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Molecular phylogeny and hemipenial diversity of South American species of *Amerotyphlops* (Typhlopidae, Scolecophidia)

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Abstract

Typhlopidae is the most diverse family of Scolecophidia, with 269 species. *Amerotyphlops* was recently erected within subfamily Typhlopinae and comprises fifteen species distributed from Mexico to Argentina and the southern Lesser Antilles. Despite recent advances, affinities among typhlopines remain poorly explored, and the phylogenetic relationships and morphology of the South American (SA) species were never accessed before. Here, we performed a phylogenetic analysis including 106 species of Typhlopidae and ten genes. Our dataset represents the most comprehensive for SA species, containing seven of eight recognized species. Corroborating previous studies, we recovered the main groups of Typhlopoidea, and for typhlopines, we recovered with strong support two clades: (a) the Greater Antilles radiation, and the (b) Lesser Antilles and SA radiation. Within the SA radiation, we recovered four main lineages: (a) a clade formed by *A. tasymicris* and *A. minuisquamus*; (b) a clade composed by *A. reticulatus* as the sister group of all other SA species; (c) a clade composed by *A. brongersmianus* as the sister group of a clade comprising all Northeast Brazilian Species (NBS); and (d) a clade of the NBS, including *A. yonenagae*, *A. arenensis*, *A. paucisquamus,* and *A. amoipira*. We supplemented our phylogenetic result with the description of hemipenial morphology for seven SA species and comment their relevance to the systematics of Typhlopinae. Hemipenes of SA *Amerotyphlops* follow the general pattern in scolecophidians (single organ with undivided sulcus). Only *A. reticulatus* and *A. minuisquamus* have organs with calcified spines. According to our results, hemipenial ornamentation have shown highly informative and could represent a potential source of systematic and taxonomic characters in that group. We also present an extensive review of the geographical distribution for all SA species. Our study represents the first integrative analysis of a poorly known evolutionary radiation of one of the most widespread SA fossorial snakes.

1 | INTRODUCTION

Fossorial snakes of the family Typhlopidae (Merrem, 1820) include 269 recognized species (Uetz, Goll, & Hallerman, 2017), representing 60% of the diversity of the infraorder Scolecophidia (McDiarmid, Campbell, & Touré, 1999; Wallach, Williams, & Boundy, 2014). Higher-level taxonomy of typhlopids was relatively stable during the last century, until Hedges, Marion, Lipp, Marin, & Vidal (2014) divided the family into four subfamilies: Afrotyphlopinae, from sub-Saharan Africa; Asiatyphlopinae, from southern and eastern Asia, Australasia, and western and southern Pacific islands; Madatyphlopinae, from Madagascar; and Typhlopinae, from the New World. Hedges et al. (2014) also set the grounds for a new generic arrangement within Typhlopinae, allocating the New World species previously grouped in the genus *Typhlops* (Oppel, 1811) in four distinct genera—*Amerotyphlops*, *Cubatyphlops*, *Antillotyphlops*, and *Typhlops* (Hedges et al., 2014). All mainland South and Middle American species were allocated in the genus *Amerotyphlops* while the West Indian species were included in the other three recognized genera (Hedges et al., 2014; but see Wallach et al., 2014 for a different taxonomic scheme).

Currently, *Amerotyphlops* comprises 15 recognized species, five of them occurring in mainland Middle America, two in the southern islands of the Lesser Antilles, and eight throughout South America (Hedges et al., 2014; Pyron & Wallach, 2014; see Table 1). From these 15 species, only *A. reticulatus*, *A. tasymicris,* and *A. minuisquamus* were already sequenced and positioned in a phylogenetic context. The specimen of *A. brongersmianus* (AMNH R-140972) sequenced by Vidal et al. (2010), and used in all other subsequent studies (Hedges et al., 2014; Marin, Donnellan, Hedges, Doughty, et al., 2013; Marin, Donnellan, Hedges, Puillandre, et al., 2013; Nagy et al., 2015; Wallach et al., 2014), was misidentified and actually corresponds to *A. minuisquamus* (see Results below).

Besides improvement in our evolutionary knowledge generated by molecular evidence, hemipenial characters have also been successfully used as key morphological traits to trace phylogenetic relationships at different taxonomic levels (Dowling, 1967, 2002; Keogh, 1996, 1999; Roze, 1982; Zaher, 1999; Zaher et al., 2009). Hemipenial morphology can be used to diagnose monophyletic clades in several taxonomic levels (Grazziotin et al., 2012; Guerra-Fuentes, Costa, Missassi, & Prudente, 2017; Zaher et al., 2009). Unfortunately, information on typhlopoid hemipenial morphology is scarce, mainly because of the difficulty to evert and prepare them in the field or in the laboratory.

Over the last forty years, only two studies—Thomas (1976) and Dixon & Hendricks (1979)—analyzed morphological characters for a broad sampling of typhlopines. Both studies proposed hypotheses of evolutionary affinities within the subfamily and defined morphologically the groups of species posteriorly allocated by Hedges et al. (2014) in different genera. Although Thomas (1976) and Dixon & Hendricks (1979) included hemipenial morphology in their analyses, they did not provide detailed descriptions of the organs, and the characters used were restricted to overall body shape and few ornamentations.

Here, we provide the first molecular phylogenetic analysis of a comprehensive sampling of South American typhlopines. We also provide a detailed description of the hemipenial pattern retrieved in seven species of *Amerotyphlops*, comparing

TABLE 1 Fifteen species belonging to the genus *Amerotyphlops* with their occurrence and reference

Species	Occurrence	References
Amerotyphlops amoipira	South America	Rodrigues & Juncá (2002)
Amerotyphlops arenensis	South America	Graboski et al. (2015)
Amerotyphlops brongersmianus	South America	Vanzolini (1972, 1976)
Amerotyphlops costaricensis	Middle America	Jimenez & Savage (1963)
Amerotyphlops lehneri	South America	Roux (1926)
Amerotyphlops microstomus	Middle America	Cope (1866)
Amerotyphlops minuisquamus	South America	Dixon & Hendricks (1979)
Amerotyphlops paucisquamus	South America	Dixon & Hendricks (1979)
Amerotyphlops reticulatus	South America	Linnaeus (1758)
Amerotyphlops stadelmani	Middle America	Schmidt (1936)
Amerotyphlops tasymicris	Lesser Antilles	Thomas (1974)
Amerotyphlops tenuis	Middle America	Salvin (1860)
Amerotyphlops trinitatus	Lesser Antilles	Richmond (1965)
Amerotyphlops tycherus	Middle America	Townsend, Wilson, Ketzler, & Luque-Montes (2008)
Amerotyphlops yonenagae	South America	Rodrigues (1991)

it with the pattern shown in other typhlopine genera and commenting on the evolution of hemipenial morphology within that group. Additionally, we review the geographic distribution data available for South American typhlopines.

2 | MATERIALS AND METHODS

2.1 | Taxon and gene sampling

We sequenced 61 DNA fragments for five genes, including four mitochondrial (*16S, 12S, cytb* and *cox1*) and one nuclear gene (*bdnf*) for nine species of blind snakes (see Supporting information Appendix S3: Table S1). From these sequenced species, the following seven are part of the South American ingroup in our analysis: *Amerotyphlops amoipira, A. arenensis*, *A. brongersmianus, A. minuisquamus, A. paucisquamus, A. reticulatus,* and *A. yonenagae*. The other two are outgroups from Cuba (*Typhlops* sp.) and Madagascar (*Madatyphlops ocularis*). We sequenced only one individual for the outgroups and for *A. yonenagae*, while we sequenced two individuals for other South American species. For tissue samples identification, we checked specimen vouchers (excepted for *Typhlops* sp. and *Madatyphlops ocularis*).

We also sequenced one alethinophidian (*Anilius scytale*) to root our phylogenetic tree. To better test the monophyly of *Amerotyphlops*, and to analyze its phylogenetic position, we sampled throughout the scolecophidian diversity by including 634 additional GenBank sequences (Supporting information Appendix S4: Table S1). These downloaded sequences are from the same five sequenced genes plus five other nuclear genes (*rag1, bmp2, nt3, prlr*, and *amel*). The sequences for the insular species *A. tasymicris* (from Grenada and Saint Vincent and Grenadines) were also downloaded from GenBank. Our sampling for the South American *Amerotyphlops* included all mainland species, but *A. lehneri* (from Venuezela); and all insular species, but *A. trinitatus* (from Trinidad and Tobago). Our final data matrix comprised 10 genes for 120 terminals, being 110 scolecophidians and 10 alethinophidians (Supporting information Appendix S4: Table S1).

