

Phylogeny and taxonomic status evaluation of Dark-sided Narrow-mouthed Frog, *Microhyla heymonsi*, (Anura: Microhylidae) from Sumatra, Indonesia

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Abstract. Eprilurahman R, Atmaja VY, Munir M, Ubaidillah R, Arisuryanti T, Smith EN, Hamidy A. 2023. Phylogeny and taxonomic status evaluation of Dark-sided Narrow-mouthed Frog, *Microhyla heymonsi*, (Anura: Microhylidae) from Sumatra, Indonesia. *Biodiversitas* 24: 1092-1103. *Microhyla heymonsi* (Dark-sided narrow-mouthed frog) was once known to be widespread along the continent of Asia from Taiwan to the mainland of east and southeast to Nicobar Islands and Sumatra. Recent studies identified that this species is cryptic species consisting of *M. heymonsi* itself and several newly described species, i.e., *M. daklakensis*, *M. heymonsi*, *M. nakkavaram*, *M. ninhthuanensis*, and the possibility of unnamed lineage. In Sumatra, *M. heymonsi* is not only distributed from the north to south of the mainland but also in the adjacent islands in Nias. Regarding the taxonomic status of Sumatran population, here we used molecular and morphological data to evaluate the taxonomic status of the Sumatran *M. heymonsi*. The phylogenetic analyses show that the Sumatran *M. heymonsi* is closest to *M. nakkavaram* and strongly supported as a member of subgroup *M. heymonsi* along with other recognized species, i.e., *M. daklakensis*, *M. heymonsi*, *M. nakkavaram*, and *M. ninhthuanensis*. The morphological assessment of Sumatran *M. heymonsi* shows that they are suitable with the morphological characteristic of *M. nakkavaram*. Both molecular and morphological data strongly support that Sumatran *M. heymonsi* is identical to the *M. nakkavaram* from Nicobar Islands. Our results expanded the distribution of *M. nakkavaram*, not only in Nicobar Islands but also in Sumatra and its adjacent islands. In addition, the phylogenetic tree showed the supported clade of Nicobar and Sumatran *M. nakkavaram* as sister taxa of the possibly undescribed species from Singapore and Peninsular Malaysia. Further study of this undescribed species is urgent.

Keywords: Microhylidae, molecular phylogeny, morphology, new record, taxonomy

INTRODUCTION

The narrow-mouthed frogs, genus *Microhyla*, showed a rapid increase in species number due to divergent lineages and a high degree of undiagnosed diversity (Yuan et al. 2016). The description of new species within the genus *Microhyla* in the last decade (2012-2023) was done in sequence by Matsui et al. (2005, 2011, 2013), Hasan et al. (2012, 2014a, 2014b), Poyarkov et al. (2014), Howlader et al. (2015), Seshadri et al. (2016), Wijayathilaka et al. (2016), Nguyen et al. (2019), Atmaja et al. (2019), Li et al. (2019), Poyarkov et al. (2020), Hoang et al. (2021), Eprilurahman et al. (2021b), and Garg et al. (2022). One of the species which is being recently separated into several species is *Microhyla heymonsi*.

Microhyla heymonsi has been described based on the specimen from Taiwan by Vogt (1911). It was described based on morphological characteristics such as the presence of a mid-dorsal line with black-shaped marking in the mid-dorsum and dark brown coloration in the ventral surface of

the foot and upper anal region. Then, this name was widely applied to other populations in the mainland of East and Southeast Asia (Sheridan et al. 2010; Teynié et al. 2010; Grosjean et al. 2015; Jang-Liaw and Chou 2015; Gorin et al. 2020; Poyarkov et al. 2020; Hoang et al. 2021), Sumatra (Lawalata 2011; Pradana et al. 2017; Akbari et al. 2021), Singapore (Sheridan et al. 2010) and Nicobar Islands (Garg et al. 2019; Garg et al. 2022) resulting in a wider distribution of the species.

Hasan et al. (2014a), Pradana et al. (2017), Garg et al. (2019), Gorin et al. (2020), Akbari et al. (2021), and Hoang et al. (2021) found the possibility of *M. heymonsi* as a species complex. While Sheridan et al. (2010) conclude they are conspecific, since there are no differences in their call characteristics, even though their molecular result showed the possibility of a species complex. Further investigation by Garg et al. (2019) and Gorin et al. (2020) confirmed that *Microhyla heymonsi* is a species complex. This species complex spread across a vast distribution range needs to be clarified and compared with the true *M. heymonsi* from the

topotype. Recent studies described several new species related to *M. heymonsi*, there is *M. pineticola*, *M. neglecta*, *M. daklakensis*, *M. ninhthuanensis* from Vietnam and *M. nakkavaram* from Nicobar Islands, India (Poyarkov et al. 2014, 2020; Hoang et al. 2021; Garg et al. 2022). Those results leave the population from Peninsular Malaysia, Singapore, Sumatra and the adjacent islands that need to be evaluated.

In Sumatra, *M. heymonsi* is commonly recorded in a wide range of habitats and altitudes (1-1552 masl). Our recent field surveys identified that this species could be found from the northern to southern of Sumatra, from the coastal to hilly areas as was previously reported (Inger and Iskandar 2005; Teynié et al. 2010; Manthey and Denzer 2014; Kaprawi et al. 2020; GBIF 2022) and also its adjacent island in Nias (Lawalata 2011). Here we examined the molecular and morphological of Sumatran *M. heymonsi* to evaluate its taxonomic status.

MATERIALS AND METHODS

Samples information

Seven new partial 16S mtDNA sequences of the *Microhyla heymonsi* from Sumatra were generated and examined along with other *Microhyla heymonsi* subgroups (Tables S1 and S2). Specimens examined in this study are the collections of Museum Zoologicum Bogoriense (MZB), the Research Center for Biosystematic and Evolution, National Research and Innovation Agency of Indonesia.

Specimens were preserved in 10% formalin and stored at 70% ethanol solution. The map of type localities and distribution of the recognized species of the examined *Microhyla heymonsi* species subgroup is provided in Figure 1.

Molecular analysis

Total DNA from liver tissue was extracted using the standard phenol-chloroform procedure (Sambrook et al. 1989). The targeted 16S mtDNA (ca. 468) gene fragment was amplified using the primer set of 16L-1 (CTGACCGTGCAAAGGTAGCGTAATCACT) and 16H-1 (CTCCGGTCTGAACTCAGATCACGTAGG) (Hedges 1994) following Matsui et al. (2011, 2013). DNA sequences were checked and edited manually using ChromasPro software (Technelysium Pty Ltd., Tewantin, Queensland, Australia). The newly generated Sumatran *M. heymonsi* sequences were deposited in GenBank with accession numbers OP363085-OP363091 (labelled as *M. nakkavaram* in Table S2). We applied Clustal W in MEGA X (Kumar et al. 2018) and aligned the new sequences together with those from GenBank (Table S2). Phylogenetic trees were reconstructed using Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses. The NJ tree analysis was calculated using p-distances with 1000 bootstrap replications in MEGA X. ML analysis was performed using the IQ-Tree Web Server (Trifinopoulos et al. 2016, available at <http://iqtree.cibiv.univie.ac.at>) with 10,000 ultrafast bootstraps (UFB) replicates and the ‘auto’ criterion to select an appropriate model of DNA evolution.

