

A new species of *Leptobrachium* Tschudi, 1838 (Anura: Megophryidae) from Bali and East Java, Indonesia

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Abstract. The fauna of Indonesia's Lesser Sunda and eastern Java areas harbour lineages of *Leptobrachium* that have been historically assigned to *L. hasseltii* Tschudi, 1838. Here, using an integrative dataset—mtDNA (12S–tRNA^{Val}–16S; 1,979 bp), adult and larval morphology, and advertisement calls—we delimit and describe a new species from Buleleng (Bali) and Kediri (East Java). Phylogenetic analyses (ML/Bayesian) recover two well-supported clades within *L. hasseltii* sensu lato: (1) West–Central Java and southern Sumatra (true *L. hasseltii*) and (2) Bali–East Java (new species), with 16S uncorrected p-distance 3.2–5.2% to *L. hasseltii*. Morphologically, the new species differs from *L. hasseltii* by the combination of brown iris with a light-blue scleral arc (eye fully open), very large oval femoral glands (dark edged), no vomerine teeth and rectal glands, distinctive dorsal pattern (dark blotches with paired transverse bars) and ventral patterning, and tadpoles having LTRF 7(1–6)/5(1–4) versus 5(1–4)/5(1–4) in *L. hasseltii*. The advertisement call is an elongated series of short barks followed by low-frequency squawks and differs markedly in temporal structure from *L. hasseltii*. We discuss biogeographic implications and provide an IUCN preliminary assessment following current guidelines.

Key words. Amphibia, bioacoustics, *Leptobrachium hasseltii*, morphology, phylogeny

INTRODUCTION

The megophryid frogs of the genus *Leptobrachium* Tschudi, 1838—often termed Asian spadefoot toads—are characterised by large heads and eyes, slender limbs, conspicuous humeral and femoral glands, variable iris/scleral colouration, and the absence of nuptial pads (Dubois & Ohler, 1998; Ohler et al.,

2004; Brown et al., 2009; Hamidy & Matsui, 2010, 2017; Matsui et al., 2010; Stuart et al., 2011; Wogan, 2012). The genus currently includes 39 recognised species distributed from the Indian subcontinent through southern China and Indochina to Sundaland and the Philippines (Frost, 2024). Phylogenetic work consistently resolves two principal clades broadly corresponding to (i) China–Indochina and (ii) Sundaland–southern Thailand, providing a framework for regional comparative taxonomy (Inger & Voris, 2001; Matsui et al., 2010).

Historically, the type species *Leptobrachium hasseltii* Tschudi, 1838 was treated as widespread, but subsequent revisions restrict it to southern Sumatra, Java, and Bali (Hamidy & Matsui, 2010, 2017; Matsui et al., 2010). Over the past two decades, integrative studies have revealed substantial cryptic diversity within *Leptobrachium*—particularly where morphology is conservative but iris/scleral colouration and advertisement calls are diagnostic—leading to descriptions of multiple new species from Indochina, the Philippines, and Sundaland (Lathrop et al., 1998; Ohler et al., 2004; Brown et al., 2010; Hamidy & Matsui, 2010; Stuart et al., 2011; Wogan, 2012; Hamidy et al., 2012). Within *L. hasseltii* sensu lato, earlier phylogeographic work recovered at least two well-supported lineages: (1) southern Sumatra + western/central Java and (2) Bali; those authors recommended focused morphological and acoustic study of the Balinese population to clarify its taxonomic status (Hamidy & Matsui, 2017). Field notes and museum records further document *Leptobrachium* from Bali's Mount Batukaru and multiple Javan localities, hinting at east–west structuring across Java

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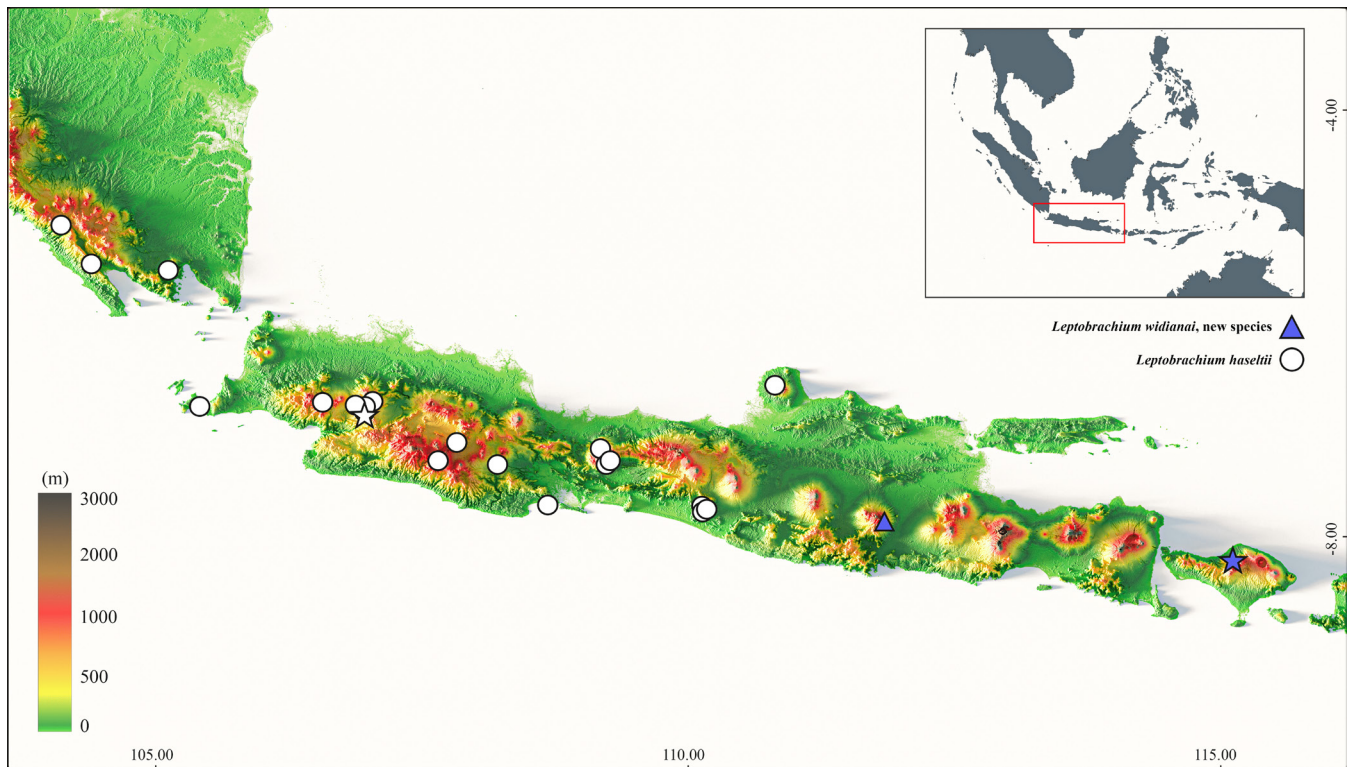


Fig. 1. Map of southern Sumatra and Java showing the distribution of *Leptobrachium* in Indonesia: *L. hasseltii* (white circles) and the new species (blue triangles), stars indicate the type localities of each species.

and possible divergence across the Bali Strait (Iskandar, 1998; MacKay, 2006; Hamidy & Matsui, 2017).

Here, we reassess the *L. hasseltii* complex across southern Sumatra, Java, and Bali using an integrative approach that combines expanded mitochondrial sampling (12S–tRNA^{Val}–16S), adult and larval morphology (including detailed oral disc and LTRF characters), and quantitative bioacoustics. In particular, our new material from East Java fills a critical geographic sampling gap between previously studied West/Central Javan and Balinese populations, providing the first comprehensive coverage of the species complex across its Sunda Arc range. Our results resolve a deeply divergent East Java–Bali lineage that we describe as a new species and refine the limits of *L. hasseltii* sensu stricto to West/Central Java and Sumatra (Matsui et al., 2010; Hamidy & Matsui, 2017; Frost, 2024).

MATERIAL AND METHODS

Field surveys and specimen collection. We conducted nocturnal field surveys at Banyuwana Amertha Waterfall (Fig. 1), Buleleng, Bali, during two expeditions: 20–21 December 2021 and 9–11 February 2023. Surveys followed established protocols for amphibian inventories in Southeast Asian tropical forests (Hamidy & Matsui, 2010; Matsui et al., 2010). Visual Encounter Surveys (VES; Heyer et al., 1994) were performed between 1900 and 2300 h, targeting streamside leaf litter, seepage zones, and flooded banks within ~300 m of the main stream.

The only previously sequenced *Leptobrachium* specimens from Bali were collected from Mount Batukaru (Hamidy & Matsui, 2017). Our new series from Banyuwana Amertha represents the first genetic samples from Buleleng, located approximately 20 to 25 km north of the Batukaru locality, thereby extending the known genetic sampling range on the island. We collected 13 adult specimens by hand, using powder-free nitrile gloves to prevent contamination and minimise disturbance to microhabitats and non-target fauna. Individuals were temporarily housed in clean, damp cloth bags until processing. Standard morphometric, habitat, and environmental data (air/water temperature, humidity, GPS coordinates, elevation) were recorded prior to euthanasia with 20% benzocaine solution, tissue sampling, fixation, and deposition in the Museum Zoologicum Bogoriense (MZB).

Immediately after capture, specimens were anaesthetised via topical application of 20% benzocaine following guidelines outlined by the American Society of Ichthyologists and Herpetologists (HACC-ASIH, 2004). Liver tissue was removed using sterile tools and placed in 95–100% ethanol for DNA analysis (Nagy, 2010). Specimens were then fixed in 10% neutral-buffered formalin for 24–48 hours and transferred to 70% ethanol for long-term storage in the Museum Zoologicum Bogoriense (MZB), accessioned under institutional protocol.

DNA extraction and phylogenetic analyses. We extracted genomic DNA using a phenol–chloroform protocol (Sambrook & Russell, 2001) optimised for amphibian tissues. We PCR-amplified a 1,979 bp concatenated fragment comprising mitochondrial 12S rRNA, tRNA^{Val}, and 16S

Table 1. Uncorrected pairwise sequence divergences (p-distances, %) for the mitochondrial 16S rRNA fragment among *Leptobrachium* (sensu stricto) species from Sundaland. Values are based on aligned sequence data generated in this study and retrieved from GenBank; p-distances were calculated using MEGA under the pairwise deletion option.

No	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	<i>L. widianai</i> , new species, Bali	0.0											
2	<i>L. widianai</i> , new species, Kediri	0.0–1.3	0.0										
3	<i>L. smithi</i>	17.1–17.5	17.5	0.0									
4	<i>L. kanowitense</i>	14.9–15.3	14.9	20.0	0.0								
5	<i>L. montanum</i>	14.8–15.1	14.8	15.1	15.3	0.0							
6	<i>L. waysepuntiense</i>	14.4–14.6	13.8	14.4	15.3	2.6	0.0						
7	<i>L. abbotti</i>	14.2–14.4	13.9	15.7	15.7	5.4	5.2	0.0					
8	<i>L. nigrops</i>	13.2–13.4	12.5	18.4	13.3	15.1	15.1	15.0	0.0				
9	<i>L. ingeri</i>	12.7–12.9	12.1	18.8	15.3	15.5	15.5	14.8	10.3	0.0			
10	<i>L. hendricksoni</i>	10.0–10.2	9.7	18.7	15.6	16.0–16.2	15.8–15.9	15.3	16.1	17.1	0.0		
11	<i>L. hasseltii</i> Sumatra	3.7–4.1	3.2–3.4	16.7–16.9	12.5–12.9	12.7–12.9	12.7–12.9	15.5	12.3–12.5	11.4–11.6	9.8–10.2	0.0	
12	<i>L. hasseltii</i> West Java	4.3–5.2	3.9–4.5	16.2–17.1	12.3–13.1	12.4–12.7	12.9–13.1	12.0–12.4	12.3–12.7	11.0–11.9	9.7–10.6	1.5–2.2	0.0
13	<i>L. hasseltii</i> Central Java	4.7–5.2	4.3–4.5	15.4–16.0	13.3–14.0	12.7–12.9	12.0–12.7	10.9–11.5	12.3–12.5	11.2–11.4	10.4	2.4–2.8	2.6–3.0

rRNA genes—markers frequently used for species-level resolution in anuran systematics (Vences et al., 2005; Brown et al., 2009). PCR employed primers and thermal profiles from Hamidy et al. (2012) (Supplementary Table 1), and sequencing was performed bidirectionally using Sanger methods following Sanger et al. (1977).

Our dataset comprised 24 *Leptobrachium* sequences, of which five samples represent four other congeners —*L. ingeri* (n = 1), *L. montanum* (n = 1), *L. waysepuntiense* (n = 1), and *L. nigrops* (n = 2)—used as outgroups, while the remaining 19 sequences represent *L. hasseltii* sensu lato. Of these, 14 sequences were obtained from Hamidy & Matsui (2017; Supplementary Table 2), while five new sequences were generated in this study from our East Java (n = 2) and Bali (n = 3) specimens. The Bali series includes newly added museum samples from eastern Bali, filling a previous geographic gap in sampling coverage on the island.

Sequences were aligned in MAFFT v.7 (Katoh & Standley, 2013) with manual adjustment in Geneious. We used PartitionFinder2 (Lanfear et al., 2017) to select optimal partitioning schemes and substitution models under AICc, which favoured GTR+G. Phylogenetic reconstruction followed a dual Bayesian and Maximum Likelihood (ML) framework: MrBayes v3.2.7 (Ronquist et al., 2012) with two independent MCMC runs (four chains) of 20 million generations (sampling every 1,000; 25% burn-in), and IQ-TREE v2 (Minh et al., 2020) with 1,000 ultrafast bootstrap replicates. Topologies were considered well-supported with posterior probabilities ≥ 0.95 and bootstrap values $\geq 95\%$, consistent with thresholds applied in recent taxonomic revisions (Hamidy & Matsui, 2017; Fauzan et al., 2023).

