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A new addition to the frog fauna of Uruguay, *Physalaemus cristinae* Cardozo *et al.*, 2023 (Anura, Leptodactylidae)

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ABSTRACT

We present the first finding in Uruguay of the recently described frog *Physalaemus cristinae*. Adult specimens were collected in two localities of north-western Uruguay, on the east bank of the Uruguay River: near the city of Bella Unión associated to temporal ponds of an agricultural area; and in an urban area within the city of Salto. The advertisement call consisted of a single and non-pulsed note, characteristic of *P. cristinae*. In addition, partial sequences of the cytochrome b gene confirmed the species identity. We consider that the presence of *P. cristinae* in north-western Uruguay, previously unregistered in a well sampled area, is due to recent cross-river dispersal from populations in the Provinces of Entre Ríos and Corrientes, Argentina, on the west bank of the Uruguay River.

Key Words: Amphibian; Geographic distribution; Uruguayan savanna; *Physalaemus*.

Uruguay is situated within the Uruguayan savanna ecoregion of eastern South America, at the southern part of the Neotropical Region (Morrone, 2014). The north-west of this country constitutes a transitional zone with the Southern Cone Mesopotamian Savanna and Espinal ecoregions of Argentina. Consequently, the geographic distribution of some vertebrate species associated to these biomes reach north-western Uruguay as the boundaries of their distributions. There are well known examples of this among amphibians, like the species *Rhinella dypticha* (Cope, 1862) (Bufonidae; Klappenbach, 1969), *Pseudis limellum* (Cope, 1862) (Gudynas and Rudolf, 1983), *Dendropsophus nanus* (Boulenger, 1889) (Langone and Basso, 1987), *Scinax nasicus* (Cope, 1862), *Leptodactylus macrosternum* Miranda-Ribeiro, 1926, and *L. podicipinus* (Cope, 1862) (Vaz-Ferreira *et al.*, 1984). A similar distribution pattern is exhibited by the recently described frog *Physalaemus cristinae* Cardozo *et al.* (2023) of the *P. cuvieri* Fitzinger, 1826 species group (Leptodactylidae), present in central-eastern Argentina and Paraguay. This species dwells from the Humid Chaco to the Espinal ecoregion more southwards, including some areas adjacent to north-western Uruguay but the species was still not recorded from this country. Herein, we present the first record of *P. cristinae* in Uruguay, based mainly on DNA sequences and acoustic analy-

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sis.Vouchers were accessioned at the herpetological collection of Museo Nacional de Historia Natural (MNHN), Montevideo, Uruguay. Four adult frogs were collected in the agricultural area of Alcoholes del Uruguay – ALUR (GRA; 30.1957ºS, 57.3637ºW), 7 km south from the city of Bella Unión, Departamento de Artigas: MNHN 9949 and MNHN 9950, 29 January 2024, males, snout-vent length (SVL) 22.1 mm and 21.7 mm respectively; MNHN 9951, 9 February 2024, female, SVL 25.0 mm; MNHN 9952, 3 March 2024, male, SVL 22.8 mm. An additional one was captured within the urban area of the city of Salto, Departamento de Salto (SGBS; 31.2408ºS, 57.5903ºW): MNHN 9999, 2 April 2025, male. The climate in this region is temperate wet with average annual temperature and precipitation of 19.8 ºC and 1600 mm respectively (Bidegain *et al.*, 2012). Land use in the surrounding landscape is dominated by intensive agriculture, mainly rice and sugar cane crops. At ALUR, frogs were detected at night during summer, around human habitations and temporary ponds of adjacent grasslands. Most captured specimens were males found calling hidden among herbaceous vegetation (Fig. 1). The specimen from Salto was captured in a temporary pond within an urban area, while calling along with *Odontophrynus asper* and *Leptodactylus latinasus*.

