# Local distribution and notes on reproduction of *Vitreorana aff. eurygnatha* (Anura: Centrolenidae) from Sergipe, Northeastern Brazil

SIDNEY FEITOSA GOUVEIA[1,4], RENATO GOMES FARIA[2] AND PATRICIO ADRIANO DA ROCHA[3,4]

[1]*Post-Graduation Program in Ecology and Evolution, Federal University of Goiás, Goiânia – GO, Brazil.*

[2]*Departament of Biology, Federal University of Sergipe, São Cristóvão – SE, Brazil.*

[3]*Post-Graduation Program in Biology, Federal University of Paraiba, João Pessoa – PB, Brazil.*

[4]*Post-Graduation Program in Ecology and Conservation, Federal University of Sergipe, São Cristóvão – SE, Brazil.*

[1]Corresponding author: sidfgouveia@gmail.com

ABSTRACT - Species of the family Centrolenidae are poorly known, especially concerning the basic features of their natural history, especially those distributed at eastern Brazil. During the rainy season of 2006, we studied the local-scale pattern of spatial distribution and some aspects of reproduction, including behaviour, of a population of *Vitreorana aff. eurygnatha* from Sergipe State, Brazil. Individuals were clumped-distributed and reproduced on vegetation overhanging streams, between 0.30 and 4.00m height. The species exhibits sexual dimorphism in size, with females slightly larger than males. Their egg clutches consisted of about 18 eggs and were laid mostly on the upper side of leaves. We also describe the overall calling pattern and present the first record of chorus leadership in Centrolenidae. Additional ecological traits plus some notes of a male-female and a male-male encounter are presented and compared to other Hyalinobatrachinae glass-frogs.

## INTRODUCTION

Glass-frogs (Anura: Centrolenidae) are amongst the largest endemic families of the Neotropical anurans, with 148 species recognized (Cisneros-Heredia et al. 2009). They are geographically distributed from Mexico to Argentina and Brazil and show recognizable ecological features concerning microhabitat use and reproductive mode (Cisneros-Heredia & McDiarmid, 2007). The latter consists of the deposition of a jellylike mass of eggs on leaves or rocks along streams, where advanced staged exotrophic larvae fall or are washed down to the water to develop (Cisneros-Heredia & McDiarmid, 2003, 2006).

Recent studies have stressed the taxonomic and phylogenetic relationship among glass-frogs (Cisneros-Heredia & McDiarmid, 2006, 2007; Guayasamin et al., 2009), but the knowledge on population ecology, behaviour and reproductive biology are remarkably scarce, particularly from those species distributed in eastern Brazil (Cisneros-Heredia & McDiarmid, 2003, 2007). Available data on the autoecology of Centrolenids generally consist of naturalistic reports (e.g. Duellman & Tulecke, 1960; McDiarmid & Adler, 1974; Greer & Wells, 1980; Bolívar et al., 1999) or notes on natural history from taxonomic comparison and description of new taxa (e.g. Ruiz-Carranza & Lynch, 1991; Cisneros-Heredia & McDiarmid, 2006). Generally they conform to the overall ecological traits of glass-frogs, but specific characterization is still limited.

