

Territorial and mating behavior in *Phyllomedusa azurea* (Anura: Hylidae) at a temporary pond in west-central Brazil

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Abstract

Territorial and mating behavior in *Phyllomedusa azurea* (Anura: Hylidae) at a temporary pond in west-central Brazil. We studied territorial and mating behavior of *Phyllomedusa azurea* in the municipality of Cocalzinho de Goiás, Goiás, which is located in central Brazil. During the mating season, male *P. azurea* engage in territorial behavior that is associated with vocalizations—advertisement, territorial, fight, or a combination of two more of these. When one male frog invades the territory of another, they commonly emit territorial calls and engage in physical combat. Three main behavioral traits were observed in mating males—viz., “male-singer,” “active search” and “male-shifter.” Males vocalize during the amplexus and oviposition. No aggression between females *P. azurea* was observed.

Keywords: Anura, Hylidae, *Phyllomedusa azurea*, mating behavior, territorial behavior.

Resumo

Comportamento territorial e de acasalamento de *Phyllomedusa azurea* (Anura, Hylidae) em uma lagoa temporária do centro-oeste do Brasil. Estudamos o comportamento territorial e de acasalamento de *Phyllomedusa azurea* no município de Cocalzinho de Goiás, Goiás, localizado no Brasil central. Durante a estação reprodutiva, o macho de *P. azurea* apresenta comportamento territorial que é associado com a emissão de vocalizações—cantos de anúncio, territorial e de luta ou uma combinação de dois ou mais deles. Quando um macho invade o território do outro, comumente emite cantos territoriais e entra em combate físico com o macho residente. Três estratégias principais de acasalamento foram utilizadas pelos machos—“macho-cantor”, “procura ativa” e “macho-deslocador.” Os machos vocalizam durante o amplexo e a ovipostura. Nenhum tipo de comportamento agressivo foi observado entre as fêmeas de *P. azurea*.

Palavras-chave: Anura, Hylidae, *Phyllomedusa azurea*, comportamento de acasalamento, comportamento territorial.

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Introduction

Territorial defense in many animals includes the advertisement of the territory's occupant by acoustic signals, threat signals over long distances, and even agonistic encounters (Bradbury and Vehrencamp 1998, Behr *et al.* 2006). In general, these tactics establish recognizable boundaries of the territory of the individual resident to potential intruders (Temeles 1994, Stoddard 1996). Anuran territorial behavior may include acoustic interactions, postures, and fighting, all of which are motivated by competition for resources, such as calling sites, spawning sites, and sexual partners (Martins *et al.* 1998, Bastos and Haddad 2002, Guimarães and Bastos 2003).

Territoriality (as well as other forms of behavior, such as complex and prolonged courtship) is more pronounced in anurans with extended breeding seasons, because males and females do not arrive synchronously at the breeding site. In this case, males must maintain a territory that is free of competitors and vocalize to attract females that are ready for oviposition (Wells 1977a, Murphy 1994). Thus, in anurans with this type of reproduction, the success of the reproductive male frog depends on the male's ability to attract females to the calling site by emitting varied and complex calls, and to defend his territory from intruding males.

In anurans with extended reproductive seasons, both the characteristics of the male's territory and vocalizations affect female preference. This suggests that the selection has favored the evolution and retention of postural displays, as well as other stereotyped behaviors that are used in complex courtships and in the defense of territories (Wells 1977a). Herein, we report data and observations on the territorial and mating behavior in a population of *Phyllomedusa azurea* (Cope, 1862).

Materials and Methods

Behavioral observations were conducted at Sítio Santa Terezinha (15°42'23.5" S, 48°49'48.3" W)

in the municipality of Cocalzinho de Goiás, state of Goiás, west-central Brazil, during the reproductive season of *Phyllomedusa azurea*, between 25 February 2006 and 11 April 2007.

Observations were made at a temporary pond with open canopy; the vegetation consisted predominantly of *Brachiaria* grasses, and trees and shrubs. The water is present in the pond during six months per year. Observations began after sunset and were completed between 24:00 h and 05:00 h. We used lanterns with white or red light; the red light was less stressful on the frogs. Methods of "animal focal" and "all occurrences" (Altmann 1974, Martin and Bateson 1986) were employed.

When first observed, each frog was weighed and its snout-vent length (SVL) measured with calipers. Individuals were marked by toe-clipping following the method of Martof (1953) with modifications; opposable fingers were not clipped. We marked the frogs to determine which males resided in the part of the pond that we studied; if an individual was found two more more times at the breeding site, it was considered to be a resident. Observations were recorded with a tape recorder, and frogs were photographed to document their behavior.

