Reassessment of *Tantilla boipiranga* (Serpentes: Colubrinae) and a preliminary approach to the phylogenetic affinities within *Tantilla*

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Abstract. *Tantilla* is one of the most diverse genera among colubrids, with 67 species arranged in six phenotypically recognized species groups. *Tantilla boipiranga* is the most recently described species within the *T. melanocephala* group, and it was described based on a small type series, collected in the rupestrian grasslands of south-eastern Brazil. The morphological diversity and the phylogenetic affinity of this species remain poorly known. Here, based on the assessment of recently collected specimens in combination with results of a molecular phylogenetic analysis, we evaluate the morphological variation within *T. boipiranga* and its phylogenetic position. Our analyses confirm *T. boipiranga* as genetically distinct from its generic counterparts, and diagnosable based on a combination of colour pattern, meristic counts, and hemipenial morphology. However, contrary to its original description, the hemipenial ornamentation does not differentiate *T. boipiranga* from the highly variable *T. melanocephala*. In our phylogenetic analysis, *T. boipiranga* is retrieved as a monophyletic group, nested within the diversity of *T. melanocephala* and sister to a clade composed by specimens from south-eastern Brazil. *Tantilla melanocephala* is recovered as a highly diverse lineage, indicating the possible presence of undescribed species. Additionally, our analysis indicates that the *T. coronata* and *T. planiceps* species group are monophyletic, while the *T. taeniata* species group is paraphyletic and the only sampled species for the *T. calamarina* group is nested within the *T. melanocephala* group. Our results suggest that the phenotypic evolution within the genus is probably more complex than previously recognized, and some colouration patterns used to define the groups can in fact represent phenotypical convergences. Moreover, our phylogenetic analysis suggests a strong signal of geographical structure in the tree topology. Three main geographical lineages were found, a North American, a South American and a Central American. The first two lineages were recovered as monophyletic, while the latter is paraphyletic, with Central American species positioned as sister groups of both, the North American and the South American clades. Although a more comprehensive phylogenetic analysis is needed, our study strongly indicates the existence of hidden diversity within the *T. melanocephala* group and that *Tantilla* represents an ideal model to evaluate the validity of phenotypical groups in snake systematics and to study the driven mechanisms of morphological evolution.

Key words. Squamata, Colubridae, Sonorini, fossorial snakes, snake biogeography, snake systematics, snake taxonomy, phylogeny, molecular phylogenetics, comparative morphology.

Introduction

Species with cryptic behaviour are usually difficult to study based on comprehensive sampling and multiple sources of evidence (Button et al. 2019). Fossorial snakes represent the typical organism that frequently challenges ecologists and taxonomists. They spend most part of their lives in inaccessible habitats, which often make their detection in the field very difficult and preclude large numbers of specimens deposited in scientific collections (Pinto et al. 2018, Koch et al. 2019).

Tantilla Baird & Girard, 1853 is an American genus of small-bodied fossorial snakes, which present broad phenotypical variability (Wilson & Mata-Silva 2015). It includes 67 species, from which 56 are currently allocated in the following six phenotypic species groups: *T. taeniata* (26 species), *T. melanocephala* (10 species), *T. calamarina* (seven species), *T. planiceps* (seven species), *T. coronata* (three species), and *T. rubra* (three species) (Townsend et al. 2013, Wilson & Mata-Silva 2015, Antúnez-Fonseca et al. 2020). The genus is distributed in the Neotropical region throughout Central and South America, from northern Argentina and Uruguay and part of the Nearctic region to the south-central United States (Mata-Silva & Wilson 2016). Despite the wide distribution and large phenotypical variation, few specimens have been deposited in scientific collections and many species are known exclusively from the holotype (Hofmann et al. 2017, Antúnez-Fonseca et al. 2020).

Recent taxonomic studies on *Tantilla* have focused mainly on the taxonomy of the *T. taeniata* group (McCranie 2011, Townsend et al. 2013, Batista et al. 2016, McCranie & Smith 2017, Antúnez-Fonseca et al. 2020). In contrast, few studies focusing on the *T. melanocephala* group have been published in the last two decades. These studies can be summarised by the following five taxonomic acts: 1) resurrection of *T. armillata* and *T. ruficeps* (Savage 2002); 2) the description of *T. boipiranga* (Sawaya & Sazima 2003); 3) the description of *T. marcovani* (Lema 2004); 4) the synonymization of *T. equatoriana* (Greenbaum et al. 2004); and 5) the synonymization of *T. marcovani* (Mata-Silva & Wilson 2016).

The *T. melanocephala* species group is the second most diverse in the genus, including the following 10 species: *T. andinista* Wilson & Mena, 1980; *T. armillata* Cope, 1876; *T. boipiranga* Sawaya & Sazima, 2003; *T. capistrata* Cope, 1876; *T. insulamontana* Wilson & Mena, 1980; *T. lempira* Wilson & Mena, 1980; *T. melanocephala* (Linnaeus, 1758), *T. miyatai* Wilson & Knight, 1987; *T. ruficeps* (Cope, 1894); and *T. petersi* Wilson, 1979. Within them, *T. melanocephala* has the largest geographic distribution, ranging from Panama to northern Argentina and Uruguay. This species also presents isolated populations on Trinidad and Tobago, Granada, Saint Vincent and the Grenadines, Colombian island of Gorgona, and Brazilian island of Arvoredo (Berg et al. 2009, Castro-Herrera et al. 2012, Wilson & Mata-Silva 2015, Monteiro-Filho & Conte 2017, Guedes et al. 2018). While *T. melanocephala* is widely distributed, the morphologically closest species *T. boipiranga* Sawaya & Sazima, 2003 is known only from Serra do Espinhaço (Espinhaço Range) and adjacent regions in the Brazilian state of Minas Gerais (Wilson & Mata-Silva 2015, Tunes et al. 2020). Such distribution encompasses ~78,200 km² from where only 24 specimens have been registered so far (Silveira et al. 2009, Tunes et al. 2020).

