



UNVEILING MISIDENTIFICATION OF FORMERLY REPORTED *Microhyla fissipes* BOULENGER, 1884 (ANURA: MICROHYLIDAE) WITH NOTES ON TWO *Microhyla* SPECIES FROM SUMATRA

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Abstract

Sumatra is widely recognized as a land bridge for the dispersal of amphibians between mainland Asia and the rest of the Indonesian Archipelago. Currently, six species of *Microhyla* are recognized in Sumatra: *M. gadjahmadai*, *M. nakkavaram*, *M. palmipes*, *M. sundaica*, *M. superciliaris*, and *M. sriwijaya*. Pradana *et al.* (2017) identified some *Microhyla* specimens from Sumatra as *M. fissipes* using molecular analysis (partial sequence of 16S mtDNA). Meanwhile, Yuan *et al.* (2016) restricted the distribution of *M. fissipes* to the northeast of the Red River Valley and Taiwan. In this study, we have revised the identification of ‘*Microhyla* sp. aff. *fissipes*’ Sumatra *sensu* Pradana *et al.* (2017) to *M. mukhlesuri*, based on both molecular and morphological analyses. Additionally, we report the first site records of *M. mantheyi* and *M. butleri* from Sumatra. We reconfirm the diagnostic characters based on their original descriptions and report on the morphological variation of the Sumatran populations of these three species.

Keywords: 16S mtDNA, morphology, *M. butleri*, *M. mantheyi*, *M. mukhlesuri*, systematics

Introduction

Sumatra is one of the Greater Sunda Islands of Indonesia. It has a unique geographic amalgamation of the four continental blocks of East Malaya, Sibumasu, West Sumatra, and Woyla terranes, influenced by complex tectonic activity (Voris 2000, Hall 2012, 2013). These processes have created diverse ecosystems in mountains, plateaus, and valleys, such as tropical rainforests, peat swamps, and mangrove forests, providing a wide variety of habitats for flora and fauna (Inger & Voris 2001, Lohman *et al.* 2011). During glacial periods, low sea levels allowed for the formation of a land bridge between Sumatra and mainland Asia, which enabled migration and genetic exchange between amphibian populations (Voris 2000, Brown & Guttman 2002).

The members of the genus *Microhyla* Tschudi, 1838 occur at various altitudes and habitats, from tropical rainforests to rice fields, and in terrestrial and sub-fossorial microhabitats (Poyarkov *et al.* 2014, Gorin *et al.* 2021). Discoveries of new *Microhyla* species have increased over the past decade. Their tiny size and cryptic morphology have led to an unclear taxonomic position for some widely distributed species. Indonesia currently has ten species of *Microhyla*, and six of them are found in Sumatra: *M. gadjahmadai* Atmaja, Hamidy, Arisuryanti *et al.*, 2018; *M. nakkavaram* Garg, Sivaperuman, Gokulakrishnan *et al.*, 2022; *M. palmipes* Boulenger 1897; *M. sundaica* Trofimets, Dufresnes, Pawangkhanant *et al.* 2024; *M. superciliaris* Parker 1928; and *M. sriwijaya* Eprilurahman, Hamidy, Smith *et al.*, 2021a (Parker 1934, Eprilurahman *et al.* 2023, Frost 2024, Trofimets *et al.* 2024).

Pradana *et al.* (2017) identified five *Microhyla* specimens from Sumatra closer to the Chinese species, *M. fissipes* based on 1.7–1.9% *p*-distances using molecular analysis (partial sequences of 16S mtDNA). Unfortunately, they concluded this finding with neither a detailed morphological examination nor an elaborating discussion on the work of Yuan *et al.* (2016). Yuan *et al.* (2016) restricted the distribution of *M. fissipes* to regions northeast of the Red River Valley as far north and east as Taiwan. Records from areas west and south of this range are considered to be *M. mukhlesuri* Hasan, Islam, Kuramoto *et al.* 2014.

The taxonomic evaluation of widely distributed *Microhyla* species in the Sundaic region has resulted in new species descriptions

for populations outside of their original biogeographic range. For instance, in Sumatra the populations of *M. heymonsi* have been revised to *M. nakkavaram* (see Eprilurahman *et al.* 2023), and *M. berdmorei* has been revised to *M. sundaica* (see Trofimets *et al.* 2024). In this study, we re-examined the Sumatran specimens formerly identified as *M. fissipes* by Pradana *et al.* (2017) [hereafter, *M. sp. aff. fissipes* Sumatra]. We also conducted a detailed exploration and identification of other Sumatran *Microhyla* specimens, which has led us to report here on new locations of two other *Microhyla* species on this island.

Material and Methods

Sampling. One specimen of *Microhyla mantheyi* (MZB Amph 31522) was collected by R.D. Eki Aprilia in June 2017 at the Hutan Harapan National Forest in Jambi. Thirty-seven specimens of three *Microhyla* species (*M. mantheyi* = 2; *M. butleri* = 15; *M. sp. aff. fissipes* Sumatra = 20) were collected in Sumatra between 2013 and 2016 under the NSF-funded project “Exploration and Speciation in the Volcanoes of the Indonesian Ring of Fire: A Large Scale Inventory of the Herpetofauna of the Highlands of Sumatra and Java (DEB-1146324)”, involving personnel and students from Brawijaya University, Broward College, Museum Zoologicum Bogoriense (MZB), Research Centre for Biosystematics and Evolution (National Research and Innovation Agency), and the University of Texas at Arlington Amphibian and Reptile Diversity Research Center (ARDRC). All the specimens examined were deposited at the MZB or UTA.

Phylogenetic Analysis. We sequenced three new *Microhyla* specimens (*M. mantheyi* = 1; *M. butleri* = 2) for this study from Sumatra (Fig. 1). Fragment sequences of 16S rRNA mtDNA were obtained following extraction and PCR methods detailed by Matsui *et al.* (2011). The sequencing results were checked manually using the Chromas Pro Software (Technelysium Pty Ltd., Tewantin, Australia). The final sequences were then aligned with those of other congeners available through the GenBank database (479 sequences; Sup. Table 1), using MEGA 11 (Tamura *et al.* 2021) with default parameters. Next, we reconstructed phylogenetic trees with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses to visualize the position of our new sequences within the *Microhyla* species complex. The best evolution model for

both ML and BI was selected using Kakusan 4 (Tanabe 2011), resulting in the Akaike Information Criterion (AIC) selecting the GTR+G4 model. ML analysis was conducted using the IQ-Tree Web Server (Trifinopoulos *et al.* 2016, <http://iqtree.cibiv.univie.ac.at>), with the 'auto' substitution model option and the 1000 Ultrafast bootstrap analysis. BI analysis was then performed using Mr. Bayes ver. 3.2 (Ronquist *et al.* 2012) with the generation replication settings set according to the sample requirements (up to the standard deviation value of the split frequencies below 0.01). Next, results were visualized using Figtree v1.4.3 (Rambaut 2016). The ML bootstrap values on the tree branches were considered significant if >70% (Hillis & Bull 1993). In the BI analysis, nodes with a BPP >95% were considered statistically significant (Leaché & Reeder 2002). We also calculated the genetic uncorrected *p*-distances between specimens using MEGA 11 (Sup. Tables 2, 3).

