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Geographical features are the predominant driver of molecular diversification in widely distributed North American whipsnakes

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Abstract

Allopatric divergence following the formation of geographical features has been implicated as a major driver of evolutionary diversification. Widespread species complexes provide opportunities to examine allopatric divergence across varying degrees of isolation in both time and space. In North America, several geographical features may play such a role in diversification, including the Mississippi River, Pecos River, Rocky Mountains, Cochise Filter Barrier, Gulf of California and Isthmus of Tehuantepec. We used thousands of nuclear single nucleotide polymorphisms (SNPs) and mitochondrial DNA from several species of whipsnakes (genera *Masticophis* and *Coluber*) distributed across North and Central America to investigate the role that these geographical features have played on lineage divergence. We hypothesize that these features restrict gene flow and separate whipsnakes into diagnosable genomic clusters. We performed genomic clustering and phylogenetic reconstructions at the species and population levels using Bayesian and likelihood analyses and quantified migration levels across geographical features to assess the degree of genetic isolation due to allopatry. Our analyses suggest that (i) major genetic divisions are often consistent with isolation by geographical features, (ii) migration rates between clusters are asymmetrical across major geographical features, and (iii) areas that receive proportionally more migrants possess higher levels of genetic diversity. Collectively, our findings suggest that multiple features of the North American landscape contributed to allopatric divergence in this widely distributed snake group.

KEYWORDS
allopatry, *Coluber*, gene flow, geographical feature, *Masticophis*, North America, phylogeography

1 | INTRODUCTION

Divergence in allopatry has long been considered the most common model of diversification (Coyne & Orr, 2004; Dobzhansky, 1940; Mayr, 1942; Zink, 2014). The concordance of species' boundaries with geogreaphical features provides the strongest evidence for allopatric differentiation (Avise et al., 1987; Coyne & Orr, 2004). Dispersal to new areas or the formation of physical barriers isolates populations (Diamond, 1977; Kirkpatrick & Barton, 1997) and can lead to significant reductions in gene flow, thus promoting lineage divergence (Futuyma & Mayer, 1980). However, the genetic signal of previous isolation can be masked by gene flow and recombination at secondary contact. Recently diverged populations experiencing secondary contact can form hybrid zones, indicating that either a barrier

no longer exists, such as glaciers, or that a barrier is permeable, such as noncontinuous mountain ranges (Coyne & Orr, 2004; Feder, Egan, & Nosil, 2012; Jordan, 1905). Thus, evidence of hybrid zones can support a scenario where historical barriers led to temporarily isolated populations (e.g. Pleistocene glacial refugia in North America). However, complete barriers to gene flow can isolate populations permanently, leading to reproductive isolation (Pyron & Burbrink, 2010). Studying species at different temporal intervals in this process can help us understand the influence of such geographical features on limiting gene flow, and how barriers contribute to species diversification. The age and permeability of these features often determine the level of genetic differentiation that occurs between isolated populations (Pyron & Burbrink, 2010).

Rivers, mountains and geographical depressions have played important roles in the diversification of North American biota, including plants, invertebrates and vertebrates (Figure 1, Table 1; Swenson & Howard, 2005). Seven geographical features correlate with divergence of multiple taxa across the continental United States, Mexico and Central America. In the eastern United States, a consistent faunal break is found at the Mississippi River (MR). In the western United States, the Pecos River (PR; dividing the Chihuahuan Desert and central plains), the Cochise Filter Barrier (CFB; the division between the Chihuahuan and Sonoran deserts), the Rocky Mountains (RM) and the Gulf of California (GC) have been identified as barriers that likely influenced the evolution of multiple plant and animal species. In Mexico, the Isthmus of Tehuantepec (IT) has been identified as an influential barrier (see Table 1). In this study, we investigate how these geographical features have promoted diversification of eight widely distributed snake species (genera *Masticophis* and *Coluber*).

Both biotic and abiotic factors regulate levels of gene flow that occur across discrete geographical features (Futuyma & Mayer, 1980; Steeves, Anderson, & Friesen, 2005). First, biotic factors such as a species' dispersal potential and ecological tolerance influence how often a species can cross a geographical feature (Pyron & Burbrink, 2010). Often, larger animals are more capable of dispersing larger distances (Sutherland, Harestad, Price, & Lertzman, 2000). The abiotic factors intrinsic to the geographical feature also determine how much gene flow can occur and thus the level of population differentiation. The abiotic isolating potential of a feature is influenced by three factors. First, the age of the feature determines how long isolation has taken place and thus the level of differentiation between populations. Second, the permeability of the feature to gene flow affects genetic divergence of allopatric populations (hard and soft barriers; Pyron & Burbrink, 2010). Finally, the intrinsic composition of a feature also influences its isolating potential. For example, rivers and mountains may isolate species differently, and some historically hard barriers today allow limited gene flow. Habitat contractions associated with Pleistocene glaciation, and the once flooded IT in Mexico would be two examples of features that once isolated populations, but today only leave an eroding signal of isolation. On the other hand, ancient features such as the MR and the RM have isolated populations since their formation, although we expect to see evidence for greater levels of historical gene flow as the features were newly formed, with low levels of contemporary gene flow (Burbrink, Fontanella, Pyron, Guiher, & Jimenez, 2008; Egge & Hagbo, 2015). These factors add additional complications to hypotheses about the level of divergence and gene flow observed between isolated populations, because an ancient, yet permeable barrier may allow greater gene flow than a younger yet less permeable barrier. Additionally, while a feature such as the CFB may isolate less vagile animals (Table 1), the high vagility of birds has allowed many species to migrate across it (Zink, Kessen, Line, & Blackwell-Rago, 2001).

A broad geographical distribution, high potential for dispersal and high species diversity make colubrid snakes ideal models to test hypotheses of diversification because they provide natural replicates to test hypotheses across distinct geographical features (Burbrink et al., 2008; Conant & Collins, 1998; Dodd & Barichivich, 2007; Halstead, Mushinsky, & McCoy, 2009; Hirth, Pendleton, King, & Downard, 1969). Whipsnakes (genera *Masticophis* and *Coluber*) are a group of colubrid snakes distributed throughout North and South America spanning several important geographical features (Conant & Collins, 1998; Pyron et al., 2011; Utiger, Schatti, & Helfenberger, 2005). In this study, we investigate the role that barriers to dispersal have played in divergence across several widely distributed whipsnake species. Using a restriction site-associated DNA sequencing (RADseq) data set, we pursue the following questions: (i) How is genetic diversity partitioned within species across the landscape? (ii) Does migration occur between populations across geographical features? We find that at least six geographical features are associated with allopatric units in whipsnakes, including the MR, the PR, the CFB, the RM, the GC and the IT. Using comparisons that involved several species, we find evidence for asymmetric rates of migration from east to west across the MR, the PR and the IT and from west to east across the RM and CFB. More extensive geographical sampling of mitochondrial DNA revealed corroborating evidence for many of the patterns observed in the nuclear data set and also several instances of intraspecific allopatric circumscription. Collectively, these results suggest that divergence in allopatry is the predominant form of evolution among whipsnake species.

2 | METHODS

2.1 | Study system and sampling

Whipsnakes (Colubridae: Colubroidea) are large, typically diurnal, slender and active snakes that occur throughout North America and into northern South America (Dodd & Barichivich, 2007). For decades, most taxonomists placed all whipsnakes, excluding *Coluber constrictor*, in the genus *Masticophis* (Ortenburger, 1923), until Utiger et al. (2005) used molecular data to demonstrate that *C. constrictor* was nested within two *Masticophis flagellum* samples. Recently, Burbrink and Myers (2015) and Pyron, Burbrink, and Wiens (2013) provided additional support for this arrangement when they found that

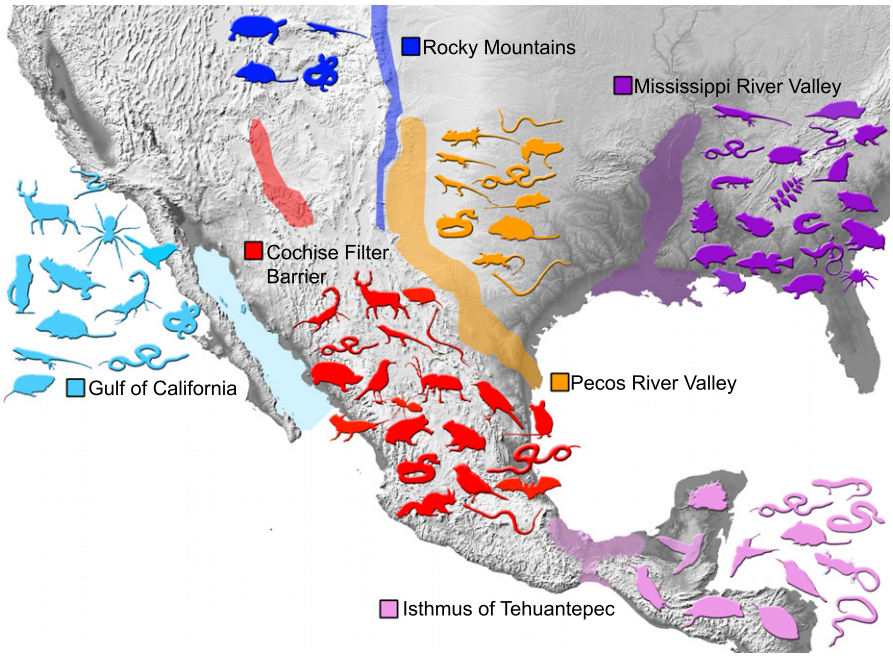

FIGURE 1 The major geographical features discussed in this study are highlighted. Next to each feature are representatives of species with allopatric divisions at these features. References are found in Table 1. [Colour figure can be viewed at wileyonlinelibrary.com]

