MAJOR ARTICLE

TAPROBANICA, ISSN 1800–427X. Vol. 14, No. 02 (2025): pp. 158–165, pls. 13–14.
Published by Research Center for Climate Change & Faculty of Mathematics & Natural Sciences, Universitas Indonesia, Depok 16424, INDONESIA.
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http://www.taprobanica.org
https://doi.org/10.47605/tapro.v14i2.367



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urn:lsid:zoobank.org:pub:E9077959-8548-42C2-9B46-53656F39EC0C

A NEW SPECIES OF THE GENUS *Smithophis* GIRI, GOWER, DAS *ET AL.*, 2019 (REPTILIA: SERPENTES: NATRICIDAE) FROM MIZORAM, NORTHEAST INDIA

Submitted: 14 March 2025, Accepted: 30 June 2025, Published: 5 August 2025 Subject Editor: Ivan Ineich

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Abstract

Based on two specimens, we describe a new species of snake in the genus *Smithophis* from Mizoram, as determined through detailed morphological and molecular analyses of the cytochrome b mitochondrial gene. The new species genetically forms a sister taxon to the clade containing *S. atemporalis*, *S. bicolor*, and *S. mizoramensis*, with a strong nodal branch support along with a deep level of genetic divergence. The species can be easily distinguished from its congeners by several morphological and colouration characters, including the presence of distinct, narrow, and incomplete transverse bands in the dorsum. Our study also precisely determines the distribution of *S. bicolor*, restricting its range to Meghalaya and removing it from the faunal list of Mizoram.

Keywords: cytochrome b, India, rain snake, Rhabdops, Smithophis bicolor, systematics, taxonomy

Introduction

Blyth (1855) originally described *Smithophis bicolor* in the genus *Calamaria*, based on a specimen provided by Mr. Robinson, with "Assam" as the type locality. In the same publication, he stated that the specimen may have originated from the Khasya Hills (now the Khasi Hills, Meghalaya) or another upland territory. The specimen had a dusky-plumbeous dorsal colouration, a buffy-white ventral surface, 17 mid-dorsal scale rows, 210 ventral scales, 75

paired subcaudals, a snout-vent length of 495 mm, and a tail length of 111 mm. Later, Boulenger (1893) assigned "bicolor" to the genus Rhabdops Boulenger, 1893, thereby extending its known distribution to the Khasya hills, Assam (now Khasi hills, Meghalaya), and Yunnan (China). He designated Rhabdops olivaceus (Beddome, 1863), a species from the Western Ghats, as the type species of Rhabdops that was later expanded (see Giri et al. 2017) to include R. aquaticus Giri, Deepak, Captain &

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Gower, 2017 from the Western Ghats. Later, phylogenetic analysis revealed that populations previously referred to R. bicolor from northeast India were genetically distinct from their Western Ghats congeners. Therefore, Giri et al. (2019) erected a new genus Smithophis, to accommodate the northeast Indian populations, reassigning S. bicolor to this new genus and describing Smithophis atemporalis Giri, Gower, Das et al., 2019 as a new species. Since the erection of Smithophis, three new species have been described, further expanding the diversity of the genus to five species. The other three species are Smithophis arunachalensis Das, Deepak, Captain et al., 2020, Smithophis linearis Vogel, Chen, Deepak et al., 2020, and Smithophis mizoramensis Mirza, Bhardwai, Lalmuanawma et al., 2024.

A recent study has provided new insights into the taxonomy of Smithophis bicolor (see Purkayastha et al. 2025) based on genetic data from a specimen collected in the Eastern West Meghalaya Khasi Hills. (25.559415°N, 91.635047°E; 1564 m a.s.l.), a locality that closely matches Blyth's (1855) probable type locality. Comparative morphological and genetic analyses revealed that S. bicolor from Meghalaya is distinct from the populations in Mizoram, which were previously considered conspecific (Giri et al. 2019). These findings indicate that the Mizoram population, previously identified as S. bicolor, represents an undescribed species within Smithophis. Here, based on freshly collected samples from Mizoram, we formally describe this population from Mizoram, previously referred to as Smithophis cf. bicolor (Giri et al. 2019) as a new and distinct species.

