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RESEARCH ARTICLE

Effects of Environmental Variation in Structuring Population Genetic Variation in the False-Water Cobras (Xenodontinae: *Hydrodynastes*)

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Abstract

Although rivers are known to promote diversification and shape phylogeographic patterns, they can also be permeable and facilitate the dispersal of species. Using multiple molecular methodological approaches, we conducted a phylogeographic investigation of two semiaquatic snake species with continental distributions across South America, testing how environmental and historical factors (e.g., potential allopatric divergence across rivers) have affected their evolutionary history. Our results show that *Hydrodynastes gigas* and *H. bicinctus* have a recent divergence time (~1.5 mya) and that both species have low genetic diversity with no geographic structure. Population genetic divergence in *H. gigas* is explained by geographic distance (isolation-by-distance), climate (isolation-by-environment), and hydrographic basin. Paleo-niche models suggest that historically stable regions of habitat suitability for both taxa are largely restricted to the La Plata basin. We suggest that life history traits of these species, for example high dispersal capabilities and generalist ecologies, have allowed for their extensive geographic distributions and population connectivity resulting in no geographic structure and low genetic variation.

Keywords Dipsadidae · Phylogeography · Squamata · Evolution · Climatic changes · Watershed · Niche modeling

Introduction

The topographic complexity and environmental heterogeneity of South America has played an important role in driving species diversification and structuring patterns of biodiversity (Hoorn et al., 2010; Rull, 2008, 2011; Turchetto-Zolet et al., 2013). Much of this diversity is structured by major biomes, large hydrographic basins, and across climatic gradients. Intricate fluvial and river drainage systems are a reflection of the complex geoclimatic history of South America (e.g., Arzamendia & Giraudo, 2009; Hoorn et al., 2010) and these large rivers can act as barriers that limit gene flow (Pirani et al., 2019). Furthermore, these rivers can differentially affect the process of diversification of species both promoting vicariant speciation and acting as secondary barriers, reinforcing species divergence (Aleixo, 2004; Bittencourt et al., 2019; Dal Vechio et al., 2019; Naka & Brumfield, 2018; Pirani et al., 2019; Vargas-Ramírez et al., 2020; Werneck et al., 2012). By contrast, phylogeographic analyses of many groups of both terrestrial and aquatic organisms have demonstrated the permeability of rivers as biogeographic barriers and therefore may have little effect in influencing patterns of lineage divergence (e.g., Carvalho et al., 2020; Colston et al., 2013; Magalhães et al., 2020;

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Pinto et al., 2019; Pirani et al., 2019). Furthermore, large river systems of South America may be facilitating gene flow by acting as corridors for dispersal among aquatic and semiaquatic species (Arzamendia & Giraudo, 2009; Fonseca et al., 2021; McCartney-Melstad et al., 2012).

Importantly, phylogeographic patterns across South America cannot be explained by a single process or during one time period (Rull, 2008, 2011; Turchetto-Zolet et al., 2013). Instead, spatial patterns of genetic variation within a taxon may be simultaneously influenced by several factors, such as geographic distance between populations (e.g., isolation-by-distance; Oliveira et al., 2018), climatic variation (isolation-by-environment; Camurugi et al., 2021), landscape attributes acting as vicariant barriers (isolation-by-resistance; Rivera et al., 2020), or vicariance caused by historical climate change (e.g., isolation-by-instability; Vasconcellos et al., 2019). All of these may contribute to the population genetic differentiation between lineages and drive species diversification (Anderson et al., 2010). Additionally, phenotypic traits may influence patterns of species distributions and impact genetic differentiation (Fouquet et al., 2015; Papadopoulou & Knowles, 2016; Paz et al., 2015; Zamudio et al., 2016). For example, aspects of life history, behavior, diet, or phenotype may be better predictors of spatial genetic structure than geographic distance, climate, or biogeographic barriers (Fouquet et al., 2015; Henderson et al., 2013; Papadopoulou & Knowles, 2016; Paz et al., 2015).

The false-water cobras (genus *Hydrodynastes*), consists of two, large (> 1.5 m in snout-vent length) semiaquatic snakes broadly distributed across South America occurring in many biomes and hydrographic basins (Carvalho et al., 2020; Murta-Fonseca et al., 2015). These two species, *Hydrodynastes bicinctus* (Hermann, 1804) and *Hydrodynastes gigas* (Duméril, Bibron and Duméril, 1854), are broadly sympatric within the Amazonas, Araguaia-Tocantins, La Plata, and Northeast South American hydrographic basins, and occur in syntopy in many locations in these basins (Carvalho et al., 2020; Murta-Fonseca et al., 2015). Several studies have revealed that widespread vertebrate species that co-occur with these snakes, across multiple environmental conditions and ecoregions, have high geographic structure, genetic diversity, and may be composed of multiple cryptic species (e.g., *Dendropsophus minutus* and the *Mabuya dorsivittata* complex; Gehara et al., 2014; Rivera et al., 2020). Although it has been shown that *H. gigas* is a single, widely distributed species with low genetic diversity (Carvalho et al., 2020), the phylogeographic patterns in aquatic snakes varies between regions, species and geographic distributions (e.g., Brandley et al., 2010; Guiher & Burbrink, 2008; Lukoschek et al., 2011), and these patterns have yet to be explored in *Hydrodynastes*. Despite the low genetic diversity (Carvalho et al., 2020), we expect lineages to be structured by watershed and that rivers exert a greater force than climate or biomes on genetic diversity. There are several aquatic and semiaquatic snake species throughout South America, however, there is no evaluation of how rivers and different environments have promoted their speciation and shaped their genetic diversity. The wide-distribution of these species provides an excellent model for investigating phylogeographic patterns across many potential biogeographic barriers and in relation to past changes in climate. Based on a multi-locus dataset and ecological niche models we infer the phylogenetic relationships and spatial and temporal population genetic structure within the false-water cobras.

