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Range extension of *Ichthyophis multicolor* Wilkinson et al., 2014 to India and first molecular identification of *Ichthyophis moustakius* Kamei et al., 2009

Hmar Tlawmte Lalremsanga[1], Jayaditya Purkayastha[2], Mathipi Vabeiryureilai[1], Lal Muansanga[1], Ht Decemson[1], Lal Biakzuala[1*]

1 Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl, Mizoram, 796004, India • HTL: htlrsa@yahoo.co.in https://orcid.org/0000-0002-3080-8647 • MV: m.vabeiryureilai@gmail.com https://orcid.org/0000-0001-8708-3686 • LM: muanapunte16@gmail.com https://orcid.org/0000-0001-8182-9029 • HD: htdecemson@gmail.com https://orcid.org/0000-0002-7460-8233 • LB: bzachawngthu123@gmail.com https://orcid.org/0000-0001-5142-3511

2 Help Earth, Guwahati, Assam, 781007, India • mail.jayaditya@gmail.com https://orcid.org/0000-0002-3236-156X

* Corresponding author

Abstract

We report a substantial range extension of *Ichthyophis multicolor* Wilkinson, Presswell, Sherratt, Papadopoulou & Gower, 2014, with new material from Mizoram State, Northeast India. The species was previously known only from its type locality more than 800 km away in Ayeyarwady Region, Myanmar. The species was identified by both its morphology and 16s rRNA gene sequence data. One of the studied individuals represents the largest known specimen for the species (total length = 501 mm; mid-body width = 18.8 mm). Brief comparisons of *I. multicolor* with the sympatric as well as parapatric congeners in the region, and first barcode data for *I. moustakius* Kamei, Wilkinson, Gower & Biju, 2009 are also presented.

Keywords

Caecilian, Ichthyophiidae, Mizoram, new record, phylogeny, 16s rRNA

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Introduction

The genus *Ichthyophis* Fitzinger, 1826 is recognized as the most speciose and widely distributed genus of the caecilian amphibians (e.g., Wilkinson et al. 2014; Taylor 1968; Gower et al. 2002). Currently, the genus is represented by 50 species, of which 13 species occur in India. Eight of these 13 species are reported from Northeast India, namely *I. alfredi* Mathew & Sen, 2009; *I. daribokensis* Mathew & Sen, 2009; *I. garoensis* Pillai & Ravichandran, 1999; *I. khumhzi* Kamei, Wilkinson, Gower & Biju, 2009; *I. moustakius* Kamei, Wilkinson, Gower & Biju, 2009; *I. nokrekensis* Mathew & Sen, 2009; *I. sendenyu* Kamei, Wilkinson, Gower & Biju, 2009; and *I. sikkimensis* Taylor, 1960 (Frost 2021).

Recently, Wilkinson et al. (2014) described a new

species of *Ichthyophis*, *I. multicolor* Wilkinson, Wilkinson, Presswell, Sherratt, Papadopoulou & Gower, 2014, based on 14 specimens from Ayeyarwady Region, Myanmar. This species is known only from its type locality and is presently not included in the IUCN Red List. We provide the first record of *I. multicolor* from Mizoram State, India, and compare this species with the congenerics reported from Northeast India. We also provide genetic data of *I. moustakius* for the first time and compare this species' molecular proximity to its congeners through molecular phylogenetic analysis.

Methods

The specimens reported here were collected after obtaining permission for herpetofaunal collection in the State of Mizoram from the Environment, Forests and Climate Change Department, Government of Mizoram (permit no. A.33011/2/99-CWLW/225). The specimens were deposited and voucher numbers were obtained from the Departmental Museum of Zoology, Mizoram University (MZMU). Measurements were taken with Mitutoyo dial vernier calipers (Model 505–671) to the nearest 0.1 mm. The body circumference and total length (TL) were measured with thread and ruler. Annular grooves were counted by using ImageJ2 software (Rueden et al. 2017) and cross-checked manually. The numbers of vertebrae were counted by using digital radiography at 56 kVp and 24 mAs (Allengers MARS 30 X-Ray Machine). Fresh liver tissue was collected and stored at −20°C in 95% ethanol in the facility of Developmental Biology and Herpetology Laboratory, Mizoram University, India. A map was produced using QGIS v. 3.16.2 software (QGIS Development Team 2020).

The abbreviations of Wilkinson et al. (2014) were used: AG = annular groove; AM = anterior most margin of mouth on upper jaw; C1 = first nuchal collar; C2 = second nuchal collar; CM = corner of the mouth; IM = inner mandibular tooth; NG1 = first nuchal groove; NG3 = third nuchal groove; OM = outer mandibular tooth, PM = premaxillary-maxillary tooth; ST = snout tip; TA = tentacular aperture; TG = dorsal transverse groove on collar; TP = tentacular papillus; VP = vomeropalatine tooth; L/H = total length divided by head length (the latter = ST–NG1 measured directly behind CM); L/T = total length divided by tail length (the latter = distance behind vent); L/W = total length divided by midbody width; W/S = width at midbody divided by maximum width of stripe at midbody; AV = anterior limit of vent; PV = posterior limit of vent; TT = tail tip and in measures N = naris; and E = eye.

