



# Advertisement calls of six glassfrog species in the Colombian Andes, and comments on priorities for future research and conservation

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**Abstract.**—Conservation initiatives are particularly important in light of the high proportion of species threatened with extinction worldwide. Advertisement calls are a valuable resource for conservation given their importance for studies on amphibian taxonomy, systematics, evolutionary biology, ecology, and monitoring strategies. However, advertisement calls are still unknown for many species. Here, advertisement call descriptions are provided for six glassfrog species (*Centrolene huilensis*, *Centrolene hybrida*, *Nymphargus orenonympha*, *Rulyrana flavopunctata*, *Rulyrana susatamai*, and *Sachatamia punctulata*) from six localities in four municipalities in the Andes of Colombia. Based on our current knowledge of the acoustics, distribution ranges, presence in protected areas, and IUCN Red List threat status, some species of glassfrogs are identified as priorities for future studies. The overall parameters measured for the advertisement calls of the species described here fall within those known for other species from the same genus, but the species studied here are differentiated from their congeners by the pulse rate, number of notes, and/or dominant frequency. To date, advertisement calls are known for approximately 60% of glassfrog species and we identified 23 priority species with unknown calls which should be the focus of future research efforts.

**Keywords.** Anura, bioacoustics, Centrolenidae, South America, threatened species, taxonomy

**Resumen.**—Las iniciativas de conservación son especialmente importantes teniendo en cuenta la elevada proporción de especies amenazadas de extinción en todo el mundo. Dada su importancia para los estudios sobre taxonomía, sistemática, biología evolutiva, ecología y estrategias de seguimiento de los anfibios, los cantos de anuncio son un recurso valioso para la conservación. Sin embargo, los cantos de anuncio siguen siendo desconocidos para muchas especies. En este trabajo se describen los cantos de anuncio de seis especies de ranas de cristal (*Centrolene huilensis*, *Centrolene hybrida*, *Nymphargus orenonympha*, *Rulyrana flavopunctata*, *Rulyrana susatamai* y *Sachatamia punctulata*) de seis localidades en cuatro municipios de los Andes de Colombia. A partir de los conocimientos sobre acústica, áreas de distribución, presencia en áreas protegidas y estado de amenaza (Listas Rojas de la UICN), se identificaron algunas especies de ranas de cristal como prioritarias para futuros estudios. Los parámetros generales medidos en los cantos de anuncio de las especies descritos están dentro de los conocidos para otras especies del mismo género, pero las especies se diferencian de sus congéneres por la frecuencia de los pulsos, el número de notas o la frecuencia dominante. Hasta la fecha, se conocen los cantos de anuncio de aproximadamente el 60% de las especies de ranas de cristal y hemos identificado 23 especies prioritarias con llamadas desconocidas hacia las que sugerimos dirigir los esfuerzos de investigación.

**Palabras clave.** América del Sur, Anura, bioacústica, Centrolenidae, especies amenazadas, taxonomía

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## Introduction

Amphibians provide valuable ecosystem services in aquatic and terrestrial environments acting in disease control, pest control, and nutrient cycling (Valencia-Aguilar et al. 2013), as well as providing an important element of terrestrial and aquatic food webs (Zipkin et al. 2020). For instance, the tadpoles of some glassfrog species are considered important for nutrient cycling in aquatic ecosystems by stimulating the fungal activity in the leaf litter (Connelly et al. 2011). Therefore, the absence of these species can alter the structures and functions of the ecosystems (Whiles et al. 2006, 2013). At the same time, amphibians are the most threatened group of vertebrates worldwide, being affected by habitat destruction, climate change, and epidemic diseases (Lips et al. 2005; Loyola et al. 2012; Pounds et al. 2006; Wake and Vredenburg 2008). Habitat fragmentation threatens 89% of Neotropical amphibians through population isolation, inbreeding, and edge effects (Stuart et al. 2008), and the proportions of declined species which exhibit recovery, stabilize at lower abundance, or continue to decline remain unknown (Scheele et al. 2019).

Considering that economic resources directed towards conservation are scarce, these efforts must be channeled towards the most vulnerable species (Barret et al. 2014). Multiple approaches have been employed to identify conservation priorities, including selection by species range, phylogenetic endemism, or coverage by protected areas (Mendoza and Arita 2014). Protected areas mitigate threats acting upon anuran populations, mainly by decreasing the deforestation due to land-use changes. Additionally, protected areas also facilitate scientific research by allowing investigators to perform long-term studies on the biology and ecology of the key species (Brooks et al. 2004; Guerra et al. 2018; Guayasamin et al. 2020; Lips et al. 2005; Loyola et al. 2012).

Detailed descriptions of advertisement calls are a valuable resource among the multidisciplinary approaches for clarifying taxonomic limits (e.g., Padial and De La Riva 2009; Köhler et al. 2017), and for studies of behavior, reproductive ecology, and evolution (Wells 2007). Advertisement calls are also an important resource for the effective conservation assessment, planning, and management of threatened species (Laiolo 2010; Sanchez-Giraldo et al. 2020). However, despite increasing efforts in the description of frog calls in neotropical species-rich countries (Hutter et al. 2014; Guayasamin et al. 2020; Viuche-Lozano et al. 2018), information on the advertisement calls is still lacking for many species (Guerra et al. 2018; Rivera-Correa et al. 2021).

The glassfrogs (family Centrolenidae) are a significant component of the neotropical amphibian communities in rivers and streams. Glassfrogs comprise a highly diverse group, currently with 12 genera and 156 species (Guayasamin et al. 2020; Frost 2021). The northern Andes in Colombia, Ecuador, Peru, and Venezuela have very high

species richness and endemism rates (Guayasamin et al. 2020; Ruiz-Carranza and Lynch 1991a; Twomey et al. 2014). Glassfrogs include riparian frogs found from sea level in tropical rainforests to 3,300 m asl in the Andean Páramos, where most species call to attract females, to display territoriality, and as part of parental care behavior (Cisneros-Heredia and McDiarmid 2007; Delia et al. 2014; Bravo-Valencia and Delia 2016; Delia et al. 2017). Nevertheless, the vocalizations of most glassfrogs remain largely unknown and are limited to approximately 92 of the 156 species (or about 59%).

The large diversity in this region is threatened by human activities such as mining, exploitation of natural resources, and land transformation, all of which have significant consequences on water quality and, therefore, on the associated diversity. More than one-third of the 156 glassfrog species are in one of the risk categories of the International Union for Conservation of Nature, including eight Critically Endangered, 21 Endangered, and 23 Vulnerable, with another 53 in the Data Deficient category (IUCN 2010). Unfortunately, the areas with higher diversity and endemism are not effectively covered within established protected areas (Mendoza and Arita 2014).