Materials and Methods

Sampling and Molecular Data

We sampled 32 individuals of *Hydrodynastes gigas* and 12 of *H. bicinctus* from 37 localities in South America (Fig. 1). We used *Oxyrhopus trigeminus* (only for the NT3 gene), *O. guibei*, and *Helicops angulatus* as outgroup taxa (Zaher et al., 2019) (Table S1). We extracted genomic DNA from muscle, liver, or scale clips using a phenol–chloroform extraction protocol (Sambrook et al., 1989). We amplified the partial sequences of the mitochondrial DNA (mtDNA) 16S ribosomal gene (16S rRNA; Palumbi et al., 2002) and cytochrome b (Cytb; Pook et al., 2000) and the nuclear DNA (nuDNA) loci Oocyte maturation factor Mos (Cmos; Lawson et al., 2005) and Neurotrophin-3 (NT3; Noonan & Chippindale, 2006) using PCR. We aligned each locus using the Muscle algorithm (Edgar, 2004) in Geneious v9.1.8 (https://www.geneious.com). To determine the most probable pair of alleles for each nuDNA gene, we used the PHASE algorithm (Stephens et al., 2001) implemented in DNASP 5.10 software (Librado & Rozas, 2009), where we only considered alleles with posterior probabilities > 0.90.

Phylogenetic Analysis

To estimate gene-trees independently for mtDNA and nuDNA we used Bayesian inference implemented in MrBayes v3.2.2 (Ronquist & Huelsenbeck, 2003) using the substitution models selected by jModelTest (Darriba et al., 2012). We ran two independent runs of four Markov chains for 20 million generations sampling every 5000 generations and discarding the first 25% of generations as burn-in. We evaluated the stability of the analysis in Tracer v1.6, ensuring that all effective sample size (ESS) values were > 200 (Rambaut et al., 2018).

We estimated a dated phylogeny with a concatenated alignment of both mtDNA loci. We used PartitionFinder2 to identify partitioning schemes and the most appropriate nucleotide replacement models (Lanfear et al., 2017) for

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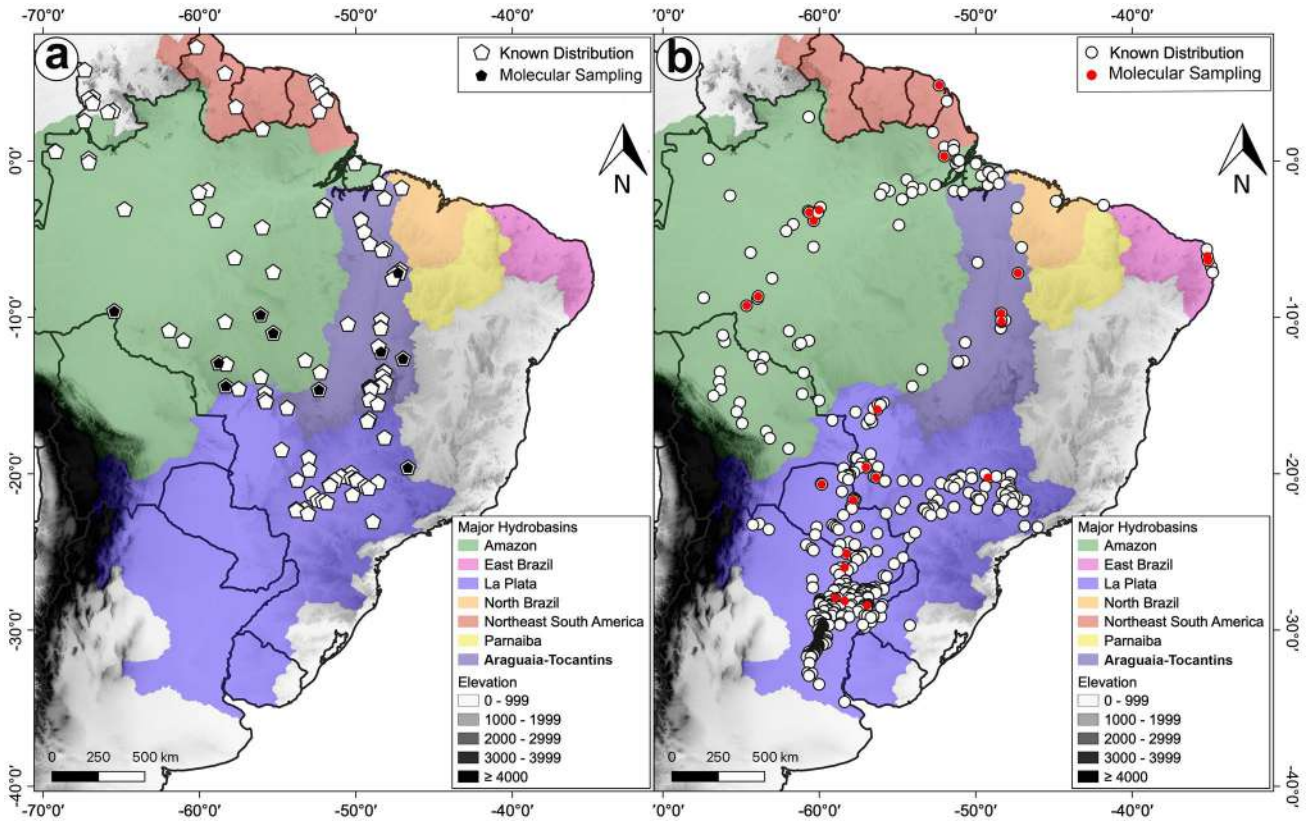

Fig. 1 The known geographic distributions of the *Hydrodynastes* species in South America are shown with open symbols, specimens sampled for molecular data are shown with inserts. **A** *H. bicinctus*; **B** *H. gigas*. The geographic extent of the major hydrobasins across South America and discussed in the test are highlighted in different colors (Color figure online)

the alignment. According to our concatenated alignment, we found two partitions evaluated by BIC. We performed a Bayesian phylogenetic analysis using BEAST v.2.6.3 (Bouckaert et al., 2019) with a Yule process tree prior, a strict clock model, and we calibrated the tree using a rate of 1.3% substitutions per million years that has been previously used in closely related snakes (Daza et al., 2009). We ran this analysis for 50 million generations, sampling every 2000 steps. We checked for stationarity by visually inspecting trace plots and ensuring that all ESS values were above 200 in Tracer v1.7.1 (Rambaut et al., 2018). The first 10% of sampled genealogies were discarded as burn-in, and the maximum clade credibility tree with median node ages was summarized TreeAnnotator v.2.6.3.