Tantilla boipiranga was described based on four specimens from the highlands of the Brazilian state of Minas Gerais, at an altitude of ~1,200 m above sea level, from the locality of "Serrote" in the municipality of Santana do Riacho. This area is characterised by the presence of the ecosystem known as "campo rupestre" (rupestrian grassland) formed by a montane fire-prone vegetation mosaic dominated by herbs and shrubs (Miola et al. 2021). Although Sawaya & Sazima (2003) suggested that the species is endemic to this rupestrian grassland, Cassimiro (2003) identified a distribution record from the municipality of Caratinga (state of Minas Gerais), in the Brazilian Atlantic Forest, 190 km east of the type locality. Silveira et al. (2009) expanded this distribution even further, registering new records to the south in the municipality of Ouro Preto and to the north in the municipality of Alvorada de Minas (state of Minas Gerais). In this study, they also suggested that *T. boipiranga* is endemic to the contact regions between Cerrado and Atlantic Forest in the southern portions of Espinhaço Range. They also showed the existence of phenotypic variation in the head colour among different populations throughout the species distribution. More recently, Tunes et al. (2020) expanded the geographic distribution of *T. boipiranga* to the north-eastern state of Minas Gerais, by registering the presence of the species in the municipality of Almenara.

The series of continuous expansion of the geographic distribution in the last two decades indicates the scarcity of our current knowledge about *T. boipiranga*. Additionally, the taxonomic status of this rare species was never evaluated in relation to the highly diverse *T. melanocephala*, and the phylogenetic position of *T. boipiranga* within the genus was never assessed before.

Here, we employ an integrative approach, combining molecular and morphological evidences, to phylogenetically evaluate and compare different populations previously assigned to *T. boipiranga* and *T. melanocephala*. We also evaluate and describe the morphological variations of *T. boipiranga* expanding its geographic distribution and adding new records for the species from six new localities. Additionally, we test the monophyly of *Tantilla* and discuss the phylogenetic and biogeographic patterns of the genus based on the topology of our molecular phylogeny.

Material and methods
Taxon sampling and specimens examined

During recent field expeditions in south-eastern Brazil (states of Minas Gerais and Espírito Santo) we collected new specimens morphologically similar to *Tantilla boipiranga*, hereafter identified as *T.* cf. *boipiranga*. Although preliminary, the association of these new specimens to *T. boipiranga* already expand considerably the known area of occurrence for the species. The collected specimen IBSP 90663 was found in the northern part of Reserva Natural da Vale (RNV) in the municipality of Linhares (19°17'40.92" S, 40°5'3.12" W), state of Espírito Santo, Brazil, on November 18, 2016. The specimen IBSP 90487 was found of Parque Estadual Alto Cariri in the municipality of Santa Maria do Salto (16°21'45.26" S, 40°3'5.34" W), state of Minas Gerais, Brazil, on December 05, 2018. Tissue

samples and the specimens were deposited in the Coleção Herpetológica "Alphonse Richard Hoge" do Laboratório de Coleções Zoológicas, Instituto Butantan (IBSP). To properly identify these specimens, we analysed other 22 individuals of *T. boipiranga* (Supplementary Table S1) from the herpetological collection of the Instituto Butantan and Coleção Herpetológica da Universidade Federal de Minas Gerais (UFMG). Comparative data from literature were obtained from Sawaya & Sazima (2003), Silveira et al. (2009) and Tunes et al. (2020) (Supplementary Table S2).

DNA sequencing and molecular analyses

We sequenced three mitochondrial (16S ribosomal RNA; cytb, cytochrome b; cox1, cytochrome c oxidase subunit 1) and three nuclear (bdnf, brain derived neurotrophic factor; c-mos, oocyte maturation factor Mos; and nt3, neurotrophin-3) gene fragments of three *T. boipiranga* and four *T. melanocephala* individuals. These sequences were deposited in GenBank (Supplementary Table S3). We extracted DNA from scales and liver tissues using the IN-VITROGEN PureLink™ Genomic DNA kit (ThermoFisher, MA, USA). We performed PCRs using the protocols described in Grazziotin et al. (2012) and Graboski et al. (2018). The primer sequences are described in Supplementary Table S4. We sequenced both strands and the contigs were assembled and edited using Geneious Prime 2020.0.5 (https://www.geneious.com, Kearse et al. 2012).

To build our molecular matrix we used the reduced molecular dataset for colubrids of Montingelli et al. (2019), and we also downloaded DNA sequences from GenBank for 13 other species of *Tantilla* and four sequences for two species provided by Batista et al. (2016) and Cox et al. (2018) (Supplementary Table S3). Our final dataset comprises 73 terminals from 61 species of colubrids, from which 17 are species of *Tantilla* and 44 are other colubrids included as outgroup. Our dataset was designed to evaluate both, the relationship among species of *Tantilla*, and the monophyly of the genus (Supplementary Table S3). Our sample comprises about 25% of the described species within *Tantilla*, including representatives of five phenotypic groups of species: 1) *calamarina* group = *T. vermiformis* (Hallowell, 1861); 2) *coronata* group = *T. coronata* Baird & Girard, 1853 and *T. relicta* Telford, 1966; 3) *melanocephala* group = *T. armillata* Cope, 1875, *T. boipiranga* Sawaya & Sazima, 2003, *T. melanocephala* (Linnaeus, 1758); 4) *planiceps* group = *T. gracilis* Baird & Girard, 1853, *T. hobartsmithi* Taylor, 1937, *T. nigriceps* Kennicott, 1860, *T. planiceps* (Blainville, 1835), *T. wilcoxi* Stejneger, 1902, *T. yaquia* Smith, 1942; 5) *taeniata* group = *T. berguidoi* Batista, Mebert, Lotzkat & Wilson, 2016, *T. impensa* Campbell, 1998; and the species without a defined group *T. alticola* (Boulenger, 1903), *T. supracincta* (Peters, 1863), and *T. tjiasmantoi* Koch & Venegas, 2016.

We aligned the sequences using MAFFT (Katoh & Standley 2013) with standard parameters as implemented in Geneious. Since the gene fragments included in our dataset did not overlap for several terminals, we split our concatenated matrix in the following five partitions: 1) mitochondrial rRNA (12S and 16S), 2) first and second codon positions of mitochondrial coding genes (cox1 and cytb), 3) third codon position of mitochondrial coding genes (cox1 and cytb), 4) first and second codon positions of nuclear genes (bdnf, c-mos, nt3 and rag1), and 5) third codon position of nuclear genes (bdnf, c-mos, nt3 and rag1). By using such partition scheme (Supplementary Table S5), we aimed to reduce problems of over-parameterization derived from the lack of phylogenetic informative sites from highly partitioned matrices (McGuire et al. 2007). We performed maximum likelihood (ML) analysis using RAxML v.8 (Stamatakis 2014) through the CIPRES portal (Miller et al. 2010) using the partitioned dataset and the GTRGAMMA model of nucleotide evolution. We conducted a rapid bootstrap analysis and search for the best scoring ML tree in the same run (option -f a). Branch support was assessed by using 1,000 pseudoreplications of bootstrap. Clade robustness (modified from Zaher et al. 2019) was classified as follows: unambiguously supported (100%), strongly supported (80%–99%), moderately supported (70%–79%) and weakly supported (below 70%).

