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Article



A new species of blue-eyed *Leptobrachium* (Anura: Megophryidae) from Sumatra, Indonesia

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Abstract

A new megophryid species of *Leptobrachium* is described on the basis of three specimens collected from Kubu Perahu, Lampung Province, Sumatra, Indonesia. The new species, *L. waysepuntiense* **sp. nov.** is distinguished from all other congeners by the colour of iris, which is totally light bluish in adult and greyish in juvenile stages. It is similar to Bornean endemic species in the absence of dark markings around groin, and particularly to *L. gunungense* in the very small size of femoral gland. It is the fourth species of *Leptobrachium* recorded from Sumatra, and its discovery underscores the under estimated diversity of amphibians on Sumatra. Variation in the pattern of iris colour in *Leptobrachium* is discussed.

Key words: Iris colour, Lampung, Leptobrachium hasseltii, Southeast Asia, Sundaland, Sumatra

Introduction

Leptobrachium is a megophryid genus proposed by Tschudi with *L. hasseltii* from Java being the type species. The genus is sometimes split into two subgenera, *Vibrissaphora* Liu, a group with adult males having spines on upper lip, and *Leptobrachium*, a group without such spines (Dubois & Ohler 1998). The first group consists of about five species ranging from southern China to northern Vietnam, whereas the second group is represented by at least 17 species, widely distributed from southern China, northern India, through Indochina, Sundaland to Philippines (Fei *et al.* 2009). However, the taxonomic relationships of these two groups as different genera, subgenera, or a single genus, is an ongoing argument (e.g., Dubois 1980; Dubois & Ohler 1998; Rao & Wilkinson 2008; Zheng *et al.* 2008).

At least seven species of *Leptobrachium sensu stricto* have been described from Sundaland: *L. hasseltii* Tschudi, *L. montanum* Fischer, *L. abbotti* (Cochran), *L. hendricksoni* Taylor, *L. nigrops* Berry & Hendrickson, *L. gunungense* Malkmus, and *L. smithi* Matsui, Nabhitabhata & Panha (also found in Thailand to northern India). However, information on the presence of this genus in Sumatra is still meagre. Iskandar & Colijn (2000) placed three species, *L. hasseltii*, *L. hendricksoni*, and *L. nigrops* in the faunal list of this island.

While examining the collection of *Leptobrachium* in Museum Zoologicum Bogoriense (MZB), we found a male specimen (MZB Amp 14592) labelled as *Leptobrachium* sp. collected in November 2004 by A. Ul-Hasanah and W. Endarwin from Way Sepunti, Lampung, southwestern Sumatra. This specimen was identified as *Leptobrachium sensu stricto* because of its lack of keratinized spines on the upper lip (Dubois & Ohler 1998) but was different from three congeneric species from the island (*L. hasseltii, L. nigrops*, and *L. hendricksoni*) in several morphological characteristics such as lack of dark markings around groin, and a dark venter covered with white dots. Through the courtesy of A. Ul-Hasanah, we obtained a photograph of this specimen in life, taken by A. Nurcahyo of the Wildlife Conservation Society of Indonesia. In this picture it was obvious that the iris colour of MZB Amp 14592 is completely light blue, unlike other light blue-eyed species (*L. chapaense* [Bourret], *L. hainanense* Ye & Fei, *L. huansen* Fei & Ye and *Vibrissaphora* spp.), in

which light colour is restricted to the upper half of the iris (Dubois & Ohler 1998; Matsui et al. 1999).

The importance of iris colour as a diagnostic character for this genus was independently stressed by Dubois & Ohler (1998) and Matsui *et al.* (1999). Indeed, two recently described species, *L. buchardi* Ohler, Teynié & David and *L. mauhoti* Stuart, Sok & Neang, both from Indochina, were thought to be distinct based on this character. All Sundaland species, except *L. hendricksoni* and *L. smithi*, have completely black irises, while the latter two species have the yellow to scarlet colour in the upper part of iris. The completely light coloured iris of the Sumatran specimen in question is not only the first record among Sundaland species, but also in all *Leptobrachium sensu lato*. Based on the record for this specimen, we conducted a short-term survey in the locality where it was collected. As a result, we collected an adult female and a juvenile, identical with the male specimen (MZB Amp 14592). Considering the completely light coloured iris as a unique character distinguishing the specimens from all previously described *Leptobrachium*, we describe them below as a new species.

