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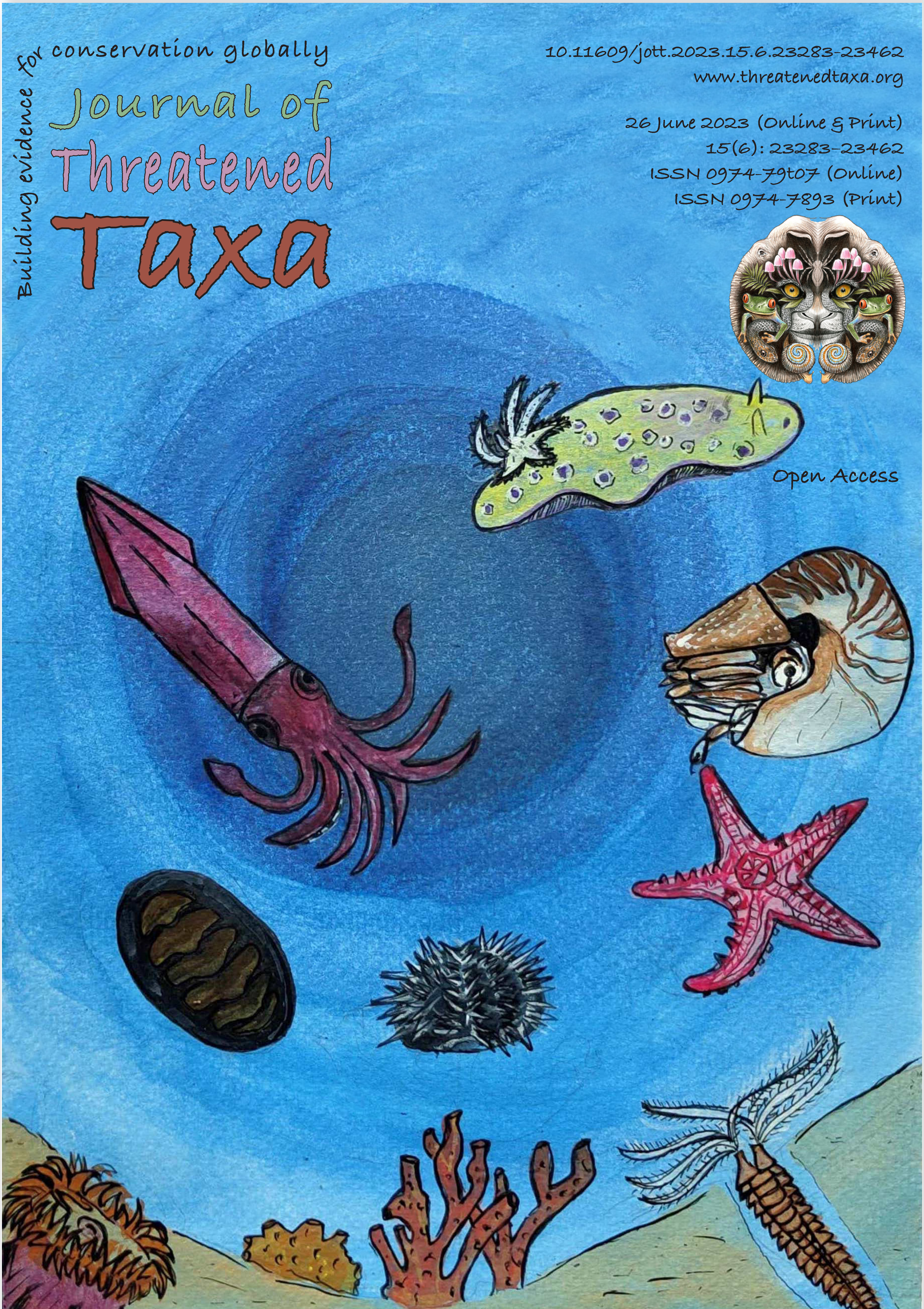

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COMMUNICATION

Addition of four new records of pit vipers (Squamata: Crotalinae) to Manipur, India

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Abstract: In spite of the potential richness in biodiversity, Manipur is still one of the least explored areas in northeastern India. In this work, we report on our finding of four pit viper species, namely, *Trimeresurus mayaae*, *T. salazar*, *T. erythrurus*, and *Protobothrops jerdonii* in Manipur during our herpetological surveys in 2022. We affirm the identity of these specimens based on the combination of standard morphological characters and mitochondrial cytochrome b gene. Our study not only reports four new records of pit vipers for the first time in Manipur, but also extends their respective known geographical ranges. This information calls for further dissemination especially to health care sector, as it is on venomous snakes that could be of medical importance as regards human snakebites.

Keywords: morphology, new state report, northeastern India, phylogenetics, *Protobothrops*, range extension, systematics, *Trimeresurus.*

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Competing interests: The authors declare no competing interests.

