

## STUDY ON MORPHOLOGICAL CHARACTERS OF INDONESIAN LITTER FROG (*LEPTOBRACHIUM*, MEGOPHRYIDAE)

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### ABSTRACT

*Leptobrachium* from Southeast Asian populations previously were recognized as a single species *Leptobrachium hasseltii* Tschudi. Taxonomic reassessment revealed that *Leptobrachium* consists of some cryptic species due to similarities in their morphological appearance but diverse in molecular characters. Among the Indonesian populations, *Leptobrachium* was split into seven species (*L. hasseltii*, *L. nigrops*, *L. waysepuntiense*, *L. hendricksoni*, *L. ingeri*, *L. abbotti*, and *L. montanum*). Specifically, *L. hasseltii* is separated into two groups; the Sumatran-Javan and the Bali populations. In this study, we analyzed the morphological data of 100 individuals of *Leptobrachium* to evaluate their morphological differences. The morphological differences expressed by analysis of variance (ANOVA) and principal component analysis (PCA) revealed significant differences between each *Leptobrachium* and four morphological characters suitable for species identification (the iris color, color pattern, humeral and femoral gland). The morphological differences also showed that the Bali population probably is undescribed species.

**Key words:** amphibia, ANOVA, litter frog, morphometry, PCA

### INTRODUCTION

*Leptobrachium* Tschudi, 1838 (type species *L. hasseltii*) is diagnosed by its broad head, slender limbs, presence of the humeral and femoral gland, and the absence of the nuptial pads (Dubois & Ohler, 1998; Hamidy & Matsui, 2012). This genus ranges from Indochina to Sundaland and consists of about 15 species (Fei et al., 2009; Frost, 2022). Sundaland includes the Malay Peninsula and the Indonesian islands of Sumatra, Java, Bali, Borneo, and smaller islands west of the Makassar and Lombok straits. All these regions are connected by shallow waters (<200 m) of the Sunda shelf exposed during the Pleistocene sea-level decline period. Sundaland's eastern boundary is the Wallace line, where the fauna of Indomalaya and Australasia meet. Studies of several lineages of Sundaland anurans have shown that species distribution and phylogeny are strongly associated with the geological history of the region (Emerson et al., 2000; Inger & Voris, 2001; Brown and Guttman, 2002; Matsui et al., 2010).

*Leptobrachium* taxonomic challenges are extended from the supraspecific category to the species level. *Leptobrachium hasseltii* was assigned to numerous Southeast Asian *Leptobrachium* populations (Inger, 1954, 1966; Berry, 1975) until Inger et al. (1995) clarified that the Bornean

populations were not conspecific with the Javanese populations, and then, a recent molecular study of the Philippine population divided *L. hasseltii* into three distinct species (*L. mangnyanorum*, *L. tagbanorum*, and *L. lumadorum*) (Brown et al., 2009). In the investigation of the Javan population, Hamidy and Matsui (2017) categorized *L. hasseltii* into two populations: 1) the Javan and Sumatran group and 2) the Bali group, based on various discoveries of *L. hasseltii* on the island of Bali. The presence of *L. hasseltii* in Bali was initially reported by Iskandar (1998), followed by subsequent findings at Mount Batu Karu by MacKay (2006) and two tadpoles in the same location (Hamidy & Matsui, 2017).

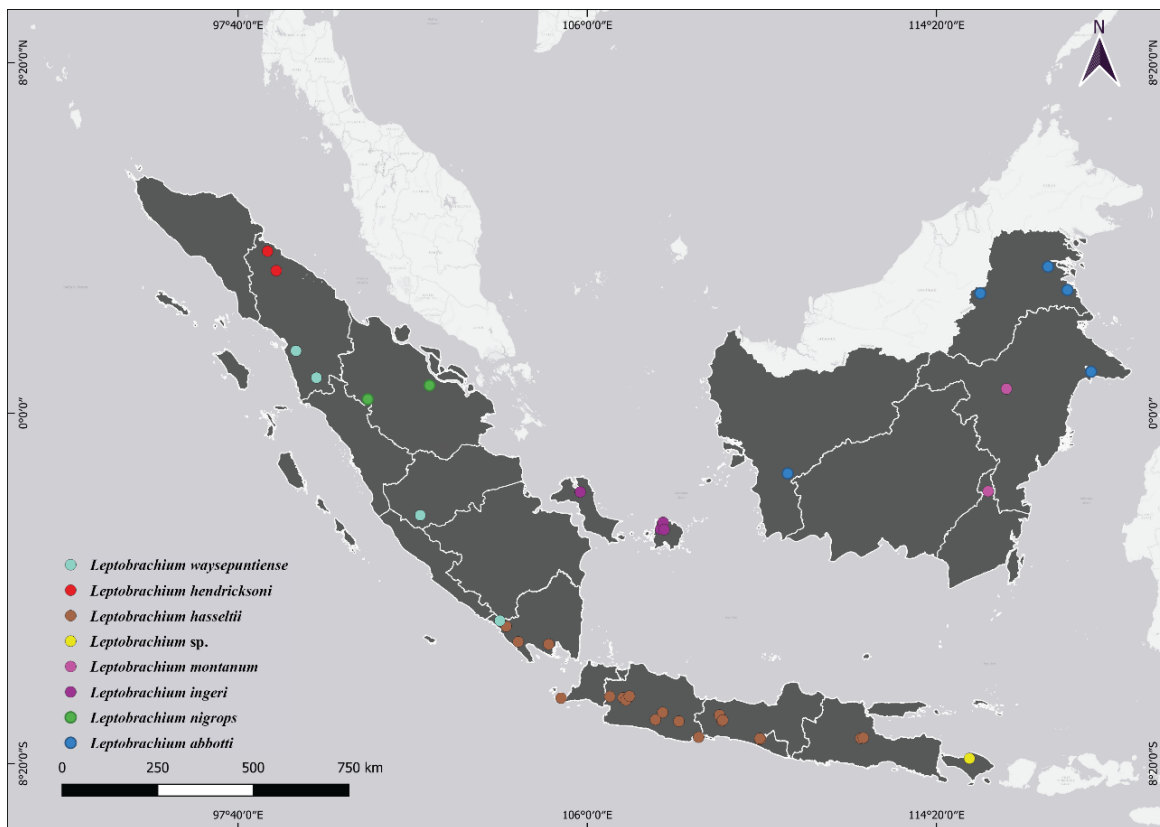
Indonesia currently has seven out of the 35 species, namely *L. hasseltii*, *L. nigrops*, *L. waysepuntiense*, *L. hendricksoni*, *L. ingeri*, *L. abbotti*, and *L. montanum* (Hamidy & Matsui, 2017; Frost, 2022). The high diversity of *Leptobrachium* in Indonesia illustrates how well-developed taxonomy is. However, it also presents challenges in distinguishing each *Leptobrachium* species morphologically since there is an issue with cryptic problems among this genus (Hamidy et al., 2012). Thus, this study aims to evaluate the morphological differences among the Indonesian *Leptobrachium* species, provide strong characteristics for identification, and solve the cryptic problem.

## MATERIALS AND METHODS

We examined a total of 100 specimens of *Leptobrachium* (consisting of seven specimens of *L. abbotti*, 67 specimens of *L. hasseltii*, five specimens of *L. hendricksoni*, six specimens of *L. ingeri*, two specimens of *L. nigrops*, a single specimen of *L. montanum*, three specimens of *Leptobrachium* sp. Bali, and nine specimens of *L. waysepuntiense*) collection of Museum Zoologicum Bogoriense (MZB), National Research and Innovation Agency (BRIN), from Indonesia (Sumatra, Jawa, Bali, and Kalimantan). The distribution of the sample used in this study is presented in Fig. 1. Morphometric measurements were conducted using a digital caliper to the nearest 0.1 mm. Measurements were taken following Matsui (1984) for 21 characters: 1) in Snout-Vent Length (SVL), distance from the tip of the snout to posterior end of the vent; 2) Head length (HL), distance of the tip of the snout to the posterior jaw angle; 3) Snout-Nostril Length (SNL), distance of the tip of the snout to the anterior border of the nostril; 4) Nostril-Eye Distance (NEL), distance between posterior edge of the nostril to anterior corner of the eye; 5) Eye Length (EL), distance from the anterior to posterior corner of the eye; 6) Snout Length (SL), distance from tip of the snout to the anterior corner of the eye; 7) Tympanum-Eye Length (TEL), distance between posterior corner of the eye to posterior border of the tympanum; 8) Tympanum Diameter (TD), distance from anterior to posterior border of the tympanum; 9) Head Width (HW), distance between posterior corners of the eyes; 10) Internarial Distance (IND), distance between the nostril; 11) Interorbital Distance (IOD), distance between the orbit across top of the head; 12) Upper Eyelid Width (UEW), distance between upper to lower edge of the eyelid; 13) Forelimb Length (FLL), distance from arm insertion to the tip of finger IV of the flexed arm; 14) Lower Arm and Hand Length (LAL), distance from the elbow to the tip of the finger IV; 15) Outer Palmar Tubercle Length (OPTL), maximum length of the outer palmar tubercle; 16) Inner Palmar Tubercle Length (IPTL), maximum length of the inner palmar tubercle; 17) Hand Length (HAL), distance from the outer palmar tubercle to the tip of the finger IV; 18)

Hindlimb Length (HLL), distance of the vent to the tip of toe IV of the flexed toe; 19) Tibia Length (TL), distance of the heel to kneel; 20) Foot length (FL), distance of the proximal edge of inner metatarsal tubercle to the tip of the finger IV; 21) Inner Metatarsal Tubercle Length (IMTL), maximum length of the inner metatarsal tubercle.

