

Habits hidden underground: a review on the reproduction of the *Amphisbaenia* with notes on four neotropical species

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Abstract. We review the information currently available on the reproduction of the *Amphisbaenia* and provide original data on the reproductive biology of four Neotropical species: *Amphisbaena alba*; *A. mertensi*; *Cercolophia roberti*, and *Leposternon infraorbitale*. In total, we compiled data for 22 species: 17 *Amphisbaenidae*, 1 *Rhineuridae*, 3 *Bipedidae*, and 1 *Trogonophidae*. The majority of the species were oviparous with the exceptions of *Loveridgea ionidesii*, *Monopeltis anchietae*, *M. capensis*, and *Trogonophis wiegmanni*. Viviparity was interpreted as a derived trait that evolved independently for at least 3 times within the *Amphisbaenia*. In most species, reproduction is synchronized with the hot and rainy season and seems to vary with latitude. Although *Amphisbaenia* eggs have been found in ant nests, it remains disputable whether this is an obligatory or even a preferable location for egg-laying. Incubation time in *A. mertensii* lasts 59 days and this is the first report encompassing egg-laying to hatching for any *Amphisbaenia* species. Nonetheless, a two months incubation period seems to be the common rule for oviparous *Amphisbaenia*. The general pattern of reproductive output in *Amphisbaenia* is characterized by a low number of eggs/embryos per clutch whose individual size is comparatively large in relation to adult body size. Eggs are markedly elongated on the long axis and arranged in-line within the abdominal cavity possibly to prevent/diminish biomechanic drawbacks of egg bearing. Hatchlings of *A. mertensi* possess an egg-tooth implanted at the upper jaw, exhibit positive geotropism, and display defensive behaviors known to be present in adults. Our review shows that our current knowledge of *Amphisbaenia* reproduction is fragmentary, often based on the examination of small samples, and heavily dependent on the publication of anecdotal observations. Future publications on this subject are encouraged.

Introduction

Amphisbaenians are rarely encountered in the field perhaps because of their fossorial habits which diminish the probability of encounter and the opportunities for observation in nature. Accordingly, *Amphisbaenia* biology is poorly known and their reproductive habits stands as the most obscure aspect of their natural history. Most reports about reproduction in *Amphisbaenia* are restricted to the description of reproductive mode, i.e., oviparous or viviparous, and reports of embryos or eggs being found (Gans, 1978; Jared et al., 1997; Montero et al., 1999; Webb et al., 2000; Barros-Filho and Nascimento, 2003). In most cases, the information provided is based on anecdotal and sparse observations, often included in taxonomic or more general studies (e.g., Bogert, 1964; Gans, 1966,

1969, 1978; Bellairs and Gans, 1983; Gans and Kraklau, 1989; Vanzolini, 1992). Since no attempt has been made in summarizing the current information available on the reproduction of *Amphisbaenia*, herein we offer a review aimed to bring together the data that has been published on this subject. Furthermore, we expand the knowledge in this area by providing original data on four species from the Neotropics: *Amphisbaena alba*, *A. mertensi*, *Leposternon infraorbitale*, and *Cercolophia roberti*.

Material and methods

Original observations

Living *Amphisbaena alba* Linnaeus, 1758 and *Amphisbaena mertensi* Strauch, 1881 were collected in Southeastern Brazil during an animal rescue operation during the construction of a hydroelectric power plant ("UHE – Miranda", at the municipality of Indianópolis, Minas Gerais State) on September 1997. Animals were transported to the Departamento de Zoologia, Universidade Estadual Paulista, in Rio Claro, São Paulo state, southeastern Brazil. In captivity they were maintained in groups of 3 to 4 individuals in plastic boxes (35 × 30 × 15 cm) covered with a layer of moist soil of approximately 5 cm deep and were fed every other week

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with commercial canned cat food (Whiskas®, Purina Co.). The observations on egg laying, incubation, and hatchling presented at the results section were taken from females belonging to this group of animals.

We complement these observations with data obtained on a few other specimens of *A. mertensi* and *A. alba* that have been collected by locals on the outskirts of Rio Claro municipality, and sent to our Department over the years.

We examined preserved specimens of *Leposternon infraorbitale* collected by us in the same rescue operation and locality mentioned above. Initially, these animals were kept in captivity in the same conditions mentioned above, but as they refused eating, they were all killed and preserved, as described below, two months after collection, i.e., in November, 1997. At this time the animals did not appear to have become emaciated.

We examined preserved individuals of *Cercolophia roberti* collected by us in the outskirts of Rio Claro municipality, São Paulo State, southeastern Brazil, while the soil was being ploughed for manioc harvest. All these animals were killed and preserved immediately after collection in August, 1982.

All animals examined were deposited in herpetological collections at the Departamento de Zoologia, Universidade Estadual Paulista (DZRC), in Rio Claro, SP, Brasil and at the Museu Nacional do Rio de Janeiro, (MN), in Rio de Janeiro, RJ, Brasil. If not already preserved, animals were killed by CO₂ inhalation, fixed in 10% formalin, and preserved in 70% alcohol. In face of the low number of animals available to examination and the non-systematic nature of collection, we restricted our reports on the reproductive characteristics of reproductively active females. The material that we had access for this study did not allow for the gathering of reliable data on non-reproductive individuals or on the reproductive state of males.