Author details: Premjit Singh Elangbam is a BCom graduate currently engaging in wildlife photography & research in Manipur. He has contributed photographs and articles in many books on flora & fauna of Manipur especially Indian birds. He has involved in many efforts in conservation research conducted by Forest Dept., Govt. of Manipur. Now, he is secretary of Wildlife Explorers Manipur, an organization fully devoted to wildlife photography based on conservation & research in Manipur. Lal Biakzuala is an emerging researcher in the field of herpetological taxonomy and systematics, natural history, molecular phylogenetics, and conservation. He is currently pursuing his PhD degree focussing on the molecular systematics of elapid snakes, and is also currently working under ICMR funded project for estimating the incidence, mortality, morbidity and economic burden due to snakebites in India. Parag Shinde is a commerce graduate and has also finished a photography school. He is passionate about reptile photography, herpetological related field investigations and animal rescuing. H.T. Decemson is currently doing his Ph.D. work on primates and also engaged as JRF under the DST-SERB project on the taxonomy, ecology and distribution of amphibians and reptiles in Mizoram. He also extended his work on the herpetofauna of Manipur State in collaboration with the Department of Forest, Sanjenthong, Imphal, Government of Manipur. M. Mathipi Vabeiryureilai is an assistant professor with an expertise in cancer biology, evaluation of medicinal plants, taxonomy and molecular phylogeny of various taxa. H.T. Lalremsanga is a professor and a pioneer in the field of herpetology in Mizoram. Currently, he is teaching post-graduate students, guiding research scholars, and overseeing major projects funded by the Department of Science and Technology - Science and Engineering Research Board (DST-SERB) and Department of Biotechnology (DBT) under the Ministry of Science and Technology, the Defence Research and Development Organisation (DRDO) under the Ministry of Defence, Indian Council of Medical research (ICMR), The Habitats Trust (THT), Noida and the National Mission for Himalayan Studies (NMHS), Government of India.

Author contributions: PSE, HD, PS did the field-based investigations and sampling; LB performed data analyses, and wrote the first draft of the manuscript with HTL; MV carried out laboratory and technical works; HTL conceived the study, review and editing, and approval of the manuscript prior submission. All authors reviewed and agreed upon the final version.

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INTRODUCTION

Pit vipers are a group of front-fanged venomous snakes that comprise various genera of vipers having heat sensitive pit between each eye and nostril. Asian pit vipers that are distributed across southern and southeastern Asia are known to inhabit several types of habitats like wet, humid and montane forests, and also found in a few dry forest habitats (Gumprecht et al. 2004). Recent phylogeographic studies have largely addressed the taxonomic status of certain groups of pit vipers like *Trimeresurus* complex (Malhotra & Thorpe 1997, 2004; David et al. 2011; Mallik et al. 2021) and *Protobothrops* (see Guo et al. 2009). Geographical diversity as well as sexual dimorphism often led to inaccurate taxonomic assignment among the closely similar pit viper species (Malhotra & Thorpe 1997, 2004). Consequently, pit vipers are still regarded to contain several taxonomically complex species in India. Recently, many such species complexes were revised including those from the *Craspedocephalus malabaricus* and *C. macrolepis* complexes (Mallik et al. 2021), the *T. medoensis* complex (Rathee et al. 2022), and the *T. albolabris* complex (Chandramouli et al. 2020; Mirza et al. 2020; Vogel et al. 2022).

Manipur is a hilly Indian state that lies at the extreme east of the northeastern region of India bordering Nagaland in the north, Mizoram in the south-east, Assam in the west, and Myanmar towards the east and south-east. In spite of the potential richness in biodiversity, zoological exploration in Manipur is rather limited (Roonwal 1948; Singh 1995; Pawar & Birand 2001) and there exist only a handful of snake faunal reports from the area till date (e.g., Mathew 2005; Sinate et al. 2021, 2022; Elangbam et al. 2022). Although Wallach et al. (2014) listed Manipur as the potential range of *T. erythrurus*, there is no confirmatory records from the area, and none of the aforementioned literature listed pit vipers from the snake fauna of Manipur except *Ovophis monticola* (see Whitaker & Captain 2008). In this paper, we provide notes on our new addition of pit vipers from Manipur based on our field documentations during our herpetological surveys in 2022.

MATERIALS AND METHODS

Sampling and morphology

We conducted our field surveys after obtaining herpetological specimen collection permit from the Forest Department, Government of Manipur (permit no. #3/22/2018-WL [Vol-II]). Morphological characters of the newly collected specimens were taken. We followed Dowling's (1951) method for counting ventrals (Ve), and we excluded the terminal scute in counting the subcaudals (Sc). All the measurements are given in millimeter, and other acronym used for the morphological characters are: snout-vent length, SVL; tail length, TaL; inter-orbital distance (measured on cephalic scale which was counted on a straight line between the middle of the supraoculars), IOD; supralabials, SL; infralabials, IL; and the bilateral characters are given as left/right. We euthanized the live specimens using MS-222 according to the standardized protocol (Conroy et al. 2009), and the procedure was approved by the Institutional Animal Ethics Committee (IAEC) (Permission No. MZU-IAEC/2018/12). All the specimens collected during this survey were deposited in the Departmental Museum of Zoology, Mizoram University, India (MZMU). For molecular analyses, we dissected the liver tissue from the freshly euthanized specimens and stored in 95% ethanol at -20°C refrigerator. Distribution map was prepared using QGIS v3.16.2.

