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Reproductive biology of the snake *Liophis anomalus* (Günther, 1858, Dipsadidae, Xenodontinae)

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We describe the reproductive pattern of *Liophis anomalus* based on examination of museum specimens from its entire distribution area. This species shows seasonal activity, although follicles in secondary vitellogenesis were found throughout the year. Our data suggest that multiple clutches occur within a single reproductive period. As in many other snakes, adult females are larger than males when sexual maturity is reached. The sexual size dimorphism index is 0.16, which is consistent with the absence of combat between males (a common feature among species belonging to the Xenodontini tribe). Testicular volume was positively correlated with male size. Clutch size averaged 7.37 eggs, was inversely correlated with mean egg size and positively correlated with female body size. Our results confirm that both phylogeny and climate influence reproductive patterns of this Xenodontini snake.

Key words: ecology, *Liophis anomalus*, reproduction, snake

INTRODUCTION

Reproduction is a major component of an organism's life history (Pizzatto, 2003; Shine, 2003). The costs of reproduction impose strong selection pressure on snakes, which have to adjust their reproductive strategies to local conditions. The fact that sexual maturity, fecundity and sexual dimorphism are conditioned by abiotic, ecological and geographical factors generates divergence in reproductive tactics among and within species (Vitt & Vangilder, 1983; Shine, 2003).

Studies on the reproductive biology of snakes in North America, Europe and Australia (e.g., Shine, 1988a; Madsen et al., 1993; Aldridge et al., 2009) are more numerous than studies from the neotropical region (e.g., Vitt & Vangilder, 1983; Martins & Oliveira, 1999; Pinto & Fernandes, 2004). While the last decade experienced an increased interest in temperate species of the new world (i.e. Aguiar & Di Bernardo, 2005; Balestrin & Di-Bernardo, 2005; López et al., 2009), we still lack a comprehensive understanding of general patterns for neotropical snakes due to high species richness (Pizzatto & Marques, 2002; Uetz, 2011).

The yellow-striped snake, *Liophis anomalus* (Günther, 1858) is distributed from northern Argentina to La Plata (Buenos Aires, Argentina), Uruguay and the state of Rio Grande do Sul in Brazil (Dixon, 1980; 1985). This species inhabits open meadows and grazing lands associated with water bodies (Dixon, 1980). Previous studies on the species have mainly focused on other aspects than its reproductive biology (Carreira, 2002; Volonteiro et

al., 2006). *Liophis anomalus* is currently in a complex taxonomic situation. It was one of >40 *Liophis* species within the Dipsadidae (Xenodontinae, Xenodontini, Dixon, 1980; Vidal et al., 2007; Curcio et al., 2009) before Zaher et al. (2009) transferred *L. anomalus* together with eight other *Liophis* species to the resurrected genus *Lygophis* Fitzinger, 1843. These modifications are not currently fully accepted by the scientific community (Curcio et al., 2009; Vidal et al., 2010). In the present study we therefore do not use the nomenclature proposed by Zaher et al. (2009), maintaining *L. anomalus*. The aim of this study is to provide data on the sexual maturity, sexual dimorphism and reproductive output of *L. anomalus* across its distribution area.

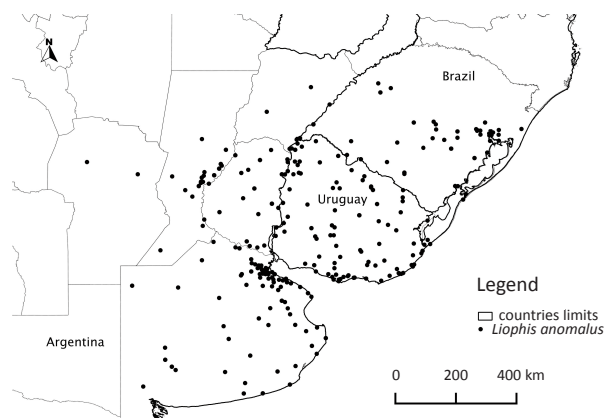


Fig. 1. Map showing the location of all examined specimens of *Liophis anomalus*.

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MATERIALS AND METHODS

A total of 567 specimens (242 adult females, 267 adult males and 58 juveniles) from the entire distribution range were examined (see Appendix and Fig. 1), but only 434 were dissected due to collections policies. The specimens were collected between 1896 and 2009, and deposited in Instituto Nacional de Limnología, Santa Fe (INALI), Museo provincial de Ciencias Naturales “Florentino Ameghino”, Santa Fe (MFA), Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires (MACN); Museo de Ciencias Naturales de La Plata (MLP); Museo Nacional de Historia Natural, Montevideo (MNHN); Zoología Vertebrados de la Facultad de Ciencias, Montevideo (ZVC-R); Universidade Federal de Rio Grande do Sul, Porto Alegre (UFRGS); Pontificia Universidade Catolica de Rio Grande do Sul, Porto Alegre (MCP), Fundação Zoobotanica de Rio Grande do Sul, Porto Alegre (MCN); and Universidade Federal de Santa Maria, Santa Maria (ZUFMSM).

