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Tadpoles of the endangered Ka Lon litter toad, *Leptobrachella kalonensis* (Anura: Megophryidae): molecular identification and morphological description

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ABSTRACT. – Tadpole identification has important implications for subsequent studies on the life history, behavior, ecology, and distribution of amphibian species. The tadpole morphology of the Ka Lon litter toad, *Leptobrachella kalonensis,* is first characterized based on DNA barcoding identification. The description is based on the collection of four tadpoles at stage 26 from a disturbed stream in Di Linh plateau, central Vietnam. DNA sequences of the mitochondrial 16S rRNA gene obtained from the tadpoles have less than 0.2% sequence divergence with the adult *L. kalonensis* in GenBank, rendering the identification unambiguous. The tadpoles of this species were discovered in a small pool in a rocky cascade stream covered by a disturbed broadleaf and bamboo forest. Tadpoles of *L. kalonensis* are found to be distinguished from those of their congeners by having a significantly elongated body, a tail twice as long as the body, a uniform brown color without a distinct pattern, an intestinal tube that is invisible due to the robustness of the rectus abdominis muscle, and an LTRF 2(1–2)/2(1–2) or 2(1–2)/2(1). A previously unknown locality for this species on the Di Linh plateau is also reported, which further verifies the IUCN Redlist's suggestions regarding the geographic range of the species.

KEYWORDS: 16S rRNA, Megophryidae, morphology, Southeast Asia, tadpoles

INTRODUCTION

The larval or tadpole stages of anuran amphibians are strikingly different from the fully developed froglets or adults. Understanding the ecological requirements of any frog species necessitates morphological and ecological knowledge of tadpoles, an issue that becomes especially essential in light of the global decline of amphibians (Stuart et al. 2004). Unfortunately, due to the difficulty of morphological identification, which involves either rearing spawn from an identified mating pair of frogs or keeping tadpoles alive until their metamorphosis, many frog species still lack detailed descriptions of their tadpoles (Grosjean et al. 2015). Recently, molecular taxonomy has provided an efficient alternative for identifying tadpoles, contributing to an increasing knowledge of the tadpole stages of more frog species (Raharivololoniaina et al. 2006; Grosjean et al. 2015; Schulze et al. 2015).

The Asian leaf litter toad genus *Leptobrachella* Smith, 1925, is a member of the Megophryidae family, which is distributed from northeastern India to southern China and Southeast Asia (Frost 2023). On the basis of a large-scale molecular analysis, this genus was merged with the genus *Leptolalax* and is currently one of the most species-diverse groups with 99 species (Chen et al. 2018; Frost 2023). More than half of the species have been discovered within the last decade due to recent increase in survey effort and the use of integrative taxonomic approaches that incorporate molecular analyses, acoustic data, and morphological comparisons (Rowley et al. 2016; Frost 2023). In contrast to the series of new species descriptions based primarily on the adult stage, studies on tadpoles of *Leptobrachella* species have been interrupted for nearly 40 years since the tadpole descriptions of *L. pelodytoides* (Smith 1917), *L. mjorbergi*, and *L. gracilis* (Inger 1985). Since 1999, descriptions of tadpoles of 16 *Leprobrachella* species have been published as part of ongoing research on tadpoles of *Leptobrachella* species. There are currently 21 descriptions of tadpole among 99 recognized species (Malkmus et al. 1999; Ohler et al. 2011; Oberhummer et al. 2014; Nguyen et al. 2018; Lyu et al. 2020; Nguyen et al. 2020; Le et al. 2021; Shi et al. 2021; Vassilieva 2021; Haas et al. 2022).

The Kalon leaf-litter toad, *Leptobrachella kalonensis* (Rowley et al., 2016), was described in 2016 and is classified as endangered by the IUCN (IUCN SSC 2021). The species inhabits disturbed evergreen forests between 200 m and 791 m in elevation and is known only from Binh Thuan province in southern Vietnam (Rowley et al. 2016). In this study, we describe for the first time the tadpole morphology of *L. kalonensis* and confirm the species' presence in areas adjacent to its native distribution.

