



## Reproductive ecology and diet of the fossorial snake *Phalotris lativittatus* in the Brazilian Cerrado

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Fossorial snakes have attracted little scientific attention in studies of natural history, despite their relevance to capture the range of evolutionary-ecological strategies of snakes. In this study, we examined 62 preserved specimens of *Phalotris lativittatus* (a member of the fossorial and poorly studied Elapomorhini tribe) to obtain information about sexual dimorphism, reproduction, seasonal activity and diet. Males were smaller than females but had longer tails, larger heads and were more heavy-bodied. Females attained sexual maturity at larger body sizes than males. Reproduction is seasonal in both sexes. Vitellogenesis started in mid-autumn, and peaked from late spring to summer. Oviductal eggs and oviposition were recorded from late spring to early summer, while hatchings occurred from late summer to autumn. Clutch size was low, a recurrent trait in fossorial snakes. Spermatogenesis began in autumn, peaked during spring and testicular quiescence occurred in summer. The ductus deferens contained sperm only in spring, when the sexual segment of the kidneys showed dense secretory granules and males were more active. Thus, we suggest that mating is likely to occur in spring. Diet is specialised in amphisbaenids, and no evidence of ontogenetic shift was detected. This is the first quantitative study on the ecology of an Elapomorhini species.

*Key words:* activity patterns, body sizes, Elapomorhini, food habits, reproductive cycles, sexual dimorphism

### INTRODUCTION

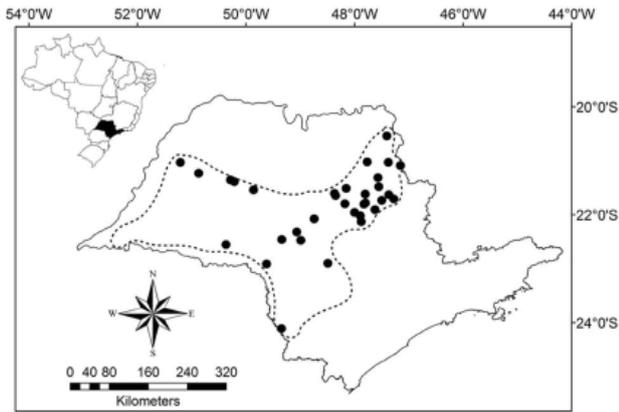
Detailed information on natural history is required for understanding patterns and processes involved in the evolution of ecological traits. For example, a description of the reproductive cycle of a number of snake taxa is required to test alternative hypotheses on the evolution of reproductive patterns (e.g., Almeida-Santos & Salomão, 2002; Aldridge et al., 2009). Although a large amount of data is available for several species, knowledge on snake ecology is mostly based on diurnal and terrestrial taxa. Accordingly, more secretive species such as fossorial taxa remain poorly studied with respect to their natural history. A recent study on the conservation status of the world's reptiles highlighted this issue. While assessing the extinction risk in a representative sample of reptiles, the authors noticed a high data deficiency in tropical regions and in fossorial or semi-fossorial reptiles (Böhm et al., 2013).

Fossorial life may impose serious challenges to morphology and thermoregulation (Pough, 1980; Shine, 1983). In turn, these characteristics may constrain opportunities to feeding and strongly affect potential reproductive output. For example, clutch size in distantly related fossorial or semi-fossorial species is often low and eggs are usually elongated (Marques, 1996; Marques & Puerto, 1998; Balestrin & Di-Bernardo, 2005; Braz et

al., 2009). Thus, the ecology of fossorial snakes is of interest *per se*, and more data are needed for a complete understanding of snake ecology.

Elapomorhini is a monophyletic group of fossorial snakes containing nearly 48 species allocated to three genera (*Apostolepis*, *Elapomorphus* and *Phalotris*) widely distributed in South America (Ferrarezzi, 1994; Graziotin et al., 2012; Uetz, 2013). They share several features, such as eye reduction, cylindrical body, short tail, fusion of cephalic scales, cranial reinforcement and small heads not distinct from the body, usually interpreted as specialisations for fossorial life (Savitzky, 1983; Ferrarezzi, 1993; Harvey, 1999). Mostly because of their fossorial lifestyle, the group is poorly represented in scientific collections (Harvey 1999; Hofstadler-Deiques & Lema, 2005), and no quantitative autecological study has so far been published. Food items identified from wild-caught individuals of Elapomorhini consisted mainly of amphisbaenids and small fossorial snakes (e.g., Lema, 1989; Zamprogno & Sazima, 1993; Ferrarezzi et al., 2005; Gomes et al., 2005; Bernarde & Macedo-Bernarde, 2006; Duarte, 2006, 2012; Hartmann et al., 2009; Mesquita et al., 2009; Barbo et al., 2011). Vitellogenic females and egg-laying were previously reported from late spring to early summer, and the clutch size is usually low, ranging from two to eight eggs (Travaglia-Cardoso et al., 2001; Leynaud, 2003; Braz et al., 2009; Barbo et al., 2011).