Tissue samples sequenced in this study are deposited in the following institutions (acronyms in parentheses): Coleção Herpetológica da Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil (CHUFPB) and Coleção de Tecidos do Laboratório de Herpetologia do Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil (CTMZ). Other Museum acronyms followed Sabaj (2016) and Frost (2018), see Supporting information Appendix S3.

2.2 | DNA sequencing

DNA was extracted following the protocol described by Hillis, Mable, & Moritz (1996). Sequences were amplified via polymerase chain reaction (PCR) using the primers for: *12S* and *16S*, as described in Zaher et al. (2009); *cytb*, as described by Grazziotin et al. (2012); *bdnf*, BDNFF (5′ GAC CAT CCT TTT CCT KAC TATG GTT ATT TCA TAC TT 3′) and BDNFR (5′ CTA TCT TCC CCT TTT AAT GGT CAG TGT ACA AAC 3′) based on Noonan & Chippindale (2006); *cox1*, MLepF1.mod, (5′ GCA TTY CCA CGA ATA AAT AAY ATR AG 3′) as described by Hajibabaei, Janzen, Burns, Hallwachs, & Hebert (2006) and COI_r928 (5′ CCT GTT GGA AYT GCR ATR ATT AT 3′) described herein.

PCRs were performed using standard protocols, with adjustments to increase the efficiency of amplification as following: the addition of 10% of Trehalose for *12S, 16S, cytb* and *cox1*, or 0.4% of Triton 100 for *bdnf*. We used the annealing temperature of 54°C for *12S* and *16S*, 56°C for *bdnf*, a touch down cycle of 60–50°C with final annealing of 54°C for *cytb* and *cox1*. Amplified fragments were purified with shrimp alkaline phosphatase and exonuclease I (GE healthcare, Piscataway, NJ), and both strands were processed using the DYEnamic ET Dye Terminator Cycle Sequencing Kit in a MegaBACE 1,000 automated sequencer (GE healthcare) following manufacturer's protocols. Both strands were quality checked, and when necessary edited manually. The consensus of both strands was generated using Geneious 7.1.8 (http://www.geneious.com, Kearse et al., 2012).

2.3 | Molecular analyses

Sequences were aligned using MAFFT 1.3.6 (Katoh, 2013) as implemented in Geneious. The *12S* and *16S* were aligned under the E-INS-i algorithm, while *cox1, cytb*, and the nuclear genes were aligned under the G-INS-i algorithm. We used default parameters for gap opening and extension. All protein-coding genes were visually checked using Geneious to verify whether all sequences follow the correct reading frame.

We used PartitionFinder 2 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016) to choose the combined sets of partitioning schemes and models of molecular evolution. We divided our matrix into 26 partitions (each coding genes were partitioned by codon positions and each rRNA was analyzed as a separate partition) and performed a search using the greedy option. We performed two different runs, as follows: run (1) allowing the program to select using Akaike information criterion with correction (AICc) only the models of molecular evolution implemented in RAxML (models GTR and GTR+G); and run (2) allowing the program to select using Bayesian information criterion with correction (BICc) the models of molecular evolution implemented in MrBayes 3.1.2 (models GTR+G, F81, F81+G, SYM, JC, HKY, K80, SYM+G, K80+G, GTR, HKY+G, JC+G). We did not allow PartitionFinder to select models with correction for proportion of invariant sites (P-Invar), as suggested by Alexander

Stamatakis in RAxML's manual, to avoid correlation between values of alpha and P-Invar.

We performed a maximum likelihood (ML) analysis using RAxML 8.2.3 (Stamatakis, 2014). The ML tree was estimated using the RAxML algorithm that conducts a rapid bootstrap analysis and searches for best scoring ML tree in the same run (option *-f a*). We run 1,000 bootstrap replicates, and the best scoring ML tree was estimated 200 times using as starting tree each 5th bootstrap tree. We also performed a Bayesian inference analysis (BI) using MrBayes 3.2.5 (Ronquist et al., 2012). We allowed four incrementally heated Markov chains (standard initial temperature with heating parameter set to 0.05) and run the chains for one million generations, sampling every 1,000th generation. Maximum clade credibility tree (MCT) and values of Bayesian posterior probability (PP) were estimated from sampled trees after discarding as burn-in the trees sampled before posterior trace convergence. We used Tracer 1.6.1 (Rambaut, Suchard, Xie, & Drummond, 2014) to check for trace convergence and values of ESS (effective sample size), and we used TreeAnnotator v 1.5.4 (Rambaut & Drummond, 2010b) to perform the burn-in and summarize the tree distribution and the parameters estimated.

2.4 | Specimens examined

We examined 326 specimens from seven South American species of *Amerotyphlops* (Supporting information Appendix S1). *Amerotyphlops lehneri* was the only mainland South American species not examined here, and for which we used the information available in the original description (Roux, 1926). We used the available information in the literature (Dixon & Hendricks, 1979; Domínguez & Díaz, 2015; Hedges et al., 2014; Pyron & Wallach, 2014; Shreve, 1947; Thomas, 1968, 1976; Thomas & Hedges, 2007; Wallach, 1998) for species from the Caribbean and Central America regions. We analyzed 19 hemipenes for the following species: *A. brongersmianus* ($n = 5$), *A. reticulatus* ($n = 8$), *A. minuisquamus* ($n = 3$), *A. amoipira* ($n = 1$), *A. arenensis* ($n = 1$), *A. paucisquamus* ($n = 1$), and *A. yonenagae* ($n = 1$) (Supporting information Appendix S2).

Species identification followed descriptions provided by Dixon & Hendricks (1979), Rodrigues (1991), Rodrigues & Juncá (2002), and Graboski, Pereira-Filho, Silva, Prudente, & Zaher (2015). All specimens examined are deposited in the following institutions in Brazil (acronyms in parentheses): Instituto Butantan, São Paulo, São Paulo (IBSP); Coleção Herpetológica da Universidade Federal da Paraíba, João Pessoa, Paraíba (CHUFPB); Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa, Minas Gerais (MZUFV); Universidade Estadual de Santa Cruz, Ilhéus, Bahia (UESC); Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso (UFMT); and Laboratório de Anfíbios e Répteis, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte (AAGARDA). Other Museum acronyms followed Sabaj (2016) and Frost (2018), see Supporting information Appendix S1.

We built a geographical dataset of 422 distribution records for the species of *Amerotyphlops* distributed in mainland South America. We included distribution records of *A. tasymicris* and *A. trinitatus* since Trinidad and Tobago, Grenada and, Saint Vincent and Grenadines were biogeographically connected to the South American mainland (Hedges, 1996). Maps were generated through the software ArcGIS v10.2.2 (ESRI, 1999). Geographical coordinates were obtained from Species Link online database (http://www.splink.org.br), based on the institution database; compiled data contained in the literature (Arruda, Almeida, Rolim, & Maffei, 2011; Ávila & Kawashita-Ribeiro, 2011; Brito & Freire, 2012; Caicedo-Portilla, 2011; Cunha & Nascimento, 1978, 1993; Dixon & Hendricks, 1979; França, Mesquita, & Colli, 2006; França & Venâncio, 2010; França, Germano, & França, 2012; Freire, 2001; Graboski et al., 2015; Guedes, Nogueira, & Marques, 2014; Loebmann, 2008; Martins, Silveira, & Bruno, 2010; Rivas et al., 2012; Roberto, Ávila, & Melgarejo, 2015; Roberto, Oliveira, Filho, & Ávila, 2017; Rodrigues, 1991; Rodrigues & Juncá, 2002; Roux, 1926; Roze, 1956; Shreve, 1947; Wallach et al., 2014), or directly taken from the localities of specimens examined in collections (see Supporting information Appendix S1 and S2). We generated lists of distribution for all South American species, providing accurate maps for all of them and commenting on the geographical pattern for the genus.