MATERIALS AND METHODS

Sample collection

Tadpoles of *Leptobrachella kalonensis* were collected on August 26, 2022, during fieldwork in Di Linh district, Lam Dong province, Vietnam (11.2985ºN, 108.0967ºE, 661 m elevation). Tadpoles were photographed alive in a small glass tank using a Canon D300 camera. Prior to preservation, tissue samples from the tail musculature were obtained. The tissue samples were stored in 99% ethanol. All voucher specimens were then preserved with 10% formalin.

Morphological examination and measurement

The staging of the tadpoles was in accordance with Gosner (1960). Dial calipers were used to measure four specimens to the nearest 0.01 mm. Morphological measurements followed Altig and McDiarmid (1999) and Pezzuti et al. (2021), including: Total length (TL) was measured from the tip of the snout to the tip of the tail; Body length (BL): from the tip of the snout to the point where the caudal muscle medial line touches the body wall; Tail length (TaL): distance between the level of the caudal muscle's medial line's contact with the body wall and the tip of the tail; Maximum tail height (MTH): maximum distance between the external ventral fin and dorsal fin; Interorbital distance (IOD): distance between the centers of the pupils; Internostril distance (IND): distance between the medial margin of the nostrils; Tail muscle height (TMH): maximum distance between the ventral and dorsal edges of the tail muscle; tail muscle width (TMW): maximum distance between the lateral edges of the tail muscle; body width (BW): maximum distance between the lateral edges of the body; body width at nostril position (BWN): maximum distance between the lateral edges of the body at nostrils level; body width at eye position (BWE): maximum distance between the lateral edges of the body at eyes level; body height (BH): maximum distance between the dorsal and ventral edges of the body; eye–snout distance (ESD): distance from the center of the eye to the tip of the snout; eye-nostril distance (END): distance from the center of the eye to the medial margin of the nostril; nostril-snout distance (NSD): distance from the medial margin of the nostril to the tip of the snout; eye diameter (ED): maximum distance between eye edges; nostril diameter (ND): maximum distance between nostril edges; snout-spiracular distance (SSD): distance from the tip of the snout to the spiracle distal edge; oral disc width (ODW): maximum distance between oral disc edges; dorsal fin height (DFH): maximum distance between external and internal edges of dorsal fin; ventral fin height (VFH): maximum distance between external and internal edges of ventral fin; spiracle length (SL): distance between the insertion of the ventral wall of the spiracle to the body and its distal edge. The Tooth formulas (LTRF) were determined according to Altig and McDiarmid's methodology (1999).

Molecular study

Genomic DNA was isolated from tissue samples using the Omega BIO-TEK tissue kit according to the manufacturer's recommendations. Approximately 550 base pairs of the 16S rRNA were amplified using the primers AH-16S_S and AH-16S_R (Grosjean et al. 2015). The 40 µl PCR reaction mixtures contained 20 µl of MyTaq PCR Mastermix (Bioline), 16 µl of ultrapure water, 0.8 µl of BSA (Euromedex), 0.6 µl of each primer (3µM), and 2 µl of DNA template. Amplifications were performed in a PTC-100 BIORAD thermal cycler. The thermal regime consisted of an initial step of 2 minutes at 92 °C followed by 35 cycles of 45 seconds at 92 °C, 45 seconds at 52 °C, and 1 minute at 72 °C, 5 minutes at 72 °C, and then a hold at 4 °C. PCR products were visualized on 1–2% agarose gels, and the most intense products were selected for sequencing by 1ST BASE, Malaysia (https://base-asia.com/). Electropherograms were inspected visually using Chromas 2.6.6, Technelysium Ltd. (https://technelysium.com.au/) and sequences were aligned using MUSCLE, implemented in MEGA7 (Kumar et al. 2016). The new sequences were then checked on BLAST (NCBI) to verify their approximate identities. Under accession number OR095097 (DL463), OR095098 (DL464), DNA sequences have been deposited in GenBank. Sequence data were obtained from GenBank for 66 additional *Leptobrachella* and two outgroups, *Oreolatax sterlingage* and *Leptobrachella* cf. *chapaense*, which were closely related to ingroup species (Rowley et al. 2015). Phylogenetic inferences based on the Maximum Likelihood (ML) framework were made using IQ-TREE (Nguyen et al. 2015) through the IQ-TREE web server (Trifinopoulos et al. 2016). The optimal partitioning models (Chernomor et al. 2016) for the ML inference were selected by ModelFinder (Kalyaanamoorthy et al. 2017) in IQ-TREE, using the minimum BIC score. Partition analysis suggested GTR+F+I+G4 as the best-fit models for ML inference (BIC = 16793.180, -lnL = 7928.781). Ultrafast bootstrap (BP) analysis for 1000 iterations (Minh et al. 2013) was carried out to determine statistical support for the nodes in ML. The trees obtained from ML were visualized using Figtree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree).

