

# *Journal of Threatened Taxa*

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*Open Access*

*10.11609/jott.2021.13.12.19675-19886*

*[www.threatenedtaxa.org](http://www.threatenedtaxa.org)*

*26 October 2021 (Online & Print)*

*Vol. 13 | No. 12 | Pages: 19675–19886*

*ISSN 0974-7907 (Online)*

*ISSN 0974-7893 (Print)*





ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher  
**Wildlife Information Liaison Development Society**  
[www.wild.zooreach.org](http://www.wild.zooreach.org)

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No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,  
Coimbatore, Tamil Nadu 641035, India  
Ph: +91 9385339863 | [www.threatenedtaxa.org](http://www.threatenedtaxa.org)  
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Caption: Stripe-backed Weasel *Mustela strigifrons*. Medium—digital, Software—procreate, Device—iPad + Apple pencil © Dhanush Shetty.



## Field identification characters to diagnose *Microhyla mukhlesuri* from closely related *M. mymensinghensis* (Amphibia: Microhylidae) and range extension of *M. mukhlesuri* up to West Bengal State, India

Suman Pratihar<sup>1</sup> & Kaushik Deuti<sup>2</sup>

<sup>1</sup> Department of Zoology, Sukumar Sengupta Mahavidyalaya, Keshpur, West Bengal 721150, India.

<sup>2</sup> Zoological Survey of India, Herpetology Division, 27 JL Nehru Road, Kolkata, West Bengal 700016, India.

<sup>1</sup>pratihar\_vu@rediffmail.com (corresponding author), <sup>2</sup>kaushikdeuti@gmail.com

**Abstract:** *Microhyla mymensinghensis* and *Microhyla mukhlesuri* were recorded from West Bengal, also analyzed 12 characters to separate the species. We also hereby first time confirm the presence of *Microhyla mukhlesuri* from the main land in India

**Keywords:** Amphibia, first record, frogs.

Twenty-seven species have been described within the last 15 years (Frost 2021), yet *Microhyla* remains one of the most taxonomically exciting groups of Asian frogs. Microhylidae is one of the most species rich family of Anura, comprising 690 species in 12 subfamilies (Frost 2020). Previous mitochondrial phylogenies have confirmed that *Microhyla mukhlesuri* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014 is sister to *M. fissipes* Boulenger, 1884 and that together they are sister to *M. mymensinghensis* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014 (Hasan et al. 2014; Howlader et al. 2016; Yuan et al. 2016). Asian congeners, *M. chakrapanii* Pillai, 1977, *M. mukhlesuri*, and *M. mymensinghensis* are nested along with *M.*

*fissipes* Boulenger, 1884 and together these four species are the sister group to the clade containing *M. mixtura* and *M. okinavensis* (Garg et al. 2019). The *M. fissipes* species group consists of two subclades (Poyarkov et al. 2019). Clade one species reported from Laos, Thailand, Myanmar, Philippines, Singapore, southern Asia, and the Andamans (*M. fissipes*, *M. mukhlesuri*, *M. mymensinghensis*, *M. chakrapanii*). And the other clade comprising species from mainland China (Garg et al. 2019).

*Microhyla mymensinghensis* was first described from Mymensingh, Bangladesh together with *M. mukhlesuri* (see Hasan et al. 2014). *Microhyla mymensinghensis* was recorded from Manipur, Meghalaya, Nagaland, Tripura, and West Bengal in India. But *M. mukhlesuri* has been listed only from Mizoram state in the northeastern India (Garg et al. 2019). No details were reported to confirm the finding. Our study confirms the presence of both species in West Bengal, by analyzing 16 morphometric characters to separate these species. We also hereby confirm the presence of *Microhyla mukhlesuri* from

**Editor:** S.R. Ganesh, Chennai Snake Park, Chennai, India.

**Date of publication:** 26 October 2021 (online & print)

**Citation:** Pratihar, S. & K. Deuti (2021). Field identification characters to diagnose *Microhyla mukhlesuri* from closely related *M. mymensinghensis* (Amphibia: Microhylidae) and range extension of *M. mukhlesuri* up to West Bengal State, India. *Journal of Threatened Taxa* 13(12): 19818–19823. <https://doi.org/10.11609/jott.7384.13.12.19818-19823>

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**Funding:** None.

