



Subterranean advertisement and duet calling behavior in *Ptychohyla legleri* (Legler's stream frog)

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Anurans use a wide variety of vocalizations to advertise and communicate with conspecifics, and the context in which they call can have significant implications on individual fitness. *Ptychohyla legleri* (Taylor, 1958) is an endangered frog for which little is known about natural history and reproductive ecology. Here we report novel observations of male *P. legleri* that were calling in synchronized pairs from concealed subterranean cavities and retreat sites along streams, advertisement behavior that we believe is previously unknown for the species. Because these calling sites resemble oviposition sites beneath waterfalls observed in captivity, concealed calling sites with flowing water may provide quality oviposition sites for females. We hypothesize that duet advertisement from concealed terrestrial sites may afford males protection from predation and/or provide amplification of outgoing calls and incoming external sounds. Future studies should examine the functional significance of duet calling from concealed locations of *P. legleri* to better understand the life history of this imperiled species.

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Anurans (frogs) have evolved a diverse repertoire of vocalizations that are used to communicate with conspecifics and promote fitness (Wells 2010). A recent review of frog vocalizations (calls) classified their calls into three categories: reproductive calls for breeding, aggressive calls to facilitate territorial interactions and defensive calls to prevent predation (Toledo *et al.* 2014). In this paper, we describe novel reproductive calling behavior for an endangered treefrog, *Ptychohyla legleri* (Taylor, 1958), in Costa Rica.

Ptychohyla legleri (Legler's Stream Frog) is a moderately sized nocturnal red-eyed treefrog that occurs near small streams in premontane wet forest on the Pacific versant of southern Costa Rica and western Panama (Duellman 2001; Savage 2002). Males are thought to advertise to females for reproduction between the months of February and July by calling from vegetation above flowing water, but males have occasionally been found advertising from rocks in streams (Duellman 2001; Savage 2002). While amplexus and oviposition behaviors are unknown for the species in the wild (Savage 2002), observations in captivity have found males to be territorial and defend small egg laying cavities under an artificial waterfall, where eggs were laid below the waterline (Proy 1993). Other than observations of wild calling males and reproduction in captivity, nothing is known about the natural history of *P. legleri*. This knowledge gap is particularly important because populations of *Ptychohyla legleri* declined precipitously after the emergence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Central America (Whitfield *et al.* 2017), and the species is listed as Endangered by the International Union for Conservation of Nature (IUCN 2014).

On 10 October 2017, we observed novel behavior of *P. legleri* at a stream at Las Cruces Biological Station, Costa Rica (ca. 8.786, -82.959) that might provide clues to the behavioral and reproductive ecology of the species. The weather was overcast and humid with no precipitation, but the site had received considerable rainfall in the days prior due to a significant tropical storm (Hurricane Nate). At 16 h 15, we heard two individuals calling from a short seepage down a vertical bank of the stream. Upon closer examination, the individuals were situated one each within two separate cavities in the soil along the stream bank. The cavities were elevated 30–50 cm above the stream with diameters of ca. 3–4 cm each and were located ca. 15 cm apart from each other. Water coursed down the stream bank around both entrances but was also present within the cavities and actively flowing from within at least one of them. The individuals were perched 3–4 cm within the cavities and were half-submerged in the water (e.g., fig. 1A). Upon shining a flashlight into each cavity, the individuals both stopped calling temporarily and retreated beneath the surface of the water. After a few minutes without exposure to a flashlight, they re-emerged from the water and resumed calling from within the cavities with the typical advertisement call (Duellman 2001; Savage 2002). The individuals chorused together, with one individual leading and the second calling in response to the first (hereafter, duetting). Shortly thereafter, we located a third individual ca. 15 m upstream that was calling from within leaf litter adjacent to the stream. A fourth individual was also calling here near and in duet synchrony with the third, but it eluded visual detection. We video recorded the first individual calling from within its cavity and audio recorded the duetting calls of the first two individuals using an iPhone 6 (Apple Inc.); video and audio files are available on the Figshare Data Repository (<https://doi.org/10.6084/m9.figshare.11797191.v1>).

We returned after dark at 19 h 00 and again observed the three individuals from the afternoon (the first two still in the same cavities; the third calling from leaf litter in the same position as before). We then captured three additional individuals. First, we located a male that we presumed was the undetected fourth individual from the afternoon; he was calling from among gravel beneath a rock (fig. 1B). The final two individuals were found ca. 15 m upstream from the third and fourth; these were calling together from underneath two rocks adjacent to the stream and were situated within small puddles with leaf-litter and twig organic detritus (fig. 1C, D). Our cumulative observations at the field site, while limited, indicate that male *P. legleri* advertise in pairs with synchronized calling behavior and that duetting pairs are uniformly spaced at distances of ca. 15 m from neighboring pairs.