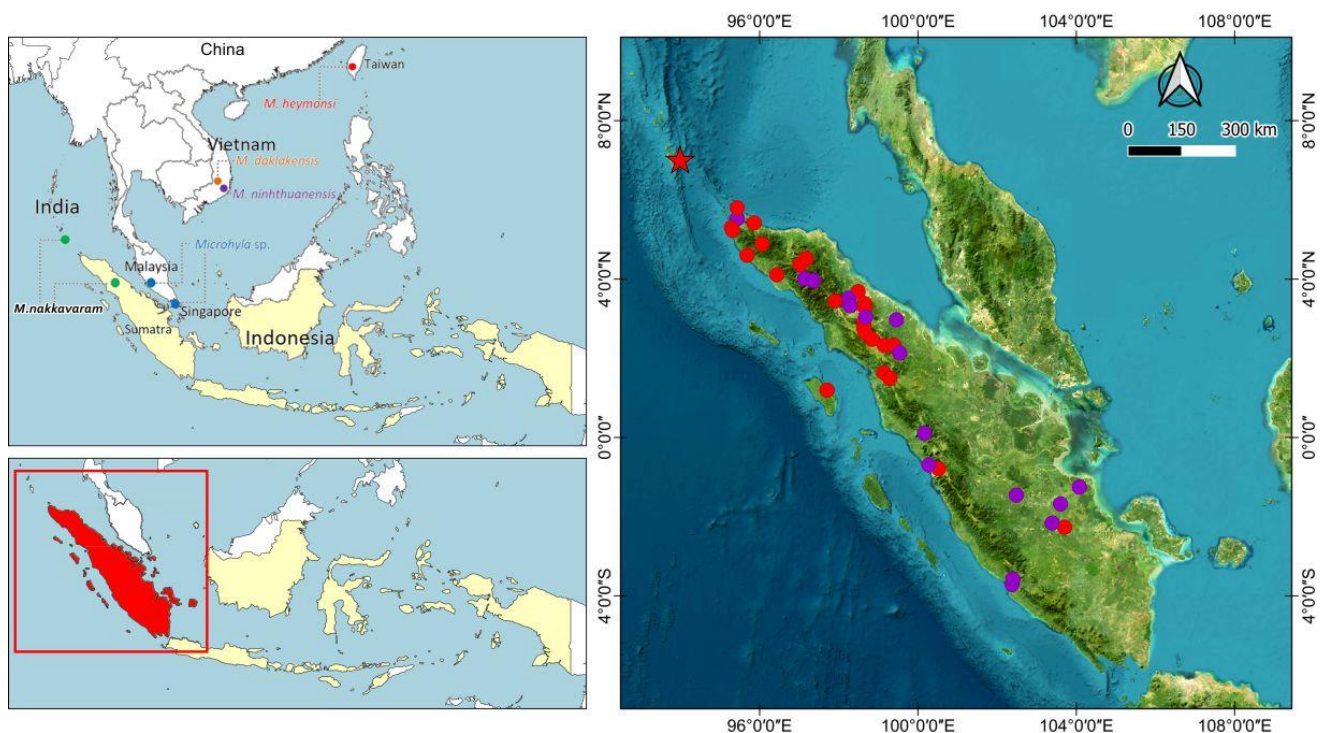


Figure 1. A. Type localities and distribution of the recognized species of the *Microhyla heymonsi* species subgroup; B. The sampling localities of *Microhyla nakkavaram* in the southern group of Nicobar Islands (India) and Sumatra Island (Indonesia). The red star indicates the species type locality (Garg et al. 2022), red dots indicate Sumatran samples included in the analysis (museum specimens: MZB & UTA) and purple dots indicate other known recorded localities (GBIF 2022: GoARK iNaturalist Project)

The BI analyses were run under the MCMC dataset according to Bayesian Information Criterion (BIC) values the best evolution model General-Time-Reversible (GTR) with empirical base frequencies (+F), invariant sites (+I) and discrete gamma shape information with four rate categories (+G4) as selected in jModelTest 2.1.10 (Darriba et al. 2012). The BI analysis was performed with two runs of four datasets Markov Chain Monte Carlo (MCMC) executed for 10 million generations, with trees sampled every 100 cycles using BEAST 2.5 (Bouckaert et al. 2019). The convergence of the runs was determined by a split frequency of <0.01 standard deviations and potential scale reduction factors of ~1.0. We discarded the first 25% of the sampled trees as burn-in and generated a majority-rule consensus tree using the remaining samples. Strong tree node support was considered when utilizing bootstrap values of 70% or more for ML and NJ analyses (Hillis and Bull 1993). The genetic distances were calculated using uncorrected p-distances in MEGA X. We considered tree nodes with BI posterior probabilities greater than 0.95 to be strongly supported, values between 0.90 and 0.95 to be moderately supported, and lower values to have no nodal support (Huelsenbeck and Hillis 1993). Nodes with UFB support values $\geq 95\%$ for ML (Minh et al. 2013) and BPP ≥ 0.95 (Leaché and Reeder 2002) were considered strongly supported.

Genetic haplotypes analysis

Twenty-seven sequences of 468 bp 16S mtDNA gene fragments of *M. heymonsi* from Nicobar Island, Sumatra and Nias Islands, Singapore, Peninsular Malaysia and Taiwan were generated using DNASp v6.12.03 (Rozas et al. 2017) and resulting 20 of haplotype variations. We then used Network v10.2.0.0 (www.fluxus-engineering.com) (Bandelt et al. 1999) to build the haplotype network with the median-joining method. The haplotype distributions for each location were then shown in an informative map to demonstrate recent genetic connectivity and distributions among populations.

Morphological evaluation

Sumatran *M. heymonsi* populations (Table S3) were morphologically studied and compared to other congeners for accurate identification. Adult subjects from the type locality (Garg et al. 2022) and Sumatran populations were used for identification and morphometrics (Table 3). Body measurements were taken to the nearest 0.1 mm using a digital slide caliper, as described by Biju et al. (2019), Garg et al. (2019), and Garg et al. (2022). Abbreviations for twenty-two morphometric characters are: snout-vent length (SVL, from tip of snout to cloaca). Head characters: Head length (HL, from the end part of the mandible to tip of snout); head width (HW, distance between the angle of the jaws); snout length (SL, from tip of snout to anterior orbital edge); eye length (EL, horizontal distance between bony orbital edges); inter upper eyelid width (IUE, the shortest distance between the upper eyelids); upper eyelid width (UEW); distance from the nostril to the tip of the snout (NS); distance from the front of the eye to the nostril (EN); internarial distance (IN, distance between the nares). Forelimb characters: Forearm length (FAL, from flexed

elbow to base of outer palmar tubercle); hand length (HAL, from the base of outer palmar tubercle to tip of a third finger); finger length (FL, maximum length of each finger FIL, FIIL, FIIL, and FIVL). Hindlimb characters: Thigh length (TL, measured from vent to knee); shank length (SHL, length from knee to heel); foot length (FOL, measured from base of inner metatarsal tubercle to the tip of the fourth toe); total foot length (TFOL, measured from heel to the tip of the fourth toe); length of inner metatarsal tubercle (IMTL); length of outer metatarsal tubercle (OMTL). Following Biju et al. (2014), the amount of webbing relative to subarticular tubercles were described by numbering the tubercles 1-3, beginning with the toe discs. Specifically, we compared the main diagnostic characteristic of Sumatran *M. heymonsi* populations with newly described *M. nakkavaram* Garg, Sivaperuman, Gokulakrishnan, Chandramouli, and Biju 2022.

RESULTS AND DISCUSSION

Phylogenetic relationship and genetic distance

The phylogenetic tree topology (Figure 2) agrees with recent studies (e.g., Biju et al. 2019; Garg et al. 2019; Gorin et al. 2020, 2021; Poyarkov et al. 2020; Eprilurahman et al. 2021b; Hoang et al. 2021; Garg et al. 2022). Sumatran *M. heymonsi* is shown to nest in *Microhyla heymonsi* subgroup, along with other four recognized species (*M. daklakensis*, *M. heymonsi*, *M. nakkavaram*, and *M. ninhthuanensis*), their relationship is possessing strong support values (NJBP 86, UFB 88, BPP 1.0) and specifically formed a well-supported group with *M. nakkavaram* (NJBP 76, UFB 98, BPP 0.99). These Sumatran *M. heymonsi* (which from now on mentioned as Sumatran *M. nakkavaram*) and Indian *M. nakkavaram* are the sister species of Peninsular Malaysia and Singapore *M. heymonsi* (NJBP 97, UFB 100, BPP 1.0), although their intraspecific relationship was not fully resolved. The distinct lineage within Sumatran *M. nakkavaram* (NJBP 62, UFB 53, BPP 0.99) shows lower support compared to the Indian clade (NJBP 99, UFB 98, BPP 1.0). The phylogenetic relationship between Sumatran and Nicobar Islands populations with Peninsular Malaysia and Singapore populations indicates that those two populations are different species, as shown by the species delimitation result using genetic distance in Table 1.

Among the subgroup *M. heymonsi*, the genetic distance between Sumatran *M. nakkavaram* and Indian *M. nakkavaram* is ranging from 1.3-2.6%, these values are below to the species delimitation proposed by Fouquet et al. (2007). Compared to other subgroup members, the genetic distance is ranging from 2.2-4.2% compared to undescribed *Microhyla* from Peninsular Malaysia and Singapore; 5.9-7.2% to *M. daklakensis*; 3.9-5.4% to *M. ninhthuanensis*; and 4.5-5.9% to the true *M. heymonsi* (Table 1). The range of intraspecific distance between Sumatran *M. nakkavaram* is inadequate to separate them as new species from Indian *M. nakkavaram*. In addition, Sumatran samples also show quite high genetic distance (1.7-2.8%) between mainland Sumatra and adjacent island populations from Nias (Figure 2). These observed interspecific p-distances are comparable to

species-level divergences between other recognized congeners *Microhyla* species (e.g., Garg et al. 2019; Gorin et al. 2020; Garg et al. 2022).

Haplotype Network

The haplotype network analysis recognized a complex ancestor or origin of *M. heymonsi* from Sumatra and Nicobar Islands populations (Figure 3). Four major haplotype groups

are named as clade Indonesia+India, Singapore+Malaysia, Vietnam and Taiwan. The topotype sample from Taiwan as the true *M. heymonsi* shows two different haplotypes H15 and H16. Indonesia and India have mixed connectivity with each other with several probabilities of genetic connection of various ancestor points evolved from neighboring populations of Singapore and Malaysia.