**Competing interests:** The authors declare no competing interests.

**Acknowledgements:** SP wishes to thank the principal of Keshpur College for this study while KD wishes to thank Dr. Kailash Chandra, Director, Zoological Survey of India for use of laboratory facilities like microscope and magnanoscope. We also like to thank to Dr. Nirupam Acharya, Vidyasagar University for his help regarding preparation of the map. We thank Debayan Gayen, junior research fellow, ZSI canning station, WB for doing the PCA analysis.

the Gangetic Plain of West Bengal in India, as it was described from Bangladesh (Hasan et al. 2014).

## MATERIALS AND METHODS

Field work was conducted during late evening in the months of May–July 2020 in Kharagpur, Medinipur Sadar (22.2500°N, 87.6500°E), Ghatal and Jhargram (22.0559°N, 87.1518°E) areas of West Midnapore district in the state of West Bengal, India. Habitats searched included marshes, pools, riverbanks and sandy soils inside grass, leaf litter and under boulders. Morphometric and meristic studies on the preserved specimens were done at the laboratory of the Herpetology Division, Zoological Survey of India, Kolkata using a LEICA EZ-4 stereo binocular microscope and a Heerburg magnanoscope. Measurements were done with Mitutoyo Digital caliper to the nearest 0.1 mm. Four specimens were collected (ZSI A 14818, ZSI A 14819, ZSI A 14871 and ZSI A 14872) and identified as *Microhyla mukhlesuri* (Image 1a, Table 1a). Five specimens were collected (ZSI A 14680, ZSI A 14681, ZSI A 14682, ZSI A 14873 and ZSI A 14874) and identified as *Microhyla mymensinghensis* (Image 1b, Table 1b). We found no *Microhyla ornata* in the sampling

site (Midnapore, West Bengal) during our survey.

For species-level analysis we examined 16 morphological characters to elucidate species identification (1) Body size (SVL) (2) Head length and head width (HL/HW) (3) extent of Tibiotarsal articulation (4) Shape of Terminal phalanges on toes (5) Shape and size of Inner and outer metatarsal, metacarpal tubercle; (6) Webbing on toes (7) length of femur and tibia (8) Dorsal spotting. (9) extent of Lateral stripe (10) pattern on the anus. (11) FAL= Forearm length, LAL= Lower arm length, HAL= Hand length, FAW= Forearm width 12. Axilla-Groin distance (A-G) (Table 1). We also have added HL, HW, FAW, TL and A-G ratios with SVL (Table no 2). We have grouped the species based on characters described by Hassan et al. (2014).

Principal Component Analysis (PCA) was performed on 16 morphometric measurements from specimens of both *Microhyla mukhlesuri* (n= 4) and *Microhyla mymensinghensis* (n= 5) using PAST 3.0 Software. Before doing the PCA, a normality test was done for all the variables. PCA factor scores for principal components (PC) with eigenvalues >1.0 were reported. Factor scores of the first two components were visualized

