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## **A New Two-Pored *Amphisbaena* Linnaeus, 1758, from Western Amazonia, Brazil (Amphisbaenia: Reptilia)**

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# A New Two-Pored *Amphisbaena* Linnaeus, 1758, from Western Amazonia, Brazil (*Amphisbaenia*: Reptilia)

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**Abstract.** Recent efforts to improve sampling of Brazilian biodiversity have yielded a number of undescribed species of amphisbaenids. Herein, we describe a new species of small, two-pored *Amphisbaena* from western Brazilian Amazonia. The new species can be distinguished from all congeners by the combination of the following characters: two precloacal pores arranged in a continuous series; snout rounded in lateral and dorsal views; tip of tail rounded; 233–250 body annuli; 20–24 caudal annuli; autotomy sites on caudal annuli 6–9; 10 dorsal and 12–14 ventral segments per annulus at midbody; absence of postmalars; suture between frontals slightly smaller than parietal and nasal sutures; and tail short relative to body length (tail length/body length = 0.10). The new species inhabits the rain forest and small patches of savanna vegetation within the Amazon Forest. A Bayesian analysis based on two mitochondrial (16S and ND2) and three nuclear (*cmos*, *BDNF* and *RAG1*) markers recovered the new species as sister to a clade formed by *A. hastata* + *A. cuiabana*; however, support for this relationship is low. Genetic divergence between populations from both sides of the middle Madeira River is low.

**Keywords.** Amphisbaenian; Fossorial reptile; New species; Phylogeny; Systematics; Taxonomy.

## INTRODUCTION

About two and a half centuries ago, Linnaeus described several genera and species of reptiles (Linnaeus, 1758), including a limbless, worm-like species with a rudimentary eye from South America that he used as the type for the genus *Amphisbaena*. It was *Amphisbaena fuliginosa* Linnaeus, 1758 from the northern portion of South America (Vanzolini, 2002; Gans, 2005). Since then, several worm-like species and genera have been described from South American lands, currently totaling > 100 species occurring in both continental and insular habitats in Central and South America and varying from wet forests to dry desert (Gans, 2005). They include species with a dorsolaterally compressed snout and differentiated scales on the thoracic region (recognized formerly as the genus *Leposternon* Wagler, 1824; for opposing taxonomic positions see Mott and Vietes, 2009, and Ribeiro *et al.*, 2011), forms with a laterally compressed snout (formerly *Anops* Bell, 1833), those with a distinctive vertical keel on the tip of the tail (formerly *Cercolophia* Vanzolini, 1992), and other forms placed in additional genera (such as *Bronia* Gray, 1865, *Mesobaena* Mertens, 1925 and *Aulura* Barbour, 1914), also with distinctive morphological features (Gans, 2005). However, and surprisingly, the striking morphological adaptations supposedly typical of each genus are probably the result of convergence, as a recent study based on DNA sequences showed that most of these genera were paraphyletic groupings of distinct lineages,

bearing similar morphological features (Mott and Vieites, 2009).

After a long period dominated by scarcity of amphisbaenians in collections, the scenario has changed and larger series of specimens are regularly obtained, resulting from either broader intensive surveys of unexplored areas or faunal monitoring/rescue efforts from large-scale engineering projects occurring across the Brazilian territory. The result is an increasing number of species being described over the last 15 years (*e.g.*, Vanzolini, 1999; Castro-Mello, 2000, 2003; Strüssmann and Carvalho, 2001; Rodrigues, 2002; Vanzolini, 2002; Ribeiro *et al.*, 2008; Ribeiro *et al.*, 2009; Mott *et al.*, 2009; Strüssmann and Mott, 2009; Pinna *et al.*, 2010; Ribeiro *et al.*, 2011; Roberto *et al.*, 2014), with many more species awaiting description.

Among the small, two-pored species, only a few have been recorded from the Amazon forest, including *A. mitchelli* Procter, 1923, *A. slevini* Schmidt, 1936, and *A. miringoera* Vanzolini, 1996 (Gans, 1963; Hoogmoed and Ávila-Pires, 1991; Mott *et al.*, 2011) in eastern Amazonia.

During an intensive herpetofaunal survey undertaken as part of the monitoring and rescue activities for the recent construction of two hydroelectric dams on the middle Madeira River, several specimens of a small species bearing two pores were found, but they could not be identified as any currently described species of *Amphisbaena*. Herein, we describe this new species, diagnose it from morphologically similar species, and address its phylogenetic relationships based on mitochondrial and nuclear markers.

## MATERIALS AND METHODS

### Morphology

Scale nomenclature follows Gans and Alexander (1962). The following measurements were taken using a ruler to the nearest 1 mm: body length, from tip of snout to cloaca (BL), and tail length, from cloaca to tip of tail (TL), measured only when the tail was complete. Observations and counting were made with the aid of a Zeiss stereomicroscope. Morphological comparisons were based on voucher specimens (Appendix) housed in the herpetological collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP) and Laboratório de Herpetologia, Universidade de São Paulo (MTR), and data in the literature (Gans, 1962, 1963, 1964a, b, 1971; Vanzolini, 1964, 1971, 1996, 1999, 2002; Gans and Amdur, 1966; Strüssmann and Carvalho, 2001; Montero and Cespedez, 2002; Rodrigues, 2002; Mott *et al.*, 2008; Ribeiro *et al.*, 2008; Ribeiro *et al.*, 2009; Strüssmann and Mott, 2009; Pinna *et al.*, 2010; Ribeiro *et al.*, 2011; Perez *et al.*, 2012).

### Phylogenetics

To infer the phylogenetic placement of the new species within *Amphisbaena*, DNA sequences were obtained for two individuals of the new species: MZUSP 101602 from the right side of the middle Madeira River and MZUSP 104237 from the left side (GenBank accession numbers KJ669327–KJ669336). These sequences were added to the dataset of Mott and Vieites (2009), composed of an additional 31 *Amphisbaena* species and the outgroup taxa *Rhineura floridana* (Baird, 1858), *Blanus strauchii* (Bedriaga, 1884), *Bipes canaliculatus* Bonnaterre, 1789, *Trogonophis wiegmanni* Kaup, 1830, and *Geocalamus acutus* Sternfeld, 1912.

DNA was extracted from tissue samples (liver or muscle) preserved in 100% ethanol (Fetzner, 1999). Two mitochondrial markers (ribosomal RNA 16S, Palumbi, 1996; protein coding ND2, Macey *et al.*, 2004) and three nuclear protein-coding genes (*cmos*, Godinho *et al.*, 2005; BDNF, Vieites *et al.*, 2007; RAG1, Mott and Vieites, 2009) were used in the phylogenetic analyses. All fragments were amplified using standard PCR protocols with annealing temperatures of 51°C for 16S; 49°C for ND2; 52°C for *cmos*; 55°C for BDNF; and 54°C for RAG1. PCR products were sequenced at the Instituto de Química da Universidade de São Paulo and Instituto de Ciências Biomédicas da Universidade de São Paulo. Resulting sequences were edited using CodonCode Aligner v. 3.7.1.1. ([www.codoncode.com](http://www.codoncode.com)) and aligned using MUSCLE (Edgar, 2004) spawned from MEGA v. 5 (Tamura *et al.*, 2011) under default settings.