Morphological data

The terminology for scale counts and cephalic shields measurements follows Peters (1964) and Peters & Orejas-Miranda (1970). Ventral counts follow Dowling (1951). Measurements were taken with a dial caliper to the nearest 0.1 mm, with the exception of the snout–vent length (SVL) and tail length (TL), which were measured with a flexible ruler to the nearest 1.0 mm. Sex was verified by a longitudinal incision at the base of the tail to verify the presence or absence of hemipenis. We prepared the right hemipenis of three specimens of *Tantilla boipiranga* (IBSP 79060, 81123, 90663) and six specimens of *T. melanocephala* (IBSP 41062, 53429, 55662, 57304, 68405, 92165) following methods proposed by Myers & Cadle (2003) and Zaher & Prudente (2003). Terminology for hemipenial morphology followed Zaher (1999).

Statistical analyses

Student's t-test was performed to evaluate the presence of sexual dimorphism in relation to the morphometric and meristic datasets. We tested the assumptions of normality and homogeneity of variance using the Shapiro-Wilk's and Levene's tests, respectively. In cases where characters showed insufficient variation to justify these assumptions, we performed the non-parametric Mann-Whitney's test. We established alpha as < 0.05 for all statistics, which were performed in R environment (R Core Team 2020). Only adults were considered for tests based on morphometric variables, and the sexual maturity was defined based on Marques & Puorto (1998) and Santos-Costa et al. (2006).

Results

Phylogenetic affinities

The concatenated matrix encompassed 5,402 base pairs of aligned sequences. The complete phylogenetic tree based on all concatenated sequences is available in Supplementary Figure S1 and a summary tree can be seen in Figure 1.

The high-level phylogenetic relationships obtained in our ML analysis (Supplementary Figure S1) is broadly similar to the topology presented by MONTINGELLI et al. (2019). The monotypic genus *Scolecophis* was positioned as the sister group of a monophyletic *Tantilla*, although with weak support of bootstrap (Fig. 1). The monophyly of *Tantilla* was strongly supported (92%) in our ML tree (Fig. 1). Within *Tantilla*, we recovered the following two weakly

supported main clades: C01, comprising *T. alticola, T. berguidoi, T. coronata, T. gracilis, T. hobartsmithi, T. impensa, T. nigriceps, T. planiceps, T. relicta, T. supracincta, T. wilcoxi* and *T. yaquia*; and C02, formed by *T. armillata, T. boipiranga, T. melanocephala, T. tjiasmantoi,* and *T. vermiformis.* Clade C01 was structured in two biogeographically delimited subclades, as follows: SC01, comprising species distributed from southern regions of USA to northern Mexico; and SC02, including species from southern Mexico to Ecuador. Within subclade SC01, the topology of our tree also suggested the presence of two other groups, one composed by species from south-eastern USA (*T. coronata* and *T. relicta*) and another including species from south-western USA and northern Mexico (all other species from SC01). In contrast, the species in subclade SC02 did not
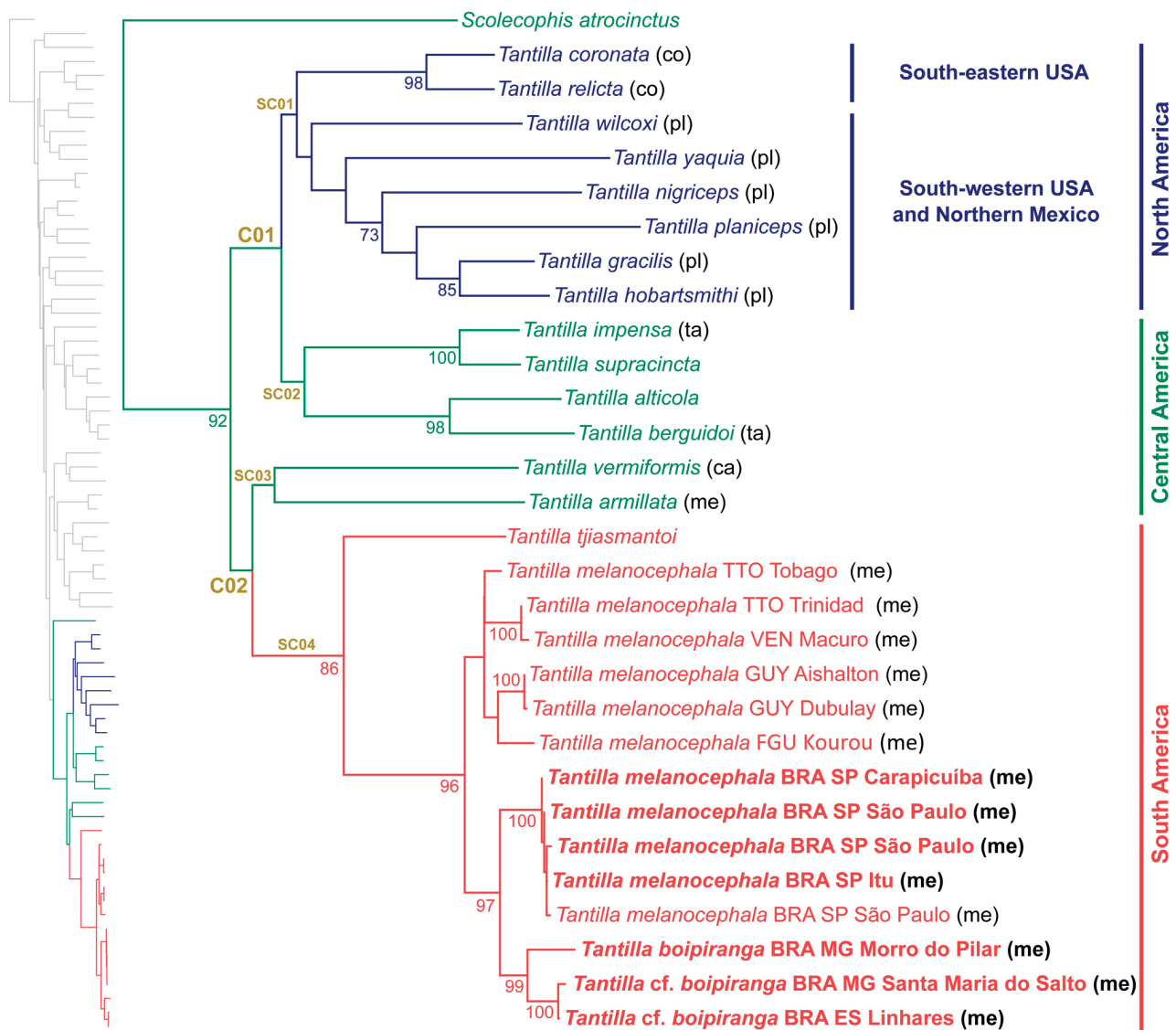

Figure 1. Maximum likelihood (ML) tree estimated using RAxML, showing the relationships within the genus *Tantilla*. The bootstrap support values are shown on each node. Bootstrap values below 70% are not shown. Terminals in bold correspond to samples sequenced in this study. co – *coronata* group; pl – *planiceps* group; ta – *taeniata* group; ca – *calamarina* group; me – *melanocephala* group.

formed structured geographical groups. These two sub-clades were weakly supported in our analysis.

