

Nesting site, clutch size and development of *Atractus reticulatus* (Serpentes, Colubridae) from Corrientes, Argentina

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ABSTRACT - *Atractus reticulatus* is an oviparous semifossorial snake whose reproductive biology is barely known. In this work we present information about the nesting sites and egg characteristics of this species from two sites in the north of the province of Corrientes, Argentina. Differences in clutch size, egg size and sex ratio between the different clutches were observed. Hypotheses are considered about oviposition, developmental period of embryos and possible association of this snake with ants of the *Odontomachus* genus.

THE study of reproductive biology is essential to the understanding of life history and adaptation by organisms to different environments. In addition, it offers fundamental information that can assist the conservation state of a species (Ibargüengoytia, 2008). There is limited data about the reproductive habits of snakes, especially that of nesting sites and clutch characteristics. Gallardo & Scrocchi (2006) presented some comparable information about oviposition period, clutch size and characteristics of eggs and neonates for eight species of neotropical Colubrids and showed intra and interspecific variation in these parameters. Despite such previous work, there remains few details on embryonic development of neotropical snakes in the wild.

Atractus reticulatus is a small, oviparous Colubrid with semifossorial habits. It occurs in Brazil, from the north of São Paulo State up to the south of Rio Grande do Sul, eastern Paraguay, and in the provinces of Misiones and north of Corrientes, in Argentina (Alvarez et al., 1992; Giraudo & Scrocchi, 2000; Carreira et al., 2005). *A. reticulatus* is scarce across most of its range and encounters are infrequent. Its ecology and biology have therefore remained poorly documented and there are few references made regarding its reproduction and development. Lavilla et al. (2000) referred to this species as 'Vulnerable' and its reproductive habits are unknown. Balestrin &

Di-Bernardo (2005) documented some aspects of the reproductive biology of *A. reticulatus* but only for the populations in the south of Brazil.

This study presents information about the reproductive habits of *Atractus reticulatus* with reference to nesting sites and clutch characteristic at two sites on the oriental border of the Iberá System, Corrientes, Argentina. We also present previously unstudied information about the embryonic development of this species.

METHODS AND MATERIALS

Four clutches of *Atractus reticulatus* (numbered one to four) were collected on 28 December 2007. Two of them were found in Paraje Galarza (28° 06' 04" S and 56° 39' 46" W, Santo Tomé Department), and the other two in Colonia Carlos Pellegrini (28° 30' 25" S and 57° 07' 15" W, San Martín Department), in Corrientes, Argentina. At the time of collection, information on the biotic and abiotic characteristics of the nest sites was recorded. The collected eggs were incubated in the laboratory on the same substrate in which they were found. The length and maximum width measurements of eggs were taken and the egg volume was calculated using the spheroid formula (Dunham, 1983). One egg from each clutch was fixed in 10% formaldehyde at the time of collection and the remaining eggs were fixed one by one every 7 or 10 days, up to the complete development of embryos. The development

Clutch	Site	UNNEC N°	Clutch Size	Diameter Length (mm)	EGGS		EMBRYOS			
					Diameter Width (mm)	Egg volume average (mm ³)	DS on day collection	Sex-ratio ♂	♀	Sam- ples
1	Cnia C. Pelleg.	10.123	3	35.5	11.8	2599.36	E. 35	1	-	1
2	Pje. Galarza	10.124	6	26.7	12.6	2214.34	E. 34	2	4	6
3	Pje. Galarza	10.125	8	24.4	12.4	1961.54	E. 26	6	1	7
4	Pje. Galarza	10.126	8	23.1	12.4	1843.80	E. 31	1	6	7

Table 1. Details of clutches and embryos of *Atractus reticulatus*.

stage (DS) of embryos was estimated according to Zehr (1962). Embryo sex was determined by the presence/absence of hemipenis. Taxonomic identification was recorded from scale pattern of the advanced embryos. The analyzed embryos were deposited in the Herpetological Collection of the Universidad Nacional del Nordeste, Corrientes, Argentina (UNNEC).