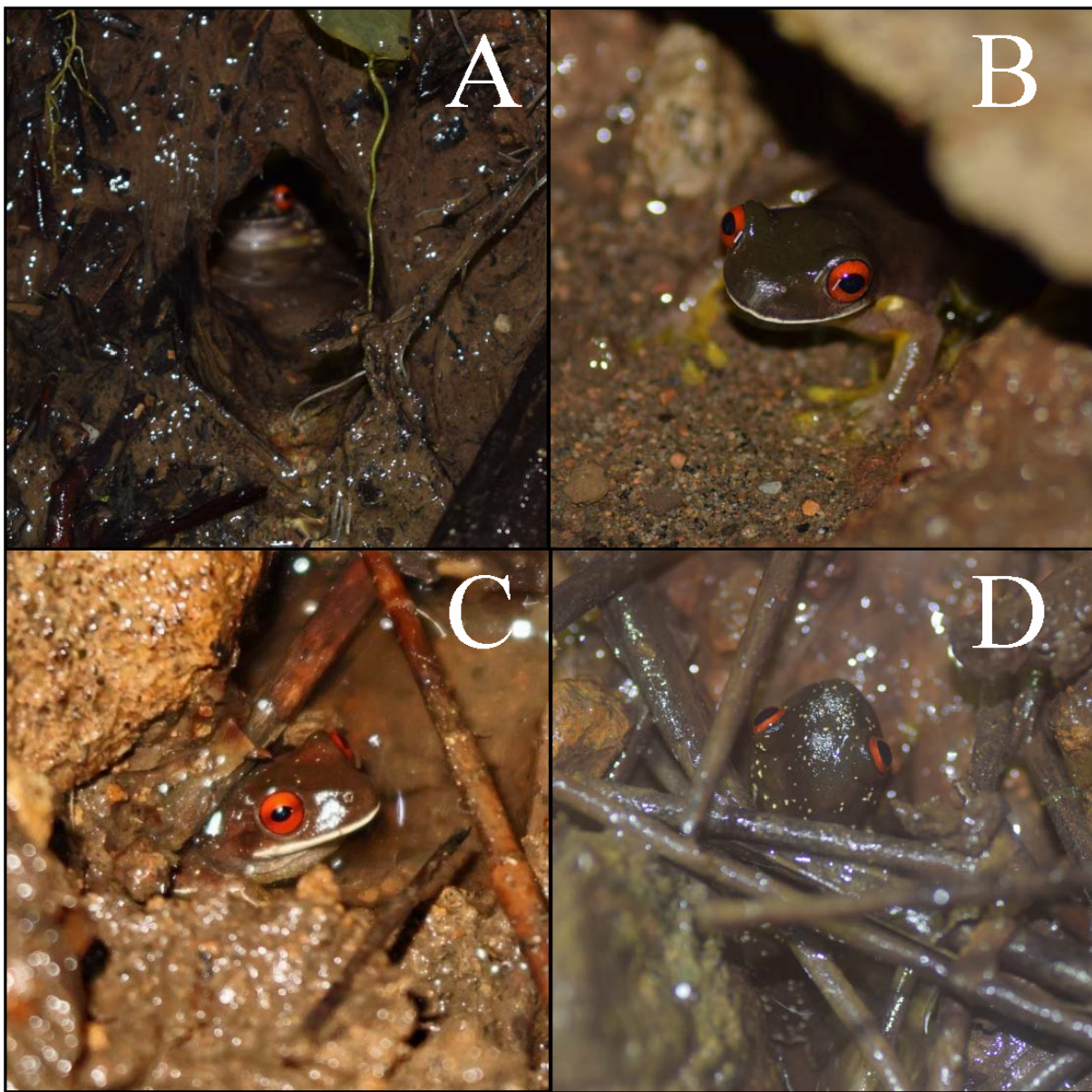


Figure 1. Male *Ptychohyla legleri* ($N = 4$) that were observed calling from (A) within a burrow, (B) beneath a rock, and (C, D) beneath a clay-twig structures in a first-order stream at Las Cruces Biological Station, Costa Rica.

We suggest that our observations of subterranean calling sites and duetting behavior represent novel and undescribed calling behavior for *P. legleri* that may provide insight about the species' reproductive biology. We observed individuals vocalizing in duets from within hidden calling sites such as subterranean cavities and retreat sites beneath rocks. It is unclear whether individuals constructed the cavities themselves (i.e., the cavities were burrowed by the frogs) or if the frogs used pre-existing cavities that formed naturally or were created by other animals. We also noted site fidelity across our two visits to the study site. While there is no literature describing territoriality of *P. legleri* in the field, the species has been noted to exhibit territorial behavior in captivity (Proy 1993), and males may be intolerant of intruders (i.e., territorial) if calling sites are quality resources for reproduction (e.g., Tsuji & Matsui 2002). While it is not uncommon for primarily nocturnal frogs to perform advertising calls diurnally during wet conditions, the use of subterranean calling sites and duetting behavior have not previously been reported for *P. legleri* to our knowledge.

Duet calling from covered terrestrial locations such as subterranean burrows may provide several benefits to individuals. For one, the subterranean calling sites observed resemble oviposition sites used by individuals in captivity (Proy 1993); wild males might also call from closed, concealed, and wet locations such as subterranean cavities or beneath rocks because these sites may be most suitable for oviposition. Calling from closed locations (i.e., cavities) could also help individuals monitor the neighboring environment by amplifying advertisement acoustics in two directions: first, by more effectively broadcasting the caller's advertisement to potential mates, and second, by amplifying external sounds from neighboring conspecifics and/or heterospecifics to a receiver (Muñoz & Penna 2016). In addition, duet calling from terrestrial or subterranean sites may help reduce predation pressure in two ways. First, calling from covered locations may provide concealment and physical protection from potential predators, such as bats, which would have more trouble collecting frogs from covered perches. Second, duet calling may decrease predation risk because it is more difficult for bat predators to detect prey frogs when frogs call synchronously (Tuttle & Ryan 1982).

In summary, we observed male *P. legleri* to advertise for females by calling in duets from concealed terrestrial locations that were uniformly spaced through the habitat. Future studies should examine the functional significance of duet calling and/or calling from concealed locations to improve our understanding of the natural history and reproductive biology of this imperiled species.

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