

**A NEW SPECIES OF *MICROHYLA* (ANURA: MICROHYLIDAE)
FROM SUMATRA, INDONESIA**

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ABSTRACT

A new species of frog in the genus *Microhyla* is described from Sumatra, Indonesia based on molecular and morphological characters. This new species was previously confused with *M. achatina*, a Javan endemic. This new species is diagnosable from its congeners by possessing a medium size (SVL in adult males 18.20–21.32 mm, in adult females 20.37–25.51 mm), a stout body, a nostril–eyelid length being about half of the snout length, having a single outer palmar tubercle, a tibiotarsal articulation reaching the center of the eye (when the hindlimbs are stretched and adpressed to the body), having finger and toe tips dilated, having the dorsum with medial longitudinal grooves, and exhibiting a very thin and short dark stripe on the temporal region above a wider cream stripe, extending from the postorbital area to insertion of forelimb. Additionally, the new species is characterized by possessing relatively little foot webbing. Uncorrected 16S rRNA sequence divergences between the new taxon and sequences for other congeneric species available ranged from 4.8 to 15.0%.

Keywords: cryptic species, mitochondrial DNA, phylogeny, taxonomy

INTRODUCTION

Narrow-mouthed Frogs of the genus *Microhyla* Tschudi, 1838 are widely distributed from the Ryukyu Islands (Japan) to the Indian Subcontinent, and including the intermediate regions of Indochina, the Sunda Shelf and Philippine islands (Matsui et al., 2005; Frost, 2017). The genus contains 41 species, six of them known to occur in Sumatra, *M. achatina* Tschudi, 1838; *M. berdmorei* (Blyth, 1856); *M. fissipes* Boulenger, 1884; *M. heymonsi* Vogt, 1911, *M. palmipes* Boulenger, 1897; and *M. superciliaris* Parker, 1928 (Parker, 1934; Frost, 2017; Pradana, 2017).

The type species of the genus is *Microhyla achatina* (Tschudi, 1838). The name *M. achatina* was previously applied to several populations from Myanmar; Peninsular Malaysia; Sumatra, Java, Bali, and the Philippines (van Kampen, 1923; McKay, 2006). Then, molecular studies proved that the populations outside of Indonesia belonged to other species (Matsui et al., 2011 & 2013; Frost, 2017) and *M. achatina* was considered a Javanese endemic (Natus, 2005; McKay, 2006). Matsui et al. (2011) firstly examined specimens from Sumatra that are morphologically look alike to *M. achatina* from Java, and these were suspected to be different

from *M. achatina*, based on large genetic distances from the later (16S rRNA 5.5–6.6%), and referred to it as *Microhyla* sp. 3. In this study, we add more sample sized and analyze the taxonomic status of *M. achatina* Sumatra based on molecular and morphological data and describe it as a new species.

MATERIALS AND METHODS

Sampling design

We examined a total of 29 partial sequences of the consecutive mitochondrial DNA genes 12S and 16S rRNA of *Microhyla* cf. *achatina* from Sumatra and members of the *M. achatina* group of Matsui et al. (2011), *M. berdmorei*, *M. fissipes*, *M. heymonsi*, *M. achatina* (from Gede Pangrango, West Java; Ungaran and Pekalongan, Central Java), *Microhyla* sp. 3, *M. orientalis*, *M. mantheyi*, *M. borneensis*, and *M. malang*, and six outgroups (four congeneric [*M. butleri*, *M. palmipes*, *M. perparva*, and *M. superciliaris*] and *Kalophrynus pleurostigma* and *Oreophryne monticola*; see Table 1). Specimens collected are presented in Fig. 1 and Table 1. Voucher specimens and tissues are stored in Museum Zoologicum Bogoriense (MZB), Research Center for Biology – Indonesian Institute of Sciences (LIPI), and at the Amphibian and Reptile Diversity Research Center (ARDRC), University of Texas at Arlington (UTA).

Molecular analyses

The methods for DNA extraction, amplification and sequencing for the 12S and 16S rRNA gene fragments are the same reported by Matsui et al. (2011, 2013). DNA sequences were edited using the ChromasPro software (Technelysium Pty Ltd., Tewantin, Queensland, Australia). All sequences, including those from Genbank (Table 1) were aligned using Clustal W in MEGA 7 (Kumar et al., 2016). Phylogenetic trees were constructed in Neighbour Joining (NJ), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. NJ analysis was conducted in MEGA 7 using p-distances and 1000 bootstrap replicates. Rate evolution models for ML and BI analyses were identified under the Akaike Information Criterion executed in Kakusan 4 (Tanabe, 2011). BI analysis was conducted in Mr. Bayes ver. 3.1.2 (Ronquist & Huelsenbeck, 2003), with a general time-reversible (GTR) model of DNA evolution and a gamma shape parameter (G). We ran the analysis for 6 million generations and sampled every 100 generations. The consensus tree was calculated after omitting the first 25% of the trees as burn-in. For ML analysis we used Treefinder ver. March 2011 (Jobb et al., 2004) with general time-reversible (GTR) and a gamma shape parameter (G). Tree nodes considered as strongly supported when having bootstrap values of 70% or more, for ML and NJ analyses (Hillis & Bull, 1993). In BI analysis, nodes with BPPs higher than 95% were considered statistically significant (Leaché & Reeder, 2002). We also calculated genetic distances of 16S rRNA gene using uncorrected p-distances with MEGA 7.

Morphology analysis

We examined specimens of *Microhyla achatina* stored in the Museum Zoologicum Bogoriense (MZB), Research Center for Biology-Indonesian Institute of Sciences (LIPI) and at the Amphibian and Reptile Diversity Research Center (ARDRC), University of Texas at Arlington (UTA) (Appendix 1). For comparisons, we also examined the type series of *M. achatina* (syntypes RMNH 1728) stored in the Naturalis Biodiversity Center (now merging the collections of the Rijksmuseum van Natuurlijke Historie, RMNH, and the Universiteit van Amsterdam, Zoologisch Museum, ZMA).

We measured 39 characters with a dial caliper to the nearest 0.01 mm. The following characters were taken (sometimes modified) from Matsui (1984, 2013): (1) snout–vent length (SVL); (2) body width (BW), greatest width of body; (3) head length (HL), from tip of snout to hind border of angle of jaw (not measured parallel with the median line); (4) head width (HW); (5) head depth (HD); (6) snout length (SL); (7) eye length (EL); (8) nostril–eyelid length (N–EL); (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) first finger length (1FL), from distal end of inner palmar tubercle to tip; (16) hand length (HAL), from proximal end of outer palmar tubercle to tip of third finger; (17) inner palmar tubercle length (IPTL); (18) outer palmar tubercle length (OPTL); (19) hindlimb length (HLL); (20) tibia length (TL); (21) foot length (FL); (22) inner metatarsal tubercle length (IMTL); (23) first toe length (1TOEL), from distal end of inner metatarsal tubercle to tip of first toe; (24) outer metatarsal tubercle length (OMTL); (25) first finger width (1FW), measured at the distal phalanx; (26–28) finger disk diameters (2–4FDW); (29–33) toe disk diameters (1–5 TDW); and (34–39) finger lengths (1–3 FLO, 2–4 FLI), for outer surface (O) of the first, inner side (I) of the fourth, and both sides of the remaining fingers, measured from tip to the junction of the neighboring finger.

