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A new highland species of dwarf litter frog genus *Leptobrachella* (Amphibia: Anura: Megophryidae) from Sarawak

Koshiro Eto*, Masafumi Matsui & Kanto Nishikawa

Abstract. A new highland species of the dwarf litter frog, *Leptobrachella itiokai*, is described from Gunung Mulu National Park, northern Sarawak, East Malaysia. It occurs syntopically with another highland species, *L. brevicrus*, but differs from it by a unique call that may be described as a chirp, with dominant frequency of ca. 10 kHz. The new species is morphologically distinguishable from congeners by the combination of the following traits: rounded ventrolateral glands usually not in series; side of body with small black spots; ventrum entirely dark-coloured with tiny light spots; male with thread-like lineae masculinae.

Key words. *Leptobrachella*, MtDNA; Phylogeny; Taxonomy; Borneo

INTRODUCTION

Within the island of Borneo, Gunung [Gn. = Mt.] Mulu National Park (NP), northern Sarawak is one of the most amphibian-rich areas. About a half of Bornean amphibians (ca. 90 species: Inger & Tan, 1996; Inger & Stuebing, 2005; Dehling, 2008; Matsui, unpublished data) are recorded from this area, of which 19 were described from there. In the case of a tiny megophryid genus *Leptobrachella* Smith, 1925, four species, including one endemic species, have been recorded from Gn. Mulu (Dring, 1983; Eto et al., 2015). Up to now this area is the only place where more than three species of *Leptobrachella* occur, and it may be safe to say that Gn. Mulu is the centre of diversity of the genus. According to the review on the genus by Dring (1983), each of *Leptobrachella* species on Gn. Mulu seems to be stratified by altitude: i.e., there are two hilly species (*L. parva* Dring, 1983: recorded mainly from Camp 1 [altitude ca. 150 m above sea level [asl]] of Mulu NP; *L. juliandringi* Eto, Matsui, and Nishikawa, 2015: Camp 2 [as *L. mjobergi* Smith, 1925: ca. 500 m asl]) and two highland species (*Leptobrachella* sp. 3 or "*baluensis*": ca. 900 m asl of the mountain; and *L. brevicrus* Dring, 1983: Camp 4 [ca. 1700–1800 m asl]). During field survey on amphibians on Mulu Camp 4, we found syntopic occurrence of two types of *Leptobrachella* that differ in their advertisement calls. One of them was identified as the endemic species *L. brevicrus* based on its morphological and acoustic traits, whereas the other could not be assigned to any named species of the genus. In this study we conducted bioacoustic, morphological, and molecular phylogenetic analyses to determine the taxonomic status of this unidentified form.

MATERIALS AND METHODS

We collected five specimens of *Leptobrachella* sp. from Sungai [= river] Tapin, close to Camp 4 of Gn. Mulu (Fig. 1). We also examined *L. mjobergi*, *L. brevicrus*, *L. parva*, and *L. juliandringi* from Sarawak containing topotypes of each species, and *L. baluensis* from Sabah for comparisons. These specimens are stored at BORNEENSIS collection, University Malaysia Sabah (BORN), Human and Environmental Studies, Kyoto University (KUHE), and Sarawak Research Collections, Forest Research Centre Sarawak (SRC) (Table 1: Appendix).

For molecular analysis, total DNA was extracted from tissues preserved in 99% ethanol. Methods for DNA extraction, subsequent amplification and sequencing of mtDNA fragments (approximately 450 base pairs [bp] of *16S* rRNA), and DNA primers used are essentially same as those reported by Eto et al. (2012). Newly obtained sequences were deposited in GenBank (Accession numbers LC137802–137810: Table 1). By using these sequences and published data from GenBank (Table 1), we reconstructed mitochondrial genealogies based on maximum likelihood (ML) method and Bayesian inference (BI). Details of analytical methods and software used are described elsewhere (Eto et al., 2012). Identical haplotypes were omitted from the phylogenetic analyses.

For specimens stored in 70% ethanol, we took 20 body measurements following Matsui (1984) with slight modification: (1) snout-vent length (SVL); (2) head length (HL); (3) snout length (SL); (4) snout-nostril length (S-NL); (5) nostril-eyelid length (N-EL) from posterior end of nostril to anterior tip of upper eyelid; (6) eye length (EL), including eyelid; (7) tympanum-eye length (T-EL); (8)

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Table 1. Samples used for DNA analysis in this study, and GenBank accession numbers. BORN: BORNEENSIS collection, University Malaysia Sabah; KUHE: Human and Environmental Studies, Kyoto University; NMBE: Naturhistorisches Museum der Burgergemeinde Bern; SP: Sabah Parks; SRC: Sarawak Research Collections, Forest Research Center Sarawak; UNIMAS: Universiti Malaysia Sarawak; ZMH: Zoological Museum of Hamburg.