A population of glass-frogs at the Brazilian State of Sergipe was first recorded by Carvalho et al. (2005). We studied this population to examine the local pattern of spatial distribution and reproductive traits related to breeding site, clutches and behaviour and compare the observed features
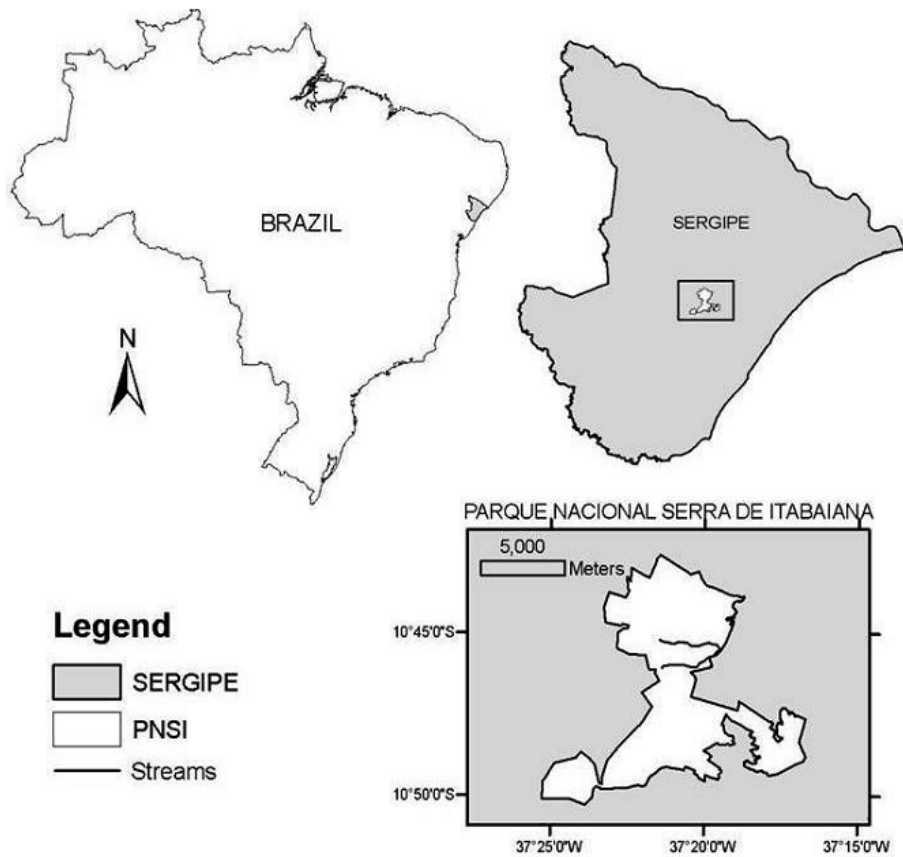
**Figure 1.** Location of the study site, PNSI, Sergipe State, Brazil.

to some other species of Centrolenidae.

### MATERIAL AND METHODS

The study was conducted at the protected area Parque Nacional Serra de Itabaiana (PNSI) (10°45'S, 37°20'W), Sergipe state, Northeast Brazil (Fig. 1). The PNSI encompasses 7,966 ha of a small and round-shaped mountain region inserted at the Atlantic Forest biome (IBGE, 2004). The local altitude ranges from 200 to 670 m and the local climate is A's according to Köppen's classification – tropical with dry and moderate summer and hydric excess at winter – with an annual precipitation between 1100 and 1300 mm (Ab'Saber, 1967).

Observations were carried out during the rainy season, once a week, between May and August

| $X_i$ | $f_i$ | $Px_i$ | $F_i$ |
|---|---|---|---|
| 0 | 141 | 0.666 | 199.988 |
| 1 | 21 | 0.270 | 48.654 |
| 2 | 10 | 0.055 | 9.864 |
| 3 | 3 | 0.007 | 1.332 |
| 4 | 4 | 0.000 | 0.126 |
| 5 | 1 | 0.000 | 0.108 |
| 6 | 0 | 0.000 | 0.000 |

Table 1. Frequency of occurrence ($f_i$) of different amounts of individual ($x_i$) per section with the associated probabilities ($Px_i$) and Chi-square statistics (F) for a Poisson distribution.

| | | Action | |
|---|---|---|---|
| Time (am) | Distance | Male | Female |
| 00:56 | 1 m | Calling on shrub | On the same plant |
| 00:57 to 01:03 | 1 m to 10 cm | Keeps calling | Moving towards the male |
| 01:04 | 10 cm | Calling | On the same leaf as the male |
| 01:06 | 0 | Clasps the female | Standing |
| 01:30 | 0 | Clasping | Displaces at nearby leaves |
| 02:52 | 0 | Clasping | Laid the clutch |
| 02:53 | 0 | Immediately released female and emitted calls | Stayed near the clutch until dawn. |

**Table 2.** Sequence of events of a male-female encounter of *Vitreorana aff. eurygnatha* at Água Fria Stream, PNSI, Sergipe. Distance between individuals are aproximations.

