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Cover: Nilgiri Large Burrowing Spider *Haploclastus nilgirinus*. Acrylic on canvas. © Aakanksha Komanduri.

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ARTICLE

Comments on the systematics and morphology of *Smithophis bicolor* (Blyth, 1855) (Reptilia: Squamata: Natricidae) based on topotypical specimens from Meghalaya, India

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Abstract: The Natricid Snake *Smithophis bicolor* has been poorly studied and understood since its initial description in the mid-1800s. This study presents a detailed morphological description of *S. bicolor* based on five examined specimens including a freshly collected mutilated specimen from Meghalaya State, India. For the first time, genetic data has been obtained and analysed, providing novel insights into the species' phylogenetic position within its genus *Smithophis*. Morphological comparisons and molecular analyses using cytochrome b mitochondrial gene reveal subtle variations that contribute to a clearer understanding of the species' taxonomic identity. This study allows us to conclude that currently *S. bicolor* is restricted to the higher elevations of Meghalaya indicating that literature records mentioning the presence of this species from Mizoram actually represents a distinct, new unnamed lineage.

Keywords: Cytochrome b, hemipenis, herpetofauna, Khasi hills, Mairang, northeastern india, phylogeny, re-description, shillong plateau, taxonomy, type locality.

Khasi: Ia u bsein bala khot u *Smithophis bicolor* ym shym la pule bad sngewthuh bha naduh ba la batai nyngkong ia u ha ka shiteng jong ki snem 1800. Kane ka jingpule ka ai ia ka jingbatai bniah ia ka dur jong u ne u bsein, katkum ki san tylli ki nongmuna ba la bishar bniah kynthup ia u bsein ba lah iap ba dang shu lum thymmai na ka jylla Meghalaya, India. Ha ka sien kaba nyngkong, la ïoh ban bishar bniah ïa ki jingtip ba ïadei bad ki gene, kaba ai ïa ki jingshem ba thymmai shaphang ka kyrdan jinglong jing man (phylogenetic position) jong une bsein hapoh ka jait jong u *Smithophis*. Ka jingïanujor ïa ka dur jong ka met bad ki jingpeit bniah da kaba pyndonkam ïa ki mitochondrial gene ba la khot cytochrome b, ki pyni ïa ki jingiapher kiba rit kiba ïarap ban sngewthuh kham shai ïa ka jingbuh kyrteng jong kine ki jait bsein. Kane ka jingpule ka ailad ia ngi ban pynkut ba mynta u *S. bicolor* u don tang ha ki jaka ba kham sha jrong jong ka Meghalaya, watla ki jingthoh ki kdew ruh ia ka jingdon jong une u jait bsein ha Mizoram hynrei u lah ban dei u ba pher, u bym pat ai kyrteng.

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Author details and **Author contributions:** See end of this article.

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INTRODUCTION

Blyth (1855) described *Smithophis bicolor* in the genus *Calamaria* Boie, 1827 based on a single specimen (holotype ZSI 7030) provided by Mr. Robinson with the type locality recorded as Assam, India. In the same piece of work, Blyth (1855: p. 287) mentioned that the specimens provided by Mr. Robinson might be from Khasya Hills (present day Khasi Hills, Meghalaya State, India) or other upland territories. The holotype ZSI 7030 was mentioned to be with a dusky-plumbeous dorsal surface and a buffy white ventral surface, 17 mid-dorsal scale rows, 210 ventrals and 75 pairs of subcaudals, snout-vent length of 495 mm and a tail length of 111 mm. Boulenger (1893) allocated *bicolor* to the genus *Rhabdops* Boulenger and stated its distribution from Khasi Hills, Assam and Yunnan. *Rhabdops olivaceus* (Beddome 1863) with distribution in the Western Ghats, India is the type species of *Rhabdops* along with the recently described *Rhabdops aquaticus* Giri et al. 2017 also known from the Western Ghats, India whereas *Rhabdops bicolor* was the only species known from northeastern India. Based on genetics and disjunct distribution in Western Ghats and northeastern side, Giri et al. (2019) erected the genus *Smithophis* to accommodate northeastern populations (*bicolor*) and also described a new species in the genus namely *Smithophis atemporalis* noting that the genus *Smithophis* is more closely related to *Opisthotropis* and *Sinonatrix* than to *Rhabdops*. Furthermore, Giri et al. (2019) in their genetic assessment of *Smithophis bicolor* used data based on materials from Mizoram State, northeastern India. Since then, three more species were added to the genus, namely, *Smithophis arunachalensis* Das et al., 2020; *S. linearis* Vogel et al., 2020 and *S. mizoramensis* Mirza et al., 2024 (Mirza et al. 2024a). Chandramouli et al. (2021) provided a morphological description of an adult male topotypic specimen of *S. bicolor* from Shillong, in the Khasi Hills. Giri et al. (2019) in their genetic assessment of *S. bicolor* used data based on materials from Mizoram, India but missed out the typical population from the Khasi Hills of Meghalaya. In this study, based on fresh collection and museum specimens (Zoological Survey of India, Northeastern Regional Centre, Shillong, Meghalaya State, India) we provide additional morphological and molecular insights for *Smithophis bicolor* from its type locality and point to the possibility of existence of an undescribed *Smithophis* from Mizoram State, India, treated as *Smithophis* cf. *bicolor* by Giri et al. (2019).