Species	Locality	Voucher	Accession Nos.	Reference
Leptobrachella sp.	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55845	LC137802	this study
Leptobrachella sp.	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55846	LC137803	this study
Leptobrachella sp.	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55895	LC137804	this study
Leptobrachella sp.	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55897	LC137805	this study
Leptobrachella sp.	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55898 (= SRC 00335)	LC137806	this study
L. "*brevicrus*"	Mulu NP, Sarawak, Borneo, Malaysia	ZMH A09365	KJ831302	Oberhummer et al., 2014
L. "*brevicrus*"	Mulu NP, Sarawak, Borneo, Malaysia	UNIMAS 8957	KJ831303	Oberhummer et al., 2014
L. brevicrus	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55844	LC137807	this study
L. brevicrus	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55847	LC137808	this study
L. brevicrus	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55896	LC137809	this study
L. brevicrus	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55935	LC137810	this study
L. parva	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55307	LC056790	Eto et al., 2015
L. parva	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55308	LC056791	Eto et al., 2015
L. parva	Mulu NP, Sarawak, Borneo, Malaysia	UNIMAS 8883	KJ831304	Oberhummer et al., 2014
L. parva	Mulu NP, Sarawak, Borneo, Malaysia	UNIMAS 8623	KJ831305	Oberhummer et al., 2014
L. baluensis	Tambunan, Sabah, Borneo, Malaysia	SP 21604	LC056792	Eto et al., 2015
L. baluensis	Kampong Lokos, Sabah, Borneo, Malaysia	SP 21687	LC056793	Eto et al., 2015
L. baluensis	Crocker Range NP, Sabah, Borneo, Malaysia	BORN 08595	LC056794	Eto et al., 2015
L. baluensis	Crocker Range NP, Sabah, Borneo, Malaysia	ZMH A13128	KJ831301	Oberhummer et al., 2014
L. juliandringi	Mulu NP, Sarawak, Borneo, Malaysia	SRC 00230	LC056779	Eto et al., 2015
L. juliandringi	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55333	LC056780	Eto et al., 2015
L. juliandringi	Lanjak Entimau, Sarawak, Borneo, Malaysia	KUHE 17554	LC056783	Eto et al., 2015
L. juliandringi	Lanjak Entimau, Sarawak, Borneo, Malaysia	KUHE 17557	LC056784	Eto et al., 2015
L. mjobergi	Gading NP, Sarawak, Borneo, Malaysia	KUHE 17064	LC056785	Eto et al., 2015
L. mjobergi	Gading NP, Sarawak, Borneo, Malaysia	KUHE 17207	LC056786	Eto et al., 2015
L. mjobergi	Gading NP, Sarawak, Borneo, Malaysia	KUHE 47876	LC056788	Eto et al., 2015
L. mjobergi	Gading NP, Sarawak, Borneo, Malaysia	KUHE 48879	LC056789	Eto et al., 2015
Leptolalax gracilis	Mulu NP, Sarawak, Borneo, Malaysia	KUHE 55624	AB847560	Matsui et al., 2014
Leptolalax heteropus	Larut, Malaysia	KUHE 15490	AB847561	Matsui et al., 2014
Leptobrachium hasseltii	Yogyakarta, Java, Indonesia	KUHE 42820	AB530424	Matsui et al., 2010

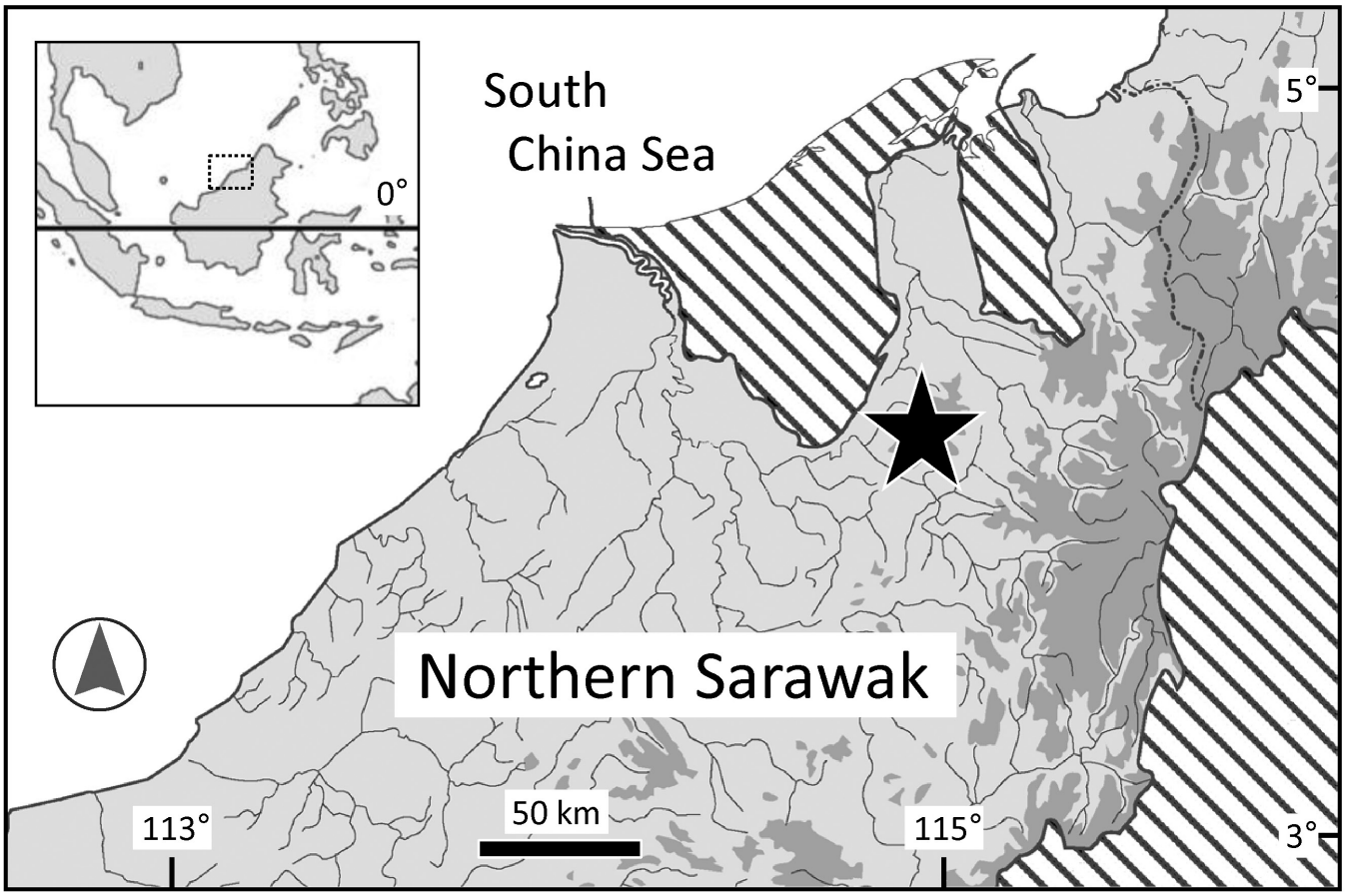

Fig. 1. Map of Sarawak, Malaysian Borneo, showing the type locality of *Leptobrachella itiokai*, new species (filled star).

maximum tympanum diameter (TD); (9) head width (HW); (10) internarial distance (IND); (11) interorbital distance (IOD); (12) upper eyelid width (UEW); (13) lower arm and hand length (LAL) from elbow to tip of third finger; (14) hand length (HAL); (15) inner palmer tubercle length (IPTL); (16) hind limb length (HLL); (17) tibia length (TL); (18) foot length (FL); (19) inner metatarsal tubercle length (IMTL); and (20) first toe length (1TOEL) from distal end of inner metatarsal tubercle to tip of first toe. We made all measurements with dial callipers to the nearest 0.05 mm under a binocular dissecting microscope. In the univariate comparisons of body measurements, characters 2–20 were converted to ratios to SVL (R).

