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Reproductive Biology and Food Habits of the Blindsnake *Liotyphlops beui* (Scolecophidia: Anomalepididae)

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Abstract. The anomalepidid *Liotyphlops beui* is a small blindsnake that is abundant in the city of São Paulo, southeastern Brazil. Data on body size, sexual size dimorphism, reproductive cycles, and food habits of this snake were obtained from the dissection of 177 specimens from this locality. The present paper compares basic aspects of the natural history of this anomalepidid with those of other Scolecophidia from Leptotyphlopidae and Typhlopidae to detect similarities or differences between these snakes. Females attain larger body size than males, whereas males have longer tails. Reproductive cycle in females is seasonal, with vitellogenesis occurring at the onset of the rainy season. Data on the diameter of deferent ducts indicate that mating occurred during this period. Clutch size ranged from 4–24 eggs. *Liotyphlops beui* feeds almost entirely on larvae and pupae of small ants, occasionally adult ants, and rarely termites. This snake gorges itself and seems to be able to eat a dozen different-sized prey in a meal. The availability of its main prey, the fire ant (*Solenopsis* spp.), may explain the high abundance of this blindsnake in the city of São Paulo. The data obtained in this study for *L. beui* indicate that the ecological traits of the three families of Scolecophidia are highly conservative.

Keywords. Atlantic Forest; Diet; Reproduction; São Paulo; Serpentes; Squamata; Southeastern Brazil.

Resumo. O anomalepidídeo *Liotyphlops beui* é uma pequena cobra-cega abundante na cidade de São Paulo, no sudeste do Brasil. Dados sobre o tamanho do corpo, dimorfismo sexual, ciclo reprodutivo e hábitos alimentares foram obtidos a partir da dissecção de 177 espécimes desta localidade. O presente trabalho compara aspectos básicos da história natural deste Anomalepididae com os de outros Scolecophidia das famílias Leptotyphlopidae e Typhlopidae para identificar semelhanças ou diferenças entre essas serpentes. As fêmeas atingem maior tamanho corporal, ao passo que os machos têm caudas mais longas. O ciclo reprodutivo das fêmeas é sazonal, com a vitelogênese ocorrendo no início da estação chuvosa. Os dados sobre o diâmetro dos ductos deferentes indicam que o acasalamento ocorre durante esse período. O tamanho da ninhada variou de quatro a 24 ovos. *Liotyphlops beui* se alimenta quase inteiramente de larvas e pupas de pequenas formigas, ocasionalmente de formigas adultas, e raramente de cupins. Esta serpente pode se empanturrar ingerindo presas de diferentes tamanhos em uma refeição. A disponibilidade de sua principal presa, a formiga lava-pé (*Solenopsis* spp.), pode explicar a alta abundância dessa serpente na cidade de São Paulo. Os dados obtidos para *L. beui* neste estudo mostram que as características ecológicas das três famílias de Scolecophidia são muito conservativas.

INTRODUCTION

The Scolecophidia, usually known as blindsnakes, differ greatly from other snakes. This group includes three families of burrowing snakes (Leptotyphlopidae, Typhlopidae, and Anomalepididae) with highly reduced eyes and a small body uniformly covered with smooth, polished, rounded scales (Shine and Webb, 1990; Greene, 1997). Leptotyphlopidae and Typhlopidae have wide distributions and occur in both the Old and New World, being the two most speciose families and totaling > 300 species (Kley, 2003b, c). In contrast, Anomalepididae is restricted to South America and includes about 20 species in four genera: *Anomalepis* Jan, 1860, *Helminthophis* Peters, 1860, *Liotyphlops* Peters, 1857, and *Typhlophis* Oppel, 1811) (Kley, 2003a).

