NORTH-WESTERN JOURNAL OF ZOOLOGY 7 (1): pp.92-97
Article No.: 111109

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The reproductive mode of *Adelophryne maranguapensis* Hoogmoed, Borges & Cascon, 1994, (Anura, Eleutherodactylidae) an endemic and threatened species from Atlantic Forest remnants in northern Brazil

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Received: 21. October 2010 / Accepted: 04. February 2011 / Available online: 14. February 2011

Abstract. The genus *Adelophryne* is presently constituted by six species of minute anurans that inhabit leaf litter and are distributed in the Amazon region and in the Brazilian Atlantic Forest. The present study aims to contribute to the knowledge of the reproductive mode of this genus, by presenting information about the oviposition site, egg and clutch characteristics, and type of development of *A.maranguapensis*. This species is listed as in danger of extinction because it is endemic to the Serra de Maranguape, a small remnant of Atlantic Forest in northeastern Brazil that is under severe pressure of anthropic degradation. The study is based on twelve *A.maranguapensis* egg clutches found on leaves of bromeliads at heights of 0.5 to 4.4 m from the ground. The clutches contained 3 to 8 eggs, each approximately 5 mm in diameter, with a vitreous surface and dark material in its interior, and were all located in bromeliad leaf axils. The eggs from one of the clutches hatched into fully formed froglets in the first 48 hours after collection, confirming that *A. maranguapensis* has direct development. These observations stress the conservation importance of the threatened natural remnants of the Serra de Maranguape, because *A. maranguapensis* uses arboreal sites to reproduction.

Keywords: *Adelophryne maranguapensis*, direct development, bromeligen.

Introduction

Amphibians have the most diversified modes of reproduction among vertebrates. This fact probably reflects evolutive patterns related to the first experiments in the conquest of terrestrial environments (Duellman & Trueb 1994). The concept of reproductive mode in amphibians was proposed by Salthe & Duellman (1973) based on the oviposition site, structural characteristics of the eggs and nests, the stage and size of newborn and the type of parental care, if any. For anurans alone, Pombal-Junior & Haddad (2007) recorded 39 different types of reproduction, of which 27 occur among anurans of the Brazilian Atlantic Forest (Haddad & Prado 2005). This great diversity of reproductive modes among amphibians of the Atlantic Forest may be related to heterogeneity of this ecosystem, which have rocky coastlines, mountain streams, wet litter, lots of epiphytes and montane forests (Kopp et al. 2010, Loebmann & Haddad 2010).

In the original description of the genus *Adelophryne*, Hoogmoed & Lescure (1984) reported females with well-developed oviducts, but had no information about the reproductive mode. Later, Ayarzaguena & Diego-Aransay (1985) found two large eggs (2mm in diameter) in a female of *A. gutturosa*, and considered this trait to be an indicator of direct development.

Hoogmoed et al. (1994), in describing *A. pachydactyla*, *A. baturitensis* and *A. maranguapensis* reported that females of the latter two species contained large eggs, and also mentioned the possible occurrence of direct development in the genus.

Thibaudeau & Altig (1999) included *Adelophryne* in a list of genera in which direct development is likely to occur, however without indicating in which category of endotrophic frogs as defined by Altig & Johnson (1989; viviparous, ovoviviparous, direct development, paraviviparous, exoviviparous or nidicolous) the genus should be included.

Hedges et al. (2008) included *Adelophryne* in a new anuran taxon of the New World named Terrarana, characterized by direct development and

ground-breeding, but they emphasized that these characteristics had not been confirmed for all genera of Terrarana, and are only assumed for some of them. Heinicke et al. (2009) reported that direct development has not yet been confirmed for most members of Terrarana, including all the species of *Adelophryne*.

Although the large size of the oviducal eggs seems to indicate the existence of direct development in this genus, some authors, rather prematurely, stated this as a fact (Reynolds et al. 2004, Silvano & Borges-Nojosa 2004a,b, Eterovick et al. 2005, Wells 2007). MacCulloch et al. (2008) were the first to record the oviposition (under artificial conditions after capture) of a large egg (4,63mm) by *A. gutturosa*. The authors suggested that this finding supported the hypothesis of direct development for the species. Thibaudeau & Altig (1999) emphasized that the size of eggs is not sufficient to conclude that any species of anuran possesses direct development, or indeed any other category of endotrophic development, because of the overlap in the sizes of endotrophic and exotrophic eggs in about 25% of species of anurans.