When our measurements and those of Dring (1983) are compared, there is slight discordance between them. Generally his SVL measurements are larger than ours when same species/localities are compared: for example, SVL measurements of male *L. juliandringi* from Mulu Camp 2 (mean: 17.9 mm) shown in Eto et al. (2015) are smaller than those of Dring (1983) (as *L. mjobergi*, 19.2 mm in median). In contrast, ratios of each character to SVL reported by Dring (1983) are generally smaller than those calculated by us, probably affected by larger SVL measurements in Dring (1983). We therefore used only part of his measurements/ratios for comparisons.

We recorded frog calls in the field using digital recorders (Olympus V-823) at 44.1 kHz/16 bits as uncompressed wave files, and analysed recordings with Raven Lite 1.0 for Windows (http://www.birds.cornell.edu/raven). Both temporal and frequency information were obtained from the audiospectrograms.

RESULTS

In the molecular analysis we obtained 458 bp of concatenated fragments of mitochondrial *16S* rRNA gene, within which 120 were variable and 101 were parsimoniously informative. After elimination of identical sequences, a total of 23 different haplotypes were analysed together with outgroups. The best substitution model chosen was J2 (Jobb, 2011) with gamma shape parameter (Γ) for ML and General-Time-Reversible (GTR) + Γ for BI, respectively. The likelihood values (-lnLs) of the ML tree and the summarised BI trees were 9746.810 and 9783.028, respectively. Nearly identical topologies were obtained in the two analyses, and only the ML tree is shown in Fig. 2.

Unexpectedly, *Leptobrachella* and Bornean *Leptolalax* (subgenus *Leptolalax*) tend to form a clade, although not fully supported (BS=63%/BPP=0.56), with continental *Leptolalax* (subgenus *Lalos*) outside of them. The species of *Leptobrachella* formed a monophyletic group against outgroups (100%/1.00), in which trichotomous relationship of *L. mjobergi* from southern Sarawak, *L. juliandringi* from central and northern Sarawak, and a clade (94%/1.00) consisted of species from Sabah and northern Sarawak (Clade

