesian posterior probability value ≥0.95). Numbers at other nodes are boostrap/Bayesian posterior probability values. Vertical lines accompanied by roman numerals correspond to the genetically distinct, geographically cohesive lineages delimited by the Poisson-Tree-Processes model. Samples in boldface are samples used in the multilocus dataset analysis

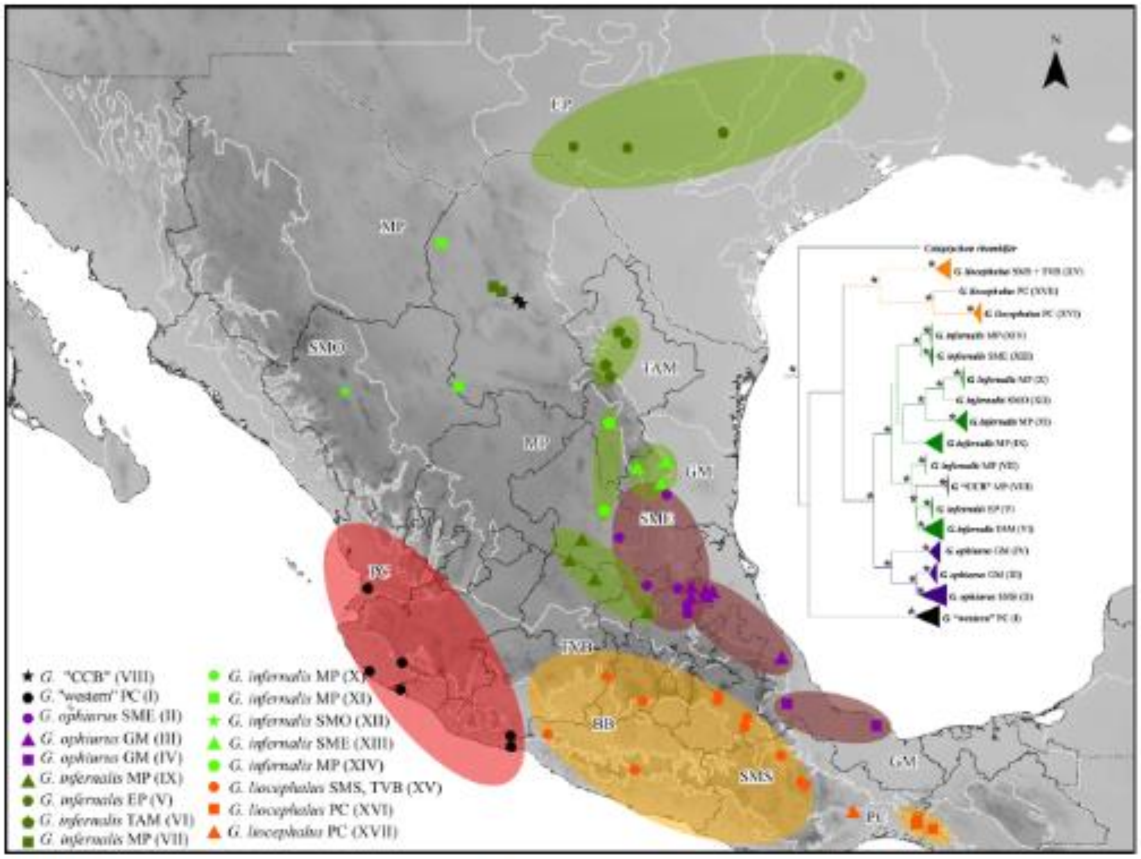

FIGURE 3 Distribution of the genetically distinct, geographically cohesive lineages delimited by Poisson-Tree-Processes model. Inset: Tree of the maternal genealogy based on Bayesian inference and maximum likelihood analyses. The asterisks represent strongly supported nodes (bootstrap value ≥70 and/or Bayesian posterior probability value ≥0.95). Selected lineages are collapsed for clarity. BB, Balsas Basin; EP, Edwards Plateau; GM, Gulf of Mexico; MP, Mexican Plateau; PC, Pacific Coast; Plio, Pliocene; SME, Sierra Madre Oriental; SMO, Sierra Madre Occidental; SMS, Sierra Madre del Sur; TAM, Tamaulipas; TVB, Trans-Mexican Volcanic Belt

geographically delimited lineages grouped into three major clades (Figures 2 & 3). The first and northernmost clade included three lineages: one from the EP (V), another one from the TAM (VI) and the other one from Sierra La Madera in central MP (VII). This clade also included the population from CCB in Coahuila (VIII), also recognized by the PTP analysis as an independent lineage hereafter referred to as G. "CCB." The second major clade included four lineages: one from southern MP (IX), one from Sierra Jimulco in central MP (X), one from Sierra La Madera in central MP (XI) and a singleton from Durango in the SMO (XII). The remaining major clade included two lineages, the first one from the SME (XIII) and the second one from the MP (XIV).

The samples of *G. liocephalus* were estimated to represent five evolutionary independent clusters by the PTP model. However, the three clusters from the TVB and SMS were considered as a single lineage (XV, see above). The remaining lineages contained the samples from Oaxaca-Chiapas in PC east of the Isthmus of Tehuantepec (XVI), and a single sample from the Mixe region, Oaxaca, in PC west of the Isthmus of Tehuantepec (XVII).

3.3 | Divergence times

Complete genetic data could not be obtained for three samples used in our multilocus analysis (Appendix S1). Specifically, *PRLR* sequences could not be obtained from *Coloptychon rhombifer*, *Barisia imbricata* and *Abronia*; thus, these samples were represented only by the mtDNA + *BDNF* data. The best-fitting models of sequence evolution for the data partitions were as follows: HKY + G for the first codon position of *ND4*, GTR + G + I for the second and third codon positions of *ND4*, TN + G for *PRLR* and JC + G for *BDNF*.

The multilocus analysis produced a phylogenetic reconstruction for *Gerrhonotus* with moderate resolution and nodal support (78% of nodes with PP > 0.95). The fossil-calibrated tree suggested that diversification in *Gerrhonotus* probably began in the late Oligocene 27 Ma (Figure 4) with a basal divergence between *G. parvus* and its sister group, including *Coloptychon*. Several divergences appear to have followed in the Miocene, including the divergences between *Coloptychon* and the species of core *Gerrhonotus* (20.8 Ma) and between *G.* "western" and the other keeled-scale species (19.5 Ma) in the early Miocene, and five splits within the *G. ophiurus* + *G. infernalis* clade and one within the *G. liocephalus* clade in the mid- to late Miocene. Our estimates placed the remaining divergences within the Pliocene (Figure 4).

3.4 | Historical biogeography

To assess the historical biogeography of the genus, the ancestral area reconstructions from the BBM analyses were combined with the divergence time estimates from the BEAST analysis of the multilocus dataset. Excluding *G. lugoi*, *G. parvus* and *C. rhombifer* (see Discussion), the Rasp analysis supported the assessment that the diversification of core *Gerrhonotus* likely began in the early Miocene in an ancestor distributed across the PC approximately 20.8 Ma. An initial divergence occurred within this ancestor approximately 19.5 Ma that separated the lineage from western Mexico from the MRCA of the other core *Gerrhonotus*. Subsequently, due to a second divergence within the last ancestor approximately 16.2 Ma, a southern and a northern clades emerged (Figures 4 & 5). The southern clade, distributed across the PC, SMS, Balsas Basin and eastern TVB, split between 2 and 11.5 Ma into three lineages of *G. liocephalus* (eastern Isthmus [XVII], western Isthmus [XVI] and SMS-TVB [XV]). Afterwards, in an important dispersal event, the MRCA of *G. ophiurus*, *G. infernalis* and *G.* "CCB" became widespread in the GM and MP during the mid-Miocene (11.7–13.4 Ma). This was followed by two vicariant events that occurred in the GM and MP during the late Miocene. The northern clade, distributed across the MP, SME, SMO, TAM, GM and EP, split between 2 and 11.7 Ma into three lineages of *G. ophiurus*, nine lineages of *G. infernalis*, and *G.* "CCB." Divergences within these regional clades appear to have happened during distinct temporal periods, including five recent events of colonization.

4 | DISCUSSION

4.1 | Historical biogeography

Based on our results, it appears that alligator lizards of the genus *Gerrhonotus* have had a relatively long history in the Mexican

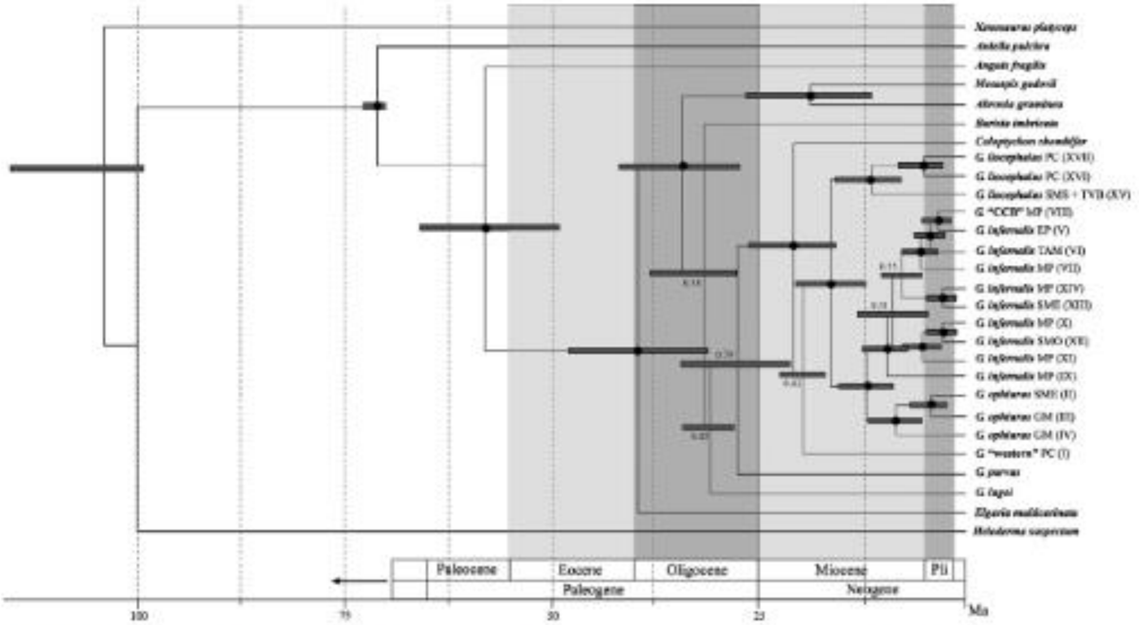

FIGURE 4 Divergence time estimates for *Gerrhonotus* estimated from the multilocus dataset using BEAST. Bars indicate 95% highest posterior densities of divergence dates, with mean estimates in millions of years ago (Ma) given at nodes. Nodes with black dots are well-supported nodes, and numbers at other nodes are Bayesian posterior probability values. EP, Edwards Plateau; GM, Gulf of Mexico; MP, Mexican Plateau; PC, Pacific Coast; Plio, Pliocene; SME, Sierra Madre Oriental; SMO, Sierra Madre Occidental; SMS, Sierra Madre del Sur; TAM, Tamaulipas; TVB, Trans-Mexican Volcanic Belt

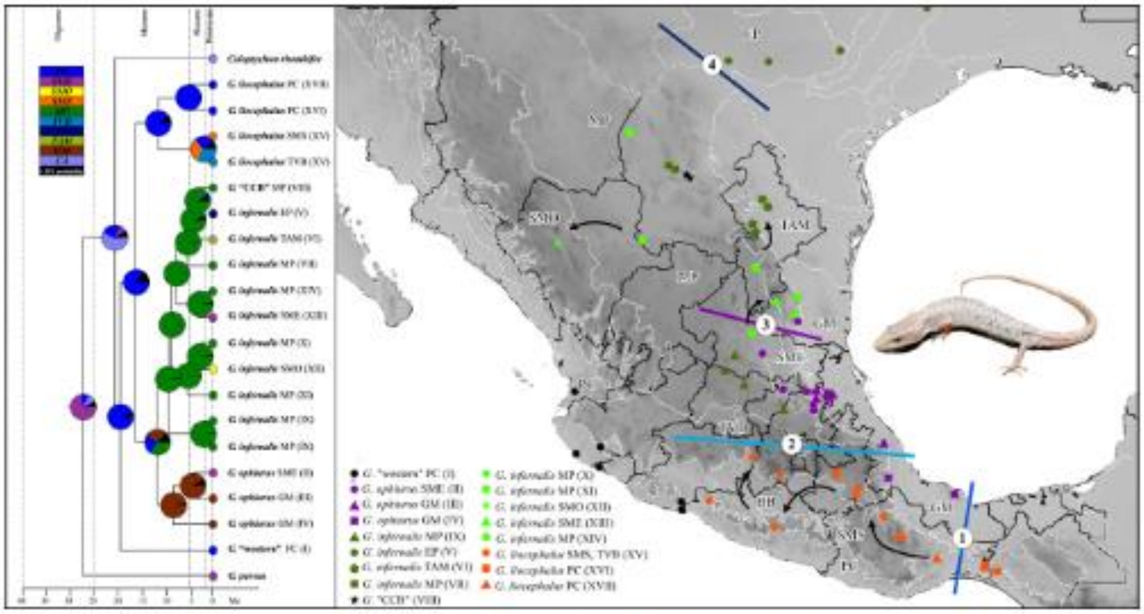

FIGURE 5 Left: Dated multilocus phylogeny for *Gerrhonotus*, showing ancestral area reconstructions. Sample localities are colour-coded to match the colours of the biogeographical regions in the inset box. Right: Geographical distribution of the lineages delimited by the Poisson-Tree-Processes model. The continuous lines and arrows indicate interpretations of historical events that affected diversification, representing vicariance and dispersion events, respectively. 1 = Isthmus of Tehuantepec, 2 = Trans-Mexican Volcanic Belt, 3 = Cerritos-Arista/Saladan barriers, 4 = Rio Grande. Abbreviations for biogeographical regions are as follows: EP, Edwards Plateau; GM, Gulf of Mexico; MP, Mexican Plateau; PC, Pacific Coast; SME, Sierra Madre Oriental; SMO, Sierra Madre Occidental; SMS, Sierra Madre del Sur; TAM, Tamaulipas; TVB, Trans-Mexican Volcanic Belt

territory. In *G. liocephalus*, the northernmost phylogroup consists of relatively undifferentiated samples from the SMS and TVB (lineage XV). These two mountain ranges are separated by the Balsas Basin, an arid lowland depression that formed in the Pliocene and Pleistocene in response to the uplifting of the TVB (Bryson, Murphy, et al., 2011). The Balsas Basin is a well-known barrier to gene flow in other taxa (Navarro-Siguenza, Peterson, Nyari, Garcia-Deras, & Garcia-Moreno, 2008) and the lack of genetic structuring in lineage XV could seem surprising. However, *G. liocephalus* inhabits a wide range of habitats (including pine forest, oak forest and desert scrub) and is distributed in the Balsas Basin between the SMS and TVB (Castro-Franco & Bustos, 2003). On the other hand, the southern phylogroup of *G. liocephalus* split in the late Miocene–early Pliocene into lineages XVI and XVII west and east of the Isthmus of Tehuantepec, respectively. Geological evidence suggests that from the late Miocene and through the late Pliocene an extensive subsidence of the eastern bloc along the Tehuantepec fault resulted in a massive reduction in the highlands and probably created a large marine embayment (Barrier, Velasquillo, Chávez, & Gaulon, 1998; Daza, Castoe, & Parkinson, 2010). Although it seems that most of the divergence events in the Isthmus occurred during the Pliocene-Pleistocene (Castoe et al., 2009), Daza et al. (2010) found a more ancient divergence in rattlesnakes, suggesting that a different geological/climatic event at the end of the Miocene (e.g. vegetation shifts; Cerling et al., 1997) may have been responsible for divergence in this group. The split between the lineages east and west of the Isthmus in the late Miocene (5–6 Ma) in *Gerrhonotus* is consistent with the latter hypothesis and with the formation of a savanna-like valley approximately 224 m above sea level and some 250 km wide at its narrowest point during the Miocene (ca. 6 Ma; Barrier et al., 1998).

