

Evolution of the rostral scale and mimicry in the genus *Xenodon* Boie, 1826 (Serpentes: Dipsadidae: Xenodontinae)

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Snakes are a stimulating life form from an evolutionary perspective. Despite the basic morphological body shape (limbless, with a tubular body), these vertebrates are extremely diverse. The Neotropical region is one of the most diverse regions for snakes in the world, with >650 known species. Within this great diversity, the genus *Xenodon* includes 12 species with interesting adaptations to terrestrial and semi-fossorial habitats. Members of this genus are mostly diurnal and terrestrial, feed mainly on anurans and exhibit Batesian mimicry of venomous snakes of the genera *Bothrops* or *Micrurus*. Here, through phylogenetic analysis and ancestral state estimation, we explore the evolution of the rostral scale and mimicry within the genus *Xenodon*. Our results suggest that the ancestral lineage of *Xenodon* had a rounded rostral scale and exhibited *Bothrops* mimicry. The evolution of the rostral scale in *Xenodon* might be related to abiotic factors, as an adaptation for open and forested habitats, and mimicry is likely to be related to biotic factors, as a defensive strategy resembling those of venomous snakes.

ADDITIONAL KEYWORDS: Batesian mimicry – *Bothrops* – forest habitats – *Micrurus* – Neotropics – open areas – South America – snakes.

INTRODUCTION

Snakes are one of the most interesting life forms from an evolutionary perspective, with their basic morphological body shape (limbless and tubular body). This vertebrate group is extremely diverse, not only ecologically but also in the number of species (Vitt & Caldwell, 2009), and is one of the most neglected groups in conservation (Guedes *et al.*, 2018). Among squamates, a snake-like body form has evolved independently ≥ 26 times (Wiens *et al.*, 2006), and snakes are the most successful of those lineages, with > 3900 species (Uetz *et al.*, 2021). Investigation of the diversity of morphology and how these changes or transitions occurred is one of the main goals of

evolutionary biology (Futuyma, 2005). For example, if one morphological character evolves multiple times, then this is evidence that it is strongly favoured by natural selection (Harvey & Pagel, 1991).

Numerous traits have evolved independently multiple times during the evolution of reptiles (e.g. loss of digits, ovoviviparity, body shape, fangs, venom, diet and size), but resulting in the same trait status or function (Brandley *et al.*, 2008; Pyron & Burbrink, 2014; Feldman *et al.*, 2015; Rabosky *et al.*, 2016; Naik *et al.*, 2021). Furthermore, ecological opportunities can trigger divergent selection and lead to new adaptations to exploit empty niches, promoting and increasing speciation and morphological diversification (Schluter, 2001; Nosil, 2012). These adaptations are especially diverse in regions with varied environments, such

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as the Neotropics (Wiens *et al.*, 2006; Grundler & Rabosky, 2014; Da Silva *et al.*, 2017).

The Neotropical region is one of the most diverse regions in the world in terms of habitat diversity, including rainforest, montane forest, savannas, montane savannas and steppes, with a great diversity of species, many of which are endemic (Olson *et al.*, 2006; Dinerstein *et al.*, 2017; Nogueira *et al.*, 2019; Azevedo *et al.*, 2020). Among this great diversity, the genus *Xenodon* currently contains 12 species and is one of most widespread snake groups in South America (Nogueira *et al.*, 2019). Some of these species were previously classified in the genus *Waglerophis* (characterized mainly by a short maxillary bone), and several others were in the genus *Lystrophis* (identified by the presence of a shovel-shaped rostral scale with a central keel; the rostral scale is the median plate on the tip of the snout bordering the mouth (Ceï, 1993; Scrocchi & Cruz, 1993). The synonymization of these two genera with *Xenodon* was proposed to maintain monophyly based on molecular analyses (Zaher *et al.*, 2009; Grazziotin *et al.*, 2012). Species of this genus are mostly diurnal and terrestrial, feeding mainly on anurans, and all species exhibit Batesian mimicry with venomous snakes of the genera *Bothrops* or *Micrurus* (Sazima & Abe, 1991; Ceï, 1993; Carreira *et al.*, 2005; Cacciali, 2009; Rabosky *et al.*, 2016).

Xenodon is distributed from southern Mexico to northern Argentina (Ceï, 1993; Carreira *et al.*, 2005; Uetz *et al.*, 2021). Within the genus, two morphological, non-monophyletic groups can easily be distinguished: species with a rounded rostral scale and species with keeled rostral scale (Table 1). The first group contains *Xenodon guentheri* Boulenger 1984, *Xenodon merremii* (Wagler, 1824), *Xenodon neuwiedii* Günther, 1863, *Xenodon rabdocephalus* (Wied-Neuwied, 1824), *Xenodon severus* (Linnaeus, 1758) and *Xenodon weneri* (Eiselt, 1963). These species are associated with forested areas and terrestrial habits, except for *X. merremii*, which is distributed mainly in open areas, with records in adjacent forested areas (Martins & Oliveira, 1998; Marques *et al.*, 2001; Giraudo, 2002; Supporting Information, Figs S1–S3).

The other group is composed by species with a keeled rostral scale resembling a shovel (the former genus *Lystrophis*), including *Xenodon dorbignyi* (Bibron, 1854), *Xenodon histricus* (Jan, 1863), *Xenodon matogrossensis* (Scrocchi & Cruz, 1993), *Xenodon nattereri* (Steindachner, 1867), *Xenodon pulcher* (Jan, 1863) and *Xenodon semicinctus* (Duméril, Bibron & Duméril, 1854), which are distributed mainly in open areas, with semi-fossorial habits (Ceï, 1993; Scrocchi & Cruz, 1993; Carreira *et al.*, 2005; Cabral *et al.*, 2015; Nogueira *et al.*, 2019; Supporting Information, Fig. S4). Among the 12 species within *Xenodon*, seven

Table 1. Species of *Xenodon*, with information on the type of rostral scale, model and habitat