DNA extraction and amplification

We employed mitochondrial cytochrome b (cytb) gene for molecular based identification. For this purpose, genomic DNA was extracted from the liver tissue using QIAamp DNA Mini Kit following the standard protocol provided. We amplified the fragment of cytb gene using a published primer pairs (Snk) (Dubey et al. 2009) under the thermal conditions of 94˚C for 3 min for initial denaturation; 35 cycles for denaturation at 94˚C for 30 sec, annealing at 49˚C for 40 sec, and extension at 72˚C for 30 sec; with the final extension of 72˚C for 5 sec, and cooling at 4°C for 15 min. The amplified products were purified using ThermoFisher ExoSAP- IT PCR product clean-up reagent and sequenced at Barcode BioSciences, Bangalore, India using Sanger's dideoxy method. The newly generated sequences were submitted to NCBI GenBank database (Benson et al. 2018) and obtained the accession numbers (OQ968475–77).

Molecular analyses

We assembled the dataset mainly based on the published datasets of Mirza et al. (2023) for *Trimeresurus* and Guo et al. (2009) for *Protobothrops*, and we obtained the published nucleotide sequences from the NCBI GenBank database (Benson et al. 2018). We used the default parameter settings of MUSCLE (Edgar 2004) for performing nucleotide alignment in MEGA 11 (Tamura et al. 2021). The flanking gaps created by the samples with shorter nucleotide sequences are treated as missing data. We estimated the uncorrected p-distance using complete deletion for gaps/missing data in MEGA 11 (Tamura et al. 2021). We partitioned the assembled datasets (1,088 bp in length for *Trimeresurus*; 847 bp in length for *Protobothrops*) by codon position. The best partitioning schemes and nucleotide evolutionary models selected by PartitionFinder v2 (Lanfear et al. 2017) under the Bayesian Information Criterion are HKY+I for cytb pos1, TRN+G for cytb pos2, HKY+G for cytb pos3 in the dataset of *Trimeresurus*; HKY+I+G for cytb pos1 and pos3, and TIM+I+G for cytb pos2 in the dataset of *Protobothrops*. By implementing the selected models, we performed the partitioned Bayesian inference (BI) phylogeny separately for the two datasets in MrBayes v3.2.5 (Ronquist et al. 2012). We conducted four independent runs with one cold and three hot chains for 10 million generations and sampling every 5000 generations. We discarded 25% of the trees as burn-in cut off after examining the trace plots generated by the MCMC runs in Tracer v1.7 (Rambaut et al. 2018).

RESULTS AND DISCUSSION

In this report, we documented a total of four pit viper species from Manipur (Image 1), and we established their identity based on the combination of mitochondrial cyt b gene and their respective morphological characters, including key diagnostic features from relevant published literature (e.g., Guo et al. 2009; Zambre et al. 2009; Yang et al. 2011; Rathee et al. 2022; Mirza et al. 2020, 2023).

Trimeresurus mayaae Rathee et al., 2022 (Image 2)

The recently discovered Maya's Pit Viper, *T. mayaae* is a species genetically close to *T. medoensis* as well as morphologically similar to *T. gumprechti* of Indochina