2.5 | Hemipenial preparations

We everted hemipenes from fresh specimens or, alternatively, from fixed specimens following the protocols described by Zaher (1999) and Zaher & Prudente (1999). In very small specimens (e.g., *Amerotyphlops arenensis* and *A. yonenagae*), we removed and opened the organ through a longitudinal slit along the side of the *sulcus spermaticus* and spread the organ flat. Hemipenial terminology followed Branch (1986), Peters & Orejas-Miranda (1970), and Passos, Caramaschi, & Pinto (2005). We photographed the hemipenis using a Leica DFC425 digital camera attached to a Leica M205a stereoscopic microscope, and performed the combination and montage of multifocal photographs using the Leica Application Suite software (LAS core version 3.8, Leica Microsystems).

We reviewed the hemipenes of seven of the eight species known to occur in South America, except *Amerotyphlops lehneri*, a very rare species restricted to Northwestern Venezuela, and from which information on hemipenial morphology is unknown. Similarly, hemipenes of the Lesser Antillean species *A. tasymicris* and *A. trinitatus* and the Middle American species *A. costaricensis*, *A. microstomus*, *A. stadelmani*, *A. tenuis*, and *A. tycherus* remain undescribed. Our hemipenial

sampling represents almost 50% of the total of current species of *Amerotyphlops*, and corresponds to almost 90% of the species occurring in South America. Although the number of species of typhlopines that Thomas (1976) examined for hemipenial morphology is higher than ours (15 vs. 7), the information presented by this author is restricted to the general shape of the hemipenial body and did not include detailed descriptions on ornamentation.

3 | RESULTS

3.1 | Phylogenetic relationships

Our concatenated alignment totalized 7,087 base pairs (859 bp for *12S*, 874 bp for *16S*, 1,134 for *cytb*, 893 for *cox1*, 709 for *bdnf*, 524 for *rag1*, 594 for *bmp2*, 639 for *nt3*, 486 for *prlr*, and 375 for *amel*). The proportion of gaps and completely undetermined characters in the concatenated alignment was

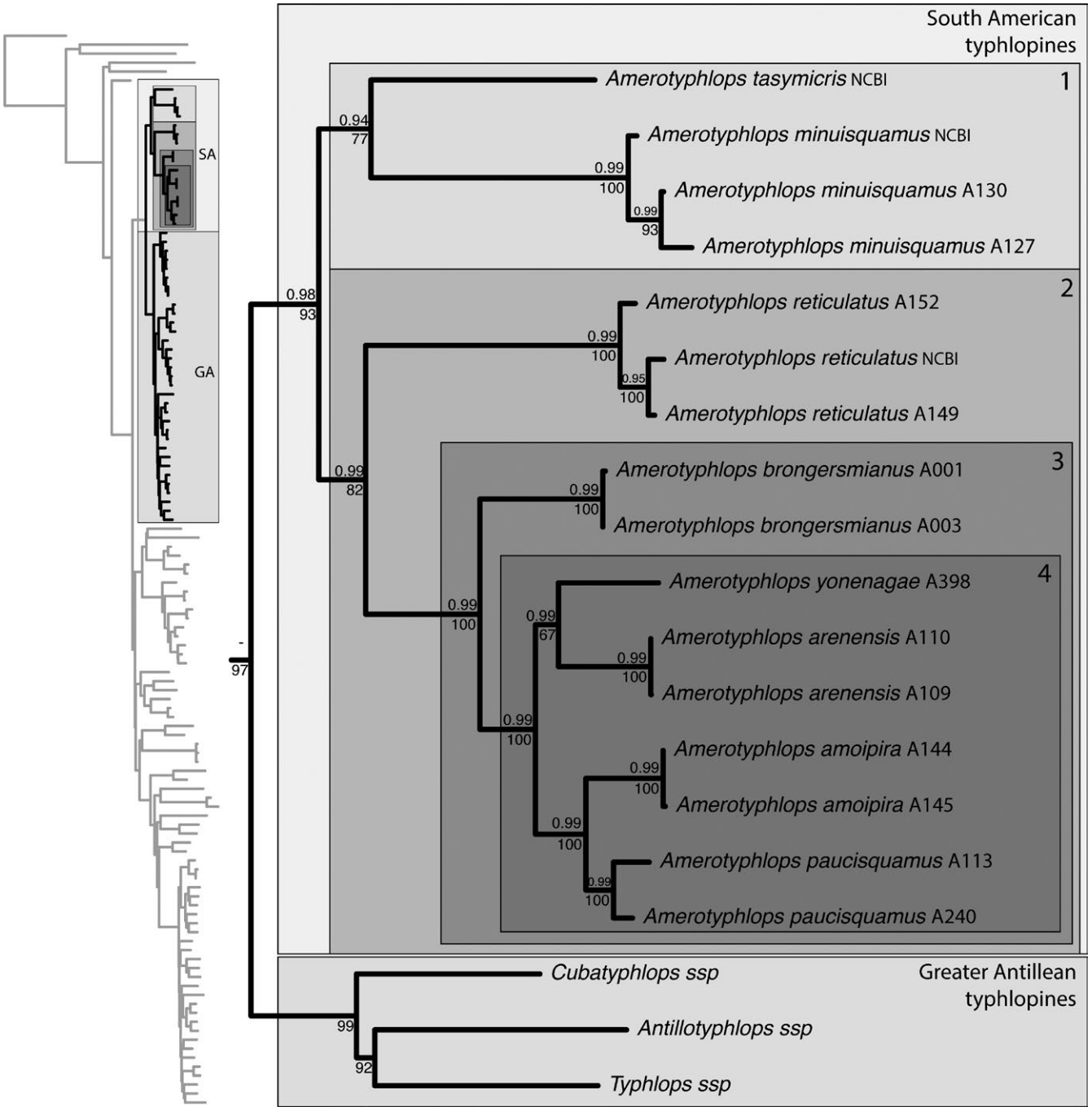

FIGURE 1 Maximum Likelihood tree of Typhlopoidea zoomed in typhlopines and particularly in the South American radiation of *Amerotyphlops*. Numbers above and below branches represent posterior probability and bootstrap values, respectively. The diversity of species for *Cubatyphlops*, *Antillotyphlops* and *Typhlops* was summarized to one terminal on the zoomed tree. Numbers on gray squares represent the four clades recovered in our analyses (see text)

52.5%. PartitionFinder selected 18 partitions with GTR+G model for ML analysis (Supporting information Appendix S5: Table S1). For BI analysis, PartitionFinder selected ten partitions and the best-fit model for each selected partition is presented in Supporting information Appendix S6: Table S1.

The resulting topologies from ML and BI analyses (Figure 1, Supporting information Appendix S7: Figure S1 and Supporting information Appendix S8: Figure S1) concerning the high-level affinities within Typhlopoidea were similar to those presented by Vidal et al. (2010); Hedges et al. (2014); Pyron & Wallach (2014) and Nagy et al. (2015). We recovered with high support values the main groups of Typhlopoidea defined by Hedges et al. (2014), as well as the following monophyletic genera (bootstrap and PP values in parenthesis): *Acutotyphlops* (100%, 0.69), *Afrotyphlops* (100%, 0.87), *Anilios* (99%, 0.95), *Argyrophis* (100%, 0.99), *Indotyphlops* (84%, 0.99), *Letheobia* (100%, 0.96), *Malayotyphlops* (99%, 0.91), *Ramphotyphlops* (80%, 0.90), *Rhinotyphlops* (86%, 0.69), and *Xerotyphlops* (100%, 0.92). The ML tree recovered a monophyletic genus *Madatyphlops*, as defined by Pyron & Wallach (2014) and Nagy et al. (2015), but with low bootstrap values (40%), while the BI analysis did not recover the genus as monophyletic, with *Madatyphlops comorensis* clustering with the Greater Antilles group, and *Madatyphlops microcephalus* clustering with *Xenotyphlops*, although with low values of PP (0.4 and 0.1, respectively).

