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RESEARCH ARTICLE

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Reproductive maturity and sexual dimorphism of a population of *Amerotyphlops brongersmianus* from a Restinga area in southeastern Brazil (Serpentes: Typhlopidae)

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Abstract

Natural history data are important for a better understanding of distinct aspects of snake biology, and this information in scarce on Scolecophidia. Here we focus on sexual maturity and sexual dimorphism within a population of *Amerotyphlops brongersmianus* from the Restinga de Jurubatiba National Park, Rio de Janeiro state, Brazil. The smallest sexually active male and female showed snout-vent length of 117.5 and 158.4 mm, respectively. Females had statistically significant larger body and head length sizes, whereas males had longer tails. Juveniles showed no sexual dimorphism for any analyzed feature. Secondary vitellogenic follicles had a more opaque, yellowish/darker aspect, being larger than 3.5 mm. We reinforce that in addition to traditional features for determining sexual maturity, morphological and histological characteristics of kidneys should be evaluated in males, as well as the morphology of the infundibulum in females. Histological data show development of seminiferous tubules and presence of spermatozoa in males, and infundibulum receptacles and uterine glands in females as a sign of sexual maturity. This type of information is essential for a more accurate description of data on sexual maturity, allowing access to information on the development of reproductive structures that are not available macroscopically.

KEYWORDS

reproductive morphology, Scolecophidia, snake anatomy

1 | INTRODUCTION

Scolecophidia Cope, 1864 comprises small-sized snakes with fossorial habits and conserved morphology (Hedges et al., 2014). Many lineages are rare in herpetological collections (Francisco et al., 2012; Passos et al., 2006), with descriptions generally based on a small number of individuals, resulting in little knowledge on issues related to the systematics and biology of this group (Francisco et al., 2018; Pinto et al., 2010; Pyron & Wallach, 2014).

Thirty-four species of Scolecophidia occur in Brazil (Costa et al., 2022), of which six are allocated in the genus *Amerotyphlops* (Typhlopidae) as proposed by Hedges et al. (2014). The type species, *Amerotyphlops brongersmianus* (Vanzolini, 1976), has brownish, yellow-brownish, or red-brownish color (Dixon & Hendricks, 1979), 20 scales

around the body (Hedges et al., 2014), and inhabits different biomes in Brazil (Wallach et al., 2014), including Restinga areas (Martins et al., 2010). Restinga areas are part of the Atlantic Rainforest, and are known to have herbaceous/shrubby coastal sand-dune habitats that cover most of Rio de Janeiro State coast (Myers et al., 2000; Rocha et al., 2007).

A. brongersmianus shows a general reproductive system similar to other snakes and representatives of Scolecophidia (Khouri et al., 2019; Siegel et al., 2011). Histological variations in reproductive organs were recorded throughout the year in a population from a Restinga area in Brazil (Khouri et al., 2019), and fecundity was found to vary in this population compared to other described populations (e.g., Ávila et al., 2006).

Anatomical and reproductive features are important to describe and characterize snake species and populations (Almeida-Santos et al., 2014; Mathies, 2011). Data on sexual maturity are fundamental for reproductive studies (Almeida-Santos et al., 2014), as well as sexual dimorphism, which might be important to recognize specific characteristics and behavior of individuals of distinct sexes. These data are also vital for studying the evolution of species through comparisons between populations (Caiacedo-Portilla, 2011).

Sexual maturity is usually defined and described with a few characters, such as testes volume and opaque ductus deferens in males, and thickened oviduct and presence of secondary follicles in females (Ávila et al., 2006; Shine & Webb, 1990; Webb et al., 2001). However, these individual morphological variations are occasionally undetectable and histological data are essential to obtain complete information on sexual maturity (Parpinelli & Marques, 2015; Shea, 2001).

Whereas characteristics of reproductive biology may interfere with snake sexual dimorphism (Weatherhead et al., 1995), body modifications might be a result of maternal quality selection or male mating success (Rivas & Burghardt, 2001; Shine et al., 1999, 2000). Studies on Scolecophidia and Typhlopidae natural history are scarce (Böhm et al., 2013), and sexual dimorphism usually follows the general snake pattern, with longer tails in males and larger body and head sizes in females (Perry, 1984; Shine, 1991, 1994, 2003). It is also known that usually in Scolecophidians, males reach sexual maturity at smaller body sizes than females (Khouri et al., 2019; Parpinelli & Marques, 2015; Shine & Webb, 1990; Webb et al., 2000). However, this information is hardly assessed due to the small size of the group, especially in younger individuals (Shine & Webb, 1990; Webb et al., 2001).

In this study, we analyze a population of *A. brongersmianus* from Restinga de Jurubatiba Natural Park, Rio de Janeiro state, Brazil, aiming to characterize sexual dimorphism and determine sexual maturity based on morphological and histological data.