In addition to morphometric analyses, we examined and scored several qualitative characters: snout shape, finger and toe tip shape, median longitudinal groove dorsally on finger and toe tips, ventral color, lateral and dorsal body color, anal region color, temporal/postorbital region and vertebral stripes, and dark stripe pattern of hind legs. We compared morphological data among populations of *M. achatina* from Java and Sumatra using a multivariate Principal Component Analysis log₁₀ transformed values (Thorpe, 1975). In univariate analysis we used converted morphometric characters (ratio to SVL; R), checked the distribution data and compared using T-Test Independent analysis. Both analyses were conducted using IBM SPSS Statistic 22 (IBM corp., 2013). We followed Savage & Heyer (1967) as modified by Myers and Duellman (1982) for the description of toe-webbing formula.

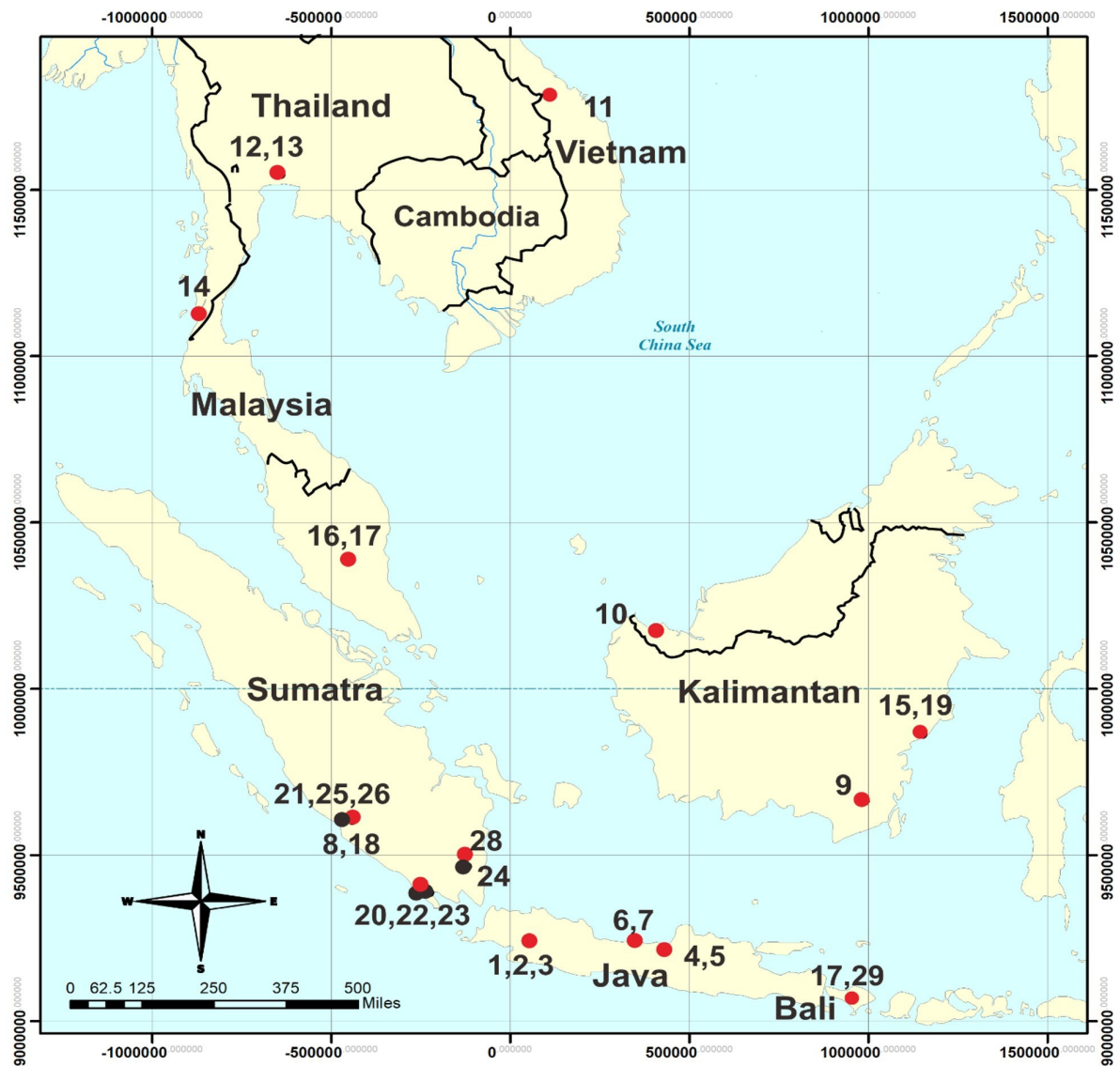


Figure 1. Map of the Sunda Shelf showing sampling localities of *Microhyla gadjohmadai* sp. nov. in Sumatra (black) and those of samples with Genbank sequences used in this study (red). Sample numbers correspond to those in Table 1 (Map modified from ArcGIS 10.2 Desktop Map, October 9, 2018).

RESULTS

We obtained 1693 bp of the 12S and 16S rRNA gene fragments for 29 samples, including outgroups. Of the 1693 nucleotide sites, 611 were variable and 443 were parsimoniously informative. The ML analysis produced a topology a high log likelihood of -9808.0088, a gamma shape parameter of 0.2071, and nucleotide frequencies of A=0.328, C=0.254, G=0.186, and T=0.232. The BI analysis had similar calculated nucleotide frequencies: A=0.348, C=0.244, G=0.171, T=0.236, and a gamma shape parameter, 0.2047. All analyses produced the same topologies and only the ML tree is shown (Fig. 2). *Microhyla cf. achatina* from Sumatra was recovered as sister to *M. achatina* from Java (supported clade NJ: 100%, ML: 99% and BI: 100%) and deeply nested with *Microhyla* sp. 3, genbank sequence from Matsui et al. (2011) (supported clade NJ: 98%, ML: 92% and BI: 99%). *Microhyla cf. achatina* from 2 localities were divided into 2 subclades, Bengkulu (NJ: 100%, ML: 100% and BI: 100%) and Lampung (NJ: 100%, ML: 100% and BI: 100%). This species is a monophyletic group, and genetically distinct from other described *Microhyla* species. Here we named Sumatran population (*Microhyla cf. achatina*) and *Microhyla* sp. 3 as *Microhyla gadjahmadai sp. nov.* (Fig. 2).