The divergence between the southern and northern lineages of *G. ophiurus* (IV and II and III, respectively) in the late Miocene (8.4–10.2 Ma) coincides with the primary development of the TVB in the late Miocene ca. 7–19 Ma (Rosas-Elguera et al., 2003). This split is spatially and temporally consistent with estimated inter-specific divergence dates in co-distributed taxa (Mexican jays, 4.4–9.4 Ma, McCormack, Peterson, Bonaccorso, & Smith, 2008; montane rattlesnakes, 5–8 Ma, Bryson, García-Vázquez, et al., 2011; and gophersnakes, 5–8.6 Ma, Bryson, Murphy, et al., 2011). A recent revision of the past two decades of research on the origin of the TVB (Gómez-Tuena, Orozco-Esquivel, & Ferrari, 2007) suggests that four major volcanic episodes during the Neogene formed most of the range. One of these episodes around 10–19 Ma resulted in the rising of mountain formations that almost completely subdivided the east portion of Mexico (Bryson et al., 2012a). These shared temporal divergences suggest uplifting of the TVB in the late Miocene broadly impacted a variety of taxa (Bryson et al., 2012b). The posterior divergence between the northern GM (III) and SME (II) lineages of *G. ophiurus* during the early Pliocene could be facilitated by the development of any of several hypothesized filter barriers (Morafka, 1977), such as the Rio Pánuco basin (Anducho-Reyes et al., 2008) and Cerritos-Arista and Saladan Filter Barriers (Bryson, García-Vázquez, et al., 2011). On the other hand, given non-identical lineage ranges, soft allopatry through ecological vicariance may also explain these distributions (Pyron & Burbrink, 2010). In addition, the geographical structure of the populations of *G. ophiurus* in southern SME suggests the existence of two lineages in this province that are separated by the Cerritos-Arista/Saladan barriers, regions characterized by the absence of temperate vegetation (Morafka, 1977) and considered of great influence in the diversification of several co-distributed taxa (Bryson, García-Vázquez, et al., 2011; McCormack et al., 2008). The potentially distinct lineage north of these barriers is represented by the haplotypes of *G. ophiurus* from southern Tamaulipas; however, the split was not proposed by PTP.

The ancestral area reconstruction suggests the dispersal to and colonization of the MP by the ancestor of *G. infernalis* and *G.* "CCB" during the late Miocene (9.5–11.1 Ma), perhaps triggered by changing ecosystems associated with wetter climate (Bryson, García-Vázquez, et al., 2011; Retallack, 2001). Under this scenario, geological barriers limiting lineage distributions and explaining the diversification within MP may not be evident, but at least one of these events may have been caused by allopatric fragmentation associated with the expansion of pine–oak woodlands across the Mexican Plateau during the Pleistocene glacial periods (Bryson et al., 2013). The confinement of the distribution of *G. infernalis* to MP is congruent with regional genetic groups seen in other highland taxa. These geographically overlapping lineages suggest similar responses to barriers across this region. However, the distributions of the sister lineages of these MP lineages vary, suggesting that the MP is accumulating lineages from geographically different sources in different taxa (Bryson et al., 2012a). On the other hand, estimated dates of divergence vary across lineages in different studies, ranging from the Miocene to the Pleistocene. This suggests that deep canyons that bisect these mountains and low-elevation xeric habitats may be acting as overlooked filter barriers that promote diversification through time (Wiens, 2004). Our analysis suggests a first split in the late Miocene (9.4–10.4 Ma). The formation of the Cerritos-Arista and Saladan Filter Barriers may have significantly affected the biogeography of *G. infernalis*. These lowland barriers essentially bisect the Sierra Madre Oriental near central San Luis Potosí and correspond to the genetic break between southern lineages in MP (IX and XIV) and the rests of the lineages (Figure 5). A geographically similar genetic break occurs within the *Sceloporus jarrovii* group (Wiens & Penkrot, 2002), *Lampropeltis* (Bryson et al., 2007) and plants of the genus *Leucophyllum* (Gandara & Sosa, 2014).

In addition, the ancestral area reconstruction for *G. infernalis* suggests recent, independent colonization events from the MP: a first event (3.5–6.8 Ma) to the TAM (VI) and a posterior colonization (2.7–4.1 Ma) to the SMO (XII) and SME (XIII). The ancestral area reconstruction also suggests a dispersal event to the EP (V) from MP (3.2–4.7 Ma); however, Gustavson (1991) found evidence that the Rio Grande began its current southern drainage at the end of the Pliocene, congruent with the split of *G.* "CCB" (VIII) and the EP lineage (V) of *G. infernalis* (3.2–4.7 Ma), which suggests a vicariant event. *Gerrhonotus* "CCB" is only known from the shores of several small lagoons in the CBB (García-Vázquez et al., submitted). This region represents a relict zone, where pluvial periods during or immediately following the Pleistocene apparently caused a gradual withdrawal of the eastern border of the Chihuahuan Desert, which permitted the colonization of a more mesic-adapted lineage from the east (Milstead, 1960). Most of these colonists presumably perished with the return of desert conditions, but some lineages (e.g. *G.* "CCB") remained as relicts clinging to a precarious existence around oases. Finally, the remaining divergences between the lineages of *Gerrhonotus* in the MP began during the early Pliocene and considerably precede the Wisconsinan pluvial period. Thus, with the exception of *G.* "CCB," the latest Pleistocene glacial period does not appear to have been a factor in the origination of the extant lineage diversity or in the shaping of among-lineage geographical distribution, contrary to traditional views for mammals (Findley, 1969), other non-avian reptiles (Morafka, 1977) and birds (Hubbard, 1973) from the Sonoran and Chihuahuan regional deserts (Riddle, Hafner, & Alexander, 2000). Additional phylogeographical studies of highland taxa with wide distributions across Mexico and subsequent analyses within a comparative framework are needed to better elucidate idiosyncratic versus general processes promoting lineage diversification across the MP and Mexican highlands (Bryson, García-Vázquez, et al., 2011).

4.2 | Composition of *Gerrhonotus*

The composition of *Gerrhonotus* and the phylogenetic placement of *G. lugoi*, *G. parvus* and *Coloptychon* have been contentious for several decades (Bryson & Graham, 2010; Pyron et al., 2013). Our analyses place *G.* lugoi as either sister to *Barisia* (Figure 2) or as an early diverging member of *Gerrhonotus* (Figure 4), suggesting that the placement of *G. lugoi* within *Gerrhonotus* is questionable. On the other hand, in our analyses *G. parvus* was the sister taxon to the core *Gerrhonotus* and *Coloptychon*, although this relationship was not always strongly supported. In previous studies, *G. parvus* also has been recovered as the sister taxon to core *Gerrhonotus* (Conroy, Bryson, Lazcano, & Knight, 2005) or to core *Gerrhonotus* and *Coloptychon* (Pyron et al., 2013). In addition, in our study *Coloptychon* and the core *Gerrhonotus* always formed a strongly supported clade exclusive of the smooth-scale *Gerrhonotus*. In the last published species-level phylogeny of Squamata, *Coloptychon* also was nested within *Gerrhonotus* (Pyron et al., 2013), and this placement was strongly supported.

Retaining *Coloptychon* would require the phylogenetic placement of *Coloptychon* as sister to core *Gerrhonotus*, a position weakly supported in our analyses. In addition, it would require erecting one new genus for *G. parvus*. Transferring *Coloptychon* into *Gerrhonotus*, thus restoring the binomen *Gerrhonotus rhombifer*, is considered herein a better decision. However, placement of *G. lugoi*, *G. parvus*, *G. farri* and *G. lazcanoi* is problematical: *G. lugoi* never formed a clade with *G. parvus* or other *Gerrhonotus*, and its phylogenetic position was weakly supported in all the analyses; the position of *G. parvus* as sister taxon of the core *Gerrhonotus* and *Coloptychon* was not always strongly supported, and *G. lazcanoi* and *G. farri* could not be included in this study. Furthermore, all of these species exhibit a unique set of morphological characters that are not present in the other species of *Gerrhonotus* (e.g. smooth dorsal scales, rostral–nasal contact and supranasals–cantholoreals contact; Bryson & Graham, 2010). Given the present uncertainty regarding the phylogenetic relationships of *G. farri*, *G. lazcanoi*, *G. lugoi* and *G. parvus*, they are kept herein in the genus *Gerrhonotus*, but this placement must be regarded as tentative. Clearly, future research with more extensive sampling of loci and taxa, including *G. farri* and *G. lazcanoi*, is needed to better ascertain the composition of the genus and the phylogenetic positions of the smooth-scale *Gerrhonotus*.

Similarly, the paraphyly of *G. infernalis*, in addition to the existence of several strongly supported, geographically delimited, and sometimes moderately divergent lineages within *G. infernalis*, *G. liocephalus* and *G. ophiurus* suggest that each of these taxa may be composed of multiple evolutionary independent lineages or species. An integrative species delimitation study of the keeled-scale *Gerrhonotus* is needed to determine the number of evolutionary independent lineages in the clade.

5 | CONCLUSIONS

Biogeographical studies seek to explain the distributions of species in terms of historical factors and contemporary ecology. The genus *Gerrhonotus* has proven to be an insightful model for studying these factors in a widely distributed group. Extreme climatic oscillations during the Pleistocene, a key driver of diversification between lineages in some taxa (León-Paniagua et al., 2007), do not appear to have substantially affected diversification in *Gerrhonotus*. Instead, Miocene and Pliocene geomorphology, perhaps in conjunction with climate change, appears to have induced allopatric divergence on a relatively small spatial scale in this genus. There is strong support for a genus *Gerrhonotus* composed of the keeled-scale species in the genus in addition to *Coloptychon rhombifer* (= *G. rhombifer*), whereas inclusion of the smooth-scale *Gerrhonotus* into the genus should be regarded as tentative. *Gerrhonotus infernalis*, *G. liocephalus* and *G. ophiurus* may each be composed of multiple evolutionary independent lineages.

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BIOSKETCHES

The authors share a general interest in the systematics and biogeography of amphibians and reptiles from Mexico.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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A New Species of *Gerrhonotus* (Squamata: Anguidae) from the Cuatro Ciénegas Basin, Coahuila, Mexico

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ABSTRACT: A new species of *Gerrhonotus*, previously confused with *G. infernalis*, is described from the Cuatro Ciénegas Basin, Coahuila, Mexico. The new species is known from the type locality only and is characterized by the following combination of characters: a cantholoreal scale usually present, a dark mark extending anteriorly from the lower temporal scales through the lower border of the orbit to the preocular or cantholoreal scales, keeled dorsal scales, dorsal body pattern usually composed of cross-bands mostly interrupted or barely discernible on middorsum, usually indiscernible vertical dark bars on the lateral fold (few, faint bars occasionally present), and black flecks scattered on the venter. The new species is geographically closest to *G. lugoi* and *G. infernalis* but morphologically most similar to *G. infernalis*.

Key words: Alligator lizards; Cryptic species; Gerrhonotinae; Taxonomy

THE ANGUID lizard genus *Gerrhonotus* Wiegmann 1828 is a conspicuous component of the herpetofauna of south-central United States and Mexico. The genus is characterized by the loss of at least one scale in the canthal/loreal series and the possession of five or fewer temporals per vertical row, 10–14 longitudinal rows of ventrals, four large and one small pairs of chinshields, and a light-on-dark dorsal cross-banding pattern (Good 1988; García-Vázquez et al. 2018). Currently, eight species of *Gerrhonotus* are formally recognized. Good (1994), in a comprehensive study of species limits in the genus, recognized four species: *Gerrhonotus lugoi* McCoy 1970 distributed in the Sierra de San Marcos y Pinos and Sierra de la Madera mountain ranges surrounding the Cuatro Ciénegas Basin (CCB) in central Coahuila, Mexico, and an isolated population in central Nuevo León (García-Vázquez et al. 2016); *G. infernalis* Baird 1859 distributed from central and west-central Texas, USA, south to central Hidalgo, Mexico, with isolated populations in south-central Chihuahua and adjacent Durango and southwestern Coahuila and adjacent Durango (Good 1994; Ramírez-Bautista et al. 2010); *G. liocephalus* Wiegmann 1828 distributed from central Guerrero and central Puebla south and east to southeastern Chiapas, with an isolated population in the Los Tuxtlas mountain range in southern Veracruz; and *G. ophiurus* Cope 1867 distributed from southeastern San Luis Potosí and southeastern Tamaulipas (García-Padilla and Villegas-Ruiz 2010) southeast to central Veracruz, Mexico. In addition, *G. parvus* Knight and Scudday 1985 was described from the Sierra Madre Oriental in west-central Nuevo León. This species was subsequently transferred to the genus *Elgaria* Gray 1838 by Smith (1986) and then placed back into *Gerrhonotus* by Conroy et al. (2005). Bryson and Graham (2010) described *G. farri* Bryson and Graham 2010, and Banda-Leal et al. (2017) described *G. lazcanoi* Banda-Leal, Nevárez-de los Reyes and Bryson 2017, both from the Sierra Madre Oriental of extreme southwestern Tamaulipas and central Nuevo León, respectively. Finally, *G. rhombifer* Peters 1876 was described from the Province of Chiriquí in western Panamá. Later, Tihen (1949) erected the monotypic genus *Coloptychon* Tihen 1949 to accommodate this species. García-Vázquez et al. (2018) placed *C. rhombifer* back into *Gerrhonotus*, however, raising the number of recognized species in this genus to eight.