Genomic DNA was extracted from the liver sample of three *I. multicolor* specimens (MZMU 1541, MZMU 1739, and MZMU 1740), and two *I. moustakius* specimens (MZMU 1758 and MZMU 1847) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the manufacturer's standard protocol. We amplified and sequenced the mitochondrial 16S ribosomal RNA gene (16s) using primers L02510 (Forward: 5′-CGCCTGTTTATCAAAAACAT-3′) (Palumbi 1996) and H3056 (Reverse: 5′-CTCCGGTTTGAACTCAGATC-3′) (Rassmann et al. 1997). The derived sequences were compared with congeners using published 16s rRNA sequence data (Gower et al. 2002, 2007; Nishikawa et al. 2012; Wilkinson et al. 2014). Sequences (maximum of 691 base pairs) were aligned with MEGA v. 7 using the MUSCLE algorithm with default parameter settings (Edgar 2004). Best fit nucleotide substitution model was selected using ModelFinder (Kalyaanamoorthy et al. 2017) based on the Bayesian Information Criterion. Phylogenetic relationships were reconstructed using maximum likelihood (ML) in IQ TREE (Nguyen et al. 2015) with 10000 ultrafast bootstrap replicates (Hoang et al. 2017). Uncorrected *p*-distance was calculated in MEGA v. 7 (Kumar et al. 2016).

Results

Ichthyophis multicolor Wilkinson, Presswell, Sherratt, Papadopoulou & Gower, 2014

Figures 1, 2; Tables 1–3

New records. INDIA – **Mizoram** • Kolasib District, Kolasib Hmar Veng; 23.3682°N, 093.1420°E; 638 m a.s.l.; 17.V. 2016; Hmar Tlawmte Lalremsanga leg.; from a roadside stream; MZMU 911, 1♂, TL 431 mm • Aizawl District, Mualpui; 24.2018°N, 093.2176°E; 833 m a.s.l.; 23.VI. 2016; Hmar Tlawmte Lalremsanga leg.; from a roadside, near riparian buffers; MZMU 913, 1♀, TL 335 mm • Aizawl District, College Veng; 24.2071°N, 093.2139°E; 874 m a.s.l.; 18.VIII. 2019; Hmar Tlawmte Lalremsanga leg.; dug out of soil during road construction; MZMU 1480, 1 ♀, TL 406 mm • Aizawl District, Tlangnuam; 23.6996°N, 092.7172°E; 997 m a.s.l.; 29.IX.2019; Samuel Lianzela leg.; from a roadside canal, near secondary forest; GenBank: MZ098157; MZMU 1541, 1 ♂, TL 501 mm • Aizawl District, Melthum; 23.6894°N, 092.7208°E; 1030 m a.s.l.; 27.VI. 2020; Lal Muansanga leg.; captured while crossing a tarmac road; GenBank: MZ098156; MZMU 1739, 1 ♀, TL 361 mm • Aizawl District, Mission Vengthlang; 23.7120°N, 092.7071°E; 934 m a.s.l.; 1 VII. 2020; Ro Malsawma leg.; GenBank: MZ098155; MZMU 1740, 1 ♀, TL 310 mm • Aizawl District, Tuirini bridge; 23.6844°N, 092.8847°E; 272 m a.s.l.; 1.X. 2020; Ht Decemson leg.; dead on road; MZMU 1956, 1 ♀, TL 339 mm • Aizawl District, Zemabawk; 23.7331°N, 092.7622°E; 860 m a.s.l.; 1.X. 2020; Gospel Zothanmawia Hmar leg.; dead on road; MZMU 1965, 1 ♂, TL 338 mm • Aizawl District, Mission Vengthlang; 23.7122°N, 092.7075°E; 943 m a.s.l.; 19.X. 2020; Ro Malsawma leg.; MZMU 2003, 1 ♀, TL 468 mm • Aizawl District, Mizoram University campus; 23.7381°N, 092.6618°E; 774 m a.s.l.; 21.X. 2020; Ht Decemson leg.; found on surface near Mizoram University park, after rain showers; MZMU 2005, 1 ♂, TL 464 mm.