Morphological analyses. Adult morphometric analyses followed the standardised protocols of Matsui (1984) and Hamidy & Matsui (2010), using a Mitutoyo digital calliper (± 0.1 mm accuracy) to measure 23 quantitative characters (see list below). Qualitative characters, including femoral and humeral gland presence, dorsal and ventral colour patterns, dermal tuberculation, and toe-webbing configuration were recorded following Savage (1975). We collected morphological data from a total of 127 preserved specimens, comprising 114 adults and 13 tadpoles (for depository, see abbreviations). Sex was determined by directly observing vocal slits in males and/or inspecting gonads when uncertain.

(1) snout-vent length (SVL); 2) head length (HL); 3) snout length (SL); 4) snout-nostril length (S-NL); 5) nostril-eye distance (N-EL); 6) eye length (EL, including eyelid); 7) tympanum-eye length (T-EL); 8) tympanum diameter (TD); 9) head width (HW); 10) internarial distance (IND); 11) interorbital distance (IOD); 12) upper eyelid width (UEW); 13) forelimb length (FLL); 14) lower arm and hand length (LAL) from elbow to tip of third finger; 15) third finger length (TFL); 16) first finger length (FFL); 17) outer palmar tubercle length (OPTL); 18) inner palmar tubercle length (IPTL); 19) tibia length (TL); 20) foot length (FL); 21) hindlimb length (HLL); 22) inner metatarsal tubercle length (IMTL); and 23) first toe length (1TOEL). Additional

Table 2. Principal component loadings of the first three axes from PCA analyses of Thorpe-adjusted morphometric characters in *Leptobranchium*. Results are provided separately for males, females, and pooled samples. Loadings indicate the relative contribution of each character to the respective principal component.

Males			
Character	PC1	PC2	PC3
Eigenvalue	3.22	1.41	0.94
% Variance	40.2	17.6	11.8
HL	-0.37	-0.08	0.26
N-EL	-0.36	0.07	0.05
HW	-0.35	-0.20	0.02
IOD	-0.33	-0.04	0.01
LAL	-0.22	0.01	-0.00
HAL	-0.30	-0.23	-0.26
TL	-0.17	0.31	-0.05
1TOEL	0.14	-0.22	-0.17
Females			
Character	PC1	PC2	PC3
Eigenvalue	3.35	1.6	0.88
% Variance	41.9	20	11
HL	-0.34	-0.06	0.28
N-EL	-0.33	0.08	0.04
HW	-0.34	-0.22	0.03
IOD	-0.31	-0.05	0.02
LAL	-0.24	0.02	-0.01
HAL	-0.31	-0.25	-0.25
TL	-0.18	0.3	-0.06
1TOEL	0.13	-0.23	-0.18
Pooled sexes			
Character	PC1	PC2	PC3
Eigenvalue	3.44	1.24	0.91
% Variance	43	15.5	11.3
HL	-0.36	-0.09	0.27
N-EL	-0.35	0.07	0.05
HW	-0.35	-0.21	0.02
IOD	-0.32	-0.05	0.01
LAL	-0.23	0.02	-0.00
HAL	-0.30	-0.24	-0.26
TL	-0.17	0.31	-0.05
1TOEL	0.13	-0.22	-0.17

qualitative morphological characters included colour pattern, dermal ornamentation (femoral and humeral glands), toe webbing states, and size and shape of subarticular tubercles.

Morphometrics and multivariate analyses. To minimise redundancy among highly correlated variables and to focus on morphologically informative traits, we selected eight representative characters (HL, N-EL, HW, IOD, LAL, HAL, TL, 1TOEL) spanning major anatomical regions (head, limbs, and trunk) for multivariate analyses. These variables were chosen based on biological interpretability and variance contribution from preliminary PCA results. To remove the confounding effects of size while retaining true shape

variation, we applied the allometric adjustment of Thorpe (1975), as optimised by Chan & Grismer (2022) in the GroupStruct R package. For each trait X , we regressed $\log(X)$ against $\log(\text{SVL})$ within each sex, and calculated adjusted values as: $\log X_{\text{adj}} = \log X - \beta [\log(\text{SVL}) - \log(\text{SVL}_{\text{mean}})]$, where β is the regression slope and is the species-specific mean SVL (OTU mean) for interspecific comparisons. This approach is widely recognised as superior to ratio or residual methods, which assume isometry or homogeneous slopes, and is particularly effective in resolving interspecific morphometric structure.

All adjusted variables were standardised (mean = 0, SD = 1) prior to multivariate analyses. Principal Component Analyses (PCA) were performed separately for males and females using the prcomp function in R, based on the correlation matrix. Biplots of the first two PCs, with 95% confidence ellipses for each species, were examined to visualise clustering and to identify the variables contributing most to separation.

To statistically test group distinctiveness, we conducted Multivariate Analyses of Variance (MANOVA) on the full set of Thorpe-adjusted and standardised morphometric variables, with species as the predictor variable. Principal Component Analysis (PCA) was used only for visualising morphospace and assessing variable loadings, while MANOVA, LDA, and PERMANOVA tested species-level separation directly on the adjusted variables. Where assumptions of parametric MANOVA were not fully met, we confirmed results with non-parametric PERMANOVA (adonis, vegan). These complementary approaches provided both visualisation and statistical support for the morphometric distinctiveness of the new species relative to congeners.

Larval morphology and image analysis. Tadpoles were staged following Gosner (1960) and Wildenhues et al. (2012). We photographed specimens in a calibrated Petri dish setup under consistent lighting, then measured 19 characters via ImageJ v1.53 (Rueden et al., 2017) using digital callipers for validation. All measures conformed to the standards established in McDiarmid & Altig (1999) and Haas & Das (2011). We recorded labial tooth row formula, oral disc morphology, and spiracular position, matching methods used in recent larval descriptions (e.g., Hamidy et al., 2012).

External body characters of tadpoles were measured using ImageJ software (Rueden et al., 2017). The measurement of 19 body characters followed McDiarmid & Altig (1999) and Haas & Das (2011). The body characters measured were: (1) eye diameter (ED); (2) body height (BH); (3) tail muscle height (TMH); (4) maximum tail height (MTH); (5) upper fin height (UFH); (6) lower fin height (LFH); (7) distance of snout to centre of spiracle (DSS); (8) body end to centre of spiracle (BES); (9) total length (TTL); (10) tail length (TAL); (11) body length (BL); (12) maximum body width (MBW); (13) oral disc width (ODW); (14) tail width at trunk-tail junction (TWTJ); (15) interorbital distance (IOD); (16) internarial distance (IND); (17) tip of snout to anterior edge of eye (TSE); (18) distance from centre of naris to centre of eye (DNE); (19) distance of naris from snout (DNS).

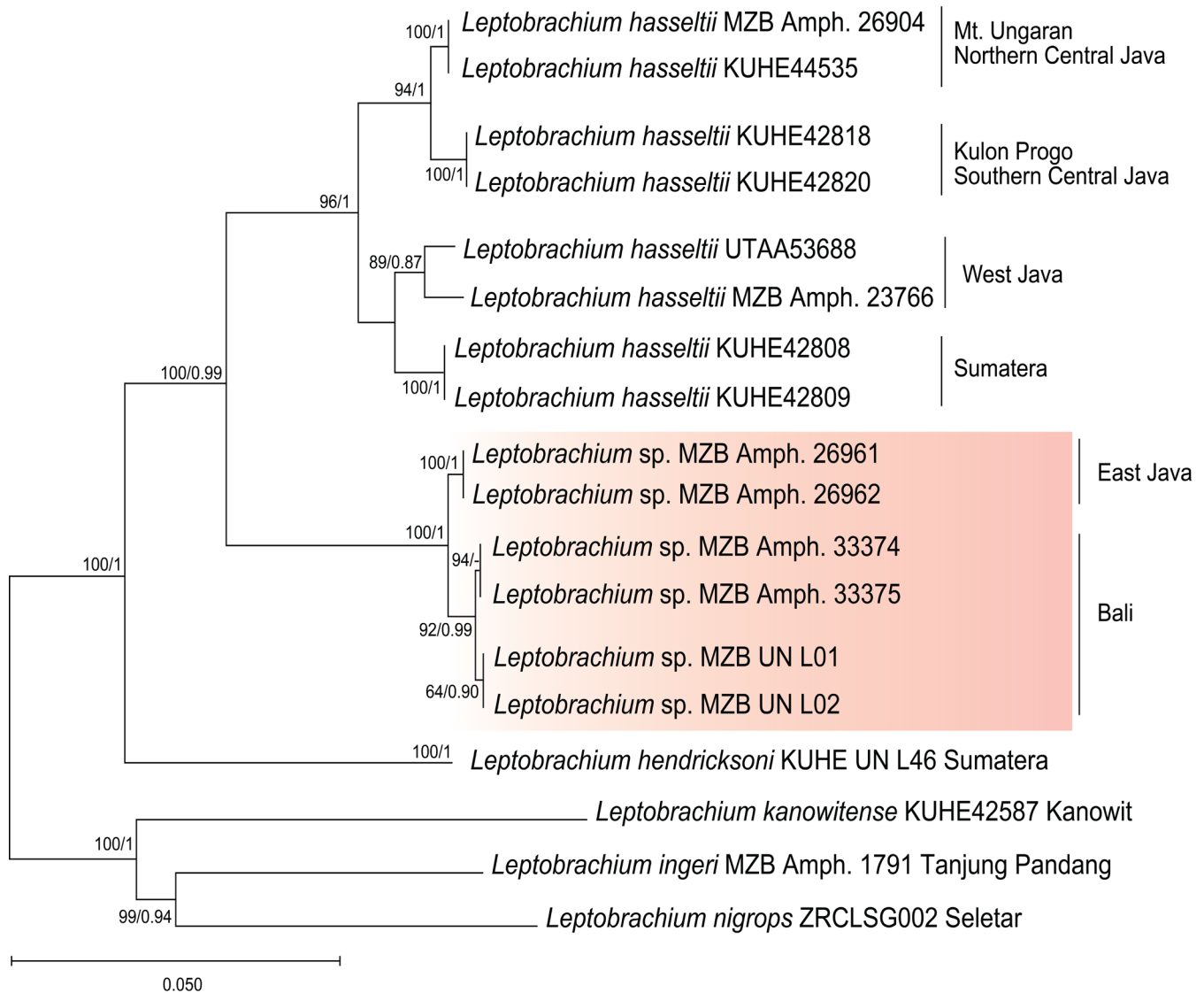


Fig. 2. Maximum-likelihood phylogram of 1,979bp of mitochondrial DNA sequences for Sundaland and Indonesian *Leptobrachium*. Numbers above branches represent maximum-likelihood bootstrap supports and Bayesian posterior probabilities (MLBS/BPP). The new species is highlighted in red.

Acoustic analyses. We recorded advertisement calls at a breeding site adjacent to a forest pool near Banyuwana Amertha Waterfall in Buleleng, Bali, at 1,100 m a.s.l. on 20 December 2021 and 9 February 2023 using a digital recorder (Sony ICD-PX470) with built-in microphones at 16 bits and 44.1 kHz as uncompressed wave files. Temperature and time of recording were logged. Three advertisement calls from a single individual (MZB Amph. 33220) were analysed in Raven Pro 1.6 using a 1024-point Hanning window FFT (www.birds.cornell.edu/raven), following acoustic parameter definitions per Köhler et al. (2017) and Pettitt et al. (2012). The calls were recorded at distances of approximately 1–2 m and an air temperature of 22.3°C. Temporal and spectral characteristics were extracted from the oscillograms (waveforms), while frequency information was obtained from the audio-spectrograms (sonograms) using Fast Fourier Transformation (1,024-point Hanning window). In defining a call and a pulse, we followed Pettitt et al. (2002), and Köhler et al. (2017) and measured the following parameters: Call duration (s; measured from beginning to the end of a

single call); Pulses per call (number of pulses per call); Pulse duration (s; measured from beginning to end of pulse); Pulse interval (s; interval between two consecutive pulses, measured from end of the first pulse to the beginning of the second pulse); Pulse rate (pulse/s; number of pulses repeated within a defined time period within a call); Calling rate (calls/s; number of calls repeated within defined time period within consecutive calls); Intercall interval (s, interval between two calls, measured from end of first call to beginning of second call); Call interval (s, interval between two consecutive calls, measured from at ends); Peak time (time within the peak of the amplitude of the pulses), and; First pulse duration (s; duration of the single first pulse, measured from the beginning of first pulse of calls to end of pulse).