Material and methods

A field survey to Way Sepunti trail, Kubu Perahu, Lampung, Sumatra (Figure 1) was undertaken between 13 and 15 February 2009. Upon collection of specimens, we took tissue sample for biochemical analysis, fixed them in 10% formalin, and later stored them in 70% ethanol at the MZB and Graduate School of Human and Environmental Studies, Kyoto University (KUHE). Measurements were made to the nearest 0.1 mm with a dial calliper under a binocular dissecting microscope mainly following Matsui (1984) for 23 morphological characters for the holotype: 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 (IMTL); and 23) first toe length (FL); 21) hindlimb length (HLL); 22) inner metatarsal tubercle length (IMTL); and 23) first toe length (1TOEL), from distal end of inner metatarsal tubercle to tip of first toe. We followed the system proposed by Savage (1975) for the description of toe webbing states.

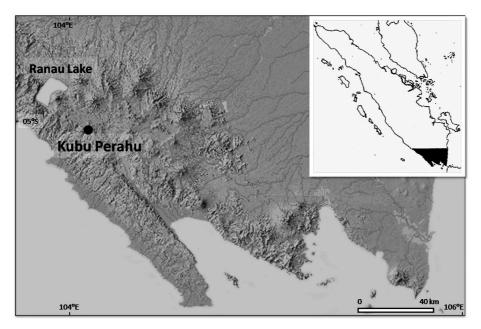


FIGURE 1. Map of southern Sumatra, showing the type locality (closed circle) of *Leptobrachium waysepuntiense* sp. nov.

Leptobrachium waysepuntiense sp. nov.

Holotype: MZB Amp 15862, an adult female collected among the leaf litter in primary forest in Way Sepunti trail (05°03'51''S, 104°02'05''E, 691 m a.s.l.; Figure 1), Kubu Perahu village, District of Liwa, Lampung Province, Sumatra, Indonesia, collected by A. Hamidy, S. Kirono, D. Susanto, Marji, Andi, and Habsi at 20:00 h on 14 February 2009 (Figures 2, 3).

Paratypes: MZB Amp 14592, an adult male collected by A. Ul Hasanah and W. Endarwin from near the type locality (05°03'51"S, 104°02'07"E, 852 m a.s.l.) in November 2004; KUHE 42805, a juvenile, collected on 13 February 2009, collectors and locality same as for holotype.

Etymology: The specific name refers to the locality where this species was collected.

Diagnosis: The new species is placed in *Leptobrachium* by having the combination of: femoral glands present; oval, flat axillary glands present; inner palmar tubercle circular, not extending along first metacarpal; vomerine teeth absent; snout and/or dermal palpebral projections absent; rictal glands and ventrolateral glandular ridges absent; spines on the upper lip absent. A medium-sized *Leptobrachium* (adult female 58.3 mm and male 50.0 mm in SVL; Table 1); iris in adult light blue with fine black reticulations, but is light grey in juvenile; dorsum uniform dark grey; dark grey laterally with white and orange dots; top of head from interorbital to parietal region, and dorsal side of fingers and toes faintly orange; greyish on belly and brownish on throat with white dots particularly on chest; no markings at groin; femoral glands very small.

Species	Mal	es		Females		
	N	Average (Range)	SD	Ν	Average (Range)	SD
L. waysepuntiense	1	50.0	_	1	58.3	_
L. hasseltii	9	43.9 (40.0–49.7)	2.83	1	59.0	_
L. hendricksoni	5	42.7 (38.0–45.1)	2.91	5	55.0 (46.1–69.0)	9.62
L. nigrops	8	31.3 (27.4–40.1)	4.18	8	40.5 (38.3–44.8)	2.32
L. montanum	10	57.9 (51.2–65.1)	4.79	2	65.1 (58.0–72.2)	_
L. abbotti	_	-	_	2	68.7 (64.8–72.6)	_
L. gunungense	4	43.9 (39.2–52.6)	5.94	4	59.4 (55.4–63.8)	3.47
L. smithi	17	41.4 (36.2–46.1)	2.49	2	55.6 (50.2-60.9)	_

TABLE 1. Morphometric variation of SVL (in mm) in *Leptobrachium* from Sundaland. For *L. smithi*, data for the topotypic population (Kao Chong) adopted from Matsui *et al.* (1999).