Species	Rostral scale	Model	Habitat	References
<i>Xenodon merremii</i>	Rounded	<i>Bothrops</i>	Open and forest	Ceï (1993); Pizzatto <i>et al.</i> (2008); Cacciali (2010)
<i>Xenodon weneri</i>	Rounded	<i>Bothrops</i>	Forest	Hoogmoed (1985); Chippaux (1986)
<i>Xenodon severus</i>	Rounded	<i>Bothrops</i>	Forest	Chippaux (1986); da Silva <i>et al.</i> (2010); Kahn (2010)
<i>Xenodon matogrossensis</i> *	Keeled	<i>Micrurus</i>	Open	Cabral <i>et al.</i> (2020)
<i>Xenodon pulcher</i> *	Keeled	<i>Micrurus</i>	Open	Ceï (1993); Scrocchi & Cruz (1993); Nenda & Cacicvio (2007)
<i>Xenodon semicinctus</i> *	Keeled	<i>Micrurus</i>	Open	Ceï (1993); Scrocchi & Cruz (1993); Nenda & Cacicvio (2007)
<i>Xenodon guentheri</i>	Rounded	<i>Bothrops</i>	Forest	Bérnils, <i>et al.</i> (2001); Abegg <i>et al.</i> (2016)
<i>Xenodon nattereri</i> *	Keeled	<i>Bothrops</i>	Open	Ceï (1993); Marques <i>et al.</i> (2015); Fiorillo <i>et al.</i> (2021)
<i>Xenodon histricus</i> *	Keeled	<i>Micrurus</i>	Open	Ceï (1993); Carreira (2002); Carreira <i>et al.</i> (2005)
<i>Xenodon dorbignyi</i> *	Keeled	Both	Open	Yanosky & Chani (1988); Ceï (1993); Carreira <i>et al.</i> (2005); Nenda & Cacicvio (2007); Tozetti <i>et al.</i> (2009)
<i>Xenodon neuwiedii</i>	Rounded	<i>Bothrops</i>	Forest	Ceï (1993); Pedrozo <i>et al.</i> (2020)
<i>Xenodon rabdocephalus</i>	Rounded	<i>Bothrops</i>	Forest	Chippaux (1986); Marques <i>et al.</i> (2015); Aguilar-López <i>et al.</i> (2021)

*Semi-fossorial species.

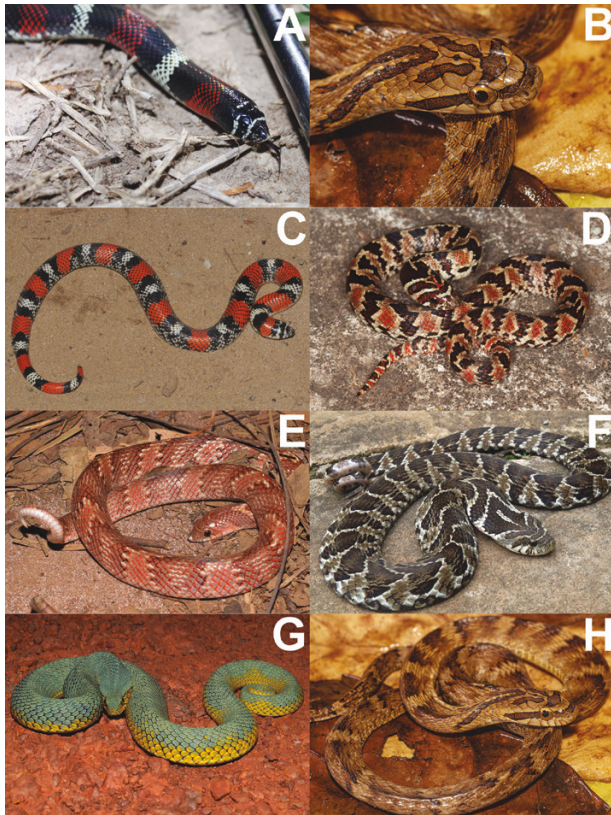


Figure 1. Mimicry and the shape of rostral scale (above the mouth) in the genus *Xenodon*. A, Lateral view of *X. pulcher* showing the keeled rostral scale and B, Lateral view of *X. newwedii* showing the rounded rostral scale. *Micrurus* (C-D) and *Bothrops* (E-H) are Batesian mimics of several species of *Xenodon*: C, *X. matogrossensis*; D, *X. histricus*; E, *X. nattereri*; F, *X. guentheri*; G, *X. wernerii*; H, *X. newwedii*. Photograph credits: Henrique Nogueira (B, H), Marcio Martins (C), Santiago Carreira (D), Diego Cavalheri (E), Karol Ceron (F), © Sébastien Sant/Parc Amazonien de Guyane (G).

are mimics of pitvipers (*Bothrops*), four are mimics of coral snakes (*Micrurus*) and one species (*X. dorbignyi*) exhibits a double mimicry of both *Bothrops* and *Micrurus* (Yanosky & Chani, 1988; Cei, 1993; Tozetti *et al.*, 2009; Alves *et al.*, 2013; Table 1; Fig. 1).

The seven species exhibiting *Bothrops* mimicry have a rounded rostral scale and terrestrial habits, except for *X. dorbignyi* and *X. nattereri*, which have keeled rostral scale and semi-fossorial habits (Cei, 1993). In contrast, all the species exhibiting *Micrurus* mimicry have a keeled rostral scale and semi-fossorial habits; the keeled scale serves as a shovel for digging (Orejas-Miranda, 1966; Cei, 1993; Scrocchi & Cruz, 1993). The members of the genus have different adaptations, with half being adapted to terrestrial habitats in forested

environments (Cei, 1993; Giraudo, 2002) and the other half adapted to a semi-fossorial habitat strongly associated with open areas (Cei, 1993; Cabral *et al.*, 2015, 2020).