2006. Visits lasted from one to four consecutive days, beginning at 18:00hrs to 00:00h local time. Two streams, Coqueiro (37°20'48"W; 10°45'57") and Água Fria (37°20'35"W; 10°45'19"S), were surveyed by walking through the stream beds.

To assess the individual pattern of spatial distribution (clumped, random or uniform) we surveyed the streams in segments of four meters lengths throughout each margin (resulting in two parallel lines of segments). This summed 360 meters along both streams, totalizing 180 sections. We then determined the presence of individuals along the streams margins, by systematically examining leaves, tree trunks and rocks, counting all individuals within each segment. During the search we recorded the substrate, height of perching, time of activity and any relevant behaviour, which were recorded ad libitum. We also recorded characteristics of the clutches and sites of oviposition, such as clutch size, height from the surface (water or ground), face of the leaf used (upper side or lower side) and size and texture of the leaves.

Six male-female pairs were captured and held in plastic bags to obtain clutches. Each individual had its snout-vent length (SVL) measured to test for sexual dimorphism and all clutches and eggs were measured to the nearest 0.01 mm immediately after deposition.

The pattern of spatial distribution was analyzed through Poisson distribution with a Chi-square goodness-of-fit test. The preference for the leaf side was tested through the Chi-square test and Student t test was used to verify sexual dimorphism (Zar, 1996). All tests were considered significant at ≤ 0.05.

**RESULTS**

During the visits the temperature varied from 20 to 27°C, and the moisture from 66 to 81%. The rain was irregular and individuals were active in the absence of rainfall. We recorded 69 individuals, which showed a clumped pattern of local distribution $\chi^2$ = 6.5148; df = 179; p < 0.001) along the streams (Table 1). Groups of frogs distanced each other from 4 to 24 m, with no more than four individuals per segment and nine individuals (including males and females) per group, considering consecutive occupied segments. Individuals used marginal vegetation along the streams as breeding sites. The height of perching varied from 0.30 to 4.00 m. The size of leaves used as calling site ranged from 7x4 cm to 28x11 cm and all leaves had a totally glabrous (smooth) limb. The main species of plant used was Inga sp. (Leguminosae), followed by Bonnetia stricta (Theaceae), Heliconia sp. (Heliconiaceae),

epiphites in tree trunks and others less frequently. The upper (adaxial) surface of the leaves was significantly preferred for egg-laying (77% of clutches, n= 62; $\chi^2$ = 22.35; df = 1; p < 0.001). The clutches consisted of gelatinous, circular, and transparent mass of individual capsules involving cream-colored to greenish eggs. Immediately after the deposition, the clutches measured approximately 10 mm in diameter, and eggs about 2.18 ± 0.23 mm. The surrounding gelatinous layers were thin and doubling their diameter once in contact with water. Clutch size varied from 10 to 25 eggs (17.77 ± 2.70 mm). Adults were not seen attending clutches.

Females were significantly larger (20.55 ± 0.79 mm) than males (18.18 ± 0.60 mm), (t = 7.41; df = 11; p < 0.001). Males were observed calling alone or forming choruses of up to six individuals, not necessarily from the same group. After the first male's call, others replied, including males from a different group distant by a few meters. The sequence of vocalizations always started by the same individual, and always followed the same order, remaining in silence from 30 seconds to 2 minutes, until the next sequence.

We observed only two encounters: one involving a male and a female (cohort followed by amplexus), and a second between two males. The male-female encounter lasted almost two hours (see full description in Table 2) similar to the time of interaction between pairs inside plastic bags. The male-male encounter lasted around 25 minutes and involved a calling male and a silent one. During the encounter, the latter frog remained in a flattened position, while the former called continually. After four minutes standing 20 cm apart from each other, the calling male hopped closer to the non-caller one, i.e., on the same leaf, remaining in an upright stance while calling for 20 minutes until it left the site. In both encounters only one type of call was heard.

## DISCUSSION

Among amphibians the pattern of clumped distribution is common and known as 'lek behaviour' (Wells, 1977). It is also widespread among birds, mammals and insect (Hoglund & Alatalo, 1995), in which males position themselves

close to each other, while attracting females, which in turn move among the males to select a mate. According to Wells (1977), this behaviour is not fully understood but might be related to the scarcity of resource (space) or due to the mechanisms of female choice. We favour the latter explanation, since we noted many vacant, but apparently suitable, microhabitats throughout the study area.