To analyze sexual dimorphism, specimens were sexed, and snout-vent length (SVL) and tail length (TL) was measured to the nearest 0.1 mm. A Sexual Size Dimorphism Index (SSD) was calculated as [mean SVL of the largest sex/mean SVL of the smaller sex] (Gibbons & Lovich, 1991; Shine, 1994); positive SSD values imply that females are the larger sex. Reproductive condition was estimated by gonadal analysis. According to Shine (1977b), females are considered mature when they present follicles in secondary vitellogenesis, oviductal eggs or folded oviducts, and males are considered mature if the deferent ducts are convoluted (cf. Shine, 1977a, 1980a; Slip & Shine, 1988). We recorded the diameter of the smallest and largest non vitellogenic follicles, and the diameter of all vitellogenic follicles and eggs. The relationship between testis volume and body size permits the description of testicular cycles in snakes, and is a good indication of male reproductive effort and spermatogenic activity (Volsøe, 1944; Shine et al., 1999; Shine, 1977a). Length and width of testis were measured to calculate testicular volume using the ellipsoid volume formula (Dunham, 1983). Testicular volume was then related to SVL and the residuals of the regressions were compared in a Kruskal-Wallis test to identify differences throughout the year (Zar, 1999).

The number of eggs in gravid females and vitellogenic follicles were also recorded. Levene and Shapiro-Wilks tests were used to test the ANOVA assumption of homoscedasticity and normality, respectively. To analyze sexual dimorphism, SVL and TL between mature males and females were compared using the Mann-Whitney *U* test. To analyze the relationships among clutch size and female SVL, a Reduced Major Axis Regression (RMA) was used. This estimator allows error to occur in both

variables (Sokal & Rohlf, 1981; Shine, 1994). Spearman Correlations were used to quantify the association between clutch size and mean egg length. The difference between right and left testes volume was analyzed using the Mann-Whitney *U* test. As no differences were found, the volume of the right testes was arbitrarily chosen for further testing. An RMA was used to compare right testes volume and mature males SVL. As *b* was different from 1, variables were log transformed and another RMA was conducted. With the new *b* value, the right testis volume was calculated as $Z_i=(X_i/Y_i)b$, where *X* and *Y* are mean of adult SVL and right testes volume respectively, and *b* is the slope of the previous RMA. A Kruskal-Wallis test was used to assess the monthly variation in testicular volume throughout the year. The significance level was established at 0.05. All analyses were performed using XLSTAT v7.5.3 (Addinsoft, 1995) and PAST v2.02 (Hammer et al., 2001).

RESULTS

Body size and sexual dimorphism

Body sizes of mature males and females of *L. anomalus* are shown in Table 1. The SVL of the smallest mature female was 307 mm, and the smallest mature male was 210 mm. However, we considered 257 mm SVL as the minimum size at maturity for males as no immature males were recorded above this threshold. Females were significantly larger than males (Mann-Whitney *U* test: *U*=12110.00; *z*=9.183; *p*<0.001). Tail length was significantly correlated with SVL in males (*r*²=0.3594; *p*<0.001) and in females (*r*²=0.4313; *p*<0.001), and male tail length relative to SVL was larger than in females (Mann-Whitney *U* test: *U*=39625.000; *z*=-11.143; *p*<0.0001). The Sexual Size Dimorphism Index (SSD) was 0.16.

Fecundity

All females presented follicles smaller than 6 mm (Fig. 2), and vitellogenic follicles were thus considered to be above this length. The number of vitellogenic follicles averaged 8.38 (SD=5.26; range=1–27; *n*=63), and had a positive and significant relationship with SVL (*r*²=0.251; *p*<0.001; Fig. 3). There was no relationship between length of vitellogenic follicles and SVL (*r*²<0.001; *p*=0.985; *X*=14.76; SD=6.39; range=6.23–31.58; *n*=62).

Clutch size averaged 7.37 eggs (SD=2.62, range 1–13, *n*=30), and was positively correlated with female SVL (*r*²=0.221; *p*=0.0102). Egg length averaged 23.66 mm (SD=4.46, range=13.94–45.57 mm, *n*=221 eggs from 30 clutches), and had no significant relationship with female SVL (*r*²=0.008; *p*=0.63). Clutch size was inversely correlated with mean egg size (*r*²=-0.492; *p*=0.006, *n*=221 eggs from 30 clutches).