MATERIALS AND METHODS

The study was conducted in Khasi hills of Meghalaya and throughout Mizoram after obtaining permission for collection within both the states from the chief wildlife warden of Environment, Forests and Climate Change Department, Government of Meghalaya and Mizoram (No. FOR./7/2021/216 for Meghalaya and A.33011/2/99-CWLW/225 for Mizoram). A road killed male specimen of *Smithophis bicolor* was collected from Mairang (ADBU-HN/HW0210), Eastern West Khasi Hills district (25.559°N, 91.635°E; altitude 1,564 m; see Image 1), Meghalaya, India by Holiness Warjri on 12 August 2024 at around 1230 h and has been deposited in the herpetological museum maintained by the Department of Zoology, Assam Don Bosco University, Sonapur, Assam, India. Furthermore, four samples (V1/ERS/ZSI-444, V1/ERS/ZSI-2592, V1/ERS/ZSI-3052 from Shillong, Meghalaya and VR/ERS/ZSI-725 from Umiam, Meghalaya) were also studied from the collection of Zoological Survey of India, Eastern Regional Station, Shillong, Meghalaya, India. Genomic DNA was extracted from ethanol (100%) preserved liver tissue of the specimen ADBU-HN/HW0210 using tissue kit (Qiagen) following manufacturer's instructions. Partial sequence of the mitochondrial cytochrome b gene was generated using the primer pair L14910 (GACCTGTGATMTGAAAAACCAYCGTTGT), H16064 (CTTTGGTTTACAAGAACAATGCTTTA) (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 (Table 1) sequences were used following Mirza et al. (2024) and were obtained from GenBank. Sequence alignment was done using MUSCLE (Edgar 2004) in MEGA7 (Tamura & Nei 1993; Kumar et al. 2016) with default parameter settings. Maximum likelihood (ML) phylogenetic tree was reconstructed using un-partitioned dataset in IQ-TREE (Nguyen et al. 2015) with the substitution model GTR+F+I+G4 selected based on the BIC scores by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in the IQ-TREE (Nguyen et al. 2015). The ML analysis was run with an ultrafast bootstrap option (Minh et al. 2013) for 1000 iterations to assess clade support. The un-corrected pairwise p-distance was calculated in MEGA7 (Kumar et al. 2016) with pairwise deletions of missing data and gaps.

Morphometrics and meristics are taken as follows: HL (Head length taken from the retro articular process of the jaw to the tip of the snout), HW (Head width measured at the widest point of the head), HD (Head