This study contributes to knowledge of the reproductive biology of the little studied genus *Adelophryne*, and confirms the occurrence of direct development in the genus based on information on the oviposition site, egg characteristics, spawning and hatching of *A. maranguapensis*, an endangered species (Borges-Nojosa 2008). *Adelophryne maranguapensis* is endemic to a small remnant of the Atlantic Forest in northeastern Brazil, which is under severe pressure by anthropic degradation.

Material and Methods

This study is part of a research program on the herpetofauna of the Serra de Maranguape, a mountainous area about 920 m above sea level, located in the state of Ceará, northeastern Brazil. The study has been carried out since the year 1995, in discontinuous periods. These observations result from sampling carried out in each month, from 1998 to 2003, and during the first half of 2010.

Active daily and nightly searches were carried out in leaf litter, under rocks, in mosses, along streambanks and lake shores, in holes in the ground, in trees, bromeliads, and other places where clutches of *A. maranguapensis* might be found. The positions of all identified nests were recorded, and the eggs were counted and measured with a digital caliper (0.1 mm accuracy). Some spawnings were collected and housed in Petri dishes containing moist cotton or pieces of paper towel, similarly to the method for culture of *Eleutherodactylus coqui* embryos, proposed by Elinson et al. (1990, 2008) and Sabo et al. (2009). Later some of the clutches were transferred to the laboratory and kept at room temperature of approximately 23°C and protected from sunlight, simulating the environment where they were found. The eggs were photographed every two days.

Three newly hatched animals were obtained from one only spawning. They were measured, euthanized, fixed in 10% formalin, and stored in vials containing 70% ethanol. All material was deposited in the Herpetological Collection of the Núcleo Regional de Ofiologia, of the Federal University of Ceará (NUROF-UFC) (CHUFC 5806-09).

Results

Clutches of *A. maranguapensis* were found at only two points, one near the Riacho Beija-Flor (03°53'44.3"S; 38°43'18.8"W, 890m), and one on the top of the mountain, known as Pico da Rajada (3°53'44.2"S; 38°43'20.8"W, 920m). To date, 12 egg masses of *A. maranguapensis* have been obtained, in the local rainy season (first quarter of each year) (Table 1). They were located only on the adaxial side of leaves of four species of bromeliads, with an average height of 1.76 m above the ground (Table 1). The clutches consisted of 3-8 eggs, translucent with a vitreous surface, and about 5mm in diameter. Three eggs (clutch 9) with embryos in advanced stages were larger (6.6, 7.4 and 6.9mm) several minutes before hatching (Table 1, Fig. 1).

The eggs from clutch 1 (Table 1) hatched into fully formed froglets 48 hours after collection, but we could not obtain any meristic data because the newborns were rapidly attacked and ate by ants. Clutches 2, 3 and 4 (Table 1) were attacked by fungus and were totally destroyed, but these latter two (3 and 4) could be observed in the laboratory for 14 days. They had embryos with a distinct head region, dorsally with discrete dark spots bordering the yolk, and undifferentiated stumps of the forelimbs and hindlimbs, of the same size (Fig. 2A). After 48 hours (April 30), the heads of the embryos began to show dark spots similar to those on the back, and their eyes were easily discernible (Fig. 2B). After another 48 hours (May 2), the embryos were colored brown, and their eyes were darker than before. The heartbeat could also be observed at this stage (Fig. 2C). After 72 hours (May 5), the anterior and posterior limbs were well developed, and it was possible to observe the edges of the disks at the ends of elongated fingers, a characteristic of the genus (Hoogmoed & Lescure 1984). At this stage, the coloration of the embryos was similar to that of newly hatched froglets

Table 1. General data for clutches of *A. maranguapensis*.

Clutch	# of eggs	Diameter[x]	Date[+]	Distance[*]	Bromeliad species	Height[#]
1	5	n.d.	28.II.1998	2.0	*Guzmania lingulata* (Linnaeus) Mez	1.5
2	3	n.d.	12.I.2003	2.0	*G. lingulata*	0.5
3	5	n.d.	26.IV.2003	2.0	*G. lingulata*	1.8
4	6	n.d.	26.IV.2003	2.0	*G.lingulata*	1.8
5	3	5.18	21.IV.2010	2.0	*G. lingulata*	1.4
6	6	5.18	21.IV.2010	10.0	*Vriesea cearensis* L. B. Smith	1.6
7	7	4.46	21.IV.2010	n.d.	*G. lingulata*	1.6
8	8	5.74	21.IV.2010	8.0	*Aechmea pernambucentris* J. A. Siqueira & Leme	4.4
9	5	5.98	21.IV.2010	2.0	*G. lingulata*	2.5
10	6	4.52	22.IV.2010	2.0	*G. lingulata*	1.5
11	6	4.51	22.IV.2010	2.0	*Guzmania sanguinea* (André) André ex Mez	1.3
12	3	5.70	22.IV.2010	3.0	*G. sanguinea*	1.3
Mean	5.5	5.15		3.36		1.76