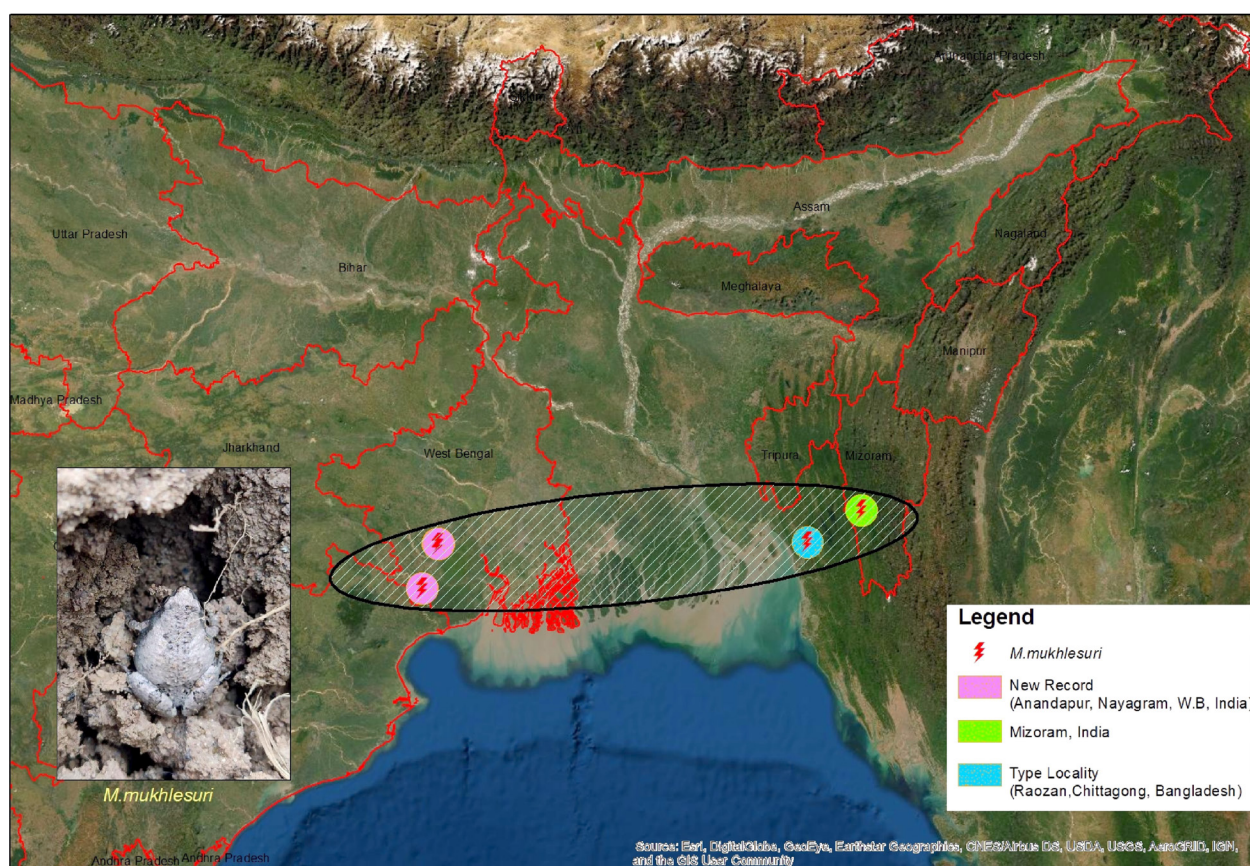


Image 1. Range extension of *Microhyla mukhlesuri* to West Bengal State.



**Table 1a.** Sixteen morphometric character values (in mm) for *Microhyla mukhlesuri*.

Specimen No	ZSI A 14819	ZSI A 14818	ZSI A 14871	ZSI A 14872	Mean
Sex	male	male	male	female	
SVL	20.86	18.92	19.84	20.76	20.09
HL	4.28	4.71	3.98	3.78	4.18
HW	4.56	5.44	4.35	4.36	4.67
SL	2.39	2.57	2.27	2.58	2.45
FAL	3	3.14	4.52	4.29	3.73
LAL	3.09	3.05	3.5	3.67	3.32
HAL	4.17	3.07	3.9	3.06	3.55
FAW	1.08	1.13	1.2	1.33	1.17
IMC	0.48	0.57	0.42	0.44	0.47
OMC	0.42	0.51	0.32	0.38	0.4
A-G	8.52	8.96	8.87	11.33	9.42
FL	8.15	8.23	8.18	8.41	8.24
TL	10.8	9.97	10.56	10.37	10.42
FoL	9.89	10.16	9.41	9.35	9.7
IMT	0.79	0.7	0.69	0.87	0.76
OMT	0.53	0.38	0.52	0.46	0.47

