

*Comments/Reflections***Nocturnal visual displays and call description of the cascade specialist glassfrog *Sachatamia orejuela*****Rebecca M. Brunner^{a,b,*} and Juan M. Guayasamin^c**^a Department of Environmental Science, Policy, and Management, University of California, Berkeley, 140 Mulford Hall, Berkeley, CA 94720, USA^b Third Millennium Alliance, Guanguiltagua N37-152 y Carlos Arosemena Tola, Edificio Lemarie, Oficina 500, Quito 170516, Ecuador^c Laboratorio de Biología Evolutiva, Instituto Biósfera, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Campus Cumbayá, Diego de Robles s/n y Pampite, Quito 170901, Ecuador

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Abstract

Although most male frogs call to attract females, vocalizations alone can be ineffective long-range signals in certain environments. To increase conspicuousness and counter the background noise generated by rushing water, a few frog species around the world have evolved visual communication modalities in addition to advertisement calls. These species belong to different families on different continents: a clear example of behavioural convergent evolution. Until now, long-distance visual signalling has not been recorded for any species in the glassfrog family (Centrolenidae). *Sachatamia orejuela*, an exceptionally camouflaged glassfrog species found within the spray zone of waterfalls, has remained poorly studied. Here, we document its advertisement call for the first time — the frequency of which is higher than perhaps any other glassfrog species, likely an evolutionary response to its disruptive acoustic space — as well as a sequence of non-antagonistic visual signals (foot-flagging, hand-waving, and head-bobbing) that we observed at night.

Keywords

anuran communication, visual signalling, advertisement call, soundscape, acoustic space, convergent evolution, glassfrog, neotropics.

Resumen

Aunque la mayoría de las ranas macho vocalizan para atraer hembras, las vocalizaciones por sí solas pueden ser señales ineficaces de largo alcance en ciertos entornos. Para llamar más la atención y contrarrestar el ruido de fondo generado por el torrente de agua, algunas especies de

ranas han desarrollado modalidades de comunicación visual, como la señalización con las extremidades y/o la cabeza, complementándola con llamadas de anuncio. Las especies que presentan estos comportamientos pertenecen a diferentes familias en diferentes continentes: un claro ejemplo de evolución convergente. Hasta ahora, la señalización visual a larga distancia no se ha registrado para ninguna rana de cristal (familia Centrolenidae). En esta publicación documentamos comportamientos de señalización en la rana de cristal *Sachatamia orejuela*. Al ser una especie con tendencia a camuflarse, encontrándose en las paredes rocosas dentro de la zona de rocío de las cascadas, *S. orejuela* sigue siendo poco estudiada, especialmente su comportamiento reproductivo. Aquí, documentamos por primera vez el canto de anuncio—cuya frecuencia es más alta que quizás cualquier otra especie de rana de cristal, probablemente como respuesta evolutiva al espacio acústico disruptivo—así como una secuencia no antagonista de señales visuales (movimiento de las extremidades anteriores y posteriores, y movimiento de la cabeza) en esta especie por la noche.

1. Introduction

Communication is crucial for mating success in frogs. To attract females, most males rely on advertisement calls, which can convey information related to a male's location and/or characteristics (e.g., body size). On a basic level, acoustic signals facilitate positive phonotaxis, enabling females to locate males across long distances (Gerhardt & Shwartz, 2001). Calls often function as form of territory delimitation as well, especially when suitable egg deposition sites are limited. Some well-studied systems have shown that a male's advertisement call can also convey information about his 'quality' in the context of female mate choice (Welch et al., 1998). For example, lower frequency variations of a frog call can be an honest signal for larger body size, which is preferred by females. Similarly, call length and pulse rates (syllable period) can convey information about performance and energetic constraints; females in playback experiments have been shown to prefer longer calls and higher pulse rates (Klump & Gerhardt, 1987).

However, vocalizations alone can be ineffective signals in certain environments. For example, loud streams and waterfalls create acoustic interference that can render a male's call difficult for listening females (or competing males) to receive. To avoid such interference, a few species produce calls in ultrasonic frequencies (Narins et al., 2007; Boonman & Kurniati, 2011). Others have evolved visual communication modalities to counter background noise, such as foot-flagging or hand-waving, where a male raises its hand and/or foot above its body in a repetitive motion (Hödl & Amezcua, 2001). Some well-known examples include *Hylodes japi* from Brazil (de Sá et al., 2016), *Micrixalus saxicola* from India (Preininger et al., 2013), and *Staurois*



Figure 1. (A) *Sachatamia orejuela*. (B) This species is found in the spray zones of waterfalls. Photos by RMB.

latopalmatus from Borneo (Grafe & Wanger, 2007). Each of these species call and visually display near rushing water, but belong to different families on different continents: a clear example of behavioural convergent evolution.