In the ML tree, American typhlopids were recovered as a well-supported clade (97%), although in the BI analysis, the group was not monophyletic because of the position of *M. comorensis* (Supporting information Appendix S7: Figure S1 and Supporting information Appendix S8: Figure S1). Both analyses retrieved two main clades within typhlopines. The first clade was formed by species from the Greater Antilles radiation (99%, 0.4), and the second by species from South America and the Lesser Antilles (93%, 0.98). We recovered the same topology of Hedges et al. (2014) for the Greater Antilles radiation, and the following genera appeared as monophyletic in our analyses: *Cubatyphlops* (100%, 0.91), *Antillotyphlops* (100%, 0.89), and *Typhlops* (99%, 0.81). The South American clade is formed only by the monophyletic genus *Amerotyphlops*. Within this genus, we found four well-supported clades, as follows: (1) a clade formed by *A. tasymicris* and *A. minuisquamus* (77%, 0.94); (2) a clade composed by *A. reticulatus* as the sister group all the other South American species (82%, 0.99); (3) a clade composed by *A. brongersmianus* as the sister group of a clade comprising all the species of *Amerotyphlops* from north-east Brazil (100%, 0.99); and (4) a clade containing the Northeastern Brazilian Species (NBS) *A. yonenagae*, *A. arenensis*, *A. paucisquamus* and *A. amoipira* (100%, 0.99). Within this last clade, *A. yonenagae* and *A. arenensis*, as well as, *A. paucisquamus* and *A. amoipira* formed two well-supported subclades (67%, 0.99% and 100%, 0.99, respectively).

3.2 | Hemipenial morphology

In this section, we described the hemipenial morphology for the seven taxa analyzed in this study following our phylogenetic tree (Figure 1).

3.2.1 | *Amerotyphlops minuisquamus* (*N* = 3; organs fully everted and inflated)

Hemipenis single, with a trumpet-shaped body (Figure 2a); slightly wider in the basal and medial portions of the body and considerably expanded apically (Figure 2a–c), forming a broad apical disk (Figure 2d); central region of the apical disk with a large, round and bulbous expansion, surrounded by a shallow canal in the lateral and asulcate sides and by two large and stout calcified spines in the sulcate side (Figure 2d); sulcus spermaticus single, protruding over the surface of the hemipenial body, originating on the medial surface of the basal region of the hemipenis, running centripetally until reach the sulcate side of the apical disk (Figure 2a–c), and draining between the two large calcareous spines (Figure 2a, d); proximal region of the asulcate side of the hemipenis with diagonal deep groove (Figure 2b); surface of hemipenial body covered with smooth flounces that become irregular near and on the surface and walls of the sulcus spermaticus (Figure 2a–c).

3.2.2 | *Amerotyphlops reticulatus* (*N* = 8; organs fully everted and inflated)

Hemipenis single, with a long cylindrical body (Figure 3a–d); hemipenial body completely covered with ornamentations, with irregular flounces on the basal region (Figure 3a–d); body covered with irregular and weakly defined calyces on the medial (highly papillated) and distal (slightly papillated) regions (Figure 3a–g); a longitudinal row of calcified and curved spines (Figure 3a, c, e, g) followed by a fleshy crest of large papillae (Figure 3a–c); sulcus spermaticus single with fleshy and protruded walls (Figure 3a), running straight to the tip but draining laterally on the distal portion of the hemipenial body (Figure 3e); distal region of lateral walls of the sulcus covered with shallow striations (Figure 3g).

3.2.3 | *Amerotyphlops brongersmianus* (*N* = 5; organs fully everted and inflated)

Hemipenis single, with a long cylindrical body (Figure 4a–b); conical in the distal region (Figure 4a, c); a tissue sheet extends from the lateral surface of the conical termination, it folds and runs transversely around the apical region, creating an irregular and asymmetrical flounce (Figure 4a–d); the region between this flounce and the lateral sheet is slightly deeper, forming a pocket on the sulcate side (Figure 4c–d); internal surfaces of the flounce and the conical termination covered with smooth

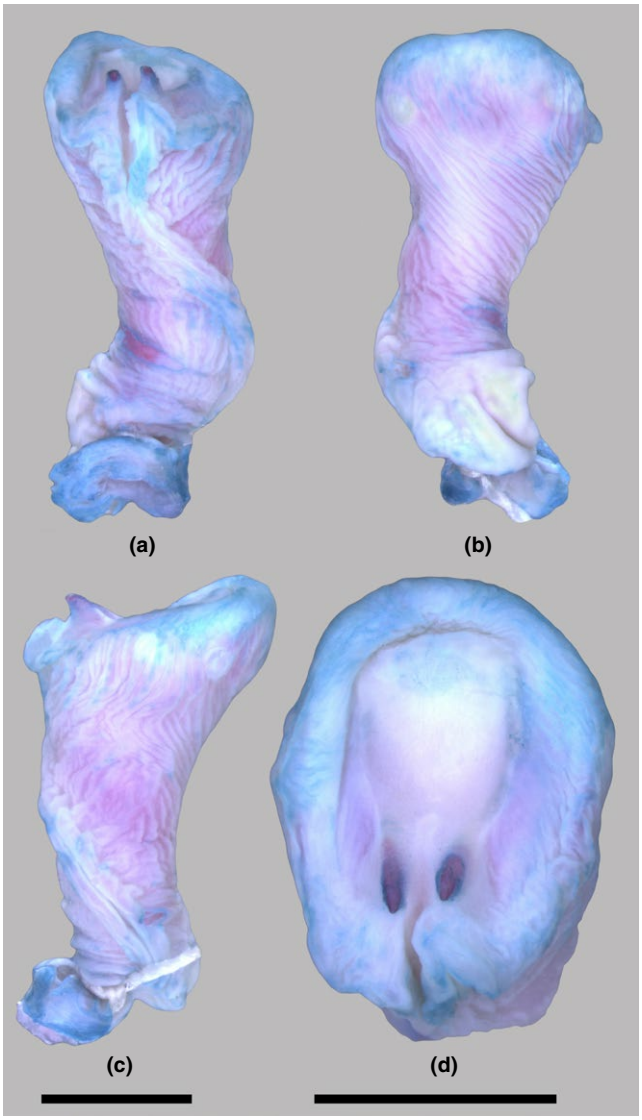

FIGURE 2 (a–d) Hemipenis of *Amerotyphlops minuisquamus* (MZUSP 21,447), in sulcate (a) asulcate (b), right views (c), and detail of the apical disk (d). Scale = 2 mm [Color figure can be viewed at wileyonlinelibrary.com]

and shallow striations (Figure 4c); sulcus spermaticus single, protruding over the surface of the hemipenial body, originating on the medial surface of the basal region of the hemipenis and running distally sinuously, reaching the flounce and draining to the pocket of the sulcate side (Figure 4a, c–d); proximal region of the asulcate side of the hemipenial body with a transversal groove (Figure 4b); medial region of the sulcate and asulcate sides (including the sulcus walls) covered with smooth and shallow striations (Figure 4a–b).

3.2.4 | *Amerotyphlops arenensis* and *A. yonenagae* (*N* = 1 each; organs dissected, not everted)

Hemipenis single, with a short cylindrical body, with a single and irregular shallow flounce on the basal region; without apical disk or calcified spines, sulcus spermaticus single, straight, with slightly protruded walls (not shown).

3.2.5 | *Amerotyphlops amoipira* (*N* = 1; organ dissected)

Hemipenis single, with short and cylindrical body; hemipenial body with a single irregular flounce on the basal region; sulcus spermaticus single, straight, slightly protruding on the surface of the hemipenial body; medial and distal surfaces of the body with small irregular striations (not shown).

3.2.6 | *Amerotyphlops paucisquamus* (*N* = 1; organ fully everted and inflated)

Hemipenis single, with a cylindrical body, broader at the base and slimmer distally (Figure 5a–b); hemipenial body with a few irregular flounces on the basal region (Figure 5a–b); sulcus spermaticus single protruding over the surface of the hemipenial body, running centripetally at the base and straight from the middle part of the organ to the apical region (Figure 5a, c–d); middle region of lateral walls of the sulcus covered with smooth and shallow striations (Figure 5a); a small sheet of tissue originates on each side of the distal region of the sulcus, runs transversally and surrounds the hemipenis to form a complete low-wall flounce on the apical region (Figure 5c–d).

3.3 | Distribution of South American *Amerotyphlops*

Our revision of the distribution records for *Amerotyphlops* from the literature, as well as the examination and reidentification of several museum specimens (see Supporting information Appendix S1 and S2) provided an updated view of the general distribution of its South American species. We provided below a succinct description of the distribution for each South American species.

Amerotyphlops tasymicris occurs in Grenada and Saint Vincent and Grenadines, the type locality is 1 mile of east Vincennes, in Saint David Parish (Thomas, 1974). Recently, the distribution record was extended to Chatham Bay, on Union Island, in Saint Vincent and Grenadines (Bentz, Rodríguez, John, Henderson, & Powell, 2011).