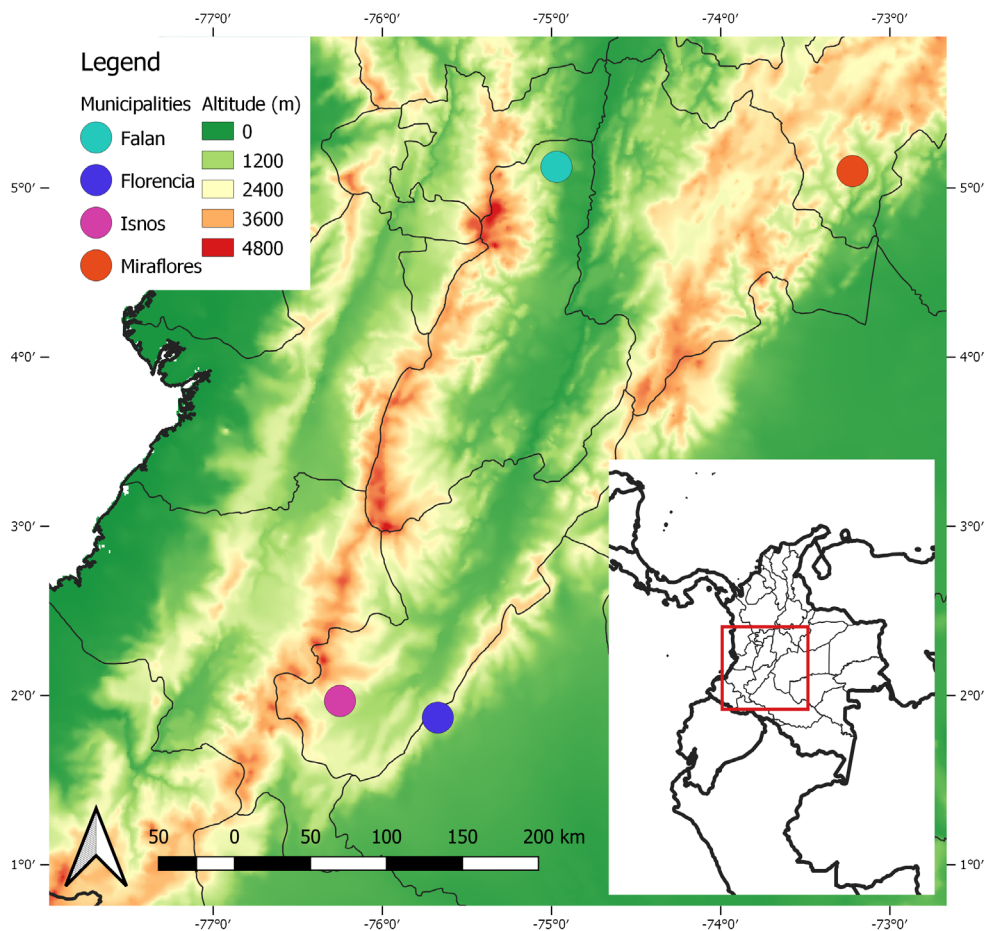
Considering the need for correctly identify glassfrog species, especially distinguishing those that are sympatric or morphologically similar, here the advertisement calls of four glassfrog species in three genera from the Colombian Andes are described for the first time, and call information for two *Rulyrana* species from additional localities are provided. Finally, considering that the description of the advertisement calls is necessary for conducting field research through active and passive searches, and that research and conservation efforts must be channeled towards the most vulnerable species, we also provide a comprehensive evaluation of the current status of glassfrog call descriptions regarding the species conservation status to identify the priority species for future studies.

## Materials and Methods

### Collection Sites

Nocturnal fieldwork occurred in April and May 2018 in the Andes of Colombia (i.e., Central and Eastern cordilleras; Fig. 1). Advertisement calls of six species were recorded from six localities in four municipalities: (1) *Centrolene hybrida*: Miraflores municipality in Boyacá department, at 1 km N of the finca el Vergel, 38 km, by road to ENE Garagoa (5.10089, -73.22038; 1,947 m; 27–29 April 2018); (2) *Centrolene huilensis*: Isnos municipality in Huila department at 5 km at NW of Istos in the road to Popayán (1.96838, -76.25021; 1,988 m; 4–5 May 2018); (3) *Nymphargus oreonympha*: Alto Gabinete, La Ruidosa stream, in Florencia municipality in Caquetá department, road Florencia-Altamira (1.86936, -75.67183; 2,073 m, 6–7 May 2018); (4) *Rulyrana flavopunctata*: Sucre

## Advertisement calls of six glassfrog species



**Fig. 1.** Fieldwork localities in the Central and Eastern Cordilleras where the advertisement calls were obtained.

sidewalk, Florencia municipality in Caqueta department, road Florencia-Altamira (1.86936, -75.67183; 1,134 m; 8 May 2018); (5) *Rulyrana susatamai*: Falan municipality, Murillo stream in Piedecuesta, Tolima department (5.1258, -74.97052; 1,133 m; 23–25 April 2018); and (6) *Sachatamia punctulata*: Falan municipality, Cuamo River at 10 km southwards from Falan head, in Tolima department (5.1258, -74.97052; 441 m; 23–25 April 2018).

At each field site, free surveys (not time or effort restricted) were conducted along the rivers and streams. At least ten calls per individual were recorded in WAV format with a digital recorder (Tascam DR-40) and a unidirectional microphone (Sennheiser K6/ME 66) at a sampling rate of 44.1 kHz and an amplitude resolution of 16 bits. Species identities were verified by comparison of morphological characters with the species diagnoses provided in Ruiz-Carranza and Lynch (1991 b,c; 1995a,b). Microhabitat details were recorded, such as calling perch height, perch type, and substrate. Surface temperatures of either male frogs or the substrate from which a male frog recently jumped were recorded using an infrared digital thermometer (Benetech GM300, resolution 0.1 °C). Snout-vent length (SVL) of calling males was measured with a digital caliper. Voucher specimens were fixed with 10% formalin. Field numbers of Angela M. Mendoza-Henao (AMMH) are reported for those specimens

already deposited in the Instituto de Ciencias Naturales (ICN), but not yet allocated museum numbers. Note that due to the COVID-19 pandemic, the ICN is shut down and numbers for AMMH specimens mentioned in the manuscript are currently not available. After allocation of museum numbers to these specimens, they can be requested from the corresponding author. Recordings used for call descriptions were deposited (in WAV format) in the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt (IAvH-CSA-34233 to 34250).

Calls were analyzed using the software Raven Pro 1.4 (<http://ravensoundsoftware.com/>) with Blackman window type, window size of 5 ms, 80% overlap, and DFT size of 1,024 samples to obtain the dominant frequency (in Hz). In cases of multiple notes per call, the inter-note silent interval was measured and for pulsed calls the pulse rate was determined. Temporal parameters were measured from the waveform (in ms). The sampling unit for the descriptive analysis was the individual. Call figures of the spectrograms were generated using Seewave v. 1.6 package (Sueur et al. 2008) in the R platform (version 3.4.3; R Development Core Team 2014). The call terminology of Köhler et al. (2017) was followed to categorize vocalization types, temporal variables, and spectral variables. In that sense, we followed the note-centered approach, and pulsed notes were defined as those notes composed by multiple short

(less than 50 ms) undividable sound units spaced with silent intervals within the note.

### Status of Glassfrogs Call Descriptions

To compile the information available in published studies (peer-reviewed papers and books) for described advertisement calls, online databases (AmphibiaWeb, BioWeb Ecuador) were searched for call information, and the Google Scholar search engine was queried with the terms “advertisement call,” “glassfrog,” “glass frog,” and “Centrolenidae.” Included here are the species calls described in the sources identified, and those from an ongoing study (Duarte-Marin et al., submitted). For each species, the information on level of threat was obtained based on their IUCN (2019) conservation status. Because many species are either classified as Data Deficient or simply Non-Evaluated, we complemented the IUCN information with two additional parameters in our comparisons: level of endemism and occurrence in protected areas.