The natural history of scolecophidians is little known, but some studies in Africa, Australia, and North America suggest that their habits are very similar (Punzo, 1974; Shine and Webb, 1990; Webb and Shine, 1993; Greene, 1997; Webb et al., 2000, 2001). However, some species present unique characteristics, and the families differ greatly in skull shape, dentition, and the transport of prey into the mouth (Webb and Shine 1993; Kley and Brainerd, 1999; Kley, 2001, 2003a, b, c; Webb et al., 2001). The family Anomalepididae is less speciose, and, consequently, information on its ecology and behavior is very scarce (Greene, 1997; Kley, 2003a). Little is known about the diet and reproduction of anomalepidid snakes, partly because of their small size and scarcity in collections (Wallach, 1998; Kley, 2003a). The anomalepidid *Liotyphlops beui* (Amaral, 1924) is a common snake in São Paulo city—one of the largest urban areas of the world—and the Instituto Butantan (IB) receives a large number of specimens throughout the year. Consequently, a relatively large number of this snake is available in the herpetological collection of the IB.

A previous study on *Liotyphlops beui* based on snakes brought each month to the IB and using live specimens monitored in captivity allowed us to characterize its seasonal and daily activity (Parpinelli and Marques, 2008). In the present study, we provide information on body size, reproductive cycles, fecundity and diet of the blindsnake *L. beui* in São Paulo city, where it is locally abundant.

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MATERIALS AND METHODS

Our study is based on the dissection of preserved specimens from the IB. We obtained data on morphology, reproduction, and diet, examining specimens from São Paulo municipality (23°32'51"S, 46°38'10"W), a large urban area (1.522,986 km²) in southeastern Brazil (population about 11.5 million), with an elevational range of 720–800 m above sea level (Atlas Ambiental do Município de São Paulo 2015; IBGE 2015). This snake can be found in fragments of secondary Atlantic forest, gardens, and empty lots in the city of São Paulo (Parpinelli, 2008). The climate of this region is Humid Tropical with two marked seasons: a dry season from April–September, with lower rainfall and temperatures, and a rainy season from October–March, with higher rainfall and temperatures (Nimer, 1989; Prefeitura do Município de São Paulo, 2002).

A total of 177 specimens from the IB collection were examined. The following data were obtained from each dissected specimen: (1) snout–vent length (SVL; mm); (2) tail length (TL, mm); (3) mouth width (MW, mm); (4) sex; (5) sexual maturity (males were considered mature if they had convoluted opaque deferent ducts; and females were considered mature if they had either ovarian follicles in vitellogenesis or oviductal eggs; Shine, 1977a, b, 1980; Shine and Webb, 1990; Marques, 1996; Hartmann et al., 2002); (6) diameter of largest ovarian follicles in females (to the nearest 0.01 mm); (7) medial (medium portion) and distal (close to cloaca) deferent duct diameter (measured with a vernier calliper to the nearest 0.1 mm); (8) stomach and/or intestine contents.