Description of Holotype (measurements in mm): Body tapering to the groin (SVL 58.3), head broad and depressed, slightly longer (HL 25.5: 43.7 % of SVL) than wide (HW 24.4: 41.9 % SVL); snout rounded from above, truncate in profile, very slightly projecting beyond lower jaw; eye large and obviously projecting from sides of head, smaller (EL 8.6: 14.8% SVL) than snout (SL 10.5: 18.0% SVL); canthi sharp, lores oblique, moderately concave; nostrils lateral, below canthus, distinctly closer to tip of snout (S-NL 4.8: 8.2% SVL) than to eye (N-EL 6.0: 10.3% SVL); internarial distance (IND 4.2: 7.2% SVL) much shorter than interorbital distance, (IOD 8.1: 13.9% SVL), latter wider than upper eyelid (UEW 7.5: 12.9% SVL); no pineal spot; tympanum distinct, diameter (TD 5.0: 8.6% SVL) about three-fifth that of eye and separated from eye by half of its diameter (T-EL 2.5: 4.3%SVL); vomerine teeth absent; tongue heart-shaped, without papillae, notched posteriorly.

Forelimb slender and long (FLL 43.2: 74.1% SVL), about three-fifths of hindlimb; fingers moderately slender, unwebbed; first finger (FFL 6.6: 11.3% SVL) slightly longer than fourth and second, third much longer (TFL 10.3: 17.7% SVL); tips rounded, not swollen; inner palmar tubercle large (IPTL 2.8: 4.8% SVL), not extending onto first metacarpal and smaller outer palmar tubercle (OPTL 2.5: 4.3% SVL); subarticular tubercles indistinct, replaced by low callous tissue.

Hindlimb slender and relatively short (HLL 71.0: 121.8% SVL); heels not meeting when legs are held at

right angles to body; tibia slightly longer (TL 20.3: 34.8% SVL) than foot (FL 19.8: 34.0% SVL); tibiotarsal articulation of adpressed limb reaching to the middle of tympanum (Table 3); third toe longer than fifth; toe tips similar to those of fingers; toe webs poorly developed, webbing formula I 2 - 2 + II + - 3 III + - 4 - IV4- -- 2 V (Figure 3C); inner metatarsal tubercle low, oval, length (IMTL 2.6: 4.5% SVL) more than half distance between tip of first toe and tubercle (1TOEL 4.1:7.0%); outer metatarsal tubercle absent; subarticular tubercles obscure, but elongate, replaced by low callous tissue.



FIGURE 2. The holotype of *Leptobrachium waysepuntiense* sp. nov. (SVL = 58.3 mm) in life (MZB Amp 15862).

Skin above nearly smooth, with minute granules scattered posteriorly, especially around waist; granules denser laterally on body and on posterior thigh; ventrally slightly granular; a very low supratympanic ridge from eye to behind tympanum; indistinct low of dermal ridges on upper surface of forelimb; a flat pectoral gland at median border of axilla behind arm insertion; minute femoral gland on posterior surface of thigh.

Colour: In life, dorsally dark brownish grey with a faint V-shaped brownish orange interorbital and parietal marking (Figure 3A); grey colour fading laterally to light grey on ventral side (Figure 3B); laterally and ventrally dotted with white and orange, especially densely on sides; white dots denser on chest and throat than on abdomen; toes orange brown dorsolaterally; iris light blue with black reticulations; light blue orbital arc surrounding iris visible when eye opened maximally; no black bars around lips; supratympanic ridge bordered by a very thin brownish orange line; forelimb dorsally vaguely barred with dark brown; posterior thigh spotted with white and orange; no dark markings around groin from posterior flank to anterior thigh. In preservative, the aspects of the colour pattern remain, but the dorsal ground colour has been darkened and orange dots have faded to white.