For the level of endemism, the range per species was obtained and four categories were defined: Local, covering less than 1,500 km<sup>2</sup> of distribution at 1–5 locations in 1–2 countries; Sub-regional, area 1,501–35,000 km<sup>2</sup> in 1–3 countries without entirely covering a whole recognized geographical region (e.g., Chocó, Northern Andes); Regional, area 35,001–200,000 km<sup>2</sup>, distributed in a large part of a country, in more than two countries, and distributed in a large part or totality of a geographical region; Global: covering more than 200,000 km<sup>2</sup>, distributed in a whole region or several geographical regions. For occurrence in

protected areas, the species ranges were compared with the polygons of the protected areas of all categories included in the World Database of Protected Areas, WDPAs (IUCN/UNEP 2010). Using Spatial Analysis tools in the ArcGIS software (ESRI 2007), the proportion of each species range contained in a protected area was determined. These values were then grouped by the criteria of Rodrigues et al. (2004): a species with a range smaller than 1,000 km<sup>2</sup> should have 100% of its range within protected areas; and a species with a range larger than 250,000 km<sup>2</sup> should have at least 10% of its range within protected areas. For species with intermediate range sizes, the critical percentage was calculated by linear interpolation from these two extremes.

The results of the three criteria (threat category, endemism, and occurrence in protected areas) were combined for each species and priority species were defined as those with local ranges (i.e., less than five known locations in less than 1,500 km<sup>2</sup>), not covered by protected areas, and in either the Critical, Endangered, Data Deficient or Non-Evaluated categories of IUCN.

### Results

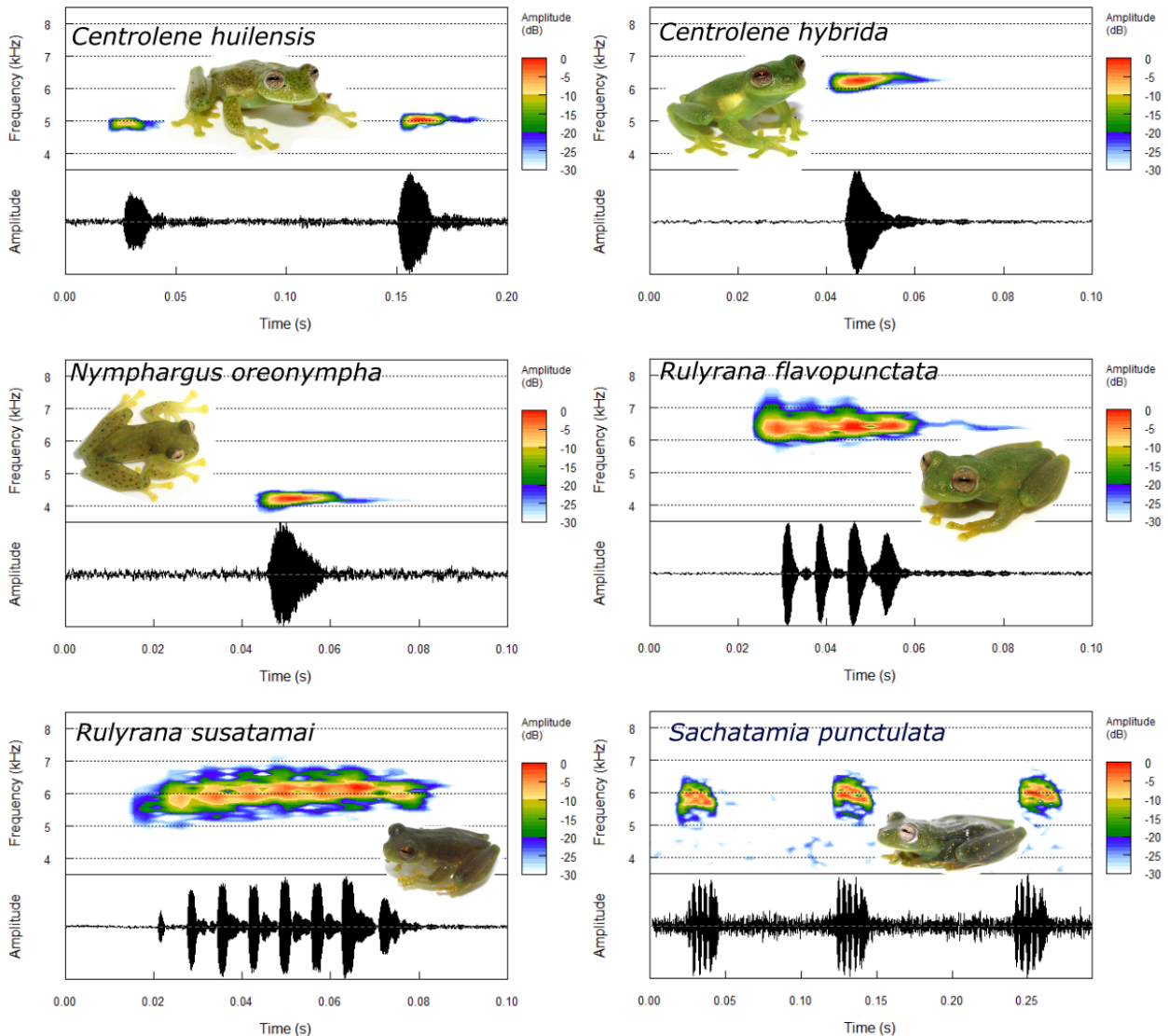
In total, 250 advertisement calls were analyzed for 19 individuals of six glassfrog species. Detailed information for the voucher code, temperature at the time of recording, number of individuals recorded, mean snout-vent length (SVL), air temperature, and measured call traits for each individual is provided in Table 1. Values in the following call descriptions are given as mean ± standard deviation. Spectrograms and oscillograms of the advertisement call

**Table 1.** Call features for the six glassfrog species described in this study. N = number of analyzed calls (recorded males). Values are mean ± SD. The call recordings from collected and non-collected specimens were deposited in the Colección de Sonidos Ambientales Mauricio Álvarez-Rebolledo.

	<i>Centrolene huilensis</i>	<i>Centrolene hybrida</i>	<i>Nymphargus oreonympha</i>	<i>Rulyrana flavopunctata</i>	<i>Rulyrana susatamai</i>	<i>Sachatamia punctulata</i>
N: calls (males)	58 (5)	40 (4)	60 (3)	18 (1)	34 (3)	40 (3)
Mean Snout-Vent Length (mm)	27.0	21.5	24.5	19.8	22.8	27.8
Collector field numbers	AMMH 174–178	AMMH 168–170	AMMH 181–183	AMMH 186	AMMH 162–164	AMMH 165–167
Call vouchers	IaVH-CSA 34233–34237	IaVH-CSA 34328–34340	IaVH-CSA 34241–34242	IaVH-CSA 34244	IaVH-CSA 34245–34247	IaVH-CSA 34248–34250
Temperature (°C)	14.7	12.5	12.6	17.0	19.5	23.5
Dominant frequency (Hz)	4,785.5 ± 336.4 (first note), 4,940.2 ± 266.3 (second note)	6,072.9 ± 106.0	4,085.0 ± 134.55	6,361.9 ± 35.6	5,967.2 ± 163.6	5,611.7 ± 213.9
Call duration (ms)	142.8 ± 4.41	227.8 ± 61.22	14.3 ± 2.2	29.7 ± 3.1	58.7 ± 6.58	183.2 ± 155.6
Number of notes	1 or 2 (1.9 ± 0.31)	1 to 5 (1.2 ± 0.68)	1	1	1	1 to 5 (2.35 ± 1.14)
Note duration (ms)	28.8 ± 1.06	19.9 ± 9.7	14.3 ± 2.2	29.7 ± 3.1	58.7 ± 6.58	17.6 ± 4.2
Internote interval (ms)	97.5 ± 12.8	1,322.3 ± 87.6	—	—	—	97.2 ± 16.1
Call type	Tonal	Tonal	Tonal	Pulsar	Pulsar	Pulsatic-harmonic
Pulses per note	—	—	—	4.06 ± 0.54	8.41 ± 0.82	3.74 ± 1.62



## Advertisement calls of six glassfrog species



**Fig. 2.** Spectrograms (top) and oscillograms (bottom) of the described glassfrogs advertisement calls. All calls are displayed at Blackman window (length = 512) and 80% overlap, at the same frequency range, and all but *S. punctulata* and *C. huihensis* are displayed at the same temporal scale.

