

SHORT COMMUNICATION

Reproductive behavior of the giant leaf frog *Phyllomedusa bicolor* (Anura: Hylidae) in the western Amazon

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The genus *Phyllomedusa* currently comprises 32 species distributed over Panama, the Pacific mountain ranges of Colombia and the eastern Andes, including Trinidad, Argentina, and Uruguay (Frost 2009). Most species are arboreal and usually walk slowly on branches and leaves, rarely leaping (Caramaschi and Cruz 2002); the terrestrial *P. atelopoides* is an exception (Duellman *et al.* 1988, Block *et al.* 2002). The vivid colors of the hidden parts of the legs in some species are an aposematic defense mechanism (Toledo and Haddad 2009). Furthermore, the skin of these animals is rich in polypeptides (Daly *et al.* 1992, Duellman and Trueb 1994). These animals deposit their eggs out of the water on open or rolled leaves (Caramaschi and Cruz 2002). After a few days, the larvae fall into the water where they continue their aquatic development as tadpoles (reproductive Mode 24 *sensu* Haddad and Prado 2005).

Phyllomedusa bicolor (Boddaert, 1772) inhabits the Amazon Basin in Brazil, Colombia, Bolivia, and Peru. It is also found in the Guianan Region of Venezuela and the Guianas (Frost 2009), and the Cerrado of the state of Maranhão (IUCN 2009) in Brazil. In the state of Acre this species is popularly known for the “frog vaccine” or “kambô”; secretions of the frog are traditionally used by Indians to treat minor injuries that are intentionally caused on the arms or legs (Souza *et al.* 2002). This vaccine also has become popular among non-indian populations (e.g., Bernarde and Santos 2009).

Although some studies and guides report some reproductive characteristics of *Phyllomedusa bicolor* (e.g., Rodriguez and Duellman 1994, Neckel-Oliveira and Wachlevski 2004, Lima *et al.* 2006, Souza 2009) and movement behavior of males (Neckel-Oliveira 1996), there is not much information on the reproductive biology of this species. We present here data on the displacement behavior of males, oviposition, and spawn of this species.

Three occurrences of amplexus in *Phyllomedusa bicolor* were recorded in the vegetation over

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lentic bodies of water. We used flashlights for these observations. Two pairs in amplexus were observed on 12 September 2008 at 23:00 h on the Fazenda Experimental Catuaba Farm (FEC), in the Municipality of Senador Guiomar and State of Acre in northern Brazil (10°04' S, 67°37'W); the frogs were on shrubby vegetation over a permanent pond at edge of an open forest with bamboo (*sensu* Silveira 2005). A third amplexant pair was observed on 8 May 2009 at 22:30 h on the Fazenda São Jorge I Farm (FSJ), Municipality of Sena Madureira and State of Acre in northern Brazil (09°26'11" S, 68°37'19" W); the frogs were on shrubby vegetation over a temporary pond in a primary forest area with selective logging. We observed the amplexus in FEC pairs until the eggs were oviposited.

Male *Phyllomedusa bicolor* found at FEC started vocalizing around 19:00 h at heights varying from 1–8 m. We observed one female moving through the vegetation toward a male that was vocalizing at a height of 4 m. As she came closer, the male climbed on her dorsum and initiated axillary amplexus without resistance. The pair remained in amplexus for 3 min, after which the female moved on branches and leaves over the surface of the water, possibly seeking a place for oviposition. The male used his hind legs to hold vegetation, possibly helping the female to maintain her balance while moving through the vegetation.

A second pair found near the first pair also moved through the vegetation, apparently seeking a place for oviposition. A solitary male that was about 3 m above this pair moved toward the amplexant pair and jumped on the frogs, grabbing the female. The male in displacement behavior placed his head between the amplexant frogs and used his hind legs to push the amplexant male, which resisted by vocalizing and pushing back with his hind limbs. The struggle was terminated when the displacing male succeeded in pushing away his opponent and initiated amplexus with the female. This fight took place over the female's body; we inferred that she accepted the displacing male,

because the female moved towards the oviposition site. The four individuals found in amplexus were measured with digital calipers (0.01 mm). The male amplexant *Phyllomedusa bicolor* were smaller than the females (males: 91 and 113 mm, females: 111 and 119 mm). Oviposition required about 2 h, and the pair of frogs stayed in amplexus for a few minutes more. The SVL of the displaced male was not measured, because the frog moved away.

