



## ECOSYSTEMS

# Morphological and biological variations of the glass snake *Ophiodes striatus* (Spix, 1825) (Squamata, Anguillidae) from southeastern Brazil

PAOLA R. DE OLIVEIRA, JULIANE FLORIANO LOPES SANTOS, JORGE ABDALA DERGAM DOS SANTOS & BERNADETE M. DE SOUSA

**Abstract:** Lizards of the *Ophiodes* genus, popularly known as glass snakes, are exclusively Neotropical. Despite their wide geographic distribution, glass snakes are poorly studied and there are many knowledge gaps in their biology and ecology to be filled. In this context, the objective of this study was to analyze possible sexual and population morphological differences of *Ophiodes striatus* specimens collected from different municipalities in the state of Minas Gerais, Brazil. A total of 63 specimens belonging to the Herpetological Collection of the Federal University of Juiz de Fora - Reptiles (CHUFJF-Reptiles), collected between 1995 and 2015 were analyzed. All the specimens were necropsied and morphometric measures related to body and head were measured. The males and females analyzed showed similar morphometric measures, and there was no size-related sexual dimorphism. Principal component analysis did not group the analyzed specimens by sex or locality. A positive correlation was observed between the snout-vent length and the vestigial limb length. The present study brings new information related to sexual dimorphism and population morphometric variation of *O. striatus*, adding information that will help in better understanding the ecology, biology and evolution of the genus in Brazil.

**Key words:** Lizards, morphometric, population variation, sexual dimorphism.

## INTRODUCTION

The lizards belonging to the *Ophiodes* Wagler, 1828 genus (Squamata, Anguillidae, Diploglossinae), popularly known as glass snakes, glass lizards or smash breaks, are exclusively Neotropical and distributed from the east of the Andes to the southeast of South America (Borges-Martins 1998, Pizzato 2005, Barros & Teixeira 2007, Montechiaro et al. 2011, Cacciali & Scott 2012, 2015, Oliveira et al. 2016, Ortiz et al. 2017, Entiauspe-Neto et al. 2017). Some morphological characteristics include a small pointed head, elongated body and tail, and an absence of external vestiges of anterior limbs with rudimentary posterior limbs (Barros

& Teixeira 2007, Montechiaro et al. 2011, Cacciali & Scott 2012, 2015).

The *Ophiodes* genus is currently composed of six species: *Ophiodes fragilis* (Raddi, 1820); *Ophiodes striatus* (Spix 1825); *Ophiodes vertebralis* Bocourt 1881; *Ophiodes intermedius* Boulenger 1894; *Ophiodes luciae* Cacciali & Scott 2015; *Ophiodes enso* Entiauspe-Neto et al. 2017. In addition to the species described above, three are morphospecies: *Ophiodes* sp. 1, *Ophiodes* sp. 2 and *Ophiodes* sp. 3 (Borges-Martins 1998, Pizzato 2005, Montechiaro et al. 2011, Cacciali & Scott 2012, 2015, Costa & Bérnils 2018, Oliveira et al. 2016, Entiauspe-Neto et al. 2017). However, taxonomic and phylogenetic review studies of

this group are lacking (Bernardo & Pires 2006, Barros & Teixeira 2007), causing it to still have several gaps.

In Brazil, the *O. striatus* species is found from mountainous areas of the southeast and center-west in the biomes Cerrado and Atlantic Forest (Barros & Teixeira 2007, Trindade et al. 2013). The morphological variations observed in *O. striatus* and its wide geographic distribution suggest the existence of a complex species (Borges-Martins 1998, Bernardo & Pires 2006, Barros & Teixeira 2007, Montechiaro et al. 2011). Studies with *O. striatus* in Brazil are limited to local information mainly on its diet, reproductive biology and herpetofauna surveys (Pizzatto 2005, Bernardo & Pires 2006, Quintela et al. 2006, Barros & Teixeira 2007, Barbo 2008, Salles & Silva-Soares 2010, Montechiaro et al. 2011, Zaher et al. 2011, Novelli et al. 2011, 2012, Sousa et al. 2010, 2012, Trindade et al. 2013, Lucas et al. 2016). Studies that analyze the ecological and biological variations of this species appear as a tool which contributes to the knowledge on the taxonomy and conservation of this group.