Batesian mimicry involves a resemblance between two species, one of which (the model) has a coloration that implies potential danger or has some other unpleasant characteristics whereas the other (the mimic) is essentially harmless (Wickler, 1968; Mallet & Joron, 1999). This mimicry often involves bright warning coloration, which a predator recognizes as unpleasant or dangerous and learns to avoid (Greene & McDiarmid, 1981). Through the resemblance to the potentially dangerous species, the mimic deceives a third species (a predator), which results in reduced predation, with the predator treating the non-dangerous prey as dangerous (Greene & McDiarmid, 1981; Mallet & Joron, 1999; Pfennig *et al.*, 2001). Mimics and models co-evolve in the classic sense of exerting reciprocal selection on one another through their respective effects on the response of the predator to signals (Brodie & Brodie, 2004; Rabosky *et al.*, 2016). In coral snakes and vipers, mimicry (involving coloration) has evolved independently, as an important defence system to avoid predation (Savage & Slowinski, 1992; Brodie & Brodie, 2004; Rabosky *et al.*, 2016). Nonetheless, little is known about the evolutionary process of mimicry in snakes (Brodie & Brodie, 2004).

The evolution of mimicry is related to the sympatric presence of both non-venomous and venomous species, the model to be imitated (Pfennig *et al.*, 2001; Brodie & Brodie, 2004; Kikuchi & Pfennig, 2010). This is an important prediction of Batesian mimicry theory (Roze, 1983; Rabosky *et al.*, 2016). Based on the dual morphology of the rostral scale and the Batesian mimicry presented by *Xenodon* species, we used ancestral character reconstruction to investigate the patterns of evolution of both the rostral scale and mimicry. *Xenodon* is an ideal genus to test evolutionary principles given that its species exhibit different adaptations, behaviours and habitat preferences. How these characters evolved in relation to ecology would help us to understand whether such characters evolved independently as adaptations to new the same environments (convergent evolution) or whether they occurred more randomly (Da Silva *et al.*, 2017; Ramm *et al.*, 2018). Such characters might have multiple origins favoured by natural selection (Harvey & Pagel, 1991), and often this used as evidence for adaptive evolution (Losos *et al.*, 2002; Ramm *et al.*, 2020).

Given that the defensive behaviours have evolved as a response to predation (Losos *et al.*, 2002; Young *et al.*, 2004; Stankowich & Campbell, 2016; Ramm

et al., 2020) and that mimicry is found in species in different Neotropical environments associated with the presence of venomous or dangerous species (Brodie, 1993; Buasso *et al.*, 2006; Kikuchi & Pfennig, 2010; Rabosky *et al.*, 2016; França *et al.*, 2017), we aim to understand how the observed pattern of mimicry in the genus evolved.

We used a time-calibrated phylogeny to understand how the evolution of the rostral scale and mimicry in the genus *Xenodon* arose. First, we predicted that the ancestral state in the genus was the presence of a rounded rostral scale (as in the other members of Xenodontini) and that evolution of the rostral scale was favoured by natural selection. The modified scale in *Xenodon* (formerly *Lystrophis*) serves as a shovel for digging in areas with sandy soils in open areas (Ceï, 1993; Carreira *et al.*, 2005; Cacciali, 2009). In contrast, the remaining species of *Xenodon* have a rounded rostral scale; apparently, this does not have a special function like the modified rostral scale.

Second, we predicted that the ancestral condition of the genus was mimicry of *Bothrops* because the *Bothrops* ancestor was widely distributed in South America, having diversified during the Middle Miocene, ~11–12 Mya (Parkinson *et al.*, 2002; Hamdan *et al.*, 2020). Moreover, at least two species of *Xenodon* exhibiting *Bothrops* mimicry are distributed in the north of South America (Supporting Information, Fig. S1), whereas the *Xenodon* species exhibiting *Micrurus* mimicry are distributed in open areas in southern South America (Supporting Information, Fig. S4). Only one species of *Xenodon* has reached Central America (*X. rabdocephalus*) (Nogueira *et al.*, 2019), and *Bothrops asper* (Garman, 1883) is the only species of *Bothrops* to have reached Central America, also being distributed in northern South America (Hamdan *et al.*, 2020) where it is associated with Amazon forest, which might support the idea of a *Xenodon* ancestor exhibiting mimicking *Bothrops*. It is noteworthy that *X. rabdocephalus* mimics both *B. asper* and *Bothrops atrox* (Linnaeus, 1758) (Myers & McDowell, 2014). Given that all the *Xenodon* species mimicking *Micrurus* are restricted to southern South America and that species of *Xenodon* exhibiting mimicking *Bothrops* are more widely distributed, and these are the only species that have reached Central America (*X. rabdocephalus*) and following the northern Plio-Pleistocene diversification (~2.2 Mya) of *B. asper*, we believe there is enough evidence that the ancestors of *Xenodon* mimicked *Bothrops* and shared its terrestrial habits. Most *Bothrops* species tend to be strongly terrestrial (de Alencar *et al.*, 2017), as are *Xenodon* species that mimic *Bothrops*. *Micrurus* are usually cryptic (Roze, 1996), similar to *Xenodon* species that mimic them; both are semi-fossorial.

MATERIAL AND METHODS

The study area was in the Neotropics, from southern Mexico to northern Argentina. We classified the species into two groups according to the presence of a rounded or keeled rostral scale (Fig. 1A, B). To clarify the concepts, we refer to a rounded rostral scale as a smooth structure without keels, not projecting horizontally or vertically (Fig. 1B) and to a keeled rostral scale as a structure heavily keeled, with a horizontal or vertical projection (Fig. 1A). Information on the shape of the rostral scale was gathered from photographs and the literature (Ceï, 1993; Scrocchi & Cruz, 1993; Giraudo, 2002; Carreira *et al.*, 2005; Alves *et al.*, 2013).

For the classification of mimicry, we used various literature sources (Greene & McDiarmid, 1981; Yanosky & Chani, 1988; Ceï, 1993; Giraudo, 2002; Carreira *et al.*, 2005; Cacciali, 2009; Alves *et al.*, 2013; Caetano *et al.*, 2016; da Silva, 2016), especially for those species exhibiting *Bothrops* mimicry, to avoid ambiguities or wrong interpretations. Species with coral snake patterns are easier to classify as *Micrurus* mimicry (Fig. 1C, D). However, for some species, such as *X. dorbignyi* and *X. histricus*, we gathered information from recent papers (Tozetti *et al.*, 2009; Alves *et al.*, 2013). For classification of mimicry, we classified the species with *Bothrops* or *Micrurus* coloration, using adult dorsal coloration only (Fig. 1C–H). The typical coloration of *X. merremii* consists of a pattern with semicircular brownish lateral marks with wavy edges skirted by thin white margins (Ceï, 1993; Giraudo, 2002; Cacciali, 2010). The typical coloration resembles that of several *Bothrops* species, and the defensive mechanism of the species also imitates that of *Bothrops* by triangulating the head and producing intimidating attacks (Ceï, 1993). Therefore, we classified *X. merremii* as exhibiting *Bothrops* mimicry.