Genomic DNA from tissue samples of two specimens was extracted after Aljanabi and Martínez (1997), and fragments of the cytochrome b gene (*Cyt-b*) were amplified by standard polymerase chain reaction (PCR) using the primers MVZ15 (5'-GACT AATGG CCCAC ACWWTA CGNAA-3'), and MVZ16 (5'-AA ATAGG AARTA TCAYT CTGGT TTRAT-3'), after Moritz *et al.* (1992). The purified amplicons were sequenced in both directions at Macrogen Inc. (Korea), and chromatograms processed using the software DNABaser v.3 (Heracle BioSoft, 2013). DNA sequences were accessioned in the GenBank database: PQ316074 (MNHN 9951), and PQ316075 (MNHN 9952). Alignments were done with Clustal W (Thompson *et al.*, 1994), executed in BioEdit (Hall, 1999) under default parameters.

The obtained sequences were studied in a phylogenetic analysis run along with a subset of the matrix data of the *Cyt-b* gene previously used by Cardozo *et al.* (2023), available from GenBank: *Engystomops freibergi* OR453824; *Physalaemus albifrons* OR453860; *P. albonotatus* OR453812-OR453816, OR453820, OR453829- OR453831, OR453842, OR453843, OR453845, OR453847, OR453854, OR453855, OR453861, OR453862; *P. barrioi* OR453825; *P. biligonigerus* OR453848; *P. cristinae* OR453826, OR453833, OR453838, OR453840, OR453849, OR453852, OR453856, OR453858: *P. cuvieri* OR453821; *P. lateristriga* OR453817; *P. lisei* OR453834; *P. nattereri* OR453863; *P. santafecinus* OR453853; *P. signifier* OR453823; *P. spiniger* OR453822. Sequence sampling mainly focused on *P. cristinae* given its known geographic proximity, and it sister taxon *P. albonotatus*. The most parsimonious trees were inferred through heuristic searches employing TNT software (Goloboff *et al.*, 2008), with 1000 addition sequences of the tree bisection-reconnection, retaining 100 trees per replication. Support values were estimated on strict consensus tree running of 1000 replicates under parsimony jackknife (Farris *et al.*, 1996) with default TNT settings, and 0.36 of removal probability.

At the site of collection of the studied specimens we could record the advertisement call of an unvouchered male with an iPhone 8 cell phone, on 28 February 2024, at 0:20 am, with 24º C environmental temperature. A single note was analysed with Sound Forge Pro 17 software, with the sonogram generated by Blackman-Harris, 2.048 FFT, 90% overlap FFT, and 10.000 resolution. Graphics were obtained with Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics, 2024).

The phylogenetic analysis indicated that the specimens collected in northern Uruguay are *P. cristinae*. Their partial sequences of the *Cyt-b* gene were recovered within a well-supported clade that grouped all analysed terminals of this species (Fig. 2). This clade was the sister of another one corresponding to *P. albonotatus*, a closely related taxon considered the sister species of *P. cristinae* by Cardozo *et al.* (2023). Both species are cryptic and similar to *P. cuvieri*, from which *P. cristinae* slightly differs due to the absence of reddish colouration in the inguinal region Cardozo *et al.* (2023). This feature was not present in our study specimens, whose major external morphological characters matched with those of *P. cristinae* as indicated by Cardozo *et al.* (2023) in the description of the species, for instance: medium size (SVL range 20.5-32.0 mm), slender body, smooth dorsal skin, head longer than wide, absence of V-shaped dorsal pattern, dorsal colouration with brown tones or entirely green, sacral region without dark ocelli, absence of a narrow white outline of the mandible or extending from the posterior corner of the eye, absence of a median