Population Assignment and Demographic History

We used the two nuDNA genes to investigate population structure within *H. gigas* using Structure v2.3.4 (Pritchard et al., 2000). We performed 10 replicate analyses over a range of number of populations (K) from 1 to 10, using a linkage model and uncorrelated allele frequencies for 50,000 generations as burn-in, followed by 50,000 generations. We chose the best value of K based on the rate of changes in the log-probability between successive K values (ΔK statistic; Evanno et al., 2005), using Structure Harvester (Earl & vonHoldt, 2012). Due to the sample size and lack of genetic variation, we did not perform this analysis for *H. bicinctus*.

To estimate changes in the effective population size in *H. gigas* and *H. bicinctus*, we conducted demographic analysis using Bayesian skyline plots in *BEAST v1.8.0 (Drummond et al., 2012). We used a concatenated mtDNA alignment in both species and a 1.3% substitution rate per million years to calibrate the molecular clock (Daza et al., 2009). We ran two independent chains of 100 million generations; parameter convergence, stationarity, and ESS values (≥ 200) were viewed using TRACER. The graphs of population dynamics through time were generated in the same program.

Species Delimitation Analyses

We applied both distance- and tree-based species delimitation analyses to assess lineage boundaries in *Hydrodynastes* (single locus delimitation), multi-rate Poisson tree processes (mPTP) and Assemble Species by Automatic Partitioning (ASAP). We ran a Maximum Likelihood tree inferred in

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RAxML (Stamatakis, 2014) via raxmlGUI 2.0 (Edler et al., 2021) with rapid bootstrap method and 1000 bootstrap iterations. We performed the mPTP (Kapli et al., 2017) on a webserver (https://mptp.h-its.org/) using the ML tree of concatenated mitochondrial DNA. This method distinguishes coalescent versus speciation processes and incorporates different levels of intraspecific genetic diversity derived from differences in either the evolutionary history or sampling of each species. The ASAP was also performed on a webserver (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html). We used the 16S and Cytb datasets, a Jukes-Cantor (JC69) model of nucleotide substitution, and Kimura 2-parameter (K2p) genetic distances. This method uses a hierarchical clustering algorithm to identify a barcode gap (i.e., difference between intra and interspecific genetic distances) (Puillandre et al., 2021).

Spatiotemporal Phylogeographic Reconstruction

We employed the concatenated mtDNA data set to reconstruct the spatiotemporal history of *Hydrodynastes gigas* using a Bayesian approach implemented in *BEAST v1.8.2 and we applied a lognormal relaxed random walk model. This method is a time heterogeneous approach and allows for variation in diffusion rates across the branches of the phylogeny, reconstructs geographic coordinates at ancestral nodes, and therefore reconstructs the dispersal of lineages continuously in space and time, taking into account gene-tree uncertainty. We implemented a coalescent prior with constant population size and an HKY+ G model of substitution. Because some sample coordinates were duplicated, we used a jitter option of 0.05 to add random noise to identical coordinates. We ran two independent chains of 50 million generations, assessed stability of the analysis in Tracer v1.6 ensuring that all ESS values were > 200 (Rambaut et al., 2018). In order to time calibrate the tree, we used a 1.3% substitution rate per million years. The resulting tree was summarized with TreeAnnotator v1.8.2, the first 10% of samples discarded as burn-in. We used SPREAD v1.0.4 to generate a kml file (Bielejec et al., 2011) which was plotted in Google Earth (http://earth.google.com).

Population Genetic Divergence

For both *H. gigas* and *H. bicinctus* we calculated the number of polymorphic sites (*S*), haplotype number (*h*), haplotype diversity (*Hd*), nucleotide diversity (π), Tajima's *D* and its p-value for each locus using DNASP (Librado & Rozas, 2009). Molecular analysis of variance (AMOVAs) was used to investigate population genetic structure between and within different hydrographic basins and biomes in South American using the mtDNA and nuDNA loci in Arlequin v3.5.2.2 with 10,000 permutations (Excoffier & Lischer, 2010). The 16S and Cmos loci were not used in this analysis for *H. bicinctus* due to a lack of variation. We constructed mtDNA and nuDNA haplotype networks for *H. gigas* and *H. bicinctus* in PopART (Population Analysis with Reticulate Trees) v1.7 using the median-joining network method in order to check haplotype sharing among hydrographic basins and species (Leigh & Bryant, 2015).

We also used redundancy analysis (RDA) to test whether genetic divergence is associated with geographic distance, climate, or the hydrographic basin for which samples were collected. We ran RDA following the approach outlined in Myers et al., (2017). Briefly, we generated normalized genetic distance matrices for each of the two species based on the mtDNA separately and for *H. gigas* for nuDNA sequences for a total of three matrices. These genetic distance matrices were then subjected to principal coordinate analysis. Climate variables were extracted for each collecting locality based on the 2.5 arc-min resolution layers from the CHELSA bioclimatic dataset (Karger et al., 2017). Hydrographic basins were used to group localities in order to determine if these basins have been responsible for promoting population genetic differentiation. Lastly, the latitude and longitude of each sample was used to test if geographic distance can predict genetic distances. We conducted a total of seven RDA analyses where predictions of population genetic structure, as measured by the PCoA matrices of genetic distances, were tested using climate, divergence between hydrographic basins, and geographic distance as explanatory variables, and all combinations of these variables including a full model with all three variables. RDA returns an r^2 value and significance is assessed using an ANOVA. These analyses were run in R using the packages 'raster','rworldmap', 'rgdal', 'ape' and 'vegan' (Bivand et al., 2021; Hijmans, 2020; Oksanen et al., 2020; Paradis et al., 2019; South, 2011). The R scripts and input files to conduct these analyses are available at https://github.com/Rhinella85/Hydrodynastes_phylogeography.

Ecological Niche Modeling

We used ecological niche models (ENMs) to estimate the potential geographic distributions of our focal species under current climate conditions and projected these on to past climate models to explore potential habitat fragmentation. Because both *Hydrodynastes* species are widespread across South America we assumed that the entire continent represents the potential area that these species can access and therefore specified South America to by our study region. The collection of localities of all snake species found within Brazil has recently been aggregated and published (Nogueira et al., 2019), and it is clear that sampling effort for many species is biased to certain regions and does not represent a random sample across the continent. This is also the case

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for both of our focal species and sampling bias is known to influence the results of ENMs (Costa et al., 2010). Therefore, we applied a similar sampling bias to select background points for our ENMs (Elith et al., 2011), following the approaches of Bell et al., (2017) and Jaynes et al., (2021) (see https://github.com/eddiemyers/Leptopelis/tree/master/ENMs_and_BG_points). To do this we selected pseudoabsence background points using a sampling effort surface based on all georeferenced snake specimen records from South America (~75,681 records; Nogueira et al., 2019). We used the number of specimens collected at a site with a resolution of 0.0083 degrees (~1 km at the equator) as an indicator of sampling effort. These data were then aggregated as a raster with a factor of 10 to produce 10 × 10 km blocks, summing all sampling points within these blocks and then disaggregating them back to 1 km resolution, preserving the sampling effort from the aggregated blocks. Sites that had no snake samples were assigned a value of 0.01. Using this raster, we selected 10,000 background points with a probability proportional to total sampling effort.

