



The phylogenomic and biogeographic history of the gartersnakes, watersnakes, and allies (Natricidae: Thamnophiini)

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ABSTRACT

North American Thamnophiini (gartersnakes, watersnakes, brownsnakes, and swampsnakes) are an ecologically and phenotypically diverse temperate clade of snakes representing 61 species across 10 genera. In this study, we estimate phylogenetic trees using ~3,700 ultraconserved elements (UCEs) for 76 specimens representing 75% of all Thamnophiini species. We infer phylogenies using multispecies coalescent methods and time calibrate them using the fossil record. We also conducted ancestral area estimation to identify how major biogeographic boundaries in North America affect broadscale diversification in the group. While most nodes exhibited strong statistical support, analysis of concordant data across gene trees reveals substantial heterogeneity. Ancestral area estimation demonstrated that the genus *Thamnophis* was the only taxon in this subfamily to cross the Western Continental Divide, even as other taxa dispersed southward toward the tropics. Additionally, levels of gene tree discordance are overall higher in transition zones between bioregions, including the Rocky Mountains. Therefore, the Western Continental Divide may be a significant transition zone structuring the diversification of Thamnophiini during the Neogene and Pleistocene. Here we show that despite high levels of discordance across gene trees, we were able to infer a highly resolved and well-supported phylogeny for Thamnophiini, which allows us to understand broadscale patterns of diversity and biogeography.

1. Introduction

The complex geological history of North and Central America during the Cenozoic has been a major driver of broadscale floral and faunal diversification and biogeographic patterns (Brown, 1904; Soltis et al., 2006; Flores-Villela and Martínez-Salazar, 2009; Rissler and Smith, 2010). This diversity is likely influenced by biogeographic boundaries that limit dispersal and gene flow (Mayr, 1963; Wang and Bradburd, 2014). Many phylogeographic studies have demonstrated the influence of major biogeographic boundaries on North and Central American biodiversity, including the Apalachee Formation (Walker and Avise, 1998; Lemmon et al., 2007; Krysko et al., 2017), the Florida Peninsular Border (McKelvy and Burbrink, 2017), the Mississippi River (Near et al., 2001; Cullingham et al., 2008; Brandley et al., 2010; Burbrink et al., 2021), the Rocky Mountains (Lamb et al., 1989; Leaché and Reeder, 2002; O'Connell et al., 2017), the Cochise Filter Barrier (Zink et al., 2001; Riddle and Hafner, 2006; Myers et al., 2019; Provost et al., 2021),

the Trans-Mexican Volcanic Belt (TMVB; McCormack et al., 2008; Morrone, 2010; Bryson et al., 2011; Mastretta-Yanes et al., 2015; Halfter and Morrone, 2017), and the Isthmus of Tehuantepec (Ranamoorthy et al., 1993; Morrone, 2014; Gray et al., 2019).

While these biogeographic boundaries are well documented across disparate taxa, their effect on diversity can be clade specific (Simpson, 1940; Fenker et al., 2021; Araya-Donoso et al., 2022). For instance, depending on timing of dispersal into North America, a lineage may be better adapted to cross river barriers than mountain barriers (Makowsky et al., 2010). Therefore, in North America, the Rocky Mountains would prove to be more impassable than the Mississippi River for some taxa. Additionally, dispersal/vicariance events can occur within a relatively short timeframe along these biogeographical boundaries. These dispersal/vicariance events may enhance speciation given ecological opportunity presented by dispersal to new areas or changes in habitat (Simpson, 1953; Yoder et al., 2010) as well as limiting secondary contact and gene flow. These rapid radiations should also increase the

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probability of incomplete lineage sorting (ILS; Maddison, 1997) resulting in greater phylogenetic uncertainty at those nodes transition between biogeographic barriers (Burbrink and Gehara, 2018; Thom et al., 2018; Singhal et al., 2021). It is therefore important to examine clades whose distributions cross these biogeographic boundaries to gain a greater understanding of how these features influence broadscale diversity.

Thamnophiini are an ecologically diverse tribe of North American snakes in the family Natricidae. They include some of the most abundant and well-studied snakes in North America, including gartersnakes, watersnakes, brownsnakes, and swampsnakes. The tribe comprises 61 currently recognized species within 10 genera and is distributed throughout North America from Alaska to Costa Rica and contains the only extant members of Natricidae in the New World (Uetz, 2021). Thamnophiini diverged from the rest of Natricidae upon dispersal to North America through Beringia in the mid-Oligocene approximately 27 Mya (Guo et al., 2012). As expected, given their wide distribution, Thamnophiini are ecologically diverse, inhabiting temperate deciduous forests, swamps, prairies, highlands, deserts, tropical forests, and now urban environments (Rossman, 1996; Gibbons and Dorcas, 2004). Furthermore, members of the clade display a wide variety of diet preferences from a generalist diet of small vertebrates to more specialized diets targeting worm-like invertebrates and crayfish (Savitzky, 1983; Grundler and Rabosky, 2021).

Thamnophiini represent an asymmetric radiation with 57% of the species diversity represented by the garter snakes (genus *Thamnophis*, 35 sp.), whereas the other major clades are much less diverse (genus *Nerodia*, 10 sp.; genus *Storeria*, 5 sp.; genus *Regina*, 2 sp.). This asymmetry is also represented in a geographic context; *Thamnophis* is generally the only genus that natively occurs west of the North American Western Continental Divide, where it spread throughout the region (Rossman, 1996; Gibbons and Dorcas, 2004; Heimes, 2016). In addition, Thamnophiini has greater phylogenetic species variability [a metric summarizing how closely related species in a community are in a phylogeny (Helmus et al., 2007)] in the Eastern Nearctic North America than in the Western Nearctic (Burbrink and Myers, 2015), with communities in the east having greater phylogenetic distance among them, relative to communities in the rest of North America. This contrasts with other squamate groups that diversified in North America during the Pleistocene, where phylogenetic species variability is greatest in Western Nearctic or in tropical Central America. This would imply that biogeographic boundaries that delineate eastern and western North America, such as the Rocky Mountains or the southwestern deserts, may be driving broadscale diversity of Thamnophiini by acting as a barrier to dispersal for most Thamnophiini lineages.