Uncorrected genetic distances (*p*-distances) were calculated using MEGA v. 5 (Tamura *et al.*, 2011). For

phylogenetic analyses, the best-fit model of evolution for each aligned marker was identified using jModelTest v. 2.1.3 software (Darriba *et al.*, 2012) and the Akaike Information Criterion (AIC). We inferred phylogenetic relationships using Bayesian analysis of the concatenated dataset of five partitions and respective best-fit substitution models using MrBayes v. 3.2 (Ronquist *et al.*, 2012) through the Cipres Science Gateway (Miller *et al.*, 2010). Two independent Bayesian runs were performed with four Markov chains, starting from a random tree. Each run consisted of 10,000,000 generations, with trees sampled every 1,000 generations. We discarded the first 25% of the trees as burn-in; convergence for independent runs and ESS values were checked using Tracer v. 1.5 (Rambaut and Drummond, 2009). The 50% majority consensus tree with clade frequencies was visualized using FigTree 1.3.1 (<http://tree.bio.ed.ac.uk/>). Scores above 0.95 were considered significant.

### Distribution

The distribution of two-pored *Amphisbaena* species was assessed through specimens examined (Appendix), data from the literature (Gans, 1963, 1964a,b, 1967; Vanzolini, 1964, 1971, 1992, 1996, 1997, 1999; Gans and Amdur, 1966; Vieira *et al.*, 1980; Hoogmoed and Avila-Pires, 1991; Cei, 1993; Azevedo-Ramos and Moutinho, 1994; Montero *et al.*, 1995; Montero, 1996; Montero and Terol, 1999; Vrcibradic and Soares, 1999; Strüssmann and Carvalho, 2001; Montero and Cespedez, 2002; Torres, 2003; Chalkidis and Di-Bernardo, 2004; Barros Filho *et al.*, 2005; Carreira *et al.*, 2005; Evers *et al.*, 2006; Barbo and Sawaya, 2008; Mott *et al.*, 2008; Silva Jr *et al.*, 2009; Strüssmann and Mott, 2009; Pinna *et al.*, 2010; Sousa *et al.*, 2010; Zacariotti and Gomes, 2010; Mott *et al.*, 2011; Dal Vechio *et al.*, 2013), and online databases (GBIF, 2013; SpeciesLink, 2013).

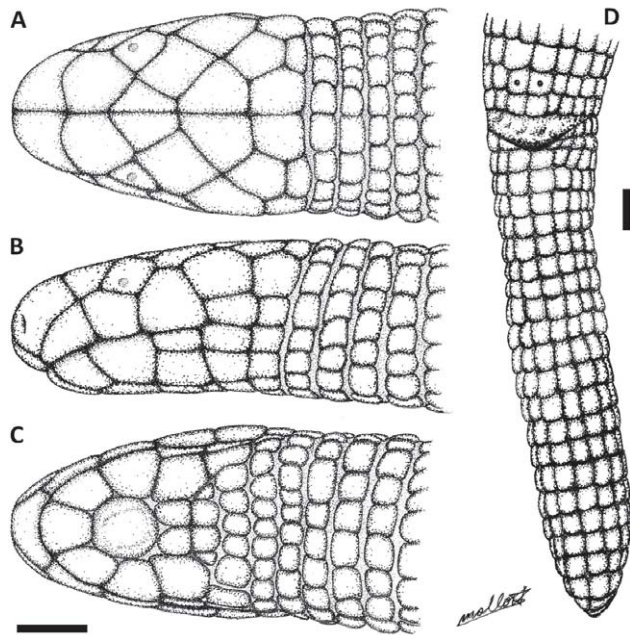
## RESULTS

### *Amphisbaena caiari* sp. nov. (Figs. 1, 2, 3C)

*Amphisbaena slevini*—Marçal *et al.*, 2011: 218 (part)

### Holotype

MZUSP 101727, collected on left side of Madeira River (ca. 08°47'S, 63°57'W, 90 m above sea level [a.s.l.]), Usina Hidroelétrica Santo Antonio, Porto Velho, Rondônia, Brazil, by biologists of Arcadis Tetraplan Consultoria Ambiental LTDA.



**Figure 1.** Holotype of *Amphisbaena caiari* sp. nov. (MZUSP 101727), showing (A) dorsal, (B) lateral and (C) ventral views of the head and (D) ventral view of the tail. Scale bar = 1 mm.

### Paratypes

MZUSP 101695–101697, 101715–101718, 101720, 101722–101726, 101728–101733, 102226–102236, 102238–102263 (ca. 08°47'S, 63°57'W, 90 m a.s.l.) from left side of Madeira River, Usina Hidroelétrica Santo Antonio, Porto Velho municipality, state of Rondônia, Brazil. MZUSP 101602 (09°35'50.43"S, 65°20'21.81"W, 113 m a.s.l., SAD69) from right side, and MZUSP 104081 (09°26'47.35"S, 64°49'29.93"W, 160 m a.s.l., SAD69) and MZUSP 104237 (09°27'13.53"S, 64°51'2.27"W, 145 m a.s.l., SAD69) from left side of Madeira River, Usina Hidroelétrica Jirau, Porto Velho municipality, state of Rondônia, Brazil. All collected by biologists of Arcadis Tetraplan Consultoria Ambiental LTDA, October 2010 to July 2012.

### Etymology

The specific epithet *caiari* is the ancient indigenous name, from the Tupi language, for the Madeira River, whose banks are inhabited by the new species.

### Diagnosis

(1) A small sized species, maximum SVL = 157 mm; (2) two preloacal pores; (3) preloacal pores arranged in a continuous series; (4) snout rounded in lateral and dorsal views; (5) tip of tail rounded; (6) body annuli 233–250; (7) caudal annuli 20–24; (8) autotomy sites on caudal annuli 6–9; (9) 10 dorsal and 12–14 ventral segments per

annulus at midbody; (10) postmalar row absent; (11) suture between frontals slightly smaller than parietal and nasal sutures; (12) tail short relative to body length (tail length/body length = 0.10).