The oviposition behavior of *Phyllomedusa bicolor* was similar in both observations at FEC, where males and females in amplexus moved through the vegetation visiting other leaves to find a place to lay the eggs. Once the oviposition site was selected, the amplexant pair folded the leaves and formed a chamber with three leaves (Figure 1A), where the eggs were protected together with a gelatinous mass about 70 cm over the surface of water. At FEC, the egg mass (Figure 1B) was enveloped by three elliptical leaves (Siparunaceae). This spawn was monitored for 14 days; the tadpoles that emerged from the eggs were seen dripping in a water pond. The tadpoles were not monitored until metamorphosis. The number of eggs in this spawn was not counted.

At FSJ, oviposition was not monitored, but the amplexant pair was seen moving through the vegetation and inspecting leaves, possibly seeking a place for oviposition (Figure 2A). The egg mass found on the following day was also 70 cm above the water surface and was enveloped by three palmate leaves (Euphorbiaceae) (Figure 2B). The spawn had 1202 eggs, all unpigmented, and with an average diameter of 2.7 ± 0.2 mm ($n = 30$).

Fighting behavior is common in many anuran species (Duellman and Savitzsky 1976, Wells 1977, 1978, Kluge 1981, Martins *et al.* 1998), including species of the genus *Phyllomedusa* (e.g., Halloy and Espinoza 2000, Abrunhosa and Wogel 2004, Wogel *et al.* 2004, 2005).

Phyllomedusa rohdei from the Atlantic Forest uses vocalization and physical interactions to defend its territory; they grab opponents by the head, waist, or nape with their hands and feet

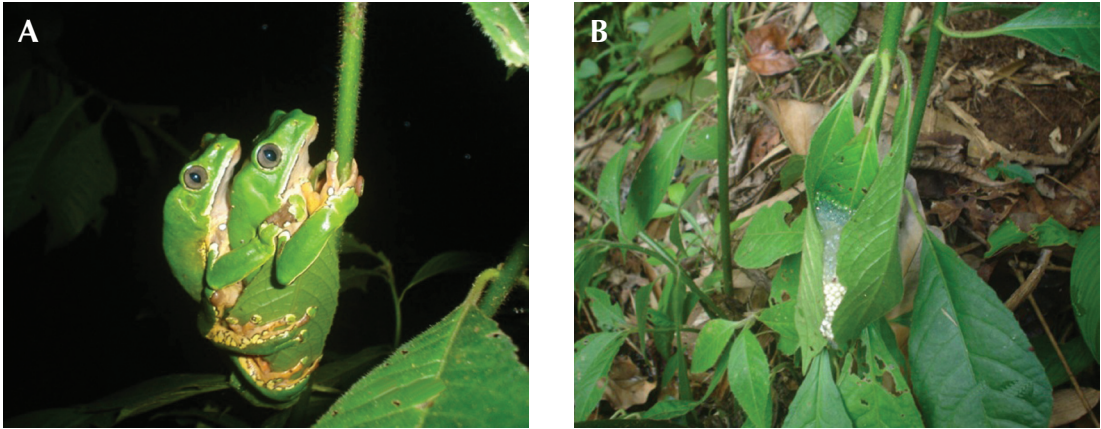


Figure 1. (A) Amplexant pair of *Phyllomedusa bicolor* building a chamber of leaves. (B) Spawn of *P. bicolor*, made of three elliptical leaves of the family Siparunaceae. Fazenda Experimental Catuaba (FEC), Municipality of Senador Guimard in the State of Acre, Brazil.