The CHELSA bioclimatic layers at 2.5 arc-min resolution (Karger et al., 2017) were used for our ENMs. Using ENMTools in R (Warren & Dinnage, 2020) we tested for correlations among these bioclim variables and removed all but one of all variables that had a Pearson's correlation > 0.7, resulting in seven variables (Annual Mean Temperature, Mean Diurnal Range, Isothermality, Temperature Seasonality, Annual Precipitation, Precipitation Seasonality, Precipitation of the Warmest Quarter). We combined the locality data from our genetic samples with those of several recent studies (Carvalho et al., 2020; Murta-Fonseca et al., 2015; Nogueira et al., 2019) for a total of 191 *H. bicinctus* and 993 *H. gigas* with the taxonomic updates of Murta-Fosenca et al. (2015) and Carvalho et al. (2020) and thinned these data so that no neighboring samples were within 20 km of one another using the R package spThin (Aiello-Lammens et al., 2015). To generate ENMs we implemented the maximum entropy approach implemented in MaxEnt v3.4.1 (Phillips et al., 2006). This requires that model parameters be optimized (e.g., Morales et al., 2017) which we did using the R package ENMeval (Muscarella et al., 2014) testing all combinations of six feature classes (Linear; Linear Quadratic; Hinge; Linear Quadratic Hinge; Linear Quadratic Hinge Product; and Linear Quadratic Hinge Product Threshold) and eight regularization multipliers ranging from 0.5 to 4.0 at 0.5 intervals. The best fit model parameters were chosen using AIC values and implemented in our models. ENMs were then constructed with MaxEnt using Biomod2 (Thuiller et al., 2020) in R with 25 evaluation runs, replicated for 5000 iterations, and using 20% of specimen localities as a training dataset for model evaluation. Variable importance for each species was measured using a jackknifing approach and model performance was assessed using response curves. Current ENMs were hindcast on to climate projects of the mid-Holocene (~6 kya; Fordham et al., 2017), the Last Glacial Maximum (~21 kya; Karger et al., 2017), and the Last Interglacial (~130 kya; Otto-Bliesner et al., 2006) at 2.5 arc-min resolution which were obtained from the PaleoClim database (Brown et al., 2018). These four binary model projections were stacked to identify regions of potential habitat stability through time (i.e. potential refugia).

Results

Phylogenetic Analysis

The best-fit models were HKY+I for 16S, HKY+G for Cytb, HKY for Cmos and K80 for NT3. Partitioning schemes and the appropriate nucleotide replacement models identified in the concatenated data were TRN+G+X for the first partition (Cytb Position 2+16S+Cytb Position 1), and HKY+G+X for the second partition (Cytb Position 3). No geographic structure for either *Hydrodynastes gigas* and *H. bicinctus* was found given the topologies of the individual gene-trees (Fig. S1). In the dated mtDNA concatenated tree, we found two genetic lineages corresponding to *H. gigas* and *H. bicinctus* that diverged during the Pleistocene (~1.5 mya) and are well-supported (Fig. 2). The phylogenetic relationships estimated were consistent among the different methodologies used.

Population and Demographic Analyzes

The ΔK statistic based on Structure runs suggested support for two populations within *H. gigas* but with no geographic component to these populations. Both populations have individuals from all sampled basins and different biomes (Fig. S2). This is likely the result of the inability of the ΔK statistic to evaluate K = 1 resulting in an overestimation of population genetic structure (Cullingham et al., 2020). The Bayesian skyline plot analysis of the concatenated mtDNA loci showed different demographic histories for the two species, with different timing of mtDNA coalescence (Fig. 3). *Hydrodynastes bicinctus* has a small effective population size (N_e), with a recent coalescent time of all alleles at approximately 130 Kya, while *H. gigas* present similar N_e, but coalesced approximately 330 Kya. Both species had relatively stable population sizes through time, however there is a trend for a population decline over the last ~20 Kya in *H. gigas*.

Species Delimitation

Both mPTP and ASAP delimitation analyses supported the existence of only two species within *Hydrodynastes:*

