



**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**  
**POSGRADO EN CIENCIAS BIOLÓGICAS**  
FACULTAD DE ESTUDIOS SUPERIORES ZARAGOZA  
**BIOLOGÍA EVOLUTIVA**

**BIOGEOGRAFÍA HISTÓRICA DEL GÉNERO *RHADINAEA* (SQUAMATA: DIPSADINAE)**

**TESIS**

**POR ARTÍCULO CIENTÍFICO**

**HISTORICAL BIOGEOGRAPHY OF THE GENUS *RHADINAEA* (SQUAMATA: DIPSADINAE)**

**QUE PARA OPTAR POR EL GRADO DE:**

**MAESTRO EN CIENCIAS BIOLÓGICAS**

**PRESENTA:**

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**MÉXICO, CD. MX. ABRIL 2021**

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

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **23 de noviembre de 2020** se aprobó el siguiente jurado para el examen de grado de **MAESTRO EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Biología Evolutiva** del estudiante **GARCÍA SOTELO URIEL ALONSO** con número de cuenta **309234100** por la modalidad de graduación de **tesis por artículo científico** titulado: **“Historical biogeography of the genus *Rhadinaea* (Squamata: Dipsadinae)”**, que es producto del proyecto realizado en la maestría que lleva por título: **“Biogeografía histórica del género *Rhadinaea* (Squamata: Dipsadinae)”**, ambos realizados bajo la dirección del **DR. DAVID NAHUM ESPINOSA ORGANISTA**, quedando integrado de la siguiente manera:

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

**A T E N T A M E N T E**  
**“POR MI RAZA HABLARÁ EL ESPÍRITU”**  
Cd. Universitaria, Cd. Mx., a 16 de abril de 2021

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And where you lead  
Sister, I will follow  
From the shadows of the past  
To the shimmers of tomorrow  
For the bond we share  
Is beyond compare  
It's unbreakable.

Army of Two – Carole & Tuesday



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## RESUMEN

Múltiples eventos geológicos y climáticos han actuado como barreras geográficas o ecológicas asociadas con eventos de especiación, jugando un papel importante en la diversificación en Norte y Centroamérica. En el presente estudio se evaluó la influencia de los eventos geológicos y climáticos durante el Neógeno y Cuaternario en la diversificación del género de culebras hojarasqueras del género *Rhadinaea* mediante datación molecular y reconstrucción de áreas ancestrales. Para ello se utilizó un set de datos multilocus que incluye 36 individuos de *Rhadinaea* con distribución en la mayoría de las provincias biogeográficas y que representan 19 de las 21 especies reconocidas actualmente, así como dos especies no descritas. Las relaciones filogenéticas fueron inferidas mediante los enfoques de Máxima Verosimilitud e Inferencia Bayesiana, la estimación de los tiempos de divergencia fue realizada utilizando un reloj molecular relajado no correlacionado, y datando el árbol con cuatro calibraciones fósiles, mientras que la reconstrucción de áreas ancestrales fue explorada utilizando un enfoque bayesiano. Como resultados se obtuvo que los análisis filogenéticos no apoyaron la monofilia de *Rhadinaea*, donde la mayoría de las especies del género se agrupan en dos clados principales definidos aquí como “Clado este” y “Clado sur” los cuales divergieron entre si a inicios del Mioceno, y su diversificación fue seguida por 14 divergencias durante el Mioceno medio al Mioceno tardío, cinco durante el Plioceno y 12 durante el Pleistoceno. El origen ancestral de *Rhadinaea* fue reconstruido en la Sierra Madre del Sur. Los análisis mostraron que la geomorfología del Mioceno y Plioceno en conjunto con cambios climáticos de la época propiciaron la diversificación de la mayoría

de las especies de *Rhadinaea*. Los cambios climáticos durante el Mioceno, así como el levantamiento de la Faja Volcánica Transmexicana, probablemente influyeron drásticamente en la diversificación de *Rhadinaea* dentro de la Sierra Madre del Sur. La formación paulatina de la Faja volcánica Transmexicana pudo haber influido en la colonización y diversificación de *Rhadinaea* en distintas áreas de Norteamérica durante el Mioceno medio; de forma similar la elevación de las tierras altas de Chiapas y Guatemala pudo haber tenido un papel importante en la colonización de Centroamérica. Durante el Plioceno y Pleistoceno ocurrieron eventos de colonización independientes por parte de *Rhadinaea* entre las áreas de la Costa del Pacífico, Desierto de Chihuahua, Depresión del Balsas, Faja Volcánica Transmexicana, Plataforma Veracruzana, Sierra Madre Occidental, Sierra Madre Oriental y Sierra Madre del Sur, probablemente impulsados por las fluctuaciones climáticas durante estas épocas.

## ABSTRACT

Multiple geological and climatic events have acted as geographic or ecological barriers associated with speciation events, playing an important role in diversification in North and Central America. In the present study, the influence of geological and climatic events during the Neogene and Quaternary in the diversification of the genus *Rhadinaea* was evaluated through molecular dating and ancestral area reconstruction. For this, a multilocus data set was used including 36 individuals of *Rhadinaea* with distribution in most of the biogeographic provinces and that represent 19 of the 21 currently recognized species, as well as two undescribed species. Phylogenetic relationships were explored using the Maximum Likelihood and Bayesian approaches, the estimation of divergence times was performed using an uncorrelated relaxed molecular clock, and dating with four fossil calibrations, while the ancestral area reconstruction was explored using a Bayesian approach. As results, it was obtained that most of the *Rhadinaea* species are grouped into two main clades defined here as "Eastern Clade" and "Southern Clade" which diverged from each other at the beginning of the Miocene, and their diversification was followed by 14 divergences during the Middle Miocene to late Miocene, five during the Pliocene and 12 during the Pleistocene. The ancestral origin of *Rhadinaea* was reconstructed in the Sierra Madre del Sur. Phylogenetic analyzes did not support *Rhadinaea* monophyly. The analyses showed that the geomorphology of the Miocene and Pliocene in conjunction with climatic changes of the time favored the diversification of the genus. Climatic changes during the middle Miocene, as well as the rise of the Trans-Mexican Volcanic Belt, probably drastically

influenced the diversification of *Rhadinaea* within the Sierra Madre del Sur. The gradual formation of the Trans-Mexican volcanic Belt may have influenced the colonization and diversification of *Rhadinaea* in different areas of North America during the middle Miocene; similarly, the elevation of the highlands of Chiapas and Guatemala may have played an important role in the colonization of Central America. During the Pliocene and Pleistocene, more recent independent colonization events by *Rhadinaea* occurred between the areas of the Pacific Coast, Chihuahuan Desert, Balsas Basin, Trans-Mexican Volcanic Belt, Veracruz platform, Sierra Madre Occidental, Sierra Madre Oriental and Sierra Madre del Sur, probably driven by climatic fluctuations during these times.

## INTRODUCCIÓN GENERAL

La biogeografía histórica se centra en el rol del espacio geográfico como impulsor de eventos biológicos como la especiación, extinción y diversificación (Cox *et al.*, 2016), las perspectivas tradicionales que se enfocan en encontrar patrones biogeográficos (ver Morrone y Crisci, 1995) habían considerado a los procesos de dispersión, duplicación y extinción como una explicación *a posteriori*. Hacia finales del siglo XX, con la aparición de la Biogeografía basada en procesos (Event-Based Biogeography; Ronquist, 1997), los procesos mencionados y la vicarianza fueron incorporados explícitamente al análisis mediante el uso de modelos deterministas (Sanmartín, 2012).

En los últimos años la forma de explicar la distribución espacial de la biodiversidad ha cambiado de forma acelerada, principalmente por la introducción de métodos moleculares en la resolución de relaciones filogenéticas y el método de reloj molecular. Gracias al creciente desarrollo de métodos moleculares para obtener filogenias, es posible estudiar los procesos que condicionaron la diversificación de las especies, contrastándolos con la escala temporal utilizando modelos probabilísticos, enfoque bajo el cual se desarrolla la Biogeografía paramétrica, que combina información filogenética y de distribución de los taxones para reconstruir su distribución ancestral. Los métodos en Biogeografía paramétrica modelan la evolución biogeográfica de un linaje como un proceso estocástico con un número finito de cambios discretos, los cuales son representados como las áreas de distribución actual del linaje en una matriz de transición, donde la probabilidad de cambio

de un estado a otro es modelada por cadenas de Markov y los parámetros de la matriz son procesos biogeográficos. De tal forma que es posible utilizar métodos estadísticos de inferencia filogenética tales como el análisis de Máxima Verosimilitud e Inferencia Bayesiana para estimar la probabilidad relativa de distribuciones ancestrales en cada nodo de la filogenia y la tasa de cambio en parámetros como la extinción o la dispersión (Sanmartín, 2012).

Los métodos propuestos en biogeografía paramétrica han sido ampliamente utilizados en diversos estudios debido a que presenta ventajas sobre otros enfoques como la consideración del grado de divergencia evolutiva entre linajes, la incorporación directa del tiempo al análisis biogeográfico y del error asociado por la integración de la estadística y probabilidad. En este sentido el estudio de patrones biogeográficos de los taxones nos permite hacer inferencias importantes sobre su origen y evolución (Sanmartín, 2012), los cuales son primordiales para entender sus relaciones y definir grupos con base a su ancestría común. Por lo cual el planteamiento de hipótesis biogeográficas dentro de estudios sistemáticos y su posterior refutación o aceptación gracias a la evidencia molecular se ha vuelto común ya que aporta información sobre las áreas que jugaron un papel importante en su diversificación.

Estos patrones biogeográficos y de diversificación a través de áreas como Norte y Centroamérica son complejos, esto se debe a que la distribución de los taxones es moldeada por distintos procesos en distintos tiempos (Ronquist, 1997; Sanmartín, 2012), por lo cual,

las rupturas biogeográficas relacionadas con la diversificación y especiación en varios grupos son idiosincráticas (Bryson *et al.*, 2017; García-Moreno *et al.*, 2004; León-Paniagua *et al.*, 2007). Un ejemplo de ello son las principales hipótesis que se proponen para taxones de herpetofauna en la serie norte-centro-sur americana, donde la zona Centroamericana juega un papel importante en la diversificación de tortugas como *Trachemys* (Fritz *et al.*, 2015), lagartos como *Norops* (Phillips *et al.*, 2015) e hílidos (Duellman *et al.*, 2016), la diversificación de estos taxones se creó data del Oligoceno hasta el Pleistoceno (Duellman *et al.*, 2016; Fritz *et al.*, 2015; Phillips *et al.*, 2015).

El género de culebras hojarasqueras *Rhadinaea* se compone actualmente a 21 especies las cuales se organizan tradicionalmente en cinco grupos morfológicos (Myers, 1974; 2011). Su distribución ocupa desde el sureste de Estados Unidos hasta Panamá con discontinuidades en su distribución a través del desierto Chihuahuense, sur de Guatemala, EL Salvador, Honduras y Nicaragua (Myers, 1974; 2011). Estas culebras son de hábitos fosoriales y se encuentran en una variedad de hábitats desde el nivel del mar hasta los 3,200 msnm, aunque muestran una clara afinidad con los bosques de pino, bosques de pino-encino y bosques nublados (Myers, 1974). En la actualidad, existen pocos trabajos sobre *Rhadinaea*, aunado a esto se sabe poco sobre la historia evolutiva y origen de este género, cuestiones que potencialmente pueden dar luz sobre sus relaciones intra e intergenéricas, las cuales se han calificado como “obscuras” (Myers, 1974). Dado lo anterior, el presente trabajo pretende poner a prueba la monofilia de *Rhadinaea*, así como dilucidar la influencia de los



eventos geológicos y ecológicos que condicionaron la distribución y diversificación del género a través de América.

## RESEARCH PAPER

### Historical Biogeography of the Genus *Rhadinaea* (Squamata: Dipsadinae)

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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 North and Central America. Here we evaluate the influence of Neogene and Quaternary geological events and climatic changes in the diversification of the colubrid snake genus *Rhadinaea* using molecular dating and ancestral area reconstruction.

Location: Southeastern United States, Mexico, and Central America.

Methods: A multilocus sequence dataset was generated for 37 individuals of *Rhadinaea* from most of the biogeographical provinces from where the genus is distributed, representing 19 of the 21 currently recognized species, as well as two undescribed species. Phylogenetic relationships were explored using Maximum Likelihood and Bayesian approaches. Divergence times were assessed based on the fossil record using a relaxed, uncorrelated, lognormal clock. Ancestral areas were inferred at divergence events across the tree using a probabilistic Bayesian approach.

