Zootaxa 5584 (2): 288–294
https://www.mapress.com/zt/

Correspondence
https://doi.org/10.11646/zootaxa.5584.2.9
http://zoobank.org/urn:lsid:zoobank.org:pub:B1562529-6980-4531-9BDA-D9BD14FE489B

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Singing tadpole: morphology and acoustic aspects of the tadpole of the Caatinga horned frog *Ceratophrys joazeirensis* (Anura: Ceratophryidae)

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The genus *Ceratophrys* Wied-Neuwied currently includes eight species found in South America. They are distributed from the Caribbean plains of Colombia and Venezuela to the Argentine Chaco and Pampas region in the southern part of the continent. Their range extends from the west coast of Ecuador and Peru, specifically in the Gulf of Guayaquil, to the east coast of Brazil (Frost 2024). The group inhabits a variety of environments, ranging from humid forests like the Amazon Rainforest in the north and the Atlantic Forest in the east of the continent to open and dry areas such as the dry forest in western Peru and the semi-arid Caatinga in northeastern Brazil (Faivovich *et al*. 2014; Frost 2024). It is known that the tadpoles of most *Ceratophrys* species exhibit carnivorous behavior (Ortiz *et al*. 2013; Schalk *et al*. 2014) and some are capable of acoustic communication (Natale *et al*. 2011; Salgado Costa *et al*. 2014). However, many aspects of larval biology remain unknown. Tadpoles of all species are described, with the exception of *Ceratophrys joazeirensis* Mercadal and *Ceratophrys testudo* Andersson. Here, we provide a description of the external morphology, as well as the acoustic aspects of the sounds emitted by the tadpoles of the scarcely known *C. joazeirensi*s.

We collected 32 tadpoles *of C. joazeirensis* on February 2 (26 tadpoles, during the nighttime) and February 4 (6 tadpoles, during the daytime) of the year 2024. The collection (Collection Authorization—ICMBio 73214-5) took place in a temporary pond located in Jussara municipality, Bahia State, in northeast Brazil (11°02'54.24"S, 41°58'17.04"W; 317 m a.s.l.). The municipality is situated within the semi-arid region, in the Caatinga biome. The pond was located within the urban perimeter and formed following heavy rains that occurred in December 2023 and January 2024. It featured emerging herbaceous vegetation, predominantly located at points further away from the center, as well as herbaceous, shrubby, and woody vegetation along the margins (area: 5804.5 m^2; temperature: 27 °C; depth: 23.15 cm). We collected the tadpoles using nets of various types and mesh sizes. Some tadpoles were reared in the laboratory until metamorphosis was completed. We euthanized the collected specimens with lidocaine. We preserved the tadpoles in 6% formalin, while the metamorphs were fixed in 10% formalin and preserved in 70% alcohol. The specimens studied are housed at the Herpetological Collection of the Universidade Federal do Recôncavo da Bahia (number: CHUFRB 0771–0792).

The characterization of the tadpole external morphology was based on 17 specimens at Gosner (1960) Stages: 27 (n = 1), 29 (n = 1), 30 (n = 3), 31 (n = 4), 33 (n = 2), 34 (n = 5) and 36 (n = 1). We obtained the morphometric measurements (in millimeters) from 11 tadpoles at Stages 31–34. Terminology follows Altig (1970) and Altig & McDiarmid (1999).

Accepted by F. Candioti: 19 Dec. 2024; published: 7 Feb. 2025

Measurements follow Lavilla & Scrocchi (1986) for total length (TL), body height (BH), body width (BW), maximum tail height (MTH), tail muscle height (TMH), eye diameter (ED), nostril diameter (ND), oral disc width (ODW), nostril-snout distance (NSD) and spiracle-snout distance (SSD); Mijares-Urrutia (1998) for dorsal fin height (DFH), ventral fin height (VFH), interorbital distance (IOD), internostril distance (IND), eye-snout distance (ESD) and eye-nostril distance (END); Altig & McDiarmid (1999) for body length (BL), tail length (TAL) and tail muscle width (TMW); Mira-Mendes *et al*. (2022) for spiracle width (SW); and Santos *et al*. (2017) for vent tube length (VTL) and vent tube width (VTW). The ratios between measurements are presented as ranges. We took the measurements using a stereoscopic microscope (Olympus SZ61) coupled with an ocular micrometer. Total length, body length, and tail length were measured with a digital caliper to the nearest 0.03 mm (Insize 1108).