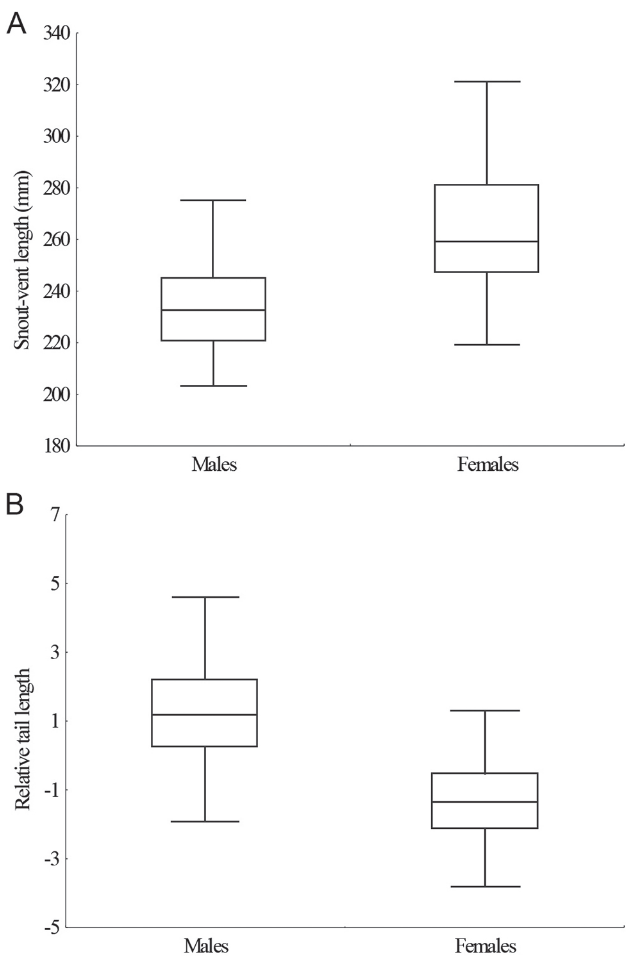

Figure 1. Sexual dimorphism in *Liotyphlops beui:* snout–vent length, mm **(A)**, and relative tail length **(B)**. Horizontal line in the box = average, box limit = 25–75% data, vertical lines = minimum and maximum values.

All statistical analyses were performed using Statistica (StatSoft, 2003) and differences were considered significant when $P < 0.05$. Sexual dimorphism in SVL was tested using Student's *t*-test, and sexual dimorphism in relative mass and relative tail length was analyzed with one-way analysis of covariance (ANCOVA), with total length (TL) and SVL as covariates, respectively (Zar, 1996). The degree of sexual size dimorphism (SSD) was calculated as 1 - (mean adult SVL of larger sex/mean adult SVL of smaller sex) (Shine, 1994). Analysis of the residual volume of testes and diameter of deferent ducts was performed using data divided into four periods: April–June (first half of the dry season), July–September (second half of the dry season), October–December (first half of the rainy season), and January–March (second half of the rainy season). We used one-way analysis of covariance (ANCOVA), with SVL as covariate, to eliminate body size effects. The identification, length, and width of each prey item were recorded. After the analyses, the contents were returned to the IB collection. The correlation (linear regression; Zar, 1996) between the MW of snakes and the length and width of the largest prey was assessed.

RESULTS

Sexual dimorphism

Mature females ($\overline{X}$ = 266 ± 27.29 mm SVL, range 219–321 mm SVL, n = 58) were significantly larger than mature males ($\overline{X}$ = 234 ± 20 mm SVL, range 178–296 mm SVL, n = 68) (t = -6.60, $P < 0.05$; Fig. 1A). However, male tails were significantly longer than female tails (F = 159.58, $P < 0.05$; Fig. 1B). The sexual size dimorphism (SSD) was 0.14.

Reproductive cycles and fecundity

Examination of ovarian follicles showed that females *Liotyphlops beui* have vitellogenic follicles at the end of the dry season and the onset of the rainy season (September–December) (Fig. 2). No individual showed oviductal eggs. The number of vitellogenic follicles (> 2.0 mm) ranged from 5–24 ($\overline{X}$ = 14.2 ± 6.24, n = 34). No correlation between the follicle size and SVL in females was found

Table 1. Identifiable prey item from the guts of *Liotyphlops beui* from São Paulo city.

Order	Family	Subfamily	Species	*n*
Hymenoptera				
	Formicidae			
		Ectatomminae		
			Gnanptogenys striatula	1
		Myrmicinae		
			Cyphomyrmex sp.	1
			Pheidole spp.	2
			Solenopsis spp.	7
			unidentified	5
Isoptera				
			unidentified	1

(R = 0.43, P < 0.05). In males, testis volume did not vary throughout the year (Fig. 3); however, medial deferent ducts showed a significant increase in diameter from the onset of the rainy season to the first half of the dry season (F = 13.12, P < 0.05; Fig. 4). Distal deferent duct diameter was significantly greater in the first half of the rainy season (F = 1.32, P < 0.05), but an increase in diameter was observed at the end of dry season and the onset of the rainy season (September–November) (Fig. 5).