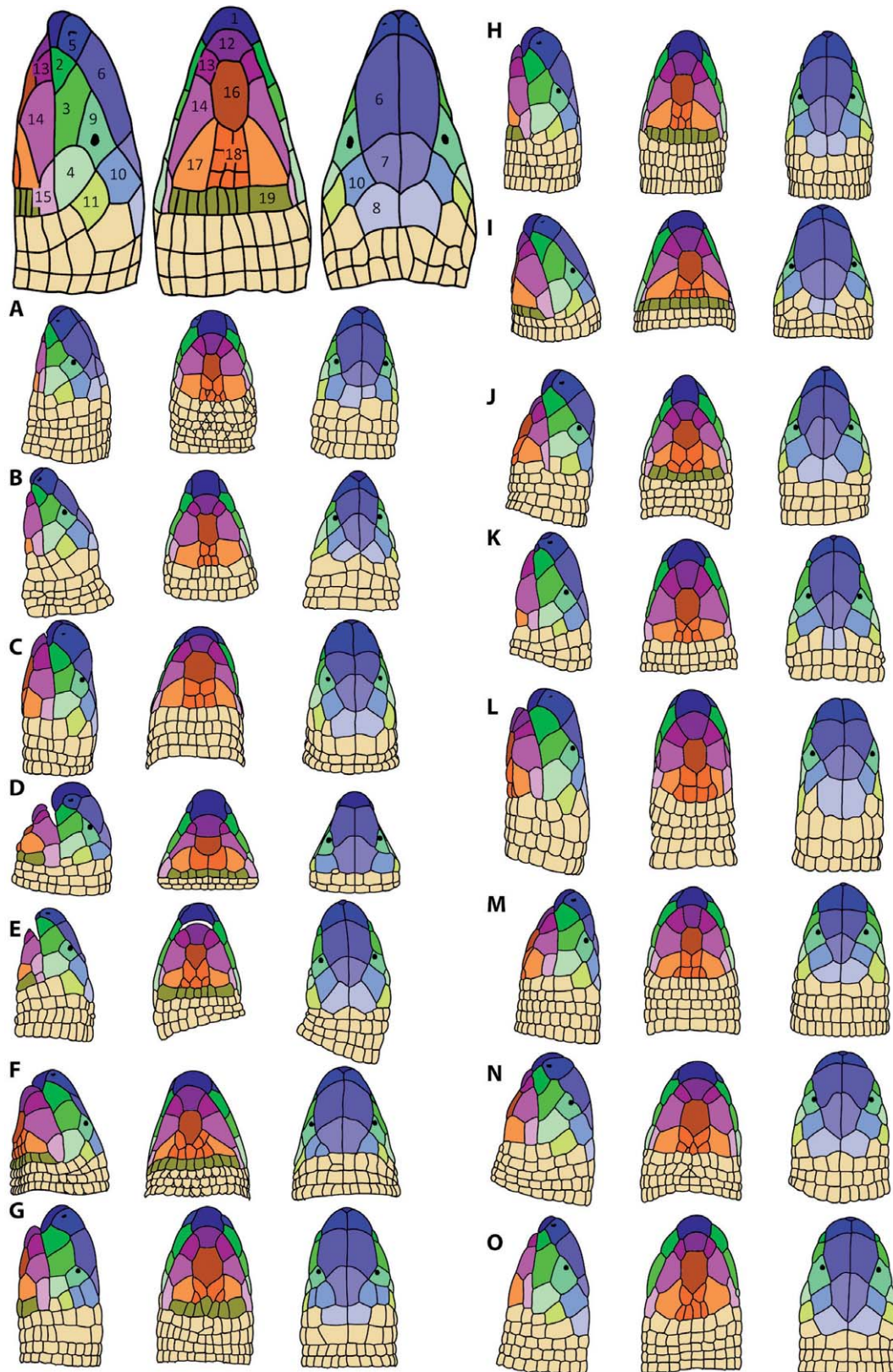
### Comparisons with other species

Characters from other species are presented in parenthesis. Among the South American two-pored *Amphisbaena* species, the rounded snout immediately distinguishes the new species from *A. cerradensis* (Ribeiro, Vaz-Silva and Santos Jr, 2008), *A. maxima* (Ribeiro, Nogueira, Cintra, Silva and Zaher, 2011) (dorsoventrally compressed, shovel-like snout), *A. acrobeles* (Ribeiro, Castro-Mello and Nogueira, 2009) and *A. bilabialata* (Stimson, 1972) (laterally compressed, with a conspicuous vertical keel). The rounded tail diagnoses it from *A. absaberi* (Strüssmann and Carvalho, 2001), *A. bahiana* Vanzolini, 1964, *A. roberti* Gans, 1964, (a sharp terminal vertical keel on tail), *A. leeseri* Gans, 1964, and *A. bilabialata* (a blunt terminal vertical keel). The presence of two pores arranged in a continuous series distinguishes the new species from *A. absaberi*, *A. anomala*, *A. bilabialata*, *A. carli* Pinna, Mendonça, Bocchiglieri & Fernandes, 2010, *A. cerradensis*, *A. hiata* Montero & Céspedes, 2002, and *A. maxima* (scales bearing pores separated from each other by one or more non-pored scales).

Among the remaining two-pored, South American species of *Amphisbaena*, the occurrence of 233–250 body annuli distinguishes the new species from *A. anaemariae* Vanzolini, 19697, *A. brevis* Strüssmann & Mott, 2009, *A. crissae* Vanzolini, 1997, *A. darwini* Duméril and Bibron, 1839, *A. dubia* Müller, 1924, *A. heterozonata* Burmeister, 1861, *A. mitchelli*, *A. neglecta* Dunn and Piatt, 1936, and *A. silvestrii* Boulenger, 1902 (< 231 body annuli) and from *A. miringoera* Vanzolini, 1971 (> 250 body annuli). The occurrence of 20–24 caudal annuli differs from counts of *A. anaemariae*, *A. brevis*, *A. dubia*, *A. heterozonata* and



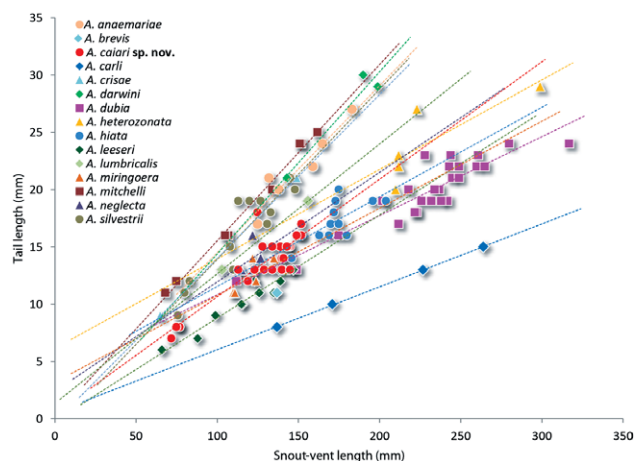
**Figure 2.** Living paratype (MZUSP 104081) specimen of *Amphisbaena caiari* sp. nov., from the left side of the Madeira River, Porto Velho, Rondônia, Brazil.



**Figure 3.** Lateral, ventral, and dorsal views of (A) *Amphisbaena anaemariae*, (B) *A. brevis*, (C) *A. caiari* sp. nov., (D) *A. carli*, (E) *A. crisaie*, (F) *A. darwini*, (G) *A. dubia*, (H) *A. hiata*, (I) *A. heterozonata*, (J) *A. leeseri*, (K) *A. lumbricalis*, (L) *A. miringoera*, (M) *A. mitchelli*, (N) *A. neglecta*, and (O) *A. silvestrii*. Adapted from their original or secondary descriptions (for references see Materials and Methods). Scales: 1 = rostral; 2, 3, 4 = supralabials; 5 = nasal; 6 = prefrontal; 7 = frontal; 8 = parietal; 9 = ocular; 10 = postocular; 11 = temporal; 12 = mental; 13, 14, 15 = infralabials; 16 = postmental; 17 = malar; 18 = postgenials; 19 = postmalar. Adapted from Gans and Alexander (1962).