For each specimen we measured snout-vent length (SVL) and tail length (TL) with a ruler to the nearest mm. Preserved museum specimens were dissected by a mid-ventral incision for the examination of the gonads. We recorded the presence of vitellogenic ovarian follicles and oviductal eggs, their number, position, and length on the longer and shorter axis (with a caliper to the nearest 0.1 mm) and mass (with an Ohaus scale to the nearest 0.001 g). Relative clutch mass (RCM) was calculated as the ratio between clutch mass and female mass after egg-laying (see Shine, 1980).

Literature review

In face of the scarceness of information on the reproductive biology of amphisbaenians, any study that had treated this subject, even if marginally, and that we were aware of was included in this review. We tabulated the selected information in an attempt to offer a quick reference source for those interested in the biology of amphisbaenians and, possibly, to identify patterns in their reproductive biology which might emerge from such a comparison. We followed the nomenclature adopted by Gans (2005).

Results

Original observations

Amphisbaena alba. One preserved female *A. alba* (SVL = 554 mm; TL = 35 mm; collected in February 1962) was found to contain seven eggs, four in the right oviduct and three on the left side. These eggs average 26.8 mm (SE ± 0.7) on the long axis and 13.7 mm (SE ± 0.6) on the short axis, and were arranged in-line on a single row within the abdominal cavity.

On September 28, 1997, a female *A. alba* (SVL = 472 mm; TL = 30 mm; post-partum body mass = 166.5 g) laid 6 soft-shelled whitish coriaceous eggs. These eggs were elongated, measuring on average 38.7 mm (SE ± 0.4) and 13.6 mm (SE ± 0.2) on the long and short axis, respectively. The average wet weight of the eggs was 4.51 g (SE ± 0.08) and the RCM was 0.16. The eggs were removed from the maintenance box and arranged in a plastic tray, covered with moist vermiculite, and kept in a climatic chamber at 30°C for incubation. After 2 months the eggs were dissected and the absence of embryos was verified.

Cercolophia roberti. Reproductive females averaged 231 mm SVL (SE ± 5.2), 16.8 mm TL (SE ± 0.4), and 6.5 g in body mass (SE ± 0.2) after the removal of the eggs upon dissection ($n = 6$, all collected in August, 1982). Clutch ($n = 6$) was composed, in average, by four eggs, varying from two to six. Based on the examination of one female containing six well-developed eggs in the oviducts, we estimated an RCM of 0.28. These eggs measured 0.7 mm (SE ± 0.02) and 2.21 mm (SE ± 0.2) on the short and long axis, respectively, and had a mean mass of 0.32 g each (SE ± 0.01). Within the abdominal cavity of the females, the eggs were arranged in-line on a single row and caused only minor disruption of the female's external body shape. Although eggs do not cause significant abdominal distension they can be seen through the thin ventral body wall of live gravid females.

Leposternon infraorbitale. Three female *L. infraorbitale* were kept in captivity for about two months after collection and were killed and preserved in November 1997 (see further details in Material and Methods). Upon dissection, these three females (mean SVL = 461 mm SE \pm 12; mean TL = 19.3 mm SE \pm 0.3; mean body mass after the removal of egg mass = 125 g SE \pm 3.5), were found to contain an average of four oviductal eggs (3, 4, and 5 each). There was a bias for eggs to be in the left oviduct (10 eggs) compared to the right oviduct (2). Within the female abdominal cavity, eggs were arranged in-line on a single row occupying the posterior region of the body just anterior to the cloaca. In two females, oviductal eggs extended from the cloaca up to almost the middle of the body, occupying approximately 35% of the total SVL length of the female's body. Eggs ($n = 10$) were elongated measuring, on average, 44.5 mm (SE \pm 5.4) and 16.3 mm (SE \pm 2.6) on the long and short axis, respectively. Eggs had an average mass of 5.2 g (SE \pm 0.09), and the average RCM was 0.17 ($n = 3$ females).

Amphisbaena mertensi. Two preserved *A. mertensi* (both with a SVL of 370 mm and TL of 60 mm; no date of collection recorded) were found to contain seven to eight shelled eggs in the oviducts (mean egg size = 14.6×6 mm SE $\pm 0.4 \times 0.2$; $n = 11$). These eggs were more or less equally distributed between the left and right oviduct and arranged in-line on a single row within the abdominal cavity.

On October 17, 1997, a female of *A. mertensi* (SVL = 360 mm; TL = 16 mm; post-partum body mass = 27.45 g) laid 6 soft-shelled, whitish coriaceous eggs. These eggs were ellipsoid measuring on average 27.3 mm (SE \pm 1.6) and 10.7 mm (SE \pm 0.5) on the long and short axis, respectively. The average weight of the eggs was 1.7 g (SE \pm 0.1) and the RCM equaled to 0.37. The eggs were removed from the maintenance box and arranged in a plastic tray, covered with moist soil, and left at room temperature (typically around $26 \pm 3^\circ\text{C}$). Out of the six eggs, four amphisbaenians, successfully

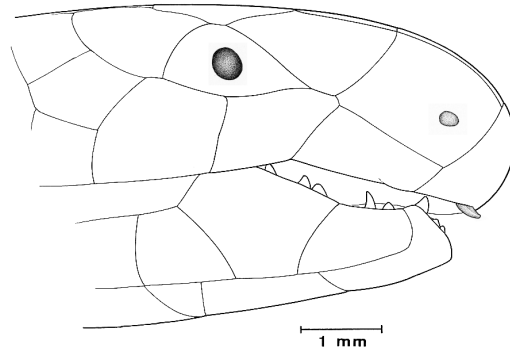


Figure 1. Schematic drawing of a newborn *Amphisbaena mertensi* showing the morphology and implantation of the egg-tooth.

hatched on December 15, i.e., 59 days after the eggs have been laid. Their general coloration was dim pinkish with the snout paler than the rest of the body, the eyes were dark colored and clearly distinguishable.