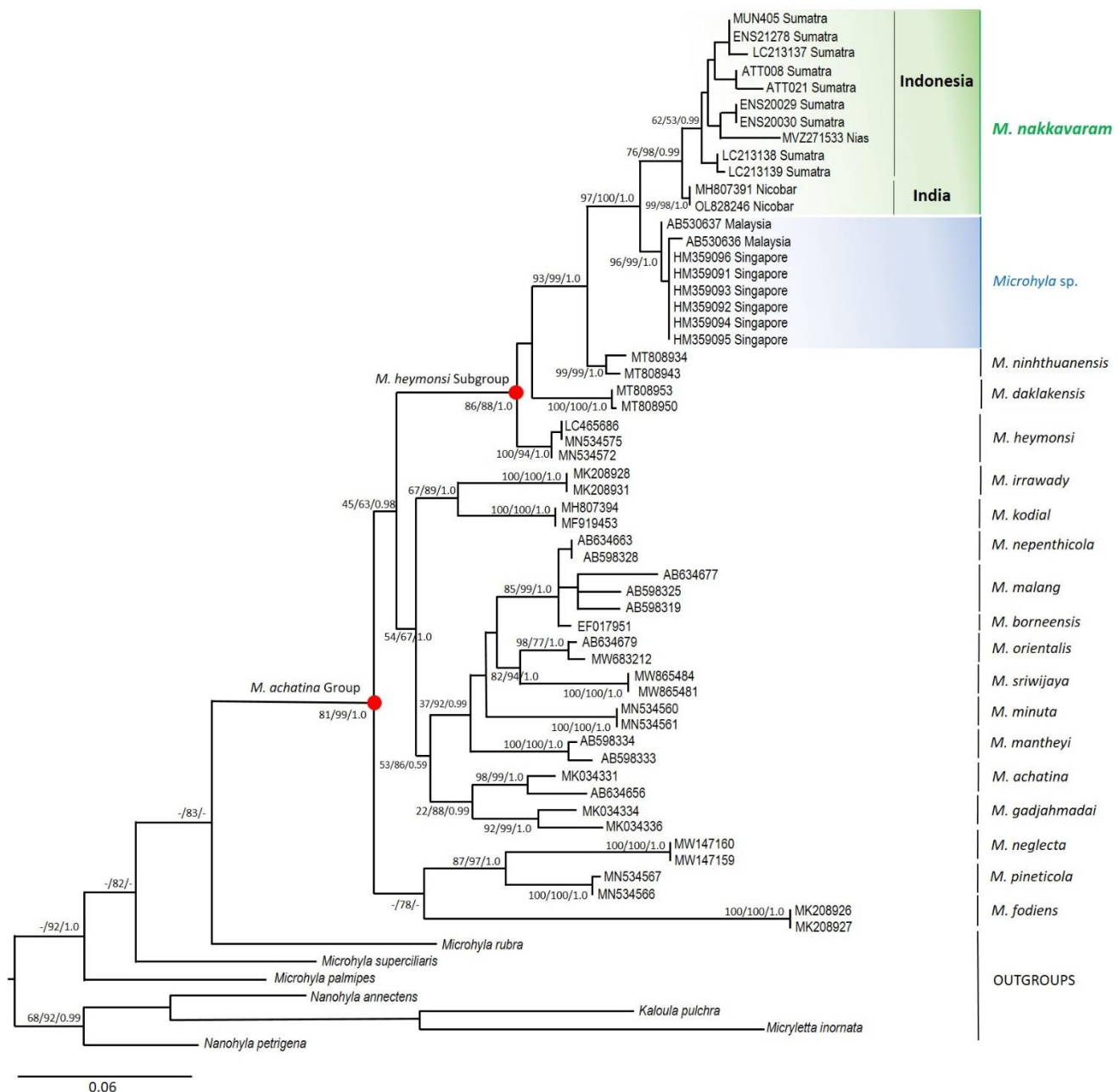


Figure 2. Maximum Likelihood tree based upon a ~468 bp fragment of 16S rRNA gene for *Microhyla heymonsi* group populations from Sundaland, along with several *Microhyla* species outside *Microhyla achatina* group. Values at branches indicate Neighbor Joining Bootstrap Proportion (NJB), Ultrafast Bootstrap Support (UFB), and Bayesian Posterior Probabilities (BPP). The Indonesian *Microhyla* samples with green highlighted indicate the newly generated sequences in this study.

Table 1. The uncorrected pairwise genetic distances (in percent) among all the recognized members of the *Microhyla achatina* species group (1-20), based on the studied fragment of the mitochondrial 16S rRNA. Comparisons with *Microhyla nakkavaram* from Sumatra, Indonesia are presented in bold. The diagonal indicates intraspecific distances. Distances are reported as mean \pm standard deviation presented in the upper part and minimum-maximum number presented in parentheses in the lower part. The number in parentheses following the species names represents the samples size. The green font show interspecific divergence range between *M. nakkavaram* (India and Indonesia) and the closest sister taxon, *Microhyla* sp. (Peninsular Malaysia and Singapore)