Eventhough *Microhyla gadjahmadai sp. nov.* formed a monophyletic clade with *M. achatina* but genetic distance is higher than threshold. The uncorrected p-distance (partial gene 16S rRNA) of *M. gadjahmadai sp. nov.* with other congeners ranged from 4.8–15.0% (included its sister taxon), while intraspecific genetic distance from 2 localities (Lampung vs Bengkulu) ranged from 0–3.0% as shown between *M. gadjahmadai sp. nov.* and *Microhyla* sp. 3 (Table 2). The threshold for amphibian species delimitation in 16S rRNA has been suggested to be $\geq 3\%$ by Fouquet et al. (2007). Therefore *M. gadjahmadai sp. nov.* can be considered as a different species from other congeners.

In morphology analysis, we examined specimens of *M. gadjahmadai sp. nov.* and its sister taxon *M. achatina*. Based on PCA result, the two species were separated by first and second axes (Fig. 3) supported by cumulative percentage 70.52% from three first component. In the univariate analysis, significant differences between two group were found: nostril–eyelid length (N–EL) ($P=0.007$) and dark stripe at temporal/postorbital region ($P=0.001$).

Systematics

Microhyla gadjahmadai sp. nov.

Synonymy:

Microhyla sp. 3: Matsui et al., 2011, p. 168 & 171

Holotype. MZB Amph 26081 (Field number ENS 18216), an adult male from Vila Hijau on Curup Lubik, Lingau Road, Rejang Lebong Regency, in the province of Bengkulu, Indonesia (3.452464° S, 102.650485° E, elevation 1085 m a.s.l., Fig. 1), collected on 17 July 2015 by E. Wostl, E. N. Smith, G. C. Sarker, and P. Thammachoti. [1.2g in weight]

Table 1. Samples of Microhylids and related species used for mtDNA analysis in this study together with information on species identification, specimen voucher number, locality, GenBank accession numbers, and references. MZB: Museum Zoologicum Bogoriense, KUIHE: Kyoto University, Graduate School of Human and Environmental Studies, MDK: Department of Conservation and Ecotourism, Faculty of Forestry, Bogor Agricultural Institutes

No.	Species	Specimen Voucher	Locality	Genbank Accession No.		Source
				12S	16S	
1	<i>Microhyla achatina</i>	MDK 24	Indonesia, Java, Gede Pangrango	AB634599	AB634657	Matsui et al. 2011
2	<i>Microhyla achatina</i>	MDK 21	Indonesia, Java, Gede Pangrango	MK034330	MK034330	This study
3	<i>Microhyla achatina</i>	MDK 22	Indonesia, Java, Gede Pangrango	MK034331	MK034331	This study
4	<i>Microhyla achatina</i>	MZB Amph 16402	Indonesia, Java, Ungaran	AB634598	AB634656	Matsui et al. 2011
5	<i>Microhyla achatina</i>	MZB Amph 16401	Indonesia, Java, Ungaran	AB598311	AB598335	Matsui 2011
6	<i>Microhyla achatina</i>	PK07	Indonesia, Java, Pekalongan	MK034337	MK034337	This study
7	<i>Microhyla achatina</i>	PK8906	Indonesia, Java, Pekalongan	MK034338	MK034338	This study
8	<i>Microhyla berdmorei</i>	MZB Amph 16413	Indonesia, Sumatra, Bengkulu	AB634602	AB634660	Matsui et al. 2011
9	<i>Microhyla berdmorei</i>	MZB Amph 15270	Indonesia, Kalimantan, Paramasan	AB634603	AB634661	Matsui et al. 2011
10	<i>Microhyla borneensis</i>	KUIHE 53165	Malaysia, Sarawak, Serapi	AB598305	AB598329	Matsui et al. 2011
11	<i>Microhyla butleri</i>	KUIHE 40591	Vietnam, A Roang, A Luoi	AB634606	AB634664	Matsui et al. 2011
12	<i>Microhyla butleri</i>	KUIHE 44203	Thailand, Bangkok	AB201178	AB201189	Matsui et al. 2005
13	<i>Microhyla fissipes</i>	KUIHE 22064	Thailand, Bangkok	AB634608	AB634666	Matsui et al. 2011
14	<i>Microhyla heymonsi</i>	KUIHE 23856	Thailand, Ranong	AB598312	AB598336	Matsui et al. 2011
15	<i>Microhyla malang</i>	MZB Amph 16364	Indonesia, Kalimantan, Balikpapan	AB634619	AB634677	Matsui et al. 2011
16	<i>Microhyla mantheyi</i>	KUIHE 52556	Malaysia, Pahang, Temerloh	AB598310	AB598334	Matsui et al. 2011

continued ...

No.	Species	Specimen Voucher	Locality	Genbank Accession No.		Source
				12S	16S	
17	<i>Microhyla orientalis</i>	MZB Amph 16259	Indonesia, Bali, Batu Karu	AB634621	AB634679	Matsui et al. 2011
18	<i>Microhyla palmipes</i>	MZB Amph 16323	Indonesia, Sumatra, Bengkulu	AB634613	AB634671	Matsui et al. 2011
19	<i>Microhyla perparva</i>	KUHE UN	Indonesia, Kalimantan, Balikpapan	AB634614	AB634672	Matsui et al. 2011
20	<i>Microhyla</i> sp. 3	MZB Amph 15291	Indonesia, Sumatra, Lampung	AB634622	AB634680	Matsui et al. 2011
21	<i>Microhyla</i> sp. 3	MZB Amph 16328	Indonesia, Sumatra, Bengkulu	AB634623	AB634681	Matsui et al. 2011
22	<i>Microhyla gadjahmadai</i> sp. nov.	MZB Amph 15292	Indonesia, Sumatra, Lampung	MK034333	MK034333	This study
23	<i>Microhyla gadjahmadai</i> sp. nov.	MZB Amph 15293	Indonesia, Sumatra, Lampung	MK034334	MK034334	This study
24	<i>Microhyla gadjahmadai</i> sp. nov.	MZB Amph 15290	Indonesia, Sumatra, Lampung	MK034332	MK034332	This study
25	<i>Microhyla gadjahmadai</i> sp. nov.	MZB Amph 16324	Indonesia, Sumatra, Bengkulu	MK034335	MK034335	This study
26	<i>Microhyla gadjahmadai</i> sp. nov.	MZB Amph 16326	Indonesia, Sumatra, Bengkulu	MK036336	MK036336	This study
27	<i>Microhyla superciliaris</i>	KUHE 52558	Malaysia, Pahang, Temerloh	AB634624	AB634682	Matsui et al. 2011
28	<i>Kalophrynus pleurostigma</i>	MZB Amph 15295	Indonesia, Sumatra, Lampung	AB634642	AB634700	Matsui et al. 2011
29	<i>Oreophryne monticola</i>	MZB Amph 16265	Indonesia, Bali, Batu Karu	AB634651	AB634709	Matsui et al. 2011