Variation: The male paratype (MZB Amp 14592) is morphologically similar to holotype, but has smaller body, fewer dark reticulations on blue iris, more rugose dorsal skin, more distinct dark crossbars on the dorsal sides of limbs, and more distinct markings between interorbital and on upper half of tympanum. It has an internal vocal sac and a pair of vocal sac openings. The juvenile paratype (KUHE 42805; Figure 4) has a light grey iris with irregular black reticulations, and a distinct orange line from canthus through margin of upper eyelid and above tympanum to arm insertion. Narrow dark brown bars each with orange spots medially are distinct on dorsal half of limbs, and toes are dorsally light brown.

Comparisons: Small number of samples limited statistical comparisons of morphometric characters, but some dimensions did not overlap between *L. waysepuntiense* and other species (Table 2). The new species tended to have longer LAL and FL than *L. hasseltii*, longer FL than *L. hendricksoni*, and longer LAL, FL, and IMTL than *L. abbotti*, all relative to SVL. In addition, the new species seemed to have larger FL/TL ratio than *L. nigrops* (Table 2). In the new species, the tibiotarsal articulation reached the centre of the tympanum, but it barely reached the posterior end of the tympanum in many specimens of *L. gunungense*, and exceeded the anterior end of the tympanum in more than half specimens of *L. montanum* (Table 3).

TABLE 2. Morphometric variation in *Leptobrachium* from Sundaland. Relative values (R) of each character to SVL (in %), and ratios of foot-tibia length and head width-head length are given in medians with ranges in parentheses. For *L. smithi*, data for the topotypic population (Kao Chong) adopted from Matsui *et al.* (1999). Juveniles and adults of both sexes combined.

Species	Ν	RHL	1	RHW		RLAL	RHLL	RTL
L. waysepuntiense	3	45.7 (43.7	7–46.0)	41.9 (41.6–	43.6)	55.6 (55.0–58.2)	121.9 (114.2–134.3)	38.2 (34.8–41.2)
L. hasseltii	10	43.0 (40.8	3–44.2)	42.3 (40.5–	43.8)	52.4 (49.3–54.6)	118.6 (110.7–126.0)	37.0 (34.7–39.3)
L. hendricksoni	10	44.8 (37.4	-46.9)	43.9 (39.1–	45.1)	56.8 (47.7–60.0)	123.9 (102.8–132.1)	38.4 (34.9–40.6)
L. nigrops	16	43.3 (41.6	5–45.8)	40.0 (38.1–	42.3)	54.1 (48.3–58.7)	127.3 (117.7–135.0)	39.5 (35.5–42.3)
L. montanum	12	44.4 (42.9	9–46.7)	42.9 (42.3–	50.4)	54.7 (50.0–61.5)	124.6 (115.5–137.7)	38.8 (34.6–43.2)
L. abbotti	2	44.3 (43.2	2–45.4)	42.3 (41.8–	42.8)	53.3 (52.1–54.4)	117.6 (112.3–122.8)	35.5 (34.5–36.5)
L. gunungense	8	44.1 (43.0)-47.6)	44.6 (42.0–	45.3)	54.6 (41.4–57.1)	119.2 (115.9–124.7)	38.0 (35.9–39.7)
L. smithi	19	44.9 (43.1	-45.8)	44.3 (41.2–	46.0)	56.0 (52.2–57.8)	123.9 (115.5–132.8)	36.5 (34.0–38.6)
continued.								
Species		Ν	RFL		RIM	TL	FL/TL	HW/HL
L. waysepuntiense		3	34.4 (34.0–	37.3)	4.2 (4.0-	-4.5)	0.91 (0.90–0.98)	0.95 (0.90–0.96)
L. hasseltii		10	32.4 (31.0–	33.7)	3.9 (3.4-	-4.4)	0.87 (0.82–0.92)	0.98 (0.94–1.03)
L. hendricksoni		10	32.0 (29.5–	33.6)	3.3 (2.3-	-4.4)	0.84 (0.79–0.87)	0.96 (0.92–1.05)
L. nigrops		16	32.0 (29.3–	34.9)	4.4 (3.3-	-5.9)	0.81 (0.76–0.85)	0.90 (0.87–1.02)
L. montanum		12	33.7 (29.0–	37.5)	3.8 (2.7-	-4.7)	0.86 (0.78–0.97)	0.97 (0.92–1.17)
L. abbotti		2	31.4 (31.3–	31.5)	2.8	-3.0)	0.89 (0.86–0.91)	0.95 (0.94–0.97)
L. gunungense		8	35.1 (32.6–	37.8)	3.7 (3.0-	-5.0)	0.92 (0.89–0.96)	1.00 (0.95–1.03)
L. smithi		19	33.3 (30.4–	36.1)	3.4 (2.8-	-4.0)	0.91 (0.82–0.95)	0.98 (0.96–1.03)