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**Fig. 1.** Geographic location of the examined specimens of *Phalotris lativittatus* (black circles) in the Cerrado Domain in São Paulo state, southeastern Brazil. Dashed line delimits the occurrence area of the species.

In this study, we investigate the ecology of *P. lativittatus* based on the examination of preserved specimens throughout its geographic distribution. We present detailed and comprehensive information on sexual maturity, sexual dimorphism, male and female reproductive cycles, clutch size, food habits and annual activity patterns. *Phalotris lativittatus* inhabits formations of the Cerrado in a restricted and highly disturbed area of São Paulo state, southeastern Brazil (Ferrarezzi, 1993; Vasconcelos & Santos, 2009; Zaher et al., 2011). The only available information on the biology of *P. lativittatus* concerns a report on egg-laying (Braz et al., 2009) and punctual data on daily activity and habitat use (Sawaya et al., 2008). Based on the limited information on Elapomorphini, we hypothesised that reproduction is seasonal, clutch size is low and diet is composed of elongate fossorial vertebrates.

## MATERIALS AND METHODS

### Data collection

We examined a total of 62 specimens of *P. lativittatus* housed in the collections of Instituto Butantan (IBSP), Museu de História Natural da Universidade Estadual de Campinas (ZUEC), and Museu Nacional do Rio de Janeiro (MNRJ). The specimens were collected throughout the Cerrado domain in São Paulo state (Fig. 1), southeastern Brazil, virtually covering the entire geographic distribution of the species (Ferrarezzi, 1993; Vasconcelos & Santos, 2009). The area is characterised by a rainy season from spring (October to December) to summer (January to March), with higher temperatures, and a dry season from autumn (April to June) to winter (July to September) also associated with lower temperatures.

For each specimen, we measured head length (to the nearest 0.01 mm), snout-vent length (SVL) and tail length (both to the nearest 1 mm). After that, a mid-ventral incision was made in most specimens ( $n=55$ ), and the following data were recorded: (i) sex (by gonadal inspection); (ii) number and diameter of the largest ovarian follicle or largest egg; (iii) length, width and thickness of the testes and diameter of ductus deferens in the distal portion (between the kidney and the cloaca); (iv)

gut contents (including both the stomach and intestines contents), which were removed, measured and identified to the lowest possible taxonomic level; and (v) body mass (to the nearest 1 g, after draining the preservative liquid and removing of gastrointestinal contents). Additional data on egg-laying, clutch size, hatching and hatchling size were obtained from published information on eggs laid by one wild-caught female (Braz et al., 2009).

Six adult males (four from spring, one from summer and one from autumn) were selected, and fragments of the right testis, ductus deferens (at the distal portion) and right kidney were collected, dehydrated in ethanol, and embedded in paraffin for histological examination. Histological sections were cut at 5  $\mu$ m and stained in hematoxylin/eosin. Sections of the testes were examined to verify testicular activity, whereas sections of the ductus deferens were evaluated for the presence or absence of spermatozoa. Sections of the kidneys were examined in search of hypertrophy of the sexual segment of the kidney (SSK). In most snakes studied, the seasonal hypertrophy of the SSK is associated with mating period (Aldridge et al., 2011).

### Data analysis

Sexual maturity in males was determined by the presence of enlarged and turgid testes and a convoluted ductus deferens (Shine, 1977; Almeida-Santos et al., 2006) in combination with the presence of spermatozoa in seminiferous tubules (by histological analyses). Females were considered mature if they had vitellogenic follicles, oviductal eggs or folded oviducts. *Phalotris lativittatus* individuals with  $SVL \leq 260$  mm were considered newborns since this value was the upper limit of body size observed at hatching (Braz et al., 2009). We used a *t*-test to examine intersexual differences in mean adult SVL and mean SVL at maturity (comparing the mean SVL of the five smallest mature individuals of both sexes; Ji & Wang, 2005). A sexual size dimorphism index (SSD) was calculated according to Shine (1994):  $(\text{mean SVL of the larger sex} / \text{mean SVL of the smaller sex}) - 1$ . This index is expressed as positive if females are the larger sex and negative if males are the larger (Shine, 1994). Sexual dimorphism in head length (HL) was analysed using ANCOVA, with trunk length (SVL minus HL) as the covariate (Nogueira et al., 2003). We also used ANCOVA to test for sex differences in tail length (SVL as the covariate) and mass (total length as the covariate).