In clade Co2 we recovered a weakly supported subclade (SC03) including species from Central America (*T. armillata* and *T. vermiformis*), which is sister to all South American species (Fig. 1). The South American species formed a strongly supported subclade (86%, SC04), in which the Peruvian species *T. tjiasmantoi* was positioned as sister to all other species. Brazilian samples of *T. melanocephala* and *T. boipiranga* grouped together (97%, Fig. 1), and the samples of *T. melanocephala* from northern South America were positioned as their sister group, forming a strongly supported clade (96%). All individuals of *T. melanocephala* from south-eastern Brazil clustered together in a unambiguously supported clade (100%, Fig. 1). The specimens of *T.* cf. *boipiranga* collected in the municipalities of Linhares and Santa Maria do Salto were grouped in a strongly supported clade sister to the specimen of *T. boipiranga* from Morro do Pilar (99%, Fig. 1).

Based on our phylogenetic result and the comparative analysis of the morphology of our expanded sampling, we are redefining below *T. boipiranga*, by integrating the morphological variability of the new records in the species description.

Tantilla boipiranga Sawaya & Sazima, 2003

Diagnosis: presence of seven supralabial scales; two postoculars scales; 143–160 ventral scales and 56–70 subcaudals in males; 153–172 ventrals and 51–59 subcaudals in females; presence of a black cephalic cap that can extend weakly to the temporal region, followed by a pale nuchal band medially divided, a black collar at the neck and a posteriorly discreet pale band, more evident laterally; presence of sub-

Table 1. Morphological variables taken from all available specimens analysed (n = 32) of *Tantilla boipiranga* (males = 18; females = 14). SD: standard deviation; SVL: snout–vent length; TAL: tail length; HL: head length; HW: head width; CN: longitudinal extension of the black collar on the neck. * Only adult individuals (males = 11; females = 10).

Variables	Males		Females	
	mean±sd	range	mean±sd	range
SVL (mm)*	314.8±52.8	237–385	351.8±66.3	246–471
TAL (mm)*	90.2±16.4	64–122	94.8±15.4	67–109
HL (mm)*	9.6±1	7.7–10.8	10.6±0.9	8.6–11.7
HW (mm)*	5.9±0.7	4.4–7.1	6.6±0.6	5.6–8.1
Gulars	3.3	2–4	3.2	2–5
Preventrals	1.5	0–3	1.5	1–3
Ventrals	152.7±4.9	143–160	161.6±5.7	153–172
Subcaudals	64.8±4.2	56–70	56±2.7	51–59
Supralabials	6.5	6–7	7	7
Infralabials	6	6	6	6
Temporals	1+1.5	1+1–2	1+1.5	1+1–2
Preoculars	1	1	1.5	1–2
Postoculars	1.9	1–2	2	2
CN	4.7±0.5	4–6	4.5±0.8	4–6

ocular black blotch; uniform dorsal reddish orange colour, sometimes presenting a discreet black vertebral longitudinal line; and presence of hemipenis elongated ornamented with large spines in the asulcate surface of distal region of hemipenial body.

Meristic and morphometric variation: The values of summary statistics for all evaluated characters are described in Table 1. Sexual dimorphism was significant for the following
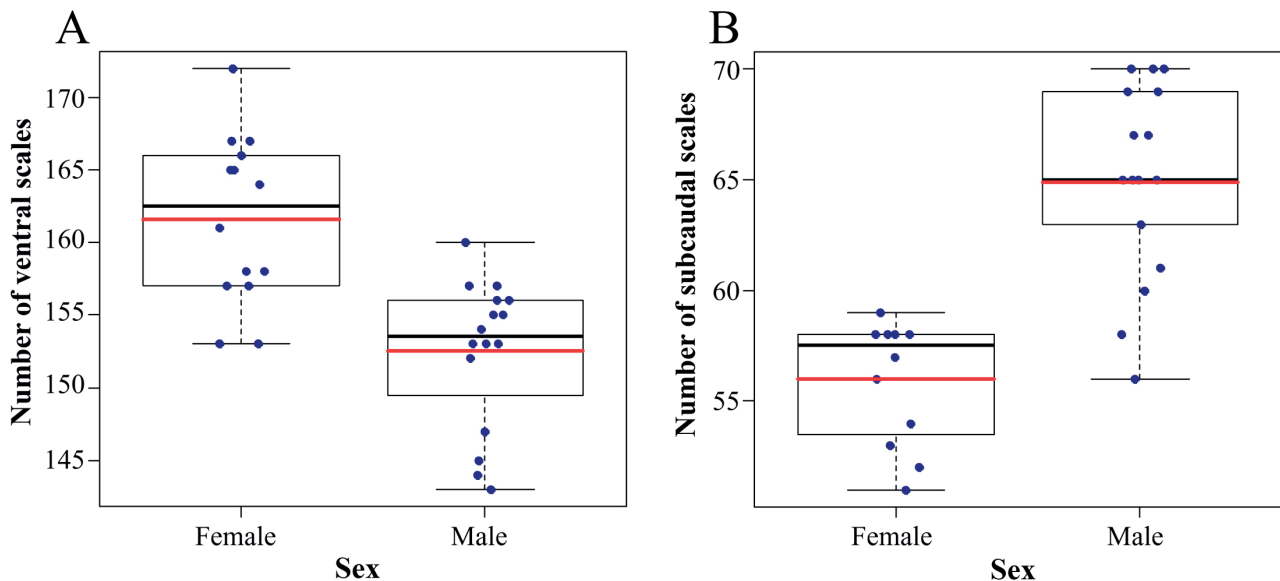

Figure 2. Meristic comparisons between females and males of *Tantilla boipiranga*. (A) ventrals; (B) subcaudals. Red line indicates the mean value and the black line the median.