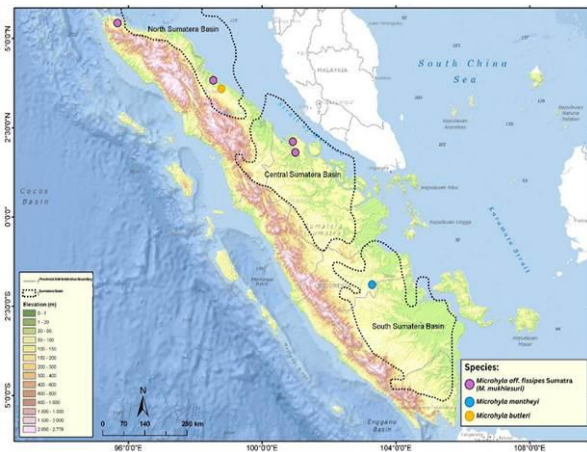


Figure 1. Sumatra map showing sampling locations for molecular analysis.

Morphological Analysis. We examined a total of 38 adult specimens of *Microhyla* consisting of: *M. butleri*, *M. mantheyi*, and *M. sp. aff. fissipes* (including specimens from Pradana *et al.* 2017). The specimens were fixed in 10% formaldehyde, stored in 70% ethanol, and later deposited at MZB-BRIN or UTA-ARDRC. We made 29 measurements using a dial calliper, recording to the nearest 0.1 mm. The following measurements from Matsui (1984) and Hasan *et al.* (2014) were taken (some modified; *M. sp. aff. fissipes* Sumatra from previous work re-measured to avoid observer data bias in the morphometric analyses): (1) snout–vent length (SVL), (2) head length (HL), (3) head width (HW), (4) snout to nostril length (S–NL), (5) nostril to eye length (NE), (6) snout length (SL),

(7) internarial distance (IND), (8) eye length (EL), (9) interorbital distance (IOD), (10) upper eyelid width (UEW), (11) forelimb length (FLL), (12) lower arm length (LAL), (13) forearm width (FAW), (14) hand length (HAL), (15–18) length of 1st to 4th finger (F1–F4), (19) hindlimb length (HLL), (20) femur length (FeL), (21) tibia length (TiL), (22) foot length (FoL), (23) inner metatarsal tubercle length (IMTL), (24) outer metatarsal tubercle length (OMTL), (25–29) lengths of 1st to 5th toe (T1–T5). We used the toe-webbing formula description method from Savage & Heyer (1967), with modifications made by Myers & Duellman (1982).

The measured samples of Sumatran *M. mantheyi* and *M. butleri* are presented in Sup. Tables 4, 5. The *M. sp. aff. fissipes* Sumatra, *M. fissipes* s.str. China, and *M. mukhlesuri* from Bangladesh as stated in Hasan *et al.* (2014), were compared using a one-way analysis of variance (ANOVA) and Principal Component Analysis (PCA). We only used 29 characteristics in this analysis to adjust to the data available from Hasan *et al.* (2014). The morphological analyses employed followed Chan & Grismer (2021) as each characteristic was adjusted with an allometric formula outlined by Thorpe (1975). We used Multivariate Analysis of Variance (MANOVA) to determine whether male and female individuals should be analysed together. All analyses were performed in Rstudio ver. 2023.12.0+369 (R Core Team 2023) using additional packages FactoMineR (Husson *et al.* 2024) and factoextra (Kassambara & Mundt 2020) for PCA analysis.

Results

New records of *Microhyla butleri* and *M. mantheyi*. The final alignment (479 bp; 304 conserved sites; 175 variable sites) consisted of 92 sequences of other *Microhyla* species and four sequences of *Nanohyla*, as outgroups. Figure 2 presents the phylogenetic tree from the ML analysis. The ML analysis resulted in a phylogeny tree with nucleotide frequencies of A: 0.317, C: 0.240, G: 0.208, T: 0.235, and a gamma shape of 0.775. The BI analysis yielded a similar topology, with a calculated overall nucleotide frequency of A: 0.347, C: 0.226, G: 0.192, T: 0.232, and a gamma shape of 0.547. The list of the sequences used in this study and their GenBank accession numbers are provided in Sup. Table 1. The phylogenetic tree obtained in this study aligns with the result of Garg *et al.* (2019) and Gorin *et al.* (2020).



Figure 2. Maximum likelihood phylogenetic tree of *Microhyla* species based on partial sequence of 16S rRNA mtDNA (479 bp). The values on the branches represent the bootstrap values for ML and posterior probabilities for Bayesian Inference, above and below diagonal, respectively.

The tree consisted of an outgroup and seven *Microhyla* groups: *M. achatina* group (blue line; ML:90 BPP: 0.92), *M. fissipes* group (purple line; ML: 99 BPP 1.00), *M. berdmorei* group (yellow line; ML: 97 BPP 1.00), *M. ornata* group (red line; ML: 97 BPP 1.00), *M. butleri* group (orange line, ML: 99 BPP 1.00), *M. superciliaris* group (green line; ML: 99 BPP 1.00), and *M. palmipes* group (cyan line).

A *Microhyla* specimen, MZB Amph 31522 from the Hutannya Harapan National Forest (Jambi), formed a clade with *M. mantheyi* from Malaysia and Myanmar (ML: 100; BPP: 1.00) (Fig. 2, OTU coloured blue) in the *M. achatina* group. The uncorrected *p*-distances based on the 16S mtDNA fragment (Sup. Tables 2, 3) between the Jambi specimen and other populations of the same species ranged between 0.84 and 1.69.

Meanwhile, the Jambi specimen differs from other *M. achatina* group species between 5.06 and 11.39 (in accordance with other populations of *M. mantheyi* vs other *M. achatina* group species 5.06–11.42). Therefore, the molecular analyses indicate that MZB Amph 31522 corresponds to *M. mantheyi*.