Amerotyphlops trinitatus occurs in Trinidad and Tobago. Until recently, this species was only known by type locality, in Trinidad, in Arima road, 3 miles above William Beebe Tropical Research Station, also known as Silma, sits in the Orinoco delta (Dixon & Hendricks, 1979; Boos, 2001; Richmond, 1965). However, in Tobago, this species seems to be widespread, occurring in Charlotteville, King's Bay, Merchiston, Parlatuvier, and Scarborough (Boos, 2001).

In mainland South America, the genus is widely distributed, occurring in dry forests of Venezuela; in both Atlantic

FIGURE 3 (a–f) Hemipenis of *Amerotyphlops reticulatus* (MPEG 23,526), in sulcate (a) asulcate (b), right (c) and left views (d), and detail of the apical region of the sulcate (e), asulcate (f), left views (g). Scale = 2 mm [Color figure can be viewed at wileyonlinelibrary.com]

and Amazon Forests; along the major open formations of Cerrado, Chaco, and Caatinga; within most Savanna enclaves within the Amazon Forest and in remnant fragments of the Atlantic Forest typically distributed throughout northeastern and northern Brazil.

Amerotyphlops lehneri occurs exclusively in the Dry Forest of Maracaibo, in El Pozón, in the state of Falcon, northern Venezuela (Figure 6) (Dixon & Hendricks, 1979; Rivas et al., 2012; Roux, 1926; Shreve, 1947; Wallach et al., 2014). This species was the only South American species for which no specimens were available for this study, and we cannot provide further information about its distribution and phylogenetic relationship among South American species.

Amerotyphlops minuisquamus (Figure 6) is distributed in the Amazonian basin, in Peru, western Brazil, Venezuela, Colombia, and the Guyana Shield. The species is also found in the Llanos, Patia Valley, and Apure-Villavicencio dry forests of Colombia (Caicedo-Portilla, 2011; Dixon & Hendricks, 1979; Roze, 1956; Wallach et al., 2014). Here, we extend the distribution of *A. minuisquamus* to Machadinho d'Oeste, in Rondônia State, Brazil, the first record of this species southern of the Amazon River.

Amerotyphlops reticulatus occurs throughout the Amazon basin in Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, and Suriname and in the adjacent ecotonal regions southern of the Amazon forest of Brazil (Figure 6). Additionally, this species occurs in gallery forests in the Cerrado biome in Brazil; Savannas of the Guyana Shield in Brazil and Guyana; in the Llanos region in Colombia and Venezuela; and in Ecuador dry forests (Ávila & Kawashita-Ribeiro, 2011; Caicedo-Portilla, 2011; Cunha & Nascimento, 1978, 1993; Dixon & Hendricks, 1979; França et al., 2006; França & Venâncio, 2010; Wallach et al., 2014).

Amerotyphlops brongersmianus is a widespread species in South America (Figure 6), it is found in Argentina, in Humid Chaco and Humid Pampas; Brazil, in South and North

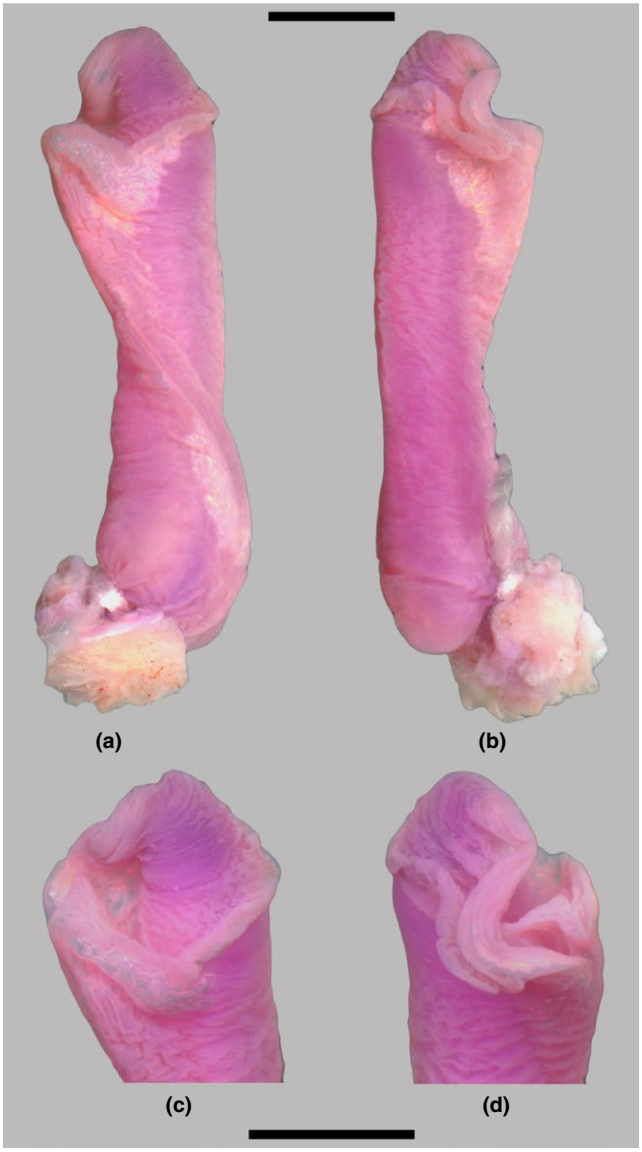

FIGURE 4 (a–d) Hemipenis of *Amerotyphlops brongersmianus* (MZUSP 14,678), in sulcate (a) and asulcate sides (b), and detail of the apical region in sulcate (c) and asulcate views (d). Scale = 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

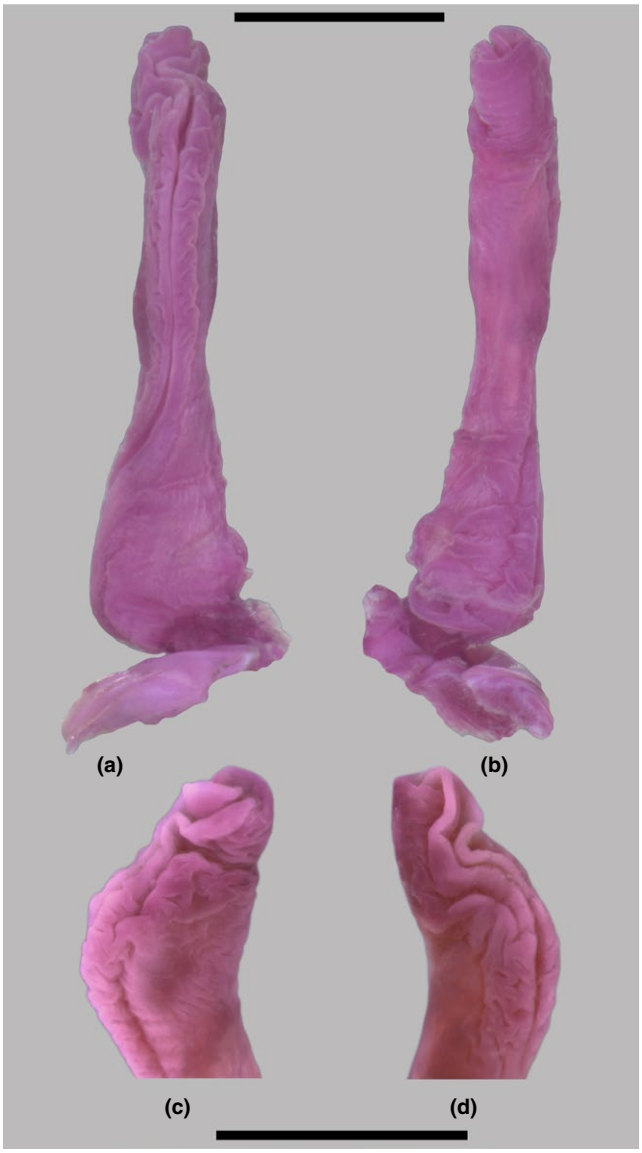

FIGURE 5 (a–d) Hemipenis of *Amerotyphlops paucisquamus* (AGARDA 2,786), in sulcate (a) and asulcate sides (b), and detail of the apical region in left (c) and right views (d). Scale = 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

Atlantic Forest, Cerrado, Savanna enclaves in the Amazon and Caatinga; Bolivia, in Beni Savanna, Central Andean Puna, and Chiquitanos Dry Forest; Colombia, in the Amazon region and in Apure-Villavicencio Dry Forest; Guyana, in Guyana Moist Forest; Paraguay, in Dry and Humid Chaco, and Atlantic Forest; Peru, in the Amazon forest; Suriname, in Guiana Freshwater Swamp Forest and Amazon-Orinoco Southern Caribbean Mangroves; Trinidad, in Lesser Antillean Dry Forest; and Venezuela, in La Costa Xeric Shrublands (Arruda et al., 2011; Dixon & Hendricks, 1979; Guedes et al., 2014; Loebmann, 2008; Martins et al., 2010; Rodrigues, 1991; Santana et al., 2008; Wallach et al., 2014).