C. constrictor was nested among samples of *Masticophis* species. Thus, until recently, most authorities recognized *Masticophis* as a junior synonym of *Coluber* (Uetz & Hošek, 2016). However, Myers et al. (2017) recovered a monophyletic *Masticophis* and recommended distinguishing *Masticophis* species from *C. constrictor*. In this study, we use the term whipsnakes to include species pertaining to both *Masticophis* and *Coluber*. Previous work on whipsnakes has used morphology to infer species boundaries (e.g., Grismer, 1990; Johnson, 1977; Ortenburger, 1923; Wilson, 1970), but this method can underestimate diversity as a result of cryptic species (Ruane, Bryson, Pyron, & Burbrink, 2014). Whipsnakes include 12 species (11 species in *Masticophis* and one species in *Coluber*) ranging across North America, with one species extending into northern South America. We used eight species of whipsnakes to test for isolating effects of North American geographical features: (i) *M. flagellum*, a group of snakes distributed from coast to coast in the southern half of the United States, and into northern Mexico (MR, PR, CFB), (ii) *M. fuliginosus*, restricted to the Baja California Peninsula in Mexico (GC), (iii) *M. mentovarius*, distributed from central Mexico to Columbia and Venezuela (IT), (iv) *M. taeniatus*, distributed from the northwestern United States to north eastern Mexico (RM), (v) *M. schotti*, distributed from southern Texas into northern Mexico, (vi) *M. lateralis*, distributed throughout California and the Baja California Peninsula in Mexico (GC), (vii) *M. bilineatus*, restricted to the Sonoran Desert in the southwestern United States and into central Mexico and (viii) *C. constrictor*, distributed across the continental United States, except the Chihuahuan Desert, which has been studied in detail previously (Burbrink et al., 2008; Conant & Collins, 1998; Richmond, Wood, Hoang, & Vandergast, 2011; Roze, 1953; Stebbins, 2003; Uetz & Hošek, 2016). Most of these species possess longitudinal stripes (*M. lateralis*, *M. taeniatus*, *M. schotti*, *M. bilineatus*), while four are predominantly uniform in dorsal coloration (*M. flagellum*, *M. fuliginosus*, *M. mentovarius* and *C. constrictor*). However, within at least one of the uniformly coloured species, coloration is highly polymorphic (*M. flagellum*). Subspecies have been described for all continental whipsnakes (four island/peninsula species are not included in this study): *M. flagellum* (*M. f. cingulum*, *M. f. flagellum*, *M. f. lineatulus*, *M. f. piceus*, *M. f. ruddocki*, *M. f. testaceus*, *M. f. fuliginosus*), *M. mentovarius* (*M. m. centralis*, *M. m. mentovarius*, *M. m. suborbatilis*, *M. m. striolatus*, *M. m. variolosus*), *M. schotti* (*M. s. schotti* and *M. s. ruthveni*), *M. taeniatus* (*M. t. girardi*, *M. t. taeniatus*), *M. lateralis* (*M. l. euryxanthis*, *M. l. lateralis*) and *M. bilineatus* (*M. b. bilineatus*, *M. b. lineolatus*, *M. b. semilineatus*), *C. constrictor* (*C. c. anthicus*, *C. c. constrictor*, *C. c. etheridgei*, *C. c. flaviventris*, *C. c. foxii*, *C. c. helvigularis*, *C. c. latrunculus*, *C. c. mormon*, *C. c. oaxaca*, *C. c. paludicola*, *C. c. priapus*). Several of these subspecies are at least partly delimited by the focal geographical features of this study (e.g. *M. f. flagellum* and *M. f. testaceus* [MR], *C. c. latrunculus* and *C. c. priapus* [MR], *M. f. testaceus* and *M. f. linatulus* [PR], *M. f. cingulum* and *M. f. lineatulus* [CFB], *M. fuliginosus* and *M. f. cingulum* [GC]; Wilson, 1970).

2.2 | Characteristics of geographical features

We conducted a literature review of putatively important geographical features in North America (Table 1). We cited studies that provided evidence of species level differentiation, population structuring or species range boundaries divided allopatrically by geographical features. Representative focal organisms from several studies are shown in Figure 1 next to the feature of interest. We primarily focused our sampling on taxonomically similar species to whipsnakes, but have also included other examples to more broadly demonstrate the contribution of these geographical features to the diversification of North American biota (Table 1). We also compiled ages of each feature from the literature (Table 3). We used the age of the origin

TABLE 1 Organisms that support the role that focal geographical features have played in facilitating divergence in allopatry

Feature	Species	Common name	Evidence	Reference
Mississippi river	*Erimystax dissimilis*	Streamline chub	Intraspecific mtDNA	Strange and Burr (1997)
	Kinosternon subrubrum	Eastern mud turtle	Intraspecific mtDNA	Walker, Moler, Buhlmann, and Avise (1998)
	Deirochelys reticularia	Chicken turtle	Intraspecific mtDNA	Walker and Avise (1998)
	Chelydra serpentina	Common snapping turtle	Intraspecific mtDNA	Walker and Avise (1998)
	Pantherophis obsoletus	Black rat snake	Intraspecific mtDNA	Burbrink, Lawson, and Slowinski (2000)
	Apalone mutica	Smooth softshell turtle	Intraspecific mtDNA	Weisrock and Janzen (2000)
	Percina evides	Gilt darter	Intraspecific mtDNA	Near, Page, and Mayden, (2001)
	Pinus taeda	Loblolly pine	Intraspecific range limits	Al-Rabab'ah and Williams (2004), Eckert et al. (2010)
	Sceloporus undulatus	Eastern fence lizard	Intraspecific mtDNA	Leaché and Reeder (2002)
	Blarina carolinensis	Southern short-tailed shrew	Intraspecific mtDNA	Brant and Orti (2002)
	Pantherophis guttatus	Rat snake	Intraspecific mtDNA	Burbrink (2002)
	Anaxyrus fowleri	Fowler's toad	Intraspecific mtDNA	Masta, Sullivan, Lamb, and Routman (2002)
	Anaxyrus woodhousii/A. americanus	North American toads	Interspecific range boundary	Masta et al. (2002)
	Blarina brevicauda	Short-tailed shrew	Intraspecific mtDNA	Brant and Orti (2003)
	Ambystoma maculatum	Spotted salamander	Intraspecific mtDNA	Zamudio and Savage (2003)
	Lithobates pipiens	Northern leopard frog	Intraspecific mtDNA	Hoffman and Blouin (2004)
	Lithobates catesbeiana	Bullfrog	Intraspecific mtDNA	Austin, Lougheed, and Boag (2004)
	Pseudacris crucifer	Spring peeper	Intraspecific mtDNA	Austin et al. (2004)
	Pseudacris nigrita	Southern chorus frog	Intraspecific mtDNA	Moriarty and Cannatella (2004)
	Juglans nigra	Black walnut	Intraspecific cpDNA	Soltis, Morris, McLachlan, Manos, and Soltis (2006)
	Eumeces fasciatus	Five-lined skink	Intraspecific microsats	Howes, Lindsay, and Lougheed (2006)
	Etheostoma caeruleum	Rainbow darter	Intraspecific mtDNA	Ray, Wood, and Simons (2006)
	Pseudacris spp.	Trilling chorus frogs	Interspecific mtDNA	Lemmon, Lemmon, and Cannatella (2007), Lemmon, Lemmon, Collins, Lee-Yaw, and Cannatella (2007)
	Procyon lotor	Raccoon	Intraspecific mtDNA	Cullingham, Kyle, Pond, and White (2008)
	Acris spp.	Cricket frogs	Interspecific mtDNA, nuDNA	Gamble, Berendzen, Shaffer, Starkey, and Simons (2008)
	Coluber constrictor	North American racer	Intraspecific mtDNA	Burbrink et al. (2008)
	Lampropeltis getula	Common kingsnake	Intraspecific mtDNA	Pyron and Burbrink (2009)
	Aphonopelma hentzi	Texas brown tarantula	Intraspecific mtDNA, eastern range limit	Hamilton, Formanowicz, and Bond, (2011)
	Mephitis mephitis	Striped skunk	Intraspecific mtDNA	Barton and Wisely (2012)
	Cryptobranchus alleganiensis	Eastern hellbender	Intraspecific microsats	Unger, Rhodes, Sutton, and Williams (2013)
	Micrurus spp.	Eastern/Texas coralsnakes	Interspecific microsats, mtDNA, SNPs	Castoe et al. (2012), Streicher et al. (2016)
	Campanulastrum americanum	American bellflower	Interspecific mtDNA, SNPs	Barnard-Kubow, Debban, and Galloway (2015)

(Continues)

TABLE 1 (Continued)

Feature	Species	Common name	Evidence	Reference
Pecos river	*Peromyscus maniculatus*	Deer Mouse	Interspecific mtDNA	Lansman et al. (1983)
	Onychomys spp.	Grasshopper Mice	Interspecific mtDNA	Riddle and Honeycutt (1990)
	Chaetodipus penicillatus	Desert pocket mouse	Western range limit	Lee et al. (1996)
	Peromyscus eremicus	Cactus Mouse	Western range limit	Walpole et al. (1997)
	Pituophis catenifer	Bullsnake	Interspecific mtDNA	Rodríguez-Robles and De Jesús-Escobar (2000), Myers et al. (2017)
	Sceloporus undulatus	Eastern fence lizard	Intraspecific mtDNA	Leaché and Reeder (2002)
	Sceloporus magister	Desert spiny lizard	Eastern range limit	Leaché and Mulcahy (2007)
	Diadophis punctatus	Ring-necked snake	Intraspecific mtDNA	Fontanella, Feldman, Siddall, and Burbrink (2008)
	Acris blanchardi	Blanchard's cricket frog	Western range limit	Gamble et al. (2008)
	Sceloporus cowlesi	White sands prairie lizard	Interspecific mtDNA, nuDNA	Leaché (2009)
	Sceloporus consobrinus	Southern prairie lizard	Interspecific mtDNA, nuDNA	Leaché (2009)
	Crotalus atrox	Western diamondback rattlesnake	Intraspecific SNPs	Schield et al. (2015)
	Rhinocheilus lecontei	Long-nosed snake	Interspecific mtDNA	Myers et al. (2017)
	Arizona elegans	Glossy snake	Intraspecific mtDNA	Myers et al. (2017)
Rocky mountains	*Xerobates agassizii*	Desert tortoise	Intraspecific mtDNA	Lamb, Avise, and Gibbons (1989)
	Peromyscus sp.	Deer mouse	Interspecific mtDNA	Riddle, Hafner, Alexander, and Jaeger (2000)
	Crotalus viridis	Prairie rattlesnake	Intraspecific mtDNA	Pook, Wüster, and Thorpe (2000)
	Sceloporus undulatus	Eastern fence lizard	Intraspecific mtDNA	Leaché and Reeder (2002)
Cochise filter barrier	*Onychomys* spp.	Grasshopper mice	Interspecific mtDNA	Riddle and Honeycutt (1990)
	Chaetodipus intermedius	Red pocket mouse	Intraspecific mtDNA	Riddle (1995)
	Chaetodipus penicillatus	Desert pocket mouse	Intraspecific mtDNA	Lee et al. (1996), Riddle (1995)
	Sciurus aberti	Tassel-eared squirrel	Intraspecific mtDNA	Lamb, Jones, and Wettstein (1997)
	Peromyscus eremicus	Cactus mouse	Intraspecific mtDNA	Walpole et al. (1997)
	Gambelia wislizenii	Long-nosed leopard lizard	Intraspecific mtDNA	Orange, Riddle, and Nickle (1999)
	Corvus corax	Common raven	Interspecific mtDNA	Omland, Tarr, Boarman, Marzluff, and Fleischer (2000)
	Crotalus viridis	Prairie rattlesnake	Interspecific mtDNA	Ashton and de Queiroz (2001)
	Toxostoma curvirostre	Curve-billed thrasher	Intraspecific mtDNA	Zink et al. (2001)
	Pipilo fuscus	Canyon towhee	Intraspecific mtDNA	Zink et al. (2001)
	Kinosternon flavescens	Yellow mud turtle	Intraspecific mtDNA	Serb, Phillips, and Iverson (2001)
	Lophocereus schottii	Senita cactus	Eastern range limit	Nason, Hamrick, and Flaming (2002)
	Myotis spp.	Vesper bats	Interspecific mtDNA	Rodriguez and Ammerman (2004)
	Phrynosoma cornutum	Texas horned lizard	Interspecific mtDNA	Rosenthal and Forstner (2004)

(Continues)

TABLE 1 (Continued)