Materials and methods

Two specimens obtained from Mizoram (MZMU1798 and MZMU2606) were euthanized using 2% Lignocaine, fixed as well, and preserved in 95% ethanol and have been deposited in the National Zoological Collection (MZMU, India, Aizawl), maintained by the Department of Zoology, Mizoram University. A small portion of the liver tissue was excised from a specimen (MZMU1798) and was preserved in absolute (99.9%) ethanol for molecular analysis, prior to fixation. All measurements were recorded to the nearest 0.1 mm using a Mitutoyo digital calliper, while total length, snout-vent length, and tail length were measured using a measuring tape. The following morphometrics and meristics were recorded: SVL (Snout Vent

Length, from the tip of snout to the anterior of the vent), TaL (Tail Length, from the posterior of the vent to tip of the tail), HL (Head Length, measured at the angle of jaw), HW (Head Width measured at the angle of jaw), HD (Head depth, measured at the angle of jaw), ED (Eye diameter, horizontal eye diameter), SW (Snout width at the tip), S-N (Snout to nostril distance, measured from tip of snout to the anterior of nostril), S-E (Snout to eye distance, measured from tip of the snout to the anterior of the eye), N-E (Nostril to eye distance), INS (Internarial space, space between the nostrils when measured dorsally), IOS (Interorbital space, space between the eyeball when measured dorsally), Ve (number of ventrals), Sc (number of subcaudal scales), SL (Number of supralabial scales), SL-E (Number of supralabials touching orbit), IL (Number of infralabial scales), DSR (Dorsal Scale Rows, (i) counted one head length behind the head, (ii) at the midbody, (iii) one head length in front of vent), PrO (Number of preocular scales), PoO (Number of post-ocular scales), Tem (Number of temporal scales), As (Anal scale entire or divided), Lor (number of loreal scale), COS (Number of circumorbital scales, scales around the orbit). The specimens were morphologically compared with all the known congeners, including those occurring in northeast India and surrounding countries. Comparisons were made by examination of the type specimens of all recognized species as well as additional referred materials, literature containing original descriptions, and literature revising species based on type specimens (see Giri et al. 2019, Das et al. 2020, Vogel et al. 2020, Mirza et al. 2024).

For this study, genetic data of one adult specimen (MZMU1798) was generated from Mizoram. Comparative data were obtained from Genbank following recent work (Giri et al. 2019, Das et al. 2020, Vogel et al. 2020, Mirza et al. 2024). Genomic DNA was extracted from ethanol (99.9%) preserved liver tissue using the (Oiagen) according manufacturer's instructions. A partial sequence of the mitochondrial cytochrome b gene was generated using the primer pair L14910 (GACCTGTGATMTGAAAAACCAYCGTTGT), H16064 (CTTTGGTTTACAAGAACAATGC TTTA) (Burbrink et al. 2000). Sequence chromatograms were quality checked, edited, and assembled into contigs using Chromas and Sequence Scanner v1.0 (Applied Biosystems). Comparative cytochrome b sequences comprising members of Smithophis

Opisthotropis species were obtained from GenBank (Sup. Table 1, following Giri et al. 2019, Das et al. 2020, Vogel et al. 2020, Mirza et al. 2024). Sequence alignment was performed using MUSCLE (Edgar 2004) in MEGA7 (Tamura & Nei 1993; Kumar et al. 2016) with parameter settings. default Α maximum (ML) phylogenetic likelihood constructed using the unpartitioned dataset in IQ-TREE (Nguyen et al. 2015) with the substitution model GTR+F+I+G4 selected based on the BIC scores by the Model Finder (Kalyaanamoorthy et al. 2017) implemented in the IQ-TREE (Nguyen et al. 2015) in the automatic setting, and a sequence of Aspidura drummondhayi from Genbank was used as an outgroup before and tree was visualized in FigTree (version 1.4.4). The ML analysis was run with an ultrafast bootstrap option (Minh et al. 2013) for 1000 iterations to assess clade support, and an ultrafast bootstrap value ranging from 90-100 represents a very strongly supported node. The uncorrected

pairwise *p*-distance was calculated in MEGA7 (Kumar *et al.* 2016) with pairwise deletion of missing data and gaps.

