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# Tremulation Display in Male Agonistic Behavior of the Black-eyed Leaf Frog (*Agalychnis moreletii*: Hylidae)

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**Abstract –** The black-eyed leaf frog (*Agalychnis moreletii*) is a critically endangered hylid frog restricted to humid lowland and lower montane forests of southern Mexico and northern Central America. Very few reports exist on wild reproductive behavior of this species. Lower body tremulation display has been reported as a relevant part of agonistic interactions between males of the related species *A. callidryas* but has never been reported in other species of *Agalychnis*. Herein, we present the first report and description of this tremulation display among males of *A. moreletii* in a lower montane forest of Guatemala. Based on our observations, we suggest that this display may serve to prevent physical confrontations among males and might play an important role in defining territories. We also suggest that the difference in fundamental frequency of the tremulations between *A. moreletii* and *A. callidryas* may serve to avoid confusing signals between species where they breed sympatrically.

**Keywords –** Agonistic behavior, Vibrational signaling, Tremulation, *Agalychnis*, Hylidae, Guatemala

The black-eyed leaf frog, *Agalychnis moreletii* (Duméril, 1853), is a moderate-sized species of hylid frog distinguished from other species of *Agalychnis* by having uniformly orange flanks and thighs and an extremely dark red eye that appears black to human sight (Duellman, 1970). The species is restricted to small areas of south-central Veracruz, Mexico, western Belize, north-western Honduras, Guatemala, and El Salvador and inhabits humid lowland and lower montane forests occurring in both pristine and disturbed habitat (Campbell, 1998; Köhler, 2011). It is listed under the Critically Endangered category of IUCN red list (Santos-Barrera, Lee, Acevedo, & Wilson, 2004) and is included under Appendix II of CITES.

Only a few published reports exist documenting breeding behavior of *A. moreletii* in the wild (Briggs, 2008; Briggs, 2010; Campbell, 1998; Duellman, 1970; Lee, 1996) or in captivity (Burger, 2012). Males descend from the forest canopy and congregate in choruses shortly after sunset and call from vegetation surrounding small lentic bodies of water such as seasonal ponds (Duellman, 1970). Advertisement ‘‘zworp’’ calls function to attract females and aggressive ‘‘chuckling’’ calls are used during intrasexual encounters (Briggs, 2010). The species exhibits pronounced sexual dimorphism in body size, with females much bigger and heavier than males (Figure 1).

In the related species of red-eyed treefrog (*A. callidryas*) a lower body vibrational display has been reported as a relevant part of agonistic interactions between males of this species at mating sites during the breeding season (Pyburn, 1970). Later research showed that this kind of vibrational display, also called tremulations, serve an important role in the aggressive interactions of red-eyed tree frogs and suggests that similar vibrational displays may be common among arboreal vertebrates (Caldwell, Johnston, McDaniel, & Warkentin, 2010). Hill and Wessel (2016) define tremulation as a shaking or trembling behavior that induces vibrations in the substrate, often through the legs, with no percussive impact. This communication via surface-borne vibrations is widely documented in arthropods, such as orthopterans (Cocroft, Shugart, Konrad, & Tibbs, 2006) and hemipterans (Kavčič, Čokl, Laumann, Blassioli-Moraes & Borges, 2013), but also had been reported in some vertebrates, such as tree frogs (Caldwell et al., 2010), salamanders (Christensen, Lauridsen, Christensen-Dalsgaard, Pedersen, & Madsen, 2015) and seals (Bishop, Denton, Pomeroy, & Twiss, 2015). The analysis of this vibrational signaling in animals, known as biotremology, allows a better understanding of communication through these signals, the propagation process in the environment and how physical variables affect this behavior (Mortimer, 2017). According to Hill (2009) vibrations carried in the substrate are considered to provide a very old and apparently ubiquitous communication channel employed in the contexts of mate location and identification, courtship and mating, maternal care and sibling interactions, predation, predator avoidance, foraging, and general recruitment of family members to work. Herein, we present the first report and description of a tremulation display among males of *A. moreletii*.

*Figure 1*. An amplectant pair of Black-eyed Leaf Frogs (*Agalychnis moreletii*: Hylidae) found in the morning during the breeding season at Reserva Natural Privada Sac Wach Já, Alta Verapaz, Guatemala. The male appears on top of the female and a clutch of eggs is visible at the upper left corner.

## Materials and Methods

The observations were conducted on six different nights throughout the month of May, which marks the end of the dry season (CONAP, 2008). The last visit to the study site on May 20th, 2017 coincided with the first rains of the year´s rainy season.