Feature	Species	Common name	Evidence	Reference
	Rhinocheilus lecontei	Long-nosed snake	Interspecific mtDNA	Myers et al. (2017), Rosenthal and Forstner (2004)
	Bufo punctatus	Red-spotted toad	Intraspecific mtDNA	Jaeger, Riddle, and Bradford (2005)
	Moneilema appressum	Longhorn cactus beetle	Intraspecific mtDNA	Smith and Farrell (2005)
	Phrynosoma spp.	Horned lizards	Interspecific mtDNA, nuDNA	Leaché and McGuire (2006)
	Crotalus atrox	Western diamondback rattlesnake	Intraspecific mtDNA; SNPs	Castoe et al. (2007), Schield et al. (2015)
	Sceloporus magister	Desert spiny lizard	Eastern range limit	Leaché and Mulcahy (2007)
	Hypsiglena torquata	North American nightsnake	Interspecific mtDNA	Mulcahy (2008), Mulcahy and Macey (2009), Myers et al. (2017)
	Thomomys spp.	Gophers	Interspecific nuDNA	Belfiore, Liang, and Mortiz (2008)
	Dilophotopsis spp.	Velvet ant	Interspecific mtDNA	Wilson and Pitts (2008), Wilson and Pitts (2010b)
	Odocoileus hemionus	American mule deer	Interspecific mtDNA	Latch, Heffelfinger, Fike, and Rhodes (2009)
	Lampropeltis getula	Common kingsnake	Intraspecific mtDNA	Pyron and Burbrink (2009), Myers et al. (2017)
	Melampodium leucanthum	Blackfoot daisy	AFLP, cpDNA	Rebernig et al. (2010)
	Pituophis catenifer	Bullsnake	Intraspecific mtDNA	Bryson, García-Vázquez, and Riddle, (2011), Bryson, Murphy, Lathrop, and Lazcano-Villareal, (2011), Myers et al. (2017)
	Gastrophryne spp.	Great Plains narrowmouth toads	Interspecific mtDNA	Streicher, Cox, Campbell, Smith, and de Sá (2012)
	Crotalus molossus	Northern black-tailed rattlesnake	Intraspecific mtDNA	Anderson and Greenbaum (2012), Myers et al. (2017)
	Pseudouroctonus minimus	Vaejovid scorpion	Intraspecific mtDNA	Bryson, Riddle, Graham, Tilston Smith, and Prendini (2013), Bryson, Savary, and Prendini (2013)
	Ammospermophilus spp.	Antelope squirrels	Interspecific mtDNA, nuDNA	Mantooth, Hafner, Bryson and Riddle (2013)
	Arizona elegans	Glossy snake	Intraspecific mtDNA	Myers et al. (2017)
	Thamnophis marcianus	Checkered garter snake	Intraspecific mtDNA	Myers et al. (2017)
	Salvadora hexalepis	Western patch-nosed snake	Intraspecific mtDNA	Myers et al. (2017)
	Masticophis flagellum	Western whipsnake	Intraspecific mtDNA	Myers et al. (2017)
	Pituophis catenifer	Bullsnake	Intraspecific mtDNA	Myers et al. (2017)
Gulf of California	*Thomomys bottae*	Pocket gopher	Interspecific mtDNA	Smith (1998)
	Polioptila spp.	Gnatcatcher	Interspecific mtDNA	Zink and Blackwell (1998)
	Urosaurus spp.	Collared lizard	Intraspecific isozymes	Aguirre, Morafka, and Murphy (1999)
	Quercos spp.	Oaks	Interspecific cpDNA	Manos, Doyle, and Nixon (1999)
	Peromyscus spp.	Deer mouse	Interspecific mtDNA	Riddle et al. (2000)
	Pituophis catenifer	Bullsnake	Interspecific mtDNA	Rodríguez-Robles and De Jesús-Escobar (2000)
	Lophocereus spp.	Senita cactus	Interspecific cpDNA	Nason et al. (2002)

(Continues)

TABLE 1 (Continued)

Feature	Species	Common name	Evidence	Reference
	Ammospermophilus leucurus	Antelope ground squirrel	Intraspecific isozymes	Whorley, Alvarez-Casteneda, and Kenagy (2004)
	Xantusia spp.	Night lizards	Interspecific mtDNA	Sinclair et al. (2004)
	Phrynosoma mcallii	Flat-tailed horned lizard	Intraspecific mtDNA	Mulcahy, Morrill, and Mendelson, (2006), Mulcahy, Spaulding, Mendelson, and Brodie (2006)
	Homalonychus sp.	Spider	Intraspecific mtDNA	Crews and Hedin (2006)
	Trimorphodon biscutatus	Western lyresnake	Intraspecific mtDNA	Devitt (2006)
	Sceloporus magister	Desert spiny lizard	Eastern range limit	Leaché and Mulcahy (2007)
	Hypsiglena spp.	Nightsnakes	Interspecific mtDNA	Mulcahy and Macey (2009)
	Odocoileus hemionus	American mule deer	Interspecific mtDNA	Latch et al. (2009)
	Crotalus atrox	Western diamondback rattlesnake	Intraspecific mtDNA	Castoe et al. (2007)
	Pseudouroctonus minimus	Vaejovid scorpion	Intraspecific mtDNA	Bryson, Riddle, et al. (2013), Bryson, Savary, et al. (2013)
	Arizona elegans	Glossy snake	Intraspecific mtDNA	Myers et al. (2017)
Ithmus of Tehuantepec	*Peromyscus aztecus*	Aztec mouse	Intraspecific mtDNA	Sullivan et al. (1997)
	Abronia spp.	Alligator lizards	Interspecific mtDNA	Chippindale, Ammerman, and Campbell, (1998)
	Reithrodontomys sumichrasti	Sumichrast's harvest mouse	Intraspecific mtDNA	Sullivan, Arellano, and Rogers, (2000)
	Habromys lophurus	Crested tailed deer mouse	Interspecific ranges	Carleton, Sanchez, and Urbano Vidales (2002)
	Bufo punctatus	Red-spotted toad	Intraspecific mtDNA	Mulcahy, Morrill, et al. (2006), Mulcahy, Spaulding, et al. (2006)
	Alouatta pigra	Black howler monkey	Upper range boundary	Baumgarten and Williamson (2007)
	Lampornis amethystinus	Amethyst-throated hummingbird	Interspecific mtDNA	Cortés-Rodríguez, Hernández-Baños, Navarro-Sigüenza, Peterson, and García-Moreno (2008)
	Habromys spp.	Deer mouse	Interspecific mtDNA	León-Paniagua, Navarro-Sigüenza, Hernández-Baños, and Morales (2007)
	Atropoides spp.	Jumping pitvipers	Interspecific mtDNA	Castoe et al. (2008)
	Cerrophidion spp.	Montane pitvipers	Interspecific mtDNA	Castoe et al. (2008)
	Campylopterus curvipennis	Wedge-tailed sabrewing	Intraspecific mtDNA, microsats	González, Ornelas, and Gutiérrez-Rodríguez (2011)
	Palicourea padifolia	Distylous shrub	Intraspecific cpDNA	Gutiérrez-Rodríguez, Ornelas, and Rodríguez-Gómez (2011)
	Pituophis lineaticollis	Gopher snake	Interspecific mtDNA	Bryson, García-Vázquez, et al. (2011), Bryson, Murphy, et al. (2011)
	Aphelocoma spp.	Scrub jays	Interspecific mtDNA	McCormack, Heled, Delaney, Peterson, and Knowles (2011)
	Bolitoglossa spp.	Tropical salamanders	Interspecific mtDNA, nuDNA	Rovito, Parra-Olea, Vásquez-Almazán, Luna-Reyes, and Wake (2012)

(Continues)

TABLE 1 (Continued)

Feature	Species	Common name	Evidence	Reference
	Bombus ephippiatus	Polymorphic bumble bee	Intraspecific mtDNA, nuDNA	Duennes, Lozier, Hines, and Cameron (2012)
	Dermatemys mawii	Central American river turtle	Intraspecific mtDNA	González-Porter et al. (2013)
	Amazilia cyanocephala	Azure-crowned hummingbird	Intraspecific mtDNA	Rodríguez-Gómez, Gutiérrez-Rodríguez, and Ornelas (2013)
	Boa constrictor	Boa constrictor	Intraspecific mtDNA, nuDNA, microsats	Suárez-Atilano et al. (2014)
	Aegolius acadicus	Northern Saw-whet owl	Southern range boundary	Withrow, Sealy, and Winker (2014)
	Rhipsalis baccifera	Mistletoe cactus	Intraspecific cpDNA, nuDNA	Ornelas and Rodríguez-Gómez (2015)
	Eugenes fulgens	Magnificent hummingbird	Interspecific mtDNA, nuDNA	Zamudio-Beltrán and Hernández-Baños (2015)
	Phaseolus vulgaris	Common bean	Intraspecific SNPs	Rodriguez et al. (2016)

of the feature to encompass its entire history. For example, the MR is an ancient feature (65 million years [My]) but has likely isolated species at different magnitudes since that time, depending on climatological conditions. For each feature, we used the following ages: MR 65 million years ago (Mya; Arthur & Taylor, 1998), PR 1.8 Mya (Havenor, 2003), CFB 1.8 Mya (Devitt, 2006; and citations therein), RM 45–36 Mya (Riddle & Hafner, 2006), GC 5.5–4.0 Mya (Lonsdale, 1991), IT 6 Mya (Barrier, Velasquillo, Chavez, & Gaulon, 1998).

2.3 | DNA extraction and mitochondrial DNA sequencing

We acquired tissue samples from across much of the range of whipsnakes, as far south as Costa Rica. Our sampling included tissues from *Masticophis bilineatus* ($n = 2$), *M. lateralis* ($n = 6$), *M. schotti* ($n = 5$), *M. taeniatus* ($n = 13$), *M. mentovarius* ($n = 34$), *M. flagellum* ($n = 69$) and a putatively undescribed Mexican lineage (*Masticophis* sp., $n = 5$) (Table S1; Figure 2a,b). We extracted DNA from muscle, liver, shed skin or whole blood stored in SDS buffer or 70% ethanol using a standard salt extraction protocol (Sambrook & Russell, 2001). We checked the quality of our extractions using a 1% agarose gel and quantified the DNA using QUBIT® 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA). We sequenced a 770 base pair fragment of the cytochrome b gene for 119 individuals using custom primers (Table S4), designed from previous *Masticophis* sequences using GENEIOUS version 7.0 (Kearse et al., 2012). Each PCR occurred in a 25 μl reaction that included 10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl2, 0.04 mM of each dNTP, 1 U *Taq* DNA polymerase, 0.5 μM each primer and 10–25 ng of DNA. The amplification protocol for all PCRs was: 94°C, 2 min; 40 cycles of 94°C 30 s, annealing temperature 54.5°C 30 s, 72°C 30 s; 72°C 10 min; final rest at 12°C. PCR purifications were performed using Sera-Mag Speedbeads (Rohland & Reich, 2012). Cycle sequencing reactions were conducted using PCR primers under the following conditions: 95°C, 2 min; 40 cycles of 95°C 15 s, annealing temperature 50°C 15 s, 60°C 4 s; final rest at 12°C. Sequencing products were resolved on an Applied Biosystems 3130XL at the University of Texas Arlington Genomics Core Facility (gcf.uta.edu; Arlington, TX, USA).