Amerotyphlops yonenagae (Figure 7) is endemic to Caatinga, living in the sand dunes habitats of the right banks of the middle the São Francisco River, occurring in the municipality of Gentio do Ouro (Santo Inácio district) and Paulo Afonso (Estação Ecológica Raso da Catarina), in state of Bahia, Northeastern Brazil (Graboski et al., 2015; Rodrigues, 1991; Wallach et al., 2014).

Amerotyphlops arenensis is endemic of upland forest fragments of Atlantic Forest in the Northeastern Brazil (Figure 7). The species was recorded in Reserva Ecológica Mata do Pau Ferro, situated at 5 km from the municipality of Areia, state of Paraíba; Reserva Particular do Patrimônio Natural Pedra D'Antas, in the municipalities of Lagoa dos Gatos and Jaqueira, in Pernambuco state; Reserva Biológica Pedra Talhada, municipality of Quebrangulo, Alagoas state (Graboski et al, 2015; Roberto et al., 2015, 2017). Here, we

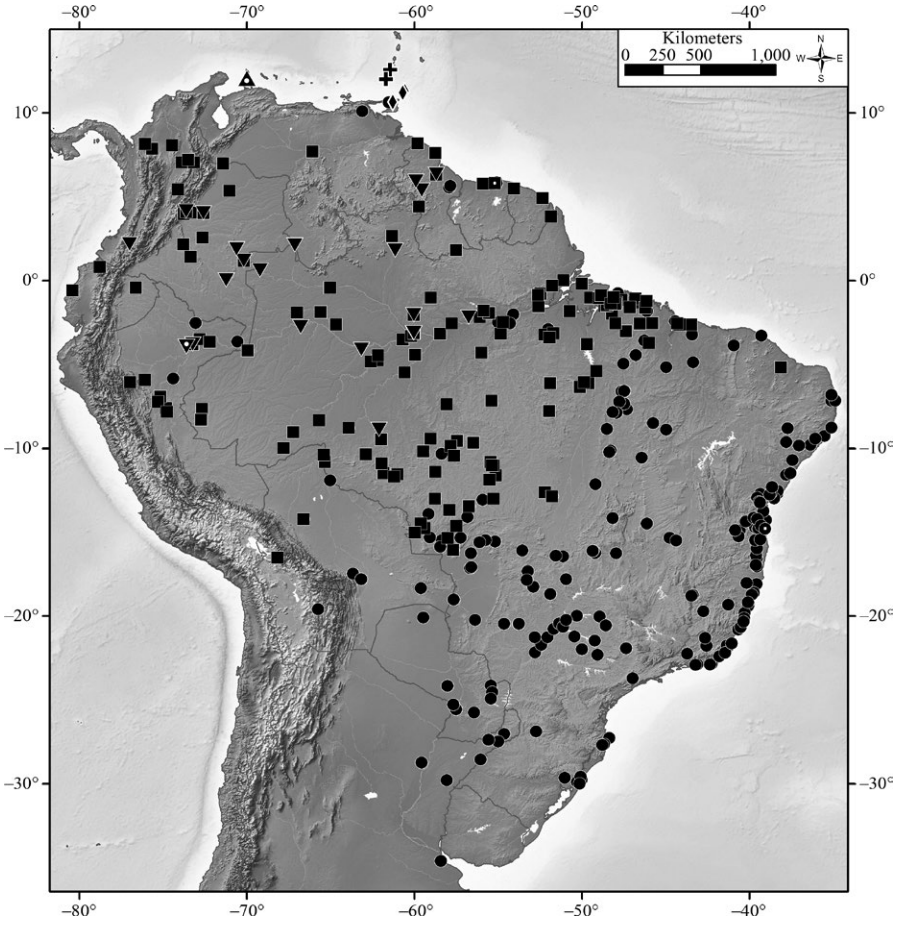

FIGURE 6 Geographical distribution of four species belonging to the genus *Amerotyphlops* from South America. Symbols are: *A. lehneri* (black triangles), *A. trinitatus* (black diamonds), *A. tasymicris* (black crosses) *A. minuisquamus* (inverted black triangles), *A. reticulatus* (black squares) and *A. brongersmianus* (black circles). Symbols with a middle white dot represent type localities. For *A. reticulatus* the type locality is based on the Neotype designation made by Dixon & Hendricks (1979).

extend the distribution of *A. arenensis* to Reserva Biológica da Mata Escura, municipality of Jequitinhonha, Minas Gerais state; and Serra do Cafundó, municipatily of Piatã, Bahia state. Fernandes, Ribeiro, Santos Dayrell, Santana, & Rocha Lima (2010), recorded *A. amoipira* in the Cerrado, at Fazenda Santa Maria de Vereda, municipality of Bonito de Minas, Minas Gerais state. Reanalyzing the described characteristics of these specimens, as pholidosis and patterns of coloration, we reidentify these individuals as being *A. arenensis.*

Amerotyphlops amoipira occurs in the psammophilous habitats of the sand dunes region of the middle the São Francisco River, in the municipality of Barra (Ibiraba district), state of Bahia. The species also occurs in the Caatinga of Alagoas state and in fragments of Atlantic forest, in dunes and sandy areas in the states of Alagoas, Rio Grande do Norte and Sergipe (Figure 7) (Brito & Freire, 2012; Graboski et al., 2015; Rodrigues & Juncá, 2002; Wallach et al., 2014). Here we extend the distribution of *A. amoipira* to Restinga de Panaquatira, municipality of São José do Ribamar, Maranhão state.

Amerotyphlops paucisquamus is endemic of the Atlantic forest of Northeastern Brazil, from state of Maranhão, Paraíba, Pernambuco, and Rio Grande do Norte; and in the Caatinga of Alagoas and Ceará state (Figure 7) (Dixon & Hendricks, 1979; França et al., 2012; Freire, 2001; Rodrigues, 1991; Rodrigues & Juncá, 2002; Wallach et al., 2014).

4 | DISCUSSION

4.1 | Systematics of Typhlopinae

Among different hypotheses concerning the systematics of typhlopines Thomas (1976), Dixon & Hendricks (1979), and Hedges et al. (2014) set the bases for an understanding of the evolutionary affinities within the group. Although studying mainly West Indian species, Thomas (1976) was the first to provide a comprehensive phylogenetic hypothesis of Typhlopinae, based on osteological, hemipenial, and soft tissue anatomy. He identified two major groups within the West Indian radiation: (a) the Biminiensis group, which includes *Typhlops biminiensis* Richmond, 1965(= *Cubatyphlops biminiensis*) and *T. caymanensis* Sackett, 1940 (= *Cubatyphlops caymanensis*); (b) the Major Antillean Radiation group (MAR) that includes most of the remaining Caribbean species. Thomas did not focus his study on mainland species of *Amerotyphlops*, although according to him the Lesser Antillean species *A. tasymicris* was closely related to *A. trinitatus* and *A. lehneri* from the continental island of Trinidad and mainland northern South American (Venezuela), respectively. Additionally, he suggested a close relationship of the Biminiensis group with the Middle American typhlopines.