Good (1994) tentatively referred certain populations of *Gerrhonotus* in western Mexico (from Durango, Sinaloa, Jalisco, and Colima) to *G. liocephalus* (his *G.* cf. *liocephalus* Samples 19 and 20). However, presumably because the limited number of available samples hindered a proper characterization of the geographic variation of morphological characters within and among *Gerrhonotus* species, Good (1994) was unable to determine with certainty whether these populations were conspecific with *G. liocephalus* or represented one or more distinct species. Recently, Castiglia et al. (2010) provided molecular evidence suggesting that populations of *Gerrhonotus* from western Jalisco might represent an undescribed species. While preparing this paper, based on the revision of a greater sample of specimens, we have gathered morphological and molecular evidence that corroborates this suggestion (A. Nieto-Montes de Oca, personal observation) and describe this species elsewhere. For the purposes of this paper, we refer to this undescribed taxon as *Gerrhonotus* cf. *liocephalus* from Western Mexico.

In a scientific meeting in 1989, A. Contreras-Arquieta (personal communication) suggested that the population of *Gerrhonotus* in the vicinity of the CCB, Coahuila, represented a new subspecies of *G. liocephalus* (*G. liocephalus aguayoi*) and provided a brief description for this taxon in the abstract for the meeting. Good (1994), however, was unable to include this taxon in his analysis of species limits in the genus and assigned all populations of *Gerrhonotus* from Coahuila to *G. infernalis*. Nonetheless, examination of several specimens of *Gerrhonotus* from the CCB and the

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surrounding mountains, as well as additional specimens of *Gerrhonotus* from Mexico and the United States, suggested that the population from the CCB actually represents a distinct, undescribed species. Herein, we formally describe this species.

MATERIALS AND METHODS

We examined a total of 16 specimens of *Gerrhonotus* from the CCB, Coahuila. All specimens were collected during fieldwork conducted in the Basin between 2008 and 2013. The specimens were fixed in 10% buffered formalin, preserved in 70% ethanol, and deposited in the herpetological collection of the Museo de Zoología of the Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC). These specimens were compared with representatives of *G. infernalis*, *G. liocephalus*, *G. lugoi*, *G. ophiurus*, and G. cf. *liocephalus* from Western Mexico (Appendix). The diagnosis is based on the specimens examined and the relevant literature (Good 1994; Bryson and Graham 2010; Lamar et al. 2015; Banda-Leal et al. 2017).

Nomenclature of scales follows Bogert and Porter (1967) and Good (1994). Scale counts were performed with the aid of a dissecting microscope. The number of transverse dorsal scale rows was determined following Campbell (1982). Measurements were taken with calipers (±0.1 mm). In the description of the holotype, bilateral characters were scored on both the left and right sides. When the condition of a given meristic or morphometric character was not identical on both sides, the conditions on the left and right sides are reported as left/right. Scale counts and measurements for *G. farri*, *G. lazcanoi*, *G. parvus*, and *G. rhombifer* were obtained from Bryson and Graham (2010), Banda-Leal et al. (2017), and Lamar et al. (2015). Institutional codes for museum and collections follow Sabaj-Perez (2016). We adhere to the Evolutionary Species Concept of Wiley and Mayden (2000) using morphological diagnosability (i.e., the presence of exclusive morphologies) as evidence to infer the existence of distinct species.

SPECIES DESCRIPTION

Gerrhonotus ***mccoyi*** sp. nov.

(Figs. 1, 2; Table 1)

Gerrhonotus infernalis Baird 1859: Good (1994), in part.

Holotype.—MZFC 29654 (field number UOGV 1438), adult male from Poza Churince, municipality of Cuatro Ciénegas, Coahuila, Mexico (26°55′11.9″N, 102°06′53.2″W; datum = WGS84), 739 m elevation, collected 9 September 2007 by U.O. García-Vázquez, M. Trujano-Ortega, and A. Contreras-Arquieta.

Paratypes.—Fifteen specimens; all from the municipality of Cuatro Ciénegas, Coahuila, Mexico: 13 (MZFC 29655–66, 29669) from the same locality as the holotype and two (MZFC 29667–68) from Pozas Azules, Rancho Pronatura (26°49′32.9″N, 102°01′20.9″W; datum = WGS84), 714 m elevation.

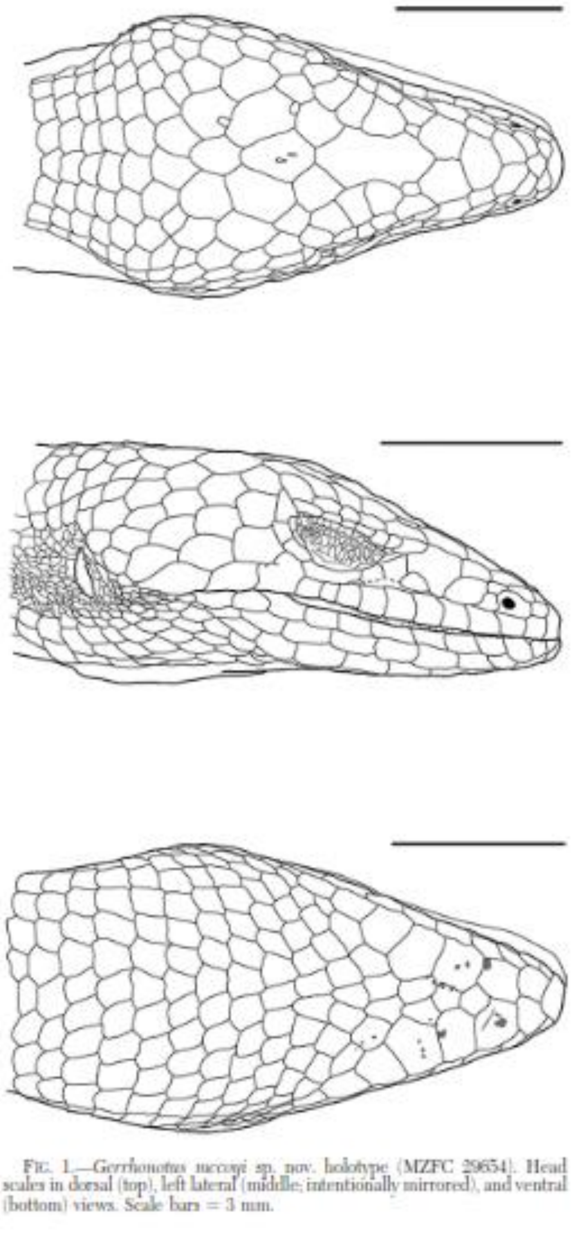

FIG. 1.—*Gerrhonotus mccoyi* sp. nov. holotype (MZFC 29654). Head scales in dorsal (top), left lateral (middle; intentionally mirrored), and ventral (bottom) views. Scale bars = 3 mm.

Diagnosis.—*Gerrhonotus mccoyi* sp. nov. can be distinguished from its congeners by a combination of characters which includes the presence of a cantholoreal scale (72%; *n* = 16), a dark mark extending anteriorly from the lower temporal scales through the lower border of the orbit to the preocular or cantholoreal scales, keeled dorsal scales, usually (75%; *n* = 16) 7–9 dorsal cross-bands mostly interrupted or barely discernible on middorsum (few cross-bands continuous, noticeable across middorsum in some specimens; midsection of cross-bands paler, narrower than the lateral sections), usually indiscernible vertical dark bars on the lateral fold (few, faint bars occasionally present), and black flecks scattered on the venter.

Comparisons with other species (Table 2).—*Gerrhonotus mccoyi* sp. nov. can be distinguished from the other species of the genus by having black flecks scattered on the

FIG. 2.—*Gerrhonotus mccoyi* sp. nov. in life. (A) Male (paratype, MZFC 29668). (B) Female, not collected. Photographs by UOG-V and P. Heimes, respectively. A color version of this figure is available online.

venter (venter immaculate or with marks other than black flecks, or dark marks restricted to the sides of the venter in the other species [venter pale gray or pale brown, distinctly mottled with white in *G. infernalis* and *G. rhombifer*; dark marks restricted to the sides of the venter rarely present in *G. liocephalus*, *G. ophiurus*, and *G. parvus*; usually a row of small dark spots on each side of the venter in *G.* cf. *liocephalus* from Western Mexico—in this latter species, the dark spots tend to merge into dark longitudinal stripes in some specimens, and a gray midventral stripe is present in some specimens]). *Gerrhonotus mccoyi* sp. nov. differs from *G. farri*, *G. lazcanoi*, *G. lugoi*, *G. parvus*, and *G. rhombifer* by having keeled dorsal scales (dorsal scales smooth in the other species); from *G. farri*, *G. infernalis*, *G. lazcanoi*, *G. lugoi*, *G. ophiurus*, *G. parvus*, and *G. rhombifer* by having a dorsal body pattern usually composed of cross-bands mostly interrupted or barely discernible on middorsum (cross-bands continuous, conspicuous across middorsum present in the other species); and from *G. liocephalus*, *G. ophiurus*, and *G.* cf. *liocephalus* from Western Mexico by usually lacking discernible dark bars on the lateral fold (dark bars on the lateral fold prominent in the other species). *Gerrhonotus mccoyi* sp. nov. can be distinguished from all of the species of the genus, except *G. liocephalus* and *G. ophiurus*, by having a dark temporal-cantholoreal mark (dark temporal-cantholoreal mark absent in the other species [present only in young specimens, vestigial in adults, in *G. infernalis* and *G. rombifer*; head completely black in *G. lazcanoi*]); and

TABLE 1.—Variation in selected morphometric characters in the type series of *Gerrhonotus mccoyi* sp. nov., including the holotype ($n = 16$). SVL = snout–vent length; HW = head width; HL = head length; EW = ear width; FL = forelimb length; HIL = hind limb length; TI = tibia length; DL = fourth toe length; TL = trunk length; T = tail length; SE = standard error. * = Holotype.

			Trait									
Specimen	Sex	Age	SVL	HW	HL	EW	FL	HIL	TI	DL	TL	T
MZFC29654*	Male	Adult	122.65	15.76	24.86	3.62	26.63	37.31	13.41	8.46	72.46	265.21
MZFC29657	Male	Adult	112.85	13.88	23.27	3.25	28.03	34.59	12.65	7.81	62.03	255.55
MZFC29669	Male	Adult	128.63	18.01	27.94	3.65	31.58	38.86	15.15	8.69	76.97	212.25
MZFC29663	Male	Adult	107.01	13.66	22.96	3.38	27.35	31.7	12.4	7.84	58.43	227.3
MZFC29655	Male	Young	62.25	7.86	13.23	1.9	14.85	17.79	7.51	5.27	36.29	128.25
MZFC29668	Male	Adult	118.36	15.38	24.79	3.47	31.73	34.65	14	8.4	67.46	200.64
MZFC29659	Male	Adult	101.77	12.64	21.3	3.25	24.3	32.61	12.49	8.03	58.86	198.33
MZFC29658	Male	Adult	108.92	13.87	22.64	3.06	27.64	33.45	12.51	8.18	65.42	210.44
MZFC29660	Male	Adult	106.86	13.7	21.93	3.21	24.75	30.51	12.2	7.62	61.01	156.46
MZFC29662	Male	Young	91.17	11.05	18.07	3.02	22.56	24.97	11.33	7	52.54	210.23
$\bar{X} \pm 1$ SE			106 ± 18.7	13.6 ± 2.7	22.1 ± 4.0	3.2 ± 0.5	25.9 ± 4.9	31.6 ± 6.2	12.4 ± 2.0	7.7 ± 1.0	61.1 ± 11.3	206.5 ± 40.9
MZFC29666	Female	Young	83.26	10.28	16.32	3.15	20.06	52.8	11.95	7.25	48.46	166.68
MZFC29661	Female	Young	79.08	9.66	16.99	2.33	20.52	24.27	8.68	6.19	47.62	164.21
MZFC29665	Female	Young	77.59	9.62	16.78	2.11	20.62	26.96	11.38	5.88	44.96	69
MZFC29664	Female	Young	87.38	10.67	18.42	3.12	20.91	26.57	10	6.79	52.63	181.13
MZFC29656	Female	Young	100.94	13.86	20.14	2.77	25.79	30.1	11.02	5.62	58.04	167.45
MZFC29667	Female	Adult	121.17	15.75	24.13	13.33	29.27	35.03	12.3	8.77	68.31	200.34
$\bar{X} \pm 1$ SE			91.6 ± 26.3	11.6 ± 3.5	18.8 ± 5.4	4.5 ± 2.6	22.9 ± 6.4	32.6 ± 9.5	10.7 ± 3.0	6.8 ± 1.8	53.3 ± 15.0	158.1 ± 57.1

TABLE 2—Character states for the diagnostic characters of *Gerrhonotus mccoyi* sp. nov. and all of the remaining species of *Gerrhonotus*. Data for *G. farri*, *G. lazcanoi*, *G. parvus*, and *G. rhombifer* obtained from Bryson and Graham (2010), Banda-Leal et al. (2017), and Lamar et al. (2015). *n* = sample size.

