

SHORT COMMUNICATION

Notes on the reproductive biology of the glass frog *Centrolene bacatum* (Anura: Centrolenidae)

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The glass frogs, Centrolenidae, comprise a diverse group of Neotropical frogs with 12 genera and at least 147 species (Guayasamin *et al.* 2009). Currently, there are 23 species within the genus *Centrolene*, however more undescribed species may exist within the genus (Guayasamin and Funk 2009, Guayasamin *et al.* 2009). *Centrolene* species are typically small (< 30 mm SVL, excluding *C. geckoideum* and *C. paezorum*), nocturnal frogs endemic to elevations of 1100–3500 m in the northern Andes (Guayasamin *et al.* 2009).

Much of the basic natural history of *Centrolene* species is currently unknown. Typically males call from the upper side of leaves and females of the genus *Centrolene* deposit their eggs on the upper sides of leaves overhanging streams in which embryos develop until hatching (Guayasamin *et al.* 2009). In several centrolenid species the males will guard the eggs from being consumed by male conspecifics or to protect the eggs from desiccation (Delia *et al.* 2010). In

some centrolenid species, males combat for calling sites (Bolivar-G. *et al.* 1999, Delia *et al.* 2010).

In June 2014 we periodically surveyed, both day and night, a forest stream along the Stream Trail at Yanayaku Biological Station (00°35' S, 77°53' W) in the Napo region of Ecuador. The stream is in primary cloud forest and was approximately between 20–50 cm deep and 2–3 meters wide. The streambed was rocky and the water flow was rapid (Figure 1).

At night we located males based on auditory cues along the stream. The calls of *C. bacatum* are a series of four or five high-pitched clicks lasting about 1 s. The call increases in volume with the clicks. Males were heard calling between 12–21°C and 66–95% humidity. Calling males were typically found on top of leaves approximately 3 m above the stream but were heard calling an estimated 5–6 m high. The abundance of calling males was greater in bamboo dense regions. The minimum calling distance between males was about 1–2 m apart; however, to our ears individuals were much farther apart.

On 25 June 2014 at approximately 20:15 h we found an amplexing pair of *C. bacatum* on

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top of a broad leaf over the stream. We did not disturb the pair for measurements, but the female was notably larger than the male. We observed them for about one hour during which time the only movement noted was to take cover from a heavy rain underneath the leaves of a fern (*Campyloneurum sphenodes*). Amplexus behavior was typical of axillary orientation in which the male is entirely on the back of the female with his hands placed upon the shoulder and partially around the neck of the female (Figure 2). The male did not appear to use the humeral spine for gripping the female, suggesting a different functional use for this structure. We returned to this site the following day but did not locate any eggs in the vicinity.

During our survey we found five egg masses above the stream. Based upon published photographs of *C. bacatum* eggs (Guayasamin *et al.* 2009), the fact that *C. bacatum* was the most prominent species calling in this location during the survey, and the presence of the amplexing pair, we believe the egg masses to be from *C. bacatum*. Clutches were found on the following identified plants: *Ocotea* sp. (Lauraceae), and *Campyloneurum sphenodes*. On average, the clutches were deposited 192.6 ± 49.0 cm above the stream. Four of the five clutches were deposited on the underside of the leaf and the minimum distance between neighboring clutches was 56 cm. Eggs were deposited in one or two layers and attached near the edge of leaves that were 44.9 ± 7.9 mm wide, but not hanging off of the leaf (Figure 3). No adults were observed near the clutches during the day or night and no clutches were predated during our survey, suggesting that no parental care is provided.

Clutches contained an average of 18 ± 2.7 embryos and had average dimensions of 17.4 ± 2.7 mm width and 23.8 ± 2.7 mm height. Photos were taken of two clutches so egg capsule and embryos size could be determined using the program ImageJ. A total of six eggs were measured, three from each of two clutches. The embryos elicited some twitching during obser-

vation and were suspected to be in developmental Stages 16 and 17 of tadpole development (Gosner 1960). The average diameter of the egg capsules was 6.0 ± 0.8 mm and average embryo diameter was 2.7 ± 0.3 mm. The embryos of one clutch elicited motor responses during observation and were estimated to be in developmental Stage 25 (Figure 3). Length of these embryos could not be determined due to bending of the tail in the capsule.

Aspects of the reproductive biology of *C. bacatum* warrant further discussion. For instance, the function of the humeral spines on males remains uncertain. While it could serve to secure the male to the female during amplexus, the axillary amplexing behavior does not seem to support this hypothesis. Some centrolenid males perform combat behaviors that involve grappling venter-to-venter and progressing to the two males grasping each other with their forearms while dangling from a leaf by one of the male's feet and the losing male eventually falling (Bolívar-G. *et al.* 1999, Guayasamin and Barrio-Amorós 2005, Delia *et al.* 2010, Dautel *et al.* 2011). It is likely the humeral spine plays a functional role in grip during combat behavior (Jungfer 1988, Hutter *et al.* 2013).

With the exception of two species for which the egg laying behavior is known, Guayasamin *et al.* (2009) reported that *Centrolene* females deposit their eggs on the top of leaves. *Centrolene bacatum* females appear to prefer oviposition under the leaves at this site, an observation also made by Guayasamin and Funk (2009). As the parents do not seem to guard the eggs, depositing the eggs beneath the leaves may reduce the likelihood of predation from aerial insects, such as wasps and katydids, which would predate the embryos (Delia *et al.* 2010).


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Figure 1. Example of an oviposition (A) and calling site (B) of *Centrolene bacatum* at Yanayacu Biological Station, Provincia Napo, Ecuador. Eggs were found attached to the underside of the leaves attached to the log over the stream.



Figure 2. A pair of *Centrolene bacatum* in amplexus at Yanayacu Biological Station, Provincia Napo, Ecuador.



Figure 3. *Centrolene bacatum* embryos at different stages of development at Yanayacu Biological Station, Provincia Napo, Ecuador. Note that both clutches are on the underside of leaves.

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