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 Sumatran <i>M. heymonsi</i> (10)	1.4\pm0.6 (0-2.8)																			
2 Indian <i>M. nakkavaram</i> (2)	1.8\pm0.4 (1.3-2.6)	0																		
3 <i>Microhyla</i> sp. (8)	3.0\pm0.3 (2.2-4.2)	2.2 \pm 0.1 (2.0-2.6)	0.2 \pm 0.1 (0-0.7)																	
4 <i>M. daklakensis</i> (2)	6.3\pm0.4 (5.9-7.2)	5.4 \pm 0.2 (5.0-5.9)	5.4 \pm 0.1 (5.2-5.4)	0.2																
5 <i>M. heymonsi</i> (3)	5.1\pm0.3 (4.5-5.9)	4.7 \pm 0.2 (4.3-5.2)	4.5 \pm 0.1 (4.4-4.6)	4.0 \pm 0.1 (3.7-4.2)	0.1 \pm 0.1 (0-0.2)															
6 <i>M. ninhthuanensis</i> (2)	4.5\pm0.3 (3.9-5.4)	3.4 \pm 0.2 (3.1-3.9)	3.8 \pm 0.1 (3.7-3.9)	4.3 \pm 0.1 (4.3-4.5)	4 \pm 0.1 (3.7-4.2)	1.1														
7 <i>M. achatina</i> (2)	9.8\pm0.4 (9.2-10.7)	9.2 \pm 0.2 (9.0-9.4)	9.7 \pm 0.2 (9.2-10.3)	7.4 \pm 0.1 (7.2-7.7)	7.3 \pm 0.1 (7.0-7.4)	8.6 \pm 0.4 (8.1-9.2)	2.6													
8 <i>M. borneensis</i> (1)	8.4\pm0.4 (7.9-9.2)	7.8 \pm 0.1 (7.8)	7.9 \pm 0.1 (7.7-7.9)	5.8 \pm 0.1 (5.7-5.9)	6.3 \pm 0.1 (6.1-6.3)	7.0 \pm 0 (7.0)	6.8 \pm 0.2 (6.5-7.0)	0												
9 <i>M. gadjahmadai</i> (2)	10.7\pm0.4 (10.1-11.6)	9.9 \pm 0.3 (9.8-10.1)	7.9 \pm 0.3 (9.4-10.3)	8.1 \pm 0.1 (7.4-8.3)	9.3 \pm 0.4 (7.9-8.3)	5.3 \pm 0.4 (8.8-9.8)	6.7 \pm 0.3 (6.3-7.0)	2.8												
10 <i>M. irrawady</i> (2)	8.9\pm0.3 (8.5-9.6)	8.3 \pm 0 (8.3)	8.3 \pm 0.1 (8.1-8.3)	6.7 \pm 0.1 (6.6-6.8)	6.3 \pm 0.1 (6.1-6.3)	8.0 \pm 0.1 (7.9-8.1)	6.5 \pm 0.5 (5.9-7.0)	7.4 \pm 0 (7.4)	6.8 \pm 0.1 (6.7-7.0)	0										
11 <i>M. kodial</i> (2)	8.5\pm0.3 (8.1-9.2)	7.9 \pm 0 (7.9)	7.7 \pm 0.1 (7.4-8.1)	5.1 \pm 0.1 (5.0-5.3)	6.1 \pm 0.1 (5.9-6.1)	7.4 \pm 0.2 (7.2-7.7)	6.7 \pm 0.1 (6.5-6.7)	5.9 \pm 0 (5.9)	7.2 \pm 0.4 (6.7-7.6)	5.0 \pm 0.1 (5.0)	0									
12 <i>M. malang</i> (3)	8.9\pm0.5 (8.1-9.8)	8.3 \pm 0.3 (7.9-8.5)	8.2 \pm 0.4 (7.4-8.5)	6.7 \pm 0.4 (6.1-7.4)	6.8 \pm 0.3 (6.3-7.2)	7.5 \pm 0.4 (7.0-8.1)	7.4 \pm 0.3 (7.0-7.8)	2.4 \pm 0.6 (1.7-3.2)	6.8 \pm 0.5 (6.1-7.4)	7.9 \pm 0.1 (7.6-8.0)	7.2 \pm 0.4 (6.7-7.8)	3.3 \pm 0.5 (2.6-3.7)								
13 <i>M. mantheyi</i> (2)	9.3\pm0.4 (8.5-10.1)	8.4 \pm 0.3 (8.1-8.8)	9.1 \pm 0.2 (8.8-9.6)	6.9 \pm 0.2 (6.6-7.2)	7.6 \pm 0.1 (7.4-7.7)	8.9 \pm 0.1 (8.8-9.0)	6.3 \pm 0.2 (6.1-6.5)	5.5 \pm 0.2 (5.2-5.6)	5.7 \pm 0.3 (5.2-6.1)	7.4 \pm 0.1 (7.4)	6.0 \pm 0.1 (5.9-6.1)	6.4 \pm 0.2 (6.1-6.7)	1.1							
14 <i>M. minuta</i> (2)	9.1\pm0.5 (8.3-9.6)	8.5 \pm 0 (8.5)	7.9 \pm 0.1 (7.7-7.9)	7.5 \pm 0.1 (7.4-7.7)	7.8 \pm 0.1 (7.7-7.9)	8.2 \pm 0.1 (8.1-8.3)	7.5 \pm 0.5 (7.0-8.0)	4.8 \pm 0 (4.8)	6.3 \pm 0.8 (5.4-7.2)	7.8 \pm 0 (7.8)	7.4 \pm 0 (7.4)	5.47 \pm 0.5 (5.0-6.1)	6.7 \pm 0.3 (6.3-7.0)	0						
15 <i>M. nepenthicola</i> (2)	9.0\pm0.4 (8.5-10.1)	8.6 \pm 0.1 (8.5-8.8)	8.6 \pm 0.1 (8.3-8.8)	6.3 \pm 0.1 (6.1-6.6)	6.8 \pm 0.1 (6.6-7.0)	8.0 \pm 0.1 (7.9-8.1)	6.6 \pm 0.1 (6.3-6.7)	0.9 \pm 0.1 (0.8-1)	6.6 \pm 0.1 (6.3-6.7)	7.3 \pm 0.1 (7.2-4)	6.2 \pm 0.1 (6.1-6.3)	2.5 \pm 0.3 (2.1-3.0)	5.6 \pm 0.2 (5.2-5.9)	5.1 \pm 0.1 (5.0-5.2)	0.2					
16 <i>M. orientalis</i> (2)	9.4\pm0.4 (8.8-10.3)	9.4 \pm 0.4 (8.5-9.4)	9.2 \pm 0.3 (8.8-9.8)	6.7 \pm 0.2 (6.3-7.0)	6.7 \pm 0.2 (6.3-7.0)	8.6 \pm 0.2 (8.3-9.0)	6.3 \pm 0.3 (5.9-6.7)	4.6 \pm 0.2 (4.3-4.8)	6.9 \pm 0.2 (6.5-7.2)	7.7 \pm 0.2 (7.4-7.9)	6.8 \pm 0.2 (6.5-7.0)	5.7 \pm 0.8 (4.5-7.0)	5.8 \pm 0.2 (5.4-6.1)	6.3 \pm 0.2 (6.1-6.5)	4.7 \pm 0.2 (4.3-5.0)	0.9				
17 <i>M. sriwijaya</i> (2)	8.7\pm0.5 (7.4-9.6)	9.5 \pm 0.1 (9.4-9.6)	8.9 \pm 0.1 (8.8-9.2)	7.8 \pm 0.1 (7.7-7.9)	7.5 \pm 0.1 (7.2-7.7)	8.9 \pm 0.1 (8.8-9.0)	8.6 \pm 0.4 (8.0-9.1)	5.5 \pm 0.1 (5.4-5.6)	7.9 \pm 0.1 (7.6-8.0)	7.5 \pm 0.1 (7.4-7.6)	8.6 \pm 0.1 (8.5-8.7)	6.5 \pm 0.5 (5.6-7.0)	6.6 \pm 0.3 (6.1-7.0)	7.1 \pm 0.1 (7.0-7.2)	5.7 \pm 0.1 (5.4-5.9)	4.5 \pm 0.4 (3.9-5.0)	0.2			
18 <i>M. neglecta</i> (2)	11.9\pm0.3 (11.6-12.5)	11.4 \pm 0 (11.4)	11.4 \pm 0.1 (11.2-11.8)	10.2 \pm 0.1 (10.1-10.3)	9.6 \pm 0.1 (9.4-9.6)	10.4 \pm 0.3 (10.1-10.7)	9.6 \pm 0.2 (9.4-9.8)	10.1 \pm 0 (10.1)	9.8 \pm 0.2 (9.6-10)	10.7 \pm 0 (10.7)	11.2 \pm 0 (11.2)	9.8 \pm 0.2 (9.6-10.1)	10.9 \pm 0 (10.9)	10.1 \pm 0 (10.1)	10.2 \pm 0.1 (10.1-10.2)	10.1 \pm 0.2 (9.8-10.3)	11.6 \pm 0 (11.6)	0		
19 <i>M. pineticola</i> (2)	9.3\pm0.3 (8.8-9.8)	8.6 \pm 0.1 (8.5-8.8)	8.7 \pm 0.2 (8.3-9.2)	8.1 \pm 0.1 (7.9-8.3)	7.7 \pm 0.1 (7.4-7.9)	8.6 \pm 0.1 (8.5-8.8)	8.6 \pm 0.2 (8.3-8.9)	7.8 \pm 0.1 (7.6-7.8)	9.0 \pm 0.3 (8.5-9.4)	8.6 \pm 0.1 (8.5-8.7)	8.4 \pm 0.1 (8.3-8.5)	7.8 \pm 0.3 (7.2-8.3)	8.2 \pm 0.1 (8.0-8.3)	7.8 \pm 0.1 (7.7-7.9)	7.7 \pm 0.1 (7.4-7.9)	8.0 \pm 0.2 (7.7-8.3)	9.5 \pm 0.1 (9.4-9.6)	6.5 \pm 0.1 (6.3-6.6)	0.2	
20 <i>M. fodiens</i> (2)	12.1\pm0.2 (11.8-12.7)	11.2 \pm 0 (11.2)	10.5 \pm 0.1 (10.3-10.9)	10.2 \pm 0.1 (10.1-10.3)	10.1 \pm 0.1 (10.1-10.3)	10.2 \pm 0.1 (10.1-10.3)	10.3 \pm 0.2 (10.1-10.5)	11.6 \pm 0 (11.6)	11.5 \pm 0.1 (11.3-11.5)	11.2 \pm 0 (11.2)	10.9 \pm 0 (10.9)	11.6 \pm 0.3 (11.1-11.8)	11.2 \pm 0 (11.2)	11.2 \pm 0 (11.2)	11.7 \pm 0.1 (11.6-11.8)	11.8 \pm 0.2 (11.6-12)	13.1 \pm 0 (13.1)	11.2 \pm 0 (11.2)	10.9 \pm 0 (10.9)	0

Four confined haplotype groups which separated the *M. heymonsi* species subgroup population were observed. Concerning the significant value of F_{ST} among populations ($F_{ST} = 0.67$; $P < 0.000$) as well as haplotype network and distribution is shown in Figure 3, four haplotype groups were differentiated in sequence by 4–6 discrete nucleotide bases between Vietnam and Taiwan population, by 5 discrete nucleotide bases between Taiwan and Singapore+Malaysia, and by 6 nucleotide bases between Singapore+Malaysia and Indonesia+India population due to the mutations.

Both of our molecular analyses supported to attest the Sumatran populations as *Microhyla nakkavaram*. The maximum threshold of the intraspecies genetic distance of *M. nakkavaram* is still under the minimum threshold of interspecies genetic distance between *M. nakkavaram* and undescribed *Microhyla* from Peninsular Malaysia and Singapore.