2 | MATERIALS AND METHODS

We analyzed 124 individuals of *A. brongersmianus* from Parque Nacional da Restinga de Jurubatiba (PARNA), municipality of Carapebus, Rio de Janeiro state (220 00′–220 25′ S, 410 50′–410 75′ W), collected every season from Summer 2010 to Autumn 2017. All specimens are housed in the herpetological collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil (Appendix 1). The following external measurements were taken with an analogical dial caliper to the nearest 0.02 mm: head length (HL), head width (HW), and tail length (TL). Snout-vent length (SVL) and body circumference of the posterior portion of the body (Circ.) were measured with a flexible ruler to the nearest millimeter. After measurements, specimens were dissected through a ventral incision in the posterior third of the body for anatomical analyses. Specimens that were previously dissected in the collection were not measured so as not to bias statistical results.

Anatomical description was based on Khouri et al. (2019) and internal organs were observed in 94 individuals (47 males and 47 females). Specimens with no obvious gonads' development were considered immatures and categorized as juveniles, whereas for those with reduced body size and also with gonads, sexual maturity was determined through evaluation of the development of kidneys and testes, combined with the color and thickness of ductus deferens in order to establish the smallest sexually mature male. We also analyzed histological data from these organs in 32 males. Length, width, and thickness of testes were measured in order to determine the testicular volume (TV), inferred from the ellipsoid formula ($TV = 4/3.\pi abc$), where a = half of the length, b = half of the width, and c = half of the thickness (Pleguezuelos & Feriche, 1999). The right kidney and caudal portion (~2 cm) of the ducti deferentia were also analyzed. Measurements were taken with a dial caliper to the nearest 0.02 mm.

Seminiferous tubules were characterized as proposed by Goldberg and Parker (1975) from one (totally regressed testes) to six (early regression). Stage 0 corresponded to nonmature males according to Khouri et al. (2019). Individuals with no or reduced TV (smaller than 2.5 mm^3) and ductus deferens smooth, translucent, and equal or smaller than 0.2 mm were considered juveniles (see Section 3). To establish the smallest sexually mature female, we assessed the development of the oviduct and

presence or absence of ovarian follicles on both sides, as well as histological data from the infundibulum, uterus, and pouch of 30 females. Secondary vitellogenesis was considered for follicles larger than 3.5 mm according to Khouri et al. (2019), and two follicles with different sizes were collected and analyzed histologically.

Relative growth rate from hatchling to maturity and from maturity to total length was estimated following Parker and Plummer (1987). As there were no newborn individuals, we analyzed the proportion between SVL of the smallest juvenile/SVL of the smallest sexually mature individual, and the proportion between SVL of the smallest sexually mature individual/SVL of the largest individual, for both sexes.

Sexual size dimorphism index (SSD) was calculated [(mean adult SVL of the largest sex/mean adult SVL of the smallest sex) − 1.0], the index being positive when females are larger, and negative if males are larger (Lovich & Gibbons, 1992). In order to verify the presence or absence of secondary sexual dimorphism we performed a Student's *t* test with significance level of 0.05 (Zar, 1999), evaluating the following variables: SVL, TL, Circ., HL, and HW. We also performed covariance analyses (ANCOVA) considering these features (except SVL) as dependent variables, sex as an independent categorical variable, and SVL as the covariate, to evaluate if the mean values of a dependent variable are distinct between sexes, independent of SVL. Specimens were considered adults or juveniles according to data obtained in the present study (see Section 3) and we tested secondary sexual dimorphism for both categories. Assumptions of homogeneity and normality were tested by Levene and Kolmogorov–Smirnov tests, respectively. In cases where characters showed no sufficient variation to justify these assumptions non-parametric tests (e.g., Mann–Whitney) were performed (Zar, 1999). All analyses were performed in R (R Core Team, 2021).

Histological techniques were applied following Junqueira et al. (1979). Sections were cut at 5 μm thick in a Leica RM2245 microtome. Slides were analyzed using a Zeiss Axio Imager.A2 microscope and the AxioVision 4 software.

3 | RESULTS

3.1 | Sexual maturity

Histological data were obtained from 30 females (2 juveniles) and 32 males (6 juveniles) (Data S1). The smallest sexually mature male measured 117.5 mm SVL (MNRJ 25359), with a ductus deferens 0.3 mm and testes of volume 2.07 mm^3 on the right side and 2.32 mm^3 on the left. Testes showed spermatozoa production, with seminiferous tubules in stage 6 and 1 (terminal stage of epithelium regression), and secretion in ductus deferens (Figure 1a,b). This specimen and all sexually active males showed a developed sexual segment of the kidneys (SSK) (Figure 1c) and kidneys were slightly texturized, this feature being noticed grossly. Seminiferous tubules were reduced (diameter = 129.61 μm; thickness = 26.29 μm) in this individual compared to other sexually active males (diameter mean size = 269.3 μm ± 89.55; thickness mean size = 46.92 μm ± 19.08). The general aspect of ductus deferens was translucent (vs. opaque in other mature males, found mostly in individuals collected during winter), although they were coiled and showed whitish secretions, which are evidence of semen production. All testes bigger than 2.5 mm^3 were associated with coiled ductus deferens. Individuals with SVL higher than 117.5 mm but with no coiled ductus deferens were also considered adults (Figure 2).