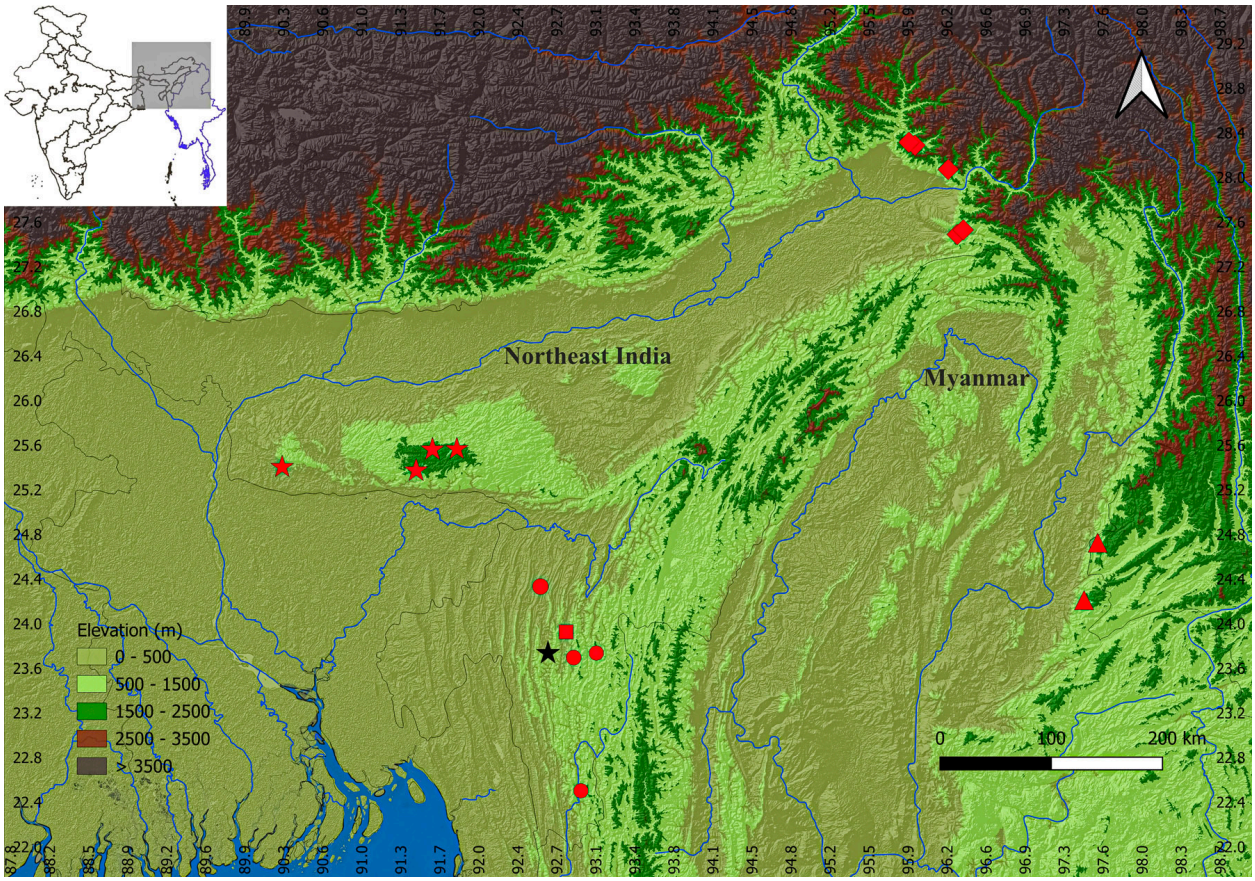

Image 1. The map showing distribution records of members of the genus *Smithophis* (red star: *S. bicolor*, red circle: *S. atemporalis*, red square: *S. mizoramensis*, black star: *Smithophis* sp. (mentioned as *Smitophis bicolor*, see Giri et al. 2019), red diamond: *S. arunachalensis*, red triangle: *S. linearis*).

depth measured at the greatest depth of the head), ED (Eye diameter taken horizontally at the outer margins of the orbit), ND (Greatest nostril diameter), NS (Nostril to snout distance taken from the anterior portion of nostril to the tip of the snout), EN (Eye to nostril distance taken from the anterior portion of orbit to the posterior margin of nostril openings), IND (Internarial distance measured between the two nostril openings), IOD (Inter orbital distance measured between the minimum distance of two outer orbital margins), PL (Greatest length of the parietal scale), SVL (Snout-vent length measured from the tip of the snout to the vent ventrally), TaL (Tail length measured from the vent to the tip of the tail ventrally), TL (Total length measured from the tip of the snout to the to the tip of the tail ventrally or dorsally), DSR (Dorsal scale rows counted in a row transversely in three sections, i.e., exactly one head length behind the neck, exactly in the midbody and exactly one head length before the vent dorsally), V (Number of ventral scales counted ventrally from the 1st scale just behind pre-ventrals till the last scale just before the anal plate), SC (Number of subcaudal scales beginning just behind the cloaca ventrally till the last scale in the tail excluding the terminal scute), SL (Number of supralabial scales counted on the upper lips), IL (Number of infralabial scales counted on the lower lips), COS (Number of circumorbital scales surrounding the orbit), Temp (Number of anterior plus posterior temporal scales in the lateral portion of the head), and Anal (Condition of the anal plate whether its divided or single).

RESULTS

Based on Cyt b (1109 bp) data generated in this study, the *Smithophis bicolor* from Mairang, Eastern west Khasi Hills, Meghalaya State was seen to show a sister relationship to *S. mizoramensis* differing in a low to moderate uncorrected p-distance of 4% whereas *S.* cf. *bicolor* from Mizoram State (Giri et al. 2019) was seen to be sister to the clade comprising *S. bicolor* (Mairang) + *S. mizoramensis*. *Smithophis* cf. *bicolor*

Table 1. List of cytochrome b sequences used in this study. New sequence entry in bold.