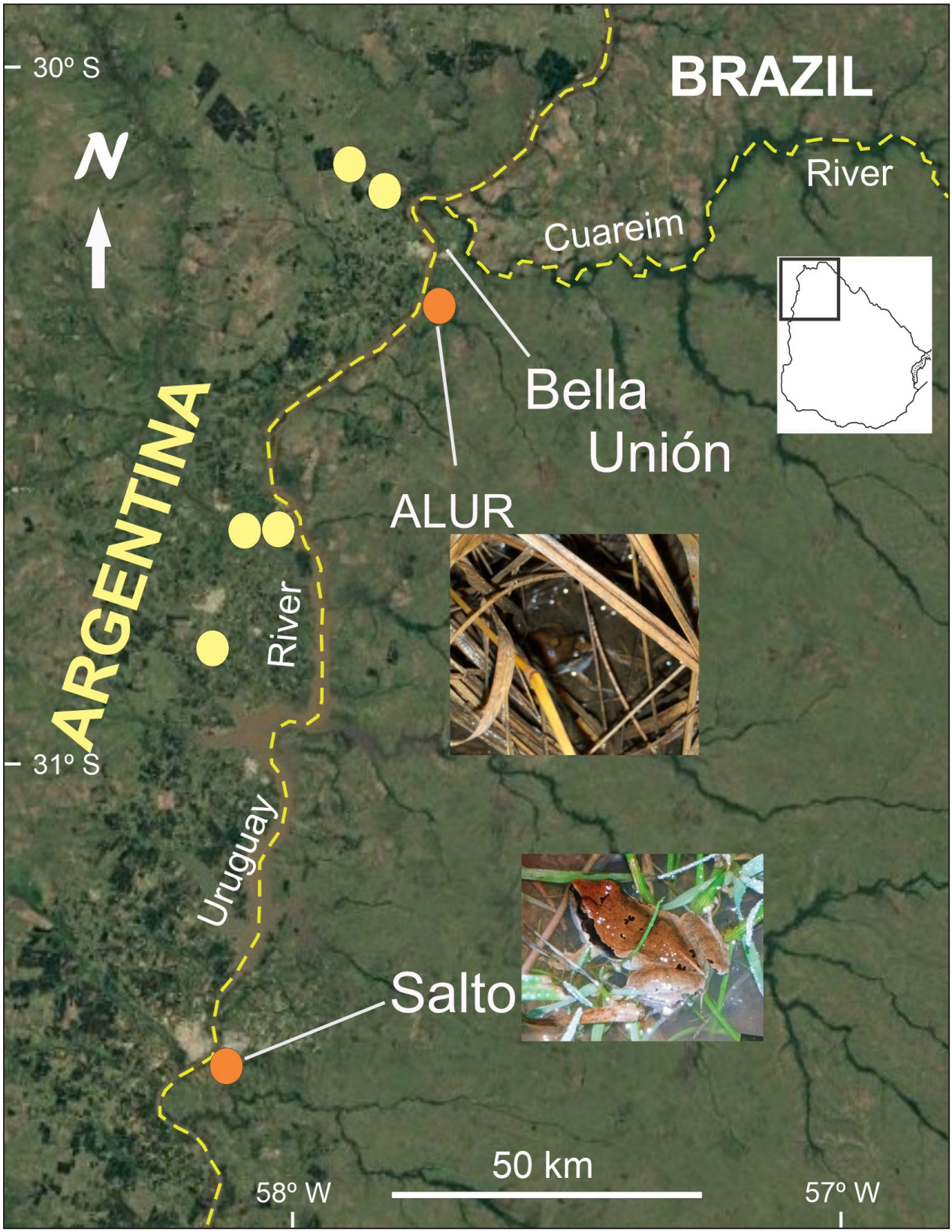

Figure 1. Location of ALUR - Alcoholes del Uruguay, and Salto, in northern Uruguay, sites of collection of *Physalaemus cristinae* (insets) in temporary ponds; orange dots. Closest known localities in Argentina, are indicated after Cardozo et al. (2023), yellow dots. Dashed lines correspond to country borders (image adapted from Google Earth Pro, 26 May 2025).

stripe on throat and chest (plus the abdomen), but sparse spotting.