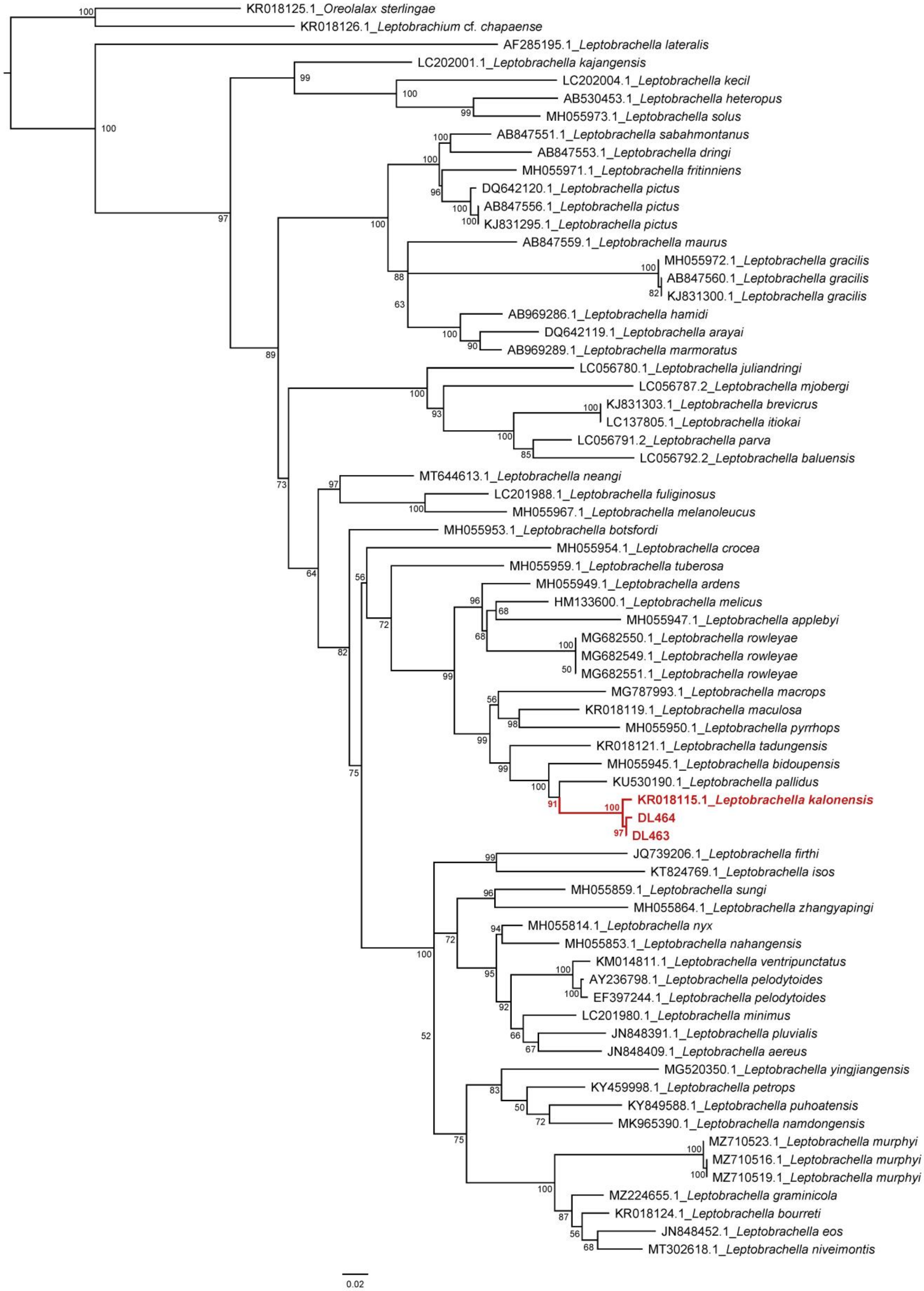

FIGURE 1. Maximum-likelihood (ML) tree based on 16S rRNA mitochondrial gene sequences for the *Leptobrachella* species and outgroups, numbers on branches are ML bootstrap values > 50%.