Legend: (x) Mean egg diameter (mm); (+) Eggs finding date; (*) Distance from nests to the water accumulated in bromeliads (cm); (#) Bromeliad height above the ground (m); (n.d.) no data.

(Fig. 2D). After this stage, embryos showed no noticeable morphological changes, and the eggs gradually changed from glassy to opaque, probably because of fungus infestation that eventually destroyed the culture of embryos. The clutches obtained in 2010 were all registered and measured during the field work, and only the spawnings 7-10 were collected.

To date the only adult female *A. maranguapensis* was collected by Hoogmoed et al. (1994), and it has SVL 17.4mm, and some of the nests found in the present study possibly were not made by only one female due to the big diameter and number of eggs (3-8) found in the clutches. The clutch 9 apparently supports this hypothesis because the embryos were in different development stages, indicating that ovipositions were not made in the same time. Three eggs from this clutch hatched in newborn froglets (mean: 4.53mm, 0.014g) in the same day of collection while one of the other embryos presented arms and legs and a big yolk quantity, and the other one was in an early stage presenting the stumps of forelimbs and hindlimbs (Fig. 3).

Discussion

This study, by monitoring the positions of clutches and recording the hatching of small, fully formed froglets from eggs of *A. maranguapensis*, confirms the occurrence of direct development in this species, and suggests that this pattern most likely also occurs in other species of *Adelophryne*. Although it is presumed that all Terrarana species have direct development, this is not confirmed for the majority of the members of the group, including all species of *Adelophryne* (Heinicke et al. 2009).

Although the egg masses of *A. maranguapensis* were not found in direct contact with the accumulated water in bromeliads, this water reservoir is likely to be an important factor in the reproduction of the species, helping to increase the relative humidity and preventing the eggs from drying, besides hosting adults during the reproductive period. The depigmentation observed in the eggs of *A. maranguapensis* is probably associated with poor lighting at the site of oviposition, as reported by Pombal-Junior (1999) for eggs of *Brachycephalus ephippium*. In both cases, the vegetation appears to function to shade the clutches from sunlight, reducing the risk of dehydration and the need for pigmentation of the eggs.

The association of some species of amphibians with bromeliads has been the subject of several studies (Laessle 1961, Peixoto 1995, Lopez et al. 1999, Schineider & Teixeira 2001, Andrade et al. 2009). The accumulation of organic matter and water in the leaf axils of tank-bromeliads makes them isolated aquatic environments (Mestre et al. 2001). Peixoto (1995) classified the amphibians found in bromeliads as bromelicolous or bromeligens, these latter being composed of species that use these plants in their reproductive cycle, and the former, those which can sometimes be found in bromeliads, but do not use them for reproduction. According to Peixoto's classification, *A. maranguapensis* is a bromeligen species. Although all the clutches were found in bromeliads, vocal activity of males has been recorded in leaf litter, indicating that this species has morphological adaptations to climbing (Reilly & Jorgensen 2011), and does not

Figure 1. A- Clutch (No. 6) of *Adelophryne maranguapensis* on *Vriesea cearensis* leaves; **B-** Translucent eggs of *A. maranguapensis* (clutch No.8), with visible embryos; **C-** Newly hatched froglet (right) (SVL 4,6 mm), and froglet about to hatch (left) (clutch No. 9).