Clutch size was estimated separately by the number of enlarged vitellogenic follicles (>20 mm) and the number of oviposited eggs obtained from the literature (Braz et al., 2009) as well as oviductal eggs counted in preserved specimens. Regression analyses were performed to determine relationships between maternal body size (SVL and mass) and clutch size (both log-transformed; King, 2000). We calculated testicular volume (TV) using the ellipsoid formula:  $TV = (4/3)\pi abc$ ; where  $a=1/2$  length,  $b=1/2$  width and  $c=1/2$  thickness (Pleguezuelos & Feriche, 1999). Preliminary analyses showed that male SVL was not correlated with both testes volume ( $r^2=0.07$ ;  $p=0.26$ ) and ductus deferens diameter ( $r^2=0.13$ ;  $p=0.14$ ). Thus, we tested for seasonal variation in testes volume and

**Table 1.** Morphological traits of adult males and females of *P. lativittatus* examined. Relative tail length is indicated as a percentage of SVL. Relative head length and relative mass are residual scores from the linear regressions of log-transformed head length against trunk length and mass against SVL, respectively. SVL=snout-vent length, TL=tail length, RTL=relative tail length, HL=Head length, RHL=relative head length, RM=relative mass.

Variable	Males			Females		
	Mean±SD	Range	<i>n</i>	Mean±SD	Range	<i>n</i>
SVL (mm)	487.9±43.9	409–587	23	647.1±72.6	507–749	24
TL (mm)	71.9±6.3	62–83	23	63.8±7.7	48–81	24
RTL	14.8±1.0	13.1–16.6	23	9.9±0.7	8.2–11.1	24
HL (mm)	15.7±1.4	13.1–18.5	22	18.0±2.2	14.4–23.9	24
RHL	0.008±0.025	-0.034–0.049	22	-0.007±0.032	-0.056–0.084	24
Mass (g)	28.4±9.5	16.0–48.0	20	50.1±23.0	22.0–97.0	23
RM	0.024±0.08	-0.122–0.196	20	-0.021±0.121	-0.267–0.203	23

ductus deferens diameter using ANOVA. Activity pattern throughout the year was inferred from the records of snake arrivals at the reception of Butantan Institute in São Paulo, southeastern Brazil (for discussion on the method see Marques et al., 2001). Seasonal variation in activity was investigated by comparing the number of snakes collected per season using a chi-squared test. To identify the influence of climatic variables on annual activity, we conducted a multiple regression with mean temperatures and precipitation as predictors, and the number of specimens collected as the dependent variable. Values of climatic variables were calculated after combining average values for each collection site ( $n=33$ ) of all specimens of *P. lativittatus* examined. To do this, we used the software DIVA-GIS v. 7.5 and the climate data set from Worldclim (v. 1.3; current climate 1950–2000) with a spatial resolution of 2.5 minutes. Taxonomy at genus and species level for the prey items followed Mott & Vieites (2009) and Ribeiro et al. (2011). All data were examined to ensure that statistical assumptions were met. Data were log-transformed when necessary to meet the assumptions of parametric statistical tests. Values are presented in the text as mean±1 SD, and the significance level for all statistical tests was set at  $\alpha=0.05$ .