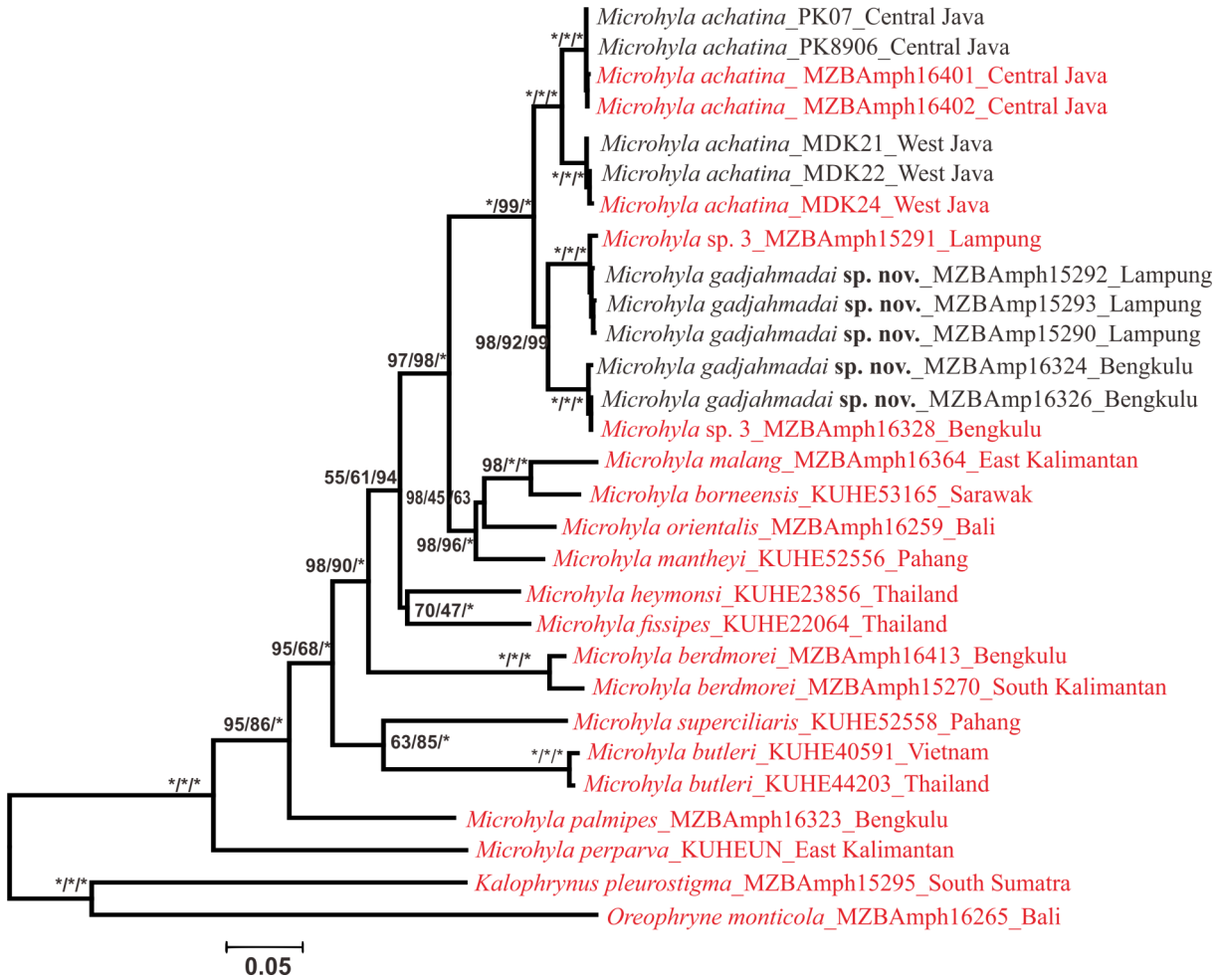


Figure 2. Maximum likelihood phylogeny from 1693 bp of the 12S & 16S rRNA mitochondrial genes for samples of *Microhyla gadjahmadai* sp. nov. and related species with outgroups. Numbers above or below branches represents bootstrap support for NJ/ML/and Bayesian Inferences (BI). Asteriks indicate significant bootstrap supports for NJ and ML ($\geq 70\%$) inferences and Bayesian Inference (BI) ($> 95\%$).

Table 2. Uncorrected p-distances (in %) from 833 bp of 16S rRNA fragment among species *Microhyla* spp.

No	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.	<i>M. achatina</i> Java	0–2.8														
2.	<i>Microhyla gadjahnadai</i> sp.nov.	4.8–6.3	0.4–2.9													
3.	<i>Microhyla</i> sp. 3	4.8–6.3	0–3.0	2.9												
4.	<i>M. malang</i>	9.0–9.5	9.0–9.4	9.4	–											
5.	<i>M. borneensis</i>	8.0–8.4	8.1–8.8	8.4–8.8	5.6	–										
6.	<i>M. orientalis</i>	7.4–7.9	8.0–8.4	8.0–8.4	8.8	6.6	–									
7.	<i>M. mantheyi</i>	8.0–8.4	7.9–8.1	8.1	7.8	7.1	6.6	–								
8.	<i>M. heymonsi</i>	10.6–11.4	11.4–12.1	11.8–12.1	10.5	9.6	9.6	10.0	–							
9.	<i>M. fissipes</i>	10.3–10.8	11.3–11.9	11.4–11.8	11.4	11.5	10.4	10.5	9.3							
10.	<i>M. berdmorei</i>	9.9–11.1	11.8–12.3	11.9–12.4	12.9–13.5	12.4–13.0	12.0–12.8	12.4–12.9	12.6–13.1	12.6–13.3	2.5					
11.	<i>M. superciliaris</i>	12.8–13.1	12.9–13.6	13.0–13.8	13.8	12.9	12.8	13.1	13.1	12.4	13.6–14.0	–				
12.	<i>M. butleri</i>	13.4–13.9	14.3–14.7	14.4–14.6	13.8–13.9	13.1–13.3	13.9–14.1	14.4–14.6	14.9	13.6	13.9–15.1	11.9–12.1	0.5			
13.	<i>M. palmipes</i>	13.9–14.1	13.5–13.9	13.6–13.9	13.9	13.6	14.6	14.0	14.3	12.0	14.4–14.9	11.5	12.5–12.8	–		
14.	<i>M. parva</i>	14.5–14.8	14.3–15.0	14.5–14.9	14.9	15.1	15.4	15.3	14.4	13.9	14.9	14.3	14.5–14.8	13.1	–	
15.	Outgroups	18.4–20.9	18.8–20.4	18.9–20.4	18.6–20.3	18.0–20.3	18.0–20.4	18.4–20.5	18.4–20.0	19.3–22.0	18.8–20.7	17.8–19.8	18.9–19.8	18.6–20.2	17.8–18.0	17.5