Results

Our molecular phylogenetic analysis, based on 1109 base pairs of the mitochondrial cytochrome b gene, recovered the Mizoram population previously assigned to Smithophis bicolor (sensu Giri et al. 2019) as a distinct, well-supported lineage (bootstrap value >90). Smithophis cf. bicolor from Mizoram is deeply nested within the monophyletic clade of Smithophis and is recovered as a sister taxon to a clade comprising S. bicolor sensu stricto and S. mizoramensis, with bootstrap support (>90) Uncorrected pairwise sequence divergence (pdistance) in the cytochrome b gene further supports its distinctiveness, with values of 9.8% from S. mizoramensis, 11.5% from S. bicolor, 12.5% from S. atemporalis, and 16.9% from S. linearis (Sup. Table 2).

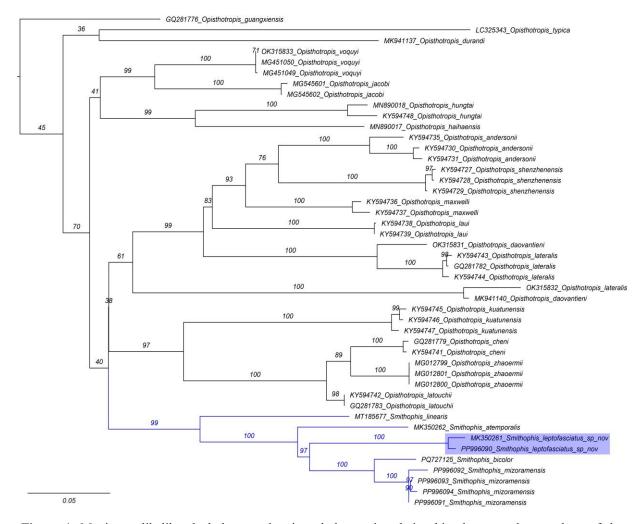


Figure 1. Maximum likelihood phylogeny showing phylogenetic relationships between the members of the genus *Smithophis* and *Opisthotropis* based on the Cytochrome b mitochondrial gene.

Taxonomy

Integrative taxonomic approach reveals a suite of diagnostic characters that differentiate the new species from all its congeners; hence, we herein describe it as a distinct species endemic to Mizoram, India.

Smithophis leptofasciatus sp. nov. (Figs. 2–4, Table 1) [urn:lsid:zoobank.org:act:7E911E63-BBA1-4F3C-9C8F-4A46DFC4B602]

Smithophis bicolor—Giri et al. 2019, Das et al. 2020, Ruatpuii et al. 2022, Mirza et al. 2024

Holotype. MZMU1798, a sub-adult male collected from Maubuang Village (23.485089°N, 92.703705°E; alt. 983 m a.s.l.), Aizawl District, Mizoram, India by Lal Muansanga at 20:35 hr on 31 July 2020.

Paratype. MZMU2606, adult female collected from Suangpuilawn Village (23.949966°N, 93.036849°E; alt. 1,087 m a.s.l.), Saitual District, Mizoram, India by J.C. Lalmuanawma and H.T. Lalremsanga at 19:45 hr on 09 October 2021.

Other material photographed. A roadkill (not preserved) and five live specimens (not collected).

Diagnosis. The new species is a small sized natricid snake having a maximum SVL of 329 mm as well as a maximum TaL of 82 mm and can be diagnosed from all its congeners based on a combination of the following characters: (1) 17 smooth dorsal scale rows throughout the body; (2) 195-201 ventrals; (3) 67-78 subcaudals, all of which are paired; (4) anal scale paired; (5) a total of five circumorbital scales surrounding and touching the eye; (6) temporal scales distinctly present; (7) absence of keeled sacral scales in both males and females; (8) dorsum colour pattern is mostly composed of a shiny dark or a black background intermixed with distinct, narrow and creamish-white or yellowish-lime transverse bands that covers a width of only one or two dorsal scales - these narrow bands nearly encircle the body since it either breaks into streaks or becomes irregular in the vertebral region, thereby forming incomplete bands; (9) ventral region in usually yellowish-lime or creamish-white in colouration; (10) underside of the tail is much darker compared to other ventral portions of the body.

Description of the holotype. The holotype is generally in good preservation condition, but the

specimen looks slightly dehydrated, possibly due to its preservation in ethanol. A sub-adult male with a somewhat cylindrical and slender body having an SVL of 204 mm, TaL of 54 mm, and a TaL/TL ratio of 0.21.