Another relevant taxonomic character useful to distinguish *P. cristinae* from *P. albonotatus* is the advertisement call. In the case of *P. albonotatus* the call consists of a single and pulsed long note (1.1–1.5 s), whereas the call of *P. cristinae* is composed by a single but non-pulsed note (0.91–1.77 s). Additional characteristics of the latter are fundamental frequency between 517–696 Hz, starting at 519–865 Hz, ending at 484–634 Hz, and the dominant frequency mainly over the first, fourth, or fifth harmonics (Cardozo *et al.*, 2023).

The advertisement call characteristics of our recorded specimen (single note) are overall coincident with that described for *P. cristinae* (Fig. 3): a non-pulsed note of 0.78 s., with descendant modulation, fundamental frequency starting at 793 Hz and ending at 607 Hz, being the dominant frequency 3243 Hz–2352 Hz. The call is noticeably longer from that of *P. cuvieri* (0.25 – 0.33 s, see Braga *et al.*, 2023) also present in Uruguay.

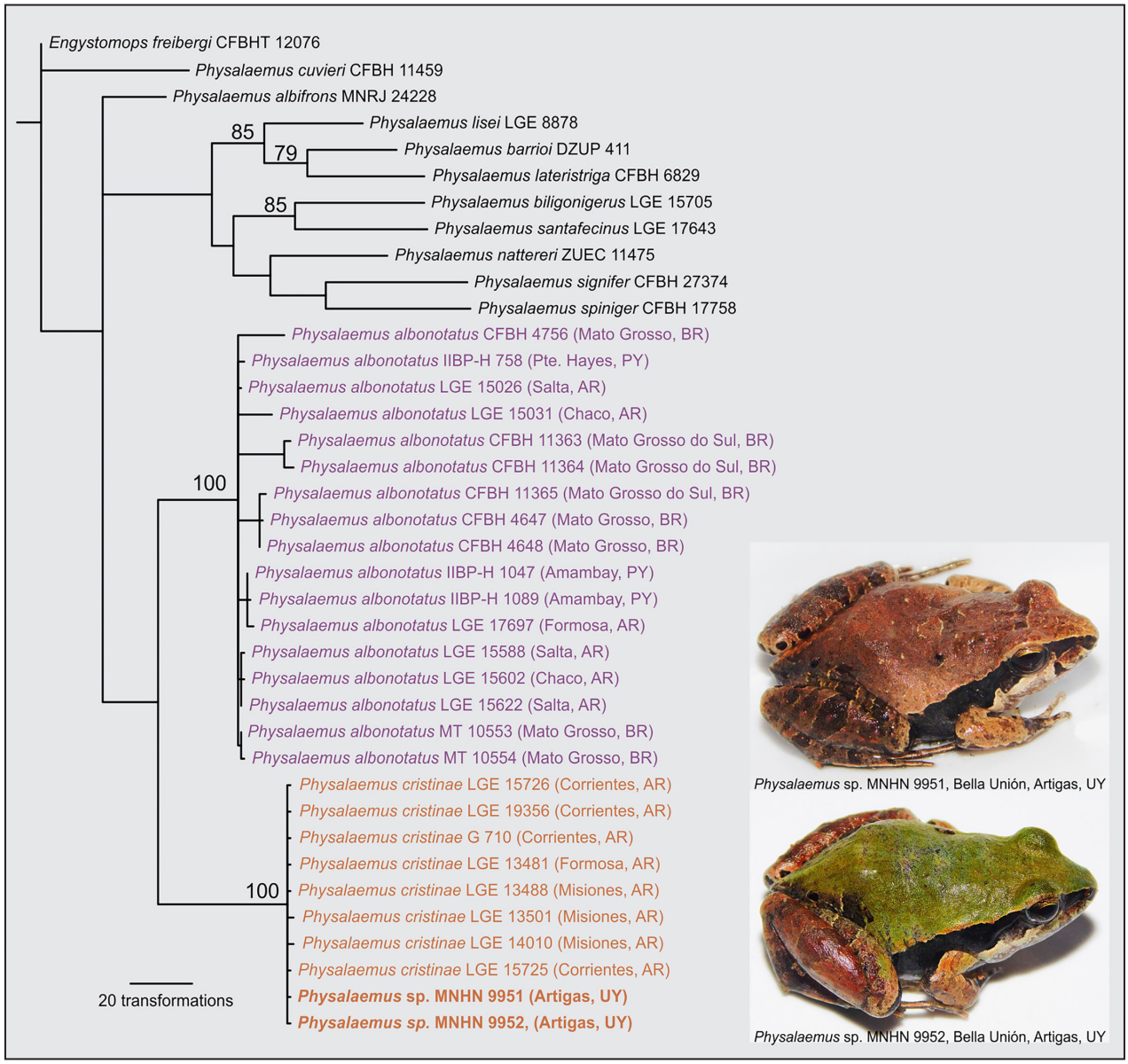

Figure 2. Phylogenetic relations of studied *Physalaemus* specimens from northern Uruguay (UY) based on partial sequences of the *Cyt-b* gene, studied against a subset of data used by Cardozo et al. (2023). The analysis targeted relationships with *P. albonotatus* and its sister taxon *P. cristinae* given their geographic proximity, including specimens from Argentina (AR), Brazil (BR), and Paraguay (PY). The figure shows the strict consensus of the 70 trees of 1032 steps obtained with TNT, branch lengths are proportional to the number of transformations, and node Parsimony Jackknife supports greater than 50 are indicated.

The presence of *P. cristinae* in north-western Uruguay is in agreement with the geographic distribution of the species depicted by Cardozo *et al.* (2023). Furthermore, according to these authors, the closest known populations of the cryptic species *P. albonotatus* are present approximately 600 km to the northwest, in Chaco Province, Argentina. Until the present work, only *P. biligonigerus* (Cope, 1861) and *P. riograndensis* Milstead, 1960, were recorded in much of our study area (Núñez *et al.