Characters	*G. mccoyi* sp. nov.	*G. farri*	*G. lazcanoi*	*G. lugoi*	*G. liocephalus*	*G. ophiurus*	*G. infernalis*	*G.* cf. *liocephalus* Western Mexico	*G. parvus*	*G. rhombifer*
n	16	1	1	2	24	14	43	6	4	6
Venter pattern	With scattered black flecks	Immaculate	Grayish beige, distinctly mottled with white	Immaculate	Immaculate (71%) or with dark marks only on sides of venter (29%)	Immaculate (25%) or with dark marks only on sides of venter (75%)	Grayish beige, distinctly mottled with white	Dark spots on sides of venter	Immaculate	Brown, distinctly mottled with white, paraventral triangular white patches
Dark temporal-cantholoreal mark	Present	Absent	Head uniformly dark	Absent	Absent (65%) or present (35%)	Present	Present in young specimens; vestigial in adults	Absent	Absent	Present only in young specimens
Dorsal pattern	Cross-bands usually interrupted or narrow, faint on middorsum (81%)	Cross-bands conspicuous on middorsum	Cross-bands conspicuous on middorsum	Cross-bands conspicuous on middorsum	Cross-bands usually interrupted or faint on middorsum (85%)	Cross-bands conspicuous on middorsum	Cross-bands conspicuous on middorsum	Cross-bands interrupted (60%) or conspicuous on middorsum (40%)	Cross-bands conspicuous on middorsum	Cross-bands conspicuous on middorsum
Dark bars on lateral fold	Usually absent (rarely faint)	Absent	Absent	Absent	Present, prominent	Present, prominent	Absent	Present, prominent	Absent	Absent
Dorsal scales	Keeled	Smooth	Smooth	Smooth	Keeled	Keeled	Keeled	Keeled	Smooth	Smooth
Number of dorsal cross-bands	7–9	12	9	10	0–8	9–11	7–11	8–9	13–14	6–8
Cantholoreal scales	Usually present (72%)	Absent	Absent	Absent	Usually present (80%)	Usually present (71%)	Usually absent (92%)	Usually present (75%)	Present	Absent

from *G. farri*, *G. infernalis*, *G. lazcanoi*, and *G. lugoi* by usually having a cantholoreal scale (cantholoreal scale usually absent [92%, $n = 39$] in *G. infernalis*, and absent in the other species).

Description of holotype (Fig. 1).—Adult male with both hemipenes partially everted. Head scales flat, smooth. Snout bluntly rounded in dorsal view, truncate in lateral view. Rostral ~1.5 × wider than tall, bordered posteriorly by one medial postrostral and one anterior internasal on each side of postrostral. Postrostral kite-shaped, 1.3 × wider than long, in narrow contact anteriorly with rostral, broad contact anterolaterally with anterior internasal and posterolaterally with supranasal on each side, and narrow contact posteriorly with posterior internasals. Anterior internasals approximately as wide as, and slightly shorter than, postrostral, each in contact laterally with first supralabial and nasal, and posteriorly with postrostral and supranasals. Supranasals 1.2 × wider than long, obliquely oriented, separated medially from each other by postrostral. Posterior internasals larger than supranasals, obliquely oriented, each in broad contact laterally with supranasal and upper postnasal, posterolaterally with canthal, and posteriorly with frontonasal. Frontonasal 1.5 × wider than long, in contact laterally with canthal on either side and posteriorly with prefrontals. Prefrontals 1.2 × wider than long, in narrow and broad contact laterally with canthal and cantholoreal, respectively; in contact posteriorly with first median supraocular, one small scale between first median supraocular and frontal, and frontal. Frontal 2.1 × longer than wide, in contact laterally with one small scale between prefrontal and second median supraocular, second and third median supraoculars, and frontoparietal on either side, and posteriorly with interparietal. Frontoparietals approximately as wide as long, each in contact laterally with third and fourth median supraoculars, posterolaterally with upper primary temporal, and posteriorly with parietal. Median supraoculars 5/5; first 1.9 × longer than wide; second as wide as long; third, fourth, and fifth 1.2–1.6 × wider than long; lateral supraoculars 3/3, much smaller than median supraoculars. Interparietal 1.3 × longer than wide, kite-shaped, enclosed by frontoparietals, parietals, and interoccipital; pineal eye poorly defined, situated on its posterior half. Parietals 1.1 × longer than interparietal, in contact anterolaterally with upper primary temporal, posterolaterally with a larger upper temporal (presumably representing fused upper secondary and upper tertiary temporals), posteriorly with occipital, and posteromedially with interoccipital. Two rows of postoccipitals; transverse scale rows on each side of postoccipitals extending laterally to upper margin of ear.

Nasals elongate antero-posteriorly, with naris situated posteriorly; separated from rostral by anterior internasal. Postnasals 2/2, subequal in size; lower ones in narrow and broad contact with second and third supralabials, respectively. Canthus rostralis rounded. Canthals 1/1, slightly longer than wide. Loreals 1/1, slightly wider than long, slightly larger than canthals. Cantholoreals 1/1, about as large as canthal and loreal combined, in contact anteriorly with canthal and loreal, posteriorly with first superciliary and upper preocular, and ventrally with fourth and fifth supralabials on left side and fifth supralabial on right side. One roughly triangular scale between cantholoreal, upper preocular, and supralabials on either side. Preoculars 1/1, approximately as wide as long; suboculars 2/2, anterior one slightly longer than wide, posterior one longitudinally elongate; postoculars 3/3. Superciliaries 7/7; first superciliary larger than remaining superciliaries. Supralabials 13/13; last three much larger and higher than anterior ones. Temporal scales in five rows. Primary temporals 4/4, lower primary temporal in contact with 11th and 12th supralabials on either side. Upper secondary and upper tertiary temporals presumably fused into one large scale in dorsal contact with parietal and occipital on either side; 3/3 and 4/4 secondary and tertiary temporals, respectively, extending ventrally from presumably fused upper secondary and upper tertiary temporal scales. Lower secondary and lower tertiary temporals in contact with 12th and 13th and 13th supralabials, respectively, on either side.

Mental approximately 1.5 × wider than long. Infralabials 12/11. Two postmentals. Six pairs of chinshields; those of first pair in broad contact with each other, those of second and third pairs separated by one and two scales, respectively.

External ear opening oval, vertically elongate (maximum width = 0.8 mm, maximum height = 3.0 mm), without lobules or spines. Dorsal scales keeled, imbricate, nearly equal in size to ventrals; in 10 longitudinal rows on neck and 14 rows at level of midbody; in 47 transverse rows from first row of nuchals to last scale row lying at least partially over posterior portion of thighs. Lateral fold well developed. Ventral scales in 40 transverse rows from anterior insertion of forelimbs to vent; in 12 longitudinal rows at level of midbody. Medial pair of precloacal scales nearly twice as large as lateral precloacal scales. Scales on dorsal surface of forelimbs smooth except for some faintly keeled scales on arms; scales on anterodorsal surface of thighs and dorsal surface of shanks smooth. Supradigital scales in one row; subdigital lamellae rounded. Subdigital lamellae on manus I 7/8, II 11/11, III 14/13, IV 16/17, V 11/11. Subdigital lamellae on pes I 8/7, II 12/12, III 15/14, IV 18/18, V 12/12. Hemipenes bifurcate distally.

Color in preservative.—Head, body, limbs, and tail ground color light brown dorsally and laterally, white ventrally. Head immaculate dorsally and laterally except for one dark brown spot on anteroventral corner of lower primary temporal scale on each side, one dark scale on temporal region on right side, and another one on nuchal region on left side. Body with eight dark, dorsal cross-bands; one at level of midneck and seven between levels of anterior insertion of arms and groin; cross-bands heterogeneous; their midsection narrower and paler than lateral sections, composed of two or three often fragmented rows of intermingled white, pale brown, or dark brown scales; their lateral sections wider, overall darker, usually composed of 2–4 (occasionally 5) short scale rows checkered with white, dark brown, and black scales; cross-bands separated from each other by 2–3 transverse scale rows. Lateral fold white; vertical dark bars indiscernible except for few (<5), barely perceptible, poorly defined bars on each side. Thighs with few, small, irregular dark spots on anterodorsal surface. Tail with four dark, dorsal cross-bands on anterior end; first at level of posterior insertion of legs, remaining ones separated from each other by two scale rows; each cross-band composed of three scale rows checkered with white, pale brown, and dark brown scales; first cross-band conspicuous; remaining ones gradually becoming fainter posteriorly.

Ventral surface of head, body, limbs, and tail immaculate white except for some irregular, scattered black flecks on belly.

Variation.—This section is based on all of the paratypes. Multistate characters: Postrostral separated from posterior internasal by supranasal on right side in MZFC 29660; separated from posterior internasals by one tiny scale in MZFC 29660. Supranasal divided on right side in MZFC 26667. Cantholoreal absent on both sides in four specimens, absent on left side in MZFC 29664. Upper primary temporal in contact with first supraocular on left side in MZFC 29665. Meristic characters: Canthals 1–3, $\bar{X} = 1.5$ (1/1 [$n = 7$], 1/2 [$n = 1$], 2/1 [$n = 1$], 2/2 [$n = 5$], 3/2 [$n = 1$]); loreals 1–3, $\bar{X} = 1.5$ (1/1 [$n = 3$], 2/2 [$n = 9$], 3/2 [$n = 1$], 3/3 [$n = 2$]); superciliaries 6–8, $\bar{X} = 6.5$ (6/6 [$n = 5$], 6/7 [$n = 3$], 7/6 [$n = 2$], 7/7 [$n = 4$], 8/7 [$n = 1$]); supralabials 12–13, $\bar{X} = 12.7$ (12/12 [$n = 1$], 12/13 [$n = 3$], 13/12 [$n = 4$], 13/13 [$n = 7$]); preoculars 1–2, $\bar{X} = 1.1$ (1/1 [$n = 13$], 1/2 [$n = 1$], 2/2 [$n = 1$]); suboculars 1–3, $\bar{X} = 2.1$ (1/2 [$n = 1$], 2/2 [$n = 10$], 2/3 [$n = 4$]); postoculars 3–4, $\bar{X} = 3.1$ (3/3 [$n = 12$], 3/4 [$n = 2$], 4/4 [$n = 1$]); primary temporals 4–5, $\bar{X} = 4.2$ (4/4 [$n = 10$], 4/5 [$n = 3$], 5/4 [$n = 2$]); secondary temporals 3–4, $\bar{X} = 3.1$ (3/3 [$n = 13$], 3/4 [$n = 2$]). Longitudinal dorsal scale rows 16 in all specimens; transverse dorsal scale rows 45–49, $\bar{X} = 47.1$; lamellae under fourth toe 17–21, $\bar{X} = 18.9$ (17/18 [$n = 2$]; 17/19 [$n = 1$]; 18/19 [$n = 3$]; 18/20 [$n = 4$]; 19/19 [$n = 1$]; 19/20 [$n = 1$]; 19/21 [$n = 1$]; 20/20 [$n = 1$]; 21/21 [$n = 1$]).

Color pattern (in preservative).—This section is based on all of the paratypes unless noted otherwise. The head, body, limbs, and tail ground color was pale to medium brown on the dorsal and lateral surfaces. A dark brown mark on the side of the head was present in all of the specimens ($n = 14$; MZFC 29656 damaged); however, it was highly variable in distinctness and extent. Usually, the mark consisted of a dark brown spot on the anteroventral corner of the lower primary temporal (gradually becoming diffuse on the rest of the scale) that extended anteriorly through the lower postocular, then narrowed into a thin line along the dorsal margin of the suboculars, and broadened again into a diffuse splotch on the upper portion of the preocular. The mark was usually evident on the lower primary temporal and lower postocular, but became barely perceptible on the dorsal margin of the suboculars and upper portion of the preocular in most of the specimens. The mark further extended to the cantholoreal in three specimens (MZFC 29655, 29662, and 29664), to the lower secondary temporal in MZFC 29668, to the cantholoreal and the dorsal margin of the supralabials adjacent to the lower primary temporal in MZFC 29661, and (if faintly) to the cantholoreal and the middle of the lower secondary temporal in MZFC 29666. In addition, the mark was barely discernible on the lower postocular on the left side in MZFC 29667, absent on the lower postocular on the right side in MZFC 29669, absent on the lower primary temporal on the left side in MZFC 29657, and absent on the lower primary temporal on the right side in MZFC 29663. A small, dark brown spot was present on the posterodorsal corner of the temporal region in 10 specimens (MZFC 29658–63 [absent on left side in MZFC 29663] and MZFC 29665–68).

Dorsal cross-bands 7–9 ($\bar{X} = 8.1$). Most or all of the cross-bands were continuous across the dorsum (their mid portion composed of 2–3 scale rows checkered with pale and dark brown scales, often flecked with white) in three specimens (MZFC 29667–69); there were no, or only few (usually 2–3, occasionally 4 or 5), cross-bands discernible across the dorsum (their mid portion usually composed of a single row of pale brown scales, thus rendering the middorsum distinctly paler than the flanks) in the remaining specimens. The chest and venter exhibited from few to numerous scattered black flecks (faint in MZFC 29655).

Etymology.—The species epithet is a noun in the genitive case and a patronym for the late Clarence Jack McCoy in recognition of his many and significant contributions to the knowledge of the amphibians and reptiles from the Cuatro Ciénegas Basin.

Distribution and ecology.—*Gerrhonotus mccoyi* sp. nov. is known only from the shores of several small lagoons in the Cuatro Ciénegas Basin, Coahuila (Fig. 3). The vegetation on the Cuatro Ciénegas Basin was described by Pinkava (1979, 1984) as composed of grasslands, sedges, and marshes, gypsum dune assemblages, desert scrub, and chaparral. The climate at the type locality is temperate (mean annual temperature = 21.4°C; mean temperatures of the coldest and warmest months are 12°C and 28°C, respectively) and arid, with annual seasonal precipitation averaging <200 mm, and a rainy season that extends from May through December (Instituto Nacional de Estadística, Geografía e Informática [INEGI] 1994). All specimens of this species were collected at night, when most of them were active on the vegetation around the pools at heights of up to 2 m (Fig. 4a). The activity of this species appears to be restricted to the period of the summer with the highest precipitation (between June and September).

Conservation.—The conservation status of the CCB lagoons has long been a matter of concern (i.e., Pinkava 1987; Breunig 2006). Currently, the Basin is considered the continent's second-smallest freshwater ecoregion (492 km^2). Because of its large number of unique (endemic) organisms and the imminent threats to their existence, it is classified, along with only 11 of North America's 76 freshwater ecoregions, in the First Priority class for conservation action by the World Wildlife Fund (Abell et al. 2000). Agricultural development and associated water extraction in the region have placed pressure on the ecological integrity of this unique ecosystem (Souza et al. 2006). Water extraction has significantly reduced the amount of habitat available for the endemic species of amphibians and reptiles that are closely associated with the humid zones in the Basin (McCoy 1984), which has reduced their distribution within the Basin (García-Vázquez et al. 2010). Unless urgent conservation strategies are implemented to regulate water extraction in the CCB, many species in the Basin could disappear.