On two instances, we were able to follow hatchling behavior. In both cases, we did not witness the beginning of the hatching process, particularly how the opening of the egg shell were made. However, we clearly noticed that the amphisbaenians emerged from the eggs tail first in both instances. Hatching lasted for about 20 minutes counting from the beginning of the observation. Immediately after hatching, the hatchlings showed a positive geotropism trying to bury themselves into the underling soil. The mean body mass of the newborns was 1.7 g (SE \pm 0.15), and SVL was 107 mm (SE \pm 2.5) with a TL of 15 mm (SE \pm 0.01).

Inspection of the newborn amphisbaenians revealed the presence of an egg-tooth implanted on the midline of the upper edge of the mouth, on the inner side of the rostral scale (fig. 1). This egg-tooth was dark colored, projected anteriorly and lightly curved. Within two weeks after hatchling the egg-tooth was lost. Newly hatched amphisbaenians responded to our finger touches on the dorsal surface of their bodies by raising the tail, tried to bite whenever handled, and, in two occasions, they defecated. Newborns of *A. mertensi* were kept in captivity, under similar conditions as described above, and

were fed with earthworms, termites and mealworms. Three months after hatching, the mean body mass of three surviving individuals had increased by 0.34 g.

Literature review

Table 1 presents a summary of the information available on the reproductive biology of 20 species of *Amphisbaenia*. We list the bibliographical source of the information, the geographical location where the observations were made, if the information was originated from a museum or field based study or, as in most cases, from anecdotal observations, and finally the main information provided. From the 21 species for which information on their reproductive biology was found 16 belong to the family *Amphisbaenidae*. The exceptions were *Rhineura floridana* (*Rhineuridae*), *Bipes tridactylus*, *B. canaliculatus*, *B. biporus* (*Bipedidae*), and *Trogonophis wiegmanni* (*Trogonophidae*). The majority of the species were oviparous with the exceptions of *Loveridgea ionidesii*, *Monopeltis anchietae*, *M. capensis*, and *T. wiegmanni*. The number of eggs per clutch never exceeded 8, and usually varied between 2 and 6.

Discussion

Our data shows that females of *L. infraorbitale* bear well-developed oviductal eggs by July coinciding with the middle of the cold and dry season in southeastern Brazil. At Bahia state, northeastern Brazil, and north from the area where we collected our specimens, Jared et al. (1997) reported on eggs of this species being found on late February, with hatching occurring in March. This timing, however, is likely to reflect a geographic/climate related difference between our study and that of Jared et al. (1997) rather than the early formation of the eggs in the females examined by us. We think that most likely, in southeastern Brazil, *L. infraorbitale* would lay eggs by the beginning

of the hot/rainy season (spring/summer) which starts by September (~1 month after we found the oviductal eggs) instead of retain eggs that are already well-formed by July until as late as March. This is corroborated by our observation in the other three species inhabiting southeastern Brazil: *A. alba* and *A. mertensi* laid eggs by September/October (see also Barros-Filho and Nascimento, 2003; Pramuk and Alamillo, 2003) with hatching occurring approximately two months later, at least for *A. mertensi*; *C. roberti* bearing oviductal eggs in August.

The data compiled from the literature, therefore, indicates a trend for reproduction to occur during the high productivity season. This seems to become more evident as we move away from the Equator. This is apparent from our own data and also from data collected in another high latitude species. For example, in Argentina, Vega (2001) reported that *Anops kingii* reproduction extends from late winter (~July) until early summer (December). At lower latitudes, it seems that the reproductive season is more variable and may extend for longer periods. For instance, in *Leposternon polystegum* and *Amphisbaena vermicularis*, which inhabit northeastern Brazil, reproduction extends from September until May (Barros-Filho and Valverde, 1996). In Mexico, Papenfuss (1982) found that hatching time in three species of *Bipes* is synchronized with the onset of the rainy season.

Amphisbaena alba seems to be a facultative inquiline of nests of the leaf-cutting ant *Atta cephalotes* in Trinidad (Riley et al., 1986) and there is mention that this species may lay eggs on ant nests (Tschudi, 1867). The association of *A. alba* and leaf-cutting ants, however, may not occur in Brazil (Riley et al., 1986; but see Goeldi, 1902; Azevedo-Ramos and Moutinho, 1994) and our observation of egg laying in captivity indicates that ant nests might not be required. This same conclusion is also applicable to *A. mertensi* which also inhabits termite nests (Pramuk and Alamillo, 2003) but laid eggs in captivity. However, since our observations were made in captivity, which may have dis-

Table 1. Summary of reproductive data currently available for different species of *Amphisbaenia*. Species are listed alphabetically. Nomenclature follows Gans (2005).

