

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

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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

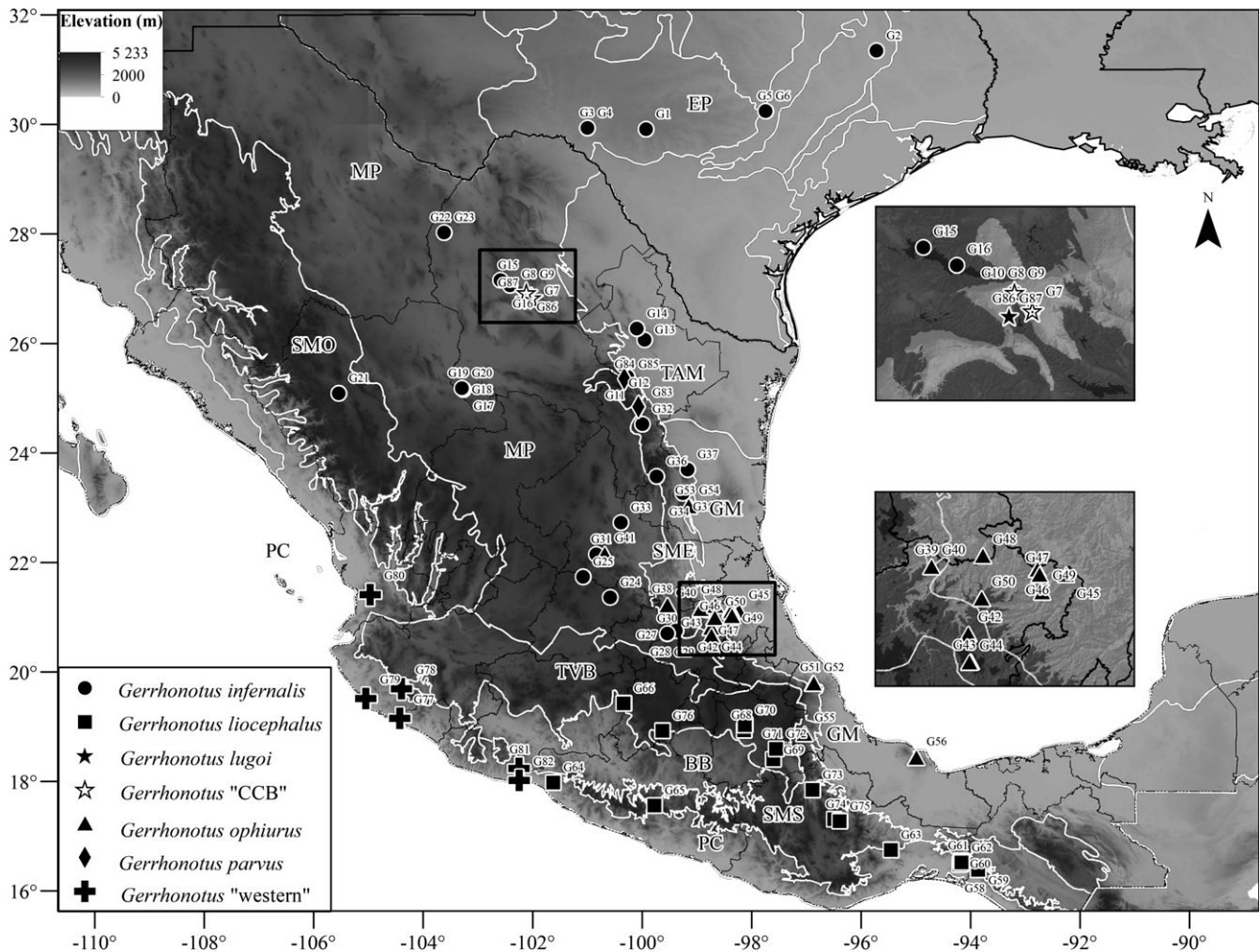
Inferring the evolutionary history of the groups in a particular region is the first step to elucidate the processes by which the fauna of that region originated (Colston et al., 2013). Multiple phylogeographical studies of species with broad distributions in the Mexican Transition Zone (MTZ) provide an illustrative case (e.g. Bryson, Pastorini, Burbrink, & Forstner, 2007; León-Paniagua, Navarro-Sigüenza, Hernández-Baños, & 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. lazcano* 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. lazcano* 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. lazcanoii*, 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 neurotrophic 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 “nrns = 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  $\geq 0.95$  and their bootstrap value was  $\geq 70\%$  (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 (Pyrón, 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 Anguils and related 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 *Bariasia* (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).