More discrete differences were found in qualitative characters (Table 4). Leptobrachium waysepuntiense differs in eye colour from all the other congeneric species including the subgenus Vibrissaphora. In most species of Leptobrachium from China and Indochina like L. chapaense, L. hainanense and L. huansen (Liu et al. 1973; Yang 1991; Dubois & Ohler 1998; Lathrop et al. 1998; Fei 1999; Fei et al. 2009), L. xanthospilum Lathrop, Murphy, Orlov & Ho and L. banae Lathrop, Orlov, Murphy & Ho, as well as species of Vibrissaphora, light blue or white colour is limited to the dorsal half of the iris, unlike L. waysepuntiense with totally light blue iris. Of the Indochinese species, L. buchardi has upper part of iris pale green (Ohler et al. 2004), and L. mauhoti has black iris surrounded by an orange-red crescent dorsally (Stuart et al. 2006). Leptobrachium hendricksoni from southernmost Thailand through Peninsular Malaysia, Sumatra to Borneo

(Berry 1975; our own observations), *L. pullum* from southern Vietnam (Smith 1921) and *L. smithi* from Thailand to southern Malay Peninsula (Matsui *et al.* 1999) share dorsal half of the iris scarlet or yellow in colour. *Leptobrachium nigrops* from Peninsular Malaysia, Sumatra and Borneo (Berry 1975; our own observations) and all Bornean species, *L. montanum, L. abbotti* (Inger *et al.* 1995; Malkmus *et al.* 2002; our own observation), and *L. gunungense* (Malkmus *et al.* 2002; our own observations) have a totally black iris. The iris of *L. hasseltii* from Sumatra to Bali, once reported as scarlet (Iskandar 1998), is actually totally black (our observations).

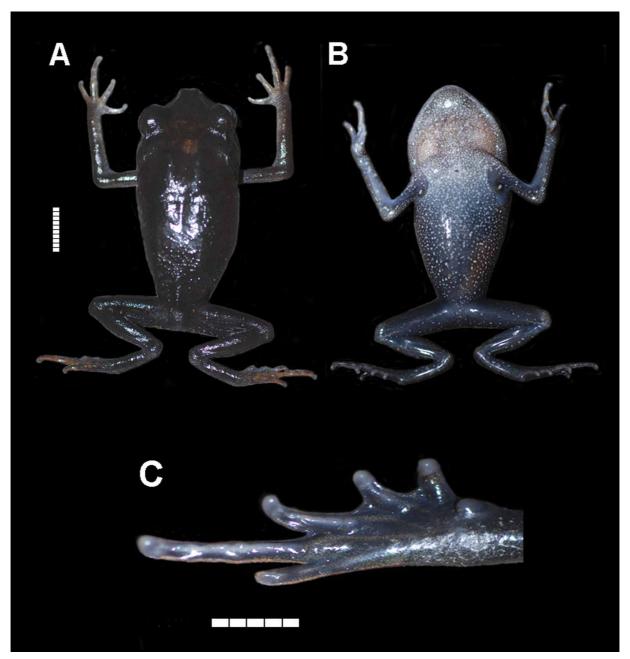


FIGURE 3. Dorsal (A) and ventral (B) views, and ventral view of the right foot (C) of the holotype of *Leptobrachium waysepuntiense* **sp. nov.** (MZB Amp 15862). Scale indicates 10 mm in A and B, and 5 mm in C.