The morphological similarity between *O. striatus* specimens makes sexual determination based on external characteristics difficult. However, studies such as Pizzatto (2005) and Montechiaro et al. (2011) determined the sexual dimorphism from external morphometric analyzes. In this context, we investigated the existence of sexual dimorphism and variation related to external morphological measurements of *O. striatus* collected from different municipalities in the state of Minas Gerais (Brazil) using morphometric data.

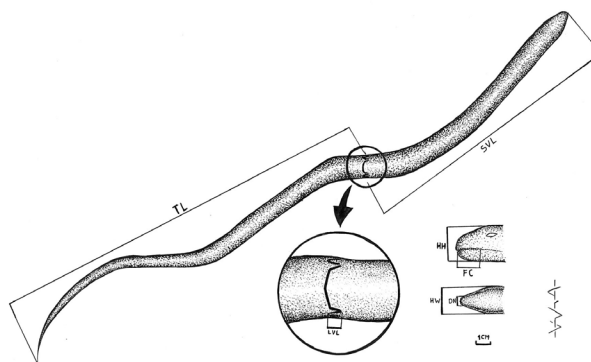
## MATERIALS AND METHODS

A total of 63 *O. striatus* specimens of the Coleção Herpetológica de Répteis da Universidade

Federal De Juiz De Fora - Reptiles (CHUFJF-Répteis) (Appendix 1) were used in the present study. The lizards were collected in fields surveys between 1995 and 2015, in the cities of Juiz de Fora, Santa Bárbara do Monte Verde, Chácara, Lavras, Ingaí, Ijaci, Ribeirão Vermelho, Coronel Xavier Chaves, Ritópolis, São João del Rei and Tiradentes, in the state of Minas Gerais (Brazil).

The sexual determination for adults and sub-adults was performed through macroscopic observation of gonadal traits by necropsy. Specimens which had body length between 47.24 mm and 63.19 mm were considered juveniles and were not necropsied; therefore, sexual identification was not performed.

The external morphometric measurements calculated were: snout-vent length (SVL), tail length (TL), length of the vestigial hind limbs (LVL – measured from left side), distance between nostrils (DN), face commissure (FC), head height (HH) and head width (HW), as shown in Figure 2. Due to the curvature of the animal's body, linear measurements of the body (SVL) and tail (TL) were measured with the line of a string. It was placed on the animal (Figure 1) and then extended to determine the size in millimeters



**Figure 1.** Relation of the morphometric variables measured in *Ophiodes striatus* belonging to the Coleção Herpetológica da Universidade Federal de Juiz de Fora - Reptiles (CHUFJF-Reptiles).

using a digital caliper. Tail length was measured on all specimens, including those having regenerated or fragmented tails (the portions being measured and then summed to define the total value of the tail length).

Some descriptive statistics were investigated such as the mean, standard deviation, and amplitude (maximum = M and minimum = m) for each variable, which were calculated by analyzing the measurements of males, females and juveniles of the whole sample. The size variation of the specimens belonging to most geographically distant (Juiz de Fora and Ritópolis) was also analyzed, aiming to investigate the population variation since these populations.

Principal Component Analysis (PCA) was performed in order to analyze whether the sample would be organized into groups. PCA was done to find out if the morphometric data showed groupings of the sample by sex (male x female) and by location (Juiz de Fora x Ritópolis). Principal Component Analysis (PCA) was performed to determine if variables influenced the formation of groups based on sex and location. PCA was performed separately for body variables (SVL, TL and LVL;  $n = 50$ ) and head (DN, FC, HH and HW;  $n = 45$ ). Both analyzes were performed to determine whether these variables influenced grouping by sex or location. The Pearson Correlation test was performed to verify the correlation between the morphometric data. Both statistical tests were performed in Statistical Program R, version 3.4.2 (Vincent 2011, Paradis & Schliep 2018, Makiyama 2018, Oksanen et al. 2019, Wickham et al. 2019).

## RESULTS

Fifty-five (55) of the 63 *O. striatus* specimens belonging to CHUFJF-Reptiles were young/adult, while eight were juveniles. Among the young/

adult specimens, it was possible to identify the sex in 50 specimens (31 males and 19 females).

From the 19 necropsied females, four were pregnant with embryos at some gestational stage, and the specimens were collected between June and September. The morphometric measures were only verified in the individuals that were in an advanced gestational stage, with complete formation (Table I). The mean litter size was 4.50 ( $\pm 1.26$ ,  $n = 4$ ). The specimen that had the largest litter ( $n = 6$ ) had SVL of 196.18 mm, while the smallest litter ( $n = 3$ ) had SVL of 192.03 mm.