The morphometric analysis used relative values (R) of each character to SVL (in %) and was done only for male *Leptobrachium* because the lack of female specimens for some *Leptobrachium* species. All ratio data were analyzed using one-way analysis of variance (ANOVA) among species. When ANOVA was significant, Tukey HSD posthoc tests were used to perform multiple pairwise comparisons among the species. Clusters in each *Leptobrachium* group were performed using Principal Component Analysis (PCA). All statistical analysis were performed in the R Core Team (2020). We also checked the differences between four morphological characters (iris color, color pattern, humeral and femoral glands) of each *Leptobrachium* species as the qualitative characters, and analyzed in descriptive as shown in Table 4.



**Figure 1.** Localities and species composition of Litter Frog (*Leptobrachium*) use in this study.(Map is created using QGIS version 3.24 TISLER).

## RESULTS

The distribution of morphological characters is summarized in Table 3. Indonesian *Leptobrachium* generally has black iris coloration (*L. hasseltii*, *L. nigrops*, *L. abbotti*, *L. ingeri*, and *L. montanum*). Notably, *L. hendricksoni* exhibits a unique iris coloration with black iris

with orange on the upper portions, while *L. waysepuntiense* showed a light blue iris with black reticulation. *Leptobrachium* sp. Bali, on the other hand, is characterized by brown iris coloration. *Leptobrachium* Indonesia typically exhibits dorsal markings ranging from without pattern to a distinct dorsal marking and varying ventral patterns, including no distinct markings (*L. hasseltii*, *Leptobrachium* sp. Bali, and *L. waysepuntuiense*), spotted (*L. hendricksoni*), large blotches (*L. abbotti* and *L. montanum*), and irregular markings (*L. ingeri* and *L. nigrops*). The femoral and humeral glands, as illustrated in Figs 6 & 7, vary from very large (*L. hasseltii* and *Leptobrachium* sp. Bali), large (*L. abbotti*, *L. hendricksoni*, and *L. nigrops*), small (*L. ingeri*), to very small (*L. montanum* and *L. waysepuntiense*).

The statistical morphometric characteristics of each species of *Leptobrachium* are summarized in Table 1. The ANOVA revealed significant differences between each *Leptobrachium* species (Table 2). *L. abbotti* and *L. hasseltii* can be easily distinguished from *L. ingeri*, with nine and 13 significantly different morphometric characters, respectively. In contrast, they have the least morphometric differences from the *L. montanum*, with only two and zero significantly different characters. *L. hendricksoni* and *L. waysepuntiense* had the most significantly different morphometric characters from *L. abbotti*, with six and nine characters. *L. hendricksoni* has at least morphometric differences from *L. montanum* with only two significantly different characters, while *L. waysepuntiense* has at least three significantly different morphometric characters from *L. hendricksoni*. *L. nigrops* has eight morphometric characters, which are significantly different from *L. hasseltii* and *L. waysepuntiense*, while it has at least two character morphometric differences from *L. montanum*, which are significantly different. Both *L. abbotti* and *L. hasseltii* can be distinguished from other *Leptobrachium* by IOD character (except *L. nigrops* and *L. waysepuntiense* in *L. abbotti*, and *L. hendricksoni* and *L. montanum* in *L. hasseltii*). *L. hendricksoni* can be easily distinguished morphometrically from each *Leptobrachium* by SNL character, while *L. ingeri* can be easily distinguished morphometrically by HW character.

Interestingly, *L. montanum* has very few significantly different characters to other *Leptobrachium*, except for *L. waysepuntiense* with three significantly different characters. Lastly, *Leptobrachium* sp from Bali, which is the closest relative to *L. hasseltii* has significant morphometric differences in six characters. The significantly different morphometric characters of each *Leptobrachium* were SNL and HW, and these two characters have the most differences.

Three principal components were retained for PCA analysis (Table 3). The first PC had an eigenvalue of 39.08, which accounted for 58.1% of the variance. The other two components (PC2 of 9.55, and PC3 of 5.77) had significantly lower eigenvalues. Overall, the variance by all three principal components was 80.9%. PCA showed clear clusters from each *Leptobrachium*, with a slight intersection between clusters *L. hasseltii* and *Leptobrachium* sp. from Bali (Fig. 3A). PC1 and PC2, with 72.3% of the variance, showed nine distinct clusters separating each *Leptobrachium*. Meanwhile, PC1 and PC3 with only 66.7% of the variance, showed ten clear clusters. Interestingly, *L. hasseltii* formed two very distinct clusters from one another (Fig. 3B), two small clusters separated from one big spherical centroid cluster. The two little clusters were formed from East Java and Sumatra *L. hasseltii* populations.

**Table 1.** Morphometric characters of *Leptobranchium abboti*, *L. hasseltii*, *L. hendricksonii*, *L. ingeri*, *L. montanum*, *L. nigrops*, *Leptobranchium* sp. Bali and *L. waysepuntitense*. SVL (Mean ± SD) and medians of other characters to SVL, followed by ranges. See text for character abbreviations

Character	<i>L. abboti</i> n=7	<i>L. hasseltii</i> n=67	<i>L. hendricksonii</i> n=5	<i>L. ingeri</i> n=6	<i>L. nigrops</i> n=2	<i>L. montanum</i> n=1	<i>Leptobranchium</i> sp. Bali n=3	<i>L. waysepuntitense</i> n=9
SVL	59.97±7.63	39.34±6.76	38.80±6.66	30.30±1.86	32.96±4.04	61.10	36.91±1.00	43.83±8.99
RHL	26.10 (23.30-28.20)	17.20 (10.90-23.00)	17.00 (13.20-20.80)	12.59 (11.70-13.80)	13.94 (13.94-14.88)	25.80	15.15 (15.02-15.15)	14.90 (14.90-24.90)
RSNL	2.80 (2.10-3.30)	1.60 (0.40-2.60)	0.50 (0.30-0.80)	1.21 (1.00-1.60)	1.44 (1.40-1.47)	3.10	1.46 (1.18-1.48)	1.10 (1.00-2.00)
RN-EL	6.20 (5.00-6.40)	3.28 (2.30-4.90)	4.10 (2.80-4.60)	2.58 (2.20-2.80)	3.06 (2.80-3.32)	5.80	3.28 (3.20-3.51)	4.40 (2.70-5.80)
RSL	9.30 (8.40-10.80)	5.60 (3.10-7.80)	5.50 (3.70-5.80)	3.93 (3.60-4.40)	5.26 (4.80-5.72)	9.40	5.24 (5.18-5.66)	6.10 (4.70-8.20)
REL	7.00 (6.00-7.70)	4.50 (2.90-7.00)	5.60 (3.80-6.80)	4.27 (3.20-4.50)	4.26 (3.80-4.72)	6.10	4.92 (4.92-5.15)	6.10 (3.80-6.70)
RTEL	3.20 (3.00-4.80)	2.00 (1.20-3.10)	1.60 (1.40-2.50)	1.17 (1.14-1.30)	1.75 (1.70-1.80)	3.10	1.64 (1.58-1.72)	2.50 (1.60-2.80)
RTD	4.10 (3.60-4.90)	2.30 (1.30-3.70)	3.00 (2.00-3.60)	2.11 (2.00-2.22)	2.65 (2.30-3.00)	4.20	2.22 (2.15-2.43)	3.20 (2.70-4.00)
RHW	26.80 (22.10-28.30)	16.50 (10.60-23.40)	17.50 (12.90-19.90)	11.11 (10.60-12.10)	13.28 (11.90-24.80)	24.60	15.04 (14.60-15.08)	21.50 (13.90-24.80)
RIND	4.30 (3.80-4.60)	3.10 (2.10-4.30)	3.10 (2.20-3.20)	2.41 (2.20-2.70)	4.00 (1.90-2.72)	4.40	3.28 (3.02-3.32)	4.00 (3.60-4.60)
RIOD	10.50 (8.00-11.50)	5.20 (3.80-7.80)	5.50 (4.10-7.10)	3.80 (3.46-4.20)	5.00 (4.10-5.90)	8.20	4.75 (4.46-5.14)	8.60 (5.00-9.80)
RUEW	7.00 (6.00-7.80)	4.70 (2.80-6.80)	5.20 (3.80-6.10)	3.49 (3.40-4.00)	4.06 (3.60-4.52)	6.90	3.85 (3.84-5.02)	4.90 (4.20-7.00)
RFL	44.60 (36.30-46.50)	27.80 (20.00-35.80)	27.90 (23.50-31.30)	22.37 (19.80-24.28)	25.02 (22.70-27.34)	43.00	27.32 (25.88-27.68)	34.50 (22.80-35.00)
RLAL	34.10 (27.80-35.70)	21.30 (15.00-28.00)	21.50 (16.50-25.60)	15.73 (14.60-17.40)	18.35 (16.70-20.00)	32.00	20.50 (20.30-20.50)	24.30 (13.40-27.10)
ROPTL	2.90 (2.60-3.00)	2.0 (1.20-2.90)	2.0 (1.30-2.10)	1.43 (1.36-1.50)	1.33 (1.26-1.40)	2.70	2.07 (1.52-2.08)	2.3 (1.80-2.60)
RIPTL	2.80 (2.70-3.10)	2.0 (1.10-2.90)	1.80 (1.50-2.000)	1.35 (1.20-1.64)	1.40 (1.20-1.60)	2.20	1.73 (1.48-1.76)	2.10 (1.50-2.80)
RHAL	16.50 (14.20-17.80)	10.60 (6.80-13.80)	9.80 (8.00-11.00)	6.77 96.40-7.56)	8.25 (8.00-13.80)	15.20	10.18 (10.10-10.18)	13.00 (8.00-13.80)
RHLL	73.20 (60.60-80.00)	46.50 (30.50-61.60)	45.20 (34.50-48.00)	36.68 (35.20-40.30)	43.14 (35.80-43.14)	73.00	46.20 (44.16-46.20)	52.00 (38.00-58.80)
RTL	24.20 (19.20-25.80)	14.30 (10.50-19.70)	15.50 (11.00-17.90)	11.84 (11.22-12.70)	13.06 (12.00-14.12)	23.00	13.62 (13.38-13.66)	18.50 (13.10-20.10)
RFL	23.00 (18.00-24.10)	15.00 (10.00-19.00)	14.10 (10.20-15.50)	10.42 (10.10-11.50)	11.14 (10.30-11.98)	21.10	14.22 (13.71-14.78)	18.20 (13.20-19.00)
RIMTL	2.10 (1.70-2.50)	1.40 (1.00-2.70)	1.30 (1.10-1.50)	1.33 (0.90-1.37)	1.28 (1.20-1.36)	1.90	1.09 (1.06-1.37)	2.00 (1.20-2.30)