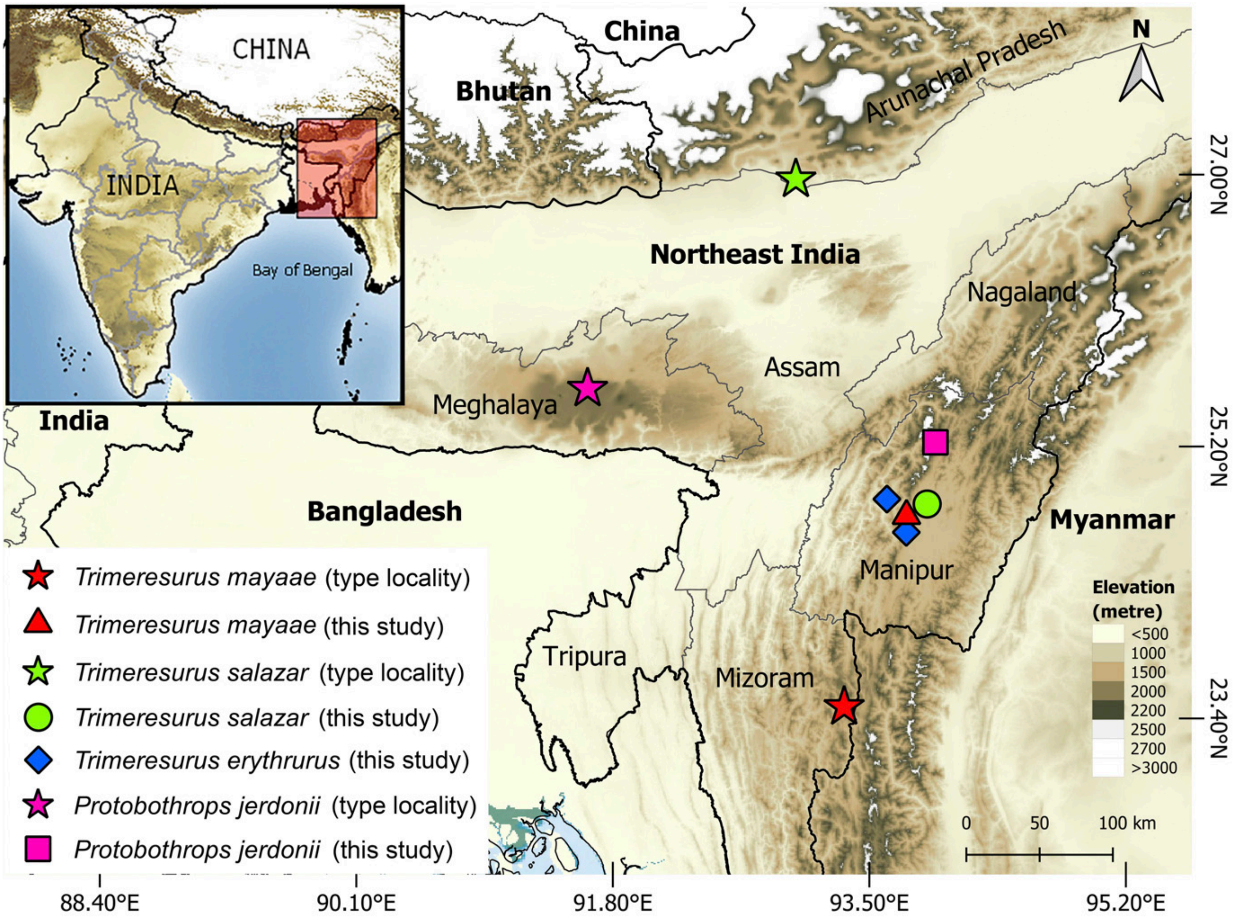

Image 1. Map showing the type localities and new collection sites of the four pit vipers in Manipur, India: *Trimeresurus mayaae* are shown in red star (type locality) and red triangle (this study); *T. salazar* in green star (type locality) and green circle (this study); *T. erythrurus* in blue diamonds (this study); *Protobothrops jerdonii* in purple star (type locality) and purple square (this study).

(Rathee et al. 2022 and references therein). Not much is known on the biology of this cryptic species, and its description is based on specimens originating from the Northeast Indian States of Mizoram and Meghalaya. This species, in fact represents the third new species of the genus from India, to be described in the past three years (Rathee et al. 2022).

On 17 July 2022, we collected both male (MZMU2971; Image 2a) and female (MZMU2970; Image 2b) individuals of *T. mayaae* from Sadu Chiru area nearby a waterfall in Kangpokpi District, Manipur (24.740°N, 93.747°E; 1,139 m). A female individual was found resting on top of tree branch at 2211 h and a male individual was found crawling on the ground about 6 m away from where a female was collected at 2237 h. The microhabitat of the collection site was predominantly covered by thorny shrubs like *Solanum incanum*, and bamboos such as *Melocanna bacifera* and *Chimonobambusa callosa.* These specimens were morphologically examined in the field and concorded to the descriptions and diagnostic keys of *Trimeresurus mayaae* fide Rathee et al. (2022). The two newly collected specimens are characterized by the absence of red and white post-ocular stripe in both specimens; iris greenish around the periphery and adjoined by a prominent rust color around the center in male, while it is greenish with partially blended by a rust color in female; snout-vent length (SVL) 430 mm in male, and 310 mm in female; tail length (TaL) 97 mm in male, and 61 mm in female; dorsal scale row (DSR) 19:19:15 in male, 21:19:15 in female; Ve 154 in male, 152 in female; Sc 58 in male, 53 in female; inter-orbital distance (IOD) measured in scale are 7 in male, and 8 in female; supralabials (SL) 9/9 (left/right) in male, 10/9 in female; infralabials (IL) 10/11 in male, 10/10 in female, and the first pair contact with each other. The new specimens updated the key diagnostic features in the lower limit of Ve in male i.e., 154 vs. 157–162 (Rathee et al. 2022) and female i.e., 152 vs. 153 (Rathee et al. 2022), and also in the lower limit of Sc in female i.e., 53 vs. 54–55 (Rathee et al. 2022). These specimens also represent the first record of the species from the Manipur State and extend the known distribution range by ca. 146 km aerial distance north-eastward from the type locality in Champhai, Mizoram and ca. 209 km aerial distance towards SE from the paratypes locality in Umroi, Meghalaya (Rathee et al. 2022) (Image 1).

Image 2 . *Trimeresurus mayaae i*n life from Sadu Chiru area nearby a waterfall in Kangpokpi District, Manipur: a—Male (MZMU2971) | b—female (MZMU2970). © Parag Shinde

Despite the fact that we are utilizing a single gene (cyt b) in this study, our reconstructed BI phylogram of *Trimeresurus* species considerably concorded to the tree topology, particularly on the clades of the subgenus "*Trimeresurus*" and "*Viridovipera*" fide Mirza et al. (2023) where they combined four mitochondrial genes (cyt b, 16S rRNA, 12S rRNA, and ND4). Accordingly, our cyt b gene tree depicted the new *T. mayaae* specimen (MZMU2970; Accession No. OQ968476) clustering alongside the type series of the species by a well-supported Bayesian posterior probability support (PP = 0.99), and is nested with the holotype from Mizoram (PP=1.00) (Figure 1). Moreover, the specimen (MZMU2970) is showing 0.0–0.7% intraspecific genetic distance with respect to the type series, and the least genetic distance was seen with the holotype (0.0%) from Mizoram, India (see Table S1).