**Table 1b.** Sixteen morphometric character values (in mm) for *Microhyla mymensinghensis*.

Specimen No	ZSIA 14680	ZSIA 14681	ZSIA 14682	ZSIA 14873	ZSIA 14874	Mean
Sex	male	male	female	male	male	
SVL	17.72	18.07	17.97	18.86	17.22	17.96
HL	3.79	3.9	3.74	3.83	3.43	3.73
HW	4.45	5.71	4.42	4.72	5.23	4.9
SL	1.97	2.41	2.35	2.5	2.22	2.29
FAL	4.48	3.71	3.8	4.65	3.55	4
LAL	3.27	3.02	3.4	4.02	3.37	3.41
HAL	3.81	3.77	3	3.86	3.44	3.57
FAW	1.11	1.2	1.02	1.45	1.34	1.22
IMC	0.45	0.42	0.44	0.68	0.55	0.5
OMC	0.37	0.34	0.38	0.55	0.48	0.42
A-G	5.62	6.55	6.14	8.53	7.6	6.88
FL	6.16	6.43	7.94	7.94	6.62	7.01
TL	10.02	9.13	10.29	9.67	9.11	9.64
FoL	8.4	7.53	8.64	8.67	8.13	8.27
IMT	0.58	0.67	0.54	0.54	0.51	0.57
OMT	0.34	0.42	0.38	0.42	0.36	0.38

on scatterplots to assess the degree of morphological differentiation between specimens of the two species.

## RESULTS

Two *Microhyla* species were collected during the present study *Microhyla mukhlesuri* (n= 4) and *M. mymensinghensis* (n= 5). The specimens were identified to belong to the genus *Microhyla* by the following morphological characters: absence of vomerine teeth, hidden tympanum, elliptical tongue, short snout, small eyes not protuberant and invisible from the ventral side, indistinct canthus rostralis and fingers free of webbing. Four specimens were identified as *Microhyla mukhlesuri* (Image 2a) based on size, extent of tibiotarsal articulation, mark on anus, forearm width, tibia length and shape of terminal phalanges (Garg et al. 2019; Hasan et al. 2014). This is the first record of *Microhyla mukhlesuri* from entire Gangetic plains of India, West Bengal (Nayagram, 22.0361°N, 87.1717°E) (Image 1) which extends its range by 494 km from its type locality (Rhozan, Chittagong, Bangladesh) and by 568 km away from Mizoram, India (which was the only record from India Garg et al. 2019). Whereas *M. mymensinghensis* (Image 2b) is identified based on size, tibia length, mark on anus and forearm width (Garg et al. 2019; Hasan et al. 2014). We found *M. mukhlesuri* differs from *M.*

*mymensinghensis* by its relatively larger adult size (Garg et al. 2019) and inverse U-shaped black mark above the anus for *M. mukhlesuri* whereas crescent-shaped black mark present above the anus in *M. mymensinghensis* as described by Hassan et al. (2014).

Identifying taxonomic characters to differentiate *M. mukhlesuri* and *M. mymensinghensis* is problematic. Both species exhibit similar extent of tibiotarsal articulation (when the hind leg is adpressed) up to the snout or between the eye and snout and a lateral stripe that also extends to near the snout. Garg et al. (2019) paid attention to the shape of terminal phalanges, though the holotype description by Hassan et al. (2014) focused on forearm width, tibial length and mark on anus. We would like to focus on five putative distinguishing characteristics (a) an inverse U shape mark on the anus for *M. mukhlesuri* (vs crescent shaped mark in *M. mymensinghensis*) (Hassan et al. 2014); (b) a larger SVL in *M. mukhlesuri* (19.92–20.86 mm) (n= 4), vs. shorter (17.22–18.86 mm) (n= 5) in *M. mymensinghensis*, (c) *Microhyla mymensinghensis* has longer Tibia (TL) than *Microhyla mukhlesuri*, (d) *Microhyla mymensinghensis* has thicker fore-arm (FAW) than *Microhyla mukhlesuri* (Hassan et al. 2014), and (e) terminal phalanges of fourth toe tip knobbed but flattened in *M. mukhlesuri* (vs. knobbed but not flattened in *M. mymensinghensis*)



