JCZ: Zoologischer Anzeiger - A Journal of Comparative Zoology 303 (2023) 10–25

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

More than meets no eyes: Taxonomic status of a *Liotyphlops* Peters, 1881 (Serpentes: Anomalepididae) blindsnake from the Atlantic Rainforest

Omar M. Entiauspe-Neto [a,b,*], Francisco L. Franco [a], Claudia Koch [c], Arthur Tiutenko [d], Juliana M. Wingert [a], Márcio Borges-Martins [a]

[a] Laboratório de Coleções Zoológicas, Instituto Butantan, 05503-90, Av. Vital Brazil, 1500, Butantã, São Paulo, SP, Brazil
[b] Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 91501-970, Porto Alegre, RS, Brazil
[c] Leibniz Institute for the Analysis of Biodiversity Change - Museum Koenig, Adenauerallee 160, 53113, Bonn, Germany
[d] Friedrich-Alexander-Universität Erlangen-Nürnberg, Schloßplatz 4, 91054, Erlangen, Germany

ARTICLE INFO

Handling Editor: Alexander Kupfer

Keywords:
Atlantic forest
Neotropical
Osteology
Phylogeny
Reptilia
Scolecophidia
Synonymy

ABSTRACT

Liotyphlops Peters, 1881 is an anomalepidid blindsnake genus that encompasses 12 species, widely distributed in Central and South America. In this study, we evaluate the taxonomic status of *Liotyphlops sousai* Santos & Reis, 2018, a species described based on a single specimen from the Atlantic Rainforest of southern Brazil, and after analyzing evidences based upon molecular systematics, external morphology and osteology, we propose its synonymy with *Liotyphlops beui* (Amaral, 1924), a common and widely distributed Anomalepidid species. We also provide comments and recommendations on the taxonomy of *Liotyphlops*, assessing the risks associated with describing a new species based on small type series.

1. Introduction

"*The amount of variation exhibited by various species of* Liotyphlops *is a formidable determent for the description of a new form based upon one specimen*" (Dixon & Kofron, 1983:259).

Scolecophidia Cope, 1864 is an infraorder of fascinating miniaturized, microphthalmic, and diverse snakes, widely distributed in all continents except Antarctica, with 451 species allocated in the families Anomalepididae Taylor, 1939, Gerrhopilidae Vidal, Wynn, Donnellan & Hedges, 2010, Typhlopidae Merrem, 1820, Leptotyphlopidae Stejneger, 1892, and Xenotyphlopidae Vidal, Vences, Branch & Hedges, 2010 (Miralles et al., 2018; Uetz and Hošek, 2020). Anomalepididae currently encompasses 20 species, distributed in most of Central and South America (Uetz et al., 2022); it can be diagnosed based on having maxillary and mandibular teeth, postorbital and ectopterygoid bones, M–shaped hyomandibular apparatus, exposed nasal gland, *levator pterigoideus profundus* muscle, and a tracheal lung (Ferrarezzi, 1994; Palci et al., 2020).

Within Scolecophids, Anomalepididae were historically regarded the only group sharing a loss of pelvic girdle remains, and an absent or vestigial 1st supralabial scale (Ferrarezzi, 1994), although recent works have challenged this view, with the discovery of pelvic remains in a single species (Palci et al., 2020); recent molecular evidence suggests Anomalepididae is paraphyletic regarding other Scolecophid families, being a sister clade to Alethinophidia Nopcsa, 1923 (Miralles et al., 2018), although other authors have recovered different results based on molecular and morphological data (Gauthier et al., 2012; Singhal et al., 2021; Strong et al., 2021).

Liotyphlops Peters, 1881, is a recondite anomalepidid genus, with 12 small–sized and fossorial species, distributed from Central America to most of South America at east of the Andes (Uetz and Hošek, 2020). Its taxonomy has been described as "chaotic", with a particularly variable morphology, with inconsistent and inaccurate descriptions, such as the brief descriptions of *Helminthophis ternetzii* (Boulenger, 1896) and *Helmintophis collenetei* (Parker, 1928), which are currently regarded as synonymous (Dixon and Kofron, 1983). The taxonomy of cisandine

* Corresponding author. Laboratório de Coleções Zoológicas, Instituto Butantan, 05503-90, Av. Vital Brazil, 1500, Butantã, São Paulo, SP, Brazil.
E-mail addresses: omarentiauspe@hotmail.com (O.M. Entiauspe-Neto), francisco.franco@butantan.gov.br (F.L. Franco), C.Koch@leibniz-zfmk.de (C. Koch), arthur@tiutenko.de (A. Tiutenko), juwingert@hotmail.com (J.M. Wingert), borges.martins@ufrgs.br (M. Borges-Martins).

https://doi.org/10.1016/j.jcz.2023.01.003
Received 11 October 2022; Received in revised form 26 December 2022; Accepted 9 January 2023
Available online 20 January 2023

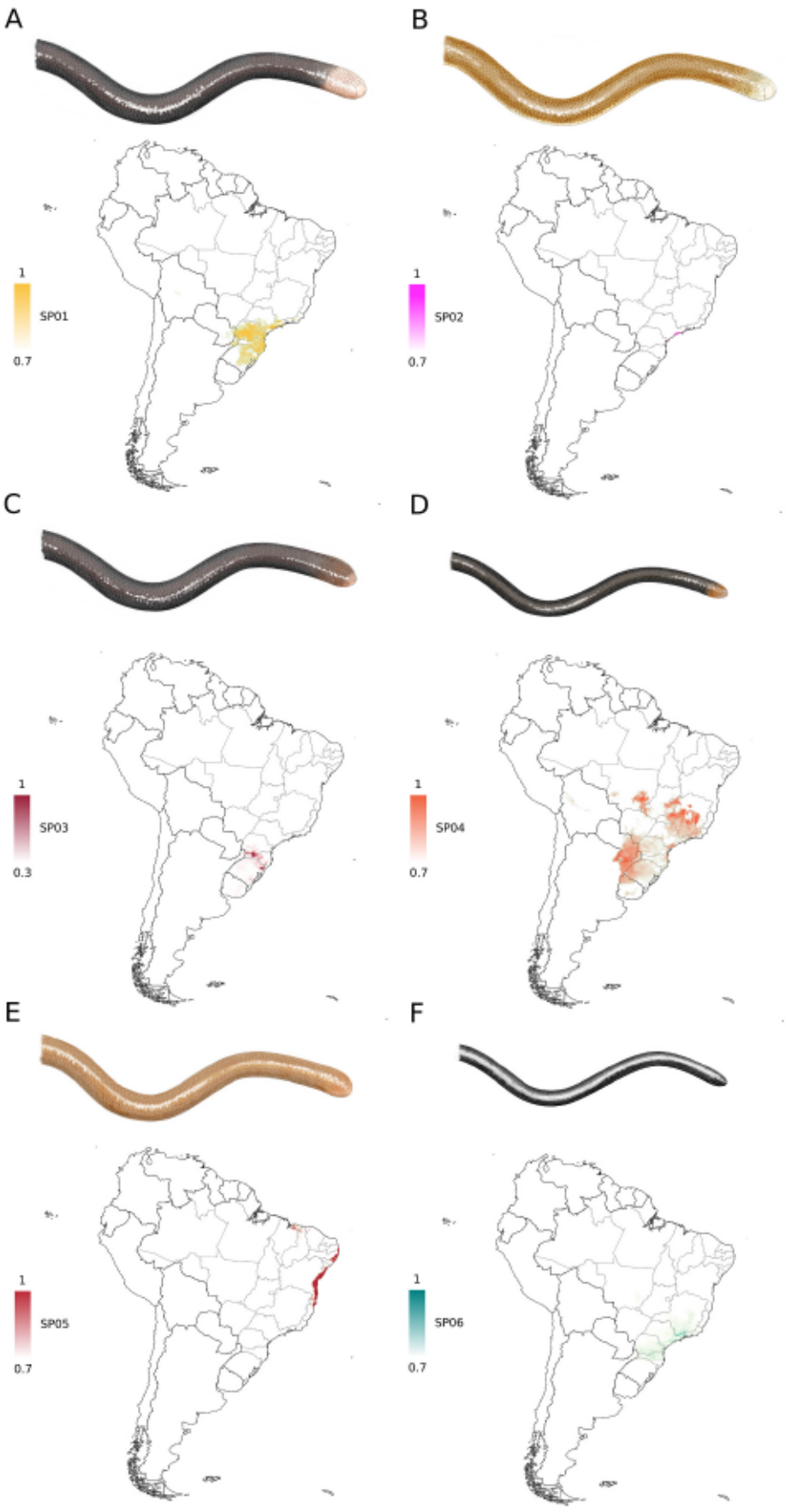

Fig. 1. Operational Taxonomic Units (OTUs) and Ecological Niche Models delimited based on *Liotyphlops* from the Atlantic Rainforest biome in South America. A) SP01, based on topotypical specimens and an additional series of *Liotyphlops beui* from the Atlantic Forest of southeastern Brazil; B) SP02, based on specimens of *Liotyphlops caissara* from lowland Atlantic Rainforest of coastal southeastern Brazil; C) SP03, based on the holotype of *Liotyphlops sousai* from the Atlantic Forest of southern Brazil; D) SP04, based on the holotype and an additional specimen of *Liotyphlops ternetzii* from the Cerrado of Brazil and Paraguay; E) SP05, based on the holotype of *Liotyphlops trefauti* from the Atlantic Rainforest of northeastern Brazil; F) SP06, based on a specimen of *Liotyphlops wilderi* from montane Atlantic Rainforest of southeastern Brazil.

Liotyphlops is unstable; Garman (1883) described *Typhlops wilderi* based on individuals from Sao Cyriaco, Brazil [corrected to Sao Cypriao, Minas Gerais, Brasil by Dixon and Kofron (1983), currently Cipriano, Minas Gerais, Brazil]. Boulenger (1893) synonymized *Liotyphlops* with *Helminthophis*. In his catalogue of snakes at the British Museum, Boulenger (1889) described *Helminthophis guentheri*, based on two specimens from Porto Real, Province Rio de Janeiro (currently in the state of Rio de Janeiro, Brazil). Later, Boulenger (1896) presented a brief description for *Helminthophis ternetzii*, based on a single specimen from Paraguay. In a short taxonomic note, Hammar (1908) suggested allocating *Typhlops wilderi* in *Helminthophis*. Amaral (1924) described *Helminthophis incertus* based on a specimen reported from Surinam [later determined as In error by Dixon and Kofron (1983)], and *Helminthophis beui* based on a series from Butantan institute, in Sao Paulo municipality, Sao Paulo, Brazil. Parker (1928) described *Helminthophis collenetei* based on a single specimen from Burity (Buriti), Mato Grosso, Brazil.

Dunn (1932) recognized *Liotyphlops* as distinct from *Helminthophis* based on characters of head pholidosis, and proposed its revalidation. Vanzolini (1948) presented a checklist of squamates from Cachoeira das Emas, in Pirassununga, Sao Paulo, Brazil, in which he described *Liotyphlops schubarti*. Dixon and Kofron (1983) presented a through revision of the group, analyzing all available type series, allocating *H. guentheri* as a junior synonym of *T. wilderi*, *H. incertus* and *H. collenetei* as junior synonyms of *H. ternetzii*, while also shifting these taxa to *Liotyphlops*. Later, Freire et al. (2007) described *Liotyphlops trefauti* from the Atlantic Rainforest of Northeastern Brazil, based on three specimens, two from Alagoas and one from Bahia. Centeno et al. (2010) described *Liotyphlops caissara* from Ilha Sao Sebastiao, an island off the coast of Ilhabela, Sao Paulo, southeastern Brazil, based on a single specimen. Santos & Reis (2018) described two *Liotyphlops* from Brazil, *Liotyphlops taylori* with a single specimen from the Cerrado of Estaçao Ecologica Serra das Araras, Porto Estrela municipality, Mato Grosso state, and *Liotyphlops sousai* with a single specimen from the Atlantic Rainforest of Usina Hidreletrica Passos Maia, Passos Maia municipality, Santa Catarina state, Brazil.

The description of *L. sousai* is particularly interesting. The species is distinguished from its congeners for having four scales contacting the posterior edge of prefrontal (Santos & Reis, 2018:507), as other congeners (except for *Liotyphlops albirostris*) would have three. Dixon and Kofron (1983) presented an interesting remark on the taxonomy of *Liotyphlops*, stating that *Occasionally, a scale(s) may contact another scale that normally does not, or vice versa, and depending upon one s concept of* Liotyphlops*, these contacts have been used to describe various species* (Dixon and Kofron, 1983:243). Prompted by this remark, we decided to investigate the taxonomic status of *L. sousai*; surprisingly enough, we discovered several individuals of *Liotyphlops beui* from the same locality as the holotype of *L. sousai* (Usina Hidreletrica Passos Maia, Passos Maia municipality, Santa Catarina state, Brazil), in the same collection, that were seemingly overlooked by the authors. This study seeks to reassess the taxonomic status of *L. sousai*, delimiting putative Operational Taxonomic Units (OTUs) from examined *Liotyphlops* specimens, and after evaluating integrative data of external and internal morphology, providing evidence for its synonymy with a common and widespread species, *L. beui*.