Species	Origin	Source	Information provided
<i>Amphisbaena alba</i>	Field study. Venezuela	Gorzula et al., 1976	Testes size increases from August to September indicating that mating happens before the rainy season.
	Anedoctal. Brazil	Tschudi, 1887	Possible occurrence of oviposition in ant nests.
	Museum based. Central Brazil	Colli and Zam- boni, 1999	Two females collected in April showed enlarged vitellogenic follicles. Reproduction is suggested to be seasonal being mostly restricted to the end of the rainy season. Males may be reproductively active for longer periods compared to females.
	Anedoctal. Southeastern Brazil	Barros-Filho and Nasci- mento, 2003	Report the posture of 8 eggs (egg size = 3.1 cm × 1.5 cm) by a recently collected female.
<i>Amphisbaena darwini</i>	Argentina and Uruguay	Berg, 1898	Eggs are laid in January. Hatching happens from February until March. Clutch sizes = 2-3 eggs. Eggs are elongated, soft-shelled, and whitish in color. Total length of hatchlings is about 80 mm. Possible occurrence of parental care. Hatchlings exhibit positive geotropism.
	Museum based. Argentina	Monteiro et al., 1999	Embryological study. Clutch size = 3-8 eggs (egg size = 2.3/0.7 mm). Oviposition occurs November and December. Egg-tooth was not noticed in the embryos examined.
	Argentina	Mangione and Monteiro, 2001	Seven eggs found in December. Embryos used to describe the morphology of endolymphatic sacs.
<i>Amphisbaena fuliginosa</i>	Anedoctal. Brazil	Tschudi, 1887	Possible occurrence of oviposition in ant nests.
<i>Amphisbaena mertensi</i>	Field study. Paraguay	Pramuk and Al- lamilo, 2003	One female collected in November was found to contain 6 eggs.
<i>Amphisbaena vermicularis</i>	Museum based. Bahia, Brazil	Barros-Filho et al., 1996	Four eggs found in a female collected in September.
<i>Anops kingii</i>	Field study. Argentina	Vega, 2001	Yolked follicles start to develop in July. Oviductal eggs are found in September. Clutch size varies from 2 to 4 eggs. Male's testes are enlarged between July and November.
	Anedoctal. Rio Grande do Sul, Brazil	Boulenger, 1885	2 eggs found in an ant nest.
	Anedoctal.	Smith et al., 1952	Comments on the morphology of the egg-tooth.
<i>Bipes biporus</i>	Field study. Mexico	Papenfuss, 1982	Females mature at a body length of 185 mm and at an age of about 45 months. Females do not reproduce every year. Clutch size vary between 1 and 4 eggs (mean = 2). Eggs are laid in July and hatchlings start to be found in September. Hatchling body length varies between 90 and 100 mm.

Table 1. (Continued).

Species	Origin	Source	Information provided
<i>Bipes canaliculatus</i>	Field study. Mexico	Papenfuss, 1982	Females mature at a body length of 185 mm and a minimum age of 45 months. Females do not reproduce every year. Mean clutch size is 2.8 eggs (1 to 4 eggs). Eggs are laid between January and April. Hatching start in April. Hatchling body length is about 90 mm.
<i>Bipes tridactylus</i>	Field study. Mexico	Papenfuss, 1982	Reproductively active females are over 115 mm total body length and at least 21 months old. Clutch is formed by 1-4 eggs (mean = 2.2) laid in January. Females reproduce every year. Hatchlings start to be found in April with a body size varying between 60-70 mm.
<i>Blanus cinereus</i>	Morocco	Bons and Saint-Girons, 1963	Sperm production seems to be concentrated between March and June. Authors suggest that reproduction may not happen every year and is likely to be oviparous.
	Iberian Peninsula	Gil et al., 1994	Female become mature at a SVL > 150 mm and are thought to reproduce every year. Number of vitellogenic follicles ranging from 2-7 (mean = 3). One female (SVL = 186.5 mm) found bearing two oviductal eggs (~24 × 5.3 mm). Gonadal volume of males peaks between March and May.
<i>Blanus mettetali</i>	Northern Africa	Schleich et al., 1996	Reproductive cycle probably biannual or longer. One female about 2 ½ years old laid a single egg (27 × 5.2 mm) in mid July. Egg was pale pink and had a soft, thin shell.
<i>Dalophia colobura</i>	Field study. Mozambique and Zambia	Loveridge, 1920	Two females collected at the end of August contained 4 eggs each. Another female laid 4 eggs in September. Egg size varied from 26/9 to 35/10 mm. Reported as <i>Monopeltis colobura</i> .
<i>Leposternon infraorbitale</i>	Anedoctal. Bahia state, Brazil	Jared et al., 1997	Six eggs (size = 60/25 mm) found in February 20 th , hatched 20 days later. Positive geotropism was reported as well the presence of an egg-tooth. Mean total length of the hatchlings was 160 mm, with a mean mass of 10.75 g.
<i>Leposternon microcephalum</i>	Anedoctal. Rio de Janeiro, Brazil	Goeldi, 1897	A clutch (number of eggs not available) was taken in March from a pile of bricks and tiles inhabited by ants (<i>Camponotus</i> sp.). Eggs were soft-shelled and contained well developed embryos.
	Museum based. Argentina and Brazil	Gans, 1971	Examination of the gross morphology of early stage embryos. Clutch size = 2-8. Size of the hatchlings ~64 mm. Information that in Santa Catarina state, south Brazil, eggs are found between September and December.
<i>Leposternon polystegum</i>	Museum based. Bahia state, Brazil	Barros-Filho and Valverde, 1996	Six eggs found in female collected in December.
<i>Loveridgea ionidesii</i>	Museum based. Liwale, Tanzania	Gans and Krak-lau, 1989	One to 2 embryos (76-77 mm) found in 3 females (all collected in October). No eggshell sign present indicating this species as viviparous. A photograph of an opened female shows embryos <i>in situ</i> and a schematic drawing of the gross anatomy of an early embryo is provided.