The phylogenetic tree supports a monophyletic relationship of the Sumatran *M. nakkavaram* with *M. nakkavaram* from the Nicobar Islands. These two populations are conspecific. Albeit the high support of nodes, those two populations show high genetic variation. Within Sumatra population itself also showed high molecular variations specifically between Sumatra's mainland and adjacent small islands west of Sumatra and the population in the south Sumatra viz. Jambi. The possibility of dispersal or even vicariant isolation often occurs over geographic barriers, resulting in the speciation of several species (Cabej 2012; Wang et al. 2018; Garg et al. 2019). Western archipelagos, an Indonesian group of islands located west of the island of Sumatra has been highlighted for its significance in the insular Southeast Asian regions

biogeographic story (Lawalata 2011, Wilting et al. 2012). This has also been revealed in the *M. heymonsi* group, which seems to have evolved from hilly to the mountainous area even to the adjacent archipelagic region (Matsui, 2005, Frost 2006, Mulcahy et al. 2018, Hoang et al. 2021). This condition is similar to the molecular and morphological variation of the rock geckos *Cnemaspis kandiana* group, which is distributed on the Nicobar Islands, Sumatra mainland and its west coast adjacent islands (Iskandar et al. 2017; Lee et al. 2019). The geographic event of the region is also related to the cause of the distinction of several species of the Sundaland (Voris 2001). The high genetic variation between the species that are distributed on the mainland of Sumatra and their adjacent island in the west was also reported on the flying lizards in the genus *Draco*, indeed some *Draco* populations in small islands in the west of Sumatra are a high possibility as distinct species from the Sumatra mainland (Lawalata 2011).

Morphological resemblance

Morphologically, *M. heymonsi* was known to have limited superficially morphological differences. The well-known specific diagnostic characteristics of *M. heymonsi* are prominent mid-dorsal line and ()-shaped marking (Vogt 1911; Jang-Liaw & Chou 2015; Garg et al. 2019; Hoang et al. 2021). Thus, sometimes leads to the misidentification of Asian species origin without detailed examination as well as with the Sumatran population of *M. nakkavaram*. *Microhyla nakkavaram* from Sumatra (Figure 4) shows high similarity in all body characteristics, meristic and measurement (Table 2) with *M. nakkavaram* from Nicobar Islands.

Table 2. Morphometric comparison between two populations of *Microhyla nakkavaram* from Sumatra Island, Indonesia and Nicobar Islands, India (all measurement in mm)

Characters	Sumatra Island, Indonesia				Nicobar Islands, India (Garg et al. 2022)		
	♂ 10		♀ 2		♂ 7	♀ 1	Value
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
SVL	14.4–22.6	19.1 ± 2.5	20.1–24.8	22.4 ± 2.4	16.8–18.3	17.5 ± 0.6	21.5
HW	5.0–8.0	6.9 ± 0.9	6.7–9.2	7.9 ± 1.2	5.4–6.0	5.7 ± 0.3	6.1
HL	3.6–7.1	5.2 ± 1.0	5.1–6.2	5.6 ± 0.5	5.0–5.7	5.5 ± 0.3	6.0
SL	1.1–2.7	1.7 ± 0.5	1.7–2.3	2.0 ± 0.3	2.2–2.5	2.4 ± 0.1	2.7
EL	1.3–2.2	1.7 ± 0.3	2.2–2.5	2.3 ± 0.2	1.4–1.8	1.6 ± 0.1	1.8
EN	0.8–1.8	1.2 ± 0.4	1.0–1.6	1.3 ± 0.3	1.2–1.4	1.3 ± 0.1	1.5
NS	0.4–0.6	0.5 ± 0.1	0.6–0.7	0.6 ± 0.1	0.8–1.1	0.9 ± 0.1	1.1
IUE	1.9–2.6	2.4 ± 0.2	2.4–2.9	2.6 ± 0.2	2.0–2.3	2.1 ± 0.1	2.3
UEW	0.8–1.1	1.0 ± 0.1	1.0–1.6	1.1 ± 0.1	0.9–1.0	1.0 ± 0.1	1.3
IN	1.3–1.7	1.6 ± 0.1	1.6–2.3	1.9 ± 0.4	1.5–1.8	1.7 ± 0.1	1.7
FAL	2.3–3.8	3.1 ± 0.5	3.7–4.4	4.0 ± 0.4	3.3–3.5	3.4 ± 0.1	4.1
HAL	3.3–5.4	4.5 ± 0.7	4.2–5.5	4.8 ± 0.7	4.3–4.7	4.5 ± 0.1	5.5
FIL	0.4–1.5	1.0 ± 0.3	0.7–1.3	1.0 ± 0.3	0.7–0.9	0.8 ± 0.1	1.5
FIIL	0.9–2.6	1.6 ± 0.4	1.2–2.1	1.7 ± 0.5	1.3–1.6	1.4 ± 0.1	2.0
FIIL	1.8–4.6	3.0 ± 0.7	2.7–3.6	3.2 ± 0.5	2.6–3.0	2.8 ± 0.1	3.6
FIVL	1.0–2.2	1.6 ± 0.4	1.3–1.9	1.6 ± 0.3	1.4–1.8	1.6 ± 0.1	2.4
TL	7.0–10.9	8.9 ± 1.0	9.1–11.8	10.4 ± 1.4	9.0–9.4	9.1 ± 0.2	11.0
ShL	7.9–12.5	10.7 ± 1.3	9.6–13.5	11.6 ± 1.9	9.9–10.3	10.0 ± 0.2	12.4
FOL	7.5–12.3	10.7 ± 1.7	10.4–13.7	12.1 ± 1.7	9.8–10.2	9.9 ± 0.2	12.1
TFOL	10.6–19.3	15.9 ± 2.7	14.3–19.9	17.1 ± 2.8	14.3–15.2	14.6 ± 0.3	18.1
IMT	0.5–1.2	0.9 ± 0.2	1.0–1.1	1.0 ± 0.1	0.6–0.7	0.6 ± 0.1	0.8
OMT	0.5–1.2	0.8 ± 0.2	1.0–1.5	1.3 ± 0.3	0.4–0.5	0.4 ± 0.1	0.6

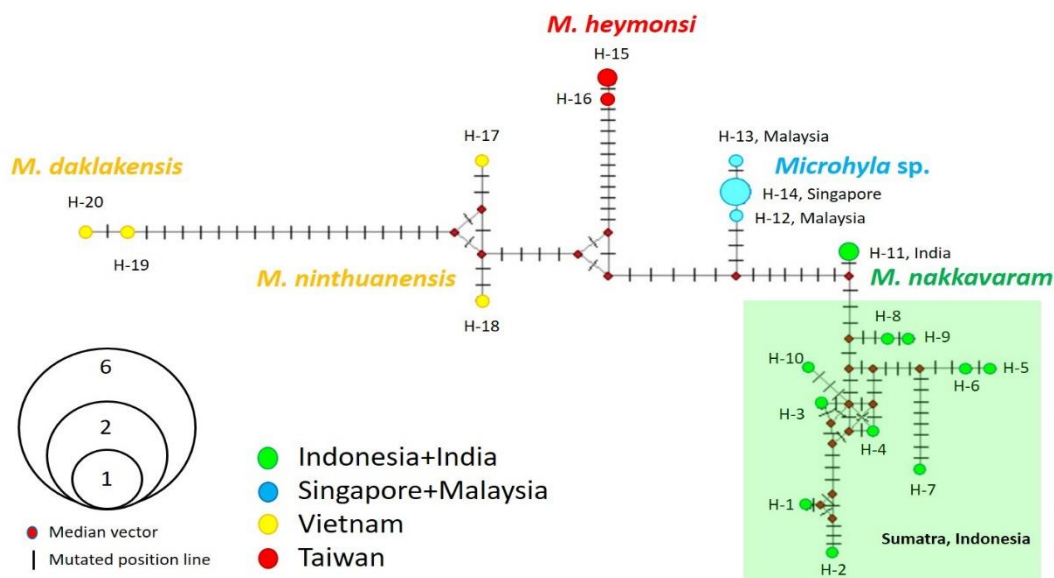


Figure 3. Median-joining haplotype network of *Microhyla heymonsi* group populations (see Table S2 for detailed samples used for this analyses)



Figure 4. Morphological appearance of Sumatran *M. nakkavaram* showing colour variation in life, all samples from Aceh in northern Sumatra: A. ENS 21278, from Mt. Bandahara, B. ENS 20030, C. ENS 20029, D. ENS 20028 from Nagan Raya

The presence of mid-dorsal line, prominent ()-shaped marking, dark brown coloration in ventral surface of foot and upper anal region, and the web toes formulae (I2-2½II2-3III3-4IV4-3V) confirmed the species identification of Sumatran populations is similar to *M. nakkavaram*. Based on its morphological characters, *M. nakkavaram* was easy to distinguish it from all those three closely related species (*M. daklakensis*, *M. heymonsi*, and *M. ninthuanensis*) by a variety combination of morphological characteristics, such as slender body shape, granular dorsal skin texture, colour pattern characteristics and body markings, the presence of two small tubercles at mid-dorsum along with ()-shaped markings, first finger (FI) longer than the second finger (FII), the presence of three distinct metacarpal tubercles on palm, and rudimentary foot webbing (Garg et al. 2019; Garg et al. 2022). The population of Sumatran *M. nakkavaram* is slightly bigger in morphology compared to the Nicobar Islands population. Detailed variation among Sumatran and Nicobar Islands *M. nakkavaram* and comparison with the other members of *M. heymonsi* subgroup are available in Table 3.