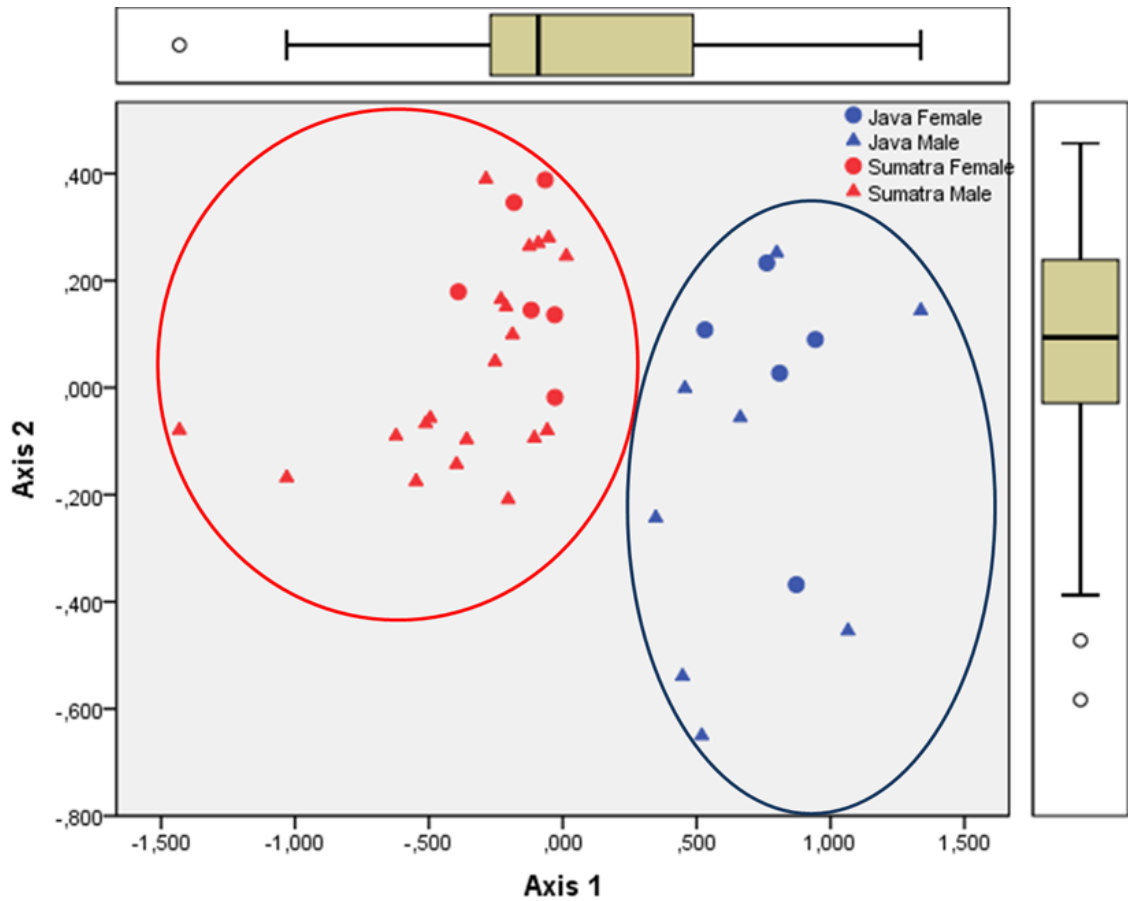


Figure 3. Scatter plot of first and second principal components showing differentiation between *Microhyla gadjahmadai* sp. nov. from Sumatra (red) and *M. achatina* from Java (blue). Circles represent females and triangles represent males. The proportion of variance explained by the first three components is 70.52% (PC1 = 55.74%, PC2 = 7.99%, PC3 = 6.96%). Notice that component 1 accounts for the species variation and component 2 for some of the variation in sex.

Paratypes. MZB Amph 26078, UTA A-65741, UTA A-65743, three adult males from Bukit Kaba, Rejang Lebong Regency, in the province of Bengkulu, Indonesia (3.49259° S, 102.63589° E, elevation 1380 m a.s.l.; 3.49210° S, 102.63516° E, elevation 1351 m a.s.l.; 3.49446° S, 102.63684° E, 1358 m a.s.l; respectively), collected on 16 July 2015 by E. N. Smith, U. Smart, and S. Sianturi. UTA A-65744, one adult male from Bengkulu Utara Regency, Road Maura Aman-Argamakmu (3.20576° S, 102.14232° E, 833 m a.s.l.), collected on 19 July 2015 by E. N. Smith, U. Smart, and G. Pradana. MZB Amph 16326–16327, two adult females and MZB Amph 16325, MZB Amph 16328, two adult males from Suro Lembak Village, Ujan Mas District, Kepahiang Regency, in the province of Bengkulu, Indonesia (3.59° S, 102.58° E, ca. 750 m a.s.l), collected on 20 July 2010 by A. Hamidy and Nurrohim. MZB Amph 16324, one adult female from Batu Ampar Village, Merigi District, Kepahiang Regency, in the province of Bengkulu, Indonesia (3.31° S, 102.33° E), collected on 18 July 2010 by A. Hamidy and Nurrohim.

Etymology. The specific epithet honors the military leader and hero Gadjah Mada, who in the fourteenth century unified the whole of the Nusantara (i.e., Malay Archipelago) under the Javanese rule of the Hindu Majapahit Empire.

Diagnosis. The new species is assigned to *Microhyla* because of having a small size (SVL < 30 mm), narrow head and mouth, tympanum hidden by skin, maxillary and vomerine teeth absent, and a reduced first finger length (Tschudi, 1838, Malkmus et al., 2002). *Microhyla gadjahmadai* **sp. nov.** is diagnosable from its congeners by having a medium size (for *Microhyla*, SVL adult males 18.2–21.3 mm, adult females 20.4–25.5 mm), stout body, a nostril–eyelid length of half the length of the snout, a single outer palmar tubercle, the tibiotarsal articulation reaching the center of eye, finger and toe tips dilated, median longitudinal grooves on dorsum, relatively reduced toe webbing (free of webbing: one and three quarter phalanges on outer surface of second toe, three phalanges on inner and outer surface of third toe, four phalanges on inner and outer surfaces of fourth toe, and two and three quarter phalanges on inner surface of fifth toe), and a thin-short dark temporal stripe over a wider cream stripe, extending from postorbital area to insertion of forelimb.