Conservation status assessment. We assessed conservation status using IUCN Red List Categories and Criteria (IUCN Standards and Petitions Subcommittee, 2022). Geographic coordinates of known populations were used to calculate Extent of Occurrence (EOO) and Area of Occupancy (AOO)

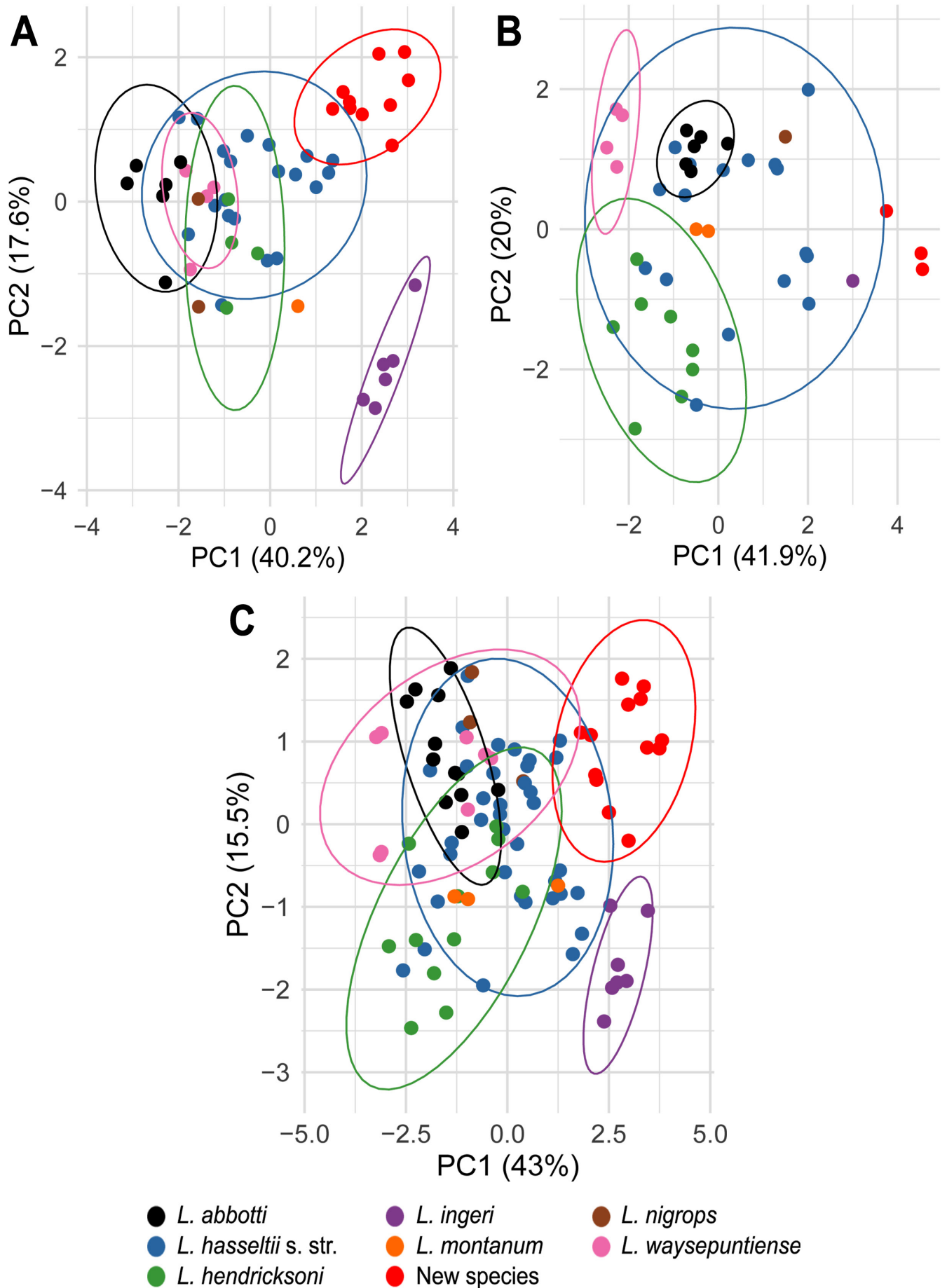


Fig. 3. Principal Component Analysis (PCA) of eight Thorpe-adjusted morphometric characters in *Leptobrachium*. Plots show the first two principal components for (A) males, (B) females, and (C) pooled samples. Each point represents an individual specimen, coloured by species, with 95% confidence ellipses illustrating group dispersion.

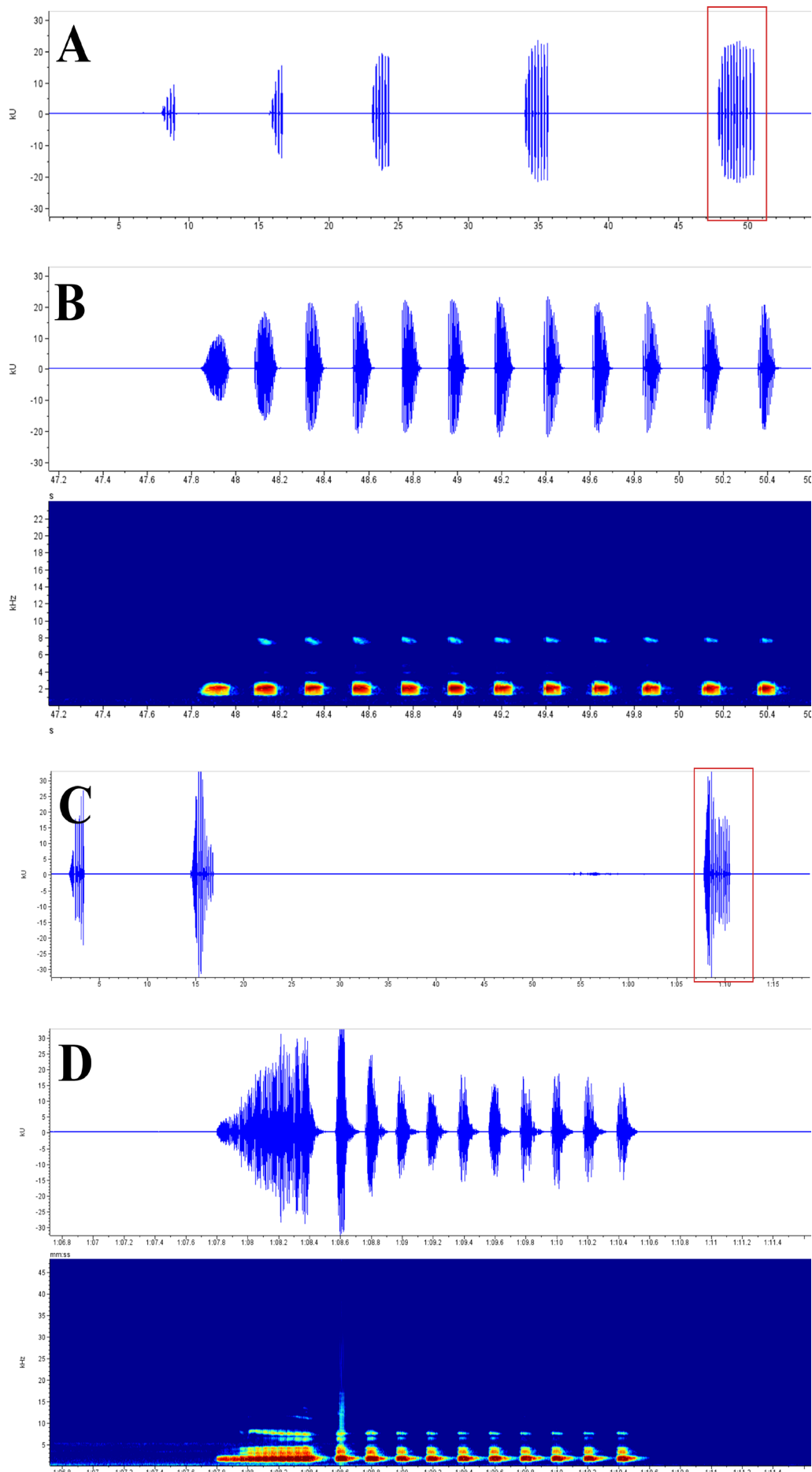


Fig. 4. Waveforms (oscillograms) and spectrograms of advertisement calls. (A, B) *Leptobrachium hasseltii* (MKB Amph. 32743) from Mt. Muria, Central Java; (C, D) *L. widianai*, new species (MKB Amph. 33374) from Buleleng, Bali.

Table 3. Summary statistics for morphometric measurements of male *Leptobrachium* (sensu stricto) from Indonesia. Snout–vent length (SVL) is presented as mean $\pm$ standard deviation (SD), with medians, ranges, and all other characters expressed as ratios relative to SVL. See text for definitions and abbreviations of morphological characters.

Characters	<i>L. hasseltii</i> n = 20	<i>L. hendricksonii</i> n = 4	<i>L. abboti</i> n = 6	<i>L. waysepuntiense</i> n = 6	<i>L. ingeri</i> n = 6	<i>L. nigrops</i> n = 2	<i>L. montanum</i> n = 1	<i>L. widianai</i> , new species n = 10
SVL	39.34 $\pm$ 6.76	38.80 $\pm$ 6.66	45.37 $\pm$ 6.04	43.83 $\pm$ 8.99	30.30 $\pm$ 1.86	32.96 $\pm$ 4.04	61.10	36.22 $\pm$ 2.65
RHL	42.2 (42.8–47.1)	43.9 (43.3–44.4)	43.6 (42.9–44.5)	44.6 (44.2–45.9)	41.5 (41.3–42.9)	43.4 (43.2–43.5)	42.2	41.7 (39.2–43.0)
RSNL	3.4 (2.5–6.1)	1.6 (1.4–1.7)	5.2 (4.7–5.7)	2.8 (2.5–2.9)	4.4 (4.3–4.8)	4.6	5.1	3.6 (3.2–4.5)
RNEL	9.3 (8.0–10.3)	9.5 (9.4–9.7)	9.4 (8.9–9.8)	9.3 (9.1–9.9)	8.6 (8.4–9.4)	9.3	9.5	8.3 (8.1–8.6)
RSL	14.1 (13.0–16.4)	12.78 (12.3–13.8)	15.9 (15.1–16.2)	13.8 (13.1–14.4)	13.2 (13.0–13.5)	15.9 (15.9–16.0)	15.4	14.7 (14.2–15.7)
REL	11.7 (8.9–14.5)	14.0 (13.8–14.4)	13.1 (12.3–13.3)	12.8 (12.7–13.1)	14.3 (13.5–14.7)	12.4 (12.1–12.6)	9.9	13.5 (12.9–13.8)
RTEL	5.3 (4.0–6.8)	4.4 (4.0–4.7)	5.9 (5.0–6.2)	4.7 (4.4–4.9)	4.0 (3.9–4.5)	5.6 (5.5–5.6)	5.1	4.5 (4.1–5.1)
RTD	6.6 (5.3–8.6)	7.5 (7.2–7.9)	7.5 (7.1–7.6)	6.8 (6.3–7.0)	7.0 (6.4–7.4)	8.4 (8.3–8.4)	6.9	6.6 (6.1–7.1)
RHW	42.7 (39.9–46.3)	43.3 (42.1–44.4)	42.5 (41.3–43.9)	43.5 (42.9–44.7)	36.9 (36.2–38.3)	40.2 (39.5–40.9)	40.3	40.4 (39.4–45.4)
RIND	8.0 (6.9–8.9)	7.8 (7.5–8.9)	7.3 (7.1–7.8)	8.3 (8.0–8.4)	7.8 (7.6–8.1)	6.4 (6.3–6.6)	7.2	8.5 (7.9–9.1)
RIOD	14.4 (10.1–16.4)	15.1 (14.3–15.3)	16.5 (16.3–16.8)	17.3 (17.0–17.9)	12.7 (12.3–13.2)	16.5 (16.5–16.6)	13.4	12.9 (11.4–13.4)
RUEW	11.5 (9.5–11.7)	12.9 (12.5–13.3)	11.2 (11.0–11.9)	10.6 (9.7–10.6)	11.5 (11.0–11.9)	12.3 (11.9–12.6)	11.3	11.0 (10.1–11.7)
RFL	70.8 (67.8–75.4)	68.7 (66.2–69.8)	76.0 (74.9–76.8)	69.7 (69.1–71.7)	74.9 (71.2–75.7)	75.9 (75.4–76.3)	70.4	71.5 (70.3–74.5)
RIAL	54.4 (46.7–57.7)	54.6 (54.1–55.7)	55.8 (52.5–56.6)	51.9 (51.8–53.5)	52.4 (52.2–52.7)	55.6 (51.8–55.8)	52.4	52.8 (51.3–53.7)
ROPTL	4.6 (3.8–5.2)	4.8 (4.4–4.9)	5.1 (5.0–5.4)	4.7 (4.5–4.8)	4.7 (4.4–4.9)	3.6 (3.5–3.6)	4.4	4.5 (4.2–5.1)
RIPTL	4.6 (4.0–6.4)	4.3 (4.2–4.6)	5.6 (5.4–5.8)	4.9 (4.3–4.9)	4.4 (4.2–4.9)	4.2 (3.9–4.5)	3.6	4.3 (4.0–4.9)
RHAL	26.3 (23.8–28.3)	24.3 (23.2–24.5)	26.6 (26.0–27.4)	26.7 (26.1–27.0)	22.2 (22.1–22.7)	28.3 (28.2–28.3)	24.9	25.7 (24.4–26.7)

Characters	<i>L. hasseltii</i> n = 20	<i>L. hendricksonii</i> n = 4	<i>L. abboti</i> n = 6	<i>L. waysepuntiense</i> n = 6	<i>L. ingeri</i> n = 6	<i>L. nigrops</i> n = 2	<i>L. montanum</i> n = 1	<i>L. widianai</i> , new species n = 10
RHLL	115.4 (112.2–121.9)	111.5 (111.1–111.7)	121.1 (119.8–121.8)	108.2 (108.1–108.9)	121.5 (121.0–121.9)	118.7 (118.4–118.9)	119.5	121.2 (120.0–123.5)
RTL	36.8 (34.7–39.8)	38.0 (37.4–39.1)	37.5 (37.3–39.1)	38.5 (37.4–38.6)	37.9 (37.6–39.0)	39.6 (39.4–39.9)	37.6	36.2 (35.4–37.8)
RFL	36.9 (34.3–38.0)	34.9 (34.0–35.7)	35.7 (33.1–36.5)	35.5 (34.6–36.9)	35.4 (34.5–36.4)	33.8 (33.4–34.2)	34.5	36.5 (35.2–38.6)
RIMTL	3.5 (2.5–4.5)	3.2 (3.1–3.2)	3.5 (3.3–3.6)	4.1 (4.1–4.5)	4.2 (4.1–4.8)	3.9 (3.8–3.9)	3.1	3.9 (3.3–4.2)
RFFL	11.0 (9.8–12.0)	10.4 (10.2–10.5)	11.6 (11.1–11.8)	10.2 (10.2–10.3)	9.1 (8.9–9.1)	9.6 (9.5–9.6)	10.4	12.0 (11.2–13.0)
RTFL	19.0 (18.0–19.0)	15.9	20.1 (18.6–21.8)	18.9 (18.7–19.0)	15.8 (15.7–16.1)	16.5	16.2	17.0 (17–18.2)
RITOEL	8.0 (7.0–8.3)	7.6 (7.6–7.7)	9.3 (9.0–9.7)	7.8 (7.5–7.9)	6.9 (6.8–7.1)	6.1	7.7	9.0 (8.0–9.0)

via GeoCAT (Bachman et al., 2011), following best practices in recent species assessments (e.g., Hamidy & Matsui, 2017; Fauzan et al., 2023).