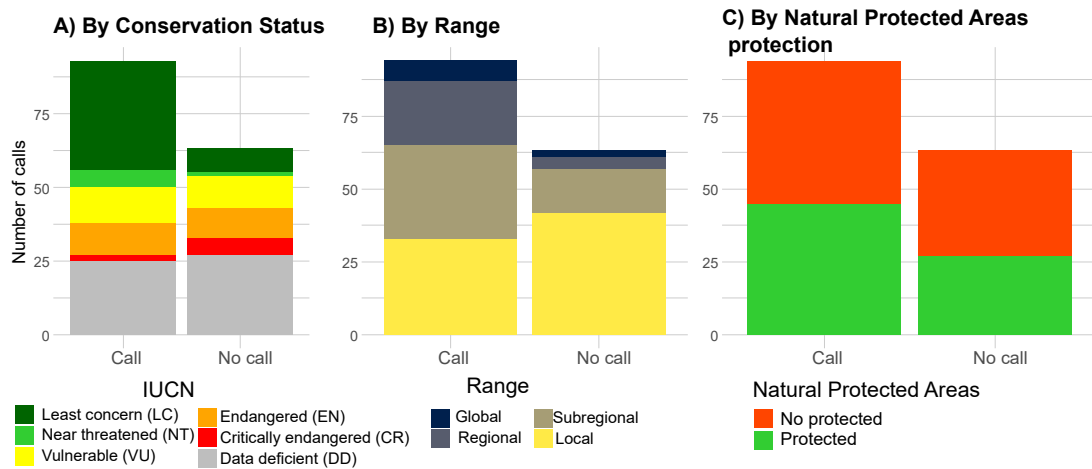
of each species are provided in Fig. 2.

*Centrolene huihensis*. Individuals were found calling from the upper side of leaves at 1.5 to 4 m above the water. Calls were obtained between 2300 h and 0130 h on clear nights with a full moon. The advertisement call is composed of one or two single-toned notes similar to a “Tic” to the human ear. The first note has a duration of  $27.63 \pm 11.37$  ms and a peak frequency of  $4,785.5 \pm 336.42$  Hz while the second one has a duration of  $30.9 \pm 11.85$  ms and a peak frequency of  $4,940.2 \pm 266.34$  Hz (Fig. 2). Both notes have limited amplitude modulation with the highest energy located near the middle of the note. The notes are separated by a silent interval of  $9.75 \pm 1.28$  ms.

*Centrolene hybrida*. Individuals call from the upper surfaces of leaves from 20 cm to 5 m above the water. Advertisement calls were obtained between 2000 h and 2350 h on clear nights in vegetation above small and medium-sized streams located on the sides of cattle-

cleared areas. The call is a toned note similar to a “Tic” to the human ear. The whole note lasted  $19.9 \pm 9.7$  ms and had a dominant frequency of  $6,072.9 \pm 106.0$  Hz (Fig. 2). In most cases, the call was composed by a single note, but in a few instances, individuals produced calls with up to five notes after a silent interval of  $1,322.3 \pm 87.6$  ms. The highest energy is displayed at the first third of the note.

*Nymphargus oreonympha*. Individuals were recorded calling at heights of up to 4 m in leaves and branches above the water in a small stream between 2200 h and 0115 h, with some males near egg depositions. Calls were obtained on nights following a heavy afternoon rain. The call is a single-toned short note ( $14.3 \pm 22.1$  ms) similar to a “Tic” to the human ear. The dominant frequency was  $3,995.9 \pm 37.8$  Hz, and low in comparison with the other calls described here. The notes have limited amplitude modulation with the highest energy located near the middle of the note. One individual showed a call with a



**Fig. 3.** Counts of glassfrog species for those with and without the call described, and categorized by: **(A)** IUCN conservation status categories; **(B)** Endemism or distribution range (see details in **Materials and Methods**); and **(C)** Occurrence in National Protected Areas.

slightly higher dominant frequency ( $4,263.3 \pm 55.8$  Hz) after a confrontation with another male. After the combat, the male emitted the call at 20 cm above the water on a branch and the second individual left the branch.

*Rulyrana flavopunctata*. The specimen calling was recorded at 0226 h from the upper side of a large Araceae-like leaf at 1 m above the water. Several males of the species were observed calling together with other individuals in the background. The call is a single-trilled note similar to a “Trii” to the human ear, lasting  $29.7 \pm 3.1$  ms, with 3–5 pulses (mean 4.06) at a rate of 137.9 pulses/s, and the dominant frequency was  $6,361.8 \pm 35.6$  Hz with no amplitude modulation (Fig. 2).

*Rulyrana susatamai*. The specimens were calling from 2000–0340 h during nights with light rain. Individuals of *R. susatamai* were calling from the tips of the upper sides of leaves, above small streams at heights of around 2–5 m. The call is a single-trilled note similar to a “Trii” to the human ear, lasting  $58.7 \pm 6.58$  ms with a dominant frequency of  $5,967.2 \pm 163.6$  Hz (Fig. 2). The note comprises 7–10 pulses at a rate of 143.3 pulses/s whose energy increases slightly along the note.

*Sachatamia punctulata*. The calls were emitted from rocks and on vegetation up to 4 m high between 0100 and 0300 h. After heavy rains, multiple amplexant individuals were also observed. The call is a series of pulsatic-harmonic notes (1–5 notes) of  $17.6 \pm 4.2$  ms, with an internote interval of  $97.2 \pm 16.1$  ms and a dominant frequency of  $5,611.7 \pm 213.9$  Hz with no amplitude modulation detected (Fig. 2). It is similar to a “Trii” to the human ear. Each note shows up to  $3.74 \pm 1.62$  pulses at a rate of 220 pulses/s.

### Status of Glassfrog Call Descriptions

Including the advertisement calls of the species described here, the number of species with advertisement call descriptions increases to 92 (59.3% of all species of glassfrogs, see Appendix 1). Regarding the species

by conservation status (Fig. 3A), 48 of the 55 of non-threatened species (Least Concern and Not Evaluated) have their calls described, while 30 of the 95 threatened species (Vulnerable, Endangered, Critically Endangered) have their calls described. Regarding the distribution categories (Fig. 3B), the call is known for 63 of the 82 species that have Sub-regional, Regional, and Global distributions; whereas only 37 of the 75 species with a Local distribution have their calls described. Fifty of the species with their calls described are effectively covered by Natural Protected Areas while the call is unknown for 35 species restricted to outside of Natural Protected Areas (Fig. 3C). By combining all these criteria, a total of 23 species whose call is undescribed are identified as priorities for bioacoustics and conservation research (Appendix 2).

