ISSN 0123 - 3068 bol.cient.mus.hist.nat. 17 (2), julio - diciembre, 2013. 127 - 140

BOLETÍN CIENTÍFICO
CENTRO DE MUSEOS
MUSEO DE HISTORIA NATURAL

NOTES ON THE NATURAL HISTORY OF THREE GLASS FROGS SPECIES (ANURA: CENTROLENIDAE) FROM THE ANDEAN CENTRAL CORDILLERA OF COLOMBIA*

*Julián Andrés Rojas-Morales[1] *;and Sergio Escobar-Lasso[2]*

Abstract

Between October 2008 and December 2010, we carried out surveys in different creeks at north of the municipality of Manizales in the Colombian Central Cordillera, in order to make observations on the natural history of three glass frogs species (*Centrolene quindianum*, *Nymphargus grandisonae* and *N. spilotus*). *Centrolene quindianum* presents clutches with 33–35 eggs (N= 2) located on the Upper of leaves at heights between 40–200 cm above water; one male was observed close to three egg-masses (< 1 m of distance of them in the same perch), suggesting the possibility of parental care. The spider *Patrera armata* (Anyphaenidae) is reported as a predator of the embryos of this species. In *N. grandisonae* both calling and oviposition occurs in the upper surfaces of large leaves, especially Araceas and Heliconias. These breeding sites are guarded territories by males for periods of up to six months. Their egg-masses have an average of 74 eggs (N = 4), located at a height and a distance from the edge of creeks between 57–218 cm ($\overline{X}$ = 113.21 ± 45.73 cm, N = 14) and 0–35 cm ($\overline{X}$ = 15.8 ± 6.7 cm, N = 14), respectively; males stay close to egg masses as they continue calling to attract females. In addition, we report for first timefly larvae (Drosophylidae) as parasitoid agent foregg-masses of *N.grandisonae*. In *N.spilotus*, we report the finding of a male with a deformity in his right leg and a female that layed eggs without the presence of a mate male.

Key words: Centrolenidae, *Centrolene quindianum*, natural history, *Nymphargus grandisonae*, *Nymphargus spilotus*, reproductive behavior.

NOTAS SOBRE LA HISTORIA NATURAL DE TRES ESPECIES DE RANAS DE CRISTAL (ANURA: CENTROLENIDAE) DE LA CORDILLERA CENTRAL DE COLOMBIA

Resumen

Entre octubre de 2008 y diciembre de 2010 se realizaron muestreos en diferentes riachuelos al norte del municipio de Manizales en la Cordillera Central colombiana, con el fin de realizar observaciones sobre la historia natural de tres especies de ranas de cristal (*Centrolene quindianum*, *Nymphargus grandisonae* y *N. spilotus*). *Centrolene quindianum* presenta nidadas de entre 33–35 huevos (N = 2), ovopositadas en el haz de las hojas a alturas entre 40–200 cm; un único macho fue

* FR: 10-V-2012. FA: 21-VIII-2013.

*Associate researcher of the División de Historia Natural, Centro de Museos, Universidad de Caldas, Manizales, Caldas, Colombia. julian.herpetologia@gmail.com

**Associate researcher of the División de Historia Natural, Centro de Museos, Universidad de Caldas, Manizales, Caldas, Colombia. Fundación R.A.N.A (Restauración de Ambientes Neotropicales Alterados), Manizales, Caldas, Colombia. biosergiobike@gmail.com

observado cerca a tres nidadas (ubicado en la misma percha a < 1 m de distancia de ellas), sugiriendo la posibilidad de cuidado parental sobre los huevos. La araña *Patrera armata* (Anyphaenidae) se reporta como depredador de los embriones de esta especie. En *N.grandisonae*, tanto la actividad decanto como la ovoposición, se da en el haz de las hojas con áreas foliares grandes principalmente aráceas y heliconias. Estos sitios reproductivos corresponden a territorios fielmente celados por los machos por períodos de hasta seis meses. Sus nidadas presentan en promedio 74 huevos (N = 4), ubicándose a una altura y a una distancia del borde de los riachuelos entre 57–218 cm ($\bar{X}$ = 113.21 ± 45.73 cm, N = 14) y 0–35 cm ($\bar{X}$ = 15.8 ± 6.7 cm, N = 14), respectivamente; los machos permanecen cerca de las nidadas y siguen cantando para atraer hembras adicionales. Además, reportamos por primera vez larvas de mosca (Drosophylidae) como agente parasitoide en las nidadas de *N. grandisonae*. En *N. spilotus* se reporta el hallazgo de un macho con una deformidad en una de sus extremidades y una hembra sin la presencia del macho amplectante.

Palabras clave: Centrolenidae,*Centrolene quindianum*, comportamiento reproductivo, historia natural, *Nymphargus grandisonae*, *Nymphargus spilotus*.

INTRODUCTION

Since description of the family Centrolenidae by TAYLOR (1951),the knowledge about the biology of glass frogs has increased considerably, especially in terms of its taxonomy and systematics (LYNCH AND DUELLMAN,1973; HEYER, 1985; RUIZ-CARRANZA AND LYNCH, 1991, 1998; AYARZAGÜENA, 1992;CISNEROS-HEREDIA AND MCDIARMID, 2007; GUAYASAMIN *et al.*,2008, 2009). The ecology and natural history of these frogs have also received attention due to the variety of reproductive behaviors, especially those associated with the use of perches and combat behavior between males, which are useful traits for systematic and phylogenetic analysis (BOLÍVAR *et al.*, 1999; GUAYASAMIN *et al.*, 2009). However, the ecological attributes of most species of centrolenids are unknown or anecdotal (see DAUTEL*et al.*, 2011), being restricted in most cases to brief ecological notes given in the descriptions of species.