*A. neglecta* (< 20 caudal annuli) and *A. mitchelli* (> 26 caudal annuli). The occurrence of the autotomic site in caudal annuli 6–9 differs from the condition found in *A. anaemariae*, *A. brevis*, *A. crissae*, *A. dubia*, *A. leeseri*, *A. neglecta* and *A. silvestrii* (autotomy site absent or in an anterior to caudal annulus 6). The presence of 10 dorsal and 12–14 ventral segments differs from all other two-pored species (> 10 dorsal or > 14 ventral segments; < 12 ventral segments in *A. crissae*), with the exception of *A. silvestrii* (10–12 dorsal and 10–14 ventral segments). Also, the new species can be distinguished from *A. darwini*, *A. heterozonata*, and *A. lumbricalis* Vanzolini, 1996 by having a fixed number of 2 pores (2–6 pores), and from *A. dubia* (0 or 2 pores). Additionally, *A. caiari* sp. nov. can be distinguished from *A. crissae*, *A. darwini* and *A. dubia* by the absence of postmalars (present) (Fig. 3). From *A. anaemariae*, *A. crissae*, *A. darwini*, *A. heterozonata* and *A. lumbricalis*, the new species differs by having a suture between prefrontals that is smaller than the suture between the frontals (suture between prefrontals larger than between frontals) (Fig. 3). In having having TL/BL = 0.10, it differs from *A. anaemariae*, *A. darwini*, *A. mitchelli*, *A. silvestrii*, and *A. crissae* (0.13–0.16 TL/BL) and *A. carli* (0.06 TL/BL) (Fig. 4).

A few additional species were also recorded in the same geographic region where the new species was found: *Amphisbaena alba* Linnaeus 1758, *A. fuliginosa*, and *A. cunhai* Hoogmoed & Avila-Pires, 1991. *Amphisbaena caiari* sp. nov. can be distinguished from those species by the presence of two pores (> 4), the occurrence of 20–24 caudal annuli ( $\geq 24$  in all species except *A. alba*, which has  $\leq 21$ ); and the presence of 10 dorsal and 12–14 ventral segments (> 14 dorsal and ventral segments). Also, the maximum known SVL of 157 mm in the new species is smaller than in other species (> 400 mm SVL).



**Figure 4.** Relationship between tail length and body length in 15 species of *Amphisbaena*. Data derived from examined specimens (Appendix) and the literature (see Materials and Methods for references).

## Description of holotype

Head not distinct from neck, snout rounded, mouth ventral, total length = 145 mm, tail length = 13.2 mm, diameter at midbody = 3 mm. Rostral as long as wide, barely visible from above, subtriangular, ventrally expanded, wider and concave posteriorly, narrowly contacting first supralabial, broadly contacting nasal; contact with supralabial as long as the diameter of nostril. Nasals, prefrontals, frontals, and parietals from both sides in broad contact along the midline. Nasals quadrangular, in broad contact with rostral, first supralabial, and prefrontal, in point contact with second supralabial. Nostrils lateral, in the anteroventral part of nasal. Prefrontals large, roughly hexagonal, and in broad contact at middorsal line, their contact narrower than the contact between both nasals, frontals, and parietals, in broad contact with nasal, frontal, and ocular, in narrow contact with second supralabial and postocular, in point contact contacting first supralabial. Frontals trapezoidal, smaller than and approximately half size of prefrontals, longer than wide, in broad contact with prefrontal and postocular and in narrow contact with parietal. Suture between frontals slightly smaller than parietal suture. Parietals subtriangular, longer than wide, in broad contact with the postoculars, temporals, and occipitals, in narrow contact with frontals. Three supralabials, first subtriangular, longer than wide, contacting rostral, nasal, second supralabial, and in point contact with prefrontal; second supralabial smaller than first supralabial, subpentagonal, contacting first supralabial, prefrontal, ocular, third supralabial, and in point contact with nasal; third supralabial largest and highest, subpentagonal, contacting second supralabial, ocular, postocular, and temporal and in posterior contact with first body annulus. Ocular quadrangular, broadly contacting second and third supralabials, prefrontal, and postocular; eye barely visible in the anterior corner of ocular. Postocular large, subquadrangular, in broad contact with frontal, parietal, ocular, and temporal, in narrow contact with second supralabial and prefrontal. First temporal largest, ventrally contacting a smaller temporal scale. Posterior margin of head formed by occipital and temporals, almost straight, in contact with second body annulus.

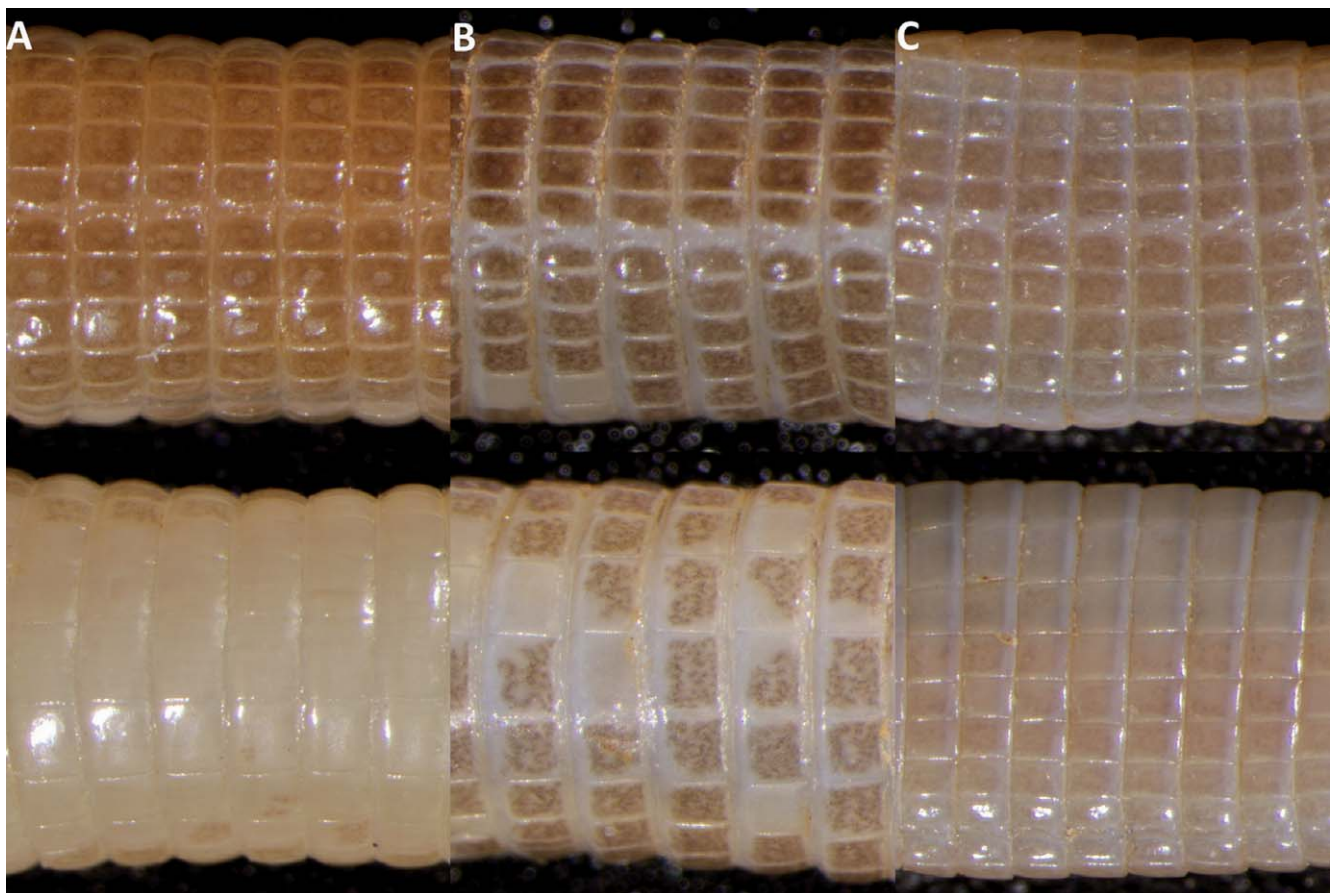
Mental trapezoidal, smaller and narrower than rostral, longer than wide, wider anteriorly, in broad contact with first infralabial and postmental; lateral margins slightly concave, converging posteriorly to a large and truncate posterior margin. Postmental large, ellipsoid, as long as wide, slightly longer than mental, in broad contact with mental, first and second infralabial, and first row of postgenials, and in point contact with malar. Two rows of postgenials posterior to postmental and between malars: first row larger, with two scales in broad contact with malar, postmental, and second row of postgenials; second row with four scales, outer ones smaller, in broad contact

