

# Rediscovering *Cycloramphus bandeirensis* (Anura: Cycloramphidae): natural history and breeding biology of a vulnerable species with a variant reproductive mode

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## Abstract

**Rediscovering *Cycloramphus bandeirensis* (Anura: Cycloramphidae): natural history and breeding biology of a vulnerable species with a variant reproductive mode.** We present here data on the breeding biology of *Cycloramphus bandeirensis*, including descriptions of the advertisement call and tadpole. Males call from beneath rocks in open grasslands above approximately 2,450 m a.s.l. at the Parque Nacional do Caparaó, southeastern Brazil. Breeding occurs from late October–December, when mist and clouds frequently cover the area. The advertisement call consists of a first note, followed by two or three shorter ones. The spawn is hidden under rocks from which the males call, and the tadpoles are exotrophic and semiterrestrial. This report of a semiterrestrial tadpole that develops in seclusion in a terrestrial habitat is a variant of Reproductive Mode 19. We compared the ontogeny of the semiterrestrial tadpole of *C. bandeirensis* to that of generalized tadpoles, and a new function is ascribed to the skin flap that is present in larvae of *Cycloramphus*. It is suggested that *C. bandeirensis* be transferred to Vulnerable category (VU, d2) of IUCN; the species has a small geographic range (< 20 km<sup>2</sup>), specialized environmental requirements, is vulnerable, has phylogenetic affinities to species that consistently have undergone local population crashes in southeastern Brazil, and is subject to plausible potential threats in its range (e.g., fires and climate change).

**Keywords:** Atlantic Forest Domain, endemic species, heterochrony, semiterrestrial tadpole, southeastern Brazil.

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## Resumo

### **Redescoberta de *Cycloramphus bandeirensis* (Anura: Cycloramphidae): história natural e biologia reprodutiva de uma espécie vulnerável com modo reprodutivo diferenciado.**

Apresentamos aqui dados sobre a biologia reprodutiva de *Cycloramphus bandeirensis*, incluindo a descrição do canto de anúncio e dos girinos. Os machos vocalizam escondidos sob rochas nos campos de altitude acima de aproximadamente 2450 m de altitude no Parque Nacional do Caparaó, sudeste do Brasil. A reprodução ocorre do final de outubro a dezembro, quando neblina e nuvens cobrem a área frequentemente. O canto de anúncio consiste de uma primeira nota, seguida de duas a três notas mais curtas. A desova ocorre sob as rochas, de onde os machos vocalizam, e os girinos são exotróficos e semiterrestres. Este registro de girino semiterrestre, que se desenvolve abrigado em ambiente terrestre, é uma variação do Modo Reprodutivo 19. Comparamos a ontogenia dos girinos semiterrestres de *C. bandeirensis* à de girinos de morfotipos mais generalistas e sugerimos uma nova função para a dobra de pele presente nos girinos de *Cycloramphus*. Sugerimos transferir *C. bandeirensis* para a categoria Vulnerável (VU, d2) da IUCN; a espécie apresenta distribuição geográfica restrita (< 20 km<sup>2</sup>), altos requerimentos ambientais, vulnerabilidade, afinidade filogenética a espécies que sofreram declínios populacionais no sudeste do Brasil e está sujeita a ameaças potenciais plausíveis na área de ocorrência (e.g., incêndios e alterações climáticas).

**Palavras-chave:** espécie endêmica, girino semiterrestre, heterocronia, Mata Atlântica, sudeste do Brasil.

## Introduction

Cycloramphidae is endemic to Brazil; the family includes *Thoropa*, *Cycloramphus*, and *Zachaenus* (Frost 2019), of which the latter two are endemic to the Atlantic Forest Domain (Heyer 1983a,b, Verdade *et al.* 2009a). The species in the family typically are forest inhabitants that are associated with forest litter, rocky outcrops, or fast-flowing streams. The external morphology of the adults ranges from semi-fossorial to terrestrial morphotypes in which the toes lack webbing and the legs are short and robust to semi-aquatic morphotypes having toe webbing, slender bodies, and longer legs (Heyer 1983a, Verdade and Rodrigues 2003, Lima *et al.* 2012). There are two tadpole morphotypes in the family—exotrophic semiterrestrial tadpoles and the endotrophic terrestrial larvae (Lutz 1944, Bokermann 1965, Heyer 1983a, b, Heyer and Crombie 1979). All semi-fossorial species are associated with forest litter and have endotrophic, terrestrial tadpoles, whereas the semi-aquatic species are associated with streams and have exotrophic, semi-terrestrial tadpoles (e.g., Heyer and Crombie 1979, Heyer 1983a, b).

*Cycloramphus* is the most speciose genus in Cycloramphidae; it contains 28 species (Frost 2019), with representatives of both terrestrial and semi-aquatic adult and tadpole morphotypes. All species of *Cycloramphus* inhabit forested areas except two species—*C. bandeirensis* and *C. organensis* that live in the open high grasslands of the Parque Nacional do Caparaó and the Parque Nacional da Serra dos Órgãos, respectively (Heyer 1983a, Heyer and Maxson 1983, Weber *et al.* 2011).

Little is known about the natural history of *Cycloramphus* (Giaretta and Facure 2003, Hartman *et al.* 2003, Lingnau *et al.* 2008, Verdade *et al.* 2009b). Advertisement calls are known for 13 species and there are descriptions of tadpoles for only nine species in the genus. The calls range from short simple metallic calls to long simple calls, and complex ones (Heyer 1983a). No correlations have been made among different habitats, adult morphotypes, or phylogeny.

The habitat and reproductive specializations in Cycloramphidae contribute to the high diversity of anuran reproductive modes (RM) in the Atlantic Forest (Haddad and Prado, 2005,