Next, two specimens from Deli Serdang, North Sumatra (MZB Amph 23116 and 24059) formed a clade with *M. butleri* from other populations (ML: 100 BPP: 1.00) (Fig. 2, OTU coloured orange) in the *M. butleri* group. The uncorrected *p*-distances based on the 16S mtDNA fragment (Sup. Tables 2, 3) between the North Sumatran specimens and other *M. butleri* ranged between 0.42 and 2.13 (between 7.01 and 7.22 with other *M. butleri* group species). These results suggest that MZB Amph 23116 and 24059 are *M. butleri*. The genetic distance between the *M. mantheyi* and *M. butleri* populations in Sumatra to other populations of the same species are relatively small (<3%) and the phylogenetic tree shows well-supported monophyletic species and group branches for these (Fig. 2).

Evaluation of *M. sp. aff. fissipes sensu Pradana et al. (2017) occur in Sumatra.* During the Sumatra expedition (2013–2016), some *Microhyla* specimens were identified as *M. fissipes* based on general morphology. Then, in 2017, Pradana *et al.* analysed the relationships of *Microhyla* from Sumatra using a 16s rRNA mtDNA fragment and concluded that *M. fissipes* from Sumatra were more closely related to *M. fissipes* (AB201185) from China (1.7–1.9%) than to *M. mukhlesuri* (AB543608–09) from Bangladesh (2.3–2.5%). However, Pradana *et al.* (2017) did not follow up with morphological examination of the specimens. *Microhyla fissipes* was first described from Taiwan (China) by Boulenger in 1884 and was previously considered to be a species distributed across Southeast Asia. However, Yuan *et al.* (2016) found that *M. fissipes* is distributed only in the northeast of the Red River Valley and Taiwan. Therefore, studies on *M. fissipes* populations outside of these locations are deemed necessary. We re-examined the Sumatran *M. fissipes* specimens published by Pradana *et al.* (2017) using molecular and morphological analyses. Figure 2 (OTU coloured purple) shows that the *M. sp. aff. fissipes* Sumatra sequences LC213133–36 and LC213145 are nested within *M. mukhlesuri* and are separated from *M. fissipes* s.str. China (ML: 95; BPP: 1.00). Moreover, the

uncorrected *p*-distances (%) between *M. sp. aff. fissipes* Sumatra and other *M. mukhlesuri* ranges between 0.42 and 2.54, and those to *M. fissipes* s.str. China from 1.69 to 1.91. These results align with those of Pradana *et al.* (2017), where the genetic distances between *M. fissipes* s.str. China and *M. sp. aff. fissipes* Sumatra are relatively low, <3% (1.70–1.90), and within the variation found between *M. fissipes* s.str. China and *M. mukhlesuri* from 1.27 to 2.97. Given the confusing variation, we conducted statistical analyses on morphological characteristics to confirm the distinctiveness of *M. sp. aff. fissipes* Sumatra.

We analysed the morphological characters of *M. sp. aff. fissipes* Sumatra, *M. mukhlesuri* from Bangladesh, and *M. fissipes* s.str. China (morphological data available in Hasan *et al.* (2014). Male and female individuals were analysed together as they showed no significant difference in a MANOVA analysis (*p*-value 0.265 > 0.05). Based on a One-Way ANOVA analysis, of 29 characters, 23 had a mean value that was significantly different between groups (Sup. Table 7). Therefore, we conducted the post hoc test Tukey HSD to compare each group: *M. sp. aff. fissipes* Sumatra vs. *M. fissipes* s.str. China had differences in HL, HW, EL, IND, IOD, S-NL, UEW, HLL, TiL, FoL, F1, F2, F3, F4, T2, T3, T4, and T5; *M. sp. aff. fissipes* Sumatra vs. *M. mukhlesuri* had differences in HW, EL, IND, IOD, S-NL, UEW, FLL, LAL, HLL, FeL, HAL, F1, F4, T1, and T4; and *M. fissipes* s.str. China vs. *M. mukhlesuri* had differences in HL, HW, IND, S-NL, UEW, FLL, LAL, HLL, TiL, FoL, FeL, HAL, F1, F2, F3, F4, T1, T2, T3, T4, and T5. The differing characters between the groups, based on the ANOVA, are presented in Sup. Table 7.

The Principal Component Analysis (Fig. 3) resulted in three PCs with a cumulative variance of 66.94% (PC1: 41.61%; PC2: 19.14%; PC3: 6.19%) (Sup. Table 8). We visualized the scatter plot of each specimen based on PC1 vs. PC2 and PC1 vs. PC3 (Fig. 3A–B). Interestingly, the *M. sp. aff. fissipes* Sumatra, *M. fissipes* s.str. China, and *M. mukhlesuri* form clusters that are distinct from each other. *Microhyla mukhlesuri* are clustered in quadrant III (Fig. 3A) based on the HW and HL, and *M. sp. aff. fissipes* Sumatra are clustered in quadrant I and II (Fig. 3A) based on the SL, S-NL, IND, UEW, and FeL. Meanwhile, the *M. fissipes* s.str. China is clustered in quadrant IV (Fig. 3A) based on the EL, TiL, FLL, and HLL.