**Table 1.** Meristic features of two-pored, non-shovel-snouted *Amphisbaena* that lack a sharp vertical terminal keel on the tail, based on examined specimens (Appendix) and the literature (for references see Materials and Methods). Pores = number of pores (slashes indicate pores separated by non-pored scales); BA = number of body annuli; CA = number of caudal annuli; AS = autotomy site (n = none; ? = unknown); DS/VVS = dorsal segments/ventral segments.

	Pores	BA	CA	AS	DS/VVS
<i>A. anaemariae</i>	2	156–170	18–20	3–5	14–16/14–16
<i>A. brevis</i>	2	139	9	?	18/14
<i>A. caiari</i> sp. nov.	2	233–250	20–24	6–9	10/12–14
<i>A. carli</i>	1/1	221–248	10–14	n	20–23/18–23
<i>A. crisae</i>	2	180–185	20–22	4–6	10/10
<i>A. darwini</i>	2–5	174–195	19–25	7–10	13–19/16–23
<i>A. dubia</i>	0,2	211–231	13–18	n	13–16/14–19
<i>A. heterozonata</i>	2–6	190–207	13–18	5–8	14–24/15–28
<i>A. hiata</i>	1/1	202–213	18–19	8	16–17/18–22
<i>A. leeseri</i>	2	216–249	14–19	5–8	10–15/12–18
<i>A. lumbricalis</i>	2,4,5,6	225–247	20–26	6–10	12–14/16–20
<i>A. miringoera</i>	2	250–264	20–24	7–8	12/12–15
<i>A. mitchelli</i>	2	203–220	26–29	6,7	12–14/14–16
<i>A. neglecta</i>	2	151–161	14–16	4–6	12–14/12–14
<i>A. silvestrii</i>	2	173–190	20–23	4–7	10–12/10–14

with first row of postgenials, malar, and first body annuli. Three infralabials: first trapezoidal, in broad contact with mental, postmental, and second infralabial; second largest, as long as wide, trapezoidal, in broad contact with first infralabial, postmental, and malar, in narrow contact with third infralabial, in point contact with postgenial;

third smallest, longer than wide, about the same size as postmental, in broad contact with second infralabial, malar, and the first body annuli. Malar pentagonal, about the same size as first infralabial and smaller than second infralabial, followed by the ventral scales of the first annulus posterior to angulus oris; postmalar row absent.

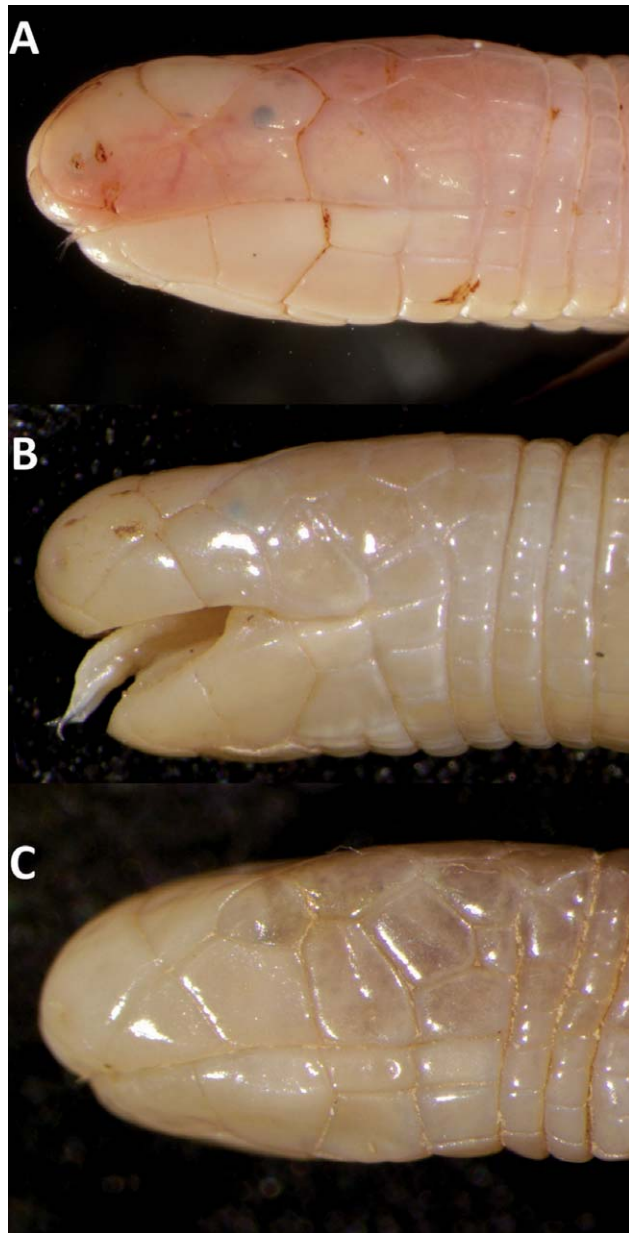


**Figure 5.** Variation in coloration of dorsum (top) and venter (bottom) in *Amphisbaena caiari* sp. nov. (A) MZUSP 101717; (B) MZUSP 101718; (C) MZUSP 101723.



Body annuli well demarcated. Lateral and middorsal sulci present. Second annulus with enlarged dorsal segments, posteriorly to parietals and temporals. Segments become regularly quadrangular toward posterior portion of body and progressively longer than wide; 249 body and 22 caudal annuli; 10 dorsal and 12 ventral segments per annulus at midbody; autotomy site on caudal annulus 8. Ventral segments larger towards midventral area.