Our findings based on both molecular and morphological analyses strongly suggested that the Sumatran *M. heymonsi* is identical to *M. nakkavaram* and can be distinguished from

other species within the *M. heymonsi* group. In addition, they also support extending the distribution of *M. nakkavaram* which was previously only known to occur in the Nicobar Islands, to Sumatra and the islands on its western coast, including Nias Island. Our results highlight the need for proper identification and taxonomic assessment. Our results are also in accordance with those of Garg et al. (2019), Gorin et al. (2020), and Garg et al. (2022), and we have treated the Peninsular Malaysia and Singapore *M. heymonsi* as different species, and both their findings and ours showed that the population is completely different from the other members of the *M. heymonsi* subgroup. A comprehensive study of the morphological assessment is urgently needed.

The results of this study confirmed the identity of Indonesian *M. heymonsi* populations as *M. nakkavaram* and expanded the distribution of the later beyond the Nicobar Islands into Sumatra and its adjacent islands. Accordingly, all known *M. heymonsi* from Indonesia should be removed from the Indonesian species list and reclassified as *M. nakkavaram*. Meanwhile, the phylogenetic tree showed the supported clade of the Nicobar and Sumatran *M. nakkavaram* as sister taxa of the possibly undescribed species from Singapore and Peninsular Malaysia.

Table 3. Morphological comparison of Sumatran *Microhyla heymonsi* from Indonesia with the other four recognized members of the *Microhyla heymonsi* species subgroup. Characters specification follows Garg et al. (2022)

Characters	<i>M. nakkavaram</i> (n=♂ 10; ♀ 2)	<i>M. nakkavaram</i> (n=♂ 7; ♀ 1)	<i>M. daklakensis</i>	<i>M. heymonsi</i>	<i>M. ninhthuanensis</i>
Snout to vent length size	♂14-23; ♀20-25	♂16-19; ♀21	♂17-21 ♀22-26	♂16-24 ♀18-27	♂19-24 ♀21-24
Body	Slender	Slender	Stocky	Stocky	Stocky
Snout profile (dorsal view)	Rounded	Rounded	Rounded	Obtusely pointed	Abruptly round
Dorsal skin texture	Shagreened with granular projections	Shagreened with granular projections	Smooth	Smooth to Shagreened	Smooth
Length of Finger I vs Finger II	FI > ½ FII	FI > ½ FII	FI > ½ FII	FI ≤ ½ F2	FI < ½ F2
Dermal grooves on toes	Present	Present	Present	Present	Present
Discs on finger & toe tips	Present	Present	Present	Present	Present
Mid dorsal line	Present	Present	Present	Present	Absent
Foot webbing	Rudimentary: I2-2½II2-3III3-4IV4-3V	Rudimentary: I2-2½II2-3III3-4IV4-3V	Rudimentary: I2-2½II2-3III3-4IV4½-3V	Small: I2-2½II2-3III3-4IV4-3V	Rudimentary: I2-2½II2-3III3-4IV4½-3V
Tibio-tarsal articulation	Up to eye	Up to eye	Below snout tip	Below snout tip	Below snout tip
Distribution	Sumatra, Indonesia (Present study)	Nicobar Islands, India (Garg et al. 2022)	Endemic to Vietnam (Hoang et al. 2021)	Asian mainland to East Asia islands: Taiwan (Vogt 1911, Jang-Liaw and Chou 2015, Tominaga et al. 2019, Gorin et al. 2020)	Endemic to Vietnam (Hoang et al. 2021)

The results of this study confirmed the identity of Indonesian *M. heymonsi* as *M. nakkavaram* and expanded the distribution not only in the Nicobar Islands but also in Sumatra and its adjacent islands. In addition, the phylogenetic tree showed the supported clade of Nicobar and Sumatran *M. nakkavaram* as sister taxa of the possibly undescribed species from Singapore and Peninsular Malaysia. Further study of this undescribed species is urgent.

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Table S1. Specimens examined

Specimens examined, MZB: Museum Zoologicum Bogoriense; UTA: The Amphibian and Reptile Diversity Research Center, University of Texas at Arlington; ENS: Field Code of Eric N. Smith.

Microhyla nakkavaram (n=68) – Indonesia: Sumatra: Province Sumatera Utara: Kabupaten Langkat, Bukit Lawang Bohorok, MZB Amph 8518-19, Kabupaten Deli Serdang, Desa Sei Glugur, 54 m, 3.52007°S, 98.57333°E, MZB 23571-3, MZB 24070-71, Desa Rambung Baru, 239 m, 3.37724°S, 98.5975°E, MZB 23571, Road from Medan to Brastagi, Deli Serdang, Desa Rambung Baru, 239 m, 3.37724°S, 98.5975°E, MZB Amph 24081, MZB Amph 23584, 542 m, MZB Amph 24078, Kabupaten Tapanuli Utara, Kecamatan Adian Koting, MZB Amph 31472-74, MZB Amph 31488, 31490, Kabupaten Tapanuli Selatan, Utainbaru, 575 m, 1.66085°S, 99.17375°E, UTA-ENS15644 (subadult), Road between Utainbarua and Sipirok, 516 m, 1.65132°S, 99.17859°E, UTA-ENS15664, MZB Amph 23576, 24074, Utainbaru, 580 m, 1.66151°S, 99.17432°E, MZB Amph 24075, Sibuali, 1004 m, 1.56463°S, 99.28416°E, MZB Amph 24083, Below Sipirok, 1089 m, 1.62017°S, 99.22588°E, MZB Amph 23580, 23585, 24077, Kabupaten Karo, Kecamatan Brastagi, brought by local collector, MZB Amph 24072-73, Peceren, 1530 m, 3.20896°S, 98.51987°E, MZB Amph 24082, Gunung Sibuatan, Above Kampung Naga Linga, 1520 m, 2.915°S, 98.47091°E, MZB Amph 23580-81, MZB Amph 24084-86 (male), Kabupaten Dairi, Desa Silalahi, 976 m, 2.79804°S, 98.52049°E, MZB Amph 23575, Kabupaten Toba Samosir, Taman Eden, Gunung Pangulubao, 1392 m, 2.60441°S, 99.04599°E, MZB Amph 23578-79, MZB Amph 24082, Kabupaten Samosir, Higher Elevations above Pangururan, 1552 m, 2.6134°S, 98.804°E, MZB Amph 23582-83, MZB Amph 24079-80, Road Dehli Serdang to Rd. to Brastagi, 143 m, 3.42196°S, 98.6246°E, UTA-ENS 17968, Kabupaten Mandailing Natal, Desa Pagar Gunung, Kotanopan, MZB Amph 32212, 32214-16, Kecamatan Puncak Sorik, Desa Sibanggor Tonga, MZB Amph 32270; Province Aceh: Kabupaten Aceh Besar, Suelewah Agam, 635 m, 5.45325°S, 95.69842°E, UTA.A.18334, Lhoknga River Resort, 5.468435°S, 95.24522°E, MZB Amph 26087 (female), Along Coastal Road S. from Banda Aceh, 18 m, 5.36774°S, 95.28398°E, MZB Amph 26088, Simpang Gilang, Along road from Takengon to Isaq, 1174 m, 4.47443°S, 96.8731°E, MZB Amph 26089, Kabupaten Nagan Raya, Town Forest property of Grand Nagan Hotel, 36 m, 4.16633°S, 96.32845°E, ENS 20020 (male), 71 m, 4.16636°S, 96.32901°E, ENS 20027-30 (male), Kabupaten Aceh Raya, Calang, 1 m, 4.66641°S, 95.5676°E, ENS 20041, Kabupaten Pidie Jaya, 333 m, 4.953583°S, 95.9745°E, ENS 21177, Kabupaten Aceh Tenggara, Lawe Penanggalan, Kecamatan Ketambe, 352 m, 3.63485°S, 97.71897°E, ENS 21278 (female), Gunung Leuser National Park, MZB Amph 4083, Kabupaten Bener Meriah, Mt. Burni Telong, 1676 m, 4.7645°S, 96.8504°E, MZB Amph 32350, Kabupaten Aceh Tengah, Kecamatan Bintang, Teluk Mengaya, 1245 m, 4.5818°S, 96.9739°E, MZB Amph 32347-49; Province Jambi: Kabupaten Batanghari, Hutan Harapan Jambi, MZB Amph 30592-93; Province Riau: Kabupaten Indragiri Hulu, Bukit Tiga Puluh, MZB Amph 7090-91; Province Sumatera Selatan: Kabupaten Ogan Ilir, Kecamatan Indralaya, MZB Amph 27225.