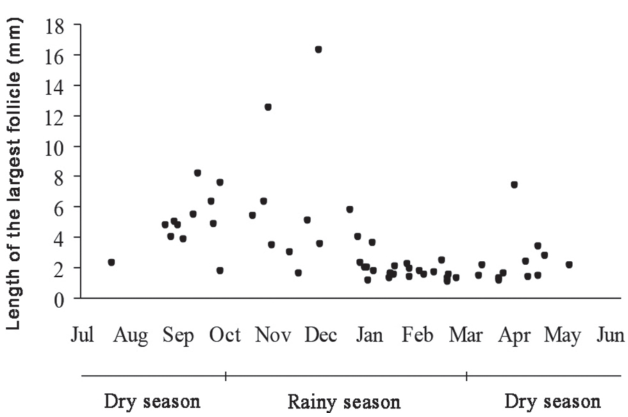

Figure 2. Seasonal variation in the diameter of the largest ovarian follicle in adults of *Liotyphlops beui* from the São Paulo city.

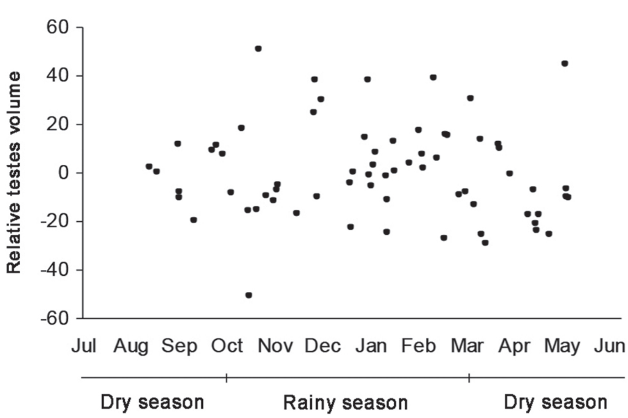

Figure 3. Seasonal variation in the relative testis volume in adults of *Liotyphlops beui* from the São Paulo city.

Food habits

Ninety-five (54%) of 177 preserved specimens of *Liotyphlops beui* contained prey remains in their guts. The proportion of snakes with prey in the gut was similar throughout the year. The remains were of ant pupae and larvae, some fragments of adult ants, and termites. The number of prey for each snake averaged 6.75 larvae (range 1–196) and 0.64 pupae (range 0–17). Among 17 identifiable prey (immature or adults), 16 were ants (Formicidae) and one was a termite (Isoptera). The scarcity of adult insects in the gut renders it difficult to identify prey to the level of genus or species. The predominant item (~ 40%) were fire ants (*Solenopsis* spp.) (Table 1).

Neither the length nor the width of the largest prey was correlated with mouth width of snake (R^2 = 0.20, P > 0.05, n = 18; r^2 = 0.14; P > 0.05, n = 18; respectively). Prey items were usually longer than the mouth width of the snakes ($\overline{X}$ MW = 2.29 mm; $\overline{X}$ length of larvae = 2.63 mm; $\overline{X}$ pupae = 2.45 mm). The length of the

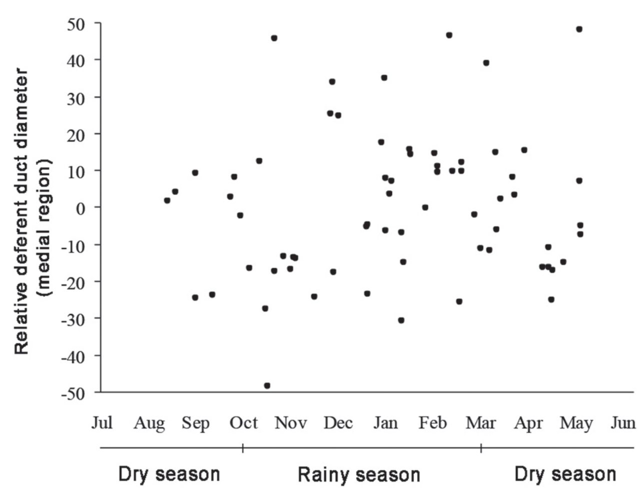

Figure 4. Seasonal variation in the relative medial deferent duct diameter in adults of *Liotyphlops beui* from the São Paulo city.