RESULTS

Phylogeny. Our molecular phylogenetic analyses, based on concatenated mitochondrial 12S–16S rRNA sequences, recovered a strongly supported monophyletic clade corresponding to *Leptobrachium hasseltii* sensu lato (MLBS = 100; BPP = 0.99; Fig. 2). Within this clade, populations from East Java and Bali—herein described as a new species—formed a reciprocally monophyletic group, recovered as the basal lineage to all other *L. hasseltii* sensu stricto populations. This lineage was strongly supported in both ML and BI analyses (MLBS = 100; BPP = 1.00) and was clearly distinct from populations of *L. hasseltii* occurring in Central Java, West Java, and Sumatra (MLBS = 96; BPP = 1.00).

These results corroborate earlier work by Hamidy & Matsui (2017), which identified two major genetic lineages within the *L. hasseltii* complex. Our data clarify this structure by demonstrating that the East Java–Bali lineage is genetically cohesive, geographically restricted, and phylogenetically distinct from all other Sundaic *L. hasseltii* sensu stricto populations.

Uncorrected pairwise sequence divergences (16S rRNA) between the East Java–Bali population and its Sundaland congeners ranged from 3.2% to 17.5% (Table 1). Its genetic distance from its closest relative, *L. hasseltii* sensu stricto, was 3.2–5.2%, exceeding the ≥3% divergence threshold widely used as a preliminary criterion for candidate species recognition in amphibians (Vences et al., 2005; Fouquet et al., 2007). This level of divergence, combined with reciprocal monophyly and strong statistical support, provides robust molecular evidence for the recognition of the East Java–Bali population of *L. hasseltii* as a distinct taxon.

Morphometric differentiation. After allometric size adjustment and standardisation of the eight selected morphometric characters (HL, N-EL, HW, IOD, LAL, HAL, TL, and 1TOEL), Principal Component Analysis (PCA) revealed clear structuring of morphometric variation among *Leptobrachium* species. In males, PC1 and PC2 had eigenvalues of 3.22 and 1.41, explaining 40.2 % and 17.6 % of total variance, respectively. In females, PC1 and PC2 had eigenvalues of 3.35 and 1.60, accounting for 41.9 % and 20.0 % of total variance. For pooled sexes, PC1 (eigenvalue = 3.44) and PC2 (eigenvalue = 1.24) together captured 58.5 % of overall variation. Only components with eigenvalues > 1 were retained for interpretation. PC1 was mainly associated with head dimensions (HL, HW, N-EL, IOD) and limb proportions (LAL, HAL), whereas PC2 was influenced primarily by tibia length (TL) and first-toe length (1TOEL) (Table 2). Biplots of the first two PCs showed that *L. widianai*, new species, formed a distinct and compact cluster with little or no overlap with congeners (Fig. 3). In the pooled-sex PCA (Fig. 3C), partial overlap among congeners

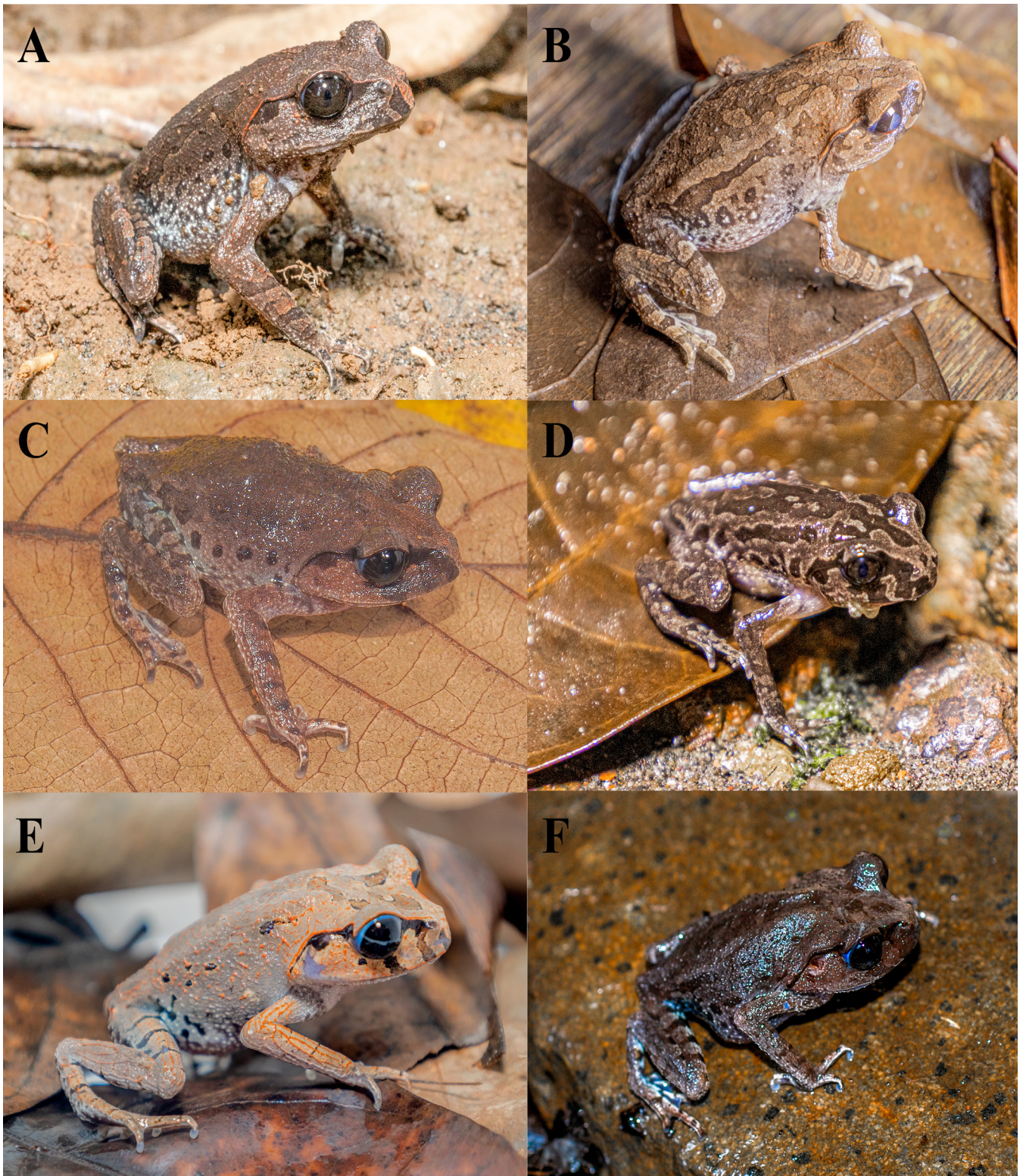


Fig. 5. Two species of Bali and Javan *Leptobrachium* in life. (A, B) Adult male *L. widianai*, new species, from Buleleng, Bali (MZB Amph. 33320, 32734); (C) adult male from Kediri, East Java (MZB Amph. 26959); (D) juvenile (MZB Amph. 32735); (E) adult male *L. hasseltii* from Mt. Slamet, Central Java (MZB Amph. 32743); (F) adult male *L. hasseltii* from Cisarua, West Java (MZB Amph. 32735).

was expected due to sexual dimorphism and the conservative morphology of the genus. Nevertheless, *L. widianai*, new species, remained clearly distinct and non-overlapping with all other species, confirming its morphometric separateness.

Multivariate analysis of variance (MANOVA) confirmed significant differences among species in multivariate

morphometric space (Supplementary Table 3). In males, the model returned Wilks' $\lambda = 5.51$, approximate $F = 5.39$, $df = 154/224$, $p < 0.001$. Equivalent results were obtained with Pillai's trace, Hotelling–Lawley, and Roy's largest root, all indicating highly significant species effects. Similar patterns were observed in females and pooled datasets, reinforcing the strong morphometric distinctiveness of the new species.

Linear Discriminant Analysis (LDA) further quantified interspecific separation (Supplementary Table 4). Cross-validated classification accuracy exceeded 90% across all datasets, with *L. widianai*, new species, correctly classified in all cases and never misassigned.

Permutation-based multivariate analysis (PERMANOVA) provided additional support for species separation (Supplementary Table 5). In males, the model explained 53.5 % of variance ($R^2 = 0.535$; pseudo- $F = 7.74$; $p = 0.001$). In females, the model explained 51.9 % of variance ($R^2 = 0.520$; pseudo- $F = 5.25$; $p = 0.001$), and in the pooled dataset 39.3 % of variance ($R^2 = 0.393$; pseudo- $F = 8.22$; $p = 0.001$). These results consistently confirm that the morphometric separation among species—particularly that of *L. widianai*, new species—is robust to parametric assumptions and supported across all analyses.

Integrated evidence for species recognition. The congruence of three independent datasets—molecular phylogeny, quantitative morphology, and bioacoustics—provides a robust framework for recognising the East Java–Bali population as a distinct evolutionary lineage. The East Java–Bali populations are genetically diagnosable, morphologically discrete, and bioacoustically divergent from *L. hasseltii* sensu stricto and all other congeners. The combination of strong node support in phylogenetic analyses, diagnostic morphometric differences, and species-specific acoustic traits meets multiple operational species criteria and justifies formal taxonomic description.

TAXONOMY

Family Megophryidae Bonaparte, 1850

Leptobrachium Tschudi, 1838

Leptobrachium widianai, new species

(Figs. 5A–C, 6; Tables 3–5)

Leptobrachium hasseltii (part) Hamidy & Matsui, 2017, p. 23

Holotype. MZB Amph. 33320, adult male, collected among leaf litter in intact primary lower montane forest along the trail to Banyuwana Amertha Waterfall, Wanagiri Village, Sukasada District, Buleleng Regency, Bali Province, Indonesia (08°12'46.18"S, 115°07'20.02"E; 1,100 m a.s.l.), at 2000 h on 9 February 2023 by Muhammad Fakhri Fauzan, Efendi Sabinhaliduna, and Dimas Bayu Nur Ramadhan.

Paratypes. Fourteen specimens from the type locality: Adult males ($n = 4$): MZB Amph. 32733, 32734, 33351, 33369 (collected 9 February 2023); Adult females ($n = 3$): MZB Amph. 22210, 26961, 26962; Subadults/Juvenile ($n = 8$): MZB Amph. 33352, 33364, 33365, 33366, 33367, 33368 (collected 9 February 2023), MZB Amph. 32735, 32736 (collected 20 December 2021). Collected by Muhammad Fakhri Fauzan, Quraisy Zaky, and Dimas Bayu Nur Ramadhan.

Diagnosis. A medium-sized *Leptobrachium* (adult male SVL 36.2 mm; adult female SVL to 54.1 mm) assigned to the *Leptobrachium hasseltii* group (subgenus *Leptobrachium* sensu Matsui et al., 2010) based on iris colour pattern, femoral gland morphology, and absence of upper lip spines. Distinguished from all congeners by the following combination of characters: (1) Iris and sclera: iris uniformly brown in adults with a narrow light blue scleral arc visible when the eye is fully open (juveniles with distinctive white spot-ring on iris); (2) Femoral glands: very large, oval (occasionally circular), conspicuous, with dark surrounding pigmentation; (3) Humeral glands: present, circular, flat; (4) Dentition and projections: vomerine teeth absent; snout without projections; no dermal palpebral projection; no rictal glands; ventrolateral glandular ridges absent; no upper lip spines. (5) Digital morphology: inner palmar tubercle circular, not extending along first metacarpal; subarticular tubercles reduced, replaced by low callous pads; fingers and toes with rounded, swollen tips, lacking circummarginal grooves; toe webbing poorly developed (I 0–2+, II 0–3+, III 2+–4, IV 4–2 V); (6) Dorsal pattern: typically dark brown with large irregular blotches and paired transverse bars; sometimes with orange vertebral line or orange dots; interorbital–parietal region with darker markings; (7) Lateral and ventral pattern: lateral surfaces dark grey with scattered white granules and a distinct black spot between axilla and groin; ventrum grey to light grey, throat darker, chest densely granular with white spots; (8) Call structure: elongated series of short “barks” followed by low-frequency “squawks,” temporally and structurally distinct from *L. hasseltii*; (9) Larvae: LTRF 7(1–6)/5(1–4) (vs. 5(1–4)/5(1–4) in *L. hasseltii*), marginal papillae conical and acute.