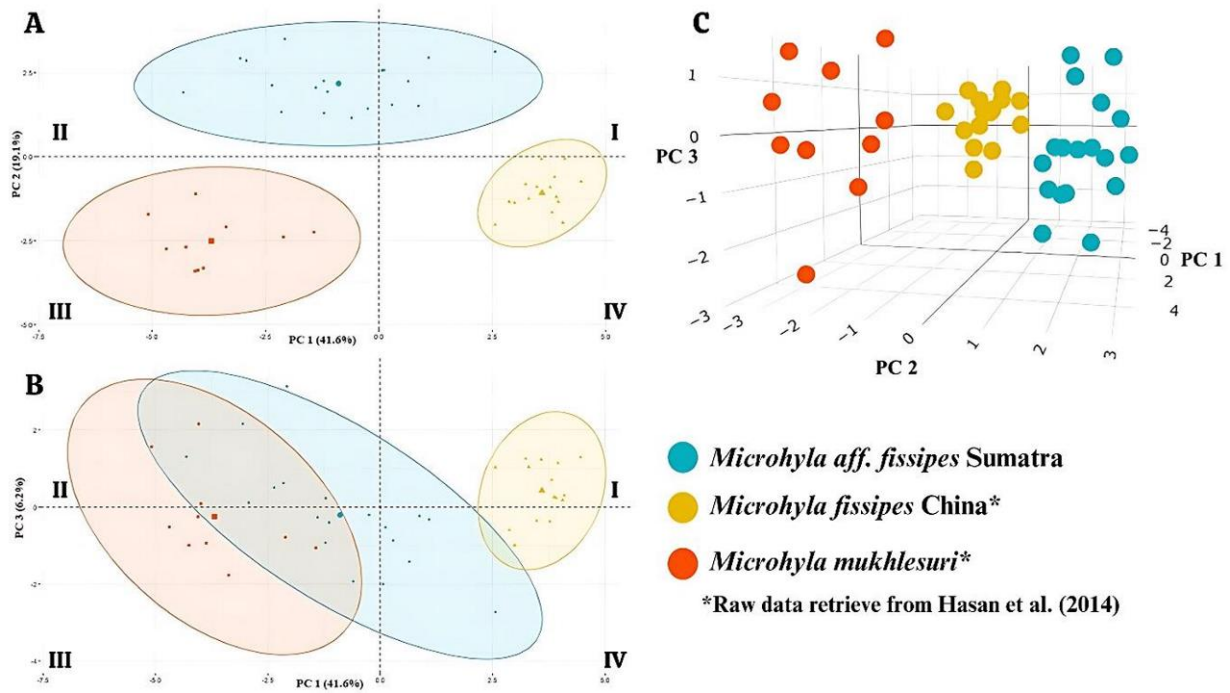


Figure 3. Scatter plots of Principal Component Analysis (PCA) of morphometric characteristics for populations of *Microhyla* sp. aff. *fissipes* Sumatra, *M. fissipes* s.str. China, and *M. mukhlesuri* from Bangladesh. The graph displays (A) PC 1 vs. PC 2 (60.7% of variance); (B) PC 1 vs. PC 3 (47.80% of variance); and (C) PC 1 vs. PC 3 (66.94% of variance).

Based on comparative morphological data in Sup. Table 6, several characteristics differentiate the three groups. The head size of *M. fissipes* s.str. China is more elongated than wide (HL: 19.0–27.4 mm and HW: 26.0–35.9 mm), in contrast to the heads of the *M. sp. aff. fissipes* Sumatra (HL: 25.8–32.7 mm and HW: 25.3–34.3 mm) and *M. mukhlesuri* (HL: 32.7–46.6 mm; HW: 30.3–45.3 mm), whose head width and length are almost equal. The eye length (EL) of the *M. sp. aff. fissipes* Sumatra (9.1–13.5 mm) is the largest compared to the others (*M. fissipes* s.str. China: 7.9–10.2 mm; *M. mukhlesuri*: 8.1–10.0 mm). *Microhyla mukhlesuri* (3.3–5.4 mm) has the shortest snout to nostril length (S-NL), followed by the *M. sp. aff. fissipes* Sumatra (4.9–6.7 mm) and *M. fissipes* s.str. China (6.8–6.9 mm). Lastly, the *M. sp. aff. fissipes* Sumatra has the longest hindlimb length (HLL) (156.2–204.3 mm), followed by *M. mukhlesuri* (147.3–174.6 mm) and *M. fissipes* s.str. China (136.5–168.7 mm).

Furthermore, the ANOVA analysis and PCA indicate that the three groups can be distinguished by their morphometric characters despite their small genetic distance values. Similar cases have also been found in other *Microhyla* species. Matsui & Tominaga (2020) described *M. kuramotoi* from the Southern

Ryukyu Islands (Japan) as a new species, although it has <3% genetic distance from *M. mixtura* (1.77%) and *M. beilunensis* (2.15%) from China (based on partial sequence of 16S gene [520 bp]). Despite the small genetic distance, several lines of evidence proved *M. kuramotoi* is a different species, such as the robustness of the ML and BI phylogenetic trees (12S & 16S gene; 869 bp), the significant differences from the morphometric analysis, and geographical distribution. Another example is *M. dabiieshanensis* from Dabie Mountain (China), which was described as a new species by Zhang *et al.* (2022). It has a small genetic distance from other microhylids, namely *M. mixtura* (2.4%) from China and *M. okinavensis* (2.0%) from the Central Ryukyu Islands. Like *M. kuramotoi*, *M. dabiieshanensis* differs significantly from other microhylids in bioacoustics characteristics.

A genetic distance of 3% in the mitochondrial 16S rRNA gene has been suggested as a practical benchmark for identifying distinct evolutionary lineages among amphibians (Fouquet *et al.* 2007). However, employing a threshold value is considered arbitrary, since it may conflict with other species definitions, such as the evolutionary species concept. The extent of intraspecific genetic divergences varies significantly between lineages due to the diverse

factors influencing population-level divergences (Whitlock 2003, Padial *et al.* 2009). Meanwhile, morphological divergence can occur for many reasons and is not always in proportion with genetic differentiation (e.g., phenotypic plasticity). Therefore, we consider the *M. sp. aff. fissipes* Sumatra to be *M. mukhlesuri*, based on our analyses: the phylogenetic tree topology where the *M. sp. aff. fissipes* Sumatra is nested in the *M. mukhlesuri* group (ML/BI: 95/1.00) and separated from true *M. fissipes*, the p-distance (%) of *M. sp. aff. fissipes* Sumatra vs. *M. mukhlesuri* ranges between 0.42 and 2.54, and the geographic restriction of *M. fissipes* s.str. China to only be found in the northeast of the Red River Valley in China and Taiwan. Further molecular (longer sequences and multiple genes) and bioacoustics analyses are required to resolve the group separation between the Sumatran population and other *M. mukhlesuri* populations.

After confirming the occurrence of *M. mantheyi* and *M. butleri* in Sumatra (based on molecular data), the following section describes the morphology of our specimens and compares it to their original descriptions. This section also provides a comprehensive description of the Sumatran *M. mukhlesuri*.

Taxonomy

M. mantheyi Das, Yaakob, and Sukumaran 2007 (Figs. 4, 5; Sup. Table 4)

Manthey's Narrow-mouthed Frog

Type locality. The road between Jemaluang and Kahang (02°16'N, 103°52-36'E), Johor, Malaysia.

Material examined. Three adult males (deposited at MZB and UTA) from: Jambi Province: *Kabupaten Kerinci*: Gunung Kunyit, 2.25181°S, 101.50123°E, 1242 m a.s.l.—MZB Amph 22398 (male, ENS 16051) and UTA A-66568 (male, ENS 16052, formerly MZB Amph 24021), collected on 24 June 2013 by Elijah Wostl, Eric N. Smith, Wahyu Trilaksono, and Gabriel Barraza; *Kabupaten Batang Hari*: Kecamatan Bajubang, Desa Bungku, Hutan Harapan National Forest [c.a. 1.90066°S, 103.25709°E, 68 m a.s.l.]—MZB Amph 31522 (male), collected on June 2017 by Eki Aprilia R. D.

Redescription. The *M. mantheyi* specimens from Sumatra align with the diagnostic characteristics of *M. mantheyi* described by Das, Yaakob, and Sukumaran (2007) based on the

following characteristics: male SVL 14.4–17.3 mm (vs. 15.0–29.2 mm); light brown snout region and darker dorsal part of the body (vs. cream coloured top of snout); a thick dark strip laterally on body, from the upper part of the arm fading to the inguinal region (vs. a dark flank stripe on the entire lateral region of the body); a thin white vertebral stripe (vs. narrow vertebral stripe); snout length (SL) 2.8–3.0 mm (vs. 2.6–3.7 mm); toe web formula $\text{I0-I110-2}\frac{3}{4}\text{III2-2IV2-0V}$ (vs. webbing below level of penultimate subarticular tubercle of toe IV); and dark marks above the vent (vs. marks above the vent).