### Discussion

The detailed descriptions of the advertisement calls for the anurans of highly diverse regions, like the northern Andes, are a valuable resource for studies in taxonomy, biodiversity monitoring, and various ecological and evolutionary aspects of anurans. Here, the descriptions of the advertisement call of six Andean glassfrog species are provided, and the call parameters are compared with those from other species in the same genus. The species which either do or do not have their calls described are assessed regarding their conservation status and the implications and challenges of the current species coverage are discussed in terms of ecology and conservation.

### Call Comparisons among Species

The overall structures of the advertisement calls of the species included in this study coincide with those known for other glassfrogs in terms of having short notes (less than 200 ms) of relatively high frequencies (over 3,000 Hz), but the specific calls can be differentiated among congeneric species. For instance, the advertisement call of *S.*

*punctulata* differs from the calls described for *Sachatamia illex* and *Sachatamia albomaculata* by having a set of multiple notes (1–5), while those of the latter species are composed only by a single note (Guayasamin et al. 2020; Kubicki 2007). The call of *N. oreonympha* has a frequency of  $4,085.0 \pm 134.55$  Hz, similar to most calls described for *Nymphargus* species (only *N. siren* has a frequency over 5,000 Hz, Guayasamin et al. 2020), however the *N. oreonympha* advertisement call can be differentiated from the others described to date (a) by having the shortest note ( $14.3 \pm 2.2$  ms vs.  $202.3 \pm 9.3$  ms in *N. bejaranoi*,  $115.0 \pm 18.0$  ms in *N. grandisonae*,  $122.0 \pm 9.0$  ms in *N. griffithsi*,  $26.0 \pm 6.0$  ms in *N. lasgralarias*,  $16.5 \pm 2.1$  ms in *N. mariae*,  $100.0 \pm 7.0$  ms in *N. manduriacu*,  $181 \pm 2.81$  ms in *N. pluvialis*,  $24.0 \pm 7.0$  ms in *N. siren*, and  $170.1 \pm 4.41$  ms in *N. truebae*); and (b) because the *N. oreonympha* advertisement call is comprised by a single tonal note while most others are pulsar (*N. bejaranoi*, *N. gradisonae*, *N. manduriacu*, *N. pluvialis*, and *N. truebae*; Catenazzi et al. 2009; Guayasamin et al. 2019, 2020; Hutter and Guayasamin 2012; Márquez et al. 1996).

*Centrolene*, with 24 species described, is one of the high diversity clades within Centrolenidae; it is also one of the genera with more advertisement calls recorded. The advertisement calls of *Centrolene* species usually consist of pulsed notes (e.g., *C. altitudinale*, *C. buckleyi*, *C. condor*, *C. geckoidea*, *C. sabini*, *C. hesperia*, *C. notosticta*, *C. peristicta*, *C. sanchezi*, and *C. venezuelense*), a combination of pulsed and tonal notes (*C. lynchi*), or tonal and pulsed notes as in "*C.*" *quindianum* (Cadle and McDiarmid 1990; Márquez et al. 1996; Grant et al. 1998; Señaris and Ayarzagüena 2005; Guayasamin et al. 2006; Dautel et al. 2011; Catenazzi et al. 2009; Almendariz-C and Batalla 2012; Salgado and Guayasamin 2018; Viuche-Lozano et al. 2018; Rios-Soto et al. 2017). This call structure differs of that observed here in *C. huilensis* and *C. hybrida*, and those described for *C. daidalea* and *C. savagei*, whose calls consist of tonal note(s) (Cardozo-Urdaneta and Señaris 2012; Vargas-Salinas et al. 2017). Likewise, we can note that among *Centrolene* species, the advertisement calls of *C. huilensis*, *C. altitudinale*, *C. hybrida*, and *C. sanchezi* are quite similar in several spectral parameters (see Señaris and Ayarzagüena 2005; Guayasamin et al. 2020). However, there are remarkable differences in some temporal parameters of the calls, i.e., the internote interval of *C. huilensis* is shorter ( $97.5 \pm 12.8$  ms) than that reported in *C. altitudinale* ( $331 \pm 6.5$  ms) and the notes of *C. hybrida* are longer ( $227.8 \pm 6122$  ms) than those described for *C. sanchezi* ( $11 \pm 2.8$  ms). In addition, in most cases the advertisement calls of *C. hybrida* exhibited a single note (mean 1.2 notes per call), while in *C. sanchezi* the mean was 5.9 notes. Although most glassfrog calls comprise high-pitched short notes of high energy without variation between the dominant frequencies of each note, the call of *C. huilensis* had variation in the dominant frequencies between the two notes. To date, this pattern is known for only a few glassfrog species, such as *C. lynchi* (Dautel et

al. 2011), *C. hesperium* (Cadle and McDiarmid 1990), and *C. sabini* (Catenazzi et al. 2009).

The delimitation of some species within *Rulyrana* is still a matter of debate. For example, Twomey et al. (2014) found a very low genetic distance between samples of the sister species *R. adiazeta* and *R. susatamai*, raising some questions regarding their taxonomic status. The species are highly similar morphologically, so a multidisciplinary approach is necessary to resolve the species limits, and an integrative approach is necessary to determine the phylogenetic relationships between *R. susatamai* and *R. adiazeta* (Twomey et al. 2014). In this sense, information for the species in their respective type localities can be used to solve such riddles. Here, the parameters of the advertisement call of *R. susatamai* obtained from the type locality (Falan municipality, Tolima department) are quite similar to those described by Galindo et al. (2020) from males of the species recorded in two localities also in Tolima department (Anzoátegui and Líbano municipalities). They will be useful for direct comparisons in order to resolve this taxonomic problem when the advertisement call of *R. adiazeta* becomes available. Concerning *R. flavopunctata*, although the male recorded here (eastern slope of eastern Cordillera in Colombia, AMMH-186, Fig. 2) is morphologically similar to males from Ecuador, the advertisement calls described by Guayasamin et al. (2020) have a longer mean note duration (40 ms Ecuador vs. 19.9 ms Colombia), greater mean number of pulses per note (7 Ecuador vs. 4 Colombia), and greater mean pulse rate (262 pulses/s Ecuador vs. 137.9 pulses/s Colombia). In this study, an unexpected variation in parameters is illustrated between two geographically distant advertisement calls. Temporal acoustic parameters, such as call duration and number of pulses, are highly influenced by climatic and social factors (Morais et al. 2012; Köhler et al. 2017). Unfortunately, the number of individuals recorded in both localities was too low to provide an explanation for these large differences, which could potentially be interpreted as individual or intraspecific variation or as evidence of undescribed morphologically similar diversity. However, the variation detected is a good example highlighting the need to include calls from different individuals at a variety of localities in taxonomical and ecological studies.

### Usefulness and Gaps in Conservation Strategies

The advertisement calls described in this study represent a reliable resource for further studies on glassfrog species. Two species, *S. punctulata* and *R. susatamai*, are considered Vulnerable and Near Threatened by the IUCN, respectively. They were previously identified as priority species for conservation considering their high phylogenetic endemism (Twomey et al. 2014; Mendoza and Arita 2014). We also described for the first time the call of the Endangered species *C. huilensis*, which was recently registered for Colombia after 30 years without records. Populations of *C. huilensis*, especially those from

Ecuador, require further evaluation regarding taxonomy and ecological monitoring (Mendoza-Henao et al. 2019). The call description provided here will facilitate species identification and detection in acoustic surveys, as a valid source of data on the species distribution which is needed for conservation (Carvajal-Endara et al. 2019).