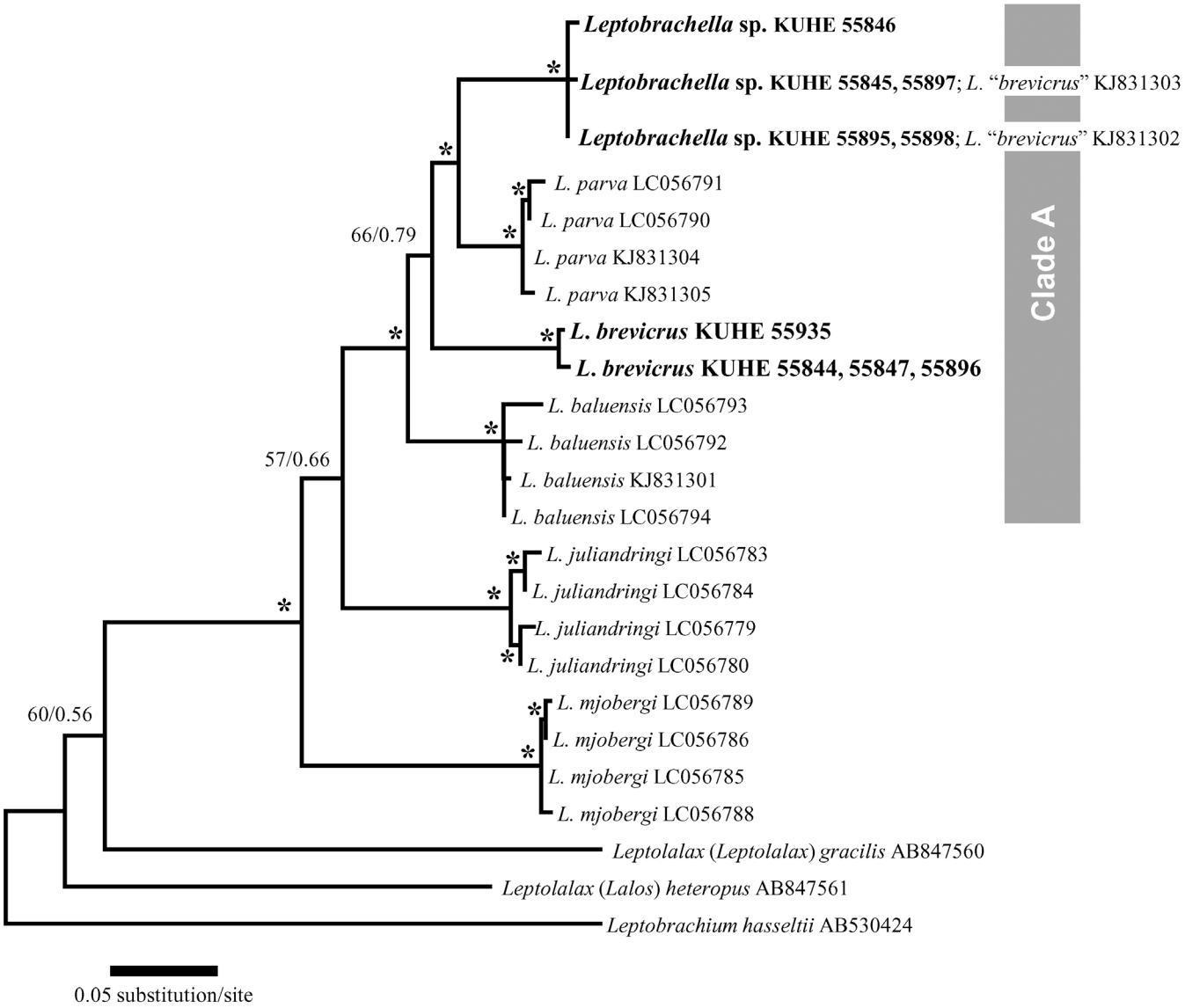

Fig. 2. Maximum likelihood (ML) tree of a 458 bp sequence of mitochondrial *16S* rRNA for samples of *Leptobrachella* (for sample details, see Table 1). Numbers above nodes represent the support values (BS/BPP). Newly obtained sequences are shown by bold face. Asterisks indicate the nodes with sufficient support (BS ≥ 70% and BPP ≥ 0.95).

Table 2. Mean and range (in parenthesis) of uncorrected p-distance (%) for fragments of *16S* rRNA among *Leptobrachella* taxa compared.

		1	2	3	4	5	6
1	*Leptobrachella* sp.	0.3 (0.2–0.5)					
2	*L. parva*	5.9 (5.8–6.0)	0.2 (0.0–0.2)				
3	*L. brevicrus*	10.7 (10.3–11.0)	8.4 (8.2–8.6)	0.7 -			
4	*L. baluensis*	9.7 (9.1–10.3)	6.3 (5.8–6.7)	8.7 (8.4–9.1)	1.3 (0.2–2.4)		
5	*L. juliandringi*	12.5 (11.8–13.4)	12,5 (12.0–13.2)	11.8 (11.3–12.2)	11.9 (11.0–13.2)	1.1 (0.2–1.9)	
6	*L. mjobergi*	13.7 (13.2–13.9)	11.8 (11.5–12.0)	12.4 (12.0–12.7)	11.0 (10.3–11.8)	12.2 (11.5–13.0)	0.5 (0.2–1.0)

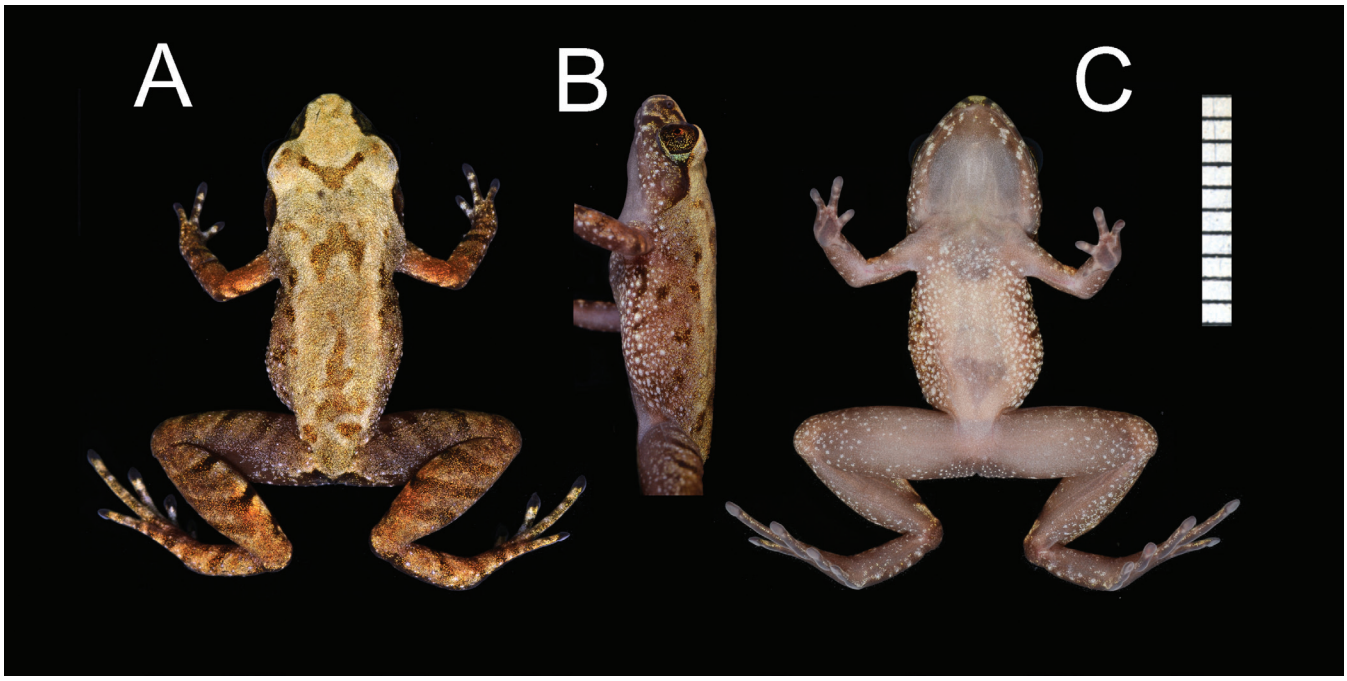

Fig. 3. A, dorsal; B, lateral; C, ventral views of male holotype of *Leptobrachella itiokai*, new species (KUHE 55895). Scale bar = 10 mm.

A: Fig. 2) was found. This topology is not identical with that reported previously (Eto et al., 2015), probably because of the increase of taxa analysed. Each species was shown to be monophyletic, but two sequences labelled as *L. brevicrus* from GenBank (KJ831302 and KJ831303: Oberhummer et al., 2014) were separated from the topotypic specimens and embedded in our *Leptobrachella* sp. Within Clade A, *L. baluensis* first diverged and the remaining species from northern Sarawak tended to form a clade, although the topology was poorly supported (66%/0.79). *Leptobrachella* sp. from Gn. Mulu formed a clade (100%/1.00) in Clade A, and was sister to *L. parva* (95%/0.95). The genetic divergence between *Leptobrachella* sp. from northern Sarawak and the other species examined ranged from 5.8 to 13.9% (Table 2).