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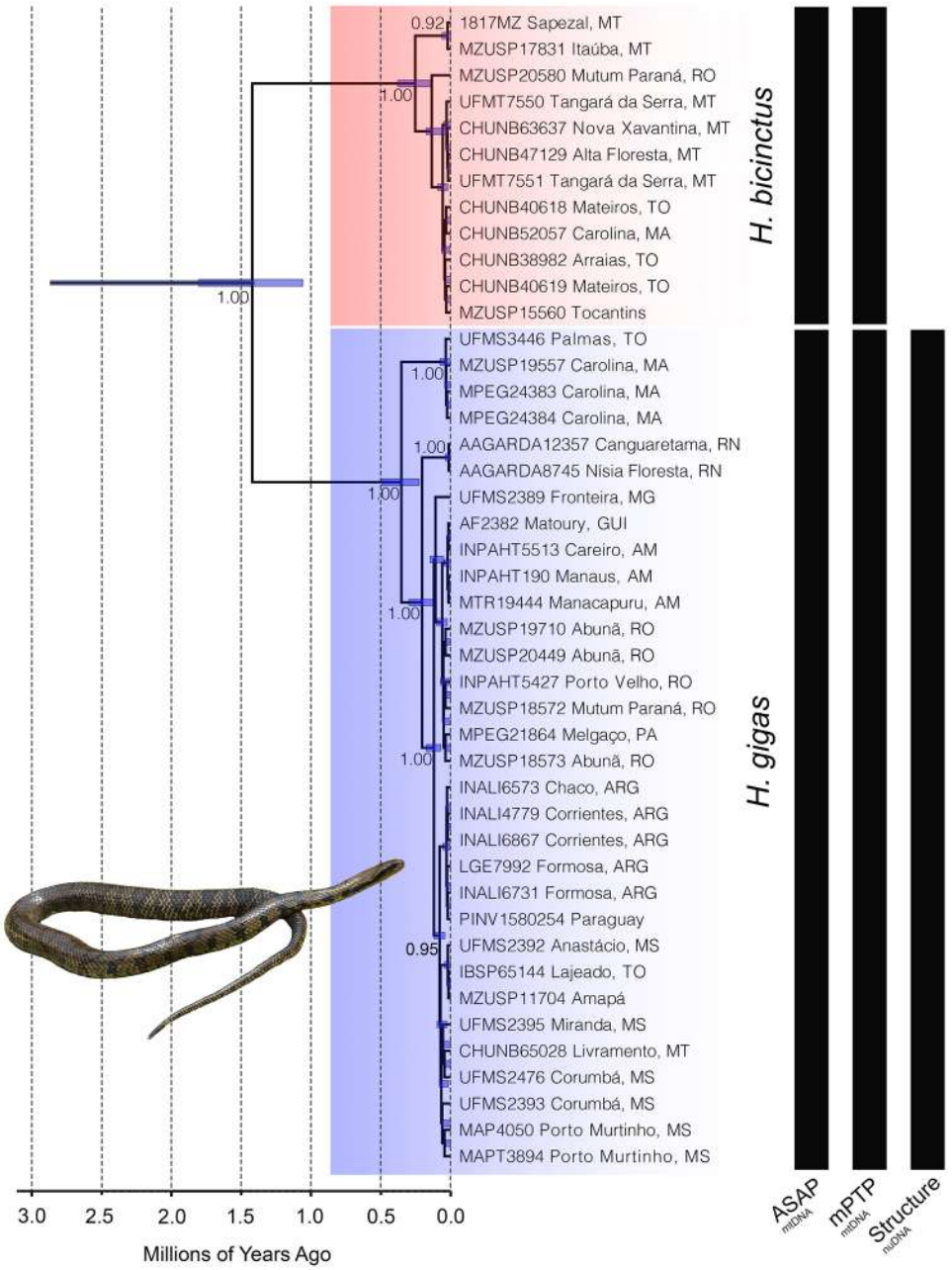

Fig. 2 Phylogenetic relationships and divergence times based on the concatenated mtDNA loci and inferred in BEAST. *Hydrodynastes gigas* is highlighted in blue and *H. bicinctus* in red. Nodal posterior probabilities are not provided for support values < 0.90. Black bars represent each evolutionary entities delimited by the following methods: ASAP (Assemble Species by Automatic Partitioning), mPTP (multi-rate Poisson tree processes) and Structure (Color figure online)

H. bicinctus and *H. gigas* (Fig. 2). The 16S and Cytb loci delimited only two species within *Hydrodynastes* regardless of which distance parameter was implemented. The best 16S partition had an ASAP score of 1.00 ($p \leq 0.05$) at a threshold distance of 0.022. The best Cytb partition had an ASAP score of 3.50 ($p \leq 0.001$) at a threshold distance of 0.068.

Spatiotemporal Phylogeographic Reconstruction

Spatiotemporal reconstruction based on the relaxed random walk model suggested that *H. gigas* originated in the Araguaia-Tocantins basin region (Fig. 4). From there, it spread to the La Plata, East Brazil and northeastern Amazon basins around 56 Kya. The ancestral population from the northeastern Amazon basin dispersed to a more southern region of the Amazon basin around 20 Kya. Furthermore, this model suggests that during the LGM *H. gigas* was widespread throughout all of the hydrographic basins where the species is currently distributed.

Population Genetic Divergence

Both *Hydrodynastes* species have low levels of genetic divergence (Table S2). For *Hydrodynastes gigas*, the

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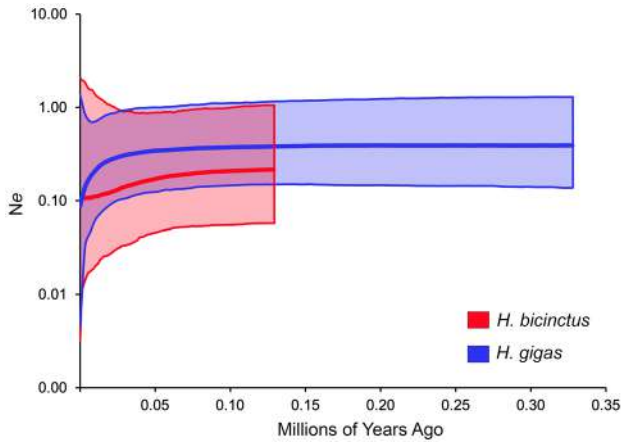

Fig. 3 Effective population sizes (Ne) through time (in millions of years) estimated from Bayesian skyline plots. *Hydrodynastes gigas* is highlighted in blue and *H. bicinctus* in red. The bolded line represents the median population size estimate and the shaded region represents the 95% higher posterior probability (Color figure online)

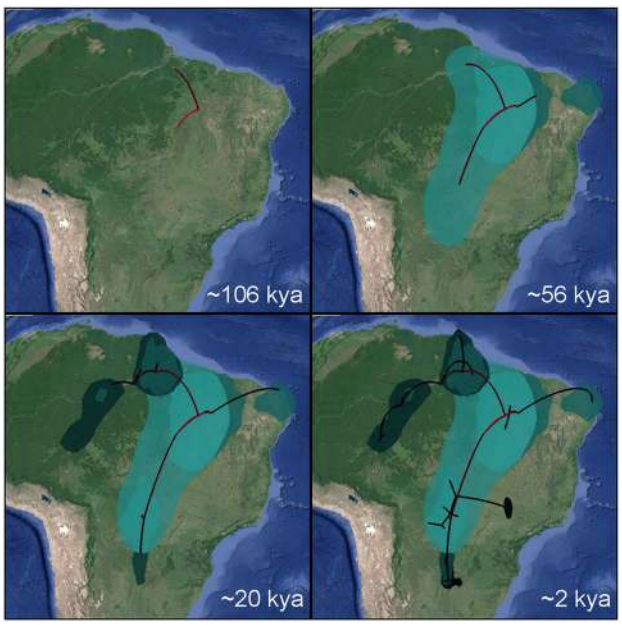

Fig. 4 Bayesian spatiotemporal diffusion of *Hydrodynastes gigas* at four time slices. Reconstructions are based on the maximum clade credibility tree estimated with a time-heterogeneous Relaxed Random Walk approach. Shading represents 80%-HPD uncertainty in the location of ancestral branches with lighter and darker shades representing older and younger diffusion events, respectively (Color figure online)

number of variable sites was highest in Cytb (25 sites), followed by NT3 (10 sites), 16S (four sites) and lowest in Cmos (one site). For *H. bicinctus* both 16S and Cmos have no variable sites, while Cytb and NT3 have 14 and five variable sites, respectively. A summary of all population genetic statistics for the *Hydrodynastes* species is presented in Table S2.