2.4 | Mitochondrial sequence processing and phylogenetic analyses

Raw sequences were assembled into contigs and edited by eye for sequencing errors in GENEIOUS version 7.0 (Kearse et al., 2012). We also downloaded 52 sequences from GenBank, including *M. flagellum* ($n = 42$), *M. bilineatus* ($n = 1$), *C. constrictor* ($n = 3$), *Drymarchon corais* ($n = 1$), *Opheodrys aestivus* ($n = 1$), *Oxybelis aeneus* ($n = 1$), *Phyllorhynchus decurtatus* ($n = 1$), *Salvadora mexicana* ($n = 1$), *Sonora semiannulata* ($n = 1$), *Spilotes pullatus* ($n = 1$), *Tantilla relicta* ($n = 1$; Table S1). For *C. constrictor*, we chose one individual from each of the three primary clades identified in Burbrink et al. (2008). Sequences were aligned using GENEIOUS aligner with default settings. Prior to phylogenetic analysis, we selected the most probable

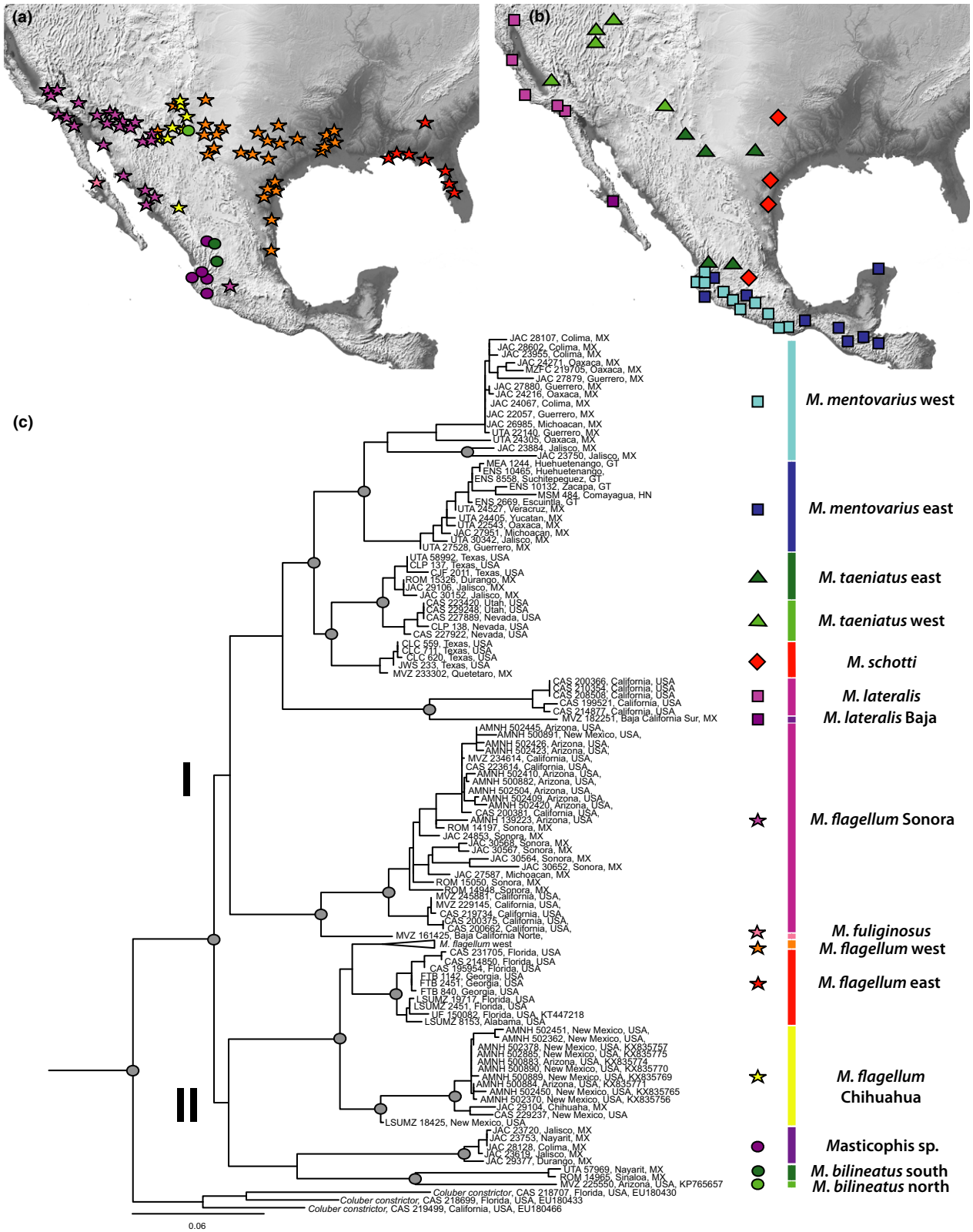

FIGURE 2 Phylogenetic analysis of eight whipsnake species based on Cytochrome b sequencing. (a) Map showing location of haplotypes for *Masticophis flagellum*, *M. bilineatus* and *Masticophis* sp. (b) Map of localities for mitochondrial haplotype groups of *M. lateralis*, *M. taeniatus*, *M. schotti* and *M. mentovarius*. (c) Maximum-likelihood phylogeny of mitochondrial whipsnake relationships. Dark grey circles represent nodes with ≥70% bootstrap support. Shapes on the map and next to the phylogeny differentiate species divisions, while different colours represent different clades [Colour figure can be viewed at wileyonlinelibrary.com]

models of nucleotide evolution for likelihood and Bayesian analyses using Bayesian information criteria implemented in PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012), partitioning by codon position.

We estimated phylogenetic relationships across all taxa using maximum likelihood (ML) in RAXMLGUI version 1.3 (Silvestro & Michalak, 2012) with 1,000 rapid bootstrap repetitions. We partitioned by codon, using GTR + Γ for each partition. We calculated mean pairwise distances (*p*-distance) among haplotype groups in MEGA version 7 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). We estimated divergence times of mitochondrial clades across geographical features to place our diversification events in a historical context. We randomly sampled one individual from each haplotype group identified in our ML phylogeny, including our eight outgroups, three *C. constrictor*, five *M. flagellum*, two *M. bilineatus*, one *Masticophis* sp., two *M. lateralis*, two *M. taeniatus*, one *M. schotti*, and two *M. mentovarius*. We estimated the phylogeny using a HKY model of evolution across each codon position. To estimate divergence times across geographical features, we used a relaxed clock lognormal clock model, a calibrated Yule tree prior and a lognormal prior on our fossil calibration points. We used two fossil calibration points, following Burbrink et al. (2008). We placed a lognormal prior on the MRCA of *Masticophis* and *Coluber* with a mean of 11 My, with a standard deviation of 0.1 (Holman, 2000). This resulted in a 95% confidence interval (CI) of 9.00–13.3 My. We also placed a lognormal prior on the root age, which encompassed all North American Colubrinae, with a mean age of 19 My and a standard deviation of 0.2. This resulted in a 95% CI of 12.6–27.6 My. This calibration corresponds to the oldest dates of the fossils *Paracoluber* (middle Miocene) and *Salvadora* (Late Miocene; Holman, 2000). We sampled 100,000,000 generations, sampling every 10,000 generations in BEAST version 2.4.5 (Bouckaert et al., 2014). We checked convergence of all parameters in TRACER (Rambaut, Suchard, Xie, & Drummond, 2014) and summarized all trees in TreeAnnotator (Bouckaert et al., 2014). We removed the first 25% of trees as burn-in and estimated the maximum clade credibility tree with median node heights.

2.5 | RADseq library generation and computational analysis

We prepared ddRADseq libraries for 132 individuals following the protocol described in Peterson, Weber, Kay, Fisher, and Hoekstra (2012). This method allows for the sequencing of thousands of orthologous loci from across the genome for large sample sets and has been successfully used in the absence of a reference genome in a variety of taxa (Eaton & Ree, 2013; Hipp et al., 2014; Streicher et al., 2014; Wagner et al., 2013).

We conducted double digests of 200–500 ng of DNA per individual using 20 units of *Sbf*I and 20 units of *Msp*I (NEB) for 8 hr at 37°C in 1x CutSmart Buffer (NEB). We ligated barcoded Illumina TruSeq adapters at 16°C for 30 min and heat-killed the enzyme at 65°C for 10 min. Each adapter included an 8-bp unique molecular identifier (UMI) that helped reduce poor quality sequence at the end of sequencing reads. We pooled up to 12 uniquely barcoded individuals into a group and labelled each group with a TruSeq single index; this double-barcoding scheme allowed us to multiplex all individuals for sequencing on a single Illumina HiSeq 2500 lane. We size selected all 11 groups using the Blue Pippin electrophoresis platform (Sage Science, Beverly, MA, USA) for fragments between 435 and 535 bp. RAD libraries were amplified using indexed Illumina® paired end PCR primers with Phusion® High Fidelity Proofreading Taq (NEB) under the following thermocycler conditions: 98°C, 30 s; 12–30 cycles of 98°C 30 s, annealing temperature 55°C 30 s, 72°C 1 min; 72°C 5 min; final rest at 12°C. We confirmed successful library preparation using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 chip kit, and final concentrations were verified using the Qubit 2.0®. We pooled our 11 sublibraries in equimolar amounts and sequenced our final library (100 bp paired end sequencing) on an Illumina® HiSeq 2500 at the University of Texas Southwestern Genomics Core facility (genomics.swmed.edu).

We processed our RAD data using the STACKS version 1.12 pipeline (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). We followed the recommended workflow which implemented the following scripts and programs: (i) process_radtags which filtered out reads below 90% quality score threshold, (ii) ustacks which set a maximum distance of 3 between "stacks," (iii) cstacks, which creates a catalogue of all of the loci within all individuals (−*n* flag; setting of 0), (iv) sstacks which searches the stacks created in ustacks against the catalogue from cstacks and (v) populations, which genotypes each individual according to the matched loci from sstacks. Following populations, we used custom python scripts to filter out invariant loci and loci with more than two haplotypes. We began by processing our RAD data for all species together, but recovered very few homologous loci (<100). Thus, we analysed each species group independently to maximize the number of homologous loci retained in each data set. To test the effect of missing data on our analyses, we generated three SNP data sets with varying amounts of missing data (50%, 20% and 10% missing data per locus) for *M. flagellum* and *M. mentovarius*. For *M. lateralis* and *M. taeniatus*, we filtered to 20% missing data per locus. This resulted in data sets ranging from 80 to 3,006 loci. At the individual level, our data sets ranged from 0 to 59% missing data per individual. The full number of loci used in each analysis is shown in Table S2.