Table S2. Samples of Indonesian *Microhyla heymonsi* and outgroups were generated for 16S mtDNA analysis in this study with detailed information on museum number, locality, GenBank accession numbers, and references. Museum and Voucher codes: VNMN: Vietnam National Museum of Nature; KUHE: Kyoto University, Graduate School of Human and Environmental Studies, Kyoto, Japan; ZMMU: Zoological Museum, Moscow Lomonosov State University; SDBDU: Sathyabhama Das Biju Delhi University; MZB: Museum Zoologicum Bogoriense; ENS: Eric N. Smith; MVZ: The Museum of Vertebrate Zoology at the University of California, Berkeley; UTA: The University of Texas at Arlington; MDK: Mirza D. Kusri; RM: Raffles Museum (Now ZRC), Singapore

Species	Museum number/ voucher code	Locality	GenBank Acc. No.	References
<i>Microhyla achatina</i> group				
Subgroup <i>Microhyla heymonsi</i>				
<i>Microhyla daklakensis</i>	VNMN06877	Dak Lak, Vietnam	MT808950	Hoang et al. (2021)
<i>Microhyla daklakensis</i>	VNMN06867	Dak Lak, Vietnam	MT808953	Hoang et al. (2021)
<i>Microhyla heymonsi</i>	KUHE 50505	Guan Zi Ling, Taiwan	LC465686	Tominaga et al. (2019)
<i>Microhyla heymonsi</i>	ZMMU A4975	Pingtong, Taiwan	MN534572	Gorin et al. (2020)
<i>Microhyla heymonsi</i>	ZMMU A5334-45	Kaohsiung, Taiwan	MN534575	Gorin et al. (2020)
<i>Microhyla nakkavaram</i>	Not preserved	Great Nicobar island, India	MH807391	Garg et al. (2022)
<i>Microhyla nakkavaram</i>	SDBDU 4200	Nicobar island, India	OL828246	Garg et al. (2022)
<i>Microhyla nakkavaram</i>	MZB Amph 30592	Jambi, Indonesia	OP363085	<i>This study</i>
<i>Microhyla nakkavaram</i>	MZB Amph 30593	Jambi, Indonesia	OP363086	<i>This study</i>
<i>Microhyla nakkavaram</i>	ENS21278	Aceh, Indonesia	OP363087	<i>This study</i>
<i>Microhyla nakkavaram</i>	ENS20029	Aceh, Indonesia	OP363088	<i>This study</i>
<i>Microhyla nakkavaram</i>	ENS20030	Aceh, Indonesia	OP363089	<i>This study</i>
<i>Microhyla nakkavaram</i>	MZB Amph 32347	Aceh, Indonesia	OP363090	<i>This study</i>
<i>Microhyla nakkavaram</i>	MVZ 271533	Nias Island, Indonesia	OP363091	<i>This study</i>
<i>Microhyla nakkavaram</i>	UTA-ENS 18334	Aceh, Indonesia	LC213138	Pradana et al. (2017)
<i>Microhyla nakkavaram</i>	MZB Amph 26807	Aceh, Indonesia	LC213139	Pradana et al. (2017)
<i>Microhyla nakkavaram</i>	MZB Amph 24084	North Sumatra, Indonesia	LC213137	Pradana et al. (2017)
<i>Microhyla</i> sp.	IABHU 21025	University Malaya, Malaysia	AB530636	Hasan et al. (2014a)
<i>Microhyla</i> sp.	IABHU 21026	University Malaya, Malaysia	AB530637	Hasan et al. (2014a)
<i>Microhyla</i> sp.	RM MIHEJS2	Singapore	HM359091	Sheridan et al. (2010)
<i>Microhyla</i> sp.	RM MIHEJS3	Singapore	HM359092	Sheridan et al. (2010)
<i>Microhyla</i> sp.	RM MIHEJS4	Singapore	HM359093	Sheridan et al. (2010)
<i>Microhyla</i> sp.	RM MIHEJS5	Singapore	HM359094	Sheridan et al. (2010)
<i>Microhyla</i> sp.	RM MIHEJS6	Singapore	HM359095	Sheridan et al. (2010)
<i>Microhyla</i> sp.	RM MIHEJS8	Singapore	HM359096	Sheridan et al. (2010)
<i>Microhyla ninthuanensis</i>	HAO185	Ninh Thuan, Vietnam	MT808934	Hoang et al. (2021)
<i>Microhyla ninthuanensis</i>	HAO73	Ninh Thuan, Vietnam	MT808943	Hoang et al. (2021)
Subgroup <i>Microhyla achatina</i>				
<i>Microhyla achatina</i>	MZB Amph 16402	Central Java, Indonesia	AB634656	Matsui et al. (2011)
<i>Microhyla achatina</i>	MDK22	West Java, Indonesia	MK034331	Atmaja et al. (2019)
<i>Microhyla borneensis</i>	VUB0588	Malaysia	EF017951	Bocxlaer et al. (2006)
<i>Microhyla gadjahmadai</i>	MZB Amph 15293	Lampung, Indonesia	MK034334	Atmaja et al. (2019)
<i>Microhyla gadjahmadai</i>	MZB Amph 16326	Bengkulu, Indonesia	MK034336	Atmaja et al. (2019)
<i>Microhyla irrawaddy</i>	ZMMU A5966	Myanmar	MK208928	Poyarkov et al. (2019)
<i>Microhyla irrawaddy</i>	ZMMU A5976	Myanmar	MK208931	Poyarkov et al. (2019)
<i>Microhyla kodial</i>	n/a	Karnatak, India	MF919453	Vineeth et al. (2018)
<i>Microhyla kodial</i>	SDBDU 2017.3674	Karnatak, India	MH807394	Garg et al. (2019)
<i>Microhyla malang</i>	BORN 9211	Sabah, Malaysia	AB598325	Matsui et al. (2011)
<i>Microhyla malang</i>	KUHE 53018	Sarawak, Malaysia	AB598319	Matsui et al. (2011)
<i>Microhyla malang</i>	MZB Amph 16364	Balikpapan, Indonesia	AB634677	Matsui et al. (2011)
<i>Microhyla mantheyi</i>	KUHE 15726	Selangor, Malaysia	AB598333	Matsui et al. (2011)
<i>Microhyla mantheyi</i>	KUHE 52556	Pahang, Malaysia	AB598334	Matsui et al. (2011)
<i>Microhyla minuta</i>	ZMMU A5048-91	Dong Nai, Vietnam	MN534560	Gorin et al. (2020)
<i>Microhyla minuta</i>	ZMMU A5048-96	Dong Nai, Vietnam	MN534561	Gorin et al. (2020)
<i>Microhyla nepenthicola</i>	KUHE 53033	Sarawak, Malaysia	AB598328	Matsui (2011)
<i>Microhyla nepenthicola</i>	KUHE 53938	Sarawak, Malaysia	AB634663	Matsui et al. (2011)
<i>Microhyla orientalis</i>	MZB Amph 16259	Bali, Indonesia	AB634679	Matsui et al. (2011)
<i>Microhyla orientalis</i>	MZB Amph 12986	East Java, Indonesia	MW683212	Eprilurahman et al. (2021a)
<i>Microhyla sriwijaya</i>	MZB Amph 31747	Belitung island, Indonesia	MW865481	Eprilurahman et al. (2021b)
<i>Microhyla sriwijaya</i>	UTA A-66215	Lampung, Indonesia	MW865484	Eprilurahman et al. (2021b)
Subgroup <i>Microhyla pineticola</i>				
<i>Microhyla neglecta</i>	ZMMU A-7308	Lam Dong, Vietnam	MW147159	Poyarkov et al. (2020)
<i>Microhyla neglecta</i>	ZMMU A-7302	Lam Dong, Vietnam	MW147160	Poyarkov et al. (2020)
<i>Microhyla pineticola</i>	ZMMU A4331-16	Lam Dong, Vietnam	MN534567	Gorin et al. (2020)
<i>Microhyla pineticola</i>	ZMMU A6029	Lam Dong, Vietnam	MN534568	Gorin et al. (2020)
Ungrouped				
<i>Microhyla fodiens</i>	ZMMU A5960	Magway, Myanmar	MK208926	Gorin et al. (2020)
<i>Microhyla fodiens</i>	ZMMU A5961	Magway, Myanmar	MK208927	Gorin et al. (2020)
Outgroups				
<i>Microhyla palmipes</i>	MZB Amph 16255	Bali, Indonesia	AB634670	Matsui et al. (2011)
<i>Microhyla rubra</i>	Not preserved	Karnatak, India	AB201192	Matsui et al. (2011)
<i>Microhyla superciliaris</i>	KUHE 53371	Negeri Sembilan, Malaysia	AB634683	Matsui et al. (2011)
<i>Nanohyla annectens</i>	KUHE 53373	Selangor, Malaysia	AB634658	Matsui et al. (2011)
<i>Nanohyla petrigena</i>	KUHE 53743	Sarawak, Malaysia	AB634675	Matsui et al. (2011)
<i>Micryletta inornata</i>	MZB Amph 27242	Aceh, Indonesia	LC208138	Alhadi et al. (2019)
<i>Kaloula pulchra</i>	KUHE35171	Kanchanaburi, Thailand	AB201194	Matsui et al. (2005)

Table S3. Morphometric measurements of the Indian (Garg et al. 2022) and Sumatran (Indonesia) *Microhyla nakkavaram* examined in the current study. HT holotype; PT paratype; ST syntypes; RS referred specimen; M male; F female. All measurements are in millimeters (mm).