Iskandar *et al.* 2014). Species of *Thoropa* and most species of *Cycloramphus* have RM 19. The females lay their eggs exposed on humid rocks, in rock crevices, or on tree roots above water; tadpoles are exotrophic and semiterrestrial, living on rock and rock crevices in a water film or in the water-land interface in the splash zones near waterfalls (Bokermann 1965, Heyer 1983a, b). All species in *Zachaenus* have RM 21, in which eggs hatch into endotrophic tadpoles that complete their development in concealed nests placed under leaf litter (Lutz 1944, Zocca *et al.* 2014, Almeida-Silva *et al.* 2019). Some species of *Cycloramphus* have RM 22, in which females lay eggs concealed on land; the female remains in the nest, which usually is placed in natural or constructed cavities. The larvae hatch into endotrophic tadpoles that climb on the female's dorsum where they complete their development (Heyer and Crombie 1979, VKV pers. obs.). Among the few anurans that have RMs 19, 21, and 22 (Haddad and Prado 2005) are *Indirana* (Ranixalidae) and *Petropedetetes* (Petropedetidae) (RM 19, Ranoidea); *Eupsophus* (RM 21, Alsodidae, Hyloidea); *Rhinoderma* (RM 22, Rhinodermatidae, Hyloidea); and *Leiopelma* (RM 22, Leiopelmatidae, Ranoidea) (Wells 2007). Detailed anatomical and ontogenetic comparisons among larvae are lacking to test homology in the cases mentioned, and similarities observed are probably result of convergence in the evolutionary history of Anura. Likewise, the lack of natural history data and a stable phylogeny for Cycloramphidae preclude speculations as to the origin of reproductive specializations and tadpole morphotypes in this family. The monophyly of the family is debated (Frost *et al.* 2006, Grant *et al.* 2017, Jetz and Pyron 2018, Streicher *et al.* 2018) and the familial internal relationships are poorly resolved (Verdade 2005, Weber *et al.* 2011, Fouquet *et al.* 2013, Barros 2016, Sabbag *et al.* 2018, de Sá *et al.* 2019).

*Cycloramphus bandeirensis* is endemic to the open, high grasslands of Parque Nacional do

Caparaó, and was last encountered in 1981 (Heyer 1983a). Few data on the species are available; however, there are unpublished field notes of William Ronald Heyer and Paulo Emilio Vanzolini housed at the library of the Museu de Zoologia da Universidade de São Paulo (MZUSP). During a series of herpetological surveys in the Caparaó Mountains, we collected new specimens and eggs of *C. bandeirensis*, which enable us to elucidate the natural history and breeding biology of Cycloramphidae.

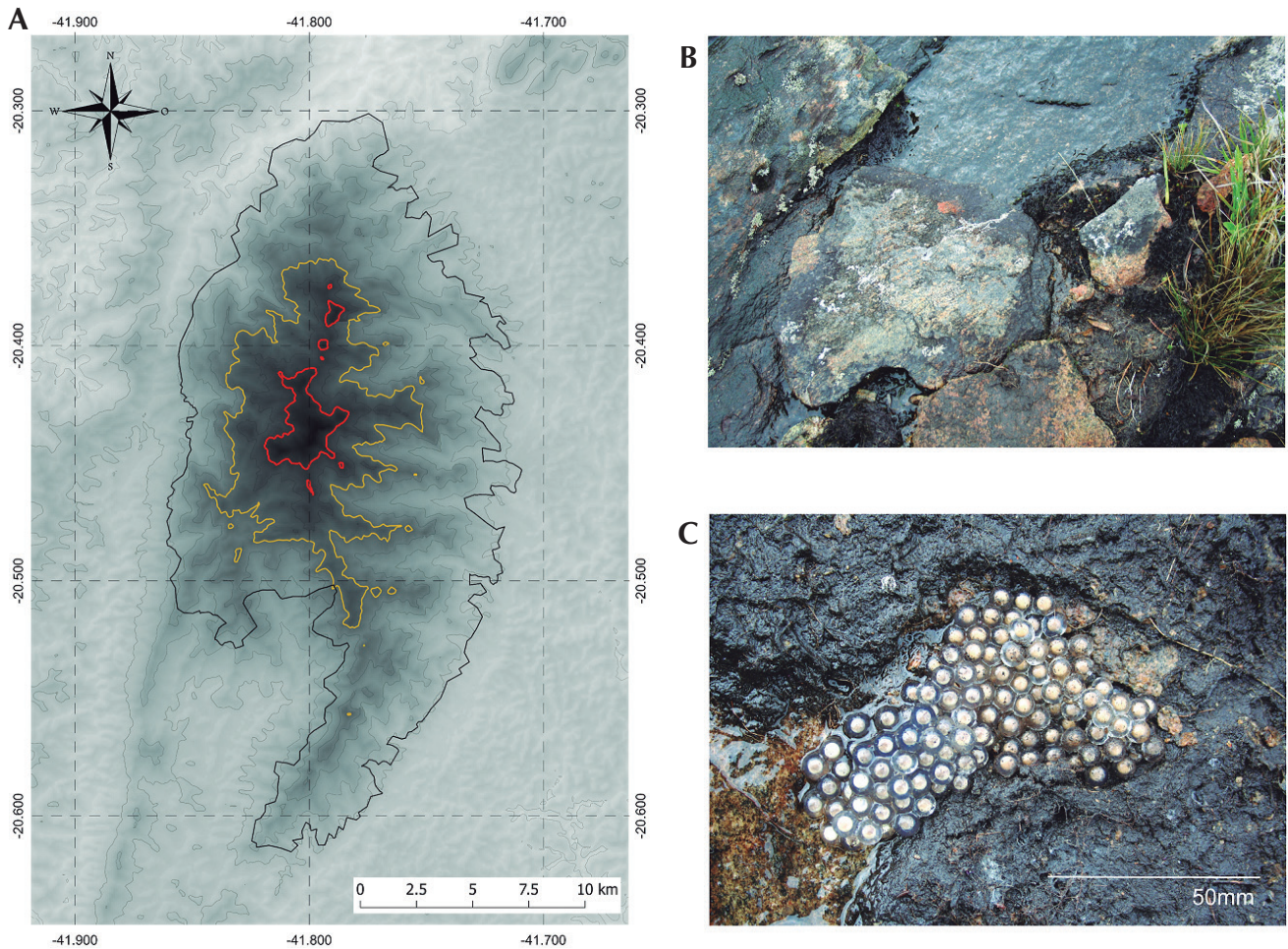
## Materials and Methods

Fieldwork was conducted at the type locality of *Cycloramphus bandeirensis* in the high grasslands of Parque Nacional do Caparaó, southeastern Brazil (Figure 1; see Rodrigues *et al.* 2009). We sampled the area between October and January from 2004–2008 for a total of 50 days during the rainy season.

Forests and high grasslands from 800–2890 m were surveyed on both the west and east sides of the park. We searched for frogs by walking in streams, as well as along the margins of the streams, in grasslands by day and at night, and turned over scattered rocks in the open field. We calculated the area (km<sup>2</sup>) of high grasslands (2000–2890 m) and the range of occurrence of *C. bandeirensis* based on elevations between 2450 and 2890 m in the park using topographic contour lines and calculating surface area in QGIS (2019).