Table 1. (Continued).

Species	Origin	Source	Information provided
<i>Monopeltis anchietae</i>	Museum based. South- ern Africa	Webb et al., 2000	Possibly viviparous. Based on counting the enlarged ovary follicles mean litter size estimated to be 2.4, range = 1-5 ($n = 5$).
<i>Monopeltis capensis</i>	Museum based. South Africa	Broadley et al., 1976	Eight females captured in December contained 1-3 embryos indicating viviparity.
	South Africa	Visser, 1967	A newborn young was expelled on January 31, after its mother had been damaged by a plowshare.
<i>Rhineura floridana</i>	Anedoctal. Gainesville, Florida, USA	Carr, 1949	Two eggs taken found in September. Premature hatchling probably caused by manipulation. Hatchlings exhibited positive geotropism. Total length of the hatchlings = 85 and 103 mm. Egg size = 38/8.9 mm.
<i>Trogonophis wiegmanni</i>	Morocco	Bons and Saint- Girons, 1963	Sperm production happens between March and June. Viviparous. Two females gave birth to 5 individuals each in September. Females are thought not to reproduce every year.
	North Africa	Schleich et al., 1996	Reproductive cycle biannual or longer. Males become mature after 2.5 years. Mating happen in June. Litter size = 2-5 individuals born by September. Total weight of the litter often equals the weight of a non-gravid female. Total length of the neonate up to 88 mm.
<i>Zygaspis quadrifrons</i>	Museum based. South- ern Africa	Webb et al., 2000	Mean clutch size = 3.3 (range 1-8). Mean SVL for adult mature female = 168 mm; Presumed hatchling body length = 75 mm. One female collected in January contained 2 shelled oviductal eggs (size = 21.5/2.8 mm).

rupted the natural behavior of the animals, it remains unconfirmed whether ant nests constitute an obligatory or even a preferable location for egg-laying in *Amphisbaenia*. Besides *A. alba* and *A. mertensi*, there are three other species – *Amphisbaena fuliginosa*, *Anops kingii*, and *Leposternon microcephalum* (see table 1) – whose eggs have been found in ant nests. Advantages attributed to the use of ant or termite nests as incubators for reptile eggs include the provision of a stable micro-environment in terms of temperature and humidity and mechanical defense against potential predators and against microbial attack (see review in Riley et al., 1985).

Although other researcher's have maintained *A. alba* in captivity for extended periods of time, no observation of reproductive activity has been reported for this species while captive (Flower, 1925; Gans, 1962; C. Jared, pers. comm.). Reproductive data from museum specimens pro-

vide little insight on the reproductive biology of *A. alba*. In a large series of *A. alba* (214 specimens), Colli and Zamboni (1999) found only 2 reproductively active females. Finally, Barros-Filho and Nascimento (2003) reported that a female of *A. alba* laid 8 eggs few hours after being captured, while maintained within a wooden collection box, which probably was caused by the stress associated with the capture. Finally, the examination of table 1 reveals that reproductive data was never obtained for animals kept in captivity on a regular basis. Whether this is due to an intrinsically low reproductive activity or inhibition of reproductive activity in captivity remains unclear.

Incubation time has rarely been reported for any *Amphisbaenia*. In *A. darwini*, egg-laying occurs in January with hatching occurring between February and early March (Klapenbach, 1960; Gallardo, 1967). In the three species of

the *Bipes* genus, incubation also lasts of about 2 months (Papenfuss, 1982). In *L. infraorbitale*, hatching occurred within one month, but embryo developmental stage, when the clutch was found in the field, was not known (Jared et al., 1997). Therefore, the present study is probably the first one to report the incubation time, from posture to hatching, for any amphisbaenid species. The two months time we recorded for the incubation of *A. mertensi* agrees with that mentioned above for *A. darwini* and for *Bipes* and is likely to be similar in other species.

We observed that the newly hatched *A. mertensi* were very prompt to bury themselves when placed over the soil. Such behavior – positive geotropism – was previously observed by Berg (1898) in *A. darwini*, by Carr (1949) in *Rhineura floridana*, and by Jared et al. (1997) in *L. infraorbitale*. As fossorial animals, the innate ability for burying themselves may provide protection against predators and/or desiccation. Indeed, amphisbaenians are known to have high rates of evaporative water loss (Krakauer et al., 1968), and the risk of desiccation may be especially high for hatchlings because their higher surface-to-volume ratio compared to adults. Predation may also be particularly high for hatchling amphisbaenids, Papenfuss (1982) estimated that between 40-60% of *Bipes* hatchlings do not survive their first year.