Identification. The specimens MZMU 1541, MZMU

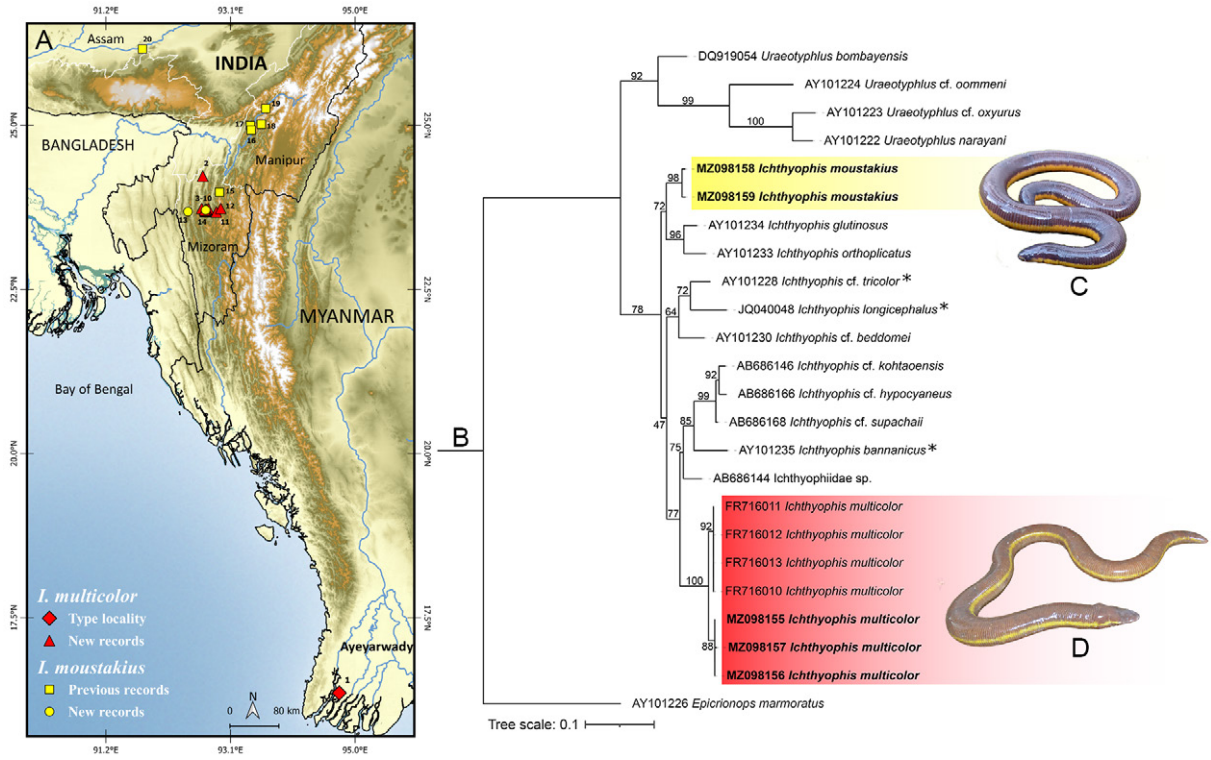

Figure 1. A. Map showing the type locality of *Ichthyophis multicolor* in Ayeyarwady, Myanmar (red diamond), and new localities from Mizoram, India (red triangles); and the new collection sites (yellow circles) and previous records (yellow squares) of *Ichthyophis moustakius* from India: (1) Ayeyarwady, (2) Kolasib Hmar veng, (3) Mizoram University campus, (4) Mualpui, (5) College veng, (6, 7) Mission vengthlang, (8) Tlangnuam, (9) Zemabawk, (10) Melthum, (11) Tuirini, (12) Tamdil, (13) Dampa Tiger reserve, (14) Thakthing, (15) Sawleng, (16) Bamgaizaeng, (17) Aziuram, (18) Nswanram, (19) Nriangluang, (20) Guwahati. **B.** Maximum likelihood 16s tree showing the inferred relationships of Northeast Indian and Myanmar *Ichthyophis multicolor* and congeners with 10000 bootstrap replicates (values at internal branches are bootstrap support values). The rhinatrematid *Epicrionops marmoratus* was used as outgroup. Sequences generated in this study are shown in bold, and * indicates species recently allocated to the genus *Epicrium* (see Dubois et al. 2021). **C.** *Ichthyophis moustakius* (MZMU 1847) in life. **D.** *Ichthyophis multicolor* (MZMU 1480) in life.

1739, and MZMU 1740 were identified based on morphology and 16s rRNA data. The individual MZMU 1541 represents the largest of the known *I. multicolor* at 501mm/18.8 mm (TL/mid-body width) vs. the largest known specimen at 402 mm/15.8 mm (see Wilkinson et al. 2014). In life, the coloration of Mizoram specimens agree well with the original description in having a brownish dorsum and a pale venter, narrow whitish markings mid-ventrally, and a pale, irregular yellowish lateral stripe on both sides bordered below by a darker longitudinal stripe that blends into the paler venter. Furthermore, in the type series, AG number 346–386 (vs. 346–385 in the Mizoram population); AG 5–8 are interrupted by the disc (vs. 5–7 in the Mizoram population); AG 3–6 are behind the disc (vs. 3–7 in the Mizoram population); PM: 36–51, VP: 35–48, OM: 30–43, IM: 26–37 (vs. 35–49, 31–38, 35–41, 23–35, respectively, in the Mizoram population); W/S: 4.5–7.2 (vs. 5.0–7.3 in the Mizoram population). An anomalous condition is documented in the specimen MZMU 1541 with no AGs behind the disc (Fig. 1D, E). Genetically, the population from Mizoram formed a sister clade to the type series of *I. multicolor*, differing from each other by an uncorrected *p*-distance of 0.14–0.17.