Although the number of studies of bioacoustics in glassfrogs has increased in recent years, there are still many species in this family for which the advertisement call is still undescribed. Six of the eight Critical Endangered species that do not have their advertisement call described are not recorded in any protected areas (Fig. 3). In addition, 23 species are under some degree of threat and thus considered priorities for conservation (i.e., assessed in an IUCN threat category, having a local distribution, and not being covered by a protected area, Appendix 1).

The current gaps in glassfrog call descriptions can be explained by several factors. Many locations where the species are reported are difficult to access. Considering that some glassfrogs are highly susceptible to changes in habitat conditions, they mostly occur in inaccessible (and unaltered) areas of the rainforest and cloud forest (e.g., *Nymphargus garciae*, Ruiz-Carranza and Lynch 1995; *Nymphargus humboldti*, Guayasamin et al. 2020; *Teratohyla adenocheira*, Harvey and Noonan 2005). In addition, the ecology of some glassfrog species makes it difficult to record their calls because they are explosive breeders. This means that they are available only for a limited time and under specific environmental conditions, and the males perch and call near waterfalls where they usually go unnoticed due to the high level of background noise (e.g., “*Centrolene medemi*,” *C. gekoidea*, and *R. adiazeta*; Ruiz-Carranza and Lynch 1991; Lynch et al. 1983). Finally, low population densities or population decreases could reduce the detectability of these species in the field (e.g., “*Centrolene acantidiocephalum*,” Ruiz-Carranza and Lynch 1989; “*Centrolene azulae*,” Flores and McDiarmid 1989), making recordings of their calls rare or non-existent.

The new call descriptions for four glassfrog species presented here, plus the call descriptions of two species in strategic localities, contribute to the knowledge of endemic amphibian species that occur in the northern Andes. In addition, our results highlight the glassfrog species that do not have their call described thus far, and which should be prioritized in bioacoustic studies due to their threat status, endemism, and lack of occurrence in protected areas. This information can be used to establish future programs that use acoustic recordings to monitor populations with automated records, and to explore the taxonomic, ecological, and behavioral aspects of other understudied species.

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**Appendix 1.** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>Celsiella revocata</i>	Yes	Vulnerable (VU)	Subregional	Yes	Señaris and Ayarzagüena 2005
<i>Celsiella vozmedianoii</i>	No	Data Deficient (DD)	Local	Yes	
“ <i>Centrolene</i> ” <i>acanthidiocephalum</i>	No	Data Deficient (DD)	Local	No	Gonzalez-Durán et al., in prep.
<i>Centrolene altitudinalis</i>	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005
<i>Centrolene antioquiensis</i>	No	Near Threatened (NT)	Subregional	No	Duarte-Marín et al., in prep.
“ <i>Centrolene</i> ” <i>azulae</i>	No	Data Deficient (DD)	Local	No	
<i>Centrolene ballux</i>	Yes	Endangered (EN)	Local	No	Márquez et al. 1996
<i>Centrolene buckleyi</i>	Yes	Vulnerable (VU)	Regional	No	Guayasamin et al. 2006
<i>Centrolene charapita</i>	No	Critically Endangered (CR)	Local	No	
<i>Centrolene condor</i>	Yes	Data Deficient (DD)	Local	No	Almenzariz-C and Batalla 2012
<i>Centrolene daidalea</i>	Yes	Vulnerable (VU)	Subregional	No	Cardozo-Urdaneta and Señaris 2012
<i>Centrolene geckoidea</i>	Yes	Critically Endangered (CR)	Subregional	No	Grant et al. 1998
<i>Centrolene heloderma</i>	Yes	Vulnerable (VU)	Subregional	Yes	Guayasamin et al. 2020

**Appendix 1 (continued).** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>Centrolene hesperia</i>	Yes	Endangered (EN)	Local	Yes	Cadle and McDiarmid 1990
<i>Centrolene huilensis</i>	Yes	Endangered (EN)	Local	No	This study
<i>Centrolene hybrida</i>	Yes	Least Concern (LC)	Subregional	Yes	This study
<i>Centrolene lemniscata</i>	No	Data Deficient (DD)	Local	Yes	
<i>Centrolene lynchi</i>	Yes	Endangered (EN)	Subregional	No	Dautel et al. 2011
" <i>Centrolene</i> " <i>medemi</i>	No	Endangered (EN)	Subregional	No	
<i>Centrolene muelleri</i>	No	Data Deficient (DD)	Local	Yes	
<i>Centrolene notosticta</i>	Yes	Least Concern (LC)	Subregional	No	Viuche-Lozano et al. 2018
<i>Centrolene paezorum</i>	No	Data Deficient (DD)	Local	No	
<i>Centrolene peristicta</i>	Yes	Least Concern (LC)	Regional	No	Salgado and Guayasamin 2018
" <i>Centrolene</i> " <i>petrophilum</i>	No	Endangered (EN)	Local	No	
<i>Centrolene pipilatum</i>	No	Endangered (EN)	Local	Yes	
" <i>Centrolene</i> " <i>quindianum</i>	Yes	Vulnerable (VU)	Subregional	No	Ríos-Soto et al. 2017
" <i>Centrolene</i> " <i>robledoi</i>	No	Least Concern (LC)	Regional	Yes	Duarte-Marín et al., in prep.
<i>Centrolene sabini</i>	Yes	Vulnerable (VU)	Local	Yes	Catenazzi et al. 2009, 2012
<i>Centrolene sanchezi</i>	Yes	Data Deficient (DD)	Local	Yes	Guayasamin et al. 2020
<i>Centrolene savagei</i>	Yes	Least Concern (LC)	Subregional	Yes	Díaz-Gutiérrez et al. 2013
<i>Centrolene solitaria</i>	No	Endangered (EN)	Local	No	Basto-Riascos et al., in prep.
<i>Centrolene venezuelense</i>	Yes	Least Concern (LC)	Subregional	Yes	Señaris and Ayarzagüena 2005
<i>Chimerella corleone</i>	Yes	Data Deficient (DD)	Local	No	Twomey et al. 2014
<i>Chimerella mariaelenae</i>	Yes	Least Concern (LC)	Regional	No	Batallas and Brito 2016
" <i>Cochranella</i> " <i>duidaeana</i>	No	Data Deficient (DD)	Local	Yes	
<i>Cochranella erminea</i>	Yes	Least Concern (LC)	Regional	No	Twomey et al. 2014
" <i>Cochranella</i> " <i>euhystrix</i>	No	Critically Endangered (CR)	Local	No	
<i>Cochranella euknemos</i>	Yes	Least Concern (LC)	Regional	Yes	Savage and Starrett 1967; Kubicki 2007
" <i>Cochranella</i> " <i>geijskesi</i>	No	Least Concern (LC)	Local	Yes	
<i>Cochranella granulosa</i>	Yes	Least Concern (LC)	Regional	No	Ibáñez 1993; Kubicki 2007
<i>Cochranella guayasamini</i>	Yes	Least Concern (LC)	Subregional	No	Twomey et al. 2014
<i>Cochranella litoralis</i>	No	Vulnerable (VU)	Subregional	Yes	
<i>Cochranella mache</i>	Yes	Endangered (EN)	Local	Yes	Ortega-Andrade et al. 2013
" <i>Cochranella</i> " <i>megistra</i>	No	Endangered (EN)	Subregional	No	
<i>Cochranella nola</i>	Yes	Near Threatened (NT)	Subregional	No	Lötters and Köhler 2000
" <i>Cochranella</i> " <i>ramirezi</i>	No	Data Deficient (DD)	Local	No	
<i>Cochranella resplendens</i>	No	Least Concern (LC)	Global	Yes	