In addition to the iris colour, *L. waysepuntiense* differs from five of the seven congeners from Sundaland as follows; *Leptobrachium waysepuntiense*, without distinct dorsal markings, is differentiated from *L. hasseltii* and *L. nigrops* with distinct blotches on back, and *L. smithi* with confluent dark markings (Matsui *et al.* 1999). Furthermore, *L. waysepuntiense* clearly differs in ventral marking from *L. hendricksoni* with many black spots and *L. abbotti* with large dark lead-grey to black spots. The remaining two species, *L. montanum*

and *L. gunungense*, with totally dark iris can be easily differentiated from *L. waysepuntiense* with light blue iris in life, but are otherwise very similar, although femoral glands are larger and more distinct in *L. montanum* than *L. waysepuntiense* (Figure 4B).

TABLE 3. Variation in the point reached by the tibio-tarsal joint when hindlimb is bent forwards along body (percentage frequency in parentheses). For *L. smithi*, data for the topotypic population (Kao Chong) adopted from Matsui *et al.* (1999).

Species	N	Sex	Beyond anterior edge of tympanum	Between anterior and posterior edge of tympanum	Behind posterior edge of tympanum
L. waysepuntiense	1	F	_	1 (100%)	_
	1	М	_	1 (100%)	_
L. hasseltii	1	F	_	_	1 (100%)
	9	М	_	6 (66.7 %)	3 (33.3%)
L. hendricksoni	5	F	5 (100%)	_	_
	5	М	3 (60%)	2 (40%)	_
L. nigrops	8	F	7 (87.5%)	1 (12.5%)	_
	8	М	_	_	8 (100%)
L. montanum	2	F	1 (50%)	1 (50%)	_
	10	М	7 (70%)	3 (30%)	_
L. abbotti	2	F	_	1 (50%)	1 (50%)
L. gunungense	4	F	_	_	4 (100%)
	4	М	_	2 (50%)	2 (50%)
L. smithi	2	F	_	2 (100%)	_
	17	М	_	16 (94.1%)	1 (5.9%)

TABLE 4. Comparison of diagnostic characters in Leptobrachium from Sundaland.

Species	Iris colour	Marking at groin	Femoral gland
L. waysepuntiense	all parts light blue with fine black reticulations	no	very small white spot
L. hasseltii	all parts black	yes	very large white blotch
L. hendricksoni	upper part orange	yes	large white blotch
L. nigrops	all parts black	yes	large white blotch
L. montanum	all parts black	no	small white spot
L. abbotti	all parts black	no	large white blotch
L. gunungense	all parts black	no	very small white spot
L. smithi	upper part yellow, orange, or scarlet	yes	medium-sized white spot

Range: Southwestern Sumatra, Indonesia. Known from the type locality at Way Sepunti trail, Kubu Perahu, Liwa, West Lampung, but probably also occurs in western of Jambi Province, central Sumatra (Matsui *et al.*, unpublished data, see below) (Fig. 1).

Natural history: Larval, acoustic and other ecological data are unknown. Way Sepunti trail, where the type series of *L. waysepuntiense* was collected, is in primary forest and located approximately 250 m from a rocky stream. On this trail, the new species was found at a higher elevation (691–852 m a.s.l.) than where *L. hasseltii* occurred (300 m a.s.l.). Other species found on this trail are: *Leptophryne borbonica* Tschudi, *Megophrys nasuta* (Schlegel), *Kalophrynus pleurostigma* Tschudi, *Limnonectes kuhlii* (Tschudi), *Odorrana hosii* (Boulenger), *Hylarana crassiovis* (Boulenger), *Huia sumatrana* Yang, *Microhyla palmipes* Boulenger,