2.6 | Inferring patterns of genomic divergence with Bayesian clustering

We sought to identify how geographical features may have influenced genetic diversity across the landscape by analysing population structure in STRUCTURE (Pritchard, Stephens, & Donnelly, 2000). We analysed each species group separately to avoid bias from uneven sampling (Puechmaille, 2016). Our sampling for each analysis included 36 *M. flagellum*, 24 *M. mentovarius*, five *M. lateralis* and four *M. taeniatus*. We ran STRUCTURE using all three missing data thresholds for *M. flagellum* and *M. mentovarius*. We analysed $K = 1$–10, with

five iterations at each *K* value. Each analysis was run for 500,000 generations with a burn-in of 100,000 MCMC generations. We used the independent allele frequency and the admixture ancestry model. We evaluated the results of our STRUCTURE analyses using the Evanno method (Evanno, Regnaut, & Goudet, 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012). We used the highest DeltaK value to identify the best value of *K* for each species group.

2.7 | *Bayesian estimation of migration across* geographical *features*

To quantify the level of isolation caused by each geographical feature, we estimated migration between populations across four features of varying permeability using MIGRATE-N Version 3.6.9 (Beerli, 2009). To generate input files, we called nuclear SNPs using default parameters in PYRAD version 3.0.5 (Eaton, 2014). We generated four input files for four population pairs (see below). Each population pair required different clustering thresholds specified in the PYRAD params file depending on the number of shared loci between the populations. The clustering thresholds and the number of loci used in each MIGRATE-N run are reported in the Table S2. We conducted five independent analyses: (i) *M. flagellum* east ($n = 12$) and west ($n = 23$) separated by the MR, (ii) *M. flagellum* west ($n = 26$) and Chihuahua ($n = 6$) separated by the PR, (iii) *M. flagellum* Sonoran ($n = 4$) and Chihuahua ($n = 6$) separated by the CFB, (iv) *M. mentovarius* east ($n = 28$) and west ($n = 20$) of the Isthmus of Tehuantepec, (v) *M. taeniatus* individuals east ($n = 6$) and west ($n = 2$) of the Rocky Mountains. We used a Bayesian inference model with uniform priors for θ (mutation scaled population size; 0–0.1) and *M* (mutation scaled immigration rate; 0–10,000). After a burn-in of 50,000 steps, we sampled 5,000 states from the Markov chain, one every 100 steps. We sampled four heated chains at four temperatures (1, 1.5, 3 and 100,000) to thoroughly search the parameter space. We calculated migrants per generation (*Nm*) by multiplying θ with *M* and dividing by four.

2.8 | Visualizing estimated effective migration surfaces

We used the program EEMS (Petkova, Novembre, & Stephens, 2016) to visualize how nuclear DNA-inferred migration rates were spatially distributed in select species of whipsnakes. EEMS estimates effective migration by visualizing regions where genetic dissimilarity decays quickly. It relates effective migration rates to expected genetic dissimilarities to clarify spatial features of population structure across the landscape. We ran six analyses with EEMS to estimate gene flow across the range of *M. flagellum* ($n = 36$) and *M. mentovarius* ($n = 24$) using our three missing data thresholds. We did not use *M. taeniatus*, *M. schotti*, *M. bilineatus*, *M. lateralis* or the identified lineage, because of small sample sizes. We ran three independent chains for each analysis, with 500 demes, for 8,000,000 MCMC iterations, with 3,200,000 iterations of burn-in and 9,999 thinning iterations. We checked convergence by analysing the trace file produced by the accompanying plotting program, REEMSPLOTS.

3 | RESULTS

3.1 | Mitochondrial phylogenetic analyses and divergence dating support allopatric divergence

Our ML analysis included wide geographical and taxonomic sampling and recovered 19 clades among all sampled species (Figure 2c). We recovered high support (≥70% bootstrap value) for relationships within species, but low support for many of the nodes between species. We found that *Masticophis* were monophyletic with respect to *Coluber constrictor*. Among these species, we recovered strong support for two large groups (excluding *C. constrictor*). The first group includes *M. flagellum* west of the CFB (including *M. fuliginosus*), *M. lateralis*, *M. mentovarius*, *M. taeniatus* and *M. schotti*. The second group included *M. flagellum* east of the CFB, *M. bilineatus* and *Masticophis* sp.

Within Clade I (Figure 2c), we recovered two clades pertaining to *M. flagellum* west of the CFB. The first clade included individuals ranging from Arizona in the east to California in the west and Sonora and Michoacán, Mexico, in the south (*M. flagellum* Sonora). The second clade included the *M. fuliginosus* sample. These two clades were sister to seven clades pertaining to *M. lateralis*, *M. mentovarius*, *M. taeniatus* and *M. schotti*, although this relationship was poorly supported. We recovered two clades within *M. lateralis*, one on mainland California, and the other on Baja California, Mexico (*M. lateralis* and *M. lateralis* Baja). Within *M. schotti*, we recovered one clade. In *M. taeniatus*, we recovered two clades from the east and west of the RM (*M. taeniatus* east and west). The western clade included individuals from Utah, Nevada, and New Mexico, while the eastern clade included individuals from Texas, and Jalisco and Durango, Mexico, with additional substructure observed between the Texas and Mexico samples. Within *M. mentovarius*, we observed two clades divided by the IT (*M. mentovarius* east and west). The western clade included individuals from the Pacific coast of Mexico, from Jalisco to Oaxaca, with additional substructure observed in Jalisco. The eastern clade included individuals from the Atlantic coast of Mexico, and nuclear Central America, as far south as northern Costa Rica. However, the eastern clade also included several individuals from western Mexico.

Within Clade II (Figure 2c), we recovered a sister relationship between *M. bilineatus* and *Masticophis* sp. Within *M. bilineatus*, we observe two clades, one from Arizona, and the other from Nayarit and Sinaloa, Mexico (*M. bilineatus* north and south). These species were sister to three clades of *M. flagellum* east of the CFB. The first clade pertained to individuals between the CFB and the PR in the Chihuahuan Desert (*M. flagellum* Chihuahua). This clade was sister to two reciprocally monophyletic clades divided by the MR, *M. flagellum* east and west. *Masticophis flagellum* east included all samples east of the MR as far north as Georgia. *Masticophis flagellum* west included all samples between the MR and the PR, although a few samples with this haplotype came from between the CFB and the PR. Table 2 shows uncorrected pairwise distances between each clade recovered in the ML analysis. Interclade divergences ranged from

TABLE 2 Mean between group divergences generated from uncorrected *p* distances among Cytochrome b haplogroups in the whipsnake species complex

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Masticophis flagellum* east															
2. *M. flagellum* west	3.4														
3. *M. flagellum* Chihuahua	4.9	5													
4. *M. flagellum* Sonora	10.7	10.3	9.7												
5. *M. fuliginosus*	8.3	9.8	8.6	7.3											
6. *M. bilineatus* north	9.5	9.6	6.6	13.3	12.5										
7. *M. bilineatus* south	10.5	8.9	7.9	14.2	13.8	5.8									
8. *Masticophis* sp.	10.6	10.9	9.4	11.6	10.3	8.9	11.2								
9. *M. mentovarius* west	9.2	9.2	7.5	12.3	11	11.6	12.9	12.5							
10. *M. mentovarius* east	8.9	8.6	8.6	11.8	10.5	12.3	11.3	12.6	8.8						
11. *M. taeniatus* west	9.9	10	7.7	11.6	10.1	10	10.9	10.3	8.8	7.4					
12. *M. taeniatus* east	9.8	9.8	8.4	12.3	10.4	10.8	10.8	11.8	9.8	8.4	2				
13. *M. schotti*	10.6	9.9	9	11.4	9.8	11.8	12.2	11.2	9.4	6.9	5.2	6.6			
14. *M. lateralis*	12.1	11.7	10.3	14.1	13.7	11.9	10	14.6	12.6	12	11.3	11.8	10.3		
15. *M. lateralis* Baja	14.6	14.7	11.9	15.2	13.4	13.4	13.4	15.2	13.7	12.8	10.7	10.5	11	8.2	
16. *Coluber coluber*	11.3	11.9	9.1	11.7	10.6	12.2	13.4	11.5	13.5	12.7	11.7	12.6	12.2	13.8	15.3

3.4% between *M. flagellum* east and west to 16.1% between *M. lateralis* Baja and *M. flagellum* Sonora.

Our Bayesian inference (BI) of phylogenetic relationships revealed similar phylogenetic structure between haplotype groups as the ML analysis with the two exceptions of *M. flagellum* Sonora and *M. fuliginosus*, and the relationship between *M. bilineatus* and *Masticophis* sp. (Figure 3). In the BI analysis, we recovered *M. flagellum* west of the CFB as sister to all species in group I, rather than group II (0.90 PP). We also recovered *M. bilineatus* as sister to *M. flagellum* east of the CFB, instead of to *Masticophis* sp. (0.68 PP). However, the relationship of *M. bilineatus* was not recovered with high support, underscoring the phylogenetic uncertainty of this species.

Our estimates of divergence dates placed the oldest divergence event between *C. constrictor* and all other species at 10.8 Mya (95% HPD 9.02–12.82; 1.00 PP; Table 3; Figure 3). We found that clades I and II diverged 8.56 Mya (6.83–10.48; 1.00 PP). Within group I, *M. lateralis* diverged from *M. mentovarius*, *M. taeniatus* and *M. schotti* 7.05 Mya (5.23–9.09; 0.98 PP). *Masticophis lateralis* clades were split by the GC 3.81 Mya (2.34–5.53; 1.00 PP). *Masticophis mentovarius* diverged from *M. taeniatus* and *M. schotti* 5.60 Mya (3.87–7.43; 0.85 PP) and was split by the IT 4.35 Mya (2.90–6.28; 0.67 PP). *Masticophis taeniatus* diverged from *M. schotti* 3.45 Mya (2.07–5.12; 0.99 PP) and was split by the RM 1.44 Mya (0.66–2.40; 1.00 PP). Within group II, we found the oldest divergence event at the CFB, where *M. flagellum* Sonora split from the eastern lineages 7.59 Mya (5.90–9.49; 0.90 PP). In addition, the Sonoran lineage of *M. flagellum* diverged at the GC 4.73 Mya (3.05–6.76; 0.99 PP). *Masticophis* sp. diverged from *M. bilineatus* and *M. flagellum* 6.50 Mya (4.89–8.38; 0.97 PP), and *M. bilineatus* diverged from *M. flagellum* 5.56 Mya (4.04–7.12; 0.68 PP). The northern and southern clades of *M. bilineatus* diverged 3.47 Mya (2.19–4.90; 1.00 PP). *Masticophis flagellum* diverged at the PR 4.15 Mya (2.77–5.84; 0.99 PP) and at the MR 1.87 Mya (1.00–2.89; 1.00 PP). This places the majority of divergence events in whipsnakes within the late Miocene and the Pliocene, with only two events occurring during the Pleistocene. However, including our 95% HPD, several events may have occurred in the early Pleistocene. Of the eight clades that are separated by geographical features, only two divergence events were older than the date of formation of the current geographical feature (CFB and PR), providing additional support for the role of geographical features in promoting diversification in allopatry. We note that the divergence events across the IT by *M. mentovarius* and across the MR by *C. constrictor* are not strongly supported in this analysis and thus should be interpreted with caution.