Haplotypic diversity was highest in Cytb and NT3 for both species. For the 16S and Cytb loci, there was no haplotype sharing between the two *Hydrodynastes* species. For *H. gigas*, the Amazon, Araguaia-Tocantins, La Plata and East Brazil watersheds have one, six, eight, and two exclusive haplotypes, respectively (Fig. 5). While *H. bicinctus* has only one haplotype of 16S that is present in all three watersheds where it occurs.

The AMOVA demonstrated that most genetic variation in *H. gigas* is partitioned between basins for three loci (16S, Cytb and Cmos), and the greatest genetic variation was within biomes for all genes (Table S3). While in *H. bicinctus* the partitioning of genetic variation amongst basins and biomes differs across loci, for example, within Cytb the genetic variation was greatest between basins and within biomes, whereas for NT3 the greatest variation was within basins and between biomes (Table S3).

The RDA analysis demonstrated that mtDNA population genetic divergence in *H. gigas* is best explained by the full model that includes climate, geographic distance, and sampled hydrographic basin ($r^2 = 0.91$, p-value < 0.05; Table S4). When analyzing the nuDNA for *H. gigas*, no models significantly explained genetic distances, however the full model ($r^2 = 0.88$, p-value $= 0.06$) and climate + sampling locality approach significance ($r^2 = 0.77$, p-value $= 0.056$). Within *H. bicinctus,* none of the chosen explanatory variables significantly explained genetic distances within either the mtDNA dataset and a lack of variation in the nucDNA dataset precluded this analysis.

Ecological Niche Model Projections

After thinning locality data, we retained 287 *H. gigas* and 98 *H. bicinctus* for ENMs. The best fit model parameters for *H. gigas* were 'H' with a regularization multiplier of 1.5, and for *H. bicinctus* the best fit combination of feature classes were 'LQH' with a regularization multiplier of 4.0. The models for both species performed satisfactorily (AUC = 0.85 for *H. gigas*; AUC = 0.89 for *H. bicinctus*) and predicted the known geographic distributions of these species (Fig. 6). The current model for *H. gigas* presents over-prediction in regions of northern South America (e.g., within the Orinoco basin) where this taxon has not been documented. Our current model for *H. bicinctus* suggests that this species is widely distributed within the Amazon basin, but is likely over-predicting suitable habitat within northeastern Brazil. The climate variables of most importance for modeling the geographic distributions of these species also differed, seasonal precipitation had a percent contribution of 29.5 for *H. gigas* and annual mean temperature had a percent contribution of 30.9 for the *H. bicinctus* model.

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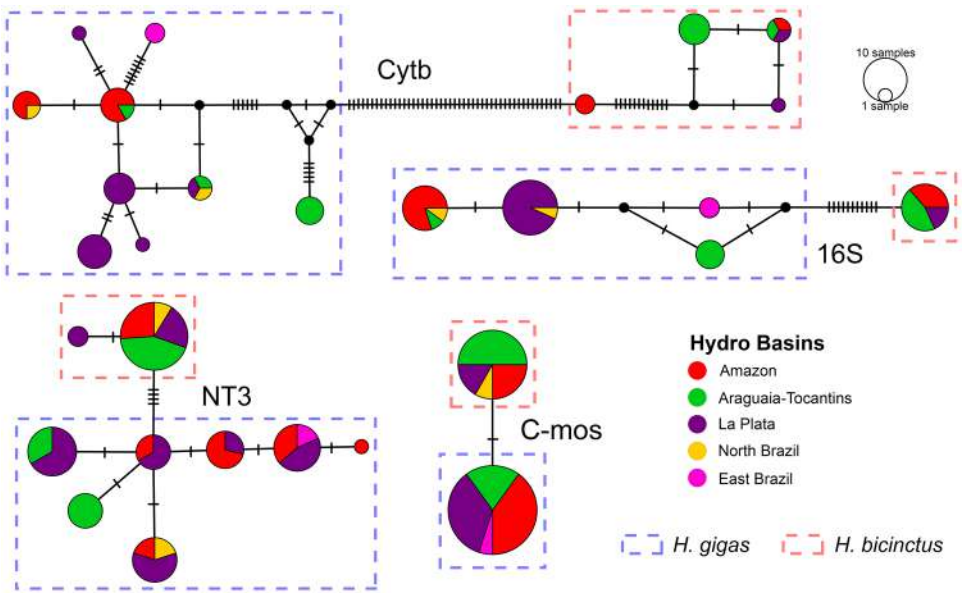

Fig. 5 Haplotype networks for each locus within the *Hydrodynastes* species. Networks were generated using PopART with the Median Joining Model. Each haplotype is represented by a circle whose area is proportional to its frequency in the dataset and each hydrobasin is represented by color (indicated in legend)