The new material of *I. multicolor* differs from *Ichthyophis* species previously reported from Northeast India in the following features as per the original descriptions by Kamei et al. (2009), Mathew and Sen (2009), Wilkinson et al. (2014), and Kamei and Biju (2016) (values in parentheses denote the ranges in the type series): It has more AGs, 346–385 (346–386) than *I. alfredi* (269–299), *I. daribokensis* (264–310), *I. garoensis* (264–309), *I. moustakius* (238–268), *I. nokrekensis* (269–300), and *I. sendenyu* (283–308). Although having an overlapping number of AGs (341–362 in the type series of *I. khumhzi*) *I. multicolor* has fewer inner mandibular teeth 23–35 (22–37) than *I. khumhzi* (40–46. *Ichthyophis multicolor* differs from *I. sikkimensis* by having a lateral yellow stripe which is absent in *I. sikkimensis*.

Ichthyophis moustakius Kamei, Wilkinson, Gower & Biju, 2009

Figures 1, 3; Tables 2, 3

Material examined. INDIA – Mizoram • Mamit District, Dampa Tiger Reserve (DTR); 23.6898°N, 092.4512°E; 263 m a.s.l.; 12.VII.2020; Ht Decemson leg.; from a tarmac road during heavy rain, near the entry gate of Teirei rest house; GenBank: MZ098158; MZMU 1758, 1 ♂, TL 196 mm • Aizawl District, Thakthing; 23.7141°N, 092.7214°E; 997 m a.s.l.; 31 VII. 2020;