2. Materials and methods

2.1. Morphological analyses

We examined a total of 102 specimens of *Liotyphlops*. A list of examined material is provided in Appendix 1. Sex determination was done with a ventral incision in the base of the tail, probing following the technique of Marais (1984), or visual inspection of hemipenes. Specimens of *Liotyphlops* were arbitrarily considered as juveniles if their snout-vent length attained under 219 mm for females and 178 mm for males, according to the maturity range defined by Parpinelli & Marques (2015). An emended diagnosis for *L. beui* is modified from the cumulative morphological character key used by Entiauspe-Neto et al. (2022). Terminology for scale counts follows Dixon and Kofron (1983). Head and tail measurements were taken with a dial caliper to the nearest 0.01 mm; for others, a flexible ruler was used. Scales were measured on the right side of the head. Species concepts follow the Unified Species Concept of de Queiroz (2007), in which existence is treated as a separately evolving metapopulation lineage as the only necessary property of species and the former secondary species criteria as different lines of evidence (operational criteria) relevant to assessing lineage separation. We approved or rejected species hypotheses based on congruence of the evaluated lines of evidence (e.g. external morphology, DNA, osteology).

2.2. Statistical analyses

We generated a dataset of quantitative and qualitative diagnostic morphological characters to be evaluated in a series of examined specimens, which underwent sex determination and were considered to be putatively adults (n 36): (1) snout-vent length (SVL), measured from tip of rostral to cloacal opening; (2) total length, measured from tip of rostral to tail tip; (3) tail length, from cloacal opening to tail tip; (4) head length, from rostral tip to corner of mouth, ventrally; (5) first row of dorsal scales; (6) last row of dorsal scales; (7) post-frontal scales in contact with prefrontal; (8) scales in contact with nasal; (9) supralabial scales; (10) infralabial scales; (11) dorsal scales in vertebral row (mid-dorsal scales); (12) mid-ventral scales; (13) subcaudal scales; (14) ratio of mid-ventral to subcaudal scales; (15) head coloration, ranging from fully white [1] to fully black [2]. This dataset is available at [Supplementary Table 1]. We evaluated the following putative Operational Taxonomic Units (OTUs, Fig. 1): SP01 (Gold, n 26), based on topotypical specimens and an additional series of *L. beui* from the Atlantic Forest of southeastern Brazil; SP02 (Orange, n 3), based on specimens of *L. caissara* from lowland Atlantic Rainforest of coastal southeastern Brazil; SP03 (Dark Purple, n 1), based on the holotype of *L. sousai* from the Atlantic Forest of southern Brazil; SP04 (Pink, n 2), based on the holotype and an additional specimen of *Liotyphlops ternetzii* from the Cerrado of Brazil and Paraguay; SP05 (Red, n 1), based on the holotype of *L. trefauti* from the Atlantic Rainforest of northeastern Brazil; SP06 (Green, n 1), based on a specimen of *Liotyphlops wilderi* from montane Atlantic Rainforest of southeastern Brazil. Considering that Santos & Reis (2018) identified a specimen of *L. beui* from the type locality of *L. sousai* (UFRGS 6275; Passos Maia, Santa Catarina state), we restricted OTU SP03 to the holotype of *L. sousai*, in order to avoid including possible intergrades or misidentified individuals to the series. Potential geographic range distribution of OTUs were evaluated with a species distribution model, based on examined vouchered specimens and literature records, implemented in MaxEnt 3.4.4k (Phillips et al., 2006), using standard bioclimatic variables summarizing patterns of precipitation and temperature, at a 2.5 arcminute resolution (Bio 4 - Temperature Seasonality, 10 - Mean Temperature of Warmest Quarter, 11 - Mean Temperature of Coldest Quarter, 12 - Annual Precipitation, 14 - Precipitation of Driest Month, 15 - Precipitation Seasonality, 16 - Precipitation of Wettest Quarter) (Hijmans et al., 2005). The resulting output was then set to 10 percentile training presence threshold, for a binary presence or absence prediction map.

Considering this dataset combines categoric, discrete and continuous variables in our multivariate analyses, we mainly used distribution-free statistical methods to analyze morphological data, following Barbo et al. (2022). To further ensure our data adhered to model assumptions, we identified outliers, collinearity, zero-variance in characters, and possible problems in our dataset through the inspection of Quantile Quantile plots, correlograms, histograms, and box plot graphs. We used a Principal Component Analysis (PCA), as a dimensionality reduction technique in order to find the underlying structure of a dataset by identifying patterns in the data and expressing the data in a new, lower-dimensional space, as an exploratory approach to visually test for morphological differences among groups. However, due to a small sample constraint,

the PCA should be seen as a strictly preliminary step, constituting an approach to dimensionality reduction of variables, in order to provide a visualization of spatial congruence among the characters from our putative operational taxonomic units. We evaluated intraspecific presence of sexual dimorphism with a Mann Whitney *U* test (*U*) for meristic (discrete) characters, and a Student *t*-test (*t*) for morphometric (continuous) characters, both with *P*-value 0.05, after evaluating the assumptions of univariate normality by using a Shapiro Wilk test, and homoscedasticity through Levene s test (Zar, 1999). Furthermore, pairwise correlation between sexually dimorphic characters was verified with a Pearson correlation test (Zar, 1999). For species that could not be distinguished with the multivariate analyses, interspecific comparisons were made with an analysis of variance (ANOVA), over characters that fulfilled assumptions of non-collinearity (0.1), univariate normality, and homoscedasticity, which was pairwise applied to OTU series of the same sex, with *P*-value 0.05. Sample sizes of each group in statistical tests are indicated as subscripts, and ranges are reported followed in parentheses by mean 1 standard deviation and sample size. All analyses were conducted in the R (v. 4.1.0) environment (R Core Team, 2013). Custom scripts used for statistical analyses are available at https://github.com/omarentiauspe/Liotyphlops.

Information of the cranial morphology of *L. beui* is based on high-resolution micro-CT scans of the paratypes (BMNH 1946.1.11.12, MCZ-R 17842, 16702). The CT scan of BMNH 1946.1.11.12 was performed with a Nikon Metrology XTH 225 ST (Nikon Metrology, Leuven, Belgium) at the Imaging Analysis Centre of the Natural History Museum (NHMUK; London, United Kingdom), with the following settings: an X-ray tube fitted with a tungsten transmission target and set to a voltage of 83 kV and a current of 41 A; no filter; 2238 projections of 500 ms exposure time each with a frame averaging of 1 recorded over a 360 continuous rotation. The magnification, resulting from the source object distance of 26.11 mm and the object-detector distance of 907.32 mm, generated data with an isotropic voxel size of 8.39 m. A filtered back projection algorithm was used for the tomographic reconstruction, using the CT-agent software (Nikon Metrology GmbH, Alzenau, Germany), producing a 16-bit uncompressed raw volume. The scan data of the specimens MCZ-R 17842 and MCZ-R 16702 were downloaded from the MorphoSource platform (https://www.morphosource.org/media/000104543; https://www.morphosource.org/media/000104546). These scans were performed with a Zeiss Xradia microXCT-400 at the University of Texas High-Resolution X-ray Computed Tomography Facility (UTCT, United States), at a voltage of 80 kV and a current of 10 W, with 1081 projections, resulting in an isotropic voxel size of 4.02 m. Finally, the datasets were rendered in three dimensions with Amira software (Thermo Fisher Scientific, Hillsboro, USA). Amira was also used for segmentation to separate and colorize the bones of one specimen (MCZ-R 17842) for better visualization.

Detailed descriptions and illustrations on the skull morphology of the congener *L. albirostris* are already available (Dunn & Tihen, 1944; Haas, 1964; List, 1966; Rieppel et al., 2009; Linares-Vargas et al., 2021). Therefore, we will not give a complete skull description of *L. beui* in the results section, but will concentrate on showing the differences or deviations from the skull of *L. albirostris*. The skull of the paratype (BMNH 1946.1.11.12) of *L. beui* shows some damage, especially in the snout region, where several bones are missing (premaxilla, right maxilla, right septomaxilla, both vomers, both palatines, both dentals, right splenial), broken (nasal, right pterygoid, left ectopterygoid, left septomaxilla, right coronoid, left splenial, anterior part of right compound bone) or dislocated. Therefore, for this specimen, only the undamaged parts of the skull were used in the comparisons to the skull of *L. sousai* (UFRGS 6274) presented in Santos & Reis (2018). Osteological terminology follows Cundall & Irish (2008) and Linares-Vargas et al. (2021).

2.3. Taxon sampling, DNA sequencing, and phylogenetic analysis

We generated DNA sequences for two specimens: 1) UFRGS 6274, holotype of *L. sousai*, from Passos Maia, Santa Catarina, southern Brazil; 2) UFRGS 6494, specimen of *L. beui*, from Erechim, Rio Grande do Sul, southern Brazil. We extracted total genomic deoxyribonucleic acid from liver or muscle tissues using a modified CTAB (hexadecyltrimethylammonium bromide) protocol (Doyle & Doyle, 1987). We used primers for the small subunit ribosomal rRNA (16S rRNA) gene described by Palumbi (1996) [5 -GCCTGTTTATCAA AAACAT-3 (16Sar) and 5 -CCGGTCTGAACTCAGAT- CACGT-3 (16Sbr)] to obtain partial mitochondrial DNA (mtDNA) sequences. Amplifications were performed in a solution with a total volume of 20 L with 13.5 L of ultra-pure water, 2.0 L of 10x PCR buffer, 1.0 L of MgCl2, 0.2 L each 10 mM primer, 0.2 L of Invitrogen Platinum Taq DNA Polymerase, and 1.0 L of DNA template (10 50 ng). The PCR reactions to 16S was performed with an initial denaturation at 95 C for 5 min, followed by 35 cycles (95 C for 30 s, 48 C for 30 s, 72 C for 60 s) and a final elongation at 72 C for 8 min. We employed the software Geneious (v.6.1; Kearse et al., 2012) to visualize and align sequences as well as a platform to export different formats. We also sampled the Scolecophidia infraorder by including other 22 additional GenBank sequences for the diversity of blindsnakes, including a topotype of *L. beui* (KR815891) from Instituto Butantan, Sao Paulo, Brazil, and two additional Caenophidia outgroups. We also included *L. beui* (SRA:SRS7160001) and *L. ternetzii* (SRA: SRS7160004) model organism samples used for target capture of UCE, AHE, and other genes from genomic DNA, for which we mapped a 16S rRNA fragment sequence (KR815891) as reference genome, and aligned sequencing reads of raw FASTQ files to the 16S rRNA reference sequence, with Geneious (v.6.1; Kearse et al., 2012) and BowTie (v.2; Langmead & Salzberg, 2012). The sequences were aligned with the MUSCLE (MUltiple Sequence Comparison by Log-Expectation) plug-in on Geneious (v.1.3; Edgar, 2004), with standard definitions and anchor optimization (maximum iterations 8, gap opening 1, anchor spacing 32). Final alignment is available at Supplementary File 1. GenBank accession codes will be provided upon acceptance of the manuscript.

The phylogenetic inferences were conducted in a maximum likelihood and bayesian inference frameworks. We estimated the best substitution model and partition schemes based on the Bayesian Information Criterion (BIC, for bayesian inference) and Akaike information criterion with correction (AICc, for the maximum likelihood inference) using PartitionFinder1.1.1 (Lanfear et al., 2012), with a linked branch lengths model and a 'greedy search, which recovered the Tamura-Nei (TrN, variable base frequencies, equal transversion rates, variable transition rates) for the BIC and the general time reversible (GTR, variable base frequencies, symmetrical substitution matrix) for the AICc scores, both with a single-codon partition (DNA, p1 1 546\3, 2 546\3, 3 546\3). The maximum likelihood framework employs the Randomized Axelerated Maximum Likelihood (RAxML v.8; Stamatakis, 2014) software, searching the best-scoring tree 100 times and conducting 1000 nonparametric bootstrap replicates (algorithm "- *f a*"). The run was performed with a General Time-Reversible model (Tavare, 1986), which accounts for six substitution rate parameters and four equilibrium, and a GAMMA (GTR) distribution for incorporating rate variation across sites. The bayesian framework employs the Bayesian Evolutionary Analysis by Sampling Trees (BEAST v. 10.1.4; Bouckaert et al., 2014) software, estimating a calibrated, partitioned gene tree using the best substitution models, a birth-death tree model, and a lognormal uncorrelated relaxed clock as priors. We calibrated our tree with a monophyletic outgroup constraint for Elapidae, and a fixed mean substitution rate of 1.07 10^{-2} 0.002 sites per million years for the 16S rRNA, as estimated for synonymous substitution rates within the mitochondrial loci, and accounting for the evolutionary rates variation among rRNA structural elements (Mindell and Honeycutt, 1990; Ochman et al., 1999; Smit et al., 2007; Eo and Woody, 2010; Hassler et al., 2022), with a normal prior distribution of divergence times. We implemented three independent runs, with 10,000,000 Markov chain Monte Carlo (MCMC) lengths. We discarded each run s initial 10%

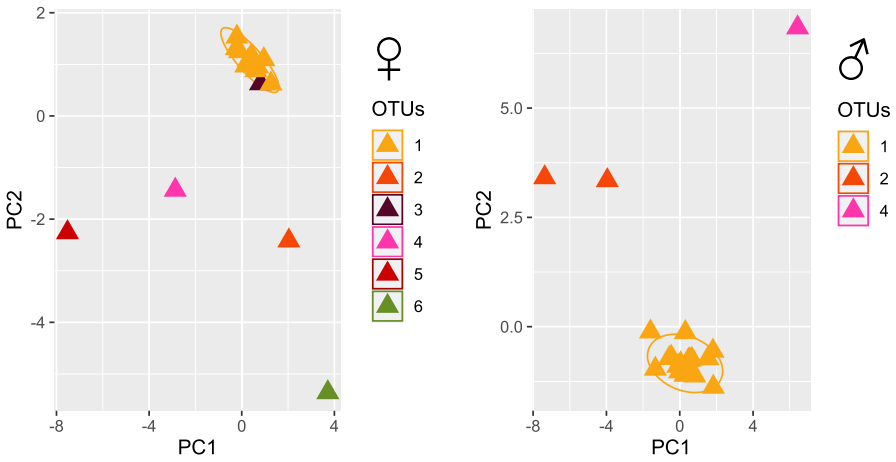

Fig. 2. Principal Component Analysis (PCA) for females (left) and males (right) of the six OTUs analyzed in this work.

generations as burn-in and combined the results with a maximum clade credibility tree in TreeAnnotator (v. 2; Bouckaert et al., 2014), and assessed for convergence of runs using on effective sample sizes (ESS 200) in Tracer (v. 2; Bouckaert et al., 2014).