In Colombia, specifically in theAndean region, glass frogs reach their maximum richness (73 of 147 spp [50 %]) (AMPHIBIAWEB, 2011), recording in some areas up to seven sympatric species (e.g. municipality of Falan, departament of Tolima; JARM pers. obs.). The coexistence of several species of centrolenids in a lotic environment (e.g. Andean creeks and streams), is an interesting aspect as it generates the question of how these species are ecologically segregated, knowing that in general most species exhibit similar behaviors and habitat requirements (see for a review MCDIARMID, 1975; RUIZ-CARRANZA AND LYNCH,1991; CISNEROS-HEREDIA AND MCDIARMID, 2007).

In this regard, field observations on the natural history of centrolenids are of great importance, since they allow to compare the varied ecological repertoire that these frogs display, especially in terms of their reproductive activity. Herein, we provide notes on the activity, reproduction and mortality of the egg-masses of *Centrolene quindianum* (RUIZ-CARRANZA & LYNCH, 1995), *Nymphargus grandisonae*

(COCHRAN & GOIN, 1970) and *N. spilotus* (RUIZ-CARRANZA & LYNCH, 1997), three species of glass frogs that live in sympatry in a locality of the Central Cordillera of the Colombian Andes.

MATERIALS AND METHODS

Study area

Surveys were conducted in five creeks, all located in the “Corregimiento El Manantial”, municipality of Manizales, department of Caldas, Colombia (5º06’N, 75º29’W, 1850–2150 m) (Fig. 1). This area is part of the Andean Orobiome on the western flank of the Cordillera Central (RODRÍGUEZ*et al.*, 2004), belonging to the Low Montane Wet Forest zone (*sensu* HOLDRIDGE, 1982 and HARTSHORN, 2002). In this area, rains are distributed usually in two periods (March–May and October–December) with 2600 mm annual average and temperature ranging between 16–20°C (CORPOCALDAS, 2002; CENICAFÉ, 2004).

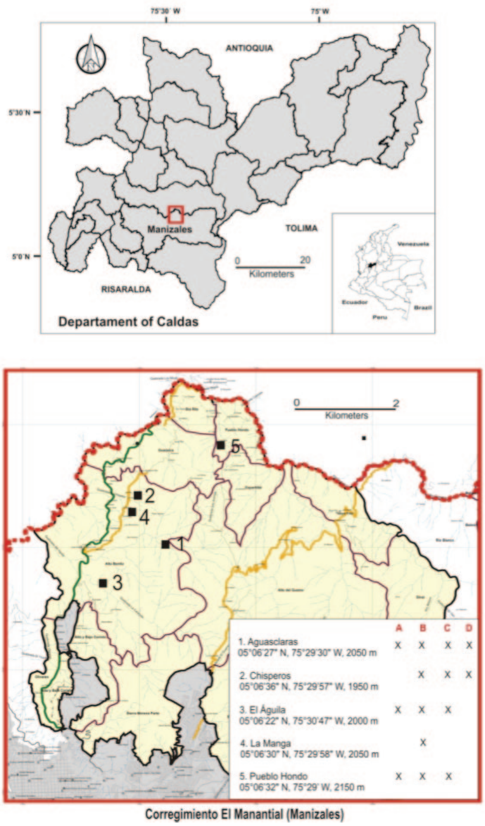

Figure 1. Study area map showing the location of the sampled creeks. At the bottom right shows a matrix of presence-absence of species in the creeks, where: (A) *Centrolene quindianum*, (B) *Centrolene savagei*, (C) *Nymphargus grandisonae* y (D) *Nymphargus spilotus*.

The surveyed creeks are within secondary forest fragments in different states of preservation, ranging from five to 20 Ha in extension. The geomorphology of these creeks is steep and unstable, forming depressions in some areas with slopes<70 degrees.Vegetation bordering the water courses consists primarily of *Heliconia latispatha*, *Calathea* sp., *Xanthosomas aggitifolium* and *Oreopanax pallidum* asociations. Both there maining natural forest as creeks themselves are still inhabited by several species of amphibians and reptiles. We have recently recorded 10 anurans, five lizards and seven snakes (unpubl. data). As for the glass frogs, the locality contains four sympatric species (*Centrolene quindianum*, *C. savagei*, *Nymphargus grandisonae* and *N. spilotus*) (Fig. 1). Of these, we excluded the information on *C.savagei*, which will be presented in other papers.

Surveys

The frog surveys were conducted intermittently between October 2008 and December 2010, totaling 54 nocturnal surveys, each of them consisting of four to seven hours between 1900 and 0200h; surveyed transects in the creeks ranged from 100 to 360 min length, and individuals were searched up to 4 min height. On each survey, the following data were recorded: reproductive activity, distance above the creek, distance to the edge of creek, perch, characteristics of the egg-masses (shape, color and numberof eggs), parental care (presence/absence and type of care), and parasites or predator of egg clutches. Considering that observations were opportunistic and infrequent in each creek, we present descriptive data on particular observations.

RESULTS

Centrolene quindianum (RUIZ-CARRANZA & LYNCH, 1995) (Fig. 2A, B). In the area, *C. quindianum* is considered a rare species because only four individuals were observed (3 males and 1 female) throughout the study period in Aguasclaras, Chisperos and Pueblo Hondo creeks. On 01 May 2010 at 2140 h, a calling male was found at 1.8m on a fern, and near it, from 30 to 100 cm apart, had three egg-masses in different stages of development. One clutch in early stage of development at 40 cm above the creek (Fig. 2C) and two others egg-masses at 2 m of height, one which the embryos were hatching and some of them being preyed by a spider (*Patrera armata*; Anyphaenidae). The egg-masses (except the one in hatching) had 33 and 35 eggs respectively (Fig. 2C, D), which have a globular form (arboreal clump *sensu* ALTIG AND MCDIARMID, 2007), resembling in shape to those of the sympatric species *Centrolene savagei* (VARGAS-SALINAS *et al.*, 2007), but varying considerably in the number of eggs per clutch (average 22 in *C. savagei*, ESCOBAR-LASSO *et al.*, in prep.). The clutch of lesser stage of development (Fig. 2C) had greeny-white colored eggs, a coloration also different from the eggs of *C. savagei*, which are cream in the early stages of development (ESCOBAR-LASSO *et al.*, in prep.).