FIGURE 2. External morphology of the *Leptobrachella kalonensis* tadpole (DL417, Stage 26) in Di Linh district, Lam Dong province, Vietnam. a. lateral view. b. dorsal view. c. ventral view. d-e. nostrils. f-g. oral discs.

RESULTS

Phylogenetic assignment

For a variety of different *Leptobrachella* species, the 16S rRNA phylogenetic topology was found to display high support in maximum likelihood bootstrap proportions greater than 70% in most nodes (Fig. 1). All newly collected specimens from the Di Linh plateau were identical to the specimen of *L. kalonensis* (98.94–99.17%), which was originally described by Rowley et al. (2016: GenBank accession number KR018115) and was a sister species of *L. pallidus* with a bootstrap value of 91% (Fig. 1).

Tadpole description

***Morphology*.—** The description of tadpole *Leptobrachella kalonensis* was based on four specimens at stage 26 (field number DL461, DL462, DL463, DL464, Fig. 2, Table 1). Specimens were deposited at the Zoology Laboratory, University of Science, Ho Chi Minh City, Vietnam. The body is found to be depressed (BH/BW = 0.66–0.76) and elliptical in dorsal view; in lateral view, it is depressed (BH/TL = 0.15–0.17). In dorsal view, the snout is nearly rounded (BWN/BWE = 0.69) and circular in lateral view. Eyes are small (ED/BWE = 0.14–0.16), dorsally located (IOD/BWE = 0.64–0.65), and dorsalaterally directed. Nostrils have an elliptical aperture, are large (ND/BL = 0.06–0.07), located dorsally, directed anterolaterally, have distance to snout 48–55% the distance between the eye and the snout, and are rimmed by a low, three- to four-lobed crest (Fig. 2d, e). The spiracle is sinistral, lateral, posterodorsally oriented, short (SL/BL = 0.07–0.09), opening in the middle third of the body (SSD/BL = 0.58–0.60), inner wall fuse to the body, with the distal portion free from the body. The lateral line system appears as a series of light dots on the lateral and dorsal surfaces of the body around the nares, eyes, flanks, and tail side. Due to the robustness of the rectus abdominis muscle, the gut tract is not visible. The vent tube opening is dextral, attaches to the ventral fin, and position at the ventral margin; the ventral wall is longer than the dorsal. Tail height is short (MTH/TAL = 0.19–0.20); musculature is robust (TMH/BH = 0.78–0.93) but do not reach the tail tip; the tail tip is observed to be nearly rounded. The dorsal fin is low in height (DFH/TAL = 0.05–0.06), parallel to the longitudinal axis, and originates in the anterior third of the tail with a low slope. Low-height ventral fin (VFH/TAL = 0.04–0.05), parallel to the longitudinal axis, beginning at the vent tube level. The dorsal fin is higher than the ventral (DFH/VFH = 1.15–1.34).

TABLE 1. Measurements (mm) of *Leptobrachella kalonensis* tadpoles from Di Linh district, Lam Dong province, Vietnam.