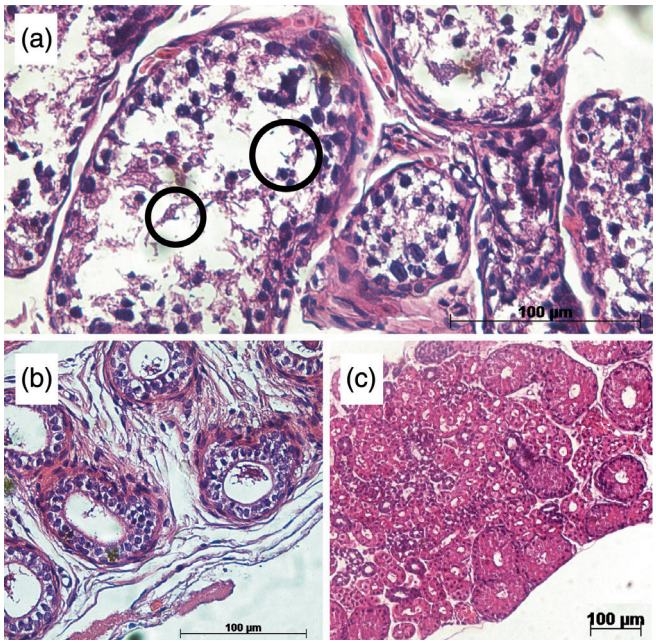

FIGURE 1 Smallest sexually mature male. (a) Seminiferous tubules; (b) sperm in ductus deferens; (c) developed sexual segment of the kidneys (SSK).

The smallest sexually active female in secondary vitellogenesis was 158.4 mm SVL (MNRJ 26533), with a slightly pleated infundibulum, opaque and pleated uterus, and the largest follicles were 4.52 and 3.8 mm on the right and left sides, respectively. We also found a female in primary vitellogenesis with 133.2 mm SVL (MNRJ 23185), smooth oviduct and follicles measuring 2.9 and 0.3 mm on the right and left sides, respectively. Both females were considered adults, the latter probably in the first year of sexual activity due to the presence of

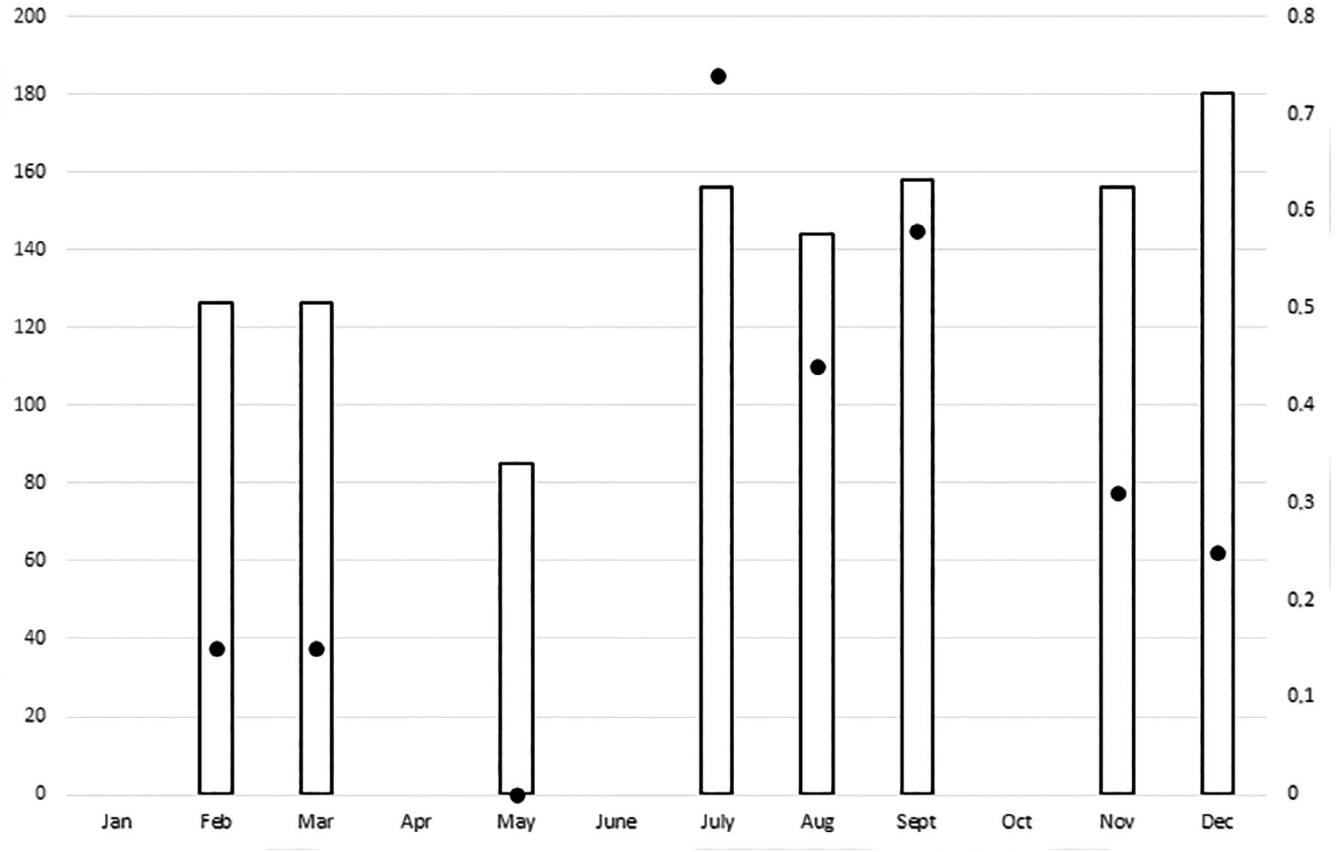

FIGURE 2 Mean snout-vent length (SVL) (bars, left axis) and mean ductus deferens diameter (dots, right axis) throughout the year. All measures are in millimeters (mm).

some developed structures in the oviduct, such as sperm receptacles and uterine glands (Figure 3a). The largest juvenile female, with translucent oviduct and no follicles, was 135.1 mm SVL (MNRJ 25297).