Results: Our analyses showed that the majority of *Rhadinaea* species nest in two main clades, herein identified as “eastern” and “southern”, these clades being recovered in all of our phylogenetic analyses. These clades probably diverged from each other in the early Miocene, and their divergence was followed by 14 divergences during the middle to late Miocene, five divergences during the Pliocene, and 14 divergences in the Pleistocene. The ancestral distribution of *Rhadinaea* was reconstructed to be across the Sierra Madre del Sur. Our phylogenetic analyses did not support the monophyly of *Rhadinaea*.

Main conclusions: Miocene and Pliocene geomorphology, perhaps in conjunction with climate change, appears to have triggered the diversification of the genus. Climatic changes during the Miocene probably induced the diversification of *Rhadinaea* in the Sierra Madre del Sur. Our analysis suggests that the uplifting of the Trans-Mexican Volcanic Belt and Chiapan-Guatemalan highlands in this same period resulted in northward and southward colonization events. This event was followed by more recent, independent colonization

events in the Pliocene and Pleistocene involving the Balsas Basin, Chihuahuan Desert, Pacific coast, Sierra Madre Occidental, Sierra Madre Oriental, Sierra Madre del Sur, Trans-Mexican Volcanic Belt and Veracruz provinces, probably driven by the climatic fluctuations of the time.

**KEYWORDS:** ancestral area reconstruction, divergence dating, diversification, Mexican Transition Zone, Pleistocenic Climate Change, Trans-Mexican Volcanic Belt, Sierra Madre del Sur

## **INTRODUCTION**

Inferring the evolutionary history of groups in a particular region is the first step in elucidating the processes by which the region's fauna originated (Colston et al., 2013). Multiple biogeographical and phylogeographical studies of groups with broad distributions through North and Central America are illustrative in this context (i.e., Bryson et al., 2011b; Burbrink et al., 2008; Fritz et al., 2012; Hofmann & Townsend, 2017; Phillips et al., 2015). Within this area, the Mexican Transition Zone (MTZ) is a complex distinguishable area where Neotropical and Nearctic biotas overlap, spanning the region from the southwestern deserts of the United States and northern Mexico to the dry and humid forests of the Nicaraguan lowlands (Morrone, 2010).

Across the North and Central American regions, multiple geological and climatic events, resulting from a complex orogeny and paleoclimatic conditions, acted as geographical or ecological barriers associated with the speciation and diversification of many taxa (e.g., Bryson, García-Vázquez & Riddle, 2011a; Daza, Castoe & Parkinson, 2010; Ferrusquía-Villafranca & González-Guzmán, 2005; Vanzolini, 1970). Some of the events that are considered of major importance or have received the most attention are: (1) The Mississippi River Basin (MR) (Burbrink, Lawson & Slowinski, 2000), which was involved in the divergence of many marine and terrestrial taxa during the Pleistocene (Soltis et al., 2006); (2) the formation over 30 million years ago (Ma) of three of the four major mountain ranges in Mexico (i.e., the Sierra Madre Occidental [SMOc], Sierra Madre Oriental [SMOr] and Sierra Madre del Sur [SMS]; Ferrusquía-Villafranca & González-Guzmán, 2005), which probably predate the origin of most extant species (Bryson, García-Vázquez & Riddle, 2012); (3) the formation of the Trans-Mexican Volcanic Belt (TVB) during the Neogene (19.5-3 Ma) (Gómez-Tuena, Orozco-Esquivel & Ferrari, 2007) in two major orogenic events: one around 19-10 Ma in western Mexico and another around 7.5-3 Ma in eastern Mexico (Ferrusquía-Villafranca & González-Guzmán, 2005), events that undoubtedly affected both the timing and tempo of biota diversification (Bryson et al., 2012a; 2012b); (4) the faulting and marine introgressions across the Isthmus of Tehuantepec (IT) in southeastern Mexico around 3 Ma (Mulcahy, Morrill & Mendelson III, 2006), a region which is a narrow lowland area that has been identified as a biogeographical barrier for many upland taxa (Castoe et al., 2009); (5) the Nicaraguan depression (ND), an area that presented different states of terrestrial conformation during the Neogene (2.5-23 Ma) (Funk, Mann, McIntosh & Stephens, 2009)

and probably presented a lowland biogeographical barrier to some taxa (Daza et al., 2010); (6) the Panama Isthmus in southern Central America, another narrow area that was completely conformed during the Pliocene (3.5 Ma), which has separated numerous taxa between Central and South America (Mendoza et al., 2019); and (7) the climatic fluctuations during the Pleistocene (0.01-2.5 Ma) (Vanzolini, 1970), which conditioned the diversification of a variety of taxa across the American continent through the repeated expansion and contraction of coniferous forests, leading isolated populations of forest-adapted taxa to speciation (Haffer, 1969; 1997).

These events, in addition to other physiographic conditions, such as river drainages within the major sierras, basins and faults, are considered to act as biogeographical barriers (Bryson et al., 2011b; Bryson et al., 2007; Daza et al., 2010; León-Paniagua et al., 2007), yet the effectiveness of these barriers in isolating lineages throughout the past several million years remains to be clarified (Bryson et al., 2012a; 2012b; García-Vázquez et al., 2018a).

The colubrid snakes of the genus *Rhadinaea* are slender, diurnal, medium- to small-sized snakes that are characterized by longitudinal dark stripes along the dorsal scales, a small subpreocular scale inserted between the corners of two supralabial scales at the anteroventral edge of the orbit, the same number of longitudinal dorsal scale rows throughout the body, and maxillary teeth without grooves posterior to the diastema (Myers, 1974; 2011; Palacios-Aguilar & García-Vázquez, 2020). Currently, 21 species of *Rhadinaea* are recognized and arranged into five morphological groups (Myers, 2011;

García-Vázquez et al., 2018b; Palacios-Aguilar & García-Vázquez, 2020). The genus is present in North and Central America from the southeastern United States to Panama, with discontinuities across the Chihuahuan Desert, southern Guatemala, El Salvador, Honduras, and central Nicaragua (Table 1) (Myers, 1974; 2011). The most speciose and widely distributed of these groups is the *decorata* group, represented by 12 species mainly distributed over the Mexican Sierras (García-Vázquez et al., 2009; García-Vázquez, 2012; García-Vázquez et al., 2018b; Luría-Manzano, Ramírez-Bautista & Canseco-Márquez, 2014; Pérez-Higareda et al., 2002; Sánchez-García et al., 2019; Torres-Carvajal et al., 2019); the *taeniata* group is endemic to Mexico and is composed of three species distributed in central Mexico (Canseco-Márquez & Gutiérrez-Mayén, 2010; Myers, 1974; García-Vázquez et al., 2018b; Myers, 1974; García-Sotelo, Martínez-Fuentes & García-Vázquez, 2018); the *flavilata* group is comprised of two species with allopatric distributions in North America (Auth, Smith & Chiszar, 1999; Lares et al., 2013; Walley, 1999); and the *calligaster* and *vermiculaticeps* groups are restricted to Central America (Myers, 1974). In addition to these 21 species, the existence of two undescribed species has been suggested based on their morphology and previous analyses (pers. obs.).

Table 1. Distribution of the species of *Rhadinaea* corresponding with the biogeographic regionalization of Mexico (Morrone *et al.*, 2017), Neotropical America (Löwenberg-Neto, 2014) and North America (Escalante *et al.*, 2013). Alleghanian region (ALLE); Balsas Basin (BB); Chocó-Darián (CH-DA); Chiapas (CHIS); Chiapas Highlands (CHIS-H); Chihuahuan Plateau (CHIH); Gatuso-Talamanca (G-T); Pacific Lowlands (PAC); Pacific coast (PAC-C); Puntarenas-Chiriquí (P-CH); Sierra Madre Occidental (SMOc); Sierra Madre Oriental (SMOr); Sierra



Madre del Sur (SMS); Trans-Mexican Volcanic Belt (TVB) and Veracruz (VER). The CHIS and CHIS-H provinces, as well as PAC and PAC-C represent homologous biogeographic regions that overlap in both regionalizations.

Group	Species	Morrone et al. (2017)									Löwenberg-Neto (2014)					Escalante et al. (2013)
		SMOr	VER	CHIH	TVB	PAC	BB	SMS	CHIS	SMOc	CHIS-H	PAC-C	G-T	P-CH	CH-DA	ALLE
decorata	<i>R. bogertorum</i>							X								
	<i>R. cuneata</i>		X					X								
	<i>R. decorata</i>	X	X			X		X	X		X	X	X	X	X	
	<i>R. forbesi</i>				X			X								
	<i>R. gaigeae</i>	X														
	<i>R. hesperia</i>			X	X	X	X	X		X						
	<i>R. macdougalli</i>		X					X								
	<i>R. marcellae</i>	X	X													
	<i>R. montana</i>	X	X													
	<i>R. myersi</i>							X								
	<i>R. nuchalis</i>							X								
<i>R. quinquelineata</i>	X			X												
taeniata	<i>R. fulvivittis</i>							X								
	<i>R. omiltemana</i>							X								
	<i>R. taeniata</i>				X		X	X								
flavilata	<i>R. flavilata</i>															X
	<i>R. laureata</i>				X			X		X						
vermiculatic eps	<i>R. pulveriventris</i>												X	X		
	<i>R. sargenti</i>												X			
	<i>R. vermiculaticeps</i>												X	X		
calligaster	<i>R. calligaster</i>												X	X		

The systematics of *Rhadinaea* has been poorly studied, and, to date, the arrangements of Myers (2011) are predominant. A recent study (Palacios-Aguilar and García-Vázquez, 2020) using a mitochondrial fragment of DNA provides a comprehensive insight into the phylogenetic relationships of this Neotropical snake genera, supporting the reciprocal monophyly of *Rhadinaea* and *Rhadinella* (a former *Rhadinaea* group separated by morphological evidence by Myers [2011]) and a close relationship with *Coniophanes* as a

sister group (as previously suggested by several authors; i.e. Bailey, 1939; Cadle, 1984; Myers, 1974; Zaher et al., 2019). Additionally, *Rhadinophanes* is a monotypic genus that is considered the sister group of *Rhadinaea* and *Coniophanes* (Cadle, 1984; Myers & Campbell, 1981), a relationship that has not been tested using molecular data. To explore their monophyly and evolutionary history, Palacios-Aguilar and García-Vázquez (2020) suggest that it is necessary to include more representatives of the genera using more molecular markers.

To date, the five groups composing *Rhadinaea* are considered based on their color patterns, which are the most informative character to distinguish them, even among species within *Rhadinaea* (Myers, 1974; 2011). However, the reciprocal monophyly of the groups is still to be assessed (García-Vázquez et al., 2018b). On the other hand, according to Myers (1974), the origin of *Rhadinaea* is related to the dispersal of an ancestor related to *Rhadinella* (former godmani group), in which geographical isolation and the subsequent evolution of terminal populations occurred, probably after unfavorable climatic changes or flooding that created barriers of lowland regions such as the IT and the ND (Myers, 1974); however, other geographical barriers corresponding to the distribution of the genus, such as the formation of the TVB or climatic events, are not discussed.

Here, we describe the phylogenetic relationships of *Rhadinaea* to evaluate the role of major orogenic events and Pleistocene climatic fluctuations on lineage diversification. Samples of the five recognized groups and two undescribed species of *Rhadinaea* were included (*R. cf.*

*marcellae* and *R. cf. taeniata*). Two mitochondrial and two nuclear loci were sequenced. We inferred the phylogenetic relationships and a time-calibrated tree 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.

## **MATERIALS AND METHODS**

### **Taxon Sampling and Laboratory Methods**

We include 37 samples of *Rhadinaea* in this study, including most of the currently recognized species of the genus (Figure 1; Appendix S1), with the exception of *R. sargenti* and *R. vermiculaticeps*, two species that are rarely found within biological collections. Samples from two undescribed species from the TVB were also included. To increase geographic representativeness for species with a wide distribution range, we included a sample per biotic region following the biogeographical regionalization of North America (Escalante, Morrone & Rodríguez-Tapia, 2013), Mexico (Morrone, Escalante & Rodríguez-Tapia, 2017) and Neotropical America (Löwenberg-Neto, 2014), except for *R. hesperia*, a species that is present in the eastern and western SMS; thus, we included a sample from each subregion.