**Table 2.** Analysis of variance (ANOVA) and Tukey honestly significant different (HSD) test on 18 morphometric characters of male individuals. Asterisk (\*) denote significantly different morphometric characters between each *Leptobrachium*, on the basis  $\alpha=0.01$ . See text for character abbreviations

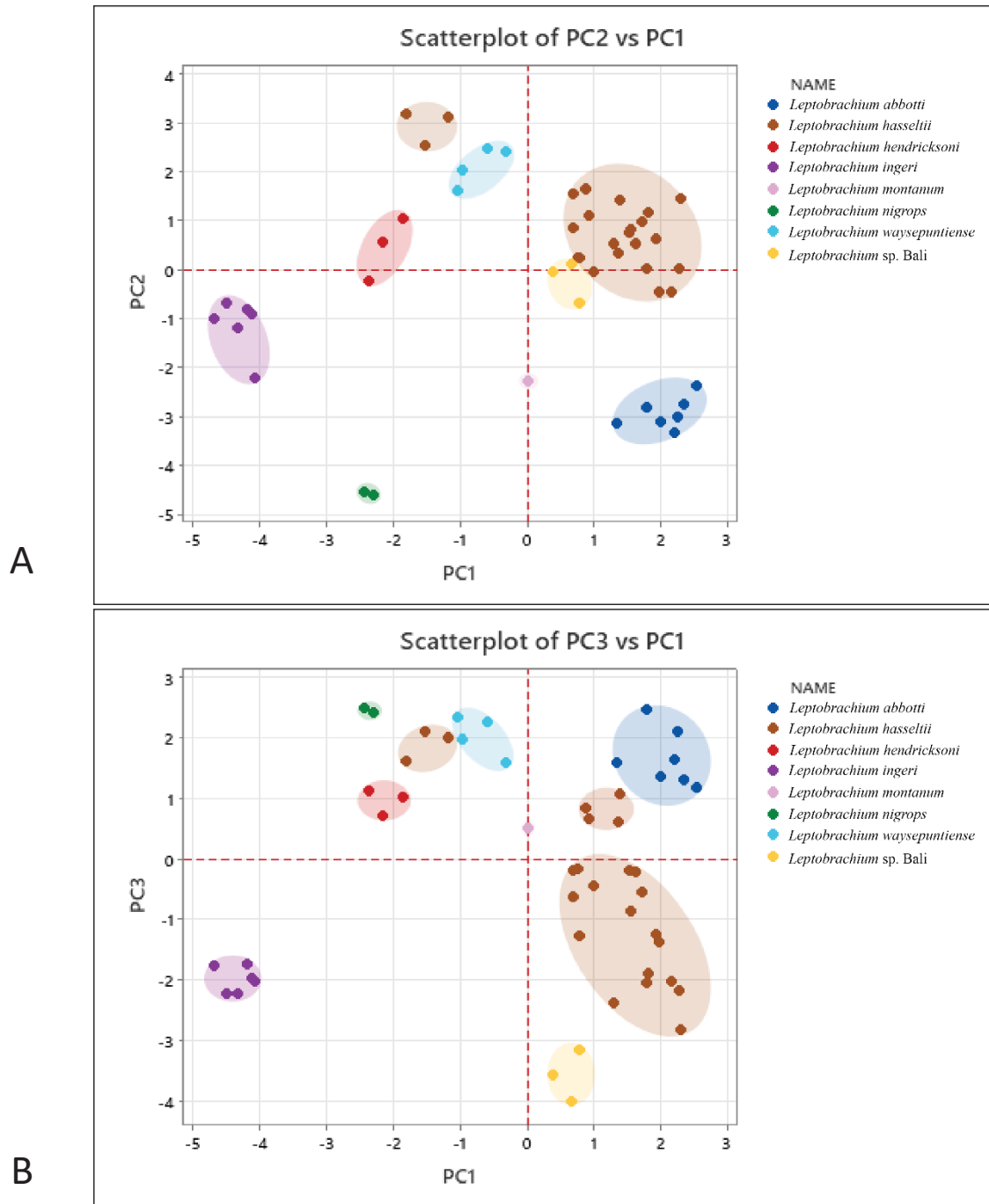
	RHL	RSNL	RNEL	RSL	REL	RTEL	RTD	RHW	RIOD	RIND	RUEW	RFL	RLAL	ROPTL	RHAL	RHLL	RTL	RIMTL
ANOVA	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Tukey HSD																		
<i>L. hasseltii</i> - <i>L. abboti</i>	0.00*	0.00*	0.00*	0.00*	0.99	0.00*	0.00*	0.01*	0.00*	0.00*	0.95	0.06	0.88	0.29	0.99	0.01*	0.00*	0.99
<i>L. hendricksoni</i> - <i>L. abboti</i>	0.90	0.00*	0.36	0.00*	0.00*	0.00*	0.99	0.95	0.00*	0.02	0.01*	0.16	0.99	0.88	0.21	0.06	0.96	0.50
<i>L. ingeri</i> - <i>L. abboti</i>	0.98	0.00*	0.00*	0.00*	0.00*	0.00*	0.98	0.00*	0.00*	0.00*	0.99	0.98	0.02	0.99	0.00*	0.84	0.95	0.02
<i>L. montanum</i> - <i>L. abboti</i>	1.00	0.87	0.93	0.99	0.42	0.51	0.98	0.00*	0.00*	0.99	0.99	0.93	0.73	0.99	0.97	0.99	0.89	0.94
<i>L. nigrops</i> - <i>L. abboti</i>	0.96	0.85	0.40	0.99	0.37	0.13	0.01*	0.00*	0.99	0.82	0.56	0.81	0.99	0.00*	0.00*	0.99	0.98	0.89
<i>L. waysepuntiense</i> - <i>L. abboti</i>	0.00*	0.00*	0.21	0.00*	0.05	0.00*	0.24	0.90	0.25	0.00*	0.61	0.00*	0.01*	0.99	0.99	0.00*	0.77	0.00*
<i>Leptobrachium</i> sp Bali - <i>L. abboti</i>	0.98	0.25	0.00*	0.15	0.02	0.00*	0.00*	0.00*	0.00*	0.00*	0.32	0.99	0.99	0.00*	0.64	0.97	0.10	0.14
<i>L. hendricksoni</i> - <i>L. hasseltii</i>	0.89	0.00*	0.91	0.20	0.00*	0.43	0.00*	0.84	0.99	1.00	0.00*	0.99	0.99	0.99	0.02	0.97	0.06	0.12
<i>L. ingeri</i> - <i>L. hasseltii</i>	0.00*	0.99	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.99	0.79	0.00*	0.08	0.78	0.00*	0.00*	0.00*	0.01*
<i>L. montanum</i> - <i>L. hasseltii</i>	0.69	0.02	0.96	0.80	0.43	0.99	0.85	0.22	0.76	0.65	1.00	1.00	0.94	0.86	0.87	0.71	0.93	0.78
<i>L. nigrops</i> - <i>L. hasseltii</i>	0.02	0.85	0.99	0.05	0.19	0.99	0.00*	0.02	0.00*	0.73	0.15	0.02	0.87	0.00*	0.00*	0.27	0.00*	0.97
<i>L. waysepuntiense</i> - <i>L. hasseltii</i>	0.88	0.00*	0.36	0.12	0.00*	0.32	0.19	0.38	0.00*	0.67	0.93	0.34	0.05	0.84	0.67	0.00*	0.00*	0.00*
<i>Leptobrachium</i> sp Bali - <i>L. hasseltii</i>	0.01*	0.99	0.92	0.99	0.00*	0.12	0.98	0.00*	0.01*	0.29	0.61	0.64	0.80	0.06	0.83	0.01*	0.97	0.01*
<i>L. ingeri</i> - <i>L. hendricksoni</i>	0.51	0.00*	0.01*	0.99	1.00	0.10	0.90	0.00*	0.03	1.00	0.04	0.03	0.28	0.98	0.60	0.00*	0.99	0.00*
<i>L. montanum</i> - <i>L. hendricksoni</i>	0.99	0.00*	0.99	0.15	0.00*	0.98	0.93	0.08	0.96	0.75	0.20	0.99	0.89	0.94	0.99	0.50	0.99	1.00
<i>L. nigrops</i> - <i>L. hendricksoni</i>	0.57	0.00*	0.99	0.00*	0.73	0.96	0.15	0.00*	0.00*	0.87	0.95	0.03	0.99	0.00*	0.84	0.19	0.77	0.16
<i>L. waysepuntiense</i> - <i>L. hendricksoni</i>	0.51	0.00*	0.99	0.99	0.32	0.99	0.24	1.00	0.00*	0.97	0.00*	0.99	0.25	0.99	0.53	0.70	0.99	0.00*
<i>Leptobrachium</i> sp Bali - <i>L. hendricksoni</i>	0.61	0.00*	0.60	0.48	0.96	0.99	0.00*	0.00*	0.36	0.68	0.00*	0.53	0.99	0.25	0.01*	0.02	0.77	0.99
<i>L. montanum</i> - <i>L. ingeri</i>	0.99	0.02	0.14	0.05	0.00*	0.11	0.99	0.03	0.95	0.66	0.99	0.73	0.99	0.99	0.59	0.99	0.99	0.08
<i>L. nigrops</i> - <i>L. ingeri</i>	0.99	0.79	0.07	0.00*	0.65	0.01*	0.00*	0.00*	0.00*	0.76	0.76	0.98	0.10	0.00*	1.00	0.99	0.74	0.95
<i>L. waysepuntiense</i> - <i>L. ingeri</i>	0.00*	0.00*	0.00*	0.93	0.14	0.00*	0.83	0.00*	0.00*	0.93	0.40	0.00*	1.00	0.99	0.00*	0.00*	0.99	0.99
<i>Leptobrachium</i> sp Bali - <i>L. ingeri</i>	1.00	0.97	0.73	0.12	0.95	0.32	0.02	0.00*	0.99	0.56	0.20	0.96	0.04	0.01*	0.00*	0.99	0.52	0.00*
<i>L. nigrops</i> - <i>L. montanum</i>	0.99	0.44	0.99	0.99	0.03	1.00	0.05	1.00	0.00*	0.99	0.76	0.49	0.64	0.38	0.72	1.00	0.68	0.58
<i>L. waysepuntiense</i> - <i>L. montanum</i>	0.40	0.00*	1.00	0.19	0.01*	0.99	0.99	0.04	0.00*	0.28	0.99	0.97	0.99	0.99	0.99	0.04	0.99	0.03
<i>Leptobrachium</i> sp - <i>L. montanum</i>	0.99	0.15	0.78	0.91	0.00*	0.91	0.69	1.00	0.99	0.12	0.97	0.98	0.61	0.10	0.52	0.99	0.99	0.99
<i>L. waysepuntiense</i> - <i>L. nigrops</i>	0.00*	0.00*	0.99	0.00*	1.00	0.99	0.00*	0.00*	0.35	0.26	0.06	0.00*	0.09	0.00*	0.03	0.00*	0.55	0.77
<i>Leptobrachium</i> sp Bali - <i>L. nigrops</i>	0.99	0.99	0.83	0.24	0.99	0.81	0.00*	1.00	0.00*	0.10	0.02	0.76	1.00	0.00*	0.00*	0.99	0.08	0.04
<i>Leptobrachium</i> sp Bali - <i>L. waysepuntiense</i>	0.00*	0.00*	0.24	0.57	0.96	0.96	0.28	0.00*	0.00*	0.97	0.98	0.08	0.03	0.01*	0.28	0.00*	0.70	0.00*