We used the latest Brazilian snake checklist, complemented with recent papers and our own data (Cacciali *et al.*, 2016; Nogueira *et al.*, 2019; França *et al.*, 2020; Pereira Filho *et al.*, 2021; Williams *et al.*, 2021), to elaborate distribution maps for each species. Habitat preferences of *Xenodon* species were obtained using natural history information on the various species. Those that are usually found in forest were classified as forest species, and species found in open areas (Pampas, dry and humid Chaco and Pantanal) were classified as open area species. The natural history references are provided in Table 1. *Xenodon merremii* was considered as occupying both forest and open areas. In addition, we gathered information about the distribution of each species from several literature sources (Ceï, 1993; Carreira *et al.*, 2005; Cacciali, 2009; Tozetti *et al.*, 2009; Alves *et al.*, 2013; Cabral *et al.*, 2015; Nogueira *et al.*, 2019; Pedrozo *et al.*, 2020). Additional

Table 2. Accession numbers

Species	12S	16S	Reference
<i>X. dorbignyi</i>	GQ457812	GQ457752	Zaher <i>et al.</i> (2009)
<i>X. guentheri</i>	JQ598849	JQ598909	Grazziotin <i>et al.</i> (2012)
<i>X. histricus</i>	GQ457813	GQ457753	Zaher <i>et al.</i> (2009)
<i>X. matogrossensis</i>	JQ598850	JQ598910	Grazziotin <i>et al.</i> (2012)
<i>X. merremii</i>	GQ457840	JQ598911	Zaher <i>et al.</i> (2009)
<i>X. nattereri</i>	JQ598851	JQ598912	Grazziotin <i>et al.</i> (2012)
<i>X. newwiedii</i>	GQ457841	GQ457779	Zaher <i>et al.</i> (2009)
<i>X. pulcher</i>	JQ598852	JQ598913	Grazziotin <i>et al.</i> (2012)
<i>X. rabdocephalus</i>	–	MH141006	Mulcahy (unpublished observations)
<i>X. semicinctus</i>	GU018156	GU018173	Vidal <i>et al.</i> (2010)
<i>X. severus</i>	JQ598853	JQ598914	Grazziotin <i>et al.</i> (2012)
<i>X. wernerii</i>	AF158468	AF158538	Vidal <i>et al.</i> (2000)
<i>E. miliaris</i>	AF158409	AF158480	Vidal <i>et al.</i> , (2000)

information was used to determine whether species were present in open or in forested areas.

PHYLOGENETIC HYPOTHESES

The latest snake phylogeny, from the study by Zaher *et al.* (2019), lists 11 of the 12 species of *Xenodon*. Nevertheless, we constructed our own calibrated tree to include all the species within the genus. We downloaded 12S and 16S mtDNA sequences of the 12 species of *Xenodon* from GenBank (Table 2), including *X. rabdocephalus*, which is absent from the tree constructed by Zaher *et al.* (2019), and used *Erythrolamprus miliaris* (Linnaeus, 1758) as an outgroup. We chose this species because *Erythrolamprus* is the sister genus of *Xenodon* and is widespread within the Neotropics. We concatenated and aligned the 12S and 16S sequences in GENEIOUS v.9.1.2 using the MUSCLE algorithm with the default parameters (Edgar, 2004). The final aligned dataset used in all analyses comprised 852 bp. We used PARTITIONFINDER 2 to identify partitioning schemes and the most appropriate nucleotide replacement models (Lanfear *et al.*, 2017). According

to our concatenated alignment, we found one partition evaluated by Bayesian information criterion, with the best model being TrN+G. For phylogenetic analysis, we performed a Bayesian phylogenetic analysis using BEAST v.2.6.3 (Bouckaert *et al.*, 2019) for 50 million generations, sampling every 2000 steps using a Yule process tree prior. We checked for stationarity by visual inspection of trace plots and ensuring that all values for effective sample size were > 200 in TRACER v.1.7.1 (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 calculated with TREEANNOTATOR v.2.6.3 (Bouckaert *et al.*, 2019).

ANCESTRAL CHARACTER RECONSTRUCTION

To evaluate the pattern of evolution of both types of rostral scales and the pattern evolution of mimicry in the genus, we tested the best models of evolution using the *fitDiscrete* function in the GEIGER package (Pennell *et al.*, 2014) for the software R v.3.6.0 (R Core Team, 2019). This function fits various likelihood models of continuous time Markov models of trait evolution for discrete characters, returning an estimate parameter along with the likelihood for univariate data (Yang, 2006). Transitions from one state to another can occur at different rates and in different directions. Three models of transition rates were used during ancestral character reconstruction: equal rates (ER), symmetric (SYM) model and all rates different (ARD). These models allow a random process whereby the probability of change between character states depends only on the current state (Yang, 2006; Pennell *et al.*, 2014). In the ER model, the rates of changes between characters are assumed to be equivalent. The SYM model allows different rates of changes between a pair of states, but changes between all states are theoretically possible (for binary data, SYM results are equivalent to ER), and in the ARD model all possible types of transitions can have different rates (Paradis *et al.*, 2004; Revell, 2014). The best models were selected based on the smallest Akaike information criterion corrected for small sample size (AICc) (Burnham & Anderson, 2007). Given that classification of the rostral scale is binary, as stated above, we used only the ER and ARD models, whereas for mimicry we used all three models (ER, SYM and ARD).