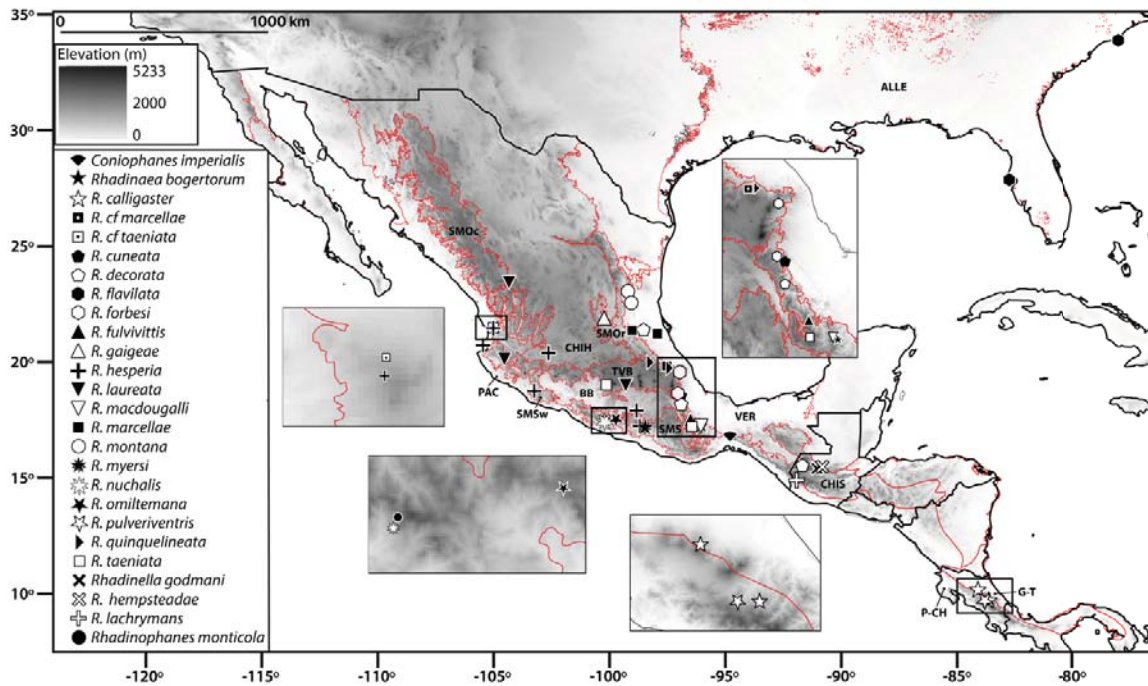


Figure 1. Sampling localities for the genetic samples used in this study (see Appendix S1). Black lines indicate political boundaries, red lines indicate biogeographic regions (Escalante et al., 2013; Löwenberg-Neto, 2014; Morrone et al., 2017). Alleghanian region (ALLE); Balsas Basin (BB); Chiapas (CHIS); Chihuahuan Plateau (CHIH); Gatuso-Talamanca (G-T); Pacific Lowlands (PAC); Puntarenas-Chiriquí (P-CH); Sierra Madre Occidental (SMOc); Sierra Madre Oriental (SMOr); Sierra Madre del Sur (SMS); western Sierra Madre del Sur (SMSw); Trans-Mexican Volcanic Belt (TVB) and Veracruz (VER).

To test the monophyly of *Rhadinaea*, we include one representative each of *Coniophanes imperialis*, *Rhadinella godmani*, *Rhadinella hempsteadae*, *Rhadinella lachrymans* and *Rhadinophanes monticola* due to the evidence that they represent close relatives to this genus (Cadle, 1984; Myers, 1974; 2011; Myers & Campbell, 1981). Finally, to time-calibrate our phylogenetic tree, sequences from three colubrid genera and two close relatives within Caenophidia were included (Grazziotin et al., 2012; Pyron et al., 2011; Zaher et al., 2019; Appendix S1).

Partial sequences of the mitochondrial gene coding for the Cytochrome b (*cytb*), NADH dehydrogenase subunit 4 (*ND4*), complete sequences of the non-coding tRNA-His and tRNA-Ser, partial sequences of the non-coding tRNA-Leu, partial sequences of the nuclear genes coding for Oocyte maturation factor (*cmos*) and Dynein axonemal heavy chain 3 (*DNAH3*) were obtained for all the 37 individuals of *Rhadinaea* and outgroups. Loci were selected since they previously showed to be informative at different levels of divergence between snakes (Bryson, et al., 2011a; 2011b; Lawson et al., 2005; Myers et al., 2017). Primer sequences for *cmos* were given by Lawson et al, (2005) and Saint et al. (1998), for *cytb* by Burbrink, Lawson & Slowinski (2000), de Queiroz, Lawson & Lemos-Espinal (2002) and Slowinski & Lawson (2002), for *DNAH3* by Townsend et al. (2008) and for *ND4* by Arévalo, Davis & Sites (1994), Frostner, Davis & Arévalo (1995). Additional internal primers were designed in this study. See Appendix S2 for primer sequences, technical details on DNA sequencing and sequence edition.

### **Phylogenetic Inference**

The phylogeny was inferred using Bayesian inference (BI) and Maximum Likelihood (ML) methods using the information of mitochondrial and nuclear genes in a concatenated matrix. For both approaches, partitioned analyses were carried out to improve phylogenetic accuracy. The best-fitting substitution models and partition schemes were selected jointly using the Bayesian Information Criterion in the software PartitionFinder 2 (Lanfear, et al.,

2016). BI analysis was conducted using MrBayes 3.2.7a (Ronquist et al., 2012) with four Monte Carlo Markov Chains (MCMC), sampling every 5,000 generations for 100 million generations. Output parameters were visualized using Tracer 1.7.1 (Rambaut et al., 2018) to ascertain stationarity and convergence. The first 25% of generations were discarded as burn-in to obtain a majority rule consensus tree using the command sumt. ML analysis was conducted using raxmlGUI (Silvestro & Michalak, 2012) under the GTRGAMMA model (Stamatakis, 2006) with 1,000 nonparametric bootstrap replicates to assess nodal support. Nodes were considered strongly supported if their Bayesian posterior probability (pp) was  $\geq 0.95$  and their bootstrap (bs) value was  $\geq 70\%$  (Huelsenbeck & Rannala, 2004).

### **Divergence Times**

Divergence dates and phylogeny were estimated simultaneously using a relaxed Bayesian molecular clock framework implemented in BEAST 2.4.8 (Bouckaert et al., 2014). For this purpose, our multilocus dataset was analyzed with an uncorrelated lognormal clock and node constraints obtained from the fossil record under lognormal distributions. The partitions and models for this analysis were estimated using bModelTest (Bouckaert & Drummond, 2017). Four fossil calibration points of Colubridae and related groups were used (See Appendix S3 for technical details on calibration points). Analyses were run for 100 million generations, samples were retained every 5,000 generations and a Yule birth-death prior was specified. Results were displayed in Tracer 1.7.1 to confirm the proper mixing and likelihood stationarity of the MCMC analyses, appropriate burn-in and adequate, effective

sample sizes (>200 for each estimated parameter). After discarding the initial 20% of the samples as burn-in, the parameter values of the posterior samples were summarized on a maximum clade credibility tree using TreeAnnotator 1.8.2 (Rambaut & Drummond, 2014), setting the posterior probability limit to 0.1 and summarized mean node heights.

### **Ancestral Area Reconstruction**

Ancestral ranges at each divergence event were reconstructed using the Bayesian Binary Monte Carlo analysis (BBM), implemented in RASP 2.0 (Yu, Harris & He, 2011). This program can determine the probability of an ancestral range at a node by averaging over a posterior set of trees and thereby accounting for phylogenetic uncertainty (Bryson, Savary & Prendini, 2013). A total of 16,000 post-burn-in trees and the condensed maximum clade credibility tree obtained in the BEAST analysis were loaded from the divergence time analysis into RASP. According to the collected locality, each sample of the phylogeny was assigned to one of the following 12 terminal biogeographical regions (Escalante et al., 2013; Löwenberg-Neto, 2014; Morrone, 2017): (1) Alleghanian Region (ALLE); (2) Balsas Basin (BB); (3) Chiapas (CHIS); (4) Chihuahuan Plateau (CHIH); (5) Pacific Lowlands (PAC); (6) Puntarenas-Chiriquí (P-CH); (7) Sierra Madre Occidental (SMOc); (8) Sierra Madre Oriental (SMOr); (9) Sierra Madre del Sur (SMS); (10) western Sierra Madre del Sur (SMSw); (11) Trans-Mexican Volcanic Belt (TVB); and (12) Veracruz (VER). The probabilities for nodes in the phylogeny were estimated. The analysis was conducted under the JC + G (Jukes-Cantor + Gamma)

model running for 10 million generations by sampling each 1,000 and using ten chains. The first 25% of the generations were discarded as burn-in.

## RESULTS

### Phylogenetic Inference

The final dataset consisted of 2,899 aligned nucleotide positions. The partitions and models that best fit the data were as follows: *cytb* third position and *DNAH3* third position, GTR+G; *cytb* second position, tRNA-His, *ND4* first position and *ND4* third position, GTR+I+G; *cytb* second position and *ND4* second position, TVM+I+G; tRNA-Ser, TVMEF+G; tRNA-Leu, TVMEF+G; *cmos* first position and *DNAH3* first position, TRN+I+G; *cmos* second position, TRN+I; *cmos* third position, HKY+I; and *DNAH3* second position, K81UF+I. All sequences were deposited in GenBank (Appendix S1). ML and BI analyses resulted in highly congruent phylogenetic trees, presenting the same topology with most clades strongly supported (Figure 2).



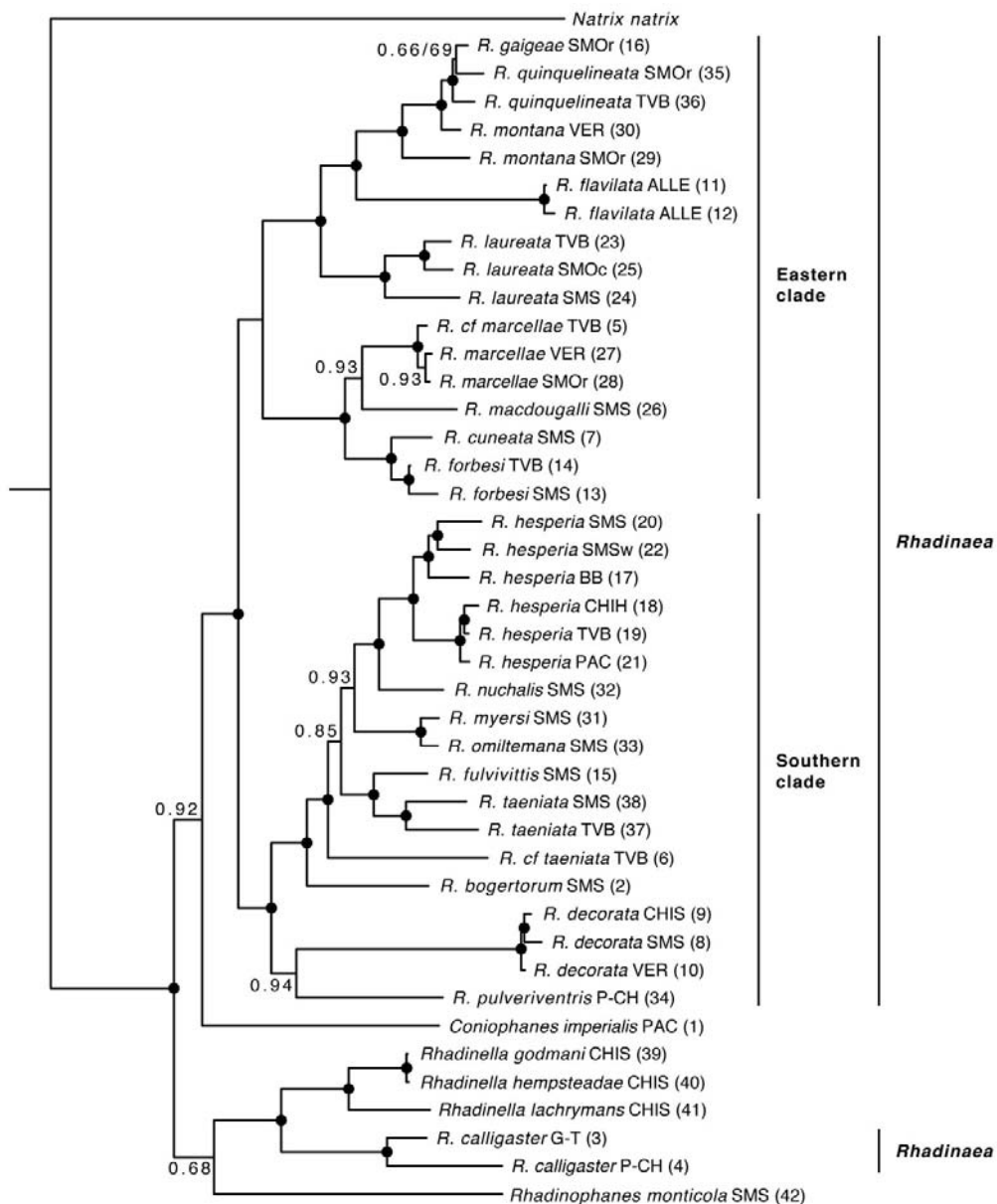


Figure 2. Phylogeny of *Rhadinaea* (BI analyses is showed) and close groups inferred from maximum likelihood and Bayesian inference analyses from the DNA concatenated matrix. Black dots represent strongly supported nodes (bootstrap value  $\geq 70$ ; Bayesian posterior probability value  $\geq 0.95$ ). Numbers at other nodes are bootstrap/Bayesian posterior probability values for poorly supported nodes in one or both analyses (marked with a slash).