Head elongated, moderately large and somewhat distinct from neck (HL/SVL 0.05); snout rather large, long as well as broad and semi-oval in shape as seen from above; eye moderately large (ED 0.90) and pupils are round; rostral scale visible from above, much wider than high and in broad contact with first supralabial. anterior portion of nasal as well as inter-nasal; nasal scale on either dorso-lateral side of the head, indistinctly pentagonal, somewhat broad and elongated; nasal scale sub-equally divided into two halves by a somewhat perpendicular suture below the irregularly circular nostrils on the right side of the head whereas on the left side, the nasal scale appears to be undivided due to the absence of any suture; nostrils lie right in the centre of the nasal scale laterally; a single internasal clearly visible from above, much wider than long and in broad contact with rostral, nasal and prefrontal scale; a single prefrontal irregularly hexagonal in shape, much wider than long and broadly in contact with internasal, nasal, loreal, two circumorbital scales (i.e. one preocular and one supraocular) and frontal; the prefrontal is larger than the internasal in both length and width; frontal large and distinct, mostly pentagonal, though irregularly hexagonal, somewhat sub-equal in length and width, about nearly one and a half times longer than prefrontal length and is in contact with prefrontal, a circumorbital scale (i.e. supraocular) and a pair of parietals; a total of five circumorbital scales surrounding the orbit which includes one preocular, one pre-subocular, one post-subocular, one postocular and one elongated supraocular; a pair of divided parietals that are large, much longer than broad, broadly in contact with each other and are also in contact with dorsal scales on the occiput, temporals (anterior as well as posterior), two circumorbital scales (i.e., a postocular and a supraocular) as well as the frontal; a distinct loreal scale on either dorsolateral side of the head; temporal scales 1+1 on both sides of the head with the anterior one being more than twice as long as the posterior one; a total of five supralabials on both the sides, all separated from the orbit by circumorbital scales, the first one being the shortest which touches the rostral, nasal, loreal and third supralabial while the fifth one being the longest which is broad contact with both anterior and posterior temporals; seven infralabials on each side, of which the fourth one is the longest and the first pair of infralabials are broadly in contact with each other behind the mental scale; mental scale is roughly triangular, wider than high; two pairs of chin shields i.e. anterior and posterior ones, both of which are nearly sub equal in size and are much longer than broad; the posterior chin shields are separated from each other by an interval of two distinct scales and mental groove is apparent; furthermore the posterior chin shields are in contact with three to four gular scales that are present before pre-ventrals. There are nine maxillary teeth, increasing in size gradually towards the rear of the jaw, with the last two being the largest and more sharply curved inward compared to the anterior teeth.

Dorsal scales somewhat rhomboid in shape, arranged in 17:17:17 rows, all of which are smooth, including the scales in the sacral region; ventral scales are 201 (plus 2 preventrals), anal plate is completely divided, and subcaudals are 78 in number, all of which are paired. Tail is moderately long, complete, having a length of 54 mm with a Tal/TL ratio of 0.21 and ends with a short tapering spine with a somewhat sharp tip.

Colouration. In life (based on Fig. 3), the dorsum of *Smithophis leptofasciatus* sp. nov. is predominantly shiny dark or black, interspersed with distinct, narrow, creamish-white or yellowish-lime transverse bands, each covering only one or two dorsal scales. These bands nearly encircle the body but become irregular or break into streaks along the vertebral region, forming incomplete bands. The ventral surface is typically yellowish-lime or creamish-white, while the underside of the tail is noticeably darker compared to the rest of the ventral region.

In preservative, the overall colouration of the specimen looks faded. The dorsum is mostly composed of a shiny, dark, or black background intermixed with distinct, very narrow, and creamish-white transverse bands that appear to be somewhat trapezoid and are irregularly Vshaped. These narrow bands originate from the ventral edges, where they are usually slightly wider (covering at least two dorsal scales), gradually becoming narrower upwards (covering only one dorsal scale) while extending towards the vertebral region transversally. These narrow bands nearly encircle the body. It either breaks into streaks or becomes irregular in the vertebral region, thereby forming incomplete bands. The ventral region is off or creamish-white in

colouration, whereas the underside of the tail is much darker, having shades of dark brown and black as compared to other ventral portions of the body. The dorsal part of the head is dark brown with shades of chocolate brown intermixed with tiny ochre-like patches.

Variation. The paratype (MZMU2606) generally matches the holotype in every aspect of morphology. Due to the lack of further specimens, no proper variation and dimorphism were observed between the sexes. However, the holotype (MZMU 1798) has a significantly higher number of subcaudals as compared to the female paratype. (78 vs 67 in the adult female paratype).