## RESULTS

### Body size and sexual dimorphism

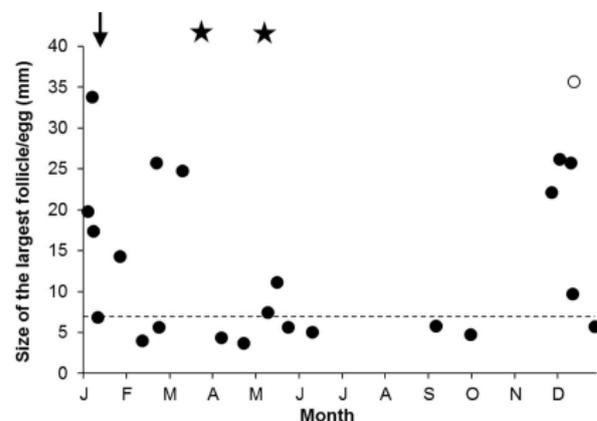
Mean SVL of adults differed between sexes ( $t=9.26$ ;  $df=45$ ;  $p<0.0001$ ). Adult females had larger mean SVLs and attained larger maximum body sizes than adult males (Table 1). The sexual size dimorphism (SSD) index was 0.33. Males exhibited higher values for all other morphological traits examined. Males of *P. lativittatus* had relatively longer tails (ANCOVA, slopes:  $F_{1,43}=0.52$ ,  $p=0.47$ ; sex:  $F_{1,44}=99.5$ ;  $p<0.0001$ ; Table 1), larger head length (ANCOVA, slopes:  $F_{1,42}=0.004$ ,  $p=0.95$ ; sex:  $F_{1,43}=8.34$ ,  $p=0.006$ ; Table 1), and were more heavy-bodied than females (ANCOVA, slopes:  $F_{1,39}=0.02$ ;  $p=0.87$ ; sex:  $F_{1,40}=5.67$ ;  $p=0.02$ ; Table 1).

### Sexual maturity

The smallest specimen examined measured 208 mm SVL. Newborn individuals (SVL<260 mm) averaged 234.5±22.0 mm SVL ( $n=6$ ). The smallest mature female had a SVL of 507 mm, whereas the smallest male had a SVL of 409 mm. The mean SVL of the smallest five individuals from each sex differed significantly ( $t=5.51$ ;  $df=8$ ;  $p=0.0006$ ), with the smallest females being larger (541.6±37.8 mm; range 507–586 mm) than the smallest males (439.4±17.1 mm; range: 409–450 mm). Thus, males attained sexual maturity at smaller body sizes than females.

### Female reproduction

Non-vitellogenic follicles were present year-round, except in July and August, when no females were collected (Fig. 2). Vitellogenic process started with follicles above 7 mm (Fig. 2). Although two females were observed in the beginning of vitellogenesis in mid-autumn (May), the peak of vitellogenesis was observed from late spring to summer (Fig. 2). One female with oviductal eggs was observed in late spring (December). A single egg-laying



**Fig. 2.** Seasonal variation in the diameter of the largest ovarian follicles or oviductal eggs in adult females of *P. lativittatus* from São Paulo state, Brazil. Solid circles=ovarian follicle, open circles=oviductal eggs, arrow=egg-laying, solid stars=hatching. Follicles above the dashed line were considered vitellogenic.

event (clutch size=5 eggs) was recorded in early summer (January) with hatchings occurring in autumn (Fig. 2; see Braz et al., 2009). Newborn snakes (SVL<260 mm) were collected in April, May, August ( $n=1$  individual per month) and October ( $n=3$ ). Clutch size based on enlarged vitellogenic follicles (>20 mm) averaged  $4.3 \pm 0.9$  eggs and showed little variation (range: 3–6 eggs;  $n=7$ ). Clutch size based on oviductal (this study) or oviposited eggs (Braz et al., 2009) ranged from 4 to 5 eggs ( $n=2$ ). Clutch size (considering both vitellogenic follicles and eggs) was not correlated with maternal SVL ( $r=0.03$ ;  $p=0.94$ ), but increased significantly with maternal body mass ( $r=0.70$ ;  $p=0.034$ ).