characters (Fig. 2): number of ventral scales (p < 0.01), ranging from 153 to 172 in females (x = 161.6, n = 14) and from 143 to 160 in males (x = 152.7, n = 18); number of subcaudal scales (p < 0.01), ranging from 51 to 59 in females (x = 56, n = 12) and from 56 to 70 in males (x = 64.8, n = 17). The following values of meristic features were highly conservative among sampled individuals (for a detailed description see Supplementary Table 1): 1) two postoculars (n = 31), rarely one (n = 1); 2) one preoculars (n = 31), rarely two (n = 1); 3) seven supralabials (n = 31), rarely six (n = 1); 4) six infralabials (n = 32); 5) one preventrals (n = 12), two preventrals (n = 17), rarely three preventrals (n = 2) or absent (n = 1);

and 7) temporals 1+1 (n = 29), rarely temporals 1+2 (n = 3). We re-evaluated the scale counts and sex determination of five individuals analysed by Tunes et al. (2020) (their values/determination are presented in parentheses): UFMG 3230 presents 2+164 ventrals and 58 subcaudals (2+175 ventrals and 52 subcaudals); UFMG 121 is a male (female); UFMG 1175 is a male (female); UFMG 3251 is a male (female); UFMG 2539 is a male and presents 2+156 ventrals and 69 subcaudals (female, 2+162 ventrals and 52 subcaudals).

Colour in life: A low degree of colour variation was found in live specimens (Fig. 3). The species showed a uniform

Figure 3. Specimens of *Tantilla boipiranga* in life. (A) *Santana do Riacho, MG, Brazil; (B) Ouro Preto, MG, Brazil; (C) juvenile, male, Taiobeiras, MG, Brazil IBSP 91609; (D) adult, female, Santa Maria do Salto, MG, Brazil IBSP 90487; and (E–F) adult, male, Linhares ES, Brazil IBSP 90663. * Type locality. Photographs by P. Martins, L. Drummond, D. Santos, A. Abegg, and T. Castro, respectively.

reddish orange dorsal colour (as Sawaya & Sazima 2003) and immaculate cream venter; nuchal band cream to orange medially divided over the parietal suture by longitudinal union that connects the black cephalic cap to the black collar on the neck. Posteriorly discreet pale band, more evident laterally (Fig. 3BCDE). The vertebral longitudinal line is vestigial (Fig. 3B) or absent. Colour variation was present only in specimen IBSP 90663, which showed a cream venter with some reddish orange macules, mainly in the cloacal region (Fig. 3F).

Colour pattern in preservative: Slight colour variation was observed in the preserved specimens. The specimens exhibited an uniform cream or orange cream colour (as Silveira et al. 2010) with the exception of specimen IBSP 88573, which presented black punctuated macules on the dorsal scales (Fig. 4F). The black cephalic cap was conspicuous and well defined in the majority of the preserved specimens with only two individuals presenting inconspicuous cephalic cap (Fig. 4AG). The cephalic cap always extends laterally through the scales in contact with the eyes (supraocular, preocular, postocular and supralabials). Frequently the cephalic cap extends throughout the whole third and fourth supralabials and rarely to half of these scales (Fig. 4F). Dorsally, the cephalic cap never exceeded the inner portion of the parietal scales. Anteriorly, the cephalic cap extends to the rostral in most of the analysed individuals and it is rarely limited to the prefrontals (Fig. 4C). Blotches in the temporal region may be absent or vestigial (Fig. 4ABCD); isolated or attached to the cephalic cap anteriorly (Fig. 4EFH); or attached to the cephalic cap laterally (Fig. 4G). All individuals presented a pale nuchal band medially divided and the black collar on the neck extends longitudinally from four to six scales in the vertebral row (Table 1).

Hemipenis morphology (right organ, n = 3): The hemipenis is elongated, unilobed, unicalyculate and noncapitate (Fig. 5). Sulcus spermaticus is simple, centrolinearly oriented, extending from the base of the hemipenis to the apex of the lobe, bordered by spinules throughout its extension. The lobe is ornamented with calyces only in asulcate and lateral surfaces. The calyces are formed by a series of spinules that are gradually arranged in transverse rows on the sulcate surface of the lobe. These spinules invade a small part of the most distal region of the hemipenial body on the asulcate surface. The distal region of the hemipenial body is ornamented with large spines, which become smaller towards the sulcus spermaticus. The proximal region of the hemipenial body is predominantly nude, with some spinules irregularly distributed. Additionally, this region is also ornamented with a distinct spine on the asulcate surface and two large spines on the limits between the sulcate and lateral surfaces. These two spines present different sizes, the most proximal is usually larger than the other.

Natural history: Inspection of the dissected specimens revealed that one female contained six eggs (IBSP 90487,

SVL 384 mm). We found stomach contents in specimens IBSP 90663 and IBSP 90487, both of which contained the remains of centipedes (Scolopendromorpha). In the field, we found two specimens (IBSP 91608 and IBSP 91609) foraging during the morning, at around 11 am, and two others (IBSP 90487 and IBSP 90663) in the afternoon, between 4 pm and 5 pm. Our data are consistent with the observations made by Sawaya & Sazima (2003) regarding the diurnal, terrestrial and cryptozoic activity of *T. boipiranga*.
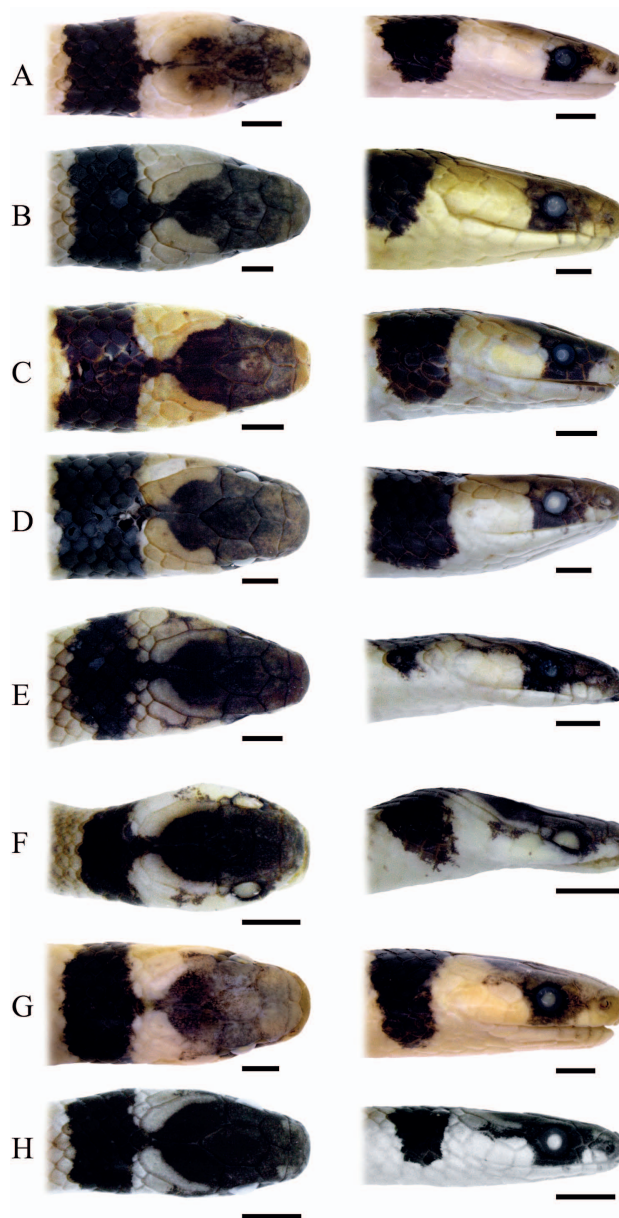