Table 1. Morphometrics and meristics for *Smithophis leptofasciatus* sp. nov. from Aizawl, Mizoram, India (L/R = left/right sides; NA = not available.

Character	Smithophis	
	leptofasciatus sp.	
	nov.	
	MZMU	MZMU
	1798	2606
	Holotype	Paratype
	(Male)	(Female)
Number of ventrals	201	195
Number of subcaudals	78	67
Number of supralabials	5/5	5/5
Supralabials touching orbit	none	none
Number of infralabials	7/7	7/7
Dorsals	17/17/17	17/17/17
Number of preoculars	1/1	1/1
Number of post-oculars	1/1	1/1
Temporals	1+1/1+1	1+1/1+1
Anal scale	divided	divided
Number of loreal scales	1/1	1/1
Snout-vent Length	204	329
Tail Length	54	82
Head Length	9.55	12.20
Head Width	5.10	6.64
Head depth	3.05	4.19
Eye diameter	0.90	0.78
Snout width at the tip	2.14	2.94
Snout-nostril distance	1.11	1.79
Snout-eye distance	2.78	3.52
Nostril-eye distance	1.85	2.12
Internarial space	2.22	2.97
Interorbital space	3.75	3.99

Comparison. Smithophis leptofasciatus sp. nov. differs from all its congeners based on a combination of the following characters: (1) Presence of 195–201 ventrals (vs 200–213 in S. mizoramensis, 183–191 in S. linearis), (2) Presence of 67–78 subcaudals (vs 59–64 in S.

Plate 13

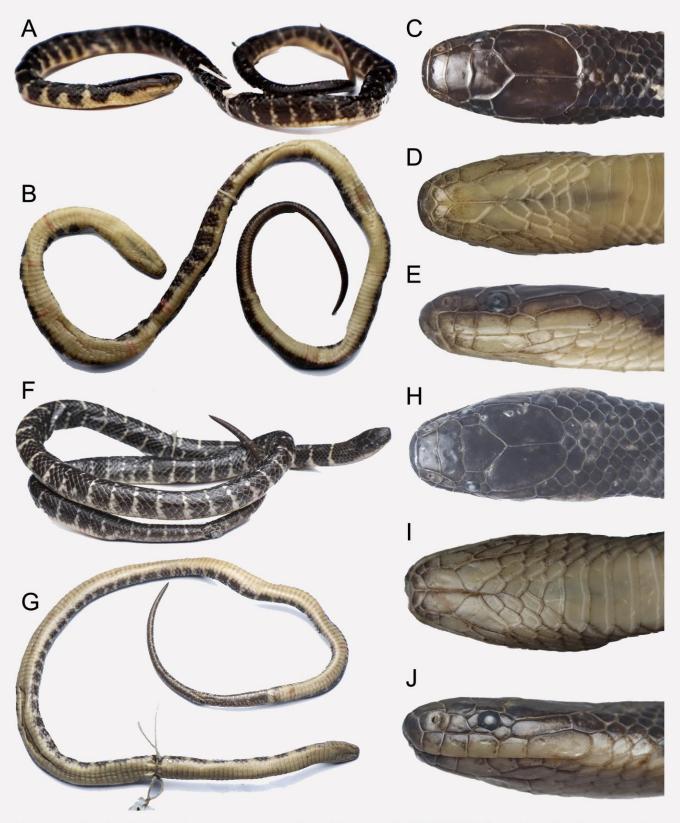


Figure 2. *Smithophis leptofasciatus* sp. nov. **(A–E)** Holotype (MZMU1798) from Aizawl, Mizoram, India: **(A)** dorsal and **(B)** ventral views of the full body; **(C)** dorsal, **(D)** ventral, and **(E)** lateral views of the head; **(F–J)** Paratype (MZMU2606): **(F)** dorsal and **(G)** ventral views of the full body; **(H)** dorsal, **(I)** ventral, and **(J)** lateral views of the head; © Photo: L. Muansanga

Plate 14



Figure 3. *Smithophis leptofasciatus* sp. nov. in life **(A)** holotype (MZMU1798), **(B)** paratype (MZMU2606), **(C-E)** uncollected specimens from Murlen, Reiek, and Lunglei, respectively

linearis), (3) Presence of distinct temporal scales (vs absent in S. atemporalis), (4) Presence of 5 circumorbital scales (vs 6–7 in S. linearis), (5) Absence of keeled sacral scales in the males (vs presence of keeled sacral scales in S. arunachalensis and S. mizoramensis), Dorsum mostly composed of a shiny dark or a black background intermixed with distinct, narrow and creamish white or lime yellowish incomplete transverse bands that covers a width of only one or two dorsal scales (vs large trapezoid bands covering at least 4–5 scales in S. arunachalensis; no such bands in S. linearis and bicolor; dorsum completely dark and ventrolateral region as well as ventrals completely gamboge yellow forming a typical bi colored appearance in S. bicolor).