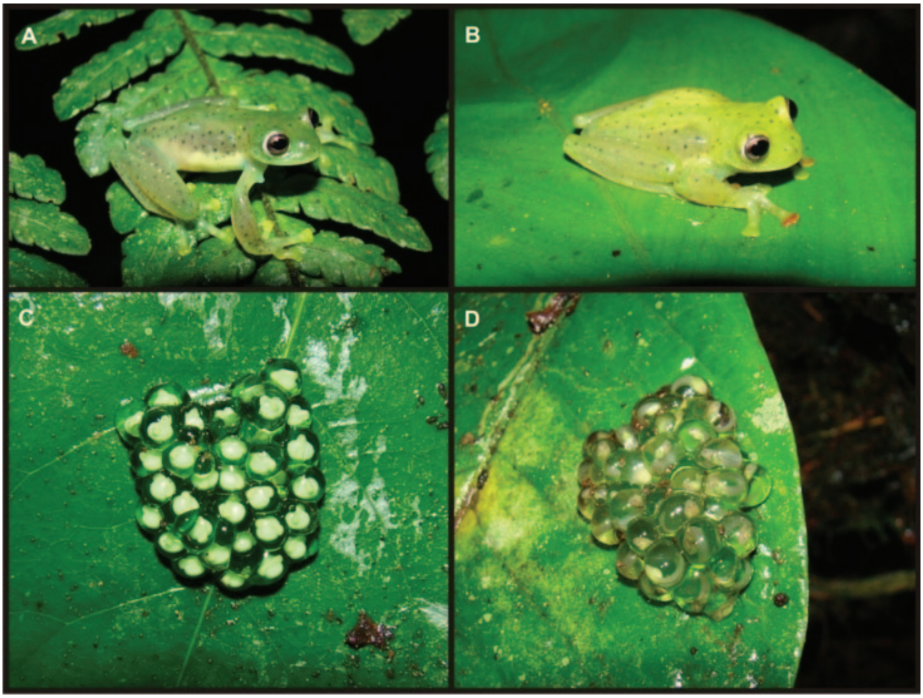

Figure 2. Male (SVL 27.9 mm) (A), and female (SVL 29.3 mm) (B) of *Centrolene quindianum* from Aguasclaras creek. Not collected. (C and D) egg-masses of *C. quindianum* in different stages of development, located on the upper of leafs.

Nymphargus grandisonae (COCHRAN & GOIN, 1970).We found populations in all surveyed creeks except in La Manga (Fig. 1), and individuals were sighted in all cases in large leaves. During surveys we observed that males are more vocally active over 3m in height, as heard on several visits and in different climatic periods, a greater number of calls in the middle of the forest. Males of *N.grandisonae* call in the upper surfaces of leaves, some of which are guarded territories up tosix months (Fig. 3A); we assume this because the frogs were repeatedly found on the same site where they were found for first time, and because were observed agonistic encounters between males in two occasions, suggesting territorial behavior (HUTTER*et al.*, in prep.). Oviposition too occurs in the upper surfaces of large leaves, noting preferences for Araceae and Heliconias(100%, N=14). For use this perches for calling and oviposition, individuals of *N.grandisonae* are generally aggregates where such vegetation is abundant. The egg-masses of *N.grandisonae* are larger compared to the other species of the El Manantial ($\bar{X}$ = 74 ± 8.6 eggs/clutch, N = 4) and up to three egg-masses maybe placed on one leaf (Fig. 3B). The height of the oviposition sites and distance to the edge of the creeks ranged from 57–218 cm ($\bar{X}$ = 113.21 ± 45.73 cm, N = 14) and 0–35 cm ($\bar{X}$ = 15.8 ± 6.7 cm, N = 14), respectively. Egg-masses of *N.grandisonae*, unlike those of *C.quindianum*, have a single layer of eggs uniformly distributed in the jelly matrix without being incontact between them (laminar array *sensu* ALTIG AND MCDIARMID, 2007). Inearly stage of development, the eggs are yellow in the vegetal pole and coffee in the animal pole and do not have a swollen jelly layer, but it appears in a more advanced ontogenetic period (Fig.4A, B).

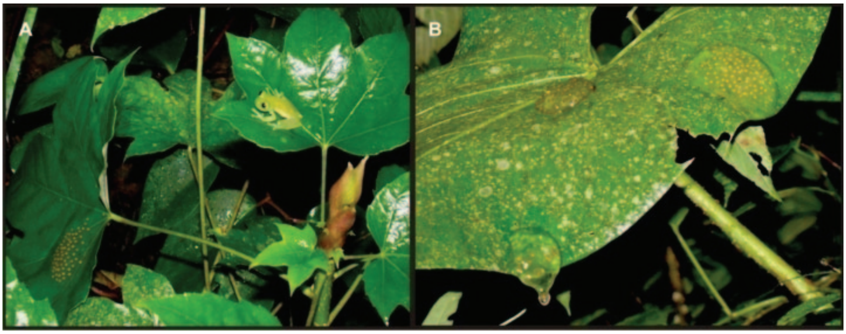

Figure 3. Male caring their reproductive perch near an egg-clutch (A). Note the direction of the male body. (B) Oviposition perch (Araceae) containing three egg-masses of *N.grandisonae* in different stages of development.