During the collection of tadpoles, we noticed that some individuals made sounds both underwater and outside the water. Therefore, we recorded the sounds emitted by nine tadpoles (Stages 34–36) using a Tascan DR40x digital recorder coupled with a Yoga HT-320A directional microphone, with a sampling frequency of 44,100 Hz and a resolution of 16 bits. Since sound emission by tadpoles of some species of the genus *Ceratophrys* and *Gephyromantis* Methuen appears to be associated with predation activities (Natale *et al*. 2011; Reeve *et al*. 2011; Salgado Costa *et al*. 2014), we recorded the underwater sounds during their feeding. To perform the recordings, we placed the microphone inside a plastic container (44 x 33 x 13 cm) filled with water (depth: 3 cm; water temperature: 28.6 ºC) along with the *C. joazeirensis* tadpoles and shortly before releasing the *Physalaemus cicada* Bokermann and *Scinax x-signatus* (Spix) tadpoles, which were used as a food source and were collected in syntopy with the *C. joazeirensis* tadpoles. To prevent the microphone from contacting the water, we covered it with a sheet of latex, following the methodology of Natale *et al*. (2011) and Salgado Costa *et al*. (2014). Three feeding/recording events were performed each lasting ten minutes. The container held nine *C. joazeirensis* tadpoles, all of which were recorded in each of the three events, with individual emitters not being distinguished. Additionally, we recorded the emitted sound by three tadpoles (Stages 34, 34 and 36), immediately after removing them from the water, over a period of one minute, with the microphone approximately 30 cm away from the tadpoles. We analyzed all the sounds from the recording that had sufficient quality to allow for reliable analysis, using Raven Pro 1.6 program (Cornell University), which generated oscillograms, spectrograms, and power graphs. We constructed the spectrograms using FFT with 512 samples and a Hann window type. We measured six acoustic parameters: sound duration (s), dominant frequency (Hz), maximum frequency (Hz), pulse duration (s), interpulse interval (s), and pulse number. Prior to the analyses, we normalized the amplitude of all acoustic recordings to 0 dB using the Audacity 3.4.2 program.

Morphology. Body shape oval in dorsal view (BW/BL = 0.55–0.64) and globular-depressed in lateral view (BH/BW = 0.63–0.78). Body length approximately 0.35–0.39 times the total length (Table 1; Fig. 1). Snout slightly pointed in dorsal view and rounded in lateral view. Nostrils large and rounded (ND/ED = 0.41–0.52), reaching more than half of the eye diameter, with a projection on marginal rim, placed dorsolaterally, with laterally directed opening, and closer to the eyes than to the snout (END/ESD = 0.14–0.26) (Fig. 2A). Eyes medium-sized (ED/BH = 0.28–0.32), dorsolaterally positioned and directed. Spiracle sinistral, lateroventral, medium to wide (SW/BH = 0.19–0.26), without forming a tube and located in the posterior portion of the body (SSD/BL = 0.63–0.71). Spiracular opening posterodorsal and visible in lateral and ventral views (SW/BH = 0.19–0.26). Vent tube medial, short and wide (VTL/TAL = 0.05–0.07; VTW/VTL = 0.50–1.00), fused to the ventral fin, oblique, with dextral opening (Fig. 2B). Tail long, 0.61–0.65 times TL and 1.59–1.88 times BL, with acute tip. Tail musculature moderately developed (TMH/MTH = 0.34–0.48; TMW/BW = 0.26–0.38), reaching more than half of the body height (TMH/BH = 0.44–0.66), gradually tapering to the tip. Dorsal fin slightly higher than the ventral fin (DFH/VFH = 0.95–1.11, mean 1.05 ± 0.95), but both fins lower than the tail musculature (DFH/TMH = 0.70–1.0, mean 0.84 ± 0.12; VFH/TMH = 0.70–1.02, mean 0.83 ± 0.10). Dorsal fin arched, beginning at the body-tail junction. Ventral fin slightly arched. Lateral line system barely visible between the nostrils and the eyes. Oral disc terminal, anteriorly positioned, large (ODW/BW = 0.55–0.65), with protruding and expanded lips, visible in dorsal, lateral, and ventral views, slightly indented at commissures and surrounded by a single row of marginal papillae without a gap (Fig. 2C). Papillae conical, elongated and regularly spaced. Submarginal papillae absent. Labial tooth row formula (LTRF) highly variable: 11(6–11)/12(1–8) (n = 3, Stages 31 and 34), 9(6–9)/10(1–7) (n = 2, Stages 27 and 30), 10(6–10)/11(1–7) (n = 2, Stages 30 and 34), 11(6–11)/11(1–7) (n = 2, Stages 31 and 33), 11(6–11)/12(1–7) (n = 2, Stages 31 and 33), 9(6–9)/10(1–6) (n = 1, Stage 29), 10(6–10)/10(1–6) (n = 1, Stage 30), 10(6–10)/12(1–8) (n = 1, Stage 31), 12(6–12)/12(undetermined) (n = 1, Stage 34), 12(7–12)/13(1–9) (n = 1, Stage 34), and 14(6–14)/14(1–10) (n = 1, Stage 36). On the anterior lip, the A1 and A2 rows are generally shorter, with A1 = A2, A1 < A2 or A1 > A2, while A3 to A5 rows have similar lengths. All other rows are always shorter, usually due to the jaw sheaths. The outermost row is progressively longer than the innermost row. On the posterior lip, the P1 to P6–P10 rows always are shorter, usually due