Species	Voucher	Genbank accession number	Location
Opisthotropis andersonii	SYS r001383	KY594735	Mt. Maofeng, Guangzhou, Guangdong, China
Opisthotropis cheni	YBU 071040 (=GP383)	GQ281779	Nanling NR, Guangdong, China
Opisthotropis daovantieni	FMNH 252009	OK315831	An Ke, Gia Lai, Vietnam
Opisthotropis durandi	NCSM 80739	MK941137	Nam Lan, Phosaly, Laos
Opisthotropis guangxiensis	GP746	GQ281776	Guangxi, China
Opisthotropis haihaensis	SYS r000537	MN890017	Quang Ninh, Vietnam
Opisthotropis hungtai 1	SYSr000538	MN890018	Mt. Wuhuang, Guangxi, China
Opisthotropis hungtai 2	SYS r000946	KY594748	Heishiding NR, Fengkai, Guangdong, China
Opisthotropis jacobi	ZFMK 100818	MG545602	Tam Dao NP, Vinh Phuc, Vietnam
Opisthotropis kuatunensis	SYS r000998	KY594745	Qixiling NR, Yongxin County, Jiangxi, China
Opisthotropis lateralis	ZMMU NAP-08678	OK315832	Xuan Son NP, Phu Tho, Vietnam
Opisthotropis latouchii	GP647	GQ281783	Fujian, China
Opisthotropis laui	SYS r001161	KY594738	Shangchuan Í., Taishan, Guangdong, China
Opisthotropis maxwelli	SYS r000841	KY594736	Nan'ao Is., Nan'ao, Guangdong, China
Opisthotropis shenzhenensis	SYS r001032	KY594729	Mt. Tiantou, Shenzhen, Guangdong, China
Opisthotropis typica	HT0794	LC325343	Malaysia
Opisthotropis voquyi	ZMMU R-16681	OK315833	Tay Yen Tu NR, Bac Giang, Vietnam
Opisthotropis zhaoermii	CIB110000	MG012801	Guzhang, Hunan, China
Smithophis atemporalis	BNHS 2366	MK350262	Mizoram, India
Smithophis cf. bicolor	BNHS 2369	MK350261	Mizoram, India
Smithophis cf. bicolor	MZMU 1798	PP996090	Aizawl, Mizoram, India
Smithophis bicolor	**ADBU-HN/ HW0210**	**PQ727125**	**Mairang, Eastern West Khasi Hills, Meghalaya, India**
Smithophis linearis	KIZ 059110	MT185677	Yunnan, China
Smithophis mizoramensis	BNHS 3766	PP996092	Suangpuilawn, Saitual, Mizoram, India
Smithophis mizoramensis	BNHS 3767	PP996093	Suangpuilawn, Saitual, Mizoram, India
Smithophis mizoramensis	BNHS 3768	PP996094	Suangpuilawn, Saitual, Mizoram, India
Smithophis mizoramensis	MZMU 2602	PP996091	Suangpuilawn, Saitual, Mizoram, India
Trimerodytes percarinata	ZMMU R-16444	OK315846	Pu Mat NP, Nghe An, Vietnam

from Mizoram differs by an uncorrected p-distance of at least 11.5% from the *S. bicolor* s. str. of Mairang, Eastern West Khasi Hills, Meghalaya State (nearly thrice as high as the divergence between *S. bicolor* s. str. and *S. mizoramensis*) (Figure 1, Table 2). Genetic divergence for the cytochrome b (cyt b) gene reveals that true *S. bicolor* is 4% to 14.3% divergent from other *Smithophis* species. Morphologically, the species *S. bicolor* differs from its sister *S. mizoramensis* mainly in body coloration as well as carination condition of the sacral scales.