Centrolenids are known to use the vegetation along streams and other bodies of water (Greer & Wells 1980, McDiarmid & Adler, 1974) and the use of high perch locations (0.30 – 4.00 m) is in agreement with other Centrolenidae (Greer & Wells, 1980), including Vitreorana eurygnatha and (1.00 - 3.00 m) (Heyer et al., 1990). Greer and Wells (1980) suggest that differential use of perch heights may influence the male reproductive success in H. fleischmanni, although other territorial and behavioural traits may interfere in female choice. The height of the clutches (0.45-4.00 m) agrees with that of adults, although it is possible that the preference of sites to lay eggs may be different from male's calling-site, as we saw with the amplected female, which was continuously searching for a definitive site for egg deposition. Information on plant selection for breeding (calling and/or egg-laying) site is limited. Greer & Wells (1980) mentioned the use of large leaves such as Dieffenbachia, bromeliads and tree-trunk epiphytes by H. fleischmanni. We suggest that differential use of specific plants reflects their local abundance (although not measured, it is evident from the predominance of Inga). But the predominant use of smooth leaves in the studied population may indicate a specific requirement related to the adult displacement and the gliding of larvae down to the water.

Clutch characteristics such as egg size and colour are similar to most Centrolenids. Certain species show differences, for example the eggs are black in *Hyalinobatrachium prosoblepon* (Starrett, 1960) and black-and-white in *H. euknemos*, (Savage & Starrett 1967). In respect to size, there is a more variation, with means ranging from two and three eggs in *H. munozorum* and *H. midas*, respectively (Crump, 1974), but 80 eggs in H. chirripoi (Kubicki, 2004).

Ruiz-Carranza & Lynch (1991) drew attention to

historical differences among groups related to leaf size preferences. Species of *Hyalinobatrachium* (sensu Guayasamin et al., 2009) tend to lay those eggs on the lower side (e.g. Crump 1974; Greer & Wells, 1980), whereas Atlantic Forest centrolenids (genus Vitreorana) use both sides (Lutz, 1947). The preference for upper leaf side found here may supports the Ruiz-Carranza & Lynch's (1991) idea, which has been phylogenetically confirmed by Guayasamin et al. (2009).

The absence of parental care also support the distinction previously discussed. In the genus *Hyalinobatrachium*, this behaviour is widespread and may involve males (McDiarmid, 1978) or female (Jacobson, 1985), and may be nocturnal (Duellman & Trueb, 1986) or diurnal-nocturnal (McDiarmid, 1978). Conversely, as far we know, no previous study has recorded this behaviour among glass-frogs from Atlantic Forest, which may indicate a reliable feature and further evolutionary distinctiveness.

Chorus formation is common in amphibians (Duellman & Trueb, 1986), including glass-frogs. Duellman (1967) cites trios in *H. fleischmanni* and Heyer et al. (1990) documented rapid and overlapping call replies, followed by a long silence in *V. eurygnatha*. However this appears to be the first record of chorus leadership within the Centrolenidae, which is characterized by the same male opening the calling sequence. It is not known, however, if chorus leaders achieve more reproductive success than non-leaders.

## REFERENCES

Ab'Saber, A.N. (1967). Domínios morfoclimáticos e províncias fitogeográficas do Brasil. *Orientação* **3**, 45-48. Departamento de Geografia, Universidade de São Paulo.

Bolívar, W., Grant L. & Osorio A. (1999). Combat behaviour in *Centrolene buckleyi* and other centrolenid frogs. *Alytes* **16**, 77-83.

Carvalho, C.M., Vilar, J.C. & Oliveira, F.F. (2005). Répteis e Anfíbios. In *Parque Nacional Serra de Itabaiana – Levantamento da Biota*, 39-61 C. M. Carvalho & J. C. Vilar (coords). Aracaju, Sergipe, Brasil: Ibama, Biologia Geral e Experimental – UFS.