Our phylogenetic analyses did not support the monophyly of *Rhadinaea* (Figure 2), showing *Rhadinaea calligaster* as the sister taxon of *Rhadinella*, a relationship strongly supported in both analyses. The rest of the genus species are grouped into two strongly supported main clades, which are defined below: An eastern clade (Eastern clade, Figure 2) composed of mostly eastern species (*Rhadinaea flavilata*, *R. forbesi*, *R. gaigeae*, *R. laureata*, *R. macdougalli*, *R. cf. marcellae*, *R. marcellae*, *R. montana* and *R. quinquelineata*, distributed across the ALLE, SMOc, SMO<sub>r</sub>, SMS, TVB, and VER; and a southern clade (Southern clade, Figure 2) composed mostly of western and southern species (*Rhadinaea bogertorum*, *R. decorata*, *R. fulvivittis*, *R. hesperia*, *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. pulveriventris*, *R. taeniata* and *R. cf. taeniata*), distributed across the BB, CHIH, CHIS, P-CH, PAC, SMS, TVB, and VER.

Within the eastern clade, *R. quinquelineata* is paraphyletic respect to *R. gaigeae* and *R. montana* is paraphyletic respect to *R. gaigeae* and *R. quinquelineata*, with a low support value in both analyses (Figure 2); these species represent the sister group of *R. flavilata*, and these last haplotypes in turn represent the sister group of *R. laureata*; the sampled haplotypes of *R. marcellae* are recovered as monophyletic with a low support value in the BI analysis (Figure 2) and represent the sister group of *R. cf. marcellae*; these species are recovered as the sister group of *R. macdougalli* with a low support value in the BI analysis (Figure 2); and these species represent the sister group of *R. cuneata* + *R. forbesi*; finally, the clade composed of *R. flavilata*, *R. gaigeae*, *R. laureata*, *R. montana* and *R. quinquelineata* represents the sister group of the clade composed by *R. cuneata*, *R. forbesi*,

*R. macdougalli*, *R. marcellae* and *R. cf. marcellae*, a relationship that is strongly supported (Figure 2).

Within the southern clade, *R. hesperia* + *R. nuchalis* represent the sister group of *R. myersi* + *R. omiltemana*, with a low support value in the BI analysis (Figure 2); all of these species in turn represent the sister group of *R. fulvivittis* + *R. taeniata* with a low support value in the BI analysis (Figure 2); *R. cf. taeniata* represent the sister group of these mentioned species and in the same way *R. bogertorum*; on the other hand, *R. decorata* is recovered as the sister group of *R. pulveriventrif* with a low support value in the BI analysis (Figure 2); finally, the clade composed by *R. bogertorum*, *R. fulvivittis*, *R. hesperia*, *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. taeniata* and *R. cf. taeniata* represent the sister group of *R. decorata* + *R. pulveriventrif*, a relationship that is strongly supported (Figure 2).

*Coniophanes* is observed as the sister group of *Rhadinaea* (except *R. calligaster*), which is a relationship strongly supported only in ML analysis. *Rhadinophanes monticola* is recovered as a sister group of *Rhadinaea calligaster* + *Rhadinella*; the relationship is only supported in the ML analysis (Figure 2).

### **Divergence Times**

Our multilocus analysis produced a reconstruction for *Rhadinaea* with moderate resolution and node support (77.8% of nodes with pp>0.95). In the calibrated tree, the same clades as

phylogenetic analyses were recovered, as well as the relationships between the clades. The dated phylogeny suggests that the diversification of *Rhadinaea* (except *Rhadinaea calligaster*) probably began in the early Miocene (20.0 Ma) (Figure 3) with a basal divergence between major clades (Eastern clade and Southern clade) in the early Miocene (17.4 Ma). Several divergences appear to have occurred during the Miocene within eastern and southern clades (Figure 3). In the eastern clade, a basal divergence between the clade composed of *R. cuneata*, *R. forbesi*, *R. macdougalli*, *R. marcellae* and *R. cf. marcellae* and the clade consisting of *R. flavilata*, *R. gaigeae*, *R. laureata*, *R. montana* and *R. quinquelineata* (15.3 Ma) is observed, followed by five splits among these species. In the southern clade, a basal divergence between the clade composed of *R. bogertorum*, *R. fulvivittis*, *R. hesperia*, *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. taeniata* and *R. cf. taeniata* and the clade consisting of *R. decorata* and *R. pulveriventris* (14.9 Ma) is observed, followed by seven splits among these species. Our estimates placed the remaining divergences within the eastern and southern clades during the Pliocene (Figure 3).

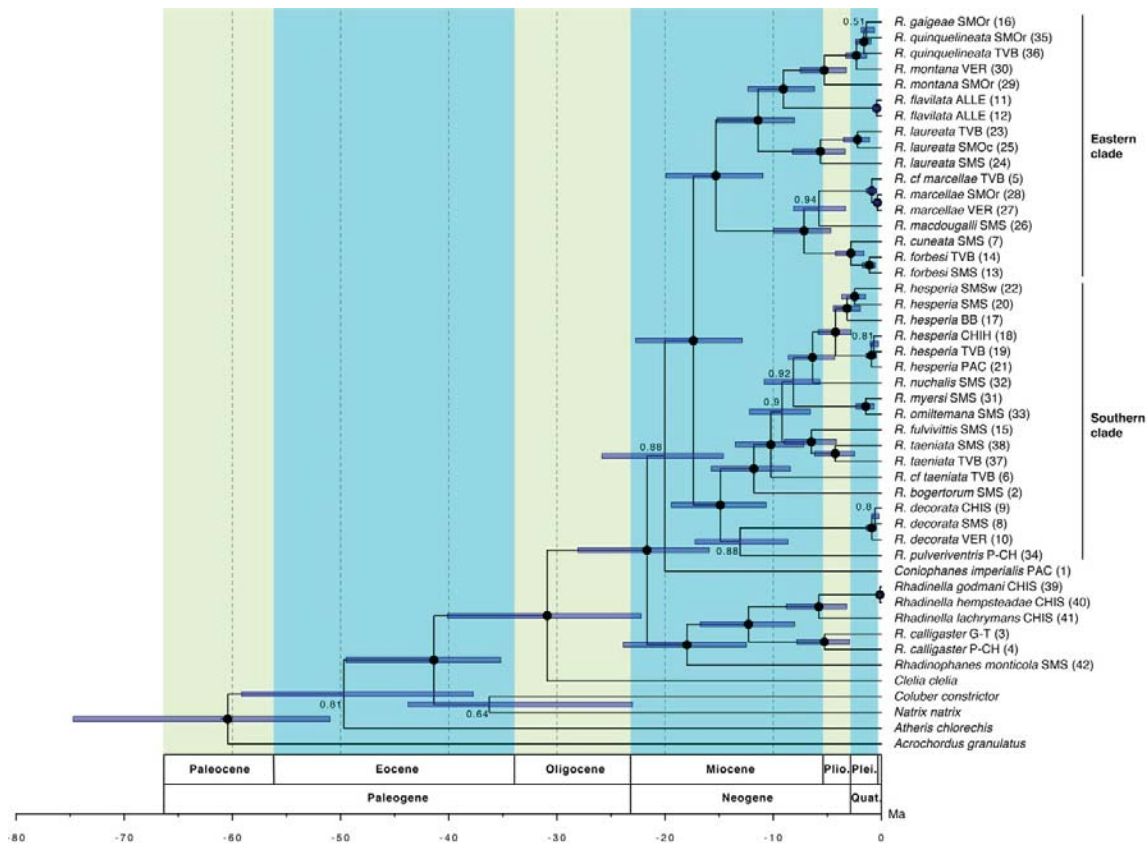


Figure 3. Divergence time estimates for *Rhadinaea* from the multilocus dataset in BEAST. Bars indicate 95% highest posterior densities of divergence dates, with mean estimates in a million years ago (Ma) at the nodes. Nodes with black dots are strongly supported nodes, and numbers near other nodes are Bayesian posterior probability values for poorly supported nodes. ALLE, Alleghanian Region; BB, Balsas Basin; CHIS, Chiapas; CHIH, Chihuahuan Plateau; PAC, Pacific Lowlands; P-CH, Puntarenas-Chiriquí; SMOc, Sierra Madre Occidental; SMOr; Sierra Madre Oriental; SMS, Sierra Madre del Sur; SMSw, western Sierra Madre del Sur; TVB, Trans-Mexican Volcanic Belt; VER, Veracruz.

The divergence time estimates also show the divergence between *Rhadinaea* + *Coniophanes* and *Rhadinaea calligaster* + *Rhadinella* + *Rhadinophanes* during the early

Miocene (21.6 Ma) (Figure 3), and the divergence between *Rhadinaea calligaster* and *Rhadinella* occurs during the middle Miocene (12.3 Ma) (Figure 3).

### **Ancestral Area Reconstruction**

The BBM analysis using the dated multilocus phylogeny obtained in BEAST was conducted excluding *R. calligaster* (see Discussion). The RASP analysis showed that the diversification of *Rhadinaea* likely began in the early Miocene in an ancestor widely distributed across the Sierra Madre del Sur (SMS), approximately 17.5 Ma (Figure 4).