Etymology. The specific epithet "leptofasciatus" is a Greek and Latin compound adjective (leptos+fasciatus) in the nominative singular given in masculine, which refers to "narrow banded" transverse body pattern on the dorsum, a distinct and distinguishing characteristic of the species. Suggested common name: narrow-banded rain snake (in English) and Ruahrul (in Mizo).

Hemipenis. The hemipenis data provided herein were from a road-killed male specimen from Aizawl, Mizoram. The hemipenis of *Smithophis leptofasciatus* sp. nov. (Fig. 4) is a relatively short, bilobed organ with well-defined lobes, exhibiting moderate calyculation and dense microspiculate ornamentation.

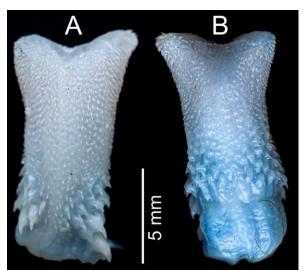


Figure 4. Hemipenis of *Smithophis leptofasciatus* sp. nov. **(A)** sulcal and **(B)** asulcal side

The lobes are symmetrically bifurcated, with the apex featuring numerous papillate and small spinulate structures. In the sulcate view, the

sulcus spermaticus is single (unbranched), extending distally along the medial surface of the hemipenial body and terminating at the base of the lobes, with well-defined, slightly flared margins bordered by small spinules that increase in density towards the apex. The asulcate surface is adorned with tightly packed spinules, becoming more prominent toward the lobes, while the basal region remains relatively smooth with a few scattered spines. Notably, the base of the hemipenis exhibits larger and more prominent spines, which are well defined and conspicuous compared to the lobular region.

Natural history. The new species seems to be semi-aquatic and nocturnal rather than diurnal or crepuscular, as they were encountered inside and close to small streams (Fig. 5), under leaf litter, and beneath rocks or decaying logs, often in shaded and humid microhabitats at night during the monsoon season. All handled specimens were very timid, gentle, showing no inclination to bite when handled. captivity, In individuals voraciously fed on earthworms. An uncollected gravid female laid a total clutch of six eggs (36-43 mm in length and 12-13 mm in width) on 16 November 2021 at Lunglei, Lunglei district, Mizoram. The eggs were oval, whitish in colour, soft, and leathery in texture. Further studies on its feeding ecology, reproductive cycle, and habitat preferences are needed to better understand its life history and conservation requirements. The species was found living in sympatry with congeners, including atemporalis and S. mizoramensis.



Figure 5. Microhabitat of *Smithophis leptofasciatus* sp. nov. at Suangpuilawn (paratype locality) © J.C. Lalmuanawma.

Habitat and distribution. Smithophis leptofasciatus sp. nov. is currently known only from moist forested landscapes in Aizawl, Mizoram, India (Fig. 6). All known specimens

were collected from tropical wet montane and tropical semi-evergreen forests at elevations ranging from approximately 900–1,200 m a.s.l. Given the ecological similarity and contiguous forested landscapes between Mizoram and the neighboring state of Tripura, it is plausible that the species may also occur in suitable habitats within Tripura. However, further herpetofaunal surveys are required to confirm its presence beyond its currently known range.

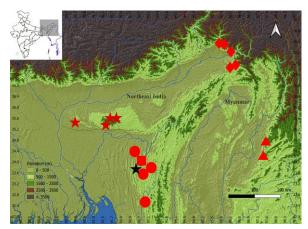


Figure 6. The distribution of the genus *Smithophis*: *S. bicolor* (red star), *S. atemporalis* (red circle); *S. mizoramensis* (red square); type locality of *S. leptofasciatus* sp. nov. (mentioned as *S. bicolor* in Giri *et al.* 2019 marked in black star); *S. arunachalensis* (red diamond); *S. linearis* (red triangle).