**Table 3.** Loading scores of morphological characters on the first three principal component analysis of Indonesian *Leptobrachium*

Character	Principal Component		
	PC1	PC2	PC3
RHL	-0,155	-0,106	-0,031
RSNL	0,072	-0,095	0,001
RNEL	-0,016	-0,090	-0,055
RSL	0,030	-0,155	-0,078
REL	0,039	0,206	-0,078
RTEL	-0,011	-0,144	-0,084
RTD	0,017	0,079	-0,156
RHW	-0,208	-0,483	-0,276
RIND	-0,034	0,043	0,057
RIOD	-0,098	-0,109	-0,441
RUEW	0,006	0,059	-0,011
RFL	0,357	0,108	-0,672
RLAL	0,130	-0,361	-0,089
ROPTL	0,007	-0,058	0,060
RIPTL	-0,003	-0,038	-0,004
RHAL	-0,020	-0,589	0,101
RHLL	0,872	-0,136	0,206
RTL	0,073	0,071	-0,392
RFL	0,047	-0,324	0,076
RIMTL	-0,019	0,064	-0,062
Eigenvalue	39.08	9.55	5.77
% Variance	58.1	14.2	8.6
Cumulative	58.1	72.3	80.9

**Table 4.** Comparison of diagnostic characters among Indonesian *Leptobrachium*

Species	Iris color	Color pattern	Humeral gland	Femoral gland
<i>L. hasseltii</i>	Black	Distinct dark markings dorsally, without a clear pattern ventrally	Very large	Very large
<i>Leptobrachium</i> sp. Bali	Brown	Distinct dark markings dorsally, without a clear pattern ventrally	Very large	Very large
<i>L. abbotti</i>	Black	No distinct dark markings, very large blotches ventrally	Large	Large
<i>L. hendricksoni</i>	Black with upper part orange	No distinct dark markings, black spot ventrally	Large	Very large
<i>L. ingeri</i>	Black	Distinct dark markings dorsally, irregular markings ventrally	Small	Small
<i>L. montanum</i>	Black	No distinct dark markings, large blotches ventrally	Very small	Very small
<i>L. nigrops</i>	Black	Distinct dark markings dorsally, irregular markings ventrally	Large	Large
<i>L. waysepuntiense</i>	Light blue with black reticulations	No distinct dark markings, without a clear pattern ventrally	Very small	Very small



**Figure 2.** Principal component analyses (PCA) based on morphometric characters of *Leptobrachium*: (A). PC1 vs PC2 with 72.3% of the variance, (B) PC1 vs PC3 with 66.7% of the variance.



## DISCUSSION

### *Morphological and morphometric traits*

*Leptobrachium* is one of the megophryd frogs, known as a cryptic species (Hamidy & Matsui, 2017). Although it is hard to distinguish cryptic species, our ANOVA and PCA analyses of *Leptobrachium* revealed substantial differences between *Leptobrachium* species. Morphological characteristics for identifying *Leptobrachium* species are their iris color, color pattern, and the presence and size of the humeral and femoral glands.

The color of the iris is believed to have evolved (Matsui et al., 2010), although no studies specifically discuss the color of the iris. The difference in the iris color on *Leptobrachium* is influenced by different color pigments on each *Leptobrachium*. Color pigments on amphibians are formed by cell type: melanophores containing black or brown, xanthophores and erythrophores containing yellow, orange or red, iridophore does not contain the original color, while gold and silver are formed by the physical effects (Winter, 1988). The iris color in almost every Sundaland *Leptobrachium* was black, besides *L. hendricksoni* which has an orange iris, and *L. waysepuntiense* with blue iris color. The color of the iris can also be seen, followed the color of the body of the frog (Glaw & Vences, 1997). Some *Leptobrachium* with black iris corresponds to the body color to dark grey. Also as in Ida's Bright-eyed Frogs, *Boophis idae* (Matellidae), has a dorsal body with a silvery and golden color followed by its golden brown iris. *Boophis microtympanum*, with a dorsal body that is predominantly green, also has a green iris (Glaw & Vences, 1997). Iris color of *Leptobrachium* can present a good diagnosis and be used to estimate the phylogenetic relationship since its variation among Sundaland species was separated into two clades (Duellman, 1975; Dubois & Ohler, 1998; Lathrop et al., 1998; Matsui et al., 1999; Matsui et al., 2010).

The color patterns of *Leptobrachium* are varied from patternless to distinct dorsal pattern, and also black spots, blotches, or no distinct ventral pattern. Body pattern is an excellent morphological character to distinguish anuran species. Dorsal and ventral patterns do not disappear even though the animal is dead or in preservation., So, the pattern is suitable for species identification for *Leptobrachium* and almost all anurans, if the pattern is consistent in one species among its populations. The typical dorsal color pattern on *Leptobrachium* is a patch that extends from the interorbital area to the parietal area (Fig. 4), several species including *L. hasseltii*, *L. abbotti*, *L. montanum*, *L. nigrops*, *L. ingeri*, and *Leptobrachium* sp. Bali has a similar dorsal pattern. *L. hasseltii* has many variations of dorsal patterns ranging from spots, blotched, or without clear patterns. Ventral patterns of *L. hasseltii*, *L. waysepuntiense* and *Leptobrachium* sp. Bali does not have a clear color pattern, *L. hendricksoni* with black spots all over the venter, while *L. abbotti* with large blotches, and *L. nigrops* and *L. ingeri* with irregular spots. *L. montanum* is known to have a ventral pattern similar to *L. abbotti* (Fig. 5). The large blotches on the ventral body are the main morphological characters that distinguish these two species from other *Leptobrachium*. Body pattern variation in each *Leptobrachium* can be influenced by the environmental conditions and adaptations as litter frogs, and forming a distinctive color pattern. Differences in color pattern are also influenced by geographic variation

among each *Leptobrachium*, as they may occupy different niches and allow ecological barriers for the variation of some species (Riyanto et al., 2010). Nesty (2013) reported that the uplands and lowlands of the Asian Common Toad, *Duttaphrynus melanostictus*, have differences in morphology. This study also shows that color pattern, along with other characters as a unique diagnostic character, is a good identification character in the genus *Leptobrachium*. The differences in femoral glands in *Leptobrachium* are found in various sizes, from just spots, large to very large (Hamidy & Matsui, 2010). The humeral and femoral glands is a definite morphological character in the type of *Leptobrachium*. The presence of the humeral and femoral glands is one of the identifications in *Leptobrachium*, although this characteristic is rarely used in the main identification of *Leptobrachium*. Differences in the size of the humeral and femoral glands in animals occur due to differences in the hormonal system in certain animals and are not influenced by different geographical locations. The difference in size occurs due to differences in volume and the amount of fluid that fills the gland, so that the size can differ for each *Leptobrachium*. Although they share the same anatomical, each species' hormonal, physiological, and behavioral conditions differ.