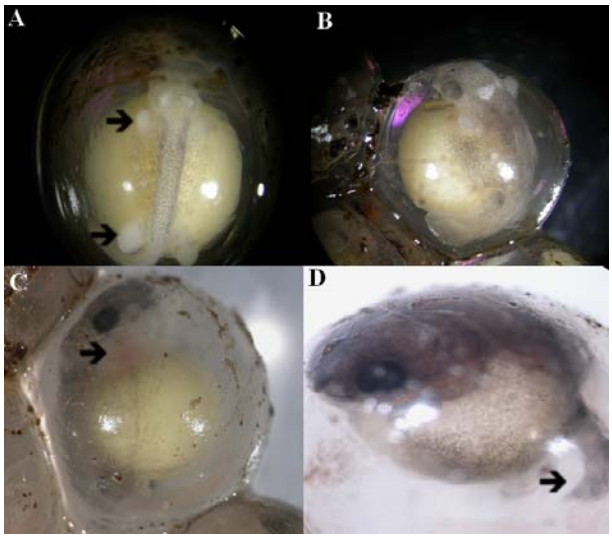

Figure 2. *Adelophryne maranguapensis*:
A- Eggs and embryos with discrete dark dorsal spots, and stumps of the forelimbs and hindlimbs (arrows);
B- Embryo with spots on the cephalic region and eyes easily discernible;
C- Embryo with a noticeable heart (arrow);
D- Embryo with developed arms and legs (arrow).

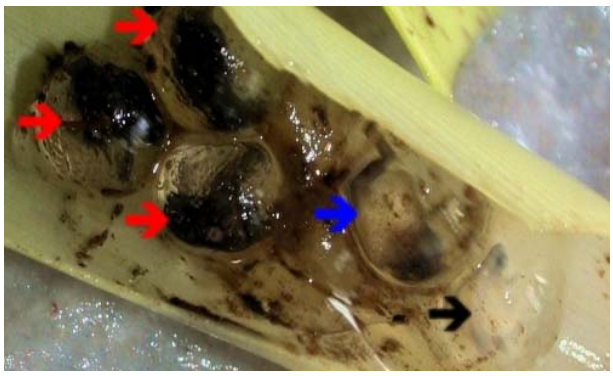

Figure 3. Clutch 9 showing *A. maranguapensis* embryos in three distinct developmental stages: Three froglets some minutes before hatching (red arrows); Embryo with distinct legs and arms but still with the yolk (blue arrow); Younger embryo with color pattern of early stages (black arrow).

occurs only near the ground as suggested by Hoogmoed et al. (1994).

The more specialized reproductive modes among the Brazilian Atlantic Forest anurans are commonly found in genera that have few species and are restricted to forest environments, such as *Brachycephalus*, *Crossodactylodes*, *Dendrophryniscus*, *Flectonotus*, *Frostius*, *Gastrotheca*, *Myersiella* and *Zachaenus* (*Ischnocnema* being an exception), and in species of small body size (Haddad & Prado 2005). The genus *Adelophryne*, which until now has only six described species, with individuals measuring less than 2 cm in SVL, seems to follow the pattern described above, having also a specialized reproductive mode.

According to the observations reported here, *A. maranguapensis* shows the reproductive mode 27 *sensu* Haddad & Prado (2005) and Pombal-Junior & Haddad (2007): arboreal eggs hatching into froglets. Haddad & Prado (2005) emphasized that populations of frogs whose reproductive modes depend on the forest moisture, tend to be suppressed by deforestation. The areas where *A. maranguapensis* is found have had their original vegetation removed for the installation of permanent crops and banana trees (Rodriguez & Smith 2002), which has caused the loss of large amounts of soil by erosion, especially in heavy rainy periods. Moreover, the practice of local people to collect bromeliads for sale as ornamental plants (Cassiano-Lima & Cascon 2008) is another major challenge for the conservation of *A. maranguapensis*, since the frogs use these plants as a breeding site. These factors indicate the importance of conserving of the natural vegetation of the Serra de Maranguape, the area of endemism of *A. maranguapensis*.

Acknowledgements. We thank the following persons and entities: The Biodiversitas Foundation, the CEPAN Foundation, the Critical Ecosystem Partnership Fund and the Municipality of Maranguape (Mayor M. Silva) for financial support; F. Monteiro and V. dos Santos Gomes for the identifying the bromeliads; A.C.O. de Queiroz Carnaval, J.C. Lima Melo, T.A. de Sousa, J. E. Vasconcelos Carlos and E. V. Nascimento (ONG Serras Verdes) for helping in some of the field trips; the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) and the Chico Mendes Institute for Conservation of Biodiversity (ICMBio) for granting collecting permits (Processes In. 02007.000965/04-10, In. 10893-1 and In. 22909-1); J. Kilder for his hospitality and help during field work; C. F. B. Haddad and C. P. de Almeida Prado for theoretical explanations; and T. Gomes dos Santos, M. Solé and J. F. de Araujo for manuscript review and suggestions. D. C. Lima is grateful to the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for the doctor's degree fellowship (proc. BDS-0017-00003.01.18/10), and S. Z. Cechin is grate to CNPq for the research fellowship (proc. nº 303359/2009-9).

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