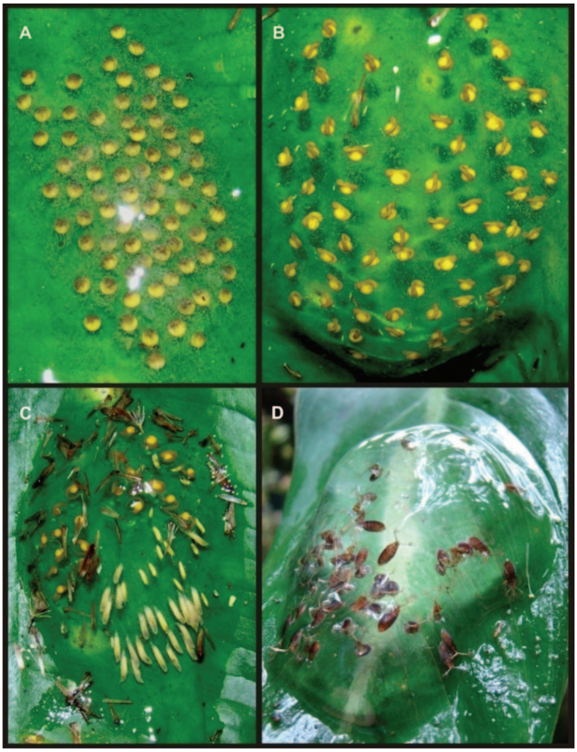

Figure 4. Egg-masses of *Nymphargus grandisonae* from the Chisperos creek, El Manantial, Manizales, Colombia. (A) recently oviposited; (B) another egg-clutch in amore advanced stage of development, where the gelatin layeris higher and it disperses the embryos; (C) parasitized egg-clutch by 40 larvae of fly (Drosophilidae) and (D) jelly layer without frog eggs, which are observed fly pupae's.

In general, males were observed in the same leaves with the egg-masses,or in adjacent leaves (Fig.3A) at 30–40 cm apart of them ($\bar{X}$ = 36±4.2 cm, N= 10 observations). On October 10, 2010 at 23:10 h, we found amale with six egg-masses in different stages of development, which were on two adjacent leaves of *O.pallidum* (Araliaceae), three in one leaf and three in another. Five males, including that one was with the six egg-masses, calling while were close to the egg-masses. The parasitoids of drosophilids larvae seems tobe common in the egg-masses of *N. grandisonae* (N = 8), whose clutches show reduction in the embryos number when they are infected (Fig.4C, D). In six of eight observations, we found that the flies larvae were present inmoderately developed embryo egg-masses (stage 19–20 *sensu* GOSNER, 1960), and there maining two, inrecently deposited egg-masses.

Nymphargus spilotus (RUIZ-CARRANZA & LYNCH, 1997) (Fig. 5A, B).This species is the least known in comparison with the other three species of the El Manantial, because it has only been observed six times in different nights, found mainly solitary maleson the upper surfaces of leaves in the Aguasclaras andChisperos creeks (Fig. 1). During the study period, we observed two interesting things about*N.spilotus*, both in Chisperos creek: one was the sighting of a female (SVL 24.9 mm) on March 28, 2009 at 1915h, egg laying on the upper surface of a leaf to 2m above the creek (Fig.5B). During 1.37 h of continuous observation, we do not found a male around and much less was observed the amplexus; one week later no eggs were encountered in this site. The other thing corresponds to the finding of a male (SVL 24.1 mm) with a deformity in his right leg, which consists in the fusion of the third and four fingers, leading to the reductionof its digits (Ectrodactyly *sensu* METEYER *et al*., 2000) (Fig. 5C). This individual was collected and deposited in the herpetological collection of the Museo de Historia Natural of the Universidad de Caldas (MHN-UC 0257). Apart from this, from October to December 2010, we conducted weekly surveys in the Chisperos creek, monitoring a male who faithfully stayed on the same perch, but never mated. During this period, this male was the only individual observed of the species.

Figure 5. (A) Male of *Nymphargus spilotus* (SVL 22.9 mm) from Aguasclaras creek. Not collected. (B) Female of *N. spilotus*(SVL 24.9 mm) egg-laying without the amplectant male presence. Not collected. (C) Deformity in the right leg of a male of *N. spilotus* (SVL 24.1 mm) (MHN-UC 0257) from Chisperos creek. Scale bar represent 5 mm.

DISCUSSION

The use of specific sites for calling and oviposition in *N.grandisonae*, seems to respond at the intrinsic requirements of their egg-masses, because the large size and the location of several of them in the same leaf, may have lead to the preference of large leaves to reproductive activity. This feature (selection of reproductive perches) is differential between some species of centrolenids (see CISNEROS-HEREDIA AND MCDIARMID, 2007) and seems to be determined by the selection made by males on certain sites, which have “good” conditions, either to the emission of calls or the egg-masses attachment (DUELLMAN AND SAVITZKY,1976; GREER AND WELLS,1980;WELLS AND SCHWARTZ,1982; VOCKENHUBER*et al.*, 2008).In *Hyalino bathrachium valerioi* for example, males also preferred as sites of calling and oviposition, large and smooth leaves, which represents better sites foreggs attachment, and offers morespace for receivingadditional egg-masses (VOCKENHUBER *et al.*, 2008). In this sense, it is understandable why different species of plants of the genera *Dieffenbachia*, *Gustavia*, *Heliconia*, *Calathea*, *Anthurium* and *Philodendron*, are used by highly territorial centrolenids whose egg-masses are placed at specific sites (e.g. *H. valerioi*, *H. fleischmanni*, *H. colymbiphyllum*; see MCDIAMID AND ADLER, 1974; GREER AND WELLS, 1980; VILLA, 1984; VOCKENHUBER *et al.*, 2008), as observed in *N. grandisonae.*