DISCUSSION

Good (1994) regarded the brief description of *Gerrhonotus liocephalus aguayoi* provided by Contreras-Arquieta as questionable because it appeared only in an abstract for a 1989 scientific conference. In addition, because no type material of *G. l. aguayoi* was designated, the populations referable to this taxon were unclear, and Good (1994) did not include it in his study. Furthermore, whereas the aforementioned description by Contreras-Arquieta suggested that *G. l. aguayoi* differed from *G. l. infernalis* by the presence of a cantholoreal scale, dorsal cross-bands with black anterior and posterior margins,

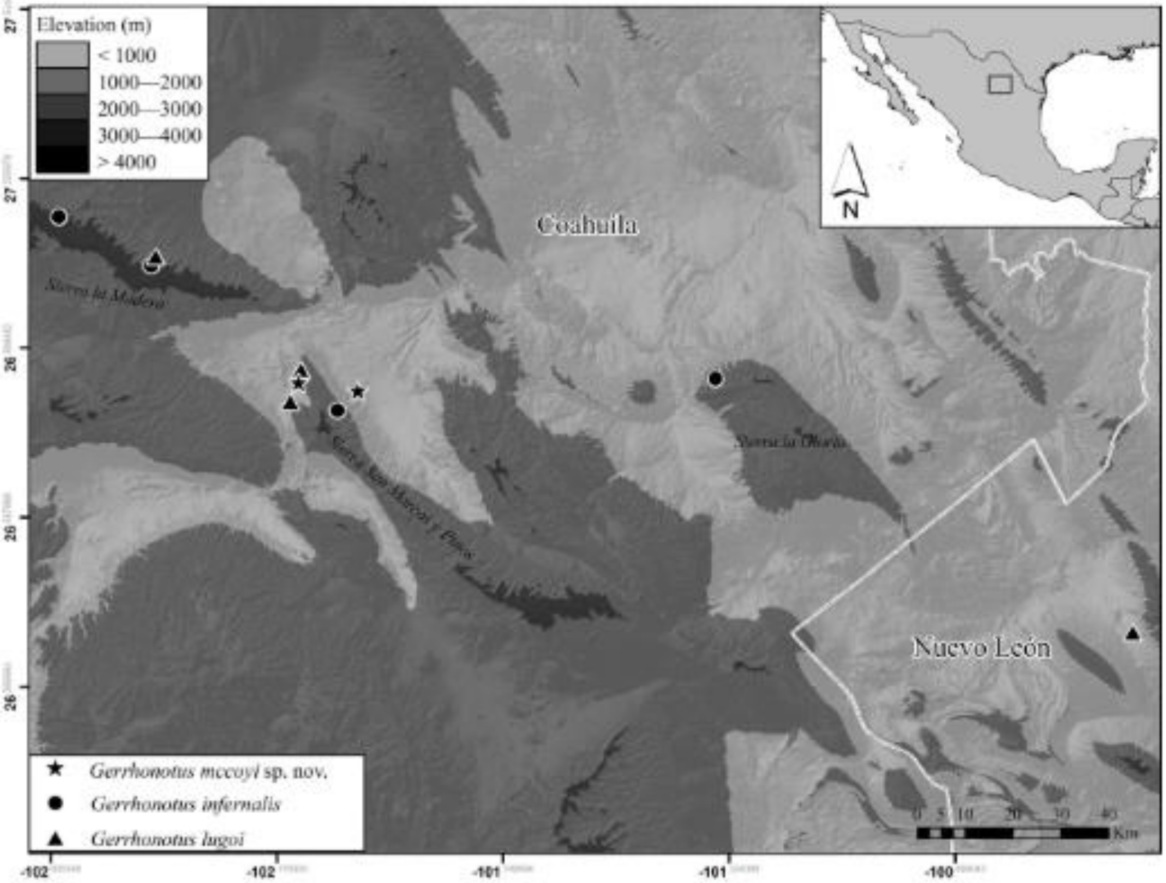

FIG. 3.—Collecting localities of *Gerrhonotus mccoyi* and other species of *Gerrhonotus* distributed in central Coahuila, México (inset). White line represents state boundaries.

and black flecks on the venter, Good (1994:198) stated that some (but not all) of the specimens from central Coahuila that he examined had a color pattern "… reminiscent of the brief description provided by Contreras-Arquieta," but that all of them had the canthal/loreal condition characteristic of *G. infernalis*. Thus, Good (1994) regarded the evidence to warrant taxonomic recognition of *G. liocephalus aguayoi* as insufficient and assigned the populations of *Gerrhonotus* from central Coahuila to *G. infernalis*.

Examination of additional specimens of *Gerrhonotus* from the CCB revealed that all of them have a ventral color pattern similar to that described by Contreras-Arquieta for *G. liocephalus aguayoi*, a pattern different from that of all the other species of *Gerrhonotus* (Fig. 4). Also, most (72%) of the specimens from the CCB possess a cantholoreal scale, which is usually absent in *G. infernalis* (Good 1994). Whereas this indicated that the population in the CCB does represent a distinct, independent lineage from *G. infernalis* and all of the other known species in the genus, it was intriguing that Good (1994) found the diagnostic characters of *G. l. aguayoi* only in some of his specimens from central Coahuila. This might be explained by Good's (1994:198) sample from "central Coahuila" which was composed of five specimens from the CCB and nine from the surrounding mountains. Unlike the specimens of the CCB, those from the Sierra La Madera and Sierra San Marcos y Pinos mountain ranges do exhibit the diagnostic characters of other populations of *G. infernalis* sensu Good (1994). Thus, it appears that Good's (1994) central Coahuila sample simply was composed of both specimens of *G. infernalis* and *G. mccoyi* sp. nov.

Even though they occur in apparent geographic proximity, *G. mccoyi* sp. nov. and *G. infernalis* are allopatric, as *G. mccoyi* sp. nov. is restricted to the halophytic vegetation along the water bodies in the lowest areas of the valley, below 750 m elevation (Fig. 4a), whereas *G. infernalis* is restricted to the higher slopes of the mountain ranges surrounding the CCB where it inhabits oak and pine–oak forests above 1300 m elevation (Fig. 4b). *Gerrhonotus lugoi*, the other species in the Cuatro Ciénegas region, is

FIG. 4.—Habitat and color pattern in life of species of *Gerrhonotus* in the Cuatro Ciénegas region, México. (A) *G. mccoyi* sp. nov. (B) *G. infernalis*. (C) *G. lugoi*. Photographs of habitats and specimen of *G. infernalis* by UOG-V; those of specimens of *G. mccoyi* sp. nov. and *G. lugoi* by P. Heimes and J. Jones, respectively. A color version of this figure is available online.

distributed at elevations intermediate between the elevational ranges of the two other species, and it is found in rocky habitats where xerophytic scrub is the dominant vegetation (Fig. 4c; McCoy 1970).

In a recent multilocus phylogeny of the genus *Gerrhonotus* (García-Vázquez et al. 2018), the four included samples of *G. mccoyi* sp. nov. and formed a strongly supported clade. *Gerrhonotus infernalis* was paraphyletic with respect to *G. mccoyi* sp. nov., however, and the latter taxon was the sister taxon of the samples of *G. infernalis* from Texas and Nuevo León and not of the closest population of *G. infernalis* (from Sierra la Madera). Thus, we consider that the paraphyly of *G. infernalis* is likely attributable to incomplete lineage sorting. Interestingly, a similar pattern is known for lizards of the genus *Scincella*: in a mitochondrial phylogeny of the *S. oligosoma* group, *S. lateralis* was paraphyletic with respect to *S. kikaapoa*, a species endemic of the CCB (García-Vázquez 2012).

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RESUMEN: Se describe una especie nueva de *Gerrhonotus*, previamente confundida con *G. infernalis*, de la Cuenca de Cuatro Ciénegas, Coahuila, México. La especie nueva se conoce sólo de la localidad tipo y se caracteriza por la siguiente combinación de caracteres: escama cantoloreal usualmente presente, una marca oscura que se extiende anteriormente desde las escamas temporales inferiores a través del borde inferior de la órbita hasta la escama preocular o cantoloreal, escamas dorsales aquilladas, patrón de coloración dorsal del cuerpo usualmente compuesto de bandas transversales típicamente interrumpidas o apenas visibles en la parte media del dorso, barras oscuras verticales en el pliegue lateral usualmente no discernibles (algunas barras tenues ocasionalmente presentes), y manchas negras pequeñas dispersas en el vientre. La especie nueva es geográficamente más cercana a *G. lugoi* y *G. infernalis*, pero morfológicamente más similar a *G. infernalis*.

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APPENDIX

Specimens Examined

Institutional codes for museum and collections follow Sabaj-Perez (2016).

Gerrhonotus infernalis.—MEXICO: COAHUILA: Ejido La Casita, Sierra la Concordia (MZFC 32946); Sierra de Jimulco, La Flor de Jimulco, Torreón (MZFC 32947, 32959); Sierra de Jimulco, El Chivo, Viesca (MZFC 32948–49); Rancho La Casita, Sierra la Madera (MZFC 32951–53); Rancho El Chupadero, Sierra La Madera (MZFC 32954). GUANAJUATO: 5 km E San Miguel de Allende (MZFC 32955). HIDALGO: Zoquizoquiapan (MZFC 4875); Zimapsin (MZFC 7776). NUEVO LEON: Monterrey (MZFC 4697); 5.3 km S La Poza, near Santa Clara (MZFC 7465); 2 km W Horsetail Falls, 14 km W Santiago on road to Laguna Sánchez (MZFC 11219); Rancho El Manzano, road Santiago-Laguna Santiago (MZFC 32956); 1.3 mi La Ciénega (MZFC 745). QUERETARO: Cadereyta de Montes, 4 km NE by road to Rancho El Árbolito (MZFC 8010–11); Cadereyta de Montes (MZFC 7825–27, 8012–13); Cadereyta de Montes, La Nopalera (MZFC 6378); Cadereyta de Montes, Mesa de León (MZFC 7777); Chichimequillas, Nueva El Marqués (MZFC 7043). SAN LUIS POTOSI: Guadalcázar, Las Lagunas (MZFC 33392). TAMAULIPAS: Miquihuana, 7 km by road from La Peña-Joya Pelona (MZFC 8429). UNITED STATES: TEXAS: 18.0 mi S of Rocksprings, at polecat E fork Nueces River (UTA 36094); 40.5 mi NNE Dryden, head of Piston canyon (UTA 2024); 6.3 mi NE of Wimberly (UTA 1613); Austin (UTA 54603); Austin: Zilcher Park (UTA 53287); Boerne (UTA 54600); ca. 8 km NW Boerne (UTA 25909); City of Austin, Mayfield Park near Mount Bonell (MZFC 6758); Clarendon Drive and Cumberland Street (UTA 44263); FM 337, 9.0 km W jct FM 187 (UTA 25905–06); Gorman Falls (UTA 30850); Hunt (UTA 44264); N side Austin, near Decker Lake (UTA 25910).

Gerrhonotus liocephalus.—MEXICO: ESTADO DE MEXICO: Villa de Guerrero, Rancho el Tejocote (MZFC 5030–31). GUERRERO: Vallecitos (MZFC 20366). OAXACA: 3 km E Guelatao (MZFC 32957); 16 km N El Jícaro (MZFC 6999); Road Santa Maria Guienagati-Santiago Lachiguiri (MZFC 16988); Ramón Escobar Balboa (MZFC 33389); Rodulfo Figueroa (MZFC 33390–91); Cerro Baúl, 19 km NW Rizo de Oro (UTA 8784, 12224, 30328); El Tejocote (UTA 6065, 6104, 10226, 12221, 19681, 22564, 22573, 30849); Huautla de Jiménez, Puerto de La Soledad (MZFC 13233). PUEBLA: 1 mi N Cacaloapan (UTA 4715); 4 km N San Juan Tepanco (MZFC 7829–30).

Gerrhonotus lugoi.—MEXICO: COAHUILA: Cuatro Ciénegas, Mountains N Nueva Atalaya (MZFC 23318, 27351).

Gerrhonotus ophiurus.—MEXICO: HIDALGO: Meztitlán, 1 km N de Zoquizoquipan (MZFC 7824, 7828); Ejido el Piñón, 15 km S Jacala (MZFC 32959); Meztitlán (MZFC 5748); Tolantongo (MZFC 6341). QUERETARO: Cadereyta de Montes (MZFC 9717). SAN LUIS POTOSÍ: Km 241, road Xilitla-San Juan del Río (MZFC 5560); Xilitla (MZFC 5939, 6106). VERACRUZ: 2.0 mi NE Catemaco, N side of Lago Catemaco (UTA 3360); Catemaco (MZFC 13879); Misantla (MZFC 32960–62).

Gerrhonotus cf. *liocephalus* (Western Mexico).—MEXICO: COLIMA: Manzanillo, 1.4–2.4 km E La Central (MZFC 32963); Minatitlán, km 2.5 camino a El Terrero (MZFC 8038). JALISCO: Chamela (MZFC 8428, 32964). MICHOACÁN: Chinicuila, close to Puerto el Caimán (MZFC 14110, 32965).

Capítulo IV: García-Vázquez, U. O., M. Trujano-Ortega, A. Contreras-Arquieta, O. Ávalos-Hernández, O. O. Escobedo-Contreras y P. Corcuera. 2019. Diversity of Amphibians and Reptiles in the Cuatro Ciénegas Basin. Pp 175–188. En: Álvarez, F. y M. Ojeda, (Eds.). Animal Diversity and Biogeography of the Cuatro Ciénegas Basin. Springer Nature Switzerland AG. (Capítulo de libro publicado en editorial Springer).

Chapter 13
Diversity of Amphibians and Reptiles in the Cuatro Ciénegas Basin

Uri Omar García-Vázquez, Marysol Trujano-Ortega, Arturo Contreras-Arquieta, Omar Ávalos-Hernández, Omar Osvaldo Escobedo-Correa, and Pablo Corcuera

Abstract We gathered and analyzed the current knowledge of the amphibians and reptiles of the Cuatro Ciénegas Basin (CCB), including their biogeographical affinities and the historical and ecological importance of the basin to the diversity of these groups in the Chihuahuan Desert (CD). The CCB has a characteristic topography with alternating highlands and basins. The degree of endemicity of the fauna within the CCB is one of the highest in North America. Morphological and genetic differentiation of these taxa suggests a quick speciation due to the isolation of the basin. Further, the restricted distribution within the CCB of some taxa indicates a high ecological dependence on aquatic microhabitats. The herpetofauna present in the CCB is composed of seven amphibian and 46 reptile species, from which 2 amphibians and 9 reptiles are endemic to the basin. Bufonidae and Colubridae are the most diverse families, while *Lithobates berlandieri* and *Aspidoscelis inornata cienegae* are the most abundant species of Amphibia and Reptilia, respectively. Twelve of the previously reported species had not been recorded recently, but we recorded five

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species for the first time in the CCB. Most of the species are distinctive of the CD; however, the endemic species are more closely related with taxa of the Tamaulipan province, Edwards Plateau, and the Sierra Madre Oriental province.