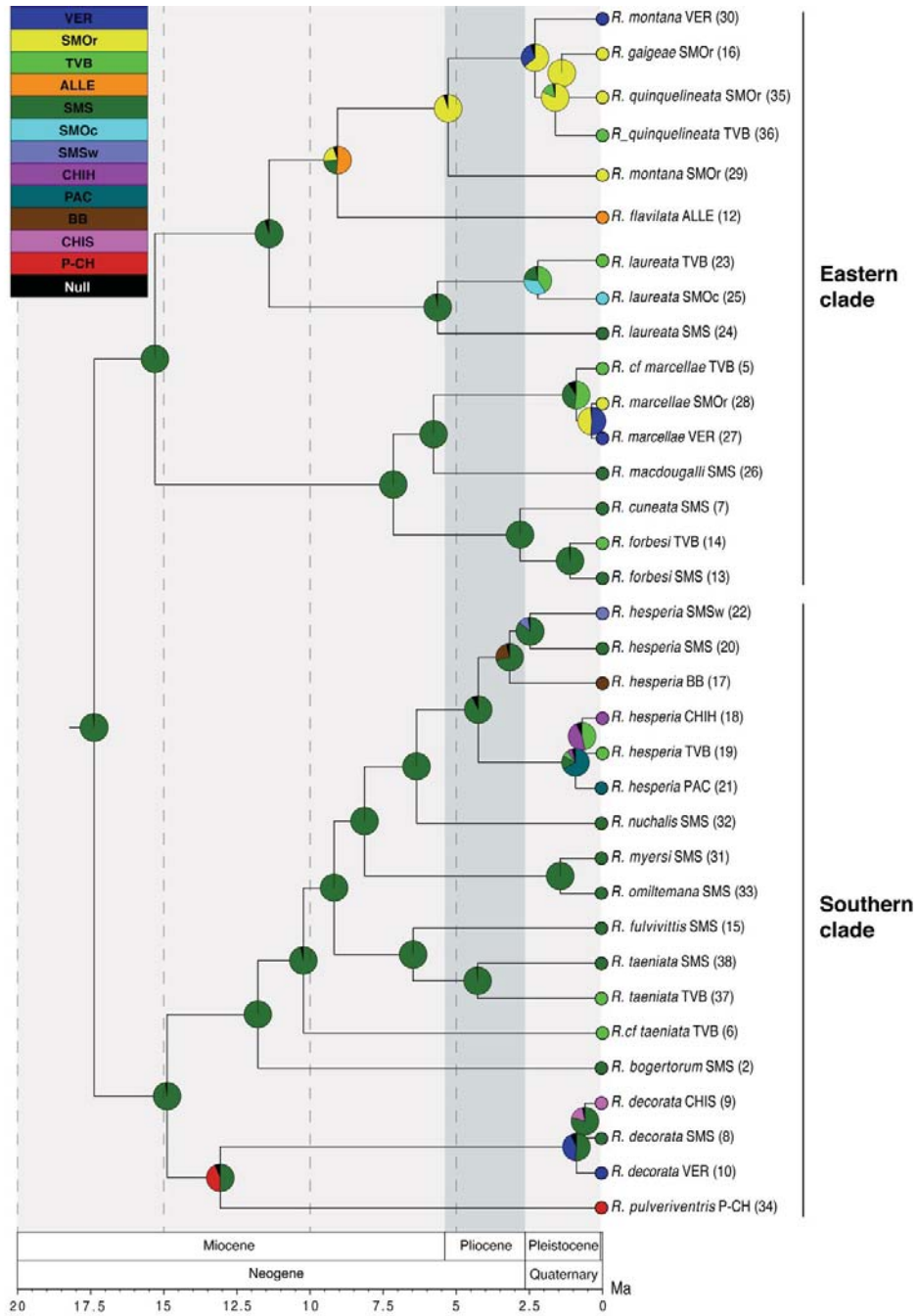


Figure 4: Dated multilocus phylogeny for *Rhadinaea*, showing BBM analysis ancestral area reconstructions. Sampled localities are color-coded to match the biogeographical regions in the inset box. ALLE: Alleghanian Region, BB: Balsas Basin, CHIH: Chihuahuan Desert, CHIS: Chiapas, P-CH: Puntarenas-Chiriquí, PAC: Pacific Lowlands, SMOc: Sierra Madre Occidental, SMOr: Sierra Madre Oriental, SMS: Sierra Madre del Sur, SMSw;

western Sierra Madre del Sur, TVB: Trans-Mexican Volcanic Belt, VER: Veracruz, Null: Null range probability. The colored pie charts in the nodes represent the probability of the ancestral area.

The eastern clade distributed across ALLE, SMOc, SMOr, SMS, TVB, and VER, split between 0.4 and 15.3 Ma into 16 lineages (Figure 4). During the Miocene, an initial vicariant event in the SMS split the most recent common ancestor (MRCA) of *R. flavilata*, *R. gaigeae* SMOr, *R. laureata* (SMOc, SMS, TVB), *R. montana* (SMOr, VER), and *R. quinquelineata* (SMOr, TVB) from the MRCA of *R. cuneata*, *R. forbesi* (SMS, TVB), *R. macdougalli*, *R. marcellae* (SMOr, VER) and *R. cf. marcellae* (15.3 Ma); followed by a colonization event by the MRCA of *R. flavilata*, *R. gaigeae* SMOr, *R. montana* (SMOr, VER), and *R. quinquelineata* (SMOr, TVB) from the SMS to ALLE during the middle Miocene (11.4 Ma); and a vicariant event in SMS between the MRCA of *R. macdougalli*, *R. marcellae* (SMOr, VER), *R. cf. marcellae* and the MRCA of *R. cuneata*, *R. forbesi* (SMS, TVB) during the late Miocene (7.1 Ma) (Figure 4); also, three colonization events are observed (Figure 4): the first one from the ALLE to SMOr involving the MRCA of *R. gaigeae* SMOr, *R. montana* (SMOr, VER) and *R. quinquelineata* (SMOr, TVB), during the late Miocene (9.1 Ma); the second one from the SMS to TVB involving the MRCA of *R. marcellae* (SMOr, VER) and *R. cf. marcellae* during the late Miocene (5.8 Ma); and from the SMS to TVB involving the MRCA of *R. laureata* (SMOc, TVB) during the late Miocene (5.6 Ma). During the Pliocene, two vicariant events took place (Figure 4): a split in the SMOr between *R. montana* SMOr and the MRCA of *R. gaigeae* SMOr, *R. montana* VER and *R. quinquelineata* (SMOr, TVB) (5.3 Ma); and a split in the SMS between *R. cuneata* and the MRCA of *R. forbesi* (SMS, TVB) (2.8 Ma). During the Pleistocene, a



vicariant event is observed in the SMOr between *R. gaigeae* SMOr and *R. quinquelineata* SMOr (1.4 Ma), as well as six colonization events (Figure 4): two from SMOr to VER (*R. montana* VER) (2.3 Ma) and TVB (*R. quinquelineata* TVB) (1.6 Ma); from TVB to SMOc involving *R. laureata* SMOc (2.2 Ma); from SMS to TVB involving *R. forbesi* TVB (1.1 Ma); from TVB to VER involving the MRCA of *R. marcellae* (SMOr, VER) (0.9 Ma); and from VER to SMOr involving *R. marcellae* SMOr (0.4 Ma).

The southern clade distributed across BB, CHIH, CHIS, P-CH, PAC, SMS, SMSw, TVB, and VER split between 0.6 and 14.9 Ma into 18 lineages (Figure 4). During the Miocene, an initial vicariant event in the SMS split the MRCA of *R. bogertorum*, *R. fulvivittis*, *R. hesperia* (BB, CHIH, PAC, SMS, SMSw, TVB), *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. taeniata* (SMS, TVB) and *R. cf. taeniata* with respect to the MRCA of *R. decorata* (CHIS, SMS, VER) and *R. pulveriventris* (14.9 Ma); followed by a dispersal event to P-CH by *R. pulveriventris* during the middle Miocene (13.1 Ma) and five vicariant events during the late Miocene inside the SMS (Figure 4): a first split between *R. bogertorum* and the MRCA of *R. fulvivittis*, *R. hesperia* (BB, CHIH, PAC, SMS, SMSw, TVB), *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. taeniata* (SMS, TVB) and *R. cf. taeniata* (11.8 Ma); a second split between the MRCA of *R. fulvivittis* and *R. taeniata* (SMS, TVB) and the MRCA of *R. hesperia* (BB, CHIH, PAC, SMS, SMSw, TVB), *R. myersi*, *R. nuchalis* and *R. omiltemana* (9.2 Ma); a third split between the MRCA of *R. hesperia* (BB, CHIH, PAC, SMS, SMSw, TVB) and *R. nuchalis* from the MRCA of *R. myersi* and *R. omiltemana* (8.1 Ma); a fourth split between *R. fulvivittis* and the MRCA of *R. taeniata* (SMS, TVB) (6.5 Ma); and a split between *R. nuchalis* and the MRCA of *R. hesperia* (BB, CHIH,

PAC, SMS, SMSw, TVB) (6.3 Ma). During the Pliocene, three colonization events from SMS to other ancestral areas took place (Figure 4): to TVB (*R. taeniata* TVB) (4.3 Ma); to PAC (MRCA of *R. hesperia* (CHIH, PAC, TVB)) (4.2 Ma); and to BB (*R. hesperia* BB) (3.2 Ma). During the Pleistocene, a vicariant event is observed in the SMS between *R. myersi* and *R. omiltemana* (1.4 Ma) and five colonization events (Figure 4): three from SMS to SMSw (*R. hesperia* SMSw) (2.5 Ma), to VER (*R. decorata* VER) (0.9 Ma), and to CHIS (*R. decorata* CHIS) (0.6 Ma); from PAC to TVB involving the MRCA of *R. hesperia* (CHIH, TVB) (0.9 Ma); and from TVB to CHIH including *R. hesperia* CHIH (0.7 Ma).

## DISCUSSION

### Phylogenetic relationships within *Rhadinaea*

Our molecular-based phylogeny generally does not support the traditional taxonomy of *Rhadinaea* based on morphology (Myers, 1974, 2011). Our analyses place *Rhadinaea calligaster* as the sister group of *Rhadinella* (Figure 2, 3). This relationship is not surprising due to the complex morphology within *Rhadinaea* members (Myers, 1974), which makes it difficult to accurately diagnose the members of this genus due to the lack of exclusive diagnostic characters and the existence of variable morphological traits such as the form of the neck collar, pigmentation and longitudinal patterns of the dorsal scales (Myers, 1974; Sánchez-García, 2019), a situation that has caused a further modification of the scientific understanding of the composition of the genus (Myers, 2011; Palacios-Aguilar & García-

Vázquez, 2020). In this particular case, Myers (1974) described the relationships of this monospecific group as obscure and mentioned that morphological features like a bilobated hemipenis, absence of a subpreocular scale and a pale bar from the eye to the corner of the mouth bespeak an ancestry from the godmani group (now *Rhadinella*). This relationship is plausible, given our results. This situation leads us to infer that *Rhadinaea* is not monophyletic, in contrast with the results obtained by Palacios-Aguilar & García-Vázquez (2020). Whereas *Rhadinaea* and *Rhadinella* are recovered as reciprocally monophyletic in their study, here we only retrieve the monophyly of *Rhadinella*, a situation that is yet to be corroborated including a larger number of taxa.

The classification of the species of *Rhadinaea* into groups defined by Myers (1974; 2011) shows inconsistencies that involve the *decorata*, *flavilata* and *taeniata* groups. Our phylogenetic hypothesis showed the *decorata* and *taeniata* groups as polyphyletic and the *flavilata* group as paraphyletic (Figure 2, 3), indicating that the relationships among these species need a further revision due to the low nodal support in the clades containing these species and that they probably do not represent natural groups. Following these observations, we also suggest that the phylogenetic position of *R. calligaster* needs to be revisited to confirm the close relationship with *Rhadinella* observed in our analyses, taking into account the evidence that suggests that this group may represent another *Rhadinella* species.

We recovered the majority of the species with more than one sample as monophyletic with strong support values, except for *Rhadinaea marcellae* (Figure 2, 3). The species that did not show a monophyletic pattern are *R. montana* and *R. quinquelineata*, shown to be paraphyletic groups (Figure 2, 3). This pattern may be due to an unclear delimitation of these species, as previously suggested by Dixon et al. (2011) with respect to the previously proposed morphological series consisting of *R. gaigeae*, *R. montana* and *R. quinquelineata* (Dixon et al., 2011; Myers, 1974), species that show a very similar morphology and are co-distributed over the SMO (Canseco-Márquez et al., 2004; Medina-Romero et al., 2016; Myers, 1974). Due to this situation, we suggest that additional studies of these taxa that include better sampling and additional loci and explicit testing of alternative species hypotheses using coalescent methods for species delimitation (E. g. Yang & Rannala, 2010; Fujita et al., 2012) to address their status and relationships adequately.

Concerning the closest relatives of the genus, it has been shown that *Rhadinaea* is closely related to *Amastridium*, *Coniophanes*, *Rhadinella* and *Tantalophis* (Daza et al., 2010; Palacios-Aguilar & García-Vázquez, 2020; Pyron et al., 2013). Pyron et al. (2013) found a strong relationship between *Rhadinaea* and *Coniophanes*, and between both genera and *Rhadinella*, according to Palacios-Aguilar & García-Vázquez (2020). In our study, these last two relationships were recovered in the analyses, even though the relationship of *Coniophanes* as the sister group of *Rhadinaea* is not well supported in the BI analysis. We attribute this poorly supported relationship to the difference in our sampling, respecting the number of individuals of each genus. Still, there is more consistent evidence of this

kinship in other works that included a larger number of *Coniophanes* samples (Palacios-Aguilar & García-Vázquez, 2020). Regarding the relationship of *Rhadinophanes monticola*, in our phylogeny it is recovered as the sister group of *Rhadinella* + *Rhadinaea calligaster* with a low support value in the BI analysis. Due to the complex morphology of this species and uncertainty in its phylogenetic position (Myers & Campbell, 1981), we cannot assess whether this species is more closely related to *Rhadinella* or *Rhadinaea calligaster* due to the low support value in our analyses (Figure 2), in which we note that *Rhadinophanes monticola* is not closely related to the main clades of *Rhadinaea* we identify herein (Figure 2, 3). This situation may be clarified, considering a broader perspective, including another related colubrid species, as in other studies (E. g. Daza et al., 2010; Pyron et al., 2013).