Members of Thamnophiini have been used as model systems in behavior (Arnold and Wassersug, 1978; Herzog and Burghardt, 1987; Arnold and Bennett, 1988), ecology (Carpenter, 1952; Jayne and Bennett, 1989; Bronikowski and Arnold, 1999), physiology (Mendonça and Crews, 1996; Robert and Bronikowski, 2010), sexual dimorphism evolution (Burbrink and Futerma, 2019), evolutionary adaptation (Arnold, 1992; Feldman et al., 2010), and arms-race coevolution (Brodie, 1989; Brodie and Brodie, 1999; Hanifin et al., 2008; Reimche et al., 2020) in squamates. However, despite the wealth of studies in this group, their evolutionary and biogeographic history remains unresolved. Previous molecular systematic studies of the group have incorporated allozyme sequences (de Queiroz and Lawson, 1994), mitochondrial DNA (mtDNA), and under ten nuclear genes to infer phylogenetic history that have led to recent taxonomic changes (McVay and Carstens, 2013; McVay et al., 2015). However, due to the limited amount of genetic data available for these studies, several major lineages of the group remain phylogenetically unresolved with low statistical support. Hallas et al. (2022) used genome-wide SNPs in their systematics study on the genus *Thamnophis* but did not include other genera in the clade. Additionally, previous systematics studies hypothesized a western Mexican origin for *Thamnophis* (Ruthven, 1908; Alfaro

and Arnold, 2001; de Queiroz et al., 2002; Hallas et al., 2022). Nevertheless, the dispersal history of the group within North America has not been analyzed using recently developed dispersal and vicariance models in a phylogenetic context or have only focused on subclades within Thamnophiini (Hallas et al., 2022).

We sequenced ultraconserved elements (UCEs) for 76 specimens representing 75% of all Thamnophiini species to investigate the evolutionary history of the tribe. We hypothesize that a phylogeny inferred from genomic data will resolve previously recalcitrant nodes in the phylogeny. Using a robust phylogeny, we conduct ancestral area estimation based on bioregions estimated from species occurrence data, to infer the biogeographic history of the tribe. Our biogeographic analyses allow us to determine if major geographic barriers, such as the Rocky Mountains, are drivers of broadscale thamnophiine diversity. Finally, we analyze levels of discordance across gene trees and compare this with biogeographic analyses to determine if levels of discordance are correlated with cladogenetic events that represent dispersal events across major geographic barriers in North America.

2. Methods

2.1. DNA extraction, target capture, and sequencing

We extracted DNA using a Qiagen DNeasy Blood and Tissue Kit, from tissues of 76 individuals representing all 10 genera and 75% of species of Thamnophiini species taken from wild caught individuals from the following collections: the American Museum of Natural History, California Academy of Sciences, Florida Museum of Natural History, and Louisiana Museum of Natural History. We targeted 5,060 UCEs with the Tetrapods-UCE-5Kv1 probe set (Faircloth et al., 2012), which has been used successfully in large-scale phylogenomic studies of vertebrate groups to resolve difficult nodes (McCormack et al., 2013; Crawford et al., 2015). Quality control, library prep, and Illumina HiSeq sequencing were completed through RapidGenomics (<https://rapid-genomics.com>) and the authors received raw FASTQ files for subsequent analyses.

2.2. Data processing

We conducted assembly, target matching, phasing, and alignment of demultiplexed Illumina reads using the PHYLUCE package (Faircloth, 2016). Filtering of low-quality reads and removal of Illumina adapters were done using Illuminaprocessor (Faircloth, 2013), and trimmomatic (Bolger et al., 2014), respectively. Assembly of the trimmed reads was done using the SPAdes assembler in PHYLUCE (Bankevich et al., 2012). To confirm species ID for each sequence, we extracted mtDNA from the assembled reads using MitoFinder (Allio et al., 2019) and ran the mtDNA through BLAST (Ye et al., 2006). To account for heterozygous loci using multispecies coalescent methods (Huang et al., 2021), we conducted allele phasing using the phyluce_workflow program, which follows the protocol outlined by Andermann et al. (2018). The phased alleles were then aligned using mafft (Katoh and Standley, 2013).

2.3. Phylogenomic analyses

We generated phylogenies based on all phased UCEs. We estimated models of substitution for each locus using ModelFinder implemented in IQ-TREE 2 (Minh et al., 2020b), which uses maximum likelihood to determine best fit models out of 22 substitution models (Kalyaanamoorthy et al., 2017). We inferred a phylogeny in IQ-TREE 2 using a partitioned concatenated dataset of all phased UCE loci across 76 individuals (152 tips). We estimated branch and tree support using the ultrafast bootstrap approximation (UFboot \geq 95%, $n = 1000$; Hoang et al., 2018) and the Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH- $alrt \geq$ 80%, $n = 1000$; Anisimova and Gascuel, 2006; Guindon et al., 2010).

In addition to the concatenated dataset, gene trees were estimated for each locus in the 54 dataset using the best fit substitution model and SH-aLRT support ($n = 1000$). As the dataset used in this study consists of thousands of loci, it is reasonable to assume that there would be high levels of gene tree discordance and ILS (Edwards, 2009). To infer species trees while accounting for ILS, we used ASTRAL-III, which reconstructs species trees from gene tree inputs while accounting for gene tree discordance (Zhang et al., 2018). ASTRAL uses dynamic programming to search for trees with the greatest number of quartet topologies with input gene trees. ASTRAL-III also computes a local posterior probability (PP), which is a Bayesian tree branch support value based on gene tree quartet frequencies ($PP \geq 95\%$, Sayyari and Mirarab, 2016). As IQ-TREE 2 forces bifurcation in its output, nodes with SH-aLRT support = 0 were collapsed to reflect gene tree topology more accurately for ASTRAL (Simmons and Gatesy, 2021).

2.4. Divergence dating

We performed divergence dating of the ASTRAL-constrained phylogeny using the penalized likelihood approach in TreePL (Smith and O'Meara, 2012) and fossil constraints (Heath et al., 2014). The ASTRAL tree topology was used to constrain the concatenated dataset in IQ-TREE 2 to produce a species tree topology with branch lengths in substitution rates (Rabiee and Mirarab, 2020). Eight calibration points were used and placed on nodes given taxonomic and age information based on the fossil record obtained through the literature (Holman, 2000) and Paleobiology Database (Alroy et al., 2008; Supplementary Table 1). Calibrations were defined as the maximum and minimum based on the lower and upper boundaries of the geologic time age of the fossils. We parameterized the penalized likelihood analysis using a cross-validation approach to choose an appropriate smoothing parameter to quantify changes in rates across the phylogeny (Sanderson, 2002). We performed cross-validation across 15 iterations of the smoothing parameter from $1 * 10^{-9}$ to $1 * 10^7$ using the thorough option to ensure that runs iterate to convergence.

2.5. Gene tree discordance

As bootstraps or posterior probabilities can overestimate support, they may not provide comprehensive measures of underlying concordance across genes and gene trees in a large dataset (Taylor and Piel, 2004; Edwards et al., 2016; Thomson and Brown, 2022; Mount and Brown, 2022). We therefore examined gene and site concordance factors (gCF and sCF, respectively). We estimated gCF and sCF values across all nodes of the concatenated and ASTRAL-constrained trees in IQ-TREE 2 (Minh et al., 2020a). We used an R script to calculate whether the concordance factors support that discordance is derived from ILS with a chi-square test. If discordance across gene trees is caused by ILS, then the number of gene trees or sites that support alternative topologies should be essentially equal (Huson et al., 2005; Green et al., 2010; Martin et al., 2015).