Two preloacal pores rounded. Anal flap semicircular with six enlarged scutes increasing in size towards midline. Tail segments longer than body segments; tip of tail smooth, rounded.



**Figure 6.** Variation in head shields and coloration in *Amphisbaena caiari* sp. nov. (A) MZUSP 104237; (B) MZUSP 101717; (C) MZUSP 101722.

Dorsal ground color pinkish; center of segments darker. Venter slightly less pigmented than dorsum. Head slightly lighter than body. Anterior portion of body pinker than the posterior portion. Tail more pigmented than the rest of the body, terminating in brownish color.

### Variation

Although the specimens are fairly homogeneous in meristic characters (Table 1), there is some notable variation on head shields (Fig. 6). Most individuals present two rows of postgenials, but a few present only one, with four scales. The second row of postgenials is formed by 2–5 scales. The contact between the second supralabial and the nasal varies from being absent to narrow and, consequently, the same condition occurs in the contact between the first supralabial and the prefrontal. The arrangement and number of temporals is also variable. The color varies from almost unpigmented on both dorsum and venter to the dorsum being pigmented, with pigmentation extending onto some ventral rows (Fig. 5) and the head (Fig. 6). Larger individuals usually present more conspicuous pigmentation than smaller ones. The tail tip also presents slight variation in shape, with the terminal row varying from slightly vertically elliptical to rounded (Fig. 7).

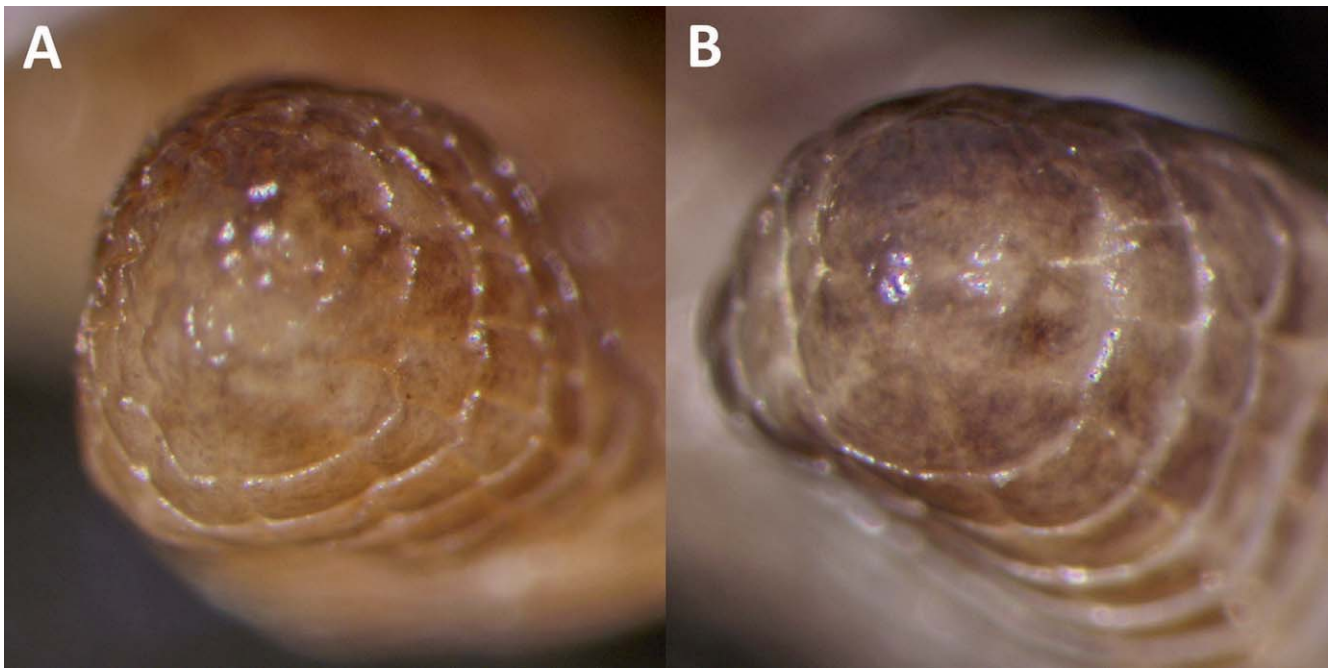
### Distribution and natural history

The new species is currently known only from the middle Madeira River. Most of the individuals were found on the left side of the Madeira River, while a single individual was found on the right side. This is the only known two pored *Amphisbaena* from the western Amazonia (Fig. 8). Little is known about its natural history. Most individuals were found in forest environments; a single specimen was found in open savanna (Fig. 9). All specimens were found buried in the soil or under fallen logs.

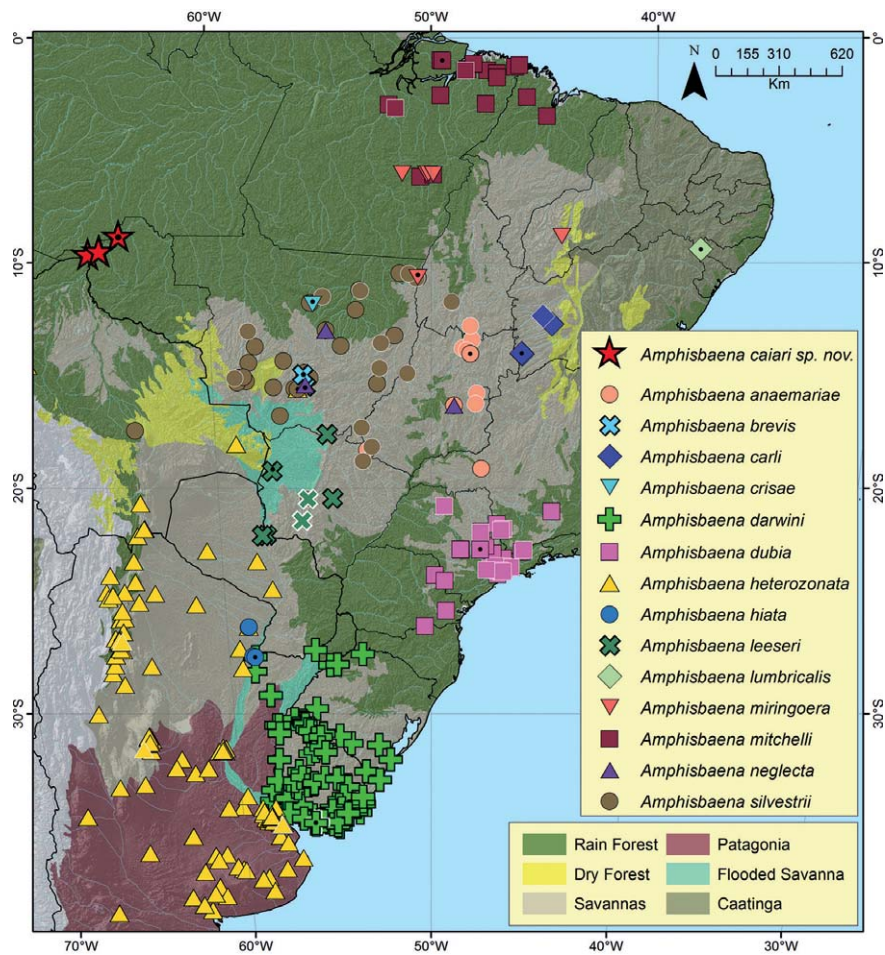
### Phylogenetics

The concatenated alignment consisted of 3211 bp: 16S = 541 bp (245 variable sites, 197 parsimony informative sites); ND2 = 767 bp (567 variable sites, 509 parsimony informative sites); *cmos* = 584 bp (184 variable sites, 103 parsimony informative sites); BDNF = 691 bp (100 variable sites, 60 parsimony informative sites); RAG1 = 628 bp (156 variable sites, 86 parsimony informative sites). The best-fit substitution models were GTR+G+I for 16S and ND2, HKY+G for *cmos*, HKY+G+I for BDNF, and GTR+G for RAG1.