Some variation was found in our specimens as compared to the original description, such as, a cream postocular stripe extending to the anterior axillary region and bordered by a dark stripe above (vs. pale postocular stripe with a dark posterior edge); cream interorbital mark continuing to the upper eyelid and posteriorly bordered dark (vs. a pale interorbital mark); a single dark spot on the mid-dorsum of all specimens; and head longer than wide HW/HL = 0.9 (vs. head wider than long HW/HL = 1.4). This species has only been recorded in the province of Jambi in Indonesia, from Gunung Kunyit, Kabupaten Kerinci, at 1242 m a.s.l. to only 68 m in a.s.l. at the Hutan Harapan National Forest. This species is not reported for Indonesia in the GBIF.org database.

M. butleri Boulenger 1900 (Figs. 4, 5; Sup. Table 5)

Butler's Narrow-Mouthed Frog

Type locality. Larut Hills at 4,000 feet, Perak, Malaysia.

Material examined. Ten adult males and 5 adult females (deposited at MZB and UTA). North Sumatra Province: *Kabupaten Deli Serdang*: Kecamatan Pancur Batu, Desa Sei Glugur, 3.52007°N, 98.57333°E, 54 m a.s.l.—MZB Amph 23115–23116 (males, ENS 15271, 15273), MZB Amph 23123 (male, ENS 15339), MZB Amph 24059–24060 (females, ENS 15334, 15336), MZB Amph 24062 (female, ENS 15340), MZB Amph 33628 (male, ENS 15272), UTA A-66563 (female, ENS 15330, formerly MZB Amph 33628), UTA A-66565 (male, ENS 15341, formerly MZB Amph 23124), UTA A-66562 (male, ENS 15274, formerly MZB Amph 24058), UTA A-66564 (male, ENS 15337, formerly MZB Amph 33629), collected on 10

January 2014 by Umilaela Arifin, Irvan Sidik, Muhammad Irfan Lubis, Wahyu Trilaksono, Kyle A. O'Connell, Utpal Smart, Elijah Wostl, and Eric N. Smith; *Kota Medan*: Vicinity of Kuala Namu Airport, 3.61447°N, 98.85047° E, 7 m a.s.l.—MZB Amph 24064–24065, UTA A-66566–67 (male, formerly MZB Amph 24063; female, formerly MZB Amph 23119), collected on 7 January 2014 by Elijah Wostl, Wahyu Trilaksono, Kyle A. O'Connell, and Irvan Sidik.

Redescription. The *M. butleri* specimens from Sumatra align with the original descriptions of the holotype of *M. butleri* provided by Boulenger (1900) and data provided by Poyarkov *et al.* (2014) and Nguyen *et al.* (2019) for Vietnamese specimens: SVL 15.3–20.2 mm in males and 19.5–23.4 mm in females (vs. 20.0–25.0 mm in males and 21.0–26.0 in females); body slender; snout rounded; EL/SL ratio 0.71–0.89 in males and 0.73–0.98 in females (vs. snout as long as orbit); IOD/UEW ratio 1.36–1.96 in males and 1.35–2.01 in females (vs. interorbital space broader than upper eyelid); fingers and toes rounded and dilated (vs. tips dilated into small but well developed disks); 1st finger/2nd finger ratio 0.67 (vs. 1st finger much shorter than 2nd); toe web formula $\text{I}1\frac{3}{4}\text{--}2\frac{1}{2}\text{II}1\frac{3}{4}\text{--}3\text{III}2\frac{1}{4}\text{--}3\frac{1}{4}\text{IV}3\frac{1}{4}\text{--}2\text{V}$ (vs. webbing basal, $\text{I}2\text{--}2\frac{1}{2}\text{II}1\frac{3}{4}\text{--}3\text{III}2\frac{1}{3}\text{--}3\frac{1}{2}\text{IV}3\frac{1}{2}\text{--}2\frac{1}{4}\text{V}$); two metatarsal tubercles small, with IMTL/FL ratio 0.06–0.09 in males and 0.07–0.09 in females (vs. 2 very small metatarsal tubercles); TiL/SVL ratio 0.51–0.62 in males and 0.54–0.63 in females (vs. tibia $\frac{1}{2}$ length of head and body); venter cream from throat to belly, pinkish chin and limbs; white middorsal line absent; dorsal region light brown with dark-brown hourglass bordered by beige (vs. teddy bear dark marking bordered with a light colour).

Variation was found in our specimens when comparing to the original description, such as tuberculate skin over dorsum of body and limbs (vs. smooth skin or with smooth warts); dark blotch from behind eye to flank (vs. lighter flecks or red); no distinct small scarlet spot on body flanks (vs. some small scarlet spots on the sides); white to cream spot (forming white line) behind eye to front of forelimb (vs. whitish oblique streak from eye to base of forelimb); ventral area white to pinkish without dark speckles (vs. whitish beneath, throat and breast speckled with dark brown). *Microhyla butleri* has only been recorded in Indonesia from the province of North Sumatra, near the city of Medan, and is not reported from Indonesia in the GBIF database.

M. mukhlesuri Hasan, Islam, Kuramoto, Kurabayashi & Sumida 2014
(Figs. 4–6; Sup. Table 6)

Mukhlesur's Narrow-mouthed Frog

Type locality. Raozan, Chittagong (22° 35' N, 91° 55' E, > 9 m asl.), Bangladesh.