Trimeresurus salazar Mirza et al., 2020 (Image 3)

The Salazar's Pit Viper, *T. salazar* is one of the recently discovered *Trimeresurus* in the past three years. The species has been confirmed so far from central-southern Nepal, Southern Bhutan (Vogel et al. 2022), Northeast Indian States of Arunachal Pradesh (type locality), Assam (Mirza et al. 2020), Meghalaya (Rathee

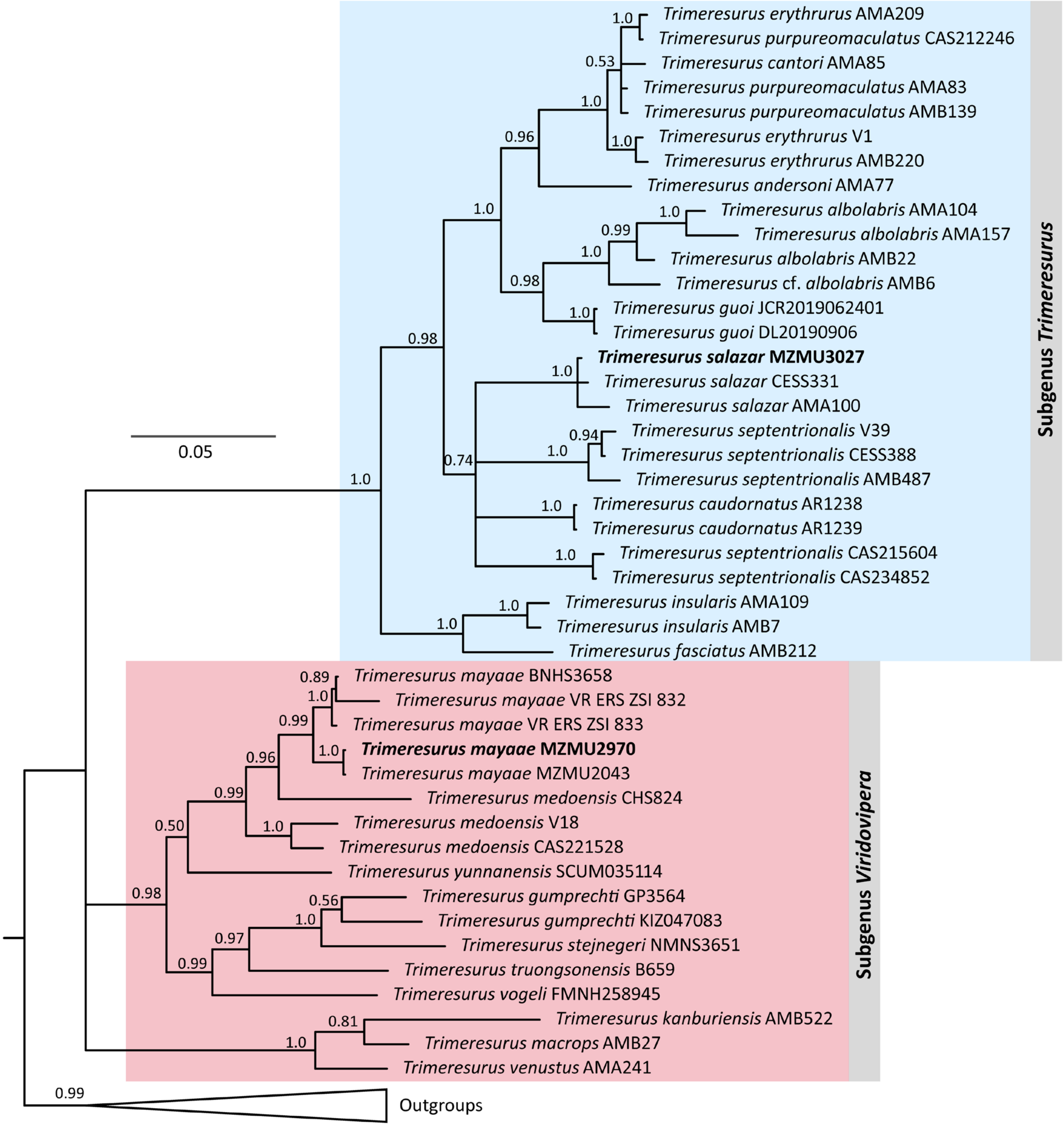

Figure 1. Bayesian inference phylogenetic tree inferred using mitochondrial cytochrome b gene of *Trimeresurus* species. The clades consisting the subgenera of *Trimeresurus* and *Viridovipera* fide Mirza et al. (2023) are shaded in blue and red, respectively. Numbers at the nodes represent the Bayesian posterior probability (PP) values. Sequences generated in this study are given in bold.

et al. 2021), Sikkim, Nagaland, and the other Indian States of Madhya Pradesh, Jharkhand, Chhattisgarh, Bihar and Northern West Bengal (Vogel et al. 2022). The species is distinguished from the congeners by the combined evidence of mitochondrial 16S and ND4 genes, external morphology, dentary and hemipenial morphology (Mirza et al. 2020).