Discussion

The type series of *Smithophis leptofasciatus* sp. nov. comprises only two catalogued specimens; however, we have additionally observed five live individuals in the field and one roadkill, all displaying the characteristic narrow-banded dorsal pattern diagnostic of S. leptofasciatus sp. nov. Notably, none of the observed individuals lacked dorsal bands. Nevertheless. acknowledge the unbanded specimen BNHS2369, reported by Das et al. (2020), which was genetically allied to S. leptofasciatus. This raises the possibility of intraspecific colour polymorphism. Although we initially suspected a potential tag mismatch given the morphological similarity of BNHS2369 to S. mizoramensis. We now consider colour polymorphism a plausible alternative explanation. This hypothesis merits further investigation, particularly in light of our limited sample size of only eight individuals. The discovery of Smithophis leptofasciatus sp. nov. from Mizoram marks the third species of *Smithophis* described from the state, following *S*. atemporalis and S. mizoramensis. This further highlights the biogeographic significance of

Mizoram in shaping the diversity evolutionary history of the genus Smithophis. The state, located within the Indo-Burma biodiversity hotspot, harbours a unique assemblage of montane and submontane forest ecosystems that provide critical microhabitats for Smithophis species. The presence of three distinct Smithophis taxa in Mizoram, each exhibiting varying degrees of morphological and genetic divergence, suggests that the region may have served as a key diversification region for the genus, potentially driven by historical biogeographic barriers which including rivers, elevational gradients, and climatic fluctuations, which need further studies.

Purkayastha et al. (2025) suggested that the position of the type locality of S. bicolor is towards Khasi Hills and recognized its current confined distribution as to Meghalaya, specifically within the Khasi and Garo Hills. The revision of S. bicolor by Purkayastha et al. (2025) clarifies previous misidentifications and aligns with morphological and molecular data that suggest S. bicolor sensu stricto is distinct from the population present in Mizoram. With the current knowledge, we remove S. bicolor from the known inventories of Mizoram and replace it with Smithophis leptofasciatus sp. nov. as detailed above. Phylogenetic analyses based on the mitochondrial cytochrome b sequences further confirm the genetic divergence of Smithophis leptofasciatus sp. nov. from S. bicolor sensu stricto, aligning with previous studies that suggested cryptic diversity within Smithophis. Earlier workers, like Ruatpuii et al. (2022) and Chinliansiama et al. (2022), reported the species distribution in the state and female oviposition based on specimens referred to as S. bicolor.

The restricted range of *Smithophis* leptofasciatus sp. nov. in Mizoram, its habitat preferences close to water sources in montane forests, and its morphological adaptations indicate a potential case of endemism, emphasizing the need for targeted conservation efforts. Given the rapid habitat degradation in Northeast India, further field surveys and ecological studies are essential to assess the species' conservation status and ecological requirements. The description of Smithophis leptofasciatus sp. nov. further highlights the of importance continued taxonomic investigations in the Indo-Burma biodiversity hotspot, where hidden herpetofaunal diversity remains to be fully explored and documented.

Author contributions

All the authors contributed equally.

Acknowledgements

We thank F. Malsawmdawngliana, Lalrinsanga, G.Z. Hmar, Romalsawma, and M. Vanlalchhuana for the assistance; V. Santra, P. Shinde, and G. Choure for contributing photos; Ibuki Fukuyama (Hokkaido University, Japan), Patrick David and Ivan Ineich (Muséum National d'Histoire Naturelle, France), and Gernot Vogel (Germany) for reviewing the manuscript.

Research permits

Collecting permit issued by the Environment, Forests and Climate Change Department, Mizoram, Government of Mizoram, India (No. A.33011/2/99-CWLW/225).

Funding information

The financial grants DST-SERB, New Delhi (CRG /2023/000805), The Habitats Trust (THT) Action Grant, Noida, Uttar Pradesh, and the Chicago Board of Trade (CBOT) Endangered Species Fund USA, and the Indian Council of Medical Research (ICMR Nos. 58/6/2/SB-CFP/AKM/2023/NCD-II and 58/6/3/SB-CFP/AKM/2023/NCD-II) to HTL and DST-SERB, New Delhi (EEQ/2023/000877) to VB.

Supplemental data

https://doi.org/10.47605/tapro.v14i2.367

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