M. berdmorei (Blyth), and *Rhacophorus* sp. Neither eggs nor larvae were found and calls were not heard in mid February.

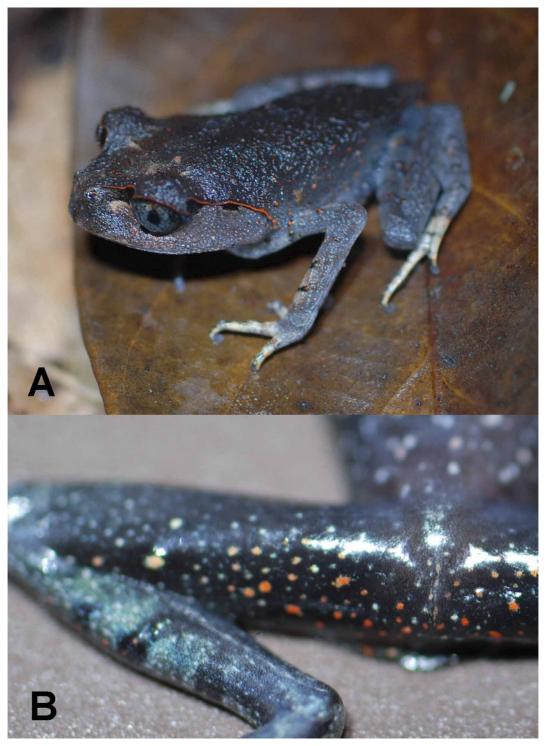


FIGURE 4. The juvenile paratype (KUHE 42805) of *Leptobrachium waysepuntiense* **sp. nov.** (SVL = 33.5 mm) in life (A) and its posteroventral view (B: TL = 13.8 mm).

Discussion

Herpetological surveys on Sumatra began long ago (localities cited in Van Kampen 1923), but the known number of frogs from Sumatra is much smaller than those of Malay Peninsula and Borneo (Inger & Voris

2001). Sumatra has a land area about 63% the size of Borneo, but has only 58% as many species; its land area is 61% larger than Malay Peninsula, but its frog fauna is 15% less than that of the Peninsula (computed from Inger & Voris' [2001] data). Moreover the ratio of endemic species in the Sumatran frog fauna (25%) is much smaller than that in Borneo (62%), Java (34%) or even Malay Peninsula (38%: Inger & Voris 2001). However, through recent intensive surveys on the island, the number of recorded Sumatran amphibians is rising. Recent discoveries of new species like *Ansonia glandulosa* (Iskandar & Mumpuni 2004) and *Xenophrys parallela* (Inger & Iskandar 2005) indicate that there remains many localities to be surveyed.

Of the Leptobrachium in Sumatra, Boulenger (1908) and Van Kampen (1923) reported a single species, Leptobrachium (as Megalophrys) hasseltii, then considered to occur throughout Indochina, Sundaland and the Philippines. Although distributional information on this genus is still limited, records of specimens in MZB and our results indicate the occurrence of at least four species within Sumatra. Leptobrachium hendricksoni and L. nigrops occur from the provinces of Aceh to Bengkulu and North Sumatra to Jambi (northern to western and central regions), whereas L. hasseltii is by now known only from Lampung (southern tip of the island), where L. waysepuntiense also occurs. However, L. waysepuntiense is almost certainly distributed in western part of Jambi, as well, because a specimen of Leptobrachium from Jambi has a mt DNA haplotype very close to this species (Matsui et al. unpublished data). Leptobrachium hendricksoni and L. nigrops have been known as lowland species that also inhabit coastal regions (Inger & Voris 2001), while L. hasseltii and L. waysepuntiense are found in higher elevations, although they seem to segregate their habitats. At Kubu Perahu, L. waysepuntiense was found at moderate elevations (691-852 m a.s.l.), whereas L. hasseltii was recorded at a lower elevation (300 m a.s.l.). Because L. hasseltii is found throughout a wider altitudinal range of 300-1500 m in west and central Java (our own observations), it may occur sympatrically with L. waysepuntiense in Lampung. However, this possibility seems unlilkely because two to three Leptobrachium from Borneo are known to segregate their ranges by inhabiting different altitudes (Inger et al. 1995; Malkmus et al. 2002), and we assume that L. waysepuntiense and L. hasseltii occur parapatrically.