Sachatamia orejuela is a relatively large glassfrog from Ecuador and Colombia that is found on rock walls or boulders within the spray zone of cascades (Guayasamin et al., 2020). Due to its dark green-grey coloration and highly reflective skin (Figure 1A), this species blends exceptionally well into the mossy, wet rock crevices of its hard-to-reach habitat (Figure 1B). *S. orejuela* has thus remained an elusive and poorly studied species; none of the literature to date includes information on its breeding biology (Duellman & Burrowes, 1989; Rada et al., 2017). Therefore, the aim of this study was to document its advertisement call and any other associated behaviours.

2. Methods and observations

Fieldwork was conducted within Mashpi Reserve (0.167082° N, 78.871437° W; 950 m a.s.l.), part of the Tropical Andes hotspot of Ecuador. At least four times per week over the course of three months (February-April 2019), our three-person team extensively surveyed known cascade sites within the reserve (approximately 250 sample hours). During this time, we observed a

total of 18 *Sachatamia orejuela* individuals on moss and/or wet rocks at four waterfalls >500 m apart, along different streams. Frogs were consistently in the spray zone, within a two- to ten-meter radius of the cascade. Most individuals ($N = 14$) were observed >3.5 meters above the water level. Those found lower ($N = 4$) were in or near hard-to-reach rock crevices over deep pools of water.

Despite extensive surveying efforts, we only heard *S. orejuela* calling at one of the four cascade sites. On 6 April 2019, between 19:15 and 22:15, we observed an *S. orejuela* individual calling from a concave rock face next to a rushing waterfall (about 3 m above the pool below). To record its call (Roland R-26 digital audio recorder, Sennheiser ME67 directional microphone; 44.1 kHz sampling rate), RMB climbed to the only accessible viewing spot for this display: a small, slippery rock ledge about 4 m across from the rock face above the same pool. In the process of recording its call, she observed cyclical visual displays that included foot-flagging, head-bobbing, and hand-waving (Figure 2) — all well after sundown. RMB took videos of these behaviours from the same ledge across from the displaying frog using the camera that was on-site at the time (Nikon 7100 with 105 mm macro lens), as these observations were not expected.

We also observed another male exhibiting the same behaviours >6.5 m away from the individual described above, on the other side of the waterfall. We visually searched for more conspecifics in the area (with and without binoculars) during the same time period, but did not hear or see any other individuals.

Call parameters were analysed in Raven Pro 1.5 (Center for Conservation Bioacoustics, Cornell Lab of Ornithology 2014) without filters or transformations.

3. Results

The *Sachatamia orejuela* advertisement call (Figure 3A; Audio 1 at 10.6084/m9.figshare.13160012) is high-pitched, even for a glassfrog (mean peak frequency = 7.433 ± 0.1203 SD; range = 7.219–7.688 kHz; $N = 13$ notes from 2 individuals; minimal amplitude modulation). Generally emitted as a single ‘peep’, each note in its call had a duration of 0.137–0.189 sec (0.167 ± 0.018 , $n = 13$). Time between individually emitted notes ranged from 12.1–57.0 sec (32.4 ± 12.9 , $N = 21$). Males would occasionally emit two notes in