3.2 | Genomic variation forms discrete allopatric clusters at multiple scales

We used the Evanno method to infer *K* values from our STRUCTURE analyses for *M. flagellum* ($K = 5$), *M. mentovarius* ($K = 2$), *M. taeniatus* ($K = 2$) and *M. lateralis* ($K = 3$). Results shown in Figure 3 correspond to the data sets with 20% missing data; results for the other missing data thresholds show similar patterns and are summarized in Fig. S1. The five clusters of *M. flagellum* individuals corresponded to samples from (i) the Baja California Peninsula (ii) west of the CFB (iii) between the CFB and the PR (iv) between the PR and the MR (v) east of the MR (Figure 4a,e). The two *M. mentovarius* clusters corresponded to individuals west and east of the IT (Figure 4b,f). *Masticophis taeniatus* clustering corresponded to samples east and west of the RM (Figure 4c,f). *Masticophis lateralis* populations divided between the California mainland and Baja California, and the southern California sample showed evidence for an intermediate

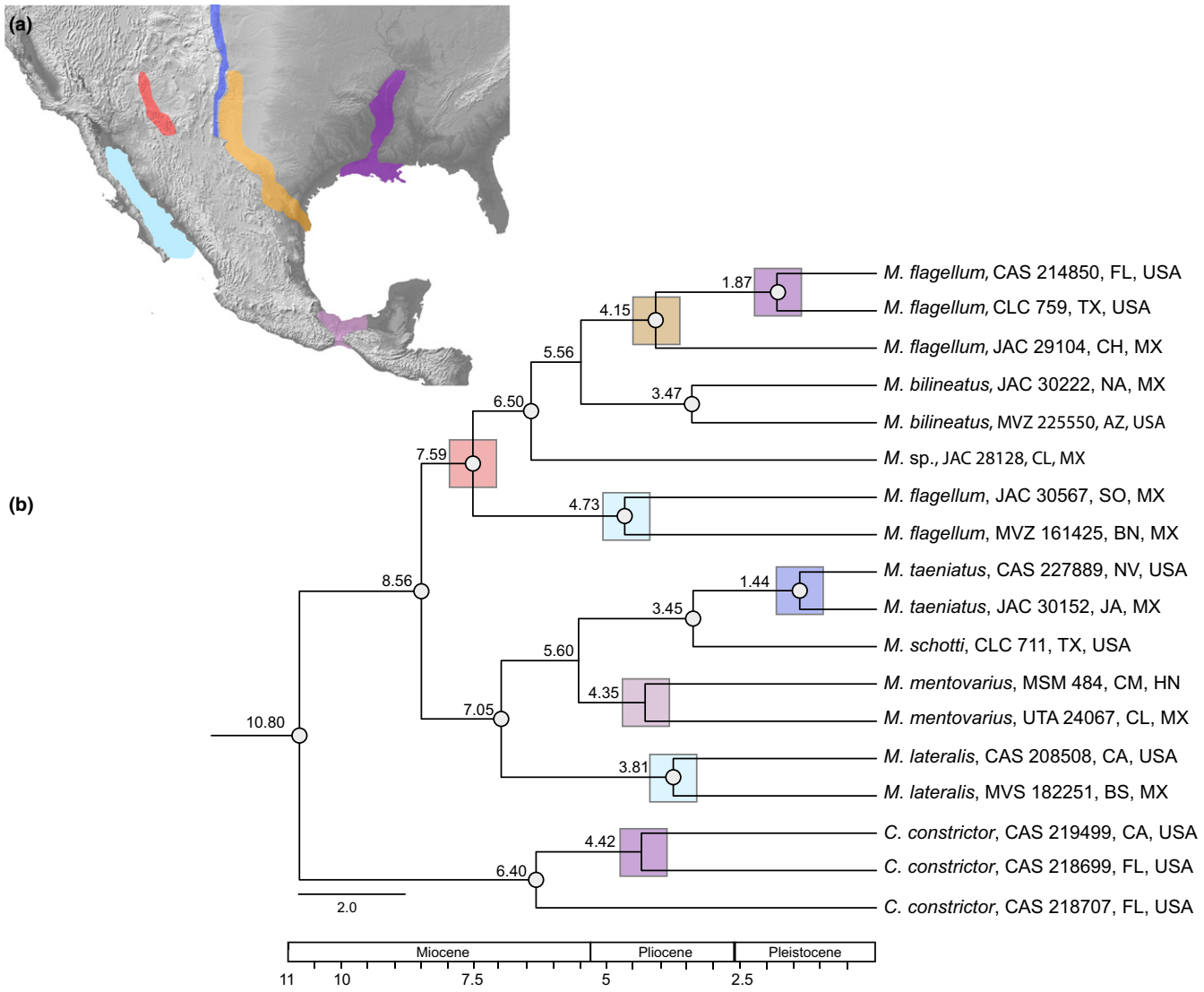

FIGURE 3 Bayesian phylogenetic analysis and divergence time estimation. (a) Map showing the geographical features of interest from Figure 1. (b) Bayesian phylogeny generated in BEAST. Nodes with ≥90% posterior probability are coloured with a grey circle. The coloured boxes behind the nodes signal phylogenetic breaks that correspond to geological features. The mean divergence time is shown above each node [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Estimated divergence times of whipsnake clades across geographical features. Clades are defined by ML haplotype groups shown in Figure 2

Feature	Haplotype groups	Divergence age (my)	95% CI	Date of formation (my)
Mississippi river	*Masticophis flagellum* east/west	1.87	1.00–2.89	65
Mississippi river	*Coluber constrictor* east/west	4.4	2.86–6.21	65
Pecos river valley	*M. flagellum* west/Chihuahua	4.15	2.77–5.84	1.8
Cochise filter barrier	*M. flagellum* Sonora/Chihuahua	7.59	5.90–9.49	1.8
Rocky mountains	*M. taeniatus* east/west	1.44	0.66–2.40	45
Gulf of California	*M. flagellum* Sonora/*M. fuliginosus*	4.73	3.05–6.76	5.5
Gulf of California	*M. lateralis* mainland/Baja	3.82	2.34–5.53	5.5
Isthmus of Tehuantepec	*M. mentovarius* east/west	4.35	2.90–6.28	6

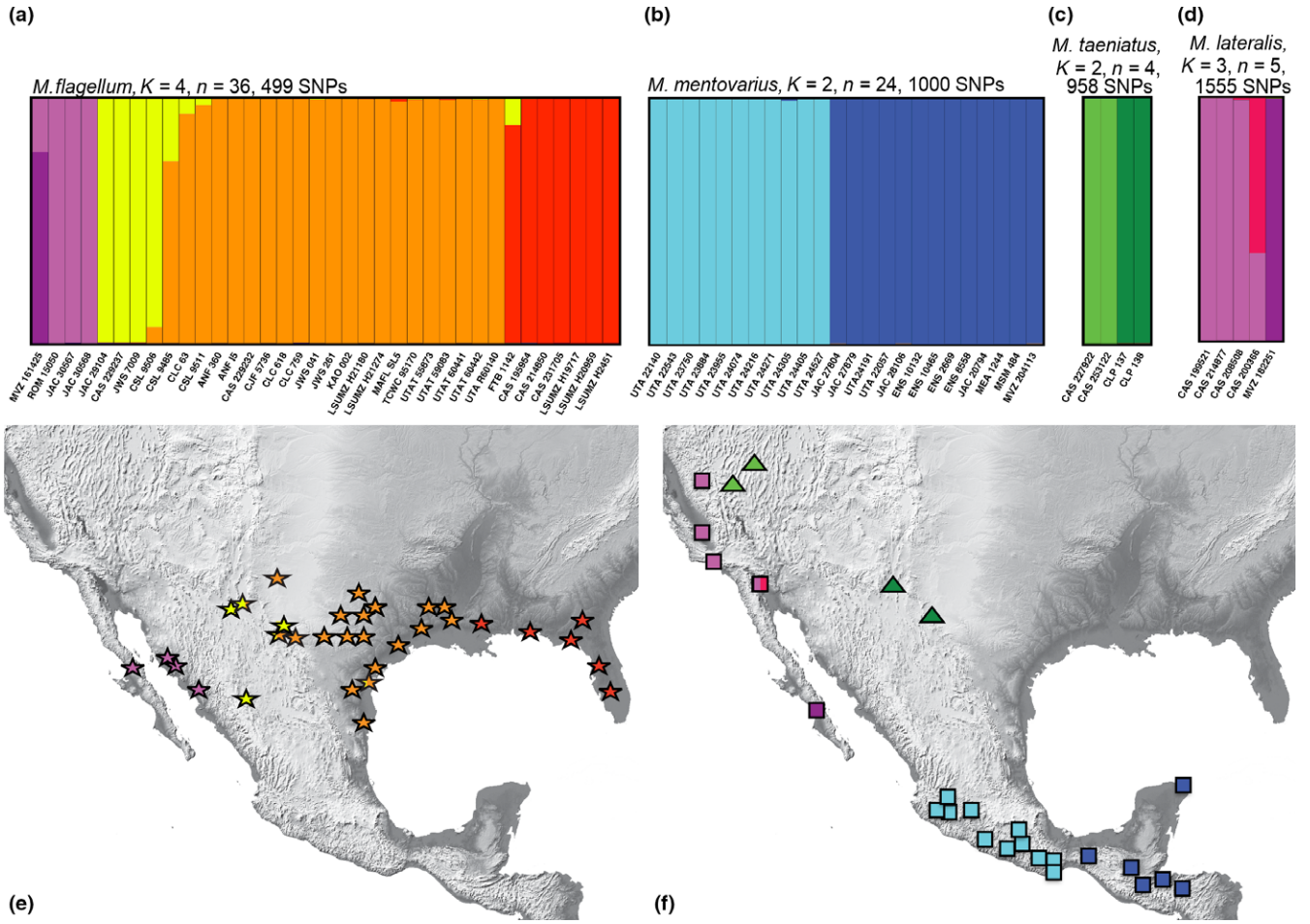

FIGURE 4 Graphical results of the nuclear analyses. (a–d) Genomic clustering results for each species group. Species name, inferred value of *K*, sample size and number of SNPs used are labelled above each STRUCTURE plot. (e) Maps showing locations of genomic clusters for *Masticophis flagellum*. (f) Map showing location of genomic clusters for *M. mentovarius*, *M. taeniatus* and *M. lateralis* [Colour figure can be viewed at wileyonlinelibrary.com]

population (Figure 4d,f). Notably, all the major genomic clusters inferred from our nuclear SNP sampling occurred on opposite sides of our focal geographical features (Figure 4). Our analyses that utilized different missing data thresholds recovered similar results. In *M. flagellum*, we found that allowing up to 50% missing data at the locus level and up to 59.6% missing data at the individual level recovered very similar results to the data set shown in Figure 4 (Fig. S1a). Allowing only 10% missing data did not recover *M. fuliginosus* as an independent population and included an additional population within *M. flagellum* west that may not correspond to real genetic structure. In *M. mentovarius*, we recovered congruent population assignments across missing data thresholds (Fig. S1b).