On the other hand, the presence of multiple egg-masses in a single oviposition site (see OSPINA-SARRIA *et al.*, 2011 for *N.grandisonae*), indicates that males with a defined territory are able to attract additional females while performing the care of their egg-masses (VOCKENHUBER *et al.*, 2008). However, it is also possible that the presence of a male close to one or more egg-masses is due to the loyalty that this presents for the site itself, rather than the eggs parental care (GREER AND WELLS, 1980; DELIA *et al.*, 2010). For *N.grandisonae* we assume this assumption, since parental care one ggs must represent a direct benefit for embryonic survival (e.g. antipredatory, hydration; MCDIARMID, 1978; JACOBSON, 1985; HAYES, 1991; VOCKENHUBER *et al.*, 2009), which apparently do not occur in this species, as observed repeatedly egg-masses affected by fly larvae, and males neither displayed egg-brooding behavior to hydrate the eggs. However, it is necessary to develop a rigorous experimental study to test this assumption, since in some centrolenids that display parental care, predation and parasitoidism also occur in some degree (see VILLA, 1977; HAYES, 1991; VOCKENHUBER *et al.*, 2008).

There are two kinds of parasitoids that attack glass frog’s embryos: beetles (Lampyridae) (GRANT *et al.*, 1998), and flies (Drosophylidae) (VILLA, 1977, 1984; GREER AND WELLS,1980; HAYES, 1991) (Table 1). In the study of VILLA (1977), the artificially inoculated drosophilidflie larvae, success fully infested egg-masses of *Agalychnis callidryas*, *Hylaebraccata* (=*Dendrosphusebraccatus*), *Centrolenella fleischmanni* (=*Hyalino bathrachium fleischmanni*) and *Centrolenella pulverata* (=*Teratohyla pulverata*), but not those of *Centrolenella granulosa* (=*Cochranella granulosa*); all these species were in sympatry in San José de la Montaña, Nicaragua. The peculiarity of this investigation was found that only *H. fleischmanni* egg-masses were infested in considerable number over other species egg-masses in the wild, suggesting that exist a preference of flies on the eggs of the former species. In the case of the El Manantial glass frogs, something similar could happen, as the fly larvae have only been found in the *N. grandisonae* egg-masses; however, the characteristics of these egg-masses, both embryos and their gelatinous matrix, and the preference of the flyto lay their eggs in them, are issues that await investigation. We adopt the term parasitoid following CRUZ-REYES AND CAMARGO-CAMARGO (2001), because the fly larvae are parasitic during early stages of development, and eventually kill the host (frog embryos) to complete its development. When VILLA (1977)described the relationship between fly larvae and frogs, he did not say if flies acted as parasiteor predator, and this led to an inconsistency to describe the relationship between flies and egg-masses in later works (e.g.VOCKENHUBER*et al.*, 2008), using both terms with no differentiation. However, both terms are erroneous since these flies act as a parasitoid (CRUZ-REYES ANDCAMARGO-CAMARGO,2001).

Table 1. Parasitoids reported affecting the egg-masses of glass frogs (Centrolenidae).

Parasitoid			Glass frogs species afected	Reference
Order	Family	Species		
Coleoptera	Lampyridae	Unidentified	*Centrolene geckoideum*	Grant*et al.* (1998)
Diptera	Drosophilidae	*Drosophila orbitalis*	*Hyalinobatrachium fleischmanni*	Villa (1977)
		Drosophila sp.	*H. fleischmanni*	Villa (1984)
		Drosophila orbitalis	*Teratohyla pulverata**	Villa (1977)
		Drosophila sp.	*H. fleischmanni*	Hayes (1991)
		Drosophila sp.	*H. fleischmanni*	Greer and Wells (1980)
		Drosophila sp.	*Nymphargus grandisonae*	Thiswork

*The parasitic infection by *Drosophila orbitalis* on the egg-masses of *T.pulverata* was artificially induced.

The egg-masses of glass frogs show interesting morphological and ecological characteristics but are little known. In this paper we describe for first time the egg-masses of *C.quindianum*, although with very limited data due infrequent observations of the species. The egg-masses of this frog are susceptible to be preyed by the spider *P.armata*, which is the main agent of embryonic mortality in the sympatric species *C.savagei* (ESCOBAR-LASSO *et al.*, in prep.). It is possible that this predator also attack in high proportions the egg-masses of *C.quindianum*, because the egg-masses characteristics of both species are similar. The lack of recurrent observations in which males of *C.quindianum*were near the egg-masses, not allow us to assert if this is their egg parental care, and for this reason, additional observations are needed to determine whether such behavior was casuistic, or otherwise corresponds to aconserved pattern.

Nymphargus spilotus is one of the many species of centrolenids that not have a basic knowledge about their biology, populational status and distribution, and hence it has been cataloged with data deficient (DD) by IUCN (WILD AND LYNCH, 2004). The only information known about the natural history of this species is provided by RUEDA-ALMONACID (2000), who stated that females of *N.spilotus* put of 16–20 cream-colored eggs, grouped in the under sides of leaves on riparian vegetation; apart from this, characteristics of their reproduction such as oviposition sites preferences, parental care and other features of their egg-masses are unknown. The few observations of this species during the study period (October 2008 – December 2010) and its presence in only two of the five creeks visited, suggest that this species is demographically rare, however, it is possible that both species, *N.spilotus* and *C.quindianum* are naturally rare or too difficult to observe, because they can stay in microhabitats located on the forest canopy. This has been considered for *Centrolene antioquiense*, a species which according to RUIZ-CARRANZA AND LYNCH (1997), call high in the trees, and also to *Centrolene peristictum*, which has been observed most commonly between 4–5 min height in northern Ecuador (RUIZ-CARRANZA AND LYNCH, 1997). The two peculiarities observed in *N. spilotus* (individual with malformation and female egg-laying alone), may suggest that this species is facing a population decline in the area. Unfortunately, the lack of observational data before 2008 cannot assert this assumption, and there for ewe recommend astrict population monitoring to determine their truly conservation status.