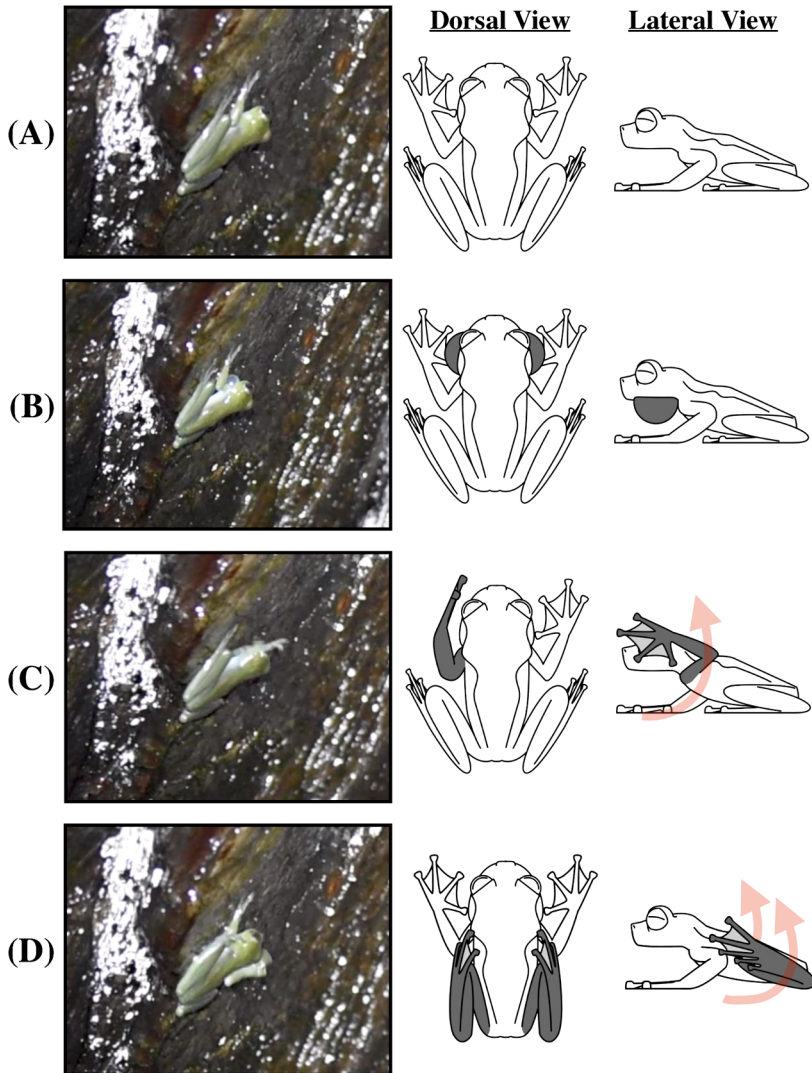


Figure 2. Visual signalling sequence of *Sachatamia orejuela*. Positions: (A) not calling or displaying, (B) vocal sacs inflated while calling, (C) arm-waving, (D) double foot-flagging. Photos from Video 3, taken by RMB. Illustrations by Robert Tyler.

quick succession (Figure 3A; between-note interval 0.455–0.548 sec, $N = 2$); although we only recorded it twice, we heard the double note call multiple times each hour. We also observed that *S. orejuela* has paired vocal sacs (Figure 3B), the first record of such in glassfrogs.