Hatching in *A. mertensi* occurred tail first, which differs from the head first mode observed in *L. infraorbitale* (Jared et al., 1997). However, we must note that the eggs of both species were uncovered to make the hatching observations possible. Under natural conditions, therefore, with the eggs buried in the soil or covered with debris, the occurrence of differences in hatching mode or even the significance of such difference is largely uncertain. Recently hatched *A. mertensi* responded promptly by raising the tail when disturbed, as adults do (Jared et al., 1998). This reveals that the use of tail display and autotomy as a defensive mechanism (see Brito et al., 2001) is already present from the early stages of *A. mertensi* ontogeny. Defecation and biting are

also defensive behaviors displayed by adult *A. mertensi* (personal observation) that were also observed in hatchlings.

An egg-tooth is a common characteristic of squamate reptiles (Smith et al., 1952) that have been reported for other amphisbaenians (Bogert, 1964; Gans, 1978; Jared et al., 1997). We noticed that *A. mertensi* hatches tail first, but because we missed the beginning of the hatching process, we could not directly confirm that this species uses the egg tooth to rupture the egg shell. Similarly, the use of the egg tooth for opening the shell is also uncertain in *L. infraorbitale*, even though Jared et al. (1997) observed that the hatching sequence in this species starts by the opening of a small hole in the egg-shell. Montero et al. (1999) reported that the egg-tooth is absent in *A. darwini heterozonata*.

In this study, we compiled published information on the reproduction of 21 Amphisbaenia species (see table 1), number that increases to 22 when we include our original observations in *C. roberti*. Out of these 22 species, only 4 are reported to be viviparous: *Loveridgea ionidesii*, *Monopeltis capensis* and *M. anchietae*, and *Trogonophis wiegmanni*. Our current knowledge, therefore, indicates that the evolution of viviparity in Amphisbaenia has been restricted to African species (see geographic ranges in Gans, 2005). On the other hand, when considered on a phylogenetical basis, it is revealed that viviparity is present in at least one representative of the main higher-level amphisbaenian clades, with the exception of the Bipedidae (see Kearney, 2003; Macey et al., 2004). More specifically, viviparity is found in the Amphisbaenidae (= *L. ionidesii*), in the Trogonophidae (= *T. wiegmanni*), and in the *Monopeltis* species which, according to Kearney (2003) is the sister group of the family Rhineuridae forming the Rhineuroidea clade. Accordingly, this phylogenetically widespread affiliation of viviparity is parsimoniously interpreted here as a derived trait that evolved independently for at least 3 occasions within the Amphisbaenia. The limited number of viviparous Amphisbaenia might

be due to their fossorial habits which would constraint the evolution of this trait, for example, body distension during gestation may hinder locomotor activity underground (Shine, 1985). Why viviparity is restricted to African species and the existence of ecological corollaries linked to the evolution of this trait – for example, are viviparous amphisbaenians less fossorial than oviparous ones? – are questions that remain to be studied.

Low reproductive output, in terms of number of eggs/embryos per clutch, seems to apply to most amphisbaenian species for which this information was available. Clutch size in amphisbaenians typically included from 2 to 6 eggs/embryos. Hatchling size, however, was usually large compared to the size of the parents resulting in a considerably high reproductive investment. For example, the RCM of *C. roberti* and *A. mertensi* equaled to 0.27 and 0.37, respectively. The RCM of *A. alba* and *L. infraorbitale* attained values around 0.17, which is not as large, but still falls within the range reported in other Squamata (e.g., Seigel and Fitch, 1984; Shine and Schwarzopf, 1992). Although some of our RCM values might be inaccurate since they were taken under different conditions (e.g., after the deposition of eggs in *A. mertensi* and in *A. alba*, and upon dissection of preserved specimens in *C. roberti* and *L. infraorbitale*; see also Martin, 1978; Vitt and Howland, 1985), in general, it seems that amphisbaenians produce a low number of eggs/embryos per clutch whose individual size is comparatively large in relation to female body size. In all oviparous species for which egg dimensions were available and also based on our original data, it is apparent that amphisbaenid eggs are markedly elongated in one axis. Furthermore, in the four species we examined the eggs were arranged “in-line” within the oviduct. These two characteristics may minimize a possible constraint between egg-bearing and fossorial life by lessening the disruption of the external body shape of the gravid females. It is noteworthy that while we were opening gravid females of *L. infra-*

orbitale and *C. roberti*, some containing large egg masses, their reproductive state was virtually undetectable externally.

Approximately 190 species of living amphisbaenian are currently recognized (Gans, 2005) and for most of them no information on their natural history is available. Our review shows that information on Amphisbaenia reproduction is fragmentary, often based on the examination of small samples, and heavily dependent on the publication of anecdotal observations. This pattern is likely to continue and the publication of any observation on Amphisbaenia reproduction should be highly encouraged. Also encouraged should be the study of species that are locally abundant and provide a rare opportunity to examine the natural history of the Amphisbaenia in greater detail (see Papenfuss, 1982). So far, we have reproductive data accumulated for 22 Amphisbaenia species and a better understanding of the reproduction of these animals will probably emerge only by the slow accumulation of isolated observations.