Currently the available information on ecological and behavioral characteristics of glass frogs is still scarce (seeDAUTEL *et al.*, 2011). Actually, in the Central Cordillera of Colombia over 1000 m of elevation, have been reported 17 glass frogs species (AMPHIBIAWEB, 2011) equivalent to 11.5% of the total family diversity. Of these species, a little over 50% are known their call and oviposition sites, but aspects such as the clutch structure, eggs number, type of parental care and territoriality, are known only for a small number of species (29.4%, 35.2%, 17.6%, 17.6%, respectively) (Table 2). Added to this, there are still gaps in knowledge about the geographical distribution of Andean centrolenids, which hinders further understanding of the diversity of this group in the Colombian Andes. For this reason, additional efforts should be made to sample new areas, as well as expeditions to the historic localities of the species, in order to obtain ecological and biogeographical information to contribute with a more robust body of data to the taxonomic and systematic analysis of Centrolenidae, and is also necessary to identify areas of high concentration of species to develop programs aimed at their conservation.

Table 2. Ecological and behavioral information available for 17 species of glass frogs of the Central Cordillera of Colombia. All species are considered Andeanas they inhabit above 1000m elevation, except *Espadarana prosoblepon*. * The females of *E.prosoblepon* stand beside or on the egg-masses for short periods (up to 131 min) after oviposition (see JACOBSON, 1985). Apart from this, no care behavior has been observed in this species.

Species	Callingsite	Oviposition site	Clutch structure	Number of eggs	Egg-attendance	Territoriality	Reference
Centrolene antioquiense	Upper leaf side	Under leaf side	–	–	–	–	GUAYASAMIN*et al.* (2009), MARCO RADA pers. comm.
C. buckleyi	–	–	–	–	Remains nearby?	–	GUAYASAMIN *et al.* (2006)
C. geckoideum	Rocks	Rocks	Laminar array	60-79	Remains near by-Egg-brooding	Yes	GRANT *et al.* (1998) RUEDA-ALMONACID (1994, 2000)
C. guanacarum	–	–	–	–	–	–	
C. huilense	–	–	–	–	–	–	
C. paezorum	–	–	–	–	–	–	
C. quindianum	Upper leaf side	Upper leaf side	Arboreal clump	33-35	Remains near by?	–	This work
C. robledoi	Upper leaf side	–	–	–	–	–	RUEDA-ALMONACID (2000)
C. savagei	Upper leaf side	Upper leaf side	Arboreal clump	17-26	Egg-brooding	Yes	Data no publ.
Espadarana prosoblepon	Upper leaf side	Upper leaf side	Laminar array	32	Not*		JACOBSON (1985), BAUTISTA *et al.* (2010)
Nymphargus garciae	–	Upper leaf side	–	–	–	–	
N. grandisonae	Upper leaf side	Upper leaf side	Laminar array	30-82	Remains near by?	Yes	OSPINA-SARRIA *et al.* (2011), This work
N. griffithsi	Upper leaf side	Upper and under leaf side	–	–	–	–	RUEDA-ALMONACID (2000) CADAVID*et al.* (2005)
N. posadae	–	–	–	–	Remains near by	–	MARCO RADA pers. comm.
N.rosadus	–	Upper leaf side	–	–	–	–	
N. ruizi	–	–	–	–	–	–	
N. spilotus	Upper leaf side	Upper and under leaf side	–	16-20	–	–	RUEDA-ALMONACID (2000), Thiswork

ACKNOWLEDMENTS

JARM thanks specially to Luis Fernando Escobar and Adriana Garrido Botero for their hospitality in "La Manga" farm during this investigation. To Paul D.A. Gutiérrez-Cárdenas for provided suggestions during the planning of this study and give us specialized literature. To Marco Rada who kindly provided unpublished data on some species included in the Table 2, and to Juan Manuel Guayasamin who commented and improved different parts of the manuscript. We are grateful to the residents of the El Manantial, especially to don Gilberto Bermeo for allowing us to work in the areas neighboring to their houses. This research is part of the Project "Ranas de cristal (Anura: Centrolenidae) desapareciendo de los Andes de Colombia: una examinación de la presencia actual, distribución y ecología reproductiva",supported by Vicerrectoría de Investigaciones y Postgrados de la Universidad de Caldas (Manizales, Colombia).

BIBLIOGRAPHY

AMPHIBIAWEB.- Information on amphibian biology and conservation. [web application]. 2013. Berkeley, California: AmphibiaWeb. <http://amphibiaweb.org/>. Accessed on December 01 2011.

ALTIG, R. & MCDIARMID, R.W., 2007.- Morphological diversity and evolution of egg and clutch structure in amphibians. *Herpetological Monographs*, 21: 1-32.

AYARZAGÜENA, J., 1992.- Los Centrolenidos de la Guayana Venezolana. *Publicaciones de la Asociación de Amigos de Doñana*, 1: 1-48.

BAUTISTA, M.H., HERNÁNDEZ-CUADRADO, E.E. & BARRAGÁN, M.G., 2010.- *Centrolene prosoblepon* (Glass Frog). Reproduction. *Herpetological Review*, 41: 61-62.