	Form 1 (P2 entired)			Form 2 (P2 divided)		
	DL461	DL464*	Average ± SD	DL462	DL463*	Average ± SD
Stage	26	26	26	26	26	26
TL	61.40	-	61.4	46.86	-	46.86
BL	16.30	15.37	15.83 ± 0.66	12.47	12.42	12.44 ± 0.03
TaL	45.10	-	45.1	34.39	-	34.39
MTH	8.91	-	8.91	6.53	-	6.53
IND	3.09	3.54	3.31 ± 0.32	2.65	2.27	2.46 ± 0.27
IOD	5.31	4.34	4.83 ± 0.69	3.96	3.76	3.86 ± 0.14
TMH	6.73	4.89	5.81 ± 1.30	4.57	4.16	4.36 ± 0.29
TMW	6.36	4.51	5.43 ± 1.30	4.24	3.08	3.66 ± 0.82
LTRF	2(1–2)/2(1)	2(1–2)/2(1)	2(1–2)/2(1)	2(1–2)/2(1–2)	2(1–2)/2(1–2)	2(1–2)/2(1–2)
BW	10.98	8.27	9.63 ± 1.92	7.47	7.14	7.30 ± 0.24
BWN	4.75	4.34	4.55 ± 0.29	4.09	3.65	3.87 ± 0.31
BWE	8.22	6.73	7.48 ± 1.05	6.21	5.82	6.01 ± 0.27
BH	7.27	6.26	6.76 ± 0.71	5.05	5.27	5.16 ± 0.15
ESD	5.17	4.84	5.00 ± 0.24	4.32	4.18	4.25 ± 0.10
END	3.13	2.90	3.01 ± 0.16	2.49	2.29	2.39 ± 0.14
NSD	2.83	2.51	2.67 ± 0.23	2.12	1.99	2.05 ± 0.09
ED	1.13	1.10	1.11 ± 0.02	0.95	0.87	0.91 ± 0.06
ND	0.93	0.86	0.90 ± 0.05	0.83	0.72	0.77 ± 0.07
SSD	9.48	9.22	9.35 ± 0.18	7.46	7.41	7.43 ± 0.03
ODW	3.65	3.47	3.56 ± 0.13	3.33	2.99	3.16 ± 0.24
DFH	2.37	-	2.37	1.91	-	1.91
VFH	1.77	-	1.77	1.66	-	1.66
SL	1.49	1.13	1.31 ± 0.25	0.95	0.86	0.91 0.06

- missing data, * indicates the cutting tail. P2 is labial teeth row P2 according to Altig and McDiarmid (1999).

The oral disc is found to be cup-like, moderately wide (ODW/BW = 0.33–0.45), and anteroventrally positioned, with margination in the upper and lower lip midline. The base of the oral funnel bore 14–17 large, round, fleshy submarginal papillae on the left and 13–16 on the right side. There are two forms of oral disc distinct from LTRF, and submarginal papillae coexisted with marginal papillae:

- Form 1 (n=2): LTRF 2(1–2)/2(1–2); a virtually formed row of 41–54 small, conical submarginal papillae extend to a continuous single row of small, conical marginal papillae from the upper to lower lip (Fig. 2f).
- Form 2 (n=2): LTRF 2(1–2)/2(1); an extremely short array of 2–5 small, conical submarginal papillae is arranged within a row of marginal papillae (Fig. 2g).

Jaw sheaths are stout, strong, and irregularly serrated; the upper jaw sheath is M-shaped, with a smaller series of serrations along the middle position and an enlarged median serration; the lower jaw sheath is V-shaped**.**

Color.— In life, the body is brown dorsally and laterally, and pale ventrally. The tail is observed to be brown, with the caudal muscle medial line being darker on the dorsal side than the ventral side. The ventral fin margin is found to be whitish. The tail tip is brown to whitish. Iris is black in color. Half of the spiracle's funnel is unpigmented, while the other half was light brown. Both the vent tube and oral disc lack pigmentation. In preservative, colors become paler than in nature.

***Comparison*.—** The *Leptobrachella kalonensis* tadpoles share the following characteristics with the genus *Leptobrachella* tadpoles described by Inger (1985), Li et al. (2011), and Vassilieva (2021): The body is found to be elongated with a moderately long tail and a well-developed muscular section; the tail fins are relatively low; the spiracle is sinistral; the vent tube is dextral; and the oral disc is cup-shaped on the ventral side.

Tadpole of *Leptobrachella kalonensis* could be distinguished from other *Leptobrachella* tadpoles based on the following traits: a markedly elongated body; a tail twice as long as the body; uniform brown coloration without a distinct pattern; an intestinal tube that was invisible because of the robustness of the rectus abdominis muscle; and an LTRF 2(1–2)/2(1–2) or 2(1–2)/2(1) (Table 2).