The infundibulum was generally grossly pleated in adults ($n = 21$), smooth in juveniles ($n = 13$), and smooth in some adults ($n = 4$). Adults with smooth infundibulum showed ovarian follicles with receptacles visible by histological analysis (Figure 3b). The uterus of juveniles was narrow, with almost no free space in the lumen, and with a reduced epithelium (Figure 3c), whereas adult females possessed pleated and opaque oviduct, developed uterine glands, and enlarged lumina (Figure 3d). Female's pouch showed no variation between juveniles and adults.

3.2 | Morphometry and sexual dimorphism

We analyzed a total of 55 females (43 adults and 12 juveniles), 69 males (60 adults and 9 juveniles) and 3 juveniles whose sex could not be determined. Raw data from these individuals are showed in Data S2.

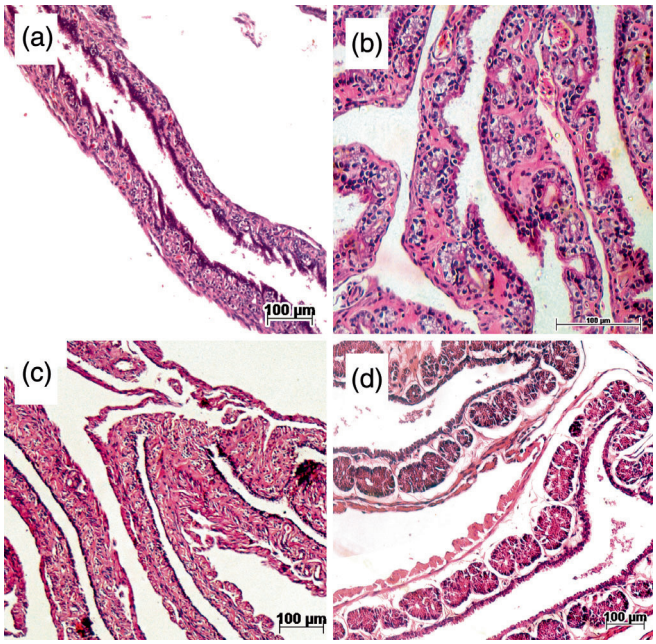

FIGURE 3 (a) Developed uterine glands in a small (133.2 mm) female (MNRJ 23185); (b) infundibulum with receptacles (MNRJ 23180); (c) uterus of a juvenile, without developed glands (MNRJ 23167); (d) uterus of a mature adult, with developed glands and enlarged lumen cavity (MNRJ 26520).

We identified size variation between juveniles from the first ($n = 18$) and second ($n = 6$) half of the year. The smallest individuals were 74.5 and 77.6 mm SVL, for females and males, respectively (Figure 4), both from February. The largest juvenile was a female collected in March with 135.1 mm SVL. In adults, the smallest female showed 133.2 mm SVL in August, and largest females measured 250.5 and 255.7 mm SVL in August and September, respectively. The smallest male measured 117.5 mm SVL in September, and largest males were 212.6 and 208 mm SVL in November and December, respectively.

The smallest juveniles measured 47 and 65% of the SVL of the smallest sexually active female and male, respectively. Females and males reached sexual maturity at 61 and 55%, respectively, of the SVL of the largest sexually corresponding adult.

The positive SSD (=0.21) shows that females are generally larger than males in this population. Females showed higher values of SVL ($u = 2061$; $p = 0.01$; $n = 103$), whereas males had longer tails ($t = -2.5484$; $p < 0.01$; $n = 103$), even considering females of the same SVL ($F_{1,99} = 38.847$; $p = 0.001$; $n = 103$). Females had longer heads ($t = 5.0407$; $p < 0.01$; $n = 101$) than males, but this difference was not significant considering SVL as a covariate ($F_{1,97} = 0.026$; $p = 0.872$; $n = 101$) (Table 1). Juveniles showed no significant sexual dimorphism for any analyzed feature. The ANCOVAs showed no significant interaction between sex and SVL for any dependent variable (for both adults and juveniles).

3.3 | Vitellogenesis

All observed follicles smaller than 3.5 mm were similar to non-vitellogenic follicles, with lighter color and occasionally translucent aspect (Figure 5a). A female (MNRJ 23168) showed right and left follicles in the stage of primary vitellogenesis measuring 2.37 and 2.74 mm, respectively (Figure 5b), with total absence of yolk and a more basophilic cytoplasm.