### Male reproduction

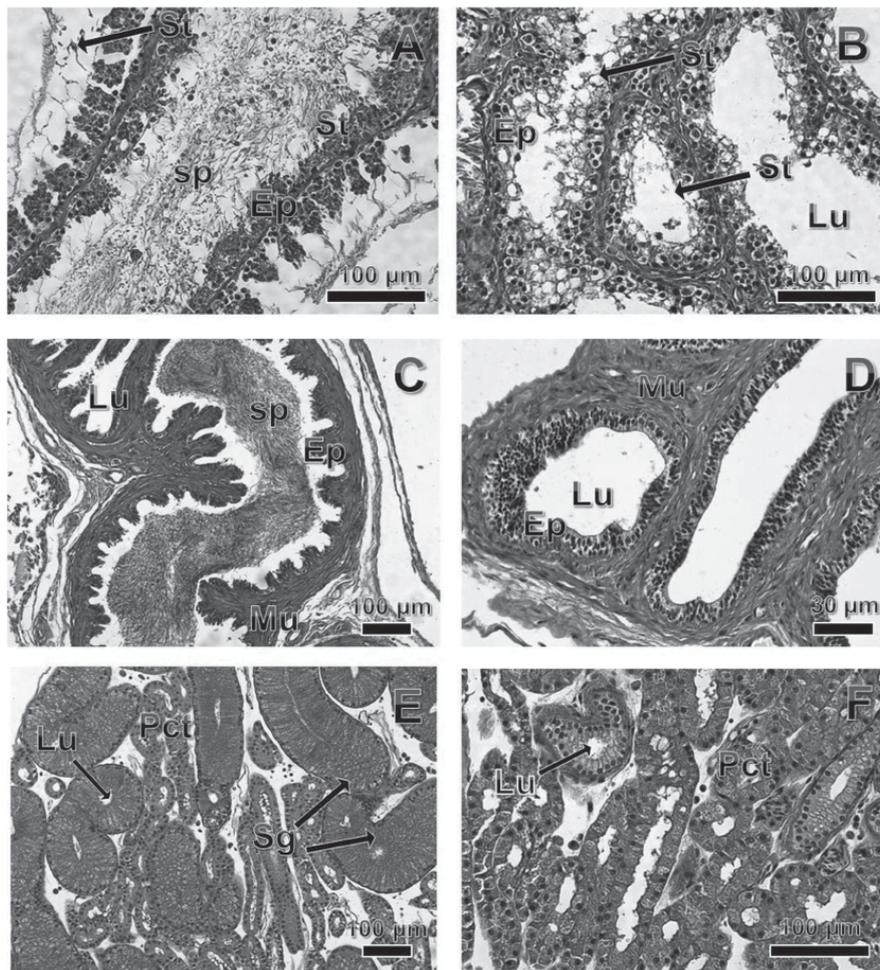
We compared variables between spring, summer and autumn because no male snake was collected in winter. Although testicular volume tended to be larger in early spring, there was no statistically significant difference between seasons (ANOVA,  $F_{2,17}=2.18$ ;  $p=0.14$ ). However, histological analyses revealed that males of *P. lativittatus*

showed a seasonal spermatogenic cycle. Spermatogenesis begun in autumn (when only spermatocytes were observed) and peaked during early spring (October–November; Fig. 3A). Testicular quiescence was observed in summer (February), when only spermatogonia were observed (Fig. 3B).

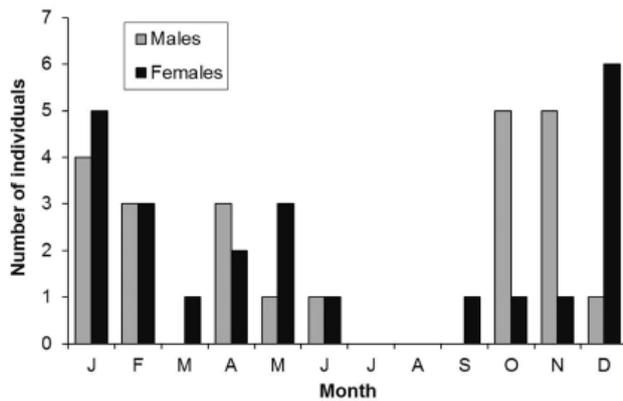
The diameter of the ductus deferens did not vary significantly between seasons (ANOVA,  $F_{2,17}=1.28$ ;  $p=0.30$ ). The ductus deferens was full with sperm in early spring (October; Fig. 3C), containing less sperm in mid-spring (November). In summer (February) and autumn (April), no sperm was observed (Fig. 3D). In spring, the SSK showed an increase in the secretion of eosinophilic granules (Fig. 3E). In summer (Fig. 3F) and autumn, the SSK showed a reduction of cytoplasmic granules, indicating a decrease in the secretory activity.