At stage 26, the tadpole of *Leptobrachella kalonensis* differs from other known *Leptobrachella* tadpoles by having an invisible gut tube (vs. visible in all known tadpoles in the genus *Leptobrachella*); visible lateral line organs (vs. invisible in *L. gracilis* and *L. petrops*); and a different LTRF with two paired teeth rows on the upper lip and two teeth rows on the lower lip: 2(1–2)/2(1–2) or 2(1–2)/2(1) (vs. 5(2–5)/4(1–3) in *L. minima*; 4(2–4)/3(1–2) in *L. aerea*, *L. bourretti*, *L. gracilis*, *L. oshanensis*, *L. petrops*, *L. rowleyae*, and *L. ventripunctata*; 3(1–3)/4(1–3) in *L. botsfordi*; 3(2–3)/3(1–2) in *L. yeae*; 1(1)/0 in *L. bidoupensis*; 0/0 in *L. itiokai* and *L. mjorbergi*) (Table 2).

In comparison to other tadpoles in the genus *Leptobrachella* lacking stage 26 examined, tadpole of *Leptobrachella kalonensis* differs from other known *Leptobrachella* tadpoles by having an invisible gut tube (vs. visible in all known *Leptobrachella* tadpoles) and clearly visible lateral line organs (vs. invisible in *L. dringi*). Moreover, the LTRF of *Leptobrachella kalonensis* tadpoles with 2(1–2)/2(1–2) or 2(1–2)/2(1) differs from 6(3–6)/4(1–3) in *L. pelodytoides* (stage unknown), 5(4)/4(1–3) to 6(2–6)/5(1–4) in *L. kajangensis* (stage 30–38), 5(2–5)/3(1–3) to 4(2–4)/3(1–2) in *L. dringi* (stage 25), 4(2–4)/3(1) in *L. bashaensis* (stage unknown), ≥ 4/3 in *L. mangshanensis* (stage unknown), 1(1)/0 in *L. arayai* (stage 30), 0/0 in *L. juliandringi* (stage unknown) and *L. baluensis* (stage unknown) (Table 2).

***Natural history*.—** *Leptobrachella kalonensis* tadpoles inhabited a small pool surrounded by enormous boulders in rocky cascading streams. This pool measured around 0.4 m wide and 0.15 m deep. The riparian vegetation consisted of bamboo forest and disturbed secondary broadleaf forest with a relatively open canopy. At night, tadpoles were active near the bottom of the pool.

DISCUSSION

In the past, research on tadpoles received less attention than research on the adults, mostly because tadpole identification based on rearing eggs or tadpoles to juveniles is time-consuming and may be inaccurate due to the diversity and complexity of anuran species in tropical forests. With the increasing development of molecular data in global systems such as GenBank and BOLD Systems, the assignment of tadpoles to adults with known DNA sequences enables quicker and more accurate tadpole identification (Grosjean et al. 2015). Herein, we employed a fragment of the 16S rRNA gene, which can be amplified and sequenced reliably to identify tadpoles, particularly *Leptobrachella* spp. (Grosjean et al. 2015; Rowley et al. 2015, 2016). The molecular data of the present study successfully supported the identification of an *L. kalonensis* tadpole. In addition, the 16S rRNA phylogenetic tree showed a close relationship between *L. kalonensis* and *L. pallidus*, *L. bidoupensis*, and *L. tadungensis*, which was consistent with the findings of Rowley et al. (2015, 2016) and Chen et al. (2018). The successful identification of *Leptobrachella kalonensis* tadpoles further confirms the effectiveness of DNA barcoding in amphibian tadpole research.

Tadpoles are essential for improving estimates of a site's anuran biodiversity since many species migrate far from their breeding waterbodies or remain dormant during the non-breeding season, making them difficult to locate. Tadpole information is also highly relevant for understanding the ecological requirements and natural history of frog species, particularly endangered species. During the 10-day fieldtrip in Di Linh, tadpoles of *Leptobrachella kalonensis* were collected without conspecific adults. Initially, it was believed that *L. kalonensis* was only known to inhabit the Song Luy Watershed Forest in Binh Thuan province, Vietnam (Rowley et al. 2016). However, this species is likely to be found in similar habitats and elevations in neighboring areas (IUCN SSC 2021). Our *L. kalonensis* tadpole specimens were collected in Lam Dong province, 12.8 km south of where the holotypes and paratypes of the species were collected (Fig. 3). The new locality of *L. kalonensis* described in this paper extends its known distribution and is consistent with the suggestions regarding the geographic range of the species in the IUCN Red list. At the new locality, *L. kalonensis* remains threatened by habitat degradation. The tadpoles of *L. kalonensis* were gathered from a stream that passes across a highway and a disturbed forest. Even during the peak of the rainy season, the stream was almost desiccated. If this drying is more severe, the tadpoles of *L. kalonensis*

TABLE 1. Comparison of key distinctive features between the *Leptobrachella kalonensis* tadpoles and other species.