Keywords Amphibian · Reptiles · Endemism · Behavior · Biogeographic affinities · Chihuahuan Desert

13.1 Introduction

The diverse herpetofauna is one of the most important elements of the Mexican fauna (Flores-Villela and Gérez 1994). An estimated 864 species of reptiles are distributed in Mexico, described in 159 genera and 40 families, which constitute 8.7% of the reptiles worldwide. Of these 864 species, 417 are lizards, 393 snakes and vipers, 48 turtles, 3 amphisbaenians, and 3 crocodiles. Also, 493 taxa are endemic to Mexico (Flores-Villela and García-Vázquez 2014). On the other hand, amphibians are one of the most diverse groups of vertebrates with 376 species in Mexico, classified into 16 families in three orders; this places Mexico as the country with the fifth highest diversity of this group. The degree of endemism is high, considering that more than 50% of the species in seven families are endemic to Mexico, including seven endemic genera, three anurans, and four salamanders (Parra-Olea et al. 2014).

Amphibians and reptiles are key groups in xeric environments, being found in very specific and particular sites. The two groups participate in the natural control of populations of potentially harmful vertebrate and invertebrate species. Amphibians, in particular, are associated with aquatic habitats, which makes them vulnerable to perturbations produced by pollution, desiccation, and global warming. They are considered great bioindicators of the ecosystem health. As for the reptiles, some species are closely associated with specific habitats and, therefore, sensitive to anthropogenic modifications (Kremen 1992; Colwell and Coddington 1994; Fitzgerald et al. 2004).

The CCB in Coahuila, Mexico, is a unique region in the CD because of its water reservoirs. It contains one of the most endemic-rich faunas of North America, which includes, among others, aquatic snails, isopod crustaceans, fishes, turtles, and lizards (García-Vázquez et al. 2010). Particularly, amphibians and reptiles are possibly the most studied groups of vertebrates in the CCB, because of their high degree of endemism. Milstead (1960) recognized three amphibian and seven reptile species from the CCB as relicts. These are mesic-adapted species in the CD, existing in the region as apparently disjunct populations considerably apart from the main portion of the range of the species. Later, McCoy (1984) reported 66 native species to the CCB (8 amphibians and 58 reptiles) and 2 introduced reptile species. Of these, 41 were typical desert species, 13 riparian, 6 semiaquatic, and 6 exclusively aquatic. In the last study of the herpetofauna from Coahuila, Lemos-Espinal and Smith (2016) reported 24 species of amphibians and 109 of reptiles, of which 4 species are endemic to the CCB. However, these authors considered *Gerrhonotus lugoi* McCoy, 1970, as endemic, a species recently reported in Nuevo León (García-Vázquez et al. 2016); additionally, they omitted in their list of endemics *Craugastor augusti fuscofemora* Zweifel, 1956, *Aspidoscelis inornatus cienegae* (Wright and Lowe 1993), and *Aspidoscelis gularis pallidus* (Duellman and Zweifel 1962); all of them exclusive to the CCB. Finally, García-Vázquez et al. (2018a) described *Gerrhonotus mccoyi* García-Vázquez, Contreras-Arquieta, Trujano-Ortega, and Nieto-Montes de Oca, 2018, a new endemic species of the CCB.

Previous studies summarize the historical records of the last 70 years; however, there are no recent ecological studies which assess the current diversity of these groups in the CCB. We present a systematic faunistic study of the amphibians and reptiles in the CCB. The diversity, abundance, species richness, origin, and biogeographical affinities are analyzed. This study will contribute to the understanding of the current vertebrate communities of the CCB and particularly of the human activities which are impacting these species.

13.2 Materials and Methods

We selected seven sites in three localities: (1) Las Teclas in Antiguos Mineros del Norte; (2) Mezquital, Poza Bonita, Poza Churince, and Poza de en Medio in Churince; and (3) Poza Escobedo and Poza Tío Cándido in Rancho Orozco. These sites were selected according to size, environmental heterogeneity, and previously observed diversity of each locality (Fig. 13.1). All localities have superficial water,

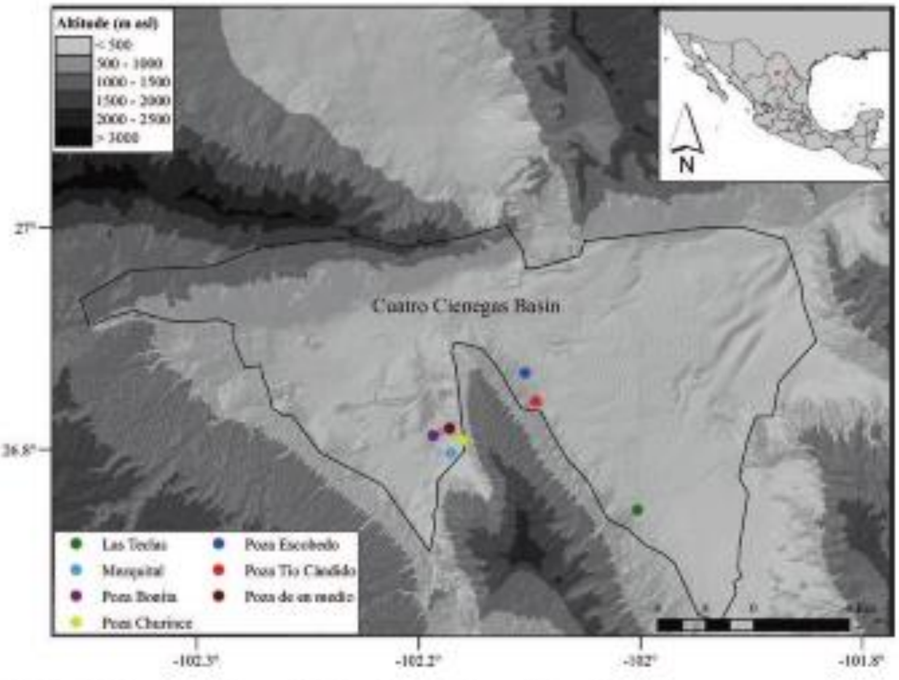

Fig. 13.1 Map of study sites within the Cuatro Ciénegas Basin, Coahuila

except Mezquital. Most localities present some degree of perturbation due to the presence of cattle, crops, and tourism. The main vegetation types in the CCB are grasslands, sedge marshes, gypsum dunes, desert scrub, and chaparral (Pinkava 1979, 1984). The mean annual temperature is 21.4 °C and ranges from 12 °C in the coldest months to 28 °C in the warm season. Annual seasonal precipitation averages 200 mm, and the rainy season extends from May through December (INEGI 1994). Most habitats present in the CCB were included: sotol, mesquite, secondary grassland, and semiaquatic vegetation associated with ponds.

13.2.1 Fieldwork

Fourteen field trips were made from 2012 to 2013 in order to determine and count the amphibians and reptiles found on each site. In addition, we included records from the literature and made sporadic trips in 2009, 2010, 2014, and 2015 to complete the taxonomic composition. Only the field records in 2012 and 2013 were included in the statistical analysis. The sampling effort involved 72 days with at least three collectors each day. To make sure to include diurnal, crepuscular, and nocturnal species, samplings were performed from 9:00 to 16:00 and 19:00 to 23:00 hours. Within each site, we sampled all of the main habitats with a procedure that included opportunistic searching and linear transect surveys that varied in length depending upon the amount of habitat available (Campbell and Christman 1982).

13.2.2 Taxonomic Determination

We identified all of the observed amphibians and reptiles with Lemos and Smith (2007) field guide and also verified the correct identifications with the specimens kept in the Herpetological Collection of the Zoological Museum "Alfonso L. Herrera" of the Facultad de Ciencias, UNAM.

13.2.3 Data Analysis

We assessed the sampling efficiency with the Jackknife 1 and Chao 1 estimators (Colwell 2006). The two estimators are reliable for relatively small sampling units (i.e., circular plots; Hortal et al. 2006). In addition, they are less dependent on sampling intensity than other estimators (Colwell and Coddington 1994; Hortal et al. 2006). We used rarefaction techniques to compare and to assess if the number of species between years was significantly different. In order to test if there were differences of the relative reptile abundance between years among sites, we used an X^2 goodness of fit test. Dominance, diversity, and evenness were calculated with the Simpson, Shannon-Wiener, and Pielou indexes. Similarities between localities with similar sampling efforts were estimated with the Bray-Curtis index. Lastly, biogeographic affinities were determined with specialized literature considering two regions, Nearctic and Neotropical (Morrone et al. 2002).

13.3 Results

The herpetofauna of the CCB is composed of 5 amphibian and 14 reptile families, including 6 genera and 11 species of amphibians and 37 genera and 61 species of reptiles, from which 2 amphibians and 9 reptiles are endemic to the CCB. Colubridae with 23 taxa, Phrynosomatidae with 11 taxa, and Bufonidae and Viperidae with 5 taxa each were the most diverse families. Seven families were represented by only one species. Teiidae was the most abundant family with 413 observed specimens, followed by Phrynosomatidae with 286, Ranidae with 166, and Scincidae with 152; seven families were represented by 10 or fewer organisms. Specifically, *Aspidoscelis inornatus cienegae* (Teiidae) and *Lithobates berlandieri* (Baird, 1859) (Ranidae) were the most dominant species on both spatial and temporal scales.

We found one taxon that had not been previously recorded in Coahuila (*Salvadora grahamiae grahamiae* (Baird and Girard,1853)), and three are new to the state and endemic for the CCB (*Eleutherodactylus* sp. nov., *Storeria* sp. nov., and *Gerrhonotus mccoyi*). On the other hand, 12 taxa that had been previously collected in the CCB were not observed in this study.

13.3.1 Species Richness

We found from 75 to 89% of the estimated species in 2012 and 74% of the estimated richness in 2013, according to Chao 1 and Jackknife 1 estimators (Table 13.1, Fig. 13.2). Because the confidence intervals between years overlapped, the number of species was not significantly different between the two years (Fig. 13.3).

Table 13.1 Number of observed species and percentage of the expected richness in the Cuatro Ciénegas Basin, Coahuila, according to the Chao 1 and Jackknife 1 estimators

Year	Observed	Chao 1 (%)	Jack 1 (%)
2012	21	88.8	75.4
2013	17	74.0	73.9

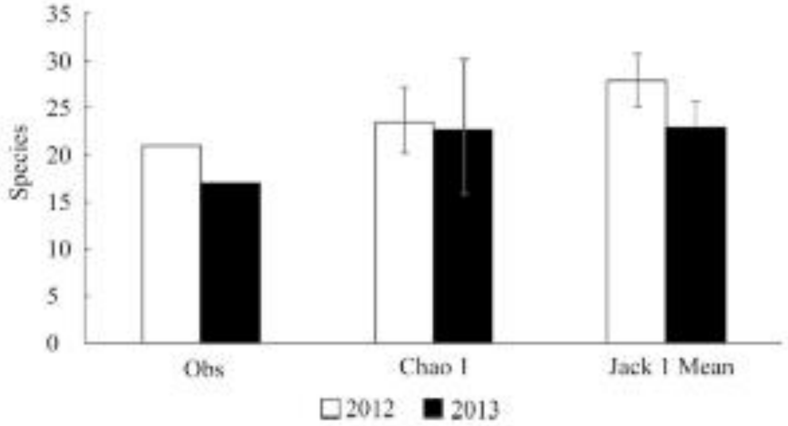

Fig. 13.2 Species richness of amphibians and reptiles from the Cuatro Ciénegas Basin, Coahuila

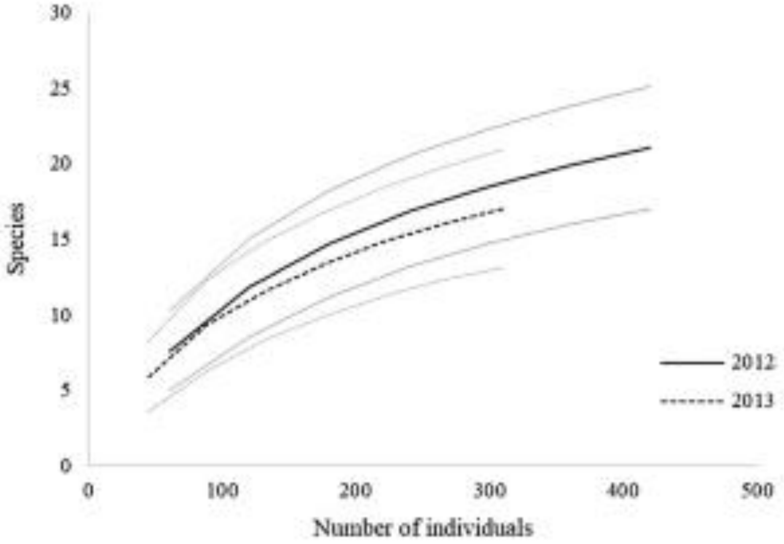

Fig. 13.3 Species observed of amphibians and reptiles from the Cuatro Ciénegas Basin, Coahuila

13.3.2 Diversity

There was a significant difference in the number of individuals among the seven sites between the two years ($X^2 = 116.03$, 6 df, $p < 0.001$). In particular, in 2013 the abundance in Churince was lower in Poza Churince ($X^2 = 41.63$, 1 df, $p < 0.001$) and higher than expected in Poza Tío Cándido ($X^2 = 71.1$, 1 df, $p < 0.001$) (Fig. 13.4; Table 13.2). Dominance was higher in Churince in the second year. Diversity showed the opposite trend.