We collected adults and eggs in the field (20°25'59" S, 41°47'54" W; 2770 m a.s.l., WGS 84). An egg mass was found under a rock in the open field; the eggs were placed in a plastic box and transported to the laboratory, where we divided the egg mass into two groups that were maintained in separate closed plastic boxes. One box was kept on a dark shelf at room temperature (22–32°C), and the other was refrigerated at 6–10°C. After hatching, tadpoles were reared until metamorphosis in a terrarium; emergent rocks were placed in water in the bottom of the





**Figure 1.** (A) Border of Parque Nacional do Caparaó (black line); high grasslands in the park (2000–2890 m; yellow line); IUCN website distribution (Silvano and Heyer 2004; green line); and high grasslands where specimens of *Cycloramphus bandeirensis* were recorded (2450–2890 m; red line). Reproductive site (B) and spawn (C) on underside of rock (Photos: J. Cassimiro). Source map modified from Ministério do Meio Ambiente (<http://www.mma.gov.br/governanca-ambiental/geoprocessamento>) with QGIS (2019).

terrarium and water was pumped over the rocks. Some of the rocks provided moist surfaces where the larvae could feed and hide. The tadpoles were mainly fed banana rind, supplemented with wet rabbit pellets, lettuce, and fish food flakes. Individuals captured in the field were euthanized with an overdose of anesthetics (GBBPEA 2013), fixed in 5% formaldehyde (eggs and tadpoles) or 10% formaldehyde (adults), and stored in formalin (eggs and tadpoles) or in ethanol 70% (adults). We deposited voucher specimens at the Museu de Zoologia da Universidade de São Paulo (MZUSP 133852, 133898, 135668–78) and Universidade Federal do ABC (ZUFABC 30–35).

### Tadpoles

The larvae are staged according to Gosner (1960). We measured 28 tadpoles between Stages 26 and 42 with an ocular grid in a stereomicroscope to the nearest 0.01 mm (Table 1). Nomenclature and measurements follow those of Altig and McDiarmid (1999): total length (TL), body length (BL), tail muscle height (TMH), tail muscle width (TMW), dorsal (DFH) and ventral fin heights (VFH), body maximum width (BMW), body maximum height (BMH), eye diameter (ED), frontonasal distance (FN), nostril major axis (N), and oral disc width (OD).

In addition we measured internarial distance (IND), eye–nostril distance (END), and interorbital distance (IO) of Scrocchi and Lavilla (1986).

#### *Advertisement Call*

Advertisement calls were recorded with a Panasonic (RQ-L31) tape recorder and a directional microphone (Yoga HT81) in October 2005 (6°C air temperature;  $N = 17$  calls from a single male MZUSP 135674). We digitized the tape recordings at a sampling frequency of 44.1 kHz and 32 bits resolution with Adobe Audition 3.0. Fourteen of the 17 calls could be successfully isolated from the background noise caused by the high wind in Audacity® version 2.1.1 (Audacity 2016) by applying successive low-pass and high-pass filters based on each call oscillogram. We used the R package Seewave to analyze these isolated calls (Sueur *et al.* 2008), setting frame width with 1024 points and Hann window. Acoustic parameters include those proposed by Kohler and collaborators (2017) as guidelines to bioacoustical descriptions focused on taxonomy—viz., call length, note length, number of notes per call, number of pulses per note, interval between notes, call rate, pulse rate, and call dominant frequency (kHz). We used note-centered terminology (e.g., fig.7D, Kohler *et al.* 2017) and calculated temporal parameters by the interval between the 10% levels at the leading and trailing edges of the oscillogram.

#### **Results**

*Cycloramphus bandeirensis* was the only species in the genus that we found at the study area, and all frogs were encountered in high grasslands at elevations above ~2,450 m. Adults and juveniles were found from the end of October to December under small (~60 cm diameter × 20 cm high), scattered rocks; none was encountered in the stream. Individuals captured and handled were not aggressive.

We found the spawn in the same microhabitat (Figure 1B, C) under a small rock in the open field and not near a stream. The egg mass collected on 05 November 2005 contained about 130 eggs (egg diameter  $6.3 \pm 0.68$  mm; yolk diameter  $4.2 \pm 0.47$  mm;  $N = 57$ ; ZUFABC 30). An adult was under the same rock, but it escaped before we could determine its sex. The first hatching in captivity occurred 11 days later (16 November 2005) in the eggs maintained in the dark at room temperature. Tadpoles from the eggs maintained in the refrigerator hatched within another 3 or 4 days. All tadpoles hatched at Stage 26 and did not feed for approximately 10 days while yolk was still visible in the gut.

Tadpoles were primarily active at night; they usually were attached to the underside of the rocks in the terrarium. They moved across the wet surfaces of the rocks by affixing their mouth to the rock and then pulling the body forward by abdominal muscular contraction. If disturbed, the tadpoles undulate the muscular tail rapidly resulting in a small jump across the surface. Some tadpoles fell in the water at the bottom of the terrarium, and although they were able to swim, they rapidly attempted to climb up a rock or the terrarium wall; if unsuccessful, they drowned. All tadpoles metamorphosed within 25 days.

*Tadpole Description* ( $N = 28$ , ZUFABC 31–35, Figure 2, Tables 1–2)

Body length less than one-third total length. Body depressed in lateral view, elliptical in dorsal view. Tadpoles in Stages 26–29 rounded and globular, both in dorsal and lateral aspects owing to the presence of yolk. Snout rounded. Nares small, dorsolateral, oval, without ornamentation, positioned closer to the snout than to the eyes. Interorbital distance approximately 25% greater than internarial distance. Eyes lateral and oriented dorsolaterally; eye diameter nearly 20% body length. Distinct meniscus in iris (Miranda-Ribeiro 1926, Barrasso *et al.* 2013)



formed by a dorsal expansion that initially develops in Stage 33, reaching full development in Stage 35. A tiny sinistral spiracle aperture at the middle third of body visible in Stage 33. Intestine reversal loop displaced to the left of the abdomen. Tail musculature width in dorsal view half body width, tapered toward a rounded tail tip. Dorsal and ventral fins low, ventral fin higher than the dorsal one. Ventral fin arising from body-tail junction in first stages of development, restricted in Stages 33–42 to distal third of tail. Skin flap covering venter of larva in Stages 33–38 after yolk consumption; anterior margin posteriorly adjacent to oral disc with flap extending entire body and vent tube (Figure 1C). Posterior margin of flap shallowly bilobate. Lateral line system not visible. Oral disc anteroventral, two-thirds width of body. Marginal papillae unpigmented, cylindrical, longer than wide, distributed in a single row interrupted by a large anterior gap. Submarginal papillae absent. In tadpoles from Stages 26–29, oral disc with denticles and poorly keratinized jaw sheath. Labial tooth row formula (LTRF) at Stage 29: 2(2)/2(1); variable from Stages 33–42: 2(2)/3(1), 2(2)/3(1,3), or 2(2)/3(1,2,3). Rows P-1 and P-2 equal in length, and P-3 two-thirds P-1. Jaw sheath chisel-shaped with eight to ten short triangular serrations, six serrations per 0.25 linear millimeter.