Description of holotype (measurements in mm). SVL 20.6; habitus stout; head as wide as long (5.8 vs 5.9); snout dorsally rounded; eye (2.0) shorter than snout (3.3), slightly longer than nostril to eyelid length (1.8); lore sloping and slightly convex; nostril distinct in lateral view, located below canthus rostralis, closer to tip of the snout (1.6) than to eye (1.8); interorbital distance (2.7) wider than internarial distance (2.2), and twice upper eyelid (1.1); tympanum hidden; supratympanic fold visible, extending from posterior corner of eye to forelimb insertion; vocal slits and vocal sacs present; dorsum and anal region with low tubercles; abdomen and inner surface of thigh smooth.

Forelimb short (FLL 12.2; 59.1% of SVL); fingers thin, free of web, with poorly developed skin fringes on both sides of fingers II–IV; finger tips weakly dilated, dorsally with median longitudinal groove; fingers lengths: **III** (2.8) > **IV** (1.9) > **II** (1.3) > **I** (0.9); single outer palmar tubercle (1.1), larger than inner (0.9); subarticular tubercles distinct and rounded, formula: 1, 1, 2, 2; nuptial pad absent.

Hindlimb long (43.1; 209.0% of SVL) about three times length of forelimb; tibia (13.0) longer than foot (10.9); tibiotarsal articulation reaching center of eye; toe III length 0.8; toe tips distinctly dilated, spatulate, dorsally with median longitudinal groove; web formula **I** 2–2¼ **II** 1¾–3 **III** 3–4 **IV** 4–2¾ **V**; subarticular tubercles prominent, somewhat oval, formula: 1, 1, 2, 3, 2; inner metatarsal tubercle oval, length (0.8) about half of first toe (2.0); outer metatarsal tubercle (0.6) smaller than inner.

Coloration. In life, dorsum light brown; cream vertebral line from snout to anal region, wider at snout tip; region from snout to mid supraorbital region cream; iris bright gold with faint black reticulation; a thin-short dark temporal stripe over a wider cream stripe, extending from postorbital area to insertion of forelimb; body dorsum with brown mark, bordered by a cream line, extending from between orbits to iliac area, laterally extending to cover posterior half of supraorbital area and suprascapular region, and extending posterolaterally from middorsum towards groin and from midsacral area towards waist; dark sepia lateral band extending from above arm to half length of flank, darker and boldly edged above, fading towards venter; two inconspicuous and thin dark bars on hindlimb, over thigh and leg; throat dark brown, with some cream peppering; chest white-cream; abdomen, inguinal area, and proximal ventral region of limbs translucent pink (Fig. 4A). In preservative, pattern did not change dramatically, only becoming darker (Figs. 5–6).

Variation. See Table 3 for morphometric data from 18 male and six female adults. Half of the examined specimens are darkly pigmented from throat to pectoral and the rest have same pattern with holotype. Stripe at lateral body: nine specimens with a stripe extends from above arm to groin and the rest to mid body. Dark bars on limbs: mostly with two clear dark bars and some specimens with one or two addition faded dark bars. Webbing formula was stable for the outer and inner side of fourth toe with four phalanges free, but slight variations are recognized in other positions e.g., in a female paratype (MZB Amph 16326), one half phalanges free on first toe, while male holotype (MZB Amph 26081) has two free phalanges. This variation is not consistent based on sex.

Range. The new species is currently known from Bengkulu (Rejang Lebong, Bengkulu Utara, and Kepahiang Regency), Lampung (Tanggamus and Lampung Tengah Regency), and South Sumatra (Muara Enim Regency and Pagar Alam City) province with elevation range from 700–1647 m a.s.l.

Comparisons. *Microhyla gadjahmadai* **sp. nov.** can be differentiated from genetically close members of the *M. achatina* group of Matsui et al. (2011) and Sumatran *Microhyla* as following characters: The new species differs from *M. achatina* by having thin-short dark stripe at temporal/postorbital region (vs. dark stripe strong and short/longer (almost reach up to shoulder) (Fig. 4A vs 4B) and N–EL half sized of SL (vs. N–EL more than half of SL) . From *M. orientalis* by having lateral band strong and clearly bordered above, also more developed fifth toe web (2.75 free phalages) [vs. a lateral dark band vaguely bordered, and less developed fifth toe web (3 free phalages)]. The new species differs from *M. borneensis* by larger body size, male SVL 18.2–21.3 mm (vs. 11.0–13.2 mm) and less developed toe webbing. Differs from *M. malang* and *M. mantheyi* by less developed toe webbing, with the fifth toe having two and three quarter phalanges free (vs. one phalange free). The new species differs from *M. heymonsi* Vogt by having two metacarpal tubercles and lacking loreal mask (vs. having three tubercles, a black band covering side of head, one or two pairs of black marks on sides of vertebral line, two black spots bisecting vertebral line). From *M. fissipes* Boulenger, *M. mixtura* Liu and Hu, *M. okinavensis* Stejneger, and *M. pulchra* (Hallowell), the new species differs by having dilated finger and toe tips with dorsal median longitudinal grooves (vs. digiti tips not dilated and grooves entirely absent or present only on toes). From *M. berdmorei* (Blyth), the new species differs by less developed toe webbing (vs. toe webbed to the tips). Finally, from *M. palmipes* and *M. superciliaris*, the new species differs by lacking a supraciliary tubercle, less developed toe webbing, and the presence of dorsal median grooves on digiti tips (vs. having a supraciliary tubercle, more developed toe webbing, and toe tips with circum marginal grooves).

Natural history. *Microhyla gadjahmadai* **sp. nov.** is distributed in southern Sumatra, inhabiting from primary forest, secondary forest and open area such as farming area (Bengkulu). The tadpole and advertisement call of this species are still unknown.

DISCUSSION

In a previous study of phylogenetic analysis of microhylids in Oriental region, Matsui et al. (2011) recognized three unidentified taxa nested within *Microhyla* group. One of them is *Microhyla* sp. 3 collected from Bengkulu and Lampung. *Microhyla* sp. 3 formed a clade with *M. achatina* supported by significant bootstrap values in all phylogenetic trees. Although in the same clade, *Microhyla* sp. 3 and *M. achatina* had large genetic distance (5.5–6.6%) (Matsui et al., 2011). In accordance with phylogenetic tree of Matsui et al. (2011), our analyses resolved the taxonomic status of *Microhyla* sp. 3. and we here described it as *Microhyla gadjahmadai* **sp. nov.** This species is a sister taxon to *M. achatina* with uncorrected

Table 3. Measurements of adult male and female specimens of *Microhyla gadjahmadai* **sp. nov.** and *Microhyla achatina*. SVL (Mean \pm 1SD, in mm) and medians of ratios (R) of other characters to SVL (R: % SVL), followed by ranges in parenthesis. See text for character abbreviations