Follicles larger than 3.5 mm showed a more opaque, yellowish/darker aspect (Figure 5c), like follicles in secondary vitellogenesis. Some females (MNRJ 25365 and MNRJ 26514) had follicles in secondary vitellogenesis larger than 4.5 mm in both infundibulum, with numerous yolk droplets all over the follicle, indicating lipid storage (Figure 5d).

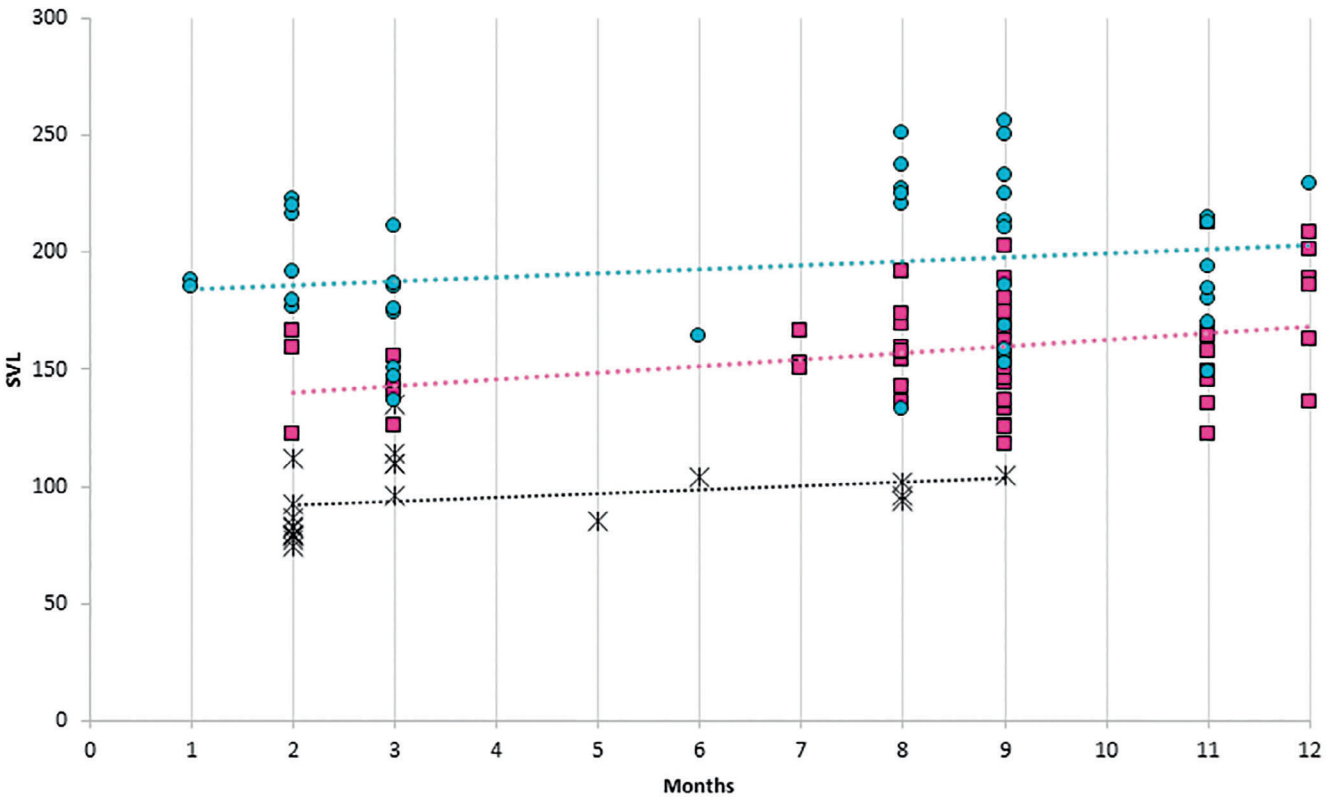

FIGURE 4 Snout-vent length (SVL) from juveniles (asterisk), females (blue circles), and males (pink square) of *Amerotyphlops brongersmianus* throughout the year.

		Mean ± SD (*n*)	Minimum–maximum	
SVL	A	(F) 192.75 ± 32.85 (43) (M) 158.03 ± 22.40 (60)	(F) 133.2–255.65 (M) 117.45–212.65	$u = 2061$; $p < 0.01$
	J	(F) 92.48 ± 17,78 (12) (M) 98.45 ± 12.23 (9)	(F) 74.45–135.1 (M) 77.6–114	n.s.
TL	A	(F) 5.41 ± 1.08 (43) (M) 5.96 ± 1.07 (60)	(F) 3.2–8.24 (M) 3.35–8.1	$t = -2.5484$; $p = 0.01$
	J	(F) 3.06 ± 0.44 (12) (M) 3.27 ± 0.56 (9)	(F) 2.4–3.9 (M) 2.4–4	n.s.
Circ.	A	(F) 23.29 ± 5.31 (40) (M) 19.55 ± 3.17 (57)	(F) 14.4–34.3 (M) 13.3–29.85	$u = 1{,}633.5$; $p < 0.01$
	J	(F) 12.16 ± 1.95 (12) (M) 12.46 ± 1.03 (8)	(F) 9.7–16.75 (M) 11.45–14.6	n.s.
HL	A	(F) 8.42 ± 1.11 (42) (M) 7.43 ± 0.86 (59)	(F) 6.6–11.4 (M) 5.35–9.95	$t = 5.0407$; $p < 0.01$
	J	(F) 5.51 ± 0.44 (12) (M) 5.41 ± 0.47 (9)	(F) 4.9–6.4 (M) 4.5–6.0	n.s.
HW	A	(F) 5.47 ± 0.85 (42) (M) 4.71 ± 0.59 (59)	(F) 3.8–6.95 (M) 3.66–6.35	$u = 1880.5$; $p < 0.01$
	J	(F) 3.23 ± 0.4 (12) (M) 3.41 ± 0.44 (9)	(F) 2.8–4.1 (M) 2.85–4.2	n.s.