Advertisement calls of six glassfrog species

**Appendix 1 (continued).** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>"Cochranella" riveroi</i>	No	Vulnerable (VU)	Local	Yes	
<i>"Cochranella" xanthoheridia</i>	No	Vulnerable (VU)	Subregional	No	
<i>Espadarana andina</i>	Yes	Least Concern (LC)	Subregional	No	Señaris and Ayarzagüena 2005; Cabanzo-Olarte and Ortega-Chinchilla 2017
<i>Espadarana audax</i>	Yes	Least Concern (LC)	Regional	Yes	Duarte-Marín et al., in prep.; Guayasamin et al. 2020
<i>Espadarana callistomma</i>	Yes	Least Concern (LC)	Regional	Yes	Guayasamin et al. 2020
<i>Espadarana durrellorum</i>	No	Least Concern (LC)	Regional	No	
<i>Espadarana prosoblepon</i>	Yes	Least Concern (LC)	Global	Yes	Jacobson 1985; Kubicki 2007
<i>Hyalinobatrachium adespinosai</i>	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2019
<i>Hyalinobatrachium anachoretus</i>	Yes	Endangered (EN)	Local	No	Twomey et al. 2014
<i>Hyalinobatrachium aureoguttatum</i>	No	Near Threatened (NT)	Subregional	No	
<i>Hyalinobatrachium bergeri</i>	Yes	Least Concern (LC)	Regional	Yes	Castroviejo-Fisher et al. 2009
<i>Hyalinobatrachium cappellei</i>	Yes	Not evaluated	Regional	Yes	Myers and Donnelly 1997, 2001; Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2011; Oliveira et al. 2015
<i>Hyalinobatrachium carlesvilai</i>	Yes	Not evaluated	Regional	No	Márquez et al. 1996; Castroviejo-Fisher et al. 2009
<i>Hyalinobatrachium chirripoi</i>	Yes	Least Concern (LC)	Subregional	Yes	Kubicki 2007; Kubicki et al. 2015
<i>Hyalinobatrachium colymbiphylum</i>	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Kubicki et al. 2015
<i>Hyalinobatrachium diana</i>	Yes	Data Deficient (DD)	Local	Yes	Kubicki et al. 2015
<i>Hyalinobatrachium duranti</i>	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005
<i>Hyalinobatrachium esmeralda</i>	Yes	Endangered (EN)	Local	No	Acosta-Galvis 2017
<i>Hyalinobatrachium fleischmanni</i>	Yes	Least Concern (LC)	Global	Yes	Jacobson 1985; Kubicki 2007; Castroviejo-Fisher et al. 2011; Greer and Wells 1980
<i>Hyalinobatrachium fragile</i>	Yes	Vulnerable (VU)	Subregional	Yes	Wen et al. 2012
<i>Hyalinobatrachium guairarepanense</i>	Yes	Endangered (EN)	Local	Yes	Señaris and Ayarzagüena 2005
<i>Hyalinobatrachium iaspidiense</i>	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2011
<i>Hyalinobatrachium ibama</i>	No	Vulnerable (VU)	Subregional	No	
<i>Hyalinobatrachium kawense</i>	Yes	Least Concern (LC)	Regional	No	Castroviejo-Fisher et al. 2011
<i>Hyalinobatrachium mesai</i>	Yes	Not evaluated	Local	Yes	Castroviejo-Fisher et al. 2011
<i>Hyalinobatrachium mondolfii</i>	Yes	Least Concern (LC)	Regional	No	Señaris and Ayarzagüena 2001; Castroviejo-Fisher et al. 2011

**Appendix 1 (continued).** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>Hyalinobatrachium muiraquitana</i>	No	Not evaluated	Local	No	
<i>Hyalinobatrachium munozorum</i>	Yes	Least Concern (LC)	Global	Yes	Guayasamin et al. 2020
<i>Hyalinobatrachium orientale</i>	Yes	Vulnerable (VU)	Subregional	No	Castroviejo-Fisher et al. 2008; Cardozo-Urdaneta and Señaris 2012
<i>Hyalinobatrachium orocostale</i>	Yes	Not evaluated	Subregional	Yes	Castroviejo-Fisher et al. 2008
<i>Hyalinobatrachium pallidum</i>	Yes	Endangered (EN)	Local	Yes	Cardozo-Urdaneta and Señaris 2012
<i>Hyalinobatrachium pellucidum</i>	Yes	Near Threatened (NT)	Local	Yes	Wen et al. 2012
<i>Hyalinobatrachium talamancae</i>	Yes	Least Concern (LC)	Subregional	Yes	Kubicki 2007; Kubicki et al. 2015
<i>Hyalinobatrachium tatayoi</i>	Yes	Least Concern (LC)	Local	Yes	Castroviejo-Fisher et al. 2007
<i>Hyalinobatrachium taylori</i>	Yes	Least Concern (LC)	Global	Yes	Señaris and Ayarzagüena 2005
<i>Hyalinobatrachium tricolor</i>	Yes	Least Concern (LC)	Regional	Yes	Castroviejo-Fisher et al. 2011
<i>Hyalinobatrachium valerioi</i>	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Kubicki et al. 2015
<i>Hyalinobatrachium vireovittatum</i>	Yes	Data Deficient (DD)	Local	No	Kubicki 2007; Kubicki et al. 2015
<i>Hyalinobatrachium yaku</i>	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2017
<i>Ikakogi ispacue</i>	Yes	Not evaluated	Local	Yes	Rada et al. 2019
<i>Ikakogi tayrona</i>	Yes	Vulnerable (VU)	Subregional	Yes	Vargas-Salinas et al. 2015
<i>Nymphargus anomalus</i>	No	Critically Endangered (CR)	Local	Yes	
<i>Nymphargus armatus</i>	No	Critically Endangered (CR)	Local	No	
<i>Nymphargus balionotus</i>	No	Vulnerable (VU)	Subregional	No	
<i>Nymphargus bejaranoi</i>	Yes	Least Concern (LC)	Regional	No	Márquez et al. 1996
<i>Nymphargus buenaventura</i>	No	Data Deficient (DD)	Local	No	
<i>Nymphargus caritiocommatus</i>	No	Data Deficient (DD)	Local	Yes	
<i>Nymphargus caucanus</i>	No	Endangered (EN)	Local	Yes	Duarte-Marín et al., in prep.
<i>Nymphargus chami</i>	No	Data Deficient (DD)	Subregional	No	Duarte-Marín et al., in prep.
<i>Nymphargus chancas</i>	No	Data Deficient (DD)	Local	Yes	
<i>Nymphargus cochranae</i>	No	Vulnerable (VU)	Subregional	Yes	
<i>Nymphargus colomai</i>	No	Vulnerable (VU)	Local	No	
<i>Nymphargus cristinae</i>	No	Data Deficient (DD)	Local	No	
<i>Nymphargus garciae</i>	No	Vulnerable (VU)	Subregional	Yes	González-Acosta et al., in prep.
<i>Nymphargus grandisonae</i>	Yes	Least Concern (LC)	Subregional	No	Hutter et al. 2013
<i>Nymphargus griffithsi</i>	Yes	Least Concern (LC)	Regional	No	Hutter and Guayasamin 2012; Arcila-Perez et al. 2017
<i>Nymphargus humboldti</i>	No	Data Deficient (DD)	Subregional	Yes	
<i>Nymphargus ignotus</i>	No	Least Concern (LC)	Subregional	No	Duarte-Marín et al., in prep.