### **Historical biogeography**

Based on our results, it appears that colubrid snakes of the genus *Rhadinaea* have had a relatively long history in North and Central America. The SMS is an extensive mountain system that has been present in the Mexican territory since its formation due to the Laramide geologic activity (DeMets & Stein 1990) and has presented significant geological changes during the Late Cretaceous to the Miocene (23- 100.5 Ma) (Nieto-Samaniego et al., 2006). This region is known to present a high biological diversity and number of endemisms (Escalante et al., 2002; Navarro-Sigüenza et al., 2009; Blancas-Calva et al., 2010) including amphibians and reptiles (Flores-Villela, 1993). About the MRCA of *Rhadinaea* and the first divergences of the main clades we herein identify inside the SMS during the Miocene (6.3 -

17.5 Ma) (Figure 4), we infer that these divergences can be due to the discontinuity of the pine-oak forests and cloud forests present in this biogeographical area to date (Rocha-Méndez et al., 2019; Santiago-Alvarado, Montaña-Arias & Espinosa, 2016), which has experienced changes during similar times (Ornelas et al., 2013; 2010). This mosaic-like landscape has been associated with centers of diversification along elevational gradients and is believed to be closely related to some divergences between some vertebrate taxa (e.g., *Eupherusa* [Rocha-Méndez et al., 2019]; *Chlorospingus* [García-Moreno et al., 2004], *Plestiodon* [Pavón-Vázquez et al., 2018], *Sarcohylla* [Caviedes-Solís & Leaché, 2018]) within the SMS, probably as a consequence of climatic changes during the Miocene that produced a long-term cooling interrupted by warm intervals, especially the middle Miocene Climatic Optimum (MCO) (17–14 Ma; Zachos et al., 2001). These divergences predate the diversification of all species inside the eastern and southern clades and points toward a close relationship between the habitat preferences of the majority of the species of *Rhadinaea* as a primarily montane genus associated with Pine-Oak and humid forests (Myers, 1974; García-Vázquez et al., 2019) and the heterogeneity of the SMS (Bryson et al., 2017; Luna-Vega et al., 1999; Santiago-Alvarado et al., 2016).

In this same period, two dispersal events from the SMS to other areas took place. First, we note inside the eastern clade a northward dispersal of the MRCA of *R. flavilata*, *R. gaigeae*, *R. montana* and *R. quinquelineata* (Figure 4), which became widespread during the middle Miocene. This event corresponds temporally with the first episode of volcanic and orogenic activity that originated the TVB in the western portion of Mexico (Bryson et al., 2012a;

2012b; Ferrusquía-Villafranca & González-Guzmán, 2005; Gómez-Tuena et al., 2007). This mountain system is known for its influence on the diversification of various montane taxa creating new montane habitats (Bryson et al., 2012b) and probably allowed further colonization of more mesic-adapted lineages (e.g. García-Vázquez et al., 2018a; Milstead, 1960) such as *R. flavilata*, in coordination with the low temperatures posterior to the MCO (Zachos et al., 2001). Secondly, we observe a dispersal event during the Miocene toward the south by *R. pulveriventris* (Figure 4). This divergence is generally consistent with the formation of the Chiapan-Guatemalan highlands of northern Central America, which formed during two different time intervals (Campbell, 1999). The uplift of the extensive northern Central American plateau occurred during the late Miocene to early Pliocene, from approximately 10–3.8 Ma (Rogers et al., 2002). Additionally, the formation in the late Pliocene of a younger chain of volcanoes along the western portion of the Central American plateau (Williams, 1960) had a significant impact on the local biota, both through extinction and resulting climatic change, creating cloud forest conditions on the windward (south) slopes and rain shadow conditions in the interior valleys (Campbell & Vannini, 1988; Campbell, 1999). In addition, an abrupt turnover from xeric, subhumid vegetation to humid forest occurs in south-central Chiapas and extends along the coast to south-central Guatemala (Campbell & Vannini, 1988; Campbell, 1999); these wetter conditions along the Pacific coast of southwestern Guatemala and southeastern Chiapas could be suitable for cooler-adapted lineages such as *Rhadinaea*. Even with this scenario as plausible, we cannot fully explain the invasion of *R. pulveriventris* toward the P-CH region nested in the Lower Central American Highlands as a single dispersal event, which is inhabited by other

unsampled species such as *R. sargenti* and *R. vermiculaticeps*, too. However, there is evidence of a similar colonization pattern in pit vipers (Castoe et al., 2009) where significant biogeographic barriers in Central America, such as the Motagua-Polotchic Fault (Marshall, 2007) and the Nicaraguan Depression (Rogers et al., 2002; Marshall, 2007), played an essential role in the diversification of several taxa (Campbell, 1999; Devitt, 2006; Parra-Olea et al., 2004; Perdices et al., 2005; Savage, 1982).

Along with these critical orogenic events, the Miocene climate change appears to have played an important role, sparking evolutionary radiations in some successful modern lineages, including colubrid snakes, segregating the species along latitudinal and altitudinal environmental gradients (Greene 1997; Van Devender 1995). These conditions probably have had influence in other colonization events during this time within the eastern and southern clades, such as the colonization of the SMO<sub>r</sub> by the MRCA of *R. gaigeae*, *R. montana* and *R. quinquelineata* (Figure 4) perhaps produced by changing ecosystems associated with the wetter climate during this period through the Northern Mexican highlands (Bryson et al., 2013; 2017; Retallack, 2001) as seen in other reptile groups (i.e. *Gerrhonotus* [García-Vázquez et al., 2018a]). Similarly, these changes could influence the dispersal toward the TVB of the MRCA of *R. laureata* TVB and *R. laureata* SMO<sub>c</sub>, and the MRCA of *R. marcellae* and *R. cf. marcellae* of the eastern clade; and *R. cf. taeniata* of the southern clade (Figure 4); this last hypothetical species is only known from the westernmost portion of the TVB in Sierra San Juan, a too complex area with a high number of endemisms (Escalante & Llorente, 1985; Miranda & Luna-Vega, 2006).



During the Pliocene, the last episode of formation of the TVB had a role (3-5 Ma) (Gómez-Tuena et al., 2007) in creating a highland that bisected the southern continuity of the SMOr (Espinosa et al., 2008) and created a complex area of highlands between the Sierra de Juarez, eastern TVB, and southern SMOr. Along with this physiographic change, a series of climatic fluctuations occurred during the Pliocene and Pleistocene (Ca. 0.1-4 Ma) (Paillard, 2017; Vanzolini, 1970); these cool intervals are believed to have caused the expansion of some Pine, Pine-Oak and humid forests to lower elevations due to temperature fluctuations, provoking an extension of the Mexican montane flora to lower elevations of at least 1000 m (McDonald, 1993; Jaramillo-Correa et al., 2009; Ornelas et al., 2013; Sosdian & Rosenthal, 2009). These orogenic and climatic factors in more recent conjunction, are considered the processes that have had a more significant impact in a high number of montane taxa (as discussed in Bryson et al., 2012b). Throughout these Pliocene and Pleistocene periods, most extant species of *Rhadinaea* among the eastern and southern clades originated and colonized other regions.

Inside the MTZ, the contact between the SMOc, SMOr, SMS, TVB and VER regions are characterized by a very complex biotic interchange, presenting a significant amount of shared floristic and faunistic elements (Espinosa et al., 2004; Marshall & Liebherr, 2000), which some of these show a recent dispersal to adjacent provinces during the Pliocene and Pleistocene (i.e. Cavender-Bares et al., 2011). In this sense, we can attribute the several colonization events inside the MTZ involving the eastern species of *R. gaigeae*, *R. forbesi*, *R.*

*laureata*, *R. marcellae*, *R. cf. marcellae*, *R. montana* and *R. quinquelineata* through some hypothesized filter barriers (Anducho-Reyes et al., 2008; Bryson et al., 2011b; Morafka, 1977), such as in other co-distributed taxa (i.e. *Hyla* [Bryson et al., 2010]; *Phrynosoma* [Bryson et al., 2012a]; *Sceloporus* [Bryson et al., 2012b]; *Gerrhonotus* [García-Vázquez et al., 2018a]; gymnosperms, angiosperms and pteridophytes [Luna-Vega et al., 1999]; *Bufo* [Mulcahy et al., 2006]). For the southern species, this colonization between biogeographical regions inside the MTZ is also present in *R. hesperia*, *R. taeniata*, *R. myersi*, *R. omiltemana* between the BB, CHIH, CHIS, PAC, TVB, VER and the eastern and western portions of the SMS; these dispersal patterns are observed in other taxa with similar distribution (e.g. *Hyla* [Bryson et al., 2010]; *Pituophis* [Bryson et al., 2011a]; *Sceloporus* [Bryson et al., 2012b]; *Sarcohylla* [Caviedes-Solís & Leaché, 2018]; *Buarremon* [Navarro-Sigüenza et al., 2008]) and are believed to be driven by the aforementioned geologic and climatic factors; about the colonization events by *R. decorata*, we observe a similar southward pattern as in other snake taxa (*Atropoides* and *Cerrophidion* [Castoe et al., 2009]); we consider this final clade is incomplete due to our sampling respect to the wide distribution of *R. decorata*; as such, the resolution of the biogeographic patterns also remains incomplete; this same problem is present within other widely-distributed species of *Rhadinaea* such as *R. taeniata* and *R. hesperia*.

Finally, regarding the vicariant events involving some of the species present in both eastern and southern clades, we attribute these divergences to distinct processes such as soft allopatry through ecological vicariance (Pyron & Burbrink, 2010), given non-identical

lineage ranges of *R. gaigeae* and *R. quinquelineata* inside the SMO and the action of lowland barriers inside these biogeographical regions such as river drainages of the Río Grande and the Río Santa Catarina (Pavón-Vázquez, et al., 2018) in the case of the divergence between *R. myersi* and *R. omiltemana* inside the SMS. These analyses are enlightening about the geographic origin and timing of most of the *Rhadinaea* species divergences and point toward an origin of the genus more related to the woodland dynamics inside the SMS than another proposed biogeographical barrier present in southeastern Mexico such as the IT and ND.

## **CONCLUSIONS**

Biogeographical studies seek to explain the distribution of species in terms of historical factors and climatic phenomena (García-Vázquez et al., 2018a). The genus *Rhadinaea* has shown 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) and Miocene and Pliocene geomorphology in conjunction with climate change appears to have induced allopatric divergence on a relatively small spatial scale in this genus, and points toward a complex origin inside the heterogeneous area of the SMS and several diversification events among the TVB and adjacent provinces, providing an insight into the historical processes responsible for the diversification in this complex system. Our result also shows the necessity of further systematic exploration of the genus, where the morphological

characters used may not be sufficient to reconstruct the evolutionary history of *Rhadinaea*. On this issue, an integrative perspective using molecular and morphological data, taking into historical account information of the species, may illuminate the path for the systematics and evolution of these poorly known colubrid snakes.

#### DATA AVAILABILITY STATEMENT

All sequence data generated in this study will be available in the public genetic sequence database GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

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### **Biosketch**

The shared interests of the authors are the biogeographic context of Mexico and biogeographic patterns present in America. U.A.G.S. and U.O.G.V research interest are herpetofauna diversity, Molecular systematics, colubrid and anguid systematics and NGS. Author contributions: U.A.G.S., U.O.G.V. and D.N.E.O. conceived the ideas; U.A.G.S. and U.O.G.V. collected and analyzed the data; U.A.G.S, U.O.G.V and D.N.E.O. led the writing.

## **MATERIAL SUPLEMENTARIO**

### **APPENDIX S1. Sampling DNA**

Collection and voucher data for *Rhadinaea* genetic samples used in this study. Institutional codes for museum and collections follow Sabaj (2016). AEVB, AMH, ANMO, CIG, DGM, ENS, GK, ISZ, JAC, JCBH, JCSG, JRM, MXC, OFV, OORH, RICB, RSB and UOGV are field identifiers for uncatalogued specimens being introduced in the MZFC. The X's present in the GeneBank accession codes represent pending submissions of sequences, the Em dash represents absent information. Alleghanian Region (ALLE); Balsas Basin (BB); Chiapas (CHIS); Chihuahuan Plateau (CHIH); Gatuso-Talamanca (G-T); Pacific Lowlands (PAC); Puntarenas-Chiriquí (P-CH); Sierra Madre Occidental (SMOc); Sierra Madre Oriental (SMOr); Sierra Madre del Sur (SMS); western Sierra Madre del Sur (SMS-W); Trans-Mexican Volcanic Belt (TVB) and Veracruz (VER).