TABLE 1. Morphometric measurements (in millimeters) of 11 tadpoles of *Ceratophrys joazeirensis* (Stages 31–34) from Jussara municipality, Bahia State, Brazil. SD = Standard deviation.

Morphometric measurements	Mean	SD	Range
Total length	53.59	4.22	45.58–59.05
Body length	19.70	1.40	17.57–21.72
Tail length	33.89	2.94	28.01–37.33
Body height	8.44	0.58	7.40–9.10
Body width	11.61	1.08	9.88–13.88
Tail muscle height	4.64	0.49	4.00–5.60
Tail muscle width	3.44	0.55	2.70–4.70
Dorsal fin height	4.05	0.39	3.20–4.50
Ventral fin height	3.90	0.37	3.20–4.30
Maximum tail height	11.50	1.08	9.50–13.00
Eye diameter	2.52	0.15	2.30–2.70
Interorbital distance	3.55	0.39	3.00–4.20
Nostril diameter	1.18	0.09	1.10–1.30
Internostril distance	2.98	0.21	2.70–3.30
Nostril-snout distance	3.65	0.56	2.90–4.40
Eye-snout distance	5.50	0.60	4.50–6.25
Eye-nostril distance	1.03	0.25	0.70–1.60
Spiracle width	1.87	0.29	1.50–2.30
Spiracle-snout distance	13.45	0.92	12.00–14.63
Vent tube length	1.80	0.27	1.30–2.20
Vent tube width	1.23	0.32	0.90–2.00
Oral disc width	6.91	0.65	5.70–7.70