Table 1. Snout-vent length (SVL) and total length (TL) in adults of *Liophis anomalus*, expressed in millimetres.

Character	Females			Males		
	<i>n</i>	Mean±SD	Range	<i>n</i>	Mean±SD	Range
SVL	194	436.24±63.03	307–584	253	376.52±58.44	257–550
TL	194	126.97±19.75	78.5–176	253	127.53±21.58	67–195

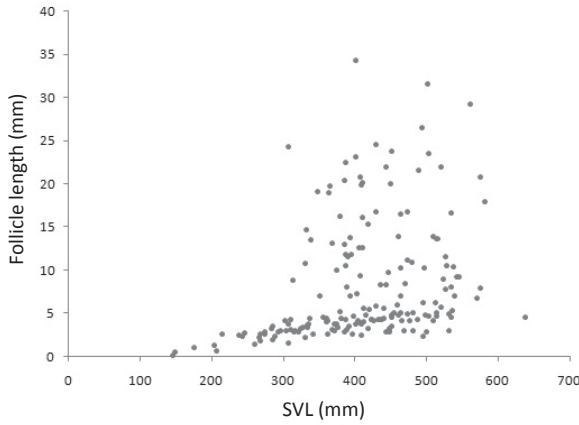


Fig. 2. Relationship between female SVL and follicle length in *Liophis anomalus* ($r^2=0.141$; $p<0.001$; $b=0.075$).

Reproductive cycle

Vitellogenic follicles were found throughout the year (except in April and June). Although there was no significant variation in the length of vitellogenic follicles along the year ($H=16.270$; $p=0.092$), those up to 15 mm were found only in the warmer season (from September to March). Gravid females ($n=30$) were found between August and February, but a higher frequency of these were registered in October ($n=11$). Mature individuals were found throughout the year, while young individuals were not found during winter (only one record of immature females in June and July, with SVLs of 267 and 285 mm respectively).

The smallest SVL recorded for an immature male was 112 mm (mean=178.5; $SD=43.93$; $n=24$) and the smallest SVL recorded for an immature female was 145 mm (mean=250.97; $SD=47.88$; $n=23$). The smallest juveniles were found in November and between February and March (Fig. 4), so it is probable that eclosion occurs during those months and juveniles can be expected to be found until April. Additionally, since six gravid females presented vitellogenic follicles, and because 47.62% of the females that presented follicles in secondary vitellogenesis had

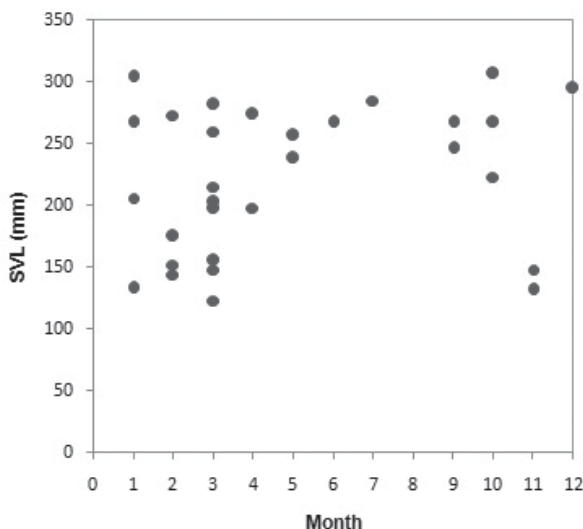


Fig. 4. Distribution of juvenile sizes (SVL, in millimetres) throughout the year (monthly).

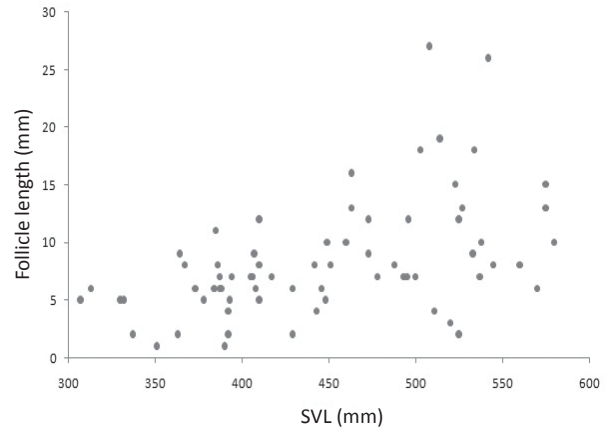


Fig. 3. Relationship between the number of vitellogenic follicles with snout-vent length in *Liophis anomalus* ($r^2=0.251$; $p<0.001$).

folded oviducts, we hypothesize that multiple clutches are laid during one reproductive season. Additionally, 10% of the gravid females were found with prey items in their stomach, suggesting that they do not stop feeding during the clutching period.