To compare gene trees to the species tree we implemented phyparts (Smith et al., 2015), which measures the number of gene trees that are either concordant or discordant at each node across the species tree. From these results we determined the level of discordance across nodes if conflict is due to one frequent alternative topology or several low-frequency topologies. Additionally, to determine spatial patterns of gene tree discordance, measured as the ratio of discordant gene trees to the total number of resolved gene trees at each node, we mapped the time-integrated estimate of gene tree discordance in each lineage leading to each extant tip in the phylogeny (Title and Rabosky, 2019; Singhal et al., 2021). We estimated range data for each species based on cleaned occurrence points obtained from digital depositories (Supplementary Table S2).

We tested whether diversity across western lineages and along discordant nodes is associated with accelerated speciation rates. We

estimated branch-specific rates in ClaDS (Maliet et al., 2019), a Bayesian approach that relies on a model where shifts in diversification rates occur at each speciation event. We fitted the ClaDS2 model, which assumes varying extinction rates and constant turnover. To detect clade-specific shifts in speciation rates a priori, we used the "search.shift" function in *RRphylo* (Castiglione et al., 2019). This function takes branch-specific rates of speciation estimated by the previous ClaDS analysis and computes the difference between the mean rates of all clades of a given size and the rest of the tree. Clade-specific differences are then compared to a null distribution of rate differences obtained through the randomization of branch rates.

2.6. Ancestral area estimation

To determine the appropriate biogeographic areas for use in ancestral area estimation, we ran Infomap Bioregions (mapequation.org/bioregions/) to identify potential bioregions in a clade based on georeferenced occurrence data using a bipartite network approach (Edler et al., 2017). This network approach has been shown to identify transition zones, here defined as areas where two bioregions connect (Ruggiero and Ezcurra, 2003; Morrone 2004; Morrone 2006), while also considering sampling biases (Vilhena and Antonelli, 2015). This approach allows us to determine valid bioregions specific to the clade of study rather than relying on ecoregions identified from other studies of unrelated communities (Omernik and Griffith, 2014). Therefore, we can more easily identify which biogeographic boundaries more heavily influence species distributions within *Thamnophis*, providing a more appropriate set of bioregions to test. Georeferenced occurrence data were collected for each species through the R package *spocc* (Chamberlain et al., 2021) and retrieved from digital depositories Global Biodiversity Information Facility (GBIF), Integrated Digitized Biocollections (iDigBio), Berkeley Ecoinformatics Engine, Biodiversity Information Serving Our Nation (BISON), and Vertnet. Data were cleaned using the R package *scrubr* (Chamberlain, 2021) to remove data with incomplete/unlikely coordinates and duplicate dates/localities. Occurrence points were further confirmed with published range maps (Rossman, 1996; Gibbons and Dorcas, 2004; Heimes, 2016). Species occurrence data are susceptible to spatial sampling bias, with sampling inclined towards easily accessible areas near urban centers, human settlements, roads, paths, and rivers (Kadmon et al., 2004; Engemann et al., 2015; Meyer et al., 2016; Zizka et al., 2021). To reduce the effect of sampling bias we thinned occurrence points spatially using the R package *spThin* (Aiello-Lammens et al., 2015) with a buffer of 5 km. The cleaned occurrence data were input into Infomap Bioregions where clustering was performed across 10 trials with a standard cluster cost of 2.5 at a 16" maximum cell size resolution to avoid overfitting of bioregion inference due to outliers in occurrence point data.

Ancestral area estimation was conducted through BioGeoBEARS (Matzke, 2013), which implements both maximum likelihood and Bayesian inference to test different models of historical biogeography along a phylogeny. Using the corrected Akaike information criterion (AICc), we compared three different range evolution models: 1) the Dispersal-Extinction-Cladogenesis (DEC; Ree and Smith, 2008) model, which allows either vicariance or partial sympatric speciation if one of the descendant lineages occurs only in a single region, 2) the Dispersal-Vicariance model (DIVA-like; Ronquist, 1997), which allows vicariant speciation even if both descendant lineages have widespread ranges, and 3) the BayArea-like model (Landis et al., 2013), which does not allow range evolution to occur during cladogenesis. We also ran each of these models with a J parameter, which accounts for founder-effect speciation of a descendant lineage where the region is not occupied by the ancestor (Matzke, 2014; 2022).

3. Results

3.1. UCE evaluation

Based on a 95% completeness matrix, we recovered 3785 loci totaling 3,372,860 base pairs and 590,629 parsimony informative sites. Our results from MitoFinder and BLAST indicated that all specimens were correctly identified. One specimen included in our dataset was identified as a hybrid between *Thamnophis sirtalis parietalis* and *T. radix* (CAS Herp-172016, RL-B14P284c). Blasting the mitochondrial locus identified this specimen as *T. sirtalis*, whereas it was grouped with the *T. radix* specimen in both the concatenated and MSC datasets.

3.2. Phylogenomic analyses

The maximum-likelihood analysis of the concatenated dataset yielded strong bootstrap support (UFboot \geq 95%) across all nodes (Fig. 1). The ASTRAL species tree showed strong local posterior probability support (PP \geq 95%) for all but two deep nodes in the phylogeny. Several nodes were discordant between the concatenated tree and the ASTRAL species tree. *Thamnophis* comprised two clades, the “Mexican” clade consisting mainly of species found within the Mexican Transition Zone (MTZ) and the “Widespread” clade mainly consisting of species found within the United States and Canada, with the ranges of three species extending into Mesoamerica (*T. eques*, *T. marcianus*, and *T. proximus*) showing strong statistical support (PP = 0.99, UFboot = 100, SH-aLRT