Species	Stage	BW/BL	TaL/BL	LTRF in stage 26	LTRF	Lateral line organs	Gut tube	Coloration	References
Leptobrachella kalonensis	26	0.54–0.67	2.76–2.77	2(1–2)/2(1) 2(1–2)/2(1–2)	2(1–2)/2(1) 2(1–2)/2(1–2)	visible	invisible	brown dorsally and laterally, pale ventrally, tail is brown	This study
L. aerea	25–38	–	1.93–2.56	4(2–4)/3(1–2)	4(2–4)/3(1–2) 4(2–4)/4(1–3) 5(2–5)/4(1–3) 6(2–6)/3(1–2) 6(2–6)/4(1–3)	visible	visible	brown to beige with dark dots	Ohler et al. 2011; Vassilieva 2021
L. arayai	30	0.39	1.81	-	1(1)/0	-	-	brownish with pale speckling	Malkmus et al. 1999; Vassilieva 2021
L. baluensis	unknown	-	2.33	-	0/0	visible	visible	uniformly dark brown above and unpigmented, semi-translucent below	Haas et al. 2022
L. bourretti	25–36	-	1.57–2.61	4(2–4)/3(1–2)	4(2–4)/3(1–2) 5(2–5)/4(1–3)	visible	visible	brown olive with lighter spots	Ohler et al. 2011; Vassilieva 2021
L. bashaensis	unknown	0.44	2.08	-	4(2–4)/3(1)	-	visible	dark brown with small, brown, irregularly shaped spot	Lyu et al. 2020
L. bidoupensis	25–36	0.45–0.49	1.71–2.34	1(1)/0	1(1)/0	visible	visible	pale brown	Vassilieva 2021
L. botsfordi	25–26	0.46–0.49	1.76–2.08	3(1–3)/4(1–3)	3(1–3)/4(1–3)	visible	visible	whitish brown to grey	Nguyen et al. 2020; Vassilieva 2021
L. dringi	25	0.47	2.61	-	5(2–5)/3(1–3) 4(2–4)/3(1–2)	invisible	visible	pigmented brown, the tail is darker coffee brown	Oberhummer et al. 2014; Haas et al. 2022
L. gracilis	25–37	0.42–0.51	1.70–2.20	4(2–4)/3(1–2) 5(2–5)/3(1–2)	4(2–4)/3(1–2) 5(2–5)/3(1–2) 7(2–7)/3(1–2)	invisible	visible	body background color gray, with a bluish-white sheen	Inger 1985; Haas et al. 2022
*L. itiokai**	25	0.35	2.8	0/0	0/0	visible	visible	translucent brown	Oberhummer et al. 2014; Haas et al. 2022
L. kajangensis	30–38	0,48–0,64	1,63–2,10	-	5(4)/4(1–3) 6(2–5)/5(2–4) 6(2–6)/5(1–4)	visible	-	dusky brown with darker mottling	Grismer et al. 2004
L. mangshanensis	unknown	0.37	1.65	-	≥ 4/3	-	-	Brown	Hou et al. 2018
L. mjorbergi	25–41	0.31–0.36	1.70–2.10	0/0	0/0	visible	visible	uniformly dark brown above and unpigmented, semi-translucent below	Inger 1985; Haas et al. 2022
L. minima	25–37	-	2.25–2.54	5(2–5)/4(1–3)	5(2–5)/4(1–3)	visible	visible	grey olive with black spots on the caudal muscle	Ohler et al. 2011
L. oshanensis	25–36	0.36–0.50	2.02–2.42	4(2–4)/3(1–2)	4(2–4)/3(1–2) 4(2–4)/4(1–3)	visible	visible	uniformly rufous	Shi et al. 2021
L. pelodytoides	unknown	~ 0.5–0.6	~ 2.0	-	6(3–6)/4(1–3)	-	-	dark brown with speckled and spotted with black	Smith 1917
L. petrops	25–45	0.48	2.56	4(2–4)3(1–2)	4(2–4)3(1–2)	invisible	visible	olive brown to orangish brown with distinct whitish golden speckling, the caudal muscle with some large black spots	Le et al. 2021
L. rowleyae	25–38	0.55–0.87	2.21–2.95	4(2–4)/3(1–2)	4(2–4)/3(1–2)	-	visible	olive-brown to grey with distinct whitish-golden speckling	Nguyen et al. 2018
L. ventripunctata	25–36	-	2.12–2.51	4(2–4)/3(1–2)	3(2–3)/3(1–2) 4(2–4)/3(1–2) 4(2–4)/4(1–3) 5(2–5)/4(1–3)	visible	-	reddish dorsally with indistinct spots or uniformly greyish brown	Ohler et al. 2011
L. yeae	25–37	0.36–0.50	1.97–2.42	3(2–3)/3(1–2)	3(2–3)/3(1–2)	visible	visible	translucent light brown	Shi et al. 2021
L. juliandringi	unknown	-	2.13	-	0/0	visible	visible	pinkish gray or uniformly dark brown	Haas et al. 2022