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References

- Azevedo-Ramos, C., Moutinho, P.R.S. (1994): Amphisbaenians (Reptilia: Amphisbaenidae) in nests of *Atta sexdens* (Hymenoptera: Formicidae) in eastern Amazonia, Brazil. *Ent. News* **105**: 183-184.
- Barros-Filho, J.D., Valverde, M.C.C. (1996): Notas sobre os *Amphisbaenia* (Reptilia, Squamata) da microrregião de Feira de Santana, estado da Bahia, Brasil. *Sitientibus* **14**: 57-68.
- Barros-Filho, J.D., Nascimento, L.B. (2003): *Amphisbaenia alba* (White-bellied Worm Lizard). Reproduction. *Herp. Rev.* **34**: 243-244.
- Brito, S.P., Andrade D.V., Abe, A.S. (2001): *Amphisbaenia mertensi* (NCN). Defensive behavior. *Herp. Rev.* **32**: 43-44.
- Bellairs, A.D'A., Gans, C. (1983): A reinterpretation of the amphisbaenian orbitosphenoid. *Nature* **302**: 243-244.

- Berg, C. (1898): Ueber die Eiablage, die Brutpflege und die Nahrung von *Amphisbaenia darwini*. Verhandlungen der Gesellschaft Deutscher Naturforscher und Aerzte (Leipzig) **69**: 164-165.
- Bogert, C.M. (1964): Little snake with hands: amphisbaenids are a taxonomic enigma. *Nat. Hist.* **73**: 18-25.
- Bons, J., Saint-Girons, H. (1963): Ecologie et cycle sexuel des amphisbeniens du Maroc. *Bull. Soc. Sci. nat. phys. Maroc* **43**: 117-170.
- Boulenger, G.A. (1885): A list of the reptiles and batrachians from the province of Rio Grande do Sul, Brazil, sent to the Natural-History Museum by Dr. H. von Ihering. *Annals and Magazine Nat. Hist.* (series 5) **15**: 191-196.
- Broadley, D.G., Gans, C., Visser, J. (1976): Studies on amphisbaenians (Amphisbaenia, Reptilia). 6. The genera *Monopeltis* and *Dalophia* in southern Africa. *Bull. Am. Mus. Nat. Hist.* **157**: 311-486.
- Carr, A.F. (1949): Notes on eggs and young of the lizard *Rhineura floridana*. *Copeia* **1949**: 77-77.
- Colli, G.R., Zamboni, D.S. (1999): Ecology of the worm-lizard *Amphisbaena alba* in the cerrado of central Brazil. *Copeia* **1999**: 733-742.
- Flower, S.S. (1925): Contributions to our knowledge of the duration of life in vertebrate animals. III. Reptiles. *Proc. Zool. Soc. London* **1925**: 911-981.
- Gallardo, J.M. (1967): Saurios argentinos. I – Observaciones sobre *Amphisbaena darwini heterozonata* Burmeister en los alrededores de Buenos Aires, Argentina. *Ciencia e Investigación* **23**: 406-411.
- Gans, C. (1962): Notes on amphisbaenids (Amphisbaenia, Reptilia). 5. A redefinition and a bibliography of *Amphisbaena alba* Linné. *Am. Mus. Novitates* **2105**: 1-31.
- Gans, C. (1966): Redescription of *Amphisbaena mertensi* Strauch, with comments on its geographical variation and synonymy (Amphisbaenia: Reptilia). *Copeia* **1966**: 534-548.
- Gans, C. (1969): Amphisbaenians – reptiles specialized for a burrowing existence. *Endeavour* **28**: 146-151.
- Gans, C. (1971): Studies on amphisbaenians (Amphisbaenia, Reptilia). 4. A review of the amphisbaenid genus *Leposternon*. *Bull. Amer. Mus. Nat. Hist.* **144**: 379-464.
- Gans, C. (1978): The characteristics and affinities of the Amphisbaenia. *Trans. zool. Soc. Lond.* **34**: 347-416.
- Gans, C. (2005): Checklist and bibliography of the Amphisbaenia of the world. *Bull. Am. Mus. Nat. Hist.* **289**: 1-130.
- Gans, C., Kraklauer, D.M. (1989): Studies on amphisbaenians (Reptilia) 8. Two genera of small species from east Africa (*Geocalamus* and *Loveridgea*). *Am. Mus. Novitates* **2944**: 1-28.
- Gil, M.J., Guerrero, F., Pérez-Mellado, V. (1994): Clutch size and reproductive frequency of *Blanus cinereus* in central Spain. *Acta Biol. Cracov. Ser. Zool.* **36**: 37-40.
- Goeldi, E.A. (1897): Die Eier von 13 brasilianischen Reptilien, nebst Bemerkungen über Lebens – und Fortpflanzungsweise letzterer. *Beobachtungen aus den Jahren 1884-1897. Zool. Jb. (Syst.)* **10**: 640-676.
- Goeldi, E.A. (1902): Lagartos do Brazil. *Bol. Mus. Emilio Goeldi* **3**: 1-62.
- Gorzula, S., Salazar, C., Rendon, D. (1976): Aspects of the ecology of *Amphisbaena alba* Linnaeus in the Venezuelan Guayana. *Brit. J. Herpetol.* **5**: 623-626.
- Jared, C., Antoniazzi, M.M., Almeida-Santos, S.M. (1997): *Leposternon infraorbitale* (Two-headed Snake). *Reproduction. Herp. Rev.* **28**: 44-45.
- Jared, C., Antoniazzi, M.M., Freymüller, E., Junqueira, L.C.U. (1998): A possible advantage of displaying the tail: a comparison between the tail and body integument structure in *Amphisbaena alba* and *Leposternon microcephalum* (Squamata, Amphisbaenia). *Ann. Sci. Natur.* **2**: 89-97.
- Kearney, M. (2003): Systematics of the Amphisbaenia (Lepidosauria: Squamata) based on morphological evidence from recent and fossil forms. *Herpetol. Monographs* **17**: 1-74.
- Klappenbach, M.A. (1960): Notas herpetológicas, V. I. *Amphisbaena muñoai* n. sp (Amphisbaenidae). *Com. Zool. Mus. Montevideo* **4**: 1-12.
- Krakauer, T., Gans, C., Paganelli, C.V. (1968): Ecological correlation of water loss in burrowing reptiles. *Nature* **218**: 659-660.
- Loveridge, A. (1920): Notes on east African lizards collected 1915-1919, with description of a new genus and species of skink and new subspecies of gecko. *Proc. Zool. Soc. London* **1920**: 131-167.
- Macey, J.R., Papenfuss, T.J., Kuehl, J.V., Fourcade, H.M., Boore, J.L. (2004): Phylogenetic relationships among amphisbaenian reptiles based on complete mitochondrial genomic sequences. *Mol. Phyl. Evol.* **33**: 22-31.
- Mangione, S., Monteiro, R. (2001): The endolymphatic sacs in embryos of *Amphisbaena darwini*. *J. Herpetol.* **35**: 524-529.
- Martin, R.F. (1978): Clutch weight/total body weight ratios of lizards (Reptilia, Lacertilia, Iguanidae): preservation induced variation. *J. Herpetol.* **12**: 248-251.
- Montero, R., Gans, C., Lions, M.L. (1999): Embryonic development of the skeleton of *Amphisbaena darwini heterozonata* (Squamata: Amphisbaenidae). *J. Morph.* **239**: 1-25.
- Papenfuss, T.J. (1982): The ecology and systematics of the amphisbaenian genus *Bipes*. *Occ. Pap. California Acad. Scienc.* **136**: 1-42.
- Pramuk, J.B., Alamillo, H. (2003): An effective technique for collecting *Amphisbaena mertensi* with notes on its natural history. *Herp. Rev.* **34**: 221-223.
- Riley, J., Stimson, A.F., Winch, J.M. (1985): A review of Squamata ovipositing in ant and termite nests. *Herp. Rev.* **16**: 38-43.
- Riley, J., Winch, J.M., Stimson, A.F., Pope, R.D. (1986): The association of *Amphisbaena alba* (Reptilia: Amphisbaenia) with the leaf-cutting ant *Atta cephalotes* in Trinidad. *J. Nat. Hist.* **20**: 459-470.
- Schleich, H.H., Kästle, W., Kabisch, K. (1996): Amphibians and Reptiles of North Africa. Koenigstein, Koeltz Scientific Publishers.
- Seigel, R.A., Fitch, H.S. (1984): Ecological patterns of relative clutch mass in snakes. *Oecologia* **61**: 293-301.
- Shine, R. (1980): "Costs" of reproduction in reptiles. *Oecologia* **1980**: 92-100.