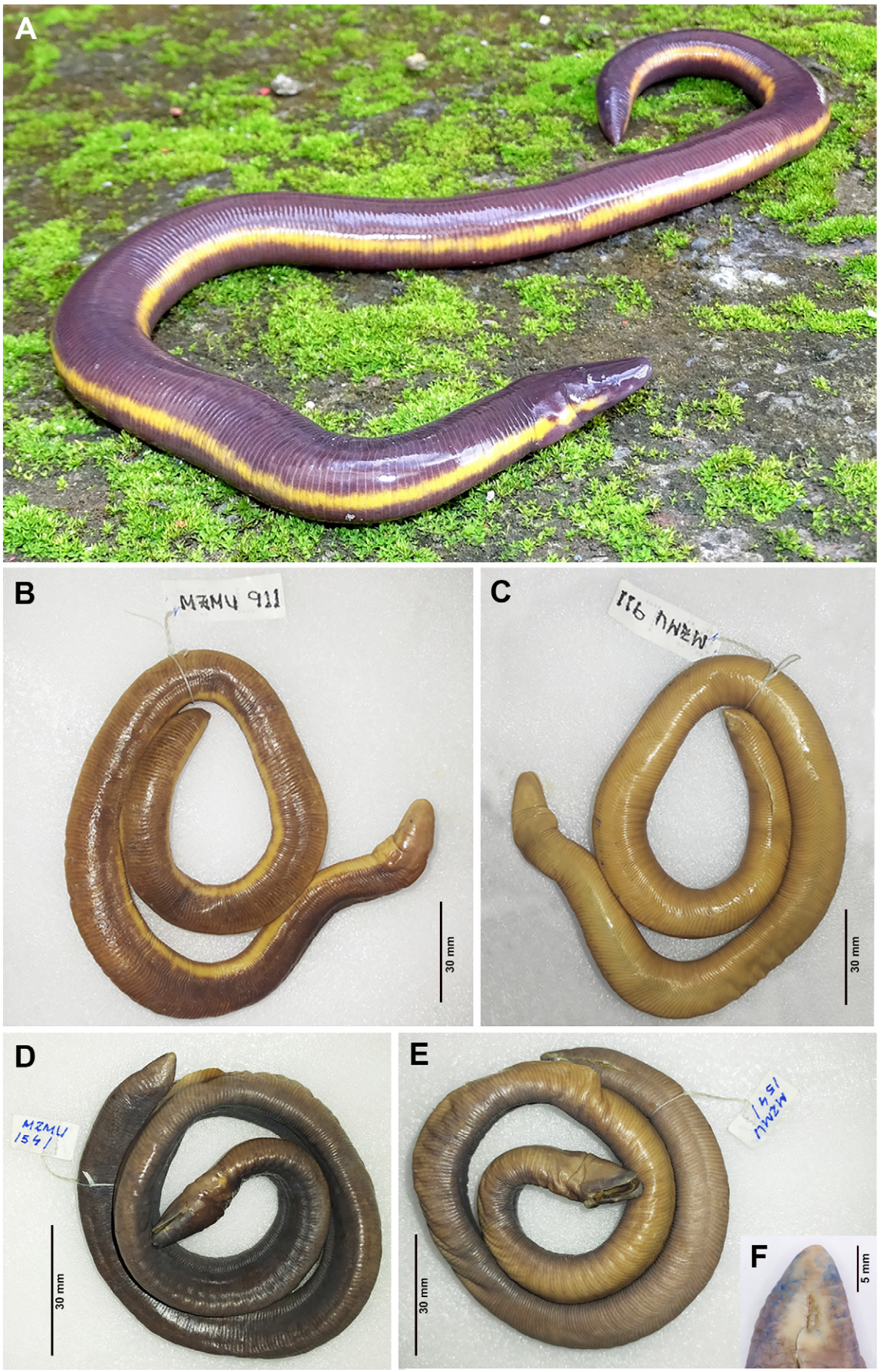

Figure 2. *Ichthyophis multicolor.* **A.** MZMU 1740 in life. **B, C.** Dorsal and ventral views of MZMU 911, preserved. **D, E.** Dorsal and ventral views of anomalous specimen, MZMU 1541, in preservation. **F.** The tail of MZMU 1541 with no AGs behind the disc.

Table 1. Morphometric (in mm) and meristic data for the newly reported specimens of *Ichthyophis multicolor* from Mizoram and ranges for the type series from Wilkinson et al. (2014). Asterisk denotes damaged head on right side, and plus (+) denotes anomalous character. Counts made on left and right are given in L / R order. See Methods for abbreviations.

Specimen	MZMU 1740	MZMU 913	MZMU 1965	MZMU 1956	MZMU 1739	MZMU 1480	MZMU 911	MZMU 2005	MZMU 2003	MZMU 1541	Wilkinson et al. (2014) CAS 212254–67	This study
Sex	f	f	m	f	f	f	m	m	f	m	m + f range	m + f range
Length	310	335	338	339	361	406	431	464	468	501	168–402	310–501
Width	9.8	13.1	17.5	10.4	11.2	13.8	17.5	15.2	18.2	18.8	6.5–15.8	9.8–18.8
Circumference	31.3	42	49.3	35.7	31.4	38	50	45.1	57.6	60	20–49	31.3–60.0
Width of stripe	1.6	2.6	2.4	2.0	1.6	2.6	3.5	2.7	3.0	3.2	1.1–2.2	1.6–3.5
Width at AV	4.5	6.3	6.9	6.4	5.6	5.6	6.8	4.0	7.1	7.4	3.2–7.9	4.0–7.4
NG1–ST	14.1	13.7	*	12.7	14.3	15	16.2	16.2	18.9	21.2	7.9–14.3	12.7–21.2
Length of C1	3.3	2.8	4.2	3.2	2.9	4.1	5.0	4.0	3.5	5.4	1.8–3.3	2.8–5.4
Length of C2	4.1	6.2	4.8	4.4	3.6	7.9	6.7	5.1	6.2	8.6	2.1–4.5	3.6–8.6
Width at CM	7.6	8.5	*	7.8	7.9	8.9	10.5	9.8	10.5	12.7	5.5–9.9	7.6–12.7
Depth behind CM	5.1	5.0	7.2	5.4	4.8	5.3	6.2	6.0	7.5	9.4	4.8–8.6	4.8–9.4
Width at NG1	8.5	10.2	*	8.1	8.1	9.7	10.7	10.5	12.5	13.1	6–10.2	8.1–13.1
E–E	5.4	6.0	*	6.0	5.5	6.5	7.5	6.7	6.3	8.2	3.7–6.7	5.4–8.2
N–N	1.8	2.0	*	1.7	2.2	2.4	2.7	2.5	2.9	3.4	1.6–2.4	1.7–3.4
E–N	5.6	5.7	5.7	4.5	5.9	5.9	7.3	6.5	7.0	8.1	3–5.3	4.5–8.1
TA–TA	6.1	6.1	*	5.7	6.3	7.2	8.0	6.7	8.0	8.7	4.2–7.3	5.7–8.7
E–TA	1.6	1.7	1.6	1.7	1.8	2.1	2.7	2.1	2.1	2.4	1–1.8	1.6–2.7
N–TA	3.7	3.8	3.9	3.3	4.2	4.5	5.5	4.4	5.3	5.7	2.1–4	3.3–5.7
E–ST	7.8	6.2	7.3	5.8	7.0	6.0	8.0	7.5	8.7	9.5	3.7–6.6	5.8–9.5
AM–ST	1.2	1.3	1.2	1.0	1.3	1.2	1.6	1.4	1.8	1.8	0.7–1.1	1.0–1.8
N–L	1.0	1.0	1.0	0.9	0.9	1.1	1.4	1.0	1.5	1.5	0.7–1.3	0.9–1.5
E–L	1.2	1.2	1.6	1.1	1.4	1.4	1.1	1.4	1.8	1.8	0.8–1.5	1.1–1.8
AV–TT	5.0	7.3	5.7	5.5	5.5	7.9	6.3	7.9	4.6	6.3	2.8–3.8	4.6–7.9
PV–TT	4.0	5.9	4.4	3.6	4.3	6.2	4.6	6.1	3.6	5.0	2.5–3.3	3.6–6.2
AGs	361	384	346	374	369	385	372	367	359	372	346–386	346–385
Vertebrae	122	126	123	120	119	125	123	127	127	124	126–132	119–127
TGs	2	1	2	1	2	1	2	2	2	1	2–4	1–2
AGs behind vent	5/5	5/5	4/4	3/3	4/4	7/7	4/4	5/5	4/4	0/0+	3–6	3–7
AGs interrupted in vent region	5/5	5 / 5	6/7	6/6	5/5	6 / 7	5 / 5	5/6	6/6	6 / 7	5–8	5–7
PMs	39	35	*	37	42	39	40	43	49	38	36–50	35–49
VPs	38	33	*	36	37	31	37	38	37	37	35–48	31–38
OMs	35	33	*	35	39	37	35	36	40	41	30–43	33–41
IMs	30	31	*	23	35	31	31	32	26	35	22–37	23–35
L/W	31.6	25.6	19.3	32.6	32.2	29.4	24.6	30.5	25.7	24.2	24.4–27.5	19.3–32.6
L/H	22.0	24.5	*	26.7	25.2	27.1	26.6	28.6	24.8	26.6	25.8–39.5	22.0–28.6
L/T	77.5	56.8	76.8	94.2	84.0	65.5	93.7	76.1	130.0	100.2	50.9–158	56.8–130
W/S	6.1	5	7.3	5.2	7.0	5.3	5	5.6	6.1	6.5	4.4–7.2	5.0–7.3
N–TA/E–TA	2.3	2.2	2.4	1.9	2.3	2.1	2.0	2.1	2.5	2.4	1.9–2.6	1.9–2.5
E–ST/E–E	1.4	1.0	*	1.0	1.3	0.9	1.1	1.1	1.4	1.2	1.0–1.1	0.9–1.4