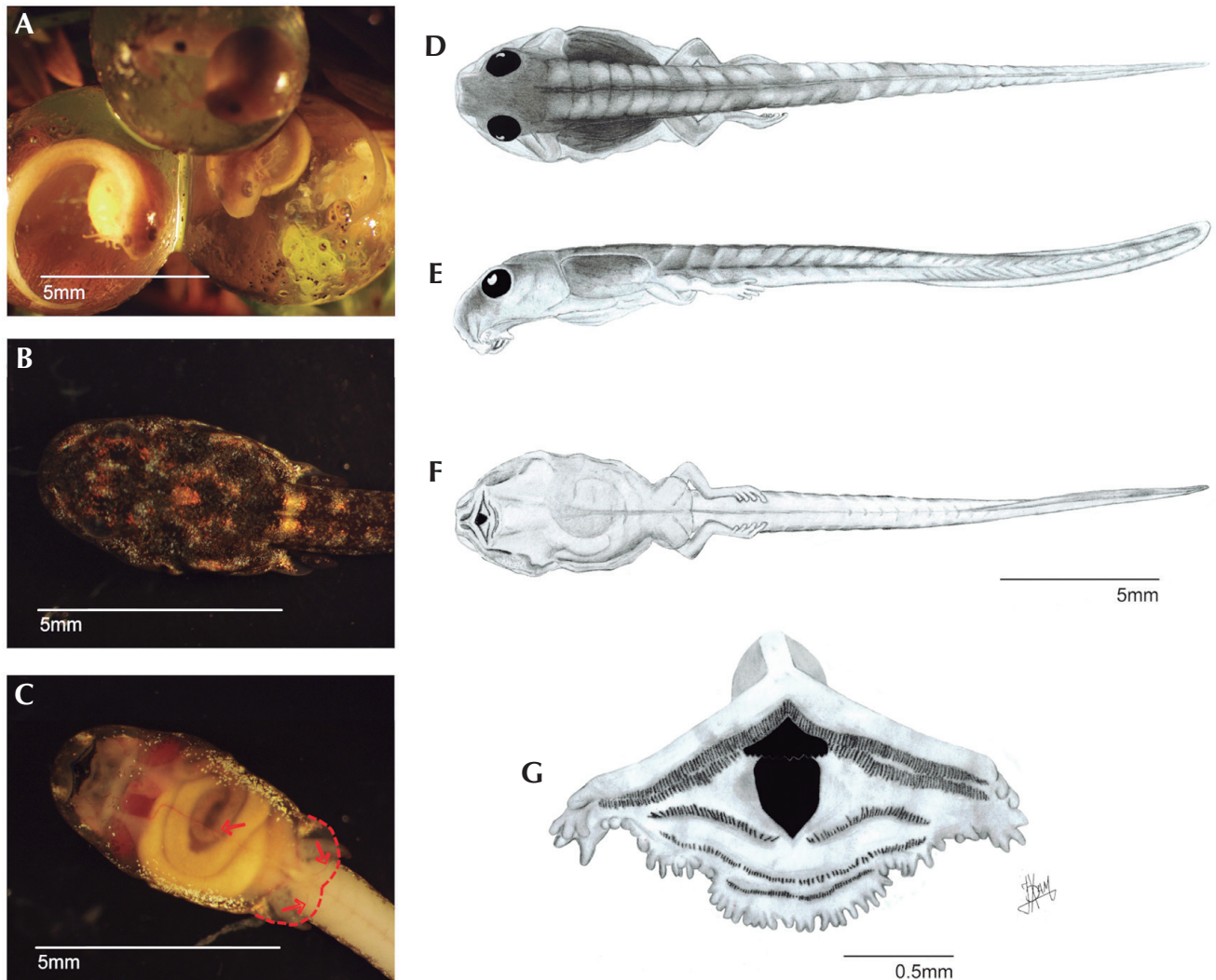
When alive, tadpoles with white and reddish metallic hues owing to presence of iridophores and erythrophores (Figure 2B, C). Iridophores distributed along the body, but especially laterally. In preservative, body and tail coloration pale cream and ochre with melanophores evenly distributed dorsally, denser on lateral body, and forming serial bands in the tail. Venter pale white covered by translucent skin flap.

#### Advertisement Call (N=14, Figure 3, Table 3)

Male *Cycloramphus bandeirensis* call from beneath scattered small rocks in humid parts of the grasslands; they are not found in streams. Calls were heard by day and early at night, usually

**Table 1.** Measurements of the tadpoles of *Cycloramphus bandeirensis* (lines). Each column indicates Gosner (1960) stage and the number of tadpoles measured (N). Range is presented when more than one tadpole was available. Abbreviations follow those from Material and Methods section.