Morphology of topotypical, Meghalaya specimens

(V1/ERS/ZSI-2592, V1/ERS/ZSI-725, V1/ERS/ZSI-3052, V1/ERS/ZSI-444, ADBU-HN/HW0210; Images 2–4): *Smithophis bicolor* is mainly characterized by having 17 smooth dorsal scale rows across the body without having any keeled sacral scales in males. It possesses temporal scales (1+1) and features 5 circum-orbital scales along with a single internasal and a single prefrontal scale. Tail is relatively short and ends with a short spine like scale. The examined specimens from Meghalaya exhibit an elongated body with a head slightly distinct from neck, little longer than broad (HW/HL 0.58–0.75), slightly flattened dorsoventrally (HL/HD 0.41–0.57). The eyes are moderately sized (ED/HL 0.12–0.19); nostrils are placed closer to snout than to the eye (NS/EN 0.69–0.88), relatively small in size (ND/ED 0.50–0.71); a pair of parietals, each parietal scale is relatively large, half or slightly more than half of head length (Parietal/HL 0.50–0.61); SVL ranges from 360–535 mm; TaL ranges from

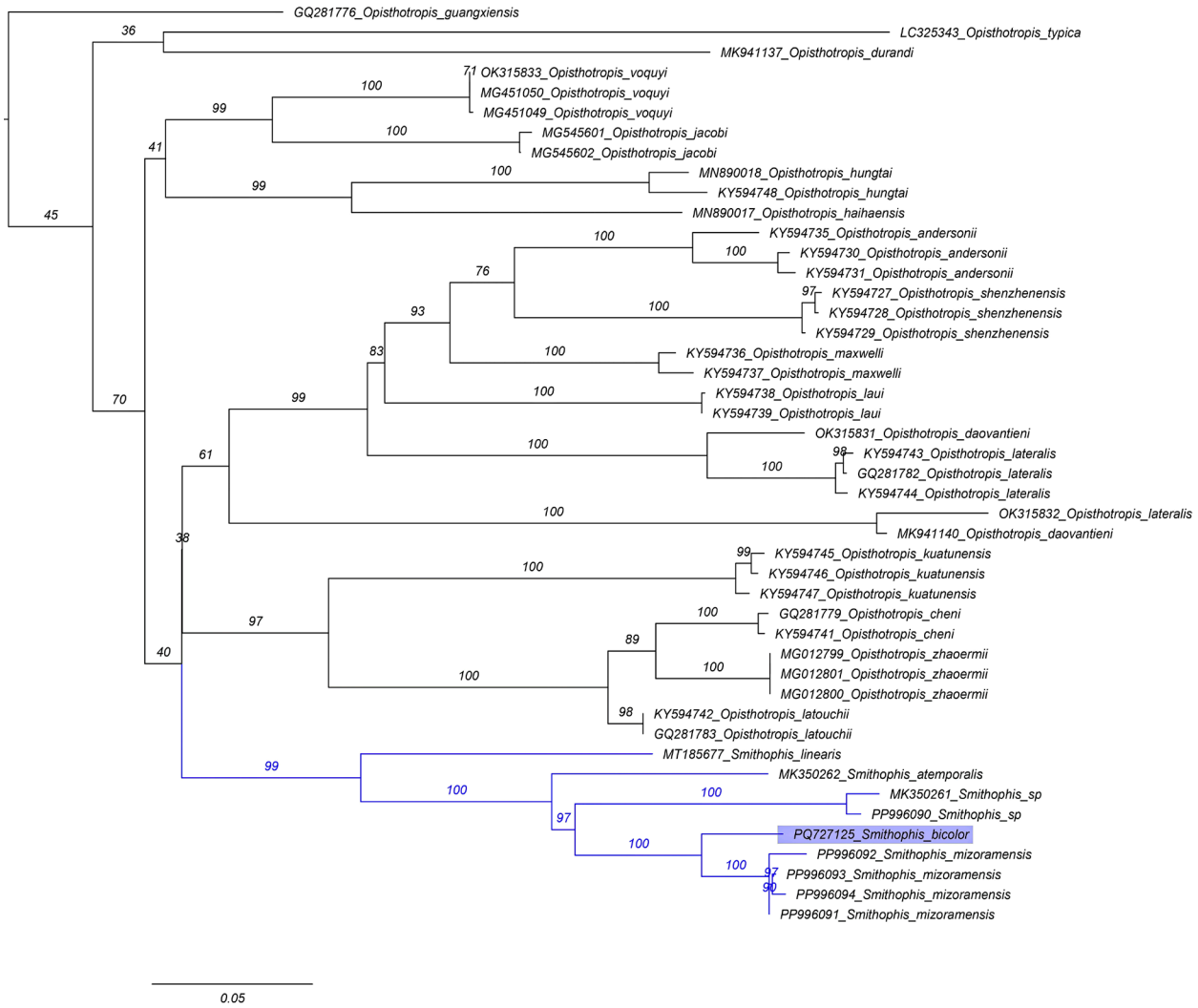

Figure 1. Maximum likelihood phylogeny showing the relationships amongst the members of genus *Smithophis* based on Cytochrome b mitochondrial gene. Preceding the species name is NCBI accession number and numbers at nodes represent bootstrap support values.