Characters	<i>Microhyla gadjahmadai</i> sp. nov.			<i>Microhyla achatina</i>		
	Male (20)	Female(6)		Male (8)	Female (5)	
SVL	20.67 \pm 0.29 (18.20–21.32)	20.87 \pm 0.49 (20.37–25.51)		22.47 \pm 1.26 (21.70–24.31)	24.57 \pm 1.94 (20.56–27.96)	
RHL	29.72 (25.99–35.23)	28.79 (24.94–33.17)		29.89 (26.46–36.34)	26.31 (25.43–29.06)	
RHW	31.45 (26.84–41.38)	31.15 (28.15–36.08)		31.70 (26.95–37.63)	29.58 (28.25–32.72)	
REL	9.61 (8.58–11.46)	8.67 (7.05–11.53)		9.95 (9.36–12.14)	8.76 (7.40–9.34)	
RN-EL	7.56 (7.39–7.89)	7.49 (6.53–7.95)		8.19 (7.98–8.48)	9.74 (7.31–8.61)	
RIND	10.77 (8.98–12.43)	10.05 (8.94–11.56)		10.58 (9.30–11.61)	9.28 (7.90–10.31)	
RIOD	12.44 (10.29–14.84)	11.90 (10.74–13.63)		12.64 (11.52–14.26)	11.55 (8.94–12.39)	
RSL	14.56 (14.03–16.13)	14.36 (13.06–14.77)		14.46 (13.74–15.07)	14.01 (13.20–14.77)	
RUEW	5.62 (4.43–6.60)	5.31 (4.55–6.67)		5.60 (4.77–6.28)	4.84 (4.26–5.43)	
RFL	61.26 (51.15–68.13)	61.18 (53.15–65.82)		61.13 (55.54–64.57)	57.65 (51.43–61.16)	
RLAL	40.82 (35.25–45.88)	40.59 (37.04–43.10)		42.14 (39.20–46.26)	40.78 (37.09–42.85)	
RIPTL	3.89 (2.58–4.92)	4.17 (2.45–5.17)		3.74 (2.39–4.93)	3.39 (2.86–3.70)	
ROPTL	3.90 (3.18–5.59)	3.41 (2.98–6.58)		4.03 (2.63–5.29)	4.51 (3.91–5.39)	
RHLL	220.21 (192.21–260.98)	219.53 (169.65–259.96)		209.68 (184.62–224.39)	195.11 (183.37–214.40)	
RTL	65.86 (60.71–72.63)	65.27 (59.74–70.43)		60.28 (55.93–65.06)	57.40 (52.79–64.25)	
RFL	53.40 (49.72–60.26)	54.74 (48.53–56.53)		51.69 (44.92–56.77)	50.20 (42.53–57.48)	
RIMTL	5.12 (3.63–8.80)	4.78 (3.57–6.21)		5.26 (3.95–6.31)	4.37 (3.75–5.51)	
RITOEL	10.26 (8.11–12.78)	10.15 (8.83–12.15)		9.82 (8.56–11.65)	9.10 (8.76–10.36)	
ROMTL	5.21 (2.91–7.66)	4.54 (3.19–7.37)		5.79 (4.08–6.75)	5.43 (4.51–6.40)	
RHW/HL	5.34 (4.07–6.85)	3.76 (3.19–5.85)		4.94 (3.71–6.08)	4.53 (3.97–5.43)	
RN-EL/SL	2.49 (2.19–2.71)	2.42 (2.35–2.51)		2.53 (2.34–2.83)	2.31 (2.23–2.55)	
REL/SL	3.24 (2.37–4.01)	2.64 (1.95–3.49)		3.35 (2.62–3.86)	2.58 (2.07–3.43)	
RLAL/FLL	3.44 (3.00–3.91)	2.81 (2.63–3.54)		3.25 (2.80–3.78)	2.84 (2.58–3.66)	



Figure 4 A. In life, dorsolateral and ventral view of male holotype of *Microhyla gadjahmadai* **sp. nov.** (MZB Amph 26081) from Rejang Lebong Regency, in the province of Bengkulu, Sumatra, Indonesia (Photographs by E. N. Smith).



Figure 4 B. In life, dorsolateral and ventral view of *Microhyla achatina* (MZB Amph 24099) from Pandeglang Regency, in the province of Banten, Java, Indonesia (Photographs by E. N. Smith).



Figure 5. Dorsal, ventral, male holotype of *Microhyla gadjahmadai* **sp. nov.**—MZB Amph 26081 (SVL 20.61 mm) and female paratype—MZB Amph 16326 (SVL 25.51 mm). Scale bar = 5 mm. (Photographs by V.Y. Atmaja).