Taxa	Sampled area according to this study	Locality	Voucher Number	sequence's Genbank accession number				Sample ID
				cmos	cytb	DNAH3	ND4	
<i>Coniophanes imperialis</i>	PAC	Mexico: Oaxaca: La Cofradía	MZFC-HE15533	XXXXXX	MT308778	XXXXXX	XXXXXX	1
<i>Rhadinaea bogertorum</i>	SMS	Mexico: Oaxaca: Totontepec	CIG 739	XXXXXX	XXXXXX	XXXXXX	XXXXXX	2
<i>Rhadinaea calligaster</i>	G-T	Costa Rica: Heredia: Volcan Barva	GK 2462	XXXXXX	XXXXXX	XXXXXX	XXXXXX	3
<i>Rhadinaea calligaster</i>	P-CH	Costa Rica: Cartago: Turrialba	ENS R calligaster	XXXXXX	XXXXXX	XXXXXX	XXXXXX	4
<i>Rhadinaea cf marcellae</i>	TVB	Mexico: Puebla: Xocayucan	ANMO 2109	XXXXXX	XXXXXX	XXXXXX	XXXXXX	5
<i>Rhadinaea cf taeniata</i>	TVB	Mexico: Nayarit: Cerro San Juan	UOGV 3664	XXXXXX	XXXXXX	XXXXXX	XXXXXX	6
<i>Rhadinaea cuneata</i>	SMS	Mexico: Veracruz: Aticpac	DGM 52	XXXXXX	XXXXXX	XXXXXX	XXXXXX	7
<i>Rhadinaea decorata</i>	SMS	Mexico: Oaxaca: Eloxochitlán de Flores Magón	OFV 1109	XXXXXX	XXXXXX	XXXXXX	XXXXXX	8
<i>Rhadinaea decorata</i>	CHIS	Guatemala: Huehuetenango: Barillas	UTAR44718	XXXXXX	MT308780	XXXXXX	XXXXXX	9
<i>Rhadinaea decorata</i>	VER	Mexico: Veracruz: Otontepec	JCSG 58	XXXXXX	MT308781	XXXXXX	XXXXXX	10
<i>Rhadinaea flavilata</i>	ALLE	USA: Florida: Laural	CAS: HERP 198634	AF471152	AF471078	—	—	11
<i>Rhadinaea flavilata</i>	ALLE	USA: North Carolina: Southport	MVZ HERP 164867	XXXXXX	XXXXXX	XXXXXX	XXXXXX	12
<i>Rhadinaea forbesi</i>	SMS	Mexico: Veracruz: Finca Santa Martha	UOGV 3068	XXXXXX	XXXXXX	XXXXXX	XXXXXX	13
<i>Rhadinaea forbesi</i>	TVB	Mexico: Veracruz: Banderillo	JRM 3800	XXXXXX	XXXXXX	XXXXXX	XXXXXX	14
<i>Rhadinaea fulvivittis</i>	SMS	Mexico: Oaxaca: San Pablo Macuilianguis	ANMO 2388	XXXXXX	XXXXXX	XXXXXX	XXXXXX	15
<i>Rhadinaea gaigeae</i>	SMOr	Mexico: San Luis Potosí: Cerro el Cabezón	RICB 340	XXXXXX	XXXXXX	XXXXXX	XXXXXX	16
<i>Rhadinaea hesperia</i>	BB	Mexico: Guerrero: Olinalá	ANMO 3631	XXXXXX	XXXXXX	XXXXXX	XXXXXX	17
<i>Rhadinaea hesperia</i>	CHIH	Mexico: Jalisco: La Barca	ISZ 531	XXXXXX	XXXXXX	XXXXXX	XXXXXX	18
<i>Rhadinaea hesperia</i>	TVB	Mexico: Nayarit: Cerro San Juan	UOGV 2910	XXXXXX	XXXXXX	XXXXXX	XXXXXX	19
<i>Rhadinaea hesperia</i>	SMS	Mexico: Guerrero: Malinaltepec	OORH 55	XXXXXX	XXXXXX	XXXXXX	XXXXXX	20
<i>Rhadinaea hesperia</i>	PAC	Mexico: Nayarit: Bahía de Banderas	RICB 556	XXXXXX	XXXXXX	XXXXXX	XXXXXX	21
<i>Rhadinaea hesperia</i>	SMS-W	Mexico: Michoacán: Coalcomán de Vázquez Pallares	AMH 698	XXXXXX	XXXXXX	XXXXXX	XXXXXX	22
<i>Rhadinaea laureata</i>	TVB	Mexico: Morelos: Huitzilac	MZFC-HE21661	XXXXXX	MT308785	XXXXXX	XXXXXX	23

<i>Rhadinaea laureata</i>	SMS	Mexico: Jalisco: Chilacayote	CIG 503	XXXXXX	XXXXXX	XXXXXX	XXXXXX	24
<i>Rhadinaea laureata</i>	SMOc	Mexico: Durango: Alemán	CIG 1556	XXXXXX	XXXXXX	XXXXXX	XXXXXX	25
<i>Rhadinaea macdougalli</i>	SMS	Mexico: Oaxaca: Totontepec	UOGV 2091	XXXXXX	XXXXXX	XXXXXX	XXXXXX	26
<i>Rhadinaea marcellae</i>	VER	Mexico: Veracruz: Rancho San Nicolasillo	JCSG 22	XXXXXX	XXXXXX	XXXXXX	XXXXXX	27
<i>Rhadinaea marcellae</i>	SMOr	Mexico: San Luis Potosí: Xilitla	ANMO 4339	XXXXXX	XXXXXX	XXXXXX	XXXXXX	28
<i>Rhadinaea montana</i>	SMOr	Mexico: Tamaulipas: Gómez Farias	CIG 605	XXXXXX	XXXXXX	XXXXXX	XXXXXX	29
<i>Rhadinaea montana</i>	VER	Mexico: Tamaulipas: Ciudad Victoria	AEVB 401	XXXXXX	XXXXXX	XXXXXX	XXXXXX	30
<i>Rhadinaea myersi</i>	SMS	Mexico: Guerrero: Metlaltónoc	ANMO 3887	XXXXXX	XXXXXX	XXXXXX	XXXXXX	31
<i>Rhadinaea nuchalis</i>	SMS	Mexico: Guerrero: Atoyac de Álvarez	MZFC-HE 22161	XXXXXX	XXXXXX	XXXXXX	XXXXXX	32
<i>Rhadinaea omiltemana</i>	SMS	Mexico: Guerrero: Omiltemi	RSB 25	XXXXXX	XXXXXX	XXXXXX	XXXXXX	33
<i>Rhadinaea pulveriventris</i>	P-CH	Costa Rica: Cartago: Río Grande Orosí	MVZ HERP 204129	XXXXXX	XXXXXX	XXXXXX	XXXXXX	34
<i>Rhadinaea quinquelineata</i>	SMOr	Mexico: Puebla: Zacatlán	UOGV 3266	—	XXXXXX	XXXXXX	XXXXXX	35
<i>Rhadinaea quinquelineata</i>	TVB	Mexico: Puebla: Hueyapan	MXC SN QUIN	—	XXXXXX	XXXXXX	XXXXXX	36
<i>Rhadinaea taeniata</i>	TVB	Mexico: State of Mexico: Valle de Bravo	UOGV 2181	XXXXXX	MT308787	XXXXXX	XXXXXX	37
<i>Rhadinaea taeniata</i>	SMS	Mexico: Oaxaca: Santa María Yavesía	MZFC-HE23859	XXXXXX	MT308788	XXXXXX	XXXXXX	38
<i>Rhadinella godmani</i>	CHIS	Guatemala: Quiché: San Juan Cotzal	ENS 7810	XXXXXX	XXXXXX	XXXXXX	XXXXXX	39
<i>Rhadinella hempsteadae</i>	CHIS	Guatemala: Quiché: Uspantán	UTAR42470	XXXXXX	MT308783	XXXXXX	XXXXXX	40
<i>Rhadinella lachrymans</i>	CHIS	Guatemala: San Marcos: San Rafael Pie de la Cuesta	UTAR42335	XXXXXX	MT308784	XXXXXX	XXXXXX	41
<i>Rhadinophanes monticola</i>	SMS	Mexico: Guerrero: Puerto del Gallo	JAC 29554	XXXXXX	XXXXXX	XXXXXX	XXXXXX	42
<i>Achrochordus granulatus</i>	—	—	NUM-Az 0375	—	NC_007400	—	NC_007400	
<i>Atheris chlorechis</i>	—	—	PEM R5297	AY611921	AY612012	—	—	
<i>Clelia clelia</i>	—	—	MHNSM 7380	GQ895803	GQ895859	—	—	
<i>Coluber constrictor</i>	—	—	CAS:HERP 236039	MF402211	MF402798	MF402397	—	
<i>Natrix Natrix</i>	—	—	MTD T 11570	—	LL999946	—	LL999896	

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## APPENDIX S2. Technical Details on DNA Sequencing and Sequence Edition

**DNA extraction, PCR, sequencing and sequence edit.** Total genomic DNA was extracted from liver, muscle, or shed skins following the main protocol of ammonium acetate by Fetzner (1999) or using the DNeasy Blood & Tissue Kit (QIAGEN). All gene regions were amplified via polymerase chain reaction (PCR) in a 25 µl reaction volume containing 0.5–1.0 µl deoxynucleoside triphosphates (dNTPs; 2–10 mM) (ThermoFisher Scientific, NV, USA), 18–19.25 µl double-distilled water, 0.2–0.5 µl each primer (2–10 mM), 2.5 µl 1 X PCR buffer, 1.2 mM MgCl<sub>2</sub> (ThermoFisher Scientific), 0.15 µl Taq DNA polymerase (ThermoFisher Scientific), and 1.0–1.5 µl template DNA. For *cmos* and *DNAH3*, DNA was denatured at 94°C for 3 min, followed by 35 cycles of: 94 °C for 45 s, 48–55 °C for 45 s, and 72 °C for 1 m. A final extension phase of 72 °C for 6 min terminated the protocol (Saint et al., 1998). For *cytb*, DNA was denatured at 94°C for 7 min, followed by 40 cycles of: 94 °C for 30 s, 46 °C for 30 s, and 72 °C for 1 m. A final extension phase of 72 °C for 7 min terminated the protocol (Burbrink et al., 2000). For *ND4*, DNA was denatured at 94 °C for 3 min, followed by 35 cycles of 93 °C for 30 s, 50 °C for 1 m, and 72 °C for 1 m. A final

extension phase of 72 °C for 7 min terminated the protocol (Forstner et al., 1995). The primers used for amplification are presented in the following table.

Used primers in the present study to amplify *cmos*, *cytb*, *DNAH3* and *ND4* genes for *Rhadinaea*. \* Primers used for sequencing.

Gene	Primer name	Primer sequence	Reference
Cyt-b	L14919*	5'-AAC CAC CGT TGT TAT TCA ACT-3'	Burbrink et al., 2000
	L15584-Z*	5'-CCA TTY CAC CCM TAY CAC TC-3'	De Queiroz et al., 2002
	H15716-Z	5'-GGT TTY ATG TGR TGT GGT GTT AC-3'	Slowinski & Lawson, 2002
	H16064*	5'-CCT TGG TTT ACA AGA ACA ATG CTT TA-3'	Burbrink et al., 2000
ND4 & tRNA's	ND4*	5'-TGA CTA CCA AAA GCTC ATG TAG AAG C-3'	Forstner et al., 1995
	Leu*	5'TRC TTT TAC TTG GAT TTG CAC CA-3'	Forstner et al., 1995
	GerrIntFwd*	5'-AAY ACT AAC TAY GAA CGA AC-3'	The present study
	GerrIntRev	5'-TGT GTT GGR AGT TTT CCT CGT TG-3'	The present study
	RhaintRev*	5'- CCT GTG AAG TTT ATR CTK GG-3'	The present study
DNAH3	DNAH3-f1	5'-GGT AAA ATG ATA GAA GAY TAC TG-3'	Townsend et al., 2008
	DNAH3-r6*	5'-CTK GAG TTR GAH ACA ATK ATG CCA T-3'	Townsend et al., 2008
C-mos	S77*	5'-CAT GGA CTG GGA TCA GTT ATG-3'	Lawson et al., 2005
	G74	5'-TGA GCA TCC AAA GTC TCC AAT C-3'	Saint et al. 1998

Double-stranded PCR 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.), as well as an ABI 3100 automated DNA



sequencer (Applied Biosystems, Inc.) by Macrogen Korea Inc., using the amplification primers (see table above). Sequences were assembled and edited in the software Sequencher 3.1.1 (Nishimura, 2000), edited sequences were aligned per gene in MEGA7 (Kumar et al., 2016), using Muscle algorithm (Edgar, 2004), and a concatenated matrix was created later using Mesquite 3.61 (Maddison & Maddison, 2019).