On 17 October 2022 at ca. 0900 h, we encountered and captured a male individual coiled among bushes of tomato *Solanum lycopersicum* inside a plantation surrounded by *Mangifera indica*, *Lantana camara*, *Psidium guajava*, and *Melocanna bacifera* at the foothills of Langol Hill Reserve Forest, near the villages of Lamdeng and Khundi, Imphal West District, Manipur (25.227262°N, 93.939305°E; 2,170 m). The new specimen is collected ca. 253 km aerial distance southeastward from the type locality at Pakke Tiger Reserve, Arunachal Pradesh (Mirza et al. 2020) (Image 1). The other nearest occurrence record of the species is most likely from Hamren, Karbi Anglong District in Assam (CESS604) ca. 174 km aerial distance north-westward from the new collection site. Previously, the locality for CESS604 was mistakenly provided as "Aizawl, Mizoram, India" by Vogel et al. (2022) while it was actually captured from the Assam-Meghalaya interstate border road (25.829°N, 92.556°E, 449 m) (Lalnunhlua pers. comm.), and this clarification warrants the removal of Mizoram as part of the known range of *T. salazar* until there is further confirmatory work. The snake remained very active while capturing and under temporary captivity for morphological examination. Based on the morphological characters, we identified the specimen as a male *T. salazar* (MZMU3027) in agreement with literature mentioning diagnostic keys (Mirza et al. 2020; Rathee et al. 2020; Vogel et al. 2022). We obtained the following morphological attributes: SVL 475 mm; TaL 110 mm; DSR 21:19:15; Ve 165, Sc 65; IOD 12; SL 11/11, and the first SL fused with nasal scale; IL 12/12, and the first pair in contact with each other. Although the lateral stripe on head, one of the taxon's male key features fide Mirza et al. (2020) is not evident in our specimen, we considered the particular character as a phenotypically plastic trait as seen in *T. mayaae* (Rathee et al. 2022) and *T. popeiorum* (Mirza et al. 2023) rather than sexually dimorphic trait, and also in consideration of the low sampling size in the original description (three

Image 3. Male *Trimeresurus salazar* (MZMU3027) in life from Langol hill Reserve Forest, Imphal West District, Manipur. Inset showing the antero-lateral side of the head. © Premjit Singh Elangbam

Image 4. a—Uncollected female *Trimeresurus erythrurus* in life from Bishnupur District, Manipur | b—uncollected road killed *T. erythrurus* from Noney town, Noney District, Manipur. Inset showing the lateral view of the head. © Premjit Singh Elangbam

males and one female) (See Mirza et al. 2020). Even so, with respect to *T. erythrurus*, the species that usually lacks the red postocular stripe in males, the specimen (MZMU3027) is distinct in having 19 mid-dorsal scale rows vs. 23 in *T. erythrurus* (Mirza et al. 2020).

Our cyt b based BI phylogram also depicted a subclade of *salazar* + *septentrionalis* +*caudornatus* that was conceived by Mirza et al. (2023); and the new *T. salazar* specimen (MZMU3027; Accession No. OQ968477) clustered with the conspecies from Meghalaya, India (CESS331) and Nepal (AM A100) with a well-supported branch (PP = 1.00) (Figure 1). We uncovered 0.2–0.5% intraspecific genetic distance among *T. salazar* samples, and our sample shows 0.2% genetic distance with respect to both of them (see Table S1).

Trimeresurus erythrurus (Cantor, 1839) (Image 4)

The Red-tailed Pit Viper, *T. erythrurus* was originally described from the "Delta Gangeticum" which is apparently from the Indian Sunderbans of West Bengal state in the present time fide Deuti et al. (2021). The species is widely distributed in parts of Southern Asia including Bhutan (Wangyal 2014; Deuti et al. 2021 and references therein), Nepal, Bangladesh, Myanmar (Mahony et al. 2009; Wallach et al. 2014; Chan et al. 2022), and the Indian states of Andhra Pradesh (Kakinada), West Bengal (Deuti et al. 2021), and Northeast India viz. Arunachal Pradesh, Assam, Mizoram, Nagaland, and Sikkim (Whitaker & Captain 2008; Wallach et al. 2014); possibly in Meghalaya and Manipur (see Wallach et al. 2014).

On 23 July 2022, at ca. 0700 h, we documented a live individual of *T. erythrurus* from a forest trail at Bishnupur District, Manipur (24.632°N, 93.746°E; 920 m; Image 1) ca. 500 km aerial distance northeast from the type locality in Sunderbans (Deuti et al. 2021). Seeing the bulged abdomen, it was evident that the snake had recently fed, possibly upon small mammal or a bird (Image 4a). Morphologically, we identified the snake as female *T. erythrurus* in having golden-yellow colored eye; greenish dorsum; white lateral stripe on the head and the ventrolateral body; total length of 595 mm; Ve 168; Sc 51; and 23 mid-dorsal scale rows which are in agreement with the published diagnostic characters of the species (Chen et al. 2020; Mirza et al. 2020; Deuti et al. 2021). We subsequently released back the snake into the wild after taking measurements and scalation data. On 4 November 2022, at ca. 1100 h, we encountered additional uncollected road killed male individual at Noney town, Noney District, Manipur (24.851°N, 93.617°E; 510 m asl.) ca. 28 km aerial distance northwest from the point of our observation of the live individual. As the snake was badly damaged and large amount of the scales were eaten off by ants, we could barely examine the snake but nevertheless, obtained the key features like white lateral stripe on the head and ventrolateral body, 23 mid-dorsal scale rows, and Ve 158 (Image 4b). Given that we could not obtain genetic data for this species in Manipur, we advocate DNA studies on the Manipur population to further corroborate the present report.