In morphological traits, the specimens of *Leptobrachella* sp. from Gn. Mulu were distinguishable from all the known congeners as described in the next section. In addition to the named species, a probable unnamed species, *Leptobrachella* sp. 3 cf. "*baluensis*", was also reported from Gn. Mulu previously (Dring, 1983). Because of general morphological similarity, Dring (1983) treated it as *L. baluensis*, although there are several differences between them (e.g., *Leptobrachella* sp. 3 has elongate lateral glands on the posterior part of flank, which are absent in *L. baluensis* from Sabah: Dring, 1983; this study). Thus we tentatively treated Dring's (1983) *Leptobrachella* sp. 3 as not conspecific with *L. baluensis* in this study. Although we could not examine specimens of his *Leptobrachella.* sp. 3, literature information suggests differentiation of our *Leptobrachella* sp. from it (see below). *Leptobrachella* sp. from Gn. Mulu has a chirp-type advertisement call (Fig 5), which is distinct from all the reported calls of other congeners. These results indicate that the *Leptobrachella* sp. from Gn. Mulu is an unnamed species, which we describe as follows:

SYSTEMATICS

Leptobrachella itiokai, new species
(Figs 3, 4, 7)

Leptobrachella brevicrus: Oberhummer et al., 2014: 66; Eto et al., 2015: 131 (part).

Etymology. The specific name is dedicated to Dr. Takao Itioka of Kyoto University, Japan, who is an entomologist and a core member of the biological researcher consortium in Sarawak.

Material examined. Holotype: KUHE 55895, an adult male from Sungai Tapin on Gunung Mulu National Park, Sarawak, Malaysia (Borneo) (04°02'19"N, 114°55'04"E, 1,445 m asl), collected on 23 August 2015 by K. Eto.

Paratypes: KUHE 55845, 55846, 55897, and SRC 00335 (former KUHE 55898), four males collected from the type locality on 22–24 August 2015 by K. Eto, K. Nishikawa, and Y. A. Rohman.

Diagnosis. A small form of the genus (males 15.2–16.7 mm in SVL); tan or pale-brown dorsally with dark brown markings, V-shape on head, W-shape on shoulder, and triangle- or inverted V-shape on sacral region; tibiotarsal articulation of adpressed limb reaching or beyond eye; tip of digits sharply pointed, forming arrow-like projection. The new species can be distinguished from all other congeners by the combination of the following characteristics: ventrolateral glands small, rounded, usually not in series; side of body with small black blotches; ventrum entirely dark-coloured with tiny light spots; male with thin, thread-like lineae masculinae, although often indistinct in preservative; toe webs poorly developed; chirpy-call ca. 10 kHz in dominant frequency.

Fig. 4. Ventral view of: A, left hand; B, foot of male holotype of *Leptobrachella itiokai*, new species (KUHE 55895). Scale bar = 5 mm.

Description of holotype (measurements in mm). Snout-vent length (SVL) 16.7; habitus moderately slender (Fig. 3A–C); head slightly longer (HL 6.2) than broad (HW 6.0); snout rounded, projecting beyond lower jaw; eye large, length (EL 2.6) slightly greater than snout length (SL 2.2); canthus distinct, slightly constricted; lore slightly oblique, concave; nostril below canthus, about midway between tip of snout and anterior tip of upper eyelid (S-NL 1.1; N-EL 1.1), slightly closer to snout than to canthus; internarial distance (IND 2.1) wider than interorbital distance (IOD 1.8) and upper eyelid (UEW 1.6); pupil diamond shaped; pineal spot invisible; tympanum distinct, diameter (TD 0.9) one-third that of eye, and separated from eye by half of tympanum diameter (T-EL 0.4); vomerine teeth absent; tongue weakly notched posteriorly; subgular vocal sac bipartite with a small opening each near jaw commissure.

Forelimb slender (LAL 6.7; HAL 3.6); fingers slender, unwebbed; finger length formula: I < II < IV < III (Fig. 4A); finger tips sharply pointed, forming arrow-like projections; no fringes of skin along fingers; inner palmar tubercle large (IPTL 1.5); subarticular tubercles indistinct, replaced by indistinct, low callous tissue; nuptial pads absent. Hindlimb moderately short (HLL 23.8); tibia moderately short (TL 8.1), heels slightly overlapping when limbs held at right angles to body; tibiotarsal articulation of adpressed limb reaching eye; foot (FL 7.2) shorter than tibia; toe length formula I < II < V < III < IV (Fig. 4B); toe tips similar to those of fingers; webbing confined to bases of toes, formula: I $1^{3}/_{4}$–2^{+} II 2–3^{+} III $2^{2}/_{3}$–4 IV $3^{4}/_{5}$–$2^{4}/_{5}$V; toes without lateral fringes; subarticular tubercles obscure but elongate, replaced by low callous tissue; inner metatarsal tubercle low, length (IMTL 1.2) nearly same but slightly shorter than first toe (1TOEL 1.3); no outer metatarsal tubercle.

Skin dorsally nearly smooth (Fig. 3A); a low supratympanic ridge from eye to axilla; a relatively large tubercle (or gland) on just behind of jaw commissure; sides relatively granular with scattered tubercles/glands (Fig. 3B); chest and abdomen smooth (Fig. 3C); pectoral gland present but small; supra-axillary gland small and rounded; lateral glands also small and rounded; a spaced ventrolateral series of glands present in left side, absent in right side; femoral gland small and indistinct; two and three femoral glands recognised on left and right thigh, respectively.

Colour. In life, dorsally tan on head and body with dark markings, V-shaped marking on upper eyelid and interorbital space, W-shaped on shoulder, and triangle-shaped on sacral region; dorsolateral line light and indistinct; groin with dark marking restricted to cloacal region; side of head darker than dorsum; dark bands below canthus rostralis and supratympanic fold, and upper part of tympanum; a light brown stripe from between nostrils to anteriormost edge of upper jaw; lips irregularly barred with dark brown and white; flank pale-brown with small black blotches; pectoral, supra-axillary, and ventrolateral glands white; darkly pigmented ventrally on head, body, and limbs, except for vocal sac region; trunk darkly pigmented with dense tiny white spots; whitish thread-like lineae masculinae visible through ventral skin; forelimb brown dorsally with dark crossbars on lower arm and fingers; hindlimb brown dorsally with dark crossbars; ventral part of hindlimb dark with tiny white spots except for base of thigh; femoral gland pale, indistinct; iris gold with black reticulation and outer margin of pupil orange.