The mean SVL of males and females was similar, and no apparent differences were observed between their sizes (Table I). A similar result was observed when the analysis was performed with the specimens belonging to the two most geographically distant (Juiz de Fora and Ritópolis) (Table II).

There was no sex grouping in either PCA (Figure 2a, b). PC1 explained 51.1% of the variation observed, and PC2 explained 31.8%, with the accumulated proportion of 82.82% when using body measures (Figure 2a). When using head measures, PC1 explained 57.1% and PC2 17.5% of the variation observed, with a cumulative proportion of 89.35% (Figure 2b).

According to the PCA data, it was also not possible to separate the populations from Juiz de Fora ( $n = 10$ ) and Ritópolis ( $n = 17$ ), indicating that both populations have similar size variation (Figure 3). PC1 explained 92.3% of the variation found in the sample, while PC2 only explained 5.5% (cumulative ratio = 97.8%) (Figure 3).

As there was no grouping of males and females in PCA, Pearson's correlation test was performed with the whole sample without distinction by sex. The correlation indicated a positive and moderate relationship ( $r = 0.46$ ) between SVL and LVL (Figure 4), meaning that the higher the SVL, the higher the LVL.

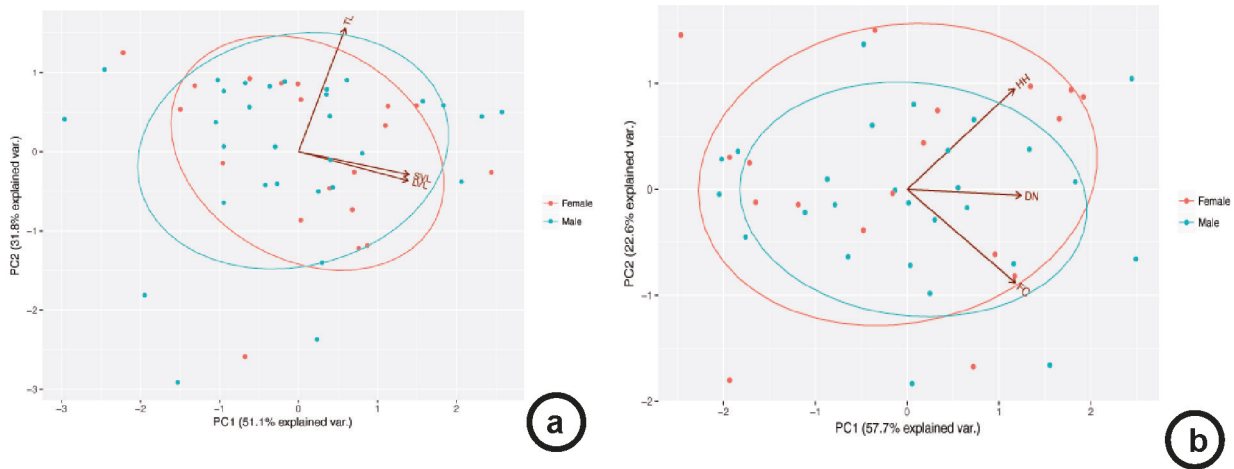
**Table I.** Means calculated for the snout-vent length (SVL), tail length (TL), length of the vestigial hind limbs (LVL), distance between nostrils (DN), face commissure (FC), head height (HH) and head width (HW) of the females, males and offspring of *Ophiodes striatus* belonging to the Coleção Herpetológica da Universidade Federal de Juiz de Fora, Minas Gerais - Reptiles (CHUFJF-Reptiles).