We used the method proposed by Borges *et al.* (2018) to test for phylogenetic signal in categorical traits. This method measures the degree of phylogenetic signal using the concept of Shannon's entropy, measuring the entropy contained in ancestral inferences using (δ) as the value of phylogenetic signal. When δ increases, the trait evolves according to the phylogeny, and when δ decreases, the trait evolves independently. Values of δ

can be any positive real number; the higher the value of δ , the higher the degree of phylogenetic signal between the trait and the phylogeny (Borges *et al.*, 2018). We performed this analysis in the software R v.3.6.0 (R Core Team, 2019), following the methods described by Borges *et al.* (2018)

We reconstructed the ancestral state using the stochastic character mapping Markov chain Monte Carlo approach (Huelsenbeck *et al.*, 2003) in the PHYTOOLS package (Revell, 2012), with the *make.simmap* function, a method for reconstruction that uses Bayesian inference. Both characters (rostral scale and mimicry) were mapped in the phylogeny, with the probability of the trait occurring among 100 stochastic trait histories (Dunoyer *et al.*, 2021). To analyse the correlation between the rostral scale and habitat, we performed a threshold model of character evolution in PHYTOOLS (Felsenstein, 2012; Revell, 2014), with

1 000 000 generations, sampled every 100 generations, and we rejected the first 200 000 generations as burn-in.

RESULTS

Our phylogenetic tree, containing all 12 species known for the genus (Fig. 2), presents two well-supported clades [posterior probability (PP) = 1.00; Fig. 2]. The first clade (PP = 1.00) contains *X. severus* as the sister taxon of *X. merremii*, which is the sister taxon of *X. wernerii* and *X. rabdocephalus*, all with a rounded rostral scale and wide distribution in South America (Supporting Information, Figs S1–S3). The relationship between *X. rabdocephalus* and *X. wernerii* shows a low support value, and *X. merremii* could also be the sister species of one or both species

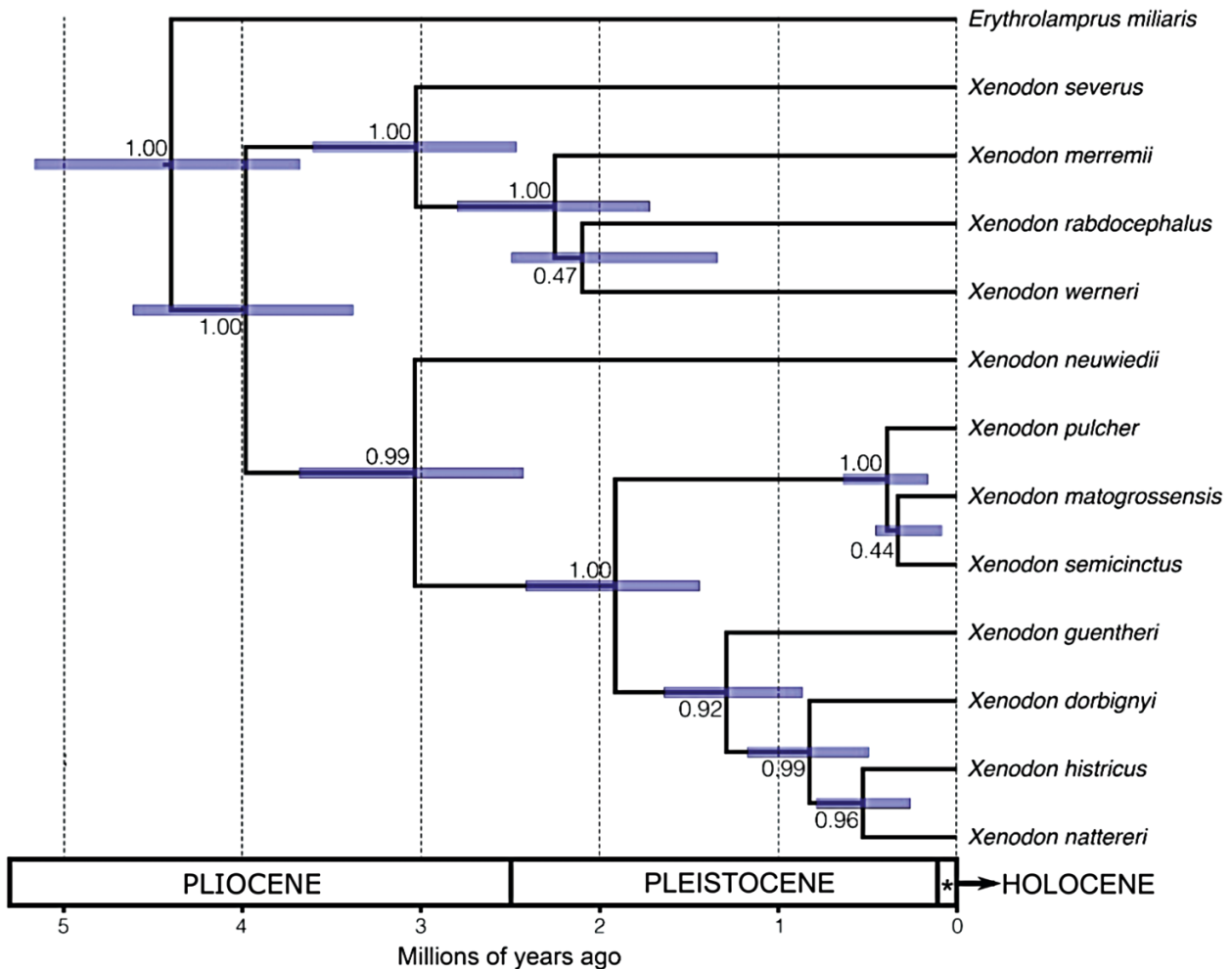


Figure 2. Species tree of *Xenodon* inferred with BEAST, including *Erythrolamprus miliaris* as an outgroup. Numbers at the nodes represent the posterior probabilities.

(PP = 1.00). The second clade is composed of two well-supported subclades (PP = 0.99), with *X. neuwiedii* as the sister species of both subclades (Fig. 2), also well supported (PP = 0.99). One subclade is composed of *X. semicinctus* as a sister species of *X. matogrossensis*, with low support, with *X. pulcher* as the sister taxon of both. The remaining subclade is also well supported (PP = 0.99) and has *X. guentheri* as the sister taxon of *X. dorbignyi*, *X. histricus* and *X. nattereri* (Fig. 2). In the second clade, all species have a keeled rostral scale, except for *X. neuwiedii* and *X. guentheri*.