Description of the holotype (all measurements in mm).

Body tapering to the groin (SVL 36.2 mm), head broad and depressed, slightly longer (HL 15.5: 41.8% of SVL) than wide (HW 14.7: 40.5% SVL); snout rounded from above, truncate in profile, slightly projecting beyond lower jaw; eye large and prominently projecting from sides of head and slightly projecting from above, smaller (EL 4.9: 13.5% SVL) than snout (SL 5.7: 15.6% SVL), pupil vertical; lips not extending beyond the lateral edge of orbit when viewed from above; canthus rostralis very sharp, lores oblique, moderately concave; nostrils lateral, below the canthus, distinctly closer to the tip of snout (S-NL 1.5: 4.0% SVL) than to the eye (N-EL 3.2: 8.8% SVL); internarial distance (IND 3.2: 8.9% SVL) much shorter than interorbital distance (IOD 4.8: 13.1% SVL), latter wider than the upper eyelid (UEW 3.8: 10.6% SVL); pineal spot absent; tympanum distinct, obliquely oval, diameter (TD 2.2: 6.1% SVL) about three-fifth of the eye and separated from the eye by half of its diameter (T-EL 1.6: 4.5% SVL), tympanic annulus weakly distinct, dorsal edge of the tympanic annulus concealed by the supratympanic fold; tongue heart-shaped, without papillae, notched posteriorly; vomerine teeth absent.

Forelimb slender and long (FLL 27.3: 75.4% SVL), about three-fifths the length of hindlimb; fingers moderately slender, unwebbed; tips rounded, swollen, relative finger lengths I

Table 4. Summary statistics for morphometric measurements of female *Leptobrachium* (sensu stricto) from Indonesia. Snout-vent length (SVL) is presented as mean $\pm$ standard deviation (SD), with medians, ranges, and all other characters expressed as ratios relative to SVL. See text for definitions and abbreviations of morphological characters.

Characters	<i>L. hasseltii</i> n = 17	<i>L. hendricksonii</i> n = 8	<i>L. abboti</i> n = 6	<i>L. waysepuntiense</i> n = 4	<i>L. ingeri</i> n = 1	<i>L. nigrops</i> n = 1	<i>L. montanum</i> n = 2	<i>L. widianai, new species</i> n = 3
SVL	54.51 $\pm$ 8.21	55.32 $\pm$ 12.34	59.97 $\pm$ 7.63	60.8 $\pm$ 11.18	41.53	34.1	60.65 $\pm$ 15.34	48.3 $\pm$ 8.97
RHL	43.9 (41.2–47.4)	45.7 (44.3–46.5)	43.0 (41.1–43.8)	45.5 (45.2–45.8)	40.6	42.2	43.5 (43.2–43.8)	38.2 (37.9–38.4)
RSNL	3.3 (2.5–4.9)	1.5 (1.3–1.8)	4.5 (4.4–4.9)	2.2 (2.1–2.5)	3.6	5.3	4.5 (4.2–4.8)	3.6 (3.5–3.9)
RNEL	9.5 (7.6–10.6)	10.3 (10.2–10.5)	10.0 (9.8–10.3)	9.4 (9.2–9.9)	8.0	8.8	10.1 (10.0–10.2)	8.0 (7.4–8.4)
RSL	13.8 (12.9–16.0)	13.1 (13.1–13.8)	16.0 (15.5–16.3)	13.5 (13.2–13.8)	13.1	14.7	15.2 (15.1–15.4)	12.7 (12.3–13.0)
REL	10.7 (9.4–12.9)	11.5 (10.1–11.9)	11.7 (11.2–12.1)	12.7 (11.8–13.3)	13.8	12.3	11.5 (11.4–11.5)	10.6 (10.5–10.9)
RTFL	5.8 (4.3–7.5)	4.9 (4.4–5.3)	5.8 (5.3–6.4)	4.9 (4.8–5.1)	3.1	5.9	7.7 (7.6–7.8)	5.7 (5.2–5.8)
RTD	6.2 (4.9–6.9)	7.8 (7.5–8.3)	7.4 (6.6–7.8)	7.0 (6.3–7.7)	7.9	7.0	7.7 (7.6–7.8)	6.6 (6.4–6.7)
RHW	42.3 (40.3–46.1)	45.0 (43.5–46.3)	44.9 (44.2–45.4)	46.0 (44.9–47.2)	39.5	39.0	42.4 (42.2–42.6)	41.4 (41.0–41.7)
RIND	7.5 (6.5–8.8)	8.4 (7.7–8.9)	7.1 (6.8–7.8)	8.6 (7.4–8.8)	7.6	6.7	7.7 (7.6–7.7)	7.3 (7.2–7.7)
RIOD	14.4 (12.5–16.9)	14.4 (13.8–15.6)	17.3 (17.0–17.4)	17.9 (17.5–18.2)	13.4	17.6	15.5 (15.3–15.7)	12.8 (12.6–13.1)
RUEW	10.9 (9.2–12.3)	12.3 (11.2–13.0)	11.8 (11.0–11.9)	12.9 (12.7–13.1)	11.8	11.7	11.6	9.4 (9.2–9.8)
RFL	72.9 (64.0–82.5)	74.8 (73.3–75.4)	73.8 (72.3–74.5)	73.4 (72.4–75.0)	72.4	67.7	73.8 (73.7–73.9)	69.6 (66.6–70.8)
RIAL	55.1 (52.9–58.0)	53.7 (52.0–55.2)	55.1 (54.1–55.3)	57.6 (57.3–58.6)	52.9	53.4	54.4 (54.1–54.6)	53.3 (52.6–54.6)
ROPTL	4.7 (3.9–5.5)	4.7 (4.2–5.9)	4.7 (4.1–4.8)	3.9 (3.5–4.9)	5.0	4.7	4.6 (4.5–4.8)	4.5 (4.3–4.7)
RIPTL	4.5 (3.8–5.6)	4.5 (3.4–5.3)	4.7 (4.6–4.9)	4.1 (3.5–4.9)	4.3	4.9	5.1 (5.0–5.2)	4.5 (4.1–4.5)
RHAL	25.7 (23.4–27.2)	25.1 (24.0–26.9)	27.9 (26.4–28.4)	26.5 (25.7–27.3)	23.6	25.8	25.7 (25.5–25.9)	24.2 (23.9–25.0)

Characters	<i>L. hasseltii</i> n = 17	<i>L. hendricksonii</i> n = 8	<i>L. abbotti</i> n = 6	<i>L. waysepuntiense</i> n = 4	<i>L. ingeri</i> n = 1	<i>L. nigrops</i> n = 1	<i>L. montanum</i> n = 2	<i>L. widianai, new species</i> n = 3
RHLL	115.7 (111.5–128.5)	114.4 (114.0–115.0)	124.0 (123.3–124.4)	115.5 (113.1–114.7)	116.2	123.2	123.4 (123.1–123.2)	114.8 (114.1–114.8)
RTL	36.3 (34.4–39.7)	39.5 (38.3–41.2)	39.0 (38.6–39.7)	38.8 (38.3–38.9)	37.5	37.8	37.4 (37.2–37.5)	36.1 (35.2–37.7)
RFL	37.1 (34.4–38.5)	34.6 (34.1–36.3)	37.1 (36.5–38.2)	38.6 (38.1–38.8)	32.8	35.2	38.6 (38.4–38.7)	34.5 (34.3–34.9)
RIMTL	3.7 (2.3–4.5)	3.7 (3.2–4.2)	3.6 (3.2–3.9)	3.5 (3.5–3.7)	4.2	4.4	3.1 (3.1–3.2)	3.5 (3.4–3.8)
RFFL	11.3 (10.0–12.0)	10.3 (9.4–10.7)	10.3 (10.0–10.6)	10.2 (10.0–10.5)	9.0	9.0	10.4 (10.3–10.5)	12.5 (11.5–13.0)
RTFL	16.0 (16.0–17.5)	16.6 (16.2–16.8)	17.6 (17.6–17.7)	17.2	15.7	16.1	16.3 (16.0–16.5)	17.3 (17.0–17.7)
RITOEL	7.0 (6.0–7.7)	6.6 (6.3–6.7)	8.7 (8.4–8.8)	7.3 (7.1–7.6)	7.0	5.8	7.7 (7.6–7.8)	8.3 (8.0–8.6)

= II < IV < III; first finger (FFL 5.5: 13.8% SVL) slightly longer than fourth and second, third finger slightly longer than first (TFL 6.6: 16.8% SVL); dermal fringe borders the lateral edges of all fingers; palmar tubercle is divided into inner and outer tubercles, inner palmar tubercle large (IPTL 1.7: 4.7% SVL) not extending into first metacarpal, outer palmar tubercle is larger than the inner palmar (OPTL 2.0: 5.5% SVL); subarticular tubercles indistinct, replaced by low callous tissue.

Hindlimb slender and relatively short (HLL 46.2: 127.6% SVL); heels do not meet when legs are held at right angles to the body; foot slightly longer (FL 14.4: 39.8% SVL) than the tibia (TL 13.3: 36.6% SVL); tibiotarsal articulation addressed limb reaches only to the posterior beginning of tympanic annulus; relative toe lengths I < II < III = V < IV; third toe being subequal to the fifth; toe tips similar to those of the fingers, circummarginal grooves absent; toe webs are poorly developed, webbing formula I 0- – 2+ II 0- – 3+ III 2+ – 4 IV 4 – 2 V (Figs. 6G, 8C); inner metatarsal tubercle low, oval, length (IMTL 2.6: 4.5% SVL) more than half the distance between the tip of the first toe and the tubercle (1TOEL 3.2: 8.0%); the outer metatarsal tubercle absent; subarticular tubercles are obscure, but elongate, replaced by low callous tissue.

The skin above is slightly rough, with minute granules or warts scattered posteriorly, especially around the waist; granules are more dense laterally on the body and on the posterior thigh; ventrally slightly granular; a very low supratympanic ridge extends from the eye to behind the tympanum; indistinct low dermal ridges are present on the upper surface of the forelimb, ventral surface of lower arm and tibia is highly tuberculate; a flat, rounded humeral gland is present at the edge border of the posterior beginning of the axilla behind the arm insertion; oval, very large femoral gland is present on the posterodorsal side of the thigh, covered by dark markings.

Colour. In life, dorsum brown with large dark blotches and paired transverse bars, often with orange highlights or vertebral line. Lateral head brown with bold black bars across upper lip, loreal, and subocular regions; parietal ridge, eyelid, and supratympanic fold edged orange. Tympanum with ~ 2/3 surface darkened. Iris brown with pale blue scleral arc visible when eye open. Ventrums grey to pale grey, mottled; throat darker; chest and throat densely speckled with white. Limbs crossbarred; posterior thigh with irregular black bars and white spotting. Femoral and humeral glands white.

In preservative, the aspects of the colour pattern remain, but the dorsal ground colour darkens, and the orange dots fade to white.

Etymology. The specific epithet honours Mr. I Made Widiana Darma Santosa, Director of the Bali Reptile Park, whose longstanding dedication to the conservation of Indonesian reptiles and amphibians — particularly those of Bali — has significantly advanced both in-situ and ex-situ conservation awareness. The name is formed as a genitive singular noun.

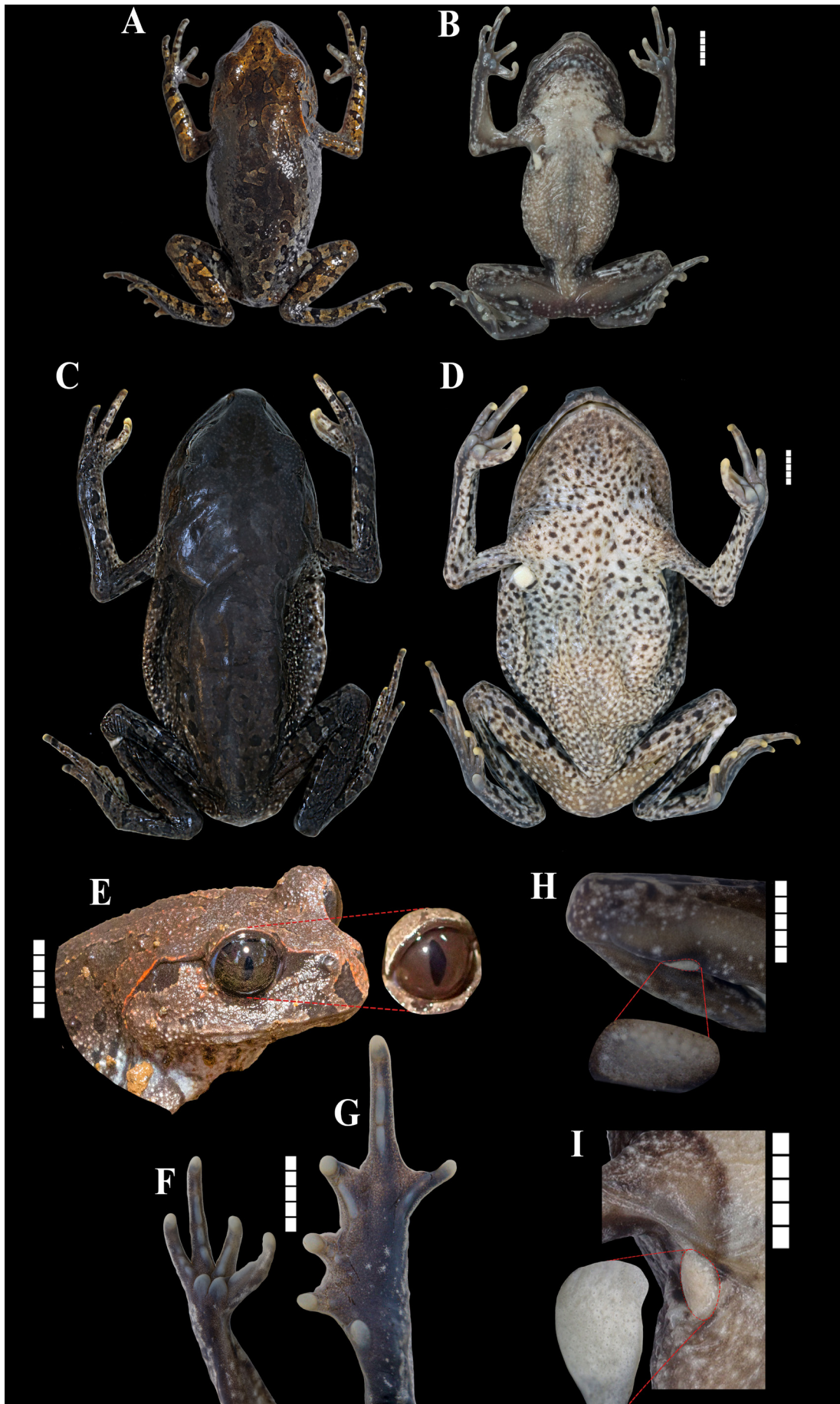


Fig. 6. Type specimens of *Leptobrachium widianai*, new species, in preservation. (A, B, E–I) Holotype (MZB Amph. 33320, male): dorsal, ventral, lateral head, eye, hand, foot, femoral and humeral glands. (C, D) Paratype (MZB Amph. 26961, female): dorsal and ventral views. Scale bar = 10 mm.