The molecular phylogenetic analysis recovered the monophyly of *Amphisbaena* with high support (Fig. 10). The posterior probability values for the major clades is low. Consequently, although *Amphisbaena caiari* sp. nov. is recovered as sister to *A. hastata* Vanzolini,



**Figure 7.** Variation in tail tip shape in *Amphisbaena caiari* sp. nov. (A) MZUSP 101717; (B) MZUSP 101722.



**Figure 8.** Distribution of the two-pored *Amphisbaena* with rounded snouts in South America, including *Amphisbaena caiari* sp. nov. Symbols with a black central dot are type localities; symbols outlined in white are localities from which we examined specimens (Appendix).

1991 + *A. cuiabana*, the evidence for this relationship is not robust. The genetic divergence between the two sequenced individuals of *A. caiari* sp. nov. (one from each side of the Madeira River) is very low for the mitochondrial (16S = 0.4%; ND2 = 1.8%) and for the nuclear (BDNF = 0.0%; *cmos* = 0.7%; RAG1 = 1.6%) loci. Uncorrected pairwise distances between *A. caiari* sp. nov. and the other *Amphisbaena* species averaged 8.6 % for 16S, 28.9% for ND2, 1.1% for BDNF, 3.3% for *cmos* and 3.5% for RAG1.

## DISCUSSION

The middle Madeira River in the region of Porto Velho, Rondônia, Brazil, is undergoing a dramatic transformation with two large hydroelectric dams being constructed. This has led to a drastic change in the landscape, with most of the original vegetation being suppressed and the bare area being permanently flooded for the reservoir. However, despite of the huge and irreversible negative impact of those activities, it provided an unparalleled opportunity to access the fauna of this area. A large-scale sampling effort both for wildlife monitoring and rescue has led to the discovery of several previously unreported species in the area, new species, such as the recently

described *Bachia scaea* (Teixeira Jr *et al.*, 2013) and the species described herein, and an unprecedented amount on data of the natural history of many species found (unpublished data; Mollo Neto *et al.*, 2013).

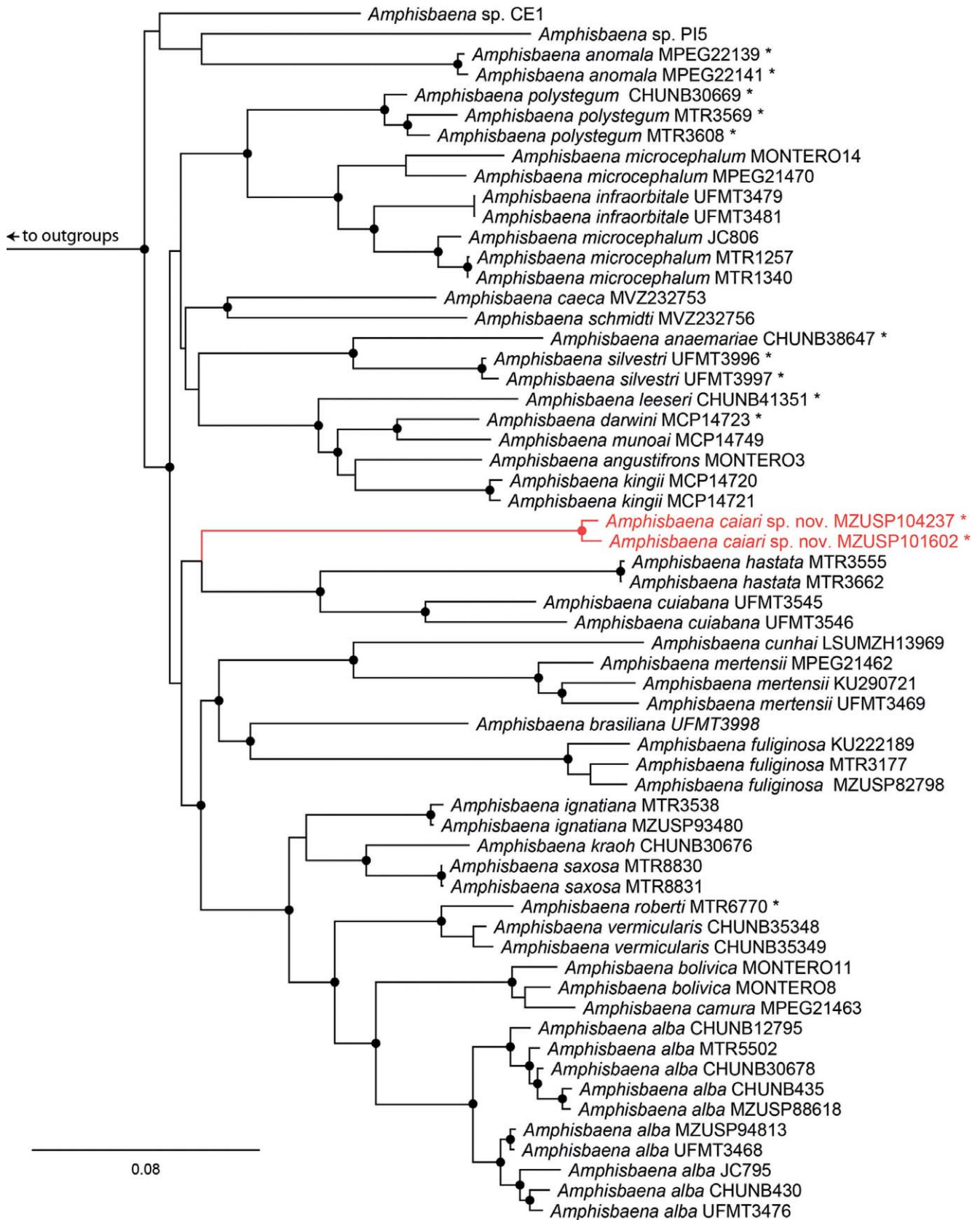
Despite the massive sampling effort, only a few specimens of both *Bachia scaea* and *Amphisbaena caiari* sp. nov. were found (less than 10 individuals), whereas the faunal rescue work resulted in more than 50 specimens of *A. caiari* sp. nov. being recovered. This difference is indicative of the strictly fossorial habits of this species and suggests that its abundance is probably greatly underestimated by standard sampling techniques. Because of this difficulty in collecting specimens, the actual distribution of *A. caiari* sp. nov. is unclear. Nevertheless, all current records lie in the middle Madeira River, indicating that it may be endemic to this region, as suspected for other recently described species (Brcko *et al.*, 2013; Teixeira Jr *et al.*, 2013).