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### **APPENDIX S3. Technical Details on Fossils Utilized for the Divergence Time Estimates**

#### **Fossils Included for the Divergence Time Estimates**

(1) *Procerophis sahnii* (Rage et al., 2008) 50.5-93.9 Ma (Ypresian to Cenomanian), to calibrate the most recent common ancestor (MRCA) of Pan-Colubroidea (*Achrochordus* + *Xenodermatidae*) as discussed in Head et al. (2016), which was defined under a lognormal distribution with an offset value of 50.5, a mean of 2.48 and a standard deviation of 1.0, resulting in a distribution with a mean of 62.4 Ma and a 95% prior credible interval from the Ypresian at 52.8 Ma to Albian at 112 Ma.

(2) Colubrid Indet. (Smith, 2013) 35.2-54 Ma (Rupelian to Ypresian), to calibrate the MRCA of Colubroidea as discussed in Zaher et al. (2019), which was defined under a lognormal distribution with an offset value of 33.9, a mean of 2.8 and a standard deviation of 0.8, resulting in a distribution with a mean of 50.3 Ma and a 95% prior credible interval from the Priabonian at 34.7 Ma to the Cenomanian at 95.2 Ma.

(3) *Paleoheterodon tihenii* (Holman, 1977) 12.5-54 Ma (Serravallian to Ypresian), to calibrate the MRCA of Dipsadidae as discussed in Zaher et al. (2019), which was defined under a lognormal distribution with an offset value of 12.5, a mean of 2.2 and a standard deviation of 1.0, resulting in a distribution with a mean of 21.5 Ma and a 95% prior credible interval from Langhian at 14.2 Ma to Selandian at 59.3 Ma.

(4) Dipsadinid Indet. (Camolez and Zaher, 2010; Onary et al., 2017) 0.129-2.58 Ma (Chibanian to Gelasian), to calibrate the MRCA of Dipsadinae, which was defined under a lognormal distribution with an offset value of 0.129, a mean of 1.5 and a standard deviation of 1.0, which resulted in a distribution with a mean of 4.61 Ma and a 95% prior credible interval from Calabrian at 0.99 Ma to Chattian at 23.3 Ma.

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## DISCUSIÓN GENERAL

En este estudio, los resultados que se obtuvieron dan pie a una serie de preguntas sobre la clasificación de *Rhadinaea* debido a que nuestra filogenia basada en caracteres moleculares, de forma general no soporta las relaciones filogenéticas tradicionales dentro del género con base a su morfología (Myers, 1974, 2011). En primer lugar, nuestros análisis muestran a la especie *Rhadinaea calligaster* como el grupo hermano del género *Rhadinella*, un género que fue resucitado por Myers (2011) debido a un arreglo del grupo godmani que formaba parte del *Rhadinaea*; al respecto, Myers (1974) señala las relaciones del grupo calligaster respecto a otros grupos como “obscuras”, y menciona que características morfológicas como el hemipene y la coloración sugerían una relación con el grupo godmani, una relación que es congruente con la filogenia molecular. Dado lo anterior, la monofilia del género no se recupera en ninguna reconstrucción efectuada con nuestros datos, en contraste con las relaciones obtenidas por Palacios-Aguilar y García-Vázquez (2020), en donde se recupera la monofilia recíproca de los géneros *Rhadinaea* y *Rhadinella*; asimismo, los grupos morfológicos definidos por Myers (1974; 2011) los cuales subdividen a *Rhadinaea* en cinco subgrupos con base en características como la coloración y distribución tampoco se recuperan. Finalmente es posible que dentro del género existan problemas intraespecíficos, como es propuesto por Dixon *et al.*, 2011 respecto a la serie *R. gaigeae-R. montana-R. quinquelineata*.

Respecto a las relaciones intergenéricas del grupo, la mayoría de las especies muestreadas en este estudio se agruparon consistentemente en dos clados fuertemente soportados (Figure 2, 3) y se recuperó al género *Coniophanes* como su grupo hermano, esta relación se ha recuperado en otros estudios filogenéticos (i. e. Bailey, 1939; Cadle, 1984; Daza et al., 2010; Palacios-Aguilar & García-Vázquez, 2020; Pyron et al., 2013). Asimismo, *Rhadinaea* + *Coniophanes* se recuperan como grupo hermano de *Rhadinaea calligaster* + *Rhadinella* + *Rhadinophanes*, lo cual provee información sobre las relaciones que guardan estos géneros de culebras, particularmente de *Rhadinophanes*, ya que ha sido considerado por algunos autores como el grupo hermano morfológico de *Rhadinaea* y *Coniophanes* (Myers y Campbell, 1981) y en este trabajo se observa más estrechamente relacionado a *Rhadinella* y *Rhadinaea calligaster* (Figure 2, 3). Estas relaciones recuperadas en conjunto sugieren que es una necesaria revisión completa del género incluyendo representantes de otros géneros mediante un muestreo más amplio y comprensivo utilizando datos morfológicos y moleculares bajo una perspectiva integrativa, esta revisión potencialmente puede arrojar información valiosa sobre el número de especies que integran al género y sus relaciones, poniendo a prueba incluso la identidad del género, como ha sido de utilidad en otros grupos (i.e. Fujita et al., 2012; Yang y Rannala, 2010).

Otro elemento importante en el proceso de clasificación y delimitación de grupos biológicos es la perspectiva histórica (Sanmartín, 2012), la cual es posible abordar integrando la datación molecular y correlacionando los eventos de diversificación con eventos geológicos y climáticos (Sullivan et al., 2000). Perspectiva bajo la cual fue desarrollada el presente



trabajo e intenta dilucidar el origen y procesos que condicionaron la diversificación de *Rhadinaea*. Respecto al análisis biogeográfico efectuado se observó que el origen del género es rastreable dentro del área ancestral de la Sierra Madre del Sur (SMS) durante el Mioceno temprano (17.5 ma). Posteriormente se observan divergencias dentro de la SMS dentro de los clados Este y Sur, las cuales pudieron ser causadas por las condiciones climáticas durante este periodo, que ocasionaron un enfriamiento constante a nivel global interrumpido por intervalos cálidos como el óptimo climático del Mioceno (OCM) (14-17 ma; Zachos *et al.*, 2001) en conjunto con la notable heterogeneidad del paisaje y vegetación de la SMS, la cual ha cambiado a lo largo del tiempo y ha afectado a una variedad de taxones asociados con hábitats similares (E. g. Caviedes-Solís & Leaché, 2018; García-Moreno *et al.*, 2004; Pavón-Vázquez *et al.*, 2018; Rocha-Méndez *et al.*, 2019).

Dentro del clado Este durante el Mioceno se observa una colonización hacia el norte por parte de los ancestros de *R. flavilata*, *R. gaigeae*, *R. montana* y *R. quinquelineata* (Figure 4) hacia las áreas de la provincia Alleganiense, la Faja Volcánica Transmexicana (FVT) y la Sierra Madre Oriental (SMOr), probablemente derivado de la primera etapa de formación de la FVT (Ferrusquía-Villafranca y González-Guzmán, 2005; Gómez-Tuena *et al.*, 2007) evento el cual propició la diversificación de varios taxones montanos (Bryson *et al.*, 2012b). Posteriormente, durante el Plioceno y Pleistoceno se observan divergencias que involucran la colonización de las regiones de la FVT, Sierra Madre Occidental (SMOc) y plataforma Veracruzana (VER), las cuales pueden haber sido derivadas de la acción de barreras filtrantes (Morafka, 1977) como la depresión del río Pánuco (Andaicho-Reyes *et al.*, 2008),

Cerritos-Arista y Saladan (Bryson *et al.*, 2011) en el caso de los ancestros de *R. gaigeae*, *R. montana* y *R. quinquelineata* en conjunto con las fluctuaciones climáticas del Pleistoceno (Vanzolini, 1970), las cuales afectaron la distribución de los bosques de pino y pino-encino, creando divergencias en varios taxones asociados a éstos hábitats (Bryson *et al.* 2012a; 2012b; García-Vázquez *et al.*, 2018a); de una forma similar, en conjunto con el último periodo de formación de la FVT (Ferrusquía-Villafranca y González-Guzmán, 2005) se relacionan las divergencias que involucran a los ancestros de *R. forbesi*, *R. laureata*, *R. marcellae* y *R. cf. marcellae* asociadas a las áreas antes mencionadas en tiempos similares (Figure 4).

Dentro del clado Sur adicional a las divergencias observadas dentro de la SMS durante el Mioceno que involucran a los ancestros de *R. bogertorum*, *R. fulvivittis*, *R. hesperia*, *R. myersi*, *R. nuchalis*, *R. omiltemana*, *R. taeniata* y *R. cf. taeniata* se observa una colonización hacia el sur por parte de *R. pulveriventris*, hacia la provincia Puntarenas-Chiriquí, la cual coincide temporalmente con el levantamiento de las tierras altas de Chiapas y Guatemala (Campbell, 1999), evento que afectó de forma similar a otros taxones (E.g. Campbell, 1999; Castoe *et al.*, 2009; Devitt, 2006; Perdices *et al.*, 2005; Savage, 1982). Posteriormente durante el Plioceno y Pleistoceno se observan divergencias que involucran la colonización de las regiones de Chiapas (CHIS), costa del Pacífico (PAC), depresión del Balsas (BB), desierto Chihuahuense (CHIH), FVT, y oeste de la SMS, las cuales pudieron haber sido ser promovidas por la actividad volcánica de la época en el caso de *R. taeniata* y las fluctuaciones Pleistocénicas en el caso de *R. hesperia* y *R. decorata*, de forma similar a otros

taxones con distribuciones coincidentes (E. g. Bryson *et al.*, 2010; 2011; 2012b; Castoe *et al.*, 2009; Caviedes-Solís y Leaché, 2018; Navarro-Sigüenza *et al.*, 2008); así como una divergencia dentro de la SMS entre *R. myersi* y *R. omiltemana*, probablemente ocasionada por barreras de tierras bajas como las cuencas de los ríos Grande y Santa Catarina, los cuales han afectado a otros taxones de forma similar (E.g. Pavón-Vázquez *et al.*, 2018). Finalmente consideramos que éste clado Sur se encuentra incompleto debido a la representatividad en nuestro muestreo de especies de distribución amplia como *R. decorata*, sin embargo, el análisis en general con las muestras incluidas para ambos clados resulta enriquecedor respecto al origen geográfico y tiempo de divergencia de la mayoría de las especies de *Rhadinaea*, el cual apunta a una estrecha relación con la dinámica y conformación de la SMS, más que con otras barreras biogeográficas propuestas presentes a lo largo de la distribución del género como el Istmo de Tehuantepec y la depresión Nicaragüense.

## CONCLUSIONES

Los estudios biogeográficos buscan explicar la distribución de las especies en términos de factores históricos y fenómenos climáticos (García-Vázquez *et al.*, 2018a). El género *Rhadinaea* ha mostrado ser un modelo informativo para estudiar estos factores en un grupo ampliamente distribuido. Las oscilaciones climáticas durante el Pleistoceno, un factor clave en la diversificación entre linajes (León-Paniagua *et al.*, 2007) y la geomorfología del Mioceno y Plioceno en conjunto con el cambio climático, parecen haber inducido divergencias alopátricas en un espacio relativamente pequeño en este género, y apunta

hacia un complejo origen dentro de la extremadamente heterogénea área de la SMS y varios eventos entre la FVT y provincias adyacentes, aportando información sobre los procesos históricos responsables de la diversificación en estos complejos sistemas montañosos. Nuestros resultados muestran la necesidad de una exploración sistemática de las relaciones del género, donde los caracteres morfológicos usados para su clasificación pueden no aportar la suficiente información para identificar un ensamblaje natural del *Rhadinaea*, una interrogante donde una perspectiva integrativa usando datos morfológicos y moleculares, tomando en cuenta la información histórica de las especies puede ser efectivo para dilucidar la sistemática y evolución de éstas culebras poco conocidas.

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