Testicular volume was correlated with SVL ($r^2=0.1233$; $p<0.001$). No statistical differences were found among testes (Mann-Whitney U test: $U=10497.0$; $p=0.549$). Testes volume varied throughout the year (Kruskal-Wallis, $H=25.470$; $p=0.008$), with its maximum between August and November.

DISCUSSION

Body size and sexual dimorphism

Sexual dimorphism is common amongst snakes (Shine, 1994; Bertona & Chiaraviglio, 2003), and can represent a common feature of a taxonomic group or can be related to specific ecological requirements (Madsen & Shine, 1993). Females tend to be the larger sex in species where male-male combat has not been recorded (Shine, 1994). The size of females is related to fecundity; a larger female might produce larger or more offspring, or have a higher reproduction frequency (Shine, 1988b; 1994). Females of *L. anomalus* were indeed larger than conspecific males, as seen in other Dipsadidae species, such as *Atractus reticulatus* (Balestrin & Di-Bernardo, 2005), *Clelia* sp. and *Boiruna* sp. (Xenodontinae, Pseudoboini) (Pizzatto, 2005). Pizzatto et al. (2008) reviewed reproductive strategies of Xenodontini and reported that SSD is moderate in members of this tribe, with values around 0.2 and 0.3. In *L. anomalus* SSD is a little lower than expected for members of the Xenodontini; male-male combat is therefore unexpected for the members of the family Dipsadidae (Bizerra et al., 2005).

The ratio between tail length and SVL is usually higher in males than it is in females (King, 1989; Shine et al., 1999). Tails of male *L. anomalus* are longer than those of females. Sexual differences in TL are common among snakes, and has been proposed to have an effect on male mating success: longer tails house a larger hemipenis, and it might also confer advantages for tail wrestling during mating balls (King, 1989; Shine, 1999).

Sexual maturity and fecundity

Male *L. anomalus* reach maturity at smaller size than females. This is a common trend among medium-sized and oviparous dipsadid species of temperate regions (Parker & Plummer, 1987; Shine, 1994). Moreover, maturation is likely to be delayed in the gender in which reproductive success increases most rapidly with body size (that is, females in species when male-male combat is absent; Shine, 1980a). Other *Liophis* species such as *L. semiaureus* and *L. miliaris* also present this pattern (Pizzatto, 2003; López et al., 2009). Delayed sexual maturity in females may allow them to reach larger body size at the time of reproduction, and to produce more eggs or larger newborns. Larger females also show higher thermal inertia, which could be an advantage for faster embryonic development (Rivas & Burghardt, 2001).

In temperate areas, a delay on reproduction might result in reproductive failure in a given year. Female snakes that inhabit tropical areas invest more energy in growth, initiating reproduction with larger bodies to increase fecundity (Pizzatto & Marques, 2006b). Males that attain sexual maturity with smaller SVLs start to reproduce earlier. Hence, in species without male-male combat, males that reach sexual maturity earlier are favoured in relation to those who invest more in growth rather than in maturation (Madsen et al., 1993). Early maturation, high mobility and reduced metabolic costs are among the advantages of smaller size in males (Madsen et al., 1993; Rivas & Burghardt, 2001). Males of *L. anomalus* could invest more energy in reproduction than in growth, which would allow them to start reproducing with smaller body size.

As was stated above, large body sizes in females confer a selective advantage because fecundity is size-dependent (Trivers, 1972; Seigel & Ford, 1987). However, larger females also may produce bigger offspring or reproduce more frequently (Shine, 1988b). We found that clutch size was positively correlated with maternal body size, and clutch size was inversely correlated with egg size. These results were also reported for the xenodontine *Oxyrhopus guibei* (Pizzatto & Marques, 2002), whereas in *L. semiaureus* and *L. poecilogyrus* clutch size was independent of female SVL. The production of smaller clutches might be balanced by a higher proportion of reproductive females with small SVL (Pinto & Fernandes, 2004; López et al., 2009).

Reproduction may influence the energy budget, decreasing food intake or consuming energy that would be available for growth. Females of *O. guibei* practically abstain from feeding while carrying oviductal eggs, a common feature for snakes because gravid females are slower than non-gravid ones (Shine, 1977a; 1980a,b; Seigel et al., 1987). In active foraging species, this may reflect a reduction in the ability of capturing prey (Shine, 1980a). Another possible interpretation would be that gravid females stop feeding because they lack physical space to accommodate prey (Gregory & Stewart, 1975; Seigel et al., 1987). However, 10% of the gravid females in our study had stomach content, suggesting that females of *L. anomalus* do not stop feeding during gestation. The same was reported for *L. poecilogyrus* (Pinto &

Fernandes, 2004), as for other colubrids (i.e. Pinto et al., 2008). As we found evidence that females of *L. anomalus* lay more than one clutch per reproductive period, they would likely not be able to cease their energy intake during pregnancy.