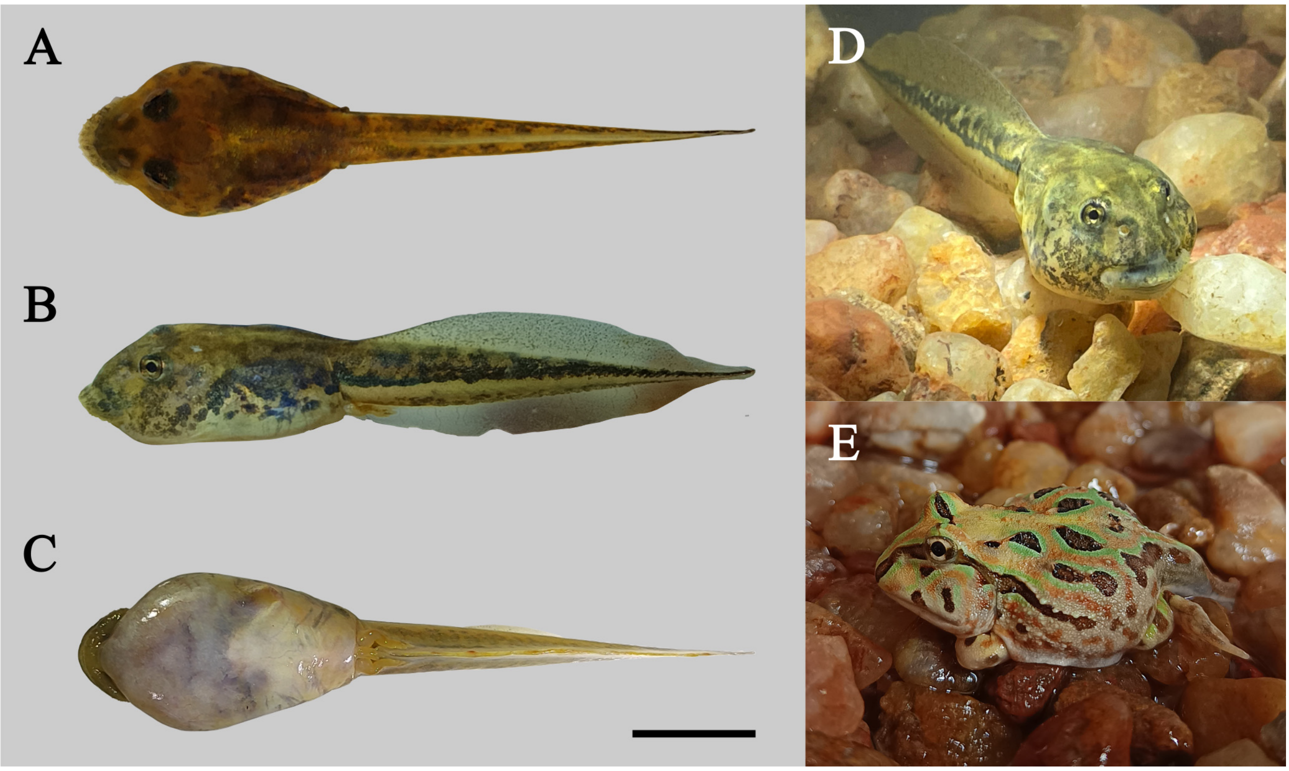

FIGURE 1. Tadpole of *Ceratophrys joazeirensis* at Stage 36. (A) dorsal view; (B) lateral view; (C) ventral view; (D) individual photographed in life; (E) metamorphic specimen. Scale bar = 10 mm.

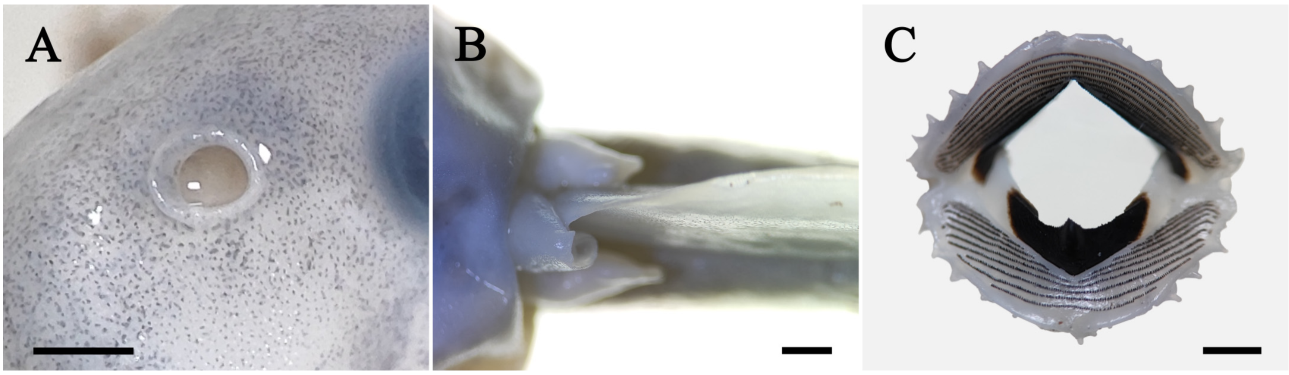

FIGURE 2. Details of the morphological structures of *Ceratophrys joazeirensis* tadpole. (A) rounded nostril (Stage 34); (B) vent tube medial with dextral opening (Stage 34); (C) oral disc, LTRF 10(6–10)/11(1–7) (Stage 30). Scale bar = 1 mm.

to the jaw sheath. The innermost row is progressively shorter than the outermost one. All other rows with similar lengths, except the penultimate and last rows which may be shorter (P9 = P10 or P9 > P10; P10 > P11; P11 = P12 or P11 < P12 or P11 > P12; P12 = P13; P13 < P14). Tooth rows are not always well organized and may be shorter, circular, fragmented, reticulated, or fused. The last tooth row on the anterior lip and the first tooth row on the posterior lip are sometimes short, fragmented, and occur on only one side. Jaw sheaths strongly keratinized and serrated; upper jaw sheath triangular, resembling an inverted "V"; lower jaw sheath more robust with a prominent acute projection in the medial portion that fits in the medial portion of the upper jaw sheath.