## Description of Study Site

The study took place in a permanent pond located in a private protected area called Sac Wach Já near San Cristobal Verapaz, in Alta Verapaz, Guatemala (15°25'17.78"N; 90°36'21.02"W; see Figure 2). Some of the most representative species of vegetation in the site are Aguacatillo (*Nectandra* sp. and *Ocotea* sp.), various species of Oaks (*Quercus* spp.), Guarumo (*Cecropia obtusifolia*), various species of palms (*Chamaedorea* spp.), Moquillo (*Saurauia oreophila*), Mountain Rosewood (*Dalbergia* sp.), Palo de Uva (*Parathesis* spp.), Mountain Cedar (*Trichilia* sp. and *Cedrela odorata*), Bacche (*Ageratina ligustrina*) and a high abundance of bromeliads, orchids and ferns. The forest that surrounds the protected area is fragmented by pine, cypress, coffee and corn plantations. The dimensions of the pond are 5.5m by 8m long, with an approximate depth of 1.5 m at the center. A dense layer of organic material from the surrounding vegetation covers the bottom of the pond. No fish are present and many tadpoles of *A. moreletii*, *Lithobates maculatus* and possibly other anurans are visible inside the water. The pond is located in a cloud forest at 1,480 m above sea level and is surrounded mainly by small trees and palms of the species *Chamaedorea tepejilote*. *A. moreletii* lay their eggs on top of these palm's leaves, positioned directly above the water level. Because the pond does not dry during the summer, tadpoles, egg clutches and calling males of *A. moreletii* have been observed all year long.

*Figure 2*. Study site at a seasonal pond in the lower montane forest at Reserva Natural Privada Sac Wach Já, Alta Verapaz, Guatemala.

## Behavioral Data Collection

The observations of agonistic behavior of this species were conducted during the night of May 20th, 2017. Ten male-male event interactions (Figure 3) were recorded using StylusTG-4 Olympus and Sony HXR-NX30E cameras from 7:00 p.m. to 12:00 a.m. These ten recorded events were performed by six different males. Females were identified based on the size difference and males were identified when they produced advertisement or territorial chuckle calls. On this night, the frogs were so abundant that several males were found sharing the same branches. The interactions were recorded using white light as we have seen that this does not affect the expression of the behavior of interest.

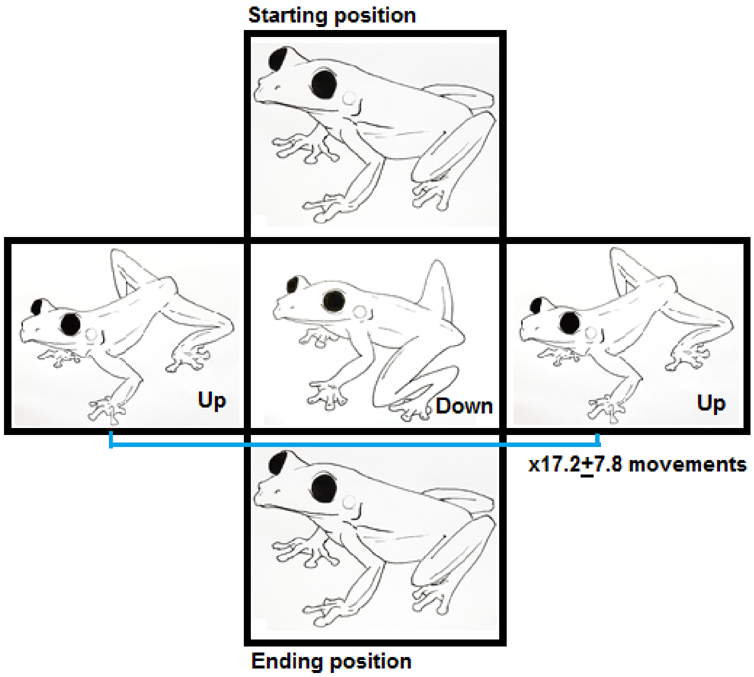


*Figure 3*. Black-eyed leaf frog (*Agalychnis moreletii*) generalized ethogram diagram of male tremulation display.

## Video Analysis

The videos were later analyzed using Movie Maker video editor to transform them to slow motion (0.125X) and we played them back at one-eighth the normal speed to observe them frame by frame and count the number of movements and the duration of each tremulation display. Reliability of movement count was performed by four independent video coders that counted each normal and slow movement display. On two of the videos, disagreements between coders were addressed by using the median of the values as our final result. On the rest, no disagreements where found. A Randolph´s free marginal kappa test was performed to calculate the agreement between the values. Frequencies (Hz) were calculated dividing the number of movements by the duration of each display in real time (1.0X). Each movement consisted of the frog lifting his lower back, then lowering it, and concluded when it reached the starting position. Other important information like the male´s direction during the display was taken from the videos.

# Results

On the night of May 20th, 2017, we found 40 active individuals of *A. moreletii*. Only three individuals were identified as females. The sex of the rest of the individuals was not assigned because the observations were made from afar. Most of the frogs were found in plants surrounding the pond at knee height, others were seen above ground in palms at approximately two meters high. Males were spotted in individual branches and leaves. Advertisement calls and territorial chuckle calls were heard upon arrival and males were active. Their activity was uninterrupted when we approached to record the behaviour. On ten occasions, agonistic interactions between males were observed. This interaction was observed when two or three males shared the same branch. Visual contact between males was observed in only one of these ten recorded events.