In preservative, colour and pattern generally faded but not obviously changed except for iris; lineae masculinae became greyish pink and hardly discernible.

Egg and larva. We could not examine any egg or larva from the type locality, but the tadpole previously reported as that of *L. brevicrus* from Sungai Tapin (ZMH A09365; Oberhummer et al., 2014) would be assigned to *L. itiokai*, because it was genealogically embedded in the new species (Fig. 2; Table 1). It has a morphology typical of the genus *Leptobrachella* and was found in gravel bed of stream (Oberhummer et al., 2014; see also discussion).

Variation. Individuals of the type series are generally similar to each other in morphology. Variations in size and body proportions are given in Table 3 together with those of the syntopically occurring *L. brevicrus*. Tibiotarsal articulation of adpressed limb reaching or extending beyond eye. Dorsal colouration varies among individuals, from tan, yellowish brown to pale brown. Size and number of black blotches on flank variable, but usually small and sparse. Lateral glands not in a series, except for some with an irregular or spaced series on sides of body. Femoral glands two to four in number, although indistinct in some.

Call characteristics. We examined two calls of the holotype (KUHE 55895) recorded at air temperature of 17.1°C, of which at least one could be the complete call (Fig. 5). The complete call consisted of a long series of short, chirpy notes

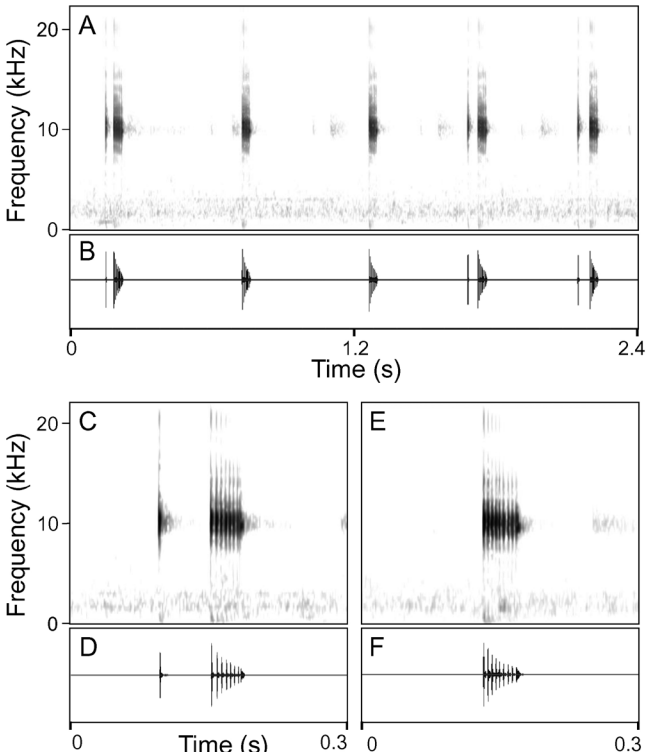

Fig. 5. Sonogram: A, C, E, 128 point window; B, D, F, wave form showing advertisement calls of holotype of *Leptobrachella itiokai*, new species (KUHE 55895) recorded at an air temperature of 17.1°C. C, D, Note type I. E, F, Note type II.

(call duration: 76.5 s; number of notes: 166), and the note repetition rate was 2.2 per s. In the calls, two note types were recognised: notes begin with a discrete pulse (type I: bottom left of Fig. 5), and those without such a pulse (type II: bottom right of Fig. 5). The note type II predominated at the beginning of calls, but was replaced by the type I note from the middle portion to the end of the call. In the note type I, each note lasted 80.1 ± 10.0 (73–106, n = 15) ms and was composed of one discrete and 7–10 subsequent, continuous pulses. On the other hand, note length and number of pulses were 35.3 ± 6.3 (25–51, n = 15) ms and 7–11, respectively, in the type II note. Note interval was 1.07 ± 0.17 (0.84–1.24) s in the beginning and 0.38 ± 0.07 (0.29–0.52) s in the middle and later. The dominant frequency of each note type was essentially same, 10.04 ± 0.03 (9.99–10.08, n = 30) kHz. Multiple harmonics and slight frequency modulation were recognised in both types of notes, and the maximum frequency was over 20 kHz.

Including the holotype, we examined a total of five calls from four individuals recorded at the type locality (air temperatures: 17.1–17.5°C), and obtained ranges of each measurement as follows: note length, 73–106 ms in type I and 21–63 ms in II; number of pulses, 7–10 with one discrete pulse in I and 5–12 in II; note interval, 0.29–1.24 s, and dominant frequency, 9.98–10.38 kHz.

Comparisons. *Leptobrachella itiokai*, new species, is similar to *L. baluensis*, *L. brevicrus*, and *L. parva* in appearance. The new species is possibly also similar to *Leptobrachella* sp. 3 "*baluensis*" from Gn. Mulu reported by Dring (1983)

Table 3. Measurements in adults of *Leptobrachella itiokai*, new species and syntopic *L.brevisrus*. Mean SVL (in mm) and medians of ratios (R) of other characters to SVL, followed by ranges in parenthesis. See text for character abbreviations.