Figure 4. Variation of head colour within preserved specimens of *Tantilla boipiranga* in dorsal (left) and lateral (right) views. (A) IBSP 90663, Linhares; (B) IBSP 90487, Santa Maria do Salto; (C) *IBSP 64088, Santana do Riacho; (D) IBSP 79060, Morro do Pilar; (E) IBSP 88572, Pedro Canário; (F) IBSP 88573, São Mateus; (G) IBSP 81123, Morro do Pilar; (H) IBSP 91609, Taiobeiras. Horizontal scale bars: 2 mm. * Paratype.

Geographic distribution: We noticed that Tunes et al. (2020) probably provided the distances between records of *T. boipiranga* and the type locality based on road distances. We correct here these values based on the estimation of the linear distances between the type locality and the geographic locality of each record as follows (distances provided by Tunes et al. 2020 in parentheses): Caratinga ~190 km (~340 km); Almenara ~462 km (~703 km); Berilo ~284 km (~471 km); Santa Luzia ~67 km (~83 km); Morro do Pilar ~23 km (~56 km); Braúnas ~95 km (~166 km). Based on the corrected values *T. boipiranga* distribution encompasses an estimated area of ~134,200 km². The species occurs between altitudes of 38 m and 1368 m above sea level, in the Brazilian states of Minas Gerais and Espírito Santo, in different vegetation formations as Bahia coastal forest, Bahia interior forest, Cerrado and Campos rupestres montane savanna (sensu Olson et al. 2001) (Fig. 6).

Discussion
Phylogenetic pattern

More than 160 years after its description, the phylogenetic relationships among species of the large genus *Tantilla*

Figure 5. Hemipenis of *Tantilla boipiranga*. Top: IBSP 79060 Morro do Pilar; Bottom: IBSP 90663 Linhares. (A, D) Asulcate, (B, E) lateral, and (C, F) sulcate views. Vertical scale bar: 2 mm.

have been poorly studied (Cole & Hardy 1981, Cox et al. 2018, Jowers et al. 2020). Traditionally, this genus is classified in six groups of species, mainly based on their colour patterns (Wilson & Mata-Silva 2014). Our tree topology reveals a phylogenetic structure that largely reflects the geographic distribution of the species and is partially similar to the phylogeny presented by Jowers et al. (2020). These authors recovered two main clades, biogeographically structured as a North-Central American clade, and a Central-South American clade. When we expanded Jowers et al. (2020) analysis based on a larger multilocus matrix, and with an increased taxon sampling (adding *T. berguidoi*, *T. supracincta*, *T. alticola*, *T. wilcoxi*, *T. boipiranga* and a much larger outgroup), we recovered their two main clades (C01 and C02, Fig. 1), although with a more complex phylogenetic structure. In our phylogenetic tree we retrieved a North American clade (SC01, Fig. 1), a South American clade (SC04) and a paraphyletic mainly Central American group (composed by SC02 and SC03, Fig. 1).

Our tree topology for the North American clade corroborates the monophyly of the *coronata* and *planiceps* species groups. Although this result has been also presented by Jowers et al. (2020), Cox et al. (2018) did not recover the monophyly of these two species groups. In the phylogenetic tree presented by the latter, *T. wilcoxi* was positioned as sister to the *coronata* group and *T. nigriceps* was allocated as the most basal species concerning both species groups, which rendered the *planiceps* group as paraphyletic. This phylogenetic hypothesis was not completely evaluated by Jowers et al. (2020) since they did not sample *T. wilcoxi* in their study. Our more comprehensive analysis supports the putative morphological synapomorphies suggested for these two North American groups of *Tantilla*. The *planiceps* species group shares a dark head cap and a light collar on nape (Cole & Hardy 1981). Additionally, *T. gracilis* and *T. hobartsmithi* were supported as sister species in our topology and they share a semicapitate hemipenis with less spines (< 30) (Cole & Hardy 1981). The putative exclusive synapomorphy for the *coronata* species group is the presence of a hemipenis with spines restricted to the lobe and an almost completely nude hemipenial body (Telford 1966).

Our ML tree recovered the *taeniata* species group as paraphyletic (SC02, Fig. 1), since *T. impensa* and *T. berguidoi* were recovered nested with *T. supracincta* and *T. alticola*. The two latter species were never classified in any of the six species group of *Tantilla* and they do not share the pale middorsal and lateral stripes that characterise the *taeniata* species group (McCranie & Smith 2017). Such group includes more than 25 species (Antunez-Fonseca et al. 2020) and until now only two species were sequenced, one by Tonini et al. (2016) and one by Batista et al. (2016). Even considering the extremely poor taxon sampling for this group, our result suggests two hypotheses: 1) the *taeniata* group may include species that do not present striped patterns, or 2) the *taeniata* group does not represent a natural assemblage of species and the striped pattern is a convergence among some species of *Tantilla*. However, to sort out these hypotheses, a more thorough study focusing on the phylogenetic

relationship among the Mexican and Central American species of *Tantilla* is needed.