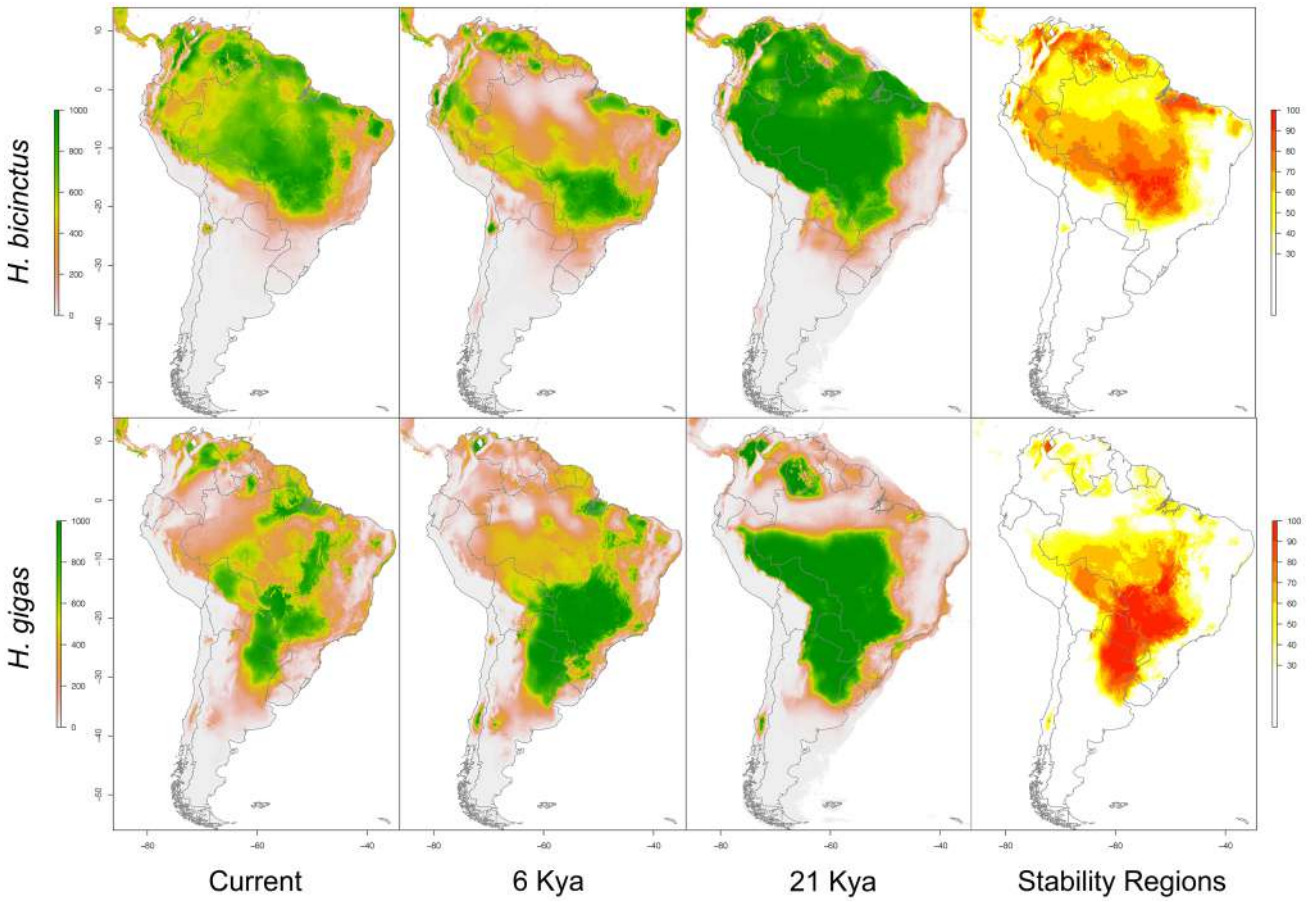

Fig. 6 Ecological niche models for both *Hydrodynastes* species based on current climatic conditions and projected onto climates at 6 kya and 21 kya. In these ENMS green colors represent higher habitat suitability. The last column represents regions of habitat suitability through time where warmer colors represent suitability in multiple time periods (Color figure online)

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The stacked suitability models for *H. gigas* suggest that the La Plata hydrographic basin has served as a region of stable habitat throughout the late Pleistocene (Fig. 6). *H. bicinctus* has a more widespread region of historical suitability based on these models, with projected habitat throughout northern La Plata, North Brazil, Orinoco, and Magdalena basins (Fig. 6). However, *H. bicinctus* does not currently occur in the Magdalena basin and is rarely collected in the Orinoco basin of northern South America (e.g., Nogueira et al, 2019).

Discussion

The false-water cobras species, *H. gigas* and *H. bicinctus*, are widespread across South America and often occur in sympatry; we find that these two species diverged recently (~1.5 mya) and have maintained relatively stable effective population sizes through time with a shared historically stable region of suitable habitat within the La Plata basin. Both taxa have low intraspecific genetic diversity and no discrete population genetic structure across their large distributions. Population genetic divergence in *H. gigas* is best explained by the interaction of geographic distance (IBD), climatic variation (IBE), and hydrographic basin, while in *H. bicinctus* none of these variables are associated with genetic distances between sampled populations.

Major river systems can promote contrasting spatial patterns of genetic divergence across codistributed species, by acting as allopatric barriers or alternatively by facilitating gene flow. Much of the discordance in how species respond to rivers may be due to species specific traits (e.g., variation in body size or range size) and biogeographic histories (Papadopoulou & Knowles, 2016). For example, phylogeographic patterns in aquatic or semiaquatic snakes vary between species even across shared geographic regions. In North American water snakes, *Nerodia rhombifer* exhibit population structure east and west across the Mississippi River (Brandley et al., 2010). However, other species with similar ecological requirements, that are codistributed with *N. rhombifer*, have very different patterns of population genetic divergence. For example, both *Nerodia erythrogaster* and *Agkistrodon piscivorus* show no genetic structure across the Mississippi River (Guiher & Burbrink, 2008; Makowsky et al., 2010).

Similar discordant patterns among ecologically similar species are observed across South America as well (Rull, 2008, 2011). The yellow anaconda (*Eunectes notaeus*) exhibits significant genetic structure associated with rivers and associated floodplains (McCartney-Melstad et al., 2012), which is in contrast to our findings of phylogeographic patterns in the false-water cobras. Phylogeographic patterns in *H. gigas* demonstrate low levels of genetic divergence, however population genetic structure is associated with a combination of geographic distance, climate, and hydrographic basin. While low sample sizes, combined with little observed genetic variation with *H. bicinctus* resulted in nonsignificant associations with factors potentially driving population genetic divergence, this taxon seemingly has similar phylogeographic patterns to its sister species (Figs. 2, 5). Furthermore, within this taxon, specimens sampled as far apart as 3170 km share the same mtDNA haplotype and previous studies have demonstrated extremely low levels of mtDNA divergence (Carvalho et al., 2020). These low levels of genetic differentiation across large geographic scales may be a reflection of the generalist biology and large adult body size of the false-water cobras (e.g.,Giraudo et al., 2014; Strüssmann & Sazima, 1990, 1993), which likely translates to having greater dispersal capabilities and higher levels of gene flow among populations. This further highlights that patterns of population genetic structure are likely associated with intrinsic characteristics of species and the importance of incorporating phenotypic traits in phylogeographic studies (Papadopoulou & Knowles, 2016). Similarly, the closely related species, *Agkistrodon piscivorus* and *A. contortrix*, have discordant phylogeographic histories, although they co-occur in most of their distributions and are in contact with the same geological and/or climatic barriers (Guiher & Burbrink, 2008). Indicating that unique characteristics in the life history traits of each species may have generated these distinct evolutionary histories (Guiher & Burbrink, 2008).