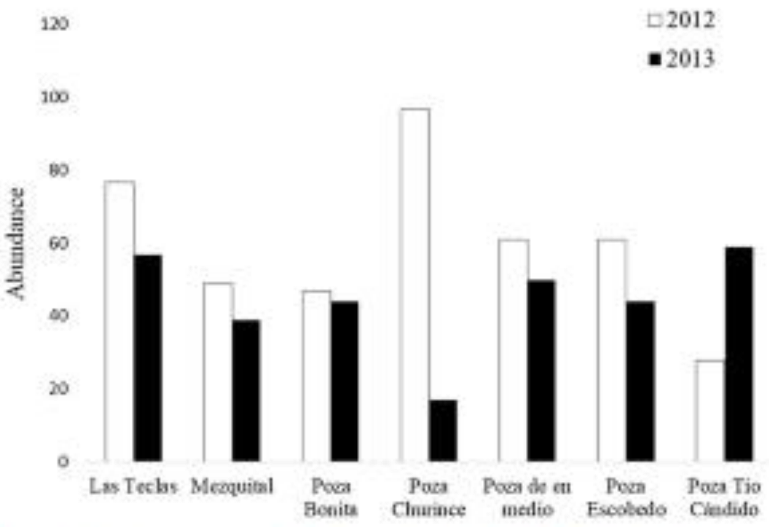

Fig. 13.4 Abundance in seven sites within the Cuatro Ciénegas Basin, Coahuila

13.3.3 Similarity

Las Teclas was the most dissimilar site according to the Bray-Curtis index. Mezquital with Poza Bonita and Poza Churince with Poza Tío Cándido shared more species with similar abundances (Fig. 13.5).

13.3.4 Biogeographic Affinity

Data of biogeographic affinity were obtained for the 68 native species and subspecies. Species composition of the CCB has an affinity with two regions. The Nearctic region is the most represented (70%, 50 species). The rest of the species have an affinity with the Neotropical region (30%, 18 species). Of the latter, 11 species have been considered relict populations (Morafka 1977; García-Vázquez et al. 2018b; this study). The Neotropical affinities include both amphibians and reptiles from six families: Eleutherodactylidae, Anguidae, Scincidae, and Trionychidae with one species each and Colubridae and Emydidae with two species. It is worth noticing that seven of these species of Neotropical affinities are endemic to the CCB (Fig. 13.6): one frog, *Eleutherodactylus* sp. nov.; one snake, *Storeria* sp. nov.; two lizards, *Scincella kikaapoa* García-Vázquez, Canseco-Marquez, and Nieto Montes de Oca, 2010, and *Gerrhonotus mccoyi*; and three turtles, *Terrapene coahuila* Schmidt and Owens, 1944, *Trachemys taylori* (Legler 1960), and *Apalone spinifera atra* Webb and Legler, 1960.

Table 13.2 Number of reptile species, abundance, dominance (D), diversity (H'), and evenness (E') in seven sites and 2 years in the Cuatro Ciénegas Basin, Coahuila

	2012							2013						
	Tc	Mez	Pb	Pc	Pm	Pe	Ptc	Tc	Mez	Pb	Pc	Pm	Pe	Ptc
Richness	7	9	8	**13**	9	4	3	9	5	8	4	8	3	4
Abundance	77	49	47	**97**	61	61	28	57	39	44	17	50	44	59
D	0.322	0.330	0.210	0.153	0.200	0.380	0.375	0.271	0.261	0.258	0.467	0.179	0.348	0.347
H'	1.355	1.457	1.762	2.127	1.852	1.126	1.040	1.559	1.456	1.590	1.006	1.893	1.077	1.128
E'	0.554	0.477	0.728	0.645	0.708	0.771	0.943	0.528	0.858	0.613	0.684	0.830	0.979	0.773

Tc Las Teclas, *Mez* Mezquital, *Pb* Poza Bonita, *Pc* Poza Churince, *Pm* Poza de en Medio, *Pe* Poza Escobedo, *Ptc* Poza Tío Cándido

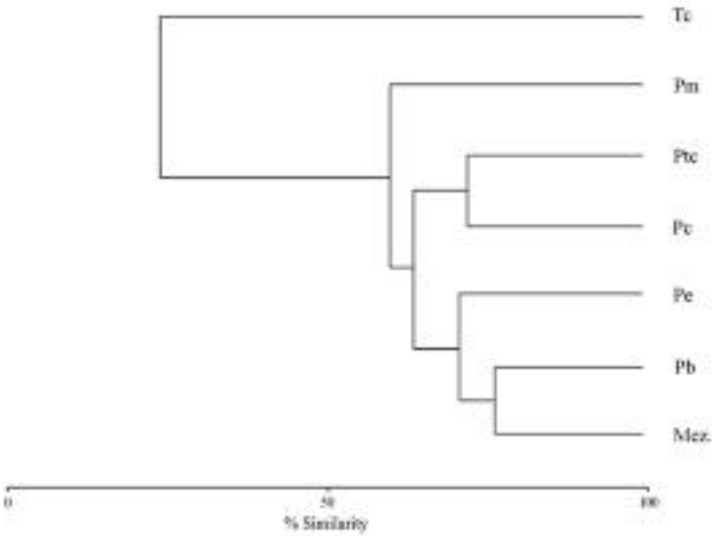

Fig. 13.5 Similarity of seven sites within the Cuatro Ciénegas Basin, Chihuahuan Desert. The Bray-Curtis similarity coefficient was used. Tc, Las Teclas; Mez, Mezquital; Pb, Poza Bonita; Pc, Poza Churince; Pm, Poza de en Medio; Pe, Poza Escobedo; Ptc, Poza Tío Cándido

Fig. 13.6 Amphibians and reptiles endemic to CCB. (**a**) *Eleutherodactylus* sp.nov., (**b**) *Gerrhonotus mccoyi*, (**c**) *Terrapene coahuila*, (**d**) *Scincella kikaapoa*

13.4 Discussion

The CCB is the site with the highest level of endemism of amphibians and reptiles within the CD and possibly the region with the highest level of endemism in the country (Lemos-Espinal and Smith 2016). Due to the high species richness of amphibians and reptiles, for many decades, the CCB was the best-sampled locality of the CD. Consequently, more than 30% of the specimens from Coahuila deposited in scientific collections are from the CCB (Lemos-Espinal and Smith 2007). However, most of the records are from a few species (v. gr. *Terrapene coahuila*, *Trachemys taylori*) and around 35% of the species are known from five or fewer specimens. By making a more general and complete sampling, here we report four taxa previously unrecorded for the CCB.

With the species recorded in the CCB, the total for the state of Coahuila increased from 133 (Lemos-Espinal and Smith 2016) to 137 species. This is important because three of the new records represent species endemic to the CCB (*Eleutherodactylus* sp. nov., *Storeria* sp. nov., and *Gerrhonotus mccoyi*), increasing the endemic taxa in the CCB to two amphibians and nine reptiles. Additionally, preliminary data suggest that there is another new species of snake to be described. Herpetofauna of the CCB presents a species richness pattern at the suborder level, different from those described by Lemos-Espinal and Smith (2016) for Coahuila. They put lizards as the richest group, but the CCB shows a higher diversity of snakes with six species more than lizards (51% of reptiles' diversity). For amphibians and tortoises, the species richness percentages within the CCB are similar to those reported for the state (Mendoza-Quijano et al. 2006; Lemos-Espinal and Smith 2016).

Based on species richness estimations, less than 50% of the historically reported species for the CCB were observed during the sampling period. However, these estimations were made with data of just seven sites, not including the edges of the basin, where one finds xeric shrubland, known to harbor a high diversity of reptiles. Therefore, in order to determine the percentage of the herpetofauna community documented in this inventory, compared with the total reported, the results (including additional records from 2010, 2011, 2014, and 2015) were compared to the historical lists of species occurring in the CCB based mainly on the reptile and amphibian checklist by Lemos-Espinal and Smith (2016). There are 12 species that are likely to occur in the CCB that were not observed (1 toad, 1 lizard, and 10 snakes). All of these 12 species that were undetected in this study are present in the region and were expected to be observed at the sites but may not have been encountered due to unfavorable environmental conditions, their cryptic nature, or low abundances making detection difficult (Davis and LaDuc 2018). Considering isolated records, this inventory may have documented 88% of the reptile and amphibian species in the CCB.

Some previously reported species that were not found are, considering their ecological requirements, presumably now absent from the CCB. These are an amphibian (*Anaxyrus debilis* [Girard, 1854]) and three reptiles (*Coleonyx reticulatus* Davis and Dixon, 1958; *Heterodon nasicus* Baird and Girard, 1852; and *Sceloporus cou-*

chi Baird, 1859). Two of these species, *A. debilis* and *S. couchi*, occupied grassland habitats and ephemeral ponds, habitats that are common in the CCB; also historical records show a great abundance of these species in the past (Lemos-Espinal and Smith 2007). Based on this evidence, these two species must have been extirpated from the CCB. Regarding the reptiles, *Coleonyx reticulatus* and *Heterodon nasicus* are known from one and two records, respectively; we consider these to be rare species that are still present in the CCB.

Species richness of these groups of vertebrates is higher in the CCB than that reported in any other studies conducted in different regions of the CD. Minton (1958), for example, found 69 species in the Big Bend Region of Texas, while Prival and Goode (2014) found between 59 and 28 species in six national parks in the CD. Recently, Cruz-Elizalde et al. (2014, 2016) reported 45 reptiles and 9 amphibians from the Real de Guadalcázar State Reserve, San Luis Potosí, Mexico. Finally, Davis and LaDuc (2018) found 50 species of herpetofauna in C.E. Miller Ranch and the Sierra Vieja in Texas. The amphibians and reptiles from the CCB represent 42% of all herpetofauna reported for the CD (Morafka 1977). All this makes the CCB a diversity hotspot within the Nearctic region.

At a smaller scale, Mezquital (Mez), Poza Bonita (Pb) and Poza Escobedo (Pe), and Poza Churince with Poza Tío Candido were the most similar localities, even if they are separated by the San Marcos and Pinos mountain range. This shows in part the homogeneity of the environment, with mainly aquatic and semiaquatic habitats around the ponds and halophyte and gypsum habitats in between the ponds. Meanwhile, the similarity between Mezquital and Poza Bonita is due to the predominance of Mezquite shrubs in both of these sites and the species associated to this habitat (i.e., *Masticophis flagellum* Shaw, 1802, and *Sceloporus magister* Hallowell, 1854). Las Teclas was the site with fewer shared species with other sites, and this could be because this site showed the greater changes in the vegetation, associated with anthropogenic activities. Even if nine species were observed, there was a great bias in the abundance and dominance, with just two species (*Lithobates berlandieri*, *Scincella kikaapoa*) comprising 82% of all records. Most of these records were associated with disturbed areas.

Species richness and abundance were higher in 2012 than in 2013. Temperature and rainfall affect the species richness, abundance, and diversity (Morafka 1977). The highest diversity and abundance were recorded in 2013 when precipitation and resource availability and quality were higher (Corcuera, unpub. data). Also, the number of records of species exclusively associated with water bodies (v. gr. *Gerrhonotus mccoyi*, *Eleutherodactylus* sp. nov., and all species of turtles) dropped 37%.

The CCB is located in the Altiplano Mexicano biogeographical province in the Nearctic region (Morrone et al. 2002); this is congruent with the biogeographic affinity of most species collected in this study; however, it is noteworthy the high number of species with Neotropical origin. In particular, the levels of morphological and genetic differentiation of these taxa suggest that they speciated rapidly because of the basin isolation (García-Vázquez et al. 2018b). This process has been analyzed in the CCB with some aquatic species (Hulsey et al. 2004; Hsiu-Ping and Hershler 2007), scorpions (Williams 1968), and amphibians and reptiles (McCoy

1984), suggesting the relictual origin of these taxa and the possibility of extensive local speciation (Meyer 1973). Besides, the high proportion of relictual species associated with the Neotropical region agrees with the general pattern of the CD. In the CD, 34% of the herpetofauna is closely related with the tropical species of the Tamaulipan province (Morafka 1977).

This study is an update on the diversity of amphibians and reptiles in the CCB. Long-term studies must be conducted in order to confirm the possible local extinction of some species in the basin and to assess the effect of water loss on the endemic species associated with water bodies (García-Vázquez et al. 2010, 2018b). In other arid habitats of the CD, amphibians and reptiles have shown great annual variations (Davis and LaDuc 2018). In this regard, future faunistic or ecological research on these communities should focus its sampling effort on the endemic species associated with aquatic habits. García-Vázquez et al. (2010) in a visit to the type locality of *Scincella kikaapoa* 2 years after the holotype and a paratype of this species were collected noted that the extension of the pond had reduced and some of the ditches connected to it had dried. During this study, additional dry ditches were observed in the region. No *Scincella*, *Gerrhonotus*, or *Eleutherodactylus* species, which inhabit only the halophilic vegetation surrounding the ponds, were found in these dry sites.

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Discusión general

Diversidad y endemismo de anfibios y reptiles en el Valle de Cuatro Ciénegas

A partir del trabajo de campo y la revisión bibliográfica, se actualizó el conocimiento de la herpetofauna del Valle de Cuatro Ciénegas (García-Vázquez et al., 2019; Capítulo 4). Al hacer un muestreo más general y completo, se encontraron cuatro taxones no registrados previamente para el VCC, lo que aumenta el número de especies a siete anfibios y 46 reptiles. Con las especies registradas en el VCC, el total para el estado de Coahuila aumentó de 133 (Lemos-Espinal y Smith, 2016) a 137 especies. Esto es importante porque tres de los nuevos registros representan especies endémicas del VCC (*Eleutherodactylus* sp. nov., *Storeria* sp. nov. y *Gerrhonotus mccoyi* [Capitulo 3]), lo que aumenta los taxones endémicos en el VCC a dos anfibios y nueve reptiles. Los anfibios y reptiles del VCC representan el 42% de toda la herpetofauna reportada para el Desierto Chihuahuense, riqueza de especies similar a la reportada en cualquier otro estudio realizado en otras regiones del Desierto (Minton, 1958; Prival y Goode, 2014; Cruz-Elizalde et al., 2016; Davis y LaDuc, 2018). Sin embargo, el nivel de endemismo es notablemente mayor (Garcia-Vázquez et al., 2019; Capitulo 4), lo que hace que el VCC sea un punto clave de diversidad dentro de la región Neártica.