TABLE 1 Variation and sexual dimorphism in *Amerotyphlops brongersmianus*.

Note: Snout-vent length (SVL), and tail length (TL), body circumference of the posterior portion of the body (Circ.), head length (HL) and head width (HW) from adults (A) and juveniles (J) females (F) and males (M). All measurements are in millimeters (mm).
Abbreviations: *n*, number of individuals; n.s., non-significant; SD, standard deviations.

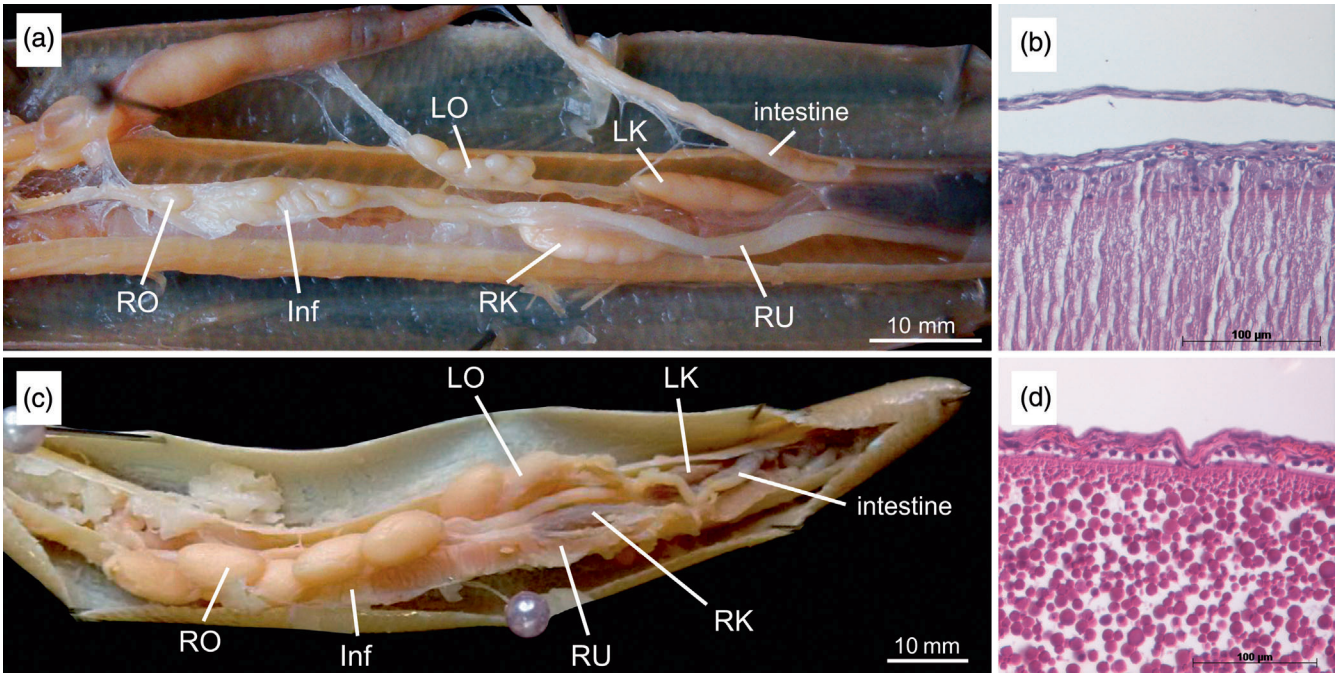

FIGURE 5 Reproductive system and follicles of females. (a) Female in primary vitellogenesis and (b) primary vitellogenesis follicle; and (c) secondary vitellogenesis female and (d) secondary vitellogenesis follicle. Inf, infundibulum; LK, left kidney; LO, left ovary; RK, right kidney; RO, right ovary; RU, right glandular uterus.

4 | DISCUSSION

Juveniles of *A. brongersmianus* with determined sex showed no sexual dimorphism. Despite our relatively small sample size for juveniles (12 females and 9 males), previous studies corroborate this result, pointing to the fact that the degree of sexual dimorphism may vary depending on body size, even intraspecifically (Abegg et al., 2020; King et al., 1999; Webb et al., 2001). Sexual dimorphism was observed in adults, corroborating other studies with Scolecophidia where dimorphism is accentuated in larger and mature individuals (Shine & Webb, 1990; Webb et al., 2000, 2001). Traditionally, sexual maturity is determined based only on testes volume and color of ductus deferens in males, and the presence of secondary follicles or eggs in females. In species that show no variation in testes volume, the diameter of ductus deferens is a good parameter (Parpinelli & Marques, 2015). However, it is worth mentioning that, aside from these traditional approaches, kidney gross anatomy, the development of seminiferous tubules, and SSK are fundamental to achieve sexual maturity in males, as these structures show reproductive importance and variation throughout the year (Khouri et al., 2019).