Table 2. Uncorrected p-distance between the members of the genus *Smithophis*.

PQ727125_*S._bicolor*								
PP996092_*S. mizoramensis*	0.040							
PP996093_*S._mizoramensis*	0.040	0.000						
PP996094_*S._mizoramensis*	0.040	0.000	0.000					
PP996091_*S._mizoramensis*	0.040	0.000	0.000	0.000				
MK350262_*S._atemporalis*	0.107	0.096	0.096	0.096	0.096			
MK350261_*S._cf. bicolor*	0.115	0.098	0.098	0.098	0.098	0.125		
PP996090_*S._ cf. bicolor*	0.115	0.098	0.098	0.098	0.098	0.125	0.000	
MT185677_*Smithophis_linearis*	0.143	0.138	0.138	0.138	0.138	0.156	0.169	0.169

110–160 mm; tail relatively short (TaL/SVL 0.30–0.44; TaL/TL 0.22–0.25); TL ranges from 470–690 mm. The meristic characteristics of the specimens are consistent across the examined specimens. All individuals possess 17–17–17 dorsal scale rows; Ventrals 209–212 in males and 192 in females; subcaudals 75–80 in males and 68 in

Image 2. Studied specimens of *Smithophis bicolor* from the collection of Zoological Survey of India, Eastern Regional Station, Shillong, Meghalaya (A—V1/ERS/ZSI 444 | B—V1/ERS/ZSI 725 | C—V1/ERS/ZSI 2592 | D—V1/ERS/ZSI 3052). © Jayaditya Purkayastha.

females; SL usually 5 and IL usually 6 or 7. One unsexed individual has 212 ventrals and 71 subcaudals, SL 5 on each side and IL 6 on each side. The circum-orbital scale count is uniform across all specimens, with five scales surrounding each eye. The temporal scales are arranged in a 1+1 pattern in all individuals and anal plate is divided in all specimens.

Sexual dimorphism

Based on our data, males of this species have ventrals ranging from 209 to 212 (versus 192 in a female), Sc ranging from 75 to 80 (*versus* 68 in a female) and Tal/TL ratio of 0.23 to 0.25 (versus 0.22 in a female).

Coloration in preservative (Image 2)

The specimens in ZSI, especially V1/ERS/ZSI 444 and 3052, have become completely faded in comparison to their coloration in life (read below), such that there is barely much difference between their dorsal and ventral colours, indicating bleaching of dorsal colour. Eyes became paler and pupil became light grey.

Coloration in life (based on live uncollected snakes; Image 3)

Smithophis bicolor we encountered had a dorsal region with uniformly shiny black above with a slightly violet tinge, whereas the ventral is uniformly (mostly) gambodge yellow in life. In the posterior half of the lateral region, a prominent, broad gambodge yellow stripe runs along the body, covering the ventrals. The dorsal and ventral coloration meet somewhat at the middle of the lateral region, giving it a typical bicoloured appearance which begins from the snout and extends up to the tip of the tail in life.

Image 3. Uncollected live specimens of *Smithophis bicolor* from Makwyarwat (Southeastern Khasi Hills, Meghalaya: A & B) and Mairang (eastern West Khasi Hills, Meghalaya, C & D). © Goldenstar Thongni and Holiness Warjri.

Hemipenial description (based on ADBU-HN/HW0210; Image 4)

Short and stout, characterized by heavy spinosity over the distal three-quarters, with the proximal one-quarter, near the base, exhibiting a somewhat calyculate texture. The spines are more pronounced and larger towards the intermediate region and the base, gradually becoming smaller and conspicuous, somewhat hair or serrations like towards the tip. The spines in hemipenis of *Smithophis bicolor* are much more pronounced than its sister taxa *S. mizoramensis*.

Comparisons

Smithophis bicolor has well defined temporal scales (1+1) vs. absent in *S. atemporalis*; *Smithophis bicolor* does not have keeled sacral scales in males vs. presence of keeled sacral scales in *S. arunachalensis* and *S. mizoramensis*. The dorsum is immaculate in *Smithophis bicolor vs.* either blotched or striped in all the other congeners. Furthermore, *Smithophis bicolor* has 4–5 circumorbital scales vs. 6–7 in *S. linearis*.

Table 3. Morphometric and meristic data of *Smithophis bicolor* from eastern and western Khasi Hills, Meghalaya State, India (-- indicates missing data). Measurements in mm.