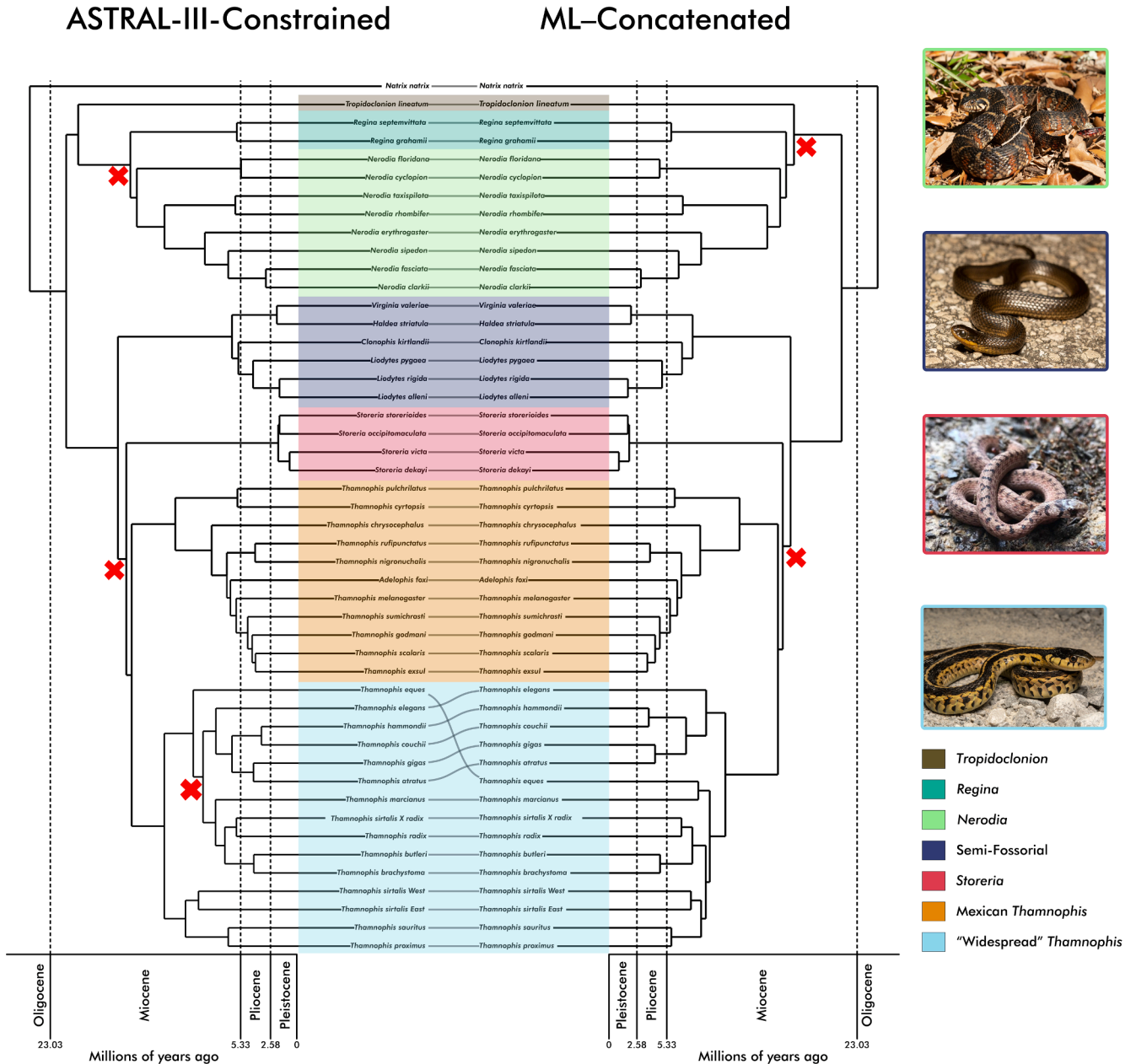


Fig. 1. Penalized likelihood fossil calibrated species tree of Thamnophiini with geological scale from constrained trees based on ASTRAL-III and concatenated analyses (left) and maximum likelihood concatenated dataset (right). Major clades are highlighted and color-coded on each phylogeny. Unhighlighted tips in both trees from the outgroup. Dashed lines are attached to identical tips on each phylogeny to highlight congruences between phylogenies. Nodes with relatively low statistical support (BS < 0.70, PP < 0.95) across both trees are marked with a red X. Photographs are from top to bottom: *Nerodia fasciata*, *Liodytes rigida*, *Storeria dekayi*, and *Thamnophis sirtalis*. Photographs for *N. fasciata*, *L. rigida*, and *T. sirtalis* were kindly provided by Court Harding. Photograph of *S. dekayi* is by Frank T. Burbrink.

= 100). Both specimens of *Adelophis foxi* were placed within the “Mexican” clade, as the sister-taxon to *T. melanogaster*, with strong statistical support (PP = 1, UFboot = 100, SH-aLRT = 99.8). One major difference between the concatenated and ASTRAL-constrained topologies was within the “Widespread” *Thamnophis* clade. In the concatenated topology, the clade containing *Thamnophis* species from the western United States formed the sister taxon to all other *Thamnophis* lineages, whereas in the ASTRAL-constrained topology the clade comprising *T. proximus*, *T. sirtalis*, and *T. sauritus* was the sister taxon to a clade comprising the other lineages of *Thamnophis*. *Regina* was monophyletic in both the concatenated and ASTRAL topologies with strong statistical support in both trees. However, the node identifying the sister-taxon relationship between *Regina* and *Nerodia* showed low support values across all metrics (PP = 0.75, BS = 75, SH-aLRT = 60.2) and had low concordance (gCF = 1.5 %, sCF = 34.1 %). *Haldea* and *Virginia* were strongly supported as sister taxa (UFboot = 100, SH-aLRT = 100), though concordance factors and posterior probabilities were relatively low (gCF = 7.96 %, sCF = 38.1 %, PP = 0.79). *Liodytes pygaea* was the sister-taxon to other species of *Liodytes* in both the concatenated and ASTRAL topologies with high statistical support (PP = 1, UFboot = 100, SH-aLRT = 100) and concordance factors (gCF = 9.911 %, sCF = 44.2 %). In both phylogenies, *Storeria* was the sister-taxon to *Thamnophis*, but overall node support was relatively low (PP = 0.87, UFboot = 77, SH-aLRT = 75.9).

A major conflict between the concatenated and ASTRAL-constrained topologies occurred in the “Widespread” *Thamnophis* clade. In the ASTRAL-constrained phylogeny, *T. eques* was the sister taxon to the clade containing both the western (*T. atratus*, *T. couchii*, *T. elegans*, *T. gigas*, *T. hammondii*) and midwestern *Thamnophis* (*T. brachystoma*, *T. butleri*, *T. marcianus*, *T. radix*) clades combined with high levels of support (PP = 1, UFboot = 100, SH-aLRT = 100); however, the western *Thamnophis* clade was weakly supported as the sister taxon to the midwestern *Thamnophis* clade (PP = 0.54). Conversely, the concatenated phylogeny placed *T. eques* as sister to *T. marcianus* (UFboot = 100, SH-aLRT = 92), and the western *Thamnophis* clade was the sister taxon to the other of the “Widespread” *Thamnophis* lineages (UFboot = 100, SH-aLRT = 100).