Many studies have pointed to the Pleistocene climatic oscillations as being a principal factor in driving the formation of new species (e.g.,Rull, 2008; Turchetto-Zolet et al., 2013). These climatic cycles have also been important in influencing the demographic histories of species globally (Hewitt, 2004). Lineages that have recently diverged from one another in the Pleistocene are expected to have low genetic diversity and weak genetic structure (e.g., Brandley et al., 2010; Gottscho et al., 2014; Grazziotin et al., 2006; Mângia et al., 2020). This is a pattern that we find in both species of *Hydrodynastes*, suggesting that recent divergence coupled with a high dispersal ability is maintaining genetic homogeneity within these species even between disparate watersheds. Because these two species of *Hydrodynastes* diverged from one another ~1.5 mya it is likely that changes in climatic conditions across South America during the mid-Pleistocene was responsible for their divergence. This period of time has been important for driving diversification in several other widespread species in South America (e.g., *Rhinella marina* and *Boana albopunctata*; Maciel et al., 2010; Prado et al., 2012).

Species with continental distributions often have highly structured genetic diversity because of factors including local adaptation within ecoregions and to heterogeneous environments or allopatric divergence across biogeographic

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barriers (e.g., Burbrink et al., 2008; O'Connell et al., 2017). In many cases, studies of these widely distributed taxa reveal cryptic species diversity (e.g., Gehara et al., 2014; O'Connell & Smith, 2018). However, to assume that all widespread species have strong genetic structure across their distributions may be unwarranted. Within the false-water cobras, while there are some unique haplotypes in several watershed basins, both species appear to be nearly panmictic with the molecular data generated here, since both species, *H. gigas* and *H. bicinctus*, present a certain ecological similarity in aspects such as habitat, feeding, reproductive mode (Giraudo et al., 2014; Silva et al., 2019). Future directions within these species should focus on generating reduced representation genomic data to test for fine-scale population structure or identify regions of the genome that are under selection for local adaptation to environmental factors like differences in temperature and climate, or even to physical–chemical elements of different rivers (Cooke et al., 2014).

Our results demonstrated that regions of suitable habitat through time occurred within the La Plata basin for both species (Fig. 6). Additionally, the highest amount of genetic diversity for *H. gigas* is found within this river basin. An association between climatically stable regions and higher levels of genetic diversity is a common pattern that has been observed in both plants and animals (e.g., Santos et al., 2014; Vasconcellos et al., 2019). It is possible that the La Plata basin has acted as a refugia for both species during recent periods of climatic oscillations. Although this region has been important for harboring genetic diversity through time, the estimated area of origin is within the Araguaia-Tocantins Basin of central Brazil. This region has also been demonstrated to be the likely area of origin and diversification for paradoxical frogs which then dispersed to both the Amazon and Paraná basins (Fonseca et al., 2021; Garda & Cannatella, 2007). The phylogeographic histories of most Neotropical taxa remain poorly understood (Beheregaray, 2008; Rull, 2008, 2011; Turchetto-Zolet et al., 2013), however, our results highlight the need for further phylogeographic studies of widespread species across South America in order to better understand which biogeographic regions have been important areas of origin for the vast biodiversity of this continent.

During the last glacial maximum both *Hydrodynastes* species had larger regions of suitable habitat across South America. However, from the Holocene to the present day these regions of suitability have been reduced and this reduction in the extent of potential geographic distribution likely affected the demographic histories of these species (Fig. 6). Our analyses suggest that both taxa have had relatively stable effective population sizes through time. Despite the wide distribution of *H. bicinctus* and the reduction of suitability regions, demographic analyses revealed no population change over time. Although it is commonly found that the impacts of glacial cycles affect the demographic dynamics of populations over time (Gehara et al., 2017) other factors can also explain demographic patterns, such as habitat availability, the ability to adapt to changes in community composition, and metabolic rate (Myers et al., 2013). Conversely, a decline in population size within *H. gigas* at approximately 20 kya is observed (Fig. 3). Many species have been shown to have decreased population sizes before the LGM, for example in both plants and animals (Brüniche-Olsen et al., 2021; Moura et al., 2014; Ony et al., 2021; Rato et al., 2021), however a finding of population decline from the end of the LGM towards present day is less often observed. Additional studies of codistributed species with aquatic affinities are needed to address whether population size declines during interglacials are a community-wide phenomena or are specific to the snake species studied here.

According to our ENM, *Hydrodynastes* could occur in the Orinoco basin, but given the recent origin of this taxon, the establishment of the current drainages of the Amazon and Orinoco basins was already established, it was connected until the middle of the Miocene (~8–10 Mya; Hoorn et al., 2022). Moreover, the Amazon basin was the last to be colonized, perhaps because these species did not manage to disperse throughout the northern region of the continent. Additionally, it is possible that the small and/or rare connections between the Amazon and Orinoco basins were not sufficient to allow for the dispersal of these species. It is also possible that mountain ranges have acted as biogeographic barriers to these taxa (e.g., the Tepuis).

Conclusion

We conclude that the genus *Hydrodynastes* has a recent history in South America and the interaction of river networks, climate and geographic distance are responsible for their current genetic differentiation and spatial structure. Since the late Pleistocene both species have had a reduction in the geographic extent of suitable habitat. We point to the need for more scientific attention, especially aquatic and semi-aquatic snakes across South America, to elucidate the role of the main rivers in the evolutionary history and ecology of this group.

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Author contributions PSC, DS, EAM, conceived the ideas, formal analysis; PSC, DS and HZ, data acquisition; PSC, data curation; and PSC led the writing with assistance from EAM, DS and HZ.

Data availability The datasets analysed during the current study are referenced in the text and publicly available.

Declarations

Conflict of interest The authors have no conflicts of interest.

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