Reproductive cycle

Reproductive cycles of males and females of *L. anomalus* were seasonal, with the highest activity during the warm season. In the southern hemisphere, more than 90% of the clutches are laid from November to January, and more than 95% of the hatchlings occur from January to March (Pontes & Di-Bernardo, 1988). In *L. anomalus*, both sexes presented gonadal activity during the first months of spring, and courtship and mating likely occurs during this period. Oviparous species need to lay their eggs during the warm period of the year as it benefits egg incubation (Vinegar, 1977), while viviparous species can elevate their body temperature and therefore retain embryos for longer periods of time (Aguiar & Di-Bernardo, 2005). Females of *L. anomalus* present a strictly seasonal reproductive activity, as gravid females were found from August to February. In temperate areas, climate is the main factor affecting reproductive patterns. Nevertheless, in *L. anomalus* vitellogenic follicles were found throughout the year, suggesting reproduction throughout the year. Amongst the Dipsadidae, ecological attributes might be insufficient to explain the duration of the reproductive cycle. In addition, phylogeny might play an important role, with members of this family being able to reproduce continuously (Pizzatto & Marques, 2002; Pizzatto, 2005; Pizzatto et al., 2008). Males of *L. anomalus* showed seasonal variation in testicular volume throughout the year, and histological analysis should be made to confirm that sperm production is strictly seasonal. Spermatogenic cycles in oviparous caenophidians are less known than follicular cycles, although both seasonal and continuous patterns can occur (Pizzatto & Marques, 2002; Shine, 2003; Pizzatto, 2005; Pizzatto & Marques, 2006a). As higher testes volumes were found between August and November and females exhibited reproductive activity during August and March, either mating occurs twice in the reproductive season or females store sperm. In some species, sperm storage either in males or females is vital for their reproductive cycle (Bull et al., 1997; Shine, 2003).

Pizzatto et al. (2008) described three types of reproductive cycles for the Xenodontini tribe that ranged from continuous cycles, to broadly seasonal cycles, to strictly season cycles (5–7 months). *Liophis anomalus* showed a strictly seasonal reproductive cycle, with behavioural activity from August to March. Six gravid females also showed vitellogenic follicles similar to other species with short reproductive periods which lay multiple clutches in one season (Peterson et al., 1993). Multiple clutches is a common trait among Xenodontini (Pizzatto, 2003; Pinto & Fernandes, 2004). Pizzatto et al. (2008) also reported that some Xenodontini species have continuous cycles close to the tropics, and seasonal cycles in higher latitudes. Geographic variation in the reproductive cycle would suggest an adaptation to the

local environment (Ballinger, 1977). Although we would need more specimens to test whether these differences occur among populations of *L. anomalus*, we would not expect this pattern because of its restricted latitudinal range.