RESULTS

Nesting Site

The landscape of both collection sites, Paraje Galarza and Colonia Carlos Pellegrini was characterised by a high degree of anthropogenic modification. This disturbance included grassland degraded by cattle, zones with plantation *Eucalyptus* spp. plantation and zones with scattered, felled tree-trunks and/or scrap. The eggs of *Atractus reticulatus* were found below *Eucalyptus* spp. trunks and were, in all cases, found inside anthills made by *Odontomachus* sp. (Formicidae: Ponerinae). The humidity recorded in the anthills varied between 40-50% and the temperature ranged from 27.4-27.8°C. Outside the anthills the ambient temperature was 32°C.

Clutch Characteristics

Table 1 summarises the information gathered. The eggs were found in groups, buried, or half buried in the substratum, cemented together at the margins or by the ends (Fig. 1). They were whitish in colour and had an elliptical form with a major average diameter of 25.3 mm and a minor average diameter of 12.4 mm. The average egg volume was 2079.8 mm³. Egg volume was variable within clutches (SD ± 269.02, ± 200.66, ± 587.85 for clutches # 2, 3 and 4 respectively) and between clutches (SD ± 322.56) (see Table 1).

Embryo Development

The development stage (DS) of embryos was variable between clutches. The embryos of clutch # 3 presented the least degree of development (DS 26) at the time of collection. Those in clutch # 4 were in intermediate stages (DS 31), whereas embryos of clutches # 1 and 2 were in the final stages of ontogenic development (DS 34 and DS 35, respectively). These embryonic stages were identified following morphologic characters according to Zehr (1962):

DS 26: maxillary and mandibular processes clearly visible; external naris not yet formed, hemipenis Anlagen present in male embryos, and trunk coils five and a half (Fig. 2).

DS 31: scales visible on the trunk but absent on the head, lateral flank muscles of the trunk separated from the thin ventral body wall.

DS 34: lateral trunk muscle sheets have met along the ventral midline in to the heart region, body pigmentation absent.

DS 35: body pigmentation visible but pattern not well developed, scales visible around the eyes and mouth, trunk muscles completely fused at the ventral midline, except on the region of the umbilical cord (Fig. 3).

The embryos of clutch # 4 incubated in the laboratory reached DS 37, which is considered close to hatching, by 23 January. These embryos showed little content of yolk and clearly exhibited the scale / pigmentation pattern that is typical of adult *Atractus reticulatus* (Fig. 4). The male-female ratio of embryos was variable in and between clutches, exhibiting no clear pattern in this parameter.



Figure 1. *Atractus reticulatus* clutch in *Odontomachus* sp. ant nest.

DISCUSSION

Reptile clutch sizes can be divided into two basic types; variable or invariable (Kratochvil & Kubicka, 2007). In the former, the number of eggs depends on the size of the female and/or the environmental conditions, whereas in the latter the females produce a constant number of eggs (Kratochvil & Kubicka, op.cit.). According to Gallardo & Scrocchi (2006), the clutch size of some Colubrids is variable between conspecific individuals. Balestrin & Di-Bernardo (2005) proposed a case of low fertility for *Atractus reticulatus* on the basis of a number of the vitellogenic follicles in mature females (2-6) and the number of eggs in pregnant females (1-3). This may relate to the small size and fossorial habits of this species. Herein we report a minimal clutch size of 3 eggs and a maximum of 8 eggs, which clearly expands the known clutch size for *A. reticulatus*.

In many species of reptiles the size of a female and the clutch size are often positively correlated (Ford & Seigel, 1989). Sinervo & Licht (1995) compared three populations of *Uta stansburiana*



Figure 2. *Atractus reticulatus* embryo at development stage 26.

and observed that females that lay large eggs tended to produce small clutches. Our data shows differences in the clutch size and egg volume among clutches of *Atractus reticulatus*. These variations could be related to differences in female size, suggesting variable fertility with a negative relationship between egg number and egg size per clutch.