	***L. itiokai* sp. nov.** **5M**	***L. brevisrus*** **4M**
SVL	15.8 (15.2–16.7)	17.5 (17.1–17.8)
RHL	37.6 (36.7–40.5)	38.4 (36.9–39.3)
RSL	12.7 (12.5–14.0)	12.5 (12.3–12.7)
RS-NL	6.8 (6.4–8.4)	7.0 (6.8–7.2)
RE-NL	6.2 (5.9–6.6)	5.6 (5.4–5.7)
REL	16.2 (15.3–16.9)	16.2 (15.4–16.5)
RT-EL	2.6 (2.3–3.0)	3.6 (2.9–4.0)
RTD	6.0 (5.5–6.5)	6.0 (5.4–6.7)
RHW	37.6 (36.2–40.1)	38.2 (36.9–39.6)
RIND	12.5 (12.2–14.4)	12.3 (11.9–12.6)
RIOD	10.5 (10.2–12.3)	11.0 (10.2–12.0)
RUEW	9.2 (8.7–9.8)	9.2 (8.1–10.0)
RLAL	41.1 (40.0–41.9)	42.8 (42.2–43.5)
RHAL	21.4 (19.9–21.7)	22.9 (22.2–23.0)
RIPTL	8.8 (8.2–8.9)	8.8 (8.3–9.3)
RHLL	165.7 (141.9–166.7)	157.0 (153.7–158.2)
RTL	52.6 (48.1–52.9)	49.4 (48.2–49.6)
RFL	45.8 (42.7–46.0)	45.3 (44.8–45.4)
RIMTL	7.3 (7.0–8.2)	6.9 (6.5–7.6)
R1TOEL	8.0 (6.9–8.3)	7.9 (7.3–8.3)

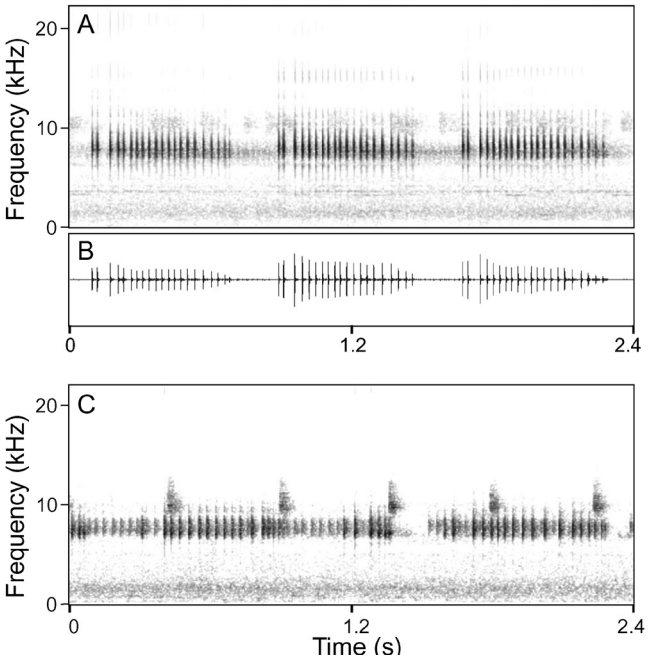

Fig. 6. A, B, Sonogram (128 point window) and wave form of advertisement call of *Leptobrachella brevicrus* (KUHE 55844) recorded at an air temperature of 17.6°C. C, Sonogram (256 point window) of a chorus of *L. brevicrus* and *L. itiokai*, new species, recorded at an air temperature of 17.5°C.

based on literature information. However, the new species can be distinguished from these species, as well as all the other congeners as follows.

By the absence of elongated lateral glands, the new species is differentiated from *L. juliandringi* (a series of elongate glands present: Eto et al., 2015) and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (elongate glands present on posterior part of flanks). The new species is also distinguishable from the other congeners from Borneo and Natuna Islands by the presence of dark pigmentation with small light spots on trunk except for *L. baluensis*, *L. brevicrus*, *L. parva*, and Dring's (1983) *Leptobrachella* sp. 3 (Dring, 1983; this study). Male of the new specie has thin, thread-like lineae masculinae, which is wide, strap-like in *L. juliandringi* (Eto et al., 2015) and not evident even on dissection in *L. brevicrus* and *Leptobrachella* sp. 3 "*baluensis*" (Dring, 1983; this study). From *L. serasanae* Dring, 1983, the new species is differentiated by the absence of wide black band on flank. From *L. palmata*, the new species is differentiated by the poor toe webs (toe webs well-developed in *L. palmata*: Inger & Stuebing, 1992). As to body size, the new species (males 15.2–16.7 mm: Table 3) overlaps *L. baluensis* from Sabah (males 14.9–15.9 mm: this study), *L palmata* (males 14.4–16.8 mm: Inger & Stuebing, 1992), *L. parva* (males 14.2–15.1 mm: this study), and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (males 15.0–16.0 mm: Dring, 1983), but is smaller than *L. brevicrus* (males 17.1–17.8 mm: this study) *L. juliandringi* (males 17.0–19.1 mm: Eto et al., 2015), *L. mjobergi* (males 15.7–19.0 mm: Eto et al., 2015), and *L. natunae* (male 17.6 mm: Günther, 1895). Although only one female specimen is known, *L. serasanae* would have the body size similar to the new species (female 16.9 mm:

Fig. 7. Dorsolateral view of male holotype of *Leptobrachella itiokai*, new species (KUHE 55895).

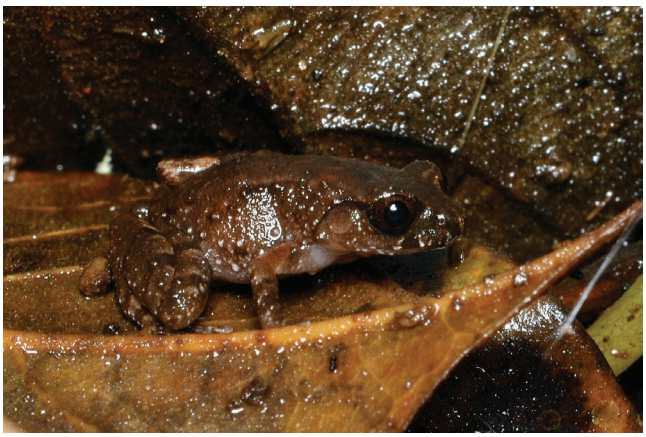

Fig. 8. Lateral view of a calling male of *Leptobrachella brevicrus* (KUHE 55844).