Results

Male *Phyllomedusa azurea* use leaves, twigs, and branches of trees, leaves of shrubs and dried grasses as calling sites. Males called from within clumps of *Brachiaria*, exposed, and even on the ground. As Costa (2008) observed, males precede females to the breeding site. Most amplexant pairs were observed around 23:00 h.

During the breeding season, 141 male and 69 female *Phyllomedusa azurea* were marked (unpubl. data), but only five males that always were found the same clump of grass were considered to be territorial. Because territorial behavior expressed by physical combat is not common in this species, it is difficult to encounter combatant males and document their behavior in the field. Of the five territorial males, one was

captured twice, one five times, one seven times, one nine times, and one ten times.

Three of the five territorial males were observed in physical combat. On 08 December 2006, two males were found fighting in a ventral embrace. They remained in the same position for several minutes, while sporadically emitting advertisement and fight calls (detailed description of calls will be published). When the males separated, one (mass = 2.5 g, SVL = 36.7 mm) remained in the bush where the fight had occurred and began to emit advertisement calls, whereas the other (mass = 2.2 g, SVL = 34.9 mm) retreated from the calling site without vocalizing.

On 05 January 2007, two males were found interacting aggressively and embraced ventrally; both frogs vocalized during this interaction. The males clasped one another by means of abdominal movements and by entwining their limbs. They separated 50 min later, with one remaining at the calling site, and the other disappearing (Figure 1). On a third occasion (23 January 2007), an invading male was chased, attacked, and driven from the territory by a resident male. The invader had been collected previously and was observed to vocalize near the border of another male's territory (Figures 2, 3).

In summary, we observed two behavioral responses when one male invaded another male's territory. (1) On noting the presence of an intruding male, the resident emitted territorial calls and then the two interacted acoustically, with both emitting territorial calls. This behavior resulted in one individual withdrawing from the site without any kind of physical combat ($n = 3$ occurrences). (2) The resident male, in the presence of the intruder, issued advertisement calls and then initiated physical combat, which continued until the intruder left ($n = 1$ occurrence). No aggressive interactions occurred between females *Phyllomedusa azurea*.

On 03 October 2006, one male was observed attempting to displace another male in amplexus. Although the solitary male was heavier and larger (mass = 3.1 g, SVL = 38.63 mm) than the



Figure 1. Male *Phyllomedusa azurea* during physical combat. Sítio Santa Terezinha, Cocalzinho de Goiás, GO, Brazil.



Figure 2. Physical combat between the resident male (red arrow) and the attacking male (blue arrow).



Figure 3. Attacking male (blue arrow) leaving the site of aggressive interaction after physical combat.

male in amplexus (mass = 2.2 g, SVL = 34.4 mm), the attempt was not successful.

Three mating strategies were observed among males—(1) advertisement vocalization for females; (2) active search by silent males that roam the environment, looking for females with which to amplex (Haddad 1991a); and (3) male-shifting, observed only once, when a male tried displace an amplexant male (Haddad 1991b). We did not observe any visual signals between males and the females before amplexus.

Initiation of amplexus was observed only once, on 25 February 2006. A male was calling from inside a clump of *Brachiaria* sp., and located a female about 40 cm away. While still calling, the male climbed toward the female and onto her back. The amplexant pair moved through the vegetation for 20 min, until both stopped and eggs were deposited and fertilized on a leaf of a *Brachiaria* above the surface of the water.

Males vocalize during amplexus ($n = 4$ pairs). During oviposition, the male and female fold the leaf with their hind limbs. Only one leaf was used to deposit and cover the egg mass, and different types of leaves, (e.g., grasses, trees and shrubs of the genera *Mimosa*, *Sida*, *Elephantopus*, *Tibouchina*, and *Brachiaria*) were used as oviposition sites. Differences between leaf sizes and the possible resulting implications were not investigated here. Because most of the area is covered with *Brachiaria* sp., most spawning occurred on this plant species. Following oviposition, the male departs, leaving the female to deposit the mass of eggless capsules that secure the surfaces of the folded leaf (Pyburn 1980) at its top. With this task completed, the female also leaves the spawning site.