3.3 | Migration occurs asymmetrically across some geographical features

Our MIGRATE-N analyses supported migration across five primary geographical features (Figure 5). All analyses reached convergence. We report the mean values for each parameter in Table S3. Across the CFB, we found that that Sonoran samples exchanged 0.800 migrants per generation (*Nm*) with the Chihuahuan clade, which exchanged 0.797 *Nm* in return. Considering the large divergence between the Sonoran clade and the other *M. flagellum*, we consider this to be low (and equal) levels of migration that helped us contextualize our other comparisons. Across the PR, we found evidence for asymmetric gene flow from east to west, with the western clade exchanging 1.20 *Nm*, and the Chihuahuan clade exchanging 0.879 *Nm*. We found a similar east to west pattern across the MR, with the eastern clade exchanging 1.07 *Nm*, and the western clade returning 0.880 *Nm*. At the RM, we found that northern *M. taeniatus* exchanged 0.860 and southern *M. taeniatus* exchanged 0.072 *Nm*. At the IT, we again found directional migration from east to west, with *M. mentovarius* east and *M. mentovarius* west exchanging 1.20 and 0.652 *Nm*, respectively. Across the MR, PR and the IT, we found stronger migration from east to west, while across the RM, we found asymmetrical rates from west to east. Across the CFB, we found symmetrical rates of migration. The full outputs of all MIGRATE-N analyses are shown in Figs. S3–S6.

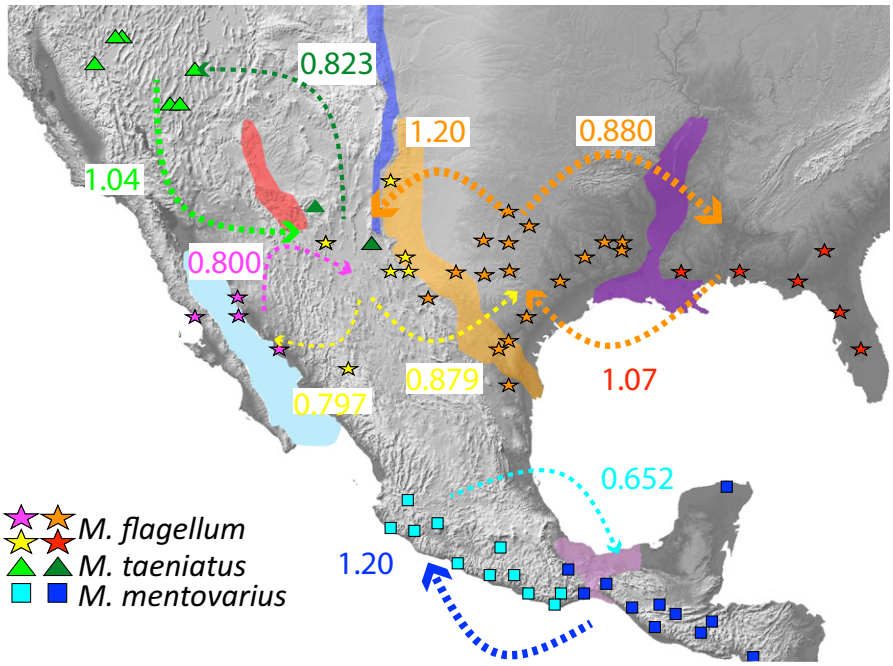

FIGURE 5 Results of the MIGRATE-N analyses. Values are given for migrants per generation between genomic clusters. Arrows are sized to indicate the strength of migration in each direction. Geographical features are shown behind each migration estimate [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Estimated effective migration reveals additional population structure and centres of genetic diversity

Estimated effective migration surfaces analyses for *M. flagellum* supported four populations, with strong barriers to gene flow at the MR, the northern PR and the CFB. We recovered evidence for weak isolation between the *M. flagellum* west and Chihuahuan population in the southern portion of their putative contact area (Figure 6a). We found no evidence for gene flow between the Sonoran population and the other three populations. We found that the centre of diversity for this species lies in the western clade, but that regions of genetic diversity existed in the Chihuahuan Desert and in the northern range of the eastern clade. We observed low levels of genetic diversity in the southern part of the eastern clade where gene flow was more prevalent, as well as in the Sonoran clade (Figure 6b). Figure S7a regresses genetic distance against Euclidian geographical distance to show that genetic distance is partitioned into four groups and is not equal across the landscape.

Our analyses of *M. mentovarius* showed a reduction of gene flow at the IT as we observed in STRUCTURE, but also showed additional population structuring on both sides of the IT (Figure 6c). The centre of diversity for this species was recovered to the west of the IT on the Pacific coast (Figure 6d). We recovered very low diversity estimates for the population east of the IT. We found that *M. mentovarius* is not isolated by distance but that genetic variation and diversity are partitioned at the Isthmus of Tehuantepec (Fig. S7b).

4 | DISCUSSION

We used genome wide SNPs and mitochondrial sequence data to evaluate major genomic divisions within whipsnakes to quantify migration and structure between clusters associated with geographical features. We found that whipsnake genomic clusters largely corresponded to geographical features, indicating that these features played a notable role in the diversification of whipsnakes. Our genomic data supported twelve clusters of whipsnakes, and our expanded mitochondrial sampling revealed extensive diversification within each species. Divergence dating suggested that most diversification events in whipsnakes occurred during the late Miocene or early Pliocene. We tested migration across four geographical features that partitioned genetic clusters and found evidence for asymmetric gene flow occurring from east to west in *Masticophis flagellum* across the MR and the PR and in *M. mentovarius* across the IT (Figure 5). We observed a west to east pattern of migration across the RM in *M. taeniatus* and symmetrical rates across the CFB in *M. flagellum* (Figure 5). However, our sampling for *M. taeniatus* was limited. Our estimated effective migration surfaces revealed strong differentiation at the MR, the CFB, the PR and at the IT (Figure 6). We observed that populations that received more migrants had higher levels of genetic diversity (Figures 5 and 6b,d). These results add more evidence of the importance of geographical features in driving diversification of North American biota by isolating populations in allopatry.

4.1 | Whipsnake phylogenetics, phylogeography and taxonomy

We recovered extensive geographical structuring within each group. While much of our nuclear clustering assigned individuals to distinct clusters with high probability, the support values for some mitochondrial relationships were low at deeper nodes. This result could be explained by an initial rapid radiation in this group, or be indicative of substitution saturation of evolving mitochondrial DNA (Rothfels et al., 2012; Streicher et al., 2014). While we observed

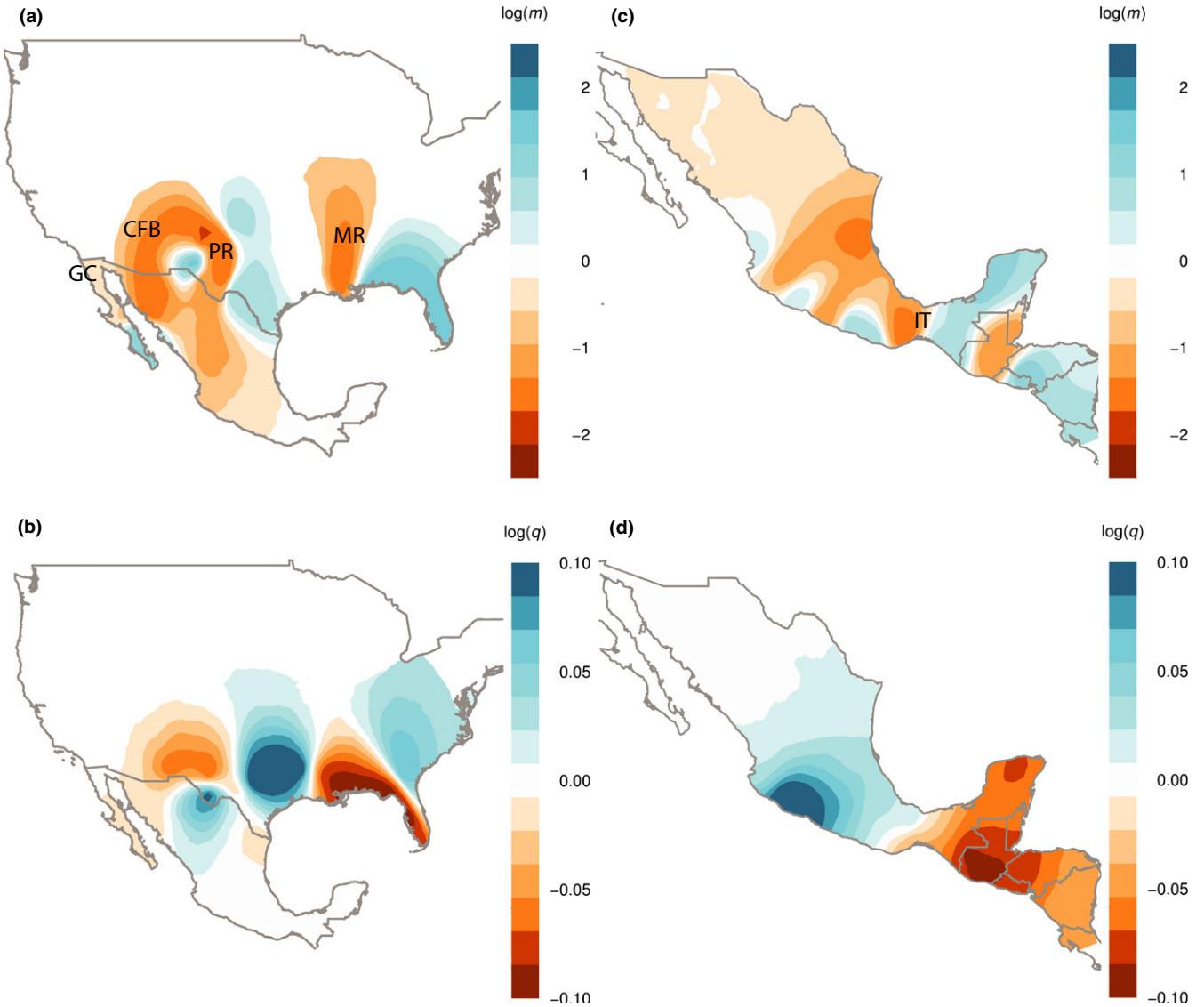

FIGURE 6 Graphical representations of estimated effective migration and diversity surfaces (EEMS). High values are represented by shades of blue; low values are represented by red-orange shades. (a, b) show results for *Masticophis flagellum*, and (c, d) show results for *M. mentovarius*. (a, c) Estimated effective migration surfaces. (b, d) Estimated effective diversity surfaces. Focal geographical features are labelled in (a) and (c): GC, Gulf of California; CFB, Cochise Filter Barrier; PR, Pecos River; MR, Mississippi River; IT, Isthmus of Tehuantepec [Colour figure can be viewed at wileyonlinelibrary.com]

substantial similarity between the groups recovered by the mitochondrial and nuclear analyses, some results were discordant. Our phylogenetic analyses with mitochondrial data recovered *M. flagellum* as nonmonophyletic with respect to *M. bilineatus* and *Masticophis* sp. (Figure 2c). More extensive sampling of *M. bilineatus* would help resolve its relationship with *M. flagellum*. Likewise, *M. flagellum* from west of the CFB clustered with group II in the ML analysis, rather than with the other *M. flagellum*, while in the BI analysis, we recovered western *M. flagellum* as sister to all other *M. flagellum*, *M. bilineatus* and *Masticophis* sp. In the light of these findings, much work remains to resolve the relationships of all whipsnake species. More extensive nuclear sampling of *M. flagellum* west of the PR, *M. bilineatus* and *Masticophis* sp. would help towards this objective.