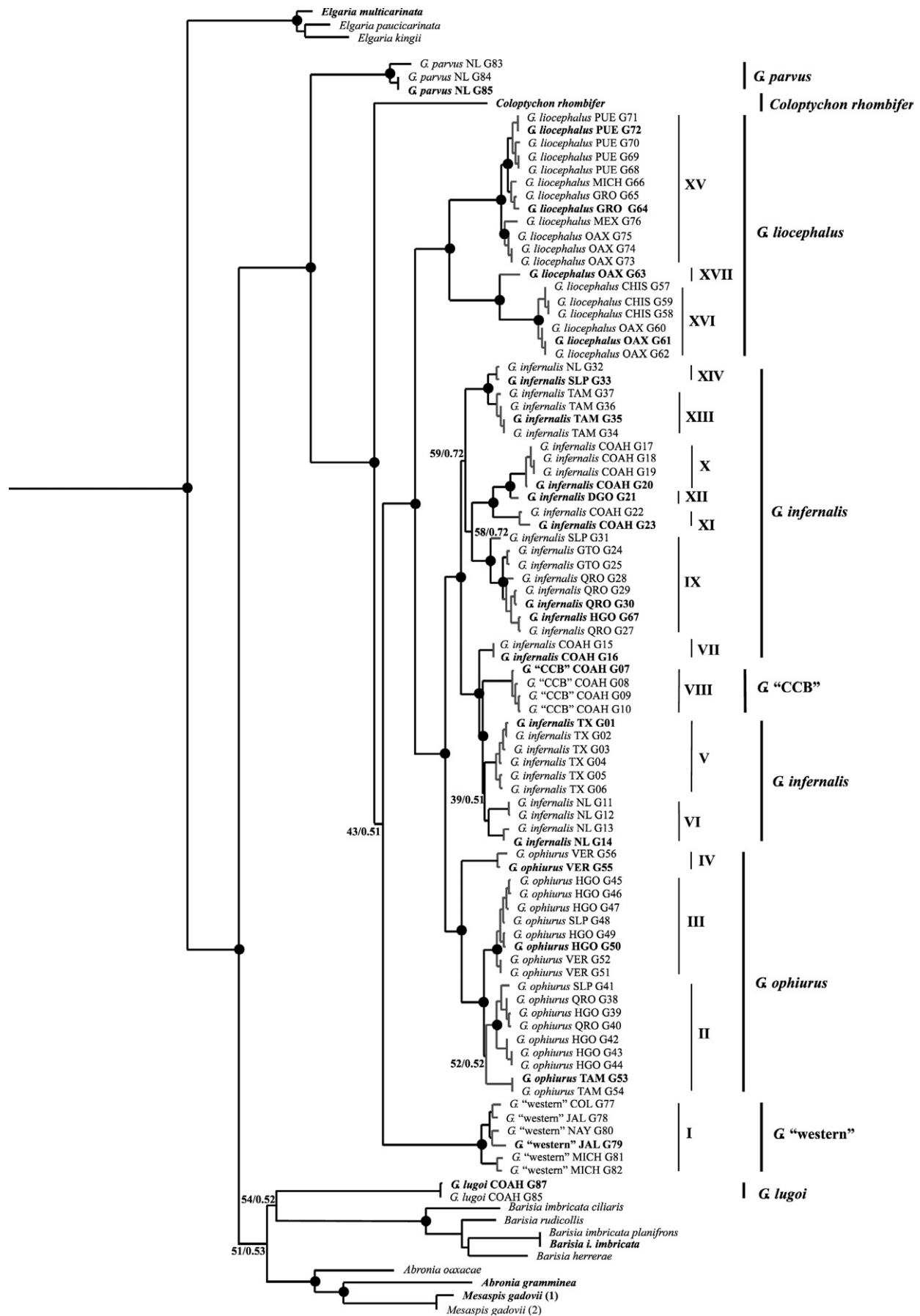
#### 3.2 | Phylogeographical estimation

*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

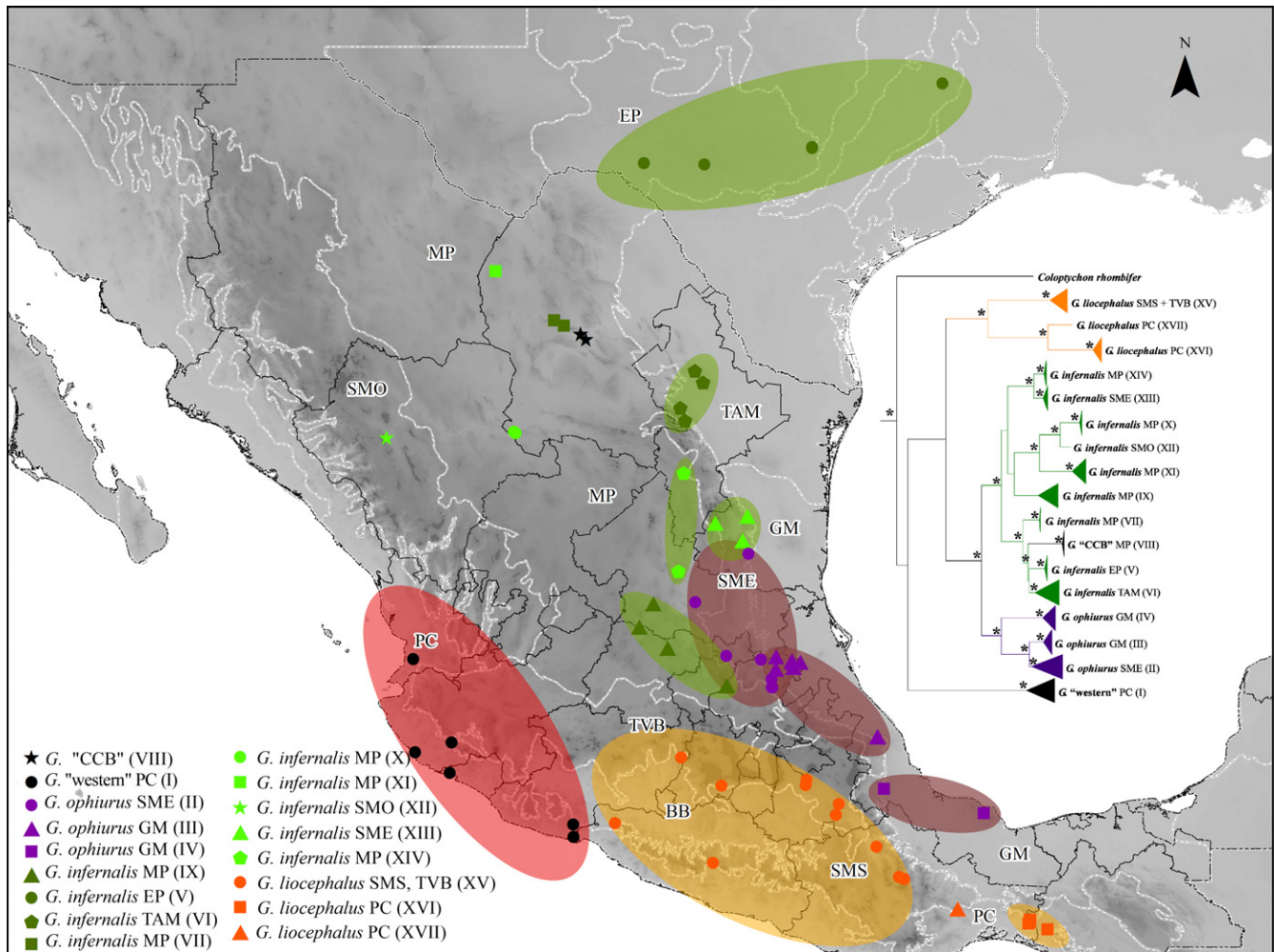
independent clusters by the PTP model, were considered as a single lineage because these clusters comprised a strongly supported, morphologically homogeneous clade and were distributed in the same biogeographical regions, yet were not segregated by region (i.e. two of the clusters were distributed both 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,