Coloration. In dorsal view, the body is golden-green with grayish spots. In lateral view, this color occupies more than two thirds of the body. The lower third has a light tone, with a creamy-white color and no stain. The venter is translucent. The surface of the oral disc is creamy-white and slightly pigmented. Tail musculature with two longitudinal strips that begin at the body-tail junction and run to the tail tip. The stripes have a golden-green color with grayish spots. The portion between the two stripes presents a light tone, with a white-creamy-golden color, without stains, similar to that presented by the final third of the body. The dorsal and ventral fins are translucent. The dorsal fin has many small dark spots distributed randomly, mainly in the first third and middle third. The ventral fin has very few spots, mainly in the last third. In preservative, the melanic pattern presented in life is maintained, but the color becomes faded. Body with many light gray and dark gray spots. The venter is beige. The stripes on the tail stand out in light gray and dark gray. The remainder of the tail musculature is beige.

Variation. Although the golden-green color with grayish spots was standard in all individuals, we were able to distinguish two groups: the first in which the dark green color stood out and the second in which the golden color stood out. Two individuals had a vent tube with a sinistral opening and two had slightly oval nostrils.

Acoustic communication. The underwater sound of *C. joazeirensis* tadpoles is a short and very audible like-metallic sound, which consists of a simple structure, composed of a single note, and it is not possible to distinguish pulses (Fig. 3A). The sound has a subtle amplitude modulation, starting abruptly and continuing with an intense reduction in energy, with the package forming a kind of arrow. The sound has high frequencies, but there is a noticeable loss of energy at those higher frequencies. The average sound duration was 0.037 s (range 0.012–0.080, n = 22), the average dominant frequency was 1924.53 Hz (1119.73–4651.17), while the average maximum frequency was 10924.03 Hz (6201.56–16968.16). We identified two different sounds emitted by *C. joazeirensis* tadpoles outside of the water, classified as agonistic sounds. The first (agonistic sound type I) was emitted more frequently and has a simple structure, being formed by a single note, without evident pulses (Fig. 3B). Audibly, the sound resembles the underwater sound, being shorter. The average sound duration was 0.003 s (0.001–0.006, n = 36), the average dominant frequency was 5361.77 Hz (1722.66–11455.66), the average maximum frequency was 12087.30 Hz (5770.90–18001.76). The second sound (agonistic sound type II) presents a single note with very evident pulses (Fig. 3C). The sound has an elliptical structure, starting small, reaching its peak amplitude halfway through the sound and then continuing to fall. In general, the pulses present in the middle of the sound had a higher concentration of energy. The average sound duration was 0.046 s (0.019–0.071, n = 20), the average dominant frequency was 2902.68 Hz (1636.52–5426.37), the average maximum frequency was 15176.60 Hz (10163.67–18604.69), the average pulse duration was 0.001 s (0.000–0.003), the average interpulse interval was 0.004 s (0.000–0.025) and the average number of pulses was 9.63 (4.00–22.00).