Uncorrected genetic distances (*p*-distances) were calculated using MEGA 11 (Tamura et al., 2013), using a d" parameter (Transitions Transversions), while assuming uniform rates among sites and homogeneous pattern among lineages. The *p*-distance calculation was made based on the proportional (*p*) differences among nucleotide sites in which two compared sequences differ, as inferred through the division of nucleotide differences by the total number of nucleotides (Tamura et al., 2013). Analyses of species delimitation was performed on the alignment using Bayesian Poisson Tree Process (bPTP) and Poisson Tree Processes (PTP) models (Zhang et al., 2013), which models speciations or branching events as analogous to number of mutations, Assemble Species by Automatic Partitioning (ASAP) model (Puillandre et al., 2021), which is an implementation of a hierarchical clustering algorithm that only uses pairwise genetic distances, avoiding the computational burden of phylogenetic reconstruction, and the Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa and Barraclough, 2013), which is a likelihood method for delimiting species by fitting within and between species branching models to reconstructed gene trees.

3. Results

3.1. Statistical analyses

Our exploratory multivariate analysis (PCA) recovered females of the OTUs SP02 (Orange, *L. caissara*), SP04 (Pink, *L. ternetzii*), SP05 (Red, *L. trefauti*), and SP06 (Green, *L. wilderi*) as distinct spatial entities, and a total overlap between the cluster of individuals from the OTUs SP01 (Gold, *L. beui*) and SP03 (Dark Purple, *L. sousai*) (Fig. 2). As for males, the OTUs SP01, SP02, and SP04 are recovered as separate entities. The univariate analyses of several variables also corroborate the overlap of both SP01 and SP03 for most of the putatively diagnostic categoric, discrete, and continuous characters, which suggest that these might represent a unique and cohesive evolutionary unit (Supplementary Fig. 1). Considering SP01 and SP03 could not be separated by the multivariate analyses, we conducted analyses of variance (ANOVA) between non-collinear characters of these two groups, which encountered statistical differences between females of OTUs SP01 and SP03 for total length ($F_{1,10}$ 15.049, P 0.05, n 11), but not for tail length ($F_{1,10}$ 0.000139, P 0.9, n 11), mid-ventral scales ($F_{1,10}$ 0.384, P 0.5, n 11), or subcaudal scales ($F_{1,10}$ 0.026, P 0.8, n 11). The differences in SVL can be possibly attributed to our assumption of specimen maturity, which restricted the sample size to adult individuals; it is likely that these differences are attributed to SP03 being a juvenile individual, which is also supported by the foramen between parietal bones, as depicted in Santos & Reis (2018), and to the minimum threshold for mature females individual being 219 mm of snout-vent length, which is higher than the values shown by the specimen of SP03 (Parpinelli & Marques, 2015).

3.2. Identity of Liotyphlops sousai

In the description of *L. sousai*, no information is given regarding the sex of the type specimen (Santos & Reis, 2018:507). We were unable to visually verify the presence of an hemipenis in the holotype of *L. sousai*. Examination of its scale counts (particularly, the number of subcaudals and ventrals to subcaudals ratio) and manual probing also corroborate that the holotype of *L. sousai* is actually a female specimen. According to the diagnosis of Santos & Reis (2018), *L. sousai is distinguished from all other* Liotyphlops, *except Liotyphlops anops*, *Liotyphlops argaleus*, *and L. trefauti*, *by having four scales contacting the posterior edge of the prefrontal (vs. three scales contacting posterior edge of prefrontal)* (Santos & Reis, 2018:507). According to the authors, the contact of four scales to the posterior edge of the prefrontal appears to be the sole diagnostic character of *L. sousai* against *L. beui*. As mentioned before, individual contact states among head scales in *Liotyphlops* are considered as highly variable, and other authors suggest not describing new species using small series due to this (Dixon and Kofron, 1983). The *four scales contacting the posterior edge of prefrontal* are defined by Dixon and Kofron (1983) as being postfrontals, and regarded as variable to their size and number in all species of *Liotyphlops*.

Closer examination of the holotype (UFRGS 6274) reveals that the contact of four postfrontal scales to the posterior edge of prefrontal is actually absent, with a seemingly aberrant ocular scale, that produces a proximal ridge, which was misinterpreted as a projection of a postfrontal scale (Supplementary Fig. 2); the left side of the head on the holotype also has three postfrontal scales contacting the prefrontal. Therefore, the diagnostic of four postfrontal scales in contact with the prefrontal scale is considered herein as unreliable and erroneous.

Of the holotype of *L. sousai*, there are CT scan images of the skull in Santos & Reis (2018). No description or raw data were made available on request to the authors. Therefore, we can only compare the skulls from the three paratypes of *L. beui* with the images from the holotype of *L. sousai* presented in Santos & Reis (2018), drawing limited conclusions regarding similarities and differences. One of the most striking differences in the skull of the holotype of *L. sousai* compared to that of *L. beui,* is the fact that there is still a rather large fontanelle between the parietal bones of *L. sousai* and there are also rather large distances between most

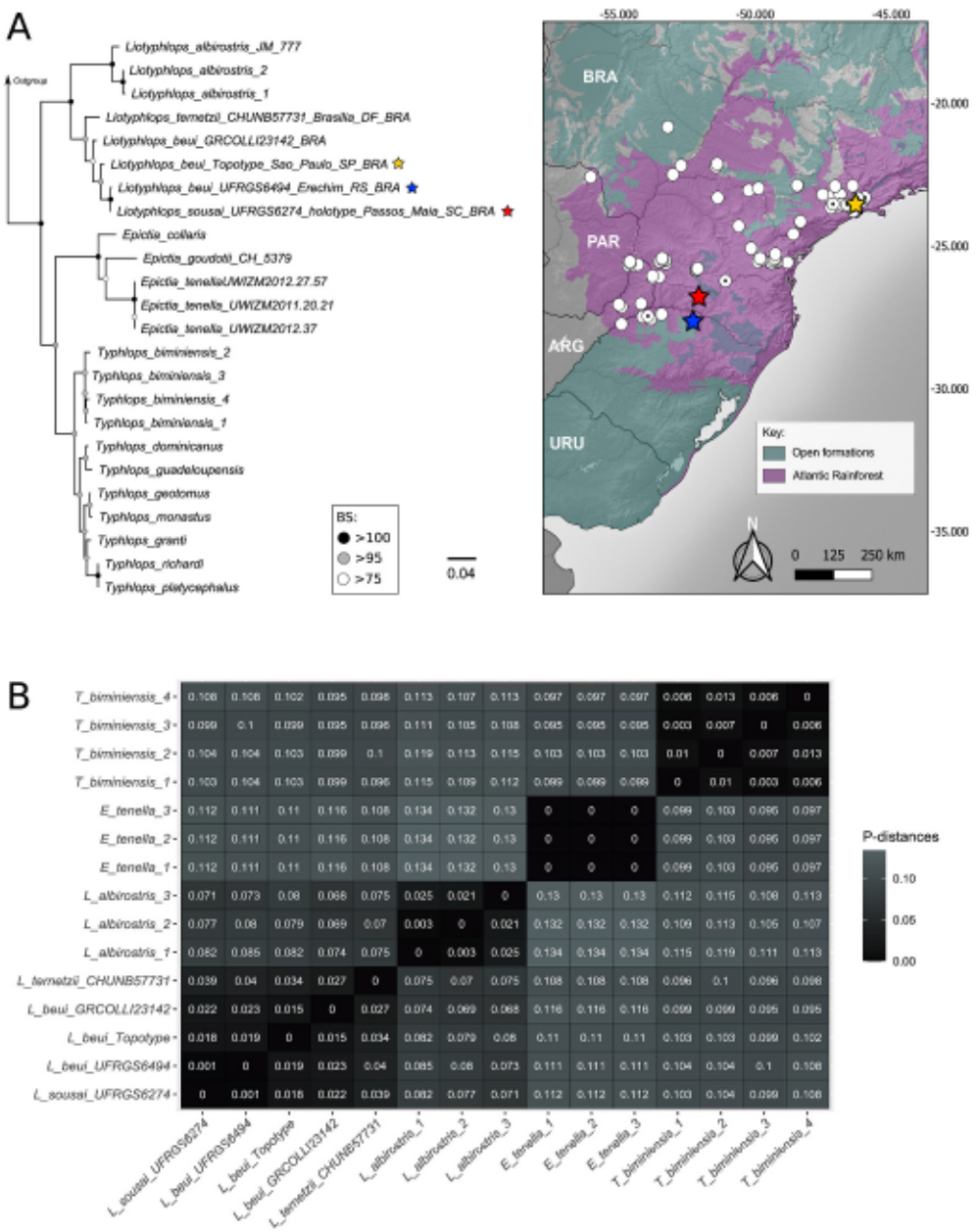

Fig. 3. Phylogenetic inferences and uncorrected *p*-distances for Scolecophidia terminals, based on the small subunit ribosomal RNA (16S rRNA) gene fragment. A) Maximum likelihood (RAxML) phylogenetic tree inference and geographic distribution of evaluated samples (white circles = *L. beui* literature records; black and white circles = *L. beui* examined records); B) Pairwise uncorrected *p*-distances for Scolecophidia terminals.

of the other cranial bones. Although possible to constitute an artifact of the CT scan method, these are typical features for a juvenile blind snake in which the ossification of the skull is not yet completed due to ontogenetic variation of dermal bones (Cundall & Irish, 2008). Since the paratypes of *L. beui* are adult specimens, we cannot compare aspects related to bone distances and sizes of foramina between these specimens and the holotype of *L. sousai*. As a further difference, in *L. sousai* the anterior surface of the nasal is not rugose thickened, as the anterior end of the nasal does not appear pointed in the dorsal view. Instead, it exhibits foramina on both sides of the midline. As similarities of *L. sousai* and *L. beui* and shared differences to *L. albirostris* can be noted the absence of a supraoccipital bone, as well as a more pronounced and longer projection of the lateral flange of the nasal.

The only noteworthy difference in *L. sousai* is the presence of a supratemporal bone, which is absent in the analyzed paratypes of *L. beui* and present in the holotype of *L. ternetzii*. According to Santos (2018), this would represent an intraspecifically variable character, considering that of four specimens of *L. beui* examined by this author (MCP 10853, MCP 16362, MCZ-R 16702, MCZ-R 17842), the two paratypes also lacked the supratemporal bone, whereas the other two specimens had it. Therefore, together with our additionally examined paratype (BMNH 1946.1.11.12), this bone was absent in three specimens and present in two. Rage (1984) describes the supratemporal as a thin and minute, rudimentary bone in Anomalepididae and Leptotyphlopidae, lacking its functional articulation with the skull. In Leptotyphlopidae, Kley (2006) reports a non-ossified ligament that is displaced between the lateral surface of the exoccipital and dorsomedial aspect of the proximal quadrate head, in accordance with the interpretation of Rage (1984), homologous to the supratemporal bone. Kley (2006) raises the possibility of the transformation of dermal bones into ligamentous tissues for the supratemporal, which could be prone to ontogenetic and intraspecific variation, and also would not be visible in the osteological analyses,

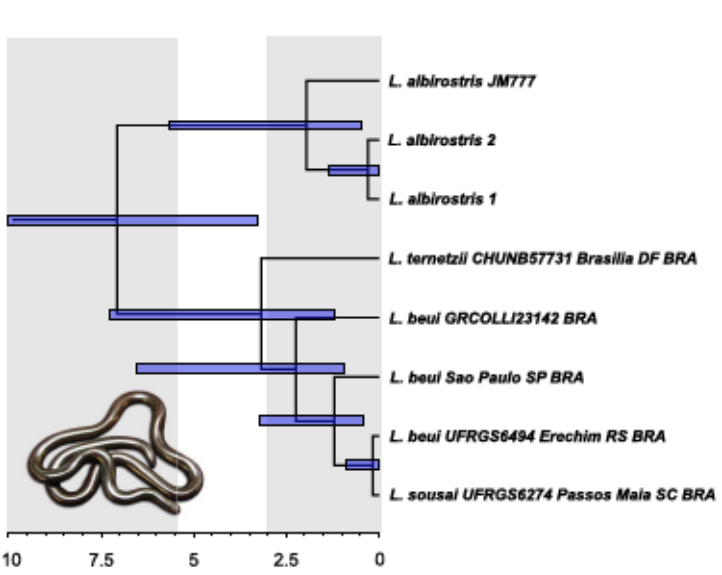

Fig. 4. Maximum clade credibility Bayesian tree, based on the small subunit ribosomal RNA (16S rRNA) gene fragment, highlighting the divergence time of terminals. Bars represent the 95% Highest Posterior Density (HPD) interval for divergence dates. Scale indicates million years ago (Myr). Full topology and posterior probability support of nodes are provided as supplementary material. Analyses of species delimitation are: Bayesian Poisson Tree Process (bPTP); Poisson Tree Process (PTP); Assemble Species by Automatic Partitioning (ASAP); Generalized Mixed Yule Coalescent (GMYC). Inset photograph: *Liotyphlops* aff. *wilderi* from Minas Gerais, Brazil. Photograph credits: Henrique C. Costa.