Material examined. Fourteen adult males and five adult females (deposited at MZB and UTA) from: Aceh Province: *Kabupaten Aceh Barat*: Grand Hotel Nagan Raya, 4.11294°N, 96.29002°E, 16 m a.s.l.—UTA A-66560 (female, ENS 20096), collected on 27 May 2016 by Eric N. Smith and Ahmad Muammar Kadafi; *Kabupaten Aceh Tenggara*: Gunung Bandahara, 3.63368°S, 97.72611°E, 672 m a.s.l.—MZB Amph 33626 (male, ENS 20904), collected on 11 June 2016 by Utpal Smart, Munir Misbahul, and Ahmad Muammar Kadafi; Gunung Mountain, 3.63384°N, 97.71634°E, 453 m a.s.l.—UTA A-66561 (male, ENS 20917), collected on 12 June 2016 by Utpal Smart, Munir Misbahul, and Ahmad Muammar Kadafi; Ketambe District, Lawe Penanggalan, 3.63509°N, 97.71955°E, 359 m a.s.l.—MZB Amph 33627 (male, ENS 21282), collected 2016; North Sumatra Province: *Kota Medan*: Road to Kuala Namu Airport, 3.59953°N, 98.84229°E, 13 m a.s.l.—UTA A-66556 (female, ENS 15187, formerly MZB Amph 23551), MZB Amph 23552–23554 (male and two females, ENS 15189, ENS 15191, ENS 15193, respectively), collected on 7 January 2014 by Umilaela Arifin, Eric N. Smith, and Utpal Smart; Medan–Belawan Highway, 3.70631°N, 98.68171°E, 7 m a.s.l.—UTA A-66557 (male, ENS 15249, formerly MZB Amph 23556), MZB Amph 23557–23558 (males, ENS 15251, ENS 15253), collected on 8 January 2014 by Eric N. Smith, Kyle A. O'Connell, and Irvan Sidik; Vicinity of Kuala Namu Airport, 3.62441°N, 98.85493°E, 10 m a.s.l.—MZB Amph 23560 (male), collected on 7 January 2014 by Elijah Wostl, Wahyu Trilaksono, Kyle A. O'Connell, and Irvan Sidik; *Kabupaten Deli Serdang*: Sei Glugur Village, 3.52007°N, 98.57333°E, 54 m a.s.l.—UTA A-66558, (male, ENS 15331, formerly MZB Amph 23559), collected on 10 January 2014 by Umilaela Arifin, Irvan Sidik, Muhammad Irfan Lubis, Wahyu Trilaksono, Kyle A. O'Connell, Utpal Smart, Elijah Wostl, and Eric N. Smith; *Kabupaten Labuhanbatu Utara*: Along Medan–Rantau Prapat highway, 2.43344°N, 99.6674°E, 19 m a.s.l.—UTA A-66559 (male, ENS 19544,

Plate 25

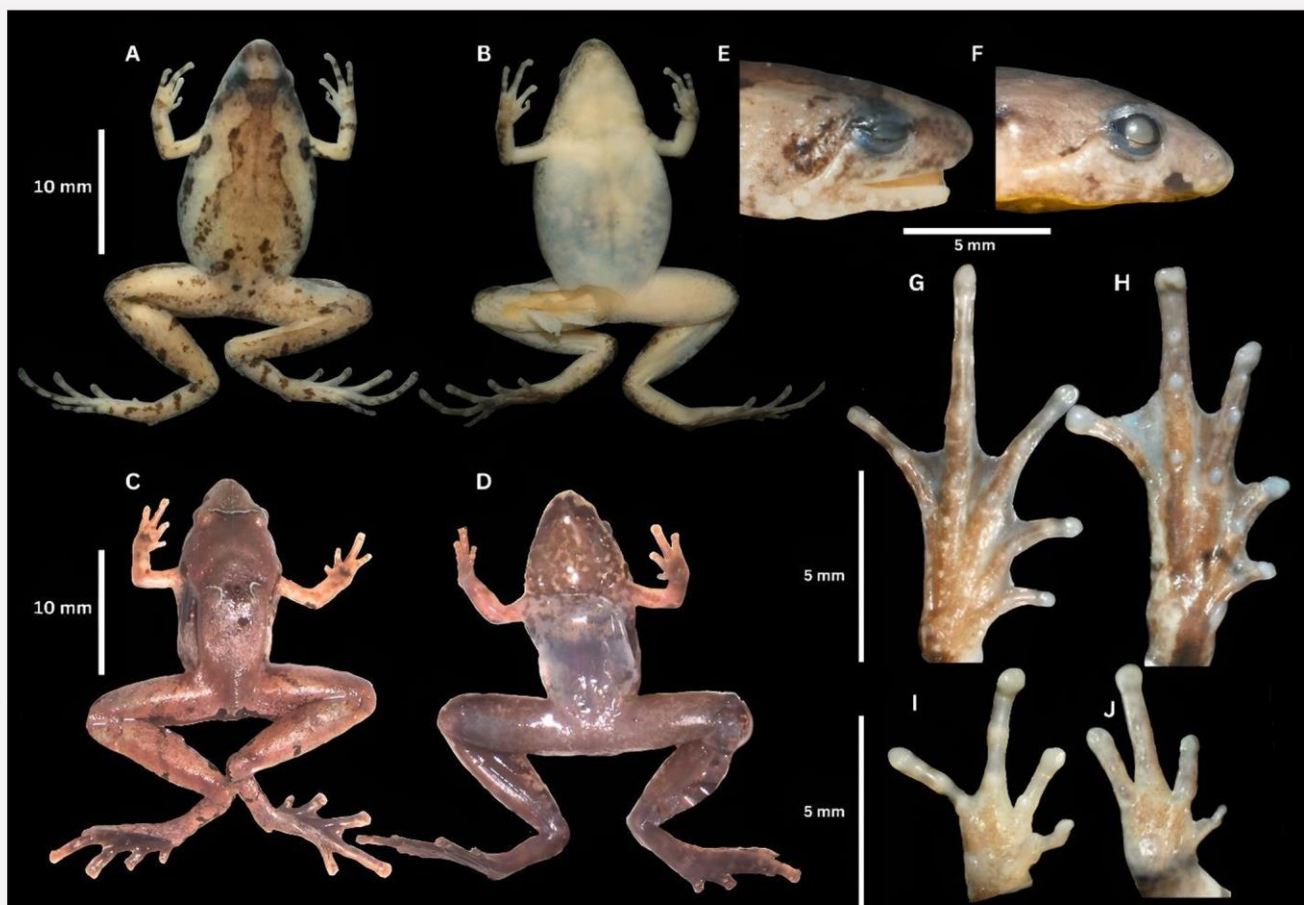


Figure 5. *Microhyla butleri* (MZB 24059, female): (A) dorsal and (B) ventral full body, (E) lateral head, (G) right foot, and (I) palm; *M. mantheyi* (MZB 22398, male): (C) dorsal and (D) ventral full body, (F) lateral head, (H) right foot, and (J) palm; © Photos: V.Y. Atmaja & E.N. Smith