3.3. Divergence dating

Our penalized likelihood analysis estimated a divergence date for crown Thamnophiini lineages at ~21.57 Mya (95% range: 21.55–21.66 Mya), which aligns with fossil data, where natricid fossils (*Neonatrix*, Holman, 1973) first appeared in North America during the Early Miocene. However, this date conflicts with the findings of McVay et al. (2015), which showed a date for the crown Thamnophiini at ~15 Mya. All divergence dates were largely consistent with the fossil record, with most lineages first appearing in the late Miocene to the late Pliocene. For each major clade: crown *Nerodia* was estimated to have originated at ~15.2 Mya (95% CI: 15 – 16.61 Mya), crown *Thamnophis* appeared at ~15.56 Mya (95 % CI: 15.21 – 16.51 Mya), the Mexican *Thamnophis* clade was estimated to be ~12.22 Mya (95% CI: 10.38 – 14.07 Mya), the “Widespread” *Thamnophis* clade ~11.97 Mya (95% CI: 9.34 – 14.99 Mya), and the “semi-fossorial” clade ~5.4 Mya (Fig. 1). These results are slightly different from previous studies, where results from McVay et al. (2015) estimated the divergence of crown *Nerodia* at ~13 Mya. Additionally, Hallas et al. (2022) estimated crown *Thamnophis* at ~12.21 Mya. Our estimates of divergence times for both the Mexican and “Widespread” clades in our study fall within the 95% highest posterior density interval of divergence date estimates in Hallas et al. (2022).

3.4. Phylogenetic discordance

Approximately 50% of gene-to-species tree discordance can be explained through ILS, including gene-to-species tree discordance at the node subtending *Regina* and *Nerodia* and the base of the western

Thamnophis clade. However, using Chi-square tests, 45.9% of gene-to-species tree discordance show statistical significance for rejecting ILS. Additionally, 39.87% of site discordance showed significance in rejecting ILS.

Across all nodes in the Thamnophiini tree, 56.35%–99.81% (mean = 91.00%) of resolved gene trees were discordant with the species tree. Additionally, the most common conflicting topology at each node only accounts for 0.13%–0.62% (mean = 0.36%) of resolved tree nodes, which indicates that discordance is primarily due to multiple low-frequency conflicting topologies (Fig. 2A).

At nodes with low branch support, discordance levels were relatively high, with the highest occurring at the node separating the western *Thamnophis* clade from the midwestern *Thamnophis* clade (99.81%). The average gene tree discordance over geographic space spanning across each species distribution ranged between 77.77% and 99.37% (mean = 91.85%; Fig. 2B). Furthermore, higher levels of discordance occur in transition zones where two bioregions connect.

Based on our ClaDS analysis we found two significant shifts in speciation rates across the ASTRAL-constrained phylogeny: one at the root of *Storeria* showing a significant increase in speciation rates ($p = 1$) and one at the root of the “widespread” *Thamnophis* clade showing a significant decrease in speciation rates ($p = 0.015$). The concatenated phylogeny had the same two shifts and a third one at the root of the (*Tropidoclonion*, (*Nerodia*, *Regina*)) clade showing a significant decrease in speciation rates ($p = 0.007$).

3.5. Ancestral area estimation

Infomap Bioregions produced four distinct bioregions based on our occurrence data (Fig. 3B). Bioregion 1 (the largest), encompassed both the Nearctic and Neotropical realms. The region consisted of the Eastern and Central Nearctic east of the Western Continental Divide including the Nearctic Northern Forests ecoregion of Canada, the Eastern Temperate Forests, the Great Plains, the Chihuahuan Desert province, and the Tamaulipas province. Additionally, it extended southward along the coast of the Gulf of Mexico into the Northern section of the Sierra Madre Oriental Province, the Veracruz, and Yucatán Peninsula provinces, and includes sections of Central America south of the Chiapas Highlands (Morrone, 2014; Omernik and Griffith, 2014). Bioregion 2 is west of Bioregion 1 and entirely within the Nearctic. This bioregion consisted of the Western United States and Canada west of the Rocky Mountains. It also extended southward to Northern Baja California and the Sonoran Province. Bioregion 3 is south of the first two bioregions and includes both Nearctic and Neotropical areas. This bioregion largely aligns with the MTZ (Morrone, 2006) and includes the southwestern United States, south of the Great Basin, and the Sonoran Desert. The bioregion also extends southward along the Pacific versant to include the Sierra Madre Occidental, TMVB, Sierra Madre del Sur, Pacific Lowlands, and Balsas Basin provinces. This bioregion also includes southern sections of the Baja California province, likely due to the presence in the occurrence dataset of *Thamnophis validus*, which de Queiroz and Lawson (2008) hypothesize dispersed to peninsular Baja California from mainland Mexico. Finally, Bioregion 4 was found solely within the Neotropics. The bioregion consisted of the Central American Nucleus south and east of the Isthmus of Tehuantepec and the Sierra Madre de Chiapas province.

The BioGeoBEARS analysis identified the DEC model as the best supported model for both the concatenated and MSC phylogenies based on the corrected Akaike information criterion (Supplementary Tables S3 and S4, Fig. 3C). Under this biogeographic model, the crown lineage of Thamnophiini originated within Bioregion 1 ~21.5 Mya. This area was consistent with fossil occurrence data showing the earliest Thamnophiini fossils in Nebraska (Holman, 1987; Voorhies et al., 1987), South Dakota (Holman, 1979), and Texas (Rage, 1984) from the Early and Middle Miocene and was consistent with previous ancestral area estimates (Guo et al., 2012, McVay et al., 2015). Most major lineages