**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  $\geq 70$ ; Bayesian posterior probability value  $\geq 0.95$ ). Numbers at other nodes are bootstrap/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  $\geq 70$  and/or Bayesian posterior probability value  $\geq 0.95$ ). 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

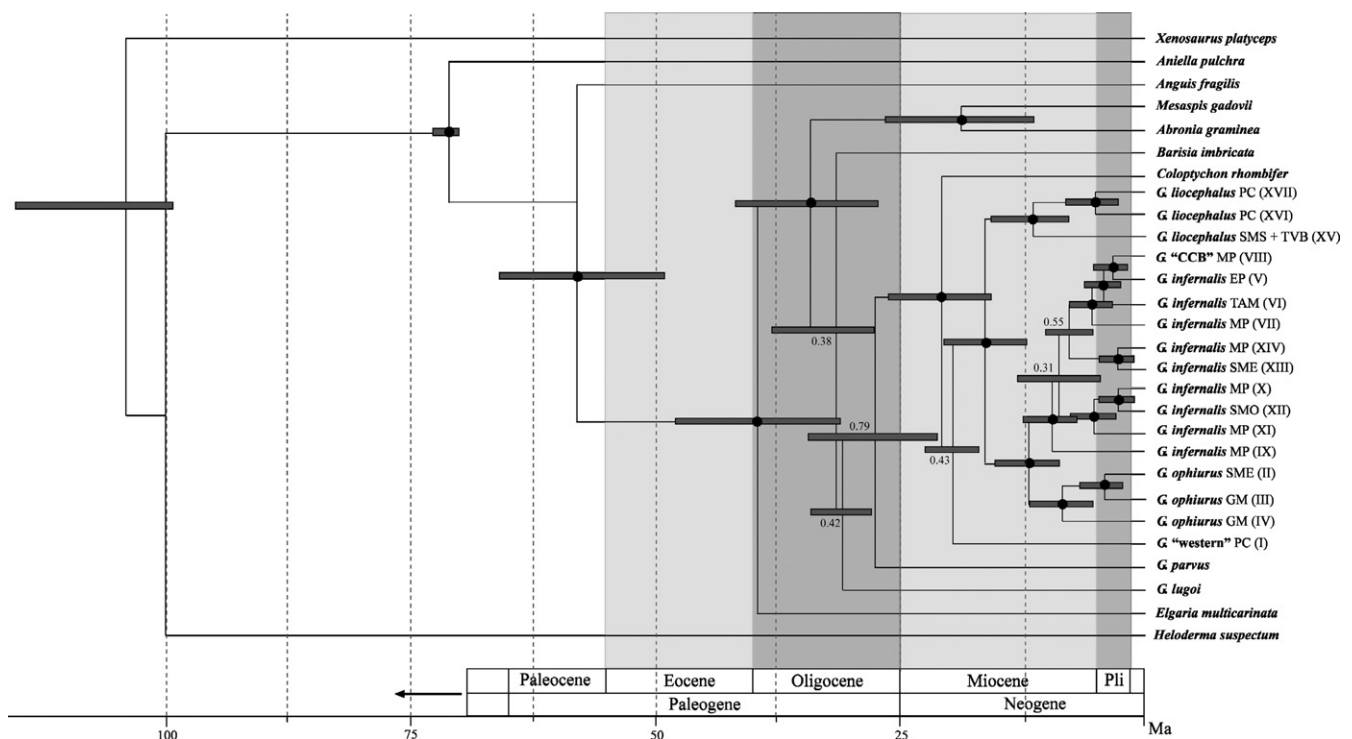
To assess the historical biogeography of the genus, the ancestral area reconstructions from the BBM analyses were combined with the divergence time estimates from the BEAST analysis of the multilocus dataset. Excluding *G. lugoi*, *G. parvus* and *C. rhombifer* (see Discussion), the Rasp analysis supported 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