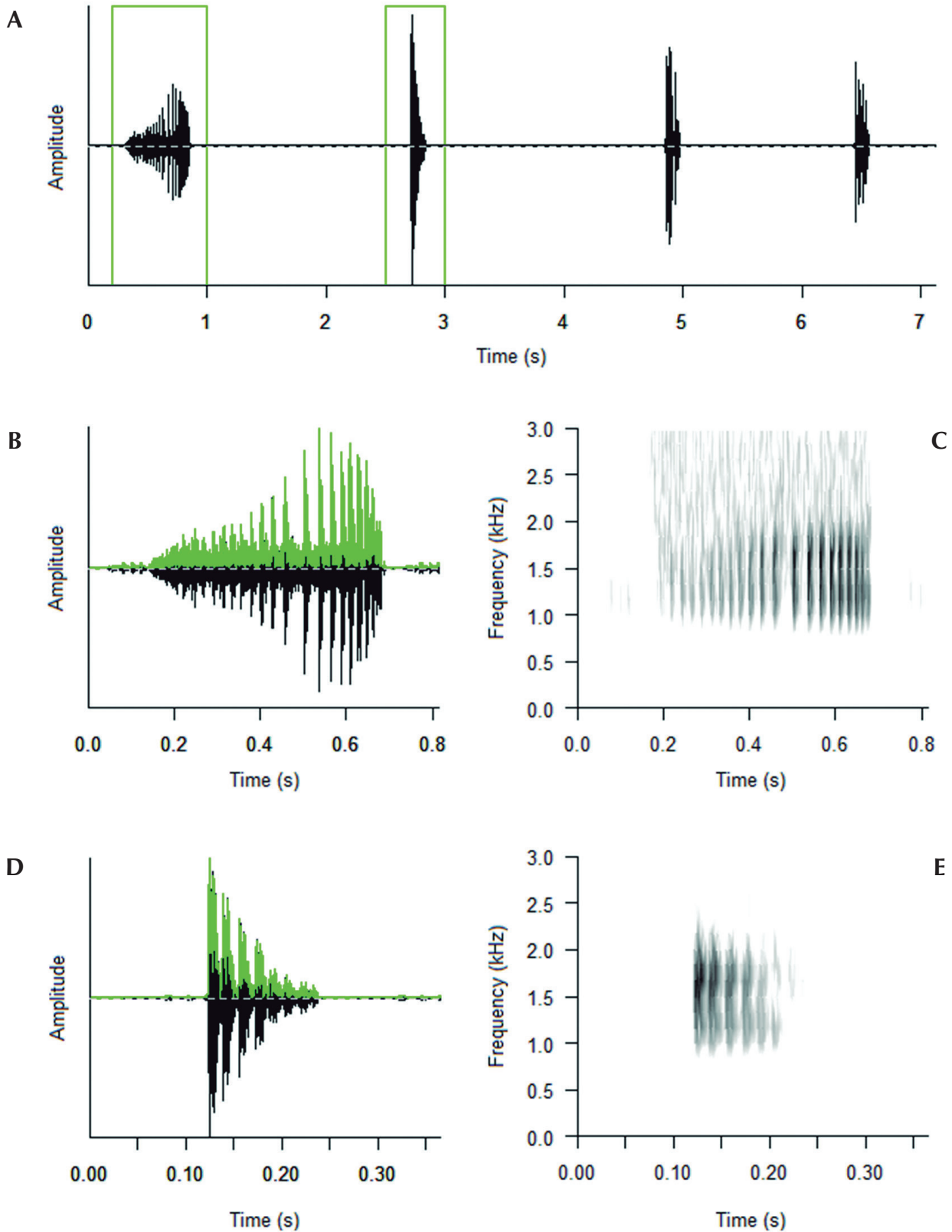
Stage	26	27 (N = 3)	28 (N = 4)	29 (N = 3)	33	34	35 (N = 3)	36 (N = 4)	37 (N = 4)	38	42 (N = 3)
TL	11.50	11.67–14.70	13.83–18.00	15.00–17.00	21.00	21.50	21.83–23.33	21.5–24.00	22.83–24.42	24.70	19.50–21.50
BL	3.20	3.29–3.60	3.43–3.90	3.58–4.13	4.34	4.95	4.88–5.45	4.60–5.16	4.60–6.14	4.60	5.34–5.66
BMW	3.10	3.28–3.49	3.01–3.34	3.12–3.23	2.59	3.23	3.03–3.85	3.31–3.75	3.54–4.11	4.00	3.26–3.41
TMW	0.80	0.88–0.96	0.9–1.03	0.89–1.30	1.45	1.33	1.47–1.61	1.57–1.80	1.6–1.82	1.70	1.66–1.81
BMH	2.40	2.53–2.89	2.37–2.86	2.44–2.70	2.31	2.34	2.61–2.79	2.41–2.88	2.51–2.71	2.70	2.32–2.76
DFH	0.13	0.19–0.26	0.21–0.25	0.18–0.23	0.20	0.15	0.20–0.22	0.20–0.23	0.21–0.24	0.20	0.16–0.20
TMH	0.91	0.67–1.26	1.08–1.14	1.07–1.22	1.29	1.22	1.22–1.49	1.14–1.42	1.31–1.49	1.50	1.13–1.42
VFH	0.160	0.19–0.29	0.26–0.30	0.26–0.34	0.29	0.16	0.27–0.39	0.25–0.30	0.27–0.34	0.30	0.16–0.25
IO	0.65	0.58–0.68	0.59–0.71	0.56–0.69	1.23	0.95	1.10–1.23	1.07–1.36	1.07–1.26	1.20	0.97–1.14
IND	0.48	0.63–0.72	0.49–0.66	0.67–0.77	0.90	0.84	0.88–0.94	0.91–1.06	0.84–0.92	0.80	0.75–0.96
END	0.20	0.32–0.40	0.25–0.41	0.34–0.46	0.62	0.61	0.63–0.63	0.62–0.78	0.60–0.77	0.70	0.58–0.70
FN	0.09	0.14–0.17	0.13–0.27	0.21–0.28	0.31	0.50	0.30–0.43	0.32–0.52	0.27–0.49	0.30	0.23–0.30
ED	0.39	0.45–0.59	0.46–0.57	0.57–0.64	0.75	0.67	0.83–0.96	0.77–1.01	0.88–1.01	0.90	0.80–0.87
N	0.03	0.02–0.03	0.02–0.05	0.03–0.05	0.11	0.10	0.10–0.12	0.09–0.12	0.09–0.10	0.10	0.08–0.10
OD	0.75	0.89–1.05	0.87–1.19	1.15–1.42	1.89	1.99	1.88–2.21	2.10–2.27	1.91–2.40	2.10	1.83–1.91



**Figure 2.** Photographs of (A) eggs of *Cycloramphus bandeirensis* and tadpole in (B) dorsal and (C) ventral view. Line drawings of the tadpole (ZUFABC 031, Stage 37): (D) dorsal, (E) lateral, and (F) ventral views; (G) oral apparatus. Dashed red line shows skin flap contour. Red arrows point to blood vessels irrigating the ventral flap.

in misty and rainy weather. Vocal activities intensify in the evening, but decrease and terminate when the temperature drops at night. In October 2006, we heard 20 males, spaced approximately 30 m from one another, calling simultaneously. The complex advertisement call consists of three or four notes (Figure 3A), with the first note differing from the following in length, amplitude modulation, and number of pulses (Figure 3B–E). The range of the first note length is 0.27–0.80 s ( $0.53 \pm 0.51$  s), whereas the

lengths of the second, third, and fourth notes range from 0.08–0.24 s ( $0.13 \pm 0.03$  s). Calls with three or four notes are equally common ( $N = 7$ , for both) lasting from 3.88–7.59 s ( $5.85 \pm 1.07$  s). The call rate is 2.45 calls/min. The interval between the first and second note is 1.13–4.37 s ( $2.36 \pm 0.83$  s), between the second and the third 1.28–2.36 s ( $1.84 \pm 0.34$  s), and between the third and fourth 1.20–1.75 s ( $1.47 \pm 0.22$  s). The first note has 9–25 distinguishable pulses ( $15.06 \pm 4.45$ ), whereas the following notes range from



**Figure 3.** Advertisement call of *Cycloramphus bandeirensis* (MZUSP 135674, SVL = 40 mm). (A) Oscillogram of the entire call, highlighting the first and second note types. (B, D). Oscillograms of first and second notes (sound envelope in green). (C, E) Spectrograms of first and second notes. Records: J. Cassimiro.