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REFERENCES

- Addinsoft. (1995). XLSTAT v7.5.3. XLstat, Your data analysis solution. Paris, France.
- Aguiar, L.F.S. & Di-Bernardo, M. (2005). Reproduction of the water snake *Helicops infrataeniatus* (Colubridae) in southern Brazil. *Amphibia-Reptilia* 26, 527–533.
- Aldridge, R.D., Goldberg, S.R., Wisniewski, S.S., Bufalino, A.P. & Dillman, C.B. (2009). The reproductive cycle and estrus in the colubrid snakes of temperate North America. *Contemporary Herpetology* 2009, 1–31.
- Balestrin, R.L. & Di-Bernardo, M. (2005). Reproductive biology of *Atractus reticulatus* (Boulenger, 1885) (Serpentes, Colubridae) in southern Brazil. *Herpetological Journal* 15, 195–199.
- Ballinger, R.E. (1977). Reproductive strategies: food availability as a source of proximal variation in a lizard. *Ecology* 58, 628–635.
- Bertona, M. & Chiaraviglio, M. (2003). Reproductive Biology, Mating Aggregations, and Sexual Dimorphism of the Argentine *Boa Constrictor* (*Boa constrictor occidentalis*). *Journal of Herpetology* 37, 510–516.
- Bizerra, A., Marques, O.A.V. & Sazima, I. (2005). Reproduction and feeding of the colubrid snake *Tomodon dorsatus* from south-eastern Brazil. *Amphibia-Reptilia* 26, 33–38.
- Bull, K.H. Mason, R.T. & Whittier, J. (1997). Seasonal Testicular Development and Sperm Storage in Tropical and Subtropical Populations of the Brown Tree Snake (*Boiga irregularis*). *Australian Journal of Zoology* 45, 479–488.
- Carreira, S. (2002). Alimentación de los ofidios de Uruguay. Asociación Herpetológica Española-Monografías de Herpetología.
- Curcio, F.F., Piacentini, V.Q., & Fernandes, D.S. (2009). On the status of the snake genera *Erythrolamprus* Boie, *Liophis* Wagler and *Lygophis* Fitzinger (Serpentes, Xenodontinae). *Zootaxa* 2173, 66–68.
- Dixon, J.R. (1980). The Neotropical colubrid snake genus *Liophis*. The generic concept. *Milwaukee Public Museum Contributions in Biology and Geology* 31, 1–40.
- Dixon, J.R. (1985). A review of *Liophis anomalus* and *Liophis elegantissimus*, and the description of a new species (Serpentes: Colubridae). *Copeia* 3, 565–573.
- Dunham, A.E. (1983). Realized niche overlap, resource abundance and intensity of interspecific competition. In: *Lizard Ecology*, 261–280. Huey, R.D., Pianka, E.R. & Schoener, T.W. (eds.). Harvard University Press, London.
- Gibbons, J.W. & Lovich, J.E. (1991). Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs* 4, 1–29.
- Gregory, P.T. & Stewart, K.W. (1975). Long-distance dispersal and feeding strategy of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in the Interlake of Manitoba. *Canadian Journal of Zoology* 53, 238–245.
- Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). PAST: Paleontological Statistics Software Package for Education and Data.
- King, R.B. (1989). Sexual dimorphism in snakes in tail length: sexual selection, natural selection or morphological constraint? *Biological Journal of the Linnean Society* 38, 133–154.
- López, S.M., Giraudo, A., Arzamendia, V. & Chiaraviglio, M. (2009). Biología reproductiva de la serpiente semiacuática *Liophis semiaureus* (Serpentes, Colubridae) en el nordeste de Argentina. *Revista chilena de historia natural* 82, 233–244.
- Madsen, T. & Shine, R. (1993). Phenotypic plasticity in body sizes and sexual size dimorphism in European grass snakes. *Evolution* 47, 1–4.
- Madsen, T., Shine, R., Loman, J. & Håkanson, T. (1993). Determinants of mating success in male adders, *Vipera berus*. *Animal Behaviour* 45, 491–499.
- Martins, M. & Oliveira, M.E. (1999). Natural history of snakes in forests of the Manaus region, Central Amazonia, Brazil. *Herpetological Natural History* 6, 78–150.
- Parker, W.S. & Plummer, M.V. (1987). Population ecology. In: *Snakes: Ecology and Evolutionary Biology*, 253–301. Seigel, R.A. Collins & Novak, S.S (eds.). McMillan Publishing Company, New York.
- Peterson, C.R., Gibson, A.R. & Dorcas, M.E. (1993). Snake thermal ecology: The causes and consequences of Body-temperature variation. In: *Snakes. Ecology and Behavior*, 241–314. Seigel, R.A & Collins, J.T. (eds.), McGraw-Hill Publishing Company, New York.
- Pinto, R.R. & Fernandes, R. (2004). Reproductive biology and diet of *Liophis poecilogyrus poecilogyrus* (Serpentes, Colubridae) from southeastern Brazil. *Phyllomedusa* 3, 9–14.
- Pinto, R.R., Fernandes, R. & Marques, O.A.V. (2008). Morphology and diet of two sympatric colubrid snakes, *Chironius flavolineatus* and *Chironius quadricarinatus* (Serpentes:

- Colubridae). *Amphibia-Reptilia* 29, 149–160.
- Pizzatto, L. (2003). Reprodução de *Liophis miliaris* (Serpentes: Colubridae) no Brasil: influencia histórica e variações geográficas. Unpublished PhD Dissertation, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brasil.
- Pizzatto, L. (2005). Body size, reproductive biology and abundance of the rare Pseudoboini snakes genera *Clelia* and *Boiruna* (Serpentes, Colubridae) in Brazil. *Phyllomedusa* 4, 111–122.
- Pizzatto, L. & Marques, O.A.V. (2002). Reproductive biology of the false coral snake *Oxyrhopus guibei* (Colubridae) from southeastern Brazil. *Amphibia-Reptilia* 23, 495–504.
- Pizzatto, L. & Marques, O.A.V. (2006a). Interpopulational variation in reproductive cycles and activity of the water snake *Liophis miliaris* (Colubridae) in Brazil. *Herpetological Journal* 16, 353–362.
- Pizzatto, L. & Marques, O.A.V. (2006b). Interpopulational variation in sexual dimorphism, reproductive output, and parasitism of *Liophis miliaris* (Colubridae) in the Atlantic forest of Brazil. *Amphibia-Reptilia* 27, 37–46.
- Pizzatto, L., Jordão, R.S. & Marques, O.A.V. (2008). Overview of reproductive strategies in Xenodontini (Serpentes: Colubridae: Xenodontinae) with new data for *Xenodon newiedii* and *Waglerophis merremii*. *Journal of Herpetology* 42, 153–162.
- Pontes, G.M.F. & Di-Bernardo, M. (1988). Registros sobre aspectos reprodutivos de serpentes ovíparas neotrópicas (Serpentes: Colubridae e Elapidae). *Comunicações do Museu de Ciências da PUCRS Série Zoologia* 1, 123–149.
- Rivas, J. & Burghardt, G.M. (2001). Understanding sexual size dimorphism in snakes: wearing the snake's shoes. *Animal Behaviour* 62, F1–F6.
- Seigel, R.A. & Ford, N.B. (1987). Reproductive ecology. In: *Snakes, Ecology and Evolutionary Biology*, 210–252. Seigel, R.A., Collins, J.T. & Novak, S.S (eds.). McMillan Publishing Company, New York.
- Seigel, R.A., Huggins, M.M. & Ford, N.B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* 73, 481–485.
- Shine, R. (1977a). Reproduction in Australian elapid snakes I. Testicular cycles and mating seasons. *Australian Journal of Zoology* 25, 647–653.
- Shine, R. (1977b). Reproduction in Australian elapid snakes II - Female reproductive cycles. *Australian Journal of Zoology* 25, 655–666.
- Shine, R. (1980a). Comparative ecology of three Australian snake species of the genus *Cacophis* (Serpentes, Colubridae). *Copeia* 1980, 831–838.
- Shine, R. (1980b). "Costs" of reproduction in reptiles. *Oecologia* 1980, 92–100.
- Shine, R. (1988a). Food habits and reproductive biology of small Australian Snakes of the genera *Unechis* and *Suta* (Elapidae). *Journal of Herpetology* 22, 307–315.
- Shine, R. (1988b). The evolution of large body size in females: a critique of Darwin's "Fecundity Advantage" model. *The American Naturalist* 131, 124–131.
- Shine, R. (1994). Sexual dimorphism in snakes revised. *Copeia* 1994, 326–346.
- Shine, R. (2003). Reproductive strategies in snakes. *Proceedings of the Royal Society London Series B* 270, 995–1004.
- Shine, R., Olsson, M.M., Moore, I.T., Lemaster, M.P. & Mason, R.T. (1999). Why do males have longer tails than females? *Proceedings of the Royal Society of London Series B* 266, 2147–2151.
- Slip, D.J. & Shine, R. (1988). The reproductive biology and mating system of diamond pythons, *Morelia spilota* (Serpentes, Boidae). *Herpetologica* 44, 396–404.
- Sokal, R.R. & Rohlf, F.J. (1981). *Biometry, the Principles and Practice of Statistics in Biological Research*, 2nd edition. San Francisco.
- Trivers, R. L. (1972). Parental investment and sexual selection. In: *Sexual selection and the descent of man*, 136–179. B. Campbell (ed.). Aldine-Atherton, Chicago.
- Uetz, P. (2011). The Reptile Database. <http://www.reptile-database.com>. Research Center Karlsruhe, Accessed 2011.
- Vidal, N., Dewynter, M. & Gower, D.J. (2010). Dissecting the major American snake radiation: a molecular phylogeny of the Dipsadidae Bonaparte (Serpentes, Caenophidia). *Comptes Rendues Biologies* 333, 48–55.
- Vidal, N., Delmas, A.S., David, P., Cruaud, C., Couloux, A. & Hedges, S.B. (2007). The phylogeny and classification of caenophidian snakes inferred from seven nuclear protein-coding genes. *Comptes Rendues Biologies* 330, 182–187.
- Vinegar, A. (1977). Evolutionary implications of temperature induced anomalies of development on snakes embryos. *Herpetologica* 30, 72–74.
- Vitt, L.J. & Vangilder, L. (1983). Ecology of a snake community in northeastern Brazil. *Amphibia-Reptilia* 4, 273–296.
- Volonteiro, O., Baletta, S. & Meneghel, M. (2006). A new genus and species of opisthogonimid (Digenea) of *Liophis anomalus* (Serpentes, Colubridae) from Uruguay. *Journal of Parasitology* 92, 1058–1063.
- Volsøe, H. (1944). Seasonal variation of the male reproductive organs of *Vipera berus* (L.). *Spolia Zoology Museum Hauniensis* 5, 1–157.
- Zaher, H., Grazziotin, F.G., Cadle, J.E., Murphy, R.W., Moura-Leite, J.C. & Bonatto, S.L. (2009). Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: a revised classification and descriptions of new taxa. *Papéis Avulsos de Zoologia. Museu de Zoologia da Universidade de São Paulo* 49, 115–153.
- Zar, J.H. (1999). *Biostatistical Analysis*. New Jersey: Prentice Hall.