Coiled and opaque ductus deferens indicate presence of spermatozoa and were present in all males with enlarged testicles (>2.5 mm^3) and other individuals from Winter and Spring (Khouri et al., 2019). The torsion of this structure points to spermatozoa storage (Almeida-Santos et al., 2006), meaning that the specimen was sexually active, even if spermatozoa production has already ended at that time. Three individuals from Spring had smooth and translucent ductus deferens (Khouri et al., 2019), but were smaller than 117.5 mm SVL, and considered juveniles.

Our results indicate that a pleated infundibulum in females represents an important indicator of sexual maturity, at least for this species, since as far as we know there is no information in the literature regarding this issue for other scolecophidians. This feature as an indicator of sexual maturity was also found in some species of the genus *Bothrops* (Barros et al., 2020; Silva et al., 2019). Based on these results we highlight that females with enlarged follicles, but with a smooth infundibulum might not have had their first reproductive event. As in other snakes, the presence of receptacles in the infundibulum, the opacity of the uterus, as well as the development of uterus glands and the presence of spermatozoa in the oviduct are also indicatives of sexual maturity (Almeida-Santos et al., 2014).

Values inferred here for sexual maturity were smaller (male = 117.5 mm; female = 158.4 mm) than those of Ávila et al. (2006) (male = 180 mm; female = 211 mm). As these authors did not determine the size for vitellogenesis, and there is no histological information on reproductive structures, this difference could be due to a distinct method of maturity determination (Barros et al., 2020). Nevertheless, Ávila et al. (2006) also found larger SVL mean sizes of males and females in the population from Mato Grosso do Sul state (227.18 ± 24.83 and 241.91 ± 20.88, respectively) compared to the present work (158.03 ± 22.20 and 192.75 ± 32.47, respectively). Therefore, a hypothesis for this maturity difference is that, considering that the average size of males and females was larger in Corumbá population, maturation size would also be larger than the population of PARNA de Jurubatiba, state of Rio de Janeiro.

According to Parker and Plummer (1987), small colubrids and elapids double their sizes from hatching to minimum mature age, and we observed a similar scenario in *A. brongersmianus*. Three newborns had a mean SVL = 57.6 (n = 3; unpubl. data), which corresponds to 36.3 and 49% of the sexually mature size of females and males, respectively. These findings indicate that males can potentially double their sizes from hatching to minimum mature age, while females could even triple their SVL.

Parker and Plummer (1987) also state that snakes attain sexual maturity with 60–75% of the maximum species size, while Shine and Charnov (1992) aim this proportion range from 50 to 90%, mostly between 60 and 70%. In *A. brongersmianus* we recorded mature males with smaller sizes than expected (55% of larger male), indicating they might mature in earlier stages than females, which is also reported for some Australian elapids (Shine, 1978). However, another interpretation is that females grow faster (61% SVL difference between smallest sexual female and larger female) during the same period compared to males, a finding also reported in a Viperidae species (Madsen & Shine, 1992). As we have no growth data over time, we reassert that more studies are needed to validate this information. It is worth noting that this rate of accuracy should be revised, as not all individuals obtain the largest size, and the proportion varies depending on the method used for maturity determination (Barros et al., 2020).

Females showed significant larger body sizes than males, as seen in other scolecophidians (Ávila et al., 2006; Caiacedo-Portilla, 2011; Parpinelli & Marques, 2015; Shine & Webb, 1990; Webb et al., 2000). Females become sexually mature with larger sizes than males, and this may be related to an increase in reproductive success and the higher reproductive costs for females (Ford & Seigel, 1989; Madsen & Shine, 1994;

Shine, 1994). Larger female scolecophidians may have proportionally larger offspring (Shine & Webb, 1990; Webb et al., 2001), but sometimes large eggs represent a smaller clutch size compared to maternal body size (Webb et al., 2000). Females also have larger head sizes, probably indicating a specialization for ingestion of larger preys (Webb & Shine, 1993a), which in turn could be related to the energetic need for growth and maturity (Taylor & Denardo, 2005), or this feature simply results from the larger body size (Webb & Shine, 1993b).

Longer tails in males, associated with the positive value of SSD, points to a conspicuous feature in many groups of snakes where male combat is absent (King, 1989; Shine, 1994; Shine et al., 1999), including scolecophidians—as far as we were able to survey the literature (Ávila et al., 2006; Parpinelli & Marques, 2015; Webb et al., 2000). Longer tails may also benefit males in aggregations (Parpinelli & Marques, 2015), a behavior ever recorded for Australians Typhlopidae (Shine & Webb, 1990), although there is no evidence of such behavior in *A. brongersmianus*.