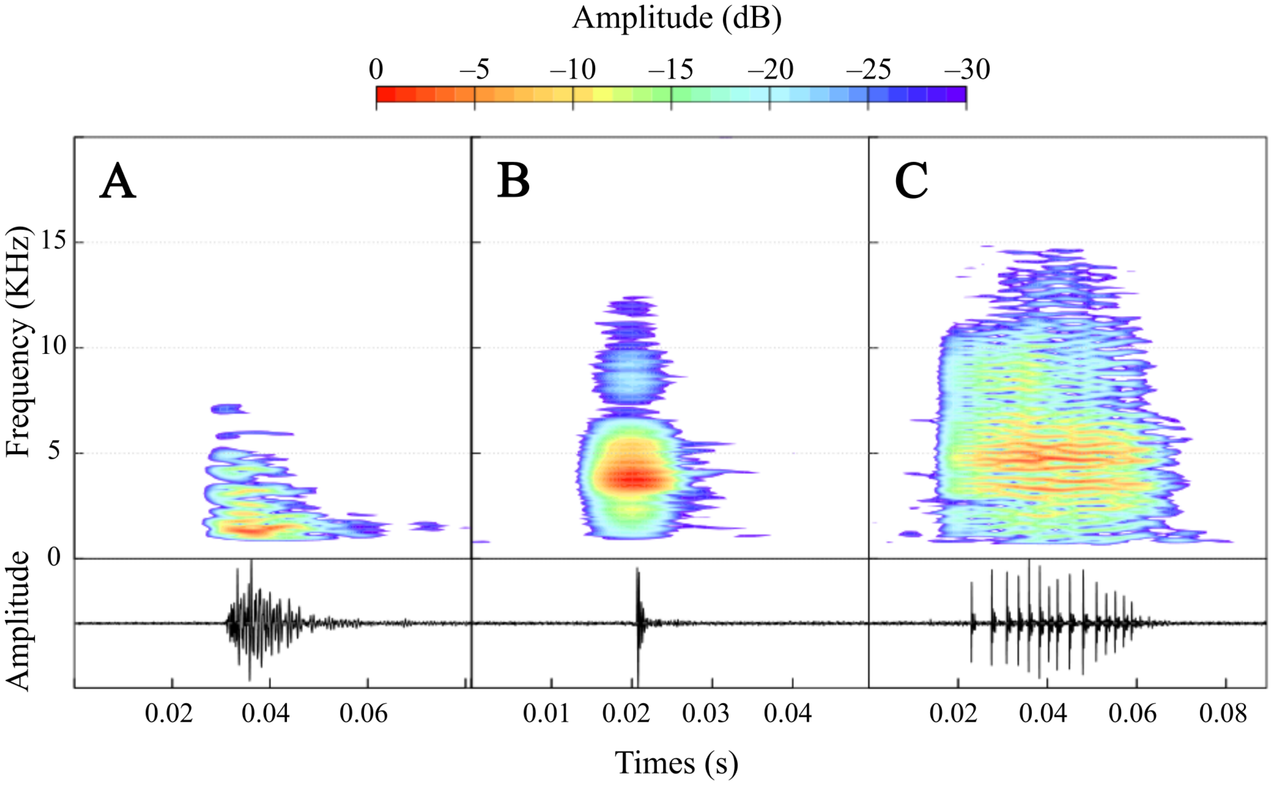

FIGURE 3. Oscillogram and spectrogram of sounds produced by tadpoles of *Ceratophrys joazeirensis*. (A) underwater sound; (B) agonistic sound type I; (C) agonistic sound type II.

Natural history notes. The sampling of *C. joazeirensis* tadpoles occurred between eight and ten days after the first observation of calling males and amplexus couples in the pond. The activity of calling males lasted only two days. The tadpoles were found close to the edge of the pond, particularly where emergent and marginal vegetation were absent or sparsely concentrated. During our field observations, we noted that the tadpoles spent most of their time at the bottom of the pond. However, they periodically visited the surface by rising vertically, slightly emerging their snouts from the water and returning to deeper regions. When threatened, tadpoles exhibited a defensive behavior descended to the bottom of the pond and remained motionless. We observed that they displayed greater activity at night compared to during the day. Additionally, after being collected and stored, we observed that *C. joazeirensis* tadpoles preyed on tadpoles of other species. We also noted instances of cannibalism, with larger individuals preying on smaller or more fragile ones.

The tadpoles of the seven described species of the genus *Ceratophrys* share several ecological and morphological characteristics. They are all exotrophic, inhabit lentic environments, are carnivorous, and exhibit cannibalistic behavior. Additionally, they exhibit marked morphological similarity. The tadpole of *C. joazeirensis* has an anterior oral disc, a sinistral spiracle without a tube, and dorsolateral eyes, which are similar to the tadpoles of *C. aurita* (Raddi), *C. calcarata* Boulenger and *C. cornuta* (Linnaeus) (La Marca 1986; Duellman & Lizana 1994; Mira-Mendes *et al*. 2022), but differs from *C. ornata* (Bell), which has dorsal eyes (Fernández & Fernández 1921). Furthermore, *C. joazeirensis* tadpoles have a vent tube medial, similar to *C. aurita*, *C. calcarata*, *C. ornata* and *C. cranwelli* Barrio (Fernández & Fernández 1921; La Marca 1986; Quinzio *et al*. 2006; Mira-Mendes *et al*. 2022), but different from *C. cornuta*, which is dextral (Duellman & Lizana 1994). The snout of *C. joazeirensis* tadpoles is slightly pointed in dorsal view and rounded in lateral view, similar to *C. aurita* and *C. calcarata* (sub-triangular) (La Marca 1986; Mira-Mendes *et al*. 2022), but different from *C. cornuta*, which is rounded in dorsal view and abrupt in lateral view (Duellman & Lizana 1994).