Table 5. Body measurements (in mm) of larval *Leptobrachium widianai*, new species, from Indonesia. See text for definitions and abbreviations of larval morphological characters.

Specimen voucher	Stage	TTL	TAL	BL	BH	BES	DNE	ED	IND	IOD	MBW	TMH	MTH	UFH	LFH	DSS	ODW	TWTJ	TSE	DNS
MZB Amph. 33564	25	73.3	46.4	29.2	12.9	15.3	4.5	2.1	4.7	7.4	16.7	7.7	14.3	5.1	3.9	14.2	6.4	7.7	7.2	4.3
MZB Amph. 33561	35	69.0	45.3	23.7	12.0	14.5	3.1	2.1	4.5	7.3	14.0	7.0	12.6	4.2	3.7	11.2	6.7	6.3	6.3	3.3
MZB Amph. 33562	36	67.0	44.0	23.0	11.3	14.9	4.2	2.1	4.3	7.1	13.4	5.9	10.0	3.9	2.9	11.6	6.8	4.9	6.4	3.4
MZB Amph. 33566	37	66.0	39.5	26.4	11.7	14.4	4.5	2.2	4.6	6.2	15.5	6.7	12.7	4.5	4.0	13.4	7.1	6.0	7.6	4.5

Fig. 7. Habitat of *Leptobrachium widianai*, new species, at Buleleng, Bali (MZB Amph. 33374).

Proposed English name: Widian's Litter Frog; Proposed Indonesian name: Katak Serasah Widian.

Tadpole morphology. (Stage 25 and Stage 37; Table 5; all measurements in mm).

The tadpoles of *Leptobrachium widianai*, new species, conform to the generalised benthic morphology typical of the genus, adapted for life in slow-flowing or lentic montane forest streams. In dorsal view, the snout is broadly rounded, blunt, and moderately elongate; in lateral profile, the head slopes smoothly into the snout without a distinct break in contour. The body is robust and large relative to tail length, with total length (TTL) measuring 73.3 mm at stage 25 and 66.0 mm at stage 37. Body outline in dorsal view is oval, widest posterior to the eyes, and clearly broader than the base of the tail. Maximum body width (MBW) constitutes 22.8% of TTL at stage 25 and 23.6% at stage 37. Tail width at trunk–tail junction (TWTJ) accounts for 10.5% of TTL at stage 25 and 9.1% at stage 37. Nostrils are distinct, relatively large, and positioned equidistant between the snout tip and the anterior margin of the eye, oriented laterally. Measurements indicate DNE $\approx$ 45% of TSE at both stages; DNS $\approx$ 60% of TSE. Eyes are dorsally positioned, low in profile, and non-protruding. The iris and scleral regions are black, densely scattered with golden iridophores; a thin ring of golden iridocytes surrounds the pupil, which is circular. Tail is elongate, comprising 63.3% of total length, with a narrowly rounded and slightly acute tip. The dorsal fin originates at the trunk–tail junction and is higher than the ventral fin; dorsal fin height (UFH) is 35.7% of maximum tail height (MTH) at stage 25 and 35.4% at stage 37. Ventral fin height (LFH) is 27.6% MTH at stage 25 and 31.1% at stage 37. The musculature is well developed, suggesting strong swimming capability. In life, body and tail are uniformly dark brown; lateral body and fins lack patterning, a useful distinguishing character from patterned larvae of some congeners.

Oral disc. Positioned anteroventrally to almost ventrally, width $<$ 50% of MBW (38.4% at stage 25; 46.0% at stage 37). Marginal papillae are complete, uniserial, relatively

Table 6. Quantitative and qualitative characteristics of the advertisement calls of *Leptobrachium widianai*, new species, and *Leptobrachium hasseltii* from Indonesia. See text for definitions of acoustic parameters and measurement protocols

Parameters	<i>Leptobrachium widianai</i> , new species			<i>Leptobrachium hasseltii</i> (MZB Amph. 32743)		
	Mean	SD	(min-max)	Mean	SD	(min-max)
Consecutive calls duration (s)	37.55	23.10	(15.38–67.77)	104.15	-	-
Call duration (s)	2.41	0.49	(1.65–3.06)	1.49	0.60	(0.79–2.62)
Pulse per call	5.00	2.21	(5–12)	7.11	2.62	(4–12)
Pulse duration (s)	0.21	0.02	(0.18–0.29)	0.11	0.02	(0.09–0.16)
Interpulse interval (s)	0.02	0.10	(0–0.81)	0.11	0.02	(0.07–0.17)
Pulse rate (pulse/s)	3.44	0.47	(2.62–3.92)	4.87	0.57	(3.72–5.89)
Calling rate (calls/s)	0.08	0.001	(0.04–0.12)	0.09	-	-
Intercall interval (s)	19.18	13.29	(7.39–50.84)	11.34	6.59	(6.4–27.06)
Peak time	0.24	0.06	(0.15–0.42)	0.41	0.10	(0.18–0.64)
First pulse duration (s)	0.75	0.08	(0.64–0.87)	0.15	0.01	(0.12–0.16)
Pulse characteristics	Heterogenous pulse, first pulse significantly differs from remaining pulse			Almost homogenous		

large, slightly conical to acute in shape, with a medial gap in the upper labium. Lower labium bears a few submarginal papillae laterally. No disc emargination present.

Oral armature. Upper jaw sheath wide; lower jaw sheath wide, V-shaped; both heavily keratinised, black, with coarse, moderately sharp serrations along cutting edges. Labial tooth row formula (LTRF) is 7(1–6)/5(1–4). Additionally, 10–12 short keratodont rows are present in the submarginal region of the lower labium—these accessory rows may aid in scraping biofilm from substrate in benthic habitats.

Other features. Vent tube dextral, short, and free from the body wall; spiracle sinistral, directed posterodorsally, free from body wall, and forming a short tube.

Remarks. The uniformly dark brown larval colouration, absence of lateral tail patterning, and specific LTRF differentiate *L. widianai* larvae from sympatric anuran species and from *L. hasseltii* larvae, which have a lower anterior tooth row count and may display pale patterning in the tail musculature.

Acoustic. Three advertisement calls from a single adult male were recorded while the individual was positioned at the water's edge on leaf litter. The advertisement call of *L. widianai*, new species, is markedly different from that of *L. hasseltii* sensu stricto, with no overlap in key temporal or structural parameters (Fig. 4, Table 6) (Call data from 10 February 2023; ambient temperature 22.8°C).

Call structure. Calls are long, complex sequences beginning with a series of short, broadband “barking” notes followed by a sequence of drawn-out, low-frequency “squawks.”

perceptually described as “wrrrrrrrrkkkkk... quakk quakk quakk quakk...”. The call appears to be given in bouts during prolonged calling sessions.

Quantitative parameters. Consecutive call duration: 37.55 ± 23.10 s (range 15.38–67.77; $n = 4$); Call duration: 2.41 ± 0.49 s (range 1.65–3.06; $n = 9$); First pulse duration: 0.75 ± 0.08 s (range 0.64–0.87; $n = 9$); Pulses per call: 5 ± 2.21 (range 5–12; $n = 14$); Pulse duration: 0.21 ± 0.02 s (range 0.18–0.29; $n = 65$); Interpulse interval: 0.02 ± 0.10 s (range 0.00–0.81; $n = 65$); Pulse rate: 3.44 ± 0.47 pulses/s (range 2.62–3.92; $n = 9$); Peak time: 0.24 ± 0.06 s (range 0.15–0.42; $n = 65$); Intercall interval: 19.18 ± 13.29 s (range 7.39–50.84; $n = 10$); Calls per consecutive call bout: 2 ± 0.55 (range 2–3; $n = 6$); Calling rate: 0.08 ± 0.001 calls/s (range 0.04–0.12; $n = 4$).

Acoustic notes. Pulse rates are not constant within a call; initial pulses are often longer and of lower amplitude, followed by shorter, more uniform pulses. This temporal pattern, combined with the transition from “bark” to “squawk,” is distinctive and has no temporal overlap in range with *L. hasseltii*. The amplitude envelope typically rises after the first pulse and remains stable until the final squawks, which often exhibit slight amplitude modulation.

Significance. In anurans, advertisement calls are key isolating mechanisms; the unique two-part call of *L. widianai*—both in temporal structure and spectral qualities—provides strong behavioural evidence of reproductive isolation from *L. hasseltii* and other sympatric megophryids.

Comparisons. *Leptobrachium widianai*, new species, is assignable to the genus *Leptobrachium* based on its

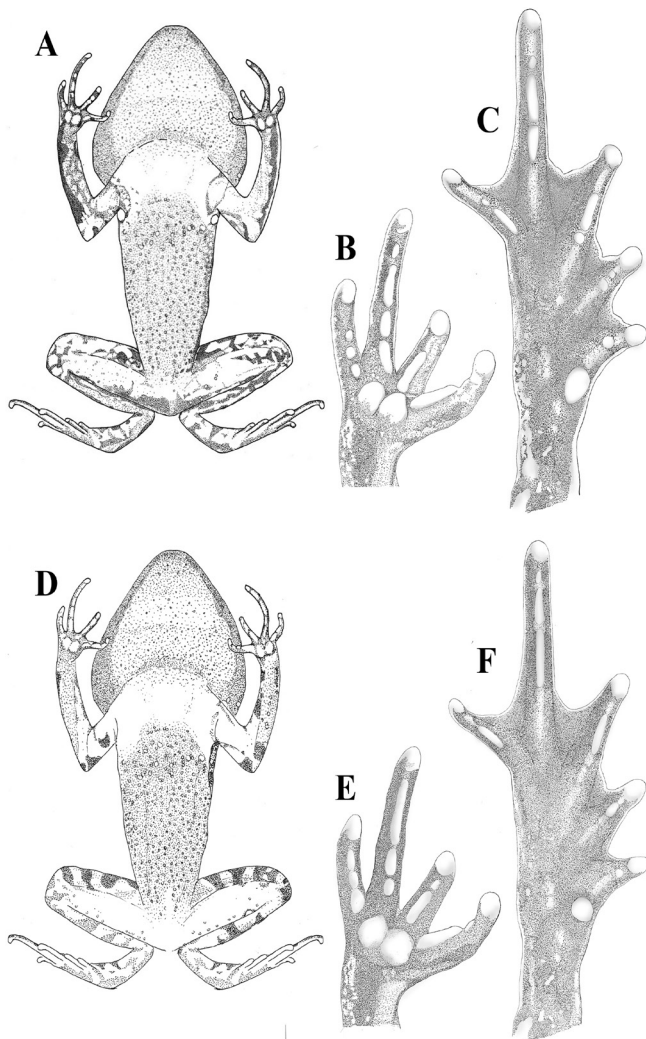


Fig. 8. Illustrations of ventral pattern and limb webbing. (A–C) *Leptobrachium widianai*, new species; (D–F) *L. hasseltii*.

combination of diagnostic adult and larval traits, including large oval femoral glands, absence of vomerine teeth, absence of dermal palpebral projections, and a typical benthic, keratinised larval morphology. Within *Leptobrachium*, it belongs to the subgenus *Leptobrachium* sensu Matsui et al. (2010), and can be readily distinguished from congeners of both subgenera (*Vibrissaphora* and *Leptobrachium* sensu stricto) by its unique set of adult, larval, and acoustic characteristics.

The new species lacks keratinised spines on the upper lip—a feature characteristic of many *Vibrissaphora* species—and is thus readily separated from *L. ailaonicum* (Yang, Chen & Ma, 1983), *L. boringii* (Liu, 1945), *L. echinatum* (Dubois & Ohler, 1998), *L. leishanense* (Liu & Hu, 1973), *L. liui* (Pope, 1947), and *L. yaoshanensis* (Liu & Hu, 1973).