**Table 2.** Comparative external morphology of the semiterrestrial *Cycloramphus* tadpoles, modified from Lima *et al.* (2010). Data extracted from direct observation and literature. (TL) total length in mm; (BL) body length; (BS) body shape in dorsal view; (ED) eye diameter; (NP) nares position relative to eyes and snout; (AF) abdominal flap; (VF) ventral tail fin; (NV) not visible; (BI) bilobate; (LTRF) labial tooth row formula. Stages follow Gosner (1960). \*Nunes-de-Almeida, Zamudio and Toledo (2016) refer to two tadpoles collected at Gosner Stage 25 along the text, but Fig. 2C shows a tadpole Stage 30; they also report spiracle “dual lateral” for *C. lutzorom*, but as a misinterpretation from the original table where they wrote down “both small” referring to both specimens.

Species	Stages	TL	BL/TL (%)	BS	ED/BL (%)	NP	AF	VF	LTRF	References
<i>C. bandeirensis</i>	26–42	11.5–24.7	20–28	elliptic	13–21	closer to snout	NV/shallowly BI	along tail, distal 1/2, distal 1/3	2(2)/2(1), 2(2)/3(1), 2(2)/3(1,3), 2(2)/3(1, 2, 3)	present paper
<i>C. boraceiensis</i>	37–42	27.2–30.7	23	elliptic	18–22	equidistant	BI	distal 1/3	2/3, 2(3)/1, 2(2)/3, 2(2)/3(1)	Heyer <i>et al.</i> (1990)
<i>C. brasiliensis</i>	41	37.5	25	-	17	equidistant	BI	distal 1/2	2/3(1)	Heyer <i>et al.</i> (1990)
<i>C. fuliginosus</i>	41	43.5	19	-	12	-	-	distal 1/2	2/3	Heyer <i>et al.</i> (1990)
<i>C. izecksohni</i>	34–42	22.0–32.0	23–29	elliptic	12–18	closer to eyes	shallowly BI/not BI	distal 1/2, distal 1/4	2/3, 2(2)/3(1)	Heyer (1983b)
<i>C. lithomimeticus</i>	37	24.3	27	elongated	14	closer to snout	BI	distal 1/2	2(2)/3(1)	Silva and Overnay (2012)
<i>C. lutzorom</i>	36	26.2	23	elliptic	15	equidistant	shallowly BI/not BI	distal 1/2	2(2)/3	Lima <i>et al.</i> (2010).
<i>C. rhyakonastes</i> *	25	22.4	24	elliptic	15	equidistant	shallowly BI	distal 1/2	2/3	Nunes-de-Almeida, Zamudio and Toledo (2016)
<i>C. valae</i>	35–36	26.4–29.3	24–29	oval	16–19	equidistant	shallowly BI	distal 1/3, distal 1/2	2(2)/3(1)	Heyer (1983b)

5–10 ( $7.07 \pm 1.18$ ) pulses (Figure 3B, D). The pulse rate in the first note is 29.82–55.56 pulses/s ( $42.9 \pm 5.94$ ), and in the following ones, 25.00–90.00 pulses/s ( $55.51 \pm 14.63$ ). The dominant frequency of the advertisement call ranges from 1.03–1.98 kHz ( $1.43 \pm 0.33$  kHz). The dominant frequency of first note is 0.99–1.59 kHz ( $1.14 \pm 0.14$  kHz), of second note is 1.03–1.97 kHz ( $1.5 \pm 0.32$  kHz), of third note is 1.05–2.36 kHz ( $1.50 \pm 0.40$  kHz), and fourth note is 1.09–1.86 kHz ( $1.39 \pm 0.28$  kHz).

## Discussion

We chose to undertake fieldwork in the open grasslands of Parque Nacional do Caparaó, based on the unpublished field notes of W. R. Heyer associated with the specimen of *Cycloramphus bandeirensis* in the collection. Heyer reported finding the single specimen collected in 1981 under a rock in the rocky fields. However, we expected to find the frogs under rocks near and along the stream running through the open grassland, given the semi-aquatic morphotype of *C. bandeirensis* (Heyer 1983a), which usually is associated with stream-dwelling species with semiterrestrial tadpoles. Contrary to our expectations, we only found the frogs and their eggs under the rocks in the rocky fields in the grasslands. All other semi-aquatic *Cycloramphus* are forest dwellers living in fast-flowing streams with exotrophic, semiterrestrial tadpoles associated with Reproductive Mode (RM) 19.

Haddad and Prado (2005) defined RM 19 as “Eggs on humid rocks, in rock crevices, or on tree roots above water; exotrophic semiterrestrial tadpoles living on rocks and rock crevices in a water film or in the water-land interface.” The eggs of *Cycloramphus bandeirensis* are attached to wet rocks, but they are hidden beneath the rocks. This is a significant departure from the habits of most stream dweller cycloramphids which leave the eggs exposed and easily visible. A second difference involves the site of egg deposition with respect to water. The eggs of *C. bandeirensis* are terrestrial; they are neither

associated with bodies of water nor wet vertical rocks. However, like RM-19 larvae, the tadpoles of *C. bandeirensis* crawl over wet rocks; thus, they meet the criterion of living in a “water film or in the water-land interface.” Together, these features distinguish the reproductive mode of *C. bandeirensis* as a variant of RM 19—i.e., terrestrial eggs concealed under humid rocks in rocky fields, and exotrophic, semiterrestrial tadpoles living under wet rocks.