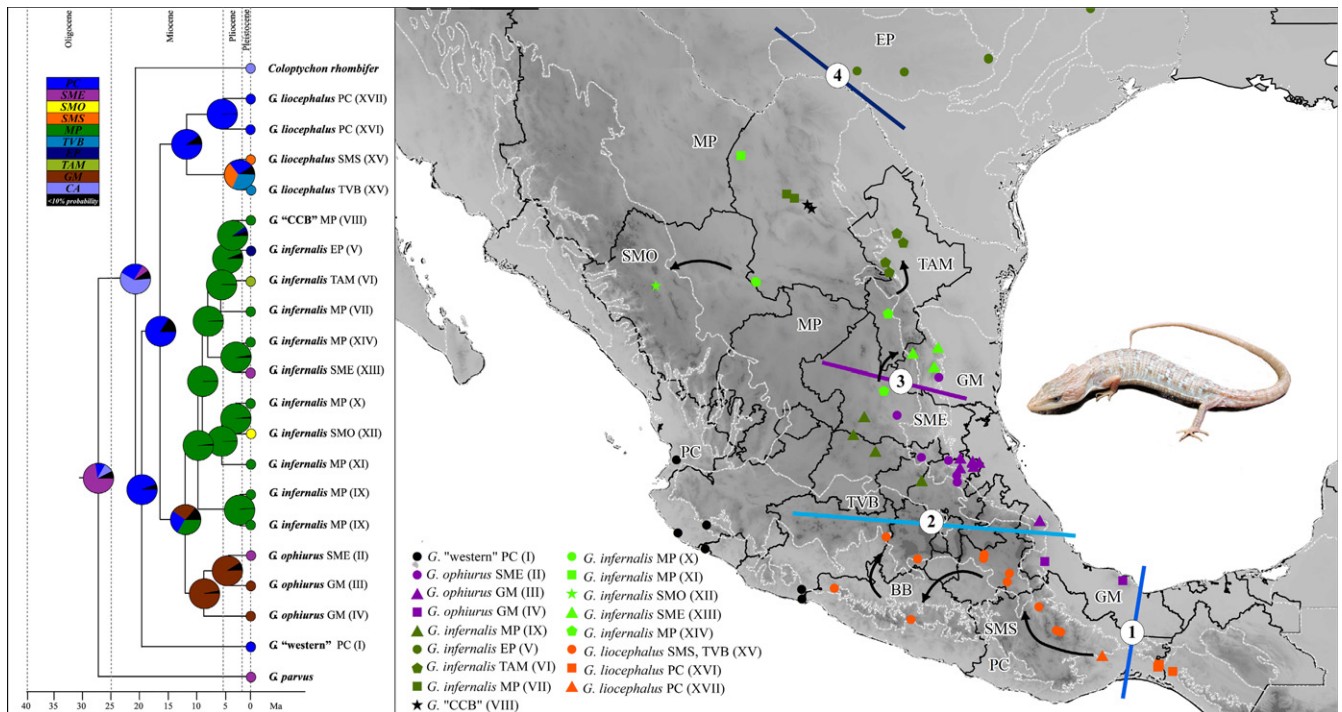
### 4.1 | Historical biogeography

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; Pli, 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 northermost 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, García-Deras, & García-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 jarrovi* 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. lazcano* 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. lazcano* 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. lazcano*, *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. lazcano*, 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. Author contributions: U.O.G.V. and A.N.M.O. conceived the project; U.O.G.V., A.N.M.O., C.J.P.V. and R.W.B. analysed the data; and all authors collected specimens and data, and shared in the writing.

## SUPPORTING INFORMATION

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

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