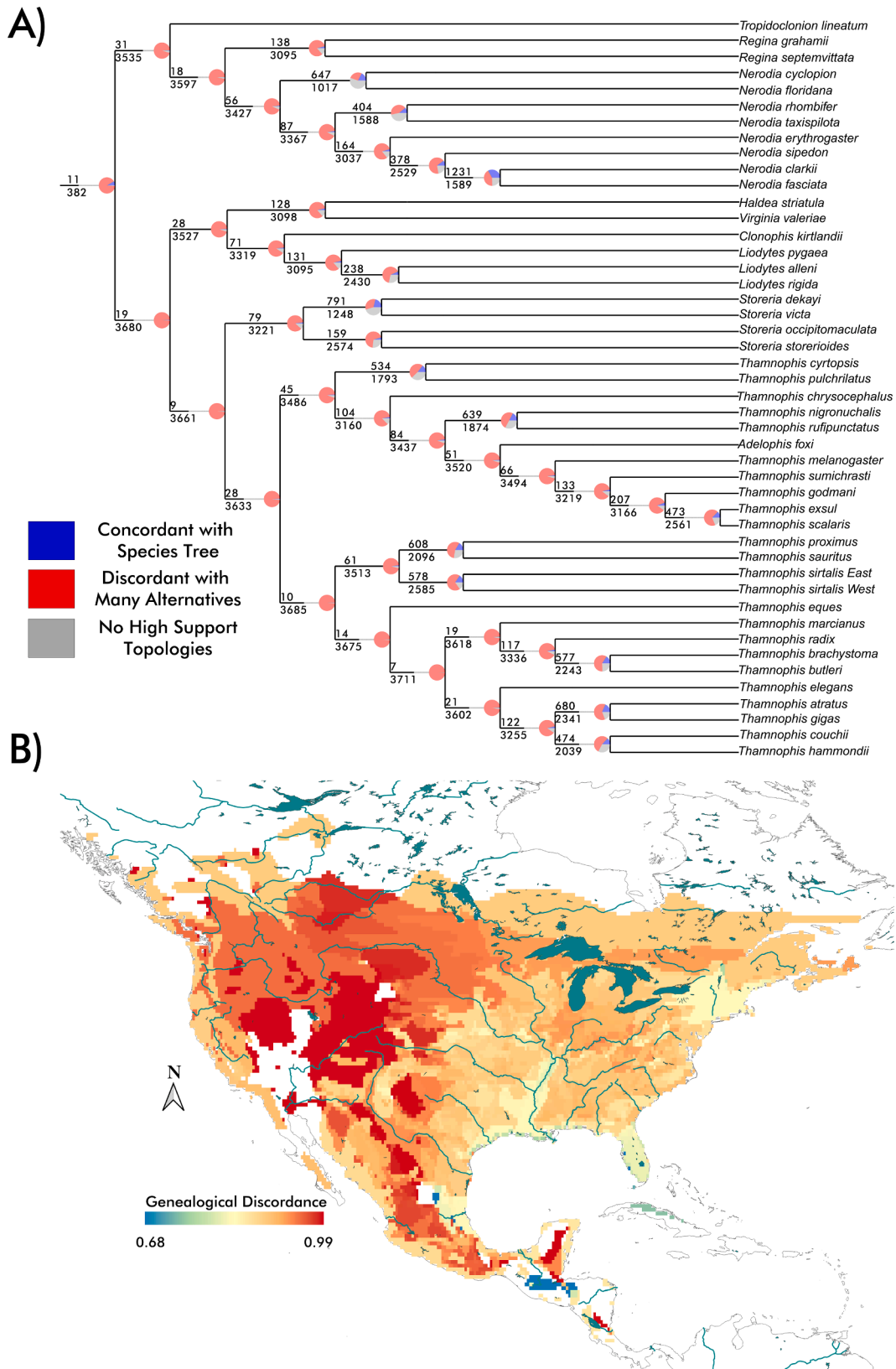


Fig. 2. A) ASTRAL-III Constrained species tree topology, with summary of conflicting and concordant gene trees. For each branch, the top number indicates the number of gene trees concordant with the species tree at that node, and the bottom number indicates the number of gene trees in conflict with the species tree at that node. The pie charts represent the proportion of gene trees that support that clade (blue), the proportion that support alternatives (red) and the proportion that inform this clade that have <50% bootstrap support (grey). B) Levels of gene tree discordance across species ranges within Thamnophiini. Genealogical discordance is described as the weighted average of gene tree discordance at nodes leading to terminals.

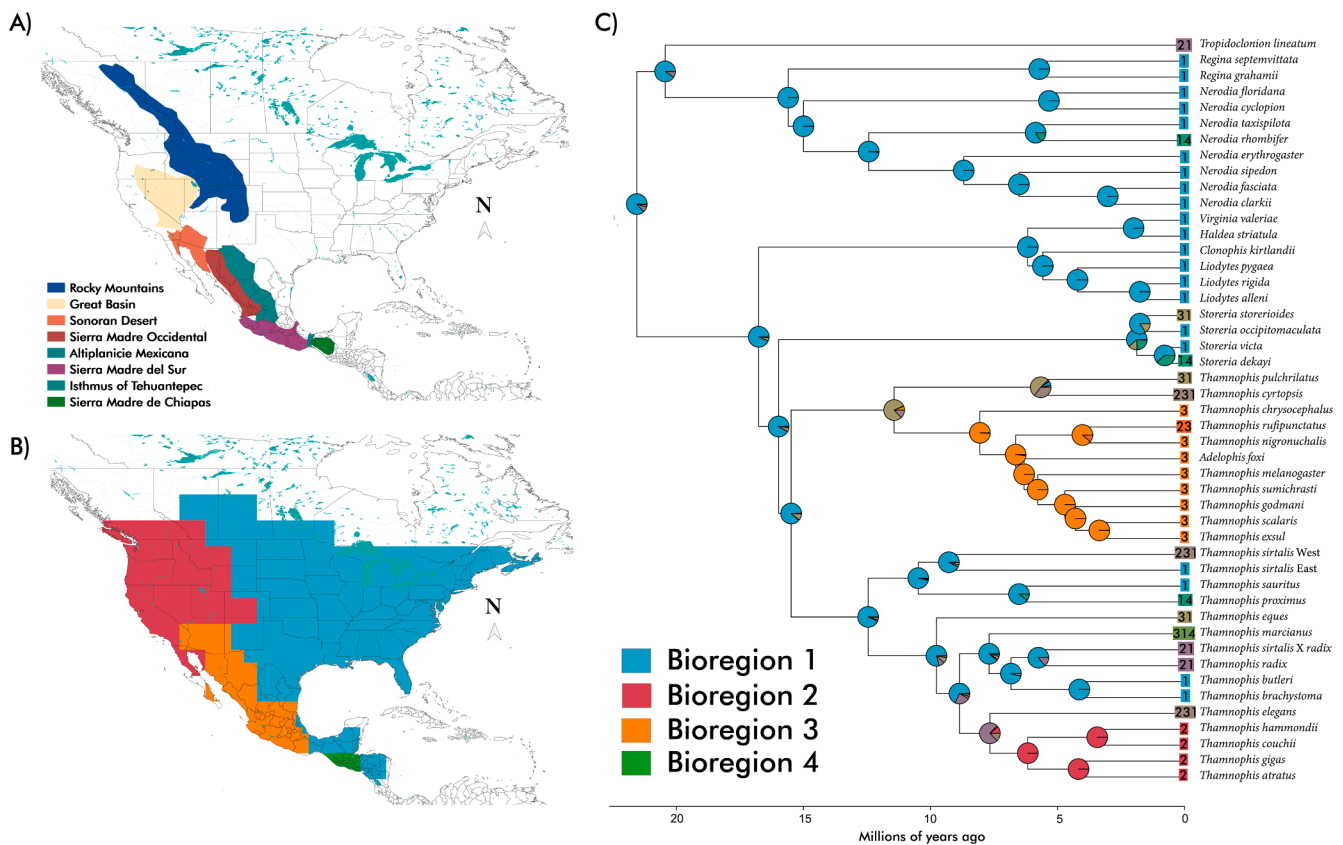


Fig. 3. Biogeographic History of Thamnophiini. A) Major geographic features that are concordant with the boundaries of the estimated bioregions. B) Bioregions map as defined by Infomap Bioregions. Bioregions are consistent with: Bioregion 1 = North America east of the Rocky Mountains, Bioregion 2 = United States west of the Rocky Mountains, Bioregion 3 = Western Mexico along the Sierra Madre Occidental, Bioregion 4 = Central American Nucleus and the Sierra Madre de Chiapas. C) Ancestral Area Estimation under the Dispersal-Extinction-Cladogenesis model from BioGeoBEARS using the ASTRAL-Constrained Species Tree. Areas are coded based on bioregions defined by Infomap Bioregions.

remained in Bioregion 1 during the Miocene until *Thamnophis* diverged from the rest of Thamnophiini approximately 15.5 Mya during the Langhian. *Thamnophis* then split into the Mexican and “Widespread” clades, and the Mexican clade dispersed into Bioregion 3 at ~11.4 Mya during the Tortonian. The “Widespread” clade remained within Bioregion 1 until the western and midwestern *Thamnophis* clades diverged ~8.86 Mya, where the western *Thamnophis* clade dispersed from Bioregion 1 to Bioregion 2. Other subsequent dispersal events occurred within individual species during the Pleistocene, including from Bioregion 1 to Bioregion 4 along the Atlantic (*Nerodia rhombifer*, *Storeria dekayi*, *T. proximus*).