Variable* (mm) Sex	SVL	TL	LVL	DN	FC	HH	HW
Female	163.21 ±25.77 M=217.21 m=117.22 (n=19)	173.24 ±68.26 M=269.24 m=34.01 (n=19)	4.21 ±0.82 M=6.28 m=2.44 (n=19)	2.23 ±0.38 M=2.82 m=1.39 (n=18)	9.20 ±1.15 M=11.54 m=7.47 (n=18)	6.07 ±0.99 M=8.00 m=4.2 (n=18)	6.80 ±0.84 M=8.57 m=5.45 (n=18)
Male	156.20 ±16.64 M=197.17 m=121.35 (n=31)	177.51 ±78.21 M=309.51 m=24.71 (n=31)	4.24 ±0.88 M=6.37 m=2.49 (n=31)	2.32 ±0.41 M=3.45 m=1.69 (n=27)	9.45 ±1.29 M=12.9 m=7.43 (n=27)	5.94 ±0.72 M=7.45 m=4.86 (n=27)	7.10 ±0.85 M=9.16 m=5.02 (n=27)
Juveniles** (n=8)	53.14 ±4.65 M=63.19 m=47.24	66.30 ±9.10 M=77.40 m=46.68	1.54 ±0.27 M=1.92 m=1.25	1.39 ±0.19 M=1.67 m=1.1	5.53 ±0.16 M=1.67 m=5.26	2.70 ±0.17 M=3.03 m=2.5	3.52 ±0.20 M=3.75 m=3.14

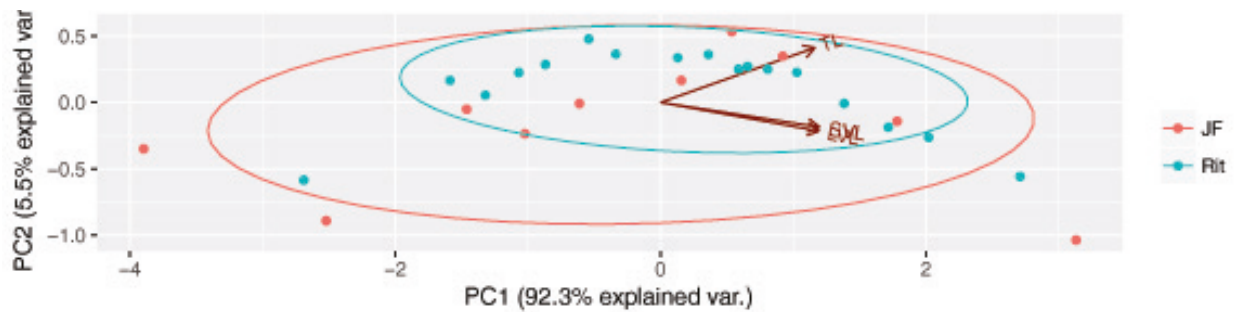
\* The number of specimens analyzed ("n" sampled) is simulated below each variable. \*\*Embryos not included in this analysis.

**Table II.** Means calculated for the snout-vent length (SVL), tail length (TL), length of the vestigial hind limbs (LVL) of the females and males specimens of *Ophiodes striatus* belonging to the Federal University of Juiz de Fora - Reptiles (CHUFJF-Reptiles) collected in previous studies in the municipalities of Juiz de Fora and Ritópolis - Minas Gerais, Brazil.

Locality / Sex Variable (mm)	Juiz de Fora		Ritópolis	
	Female (n=7)	Male (n=3)	Female (n=7)	Male (n=10)
SVL	160.64 ±22.57 M=196.18 m=129.47	136.74 ±9.72 M=146.45 m=127.01	169.70 ±18.27 M=192.03 m=138.91	154.88 ±15.83 M=187.11 m=139.5
TL	152.95 ±81.86 M=238.97 m=34.01	135.55 ±102.96 M=246.02 m=42.25	190.93 ±67.73 M=269.24 m=96.68	182.45 ±71.08 M=293.67 m=45.14
SVL	4.34 ±0.93 M=6.28 m=3.58	4.24 ±0.82 M=4.12 m=2.49	4.07 ±0.60 M=4.94 m=3.29	4.50 ±0.99 M=6.37 m=3.58



**Figure 2.** Graph of Principal Component Analysis (PCA) showing the variation of the snout-vent length (SVL), tail length (TL) and length of the vestigial hind limbs (LVL) (a, n = 50) and distance between the nostrils (DN), face commissure (FC), head height (HH) and head width (HW) (b, n = 45) (morphometric variables signaled by the brown arrow) in relation to sex (male in blue and female in pink) of *Ophiodes striatus*.