As in many frogs, especially those laying eggs in terrestrial environment, reproduction in *Cycloramphus bandeirensis* occurs during the rainy season from late October–December (Gomez-Mestre *et al.* 2012). Likewise, other cycloramphids with terrestrial eggs and tadpoles breed in the rainy season (Heyer and Crombie 1979, Mollo-Neto *et al.* 2016), and among the cycloramphids inhabiting forest streams and having semiterrestrial tadpoles, the breeding season also may be restricted to the rainy season (Giaretta and Facure 2004) or occur throughout the year (e.g., *C. dubius*; Giaretta and Cardoso 1995).

The spawn of *Cycloramphus bandeirensis* with 130 eggs is larger than that of its congeners: 30–40 eggs in *C. faustoi* and *C. stejnegeri* (Heyer and Crombie 1979, Brasileiro *et al.* 2007); 50 eggs in *C. boraceiensis* (Heyer *et al.* 1990); 70–80 in *C. dubius* (Giaretta and Cardoso 1995); and 80–100 in *C. fuliginosus* (Lutz 1929). In *Thoropa miliaris* (Spix 1824), there may be more than one spawning per breeding site (Giaretta and Facure 2004); this may be a possibility in *C. bandeirensis*. Males of stream-dwelling *Cycloramphus* are territorial and actively defend their eggs by biting (Giaretta and Cardoso 1995, Giaretta and Facure 2003; Hartman *et al.* 2003). In the species that dwell in forest-litter, at least one adult stays with the egg clump (Heyer and Crombie 1979, Brasileiro *et al.* 2007). While we cannot state that parental care occurs in *C. bandeirensis*, the fact that an adult was found with the egg mass suggests that this may be the case.

At hatching, the tadpoles of *Cycloramphus bandeirensis* have the features associated with

**Table 3.** Characteristics of advertisement calls for species of *Cycloramphus* with formally described calls. Modified from Lima *et al.* (2010) and including *C. bandeirensis*. The call type follows Heyer (1983a). \*indicates we reclassified the call type based on its length.

Species	Habitat	Call type	Call length (s)	Dominant frequency (kHz)	References
<i>C. bandeirensis</i>	high grassland	C	5.09–7.59	1.03–1.97	present paper
<i>C. bolitoglossus</i> .	litter	B	3.95–4.48	1.32–2.44	Lingnau <i>et al</i> 2008
<i>C. boraceiensis</i>	stream	A	0.03–0.06	0.50–3.50	Heyer and Mello (1979), Heyer (1983a), Heyer <i>et al.</i> (1990)
<i>C. brasiliensis</i>	stream	C	1.00	0.54–1.80	Heyer (1983a)
<i>C. dubius</i>	stream	A	0.20	1.20–3.10	Giaretta and Cardoso (1995)
<i>C. eleutherodactylus</i>	forest floor	B	4.04–4.34	0.80–2.20	Brasileiro <i>et al.</i> (2007)
<i>C. faustoi</i>	forest floor	B	2.53–4.17	0.90–2.20	Brasileiro <i>et al.</i> (2007)
<i>C. izecksohni</i>	stream	A	0.02–0.03	1.30–2.7	Heyer (1983b)
<i>C. juimirim</i>	stream	B	0.40–0.70	1.20–1.70	Haddad and Sazima (1989)
<i>C. lutzorum</i>	stream	A*	0.05–0.06	0.98–1.39	Lima <i>et al.</i> (2010)
<i>C. ohausi</i>	stream	B	1.20–1.50	0.80–1.00	Heyer (1983a)
<i>C. rhyakonastes</i>	stream	A	0.04–0.05	1.0–2.5	Heyer (1983a)
<i>C. semipalmatus</i>	stream	A	0.02–0.04	0.20–5.00	Heyer (1983a), Heyer <i>et al.</i> (1990)
<i>C. valae</i>	dripping rock	B	1.30–1.90	1.25–2.25	Heyer (1983b)

semiterrestrial larvae—viz., the body shape, amount of yolk, presence of caudal fins, and conspicuously keratinized denticles and jaw sheath. Their nocturnal activity, apparent preference for attaching to the ceiling of the rocky refuges, and mode of locomotion are noteworthy. The tadpoles move on the rocks by affixing their mouth to the surface and contracting the abdomen to pull the body forward. When disturbed, they quickly undulate the muscular tail to propel the body forward. Veeranagoudar *et al.* (2009) described a similar, but more vigorous movement, as “flipping behavior” for the semiterrestrial tadpoles of *Indirana beddomii*

(Günther, 1876) (Ranixalidae). The tadpoles of *Thoropa* Cope, 1865 use similar movements to avoid predation by trap-jaw ants (genus *Odontomachus*) (Facure and Giaretta 2009).

Semiterrestrial tadpoles have an abdominal flap of skin that without suction, supposedly facilitates adherence to rocks (Wassersug and Heyer 1983, Altig and McDiarmid 1999). The tadpoles of *Cycloramphus bandeirensis* have the abdominal flap; however, we observed that in the presence of a film of running water, the water flows beneath the flap, moving the body of the tadpole. These observations are consistent with the shallow-water microhabitat preferences and



behavior of the semiterrestrial tadpoles of *Thoropa miliaris* (Rocha *et al.* 2002). These observations, together with the elaboration of the abdominal blood vessels (Figure 3C) in the flap and the fact tadpoles drown if submerged, suggest that the flap may have an alternate function in larvae of *C. bandeirensis*.

Burggren and West (1982), studying bimodal gas exchange in tadpoles of *Lithobates catesbeianus* (Shaw, 1802), demonstrated that gas exchange through the skin contributes more to oxygen uptake than through the gills in aquatic tadpoles in initial stages, and that the contribution of the skin increases as that of the gills decreases during development. Semiterrestrial tadpoles of cycloramphids usually have relatively small branchial baskets and gill filters (Wassersug and Heyer 1983). Moreover, the larvae are not submerged such that they could maintain a high influx of water to branchial chambers; this may indicate that they rely more on cutaneous rather than gill respiration. We propose the abdominal flap of cycloramphid semiterrestrial tadpoles increases the surface area of the larvae, thereby accommodating cutaneous gas exchange.

Morphologically, the tadpoles of *Cycloramphus bandeirensis* resemble other semiterrestrial cycloramphid larvae. The positions of eyes and nares, snout shape, LTRF, shape of the skin flap edge, and the extension of caudal fins (Table 2) characterize larvae in the semiterrestrial guild. The LTRFs vary during development, mainly with respect to the presence of gaps in the posterior rows of denticles. The presence and development of the iris meniscus, the skin flap, spiracle, and reduced caudal fin are most obvious in tadpoles of Stages 33–38. Unfortunately, there is little information on the ontogeny and phenotypic variation in descriptions of tadpoles of *Cycloramphus* (e.g., Lima *et al.* 2010, Nunes-de-Almeida *et al.* 2016).