Regarding the seven species included in the *calamarina* species group (Townsend et al. 2013) we sampled only *T. vermiformis*. Although our analysis indicates that the *calamarina* group is positioned nested within the *melanocephala* species group (C02, Fig. 1), *T. vermiformis* does not represent the set of species originally included in the *calamarina* group (Wilson & Meyer 1981, Wilson 1999). Holm (2008) based only on morphological patterns ex-

plicitly disagrees on the association between *T. vermiformis* and the *calamarina* group. Our result seems to corroborate Holm (2008) by indicating that *T. vermiformis* represents a basal lineage within the *melanocephala* species group, sister to *T. armillata* (SC03, Fig. 1). However, the association between *T. armillata* and *T. melanocephala* was never tested before, and without a more comprehensive taxon sampling it is not possible to corroborate the monophyly of the *calamarina* species group or the relationship between *T. vermiformis* and *T. armillata*.
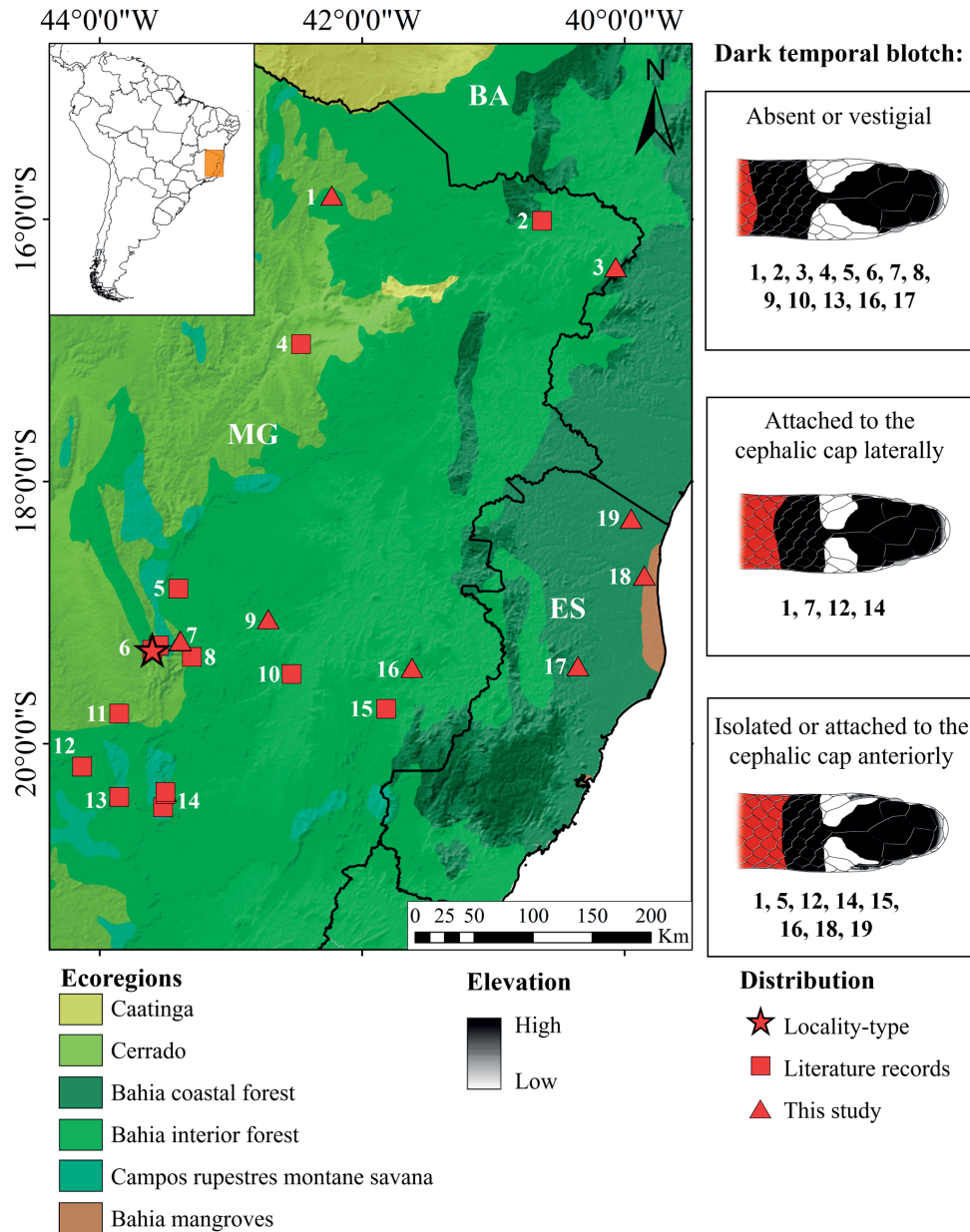

Figure 6. Geographic distribution of *Tantilla boipiranga*. Municipalities: (1) Taiobeiras; (2) Almenara; (3) Santa Maria do Salto; (4) Berilo; (5) Alvorada de Minas; (6) *Santana do Riacho; (7) Morro do Pilar; (8) São Sebastião do Rio Preto; (9) Braúnas; (10) Ipatinga; (11) **Santa Luzia; (12) Brumadinho; (13) Congonhas; (14) Ouro Preto; (15) Caratinga; (16) Alvarenga; (17) Linhares; (18) São Mateus; (19) Pedro Canário. * Type locality; ** Unknown. BA – state of Bahia; MG – state of Minas Gerais; ES – state of Espírito Santo.

Additionally, *T. tjiasmantoi* was recovered as the sister group of all South American species allocated in the *melanocephala* group (SC04, Fig. 1). This species has a colour pattern very different from that used to define the *melanocephala* group (see Wilson & Mena 1980), being morphologically similar to the South American species *T. semicincta* (phenotypical group not defined), presenting a pattern of transverse bands along the body (Koch & Venegas 2016). This result may suggest that the colour pattern used to define the *melanocephala* group cannot be found in all its representatives. A different interpretation of our tree topology can indicate that *T. armillata*, *T. vermiformis* and *T. tjiasmantoi* do not belong to the *melanocephala* group and its definition should be revised.

Regarding the *rubra* species group (sensu Wilson & Mata-Silva 2014) we suspect that the group may be non-natural, since the morphological pattern and the geographic distribution of *T. bocourti*, *T. cucullata*, and *T. rubra* are closely related to those of the species included in the *planiceps* group (Cole & Hardy 1981, Dixon et al. 2000). However, as we did not sample any of these species, the hypotheses are open to be tested in a molecular phylogenetic framework.