Sailo Saitluangpuia leg.; from a roadside; GenBank: MZ098159; MZMU 1847, 1 ♀, TL 349 mm.

Identification. Morphology-based identification was based on the published literature (Taylor 1960; Pillai and Ravichandran 1999; Mathew and Sen 2009; Kamei et al. 2009), and this work provides the first 16s genetic data for the species. *Ichthyophis moustakius* differs from Northeast Indian congeners in having a unique moustache-like, arched, yellow stripe extending beyond its tentacles which, coupled with the number of AGs on its body (Kamei et al. 2009) serve to distinguish it. In dorsal view it has a U-shaped head, gently tapering anterior to the TAs (see also Chaitanya et al. 2017). The following morphological characters of the new material are also consistent with the identification as *I. moustakius* (values in the bracket denote the ranges in the type series): AGs 280 and 286 (238–292), AGs interrupted by disc 5/5 and 5/4 (3/3, 4/3, 4/4, 4/5, or 5/5), AGs behind disc 7/7 and 6/6 (4/4, 5/5, 5/6, or 6/5) in the specimens MZMU 1758 and 1847, respectively.

Phylogeny. From the 16s rRNA tree (Fig. 1B), it is evident that *I. multicolor* from the type locality and from Mizoram are conspecific. Both populations differ from one another by an uncorrected *p*-distance of 0.014–0.017. *Ichthyophis multicolor* as a whole (Mizoram+Myanmar population) is the sister taxon to the sampled Southeast Asian *Ichthyophis* such as *I.* cf. *hypocyaneus* (Van Hasselt in Boie, 1827), *I.* cf. *kohtaoensis* Taylor, 1960, *I.* cf.

Table 2. Genetic sequences (16s rRNA) used for phylogenetic analysis.