*, 2004). These species are morphologically very different from *P. cristinae*, much smaller SVL in *P. riograndensis* (14-26 mm; Barrio, 1965), and more robust body and shovel-like metatarsal tubercles in *P. biligonigerus* (Barrio, 1965; Nascimento *et al.*, 2005; Lourenço *et al.*, 2015). It must be noted that the authors intensively surveyed a large geographic area of northern Uruguay in the surroundings of Bella Unión from 1999 to 2004 (Borteiro, 2005; Borteiro and Kolenc, 2007; Borteiro *et al.*, 2008), with additional sporadic surveys up to 2019 (Laufer *et al.*, 2021). *Physalaemus* species recorded during these surveys were only *P. biligonigerus*, and mainly *P. riograndensis*. At ALUR (formerly CALNU) and nearby areas, we knew of only the occurrence of the latter associated to flooded sugar cane crops (Borteiro and Kolenc, 2007). It is unlikely that *P. cristinae* would have passed unnoticed given its noticeable advertisement call and distinctive external morphology. The same could be said about the city of Salto, where *P. cristinae* was found within the urban area of the city. We consider that its presence in north-western Uruguay could be explained by recent cross-river dispersal. The new localities reported herein at ALUR and Salto are about 125 km distant from each other, but very close to the Uruguay River shore (2.0 and 0.8 km respectively), which leads to hypothesize that at least two different dispersal events of *P. cristinae* across this river may have occurred in recent years. This is congruent with the several close localities with confirmed presence of *P. cristinae* in the Province of Entre Ríos and Corrientes, Argentina, on the west bank of the Uruguay River (Cardozo *et al.*, 2023). It must be noticed that a severe drought affected the region between 2019 and 2022 (Besnier *et al.*, 2024; Rivera, 2024), and the extreme low water level of the Uruguay River may have favoured the dispersal of amphibians. The environments at both sides of this river are comparable, and also noticeable similarity among the diversity their anuran faunas was early pointed by Gudynas (1984). Our findings reinforce the hypothesis that the Uruguay River is not a barrier for the anuran fauna (Gudynas, 1984). The extent of the current distribution of *P. cristinae* in Uruguay,