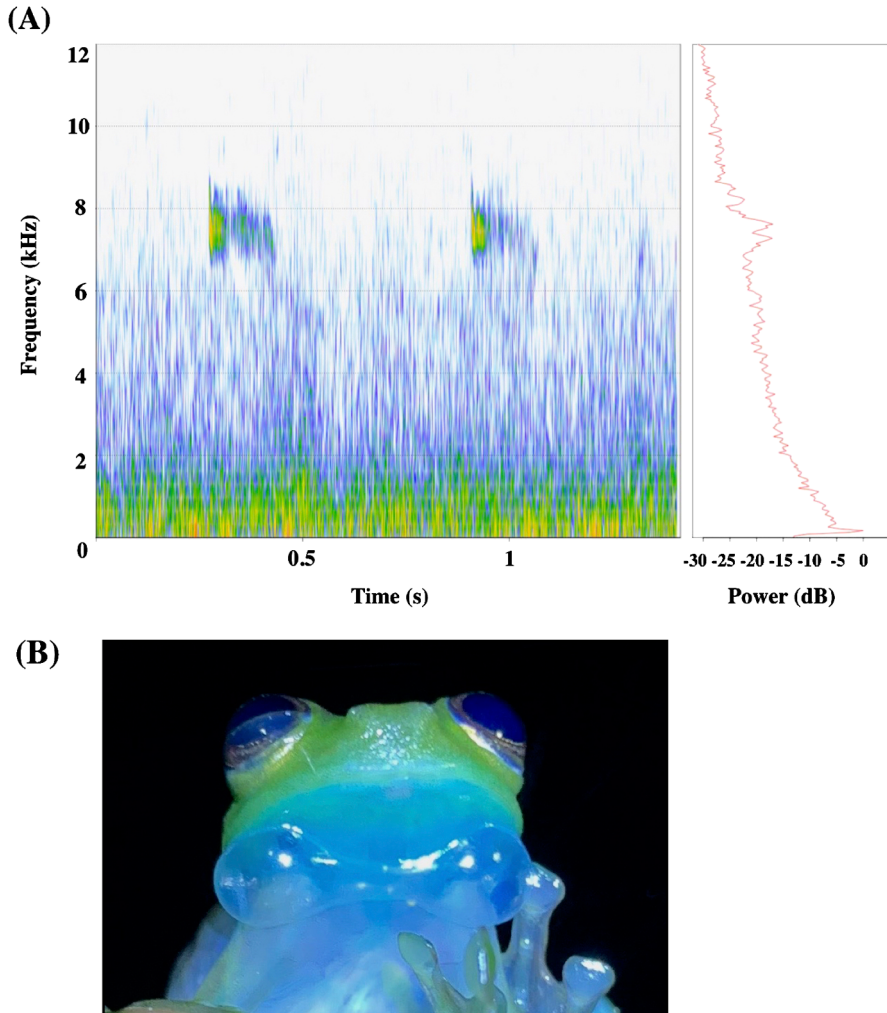


Figure 3. (A) Spectrogram (frequency vs. time; warmer colors indicate higher amplitude) and power spectrum (frequency vs. power) of the *Sachatamia orejuela* advertisement call within its loud cascade habitat. The majority of the power (dB) is located ≤ 2 kHz (noise generated by the waterfall) and between 7–8 kHz (the double-note *S. orejuela* call, which registers above much of the acoustic interference). Spectrogram was generated with a window length of 128 samples. Power spectrum was generated with a window length of 1024 samples; all powers (dB) are relative to 0, which was set as the maximum power of the recording. (B) A close-up of the *S. orejuela* paired vocal sac, the first record of such for glassfrogs.

In addition to calling, the same individual would occasionally wave its leg(s), wave its arm(s), and bob its head. The male would rotate through one of four signals every 5 to 60 seconds (20 seconds on average, based on observations of two individuals over the course of >2 hours). Although the signal sequence was not consistent, the most common pattern was as follows: calling bout/vocal sac inflation (2–4 times), head bob, hand wave, leg wave (Figure 2). It repeated these behaviours over the course of two hours, interspersed with ‘rest’ periods where it would vocalize without foot or hand movements. Video 1 at 10.6084/m9.figshare.13160012 clearly shows each of these behaviours in succession, with shakiness edited out (RMB had to balance on one foot while on the adjacent ledge). Video 2 at 10.6084/m9.figshare.13160012 shows the same succession at half speed for better visualization. The full-length, unedited recording is provided in Video 3 at 10.6084/m9.figshare.13160012.

4. Discussion

Since *Sachatamia orejuela* vocalizes near loud cascades, it is unsurprising that its call is high-pitched. Waterfalls are a source of wide-spectrum noise, but the lower frequencies contain the highest amplitudes (see power spectrum in Figure 3A). Therefore, higher frequency sounds, like the *S. orejuela* call, experience less interference. A recent review found the mean peak frequency across 72 glass frog species to be 4.88 kHz (range: 2.71–7.41 kHz) (Sulbarán et al., 2019); the peak frequency of the *S. orejuela* call is above this mean by at least 2 kHz, and in many cases higher than the upper limit of the peak frequency range in other species (e.g., 7.688 kHz).

As a comparison, the call of a sympatric glassfrog species, *Espadarana prosoblepon*, has a peak frequency (i.e., the frequency with the highest amplitude) of 5.758 ± 0.232 kHz (Jacobson, 1985), while *S. orejuela* has a peak frequency well above 7 kHz (both vocalize in Audio 2). This difference is especially interesting given that *S. orejuela* is larger (mean SVL = 31.35 ± 1.68 mm; mean mass = 2.52 ± 0.49 g; $N = 9$) than *E. prosoblepon* (mean SVL = 24.77 ± 1.84 mm; mean mass = 0.844 ± 0.22 g; $N = 60$), based on individuals we caught during the same field season. In bioacoustics, an individual’s size is typically inversely correlated with the peak frequency of its call (Wilkins et al., 2013), meaning that larger species tend

to make sounds with lower frequencies — yet *S. orejuela* calls with a much higher frequency than the smaller *E. prosoblepon*. This suggests that *S. orejuela* may have evolved higher-pitched calls in order to communicate near loud waterfalls, thus being able to occupy an otherwise inaccessible acoustic niche. *E. prosoblepon* calls much farther away above stream sections where lower frequencies can propagate with less attenuation.