Image 2: a—*Microhyla mukhlesuri* (top) from Jhargram, West Bengal on 30 June 2020 during evening observation, in preservation | b—*M. mymensinghensis* (bottom) from Midnapore, West Bengal on 29 June 2020 during night, in life. © Suman Pratihari

(Garg et al. 2019) (Table 1). Based on these characters we have identified and classified the specimens examined in this study.

In addition to aforementioned described characters, we identified a few supplementary characters to differentiate two species more confidently: (a) shape of inner metacarpal tubercle elongated in *M. mukhlesuri* vs. rounded in *M. mymensinghensis*; (b) large inner metatarsal tubercle, i.e., about 0.76 ( $\pm 0.08$ ) mm in *M. mukhlesuri* vs about 0.57 ( $\pm 0.06$ ) mm in *M. mymensinghensis*; (c) most significantly, axilla-groin distance 47% of SVL in *M. mukhlesuri* vs 38% of SVL in *M. mymensinghensis*. Both these species were observed co-occurring in humanized / agricultural habitats in the sampled areas (Image 3a, b).

*Microhyla mukhlesuri* showed differences with *M. mymensinghensis* in terms of morphology. Principal Component Analysis (PCA) for the specimens of both the species (*M. mukhlesuri*,  $n=4$ ; *M. mymensinghensis*,  $n=5$ ) recovered two Principal Components (PC) with eigenvalues  $> 1.0$  that accounted for 85.8% of the total variance. PC1 explained 74.09% variance with highest loadings for A-G, TL, SVL and FoL. PC2 explained 11.71% variance with highest loadings for A-G, and HW. The remaining factors explained 14.2% of the variations. For the combined data set for both male and female projections of the factor planes 1 and 2 showed distinct clusters for the two species (Figure 1).