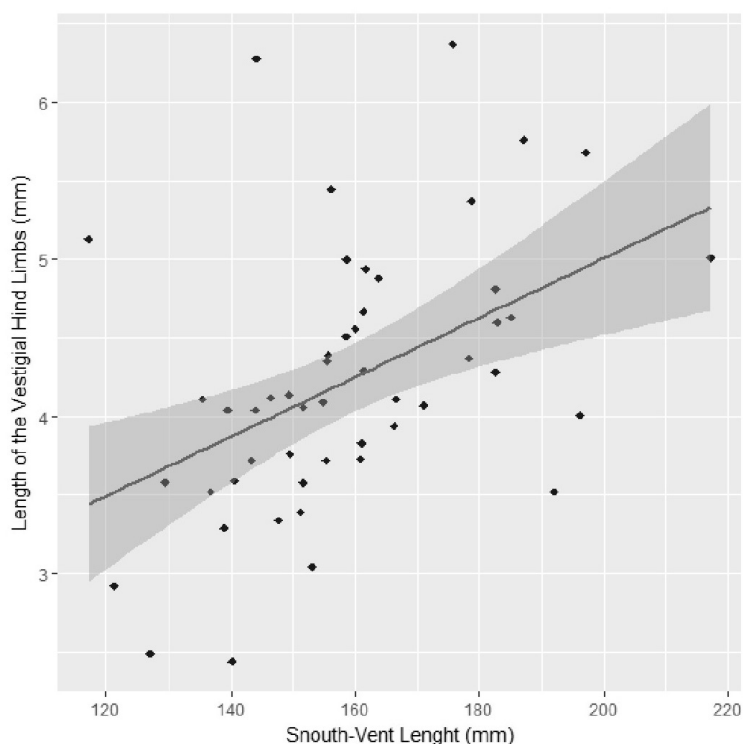
**Figure 3.** Graph of Principal Component Analysis (PCA) showing the variation of the snout-vent length (SVL), tail length (TL) and length of the vestigial hind limbs (LVL) (morphometric variables indicated by the brown arrow) in relation to the population variation (Ritópolis specimens in blue and Juiz de Fora specimens in pink) from *Ophiodes striatus* (n = 27).

**DISCUSSION**

*Ophiodes striatus* morphometric data analysis indicated the non-existence of sexual dimorphism associated with body size. However, the studies by Barros & Teixeira (2007) and Montechiaro et al. (2011) with with different populations of *O. striatus* from Brazil (Espírito Santo and Rio Grande do Sul, respectively) recorded significant differences between females and males, indicating a possible dimorphism related to body size (SVL) and of the tail (TL). The females were significantly larger than males

in both studies, which is common in many lizard species belonging to different families (Pianka & Vitt 2003). Our results on the existence of sexual size dimorphism in *O. striatus* stand in contrast to that of previous studies and suggest that sexual size dimorphism cannot be used to distinguish all populations of *O. striatus*. Analyzes using other parameters, besides the variables used in this study, may be an alternative to clarify the determination of external sexual dimorphism in this species.

No difference was observed in the tail length between the sexes, differing from that



**Figure 4. Pearson's Correlation between snout-vent length (SVL - x) and length of the vestigial hind limbs (LVL - y) of *Ophiodes striatus* (n = 50). Correlation (x, y) = 0.4587 (45.87%).**

found by Montechiaro et al. (2011). We point out that this could be due to many males having undergone autotomy. Males with longer tails were expected according to the Morphological Restriction hypothesis of King (1989), as longer tails in males are usually related to the accommodation of hemipenis and its retractor muscles (Montechiaro et al. 2011). No difference was found in measures related to the head (labial commissure and head width). However, males with larger heads than females may be related to the aggressive interactions between males (Blackburn & Vitt 1992); moreover, males and females have different eating habits, preferentially feeding on different prey items (Montechiaro et al. 2011).

The study of vestigial limb size variation is not common, despite its great evolutionary and adaptive meaning for the genus. Montechiaro et al. (2011) also observed that males had larger vestigial members than females, which was not observed in the analyzes of this study. However,

body length (SVL) and vestigial hind limbs (LVL) are positively correlated; meaning that the limbs grow simultaneously along with the body, being this fact an indication of functionality of this structure. This characteristic may not only be related to the use of these structures for sexual stimulation during mating, but also to the use in fighting between males (Carpenter et al. 1978, Montechiaro et al. 2011). Another function for this structure is currently registered in which both sexes used this limb to facilitate their locomotion over smooth surfaces (Oliveira et al. in preparation).