Museum no.	V1/ERS/ ZSI-2592	V1/ERS/ ZSI-725	V1/ERS/ ZSI-3052	V1/ERS/ ZSI-444	ADBU-HN/ HW0210
Location	Fruit garden, Shillong	Tripura Castle Road, Shillong	Risa Colony, Shillong	Umiam, Umsaw, NEPA Campus	Mairang, Eastern West Khasi hills
Sex	Unsexed	Male	Female	Male	Male
HL	12.54	10.9	14.74	9.8	11
HW	8.28	7.52	9.79	7.48	7.5
HD	7.09	5.8	7.51	5.5	5.1
ED	1.8	1.9	1.51	1.62	1.7
ND	1.27	0.9	0.9	1.18	0.9
NS	1.94	1.6	1.9	1.43	1.8
EN	2.17	1.87	2.6	2.15	2.2
IND	3.48	3.4	3.45	3.2	3.4
IOD	5.8	5.34	5.77	5.2	4.8
PL	7.63	6	7.21	6.8	6.5
SVL	520	385	535	360	360
TaL	160	113	155	120	110
TL	680	498	690	480	470
DSR	17/17/17	17/17/17	17/17/17	17/17/17	17/17/17
V	212	209+	192	212	209
SC	71	75	68	80	76
SL (L/R)	5/5	5/5	5/5	5/5	5/--
IL (L/R)	6/6	6/6	6/6	7/6	--
COS	5	5	5	5	5
Temp	1+1	1+1	1+1	1+1	1+1
Anal	Divided	Divided	Divided	Divided	Divided

Natural history and distribution (Image 5)

Based on our current data, *Smithophis bicolor* is a terrestrial to semi-aquatic species which mainly prefers to live in close proximity to streams and other water bodies surrounded by semi-evergreen, evergreen, moist deciduous, montane forests and at times even moderately disturbed hilly areas. Based on the specimens, the species is found in areas of higher elevation ranging 1,000–1,700 m. All the freshly observed specimens from Meghalaya State were seen near streams, boulders with or without mosses, loose soils or roadside edges with or without mosses within wet montane or wet semi-evergreen or evergreen forests, particularly during the day. It has been observed that this species (just like other congeners) is often seen or becomes active during heavy rainfall. The current understanding based on the available data allows us to conclude that *Smithophis bicolor* is currently restricted to the higher elevations of Meghalaya State, particularly in the Khasi Hills and its adjacent areas, i.e., Garo hills whereas those reported as *S. bicolor* s. lat. from regions outside Meghalaya State might represent a different species.

DISCUSSION

Despite being one of the earliest described species of the genus *Smithophis*, *S. bicolor* lacked any new information until recent times (Chandramouli et al. 2021). This study not only gives genetic data from the type locality of *S. bicolor* but also provides expanded morphological description of the species based on multiple specimens. The examination of additional topotypic specimens of *S. bicolor* from Meghalaya resulting in this expanded morphological description makes the species better characterised in terms of its morphology. This provides a concrete dataset for researchers to describe as well as compare new and