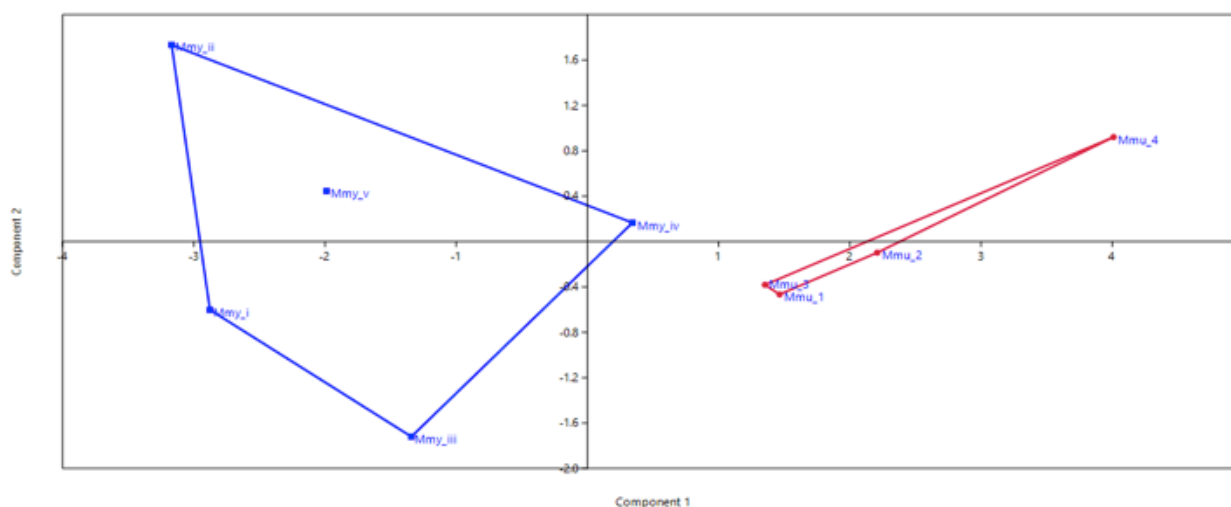


Figure 1. Principal component analysis (PCA) Scatter plot depicting morphometric differences between *Microhyla mukhlesuri* and *Microhyla mymensinghensis*.



Image 3. Habitat of (a) *Microhyla mukhlesuri* (left) and (b) *M. mymensinghensis* (right). © Chandan Dandapat

Table 2. Ratios of morphometric character values for *Microhyla mukhlesuri* and *Microhyla mymensinghensis*. For character abbreviations see Materials & Methods.

Ratios	<i>Microhyla mymensinghensis</i>	<i>Microhyla mukhlesuri</i>
HL/SVL	.21±.01	.22±.02
HW/SVL	.27±.04	.24±.03
FAW/SVL	.07±.003	.06±.005
AG/SVL	.38±.04	.47±.06
TL/SVL	.54±.02	.51±.01
HL/HW	.76±.09	.89±.03

## DISCUSSION

Previously, *Microhyla 'ornata'* like species were grossly grouped with four known species—*M. mukhlesuri*, *M. mymensinghensis*, *M. nilphamariensis*, and *M. ornata*. In 2018 two well-supported species groups were established—(1) *M. mukhlesuri* and *M. mymensinghensis*, along with *M. fissipes* from southeastern and eastern Asia, and (2) *M. nilphamariensis* and *M. ornata*, along with *M. taraiensis* from Nepal (Garg et al. 2018). A prominent lateral stripe extends from belly to nostril in *M. mukhlesuri* and *M. mymensinghensis* whereas a less prominent lateral stripe extends from shoulder to belly in *M. nilphamariensis* and *M. ornata*.

An inverse U shape mark on the anus for *M. mukhlesuri* (vs crescent shaped mark in *M. mymensinghensis*); larger SVL in *M. mukhlesuri* (19.92–20.86 mm) vs. shorter (17.22–18.86 mm) in *M. mymensinghensis* and terminal phalanges of toes knobbed but tip flattened in *M. mukhlesuri* (vs. knobbed but not flattened in *M. mymensinghensis*) are used to group the two species. In addition to this we have identified axilla-groin distance,

size of metatarsal tubercle and shape of metacarpal tubercle to differentiate these two species more confidently. The PCA results reveal two different clusters on the scatterplot, representing two different species – *Microhyla mukhlesuri* (n= 4) and *M. mymensinghensis* (n= 5).

*Microhyla mukhlesuri* has been reported only from Mizoram state in the northeast India (Garg et al. 2019). With two males and two female specimens we hereby for first time confirm the presence of *Microhyla mukhlesuri* from the Gangetic Plain in West Bengal, apart from Mizoram. We are optimistic about the wider distribution of *Microhyla mukhlesuri* in the Gangetic plains. Nevertheless we do encourage genetic studies of these *Microhyla* frogs, especially after finding geographic contact zones where two similar-looking, genetically-allied congeners *Microhyla mukhlesuri* and *M. mymensinghensis* co-occur, throwing open necessity for further fine-scale diagnosis, preferably morphological, between them.

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ravi@threatenedtaxa.org

**Journal of Threatened Taxa** is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64



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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

October 2021 | Vol. 13 | No. 12 | Pages: 19675–19886

Date of Publication: 26 October 2021 (Online & Print)

DOI: 10.11609/jott.2021.13.12.19675-19886

[www.threatenedtaxa.org](http://www.threatenedtaxa.org)

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