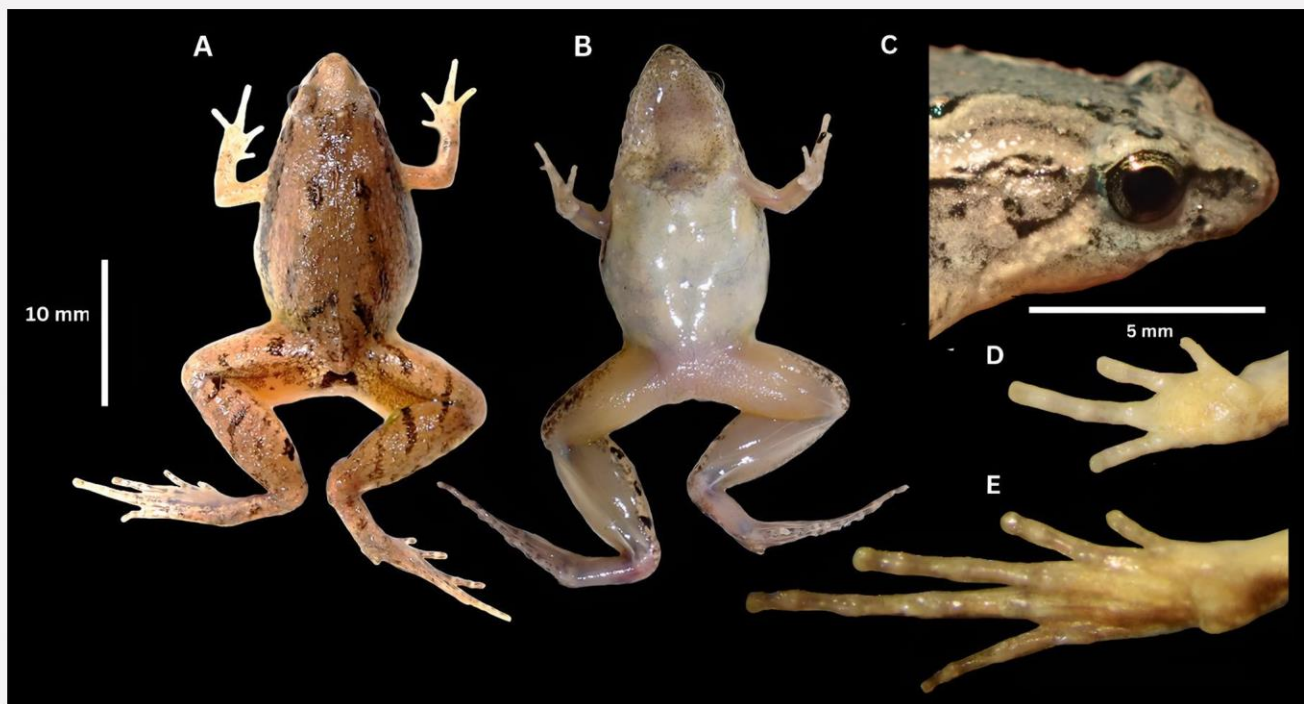


Figure 6. *Microhyla mukhlesuri* (MZB 28443, female): (A) dorsal and (B) ventral full body, (C) lateral head, (D) palm, and (E) right foot; © Photos: V.Y. Atmaja & E.N. Smith

formerly MZB Amph 26090), collected on 12 August 2015 by Eric N. Smith, Pannupong Thammachoti, Goutam C. Sarker, Elijah Westl, Irvan Sidik, Ahmad Muammar Kadafi, and Ipul; Riau Province: *Kabupaten Rokan Hilir*: Kecamatan Rimba Melintang, Jumrah, Road to Bagansiapiapi, 1.79011°N, 101.01868°E, 14 m a.s.l.—MZB Amph 28443 (female, ENS 19604), collected on 13 August 2015 by Eric N. Smith, Goutam C. Sarker, Elijah Westl, and Irvan Sidik; South Sumatra Province: *Kabupaten Ogan Ilir*: Kecamatan Indralaya, Sakatiga, 3.249601°S, 104.682105°E, 10 m a.s.l.—MZB Amph 27223, 27226–27228 (male), collected on 9 March 2016 by Deni Setyawan, M. Iqbal Catur, and Prasetyo.

Redescription. The *M. mukhlesuri* specimens from Sumatra align with the diagnostic characteristics provided by Hasan *et al.* (2014, in parentheses), namely, small frogs with SVLs of 18.38–22.78 mm for males and 17.99–21.04 mm for females (vs. 16.5–21.0 mm in males and 17.3–18.4 mm in females), webbed toes; (vs. distinct rudimentary web between the toes and subarticular tubercles); HL/HW ratio of 0.95–1.11 in males and 0.93–1.02 in females (vs. head length and width subequal); HL/SVL ratio of 0.26–0.33 in males and 0.28–0.33 in females (vs. average 0.39 ± 0.046); HW/SVL ratio of 0.26–0.30 in males and 0.25–0.34 in females (vs. average 0.37 ± 0.033); UEW/SVL ratio of 0.05–0.07 in males and 0.06–0.07 in females (vs. average 0.06 ± 0.007); HAL/SVL ratio of 0.19–0.29 in males and 0.23–0.28 in females (vs. average 0.22 ± 0.012); F1/SVL ratio of 0.03–0.06 in males and 0.04–0.07 in females (vs. average 0.04 ± 0.00); F2/SVL ratio of 0.06–0.09 in males and females (vs. average 0.08 ± 0.015); IMT/SVL ratio of 0.04–0.05 in males and 0.03–0.06 in females (vs. average 0.04 ± 0.011); and TIL/SVL ratio of 0.49–0.58 in males and 0.49–0.56 in females (vs. average 0.54 ± 0.026).

Some variation was found in our specimens compared to the original descriptions, such as tibiotarsus articulation reaching the eyes (vs. tibiotarsus articulation from between the eyes to the tip of the snout); FAW/SVL ratio of 0.05–0.06 in males and females (vs. average 0.04 ± 0.005); FLL/SVL of 0.51–0.65 in males and 0.55–0.65 in females (vs. average 0.48 ± 0.019); cream-colored lateral body with a black stripe running from the snout to the eye, then becoming a dotted line from the upper arm to the mid-body (vs. a black band begins at the tip of the snout, runs through the eyes, is briefly interrupted at the back corners of the eyes, and then merges before

reaching the groin). Regarding the distribution of *M. mukhlesuri* in Indonesia, it was found in the provinces of Aceh, North Sumatra, South Sumatra, and Riau during our NSF-sponsored Sumatra field trips (2013–2016). Additionally, the GBIF.org (2024) database reports *M. fissipes* in the provinces of West Sumatra and Jambi, but these likely belong to *M. mukhlesuri*.

Discussion

Several studies have been conducted to reconstruct and determine the origins of the ancestors of the genus *Microhyla* (Matsui *et al.* 2011, Peloso *et al.* 2016, Garg & Biju 2019, Gorin *et al.* 2020). Garg & Biju (2019) suggest that the Microhylinae may have originated in the Indian Subcontinent (ISC) during the Paleocene, spreading to mainland Asia after the ISC collision with mainland Asia (out of India hypothesis). However, this differs from the results of Gorin *et al.* (2020) who found that the ancestors of *Microhyla* I (*Microhyla* spp.), *Microhyla* II (becoming the new genus *Nanohyla*), and *Glyphoglossus* inhabited Eastern Indochina in the middle Eocene (45–43 million years ago). Then, *Microhyla* spread to East Asia, West Indochina, India, and Southeast Asia (including Borneo, Sumatra, Java, and Bali).