In addition, our calibrated time tree (Fig. 2) indicates the first divergence between the two major clades ~3.98 Mya [95% highest posterior density (HPD) = 3.38–4.61 Mya], during the middle Pliocene. The beginning of the divergence within both subclades occurs in similar periods. The first clade, containing *X. severus*, *X. merremii*, *X. rabdocephalus* and *X. weneri*, started ~3.03 Mya (95% HPD = 2.47–3.61 Mya), at the end of the Pliocene, and the other divergences occurred at the beginning of the Pleistocene. The second, and more diverse, subclade containing the remaining species started its divergence ~3.04 Mya (95% HPD = 2.43–3.68 Mya), with other divergences from the beginning to the end of the Pleistocene.

Our stochastic character mapping suggests that the ancestor of *Xenodon* had a rounded rostral scale and exhibited *Bothrops* mimicry (Fig. 3A). The keeled rostral scale appears for the first time ~2 Mya, during the Pleistocene, in the subclade containing *X. pulcher*, *X. semicinctus* and *X. matogrossensis*, and then in the subclade containing *X. dorbignyi*, *X. histricus* and *X. nattereri*, with one change ~1 Mya in *X. guentheri* (Fig. 3B). The *Bothrops* mimicry pattern was constant around the evolution of the genus, with only two changes, which appear for the first time ~2 Mya in the subclade containing *X. pulcher*, *X. semicinctus* and *X. matogrossensis*, and appear again in *X. dorbignyi* and *X. histricus* ~1 Mya, during the Pleistocene (Fig. 3B).

The best model for both rostral scale and mimicry pattern was the ER model (lowest AICc; Table 3). We found a strong phylogenetic signal for the rostral scale ($\delta = 5.04$, $P = 0.02$; when $P < 0.05$, there is evidence of a phylogenetic signal between the trait and the phylogeny), meaning that species with a keeled rostral scale are closely related species, which tend to resemble each other for the specific trait, evolving in close association within the phylogeny. In the case of mimicry ($\delta = 1.8$, $P = 0.48$, $P > 0.05$), we found a weak phylogenetic signal or no evidence of phylogenetic signal, indicating that this trait evolved independently from the phylogeny. We found a positive correlation between the type of rostral scale and the habitat inhabited by the species, with a correlation coefficient of $r = 0.5$.

DISCUSSION

As in previous studies (Zaher *et al.*, 2009; Grazziotin *et al.*, 2012), we found two well-supported clades, along with the remaining two subclades (Fig. 2). Some differences can be found in the position of *X. pulcher* as the sister species of *X. semicinctus* and *X. matogrossensis*. Additionally, differences can be observed in the position of *X. guentheri*, *X. weneri* and *X. rabdocephalus* (Fig. 2).

We found that the rostral scale evolved in close relationship within the phylogeny of the genus (strong phylogenetic signal), unlike the weak phylogenetic signal or lack of phylogenetic signal showing that mimicry evolved independently from the phylogeny. The reconstruction and selection of the ER model showed the same rate of transitions between both traits (rostral scale and mimicry). The evolution of the rostral scale and mimicry is unlikely to have been gradual; probably, the rapid transition between the clades might explain these transitions, also these transitions have been occurring too fast to be gradual. The differences between ER and ARD models (AICc) for the rostral scale are small, and either of the models could be correct; however, Δ AICc and wAICc (Table 3) support the ER model for rostral scale evolution. In ARD models, every type of transition can have different rates.

Mimicry is present widely in almost all genera of Colubridae and Dipsadidae, especially *Micrurus* (Rabosky *et al.*, 2016); however, only a few genera have all the species of their genus exhibiting mimicry. For example, the Elapomorhini tribe (*Apostolepis*, *Phalotris*, *Coronelaps* and *Elapomorphus*) has species that mimic species of *Micrurus* (Ceil, 1993; da Silva, 2016). Nonetheless, all members of *Xenodon* exhibit mimicry of *Bothrops* or *Micrurus*, which made them an interesting subject of study; that is why we only analysed the evolution of mimicry between the species and did not include other genera of snakes.

Our results suggest that species in the genus *Xenodon* evolved from an ancestor with a rounded rostral scale and a *Bothrops* colour pattern. The evolution of the rostral scale and mimicry in the genus began ~4 Mya, in the Plio-Pleistocene, a period with transitions from humid and warmer forest-like habitats to cooler and drier savannah-like habitats (Hooghiemstra & Cleef, 1995). This period was characterized by a marked and pronounced dry season, associated with drastic lowering of global temperatures and increasing aridity, resulting in the replacement of lowland rainforests by savannah woodlands (Jacobs, 2004; Plana, 2004).

Events related to extreme environmental heterogeneity have been reported in a range of Neotropical fauna, which are hypothesized to have driven the differentiation of the open biome