4. Discussion

4.1. Historical biogeography and gene discordance

We produced a robustly estimated phylogeny of Thamnophiini using genome-scale data. These results support monophyly of most genera, an origin for the group, and subsequent diversification in the Early and Middle Miocene. Furthermore, our main biogeographic conclusions show multiple instances of dispersal from the bioregion that includes the United States and Canada east of the Rocky Mountains and the Atlantic Versant to Mexico (Bioregion 1), to the western and tropical bioregions. While only three nodes had traditionally low support, we did detect key areas showing major gene discordance that occurs at biogeographically important transition zones in the Nearctic, such as the Rocky Mountains, the Altiplanic Mexicana, and the Great Basin. Gene tree discordance is common throughout the evolutionary history of Thamnophiini but was especially high in these transition zones (up to 99.37%, greater than

99% of other nodes in the phylogeny). This level of discordance could indicate short intervals of time between successive branching events occurring as lineages disperse westward, which would provide greater opportunities for incomplete lineage sorting to occur (Degnan and Rosenberg, 2006; Pease et al., 2016; Cloutier et al., 2019; Roycroft et al., 2020; Singhal et al., 2021).

However, our ClADS analyses did not show any significant shifts in speciation rates across discordant nodes where these transitions occur. Instead, we found shifts in speciation rates across the root of the (*Tropidoclonion*, (*Nerodia*, *Regina*)) clade and the root of the “widespread” *Thamnophis* clade, both showing a significant decrease in rates. This suggests that the biogeographic transitional areas also showing gene discordance are not associated with rapid rates of speciation on each of those areas.

Our ancestral area estimation analyses supported a dispersal-extinction cladogenesis (DEC) biogeographic model and an origin of the crown lineage within Bioregion 1 ~21.56 Mya. Natricidae dispersed to North America through Beringia ~27 Mya (Guo et al., 2012), which suggests a northwest to south dispersal direction for the group. The unidirectional dispersal from the Old World to the New World across Beringia is a commonly observed pattern of dispersal across taxa (Sanmartín, 2001) and has been shown in various squamate groups such as ratsnakes (Burbrink and Lawson, 2007; Chen et al. 2017), pit vipers (Wüster et al., 2008; Alencar et al., 2016), sibynophiines (Chen et al., 2013), eublepharid geckos (Gamble et al., 2011), and *Plestiodon* skinks (Brandley et al., 2011). Additionally, fossil evidence shows Thamnophiini occurring within the central Nearctic as early as the Early Miocene (Holman, 2000). Therefore, both ancestral area estimation analyses and the fossil record support an origin for extant Thamnophiini

in the Central Nearctic in the Early Miocene, and individuals dispersed through the continent to the west and south.

While previous systematic studies hypothesized a western Mexico origin for *Thamnophis* (Ruthven, 1908; Alfaro and Arnold, 2001; Hallas et al., 2022), our ancestral area estimation analyses, which includes all other genera of Thamnophiini, support an origin for *Thamnophis* within Bioregion 1~15.49 Mya. Because Bioregion 1 also includes eastern Mexico and portions of the Sierra Madre Oriental, a Mexican origin could potentially still be correct, although such a hypothesis is not yet supported in the fossil record. The oldest fossils of *Thamnophis* have been dated to the Medial Barstovian (16.3–13.6 Mya) and were found in Nebraska (Holman, 1987; Voorhies et al., 1987). Additionally, there is fossil evidence of other naticrid genera (*Nerodia*, *Neonatrix*) during this period, supporting an origin for Thamnophiini within this area. This would make the origin of Thamnophiini coincident with the origin and dominance of grasslands throughout the central Nearctic (Rage, 1984; Holman, 2000; Sanmartín, 2001; Guo et al., 2012). We note that the fossil record and the effort applied to finding fossils may not be comparable with those in the US. We found that *Thamnophis* comprises two geographically distinct groups, the Mexican clade, and the “Widespread” clade. These two clades were consistently recovered in previous systematic studies of Thamnophiini (de Queiroz and Lawson, 1994; de Queiroz et al., 2002; McVay et al., 2015; Hallas et al., 2022). The Mexican clade is composed of species distributed throughout the MTZ and into nuclear Central America. The ancestral area for this clade is estimated to be a transition zone between Bioregion 1 and Bioregion 3~11.43 Mya. This transitional zone would encompass several of the major features that define the MTZ, including the Altiplancie Mexicana, the Sierra Madre Occidental, and the TMVB. The MTZ is biogeographically complex, especially along the TMVB, which has a sky-island dynamic between the pine-oak forested highlands and the lowlands (Mastretta-Yanes et al., 2015). During the mid-to late Miocene, the uplift of the Altiplancie Mexicana and the TMVB, produced numerous vicariant and isolating events that could have influenced diversification across a wide range of taxa (Edwards and Bradley, 2002; McCormack et al., 2008; Bryson et al., 2011; Rocha-Méndez et al., 2019; Everson et al., 2021). Based on our analyses, the separation of the Mexican clade from the “Widespread” clade would have occurred during this geologically and environmentally unstable time-period, and as a result these fluctuations in climate and topography may have influenced diversification within the clade. Phylogeographic-scale studies of species within this clade might help to clarify the role that these vicariant events had in shaping diversity of the clade.

The “Widespread” *Thamnophis* clade primarily consists of species found in temperate North America, but also contain species whose ranges extend into the Neotropics (*Thamnophis eques*, *T. marcianus*, *T. proximus*; de Queiroz et al., 2002; McVay et al., 2015; Hallas et al., 2022). The main geographic break in the phylogeny isolates the western *Thamnophis* species from the midwestern *Thamnophis* species ~8.86 Mya. The ancestral area for the western *Thamnophis* clade is estimated to be a transition zone between Bioregion 1 and Bioregion 2~7.66 Mya, which is located along the Rocky Mountains. This is concordant with results presented in Hallas et al. (2022), which estimated the ancestral area for western *Thamnophis* in the same region within the same time-frame. Our results also support the assertion presented in Hallas et al. (2022) that the separation of the western and midwestern *Thamnophis* clades is due to a vicariant event. The area includes the transition to the more mesic environments of the eastern temperate forests and Great Plains to the more xeric environments immediately east of the Rocky Mountains (Axelrod, 1985). While the Rocky Mountains had already formed by the time Thamnophiini first appeared in North America, the separation of the western and midwestern clades occurred during this period of aridification and expansion of grasslands (Keeley and Keeley, 1989; Zamudio et al., 1997; Pook et al., 2000). This indicates that the initial divergence was associated with a change from a mesic to xeric environment.