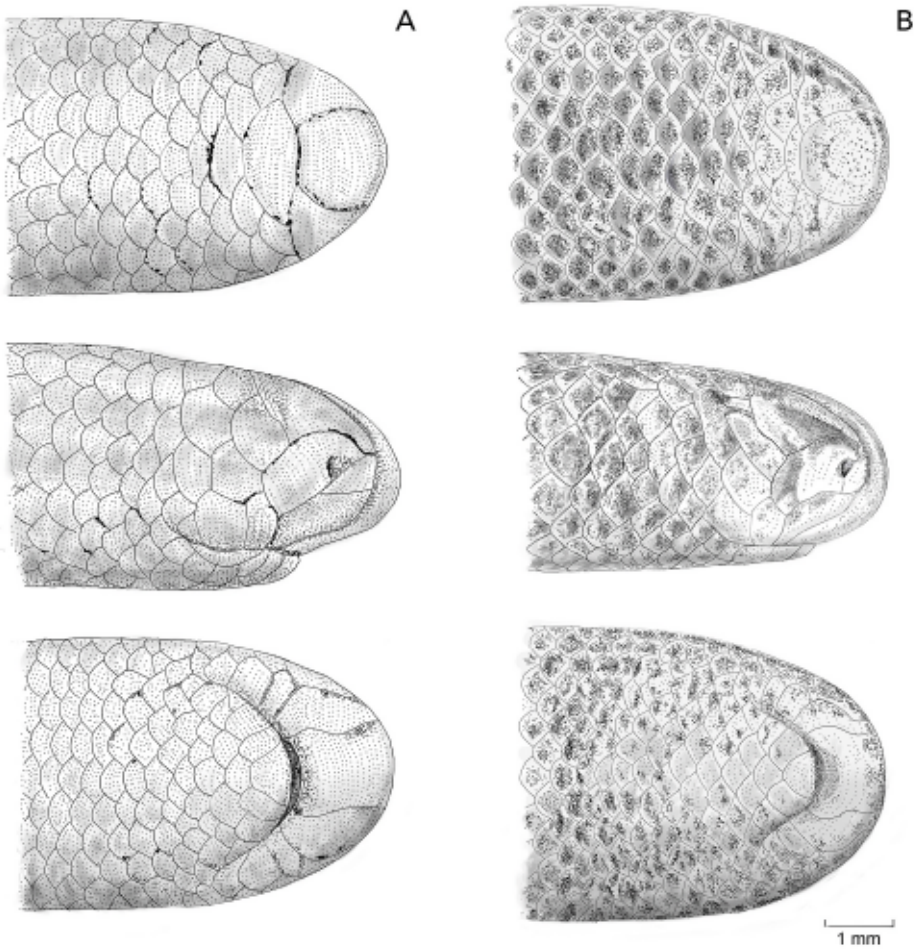

Fig. 5. Head scalation of *Liotyphlops beui* (A, MCZ-R 17842, paratype) from Instituto Butantan, São Paulo, São Paulo state, southeastern Brazil, and *Liotyphlops sousai* (B, UFRGS 6274, holotype), from Passos Maia, Santa Catarina state, southern Brazil.

requiring histological analyses for their visualization. Furthermore, Rieppel et al. (2009) reports different degrees in ossification for the supratemporal in anomalepidids, ranging from the much reduced supratemporal of *Typhlophis squamosus* (Schlegel, 1839) to absent in *Anomalepis aspinosus* Taylor, 1939. Palci (2014) also reports a variable presence of supratemporal bones in the genus *Anomalepis*, that was

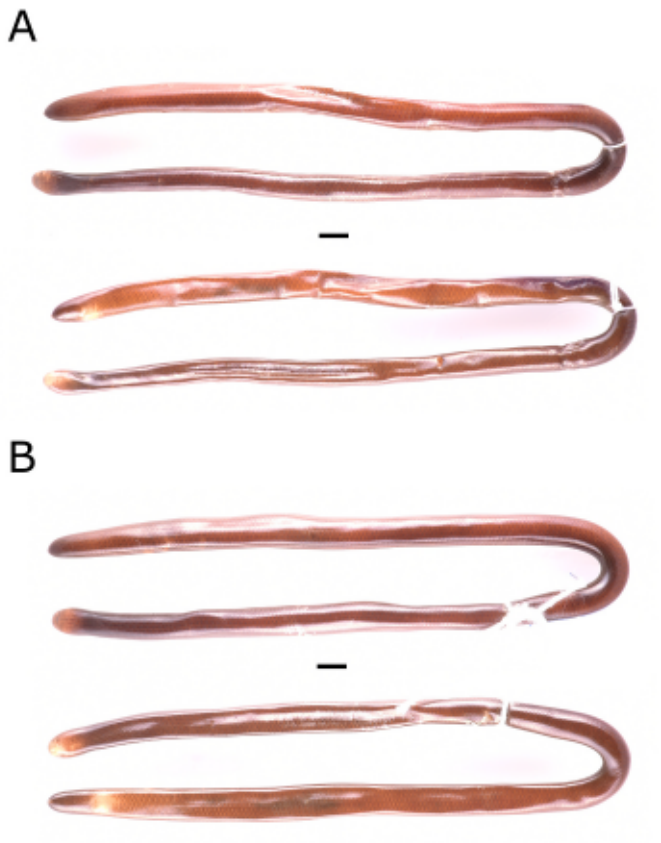

Fig. 6. Specimens of *Liotyphlops* from Passos Maia, Santa Catarina state, southern Brazil. A) Dorsal and ventral views of UFRGS 6274, juvenile female holotype of *Liotyphlops sousai*; B) Dorsal and ventral views of UFRGS 6275, juvenile male identified as *Liotyphlops beui* by Santos and Reis (2018). Scale bar = 5 mm.

previously reported as lacking a supratemporal bone (McDowell and Bogert, 1954; Haas, 1968; Rieppel et al., 2009). Rieppel (1979) suggests that neoteny, paedomorphosis in ontogenetic ossification, can be attributable for the disappearance of the lateral wing of the basisphenoid in Scolecophidia. Therefore, we find it likely that the visualization of the supratemporal bone in scolecophids, possibly related to different degrees in ossification, demineralization due to exposure to formalin, or even visualization technique artifacts, is prone to intraspecific variation.

Our final alignment totalized 490 base pairs of the 16S rRNA partial gene fragment, for 24 Scolecophidia terminals and two outgroups (Supplementary File 1). Our maximum likelihood phylogenetic inference recovers a final tree topology with a score lnL = −2336.628103, supporting the monophyly of the genera *Liotyphlops*, *Typhlops*, and *Epictia*, with a strongly supported clade (bootstrap support >90) containing *L. sousai* (UFRGS 6274) from Santa Catarina and L. *beui* (KR815891.1, UFRGS 6494) from São Paulo and Rio Grande do Sul (Fig. 3A). The observed uncorrected *p*-distances also provide no support for genetic divergence among these taxa, ranging from 0 to 1% (Fig. 3B). Similarly, intraspecific genetic variation for *Typhlops biminiensis* (Richmond, 1955) are also recovered as ranging from 0 to 1%, in *Epictia tenella* Klauber, 1939 as 0%, and in *L. albirostris* (Peters, 1858) as ranging from 0 to 2%. Our ASAP, GMYC, PTP, and bPTP species delimitation analyses also support a single evolutionary entity between *L. sousai* and *L. beui*. Our Bayesian inference also recovers a single clade with *L. sousai* and *L. beui*, with terminals likely diverging during the early Pleistocene, an interspecific divergence similar to what is observed in *L. albirostris* (Fig. 4). These results consistently support a conspecific relationship between *L. sousai* and *L. beui*.

Another issue is raised by the sympatry of *L. sousai* and *L. beui*. Although there is no explicit mention to this in the work of Santos & Reis (2018), another specimen of *Liotyphlops* was also found in the same locality as the type of *L. sousai*, the Usina Hidrelétrica Passos Maia, at Passos Maia municipality, Santa Catarina, Brazil, as indicated by the appendix of examined material. The single mentioned specimen (UFRGS 6275), also from Usina Hidrelétrica Passos Maia, was identified by Santos & Reis (2018) as *L. beui*. Further examination in the same collection revealed additional specimens from the same locality, and that these would represent a series (UFRGS 6272, 6273, 6274 [holotype of *L. sousai*], 6275) collected on the same day.

Direct examination of the series of *Liotyphlops* specimens from Usina Hidrelétrica Passos Maia (UFRGS 6272, 6273, 6274 [holotype of *L. sousai*], 6275) reveals no consistent character for its interspecific diagnosis. A comparison of size and coloration between the holotype of *L. sousai* (UFRGS 6274) and a sympatric *L. beui* (UFRGS 6275) (Figs. 5–6) reveals identical phenotypes. Furthermore, a comparison between morphological diagnostic characters reveals a complete overlap in pholidosis between *L. sousai* and *L. beui* (Table 1). Therefore, we propose to allocate *L. sousai* as a junior synonym of *L. beui*. We also provide a redescription for a topotypical specimen of *L. beui* below.

3.3. Redescription of L. beui

L. beui (Amaral, 1924)
(Figs. 1, 4–6, 9–12)

3.3.1. Synonymy

Helmintophis beui Amaral, 1924:25. Holotype: IBSP 1806 (lost), from Instituto Butantan (761 m above sea level), São Paulo municipality, São Paulo state, Brazil. Paratypes: IBSP 281 (lost), 282 (Lost), 652 (lost), 1041 (lost), MCZ 16702, 17842, BMNH 1946.1.11.12, also from Instituto Butantan, São Paulo municipality, São Paulo state, Brazil.

L. sousai Santos & Reis, 2018:507. Holotype: UFRGS 6274 (juvenile female), from Usina Hidrelétrica Passos Maia (800 m above sea level), Passos Maia municipality, Santa Catarina state, Brazil. [New synonymy].

3.3.2. Heterochresonymy

L. ternetzii [non *Helmintophis ternetzii* Boulenger, 1896:584]: Amaral,

Table 1
Selected diagnostic characters for *Liotyphlops* species from the Atlantic Rainforest, sampled in this study (*n* = number of specimens with scale counts, * = junior synonym of *Liotyphlops beui*, ** = species with marginal records in Atlantic Rainforest). For raw values, refer to Supplementary Table 1.

Species	Postfrontals contacting prefrontal	Supralabials	Infralabials	Anterior dorsal rows	Posterior dorsal rows	Dorsalson vertebral row	Mid-ventral rows	Subcaudal
L. beui (*n* = 30)	3	4	3	22–24	20	395–446	375–434	10–20
L. caissara (*n* = 2)	3	4	3	22–23	20	463–510	274–308	10–14
L. sousai * (*n* = 1)	3	4	3	24	20	439	427	13
L. ternetzii ** (*n* = 6)	3	4	3	22–26	22	438–473	455–485	10–15
L. trefauti (*n* = 2)	4	3–4	3–4	22	22	520–548	499–532	8–9
L. wilderi (*n* = 3)	3	3–4	4	22–24	22	304–358	284–383	12–19