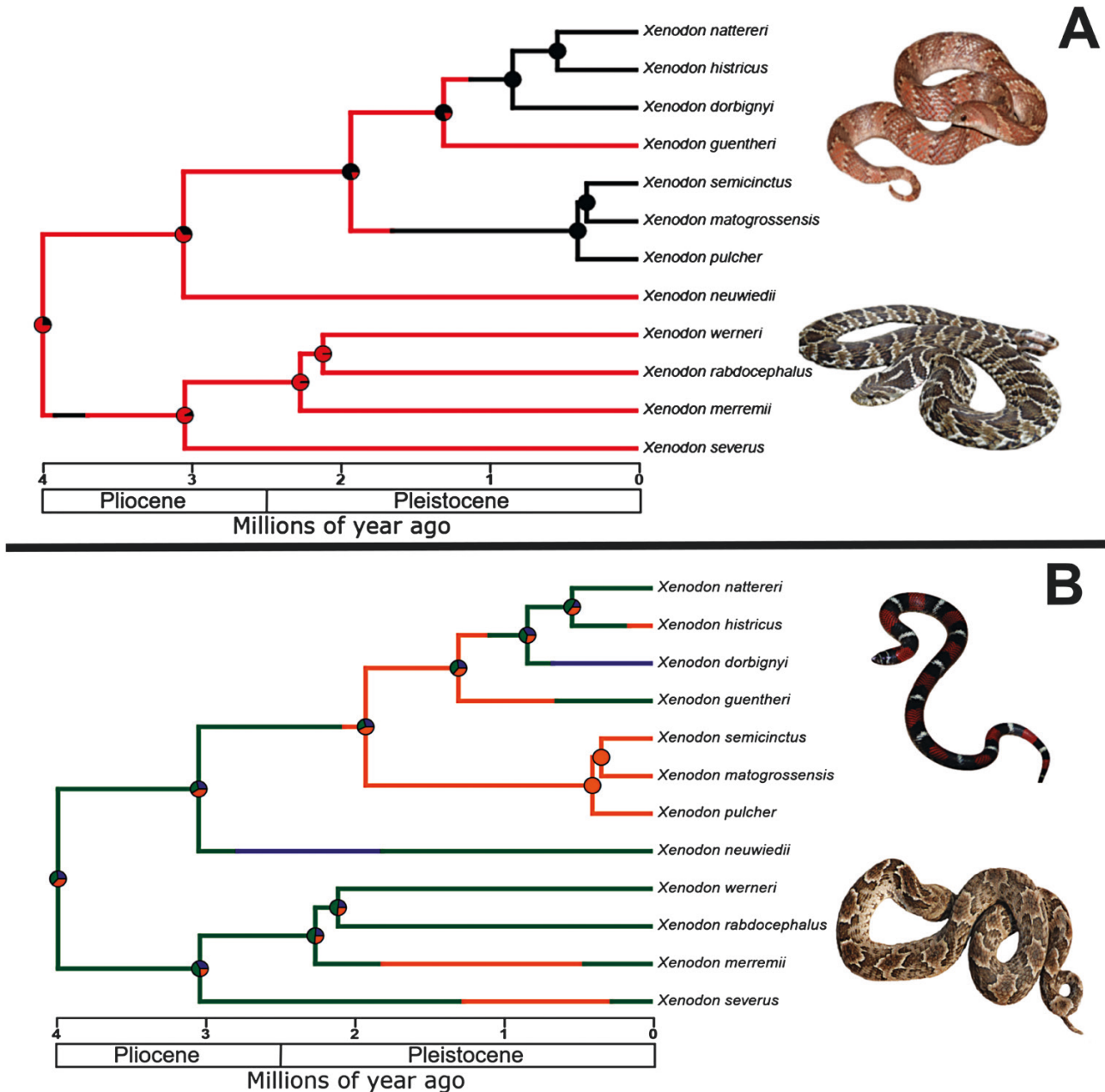


Figure 3. Stochastic character mapping reconstruction. A, reconstruction of rostral scale evolution. Black branches = keeled rostral scale; red branches = normal rostral scale. B, reconstruction of mimicry evolution. Green branches = *Bothrops* mimicry, orange branches = *Micrurus*; blue branch = mimicry of both. The pie charts at the nodes represent the probability of the ancestral state. This figure is illustrated with four *Xenodon* species: A, *Xenodon nattereri* at the top and *Xenodon guentheri* at the bottom; and B, *Xenodon pulcher* at the top and *Xenodon merremii* at the bottom.

communities from adjacent rainforests and from each other, and consequently, the biota of South America (Grau *et al.*, 2005; Bryson *et al.*, 2011; Werneck, 2011; Daza *et al.*, 2012; Sobral-Souza & Lima-Ribeiro, 2017). Therefore, the habitats where the species of *Xenodon* occur were affected directly by these events. These changes in climate and habitats

are likely to have shaped the diversification of the species and the evolution of the keeled rostral scale of the genus, probably as an adaptation to dry and open environments. The maintenance of the rounded rostral scale in *X. guentheri* is probably attributable to evolutionary convergence; there was no selection to change to a keeled rostral scale, owing to its habits,

Table 3. Akaike information criterion of the three models of transition rates tested for rostral scale and mimicry

Model	AICc	Δ AICc	wAICc	Log likelihood ($-\ln L$)	Parameters
Rostral scale					
ER	14.18	0	0.38	-5.89	1
SYM	–	–	–	–	–
ARD	15.31	1.31	0.22	-4.99	2
Mimicry					
ER	24.70	0	0.95	-11.15	1
SYM	30.68	5.98	0.04	-10.84	3
ARD	46.89	22.19	0	-9.04	6

Bold values indicate the best models for each trait.

Abbreviations: ARD, all rates different; AICc, Akaike Information Criteria with correction; ER, equal rates; SYM, symmetric; Δ AICc, Delta Akaike Information Criteria with correction; wAIC, Weight Akaike Information Criteria.

because the species has more terrestrial habits than semi-fossorial ones.

Overall, species with a keeled rostral scale exhibit a distribution associated with open areas, such as Cerrado, Chaco, Pantanal and Pampas (Supporting Information, Fig. S4). In contrast, species without keeled rostral scales are, in general, associated with forested areas, such as Amazon Forest and the Atlantic Forest, except for *X. merremii*, which has a wide distribution associated with open and forested habitats (Supporting Information, Figs S1–S3). The distribution of semi-fossorial species (those *Xenodon* species with a keeled rostral scale) in open areas is often related to habitat characteristics, such as temperature and precipitation (Kinlaw, 1999; Wu *et al.*, 2015; Oliveira & Scheffers, 2019), and those species have adaptations to live underground, making them habitat specialists (Kinlaw, 1999; Greenville & Dickman, 2009). These adaptations, however, constrain many aspects of their ecology, creating challenges for moving underground over long distances (Papenfuss, 1982; Webb *et al.*, 2000; Martín *et al.*, 2021), which is to be expected owing to the energetic costs of burrowing (Dial *et al.*, 1987). In the case of *Micrurus*, there is a strong relationship between the co-occurrence of coral snakes and their mimics (Savage & Slowinski, 1992; Rabosky *et al.*, 2016; França *et al.*, 2017), especially with triadial coral snake species, restricted to South America (da Silva, 2016). The presence of a keeled rostral scale in the genus *Xenodon* is related to semi-fossorial habits and has evolved as a strategy for adaptations to the new environment, with a positive correlation between diversification and colonization of new habitats.