Interestingly, our biogeographic analyses demonstrated that for taxa not within *Thamnophis*, the Western Continental Divide as represented by the Rocky Mountains and the MTZ presents a formidable barrier to dispersal or permanent colonization. Out of all the non-*Thamnophis* species, only one taxon, *Storeria storerioides*, occurs west of the Western Continental Divide. There are two species that occur in the Neotropics Bioregion 4 (*Storeria dekayi*, *Thamnophis proximus*) that most likely dispersed from Bioregion 1 along the versant to the Atlantic. The transition from mesic to xeric environments presented by the Western Continental Divide may be impassable to non-*Thamnophis* lineages that are adapted to mesic environments. Alternatively, it is possible that members of these typically eastern genera have since gone extinct. We note that some eastern taxa such as *Nerodia* have been introduced in the west and persevere (Rose and Todd, 2014). Therefore, it does not appear that niches for eastern taxa do not exist in the west. However, there is evidence that some of these introduced populations are negatively impacted by prolonged droughts (Rose and Todd, 2017). As such, it is likely that non-*Thamnophis* taxa were not able to disperse through these dry areas to occupy equivalent niches in the west. The transition to higher elevations along the Western Continental Divide may also prove to be impassable for non-*Thamnophis* lineages. For instance, the Mexican clade occurs primarily in highland habitats at elevations up to 4,200 m (Rossman, 1996; de Queiroz et al., 2002; Heimes, 2016), whereas non-*Thamnophis* lineages are primarily in low-elevation environments. Therefore, it is likely that *Thamnophis* contains adaptations to xeric and high-elevation environments that are not present in non-*Thamnophis* lineages.

4.2. Taxonomy

Contrary to previous phylogenetic studies of the group, our results show a markedly different topology with respect to the placement of the “semi-fossorial” clade. The following genera are shown to be monophyletic with high levels of support from both the ASTRAL-constrained and concatenated trees: *Nerodia*, *Storeria*, *Regina*, and *Liodytes*. Both topologies showed *Haldea striatula* and *Virginia valeriae* as sister taxa, which contrasts with the topology presented in McVay et al. (2015), where *H. striatula* was the sister taxon to *Storeria*. Traditionally, and prior to McVay et al. (2015), both *H. striatula* and *V. valeriae* were grouped in the genus *Virginia*. The only major diagnostic characters between the two species are the presence of keeled scales in *H. striatula* versus smooth scales in *V. valeriae*, and minor differences in labial scale counts (Rossman and Wallach, 1991). Additionally, the two species do not exhibit markedly different ecologies; both species exhibit a semi-fossorial lifestyle, burrow in loose soil and leaf litter, and have a largely vermivorous diet. Given the ecological and phenotypic similarity and phylogenetic relationship of the two sister species, returning *Haldea striatula* to *Virginia* is preferred because it stabilizes the group consistent with the generic taxonomy first used for both species in Garman, 1884.

Furthermore, *Liodytes pygaea* is strongly supported as the sister-taxon to *L. alleni* and *L. rigida*, also in contrast with McVay et al. (2015), which grouped *L. pygaea* and *L. rigida* as sister species. Additionally, *L. pygaea* also exhibits greater phenotypic and ecological differences with the other *Liodytes*, including paired internasals and a diet consisting mainly of small fish, amphibians, and earthworms (Gibbons and Dorcas, 2004) and was placed in the monotypic genus *Seminatrix* by Cope (1895) up until 2013. Given our phylogenetic results and the notable phenotypic and ecological differences between the species, we recommend that *L. pygaea* be restored as *Seminatrix pygaea*.

Our phylogenomic analyses show strong statistical support for the placement of *Adelophis foxi* within *Thamnophis*, which supports the findings of previous systematics studies of Thamnophiini (de Queiroz et al., 2002; McVay and Carstens, 2013; McVay et al., 2015; Hallas et al., 2022). Additionally, *Adelophis* has an undivided anal plate, which is an important synapomorphic character for *Thamnophis* (Rossman and Blaney, 1968). We hesitate to synonymize *Adelophis* in full as

Thamnophis, given that the rare and type-bearing taxon for this genus, *A. copei*, was not included in this study or in any previous phylogenetic studies. However, based on the evidence presented here and in previous studies, we recommend that *A. foxi* itself should be placed within *Thamnophis* and synonymized as *Thamnophis foxi*.

5. Conclusion

We produced the largest genome-scale dataset yet for *Thamnophiini*, which consisted of 3,735 UCE loci, and used these data to infer a revised and mostly resolved phylogeny for *Thamnophiini*. While high levels of gene tree discordance were apparent across loci, the resulting species tree still yielded high branch support for most nodes. Ancestral area estimation identified the Western Continental Divide as a major driver of broadscale diversification, indicating that it may be an impassable barrier for almost all non-*Thamnophis* lineages. As a result, *Thamnophis* lineages that disperse across the Western Continental Divide fill ecological niche space that are occupied by non-*Thamnophis* lineages in the Eastern Nearctic (Rossman, 1996; Hallas et al., 2022). Several nodes in our species tree contrasted with previously published topologies of *Thamnophiini*. Based on our results, we recommend that *Haldea* be synonymized with *Virginia*, *Adelophis foxi* be placed in the genus *Thamnophis*, and *Liodytes pygaea* be restored to *Seminatrix pygaea*. Finally, while we were able to infer important patterns in the evolutionary and biogeographic history of the group, a more robust genomic dataset would be needed to test emerging hypotheses of introgression and adaptation presented by this study.

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Data availability:

All molecular data used in this study have been deposited on relevant genetic databases and on figshare at <https://figshare.com/s/eafcc501a54eaad0c96d>. Occurrence data collected for this study are available on GBIF at <https://doi.org/10.15468/dd.n38jbr>.

CRedit authorship contribution statement

Leroy P. Nuñez: Conceptualization, Resources, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Visualization. **Levi N. Gray:** Resources, Supervision, Writing – review & editing. **David W. Weisrock:** Conceptualization, Resources, Supervision, Writing – review & editing. **Frank T. Burbrink:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107844>.

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Further reading

Science Analytics and Synthesis Program of the U.S. Geological Survey (USGS), Biodiversity Information Serving Our Nation (BISON): U.S. Geological Survey.