Despite the similarities between the tadpoles of different species of *Ceratophry*s, we can also identify striking differences, primarily related to the oral disc and body size. Among the labial tooth row formulae reported in the genus, *C. joazeirensis* stands out for having one of the highest numbers of tooth rows on both the anterior and posterior lips (9–14/10–14), a characteristic shared only with *C. cornuta*, which can have up to 13 rows on the anterior lip (Duellman & Lizana 1994) and with *C. calcarata* and *C. ornata*, which can have up to 10 rows on the posterior lip (Fernández &

Fernández 1921; La Marca 1986). When considering the total set of tooth rows, *C. joazeirensis* has the highest number, reaching 22 to 28 rows, followed only by *C. cornuta*, which can have up to 21 (Duellman & Lizana 1994). Comparing the size of tadpoles at similar developmental stages reveals that *C. joazeirensis* possesses one of the largest average total lengths (53.59 mm) among congeners, surpassed only by *C. stolzmanni* Steindachner (54.90 mm in *ex situ* development) (Almeida-Reinoso *et al*. 2023). In relation to *C. aurita*, the sister taxon of *C. joazeirensis* (Faivovich *et al*. 2014), this comparison reveals clear differences, with *C. aurita* having up to 17 rows of teeth (Wassersug & Heyer 1988) and reaching an average length of only 32.69 mm (Mira-Mendes *et al.* 2022).

The sound production by anuran larvae, particularly in species with macrophagous and carnivorous tadpoles, is intriguing and appears to have evolved convergently in *Ceratophrys* (*C. cranwelli* and *C. ornata*; Natale *et al*. 2011; Salgado Costa *et al*. 2014) and *Gephyromantis* (*G. kintana* Cocca, Andreone, Belluardo, Rosa, Randrianirina, Glaw and Crottini; Reeve *et al.* 2011). These are the only genera in which sound production by tadpoles has been reported. The similarity in the underwater sound emitted by *C. joazeirensis* tadpoles to that of other species, characterized by a short, metallic click sound with a simple structure and high frequency (Natale *et al*. 2011; Reeve *et al*. 2011; Salgado Costa *et al*. 2014), suggests a common function across different taxa. Such vocalization seems to serve multiple functions, potentially signaling aggressiveness during feeding or functioning as a defense mechanism against predation by conspecific tadpoles (Natale *et al*. 2011; Reeve *et al*. 2011; Salgado Costa *et al*. 2014). While the exact nature of this sound emission remains to be fully elucidated, our preliminary observations indicate a potential association with prey capture behavior, contrasting with the conclusions of Natale *et al*. (2011) and Salgado Costa *et al*. (2014), who found that *C. ornata* and *C. cranwelli* tadpoles emit sound as a defense mechanism. Therefore, we encourage further field and laboratory work aimed at studying the sounds produced by *C. joazeirensis* tadpoles to gain a better understanding of the types of sounds that they can produce and the nature of the sound emission.

We are very grateful to Jovina, Everaldo, and the entire Gama family for their support and assistance in our research that culminated in this manuscript. We are also very grateful to Zélia and Liliane for their support. We thank Mr. Braulino for allowing us to enter his property to access the pond and ICMBio for granting the animal collecting permit. We also thank Florencia Vera Candioti and one anonymous reviewer for your valuable comments and suggestions on the manuscript. ArSP thanks FUNBIO—Fundo Brasileiro para a Biodiversidade for the research grant. DOM thanks FAPESQ—Fundação de Apoio à Pesquisa do Estado da Paraíba for a PRONEX and a Universal Grant. DOM also thanks CNPq for a research fellowship. MFN acknowledges the CNPq for Research Productivity Fellowship (314496/2021-1).

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