It is further separated from all bicolour-eyed *Vibrissaphora* (e.g., *L. banae*, *L. buchardi*, *L. chapaense*, *L. guangxiense*, *L. hainanense*, *L. huashen*, *L. leucops*, *L. ngoclinhense*, *L. pullum*, *L. xanthops*, *L. xanthospilum*) and several bicolour-eyed species of the subgenus *Leptobrachium* (*L. hendricksoni*, *L. smithi*, *L. rakhinensis*, *L. tenasserimense*, *L. sylheticum*) by

its uniformly brown iris in adults (vs. distinctly bicoloured iris in these taxa).

L. widianai, new species, is separable from blue-eyed taxa (*L. waysepuntiense*, *L. bompu*) and black-eyed taxa (*L. hasseltii*, *L. nigrops*, *L. ingeri*, *L. kanowitense*, *L. montanum*, *L. abbotti*) by its brown iris with a pale blue scleral arc (visible when the eye is fully open), a character absent in all these species. It also differs from other black-eyed congeners with alternative scleral colours—orange in *L. mouhoti*, deep blue in *L. lumadorum*, *L. mangyanorum*, and *L. tagbanorum*.

Among Indonesian *Leptobrachium*, the species most similar to *L. widianai*, new species, is its sister taxon *L. hasseltii*. It differs from *L. hasseltii* in: adult male size: SVL < 39 mm (vs. > 39 mm); lateral pattern: irregular lateral markings (vs. distinct lateral spot); limb pattern: 2–3 bars on arms and femur (vs. 3–4 or 4–5 stripes); irregular dorsal blotches with paired transverse bars (vs. spots to large irregular markings); webbing formula: I 0–2+, II 0–3+, III 2+–4, IV 4–2 V (vs. I 1–2+, II 0–3+, III 1–4, IV 4–2 V); tibiotarsal reach: reaching posterior edge of tympanum (vs. barely reaching in many *L. hasseltii*); morphometric ratios: shorter head (RHL 39.2–43.0 vs. 42.8–47.1), smaller inner palmar tubercle (RIPTL 4.0–4.9 vs. 4.0–6.4), longer hindlimbs (RHLL 120.0–123.5 vs. 112.2–121.9), shorter third finger (RTFL 17.0–18.2 vs. 18.0–19.0), longer first toe (R1TOEL 8.0–9.0 vs. 7.0–8.3); Skin texture: rugose and tuberculate dorsum (vs. nearly smooth); Colour in life: brown dorsum with yellowish–orange highlights (vs. grey to dark brown).

From *L. hendricksoni*, it differs in having a brown iris (vs. black with orange upper half), presence of dark lip markings (vs. absent), and irregular ventral markings (vs. scattered black spots); from *L. nigrops*: larger body size (SVL > 35 mm vs. < 35 mm) and unpointed fingertips (vs. pointed); from *L. montanum* and *L. abbotti*: irregular ventral markings (vs. irregular large blotches); from *L. waysepuntiense*: brown iris (vs. light blue with black reticulations), dark lip markings (vs. absent), groin with dark markings (vs. none), very large femoral gland (vs. small); from *L. ingeri*: larger body size (SVL > 35 mm vs. < 35 mm) and unpointed fingertips (vs. pointed).

Larval distinctions. Tadpoles of *L. widianai*, new species, differ from those of *L. hasseltii* in: (1) labial tooth row formula (LTRF): 7(1–6)/5(1–4) vs. 5(1–4)/5(1–4); (2) colour: uniformly dark brown (vs. light brown); (3) pattern: absence of lateral body and fin pattern (vs. often with yellow spotting) and; (4) Marginal papillae: conical and acute (vs. bluntly conical).

Acoustic distinctions. Acoustically, *L. widianai*, new species, has a two-part call—short bark-like notes followed by low-frequency squawks—with longer, more variable intercall intervals and lower initial pulse amplitude than *L. hasseltii*. Key differences from *L. hasseltii* calls (West Java populations: TNHC 67277, TNHC 67279; Central Java: MZB Amph. 32743) include: (1) pulses per call:



Fig. 9. Dorsal and lateral views of tadpoles. (A, B) *L. widianai*, new species, stages 25 and 37; (C) *L. hasseltii*, stage 38. (D, E) Labial tooth row illustrations of *L. widianai*, new species, and *L. hasseltii*; (F) habitus in life of *L. widianai* tadpoles. Scale bar = 10 mm.

5–12 in *L. widianai* vs. 4–18 in *L. hasseltii* (depending on population); (2) pulse duration: shorter than West Java *L. hasseltii*, longer than Central Java *L. hasseltii*; (3) interpulse intervals: consistently shorter in *L. widianai*; (4) Amplitude envelope: *L. widianai*'s first pulse 10–70% of peak amplitude (vs. 85–95% in *L. hasseltii*), with gradual amplitude increase across call. These acoustic differences, coupled with consistent morphological and larval distinctions, provide strong evidence for the specific separation of *L. widianai*, new species, from all congeners.

Distribution. *Leptobrachium widianai*, new species, is currently known only from two localities in Indonesia: the type locality at Banyuwana Amertha Waterfall, Buleleng Regency, Bali Island (8°12'46.18"S, 115°07'20.02"E; 1,100 m a.s.l.) and Dholo Waterfall, Kediri Regency, East Java (Fig. 1). These sites are separated by the Bali Strait and are located within montane forest belts on the northern slopes of the respective islands. No records exist from lowland or coastal areas, and no confirmed occurrences are known from intervening regions such as Lombok or the Ijen highlands of easternmost Java, despite the presence of apparently suitable habitat. This suggests either a genuine disjunction or substantial under-sampling in intermediate areas.

Given its occurrence in both Bali and eastern Java, *L. widianai* appears to be a Sunda Arc endemic with a restricted highland distribution, potentially representing a relict montane lineage isolated during Pleistocene sea-level fluctuations. The altitudinal range is presently documented only above 1,000 m a.s.l., in contrast to *L. hasseltii*, which occupies a wider elevational band (300–800 m a.s.l.) in Java and Sumatra.

Natural history. At Banyuwana Amertha Waterfall, the type series of *Leptobrachium widiana*, new species, was collected within primary lower montane rainforest at approximately 1,100 m a.s.l., ~300 m from a clear, rocky stream. The habitat is characterised by dense evergreen canopy cover (>80%), high ambient humidity, and a thick accumulation of moist leaf litter. Calling males were associated with damp microhabitats, including leaf litter and moss-covered substrates adjacent to small seepages and narrow forest trails, typically in shaded and sheltered positions.

Larvae were abundant in a shallow, shaded pool at the base of the main waterfall. This pool was fed by slow-moving, well-oxygenated water, with a gravel–sand substrate interspersed with leaf litter. Tadpoles were benthic and cryptically coloured, resting on or just above the substrate, and were observed grazing on detritus and periphyton. The occurrence of numerous larvae in early February suggests that breeding is timed to the wet season (November–April), as in other *Leptobrachium* species in Southeast Asia.

Males vocalised primarily during the early evening (approximately 1800–2100 h), often from concealed positions under leaf litter or low vegetation. Calls consisted of the distinctive two-part sequence (short barking notes followed by low-frequency squawks) described in the Acoustic section.

No egg masses were located during surveys, indicating that oviposition sites may be concealed beneath submerged debris or within fine sediment in shallow pools.

In addition to the type locality, specimens from Dholo Waterfall, Kediri Regency, East Java, were collected at 1,050 m a.s.l. in disturbed lower montane forest near a cascading stream with mixed rock and sand substrate. Calling males were observed on damp soil and leaf litter along the stream margin, and several subadults were active on vegetation up to 0.5 m above ground.

Previously sequenced specimens from Mount Batukaru, Tabanan Regency, Bali, were collected at 1,150 m a.s.l. in relatively undisturbed montane forest. Individuals there were found at night on the forest floor close to seepages and small pools within 20 m of a stream. Although no larvae were recorded from Batukaru during those surveys, calling activity was noted between 1900 and 2100 h, suggesting that the breeding phenology is similar across both Bali localities.

The species occurs in sympatry with *Chalcorana chalconota* (Schlegel, 1837) and *Microhyla palmipes* (Boulenger, 1897), but microhabitat partitioning is evident: *L. widianai* was confined to the forest floor and breeding pools, *C. chalconota* frequented more open stream margins, and *M. palmipes* occupied wetter leaf-litter zones in swampy depressions.

Conservation status. Based on current knowledge, *Leptobrachium widianai*, new species, is endemic to Bali and eastern Java, with a confirmed extent of occurrence (EOO) of 3,156.06 km². It is restricted to high-elevation (>1,000 m a.s.l.) montane forests, a habitat type that is fragmented and under increasing anthropogenic pressure. Both known localities are subject to ongoing habitat degradation from agricultural expansion, infrastructure development, and particularly tourism-related disturbance, including trail widening, vegetation clearing, and increased human traffic (Gaveau et al., 2018; Voigt et al., 2021; Megantara et al., 2022).

Although parts of its range occur within areas that benefit from some degree of formal protection, enforcement is variable, and high-elevation habitats remain vulnerable to degradation. The combination of its small EOO (<5,000 km²), restriction to a narrow altitudinal band, presence of continuing threats, and inferred decline in habitat quality qualifies *L. widianai*, new species, for listing as Endangered under the IUCN Red List criteria B1ab(iii) (IUCN Standards and Petitions Committee, 2022).

Given that this species is known from only two sites and appears to be a habitat specialist with potentially fragmented populations, immediate conservation attention is warranted. Targeted surveys in other highland areas of Bali and eastern Java are recommended to clarify its true distribution, population size, and ecological requirements, which will inform any future reassessment of its conservation status.

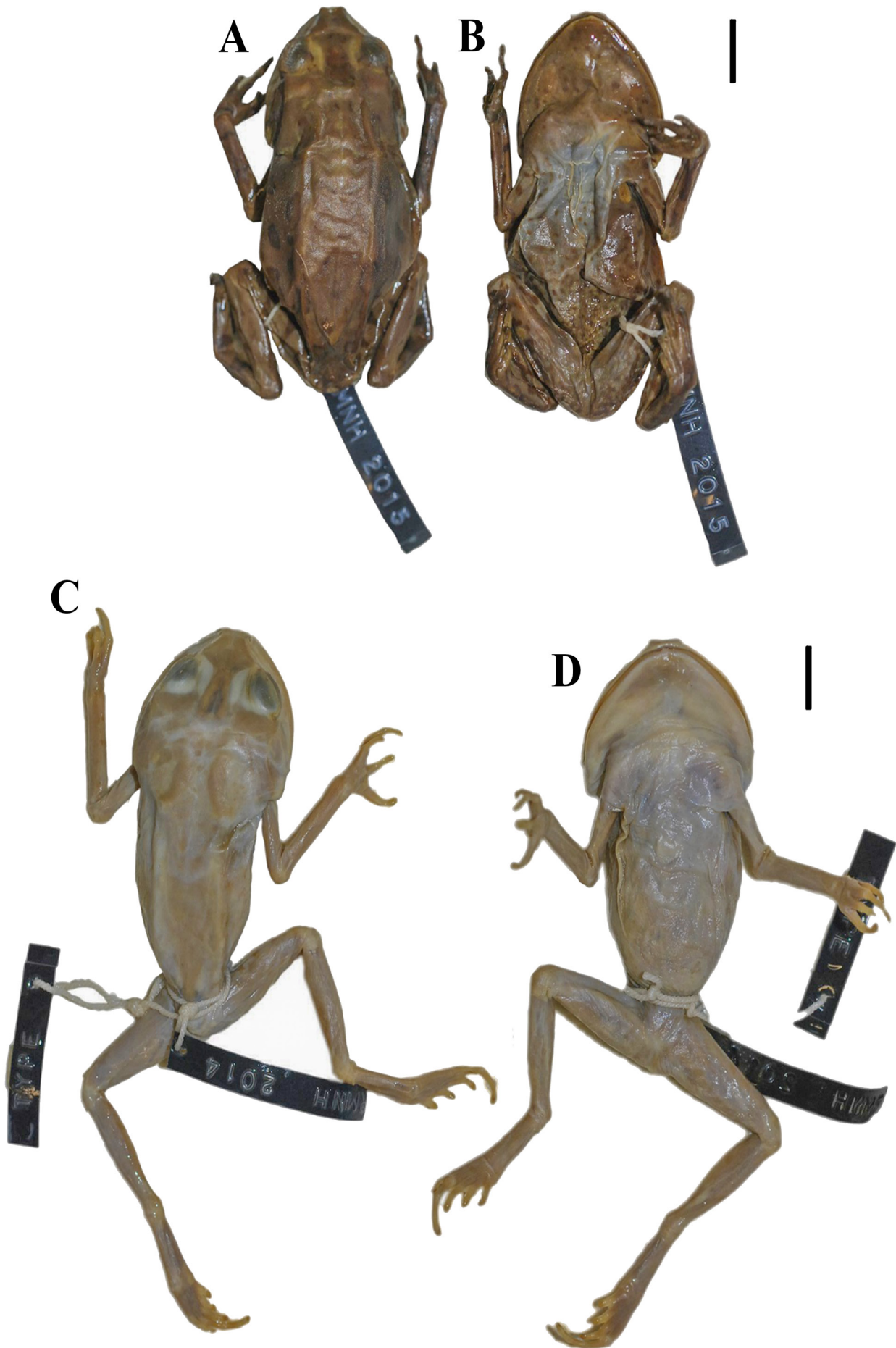


Fig. 10. Type specimens of *Leptobrachium hasseltii* in preservation. (A, B) Lectotype (RMNH 2015): dorsal and ventral views; (C, D) paralectotype (RMNH 2014): dorsal and ventral views. Scale bar = 10 mm.