BOLÍVAR, W., GRANT, T. & OSORIO, L.A., 1999.- Combat behaviour in *Centrolene buckleyi* and other centrolenid frogs. *Alytes*, 16: 77-83.

CADAVID, J.G., ROMAN-VALENCIA, C. & GÓMEZ, A.F., 2005.- Composición y estructura de anfibios anuros en un transecto altitudinal de los Andes centrales de Colombia. *Revista del Museo Argentino de Ciencias Naturales*, 7: 103-118.

CARDOZO-URDANETA, A. & SEÑARIS, C., 2012.- Vocalización y biología reproductiva de las ranas de cristal *Hyalinobatrachium pallidum* y *Centrolene daidaleum* (Anura, Centrolenidae) en la sierra de Perijá, Venezuela. Memoria de la Fundación La Salle de Ciencias Naturales 2012 174: 87-105.

CASTROVIEJO-FISHER, S., GUAYASAMIN, J.M., GONZALEZ-VOYER, A. & VILA, C., 2013.- Neotropical diversification seen through glassfrogs. *Journal of Biogeography* 40: 1-15.

CENTRO NACIONAL DE INVESTIGACIÓN DE CAFÉ. FEDERACIÓN NACIONAL DE CAFETEROS DE COLOMBIA, CENICAFÉ., 2004.- *Anuario meteorológico cafetero.* Cenicafé, Chinchiná, Colombia.

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 glassfrogs. *Zootaxa*, 1572: 1-82.

CORPOCALDAS., 2002.- *Agenda para la gestión ambiental del municipio de Manizales.* Subdirección de planeación y sistemas. Manizales, Colombia.

CRUZ-REYES, A. & CAMARGO-CAMARGO, B., 2001.- *Glosario de Términos en Parasitología y Ciencias Afines.* Instituto de Biología, UNAM. México D.F, México.

DAUTEL, N., MALDONADO, A.L.S., ABUZA, R., IMBA, H., GRIFFIN, K. & GUAYASAMIN, J.M., 2011.- Advertisement and combat calls of the glass frog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behaviors. *Phyllomedusa*, 10: 31-43.

DELIA, J., CISNEROS-HEREDIA, D.F., WHITNEY, J. & MURRIETA-GALINDO, R., 2010.- Observations on the reproductive behavior of a Neotropical glassfrog, *Hyalinobatrachium fleischmanni* (Anura: Centrolenidae). *South American Journal of Herpetology*, 5: 1-12.

DÍAZ-GUTIÉRREZ, N., VARGAS-SALINAS, F., RIVERA-CORREA, M., ROJAS-MORALES, J.A., ESCOBAR-LASSO, S., VELASCO, J.A., GUTIÉRREZ-CÁRDENAS, P.D.S. & AMÉZQUITA, A., 2013.- Description of the previously unknown advertisement call and tadpole of the Colombian endemic glassfrog C*entrolene savagei* (Anura: Centrolenidae). *Zootaxa* 3686: 289-296.

DUELLMAN, W.E. & SAVITZKY, A.H., 1976.- Aggressive behaviour in a centrolenid frog, with comments on territoriality in anurans. *Herpetologica*, 32: 401-404.

ESCOBAR-LASSO, S. & ROJAS-MORALES, J.A., 2012.- Antipredatory behaviors of the Colombian endemic glassfrog *Centrolene savagei* (Anura: Centrolenidae). *Boletín Científico Museo de Historia Natural de la Universidad de Caldas*, 16: 226-232.

GOSNER, K.L., 1960.- A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16: 183-190.

GRANT, T., BOLIVAR-G, W. & CASTRO, F., 1998.- The advertisement call of *Centrolene geckoideum. Journal of Herpetology*, 32: 452-455.

GREER, B.J. & WELLS, K.D., 1980.- Territorial and reproductive behaviour of the Tropical American frog *Centrolenella fleischmanni. Herpetologica*, 36: 318-326.

GUAYASAMIN, J.M., BUSTAMANTE, M.R., ALMEIDA-REINOSO, D. & FUNK, W.C., 2006.- Glass frogs (Centrolenidae) of Yanayacu Biological Station, Ecuador, with the description of a new species and comments on centrolenid systematic. *Zoological Journal of the Linnean Society*, 147: 489-513.

GUAYASAMIN, J.M., CASTROVIEJO-FISHER, S., AYARZAGÜENA, J., TRUEB, L. & VILÀ, C. (2008).- Phylogenetic relationships of glassfrogs (Centrolenidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 48: 574-595.

GUAYASAMIN, J.M., CASTROVIEJO-FISHER, S., TRUEB, L., AYARZAGÜENA, J., RADA, M. & VILÁ, C., 2009.- Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni. Zootaxa*, 2100: 1-97.

HARTSHORM, G.S., 2002.- Biogeografía de bosques neotropicales: 59-81 (in) GUARIGUATA, M.R. & KATTAN, G.H. (eds.) *Ecología y conservación de bosques Neotropicales.* Ediciones LUR, Cartago, Colombia.

HAYES, M.P., 1991.- A study of clutch attendance in the Neotropical frog *Centrolenella fleischmanni* (Anura: Centrolenidae): Tesis Ph.D., University of Miami, Miami, Florida.

HEYER, W.R., 1985.- Taxonomic and natural history notes on frogs of the genus *Centrolenella* (Amphibia: Centrolenidae) from southeastern Brazil and adjacent Argentina. *Papéis Avulsos de Zoologia*, 36: 1-21.

HOLDRIDGE, L.R., 1982.- *Ecología basada en zonas de vida.* IICA, San José, Costa Rica.