Patrones geográficos de las especies de reptiles endémicas del VCC con afinidad templada

Las filogenias estimadas para *Gerrhonotus, Scincella* y *Terrapene* revelaron una estrecha relación entre las poblaciones en el VCC y las poblaciones que se encuentran principalmente en el este de los Estados Unidos, mientras que en el caso de *Storeria* sp., ésta muestra una mayor afinidad geográfica con la Sierra Madre Oriental (García-Vázquez et al., 2018a; Capítulos 1 y 2). En congruencia con nuestros resultados, se han encontrado patrones similares en peces y escorpiones endémicos. Específicamente, se recuperó una relación hermana entre especies endémicas del VCC y especies del sureste de los Estados Unidos para los géneros de peces *Etheostoma* y *Herichthys* (Hulsey at al., 2004; Near et al., 2011). Además, Ennen et al. (2017) encontraron una fuerte asociación entre las tortugas de la costa de Texas y la provincia acuática de las planicies altas del sur (que incluye la cuenca de Cuatro Ciénegas) y las provincias del este de los Estados Unidos. Por otro lado, se ha observado una estrecha relación entre numerosos taxones endémicos del VCC y las especies de la Sierra Madre Oriental, incluyendo peces (e. g. *Cyprinella*, Schönhuth et al., 2006; *Cyprynodon*, Haney et al., 2009; *Gambusia*, Lydeard et al., 1995; y *Xiphophorus*, Kang et al., 2013) y escorpiones (ej. *Chihuahuanus*, Gonzalez-Santillan y Prendini, 2015). Dada la presencia de especies asociadas a los bosques de coníferas de la Sierra Madre Oriental (Pinkava, 1984) y la historia geológica de la zona (Chávez-Cabello et al., 2005), algunos autores consideran a las montañas que rodean el VCC como la zona más septentrional de la Sierra Madre Oriental (Meyer, 1973; Espinosa et al., 2014).

Origen de las especies endémicas del Valle de Cuatro Ciénegas

El Valle de Cuatro Ciénegas en Coahuila, México, es una región única en el desierto Chihuahuense debido a sus depósitos de agua. Contiene una de las mayores faunas endémicas de América del Norte, que incluye, entre otros, caracoles acuáticos, crustáceos isópodos, peces, tortugas y lagartijas (Álvarez y Ojeda, 2019). El origen de estas especies endémicas ha sido estudiado de manera independiente, y existen varias teorías que involucran edades que van desde el Mesozoico Tardío hasta el Pleistoceno Tardío (Milstead, 1967; Taylor, 1966; Miller, 1968; Espinoza-Pérez y Lambarri-Martínez, 2019). En particular, los anfibios y reptiles son posiblemente los grupos de vertebrados más estudiados en el VCC, debido a su alto grado de endemismo. De los nueve taxones de reptiles endémicos al VCC, cuatro son considerados como relictuales y con afinidades a los bosques templados y zonas de mayor humedad (García-Vázquez et al., 2019). Si bien no existen trabajos que aborden el origen de estas especies, algunos autores consideran que el VCC representa una zona de relicto, donde los períodos pluviales durante o inmediatamente después del Pleistoceno aparentemente causaron una retirada gradual de la frontera oriental del Desierto de Chihuahua, lo que permitió la colonización de linajes adaptados a climas templados (Milstead, 1960; Morafka, 1977). Presumiblemente, la mayoría de los linajes perecieron con el regreso de las condiciones de desierto, pero algunos permanecieron como relictos, lo cual promovió procesos de especiación (Morafka, 1977). Esta hipótesis se basa en que cada sucesión de eventos de avance y retroceso de glaciares pudo haber provocado la contracción, aislamiento, y expansión de las áreas de distribución de las especies, con la consecuente diferenciación–especiación (Moreno-Letelier y Piñero, 2009), y en general representa una de las explicaciones más aceptadas para el origen de las especies y patrones de distribución en América del Norte (Riddle et al., 2000). En particular, este fenómeno se ha documentado para mamíferos (Findley, 1969), reptiles (Morafka, 1977) y aves (Hubbard, 1973).

Los resultados obtenidos en este estudio (Capitulo 1) son congruentes con eventos de especiación promovidos por aislamiento dentro del VCC (Milstead, 1960), sin embargo, nuestros resultados sugieren que la divergencia de los linajes endémicos del VCC ocurrió durante el Neógeno, lo que indica que los ciclos glaciales del Pleistoceno no jugaron un papel central en su origen. En su lugar, la diversificación de los linajes del VCC fue probablemente el resultado de una variedad de factores asociados con la formación de nuevos hábitats, cambios climáticos importantes, eventos de orogenia durante el Mioceno y cambios globales en la vegetación (Zachos et al., 2001; Leavitt et al., 2012).

Por otra parte, determinar si los ensambles de especies experimentaron respuestas evolutivas compartidas a eventos geológicos históricos y de cambio climático es un objetivo central pero desafiante de la filogeografía comparativa (Arbogast y Kenagy, 2001; Bagley et al., 2018). Sin embargo, el método hABC para la filogeografía comparativa utilizada en este estudio incorpora la variación intrínseca en la coalescencia ancestral y las historias demográficas entre taxones (Hickerson et al., 2006; 2007; Topp et al., 2013), y usando HABC en conjunto con los análisis de tiempo de divergencia detectamos dos eventos de divergencia no simultáneos: uno para *Terrapene coahuila* durante el Mioceno tardío y un segundo evento para el origen de *Gerrhonotus mccoyi*, *Scincella kikaapoa* y *Storeria* sp. VCC durante el Plioceno (Capítulo 1).

Hipótesis biogeográfica

El origen de los reptiles endémicos del VCC parece estar relacionado con los procesos de aridificación del hemisferio norte durante el Neógeno (Morafka, 1977) y con eventos orogénicos durante el Mioceno (Bryson et al., 2013), aunque no es claro en qué medida influyó cada evento en particular. En este trabajo (capítulos 1 y 2) se plantea una hipótesis biogeográfica tomando en cuenta los tiempos de divergencia y el origen asincrónico de las especies evaluadas.

Uno de los principales eventos que moldearon la distribución de la fauna durante el Mioceno en el este de Norteamérica fue la expansión de las sabanas que, como algunos autores sugieren, diferían de las sabanas actuales en la presencia generalizada de árboles de origen templado (Webb, 1977). La expansión en el Mioceno tardío de la sabana arbolada alrededor de gran parte de la costa del Golfo también produjo una disyunción importante entre el bosque templado del este de los Estados Unidos y el norte de México (Estes, 1970; Martin, 1958; Martin y Harrel, 1957; Webb, 1977). La extensión de la sabana, asociada a la fragmentación del bosque templado y el aislamiento del VCC causado por los eventos de orogenia en el Mioceno (Chávez-Cabello et al., 2005; Wilson y Pitts, 2010), pudieron haber brindado oportunidades para una rápida diversificación del antepasado de *Terrrapene coahuila* que originalmente pudo haberse distribuido en todo el este de México y de Estados Unidos.

Un segundo pulso de diversificación que involucra a tres de las especies analizadas parece haber ocurrido durante el Plioceno. Si bien el evento de divergencia fue sincrónico, los procesos anteriores a las divergencias no pueden explicarse a partir del mismo fenómeno (Capitulo 1). Por una parte, tomando en cuenta la distribución de las especies hermanas y los tiempos estimados, divergencias más recientes, como en el caso de *Gerrhonotus macoyi* y *Scincella kikaapoa*, pudieron estar fuertemente influidas por la expansión del drenaje sur del Río Bravo al final del Plioceno (García-Vázquez et al. 2018a; Capitulo 2). Se ha demostrado que esta expansión está asociada con la dispersión y los sucesivos eventos de aislamiento entre la Meseta Mexicana y las sierras circundantes (2.7–4.1 Ma) (Jaeger et al., 2005; Bryson et al., 2011a), incluidas las Sierras que rodean el VCC (Chávez-Cabello et al., 2005).

En el caso de *Storeria* sp., cuya afinidad geográfica es con la Sierra Madre Oriental, diversos autores han propuesto que las montañas que rodean al VCC representan las formaciones más norteñas de la Sierra Madre Oriental (Meyer, 1973; Espinosa et al., 2014) y que, asociado al cambio climático en el área, marcado por un cambio dramático en las condiciones atmosféricas durante el Plioceno temprano, estas formaciones proveían las condiciones adecuadas para la presencia de taxones de afinidad templada y de amplia distribución, y que más tarde, durante el Plioceno tardío, dichos taxones fueron aislados por la reducción de los bosques templados y expansión de las regiones desérticas (Retallack, 1997; Bryson et al., 2013). De manera consistente con esta propuesta, varios taxones de afinidad templada muestran rupturas genéticas evidentes en tierras bajas desprovistas de bosque de coníferas (Farjon y Styles, 1997;

Anducho-Reyes et al., 2008; McCormack et al., 2008; Moreno-Letelier y Piñero, 2009; Bryson et al., 2011a, b, c; 2012). Entre las causas que dividen las especies de afinidad templada en el norte de México, también se ha propuesto un cambio dramático en las condiciones atmosféricas durante el Plioceno tardío (Retallack, 1997; Bryson et al., 2013), así como la aparición de barreras de filtro como los ríos principales del norte del país (Bryson et al., 2011c).

Finalmente, y en aparente contradicción con lo expresado arriba, las altas estimaciones del flujo de genes entre las poblaciones endémicas de VCC y sus respectivos grupos hermanos sugieren una divergencia reciente (Ornelas et al., 2013). Esto podría explicarse porque, independientemente del momento de la diversificación de especies, durante el Pleistoceno muchos taxones se desplazaron a gran parte de sus distribuciones actuales mientras seguían su hábitat adecuado a medida que los glaciares avanzaban y retrocedían repetidamente, lo que podría haber borrado u oscurecido las señales de aislamiento histórico previamente adquiridas (Bryson et al., 2011a). Se han observado patrones similares de subdivisiones genéticas profundas borrosas por contacto secundario en otros taxones de afinidad templada de América del Norte (Moreno-Letelier y Piñero, 2009; Ornelas, et al., 2010; Bryson et al., 2011a, b).

Conservación de los anfibios y reptiles del Valle de Cuatro Ciénegas

El estado de conservación del VCC ha sido motivo de preocupación durante mucho tiempo (ej. Pinkava 1987; Breunig 2006; Souza et al., 2006). Debido a su gran número de especies endémicas y las amenazas inminentes a su existencia, se clasifica, junto con solo 11 de las 76 ecorregiones de agua dulce de América del Norte, en la clase de primera prioridad para la acción de conservación del Fondo

Mundial para la Naturaleza (Abell et al., 2000). El desarrollo agrícola y la extracción de agua asociada en la región han ejercido presión sobre la integridad ecológica de este ecosistema único (Souza et al., 2006). Con base en las estimaciones de riqueza de especies (García-Vázquez et al., 2019; Capitulo 4), se observó que más del 40% de las especies de anfibios y reptiles históricamente reportadas para el VCC no se han observado de manera reciente, por lo que se puede presumir que algunas especies reportadas previamente y que no se han encontrado en trabajo de campo reciente han sido extirpados del VCC, considerando sus requisitos ecológicos. Dos anfibios, *Anaxyrus debilis* y *Scaphiophus couchi*, ocuparon hábitats de pastizales y estanques efímeros, hábitats que son comunes en el VCC, por lo que no es clara la razón por la cual están ausentes en el Valle, a pesar de que hay evidencia de una gran abundancia de estas especies en el pasado (Lemos-Espinal y Smith, 2007). Con respecto a los reptiles, *Coleonyx reticulatus* y *Heterodon nasicus* se conocen de uno y dos registros, respectivamente; por lo que podría tratarse de registros esporádicos de especies que no habitan normalmente en el VCC.

Por otra parte, la extracción de agua ha reducido significativamente la cantidad de hábitat disponible para las especies endémicas de anfibios y reptiles que están estrechamente asociadas con las zonas húmedas en la cuenca (McCoy 1984), lo que ha reducido su distribución dentro de la misma (García-Vazquez et al., 2019). Como muestra de ello, durante el trabajo de campo se visitaron sitios donde en años anteriores se podían observar de manera relativamente frecuente organismos de especies de afinidad templada, tales como *Scincella* y *Gerrhonotus* (García-Vázquez et al., 2010; 2018b), pero en algunos de estos sitios, incluida la localidad tipo de *Scincella kikapoa*, se observó que la extensión de los cuerpos de agua se había reducido y algunas de las zanjas que conectaban las pozas se habían secado (García-Vázquez et al., 2018b; Capitulo 3). Durante este estudio, se observaron zanjas secas adicionales en la región. En estos sitios secos no se encontraron ejemplares de *Scincella, Gerrhonotus, Storeria* y/o *Terrapene*, que habitan sólo la vegetación halófila que rodea los estanques.

Conclusiones generales.

Existe una mayor relación geográfica entre las poblaciones de *Gerrhonotus*, *Scincella* y *Terrapene* del VCC con las poblaciones que se encuentran principalmente en el este de los Estados Unidos, mientras que las poblaciones de *Storeria* sp. muestran una mayor afinidad geográfica con la Sierra Madre Oriental.

La diversificación dentro del VCC no fue sincrónica, pues se documentarion al menos dos eventos de divergencia no simultáneo: uno para *Terrapene coahuila* durante el Mioceno tardío y otro para el origen de *Gerrhonotus macoyi*, *Scincella kikaapoa* y *Storeria* sp. VCC durante el Plioceno.

A diferencia de la hipótesis más extendida de divergencias durante el Pleistoceno, nuestros resultados sugieren que la divergencia de los linajes endémicos del VCC ocurrió durante el Neógeno, lo que indica que los ciclos glaciales del Pleistoceno no jugaron un papel central en su origen.

La diversificación de los linajes endémicos de reptiles del VCC con afinidad templada fue probablemente el resultado de una variedad de factores asociados con la formación de nuevos hábitats, cambios climáticos importantes, eventos de orogenia durante el Mioceno y cambios globales en la vegetación. Entre estos factores, la extensión de la sabana, asociada a la fragmentación del bosque templado y el aislamiento del VCC causado por los eventos de orogenia en el Mioceno y la reducción de los bosques templados y expansión de las regiones desérticas durante el Plioceno, jugaron un papel central en los procesos de divergencia.

Las altas estimaciones del flujo de genes entre las poblaciones endémicas de VCC y sus respectivos grupos hermanos sugieren contacto secundario durante el Pleistoceno, asociado a la expansión y retracción de los bosques.

Si bien no fue el objetivo central de este trabajo, durante el desarrollo del mismo se observó un fuerte impacto sobre la conservación de los grupos estudiados, por lo que a menos que se implementen estrategias de conservación urgentes para regular la extracción de agua en el VCC, estas especies podrían desaparecer.

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