Protobothrops jerdonii **(Günther, 1875) (Image 5)**

The widely distributed *P. jerdonii* is presently found in southwestern China, northeastern India, Nepal, northern Myanmar as well as northern Vietnam (see Guo

Image 5. Male *Protobothrops jerdonii* (MZMU3011) in life from Chawangkining, near Zaimeng Lake, Kangpokpi District, Manipur. © Parag Shinde

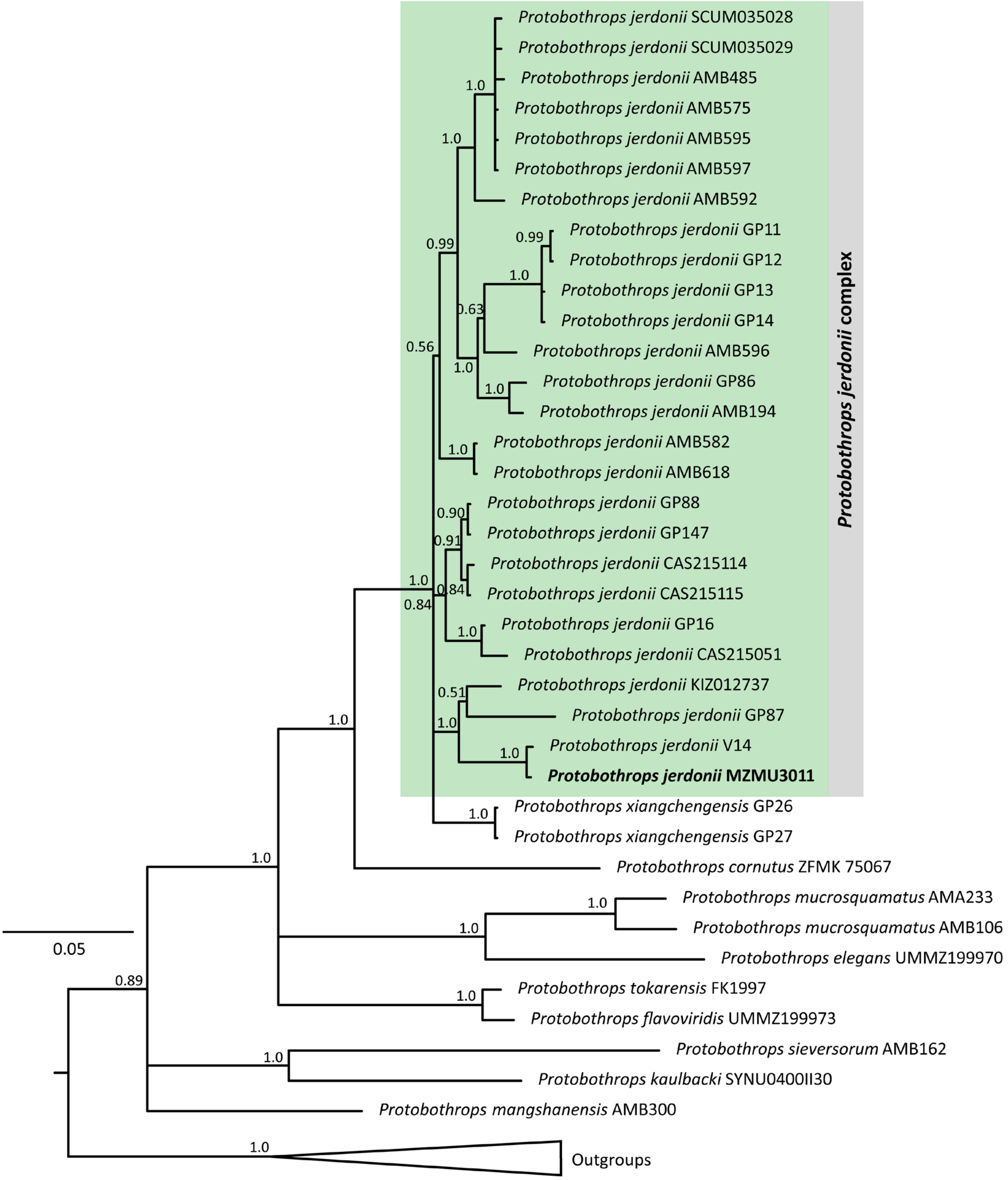

Figure 2. Bayesian inference phylogenetic tree inferred using mitochondrial cytochrome b gene of *Protobothrops* species. Numbers at the nodes represent the Bayesian posterior probability (PP) values. Sequence generated in this study are given in bold.

et al. 2009). The high level of variation in their ecology as well as morphology, particularly in the scale counts and coloration has led to a controversial intraspecific taxonomic treatment within *P. jerdonii* (Guo et al. 2009). This has led some authors to recognise the species as monotypic without any subspecies, while certain other authors treat the taxon to be polytypic (see Guo et al. 2009) with a total of five species or subspecies proposed so far within the *P. jerdonii* complex. They are, apart from the nominotypical form *jerdonii* Günther, 1875; *xanthomelas* Günther, 1889; *melli* Vogt, 1922; *meridionalis* Bourret, 1935; and *bourreti* Klemmer, 1963. Among these, only *jerdonii*, *bourreti* and *xanthomelas* were considered as valid subspecies by several authors like Hoge & Romano-Hoge (1983), Golay et al. (1993), Orlov et al. (2001), and Gumprecht et al. (2004).