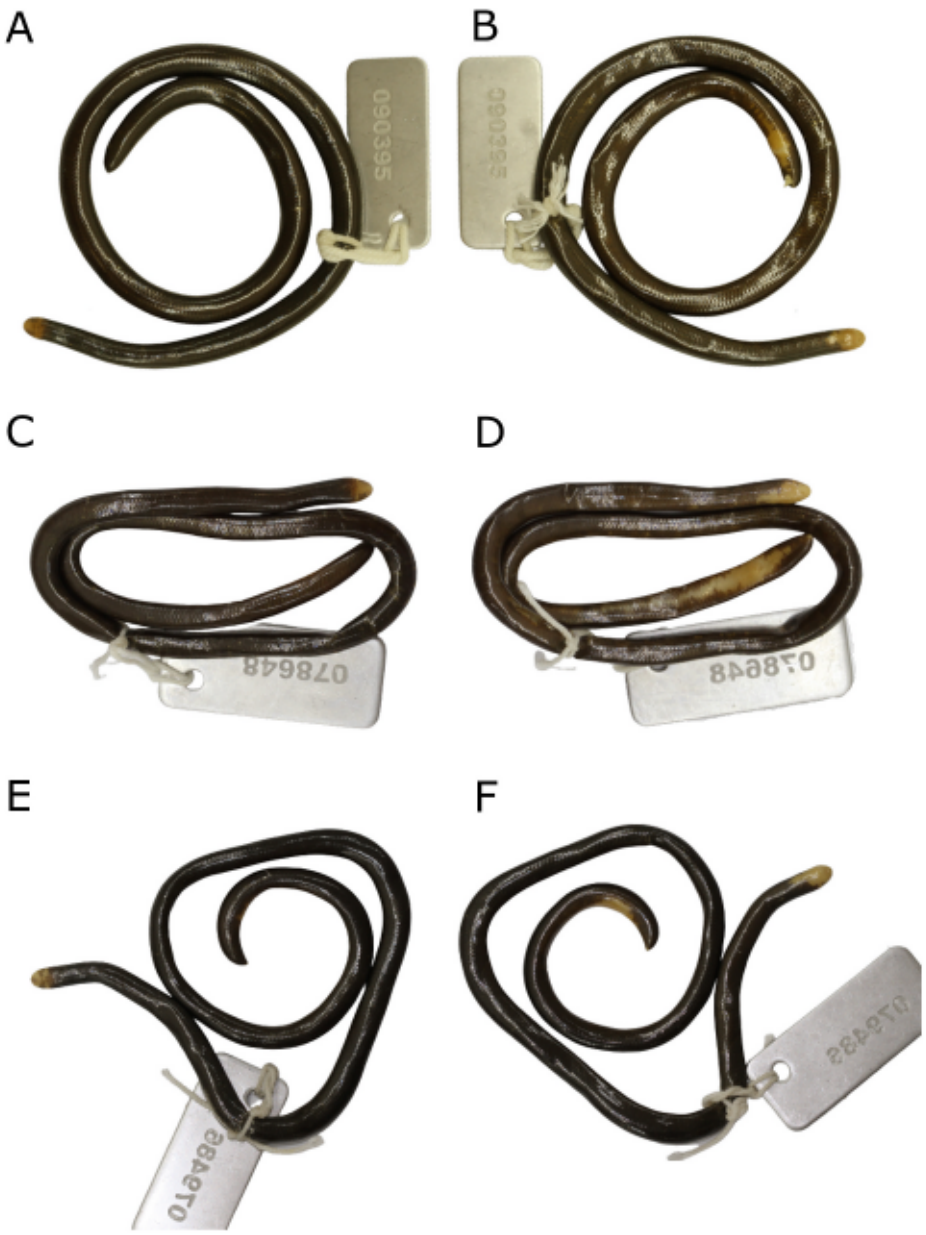

Fig. 7. Morphological variation of *Liotyphlops beui*. A-B) Adult female topotype (IBSP 90395) from Instituto Butantan, São Paulo, São Paulo state, Brazil; C-D) Adult female (IBSP 78648) from São Paulo, São Paulo state, Brazil; E-F) Adult male (IBSP 79486) from Carapicuíba, São Paulo state, Brazil. Collection tag = 50 mm. Photograph credits: Bruno Rocha.

1924; Santos & Reis, 2018 (In error).

3.3.3. Diagnosis

This species can be diagnosed based on the following combination of characters: (1) anterior dorsal scale rows 22–24, usually 22; (2) midbody dorsal scale rows 20–22, usually 20; (3) posterior dorsal scale rows 20; (4) dorsal scales in vertebral row 395–446 (in males 395–420, in females 410–446); (5) mid-ventral scales 375–434 (in males 375–402, in females 395–434); (6) subcaudal scale rows 10–20 (in males 16–20, in females 10–15); (7) supralabials four; (8) infralabials three; (9) postfrontal scales contacting prefrontal three; (10) two scales in contact with posterior edge of nasal scale; (11) dorsal coloration uniformly dark brown or black, head and up to three first dorsal scale rows pinkish; (12) ventral coloration uniformly dark brown or black, head, gular region, and infralabials white or pink, cloacal region and ventral surface of tail white; (13) SVL 173–294 mm (in males 173–275 mm, in females 191–294 mm); (14) Tail length 4.2–12.5 mm (in males 6.7–12.5 mm, in females 4.2–6.6 mm).

3.3.4. Comparisons

Characters for other species are contained within parentheses, and our data has been supplemented with those provided by Dixon and Kofron (1983). *L. beui* is likely to be confused with another similar congener, *L. ternetzii*, that also has a black dorsal coloration and is widely distributed from Uruguay and Argentina to northern Brazil. There appears to be no difference among these species in skull morphology (O.M. Entiauspe-Neto, pers. obs.). While it has been suggested that both species could be distinguished based on head coloration, which should be pink or white in *L. beui* and black in *L. ternetzii*, an analysis of both types (BMNH 1946.1.11.77 - holotype of *Helminthophis ternetzii*; BMNH 1946.1.11.12 - paratype of *H. beui*) reveals that both specimens present a similar, white head coloration in preservative, with a distinctly colored light brown body. Until an integrative revision incorporating molecular data is made, the taxonomic status of these two

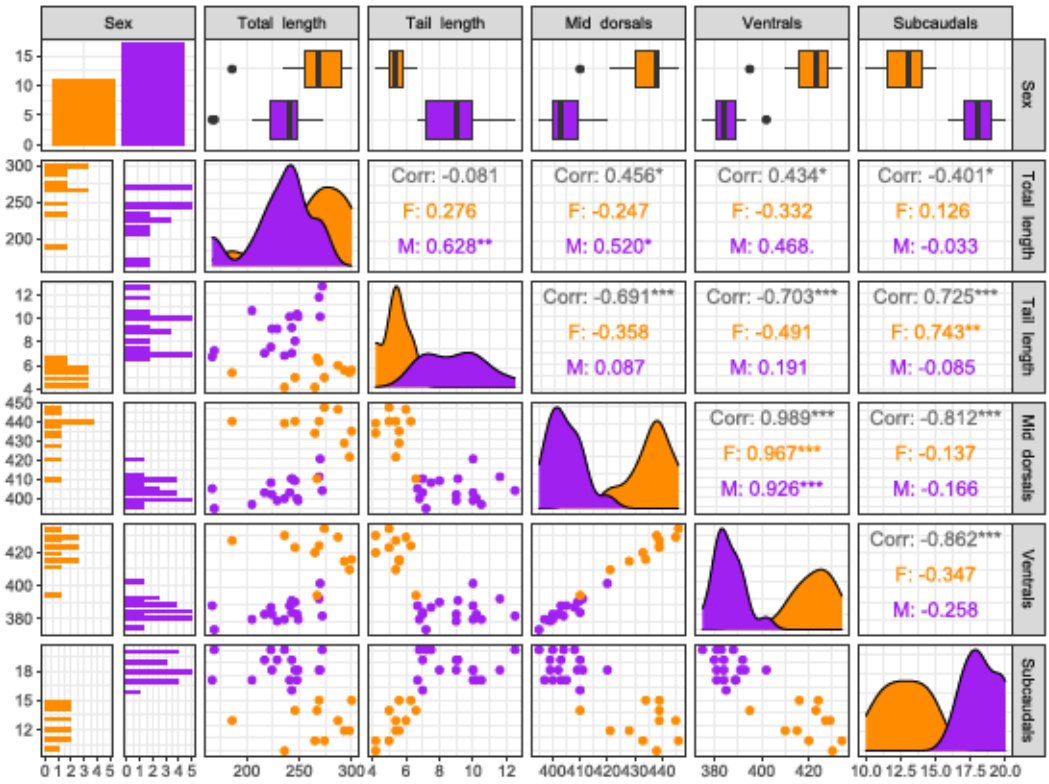

Fig. 8. Boxplots, scatterplots, histograms, density plots, and pairwise comparison of sexually dimorphic characters of external morphology in males (M, purple) and females (F, orange) of *Liotyphlops beui*. Outliers are indicated as black circles. Pearson correlation (Corr) values: * = 0.05; ** = 0.01; *** = 0.001.

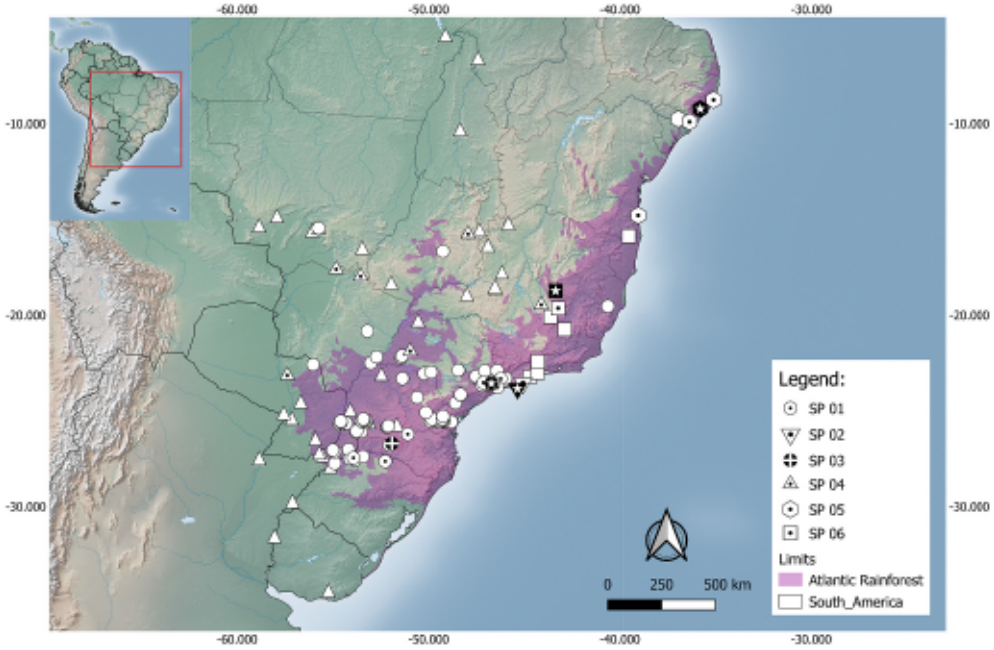

Fig. 9. Geographic distribution of Operational Taxonomic Units (OTUs) delimited based on *Liotyphlops* from the Atlantic Rainforest biome (purple), in South America. SP01: *Liotyphlops beui* from the Atlantic Forest of southeastern Brazil; SP02: *Liotyphlops caissara* from lowland Atlantic Rainforest of coastal southeastern Brazil; SP03: *Liotyphlops sousai* from the Atlantic Forest of southern Brazil, considered herein a synonym of *L. beui*; SP04: *Liotyphlops ternetzii* from the Cerrado of Brazil and Paraguay; SP05: *Liotyphlops trefauti* from the Atlantic Rainforest of northeastern Brazil; SP06: *Liotyphlops wilderi* from montane Atlantic Rainforest of southeastern Brazil. Filled symbols represent type localities and dots represent examined specimens. Literature records are indicated with hollow symbols and follow Dixon and Kofron (1983), Nogueira et al. (2019).

species is only weakly supported through external morphology. In light of this, *L. beui* can be distinguished from *L. ternetzii* by having posterior dorsal rows 20 (22 in *L. ternetzii*) and dorsal scales in vertebral row 395–446 (463–510 in *L. ternetzii*) (Dixon and Kofron, 1983; Centeno et al., 2010). However, it should also be noted that different values for these counts in literature can be attributed to possible misidentifications (e.g. reports of posterior dorsal rows 22 in *L. beui* by Santos & Reis, 2018).

Furthermore, *L. beui* can be distinguished from *L. schubarti* Vanzolini, 1948 by having the nasal scale in contact with the second supralabial (nasal separated from second supralabial by an accessory scale in *L. schubarti*). It also differs from *L. albirostris* (Peters, 1858) and *L. wilderi* (Garman, 1883) by having a single scale contacting the posterior edge of nasal, between second supralabial and prefrontal (two scales contacting

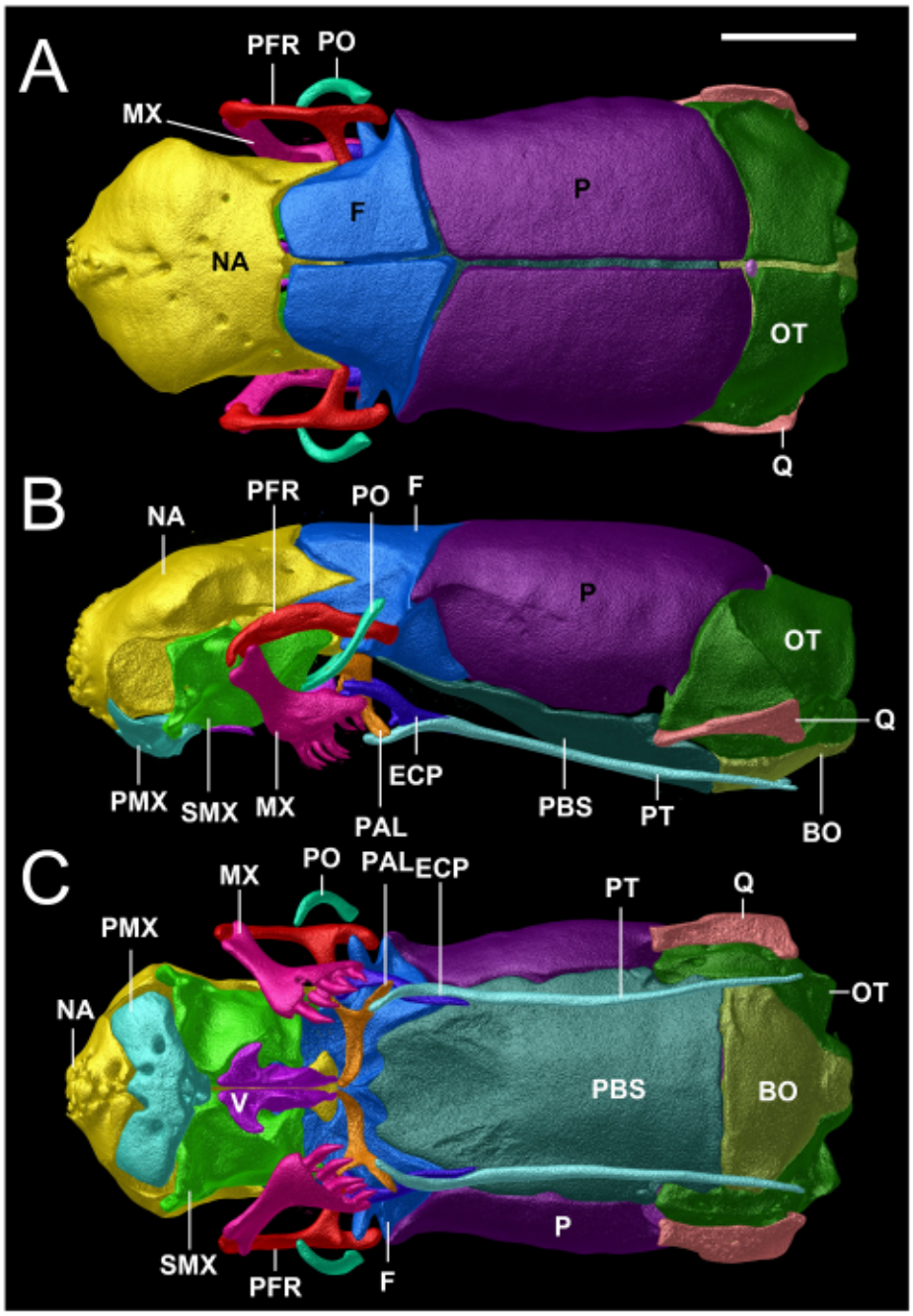

Fig. 10. Dorsal (A), lateral (B), and ventral (C) views of the skull of *Liotyphlops beui* (MCZ 17842, paratype) based on µCT imagery. Different skull elements are digitally colored and the mandible is removed for better visualization. Abbreviations: BO = basioccipital; ECP = ectopterygoid; F = frontal; MX = maxilla; NA = nasal; OT = otico–occipital complex; P = parietal; PAL = palatine; PBS = parabasisphenoid; PFR = prefrontal; PMX = premaxilla; PO = postorbital; PT = pterygoid; Q = quadrate; SMX = septomaxilla; V = vomer. Scale bar = 1 mm.