Cisneros-Heredia, D.F. & McDiarmid, R.W. (2003). Ecuadorian Glass Frogs: Current state of knowledge, new research trends and conservation. Abstracts, *Joint Meet. Ichthyol. Herpetol.*, Manaus, Brasil.

Cisneros-Heredia, D.F. & McDdiarmid, R.W. (2006). A new species of the genus *Centrolene* (Amphibia: Anura: Centrolenidae) from Ecuador with comments on the taxonomy and biogeography of glass-frogs. *Zootaxa* **1244**, 1–32.

Cisneros-Heredia, D.F. & McDiarmid, R.W. (2007). Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glass-frogs. *Zootaxa* **1572**.

Crump, M.L. (1974). Reproductive strategies in a tropical anuran community. *Mus. Nat. Hist. Univ. Kansas, Mis. Pub.* **61**.

Duellman, W.E. (1967). Social organization in the mating calls of some neotropical anurans. Am. Midl. Nat. **77**, 156-163.

Duellman, W.E. & Savitsky, A.H. (1976). Aggressive behaviour in a centrolenid frog, with comments on territoriality in anurans. *Herpetologica* **32**, 401-404.

Duellman, W.E. & Trueb, L. (1986). *Biology of Amphibians*. The Johns Hopkins Univ. Press. Baltimore and London.

Duellman, W.E. & Tulecke, J.B. (1960). The Distribution, Variation, and Life History of the Frog *Cochranella viridissima* in Mexico. *Am. Midl. Nat.* **63**, 392-397.

Greer, B.J. & Wells, K.D. (1980). Territorial and reproductive behaviour of the tropical American tree frog *Centrolenella fleischmanni*. *Herpetologica* **36**, 318-326.

Guayasamin, J.M., Castroviejo-Fisher, S., Trueb, L., Ayarzagüena, J. Rada, M. & Vilà, C. (2009). Phylogenetic systematics of Glassfrogs

(Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* **2100**, 97 pp.

Heyer, W.R., Rand, A.S., Cruz, C.A.G., Peixoto, O. L. & Nelson, C.E. (1990). Frogs of Boracéia. São Paulo, *Arq. Zool. Univ. São Paulo* **31**, 235-410.

Hoglund, J. & Alatalo, R. (1995). *Leks*. Princeton University Press, Princeton, NJ.

IBGE – Instituto Brasileiro de Geografia e Estatística. 2004. *Mapa de Biomas do Brasil, primeira aproximação*. Rio de Janeiro, Brasil: IBGE.

Jacobson, S.K. (1985). Reproductive behaviour and male mating success in two species of glass frogs (Centrolenidae). *Herpetologica* **41**, 396-404.

Kubicki, B. (2004). Rediscovery of *Hyalinobatrachium chirripoi* (Anura: Centrolenidae) in southeastern Costa Rica. *Rev. Biol. Trop.* **52**, 215-218.

Lutz, B. (1947). Trends towards non-aquatic and direct development in frogs. *Copeia* **4**, 242–252.

McDiarmid, R.W. & Adler, K. (1974). Notes on Territorial and Vocal Behaviour of Neotropical Frogs of the Genus *Centrolenella*. *Herpetologica* **30**, 75-78.

McDiarmid, R.W. (1978). Evolution of parental care in frogs. In *The Development of Behaviour: comparative end evolutionary aspects*, 127-147. Burghardt, G.M. & Beckoff, M. (eds). New York, Garland.

Ruiz-Carranza, P.M. & Lynch, J.D. (1991). Ranas Centrolenidae de Colombia I. Propuesta de una nueva clasificación genérica. *Lozania* **57**: 1-30.

Savage, J.M. & Starrett, P.H. (1967). A new fringe-limbed tree-frog (Family Centrolenidae) from lower Central America. *Copeia* **3**, 605-609.

Starrett, P.H. (1960). Descriptions of tadpoles of Middle American frogs. *Misc. Pub. Mus. Zool. Univ. Michigan* **110**.

Wells, K.D. (1977). The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666-693.

Zar, J.H. (1996). *Biostatistical analysis*. New Jersey: Prentice Hall.Ab'Saber, A.N. (1967).