The iris with white, light blue, or lime in the upper part and dark brown in the lower part in colour is found in species of *Leptobrachium* from Southern China and Indochina, and is a character shared with *Vibrissaphora* (e.g., Dubois & Ohler 1998; Fei *et al.* 2009; Ho *et al.* 1999; Lathrop *et al.* 1998; Matsui *et al.* 1999; Ohler *et al.* 2004; Rao *et al.* 2006; our own observations). As far as we are aware, there are no species of *Leptobrachium* with both parts of iris blue-coloured except for *L. waysepuntiense*. Furthermore, this is the only species with blue-coloured iris among *Leptobrachium* from Sundaland (e.g., Inger *et al.* 1995; Malkmus *et al.* 2002; our own observations).

In *L. waysepuntiense*, however, iris colour differs between adults (light blue) and juveniles (light grey). Intraspecific variation in iris colour has been reported for some species. Matsui *et al.* (1999) reported that the colour of the upper iris varies from light yellow to reddish in *L. smithi*, while a completely orange-coloured iris is found in *L. hendricksoni* whose iris is usually orange only in the upper part, in both adults and juveniles (our own observations of the species from Peninsular Malaysia). Ontogenetic change as found in *L. waysepuntiense* has been suggested only in *L. chapaense* from Vietnam (Bain & Nguyen 2004). According to these authors, upper part of iris is white in adults, but is off-white to yellow on in juveniles. Although available information is meagre, iris colour seems to vary ontogenetically. Notwithstanding these intraspecific variations, the eye colour seems an important character to diagnose species of *Leptobrachium* that are morphologically very similar.

Matsui *et al.* (1999) surmised that the blue-coloured iris might represent a primitive state in *Leptobrachium sensu stricto*. However, it seems difficult to estimate the phylogenetic relationship of *L. waysepuntiense* with the other species based on iris colour alone. On the other hand, *L. waysepuntiense* shares absence of markings at groin with all Bornean endemics, and other distinct characteristics with some of them; very small femoral gland with *L. gunungense* (Table 4), and an orange line along the supratympanic fold in juvenile with *L. montanum* and *L. abbotti*. From this limited information, we assume that *L. waysepuntiense* is closer to some Bornean species than to other Sumatran species (i.e. *L. hasseltii, L. nigrops*, and *L. hendricksoni*). This hypothesis, together with the evolution of iris colour, will be tested by molecular phylogenetic analysis, which is now underway.

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Appendix

Specimens of *Leptobrachium* examined for comparisons. The samples are deposited at the herpetological collection of the Museum Zoologicum Bogoriense (MZB Amp); Graduate School of Human and Environmental Studies, Kyoto University (KUHE); Department of Wildlife and National Parks Peninsular Malaysia (DWNP); and Sabah Park Collection (SP):

Leptobrachium abbotti (N = 2): KUHE 39294, 39296 from Mt. Kinabalu, Sabah.

- *Leptobrachium gunungense* (N = 8): KUHE 39377; SP 26745–26747, 26751, 26754, 26755, 26760 from Mt. Kinabalu, Sabah.
- *Leptobrachium hasseltii* (N = 10): MZB Amp 14590, KUHE 42807–42810 from Lampung, Sumatra; KUHE 42818–42822 from Central Java.
- *Leptobrachium hendricksoni* (N = 10): DWNP A 0021, KUHE 52585–52588 from Selangor; DWNP 01233 from Perak; KUHE 52298, 52384 from Trengganu; KUHE 52405, 52406 from Kelantan.
- *Leptobrachium nigrops* (N = 16): DWNP 00126, 00151, 00172 from Pahang; KUHE 42254, 42240–42244, 42475 from Selangor; KUHE 42574, 42575, 52587–52590 from Kanowit, Sarawak.
- *Leptobrachium montanum* (N = 12): KUHE 39204 from Mt. Kinabalu, Sabah; SP B22006–22008: Tawau, Sabah; KUHE 17306 from Matang, Sarawak; KUHE 42811, 42812 from Paramasan, South Kalimantan; KUHE 42814–42818 from Belantikan, Central Kalimantan.