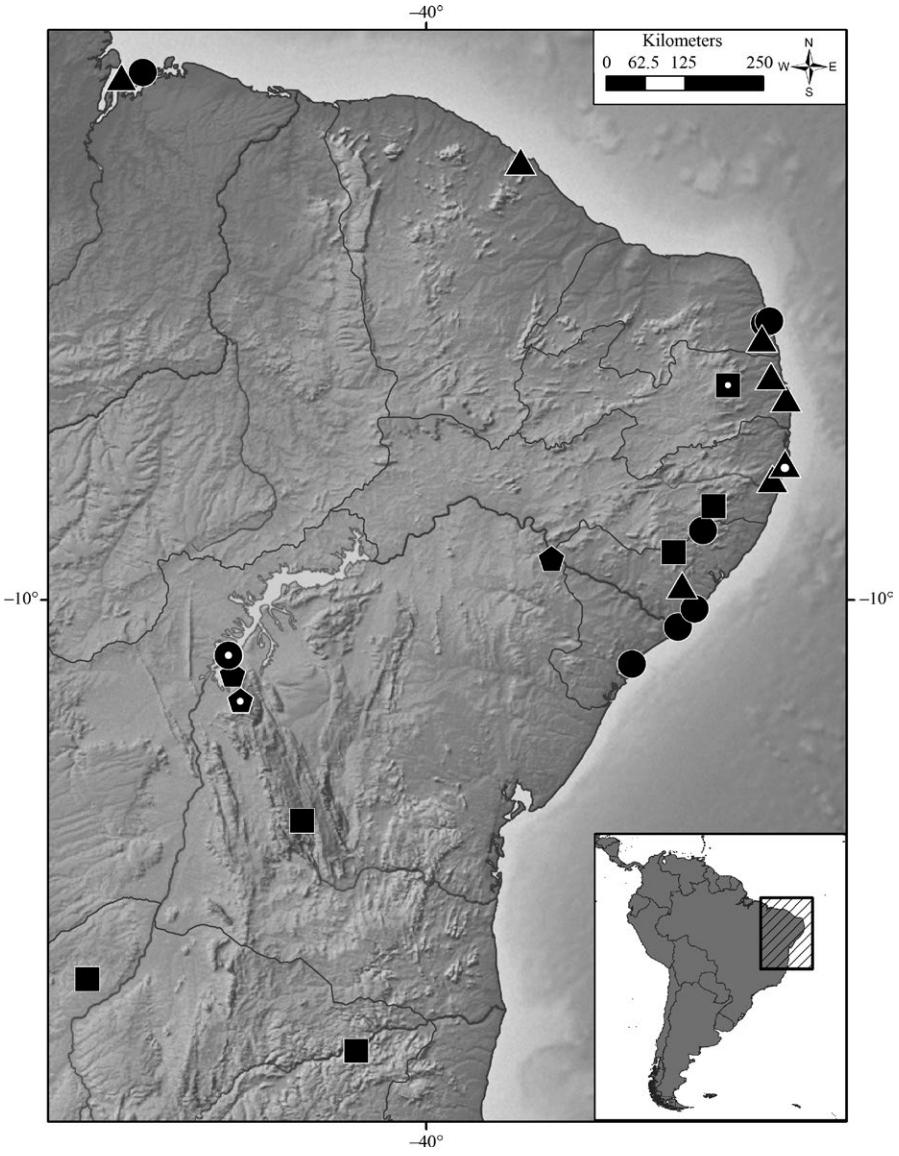

FIGURE 7 Geographical distribution of four species belonging to the genus *Amerotyphlops* from Northeastern Brazil, South America. Symbols are: *A. arenensis* (black squares), *A. amoipira* (black circles), *A. yonenagae* (black pentagons) and *A. paucisquamus* (black triangles). Symbols with a middle white dot represent type localities

Shortly after Thomas' contribution, Dixon & Hendricks (1979) published a second major phylogenetic hypothesis for typhlopines. Through a study focusing mainly on mainland species, and based on external and hemipenial morphology, they recognized three distinct groups: (a) the "Caribbean Arc" group, which include the Biminiensis group of Thomas, all continental species of Middle America, *A. tasymicris* from the Lesser Antilles, and *A. trinitatus* and *A. lehneri* from northern South America; (b) the MAR group of Thomas; and (c) the South American group, formed by the two continental species known at the time (*A. reticulatus*, *A. brongersmianus*) and two new species described in their study (*A. minuisquamus*, and *A. paucisquamus*). Dixon & Hendricks (1979) were also the first to suggest a hypothesis of relationship specifically for South American mainland typhlopines. They described the existence of the following two species groups: a northern group composed by *A. reticulatus* and *A. minuisquamus*; and a southern group formed by *A. brongersmianus* and *A. paucisquamus*. They based their suggestion on the sharing traits of general color pattern and scale row reductions.

The main difference between these two morphological studies was the relative phylogenetic importance given to the nasal scale suture (see Table 2). Although Thomas (1976) analyzed several internal and external characters, he did not comment on this trait, since all West Indian species share a complete nasal suture. On the other hand, Dixon & Hendricks (1979) allocated in their Caribbean Arc group all species with a complete nasal suture and a contact between the preoculars and the 2nd and 3rd supralabials (Table 2). Dixon & Hendricks's proposal included in the same group species from four main different biogeographic realms: (a) the South American continent, (b) the Middle American mainland, (c) the Lucayan Archipelago (Bahamas, and Turks and Caicos Islands), and the (d) Antilles (Greater and Lesser). Although Thomas also commented about the overall similarities between the Biminensis group and *A. tasymicris*, he strongly

TABLE 2 Variation of some selected characters for species of *Amerotyphlops*, *Antillotyphlops, Cubatyphlops*, and *Typhlops*

Species	MD (Min–Max)	PROC	NS	PFS	References
Amerotyphlops paucisquamus	162–209	2nd & 3rd supralabial	Incompletely	Rectangular	Graboski et al. (2015) and this study
Amerotyphlops arenensis	204–225	2nd & 3rd supralabial	Incompletely	Rectangular	Graboski et al. (2015) and this study
Amerotyphlops amoipira	203–241	2nd & 3rd supralabial	Incompletely	Rectangular	Graboski et al. (2015) and this study
Amerotyphlops brongersmianus	195–287	2nd & 3rd supralabial	Incompletely	Rectangular	Dixon & Hendricks (1979) and this study
Amerotyphlops minuisquamus	190–253	2nd & 3rd supralabial	Incompletely	Rectangular	Dixon & Hendricks (1979) and this study
Amerotyphlops reticulatus	223–299	2nd & 3rd supralabial	Incompletely	Rectangular	Dixon & Hendricks (1979) and this study
Amerotyphlops yonenagae	250–277	2nd & 3rd supralabial	Incompletely	Rectangular	Graboski et al. (2015) and this study
Amerotyphlops trinitatus	389–389	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops tasymicris	429	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops stadelmani	341–369	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops tycherus	395–395	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops tenuis	347–429	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops costaricensis	390–413	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops microstomus	487–566	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Amerotyphlops lehneri	289–331	2nd & 3rd supralabial	Completely	Rectangular	Dixon & Hendricks (1979)
Cubatyphlops caymanensis	351–408	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops paradoxus	455–472	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops perimychus	453–496	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops anousius	465–513	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops epactius	473–505	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops biminiensis	454–537	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops contorhinus	502–502	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops notorachius	475–529	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops anchaurus	514–514	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops satelles	514–527	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops arator	578–579	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Cubatyphlops golyathi	629–629	2nd & 3rd supralabial	Completely	Rectangular	Hedges et al. (2014)
Antillotyphlops monensis	299–345	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops richardi	300–369	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops geotomus	329–367	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops platycephalus	350–365	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops naugus	345–390	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops monastus	351–394	3rd supralabial	Completely	triangular	Hedges et al. (2014)
Antillotyphlops granti	370–386	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops hypomethes	363–407	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops catapontus	376–409	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops annae	400–405	3rd supralabial	Completely	Triangular	Hedges et al. (2014)

(Continues)

TABLE 2 (Continued)

Species	MD (Min–Max)	PROC	NS	PFS	References
Antillotyphlops guadeloupensis	393–430	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Antillotyphlops dominicanus	434–499	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops pachyrhinus	243–257	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops silus	254–261	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops titanops	231–264	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops lumbricalis	256–271	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops schwartzi	237–282	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops tetrathyreus	246–294	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops oxyrhinus	265–297	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops leptolepis	250–308	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops agoralionis	291–310	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops proancylops	283–312	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops sylleptor	305–324	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops hectus	284–328	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops eperopeus	305–329	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops pusillus	245–332	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops syntherus	299–353	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops rostellatus	314–358	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops jamaicensis	373–436	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops sulcatus	371–447	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops gonavensis	399–455	3rd supralabial	Completely	Triangular	Hedges et al. (2014)
Typhlops capitulatus	358–457	3rd supralabial	Completely	Triangular	Hedges et al. (2014)

Note. MD, number of middorsal scales; NS, nasal suture (incompletely or completely divided); PROC, contact between preocular with supralabial and PFS, prefrontal shape.

stated against their affinities by saying that "…*tasymicris* is a relative of the South American species *lehneri* and *trinitatus* and is unrelated in any close fashion to any of the other Antillean species," (Thomas, 1976; page 71).