The color of the iris, color pattern, humeral and femoral glands are identification characteristic of *Leptobrachium*. However, several variations in the morphological characters themselves make it difficult to depend on only one of these morphological characters, so it is not wise to identify *Leptobrachium* based only on one character. The combination of all characters and the addition of morphometric analysis will provide a better identification.

Morphometric analyses showed that several characters could be the main characters to distinguish *Leptobrachium* species (Table 2). Morphometric characters are used as a reference in identifying *Leptobrachium*. Differences in the size of certain characters can indicate a very significantly different species. The morphometric results showed that *Leptobrachium* was significantly different from other species, indicated by a highly segregated cluster between *Leptobrachium* species. However, the PCA graph of *L. hasseltii* shows segregated populations, and it is possible for new species or subspecies of two or more populations from *L. hasseltii*. Very significant differences between populations are shown in the clusters, but this requires further clarification.

We also tested morphological and morphometric analyses of *Leptobrachium* sp. from Bali to prove a separate clade from *L. hasseltii* and *Leptobrachium* sp. Bali (Hamidy & Matsui, 2018). In our PCA results, the clusters of two populations were separated (Fig. 3), and our ANOVA results (Table 2) showed different characters between them (EL, HL, HLL, HW, IMTL, and IOD). We suggest that populations of *Leptobrachium* sp. from Bali be described as a new species of *Leptobrachium*.

### **Indonesian *Leptobrachium sensu stricto***

*Leptobrachium* is generally characterized by the size of the head and eyes, the absence of spines above the lip, and the presence of femoral and axillary glands (Hamidy, 2010). *Leptobrachium* among Indonesian populations has several differences in each morphological character, consisting of the combination of the iris color; parietal, lateral, and dorsoventral body pattern and color; groin, limbs, toes pattern and color; femoral and axillar gland.

*Leptobrachium hasseltii* Tschudi, 1838

Diagnosis : *L. hasseltii* iris color is totally black without reticulations, and surrounded by light blue sclera when the eye is opened maximally, although at first reported *L. hasseltii* has a scarlet-colored iris (Iskandar, 1998), but in this research, we could not found *L. hasseltii* species which has scarlet colour; black bars around lips; supratympanic ridge often bordered by a very thin brownish orange line; the dorsal ground coloration varied from dark brownish grey to grey color, laterally fading to light grey; ventral body light gray with darker coloration towards the groin; dorsal body covered varied from a large dark marking to only black, often with brownish orange spot and line on the interorbital to parietal regions; the lateral and ventral body are covered with white granules, denser on throat and lateral body; toes are light gray or brownish dorsolaterally, usually covered by black bars dorsally, often with 3-4 striped; posterior thigh spotted with white; with dark markings around groin; the femoral gland very large, covered by dark marking (Figs 4A & 5A).

Small to medium-sized *Leptobrachium*, Body tapering to the groin (SVL  $39.34 \pm 6.76$ ), head broad and depressed, slightly longer (HL 17.20 (10.90-23.00)% of SVL) than wide (HW 16.50 (10.60-23.40)% SVL); eye large, and smaller (EL 4.50 (2.90-7.00)% SVL) than snout (SL 5.60 (3.10-7.80)% SVL); nostrils distinctly closer to tip of snout (SNL 1.60 (0.40-2.60)% SVL) than to eye (NEL 3.28 (2.30-4.90)% SVL); internarial distance (IND 3.10 (2.10-4.30)% SVL) much shorter than interorbital distance (IOD 5.20 (3.80-7.80)% SVL), latter wider than upper eyelid (UEW 4.70 (2.80-6.80)% SVL); tympanum diameter (TD 2.30 (1.30-3.70)% SVL) about three-fifth of eye and separated from eye by half of its diameter (TEL 2.00 (1.20-3.10)% SVL).

Forelimb slender (FLL 27.80 (20.00-35.80)% SVL); inner palmar tubercle large (IPTL 2.0 (1.10-2.90)% SVL), and subequal outer palmar tubercle (OPTL 2.0 (1.20-2.90)% SVL). Hindlimb slender and relatively short (HLL 46.50 (30.50-61.60)% SVL); foot slightly longer (FL 15.00 (10.00-19.00)% SVL) than tibia (TL 14.30 (10.50-19.70)% SVL); inner metatarsal tubercle length (IMTL 1.40 (1.00-2.70)% SVL).

*Leptobrachium* sp. Bali

Diagnosis: *Leptobrachium* sp. Bali iris color is brown, with white spot-ring in juveniles, with white sclera; dorsal coloration dark brown with distinct markings, sometimes with orange line and dots, and very rough granules; ventral body darker on the throat and more greyish on the belly with white granules from throat to groin, granules denser at chest; dark grey laterally with white granules and black spot from armpit to groin; top of head from interorbital to parietal region, and dorsal side of arm faintly orange; toes are brownish dorsolaterally, covered by faint irregular dark bars dorsally, often with 2-3 stripes; groin covered with dark markings, usually elongated along the body; femoral glands very large, surrounded by dark markings (Figs 4B & 5B).

Small to medium-sized *Leptobrachium*, Body tapering to the groin (SVL  $36.91 \pm 1.00$ ), head broad and depressed, slightly longer (HL 15.15 (15.02-15.15)% of SVL) than wide (HW 15.04 (14.60-15.08)% SVL); eye large, smaller (EL 4.92 (4.92-5.15)% SVL) than snout (SL 5.24 (5.18-5.66)% SVL); nostrils distinctly closer to tip of snout (SNL 1.46 (1.18-1.48)% SVL) than to eye (NEL 3.28 (3.20-3.51)% SVL); internarial distance (IND 3.28 (3.02-3.32)% SVL)

SVL) shorter than interorbital distance (IOD 4.75 (4.46-5.14)% SVL), latter wider than upper eyelid (UEW 3.85 (3.84-5.02)% SVL); tympanum diameter (TD 2.22 (2.15-2.43)% SVL) about half of eye and separated from eye by half of its diameter (TEL 4.92 (4.92-5.15)% SVL). Forelimb slender and long (FLL 27.32 (25.88-27.68)% SVL); inner palmar tubercle large (IPTL 1.73 (1.48-1.76)% SVL), smaller than outer palmar tubercle (OPTL 2.07 (1.52-2.08)% SVL). Hindlimb slender and relatively short (HLL 46.20 (44.16-46.20)% SVL); foot slightly longer (FL 14.22 (13.71-14.78)% SVL) than tibia (TL 13.62 (13.38-13.66)% SVL); inner metatarsal tubercle length (IMTL 1.09 (1.06-1.37)% SVL).

*Leptobrachium hendricksoni* Taylor, 1962

Diagnosis: *L. hendricksoni* iris color is black with orange on the upper half, varied to being orange in juvenile (Matsui et al., 2010), and surrounded by light blue sclera when the eye is opened maximally; dark markings around lips without bars; supratympanic ridge covered with black; the dorsal coloration grey fading laterally to light grey to white on ventral body, sometimes without dorsal markings, or dark gray marking in the interorbital to parietal areas, or covered with a black spot around the dorsal body; laterally white to light gray with black spots from forelegs to groin; white on the belly and throat with lots of black spots on the chin to the groin, black spots concentrating and denser on the belly around the flanks; no markings on groin; forelimbs are dark brown faintly covered by black bars dorsally; hind limbs often covered with black bars or spots; the femoral gland large, covered by dark marking (Figs 4C & 5C).

Small to medium sized *Leptobrachium*, Body tapering to the groin (SVL 38.80±6.66), head broad and depressed, slightly longer (HL 17.00 (13.20-20.80)% of SVL) than wide (HW 17.50 (12.90-19.90)% SVL); eye large, slightly larger (EL 5.60 (3.80-6.80)% SVL) than snout (SL 5.50 (3.70-5.80)% SVL); nostrils distinctly closer to tip of snout (SNL 0.50 (0.30-0.80)% SVL) than to eye (NEL 4.10 (2.80-4.60)% SVL); internarial distance (IND 3.10 (2.20-3.20)% SVL) much shorter than interorbital distance (IOD 5.50 (4.10-7.10)% SVL), latter wider than upper eyelid (UEW 5.20 (3.80-6.10)% SVL); tympanum diameter (TD 3.00 (2.00-3.60)% SVL) about half of eye and separated from eye by half of its diameter (TEL 1.60 (1.40-2.50)% SVL).