posterior edge of nasal, between second supralabial and prefrontal in *L. albirostris* and *L. wilderi*). It furthermore differs from *L. wilderi* by having dorsal scales in vertebral row 395–446 (dorsal scales in vertebral row 304–358 in *L. wilderi*). From other two species, *L. anops* (Cope, 1864) and *L. argaleus* Dixon and Kofron, 1983, *L. beui* can be distinguished by having dorsal scales in vertebral row 395–446 and usually three postfrontal scales contacting prefrontal (four postfrontal scales contacting prefrontal, posterior dorsal scale rows 22–24 in *L. anops* and *L. argaleus*). Other congener which bears great resemblance to *L. beui* is *L. caissara* Centeno, Sawaya, & Germano, 2010, from which it can be separated by having a black venter and two scales in contact with the posterior edge of nasal between second supralabial and prefrontal (white venter, one scale in contact with the posterior edge of nasal between second supralabial and prefrontal in *L. caissara*). It can be distinguished from another congener which occurs in the northernmost range of the Atlantic Forest, *L. trefauti* Freire, Caramaschi, & Argôlo, 2007, by having dorsal scales in vertebral row 395–446, a uniformly black dorsal and ventral coloration, and usually three postfrontal scales contacting prefrontal (four postfrontal scales contacting prefrontal, dorsal scales in vertebral row 520–543, uniformly brown dorsal and ventral coloration, four postfrontal scales contacting prefrontal in *L. trefauti*). Lastly, it differs from *L. taylori* Santos & Reis, 2018, by having three infralabials (two infralabials in *L. taylori*).

3.3.5. Redescription

Redescription is based on a topotypical specimen (IBSP 90395, Fig. 7A–B), an adult female, from Instituto Butantan, São Paulo municipality, São Paulo state, Brazil. Snout-vent length 258 mm, head length 5.5 mm (2.13% of SVL), tail length 8 mm (3.10% of SVL). Rostral large, longer than wider, contacting nasals anterolaterally, prefrontals laterally, and frontal posteriorly; prefrontals paired, triangular, contacting rostral anterolaterally, nasal ventrally, and frontal

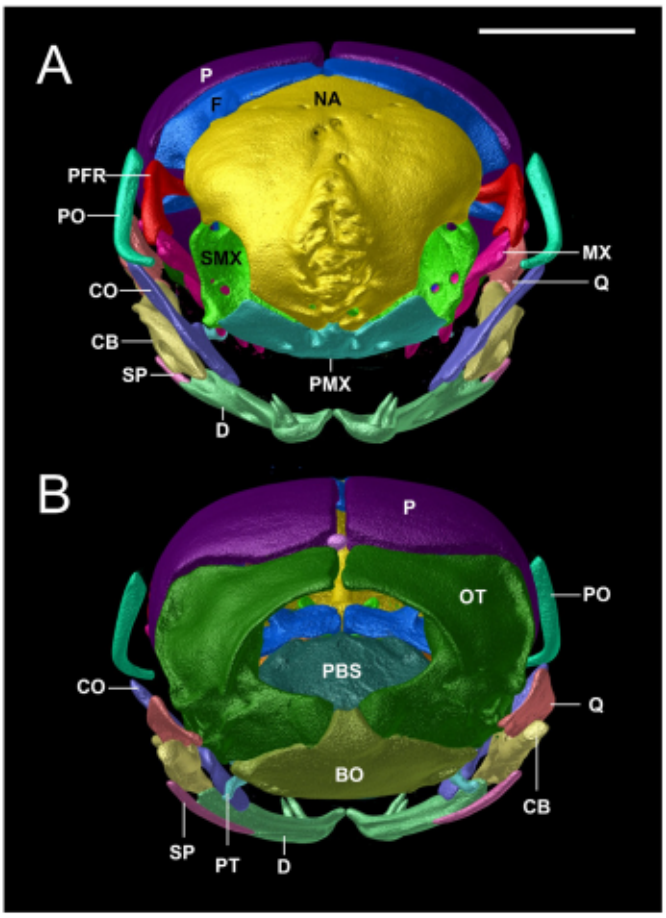

Fig. 11. Anterior (A) and posterior (B) views of the skull and lower jaw of *Liotyphlops beui* (MCZ 17842, paratype) based on μCT imagery. Different skull elements are digitally colored to improve visualization. Abbreviations: BO = basioccipital; CB = compound bone; CO = coronoid; D = dentary; F = frontal; MX = maxilla; NA = nasal; OT = otico-occipital complex; P = parietal; PBS = parabasisphenoid; PFR = prefrontal; PMX = premaxilla; PO = postorbital; PT = pterygoid; Q = quadrate; SMX = septomaxilla; SP = splenial. Scale bar = 1 mm.

dorsoposteriorly; posterior edge of both prefrontals not contacting posterior edge of rostral; nasal plate divided, contacting rostral anteriorly, prefrontal dorsally, and supralabials 1–2 ventrally; eyespot inconspicuous, not clearly visible; supralabials 3/3; postfrontals five, three contacting prefrontal; infralabials 4/4; dorsal scale rows 24/20/20, smooth, cycloid; vertebral scales 394; midventral scales 353; subcaudal scales 14.

3.3.6. Coloration

In life, specimens have a uniformly dark brown or black dorsal coloration, with a pinkish or beige head coloration, that extends up to the first five dorsal scale rows. Ventral coloration uniformly dark brown, except for cloacal region, subcaudals, gular region, and infralabials that are white or pink. Body scales have a light brown outline on bottom and dark brown or black coloration on apex. In preservative, pink coloration becomes white, and black becomes light brown.

3.3.7. External morphology variation

We detected sexual dimorphism, in which females of *L. beui* attain higher total length ($t_{17,11} = 2.7122$, $P < 0.05$, $n = 28$), smaller tail length ($t_{17,11} = 6.2525$, $P < 0.0001$, $n = 28$), lower dorsal scales in vertebral row ($U_{17,11} = 3$, $Z = -4.2$, $P < 0.00001$, $n = 28$), lower mid-ventral scales ($U_{17,11} = 1$, $Z = -4.3$, $P < 0.00001$, $n = 28$), and lower subcaudal scales ($U_{17,11} = 0$, $Z = 4.3$, $P < 0.00001$, $n = 28$). All sexually dimorphic characters were found to be correlated (Fig. 8). Total length ranges from 167 to 300 mm (245 ± 35, $n = 28$), in males ranges from 167 to 272 mm (232 ± 30, $n = 17$), in females ranges from 186 to 300 mm (265 ± 33, $n = 11$). Snout-vent length ranges from 173 to 294 mm (249 ± 32, $n = 28$), in males ranges from 173 to 275 mm (241 ± 31, $n = 17$), in females ranges from 191 to 294 mm (260 ± 30, $n = 11$). Tail length ranges from 4.2 to 12.5 mm (7.5 ± 2.2, $n = 28$), in males ranges from 6.7 to 12.5 mm (8.9 ± 1.7, $n = 17$), in females ranges from 4.2 to 6.6 mm (5.3 ± 0.7, $n = 11$). Head length ranges from 3.4 to 5.6 mm (4.7 ± 0.6, $n = 28$), in males ranges from 3.5 to 5.6 mm (4.8 ± 0.6, $n = 17$), in females ranges from 3.4 to 5.5 mm (4.5 ± 0.6, $n = 11$). First row of dorsal scales range from 22 to 24 (22 ± 0.8, $n = 28$), and the last row of dorsal scales are 20 ($n = 28$). Postfrontal scales in contact with the frontal scale are three ($n = 28$). Scales in contact with the nasal are two ($n = 28$). Supralabial scales are four ($n = 28$), infralabial scales are three ($n = 28$). Dorsal scales in vertebral row range from 395 to 446 (415 ± 16, $n = 28$), in males range from 395 to 420 (404 ± 6.2, $n = 17$), in females range from 410 to 446 (433 ± 10, $n = 11$). Mid-ventral scales range from 375 to 434 (399 ± 18, $n = 28$), in males range from 375 to 402 (386 ± 6, $n = 17$), in females range from 395 to 434 (420 ± 11, $n = 11$). Subcaudal scales range from 10 to 20 (16 ± 3, $n = 28$), in males range from 16 to 20 (18 ± 1, $n = 17$), in females range from 10 to 15 (12 ± 1, $n = 11$). Head coloration in all specimens has white pigmentation ($n = 28$), ranging from fully (up to three dorsals) to mostly (up to one dorsal, or not reaching dorsals) white.

3.3.8. Geographic distribution

L. beui has been recorded from Argentina, Brazil, and Paraguay (Fig. 9), mostly from Atlantic Rainforest, although there have been extralimital records from the Cerrado, Pantanal, and Chiquitano Dry Forest, at low to moderate altitudes (up to 1000 m above sea level) (Dixon and Kofron, 1983; Nogueira et al., 2019). Records of L. *beui* are largely concentrated in southeastern and southern Brazil, in the states of São Paulo, Paraná, and Rio Grande do Sul. Isolated records from its northernmost range, in the Cerrado of Mato Grosso and Goiás states in Brazil, should be carefully reviewed regarding their identity and provenance, as these are separated from its core distribution in southeastern Brazil by at least 500 km. This species has an estimated Extent of Occurrence (EOO) of 1,295,329,935 km^2.

In its core geographic distribution, *L. beui* is sympatric with *L. ternetzii* in northwestern Rio Grande do Sul, western Paraná, and western São Paulo. The latter species appears to be largely associated with the Cerrado and Chaco Dry Diagonal open areas, widely distributed from Pará, in northern Brazil, southwards into Uruguay. Other two congeners which *L. beui* might be sympatric with in the Atlantic Rainforest are L. *caissara*, which occurs in the coastal lowlands and marine islands of São Paulo, and *L. wilderi*, which occurs in coastal lowlands and montane forests of Rio de Janeiro and Minas Gerais. Another congener, *L. trefauti*, is recorded from the northernmost portion of the Atlantic Rainforest, in northeastern Brazil, where *L. beui* has not been recorded.