The overall process of mating and oviposition takes about 02:30 h and includes the following steps—(1) Encounter of male and female; (2) search for an oviposition site; and oviposition, accomplished by body contractions to produce spawn from the male and female and jelly capsules from the female to seal the leaf. However, we observed amplexus from its initiation

only once; therefore, the time for courtship, mating, and oviposition could be longer than indicated. Egg deposition itself does not take long; in our three observations, the times required were 48, 51, and 80 min.

The female was not observed to perform any type of signaling at the beginning of amplexus. Abdominal contractions of the both the male and the female's bodies preceded the extrusion of the gametes. The male grasps the female to keep his cloaca positioned above that of the female. Schematic representations and details of the sequence of events characterizing the oviposition behavior of three pairs are illustrated in Figures 4–6. Parental care was not observed.

Discussion

During reproductive activity, male *Phyllomedusa azurea* are territorial and defend their calling sites through physical interactions and aggressive vocalizations (territorial and fight calls). Shine (1979) suggested that no sexual dimorphism occurs when there is male-male combat and that if sexual dimorphism does occur, the males tend to be larger than females. In *P. azurea*, this hypothesis is not supported, as there is sexual dimorphism—i.e., the females are larger and heavier (Costa 2008)—and the males engage in physical combat. As stated by Halliday and Tejedo (1995), sexual dimorphism may result from other factors, such as the rate of sexual maturation and age at sexual maturity.

Male *Phyllomedusa azurea* occupy the same locations at the pond on consecutive nights; this can be considered as territorial behavior (Wells 1977b), because there is competition for limited resources, such as calling, breeding, and feeding sites. Territoriality also can occur if the presence of sufficient resources is unpredictable, as would be the case with the arrival of receptive females (Wells 1977a, Costa 2008). Territoriality occurs more frequently in anurans with an extended reproductive season, in which females arrive the breeding sites irregularly throughout the season (Wells 1977a). Wells (1977a) also suggested that