On 18 July 2022 at 1949 h, we encountered the specimen (MZMU3011) at Chawangkining village nearby Zaimeng Lake, Kangpokpi District, Manipur (25.204°N, 93.940°E; 1,725 m). The snake was captured while crawling across a forest track covered by herbs and thorny shrubs like *Mikania micrantha*, *Mussaenda* spp., *Girardinia diversifolia*, and *Rubus* spp., and surrounded by bamboos such as *Melocanna bacifera* and *Chimonobambusa callosa.* The collection site of the new specimen is situated ca. 235 km aerial distance towards east from the type locality in Khasi Hill, Meghalaya fide Wallach et al. (2014) (Image 1). In comparing the descriptions and diagnostic keys from literature (e.g., Zambre et al. 2009; Yang et al. 2011), we identified the specimen as male *P. jerdonii* in having SVL 414 mm; TaL 77; Ve 171; Sc 66; SL 7/8; IF 11/11; IOD 8; DSR 21:21:16. In considering the subspecies recognized by Gumprecht et al. (2004), the Ve of our specimen (171) falls within the range of *P. jerdonii jerdonii* (160–170) fide Yang et al. (2011), the Sc of our specimen (66) enter the Sc range of both *P. jerdonii xanthomelas* (54–67) and *P. jerdonii bourreti* (65–72) fide Yang et al. (2011). Moreover, the basal body color of our specimen is yellowish brown, and a series of rhomboidal or irregularly shaped reddish-brown patches with black margins are present along the dorsal body. Based on the morphological data alone, we cannot certainly assign the new specimen (MZMU3011) to any of the three nominal subspecies following the recent most diagnostic keys fide Yang et al. (2011). Thus, we for now refrain from making subspecies level taxonomic identification for the specimen.

Our reconstructed cyt b gene tree also depicted paraphyly amongst *P. jerdonii* as conceived by Guo et al. (2009) where they sequenced four mitochondrial genes (cyt b, 12S rRNA, 16S rRNA, and ND4). Our sample (MZMU3011; Accession No. OQ968475) nested alongside the conspecific sequences from Northeast India (V14) with a high branch support (PP = 1.00), and these two formed a subclade with the other samples from China (GP87 and KIZ012737) with high support (PP = 1.00) (Figure 2). Our estimated genetic distance also showed 0.0–3.3% intraspecific distance between ours and the other *P. jerdonii* samples; the least genetic distance (0.0%) is seen with the Northeast Indian sample while the highest (3.3%) is seen with respect to the samples from Huili, Sichuan, China (GP11–GP14) (see Table S2).

CONCLUSION

As per the current knowledge, only *O. monticola* has been listed as present in Manipur (Whitaker & Captain 2008). In this work, we report the occurrence of four new records of pit vipers in Manipur for the first time, and simultaneously provide new information on their respective geographical distribution ranges. Considering the topology in our phylogenetic reconstruction and our *P. jerdonii* sample (MZMU3011) that disclosed the overlapping of the conventional taxonomic keys for the subspecies fide Yang et al. (2011), we are convinced that our study corroborated the taxonomic statement on the species by Guo et al. (2009) where they considered *P. jerdonii* as a monotypic and paraphyletic species. In the scenario of *T. mayaae*, both ours and the published phylogenies (Rathee et al. 2022; Mirza et al. 2023) depicting the nesting of *T. mayaae* next to *T. medoensis*. Nonetheless, combining the meristic data of the type series (Rathee et al. 2022) and the new specimens, *T. mayaae* is morphologically distinct in terms of the Ve counts of male i.e., 154–162 vs. 138–149 in *T. medoensis*, and female i.e., 152–153 vs. 141–143 in *T. medoensis*; also in the mid-dorsal scale row i.e., 19–21 in *T. mayaae* vs. 17 in *T. medoensis*.

The present work, however, provides evidence for the occurrence of four additional species of pit vipers in Manipur for the first time, and simultaneously provides new information on their respective geographical distribution ranges. Thus, currently, five species of pit vipers including *P. jerdoni*, *T. salazar*, *T. mayaae*, *T. erythrurus*, and *O. monticola* are known from Manipur (Whitaker & Captain 2004; this work). We emphasise that this new data on the occurrence of four more species of pit vipers in Manipur may be disseminated well in health care sectors. This is so because these venomous snakes may be of medical importance in terms of potential snakebites on humans (e.g., Kanwar 2018; Rai et al. 2021; Ravikar et al. 2023), more so in forest fringe areas.

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