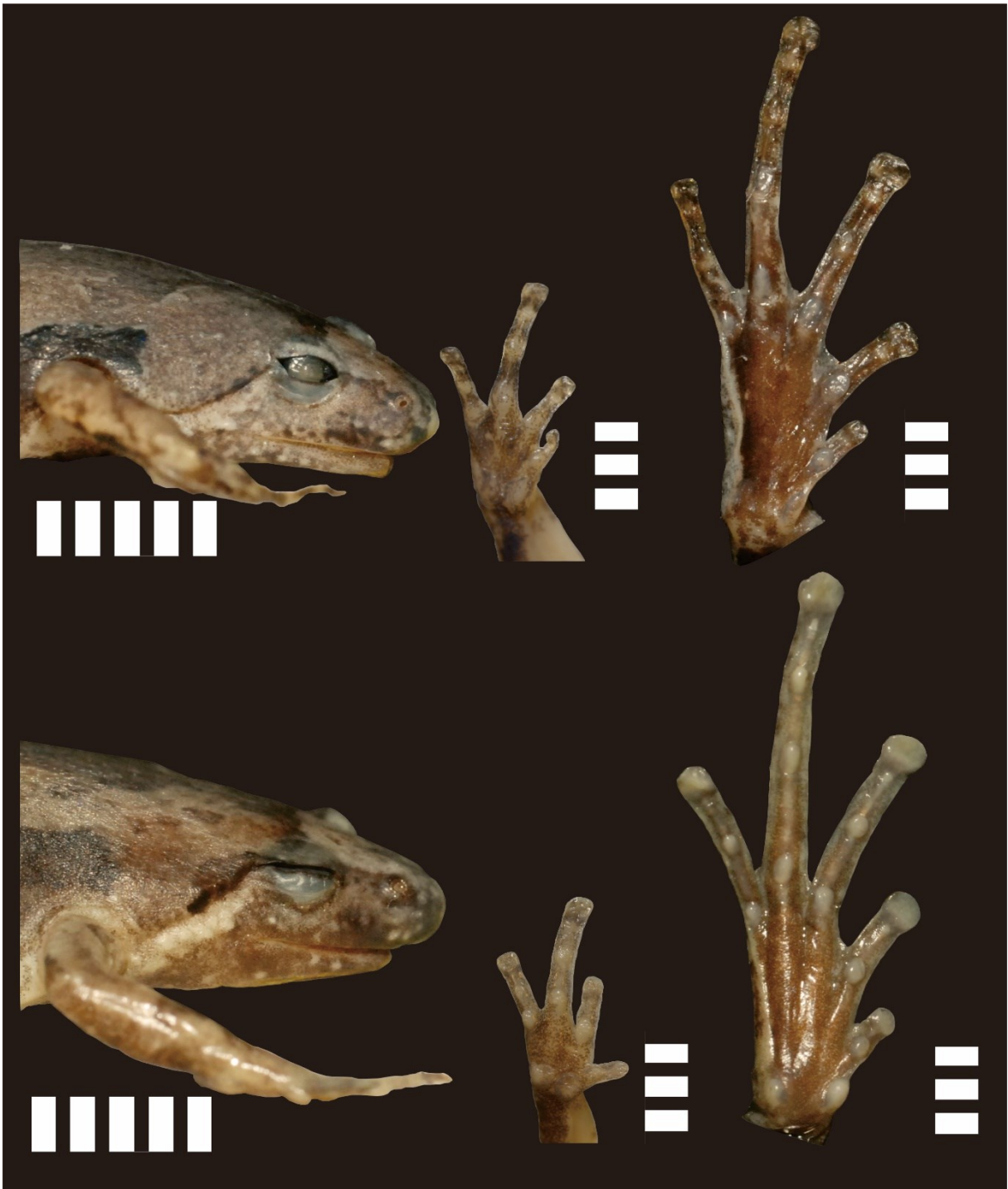


Figure 6. Lateral head, ventral right hand and ventral right foot views of holotype of *Microhyla gadjahmadai* sp. nov.—MZB Amph 26081 (SVL 20.61 mm) and female paratype—MZB Amph 16326 (SVL 25.51 mm). Scale bar = 5 mm. (Photographs by V.Y. Atmaja).

p-distance ranging from 4.8–6.3%. Together with *M. achatina*, *M. gadjahmadai* **sp. nov.** is closer to the *M. borneensis* subgroup (suggested in Matsui et al., 2013): *M. borneensis*, *M. malang*, *M. orientalis*, and *M. mantheyi* (8.0–9.4%) than to other Sumatran *Microhyla* (*M. berdmorei*, *M. fissipes*, *M. heymonsi*, *M. palmipes* and *M. superciliaris*).

Sumatra had been explored for its amphibian diversities for long decades, but new species are continuously discovered from this Island until now. At least two new endemic genera (*Sigalegalephrynus* and *Sumaterana*) and 14 species of frogs have been described from this island in the last five years (Matsui et al., 2014; Riyanto & Kurniati, 2014; Streicher et al., 2014; Hamidy & Kurniati, 2015; Smart et al., 2017; Wostl et al., 2017; Arifin et al., 2018a & 2018b; Munir et al., 2018). The finding of *Microhyla gadjahmadai* **sp. nov.** has contributed to increase the number of amphibian species in Sumatra to 95.45% of which (42 species) are endemic to the Island. The unique geological formation of Bukit Barisan Mountains may have a causal relationship with speciation and endemism across the region (Hamidy & Kurniati, 2015).

Over the two decades (from 1990–2010), tropical rain forest decreases by 40% in Sumatra Island (Margono et al., 2012). The species undergo climate change, habitat destruction (loss, degradation, and fragmentation) and resulting extinction of the species. Furthermore, the right conservation management plans are needed to improve the protection of habitat and species that have been or have not been described.

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APPENDIX 1. Comparative material examined

Microhyla achatina (Java, Indonesia, N = 13): Banten Province: Pandeglang Regency, Pulosari Mountain 6.32965° S, 105.96696° E, 682 m a.s.l.—MZB Amph 23095–23096 (female); West Java Province: Sukabumi Regency, Vicinity of Cisolok Hot spring, 6.93331° S, 106.45305° E, 99 m a.s.l.—MZB Amph 23097 (female); *Central Java Province*: Karanganyar Regency, Ngargoyoso District, Suku Temple, 7.63129° S, 111.1352° E, 1169 m a.s.l.—MZB Amph 27115 (male); Banyumas Regency, Baturaden District, Kemutuk Village, Baturaden Botanical Garden, 7.31306° S, 109.2334° E, 650 m a.s.l.—MZB Amph 27116–27118 (male); Banyumas Regency, Baturaden District, Kemutuk Village, Baturaden Botanical Garden, 7.31248° S, 109.23578° E, 724 m a.s.l. MZB Amph 27119–27120 (female); *East Java*: Semeru Mountain 8.00921° S, 112.26048° E, 1332 m a.s.l. —MZB Amph 27121–27124 (male);

Microhyla gadjahmadai **sp. nov.** (Sumatra, Indonesia, N = 23; referred specimens not included in type series): *Lampung*: Tanggamus Regency, Tanggamus Mountain, above Gisting, 5.42306° S, 104.69196° E, 1117 m a.s.l.—UTA A-65746 (male); Tanggamus Regency, Semaka District, Sukaraja Village, Taman Nasional Bukit Barisan Selatan, 5.26545° S, 104.21573° E—MZB Amph 3454 (male); Lampung Tengah Regency, Way Seputih District, 4.48307° S, 105.25599° E—MZB Amph 15290 (male); Tanggamus Regency, Semaka District, Sedayu Village, 5.30401° S, 104.2899° E—MZB Amph 15292 (male); Tanggamus Regency, Hill top above Data Rajan, 5.35986° S, 104.63287° E, 1103 m a.s.l.—MZB Amph 22387 (male); Tanggamus Regency, Ulubelu District, NE of town of Ngarip, 5.30636° S, 104.54826° E, 877 m a.s.l.—MZB Amph 22388 (male); Tanggamus Regency, Ulubelu District, NE of town of Ngarip, 5.31567° S, 104.54343° E, 837 m a.s.l.—MZB Amph 22392 (male); Tanggamus Regency, Hulubelu Mountain, 5.35895° S, 104.62708° E, 921 m a.s.l.—MZB Amph 22394, 22400 (male); Tanggamus Regency, Tanggamus Mountain, above Gisting 5.42306° S, 104.69196° E, 1117 m a.s.l.—MZB Amph 22395, 22397, 22399 (male); Tanggamus Regency, Semaka District, Sedayu Village, 5.30401° S, 104.2899° E—MZB Amph 15291, 15293 (female); *South Sumatera Province*: Maura Enim Regency, Patah Mountain, near Segamit Village, 4.21989° S, 103.471255° E, 1624 m a.s.l.—MZB Amph 26080 (male); Maura Enim Regency, Patah Mountain, near Segamit Village, 4.21715° S, 103.42165° E, 1826 m a.s.l.—UTA A-65745 (female); Pagar Alam City, Dempo Mountain, 4.01386, 103.1876, m a.s.l —MZB Amph 26079 (female).