Forelimb slender and long (FLL 27.90 (23.50-31.30)% SVL); inner palmar tubercle large (IPTL 1.80 (1.50-2.00)% SVL), and larger outer palmar tubercle (OPTL 2.0 (1.30-2.10)% SVL). Hindlimb slender and relatively short (HLL 45.20 (34.50-48.00)% SVL); foot slightly shorter (FL 14.10 (10.20-15.50)% SVL) than tibia (TL 15.50 (11.00-17.90)% SVL); inner metatarsal tubercle length (IMTL 1.30 (1.10-1.50)% SVL).

*Leptobrachium nigrops* Berry & Hendrickson, 1963

Diagnosis: *L. nigrops* iris color is completely black, surrounded by a bright blue sclera visible when the eye is fully open; the supratympanic ridge is covered with a thin dark band; the face is covered by dark markings, with light bars behind eyes; the dorsal coloration brown with well-defined dark brown markings interorbitally extending to the cloaca; pointed fingertips; lateral body with black spots extending from belly to groin; groin with irregular blotches; forelimbs with black bars dorsally, and ventral side covered by irregular blotches; hind limbs

are covered by dark brown thin bars dorsally, including the fingers and toes; the webs of the feet are well formed; femoral gland large, covered by dark markings (Figs 4G & 5G).

Small-sized *Leptobrachium*, Body tapering to the groin (SVL 32.96±4.04), head broad and depressed, slightly longer (HL 13.94 (13.94-14.88)% of SVL) than wide (HW 13.28 (11.90-24.80)% SVL); eye large, smaller (EL 4.26 (3.80-4.72)% SVL) than snout (SL 5.26 (4.80-5.72)% SVL); nostrils distinctly closer to tip of snout (SNL 1.44 (1.40-1.47)% SVL) than to eye (NEL 3.06 (2.80-3.32)% SVL); internarial distance (IND 4.00 (1.90-2.72)% SVL) shorter than interorbital distance (IOD 5.00 (4.10-5.90)% SVL), latter wider than upper eyelid (UEW 4.06 (3.60-4.52)% SVL); tympanum diameter (TD 2.65 (2.30-3.00)% SVL) about half of eye and separated from eye by half of its diameter (TEL 1.75 (1.70-1.80)% SVL).

Forelimb slender and long (FLL 25.02 (22.70-27.34)% SVL); inner palmar tubercle large (IPTL 1.40 (1.20-1.60)% SVL), larger than outer palmar tubercle (OPTL 1.33 (1.26-1.40)% SVL). Hindlimb slender and relatively short (HLL 43.14 (35.80-43.14)% SVL); foot shorter (FL 11.14 (10.30-11.98)% SVL) than tibia (TL 13.06 (12.00-14.12)% SVL); inner metatarsal tubercle length (IMTL 1.28 (1.20-1.36)% SVL).

#### *Leptobrachium montanum* Fischer, 1885

Diagnosis: *L. montanum* iris color is completely black without reticulation; supratympanic ridge covered with a thin black line; dark brown markings behind eyes from interorbital area to the parietal area; the dorsal coloration dark brown; lateral body white covered by dark brown blotches, and white granules from the arm insertion to the groin; ventral body white, covered by irregular blotches on the belly, or with very large dark blotches on the almost entire belly to the cloaca, ventral with white granules, black spots on the throat; forelimbs with thin black or dark brown bars up to fingertips dorsally, on the ventral side faintly covered by brown bands extending from palm to elbows; hind limbs are covered by dark brown thin bars dorsally, while the ventral side covered by irregular blotches; groin and hips are covered by dark marking; femoral gland large, covered by dark markings (Figs 4D & 5D).

Large-sized *Leptobrachium*, Body tapering to the groin (SVL 61.10), head broad and depressed, longer (HL 25.80% of SVL) than wide (HW 24.60% SVL); eye large, much smaller (EL 6.10% SVL) than snout (SL 9.40% SVL); nostrils distinctly closer to tip of snout (SNL 3.10% SVL) than to eye (NEL 5.80% SVL); internarial distance (IND 4.40% SVL) much shorter than interorbital distance (IOD 8.20% SVL), latter wider than upper eyelid (UEW 6.90% SVL); tympanum diameter (TD 4.20% SVL) about two-third of eye and separated from eye by half of its diameter (TEL 6.10% SVL).

Forelimb slender and long (FLL 43.00% SVL); inner palmar tubercle large (IPTL 2.20% SVL), smaller than larger outer palmar tubercle (OPTL 2.70% SVL). Hindlimb slender and relatively short (HLL 73.00% SVL); foot slightly shorter (FL 21.10% SVL) than tibia (TL 23.00% SVL); inner metatarsal tubercle length (IMTL 1.90% SVL).

#### *Leptobrachium waysepuntiense* Hamidy & Matsui, 2010

Diagnosis: *L. waysepuntiense* iris color is light blue with black reticulations, while light blue in juvenile, and surrounded by light blue sclera when eye opened maximally; no black

bars around lips; supratympanic ridge bordered by a very thin brownish orange line; the dorsal coloration varied from dark brownish grey to grey color fading laterally to light grey on the ventral side, a dorsal body without a clear pattern and with brownish orange V pattern in the interorbital to parietal regions; 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; toes brownish color with orange dorsolaterally; the forelimbs faintly covered by dark brown bars; The femoral gland very small white spotted on the posterior surface of thigh (Figs 4F & 5F).

Medium-sized *Leptobrachium*, Body tapering to the groin (SVL 43.83±8.99), head broad and depressed, shorter (HL 14.90 (14.90-24.90)% of SVL) than wide (HW 21.50 (13.90-24.80)% SVL); eye large, subequal (EL 6.10 (3.80-6.70)% SVL) than snout (SL 6.10 (4.70-8.20)% SVL); nostril distinctly closer to tip of snout (SNL 1.10 (1.00-2.00)% SVL) than to eye (NEL 4.40 (2.70-5.80)% SVL); internarial distance (IND 4.00 (3.60-4.60)% SVL) much shorter than interorbital distance (IOD 8.60 (5.00-9.80)% SVL), latter wider than upper eyelid (UEW 4.90 (4.20-7.00)% SVL); tympanum diameter (TD 3.20 (2.70-4.00)% SVL) half of eye and separated from eye by half of its diameter (TEL 2.50 (1.60-2.80)% SVL).

Forelimb slender and long (FLL 34.50 (22.80-35.00)% SVL); inner palmar tubercle large (IPTL 2.10 (1.50-2.80)% SVL), smaller than outer palmar tubercle (OPTL 2.3 (1.80-2.60)% SVL). Hindlimb slender and relatively short (HLL 52.00 (38.00-58.80)% SVL); foot slightly shorter (FL 18.20 (13.20-19.00)% SVL) than tibia (TL 18.50 (13.10-20.10)% SVL); inner metatarsal tubercle length (IMTL 2.00 (1.20-2.30)% SVL).

#### *Leptobrachium abbotti* (Cochran, 1926)

Diagnosis: *L. abbotti* iris color is completely black without reticulation; dark brown markings behind eyes from interorbital to the parietal area; the supratympanic ridge is covered with a thick black line; the dorsal coloration dark grey to brownish; lateral body covered by dark brown large blotches from the arm insertion to the groin; ventral body covered by dark brown very large blotches starting from the chin, throat, stomach down to the hind limbs; forelimbs with black or dark brown bars up to fingertips dorsally, on the ventral side dark brown blotches and bands extending from palm to elbows; groin are covered by dark marking; the femoral gland small white spotted, covered by dark marking (Figs 4E & 5E).

Large-sized *Leptobrachium*, Body tapering to the groin (SVL 59.97±7.63), head broaddepressed, slightly shorter (HL 26.10 (23.30-28.20)% of SVL) than wide (HW 26.80 (22.10-28.30)% SVL); eye large, and smaller (EL 7.00 (6.00-7.70)% SVL) than snout (SL 9.30 (8.40-10.80)% SVL); nostrils distinctly closer to tip of snout (SNL 2.80 (2.10-3.30)% SVL) than to eye (NEL 6.20 (5.00-6.40)% SVL); internarial distance (IND 4.30 (3.80-4.60)% SVL) much shorter than interorbital distance (IOD 10.50 (8.00-11.50)% SVL), latter wider than upper eyelid (UEW 7.00 (6.00-7.80)% SVL); tympanum diameter (TD 4.10 (3.60-4.90)% SVL) about two-third of eye and separated from eye by about half of its diameter (TEL 3.20 (3.00-4.80)% SVL).

Forelimb slender and long (FLL 44.60 (36.30-46.50)% SVL); inner palmar tubercle large (IPTL 2.80 (2.70-3.10)% SVL), and subequal to outer palmar tubercle (OPTL 2.90 (2.60-3.00)% SVL). Hindlimb slender and relatively short (HLL 73.20 (60.60-80.00)% SVL); foot slightly shorter (FL 23.00 (18.00-24.10)% SVL) than tibia (TL 24.20 (19.20-25.80)% SVL); inner metatarsal tubercle length (IMTL 2.10 (1.70-2.50)% SVL).