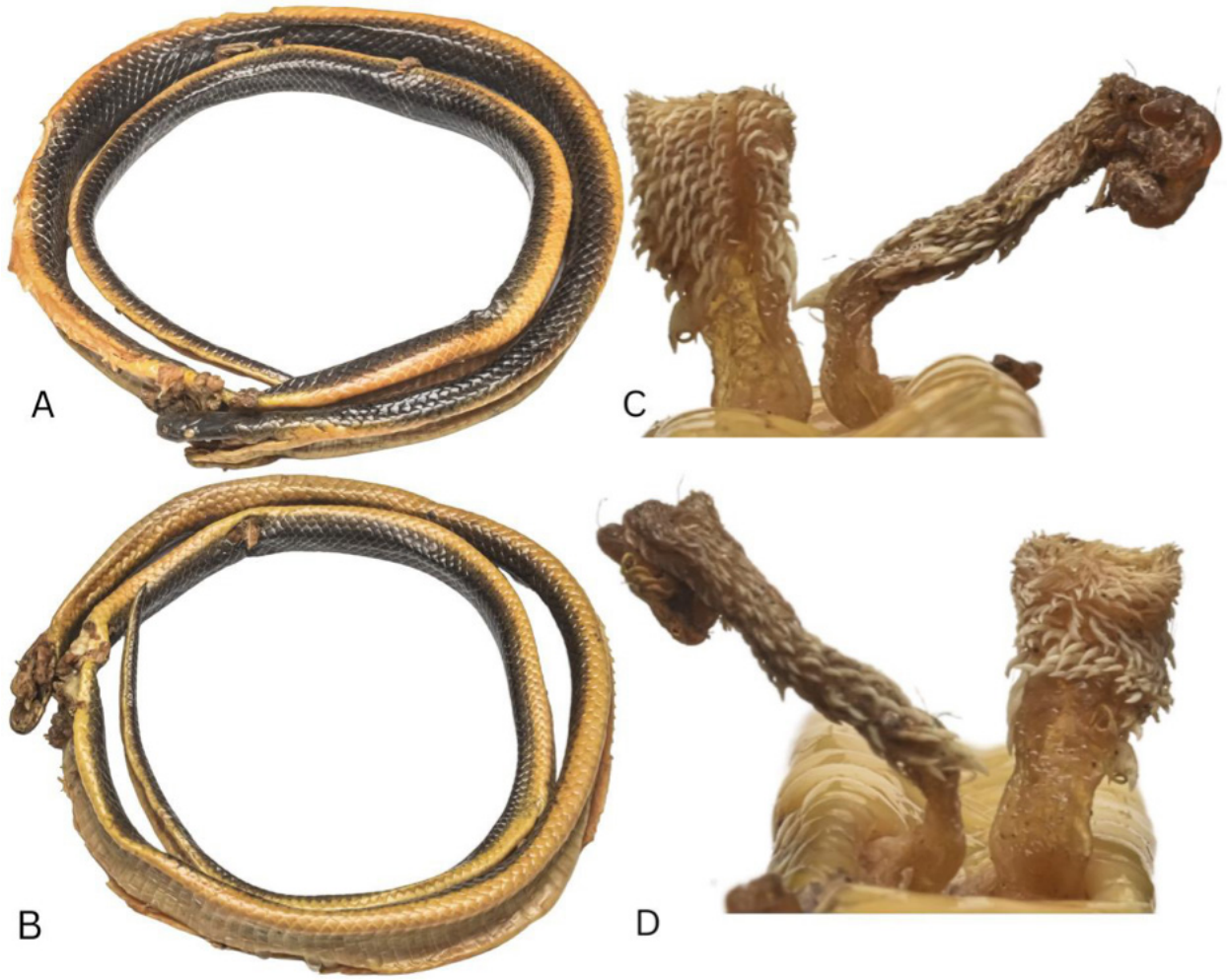

Image 4. Mutilated specimen (ADBU-HN/HW0210) of *Smithophis bicolor* from Mairang (Eastern West Khasi Hills, Meghalaya: A&B)—along with its hemipenis | C—sulcal surface | D—asulcal surface. © Sanath Chandra Bohra.

previously described species with the nominal taxon in the future. Furthermore, the population previously identified as *S. bicolor* from Mizoram (Giri et al. 2019) which represents a genetically and morphologically distinct lineage, will be formally described as a new species in our forthcoming publication in preparation.

Meghalaya State, which is rich in terms of biodiversity forms a part of the Indo-Burma biodiversity hotspot which lies on the southern bank of river Brahmaputra and has been relatively well surveyed historically, particularly during the time of British India. Since then, after a long time the state has witnessed a significant increase in the number of new herpetofaunal species descriptions in the past two decades alongside range extensions regarding certain species (e.g., Das et al. 2010; Mahony et al. 2011, 2013, 2018, 2020; Purkayastha & Matsui 2012; Datta-Roy et al. 2013; Kamei et al. 2013; Agarwal et al. 2018b; Purkayastha et al. 2020a,b; 2021; 2022; Rathee et al. 2022; Mirza et al. 2024b). Despite being a described species, further research and field work are necessary to properly understand the accurate distribution range, ecology, reproductive biology and conservation biology of *S. bicolor*. The Khasi Hill is the "type locality" for snake species like *Stoliczkia khasiensis* Jerdon, 1870 which is known just from a single specimen and since its description in 1870, the species was never reported for the second time as a result of which it has been considered as a lost species. This highlights the importance of conducting continuous as well as comprehensive herpetofaunal surveys throughout Khasi Hills and its adjoining hill ranges before the depletion of forest cover due to anthropogenic pressures.

We suggest further field investigations involving wide samplings throughout the given range of the genus *Smithophis* to understand the potential reason behind the diversification of these natricids that will definitely

Image 5. *Smithophis bicolor* in Makyrwat, southwestern Khasi Hills, Meghalaya, India. © Goldenstar Thongni and Holiness Warjri.

help to uncover further new unnamed populations in the region.

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