The BEAST chronogram (3207 bp-long mtDNA + nuDNA dataset) reconstructed by Gorin *et al.* (2020) suggests an origin and timing of the diversification of each *Microhyla* species. The *Microhyla* distributed in Indonesia entered through the Malay peninsula and simultaneously moved towards Sumatra (and eastward to Bali) and Borneo (e.g. *M. borneensis*, *M. nepenthicola*, and *M. malang*). The colonization of *Microhyla* species into Indonesia corresponds to the period when Sundaland was still united and subaerial (Pleistocene period). They then became isolated on the islands that were created when the ice age ended. This occurrence explains why the species found in mainland Asia are also found in Indonesia, and some species are endemic to Indonesia, such as *M. achatina* (Java and Bali), *M. gadjahmadai* (Sumatra), *M. orientalis* (Java, Bali, and introduced onto Sulawesi and Timor), *M. palmipes* (Sumatra, Java, and Bali), and *M. sriwijaya* (Sumatra) (Manthey *et al.* 2016, Atmaja *et al.* 2019, Eprilurahman *et al.* 2021b, 2021b, Frost 2024).

Microhyla mantheyi is included in the *M. achatina* Group and has the same recent ancestor (MRCA) as *M. borneensis*, *M. nepenthicola*, *M. malang*, *M. orientalis*, and *M. sriwijaya*, which

originated from western Indochina and then diversified towards peninsular Malaysia (Garg *et al.* 2019, Gorin *et al.* 2020). The BEAST chronogram based on mitochondrial and nuclear sequences presented by Gorin *et al.* (2020) shows that *M. mantheyi* is sister to *M. orientalis* (divergence time estimated to be 1.79–4.77 Mya), from Java, Bali, Timor, and Sulawesi (Sumatran populations previously considered *M. orientalis* have been described as *M. sriwijaya*). However, the mitochondrial topologies reconstructed by Firdaus *et al.* (2018), Eprilurahman *et al.* (2021a) and Gorin *et al.* (2020) reveal that *M. mantheyi* is sister to a clade containing *Microhyla* from Borneo (i.e., *M. borneensis*, *M. nepenthicola*, *M. malang*), as well as *M. minuta* (from Vietnam), *M. orientalis* and *M. sriwijaya*. *Microhyla mantheyi* was first described from a specimen originating from Johor, Malaysia by Das *et al.* (2007). To date, *M. mantheyi* has been recorded from the Malay Peninsula (in Myanmar, Thailand, Malaysia, and Singapore; Frost 2024, GBIF.org. 2024). Today *M. borneensis* is considered to be endemic to Borneo (Dring 1979, Das *et al.* 2007). The differences in the placement of the seven species mentioned above in the phylogenetic tree topologies presented by Gorin *et al.* (2020), Firdaus *et al.* (2018) and Eprilurahman *et al.* (2021b) could be due to the number of genes used and their sequence lengths. Further work involving more molecular data is needed to clarify the relationships among these taxa.

Microhyla butleri belongs to the *M. butleri* group, which originated from western Indochina and then colonized eastern Indochina (the origin of the ancestors of *Microhyla*, *Nanohyla*, and *Glyphoglossus*) (Garg *et al.* 2019, Nguyen *et al.* 2019, Gorin *et al.* 2020). *Microhyla butleri* then diversified to the east (mainland East Asia) and the west (West Indochina), then to the Malay Peninsula and Sumatra. Meanwhile, *M. aurantiventris* remained in eastern Indochina. Currently, *M. butleri* is reported to have a wide distribution: China, Taiwan, Cambodia, India, Laos, Myanmar, Thailand, Vietnam, Malaysia, and Singapore (Pham *et al.* 2019, Zug 2022, Figueroa *et al.* 2023, Frost 2023). As a result of the present study, Sumatra (and Indonesia by extension) can be added to this distribution list.

M. mukhlesuri was described as a separate species from *M. fissipes* by Hasan *et al.* (2014). It is found in Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Thailand, and Vietnam (Hong *et al.* 2021, Le *et al.* 2021,

Pratihari & Deuti 2021, Zug 2022, Frost 2024). However, the Bayesian GMYC analysis (a method for establishing divergence thresholds from species delimitation in phylogenetic trees) of a 2478 bp mtDNA fragment by Gorin *et al.* 2020, revealed several complex species-level lineages in *M. mukhlesuri* originating from Myanmar, Thailand, Vietnam, Laos, and peninsular Malaysia. Moreover, this study's phylogenetic trees also show a polytomy between the *M. mukhlesuri* populations, including the Sumatran population nested within it. In several cases, after being re-evaluated genetically, morphologically, and vocally, *Microhyla* species with a wide distribution have been split into several species. For example, *Microhyla heymonsi*, initially wide-ranging from China, Southeast Asia, India, and into Sumatra was recognized by Garg *et al.* (2019) as several highly divergent intraspecific lineages. Later, the population of *M. heymonsi* s.l. in the Great Nicobar Islands (India), was described as a distinct species, *M. nakkavaram*, by Garg *et al.* (2022). Eprilurahman *et al.* (2023) later expanded its distribution to Sumatra where those populations were previously identified as *M. heymonsi* s.l. Therefore, integrative studies are essential to clarify the taxonomic status and the variation of *M. mantheyi*, *M. butleri*, and *M. mukhlesuri* across their respective ranges.

Sumatra is known for its high biodiversity, which consists of many amphibian species, including a diversity of *Microhyla* species. During the Pleistocene, when sea levels were lower, Sumatra was part of the larger Sundaland and mainland Southeast Asia, a large subaerial landmass allowing the spread of *Microhyla* species in the region. After the sea levels rose and the land separated into islands, Sumatra remained an important point in the distribution of this genus. Diverse environments allowed *Microhyla* species to find suitable ecological niches. The potential for discovering new species or new distributions of already known species in Sumatra is still high. Studying the genus *Microhyla* as a model for understanding the biogeography of Indonesia provides opportunities to explore how ecological, geographic, and evolutionary factors interact to shape the distribution and adaptation of species. Our research has enriched our knowledge of *Microhyla* towards this goal.

Author contributions

VYA, RE, and AH conceived and designed the

study; ENS and the team conducted the fieldwork and collected specimens; VYA and MM performed the isolation and amplification of genetic material; VYA, RE, and MM analyzed the data; AH, ENS, TA, and RU validated the molecular and morphological analyses; VYA, RE, and ENS wrote the manuscript; all the authors reviewed and edited the manuscript; ENS and AH secured funding for the study.

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Supplement data

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