Mimicry (involving coloration) has evolved independently in colubrids (Rabosky *et al.*, 2016),

as an important defence system to avoid predation (Savage & Slowinski, 1992; Brodie & Brodie, 2004). This represents an important driver in the evolution of mimicry of coral snakes by *Xenodon* species, especially for those of the *Micrurus frontalis* group, which are widely distributed in central and southern South America (Da Silva & Sites, 1999; Di-Bernardo *et al.*, 2007). Species of the *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) group occur in sympatry with *Xenodon* species that mimic coral snakes. *Xenodon pulcher*, *X. semicinctus* and *X. matogrossensis* live in sympatry with *Micrurus pyrrhocryptus* and, in some regions, with *M. frontalis* and *Micrurus baliocoryphus* (Nogueira *et al.*, 2019), whereas *X. dorbignyi* and *X. histricus* occur in sympatry with *Micrurus altirostris*, *M. baliocoryphus* and *M. frontalis* (Nogueira *et al.*, 2019).

Regarding *Bothrops* mimicry, the same concepts apply, and there is a relationship between the presence of *Bothrops* and species that mimic them (Brodie & Brodie, 2004). Additionally, this mimicry is accompanied not only by coloration, but also by similar behaviours (Brodie & Brodie, 2004). The *Xenodon* species exhibiting *Bothrops* mimicry are more widespread, and the distribution of these species could be related to the potential model they imitate. *Xenodon rabdocephalus*, the only species of the genus with a distribution reaching Central America, apparently imitates *B. asper*, the only species of *Bothrops* in Central America (Hamdan *et al.*, 2020). *Bothrops asper* belongs to the *B. atrox* clade that began its diversification ~3.02–3.32 Mya (Hamdan *et al.*, 2020). Recent studies have suggested that the first lineage within the *B. atrox* group to diversify was *B. asper*, with the formation of the Panamanian bridge (Hamdan *et al.*, 2020). This information supports our results indicating that the ancestor of *Xenodon* was a species with *Bothrops* mimicry, following the distribution of *Bothrops* and *Xenodon* in northern South America.

Another interesting example of mimicry that corroborates our findings is the case of *X. weneri*. This species is restricted to northern Amazonia (Nogueira *et al.*, 2019), and it perfectly imitates *Bothrops bilineatus* (Fig. 1G), a *Bothrops* species with a disjointed distribution in Amazonian Forest and the Atlantic Forest (Nogueira *et al.*, 2019). The clade to which *B. bilineatus* belongs (*Bothrops taeniatus* clade) diverged during the Pliocene ~2–5 Mya in the Amazonian Forest (Hamdan *et al.*, 2020), which could be one of the reasons for the evolution of mimicry in *X. weneri*, because mimicry coloration could have evolved as a defence system to avoid predation and is often associated with the presence of the model being imitated, in this case species of *Bothrops*; both are drivers in the evolution

of *Bothrops* (Savage & Slowinski, 1992; Brodie & Brodie, 2004).

This mimicry of *Bothrops* and *Micrurus* is accompanied by behavioural postures, such as the triangulation of the head, tail rattling, hiding the head, tail display, dorsoventral body compression, coiling and s-coiling of the body (Sazima & Abe, 1991; Cei, 1993; Brodie & Brodie Jr, 2004; Cacciali, 2010), evolving independently as a defence system to avoid predation (Brodie & Brodie, 2004). All these components together are important strategies for survival, because predators react to any signals associated with venomous snakes, whether these are coloration patterns or behavioural actions, relating to congenial responses (Brodie & Brodie, 2004; França *et al.*, 2017). Finally, evolution of venomous snakes can contribute to diversity within groups of snakes (e.g. families, genera) (Greene & McDiarmid, 2005; Rabosky *et al.*, 2016; França *et al.*, 2017), and mimicry is another strong ecological force contributing to the speciation and radiation of the diversity of snakes (Brodie & Brodie, 2004).

In species that are Batesian mimics, with semi-fossorial habits and with a preference for open areas, the rostral scale was modified as a shovel for burrowing. In contrast, in species with terrestrial habits, the rostral scale remained rounded, probably owing to their association with forested habitats (Cei, 1993; Martins & Oliveira, 1998; Cabral *et al.*, 2020). In conclusion, the evolution of the rostral scale in *Xenodon* might be related to abiotic factors, as an adaptation for open and forested habitats, whereas mimicry is related to the presence of venomous species, such as *Bothrops* or *Micrurus*, because imitating them serves as a defensive strategy.

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DATA AVAILABILITY

The data and script supporting this study are available at: <https://github.com/Rhinella85/Xenodon-Evolution>

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Distribution of *Xenodon* species with a rounded rostral scale in South America and their relationships with the ecoregions, adapted from [Dinerstein et al. \(2017\)](#). White cross, *Xenodon severus*; black cross, *Xenodon weneri*; black diamond, *Xenodon rabdocephalus*.

Figure S2. Distribution of *Xenodon* species with a rounded rostral scale in South America and their relationships with the ecoregions, adapted from [Dinerstein et al. \(2017\)](#). Black star, *Xenodon merremii*.

Figure S3. Distribution of *Xenodon* species with a rounded rostral scale in South America and their relationships with the ecoregions, adapted from [Dinerstein et al. \(2017\)](#). Black triangle, *Xenodon neuwiedii*; white circle, *Xenodon guentheri*.

Figure S4. Distribution of *Xenodon* with a keeled rostral scale in South America and their relationships with the ecoregions, adapted from [Dinerstein et al. \(2017\)](#). White triangle, *Xenodon nattereri*; white circle, *Xenodon histricus*; black triangle, *Xenodon matogrossensis*; black square, *Xenodon pulcher*; white square, *Xenodon semicinctus*; black circle, *Xenodon dorbignyi*.