DISCUSSION

Historical context of *Leptobrachium hasseltii* and type locality considerations. *Leptobrachium hasseltii* was first described by Tschudi (1838) from “Java,” based on two syntypes (RMNH 2014–15) collected by H. Boie and H. Macklott and brought to Europe by Salomon Müller (Klaver, 2007). Dubois & Ohler (1998) subsequently designated RMNH 2015 as the lectotype and RMNH 2014 as the paralectotype (Fig. 10). While the original description gave no precise locality, the historical collecting patterns of Dutch naturalists during the colonial period strongly suggest that the type specimens originated from West Java, most likely from Mt. Salak, Mt. Gede, or Mt. Pangrango—three major highland localities frequently visited for zoological collection at that time. This hypothesis is further supported by our examination of modern *L. hasseltii* specimens from West Java, which closely match the lectotype morphology and colour pattern.

Significance of genetic divergence within *Leptobrachium*. Genetic distance metrics have been widely used to detect and delimit cryptic diversity in *Leptobrachium*, both within Sundaland (Matsui et al., 2010; Hamidy et al., 2011) and in the Philippines (Brown et al., 2009). Although interspecific divergences can vary depending on gene region and sequence length, the uncorrected mitochondrial 16S rRNA distances of ~3% have been widely used as an initial flag for candidate species in anurans, to be tested against morphology and bioacoustics (Vences et al., 2005; Fouquet et al., 2007). Parallel consensus standards in anuran bioacoustics now enable repeatable, cross-study comparisons of call structure and spectra—key for delimitation in groups with subtle external differences (Köhler et al., 2017). In the Sunda Arc, alternating Pleistocene lowstands and highstands repeatedly connected and isolated Java and Bali; combined with elevational specialisation, this history is often invoked to explain pronounced east–west structure in forest-dependent herpetofauna (Voris, 2000; Inger & Voris, 2001; Lohman et al., 2011).

However, the genus contains examples that challenge a simple threshold approach. Very low divergences (1.8–2.1%) occur among three orange-sclera species of southern Indochina (*L. pullum*, *L. mouhoti*, *L. lunatum*) that are nonetheless diagnosable by morphology and calls (Stuart et al., 2020). Similarly, *L. montanum* and *L. abbotti* in Borneo differ by 2.4–3.2% but are morphologically and ecologically distinct (Hamidy & Matsui, 2014). The blue-eyed *L. waysepuntiense* exhibits only 2.6% divergence from *L. montanum*, yet is clearly diagnosable by iris colour (Hamidy & Matsui, 2010). Even lower divergence (0.9%) separates *L. hainanense* and *L. guangxiense*, which differ in iris colouration and other traits (Pawangkhanant et al., 2018).

Patterns of divergence in *L. hasseltii* and the status of *L. widianai*, new species. Within *L. hasseltii* sensu lato, our data show marked genetic structuring between island and regional populations. Populations from Sumatra and West Java differ by only 1.5–2.2%, whereas Central Java populations differ

from both West Java and Sumatra by 2.4–3.4%. In contrast, the East Java–Bali lineage (*L. widianai*, new species) is more divergent from *L. hasseltii* sensu stricto, with uncorrected p-distances of 3.7–5.2%—well above the widely cited 3% threshold and comparable to divergences between recognised *Leptobrachium* species elsewhere in the region.

Importantly, these genetic distinctions are corroborated by multiple independent datasets. Morphological analyses (PCA) reveal a distinct morphospace for *L. widianai*, with differences in relative limb length, head proportions, and dorsal texture. Acoustic analyses likewise detect non-overlapping differences in call structure, temporal parameters, and amplitude envelope between *L. widianai* and *L. hasseltii*. Together, these lines of evidence indicate that *L. widianai* represents a discrete evolutionary lineage, reproductively isolated and diagnosable by multiple character systems.

Our findings also suggest that the Central Java populations of *L. hasseltii*—which show intermediate genetic distances and may possess unique acoustic or morphological features—could represent an as-yet undescribed lineage. This warrants targeted sampling and integrative analysis to fully resolve species boundaries within the complex.

Morphometric distinctiveness of the new species. Our morphometric analyses demonstrate that the new species is clearly distinct from congeners in both sexes, even after rigorous allometric correction. The species consistently occupies a unique region of morphospace, with differentiation driven largely by head proportions (relatively narrower heads, shorter nostril–eye distances, and reduced internarial widths) and limb elements (forelimb and tibia lengths). These quantitative differences are corroborated by qualitative characters, including colour pattern, dermal ornamentation, and toe webbing configuration, further reinforcing its distinctiveness. The strong separation recovered in PCA, the highly significant results of MANOVA and PERMANOVA, and the near-perfect classification success in LDA together provide robust evidence that the new taxon represents an independent evolutionary lineage rather than intraspecific variation. In combination with diagnostic qualitative traits, these findings support its formal recognition as a new species within the *Leptobrachium* complex.

Biogeographic and faunal implications. The discovery of *L. widianai*, new species, provides new insight into anuran biogeography within the Sunda Arc. The East Java–Bali lineage appears to be a highland specialist restricted to elevations above ~1,000 m, in contrast to the broader elevational range of *L. hasseltii* sensu stricto, which occurs from ~300–800 m. This distribution suggests that highland habitat preference may have played a role in isolating *L. widianai*, particularly during warmer interglacial periods when lowland forests contracted and montane habitats became fragmented (Stewart et al., 2010).

The present-day separation of East Java–Bali populations from those in Central and West Java is likely maintained by a combination of elevational preferences and historical

biogeographic barriers. The lowland corridors of eastern Java and the Bali Strait, which separates Bali from Java, represent substantial ecological and dispersal barriers for a cool-adapted montane taxon (Inger & Voris, 2001). It is plausible that initial divergence was driven by climatic oscillations during the Pleistocene, when cooler glacial periods may have facilitated intermittent forest connectivity, followed by isolation during warmer periods (Oliveros & Moyle, 2010; Stewart et al., 2010; Lohman et al., 2011; O'Connell et al., 2018).

Similar east–west population structuring has been documented in other montane or forest-dependent taxa in Java, including amphibians (e.g., *Leptophryne javanica*, Hamidy et al., 2018; *Nyctixalus margaritifer*), reptiles (*Gekko gekko* complex; Wood et al., 2012), and several montane bird species (Oliveros et al., 2011), where lineages are split across central or eastern Java due to combined effects of historical climate shifts, topographic barriers, and habitat specialisation (Amarasinghe et al., 2020). These parallels suggest that the diversification of *L. widianai* and *L. hasseltii* sensu stricto may form part of a broader biogeographic pattern in which elevational gradients and the physiographic division of Java and Bali interact to promote speciation in montane forest fauna. Conversely, several widespread lowland taxa show shallow Java–Bali structure or signs of recent dispersal, including the common tree frog *Polypedates leucomystax* and the narrow-mouthed frog *Kaloula baleata* (Brown et al., 2010; Reilly et al., 2019).

Amphibian diversity context in Java and Bali. Java supports one of the most intensively documented amphibian faunas in Southeast Asia, with 48 recognised species (Frost, 2024). Survey intensity, however, has been uneven: Western and Central Java have benefited from decades of focused research, yielding both new distribution records and novel species descriptions. Recent examples include *Polypedates macrotis* (Munir et al., 2021), *Chalcorana megalonesa* (Sabinhaliduna et al., 2022), *Chirixalus trilaksonoi* (Riyanto & Kurniati, 2014), *Leptophryne javanica* (Hamidy et al., 2018), *Theloderma pseudohorridum* (Kurniawan et al., 2023), and *Chirixalus pantaiselatan* (Munir et al., 2021). Eastern Java surveys have been more limited but have yielded significant finds, including new locality records for *Nyctixalus margaritifer* (Priambodo et al., 2021), *Polypedates pseudotilophus* (Kurniawan et al., 2020), and *Leptophryne borbonica* (Erfanda et al., 2019).

In contrast, Bali remains under-surveyed. Aside from the description of *Microhyla orientalis* (Matsui et al., 2013), few systematic amphibian studies have been conducted, and most checklists are based on incidental observations. The description of *L. widianai*, new species, from Bali thus represents a substantial addition to the island's known herpetofauna, highlighting the importance of targeted surveys in smaller, less-studied islands of the Sunda Arc.

Concluding remarks. The integrative evidence presented here—molecular, morphological, and acoustic—unequivocally supports the recognition of *Leptobrachium widianai*, new

species, as a distinct species endemic to the highlands of East Java and Bali. This discovery not only refines our understanding of the *L. hasseltii* complex but also underscores the role of montane isolation in generating amphibian diversity in the Sunda Arc. Given the species' restricted range and ongoing habitat threats, its conservation status warrants urgent attention.

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SUPPLEMENTARY MATERIAL

All supplementary material can be found at <https://doi.org/10.6084/m9.figshare.31095712>

APPENDIX 1: SPECIMENS EXAMINED

Leptobrachium hasseltii (Sumatra, Indonesia, n = 6): Kubu Perahu, Taman Nasional Bukit Barisan Selatan, MZB Amph. 14589–14590, 3470–3473; Camp Granit, Taman Nasional Bukit Tiga Puluh, MZB Amph. 7093; Labuhan Mandi, Way Krui, Bandar Lampung, Kabupaten Pesisir Barat, MZB Amph. 32291. (Java, Indonesia, n = 91): Taman Hutan Raya Soerjo, Kecamatan Klojen, Kota Malang, MZB Amph. 16661; Grojogan Sewu, Jatimulyo Village, Kecamatan Tawangmangu, Kabupaten Karanganyar, MZB Amph. 31011–31012, 31026; Gua Sekantong, Logoguwo Village, Kecamatan Kaligesing, Kabupaten Purworejo, MZB Amph. 28185; Kalipagu, Kecamatan Baturaden, Kabupaten Banyumas, MZB Amph. 15406–15414; Mudal river, Kecamatan Girimulyo, Kabupaten Kulonprogo, MZB Amph. 14517; Taman Nasional Gunung Gede Pangrango, Kecamatan Kadudampit, Kabupaten Sukabumi, MZB Amph. 926, 6631–6633, 6635–6636, 6640, 6643, 6647, 6656, 6659, 6661–6662, 6665, 8194, 15697; TNHC 67277 and TNHC 67279; PPKA Bodogol, Taman Nasional Gunung Gede Pangrango, MZB Amph. 8194–8197; Cibeureum river, Taman Nasional Gunung Halimun Salak, Cisarua Village, Kecamatan Cigudeg, Kabupaten Bogor, MZB Amph. 6341; Citalahab Village, Cikaniki, Cibunar, Taman Nasional Gunung Halimun Salak, MZB Amph. 7599, 7601, 5583, 5731, 4028, 4642, 4673–4676, 10939–10940, 10942, 10946–10948, 10950, 15102–15103, 15986; Cigenter, Cidaun, Ujung Kulon, Kabupaten Pandeglang, MZB Amph. 15689–15690, 885–890, 935, 981–983; Situ Pangkalau, Kamojang, Kabupaten Bandung, MZB Amph. 2391; Tegal, Cikamal, Pangandaran, Kabupaten Ciamis, MZB Amph. 2510, 2821, 3172, 14571; Sukabumi Selatan, MZB Amph. 3946–3947, 15687–15688; Taman Wisata 2, Cagar Alam Pangandaran, MZB Amph. 8388.

Leptobrachium hendricksoni (Sumatra, Indonesia, n = 15): MZB Amph. 15698–15702; Rambung Baru Village, Deli Serdang, MZB Amph. 23909–23911, 23843; Besilam Langkat river, Aceh, MZB Amph. 6913–6917.

Leptobrachium waysepuntiense (Sumatra, Indonesia, n = 11): Tanjung Dalam, Kabupaten Menangin, MZB Amph. 21817, 23873; Batang Gadis river, Kabupaten Mandailing Natal, MZB Amph. 23877; Kejora Martabe, Kabupaten Tapanuli Selatan, MZB Amph. 11310–11313; Danau Ranau, Kabupaten Lampung Barat, MZB Amph. 22237, MZB Amph. 22238; Hutan Lindung Bukit Bagus, Kecamatan Tais, Kabupaten Bengkulu Selatan, MZB Amph. 4038.

Leptobrachium abbotti (Kalimantan, Indonesia, n = 13): Rian River, Bulungan, MZB Amph. 9451–9453; Peridan Village, Kecamatan Sangkulinang, Kabupaten Kutai Timur, MZB Amph. 22136–22139; Paraye Village, Taman Nasional Kayan Mentarang, Kecamatan Kerayon, Kabupaten Nunukan, MZB Amph. 9119–9120; Sebadak Raya Village, Ketapang, MZB Amph. 21818; Pensiangan River, Kecamatan Malinau, Kabupaten Malinau, MZB Amph. 10747; Tekalan River, Taman Nasional Bentuang Karimun, Kabupaten Sekadau, MZB Amph. 6833; Pait River, Taman Nasional Bentuang Karimun, Kabupaten Sekadau, MZB Amph. 3486.

Leptobrachium ingeri (Belitung, Indonesia, n = 9): Riau Silip, Berbura, Taman Nasional Gunung Maras, MZB Amph. 32787–32789, 32812, 32820; Sijuk Belitung, MZB Amph. 31844–31845, 31834; Bulu Tumbang, Tanjung Pandan, MZB Amph. 11791.

Leptobrachium montanum (Kalimantan, Indonesia, n = 3): Sinangao, MZB Amph. 5980–5981; Bulota river, Kecamatan Tabang, Kabupaten Kutai, MZB Amph. 15617.

Leptobrachium nigrops (Sumatra, Indonesia, n = 3): Peatland Forest, Taman Nasional Zamrud, MZB Amph. 31136; Tiwi River, Kuto Tuo, MZB Amph. 16745–16746.