3.3.9. Osteological comparisons

Osteological description is based upon adult paratypes of *L. beui* (BMNH 1946.1.11.12, MCZ-R 17842, 16702, Figs. 10–12). Comparisons are made to the osteological description of *L. albirostris* (Rieppel et al., 2009; characters in parenthesis). In L. *beui*, the lateral flange of the nasal forms a more pronounced and longer projection that overlaps the laterally descending frontal flange (lateral flange of the nasals forms a less pronounced, smaller projection, slightly overlapping the laterally descending frontal flange in *L. albirostris*); the foramina on the external surface of the nasal tend to be concentrated along the midline of the dorsal surface, with its anterior surface of nasal without foramina, rugose thickened (foramina on the external surface of the nasal are concentrated on both sides of the midline on its anterior and dorsal surfaces in *L. albirostris*); the anterior end of nasal is pointed (straight in *L. albirostris*); the anterior end of the vomer remains separate from the

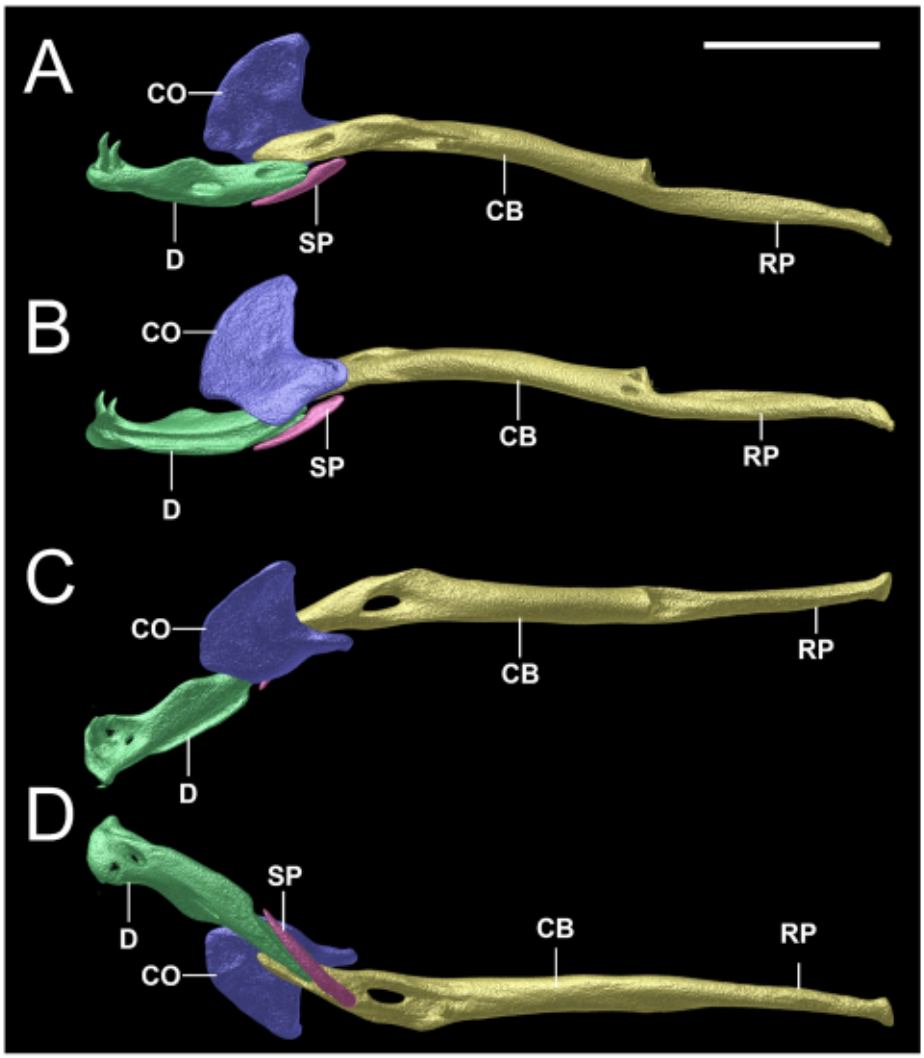

Fig. 12. Lateral (A), medial (B), dorsal (C), and ventral (D) views of the lower jaw of *Liotyphlops beui* (MCZ 17842, paratype) based on μCT imagery. Different skull elements are digitally colored to improve visualization. Abbreviations: CB = compound bone; CO = coronoid; D = dentary; RP = retroarticular process of compound bone; SP = splenial. Scale bar = 1 mm.

premaxilla, with vomers unfused along their entire length (fused on posterior part in *L. albirostris*); the paired frontals meet the paired parietals in a broad V-shaped suture (U-shaped suture in *L. albirostris*); the postorbital element contacts the lateral surface of the prefrontal slightly anterior to the prefrontal-frontal articulation (separated from the prefrontal in *L. albirostris*); no foramina in the dentigerous process of maxilla (foramina present in dentigerous process of maxilla in *L. albirostris*); the posterolateral process of palatine contacts anterior region of pterygoid (separated from pterygoid in *L. albirostris*); an ossified supratemporal and supraoccipital are both absent (present in *L. albirostris*); trigeminal foramen larger, formed by the parabasisphenoid, parietal and prootic (trigeminal foramen smaller in *L. albirostris*, formed by parietal and prootic); ventral surface of basioccipital indented at both sides of the anterolateral region (smooth in *L. albirostris*); ventral margin of coronoid not in contact with splenial (identified as "angular" by Rieppel et al. (2009), contacting splenial in *L. albirostris*); splenial visible in lateral and medial views, posterior surface not obscured in medial view by the compound bone and coronoid (posterior surface obscured by compound bone and coronoid in *L. albirostris*).

4. Discussion

The number of *Liotyphlops* species is reduced here to 11, and the ones recorded to the Atlantic Rainforest to five (Fig. 13). Like L. *sousai*, other congeners were also described based on small type series, such as *L. schubarti* ($n = 1$), *L. trefauti* ($n = 3$), and *L. taylori* ($n = 1$), which are still known from fewer than ten individuals (Vanzolini, 1948; Freire et al., 2007; Santos & Reis, 2018; Nogueira et al., 2019). It should also be noted that these descriptions were based exclusively upon characters of external morphology; although the description of *L. taylori* is followed by high-resolution images of its skull, no comparisons among species or osteological descriptions are provided. As mentioned by Dixon and Kofron (1983), small type series should be avoided for description of new species, as these are particularly prone to deleterious sampling biases, such as an incomplete assortment of interspecific variation or an insufficient diagnostic characterization of the putatively new taxon. Although head scutellation characters have been widely used in the taxonomy of *Liotyphlops*, and are certainly useful for identifications based on external morphology, these should ideally be coupled with other lines of evidence, which are here shown to reduce subjectivity in delimitation of species. We urge authors to refrain from taking taxonomic decisions based on limited samples or without support from several lines of evidence, which should include data from osteology,

Fig. 13. Species of *Liotyphlops* from the Atlantic Rainforest. A) *Liotyphlops beui* from Três Passos, Rio Grande do Sul, Brazil (IBSP 92768); B) *L. beui* from same previous locality (unvouchered); C) *Liotyphlops ternetzii* from Itiquira, Mato Grosso, Brazil (UFRGS 6458); D) *Liotyphlops wilderi* from Itapebi, Bahia, Brazil (MNRJ 15657); E) *Liotyphlops caissara* from Ilha Anchieta, São Paulo, Brazil (IBSP 89927); F) *Liotyphlops trefauti* from Traipu, Alagoas, Brazil (MUFAL 9424). Photograph credits: Arthur Abegg (A, B, E), Márcio Borges-Martins (C), Marco Antonio de Freitas (D), Ubiratan Gonçalves (F).

DNA sequences, and hemipenial morphology.

Several taxonomic groups of Neotropical snakes appear to be impaired by limited sampling or rarity. Similar patterns of rarity and low representation in scientific collections are also shared by species of the genera *Apostolepis* Cope, 1864, *Atractus* Wagler, 1830, *Coronelaps* Hofstadler-Deiques, 2010, *Tantilla* Baird & Girard, 1853, which bear taxa known exclusively upon small series (Ferrarezzi, 1993; Myers, 2003; Entiauspe-Neto et al., 2022). It is also known that, like in *Liotyphlops*, some species of the aforementioned genera share fossorial, cryptozoic habits, and nocturnal or cathemeral activity, which are likely to impair its visualization and collection by humans (Ferrarezzi, 1993; Myers, 2003). Myers (2003) mentions three possible causes for appearance of rarity in Neotropical snakes: (1) lower population density; (2) secretive habits; (3) small geographic ranges or habitat specialization. While there are no published assessments on population density of *Liotyphlops* species, all species appear to have secretive fossorial habits, and at least *L. caissara*, *L. taylori*, *L. trefauti*, *L. schubarti*, and *L. wilderi* are known for small geographic ranges.

A close relationship between *L. beui* and *L. ternetzii* is supported by our molecular analyses, which repeatedly recover these taxa as a sister-group. It is also noteworthy that there are extensive zones in which *L. beui* and *L. ternetzii* are suggested to be sympatric. Considering that these species are only weakly distinguished based on external morphology (posterior dorsal scale rows, dorsal scales in vertebral row), it is possible that these taxa might be synonymous or have a sister–group relationship (Dixon and Kofron, 1983). The diagnosis between *L. beui* and *L. ternetzii* also appears to diverge among authors, as some authors are able to distinguish both species based on scale counts (e.g. Dixon and Kofron, 1983; Giraudo, 1994; Centeno et al., 2010), and others could not (e.g. Santos & Reis, 2018). Some possible explanations, added to the ones aforementioned, also include possible misidentified individuals, or even intergradant specimens, which may occur within the contact zones and have intermediate characteristics. These potential issues should be evaluated with an evaluation of the taxonomic identity of *L. beui* and *L. ternetzii* in their whole range, added to approaches of population genetics, in order to test for hybridization and gene flux among specimens attributed to both taxons.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Omar M. Entiauspe-Neto reports financial support was provided by CAPES.

Data availability

https://github.com/omarentiauspe/Liotyphlops

Acknowledgements

We would like to thank Editor Alexander Kupfer, Angele Martins (MNRJ), Robert C; Jadin (UW-Eau Claire), and one anonymous reviewer for their suggestions and constructive criticism on our manuscript. We would also like to thank curators S. Castroviejo and G. M. F. Pontes (MCP), J. Hanken and J. Martinez (MCZ), V. Germano and F. G. Grazziotin (IBSP), and P. Campbell (BMNH) for kindly allowing access to specimens under their care. We are also deeply indebted to A. Abegg (IBSP), H. C. Costa (UFJF), B. Rocha (IBSP), U. Gonçalves and C. N. S. Palmeira (MUFAL), and M. A. Freitas (ICMBio), who kindly provided photographs and data from *Liotyphlops* specimens. CK is grateful to Vincent Fernandez and Brett Clark (NHMUK), for their assistance in scanning the paratype (BMNH 1946.1.11.12) of *L. beui* during her SYNTHESYS (Synthesis of Systematic Resources funded by the European Commission) funded research stay at NHMUK (GB-TAF-8426). We thank Jessie Maisano for granting access to the CT scan data of the paratypes (MCZ-R 17842, 16702) of *L. beui* available on the MorphoSource platform. OME-N thanks Fundaçao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP, 2021/13671 7) and Coordenaçao de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, 88887.667258/2022 00).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2023.01.003.

8. Appendix I

Material examined. Coordinates are given in WGS 84, World Geodetic System (EPSG:4326) datum. Specimens marked with an asterisk (*) could not be found in their respective collections during the time of writing (08 June 2022), and are likely to be either missing or were destroyed during the Butantan Institute Fire in 2010.

L. beui (Amaral, 1924) (*n* 75): **BRAZIL:** PARANA: *Uniao da Vitoria*, 26.22261763902104 S, 51.086770741810916 W (MCP 16360); RIO GRANDE DO SUL: *Erechim*, 27.638363223869092 S, 52.26716932850961 W (UFRGS 6494), *Tres Passos*, 27.4560 49050320402 S, 53.930360360350232 W (IBSP 92768, UFRGS 7096, 7097); SANTA CATARINA: *Usina Hidreletrica Passos Maia, Passos Maia*, 26.780814996967333 S, 52.06075943604652 W (UFRGS 6272, 6273, 6274 - holotype of *L. sousai*, 6275); SAO PAULO: *Unknown locality* (IBSP 83938), *Carapicuíba*, 23.5447828651887 S, 46.83819612391236 W (IBSP 79486, 81227, 88396), *Cotia*, 23.602598317980657 S, 46.91751639890515 W (IBSP 78274, 87449), *Guararema*, 23.414011254269383 S, 46.0354 036649803 W (IBSP 81283), *Ibiúna*, 23.66063583826207 S, 47.21189326084941 W (IBSP 80644, 84488, 87930), *Jacareí*, 23.30406153793857 S, 45.96400718555779 W (IBSP 79321), *Osasco*, 23.54042363264598 S, 46.79370348425242 W (IBSP 78672, 83634, 84494, 84576, 91867), *Santa Isabel*, 23.322737297876927 S, 46.22422290745215 W (IBSP 80680), *Santo Andre*, 23.775387835516508 S, 46.39159152886798 W (IBSP 79327), *Sao Paulo*, 23.548067038910187 S, 46.6217251404256 W (IBSP 62810, 78258, 78318, 78318, 78543, 78570, 78645, 78648, 79719, 79720, 79844, 81333 (Bairro Vila Jaguara), 81509, 81650, 81717, 81899, 83520, 84693, 84694, 84748, 84961, 85472, 85493, 85494, 87323, 87533, 88329, 88907, 89347, 89532, 91834, 92321, 92448, 92465, 92466, 78318), *Instituto Butantan in Sao Paulo*, 23.567026259684827 S, 46.71888409994317 W (IBSP 88907, 84694, 78648, 90395, 92448, topotype, MCZR-16702, 17842, 17843, BMNH 1946.1.11.12 - paratypes of *H. beui*), *Sao Roque* 23.5309028178708 S, 47.13687966384641 W (IBSP 81149, 87414).

L. caissara Centeno, Sawaya, & Germano, 2010 (*n* 3): **BRAZIL:** SAO PAULO: Ubatuba, Parque Estadual da *Ilha Anchieta*, 23.550082799793074 S, 45.066694759063566 W (IBSP 89927), *Ilha Sao Sebastiao*, 23.833729197885763 S, 45.36068313480343 W (IBSP 76774), *Location withheld at request of collector* (IBSP 81283).

L. ternetzii Boulenger, 1896 (*n* 15): **PARAGUAY:** *Unknown locality* (BMNH 1946.1.11.77 - holotype of *Helminthophis ternetzii*); SAN PEDRO: *Primavera*, 23.149219648433366 S, 57.38634608052616 W (BMNH 1955.1.5.93, 1956.1.16.34, 1960.1.2.72, 1956.1.3.34, 1956.1.3.33); **BRAZIL:** DISTRITO FEDERAL: *Brasília*, 15.773891586031407 S, 47.93388530889805 W (IBSP 81161, MCP 18381, 18058); MATO GROSSO: *Burity (Buritĺ, Alto Araguaia)*, 17.978286105793774 S, 53.54978208652379 W (BMNH 1946.1.10.73 - holotype of *Helminthophis collenetei*); MINAS GERAIS: *Unknown locality* (UFMG 0055, 2654, 1682), *Prudente de Moraes*, 19.462258859998517 S, 44.11304113612916 W (IBSP 83613); MATO GROSSO: *Itiquira*, 17.22347213649225 S, 54.1407081 18935095 W (UFRGS 6458); SAO PAULO: *Sagres*, 21.885229362316583 S, 50.959008501068325 W (IBSP 84175).