- missing data

may not be able to mature successfully. The description of the tadpoles of *L. kalonensis* and their habitats provides important new information about the reproductive biology of this endangered species. The adult males were observed calling in June (Rowley et al. 2016). In this study, tadpoles were collected in August, indicating that tadpoles may be present in the stream at least from June to August. Because tadpoles were encountered at only one site with a limited sample size (n = 4), it is recommended that further research be conducted to better understand the distribution, reproductive behavior, and ecology of the species.

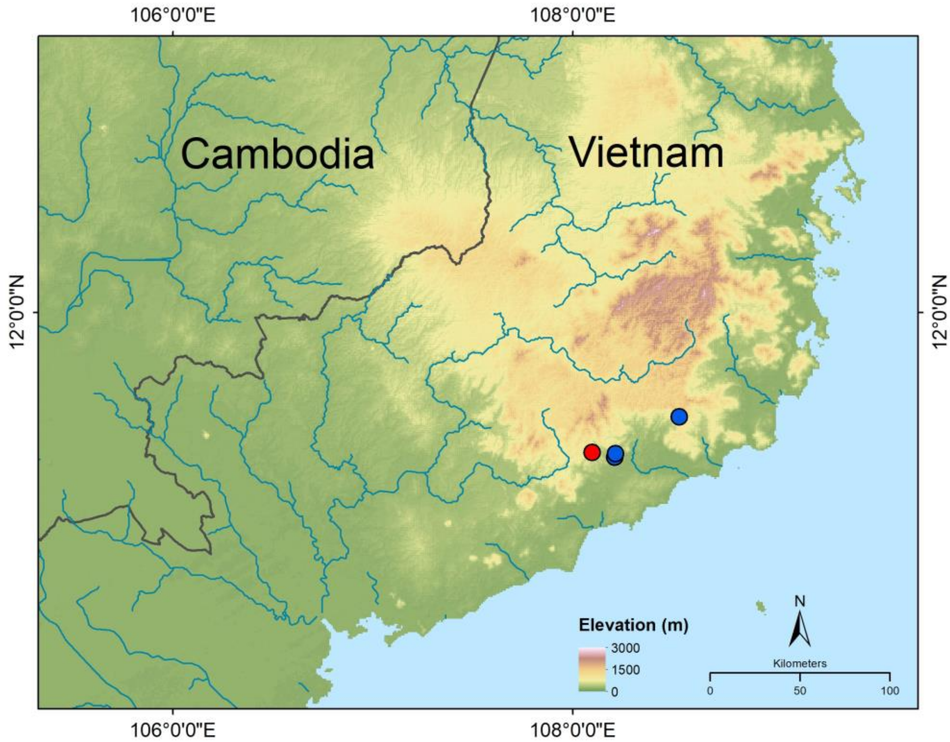

FIGURE 3. Localities where *Leptobrachella kalonensis* were collected. The blue circles show known localities of the species in Rowley et al. 2016. The red circle is new locality of the species in Di Linh, Lam Dong province, Vietnam.

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