Biogeographic patterns

Affinities recovered in our analyses within *Tantilla* point to a historical scenario of diversification of two main lineages, likely distributed initially in a region between Mexico and Central America, where much of the diversity of the genus is currently found (Wilson & Mata-Silva 2014). This pattern is similar to those found in other colubroids (Daza et al. 2009, Fontanella & Siddall 2010). One of these lineages irradiated to North America and another to South America, both largely speciating in these two regions. The two main lineages probably maintained the highest rate of speciation in Central America, since the two most diverse groups are the *calamarina* and *taeniata* species groups (represented by SC02 and SC03 in Fig. 1). Jowers et al. (2020) suggested that the time to the most recent common ancestor (TMRCA) of all species of *Tantilla* is dated to the Middle Miocene, at approximately 12 Mya. This date also represents the TMRCA of both main lineages. The same study indicates that the South American lineage started to diversify around 8.3 Mya and the *melanocephala* complex around 2.3 Mya. These time estimations combined with our results suggest that all the main cladogenic events within *Tantilla* happened before the complete closure of the Central American Seaway, around 3.5 Mya (Coates et al. 1992). If the species *T. tjiasmantoi* from Peru and the *melanocephala* complex diverged at 8.3 Mya (Jowers et al. 2020), this diversification happened before the the complete formation of land connection between South and Central America. Such scenario suggests that *Tantilla* was distributed in South America during the late Miocene (11.6 to 5.3 Mya), before the final rise of the Isthmus of Panama. An alternative scenario can be achieved if the lineage

of *T. tjiasmantoi* diversified from the *melanocephala* complex in Central American land masses, before the closure, and dispersed to South America together with the *melanocephala* complex, followed by its extinction in Central America. Both scenarios although plausible, involve several assumptions that are hard to test based on our dataset.

Although 3.5 Mya is the most accepted date for the closure of the Central American Seaway, Montes et al. (2015) argue for an earlier closure, between 15 and 13 Mya. If this alternative hypothesis is correct, the relative time of the cladogenic events estimated in our tree topology combined with the divergence times estimated by Jowers et al. (2020) agree with the geological scenario, suggesting that during the Late Miocene, the diversification of *Tantilla* happened in the South American continent.

However, to properly test all these biogeographic hypotheses, the phylogenetic affinities among the species of the *melanocephala* group and all other South American species need to be evaluated and historical biogeographic model-based analysis must be implemented.

The *melanocephala* species group

Our first attempt to clarify the taxonomy of the *melanocephala* group through the phylogenetic analysis of DNA sequences recovered *T. boipiranga* nested within the diversity of *T. melanocephala*, as the sister group of a clade formed by individuals from the Brazilian state of São Paulo. This result extends the recent contribution of Jowers et al. (2020) that sequenced five samples of *T. melanocephala* from northern South America and included only one sample from Brazil. Although their limited geographic sampling, the calibrated phylogenetic tree presented by Jowers et al. (2020) suggests that all northern South American samples grouped together in a clade. Our analysis based on an increased geographic sampling corroborates this result.

However, the inclusion of other populations and species from the *melanocephala* group unravelled the hidden diversity of *T. melanocephala*. This species is widely distributed, occupying a wide range of different environments and altitudes. Our results suggest the probable existence of more than one undescribed taxon, which are currently considered as morphotypes of *T. melanocephala*. Only a broadly and comprehensive study can shed light on the taxonomy of this continental species.

Our integrative analysis corroborates the current taxonomic status of *T. boipiranga*. All individuals identified as *T. boipiranga* clustered together in our phylogenetic tree, which supports the expansion of its geographic distribution. *Tantilla boipiranga* occurs in high and low elevations, limited to the east by the Espinhaço Range, in the states of Minas Gerais and Espírito Santo (Fig. 6). Based on the distributional records close to the limits among the states of Bahia, Minas Gerais and Espírito Santo (municipalities of Santa Maria do Salto and Pedro Canário), we consider that the species probably can be also found in the southern region of the state of Bahia.

Although its conservative general colour pattern, the species shows some intrapopulation variation in head colour (Fig. 6), and the temporal blotch laterally connected to the cephalic cap was found only in individuals from Espinhaço Range. The colour pattern observed in the type series was found in the new records presented here (municipalities of Santa Maria do Salto and Linhares), and despite the low variability, we observed the existence of a clinal variation on the number of ventral and subcaudal scales (more evident in females).

Our evaluation of the hemipenial morphology of *T. boipiranga* indicates a similar pattern to that described by Sawaya & Sazima (2003). However, the hemipenial differences between *T. boipiranga* and *T. melanocephala*, as suggested by these authors, was not evident in our analysis. Sawaya & Sazima (2003) indicated that *T. boipiranga* presents larger spines on the asulcate surface that are longer, stouter, and have proportionally narrower bases than in *T. melanocephala*. In addition, they commented that in *T. boipiranga* these spines are arranged irregularly and in *T. melanocephala* some spines form transverse rows. Based on six hemipenis of *T. melanocephala* and three of *T. boipiranga* we did not detect any of these differences. Despite being more elongated in *T. boipiranga*, we believe that the shape of the hemipenis alone is not a decisive character for the immediate diagnosis for the species.

Even after decades of advances in molecular tools and their remarkable ability to unveil the biodiversity (Hajibabaei et al. 2007), these methods have not been used in any attempt to investigate and clarify the taxonomy of *Tantilla* in Brazil. Although preliminary, our analysis allowed the phylogenetic positioning of *T. boipiranga*, the description of its morphological diversity, and contributed to a preliminary approach to the understanding of the systematics and biogeography of the genus.

Moreover, we believe that our study highlights the importance of long time field surveys in South America, where secretive and fossorial species can be collected. These studies are essential to elucidate unanswered questions related to taxonomy, natural history, biogeography and phylogenetic relationships of poorly known organisms. Indeed, our study was only possible due to the existence of protected areas in Brazil, where field surveys can still be properly performed. These state-managed areas – as the one surveyed in the present study – are crucial to obtain key information about Brazilian biodiversity, mainly because much of the ecosystems in south-eastern Brazil are already severely impacted by anthropic actions (Carvalho 2019, Rotta et al. 2020). These protected areas play multiple roles for the maintenance of the biodiversity and retain great potential to preserve the genetic and morphological diversity of rare species like *T. boipiranga*.

Acknowledgments

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Supplementary data

The following data are available online:

Supplementary Figure S1. Maximum likelihood tree based on the complete concatenated matrix.

Supplementary Table S1. Morphometric and meristic data from examined specimens of *Tantilla boipiranga*.

Supplementary Table S2. Records for *Tantilla boipiranga* and specimens examined in this study.

Supplementary Table S3. Accession numbers for all sequences analysed and voucher numbers for sequenced samples.

Supplementary Table S4. Primers used in the present study to amplify gene fragments.

Supplementary Table S5. Partitions and substitution models used in the RAxML analysis.