Dring, 1983). In the new species, relative length of tibia (RTL = 48.1–52.9%: Table 3) overlaps *L. brevicrus* (48.2–49.6%), *L. natunae* (46.1–49.4%: Dring, 1983), and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (50.0–52.7%: Dring, 1983), but is shorter than *L. baluensis* (54.2–56.9%: this study), *L. juliandringi* (50.0–58.1%: Eto et al., 2015), *L. mjobergi* (50.1–59.9%: Eto et al., 2015), *L. parva* (55.9–58.4%: this study), and *L. serasanae* (59.2%: Dring, 1983).

Other than the morphological traits, *L. itiokai* has a unique chirpy-call (Fig. 5), which is distinct form buzz-like advertisement calls of *L. brevicrus*, *L. mjobergi*, and *L. parva* (Dring, 1983; Eto et al., 2015; Fig. 6). *Leptobrachella juliandringi* and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" also have a chirpy-call, but the former has lower (7.00–8.20 kHz at air temperatures of 21.8–23.5°C: Eto et al., 2015), and the latter has higher (12–14 kHz at 22°C: Dring, 1983) dominant frequency, than the new species.

Range. Known only from the type locality, Sungai Tapin on Gn. Mulu, state of Sarawak, east Malaysia.

Natural history. In the type locality, males seem to prefer calling on leaves or branches (ca. 0.2 to 0.6 m above the ground or the water surface: Fig. 7) near steep streams. This habit contrasts to that of syntopic *L. brevicrus*, which calls

in hidden place such as among rocks or dead leaves and at the base of bushes (Fig. 8: Dring, 1983; this study). The oviposition site is unknown but perhaps in gravel portions along streams or water spring, because calling males were often observed near such an environment.

DISCUSSION

Leptobrachella itiokai, new species, occurs syntopically with *L. brevicrus* on Gn. Mulu. These two species are observed often in the same place, but seem to be isolated reproductively by the difference of their advertisement calls (Figs 5, 6), *L. itiokai* with a chirpy-call while *L. brevicrus* calls like buzz. Differentiation of the preference of calling site as mentioned above could also work to avoid interspecific mating. Syntopic occurrence of two or more *Leptobrachella* species was previously unreported (Eto et al., 2015), whereas such a distributional pattern is often observed in closely related genus *Leptolalax* (e.g., Matsui et al., 2014). Other than *L. itiokai* and *L. brevicrus*, we also observed syntopic occurrence of *L. juliandringi* and *L. parva* on Gn. Mulu (Eto, personal observation). Therefore, such a distributional pattern would be actually not uncommon in the genus. Because species of *Leptobrachella* are small in body size and are sometimes difficult to be found and identified, the number of species in the previous studies might be underscored.

Oviposition site and, larval morphology and ecology of *L. itiokai* are not known well. Oberhummer et al. (2014) noted its tadpole from Sungai Tapin, which was originally identified as *L. brevicrus* but is phylogenetically assigned to the new species (Fig. 2). They described morphological and ecological traits based on a total of six tadpoles, but they have examined DNA sequence from only one specimen. Because *L. brevicrus* also occurs syntopically with *L. itiokai* in Sungai Tapin, tadpoles assigned to both of the two species might be included in their data set. Thus, larval traits described by Oberhummer et al. (2014) require re-examination.

Present study showed that five *Leptobrachella* species occur on Gn. Mulu, reconfirming high amphibian diversity in this area. The obtained phylogenetic relationships (Fig. 2) showed that three (*L. brevicrus*, *L. itiokai*, and *L. parva*) of four *Leptobrachella* species examined from Gn. Mulu were embedded in Clade A, and only *L. juliandringi* was not grouped in it. Clade A mainly consists of highland species except for one hilly species, *L. parva*. The topology suggested that the common ancestor of the clade would be highland species, where it first diverged into ancestors of three lineages, *L. baluensis*, *L. brevicrus*, and *L. itiokai* + *L. parva*. The divergence between the ancestor of *L. baluensis* and the others would have been caused by geographic factors, because they are now isolated in distribution. What made the divergence between the ancestors of *L. brevicrus* and *L. itiokai* + *L. parva* is unclear, but it would have arisen in some region around Gn. Mulu, because *L. brevicrus* and *L. itiokai* are now restricted to this area. Finally, *L. itiokai* and *L. parva* diverged, and only the latter has adapted to environments of lower area. Another hilly species, *L. juliandringi* is phylogenetically remote from them. This species occurs widely in Sarawak (Eto et al., 2015), and might have invaded Gn. Mulu from some other regions. Phylogenetic position of the remaining species recorded from Gn. Mulu, *Leptobrachella* sp. 3 "*baluensis*" (Dring, 1983), is unknown, but would provide crucial information in clarifying the diversification process of the genus *Leptobrachella* on Gn. Mulu.

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APPENDIX

Specimens examined for morphological comparisons. Voucher specimens are stored at BORNEENSIS collection, University Malaysia Sabah (BORN), Human and Environmental Studies, Kyoto University (KUHE), and Sarawak Research Collections, Forest Research Centre Sarawak (SRC).

Leptobrachella baluensis: BORN 22842 and 22865: Kinabalu NP, Sabah; BORN 23426, 23427, and 23475: Crocker Range NP, Sabah.

L. brevicrus: KUHE 55844, 55847, 55896, and 55935 from Sungai Tapin, Gn. Mulu, Sarawak.

L. juliandringi: KUHE 10628, 10671, 10672, 10675, 10679, and 10697 from Sungai Beloh, a tributary of Sungai Katibas, Bukit Lanjak, Sarawak; KUHE 48916–48919, 55333, 55334, 55337–55341, and SRC 00230 (former KUHE 49815) from Camp 2 of Mulu NP, Gn. Mulu, Sarawak.

L. mjobergi: KUHE 17131, 17207–17210, 47872–47874, 47876, 48879, 49701, 49705, 49706, and 49712 from Gn. Gading, Sarawak.

L. parva: KUHE 48949–48952, 48970, 48980, and 55308 from Camp 1 of Mulu NP, Gn. Mulu, Sarawak.