*Leptobrachium ingeri* Hamidy, Matsui, Nishikawa & Belabut, 2012

Diagnosis: *L. ingeri* iris color is completely black, surrounded by a bright blue sclera visible when the eye is fully open; cheeks and anterior to the eyes are covered by dark brown markings; the upper part of the tympanum and a supratympanic ridge is covered with a dark band; the dorsal coloration brown with dark brown blotches in the interorbital areas, one centrally followed by longitudinal bands and one laterally followed by intersecting bands; lateral body with black dots spreading from jaw to groin, dorsolateral body brown covered by dark spots fading lighter towards belly; ventral body covered by irregular blotches, denser on belly; forelimbs and hindlimbs with black bars, including the fingers and toes on the dorsal side; the thighs covered by black bands, continues to the hips; groin covered by irregular blotches; pointed fingertips; the webs of the feet are well formed; large white femoral gland (Figs 4H & 5H).

Small-sized *Leptobrachium*, Body tapering to the groin (SVL 30.30±1.86), head broad and depressed, longer (HL 12.59 (11.70-13.80)% of SVL) than wide (HW 11.11 (10.60-12.10)% SVL); eye large, larger (EL 4.27 (3.20-4.50)% SVL) than snout (SL 3.93 (3.60-4.40)% SVL); nostrils distinctly closer to tip of snout (SNL 1.21 (1.00-1.60)% SVL) than to eye (NEL 2.58 (2.20-2.80)% SVL); internarial distance (IND 2.41 (2.20-2.70)% SVL) much shorter than interorbital distance (IOD 3.80 (3.46-4.20)% SVL), latter wider than upper eyelid (UEW 3.49 (3.40-4.00)% SVL); tympanum diameter (TD 2.11 (2.00-2.22)% SVL) about half of eye and separated from eye by half of its diameter (TEL 1.17 (1.14-1.30)% SVL).

Forelimb slender and long (FLL 22.37 (19.80-24.28)% SVL); inner palmar tubercle large (IPTL 1.35 (1.20-1.64)% SVL), smaller than outer palmar tubercle (OPTL 1.43 (1.36-1.50)% SVL). Hindlimb slender and relatively short (HLL 36.68 (35.20-40.30)% SVL); foot shorter (FL 10.42 (10.10-11.50)% SVL) than tibia (TL 11.84 (11.22-12.70)% SVL); inner metatarsal tubercle length (IMTL 1.33 (0.90-1.37)% SVL).

**Key of morphological determination to Indonesian *Leptobrachium***

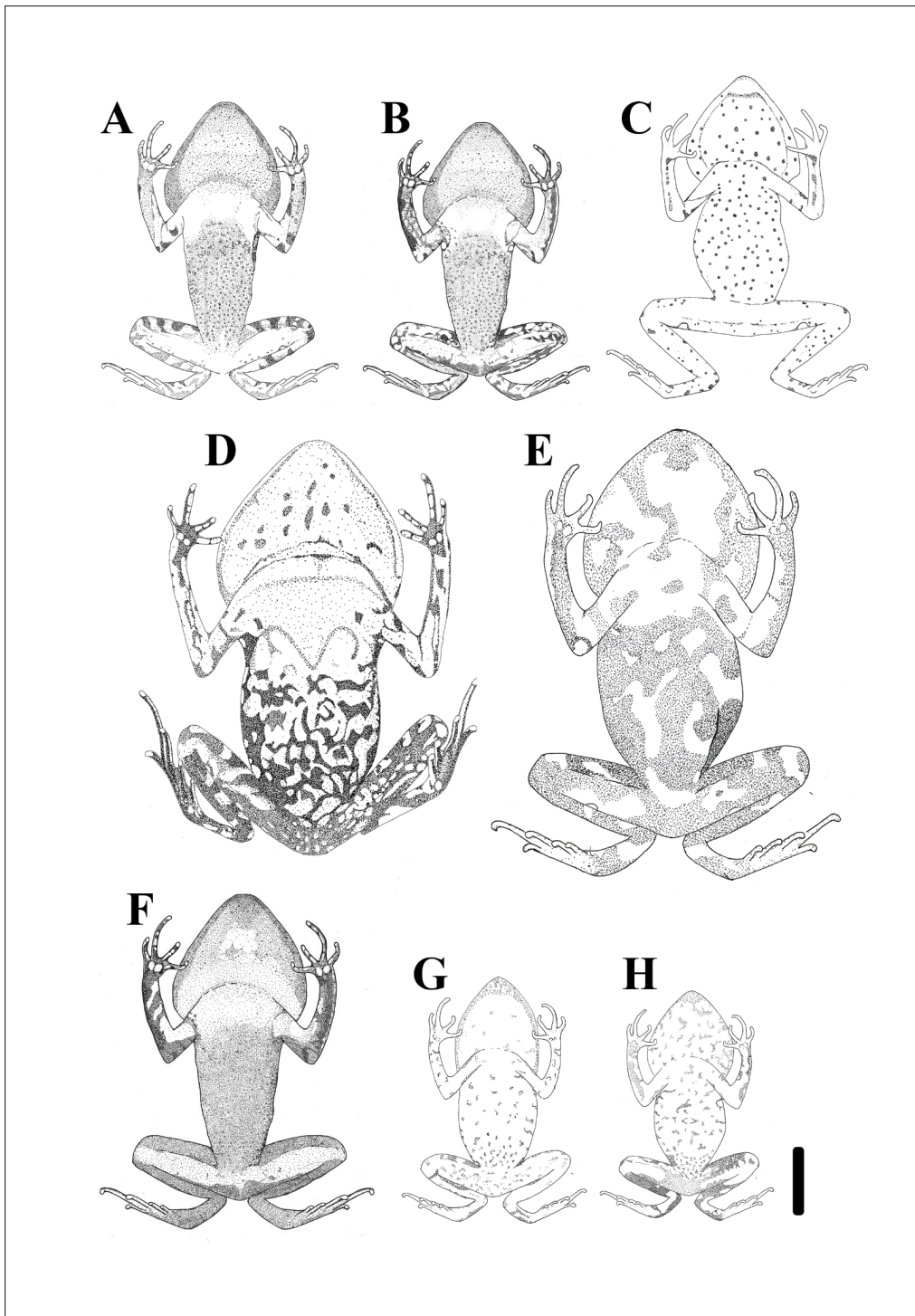
- 1a Rough skin, with warts and granules all over the body; parotoid glands behind eyes (Bufonidae)
- 1b The skin is generally smooth, with small granules; without parotoid glands (2)
- 2a The limbs are relatively short (3)
- 2b The limbs are relatively long Ranidae,  
Rhacophoridae,  
Dicroglossidae,  
Hylidae,  
Pipidae
- 3a Body small to large, robust; broad fingertips, ‘T’ shape or without extended fingertips; head and snout relatively small and narrow; small eyes Microhylidae

- |     |   |                                    |
|-----|---|------------------------------------|
| 3b  | Body small to large, slender; finger and toe without broadened tips, snout and head relatively wide; large eyes   | Megophryidae<br>(4)                |
| 4a  | Eyelids with pointed tips; have a pair of dorsolateral folds; body stocky, color like leaf litter   | <i>Megophrys</i>                   |
| 4b  | Eyelids without tips; body and limbs slender; without dorsolateral folds, body pattern with dark blotches, bars, dots or spots; femoral and humeral gland present   | <i>Leptobrachium</i><br>(5)        |
| 5a  | Iris completely black   | (6)                                |
| 5b  | Iris color other than black   | (7)                                |
| 6a  | Ventral body with distinct markings   | (8)                                |
| 6b  | Ventral body without distinct markings  | (9)                                |
| 7a  | Iris light blue with black reticulation; dorsal and ventral body without distinct markings, yellow 'V' markings in the interorbital to parietal areas; body coloration gray to dark with many orange granules | <i>L.</i><br><i>waysepuntiense</i> |
| 7b  | Iris color orange or sometimes black with orange on the upper half; dorsal with markings from the interorbital areas; ventral and lateral body all covered with black spots                                   | <i>L. hendricksoni</i>             |
| 8a  | Fingertips pointed  | (10)                               |
| 8b  | Fingertips not pointed  | (11)                               |
| 9a  | Dorsal with markings from the interorbital to parietal area; femoral glands are very large white covered with dark markings   | <i>L. hasseltii</i>                |
| 9b  | Dorsal with markings from the interorbital area extending to cloaca; laterally with irregular markings; femoral glands are very large white surrounded by dark markings                                       | <i>Leptobrachium</i><br>sp. Bali   |
| 10a | Dorsal with markings from the interorbital area extending to cloaca; ventral with irregular markings; femoral gland large, covered by dark markings   | <i>L. nigrops</i>                  |
| 10b | Common characteristics like <i>L. Nigrops</i> , but with a smaller body size; ventral body covered by irregular markings; groin covered by irregular blotches; femoral gland large                            | <i>L. ingeri</i>                   |
| 11a | Ventral with large dark brown blotches and bands extending from palm to elbows; groin is covered by dark marking; the femoral gland small white spotted, covered by dark marking                              | <i>L. abbotti</i>                  |
| 11b | Ventral covered by irregular blotches; groin and hips are covered by dark marking; femoral gland large, covered by dark markings  | <i>L. montanum</i>                 |