### Seasonal abundance

Adult individuals of *P. lativittatus* ( $n=47$ ) were collected in all months except July and August, but were significantly more abundant in the rainy season than in the dry season



**Fig. 3.** Transverse sections of the testes, distal ductus deferens and kidney of male *P. lativittatus* stained with Hematoxylin-Eosin in spring (October) and summer (February). A) Testis in spring showing an active seminiferous epithelium and a large amount of spermatozoa in the seminiferous lumen; B) Testis in summer showing inactive seminiferous epithelium and no spermatozoa in the seminiferous lumen; C) Ductus deferens in spring showing a large amount of spermatozoa in the lumen; D) Ductus deferens in summer with no spermatozoa in the lumen; E) Kidney hypertrophied during the secretory phase in spring; F) Kidney in summer during the post-secretory phase. Ep: Epithelium; Lu: Lumen; Mu: muscle; Pct: proximal convoluted tubule; Sg: Secretory granules; Sp: spermatozoa, SSK: sexual segment of the kidney; St: Seminiferous tubule.



**Fig. 4.** Seasonal abundance of adult males ( $n=23$ ) and females ( $n=24$ ) of *P. lativittatus* based on animals deposited in herpetological collections examined.

( $\chi^2=11.25$ ;  $df=1$ ;  $p=0.0008$ ; Fig. 4). Multiple regression showed that both mean temperature and precipitation explained about 63% of the variation in the number of adult individuals collected throughout the year ( $r^2=0.63$ ;  $F=7.65$ ;  $p=0.011$ ). However, both climatic variables had the same contribution to the observed variation in the number of captures (mean temperature:  $t=0.27$ ;  $p=0.80$  and precipitation:  $t=1.95$ ;  $p=0.08$ ).

Adult males ( $n=23$ ) were collected in all months except March and during the winter (July–September; Fig. 4). Number of males collected varied throughout the year and peaked in early to mid-spring (October–November;  $\chi^2=22.39$ ;  $df=11$ ;  $p=0.021$ ). Adult females ( $n=24$ ) were collected in all months except July and August (early and mid-winter; Fig. 4). Number of females collected varied throughout the year and peaked in late spring and early summer (from December to January;  $\chi^2=20.00$ ;  $df=11$ ;  $p=0.045$ ). The number of males collected outnumbered females in early to mid-spring (October and November), whereas the number of females outnumbered males in December (Fig. 4).

### Food habits

Eleven (20%) of the 55 dissected specimens (six females, two males, three immatures) contained identifiable prey in their guts. Nine specimens had only vestigial contents that corresponded to small skin pieces of amphisbaenids. One adult male (SVL=460 mm) contained a partially-digested *Leposternon microcephalum*, and one immature female (SVL=238 mm) contained an intact *Amphisbaena roberti* (total length=101 mm, i.e. 42.4% of the female size). In both cases, prey items were ingested head-first. We could not analyse the relationship between prey and predator size in all cases because gut contents were mostly vestigial. The frequency of individuals with gut contents was higher in the rainy season (spring: 54.5%;  $n=6$  and summer: 36.4%;  $n=4$ ) than in the dry season (autumn: 9.1%;  $n=1$ ;  $\chi^2=22.27$ ;  $df=1$ ;  $p<0.0001$ ).

## DISCUSSION

Our work provides the first quantitative data on the ecology of an Elapomorphini snake. We found support

for the proposed hypotheses of reproductive seasonality, low clutch size and food habits. Moreover, we present additional information on morphology, sexual dimorphism and sexual maturity.

*Phalotris lativittatus* was sexually dimorphic in all morphological traits evaluated. Sex differences in morphological traits are widespread among snakes (Shine, 1993). Females of *P. lativittatus* were longer and had shorter tails than males, which is consistent with most snakes studied (Shine, 1993, 1994). The larger body size in adult females is often related to the absence of male-male combat behaviour (Shine, 1978, 1994). The SSD index for *P. lativittatus* suggests that there is no male combat in the species (Shine, 1994), as is the case in other Neotropical Xenodontinae (e.g., Bizerra et al., 2005; Marques et al., 2006; Pizzato et al., 2008). In the absence of male combat, females are usually larger than males probably due to selection for higher fecundity (Shine, 1994). Although the explanation for relatively longer tails in males (as observed in *P. lativittatus*) is often related to the presence of the hemipenes and retractor muscles (King, 1989), the exact cause of the evolution of longer tails in males remains unclear (Shine et al., 1999).

Unexpectedly, head length and body mass were relatively larger in males than in females. This is the opposite of the most common condition in snakes (Shine, 1991). Sexual selection may explain these results only if these traits are advantageous in agonistic contests for accessing females during mating periods (Shine, 1993). However, considering the mating systems and the types of male-male fights known to date for other snake taxa (Greene, 1997), it seems unlikely that these findings are a sexually selected trait (Shine, 1991). Head-size dimorphism in snakes is often associated with intersexual divergence in diet (Shine, 1991; Vincent et al., 2004). Unfortunately, we could not address sex differences in prey size or prey types because of the low number of fed males in our sample and because most food items were vestigial.