Except for a single specimen collected on the right side of the Madeira River, which was found in a savanna patch (Fig. 9), all other individuals were obtained at the left side within the forest. Although the Madeira River is a classic riverine barrier to dispersion, separating sister-lineages of numerous closely-related species (Cracraft, 1985; Ayres and Cluttonbrock, 1992; Avila-Pires, 1995; Haffer, 1997; Fernandes *et al.*, 2012; Ribas *et al.*, 2012; Tsuji-Nishikido *et al.*, 2012; Teixeira Jr *et al.*, 2013), this seems not to be the case for *Amphisbaena caiari* sp. nov., a presumably low disperser due to its fossorial habits. This is clearly shown by the fact that the single specimen from the right side falls well within the morphologic variation observed among all other specimens and is molecularly identical to them. It is unknown if this is the result of recent dispersal across Madeira River or reflects an ancient distribution, before the establishment of its current course. Amphisbaenians have been reported crossing large water bodies in the Amazon (Maschio *et al.*, 2009), but in both cases large species were involved (*Amphisbaena alba* and *A. amazonica* Vanzolini, 1951). In fact, current phylogenetic hypotheses of American amphisbaenians suggest some events of transatlantic crossing (Vidal *et al.*, 2008). In the case of *A. caiari* sp. nov. the small size together with the water flow of the Madeira River suggest that it would be difficult for this species to cross the river.

Little was known on phylogenetic relationships of South American amphisbaenid species, until a broad molecular study revealed a paraphyletic *Amphisbaena* and led to the placement of *Bronia*, *Anops*, *Aulura*, *Leposternon*, and *Cercolophia* in the synonymy of *Amphisbaena* (Mott and Vieites, 2009; for an opposing taxonomic view, see Ribeiro *et al.*, 2011). As in our analysis, Mott and Vieites (2009) did not recover the small two pored *Amphisbaena* species as a monophyletic group. However, in both analyses most of the South America species are absent, resulting in basal polytomies and poorly supported nodes.



**Figure 9.** Habitat occupied by *Amphisbaena caiari* sp. nov. **(A)** The shaded rain forest where most of the individuals were found, on the left side of the Madeira River. **(B)** The open savanna on the right side of the Madeira River where one individual was found.



**Figure 10.** Phylogenetic relationship of *Amphisbaena*, including *A. caiari* sp. nov. based on Bayesian analysis of mitochondrial (16S, ND2) and nuclear (BDNF, RAG1, *cmos*) DNA. Dots on nodes indicate posterior probabilities higher than 0.95. Scale bar represents number of substitutions per site. Asterisks mark species bearing at least two pores.

The placement of *Amphisbaena caiari* sp. nov. is yet not resolved; although it groups with *A. hastata* and *A. cuia-bana*, support for this group is low. The inclusion of other small two-pored Amazonian species, such as *A. mitchelli* and *A. slevini*, and broader overall coverage of species may add some clarity for its positioning.

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## APPENDIX

### Specimens examined

*Amphisbaena anaemariae*: BRAZIL: **São Paulo**: Parque Estadual do Morro do Diabo, Teodoro Sampaio: MZUSP 96810; **Goiás**: Campinaçu: MZUSP 103743; Luiziania: MTR 11453, MTR 115454; São Salvador do Tocantins, UHE São Salvador: MZUSP 99394; UHE Cana Brava: MZUSP 97217; UHE Serra da Mesa: MZUSP: 97047, MZUSP 97171.

*Amphisbaema carli*: BRAZIL: **Bahia**: São Desidério: MZUSP 100658.

*Amphisbaema cunhai*: BRAZIL: **Rondônia**: Porto Velho: MZUSP 101719, MZUSP 101721, MZUSP 101734, MZUSP 102237.

*Amphisbaena darwini*: BRAZIL: **Santa Catarina**: Lauro Müller, Novo Horizonte: MZUSP 21467; URUGUAY: **Montevideo**: Puntas de Manga: MZUSP 82343–82344.

*Amphisbaena dubia*: BRAZIL: **Minas Gerais**: Poços de Caldas: MZUSP 13727, MZUSP 93983; **São Paulo**: Aurora: MZUSP 6439; Campos do Jordão: MZUSP 2527; Cascata: MZUSP 6432; Cotia: MZUSP 100372; Diadema: MZUSP 95082; Embu: MZUSP 6461; Guarulhos: MZUSP 87605; Mairinque: MZUSP 6433; Mogi da Cruzes: MZUSP 44696; Santana de Parnaíba: MZUSP 77048; Santo Amaro: MZUSP 60424; São Bernardo do Campo: MZUSP 11870; São José do Rio Preto: MZUSP 6442; São Manoel: MZUSP 6520; São Paulo: MZUSP 77051, MZUSP 83031, MZUSP 89663; Pirituba: MZUSP 91505.

*Amphisbaena fuliginosa*: BRAZIL: **Rondônia**: Porto Velho: MZUSP 102785.

*Amphisbaena heterozonata*: ARGENTINA: **Buenos Aires**: La Plata: MZUSP 59215, MZUSP 59217; Córdoba: Pampa de Achala: MZUSP 82358; Tucumán: San Miguel de Tucumán: MZUSP 13744–13745.

*Amphisbaena hogei*: BRAZIL: **São Paulo**: Caucaia do Alto: MTR 15328.

*Amphisbaena leeseri*: BRAZIL: **Mato Grosso**: Aquidauana: MZUSP 82539–82540; Guia Lopes da Lagoa: MZUSP 73313.

*Amphisbaena mitchelli*: BRAZIL: **Pará**: Belém: MZUSP 7140; Juruá, Rio Xingu: MZUSP 67714.

*Amphisbaena silvestrii*: BRAZIL: **São Paulo**: Santa Rita: MZUSP 100897; **Goiás**: Aruanã: MZUSP 2525; Gurupi: MZUSP 57037; Parque Nacional das Emas: MZUSP 87733; **Mato Grosso**: Alto Araguaia, Fazenda Granada: MZUSP 99258; Barra do Tapirapés: MZUSP 10069, MZUSP 9010; Canarana, Fazenda Peixe-Boi: MZUSP 88864; Claudia, Fazenda Iracema: MZUSP 81778; Garapu, Alto Xingu: MZUSP 3286; Jacaré, Alto Xingu: MZUSP 6412–6413; Porto Velho, Rio Tapirapés: MZUSP 9743, MZUSP 9757; UHE Guaporé: MZUSP 97884; Utiarití: MZUSP 36857; Xavantina: MZUSP 3367; **Mato Grosso do Sul**: Aquidauana: MZUSP 82541; Fazenda Bela Vista, Rio Paraguai: MZUSP 62398; Parque Estadual Nascentes do Taquari: MZUSP 88659.

*Amphisbaena slevini*: BRAZIL: **Amazonas**: Manaus: MZUSP 10913; Reserva INPAWWF: MZUSP 60905–60906, MZUSP 66148.