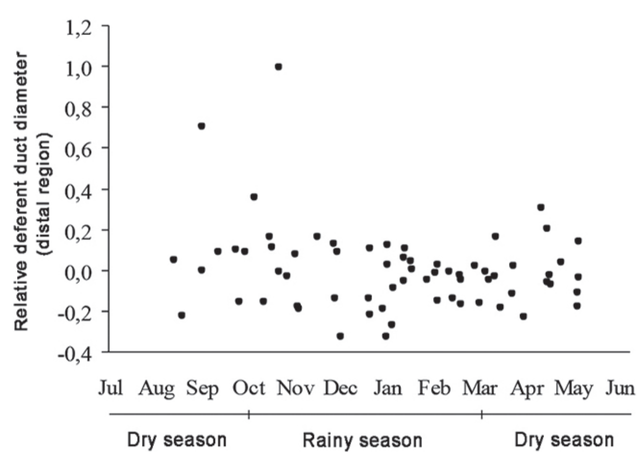

Figure 5. Seasonal variation in the relative distal deferent duct diameter in adults of *Liotyphlops beui* from the São Paulo city.

largest prey (4.87 mm) was almost twice the mouth width of the snakes (4.87 mm).

DISCUSSION

The small body size of *Liotyphlops beui* (averaging ca. 250 mm SVL) is a common feature among anomalepidids, as well as other scolecophidians, with the exception of *Afrotyphlops schlegelii* (Bianconi, 1847) (Typhlopidae), which reaches a length of almost 1 m (Dixon and Kofron, 1983; Greene, 1997). Females being larger than males has also been reported in other scolecophidians and in most snakes (Shine, 1978; Shine and Webb, 1990; Webb et al., 2000). The sexual dimorphism and the value of SSD in *L. beui,* as well as in other scolecophidians, suggest the absence of male-male combat in this group (Shine and Webb, 1990; Shine, 1994; Shine, 2003). Aggregations of leptotyphlopids and typhlopids have been observed in the field (McCoy, 1960; Shine and Webb, 1990). The fact that male-male combats were not observed in the field in such aggregations corroborates that this behavior is absent in scolecophidians.

The larger relative tail length of the male in *Liotyphlops beui* was also observed in other scolecophidians (Shine and Webb, 1990; Webb et al., 2000). Longer tails in males is usually associated with the presence of a hemipenis and muscles to retract it. However, this condition may also confer advantage in tail wrestling during courtship (King, 1989; Shine et al., 1999). This advantage may be applied to scolecophidians in general, since aggregation of males in these snakes occurs during the mating period (McCoy, 1960; Shine and Webb, 1990).

In a previous study, we proposed that mating in *Liotyphlops beui* should occur at the end of the rainy season, since male surface activity peaked in March (Parpinelli and Marques, 2008). However, data on testis volume and deferent duct diameter obtained in this study did not confirm this hypothesis. Testis volume did not vary seasonally, as in Typhlopidae (Shea, 2001), but the deferent duct diameter showed significant differences throughout the year, and deferent duct diameter is a good indication of the mating season (Almeida-Santos et al., 2006). We hypothesize that *L. beui* produce sperm throughout the year, storing it in the medial portion of the deferent ducts during the rainy season and the first half of the dry season. Then, at the end of the dry season and onset of the dry season, sperm migrates to the distal portion of the deferent ducts, close to cloaca. Thus, mating may occur when females have vitellogenic follicles, as recorded for Typhlopidae (Shea, 2001). Nevertheless, the possibility of two mating periods due to the male peak in March cannot not be ruled out (cf. Aldridge et al., 2009).

Although none of the examined females had oviductal eggs, all anomalepidids are believed to be oviparous (Kley, 2003a, b, c). Moreover, eggs of another anomalepidid, *Liotyphlops albirostris,* were found in an ant nest (Bruner et al., 2012). Large follicles were found in *L. beui* only at the onset of the rainy season. For several snake species from southeastern Brazil, vitellogenesis has only been observed during the first half of the rainy season (Marques, 1996; Marques and Puorto, 1998). Thus, we assume that egg-laying in *L. beui* occurs in the middle of the rainy season. The seasonal timing of reproduction in *L. beui* was also recorded for other scolecophid snakes from subtropical areas (Shine and Webb, 1990; Webb et al., 2000, 2001; Avilla et al., 2006).