Ávila et al. (2006) found sexual dimorphism in *A. brongersmianus* only for TL (longer tail in males), and even though SVL, HL, and HW were larger in females, these were not statistically significant. Here we found the same results for TL, but males also showed larger SVL. Besides, we found HL significantly higher for females only when SVL was not considered as a covariate. The slightly differences considering SVL might be a consequence of the smaller sample size of Ávila et al. (2006) (males = 22; females = 11) or due to different features of individual size of representatives of that population as previously discussed.

According to Guraya (1963), the basophilic aspect in primary follicles is due to protein and RNA composition, while lipidic components increase according to follicle development until reaching secondary vitellogenesis. Based on morphological and histological data, we confirmed that secondary vitellogenesis in *A. brongersmianus* initiates within follicles from 3.5 mm (Khouri et al., 2019). All females with spermatozoa in their oviducts (an indication of mating) showed at least one follicle larger or with this same size (see Khouri et al., 2019, p. 2493).

Australian species of *Anilios* (Shine & Webb, 1990) have secondary follicles measuring >5 mm, while the parthenogenic species *Indotyphlops braminus* is vitellogenic with follicles >2 mm (Kamosawa & Ota, 1996). These species are respectively larger and smaller than *A. brongersmianus*. Ávila et al. (2006) and show no follicle size information, although as discussed in Khouri et al. (2019), these authors could have mistakenly identified the vitellogenic follicles as eggs. Considering this possibility, four large follicles (called eggs by the authors) had a mean size of 8.27 mm, similar to data of large follicles here presented (see Ávila et al., 2006).

Based on the aforementioned results, one can conclude that in addition to the traditional characteristics for determining maturity, histology and other morphological aspects are also important. Equally, it is worth noting that the smallest adult will not necessarily be bigger than the largest juvenile, as we can see in males from the PARNA de Jurubatiba population. With greater knowledge on sexual dimorphism and maturity, studies on snake populations can be more cohesive and biologically relevant.

AUTHOR CONTRIBUTIONS

Rebeca Stella Khouri: Investigation; conceptualization; writing – original draft; methodology; visualization; writing – review and editing; data curation; project administration; formal analysis. **Selma Maria Almeida-Santos:** Conceptualization; writing – original draft; validation; writing – review and editing; supervision; methodology; visualization; project administration. **Daniel Silva Fernandes:** Supervision; resources; data curation; formal analysis; methodology; validation; visualization; writing – review and editing; writing – original draft; funding acquisition; investigation; conceptualization; project administration.

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ETHICS STATEMENT

Specimens were collected under permit #38378-11, granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

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APPENDIX 1

Voucher specimens of *Amerotytplhops brongersmianus* analyzed in this study ($n = 124$): PELD 1740, PELD 1711, PELD 1839, PELD 1907, PELD 1977, PELD 1999, PELD 2000, PELD 2041, PELD 2060, PELD 2076, PELD 2077, PELD 2078, MNRJ 23166, MNRJ 23167, MNRJ 23168, MNRJ 23169, MNRJ 23170, MNRJ 23171, MNRJ 23172, MNRJ 23173, MNRJ 23174, MNRJ 23175, MNRJ 23176, MNRJ 23177, MNRJ 23178, MNRJ 23179, MNRJ 23180, MNRJ 23181, MNRJ 23182, MNRJ 23184, MNRJ 23185, MNRJ 23186, MNRJ 24810, MNRJ 24811, MNRJ 24812, MNRJ 24813, MNRJ 24814, MNRJ 24815, MNRJ 24816, MNRJ 24817, MNRJ 24818, MNRJ 24819, MNRJ 24820, MNRJ 25283, MNRJ 25284, MNRJ 25285, MNRJ 25286, MNRJ 25287, MNRJ 25288, MNRJ 25289, MNRJ 25290, MNRJ 25291, MNRJ 25292, MNRJ 25293, MNRJ 25294, MNRJ 25295, MNRJ 25296, MNRJ 25297, MNRJ 25298, MNRJ 25299, MNRJ 25356, MNRJ 25357, MNRJ 25358, MNRJ 25359, MNRJ 25360, MNRJ 25361, MNRJ 25362, MNRJ 25363, MNRJ 25364, MNRJ 25365, MNRJ 25366, MNRJ 25367, MNRJ 25368, MNRJ 25914, MNRJ 25915, MNRJ 26220, MNRJ 26495, MNRJ 26496, MNRJ 26497,

MNRJ 26498, MNRJ 26499, MNRJ 26500, MNRJ 26501, MNRJ 26502, MNRJ 26503, MNRJ 26504, MNRJ 26505, MNRJ 26506, MNRJ 26507, MNRJ 26508, MNRJ 26509, MNRJ 26510, MNRJ 26511, MNRJ 26512, MNRJ 26513, MNRJ 26514, MNRJ 26515, MNRJ 26516, MNRJ 26517, MNRJ 26518, MNRJ 26519, MNRJ 26520, MNRJ 26521, MNRJ 26522, MNRJ 26523, MNRJ 26524, MNRJ 26525, MNRJ 26526, MNRJ 26527, MNRJ 26528, MNRJ 26529, MNRJ 26530, MNRJ 26531, MNRJ 26532, MNRJ 26533, MNRJ 26534, MNRJ 26535, MNRJ 26536, MNRJ 26537, MNRJ 26538, MNRJ 26539, MNRJ 26540, MNRJ 26541, and MNRJ 26542.