Information is more likely to reach its intended receiver(s) when paired with multiple modes of signalling. We observed two *S. orejuela* individuals calling, bobbing, and limb-flagging at night, >6.5 metres away from one another (and on different sides of the waterfall); no other conspecifics were visible or audible. Descriptions of nocturnal visual signalling have been limited to situations in which conspecifics are in close proximity. For example, species in the family Hylidae (treefrogs) wave their limbs reciprocally during courtship (Hartmann et al., 2004). In Centrolenidae (glassfrogs), the only records of nocturnal visual signals — rocking back and forth (*Hyalinobatrachium fleishchmanni*; McDiarmid & Adler, 1974) or limb-lifting (*Vitreorana uranoscopa*; Hartmann et al., 2005) — involve direct territorial challenges. However, our observations of visual signals in *S. orejuela* bear greater resemblance to diurnal foot-flagging displays, which generally serve as a territorial spacing mechanism (Preininger et al., 2009) in the presence of acoustic interference — not as a directly antagonistic or aggressive response to an encroaching male. If further research confirms this function, our observations add Centrolenidae to the short list of anuran families that have evolutionarily converged to counter background noise with visual signals.

Non-antagonistic visual displays are rarely observed at night. Since they communicate by waterfalls, which generally have less canopy cover, it is possible that *S. orejuela* relies on moonlit evenings for visual displays (e.g., Grant et al., 2009). However, recent experiments have demonstrated that frogs are able to distinguish blue from green light in almost complete darkness (Yovanovich et al., 2017), confirming that anuran vision functions remarkably well in the dark. Our observations emphasize the need for further research on long-range visual display systems, particularly at night. Similar behaviours may have been overlooked in other nocturnal species inhabiting disruptive acoustic spaces.

In addition to bobbing and limb-waving, vocal sac inflation is considered a visual signal in anuran communication (Wells, 2010). For example, fe-

males of *Engystomops pustulosus* prefer the coupling of call and vocal sac inflation to the same sound stimulus without the visual vocal sac component (Rosenthal et al., 2004). *Sachatamia orejuela* has paired vocal sacs (Figure 3B) — the first record of such in a glassfrog. This modification may have evolved to maintain its visual role in intraspecific communication, as the lateral extension of each vocal sac likely enhances visibility from multiple angles (e.g., directly above the calling male). If this is the case, it stands to reason that other behaviours evolved to enhance signal effectiveness as well.

Although it is not clear why the observed males did not maintain the flagging signals over the entire duration of our observations, it is likely related to energy expenditure and/or predation avoidance. Calling is already an energetically expensive endeavour for male frogs. Presumably the addition of visual signals presents even more of an energy trade-off, and is thus not sustainable to maintain for long periods of time. It is also possible that the observed male began to incorporate visual signals in response to the communication efforts of the other calling male nearby. Presumably visual cues also increase predation risk, although their habitats are incredibly slippery and hard to access; waterfall spray may even disrupt bat echolocation (e.g., Halfwerk et al., 2014).

Future studies should attempt to address whether these behaviours constitute true multimodal signalling by testing their communicative relevance in intra-specific interactions (Feng et al., 2006; Hödl & Amezcuita, 2001). It is possible that the behaviours we observed do not elicit a behavioural or physiological response in females or competing males; although some studies have found this to be the case in certain displays of presumed aggression (Furtado et al., 2019), the flagging behaviours of *S. orejuela* did not appear to occur in close proximity to another male. Further work on this species should also investigate whether its calls contain harmonics in ultrasonic frequencies by recording with a sample rate higher than 44.1 kHz.

These observations inspire further study into how the acoustic environment affects the evolution of multimodal signalling. Increasing our understanding of how species overcome signal interference through other modes of communication may inform future studies of adaptation and conservation.

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Supplementary material

Video 1. Representative clips of the acoustic and visual signals (head bobbing, hand-waving and foot-flagging) of *Sachatamia orejuela*. See 10.6084/m9.figshare.13160012.

Video 2. Same footage as Video 1, but at 50% speed. See 10.6084/m9.figshare.13160012.

Video 3. Full-length, unedited recordings of *Sachatamia orejuela* visual and acoustic signalling. Note that this includes many times when the camera is shaky or goes out of focus; the only accessible viewing spot for the *Sachatamia orejuela* display was a small, slippery rock ledge that required RMB to balance on one foot while filming. See 10.6084/m9.figshare.13160012.

Audio 1. Advertisement call of *Sachatamia orejuela* (10.6084/m9.figshare.13160012). Single-note calls occur at approximately 0:01, 0:45, 1:02, 1:30, and 1:42. An example of its double-note call occurs at 2:03.

Audio 2. Single-note advertisement call of *Sachatamia orejuela* (at approximately 0:09), followed by the characteristic double ‘click’ call of *Espadarana prosoblepon* (at approximately 0:15), a sympatric glassfrog species (10.6084/m9.figshare.13160012).