After these seminal papers, Hedges et al. (2014)—mainly based on molecular data—enhanced significantly our knowledge on typhlopoid systematics. However, regarding the major American groups of typhlopids, this study arrived to almost the same conclusions advanced by Thomas (1976) and Dixon & Hendricks (1979) 35 years earlier. The molecular analyses of Hedges et al. (2014) as well as the study of Pyron & Wallach (2014) recovered two strongly supported clades: the first one including most of the West Indian species and corresponding the MAR and *biminensis* groups of Thomas; the second one encompassing the South American typhlopines and *A. tasymicris* from the Lesser Antilles.

Our results corroborate, through a more comprehensive mainland sampling, both West Indian and South American radiations of typhlopines. The position of *A. tasymicris* nested within the South American clade as the sister group of *A. minuisquamus* is supported in our analyses by high values of bootstrap and PP (77%, 0.94; Figure 1) agreeing with the conclusions of Thomas (1976). Our results also corroborate the close relationship between *A. brongersmianus* and *A. paucisquamus* as suggested by Dixon & Hendricks (1979). Additionally, we expand here the understanding of their southern species group with the inclusion of all other NBS (*A. yonenagae*, *A. arenensis* and *A. amoipira*). However, since *A. minuisquamus* is not the sister group of *A. reticulatus* in our tree topology, we did not find corroboration for their northern species group. Otherwise, we showed the existence of two different lineages of *Amerotyphlops* from northern South America. The first lineage composed by *A. minuisquamus* and *A. tasymicris*, and the second including only *A. reticulatus*.

Unfortunately, our analyses cannot bring light on the debate about the phylogenetic position of the Middle American typhlopines since there are no available sequences for these species. The morphological evidence used by Hedges et al. (2014) and Pyron & Wallach (2014) to include Middle American typhlopines in *Amerotyphlops* is mostly based on the contact between preocular and supralabial scales, and on

the shape of preocular (that is less triangular in *Amerotyphlops* when compared to the West Indian relatives). However, it is noteworthy that Middle American typhlopines present a high mean number of dorsal scales (357–530), which overlaps the mean dorsal scale counting of *A. tasymicris*, *A. trinitatus,* and *A. lehneri* (320–429) but are sharply different to those present in other mainland South American typhlopines (191–299). The mean dorsal scale counting suggest that the latter three species could be associated with the Middle American typhlopines, as suggested by Dixon & Hendricks, but such association is still prone to the lack of validation through a phylogenetic analysis.

Concerning the West Indians typhlopids, the studies of Hedges et al. (2014) and Pyron & Wallach (2014) recovered very similar topologies, but they disagree regarding the use of the generic names *Antillotyphlops, Cubatyphlops* and *Typhlops* for the species of the Greater Antilles. The topology presented by Pyron & Wallach does not support the genera *Antillotyphlops* and *Cubatyphlops* proposed by Hedges et al. (2014), thus the authors suggested the synonymization of *Antillotyphlops* and *Cubatyphlops* with *Typhlops*. However, Nagy et al.'s (2015) reanalysis of the sequences used by Hedges et al. (2014) and Pyron & Wallach (2014) showed that synonymization of *Antillotyphlops* and *Cubatyphlops* with *Typhlops* was unsupported. Although the phylogenetic position of *C. biminiensis* is still open to debate, current phylogenetic knowledge and the available morphological evidence suggest that the three genera proposed by Hedges et al. (2014) are valid. Our own results further corroborate the monophyly and validity of the three genera proposed by them.

4.2 | Comparative hemipenial morphology

Hemipenes of South American *Amerotyphlops* follow the general pattern observed in scolecophidians: single organs with an undivided sulcus spermaticus (Branch, 1986; Wallach, 1998). All species described here retain hemipenes with more or less conspicuous flounces on the surface of the hemipenial body and a sulcus spermaticus with protruded walls. The organs of *A. minuisquamus* and *A. reticulatus* are unique in presenting large calcified spines, while this character is absent in other species. The distal region of the hemipenis of *A. minuisquamus* is expanded, forming a broad apical disk with a large, round, and bulbous expansion in the middle, a characteristic that is known to occur only in this species. The hemipenis of *A. reticulatus* shows a more complex ornamentation when compared with its congeners (Figure 3a–g).

Thomas (1976) defined two general shape patterns—expanded and attenuate—after analyzing the hemipenes of 17 Antillean typhlopines. The expanded pattern included two additional subcategories—trumpet-shaped and oblique (characterized by a differentiated flattened region on one side of the organ)—while the attenuate pattern referred to slender and filiform organs with no apparent terminal expansions (Thomas, 1976). These two general shape patterns are also present in South American *Amerotyphlops,* since most species examined in this study (six out of seven) are similar in morphology to the attenuate pattern, an only one (*A. minuisquamus*) shows the trumpet-shaped expanded pattern (Figure 2a–d). Likewise, the species of the Antillean genera *Antillotyphlops* and *Typhlops* exhibit both shape patterns, and only the members of the Antillean genus *Cubatyphlops* exhibit just the attenuated morphology.

Currently, the description made by Dixon & Hendricks (1979) of calcified ornamentations (row of spines) in the organ of *Amerotyphlops reticulatus* is the only record of this kind of structure in the genus (*Typhlops* sensu-*lato*). The presence of two calcified spines in the apical disk of the organ of *A. minuisquamus* represents the second record of such structure for the genus. However, these ornamentations are apparently not homologous since their arrangement and position in the organ are significantly distinct. Most South American species of *Amerotyphlops* we sampled for hemipenes (except *A. minuisquamus*) form a well-supported clade (*A. reticulatus* +A. *brongersmianus* +NBS), and can be characterized by the presence of a single sulcus spermaticus that protrudes over the surface of the hemipenial body. For instance, this characteristic could be considered as a putative shared derived character for this clade; however, this hypothesis must be submitted to rigorous test and new information on the hemipenes of the remaining congeners should be compared with the descriptions presented here.

According to previous studies (Dixon & Hendricks, 1979, Hedges et al., 2014, Pyron & Wallach, 2014; Thomas, 1976), the hemipenis general shape did not seem to represent an informative trait in the phylogenetic relationship of the major American groups of typhlopids. On the other hand, our results show that the micro- and macro-ornamentation of the *Amerotyphlops* hemipenis is highly diverse, potentially informative for systematics, and could represent a source of synapomorphies for some groups of species.

4.3 | Distribution of South American *Amerotyphlops*

Even considering the extensive distributional records obtained in the present review, we consider the geographic distribution of most South American species of *Amerotyphlops* as being far from completely understood. This is not a surprise, since we still largely ignore major parameters affecting distribution of fossorial squamates (Camacho et al., 2015). Furthermore, we still need basic data regarding the abiotic and biological factors promoting

interruption of gene flow and favoring speciation in organisms living below the surface. In our view, these two conditions are central to a sound biogeographical analysis. In fact, the derived clade only composed by NBS probably indicates that these species suffered some early and complex process of isolation and peripheral speciation. Moreover, real distribution of most NBS can be underestimated and might be larger than the area delimited by our reviewed records. Examples of such underestimation are *A. amoipira* and *A. arenensis*, whose distributions were considered very restricted until recently (Brito & Freire, 2012; Graboski et al, 2015; Roberto et al., 2015, 2017; Rodrigues & Juncá, 2002). Many of the geographical gaps in the distribution of these species are in unexplored areas of northeastern Brazil. Even well-sampled species, as *A. brongersmianus*, show important gaps in their Amazonian Forest and Cerrado ranges. Such distributional gaps are located in extensive, poorly sampled and environmentally complex areas, which cannot be compared to probably real distributional gaps located in areas such as the Araucaria Forest and the Uruguayan Savanna that are densely sampled in museum collections.

Only a few species, such as *A. minuisquamus*, *A. reticulatus*, and *A. brongersmianus* have a reasonable geographical record to explore some association with South American biomes, the other five species present deficient data not allowing further inferences about the process that determined their present and past distribution.

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