Head size and stoutness directly affect the ability of fossorial squamates to penetrate the substrate and to move within underground tunnels (Gans, 1974; Navas et al., 2004). For example, in the amphisbaenid *Leposternon microcephalum*, individuals with narrower heads and more slender bodies exert less force and burrow faster than conspecifics with larger heads or with stouter bodies (Navas et al., 2004). Therefore, males and females may differ in digging ability as a result of differences in body shape (head size and stoutness). Additional data for other Elapomorphini are needed to test whether the direction of sexual dimorphism observed in *P. lativittatus* is a general pattern in the tribe. Unpublished data for at least one congeneric species (*P. mertensi*) seem to be consistent with the results found here (C.M. Herédias-Ribas, pers. comm.).

Females of *P. lativittatus* mature at larger body sizes than males, a pattern commonly reported for other snakes (Parker & Plummer, 1987). Clutch size is typically correlated to maternal body size (Seigel & Ford, 1987; Shine, 1994). Hence, late sexual maturity in females may entail in advantages to fecundity by allowing larger

females produce larger clutches (Seigel & Ford, 1987; Shine, 1994). In our study, clutch size was not correlated to maternal SVL, but to maternal body mass. Failure to find this relationship is likely due to the narrow range of clutch size together with the small sample size of reproductive females.

The data presented here are consistent with the hypothesis that clutch size is low in Elapomorphini, and are similar to previously published data for the species (Braz et al., 2009) and other representatives of the tribe (Travaglia-Cardoso et al., 2001; Leynaud, 2003; Barbo et al., 2011). Low clutch size is also characteristic of several unrelated fossorial or semi-fossorial squamates (e.g., Marques, 1996; Balestrin & Di-Bernardo, 2005; Andrade et al., 2006). Therefore, morphological constraints imposed by fossoriality and the decrease in costs associated with the movement of gravid females within underground tunnels may play an important role in determining low fecundity. Some dipsadid snakes with similar or even smaller body sizes produce larger clutches than *P. lativittatus* (e.g., 12.5 eggs in *Tomodon dorsatus*: Bizerra et al., 2005; 10.0 eggs in *Liophis miliaris*: Pizzatto & Marques, 2006; 8.5 eggs in *Xenodon newiedii*: Pizzatto et al., 2008). However, hatchlings of *P. lativittatus* are markedly larger than the hatchlings of these terrestrial species (e.g., 146 mm in *Tomodon dorsatus*: Bizerra et al., 2005; ~156 mm in *Liophis miliaris*: Pizzatto & Marques, 2006; 180 mm in *Xenodon newiedii*: Pizzatto et al., 2008). Thus, one alternative explanation for the small clutch size of *P. lativittatus* is a trade-off between clutch size and hatchling size (Shine, 1994; Marques & Muriel, 2007).

As predicted, the reproductive cycles of both male and female *P. lativittatus* are seasonal, beginning in autumn, but exhibiting an identifiable peak in spring. Vitellogenesis is initiated in mid-autumn, while ovulation, gravidity and egg-laying were observed only in late-spring and early summer. The timing of gravidity and egg-laying was coincident with an increase in female activity, which may reflect searching for appropriate thermoregulation or nest-site selection. Gravidity and egg-laying in late spring and early summer were also reported for other Elapomorphini snakes, such as *Elapomorphus quinquelineatus* (Travaglia-Cardoso et al., 2001), *Phalotris punctatus* (Leynaud, 2003) and *Apostolepis dimidiata* (H.B. Braz, pers. obs.). Thus, reproductive seasonality in females appears to be conservative in the tribe. Furthermore, the reproductive cycle of female *P. lativittatus* is remarkably similar to that of other fossorial snakes from southeastern Brazil (e.g., Marques, 1996; Marques & Puerto, 1998). It is possible that their fossorial environment imposes constraints on reproductive activity. Observation of hatchlings (Braz et al., 2009) and collection dates of supposed newborns (SVL < 260 mm) indicate that recruitment occurs at the end of the rainy season and beginning of the dry season. A peak of recruitment during this period is also observed in several other snakes from southeastern Brazil (e.g., Marques, 1996; Pizzatto et al., 2008). The presence of presumably newborn individuals in early spring (October) may be explained by a low growth rate during winter or

by the fact that these individuals had smaller body sizes at hatching than the lowest value observed here.