Aside from the ontogenetic variation described above, we noted that the development of the oral disc and limbs in tadpoles of *Cycloramphus bandeirensis* are not consistent with the descriptions of Gosner (1960) stages for

generalized tadpoles. *Cycloramphus bandeirensis* tadpoles hatched with internal gills and small limb buds (Stage 26), but the oral disc is not fully developed until Stage 27 (oral disc fully developed in Stage 25). Similarly, when the forelimbs emerged (Stage 42), parts of the oral disc were keratinized (Stage 39 of Gosner). Variations in developmental rates of fore- and hind limbs in stream tadpoles were reported for *Arthroleptides martiensseni* Nieden, 1911 “1910” (Petropedetidae) (Drewes *et al.* 1989) and *Indirana beddomii* (Ranixalidae) (Veeranagoudar *et al.* 2009). Similarly, a delay in the development of oral apparatus was reported for the endotrophic terrestrial tadpoles of *Eupsophus emiliopugini* Formas, 1989 (Alsodidae) relative to that of exotrophic species (Formas 1989, Candioti *et al.* 2011). These apparent heterochronic shifts in development of different types of frog larvae require further investigation.

Comparative analyses of the advertisement calls in *Cycloramphus* are hampered by two different kinds of descriptions—i.e., call-centered descriptions versus note-centered. There are short simple calls, long simple calls, and complex calls, that approximately correspond to Types A, B, and C proposed by Heyer (1983a). Only *Cycloramphus bandeirensis* and *C. brasiliensis* (Steindachner 1864) are known to have a complex advertisement call (Type C, Heyer 1983a), which includes characteristics of Type-A (one or two short metallic notes) and Type-B (one long note) calls of Heyer (1983a). The advertisement calls of seven species of *Cycloramphus* were described after 1983; those of *C. bolitoglossus* (Lingnau *et al.* 2008), *C. eleutherodactylus*, *C. faustoi* (Brasileiro *et al.* 2007), *C. juimirim* (Haddad and Sazima 1989), and *C. valae* (Heyer 1983b) are long, Type-B calls. Although the advertisement call of *C. lutzorum* was described as Type B (Lima *et al.* 2010), it actually is Type A; the authors used a scale of milliseconds, giving the impression of a long note, but the note length is 0.05–0.06 s, as observed in *C. boraceiensis*, for example (Table

3). There is no clear association of types of advertisement calls with taxonomy, habitat, or phenotypic characters; thus far, stream-dwelling species of *Cycloramphus* have simple long, short, or complex calls, whereas all the forest-litter *Cycloramphus* have long simple calls.

Without a robust phylogenetic framework, we cannot discuss evolution of ecomorphotypes, reproductive specialization, or the association of call types with different clades. Cycloramphidae includes species with RMs 19, 21, 22, and a variant to RM 19, two larval morphotypes, and three types of advertisement calls. From the unpublished academic works available, RMs 21 and 22 would have risen once in the group from an ancestor with RM 19 (Verdade 2005, Barros 2016). Alternately, the molecular data points to multiple origin of terrestrial reproduction (RMs 21 and 22) (de Sá *et al.* 2019).

#### Conservation Status

*Cycloramphus bandeirensis* is classified as Data Deficient by the IUCN (Silvano and Heyer 2004). Our data and those from later expeditions (Zornosa-Torres *et al.*, 2019) indicate the species is endemic to the open rocky fields of the high grasslands of Parque Nacional do Caparaó. The total area of the high grassland of the park (2000–2890 m a.s.l.) is 78.7 km<sup>2</sup> (yellow line, Figure 1A). However, we only found specimens above ~2450 m; therefore, the extent of occurrence of the species would be 9.3 km<sup>2</sup> (red line, Figure 1A). According to the IUCN website, the species only occurs between elevations of 2450 and 2740 m (Silvano and Heyer 2004), which is consistent with our findings. However, the polygon on the map does not match altitudinal isoclines; thus, it includes areas of forest below 2000 m with areas of high grassland (green line, Figure 1A).

The national park is protected area but includes camping facilities at altitudes of ~2370 m in “Terreirão,” and ~2200 m in “Casa Queimada.” Excursions to Bandeira Peak are common, but seemingly do not threaten the frogs. Although

*Cycloramphus bandeirensis* occurs within a preserve, the species has stringent environmental requirements involving high and continuous levels of humidity for reproduction associated with its preferred microhabitat isolated on a mountaintop. It is a vulnerable species under the rarity indicator proposed by Toledo *et al.* (2014). The species also is related to cycloramphids that have undergone local population crashes in southeastern Brazil [e.g., *Thoropa lutzii* Cochran, 1938, *T. petropolitana* (Wandolleck, 1907), *Cycloramphus boraceiensis*, *C. dubius*, and *C. semipalmatus* (Miranda-Ribeiro, 1920)]; see Verdade *et al.* (2011) for a summary on the decline of amphibians in Brazil. Cycloramphid frogs are considered evolutionarily distinct with a low diversification rate, and thus, more susceptible to human-driven environmental changes (Greenberg *et al.* 2018).

From the usual potential factors leading frogs to population crashes worldwide, the climate change is a plausible threat to Atlantic Forest frogs, especially for those restricted to small mountaintops. It is predicted that *Cycloramphus juimirim*, *C. lutzorum*, and *C. semipalmatus* will lack climatically suitable habitats by 2050/2070 (Vasconcelos *et al.* 2018). Although there are no specific projections as to the effects of climate change on the Brazilian high grassland ecosystems and their species, projections for other high-altitude ecosystems in Latin America include habitat reduction, species shift distribution, population declines, modifications in phenology, and increase in species invasions. [See Scarano *et al.* (2016) for summary of the plight of Brazilian high grasslands.] Additionally, fires are also plausible potential threats in high grasslands (Garey and Provete 2016). The species within the park seem free from chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) (Rodríguez *et al.* 2014), but the *Bd* cannot be ruled out as a potential threat (Garey and Provete 2016).

The information provided herein justifies withdrawal of *Cycloramphus bandeirensis* from the IUCN DD category, which is intended for species lacking adequate information to allow

direct or indirect assessment of risk of extinction based on distribution or population status (IUCN 2018). We propose the transfer *Cycloramphus bandeirensis* to the Vulnerable category (VU, d2) of IUCN, based on the restricted distribution and plausible threats (e.g., fires, and climatic global change) to the species that might lead to population declines in less than a century.

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