Species	Genebank accession number	Locality	Reference
Caudacaecilia cf. *asplenia*	AB686161	Malaysia, Sabah, Tawau	Nishikawa et al. 2012
Epicrionops marmoratus	AY101226	Ecuador, Cotopaxi, San Francisco de las Pampas	Gower et al. 2002
Ichthyophiidae sp.	AB686127	Malaysia, Sabah, Ulu Senagan	Nishikawa et al. 2012
Ichthyophiidae sp.	AB686157	Malaysia, Sabah, Ulu Senagan	Nishikawa et al. 2012
Ichthyophiidae sp.	AB686144	Malaysia, Pahang, Temerloh	Nishikawa et al. 2012
Ichthyophis bannanicus	AY101235	China, Yunnan, Longlin	Gower et al. 2002
Uraeotyphlus bombayensis	DQ919054	India, Tamil Nadu, Kanyakumari	Gower et al. 2006
Ichthyophis cf. *hypocyaneus*	AB686166	Indonesia, Central Java, Pekalongan	Nishikawa et al. 2012
Ichthyophis cf. *beddomei*	AY101230	India, Kerala, Wayanad District, near Periy	Gower et al. 2002
Ichthyophis cf. *kohtaoensis*	AB686146	Malaysia, Johor, Ledang	Nishikawa et al. 2012
Ichthyophis cf. *mindanaoensis*	AB686120	Philippine, Mindanao	Nishikawa et al. 2012
Ichthyophis cf. *supachaii*	AB686168	Malaysia, Terengganu, Hulu Terengganu	Nishikawa et al. 2012
Ichthyophis cf. *tricolor*	AY101228	India, Kerala, Idukki District, near Vandiperiy	Gower et al. 2002
Ichthyophis glutinosus	AY101234	SriLanka, Central Province, near Peradeniy	Gower et al. 2002
Ichthyophis longicephalus	JQ040048		Unpublished
Ichthyophis moustakius (MZMU 1758)	MZ098158	India, Mizoram, Mamit District, Dampa Tiger Reserve	Present study
Ichthyophis moustakius (MZMU 1847)	MZ098159	India, Mizoram, Aizawl District, Thakthing	Present study
Ichthyophis multicolor	FR716010	Myanmar, Ayeyarwady Region, Mwe Hauk Village	Wilkilson et al. 2014
Ichthyophis multicolor	FR716013	Myanmar, Ayeyarwady Region, Mwe Hauk Village	Wilkilson et al. 2015
Ichthyophis multicolor	FR716012	Myanmar, Ayeyarwady Region, Mwe Hauk Village	Wilkilson et al. 2016
Ichthyophis multicolor	FR716011	Myanmar, Ayeyarwady Region, Mwe Hauk Village	Wilkilson et al. 2017
Ichthyophis multicolor (MZMU 1739)	MZ098156	India, Mizoram, Aizawl District, Melthum Village	Present study
Ichthyophis multicolor (MZMU 1740)	MZ098155	India, Mizoram, Aizawl District, Mission Vengthlang	Present study
Ichthyophis multicolor (MZMU 1541)	MZ098157	India, Mizoram, Aizawl District, Tlangnuam	Present study
Ichthyophis orthoplicatus	AY101233	SriLanka, Province of Uva, near Passar	Gower et al. 2002
Uraeotyphlus cf. *oommeni*	AY101224	India, Kerala, Idukki District, near Vandiperiy	Gower et al. 2002
Uraeotyphlus cf. *oxyurus*	AY101223	India, Kerala, Kannur District, near Payyanu	Gower et al. 2002
Uraeotyphlus narayani	AY101222	India, Kerala, Kottayam District, Kannam	Gower et al. 2002

supachaii Taylor, 1960, *I. bannanicus* Yang, 1984, and an unknown Ichthyophiidae sp.). *Ichthyophis moustakius* was seen to be more closely related to Sri Lankan species (*I. glutinosus* and *I. orthoplicatus*).

Discussion

Dubois et al. (2021) recently allocated *I. asplenius* Taylor, 1965, *I. bannanicus*, *I. biangularis* Taylor, 1965, *I. larutensis* Taylor, 1960, *I. longicephalus* Pillai, 1986, and *I. tricolor* Annandale, 1909 to the genus *Epicrium* Wagler, 1828, and found that *Epicrium* and *Ichthyophis* are sister taxa, and they together (*Epicrium* + *Ichthyophis*) are sister taxa to *Uraeotyphlus* W. Peters, 1879. Dubois et al. (2021) also allocated *Ichthyophis bombayensis* Taylor, 1960 to U*raeotyphlus*. Although Dubois et al (2021) did not mention the generic allocation of *I. multicolor* and *I. moustakius*, it is very likely that *I. multicolor* belongs to the genus *Epicrium* and that *I. moustakius* should remain in the genus *Ichthyophis* along with its Sri Lankan counterparts, *I. glutinosus* and *I. orthoplicatus* (Fig. 1).

In both molecular and morphological aspects, the new specimens of *I. multicolor* from Mizoram substantially agree with the original species descriptions provided by Wilkinson et al. (2014). Our new records of *I. multicolour* extend the range of this species by 840 km northwest from the type locality. The Mizoram records also represent a substantial extension in elevational range, from 10 m in Myanmar to 272–1030 m in Northeast India. This large horizontal and vertical range is unusual for caecilians, but is not entirely exceptional within Ichthyophioidea, with *U. bombayensis* distributed along 1,500 km of peninsular India from close to sea level to the hills of the Western Ghats (Gower et al. 2007). Our study also found preliminary evidence for a potentially close relationship between Northeast Indian *I. moustakius* and Sri Lankan *I. glutinosus* and *I. orthoplicatus*—which supports the understanding (see Agarwal et al. 2020) that Northeast India has the elements of Sri Lankan biogeographic realms in addition to Indo-Malayan, Indo-Chinese, and Indian biogeographical realms.