Although both testicular volume and ductus deferens diameter did not vary between seasons, the reproductive cycle of male *P. lativittatus* is seasonal, as shown by histological analysis. The incongruence between morphometric and microscopic analyses reinforces the need to rely on histological evidence to correctly describe male reproductive cycles (Mathies, 2011; Rojas et al., 2013). The spermatogenic cycle started in the autumn (but with no sperm in ductus deferens) and peaked during the spring. We did not histologically assess the testes and ductus deferens in the winter since no males were collected in this season. Nevertheless, given that males of *P. lativittatus* are apparently inactive during winter and that spermatogenesis is apparently affected by temperature (Aldridge & Duvall, 2002), it is likely that spermatogenic activity continues slowly or even stops. The seasonal hypertrophy of the SSK is often associated with mating period (reviewed in Aldridge et al., 2011). In male *P. lativittatus*, the hypertrophy of the SSK was observed in spring, which could be indicative of the mating period. The presence of males with ductus deferens with some emptied regions in mid-spring (November) suggests that these individuals may have recently copulated. Possible mating in spring is also supported by the encounter of a couple in early spring (October) and by the increase in male activity observed in this period. Increase in male activity has been associated with mate searching in a number of snake species (Gibbons & Semlitsch, 1987; Gregory et al., 1987; Almeida-Santos et al., 2006). Thus, the spermatogenesis, hypertrophy of the SSK, and copulation appear to be concomitant events in *P. lativittatus* (occurring in spring), defining the male reproductive cycle as associated (Crews, 1984). Few histological studies on male reproductive cycles of other dipsadid snakes are available (e.g., Rojas et al., 2013). Therefore, it is difficult to draw general comparisons among taxa. However, the reproductive pattern observed in male *P. lativittatus* is similar to that described for fossorial snakes from warmer climates of North America, in which spermiogenesis and hypertrophy of the SSK occur in spring (e.g., Goldberg & Rosen, 1999). In conclusion, we suggest that both males and females of *P. lativittatus* exhibit an associated reproductive pattern synchronised at the beginning of the rainy season (cf. Crews, 1984; Schuett, 1992).

Our observations on the diet of *P. lativittatus* are consistent with the hypothesis that this species preys on elongated fossorial vertebrates. Our data further indicate that the diet of *P. lativittatus* is specialised in amphisbaenids during the entire life cycle. We were able to identify only two prey items to the level of species. However, considering the number of amphisbaenid species that co-occur with *P. lativittatus* in São Paulo state (~8 species; Zaher et al., 2011), the prey diversity may not be high. To the best of our knowledge, a survey of the literature on food items of Elapomorphini (excluding captive reports) revealed that only amphisbaenids were reported as food item of *Phalotris* (Lema, 1989; Bernarde & Macedo-Bernarde, 2006; Duarte, 2006; this study)

and *Elapomorphus* (Lema, 1989; Zamprogno & Sazima, 1993; Hartmann et al., 2009; Duarte, 2012). However, for *Apostolepis*, there are recurrent reports of predation on both amphisbaenids (Ferrarezzi et al., 2005; Gomes et al., 2005; Sawaya et al., 2008) and small fossorial or semi-fossorial snakes (Mesquita et al., 2009; Barbo et al., 2011). Recent molecular phylogenies have recovered the genus *Phalotris* as a sister group of the clade formed by *Elapomorphus* and *Apostolepis* (Grazziotin et al., 2012). Although information on food habits of other Elapomorphini is still limited, taken collectively with our results they suggest that preying on amphisbaenids is a plesiomorphic trait in the tribe, and that the inclusion of other snakes as food item may have occurred only in the genus *Apostolepis*. Further detailed studies on the food habits of other Elapomorphini snakes could test this hypothesis.

As well as other species of the tribe, individuals of *P. lativittatus* are found active on the surface during the day and at night (Sawaya et al., 2008; Hartmann et al., 2009). However, the amphisbaenid specialisation suggests that *P. lativittatus* actively forages within underground tunnels, probably following odour trails. Although the encounter and capture of the prey items are likely to occur underground, it remains unknown whether prey ingestion also occurs in this microhabitat (e.g., Duarte, 2012). Higher food availability may explain the higher proportion of fed individuals in rainy (spring/summer) than in dry season (autumn/winter).

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