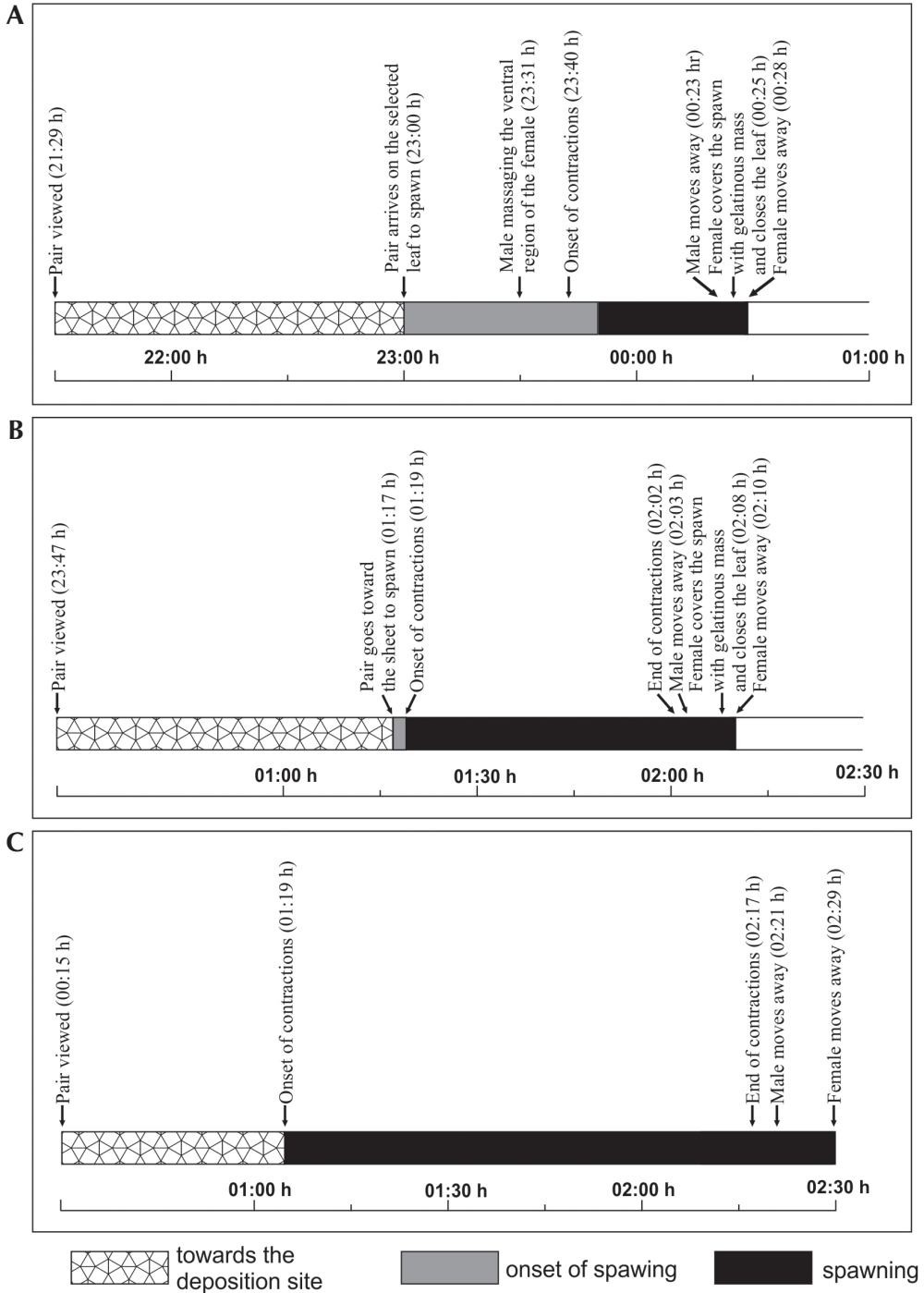


Figure 4. Ethogram of the oviposition behavior of the pairs of *Phyllomedusa azurea*. Summarized sequence of the spawning observed. (A) 09 December 2006, (B) 05 January 2007, (C) 08 January 2007.