Museum no.	Status	Sex	SVL	HW	HL	SL	EL	EN	NS	IUE	UEW	IN	FAL	HAL	FIL	FIIL	FIHL	FIVL	TL	ShL	FOL	TFOL	IMT	OMT	
<i>Microhyla nakkavaram</i> (all the samples collected from Sumatra Island, Indonesia; <i>This study</i>)																									
ENS 20020	RS	M	18.1	7.0	4.3	1.6	1.5	1.0	0.6	2.5	1.0	1.4	3.0	4.4	1.1	1.5	3.0	1.5	8.5	10.3	10.2	15.0	1.2	1.2	
ENS 20028	RS	M	20.1	7.3	5.4	1.7	2.2	1.1	0.5	2.6	1.1	1.7	3.3	4.9	1.0	1.6	2.9	1.8	8.9	11.1	12.2	17.3	1.1	1.1	
ENS20030	RS	M	19.5	7.7	5.3	2.0	2.0	1.3	0.5	2.5	1.0	1.6	3.4	4.8	1.0	1.7	3.2	2.2	9.0	11.6	12.3	19.3	1.0	0.9	
ENS15791	RS	M	16.0	5.9	4.1	1.3	1.3	0.8	0.5	2.1	0.9	1.6	2.6	3.7	0.8	1.4	2.3	1.4	8.3	9.4	9.2	13.4	1.0	0.8	
ENS 16814	RS	M	18.3	6.2	4.9	1.1	1.3	0.8	0.4	2.4	0.8	1.6	2.6	4.0	0.9	1.5	2.7	1.6	8.0	9.3	8.3	12.5	0.8	0.8	
ENS 17968	RS	M	14.4	5.0	3.6	1.5	1.8	1.0	0.6	1.9	0.8	1.3	2.7	3.3	0.4	1.0	1.8	1.0	7.0	7.9	7.5	10.6	0.5	0.5	
ENS20029	RS	M	18.2	6.9	5.4	1.4	1.3	1.0	0.4	2.6	0.9	1.7	2.3	4.1	0.8	1.8	2.9	1.8	9.6	11.7	11.6	16.9	0.5	0.6	
MZBAmph30592/ATT008	RS	M	22.6	7.8	7.1	2.2	1.8	1.7	0.5	2.5	1.1	1.7	3.5	5.4	1.5	2.6	4.6	3.08	8.7	11.1	11.2	17.9	0.7	0.5	
MZBAmph32347/ATT021	RS	M	21.6	8.0	6.0	2.7	2.0	1.8	0.5	2.3	1.0	1.6	3.4	4.9	0.8	0.9	3.2	1.0	9.7	11.6	12.3	17.8	0.9	0.8	
MZBAmph30594/MUN405	RS	M	21.8	7.4	5.8	2.0	1.7	1.7	0.4	2.6	1.1	1.5	3.8	5.3	1.2	1.8	3.4	2.2	10.9	12.5	12.1	18.2	1.0	0.9	
		Min	14.4	5.0	3.6	1.1	1.3	0.8	0.4	1.9	0.8	1.3	2.3	3.3	0.4	0.9	1.8	1.0	7.0	7.9	7.5	10.6	0.5	0.5	
		Max	22.6	8.0	7.1	2.7	2.2	1.8	0.6	2.6	1.1	1.7	3.8	5.4	1.5	2.6	4.6	2.2	10.9	12.5	12.3	19.3	1.2	1.2	
		Mean	19.1	6.9	5.2	1.7	1.7	1.2	0.5	2.4	1.0	1.6	3.1	4.5	1.0	1.6	3.0	1.6	8.9	10.7	10.7	15.9	0.9	0.8	
		SD	2.5	0.9	1.0	0.5	0.3	0.4	0.1	0.2	0.1	0.1	0.5	0.7	0.3	0.4	0.7	0.4	1.0	1.3	1.7	2.7	0.2	0.2	
ENS21278	RS	F	24.8	9.2	6.2	1.7	2.2	1.0	0.6	2.9	1.1	2.3	4.4	5.5	1.3	2.1	3.6	1.9	11.8	13.5	13.7	19.9	1.1	1.0	
ENS 18654	RS	F	20.1	6.7	5.1	2.3	2.5	1.6	0.6	2.4	1.0	1.6	3.7	4.2	0.7	1.2	2.7	1.3	9.1	9.7	10.4	14.3	1.0	1.5	
		Min	20.1	6.7	5.1	1.7	2.2	1.0	0.6	2.4	1.0	1.6	3.7	4.2	0.7	1.2	2.7	1.3	9.1	9.7	10.4	14.3	1.0	1.0	
		Max	24.8	9.2	6.2	2.3	2.5	1.6	0.6	2.9	1.1	2.3	4.4	5.5	1.3	2.1	3.6	1.9	11.8	13.5	13.7	19.9	1.1	1.5	
		Mean	22.4	8.0	5.6	2.0	2.3	1.3	0.6	2.6	1.0	1.9	4.0	4.8	1.0	1.7	3.2	1.6	10.4	11.6	12.1	17.1	1.0	1.3	
		SD	2.4	1.2	0.5	0.3	0.2	0.3	0.0	0.2	0.0	0.4	0.4	0.7	0.3	0.4	0.5	0.3	1.3	1.9	1.7	2.8	0.0	0.3	
<i>Microhyla nakkavaram</i> (all the samples collected from Campbell Bay, India; Garg et al. 2022)																									
ZSI/ANRC/T/14215	HT	M	17.7	5.7	5.4	2.3	1.7	1.2	0.9	2.0	1.0	1.7	3.4	4.5	0.8	1.4	2.7	1.6	9.1	9.9	9.8	14.3	0.6	0.4	
ZSI/ANRC/T/14216	PT	M	16.9	5.5	5.3	2.3	1.5	1.2	0.8	2.1	0.9	1.6	3.3	4.4	0.7	1.4	2.7	1.5	9.0	10.0	9.8	14.5	0.6	0.4	
ZSI/ANRC/T/14217	PT	M	17.5	6.0	5.7	2.4	1.8	1.3	0.9	2.2	0.9	1.7	3.4	4.5	0.8	1.4	2.8	1.6	9.1	10.1	9.9	14.6	0.7	0.5	
ZSI/ANRC/T/14218	PT	M	17.1	5.9	5.5	2.3	1.4	1.2	0.8	2.1	1.0	1.6	3.3	4.4	0.7	1.5	2.8	1.6	9.1	9.9	9.8	14.5	0.6	0.4	
ZSI/ANRC/T/14219	PT	M	16.8	5.4	5.0	2.2	1.4	1.2	0.8	2.0	0.9	1.5	3.3	4.3	0.7	1.3	2.6	1.4	9.0	9.9	9.8	14.5	0.6	0.4	
ZSI/ANRC/T/14220	PT	M	18.3	5.8	5.6	2.5	1.6	1.4	1.1	2.2	1.0	1.8	3.5	4.7	0.9	1.5	2.9	1.6	9.3	10.3	10.2	15.2	0.7	0.5	
SDBDU 2019.4203	RS	M	18.0	5.9	5.7	2.5	1.6	1.4	1.0	2.3	1.0	1.8	3.3	4.6	0.9	1.6	3.0	1.8	9.4	10.2	10.0	14.9	0.7	0.5	
		Min	16.8	5.4	5.0	2.2	1.4	1.2	0.8	2.0	0.9	1.5	3.3	4.3	0.7	1.3	2.6	1.4	9.0	9.9	9.8	14.3	0.6	0.4	
		Max	18.3	6.0	5.7	2.5	1.8	1.4	1.1	2.3	1.0	1.8	3.5	4.7	0.9	1.6	3.0	1.8	9.4	10.3	10.2	15.2	0.7	0.5	
		Mean	17.5	5.7	5.5	2.4	1.6	1.3	0.9	2.1	1.0	1.7	3.4	4.5	0.8	1.4	2.8	1.6	9.1	10.0	9.9	14.6	0.6	0.4	
		SD	0.6	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.1	
DOSMB05003	RS	F	21.5	6.1	6.0	2.7	1.8	1.5	1.1	2.3	1.3	1.7	4.1	5.5	1.5	2.0	3.6	2.4	11.0	12.4	12.1	18.1	0.8	0.6	