HUTTER, C.R., ESCOBAR-LASSO, S., ROJAS-MORALES, J.A., Gutiérrez-Cárdenas, P.D.A., IMBA, H. & GUAYASAMIN, J.M., 2013.- The territoriality, vocalizations and aggressive interactions of the red-spotted glassfrog, *Nymphargus grandisonae*, Cochran and Goin, 1970 (Anura: Centrolenidae). *Journal of Natural History* 47: 3011-3032.

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

LYNCH, J.D. & DUELLMAN, W.E., 1973.- A review of the centrolenid frogs of Ecuador, with descriptions of new species. *The University of Kansas Museum of Natural History Occasional Papers*, 16: 1-66.

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., 1975.- Glass frog romance along a tropical stream. *Terra*, 13: 14-18.

MCDIARMID, R.W., 1978.- Evolution of parental care in frogs: 127-147 (in) BURGHARDT, G.M. & BEKOFF, M. (eds.) *The Development of Behavior: Comparative and Evolutionary Aspects.* Garland STPM Press, New York.

METEYER, C.U., LOEFFLER, I.K., BURKHART, J.G., CONVERSE, K.A., GREEN, E., HELGEN, J.C., KERSTEN, S., LEVEY, R., EATON-POOLE, L. & FALLON, J.F., 2000.- Hind limb malformations in free–living Northern leopard frogs (*Rana pipiens*) from Maine, Minnesota and Vermont suggest multiple etiologies. *Teratology*, 60: 151-171.

OSPINA-SARRIA, J.J., BOLÍVAR-G, W., MÉNDEZ-NARVÁEZ, J. & BURBANO-YANDI, C., 2011.- The tadpole of *Nymphargus grandisonae* (Anura, Centrolenidae) from Valle del Cauca, Colombia. *South American Journal of Herpetology*, 6: 79-86.

RODRÍGUEZ, D., ARMENTERAS, D., MORALES, M. & MORENO, M., 2006.- *Ecosistemas de los andes colombianos.* Segunda edición. Instituto de investigaciones de recursos biológicos "Alexander Von Humboldt", GEMA, Bogotá, Colombia.

ROJAS-MORALES, J.A., ESCOBAR-LASSO, S. & GUTIÉRREZ-CÁRDENAS, P.D.A., 2011.- Contribución al conocimiento de los anfibios de la región centro-sur de caldas: primeros registros de Ranas de Cristal (Anura: Centrolenidae) para el municipio de Manizales, Colombia. *Boletín Científico Museo de Historia Natural de la Universidad de Caldas*, 15: 75-83.

RUEDA-ALMONACID, J.V., 1994.- Estudio anatómico y relaciones sistemáticas de *Centrolene geckoideum* (Salientia: Anura: Centrolenidae). *Trianea*, 5: 133-187.

RUEDA-ALMONACID, J.V., 2000.- *La herpetofauna de los "Bosques de Florencia" Caldas: una visión integrada sobre su composición, diversidad y relaciones ecológicas.* Corporación Autónoma Regional de Caldas CORPOCALDAS, Santafé de Bogotá.

RUIZ-CARRANZA, P.M. & LYNCH, J.D., 1991.- Ranas Centrolenidae de Colombia I: propuesta de una nueva clasificación genérica. *Lozania*, 57: 1-30.

RUIZ-CARRANZA, P.M. & LYNCH J.D., 1997.- Ranas Centrolenidae de Colombia X: los centrolénidos de un perfil del flanco oriental de la Cordillera Central en el Departamento de Caldas. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 21: 541-553.

RUIZ-CARRANZA, P.M. & LYNCH, J.D., 1998.- Ranas Centrolenidae de Colombia XI. Nuevas especies de ranas cristal del género *Hyalinobatrachium. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 22: 571-586.

TAYLOR, E.H., 1951.- Two new genera and a new family of tropical American frogs. *Proceedings of the Biological Society of Washington*, 64: 33-40.

VALENCIA-AGUILAR, A., CASTRO-HERRERA, F. & RAMÍREZ-PINILLA, M.P., 2012.- Microhabitats for Oviposition and Male Clutch Attendance in *Hyalinobatrachium aureoguttatum* (Anura: Centrolenidae). *Copeia* 4: 722-731.

VARGAS-SALINAS, F., LÓPEZ-A, F.A. & MUÑOZ-G, J.N., 2007.- *Cochranella savagei*. Reproduction. *Herpetological Review*, 38: 436-437.

VILLA, J., 1977.- A symbiotic relationship between frog (Amphibia, Anura, Centrolenidae) and fly larvae (Drosophilidae). *Journal of Herpetology*, 11: 317-322.

VILLA, J., 1984.- Biology of a Neotropical glass frog, *Centrolenella fleischmanni* (Boettger), with special references to its frogfly associates. *Milwaukee Public Museum Contributions in Biology and Geology*, 21: 1-17.

VOCKENHUBER, E.A., HÖDL, W. & KARPFEN, U., 2008.- Reproductive behaviour of the glass frog *Hyalinobatrachium valerioi* (Anura: Centrolenidae) at the tropical stream Quebrada Negra (La Gamba, Costa Rica). *Stapfia*, 88: 335-348.

VOCKENHUBER, E.A., HÖDL, W. & AMÉZQUITA, A., 2009.- Glassy fathers do matter: egg attendance enhances embryonic survivorship in the glass frog *Hyalinobatrachium valerioi*. *Journal of Herpetology*, 43: 340-344.

WELLS, K.D. & SCHWARTZ, J.J., 1982.- The effect of vegetation on the propagation of calls in the Neotropical frog *Centrolenella fleischmanni*. *Herpetologica*, 38: 449-455.

WILD, E. & LYNCH, J., 2004.- *Nymphargus spilotus*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <http://www.iucnredlist.org/>. Accesed on 01 September 2011.