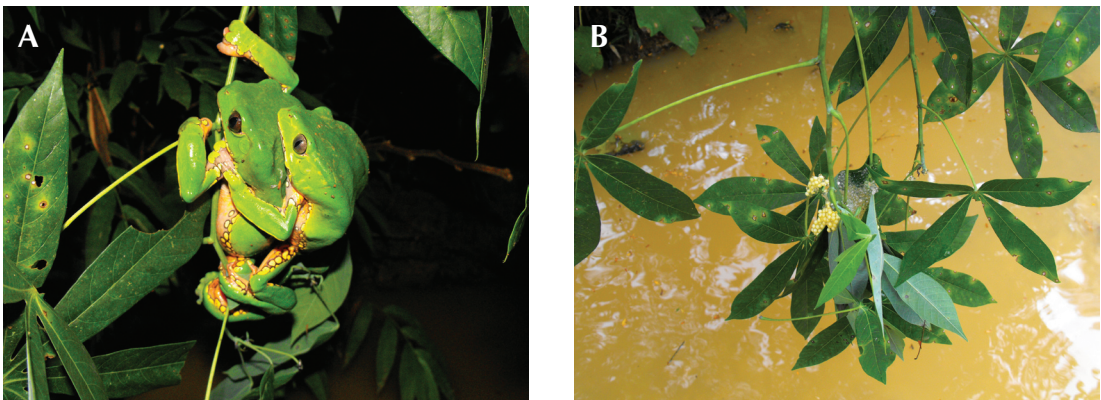


Figure 2. (A) Amplexant pair of *Phyllomedusa bicolor* inspecting leaves, possibly in order to find a place for oviposition. (B) Spawn of *P. bicolor* found on the following day, made of three palmate leaves of the family Euphorbiaceae. Fazenda São Jorge I (FSJ), Municipality of Sena Madureira in the State of Acre, Brazil.

(Wogel *et al.* 2004). In this species, three attempts by solitary males to displace males in amplexus also were observed. During this event, the male in displacement behavior used the vegetation around as a support and placed his head between the members of the amplexant pair (Wogel *et al.* 2004).

In *Phyllomedusa burmeisteri*, four fights were observed. In one of them the attacking male

embraced the intruder as in axillary or inguinal amplexus and used his nuptial callosities to squeeze the vocal sac and the head of the opponent (Abrunhosa and Wogel 2004). After losing a fight, a male exhibited marks and scratches on his head (Abrunhosa and Wogel 2004). Here, the male of *P. bicolor* found in amplexus at FSJ bore scratches that were probably injuries resulting from a dispute for a female.


During fights, males become more vulnerable to predators (Wells 1978). Shine (1979) reported that predation risks may be smaller in species with large body sizes or with chemical defenses. The large body size of *P. bicolor*, considered one of the largest hylid species (Caramaschi and Cruz 2002), together with the production of peptides on its skin (Daly *et al.* 1992) may make the species less susceptible to predatory attacks.

The reproductive behavior of *Phyllomedusa bicolor* fits Mode 24 proposed by Haddad and Prado (2005). The species has big eggs and the largest spawn found among the arboreal frogs in the Amazon (Neckel-Oliveira and Wachlevski 2004).

Neckel-Oliveira and Wachlevski (2004), in a study involving three species of the genus *Phyllomedusa* (*P. bicolor*, *P. tarsi* and *P. tomopterna*), found 31 egg masses of *P. bicolor*. Of these, 19 had an average of 859.5 eggs, and used an average of 2.3 leaves to envelop the eggs, which developed in 11.5 days. We found similar values in the present study. Spawn of both localities were found 70 cm above water enveloped by three leaves. The spawn found at FSJ comprised 1202 eggs and in the one found at FEC, the tadpoles emerged in 14 days.

Although spawn were enveloped with three leaves, different leaf shapes were used (elliptical and lanceolate) providing different spatial arrangements. Predation of spawn of *Phyllomedusa* is an extremely common event (Neckel-Oliveira and Wachlevski 2004) and spawn of *P. bicolor* is eaten by flies, beetles, and even mammals (Neckel-Oliveira and Wachlevski 2004). It is possible that the different types of spatial arrangements found here are an efficient camouflage against predators or a better way to protect the spawn against dehydration.

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