When studying an adult *O. striatus* female collected at the Universidade Federal de Ouro Preto (Minas Gerais, Brazil), Bernardo & Pires (2006) found fourteen juveniles with mean SVL of 4 mm. In analyzing different biological aspects of *O. striatus* from Santa Teresa (Espírito Santo, Brazil), Barros & Teixeira (2007) observed a mean clutch size of 6.0 (ranging from three to eleven embryo/fetuses). In *O. striatus* specimens

studied by Montechiaro et al. (2011) belonging to different museums and collections of Rio Grande do Sul (Rio Grande do Sul, Brazil), the mean clutch size was 7.96 ( $n = 51$ ), mean SVL was 51.64 mm ( $n = 22$ ). The mean litter size calculated for *O. striatus* of CHUFJF-Reptiles was 4.50 ( $n = 18$ ), lower than that observed in the previously described studies.

Although a separation between the Juiz de Fora and Ritópolis populations was expected, this was not found (Figure 4). Morphological variations in natural populations are driven by evolutionary forces (Oliveira et al. 2016), and given that these populations are geographically distant, similar sizes of measured traits of individuals within these populations indicates their consistency as distinct populations of the same species. However, this fact will only be confirmed by complementary studies, such as genetic studies.

The present study presents new information on the biological characteristics of *O. striatus*. Despite the diversity of analyzed locations, no significant variation was observed. The results showed that there is no sexual dimorphism related to size, although it has been observed in previous studies. Our study indicated that the use of morphometric analysis of external morphology may not be applicable to sexual dimorphism in some species of reptiles such as the present study, since the external morphology may be influenced by different biotic and abiotic factors.

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## APPENDIX 1

Specimens examined in the present study belonging to the Herpetological Collection of the Federal University of Juiz de Fora - Reptiles (CHUFJF-Reptiles)

Brasil: Minas Gerais: Ritópolis (CHUFJF – Reptiles: 205, 310, 343, 455, 456, 1641, 1642, 1643, 1644, 1645, 1646,1647, 1648, 1649, 1650, 1651, 1652,1653); São João Del Rei (CHUFJF – Reptiles: 1098, 1099); Tiradentes (CHUFJF – Reptiles: 776, 777, 784, 785); Juiz de Fora (CHUFJF – Reptiles: 28, 29, 35, 36, 52, 294, 309, 961, 922, 1066); Reserva Particular do Patrimônio Natural da Serra Negra - RPPNSN (CHUFJF – Reptiles: 1654, 1668); Reserva Biológica UniLavras - Boqueirão (CHUFJF – Reptiles: 1334, 1337, 1346,1392, 1395, 1401, 1402, 1403, 1404, 1405, 1406, 1415, 1430); Lavras (CHUFJF – Reptiles: 1173, 1376, 1391, 1413); Ribeirão Vermelho (CHUFJF – Reptiles: 1387, 1422, 1423, 1424); Coronel Xavier Chaves (CHUFJF – Reptiles: 1108) ; Ijaci (CHUFJF – Reptiles: 1443); Chácara (CHUFJF – Reptiles: 913, 914, 915, 916).



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**PAOLA R. DE OLIVEIRA<sup>1,2</sup>**

<https://orcid.org/0000-0001-6725-7693>

**JULIANE FLORIANO LOPES SANTOS<sup>1</sup>**

<https://orcid.org/0000-0001-7481-3109>

**JORGE ABDALA DERGAM DOS SANTOS<sup>3</sup>**

<https://orcid.org/0000-0003-1395-1377>

**BERNADETE M. DE SOUSA<sup>1,2</sup>**

<https://orcid.org/0000-0002-0815-7876>

<sup>1</sup> Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza, Universidade Federal de Juiz de Fora, Campus Universitário, São Pedro, 36036-900 Juiz de Fora, MG, Brazil

<sup>2</sup> Universidade Federal de Juiz de Fora, Laboratório de Herpetologia, Campus Universitário, São Pedro, 36036-900 Juiz de Fora, MG, Brazil

<sup>3</sup> Universidade Federal de Viçosa, Departamento de Biologia Animal, Centro de Ciências Biológicas e da Saúde, Campus Universitário, Centro, 36570-000 Viçosa, MG, Brazil

Correspondence to: **Paola R. de Oliveira**

*E-mail: paolarooliveira91@gmail.com*

**Author contributions**

PRO, JADS and BMS conceived and designed the study; PRO conducted data collection and analysis; PRO and JFLS conducted the statistical analyses; all authors wrote, read and approved the final version of the manuscript.