4.2 | Migration occurs asymmetrically across geographical features in whipsnakes

Our two migration analyses show largely concurrent results that differ in scale. Lower rates of migration inferred by MIGRATE-N appear as much darker breaks in the EEMS analyses (Figures 5 and 6a,d). Not surprisingly, the populations with the highest levels of genetic diversity are those that receive more migrants in both *M. flagellum* and *M. mentovarius*. However, the western clade of *M. flagellum* appears to have contributed higher levels of migrants to the Chihuahuan clade than to the eastern clade, yet shows little evidence for migration in Figure 6a. Our data also suggest that the Sonoran clade exchanges few migrants with the Chihuahuan clade (Figure 6a). Low

migration may explain the ~10% mitochondrial divergence between the Sonoran clade and other *M. flagellum*.

Migration patterns observed in *M. mentovarius* are consistent with the distribution of mitochondrial haplotypes, where the eastern haplotype was present to the west of the IT, but the western haplotype was not recovered east of the IT. This may suggest that eastward migration (inferred using MIGRATE-N) has carried the eastern mtDNA haplotype westward. The higher level of genetic diversity inferred in the west by EEMS (Figure 6d) suggests that westward-biased migration has increased genetic diversity in the west disproportionately (Figure 5). Unlike the MR, the CFB and the CD, the IT changed from a shallow embayment to a land bridge within the last 2 my, allowing for previously isolated populations to experience secondary contact (Barrier et al., 1998).

Other recent studies have also inferred migration in reptiles using molecular data. Grummer et al. (2015) estimated migration between fence lizard populations in the Mexican highlands (using IMA2; Hey, 2010), and Suárez-Atilano, Burbrink, and Vázquez-Domínguez (2014) used MIGRATE-N to estimate migration between *Boa constrictor* populations using microsatellite markers and found high levels of gene flow between them (7.78–31.1 *Nm*). Alternatively, Ruane et al. (2014) used MIGRATE-N to estimate rates of migration between milksnake species, rather than populations, and recovered very low rates of gene flow (0.00–1.22 *Nm*). The rates observed in our study are considerably lower than those found in Suárez-Atilano et al. (2014), but higher than those found in Ruane et al. (2014), reflecting the various levels of divergence that we investigated across geological features. Similar studies have also inferred high rates of migration between bird populations, including mallards (0.42–8.26 *Nm*), flamingos (0.40–7.88 *Nm*) and black-footed albatrosses (0.02–4.5 *Nm*; Dierickx, Shultz, Sato, Hiraoka, & Edwards, 2015; Geraci et al., 2012; Kraus et al., 2012). While it is unsurprising that rates of migration are higher in flying vertebrates than in less mobile snakes from this study, rates of migration estimated for birds were lower than those estimated for boas (Suárez-Atilano et al., 2014). More studies of migration using genomic data are needed to identify typical rates of migration between reptile populations and lineages. However, migration among whipsnake groups provides evidence that migration associated with geographical features may be lower than in other species.

4.3 | Geographical features promoted diversification at multiple timescales in whipsnakes

To place our findings in a historical context, we compared the influence of each geographical feature on whipsnakes to that of past studies. Certain features (i.e. MR, IT) consistently separated populations or sister species into discrete groups. This could be due to the nature of the features, as these two represent water crossings, while the CFB and PR represent habitat transitions and generally more recent histories of isolation. Our study found support for six geographical features that limit gene flow in North American biota (Figure 1).

The Mississippi River has exerted a strong isolating force on a variety of taxa including plants, amphibians, fish, reptiles and mammals (Figure 1, Table 1). The MR serves as the primary isolating boundary for *M. flagellum* in the east. The MR formed as long ago as 65 my (Arthur & Taylor, 1998), implicating this as an ancient isolating boundary for snakes (Castoe et al., 2012; Streicher et al., 2016). We found that whipsnakes diverged across this feature 1.87 Mya, which is the most recent diversification event in *M. flagellum*. Yet despite this recent divergence, we recover completely sorted lineages in the mitochondrial and nuclear data sets at this feature. This differs from the older divergence at the PR that shows higher levels of admixture or incomplete lineage sorting (ILS; Figures 2a and 4a). *Coluber constrictor* also exhibits lineage divergence at the MR. Burbrink et al. (2008) found that *C. constrictor* diverged 6.09 Mya at the MR, while our divergence time estimates placed this split at 4.42 Mya. This discrepancy may be due to our reduced lineage sampling for *C. constrictor*, despite utilizing the same calibration points. However, our estimates fall well within the confidence limits of this split estimated by Burbrink et al. (2008). In *C. constrictor*, Burbrink et al. (2008) did not find the northern end of the MR to be an effective barrier. For *M. flagellum*, which does not extend as far north, the MR remains a consistent barrier. Thus, we found that the MR serves as a strong barrier for many taxa and that *M. flagellum* and *C. constrictor* have diverged across it at different times and have exhibited distinct biogeographical histories.

The Cochise Filter Barrier has a complex geological and climatological history, which may have isolated species asynchronously (Myers, Hickerson, & Burbrink, 2017). The uplift of the Sierra Madre Occidental during the late Miocene, the formation of the Sonoran and Chihuahuan deserts during the Pliocene, and isolation in Pleistocene glacial refugia may have all influenced species diversification at this feature (Axelrod, 1979; Moore & Jansen, 2006; Morafka, 1977; Wilson & Pitts, 2010a). Myers et al. (2017) identified 12 snake population or species pairs that were genetically differentiated at the CFB. They found that most snake species diverged at this feature during the Pleistocene or Pliocene, but that two species diverged ~6 Mya (*M. flagellum* and *Hypsiglena torquata*). Our divergence dating suggested that *M. flagellum* diversified at the CFB at 7.59 My, an event likely influenced by Miocene mountain building, and later reinforced by Pliocene Desert formation. This Miocene diversification differs from most other codistributed snakes, which diverged at the CFB due to Pleistocene glacial cycles or Pliocene aridification (Myers et al., 2017). In fact, divergence at the CFB is the oldest within-species divergence event observed in whipsnakes, supporting a Sonoran origin and subsequent eastward colonization for *M. flagellum*. Interestingly, the opposite pattern was observed in *C. constrictor*, which seems to have an eastern origin followed by westward expansion (Burbrink et al., 2008).

The Pecos River separates the Chihuahuan Desert from the North American Grasslands (Morafka, 1977). This region between the PR and the CFB has been largely shaped by Pleistocene era processes, as glacial cycles created refugia that separated the Sonoran

and Chihuahuan Deserts (Riddle & Hafner, 2006). However, the formation of the Chihuahuan Desert during the Pliocene may have also isolating species into discrete habitats (Wilson & Pitts, 2010a). The PR inhibits gene flow of several other taxa, including rattlesnake populations (*Crotalus atrox*; Schield et al., 2015), several species of fence lizards (*Sceloporus magister*, *S. undulates*, *S. cowlesi*, *S. consobrinus*; Leaché, 2009; Leaché & Mulcahy, 2007; Leaché & Reeder, 2002) and several species of mice (*Chaetodipus penicillatus*, *Peromyscus maniculatus*, *Peromyscus eremicus* and *Onychomys* spp.; Lansman, Avise, & Aquadro, 1983; Lee, Riddle, & Lee, 1996; Riddle & Honeycutt, 1990; Walpole, Davis, & Greenbaum, 1997). While past studies have found that individuals occupying the Chihuahuan Desert region are most closely related to either Sonoran Desert or Colorado Plateau populations (west of the PR; Leaché & Mulcahy, 2007), our study found that the Chihuahuan clade of *M. flagellum* was most closely related to the western and eastern clades (east of the PR; Figure 3a). This difference suggests that either the PR is more permeable to whipsnakes than the CFB, or it could reflect the more recent divergence between the western, eastern and Chihuahuan clade. We also recovered higher rates of migration (and admixture or ILS) across the PR than the CFB (Figures 2a, 4a, and 5). Finally, we found that the timing of divergence of this clade (4.15 Mya) likely corresponds to Pliocene desertification, rather than the more recent formation of the PR.

Our mitochondrial and nuclear clustering analyses found that populations of *M. taeniatus* on either side of the RM were genetically distinct. We also found asymmetrical rates of migration between these two populations from west to east. Our divergence dating of the two *M. taeniatus* clades estimated very recent divergence for these clades, suggesting that the ancient formation of the feature did not separate an already widespread species. However, we emphasize caution in the interpretation of our results regarding *M. taeniatus* due to the small sampling sizes. Our mitochondrial and nuclear data sets are consistent with the RM having isolated this species, but more complete sampling is necessary.

The Gulf of California separated the Baja California Peninsula from western Mexico 5.5–4.0 Mya (Lonsdale, 1991). This barrier has isolated many taxa: mammals, birds, snakes and insects, and many species are endemic to the peninsula (Castoe, Spencer, & Parkinson, 2007; Grismer, 2000; Rodríguez-Robles & De Jesús-Escobar, 2000). Our mitochondrial data were consistent with past studies in that both our species sampled from the peninsula, *M. lateralis* and *M. flagellum*, had unique haplotypes found there. Our divergence dating estimated divergence at this barrier for *M. flagellum* at 4.73 My, and at 3.81 Mya for *M. lateralis*. Both these data estimates are after the formation of the feature, indicating that these species likely invaded the peninsula from the North after it had separated from the mainland.

The Isthmus of Tehuantepec has been implicated in the diversification of birds, amphibians, reptiles, mammals and plants (Table 1). The IT was submerged until the late Miocene or early Pliocene (~6 Mya; Barrier et al., 1998; Ornelas et al., 2013). Therefore, unlike the MR, the IT represents an ancient barrier that likely no longer isolates terrestrial species. We found that the IT separates two distinctive nuclear clusters of *M. mentovarius* (Figure 3b). Our migration analyses suggested that migration has proceeded largely from east to west across this feature (Figures 5 and 6c). For this reason, we expected smaller effective population sizes in the west, but our MIGRATE-N analysis estimated equal effective population sizes (Table S3). This may indicate that further population subdivision occurs to the south of Guatemala and Honduras, but we lacked substantial sampling there.

Our study has emphasized the role of geographical features such as rivers, an isthmus, mountains, and depressions as forces of diversification based on their ability to divide populations into isolated units. We quantified the influence specifically of the Mississippi River, the Pecos River, the Cochise Filter Barrier, the Rocky Mountains and the Isthmus of Tehuantepec. We found that each of these features has likely played a role in the diversification of snake species that are distributed across them. This study supports the tenant that allopatric divergence is the predominant mode of diversification for terrestrial vertebrates, even among relatively vagile and widely distributed animals like whipsnakes.

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DATA ACCESSIBILITY

Mitochondrial sequence data is available on GenBank with Accessions KT713652–KT713738. Genomic sequences are available on the Sequence Read Archive SRS1047195–SRS1047343. See Table S1 for full details.

AUTHOR CONTRIBUTIONS

KAO, JWS, ENS and MFK conceived the ideas for this project. KAO and JWS conducted laboratory work, KAO analysed the data, and KAO, JWS, and MFK wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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