## Advertisement calls of six glassfrog species

**Appendix 1 (continued).** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>Nymphargus lasgralarias</i>	Yes	Endangered (EN)	Local	Yes	Hutter and Guayasamin 2012
<i>Nymphargus laurae</i>	No	Critically Endangered (CR)	Local	No	
<i>Nymphargus lindae</i>	No	Endangered (EN)	Local	No	
<i>Nymphargus luminosus</i>	No	Endangered (EN)	Local	No	
<i>Nymphargus luteopunctatus</i>	No	Endangered (EN)	Local	No	
<i>Nymphargus manduriacu</i>	Yes	Critically Endangered (CR)	Local	Yes	Guayasamin et al. 2019
<i>Nymphargus mariae</i>	Yes	Least Concern (LC)	Regional	No	Guayasamin et al. 2020
<i>Nymphargus megacheirus</i>	No	Endangered (EN)	Local	Yes	
<i>Nymphargus mixomaculatus</i>	No	Critically Endangered (CR)	Local	Yes	
<i>Nymphargus nephelophila</i>	No	Data Deficient (DD)	Local	No	
<i>Nymphargus ocellatus</i>	No	Data Deficient (DD)	Local	No	
<i>Nymphargus oreonympha</i>	Yes	Data Deficient (DD)	Local	No	This study
<i>Nymphargus phenax</i>	No	Endangered (EN)	Local	No	
<i>Nymphargus pluvialis</i>	Yes	Data Deficient (DD)	Local	No	Catenazzi et al. 2009
<i>Nymphargus posadae</i>	No	Least Concern (LC)	Regional	No	
<i>Nymphargus prasinus</i>	No	Vulnerable (VU)	Subregional	No	
<i>Nymphargus rosada</i>	No	Vulnerable (VU)	Subregional	No	Duarte-Marín et al., in prep.
<i>Nymphargus ruizi</i>	No	Vulnerable (VU)	Subregional	Yes	
<i>Nymphargus siren</i>	Yes	Vulnerable (VU)	Subregional	Yes	Guayasamin et al. 2020
<i>Nymphargus spilotus</i>	No	Near Threatened (NT)	Local	Yes	Duarte-Marín et al., in prep.
<i>Nymphargus sucre</i>	No	Critically Endangered (CR)	Local	No	
<i>Nymphargus truebae</i>	Yes	Critically Endangered (CR)	Local	No	Catenazzi et al. 2009
<i>Nymphargus vicenteruedai</i>	No	Data Deficient (DD)	Local	Yes	
<i>Nymphargus wileyi</i>	No	Data Deficient (DD)	Local	Yes	
<i>Rulyrana adiazeta</i>	No	Vulnerable (VU)	Subregional	No	
<i>Rulyrana flavopunctata</i>	Yes	Least Concern (LC)	Regional	No	Guayasamin et al. 2020
<i>Rulyrana mediarmidi</i>	No	Data Deficient (DD)	Subregional	No	
<i>Rulyrana saxiscandens</i>	Yes	Endangered (EN)	Local	No	Twomey et al. 2014
<i>Rulyrana spiculata</i>	Yes	Near Threatened (NT)	Subregional	No	Catenazzi et al. 2009
<i>Rulyrana susatamai</i>	Yes	Near Threatened (NT)	Subregional	No	Galindo et al. 2020; This study
<i>Sachatamia albomaculata</i>	Yes	Least Concern (LC)	Regional	No	Kubicki 2007
<i>Sachatamia electrops</i>	No	Endangered (EN)	Local	No	
<i>Sachatamia ilex</i>	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Guayasamin et al. 2020
<i>Sachatamia orejuela</i>	No	Least Concern (LC)	Subregional	No	Duarte-Marín et al., in prep.
<i>Sachatamia punctulata</i>	Yes	Vulnerable (VU)	Subregional	No	This study
<i>Teratohyla adenochaira</i>	No	Data Deficient (DD)	Local	Yes	
<i>Teratohyla amelie</i>	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2020

**Appendix 1 (continued).** Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
<i>Teratohyla midas</i>	Yes	Least Concern (LC)	Global	Yes	Araújo et al. 2018; Guayasamin et al. 2020
<i>Teratohyla pulverata</i>	Yes	Least Concern (LC)	Regional	No	Ibañez et al 1999; Savage 2002
<i>Teratohyla spinosa</i>	Yes	Least Concern (LC)	Regional	No	Ibañez et al 1999; Savage 2002; Kubicki 2007
<i>Vitreorana antisthenesi</i>	Yes	Vulnerable (VU)	Subregional	Yes	Wen et al. 2012
<i>Vitreorana baliomma</i>	Yes	Not evaluated	Local	Yes	Lee Bang et al. 2019
<i>Vitreorana castroviejo</i>	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
<i>Vitreorana eurygnatha</i>	Yes	Least Concern (LC)	Global	No	Heyer et al. 1990; Santana et al. 2015
<i>Vitreorana franciscana</i>	Yes	Not evaluated	Local	Yes	Santana et al. 2015
<i>Vitreorana gorzulae</i>	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
<i>Vitreorana helenae</i>	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
<i>Vitreorana parvula</i>	No	Data Deficient (DD)	Local	No	
<i>Vitreorana ritae</i>	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005; Cisneros-Heredia 2013
<i>Vitreorana uranoscopa</i>	Yes	Least Concern (LC)	Global	Yes	Heyer 1985; Haga et al. 2014

**Appendix 2.** List of highly threatened (IUCN classifications Endangered (EN) or Critically Endangered (CR)) and understudied (IUCN categories Data Deficient (DD) or Not evaluated) glassfrog species with undescribed calls and a range that either includes less than five known locations in less than 1,500 km<sup>2</sup> or does not overlap with any protected areas. Asterisks indicate species for which the calls are currently being described.

Highly threatened species (EN or CR)	Understudied species (DD or Not evaluated)
<i>Centrolene charapita</i>	" <i>Centrolene</i> " <i>acanthidiocephalum</i> *
" <i>Centrolene</i> " <i>petrophilum</i>	" <i>Centrolene</i> " <i>azulae</i>
<i>Centrolene solitaria</i> *	<i>Centrolene paezorum</i>
" <i>Cochranella</i> " <i>euhystrix</i>	" <i>Cochranella</i> " <i>ramirezi</i>
<i>Nymphargus armatus</i>	<i>Hyalinobatrachium mairaquitana</i>
<i>Nymphargus laurae</i>	<i>Nymphargus buenaventura</i>
<i>Nymphargus lindae</i>	<i>Nymphargus colomai</i>
<i>Nymphargus luminosus</i>	<i>Nymphargus cristinae</i>
<i>Nymphargus luteopunctatus</i>	<i>Nymphargus nephelophila</i>
<i>Nymphargus phenax</i>	<i>Nymphargus ocellatus</i>
<i>Nymphargus sucre</i>	<i>Vitreorana parvula</i>
<i>Sachatamia electrops</i>	