At present, *I. multicolor* is not evaluated by the IUCN, as there is scant of information on the biology of this species. However, Wilkinson et al. (2014) suggested that this species should be categorized as Data Deficient given that little is known about its geographic range or environmental requirements and tolerances. With the current range extension, this lack of knowledge becomes more apparent, and we reiterate Wilkinson et al.'s (2014) suggestion that *I. multicolor* is a Data Deficient species.

Table 3. Uncorrected *p*-distance (16s rRNA) among *Ichthyophis* species. GenBank accession numbers are in parenthesis.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*Ichthyophis multicolor* (MZ098155)																						
2	*Ichthyophis multicolor* (MZ098156)	0.000																					
3	*Ichthyophis multicolor* (MZ098157)	0.002	0.000																				
4	*Ichthyophis multicolor* (FR716010)	0.014	0.017	0.017																			
5	*Ichthyophis multicolor* (FR716011)	0.014	0.017	0.017	0.000																		
6	*Ichthyophis multicolor* (FR716012)	0.014	0.017	0.017	0.000	0.000																	
7	*Ichthyophis multicolor* (FR716013)	0.014	0.017	0.017	0.000	0.000	0.000																
8	*Ichthyophis moustakius* (MZ098158)	0.076	0.088	0.087	0.074	0.074	0.074	0.074															
9	*Ichthyophis moustakius* (MZ098159)	0.088	0.106	0.091	0.087	0.087	0.087	0.087	0.009														
10	*Uraeotyphlus bombayensis* (DQ919054)	0.104	0.118	0.112	0.103	0.103	0.103	0.103	0.106	0.122													
11	*Uraeotyphlus* cf. *oommeni* (AY101224)	0.158	0.170	0.171	0.154	0.154	0.154	0.154	0.152	0.175	0.123												
12	*Uraeotyphlus narayani* (AY101222)	0.166	0.177	0.180	0.168	0.168	0.168	0.168	0.155	0.177	0.129	0.112											
13	*Uraeotyphlus* cf. *oxyurus* (AY101223)	0.171	0.181	0.189	0.169	0.169	0.169	0.169	0.153	0.176	0.132	0.112	0.045										
14	*Ichthyophis longicephalus* (JQ040048)	0.077	0.087	0.081	0.076	0.076	0.076	0.076	0.079	0.090	0.120	0.161	0.183	0.185									
15	*Ichthyophis* cf. *tricolor* (AY101228)	0.083	0.097	0.092	0.080	0.080	0.080	0.080	0.075	0.089	0.107	0.142	0.166	0.166	0.057								
16	*Ichthyophis* cf. *beddomei* (AY101230)	0.089	0.104	0.099	0.084	0.084	0.084	0.084	0.066	0.080	0.111	0.133	0.164	0.163	0.072	0.057							
17	*Ichthyophis glutinosus* (AY101234)	0.073	0.083	0.081	0.074	0.074	0.074	0.074	0.046	0.056	0.106	0.158	0.154	0.156	0.087	0.078	0.076						
18	*Ichthyophis orthoplicatus* (AY101233)	0.083	0.098	0.093	0.088	0.088	0.088	0.088	0.060	0.073	0.114	0.158	0.170	0.172	0.089	0.076	0.078	0.039					
19	*Ichthyophis* cf. *hypocyaneus* (AB686166)	0.080	0.097	0.089	0.084	0.084	0.084	0.084	0.073	0.082	0.124	0.153	0.164	0.159	0.099	0.090	0.092	0.088	0.094				
20	*Ichthyophis* cf. *kohtaoensis* (AB686146)	0.072	0.090	0.080	0.082	0.082	0.082	0.082	0.065	0.073	0.112	0.142	0.160	0.154	0.085	0.078	0.086	0.076	0.082	0.017			
21	*Ichthyophis* cf. *supachaii* (AB686168)	0.066	0.080	0.074	0.076	0.076	0.076	0.076	0.063	0.071	0.112	0.142	0.164	0.158	0.088	0.078	0.082	0.072	0.082	0.015	0.014		
22	*Ichthyophis bannanicus* (AY101235)	0.065	0.078	0.069	0.064	0.064	0.064	0.064	0.079	0.093	0.114	0.138	0.154	0.156	0.075	0.079	0.074	0.087	0.089	0.060	0.062	0.064	
23	Ichthyophiidae sp. (AB686144)	0.053	0.063	0.058	0.054	0.054	0.054	0.054	0.062	0.069	0.117	0.145	0.164	0.161	0.077	0.075	0.068	0.068	0.077	0.061	0.055	0.052	0.062

Figure 3. *Ichthyophis moustakius*. **A.** MZMU 1847 in life. **B.** MZMU 1847 in preservation. **C.** Dorsolateral view of the head of MZMU 1758 with the characteristic moustache-like, arched, yellow stripe between tentacular aperture and nostril.

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Authors' Contributions

HTL, JP, and LB conceived the research question, and wrote and revised the manuscript. MV, LM, and HD undertook the field surveys and helped with the first draft of the manuscript. All authors read and approved the submitted manuscript.

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