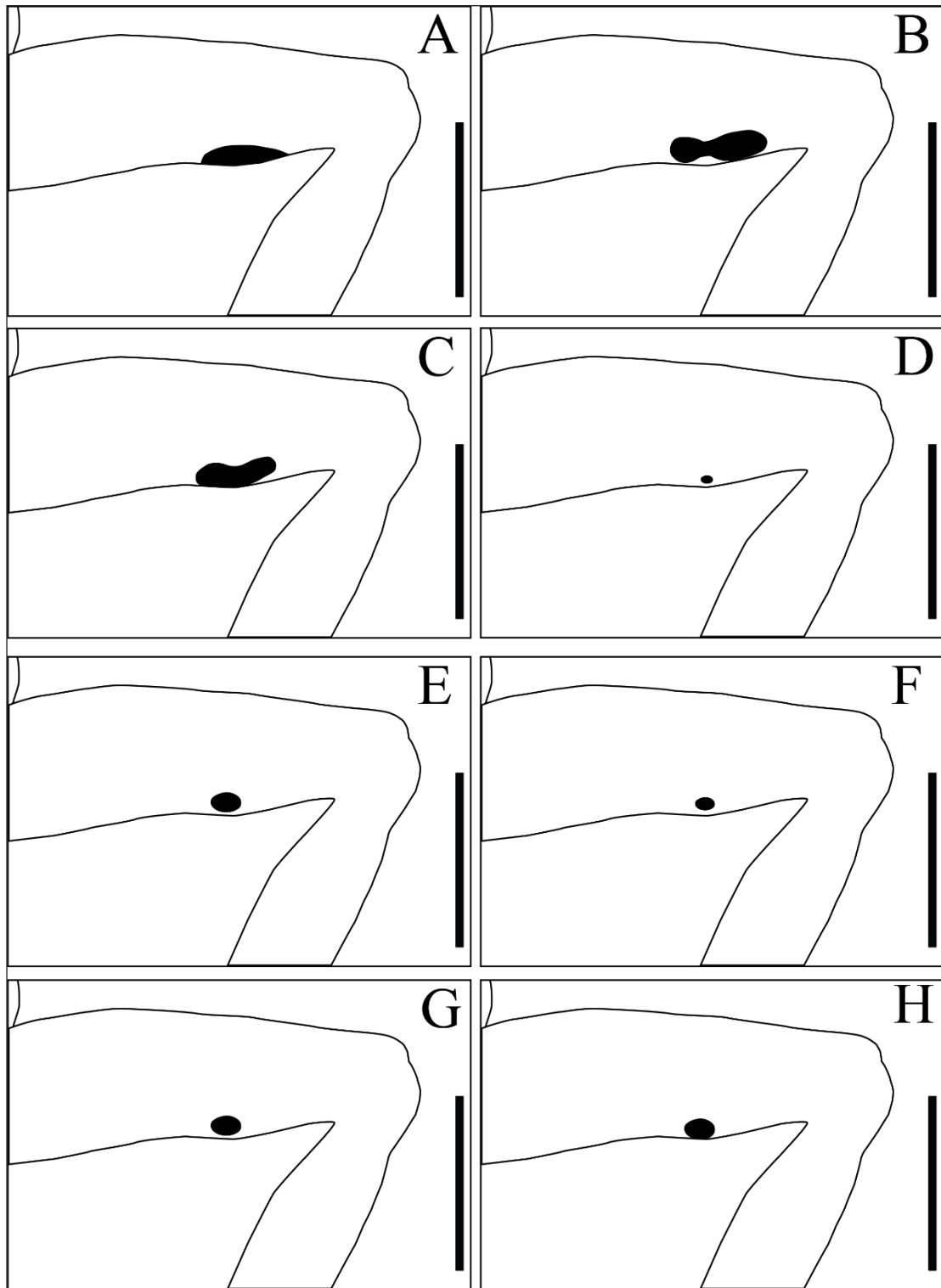




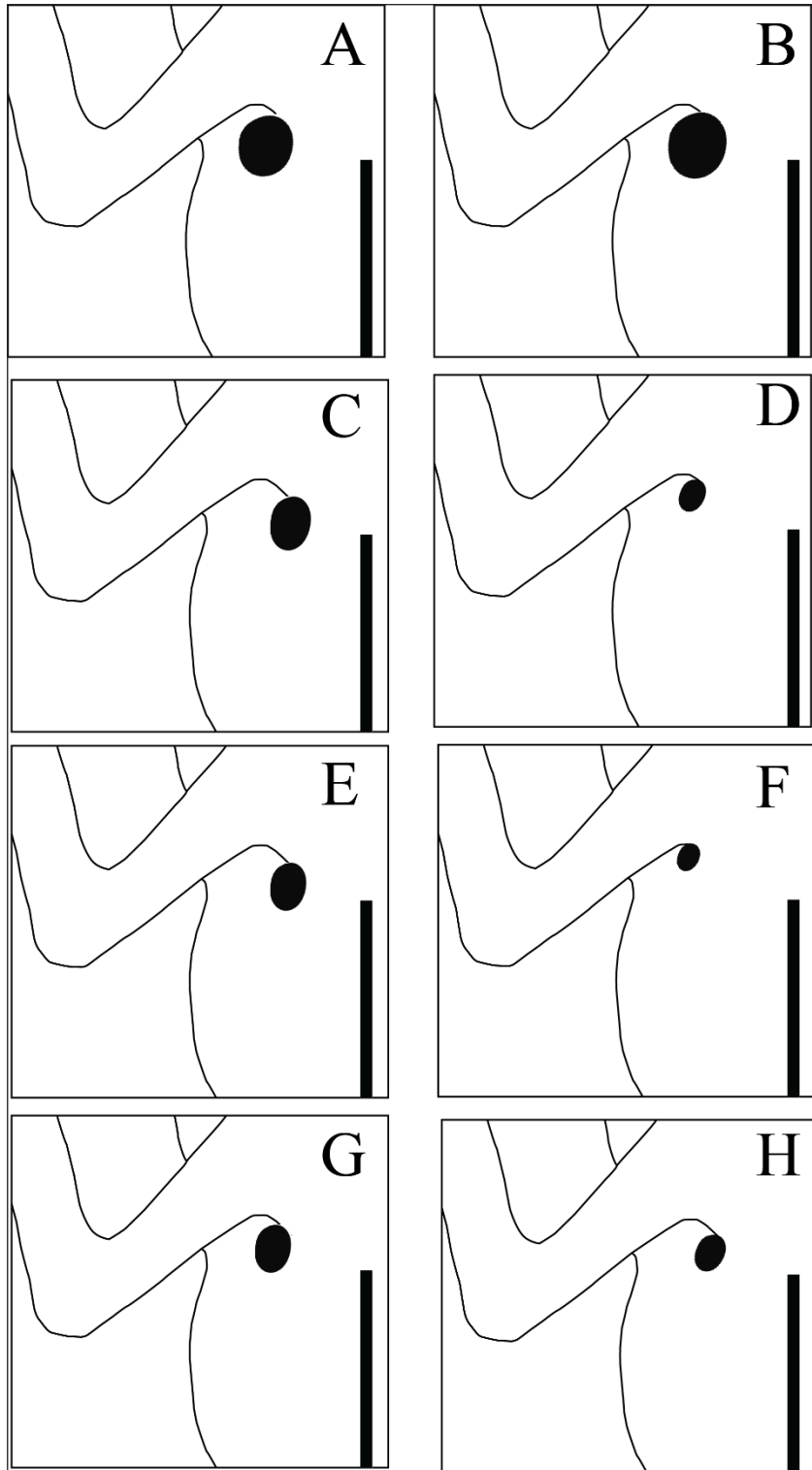
**Figure 4.** *Leptobrachium* species are distributed over Indonesia. (A) *L. hasseltii* (B) *Leptobrachium* sp. Bali (C) *L. hendricksoni* (D) *L. montanum* (E) *L. abbotti* (F) *L. waysepuntiense* (G) *L. nigrops* (H) *L. ingeri*. (A-G) Photographed by Amir Hamidy (H) Photographed by Koshiro Eto.



**Figure 5.** Ventral pattern of Indonesian *Leptobrachium*: (A) *L. hasseltii* (B) *Leptobrachium* sp. Bali (C) *L. hendricksoni* (D) *L. montanum* (E) *L. abbotti* (F) *L. waysepuntiense* (G) *L. nigrops* (H) *L. ingeri*. Scale bar 10 mm.



**Figure 6.** Illustrations of femoral gland size of Indonesian *Leptobrachium*: (A) *L. hasseltii* (B) *Leptobrachium* sp. Bali (C) *L. hendricksoni* (D) *L. montanum* (E) *L. abbotti* (F) *L. waysepuntiense* (G) *L. nigrops* (H) *L. ingeri*. Scale bar 10 mm



**Figure 7.** Illustrations of humeral gland size of Indonesian *Leptobrachium*: (A) *L. hasseltii* (B) *Leptobrachium* sp. Bali (C) *L. hendricksoni* (D) *L. montanum* (E) *L. abbotti* (F) *L. waysepuntiense* (G) *L. nigrops* (H) *L. ingeri*. Scale bar 10 mm.

## CONCLUSION

The difficulty to distinguish cryptic species is due to the high species variation or inter-species variation in the same genus, which occurs in *Leptobrachium*. By identifying Indonesian *Leptobrachium* morphological character differences, we can provide some characteristics that can be used in distinguishing each *Leptobrachium* in Indonesia, such as combinations of the iris color, body pattern, and size of the femoral gland. Despite it is still being challenging, adding several morphometric characters can be useful to distinguish each *Leptobrachium*. Morphological and morphometry have been a perfect combination in distinguishing an anuran species for a long time, and their use is also great in *Leptobrachium*. However, there are limitations in predicting the relationship between each *Leptobrachium*.

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