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APPENDIX

URUGUAY. Facultad de Ciencias (Montevideo, Uruguay). Uruguay: ZVC-R 64, 86, 131, 158, 217, 233, 250, 282, 287, 340, 341, 351, 453, 482, 490, 534, 565, 787, 801, 862, 945, 956, 1034, 1035, 1080, 1145, 1149, 1344, 1390, 1391, 1398, 1503, 1622, 1627, 1642, 1670, 1672, 1674, 1714, 1723, 1729, 1730, 1734, 1812, 1834, 1912, 1914, 1989, 2000, 2001, 2002, 2003, 2013, 2347, 2431, 2440, 2454, 2455, 2487, 2502, 2746, 2783, 2808, 2832, 2887, 2888, 2890, 2891, 2928, 2997, 3316, 3347, 3391, 3734, 3959, 3969, 3995, 4027, 4185, 4212, 4218, 4242, 4243, 4425, 4426, 4431, 4432, 4454, 4465, 4478, 4611, 4692, 4776, 4782, 4794, 4801, 4813, 4822, 4880, 4903, 4915, 4916,

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ARGENTINA. Instituto Nacional de Limnología (INALI-CONICET-UNL) (Santa Fe, Argentina). A 167, 215, 328, 367, 843, 900, 944, 1093, 1095, 1308, 1345, 1348, 1354, 1603, 1613, 1621, 1637, 1707, 1715, 1727, 1741, 1848, 1886, 1901, 1903, 1963, 2052, 2068, 2069, 2148, 2150, 2158, 2159, 2252, 2256, 2260, 2482, 2629, 2680, 2729, 2730, 2766, 2792, 2946, 3032, 3370, 3407, 3509, 3537, 3570, 3624, 3661, 3728, 3804, 3998, 4001, 4030, 4112, 4140, 4141, 4294, 34956. Museo Provincial de Ciencias Naturales "Florentino Ameghino" (Santa Fe, Argentina): 12, 17, 120, 170, 171, 330, 322, 427, 680, 686, 699. Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires (MACN). Argentina: 38, 263, 342, 521, 582, 927, 1139, 1215, 1558 (2), 1566, 1937, 1938, 1962, 1995, 2018, 2036, 2210, 2400, 2483, 2551, 2558, 2592, 2633, 2645, 2714, 2801, 3248 (2), 3453, 3578, 3639, 3849, 4148, 4256, 4356, 4425, 4426, 4490, 4546, 4547, 4764 (2), 4769, 5061, 5912, 5913, 5914, 5915, 5916, 5961, 6980, 6981, 6982, 7037, 7038, 7168, 7744, 8569, 8850, 8914, 8991, 9089, 11441, 17899, 21077, 21079, 21457, 21723, 22164, 23372, 23387, 24444, 24469, 24661, 24878, 25010, 25065, 27306, 27841, 27842, 27986, 27987, 29988, 27989, 28174, 28175,

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BRAZIL. Universidade Federal de Rio Grande do Sul (Porto Alegre, Brazil): 78, 79, 80, 206, 709, 710, 711, 712, 713, 714, 715 (2), 716, 717, 718, 719, 720, 721, 722, 723, 724, 726, 727, 787, 3566, 4597, 4603, 5218. Pontifícia Universidade de Rio Grande do Sul (Porto Alegre, Brazil): MCP 1444, 2699, 5660, 5661, 5662, 5789, 5821, 12573, 12595, 13246, 13692, 13809, 13810, 13811, 13812, 13835, 14240, 14250, 14252, 14254, 14256, 18473. Fundação Zoobotânica de Rio Grande do Sul (Porto Alegre, Brazil): MCN 207, 1161, 1669, 1822, 2004, 2088, 2440, 2510, 2625, 2845, 2887, 3167, 3305, 4191, 4486, 4498, 4534, 4535, 4732, 5392, 6234, 6382, 6503, 6791, 7021, 7544, 7647, 8345, 8681, 8745, 9286, 9449, 9664, 9863, 13954, 14867. Universidad Federal de Santa María (Santa María, Brazil): ZUFMS 0026, 0854, 2425.