L. schubarti Vanzolini, 1948 (*n* 1): **BRAZIL:** SAO PAULO: *Pirassununga*, 21.99849468592157 S, 47.426920380987184 W (IBSP 78314).

L. trefauti Freire, Caramaschi, & Argolo, 2007 (*n* 4). **BRAZIL:** ALAGOAS: Teotonio Vilela, 9.906042662565511 S, 36.360745470 67437 W (CHP-UFRPE, unvouchered); BAHIA: *Ilheus*, 14.7943608 02672307 S, 39.046523003559216 W (MZUESC 4095, 5800); PERNAMBUCO: *Tamandare*, 8.758004068840062 S, 35.1073467 0501041 W (CHP-UFRPE 0653).

L. wilderi (Garman, 1883) (*n* 4). **BRAZIL:** MINAS GERAIS: *Itabira*, 19.642353194160638 S, 43.227548881506536 W (UFMG 1807), *Alvorada de Minas*, 18.731735663885786 S, 43.3642959 86211665 W (MCZ R-5126, FMNH 73387, syntypes of *Typhlops wilderi*); RIO DE JANEIRO: *Porto Real*, 22.437846431356043, 44.323 01273906716 (BMNH 1946.1.11.3, holotype of *Helmintophis guentheri*).

References

Amaral, A., 1924. Helminthophis. Proc. New Engl. Z. Club. 9, 25 30.
Barbo, F.E., Booker, W.W., Duarte, M.R., Chaluppe, B., Portes-Junior, J.A., Franco, F.L., Grazziotin, F.G., 2022. Speciation process on Brazilian continental islands, with the description of a new insular lancehead of the genus *Bothrops* (Serpentes, Viperidae). Syst. Biodivers. 20, 1 25.
Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, 1 28.
Boulenger, G.A., 1889. XLVIII. descriptions of new Typhlopiæ in the British Museum. J. Nat. Hist. 4, 360 363.
Boulenger, G.A., 1893. In: Catalogue of the Snakes in the British Museum, vol. 1. Taylor & Francis, London, p. 448.
Boulenger, G.A., 1896. In: Catalogue of the Snakes in the British Museum, vol. 3. Taylor & Francis, London, p. 727.
Centeno, F.C., Sawaya, R.J., Germano, V.J., 2010. A new species of *Liotyphlops* (Serpentes: Anomalepididae) from the Atlantic coastal forest in southeastern Brazil. Herpetologica 66, 86 91.
Cope, E.D., 1864. On the characters of the higher groups of Reptilia Squamata and especially of Diploglossa. Proc. Acad. Nat. Sci 15, 224 231.
Cundall, D., Irish, F., 2008. The snake skull; pp. 349 692. In: Gans, C., Parsons, T.S. (Eds.), Biology of the Reptilia, vol. 20. Academic Press, London.
Dixon, J.R., Kofron, C.P., 1983. The central and SouthSouth American Anomalepid snakes of the genus *Liotyphlops*. Amphibia-Reptilia 4, 241 264.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11 15.
Dunn, E.R., 1932. Notes on blind snakes from Lower Central America. Proc. Biol. Soc. Wash. 45, 173 175.
Dunn, E.R., Tihen, J.A., 1944. The skeletal anatomy of Liotyphlops albirostris. J. Morphol. 74, 287 295.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792 1797.
Entiauspe-Neto, O.M., Koch, C., Guedes, T.B., Paredero, R.C., Tiutenko, A., 2022. Unveiling an enigma from the Cerrado: taxonomic revision of two sympatric species of *Apostolepis* Cope, 1862 (Dipsadidae: Xenodontinae: Elapomorphini) from central Brazil. Eur. J. Taxon. 817 (1), 143 182.
Eo, S.H., Woody, J.A., 2010. Evolutionary rates of mitochondrial genomes correspond to diversification rates and to contemporary species richness in birds and reptiles. Proc. Royal Soc. B. 277, 3587 3592.

Ferrarezzi, H., 1993. Sistematica Filogenetica de *Elapomorphus, Phalotris* e *Apostolepis* (Serpentes: Colubridae: Xenodontinae). Master of Science Dissertation. Universidade de Sao Paulo.
Ferrarezzi, H., 1994. Uma sinopse dos generos e classificaçao das Serpentes (Squamata): I. Scolecophidia e Alethinophidia nao Colubrídeos; pp. 69 80. In: Nascimento, L.B., Bernardes, T.A., Cotta, G.A. (Eds.), Herpetologia Do Brasil 1. Editora PUC-MG Fundaçao Ezequiel Dias. Fundaçao Biodiversitas. Belo Horizonte.
Freire, E.M.X., Caramaschi, U., Argolo, A.J.S., 2007. A new species of *Liotyphlops* (Serpentes: Anomalepididae) from the Atlantic Rain forest of northeastern Brazil. Zootaxa 1393, 19 26.
Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. Syst. Biol. 62 (5), 707 724.
Garman, S., 1883. On certain reptiles from Brazil and Florida. Sci. Observer 4 (5), 6.
Gauthier, J.A., Kearney, M., Maisano, J.A., Rieppel, O., Behlke, A.D., 2012. Assembling the squamate tree of life: perspectives from the phenotype and the fossil record. Bull. - Peabody Mus. Nat. Hist. 53 (1), 3 308.
Giraudo, A.R., 1994. Comentarios sobre las especies del genero *Liotyphlops* Peters (Serpentes: Anomalepididae) presentes en la Provincia de Misiones (Republica Argentina). Cuad. Herpetol. 8 (2), 229 233.
Haas, G., 1964. Anatomical observations on the head of *Liotyphlops albirostris* (Typhlopidae, Ophidia). Acta Zool. 45, 1 62.
Haas, G., 1968. Anatomical observations on the head of *Anomalepis aspinosus* (Typhlopidae, Ophidia). Acta Zool. 49, 63 139.
Hassler, H.B., Probert, B., Moore, C., Lawson, E., Jackson, R.W., Russell, B.T., Richards, V.P., 2022. Phylogenies of the 16S rRNA gene and its hypervariable regions lack concordance with core genome phylogenies. Microbiome 10, 1 18.
Hammar, A.G., 1908. LI. note on the type specimen of a blind snake, *Helminthophis wilderi* (Garman), from Brazil. Ann. Mag. Nat. Hist. 1, 334 335.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 1965 1978, 1978.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647 1649.
Kley, N.J., 2006. Morphology of the lower jaw and suspensorium in the Texas blindsnake, *Leptotyphlops dulcis* (Scolecophidia: Leptotyphlopidae). J. Morphol. 267, 494 515.
Lanfear, R., Calcott, B., Ho, S.Y., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29 (6), 1695 1701.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9 (4), 357 359.
Linares-Vargas, C.A., Bolívar-García, W., Herrera-Martínez, A., Osorio-Domínguez, D., Ospina, O.E., Thomas, R., Daza, J.D., 2021. The status of the anomalepidid snake *Liotyphlops albirostris* and the revalidation of three taxa based on morphology and ecological niche models. Anat. Rec. 304, 1 15.
List, J.C., 1966. Comparative osteology of the snake families Typhlopidae and Leptotyphlopidae. Ill Biol. Monogr. 36, 1 112.
Marais, J., 1984. Probing and marking snakes. J. Herpetol. Assoc. Afr. 30 (1), 15 16.
Miralles, A., Marin, J., Markus, D., Herrel, A., Hedges, S.B., Vidal, N., 2018. Molecular evidence for the paraphyly of Scolecophidia and its evolutionary implications. J. Evol. Biol. 31, 1782 1793.
McDowell, S.B., Bogert, C.M., 1954. The systematic position of *Lanthanotus* and the affinities of the anguinomorphan lizards. Bull. Am. Mus. Nat. Hist. 105, 1 142.
Merrem, B., 1820. Versuch eines Systems der Amphibien I (Tentamen Systematis Amphibiorum). J. C. Kriegeri, Marburg, p. 191.
Mindell, D.P., Honeycutt, R.L., 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. Annu. Rev. Ecol. Evol. Syst. 21, 541 566.
Myers, C.W., 2003. Rare snakes five new species from eastern Panama: reviews of northern *Atractus* and southern *Geophis* (Colubridae: Dipsadinae). Am. Mus. Novit (3391), 1 47, 2003.
Nogueira, C.C., Argolo, A.J., Arzamendia, V., Azevedo, J.A., Barbo, F.E., Bernils, R.S., Martins, M., 2019. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. S. Am. J. Herpetol. 14, 1 274.
Nopcsa, F., 1923. Eidolosaurus und Pachyophis. Zwei neue Neo-com-Reptilien. Palaeontographica 65, 99 154.
Ochman, H., Elwyn, S., Moran, N.A., 1999. Calibrating bacterial evolution. Proc. Natl. Acad. Sci. U.S.A. 96, 12638 12643.
Palci, A., 2014. On the Origin and Evolution of the Ophidia. Doctoral dissertation. University of Alberta]. Era Library University of Alberta.
Palci, A., Hutchinson, M.N., Caldwell, M.W., Smith, K.T., Lee, M.S., 2020. The homologies and evolutionary reduction of the pelvis and hindlimbs in snakes, with the first report of ossified pelvic vestiges in an anomalepidid (*Liotyphlops beui*). Zool. J. Linn. Soc. 188, 630 652.
Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), "Molecular Systematics , second ed. Sinauer, Sunderland, MA, pp. 205 247.
Parker, H.W., 1928. XI. notes on reptiles and batrachians from Matto Grosso and E. Bolivia. Ann. Mag. Nat. Hist. 2, 96 99.
Parpinelli, L., Marques, O.A.V., 2015. Reproductive biology and food habits of the blindsnake *Liotyphlops beui* (Scolecophidia: Anomalepididae). S. Am. J. Herpetol. 10 (3), 205 210.
Peters, W.C.H., 1858. Vier neue amerikanische Schlangen aus der Familie der Typhlopinen und darüber einige vorlaufige Mittheilungen. Monatsber. konigl. Akad. Wiss. Berlin. 1857, 402, 1857.
Peters, W.C.H., 1881. Einige herpetologische Mittheilungen. 1. Uebersicht der zu den Familien der Typhlopes und Stenostomi gehorigen Gattungen oder Untergattungen. 2. Ueber eine neue Art von Tachydromus aus dem Amurlande. 3. Ueber die von Herrn Dr. finsch aus Polynesien g Sitzungs-Ber. Gesellsch.Naturforsch. Freunde Berlin, 1881 (4), 69 72.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231 259.
Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. Mol. Ecol. Res. 21 (2), 609 620.
R Core Team, 2013. R: A Language and Environment for Statistical Computing.
Rage, J.C., 1984. Serpentes. Handbuch der Palaontologie, part 11. G. Fischer Verlag, Stuttgart.
Rieppel, O., 1979. A cladistic classification of primitive snakes based on skull structure. J. Zool. Syst. Evol. Res. 17, 140 150.
Rieppel, O., Kley, N.J., Maisano, J.A., 2009. Morphology of the skull of the white-nosed blindsnake, *Liotyphlops albirostris* (Scolecophidia: Anomalepididae). J. Morphol. 270, 536 557.
Santos, F.J.M., 2018. A Revision of the Small Snakes of the Family Anomalepididae (Reptilia: Squamata: Serpentes), Using High Resolution Computerized Tomography. PhD dissertation. Pontificia Universidade Catolica do Rio Grande do Sul, Brazil, Porto Alegre, p. 164.
Santos, F.J.M., Reis, R.E., 2018. Two new blind snake species of the genus *Liotyphlops* (Serpentes: Anomalepididae), from central and SouthSouth Brazil. Copeia 106, 507 514.
Singhal, S., Colston, T.J., Grundler, M.R., Smith, S.A., Costa, G.C., Colli, G.R., Rabosky, D.L., 2021. Congruence and conflict in the higher-level phylogenetics of squamate reptiles: an expanded phylogenomic perspective. Sysy. Biol. 70 (3), 542 557.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312 1313.
Stejneger, L., 1892. Notes on a collection of birds made by Mr. Harry V. Henson in the island of Yezo, Japan. Proc. U. S. Natl. Mus. 15, 289 359.
Strong, C.R., Palci, A., Caldwell, M.W., 2021. Insights into skull evolution in fossorial snakes, as revealed by the cranial morphology of *Atractaspis irregularis* (Serpentes: Colubroidea). J. Anat. 238 (1), 146 172.
Smit, S., Widmann, J., Knight, R., 2007. Evolutionary rates vary among rRNA structural elements. Nucleic Acids Res. 35, 3339 3354.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725 2729.
Tavare, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lect. Math. Life Sci. 17, 57 86.
Uetz, P., Hosek, J., 2020. The Reptile Database. Available from. http://www.reptile-database.org. (Accessed 11 September 2020).
Vanzolini, P., 1948. Notas sobre os ofídios e lagartos da cachoeira de Emas, no Município de Pirassununga, Estado de Sao Paulo: notes on ophidians and lizards in Emas, Pirassununga, Sao Paulo State, Brazil. Rev. Bras. Biol. 8, 377 400.
Zar, J.H., 1999. Biostatistical Analysis. Pearson Education.
Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869 2876.