- Shine, R. (1985): The evolution of viviparity in reptiles: an ecological analysis. In: *Biology of the Reptilia* Vol. 15C, p. 605-694. Gans, C., Billett, F., Eds, New York, C. John Wiley and Sons.
- Shine, R., Schwarzkopf, L. (1992): The evolution of reproductive effort in lizards and snakes. *Evolution* **46**: 62-75.
- Smith, M.A., Bellairs A.D'A., Miles, E.W. (1952): Observations on the premaxillary dentition of snakes with special reference to the egg-tooth. *J. Linn. Soc. Zool.* XLII: 260-268.
- Tschudi, J.J. (1867): *Reisen durch Südamerika*, vol. 3. Leipzig, Brockhaus.
- Webb, J.K., Shine, R., Branch, W.R., Harlow, P.S. (2000): Life underground: food habits and reproductive biology of two amphisbaenian species from southern Africa. *J. Herpetol.* **34**: 510-516.
- Vanzolini, P.E. (1992): *Cercolophia*, a new genus for the species of *Amphisbaena* with a terminal vertical keel on the tail (Reptilia, Amphisbaenia). *Pap. Avul. Zool.* **37**: 401-412.
- Vaz-Ferreira, R., Covelo de Zolessi, L., Achával, F. (1970): Oviposición y desarrollo de ofidios y lacertílios em hormigueros de *Acromyrmex*. *Physis* **29**: 431-459.
- Vega, L.E. (2001): Reproductive and feeding ecology of the amphisbaenian *Anops kingii* in east-central Argentina. *Amphibia-Reptilia* **22**: 447-454.
- Visser, J. (1967): First report of ovoviviparity in a southern African amphisbaenid, *Monopeltis c. capensis*. *Zool. Afric.* **3**: 111-113.
- Vitt, L.J., Howland, J.M. (1985): The effect of formalin fixation on weight of lizard eggs. *J. Herpetol.* **19**: 298-299.

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