The majority of oviparous reptiles retain eggs in the oviduct for approximately half of ontogenetic development (Shine, 1983). Nevertheless, embryonic retention in Squamata is a generalised condition and it has been stated that this circumstance has favoured the evolution and extension of viviparity in this clade (Shine, 1995). Studies on the embryonic development of *Liolaemus tenuis tenuis* (Lemus et al., 1981) and *Polychrus acutirostris* (Alvarez et al., 2005) have reported embryos in DS 27 on the first day of oviposition. A study of oviductal eggs of the snake *Mastigodryas bifossatus* has revealed that development occurs inside the uterus, at least up to DS 18 (pers. obs.). The snakes *Clelia rustica* and



Figure 3. *Atractus reticulatus* embryo at development stage 35.



Figure 4. Advanced embryo of *Atractus reticulatus* prior to hatching.

Liophis vanzolinii egg's were analyzed a few hours after oviposition and contained embryos in DS 25 and 26 respectively (pers. obs.). This variable evidence possibly suggests, for these species, that long embryonic retention may be frequent within the Colubridae. If *Atractus reticulatus* shares this embryonic retention character, the period of oviposition could be estimated. In clutch # 3, embryos were in the early stages of development, indicating recent oviposition, and were deposited by the end of December. By this time, embryos from clutches # 1 and 2 were in advanced development stages, thus we were able to predict that these eggs were deposited by the end of November. This information concurs with Balestrin & Di-Bernardo (2005) for *A. reticulatus* from the south of Brazil, where they observed eggs of this species seasonally from November until January. Data relating to embryonic condition at the moment of oviposition is scant and is a factor not always taken into account in reproductive biology studies.

We consider this information herein to be a useful tool for rough estimation of oviposition period and for furthering knowledge of the reproductive pattern of reptiles.

Fisher & Bennett (1999) hypothesized that in natural stable populations the ratio of sexes would be 1:1. The bias towards females, known for many turtle and crocodile populations, has been related to temperature dependant sex determination (Bull & Charnov, 1989), although this deviation has not yet been clearly explained in other reptile groups (Freedberg & Wade, 2001). The differences observed in proportion of sexes between and within the clutches in this study are interesting but we do not have substantial data with which to replicate observations and speculate about this factor.

The associations between reptiles and arthropods have been commonly characterised as a predator-prey relationship. Snakes of the families Colubridae, Elapidae and Leptotyphlopidae, and lizards of the family Teiidae and some Amphisbaenidae, live in nests of leaf-cutting ants of the *Acromyrmex* and *Atta* genus. In some circumstances they also use these nests as oviposition sites for incubation (Vaz-Ferreira et al., 1970, 1973; Agosti et al., 2000). A similar

relationship of this kind could be postulated for *Atractus reticulatus* and *Odontomachus* spp. ants. These ants usually build their nests at ground level, in layers of fallen leaves, earth and decomposed wood or under trunks and stones (Fernandez, 2003). Such refugia would seem suitable for the development of the snake embryos. This is most likely due to the microclimatic conditions that facilitate a warm and humid environment that would possibly be more moisture retentive compared with the external ambient environment. This relationship invites further study. Additional data about the seasonal variations of the reproductive system and ontogenetic studies in *Atractus reticulatus* would greatly contribute to knowledge of its reproductive biology and ontogenetic development.

ACKNOWLEDGEMENTS

We thank Lic. Alejandra Hernando and Dr. Maximiliano Tourmente for their suggestions and corrections of this manuscript. We also thank the Editor for improvements to the text.

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APPENDICES

- UNNEC 10123: Ea. “El Estribo”. Colonia Carlos Pellegrini, Dpto. San Martín, Corrientes, Argentina.
- UNNEC 10124: Ea. “El Estribo”. Colonia Carlos Pellegrini, Dpto. San Martín, Corrientes, Argentina
- UNNEC 10125: Ea. “El Guaruyá”. Paraje Galarza, Dpto. Santo Tomé, Corrientes, Argentina.
- UNNEC 10126: Ea. “El Guaruyá”. Paraje Galarza, Dpto. Santo Tomé, Corrientes, Argentina.