These agonistic interactions between males consisted of males approaching one another in an aggressive manner to remove the other male from its calling site. The first observation of tremulation display was recorded at 7:46 pm, when one male appeared to be following the other male’s path, jumping on the same spots and climbing on the same direction. We recorded a total of six different males performing the tremulation display (Figure 3). The average frequency of the tremulation display was 7.3 ± 0.9 Hz. Each encounter lasted an average of 2.4 ± 1.1 s with 17.2 ± 7.8 up and down tremulation movements per act (Table 1), with a free marginal kappa value of 0.75 for the measurement of agreement. A rule of thumb is that a kappa of .70 or above indicates adequate interrater agreement.

Table 1

*Metrics of Tremulation Display in Six Male Black-eyed Leaf Frogs A. moreletii*

| Video code | Male ID | Frequency (Hz) | Duration (s) | No. of movements |
|---|---|---|---|---|
| 1.1 | 1 | 6.71 | 1.64 | 11 |
| 1.2 | | 8.05 | 0.87 | 7 |
| 2.1 | 2 | 7.17 | 2.65 | 19 |
| 3.1 | 3 | 8.70 | 1.61 | 14 |
| 3.2 | | 7.46 | 2.28 | 17 |
| 4.1 | 4 | 8.24 | 1.82 | 15 |
| 5.1 | 5 | 7.03 | 4.41 | 31 |
| 5.2 | | 5.66 | 1.59 | 9 |
| 5.3 | | 6.05 | 3.47 | 21 |
| 6.1 | 6 | 7.61 | 3.68 | 28 |
| Mean | | 7.3 ± 0.9 | 2.4 ± 1.1 | 17.2 ± 7.8 |

Each act consisted of the male extending both hind limbs, uplifting his hind end followed by elevating and lowering his hind end repeatedly (Figure 4). Some encounters were followed by aggressive interactions of one male grappling another (links to example videos appear here and here). We also observed males kicking other males, some of which ended up falling off the leaves. Several tremulation displays were observed accompanied by kicks, pushes and low "chuckle" calls between males. One amplectant male was observed doing a tremulation display while being on top of a female. The displays were performed regardless of the light condition, under white light or infrared capable cameras.

*Figure 4*. A male black-eyed leaf frog (*Agalychnis moreletii*) performing a tremulation display to another male. In the background, two egg clutches can be observed on top of the palm leaves.

## Discussion

Tremulation displays are present and play an important role in the agonistic behavior of *A. moreletii* and may have many purposes in the communication between individuals. Because we observed that only one of the ten events recorded had visual contact between males, we consider that substrate-borne vibrational signals transmitted via plant stems and leaf stalks are the most important form of communication of this tremulation display. Vibrations in the substrate may carry important information about the males (Caldwell et al., 2010) as it is known that tremulations tend to produce low-frequency vibration signals in a narrow range that vary with the morphology of the sender, as well as with the body motions that create the signals (Hill, 2009). Intensity and duration of the tremulations may serve as an honest signal of the male's physical health and vitality. In most of the aggressive interactions observed, tremulations were produced before wrestling. According to Martins, Pombal, and Haddad (1998) a male frog should fight only in extreme situations, and anurans make use of other activities to avoid the risks and costs of physical combats, such as being seriously wounded or preyed upon. Based on our observations, we suggest that the displays of *A. moreletii* may serve to prevent physical confrontations.

Consistent with the observations of *A. callidryas* (Pyburn, 1970), males of *A. moreletii* use both territorial chuckle calls as well as tremulations both with and without the direct presence of females. However, the frequency and intensity of these behaviors were increased when a female was near. This suggests that the behavior observed in *A. moreletii* might play an important role in defining territories or calling locations, as it has been reported that the tremulation in *A. callidryas* can play part in an aggressive encounter between adult males following aggressive calling and posturing in high density situations (Gonzalez & Briggs, 2011).

These events were observed during the beginning of the rainy season, whereas no activity was recorded during previous observations at the same study site in the dry season. Thus, we infer that this type of behavior increases or might even be exclusive of this season.

The presence of tremulation in *A. moreletii* might have the same function as the use of substrate vibration as a form of communication that had been previously reported by Caldwell, and colleagues (2010) for *A. callidryas*. The frequency we observed in *A. moreletii* ($7.3 \pm 0.9$ Hz) is lower than the frequency reported by Caldwell et al. (2010) for *A. callidryas* ($12.1 \pm 1.9$ Hz). As there are some areas where both species breed sympatrically (Briggs, 2008), this difference in the fundamental frequency of the tremulations may serve to avoid confusing signals between species sharing the same breeding habitat. This is especially important as it has been reported that species communicating via tremulations transmitted through plant stems and branches face important limitations on this vibrational communication system for simultaneously detecting several conspecific and/or heterospecific signals, and discriminating between them (De Groot, Čokl, & Virant-Doberlet, 2011). This is the first report of tremulation display among males in *A. moreletii*.

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