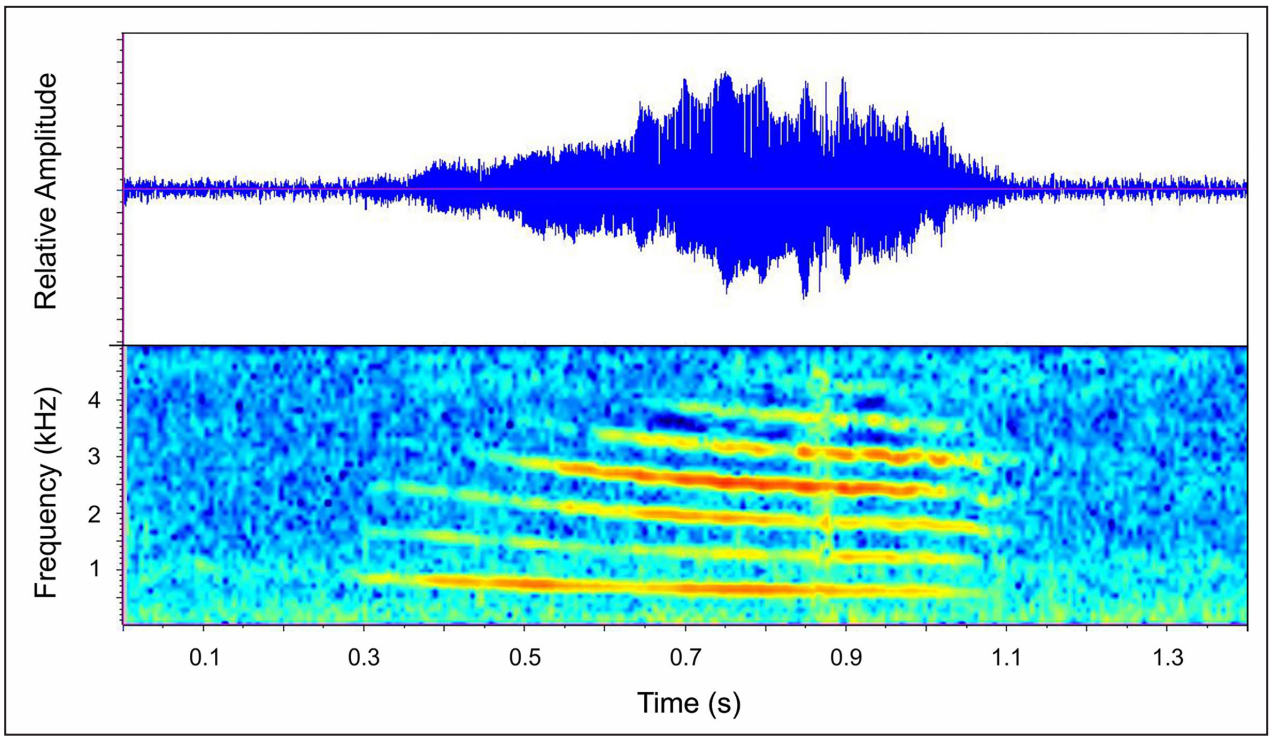

Figure 3. Advertisement call of *Physalaemus cristinae* from ALUR, Bella Unión, Uruguay: oscillogram (top) and spectrogram (bottom), 28 February 2024, 0:20 am, 24º C.

and the fate of these most likely recently established populations (including extinction or expansion) deserves future studies. A recent southwards population expansion was proposed for several frog species in Uruguay (Laufer et al., 2021), but baseline data is always difficult to ascertain. The starting point of the *P. cristinae* story in Uruguay is now being set.

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