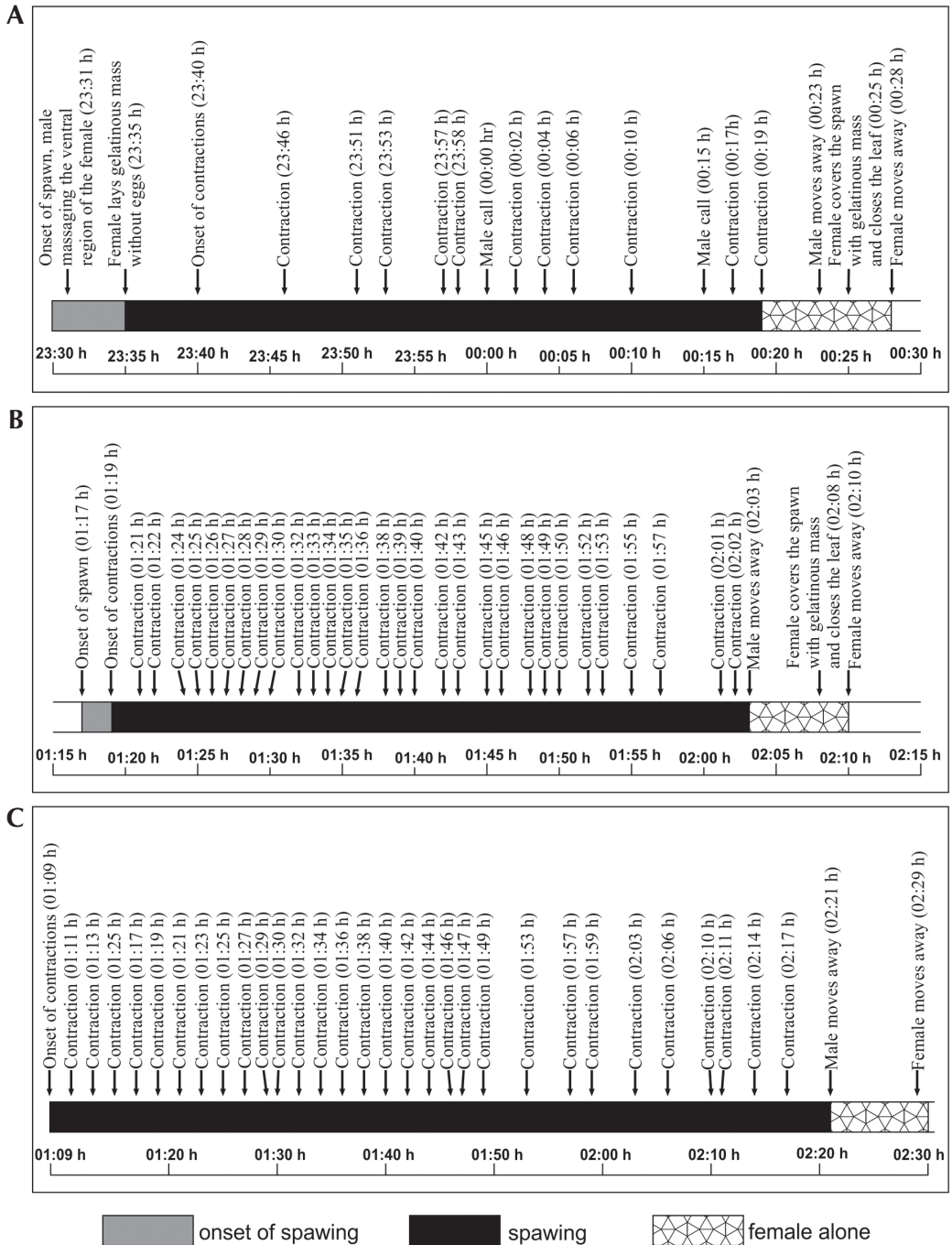


Figure 5. Ethogram of the oviposition behavior of the pairs of *Phyllomedusa azurea* that were observed during the study. Detailed sequence showing the events of abdominal contractions by males and female to expel the gametes and vocalization during oviposition. (A) 09 December 2006, (B) 05 January 2007, (C) 08 January 2007.



Figure 6. Oviposition in *Phyllomedusa azurea*. (A) Moving through the vegetation; (B) contraction during oviposition; (C) male and female depositing gametes while folding the leaf; (D) male vocalizing during amplexus; (E) male leaving the spawning site after oviposition; (F) female depositing the last layer of gelatinous capsules and closing the leaf; (G) female leaving.

territorial behavior evolved because it was inefficient for males to search for mates continuously when females arrive at a breeding site asynchronously or sporadically. These hypotheses are applicable to males and female *Phyllomedusa azurea*, which join the breeding chorus asynchronously, with the male defending the vocalization and oviposition territories. However, the strategy of an active search pattern also is observed for this species.

According to Wells (1977b), male frogs use three strategies to obtain a territory. (1) With the onset of calling activity, the male occupies a site and defends it from intruding males. (2) The male occupies a site by force, expelling its original owner. (3) The male (usually a satellite male) occupies a site that has been abandoned. In *Phyllomedusa azurea*, the first and second strategies are employed, but the third was not because no satellite males were observed in the chorus.

The phenomenon of male *Phyllomedusa azurea* calling during amplexus may represent an attempt to hide the female from “shifter males.” If the latter notice the presence of the females they could intercept them. Thus, vocalization during amplexus might bluff “shifter males” by giving them the impression that only a single male, rather than a pair, is present at the breeding site (Bastos and Haddad 2002).

Matos *et al.* (2000) observed that male *Phyllomedusa hypochondrialis* returned to the same territories on consecutive nights and that vocal interactions and fights between the males of adjacent territories were common. Other species of the subfamily Phyllomedusinae also exhibit aggressive territorial behavior with physical fighting; these include *P. burmeisteri* (Abrunhosa and Wogel 2004), *P. boliviana* (Vaira 2001), *P. rohdei* (Wogel 2001), and *Agalychnis lemur* (Jungfer and Weygoldt 1994). The typical aggressive territorial behavior of *P. azurea* consists of changing from an advertisement call to a territorial call, followed by the chasing the male intruder, acoustic and physical combat, expulsion of the invader male from the territory,

and then maintaining advertisement calls after the combat. Furthermore, although it was not common, male *P. azurea* also returned to the same place, as was observed in *P. hypochondrialis* (Matos *et al.* 2000).

The process of oviposition resembles that of the small species of the genus *Phyllomedusa*, in which eggs are deposited together with gelatinous capsules into a single leaf that is closed during oviposition with combined efforts of both the male and female (Pyburn and Glidewell 1971, Vaira 2001, Abrunhosa and Wogel 2004, Wogel 2006). This type of oviposition is more complex than that characterizing other species of Phyllomedusinae, such as *Agalychnis callidryas* and *A. dacnicolor* (Pyburn 1970), in which the spawn is deposited on open leaves. Enclosing the egg mass reduces its exposed surface, providing greater resistance to drying. The deposition of the gelatinous capsules on the upper and lower ends of the folded leaves provides added protection and an extra source of moisture (Pyburn 1970).

The time involved in mating and oviposition in *Phyllomedusa azurea* is not long in comparison to these processes in other species. In *Agalychnis dacnicolor*, for example, oviposition can last up to 6 h (Bagnara *et al.* 1986) and may be interrupted by intervals up to 1 min long between each contraction; this was commonly observed in *P. azurea*.

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