Cunha and Nascimento (1978) reported that two species of Amazonian anomalepidids feed on juvenile ants and their eggs. Our data show that *Liotyphlops beui* feeds predominantly on ant pupae and larvae, but not on ant eggs. However, ant eggs were recorded as food items of other scolecophidians (Webb and Shine, 1993; Webb et al., 2000). The specialization on immature stages of ants has also been recorded for most leptotyphlopids and typhlopids (Webb and Shine, 1993; Webb et al., 2000; Avila et al., 2006). Apparently, ant larvae and pupae are preferred because they do not contain as much indigestible chitin and, therefore, are much more nutritious than the adults stages (Webb et al., 2001).

Data on the food habits of *Liotyphlops beui* and several other species of scolecophidians disagree with anecdotal reports that blindsnakes feed mainly on termites and other small insects (see Worrel, 1963; Amaral, 1978). Apparently, only a few species of scolecophidians have a broad diet including several types of insects, as well as other arthropods (e.g. Punzo, 1974). Some authors define “ants and termites” (e.g., Broadley, 1983; Branch, 1998) as a single prey resource. However, the available data refute this assumption. When examining the specimens of *L. beui,* we were able to identify only a single termite, despite the abundance of these insects in São Paulo city, including subterranean species (Milano and Fontes, 2002). Moreover, termites are also usually infrequent or absent in the diet of other scolecophidians, even in Africa where this prey type is abundant (Webb and Shine, 1993; Webb et al., 2000).

Ants can bite or sting, and the small fire ants (*Solenopsis* spp.), the most abundant prey of *Liotyphlops beui,* are able to subdue relatively large invertebrates and vertebrates (Holway et al., 2002; Webb and Shine, 1993). *Liotyphlops beui,* as well as other scolecophidians, has polished scales that might provide adequate protection from relatively small ants (e.g., Webb and Shine, 1993). Moreover, leptotyphlopids use cloacal secretions to repel ant attacks (Gehlbach et al., 1968; Watkins et al., 1969). It would be of great interest to conduct laboratory experiments to test if other scolecophidians (including species of Anomalepididae) use cloacal secretions to avoid or deter ant attacks.

Liotyphlops beui does not seem to select the size of its prey but it seems to prefer soft-bodied prey, such as immature stages of ants. This snake, as well as other scolecophidians, consumes a large amount of prey per meal (Shine and Webb, 1990; Webb and Shine, 1993; Webb et al., 2000). Leptotyphlopids and typhlopids quickly ingest their prey (Kley, 2001) and anomalepidids probably also have this ability. For scolecophidians, large meals may be associated with the clumped nature of the prey (ant nests), allowing the opportunity for binge feeding (Webb et al., 2000). The number of individuals of *L. beui* containing prey (over 50%) was higher than the number found for other scolecophidian species (cf. Webb and Shine, 1993 and Webb et al., 2000) and other sympatric snakes (cf. Barbo et al., 2011). This high proportion of snakes with gut contents is probably associated with high availability of resources, since *Solenopsis* spp. and other ants are very common in the São Paulo city (Piva and Campos, 2012). *Liotyphlops beui* is one the most common snakes sampled in São Paulo, occurring mainly in anthropized areas to the west of the city (cf. Barbo et al., 2011), which are very urbanized and highly altered. Thus, this anomalepidid snake can be considered synanthropic.

Our study on *Liotyphlops beui* showed that there are many similarities between *L. beui* and other blindsnakes from the Leptotyphlopidae and Typhlopidae, indicating that the ecological attributes of these three families are highly conservative and should reflect the basic pattern of their common ancestor. The ecological traits of Scolecophidians also allow for the survival of these blindsnakes in various environments, including disturbed areas.

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