Yu et al. *Zool. Res. Divers. Conserv.* 2025, 2(2): 114−124
https://doi.org/10.24272/j.issn.2097-3772.2024.025
https://cstr.cn/32249.14.zrdc.2024.025

ZRDC Zoological Research: Diversity and Conservation

Article Open Access

A new species record of *Ichthyophis moustakius* Kamei, Wilkinson, Gower, and Biju, 2009 (Gymnophiona: Ichthyophiidae) from China

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ABSTRACT

A new species record of *Ichthyophis moustakius* is documented from southern Gaoligong Mountain, Yunnan Province, China, based on integrative taxonomic evidence from morphological and molecular analyses. Phylogenetic reconstruction using mitochondrial DNA placed the newly collected Chinese specimens within a strongly supported clade comprising recognized *I. moustakius* specimens from India. Uncorrected pairwise distances of 16S rRNA gene sequences between Chinese and Indian specimens ranged from 1.2% to 2.6%, indicating minimal genetic divergence. Morphological assessments revealed concordance between the Yingjiang specimens and those from the type locality in Manipur, India. Furthermore, cytochrome b (cyt *b*) gene analysis excluded the possibility of misidentification with *Ichthyophis kohtaoensis*. These findings extend the known distribution of *Ichthyophis* in China, raising the number of recognized species to three, and offer detailed morphological characterizations of both adult and larval stages.

Keywords: *Ichthyophis moustakius*; Gymnophiona; New record; Gaoligong Mountain; China

INTRODUCTION

The Gaoligong Mountain range in western Yunnan, China, lies at the biogeographic convergence of three key biodiversity hotspots, including Indo-Burma, the Himalayas, and the Mountains of Southwest China. This montane region serves as a critical ecological corridor, supporting exceptional faunal richness and endemism (Li et al., 2024; Myers et al., 2000; Wang et al., 2024; Wu et al., 2024a). To date, 84 amphibian species have been documented in the Gaoligong region, with only a single caecilian species, *Ichthyophis kohtaoensis* Taylor, 1960, recorded in the southern areas of Yingjiang and Longling counties (AmphibiaChina, 2025; Fei et al., 2006; Gower et al., 2002; Yang, 1991; Yang & Rao, 2008; Yuan

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Received: 26 May 2025; Accepted: 26 June 2025; Online: 26 June 2025
Foundation items: This work was supported by the National Key R & D Program of China (2022YFC2602500), Science and Technology Basic Resources Investigation Program of China (2021FY100200), Second Tibetan Plateau Scientific Expedition and Research (STEP) Program (2024QZKK0200), National Natural Science Foundation of China (NSFC 32100371), Yunnan Revitalization Talent Support Program Yunling Scholar Project & Young Talent Project, Yunnan Forestry and Grassland Administration (2022GF258D-10), Yunnan Applied Basic Research Projects (202301AT070312 and 202301AT070431), Major Science and Technique Program (202102AA310055) and Key R & D Program (202103AC100003 and 202301AT070431) in Yunnan Province, China's Biodiversity Observation Network (Sino-BON), CAS Special Research Assistant (SRA) Program, Biological Resources Program, CAS, and Animal Branch of the Germplasm Bank of Wild Species, CAS (Large Research Infrastructure Funding), and Program for New Records and Rediscoveries of Rare Species by Tencent Foundation and Shanshui Conservation Center.

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et al., 2022; Wang et al., 2024). Earlier records referred to Chinese caecilians as *Ichthyophis bannanicus* Yang, 1984; however, subsequent morphological and molecular studies led Nishikawa et al. (2012) to synonymize *I. bannanicus* with *I. kohtaoensis*, a taxonomic revision followed in the present study.

Caecilians are the least explored lineage among amphibians, largely due to their fossorial lifestyle and cryptic habits (Wilkinson, 2012). Although higher-level phylogenetic relationships within Gymnophiona are well-established, their species-level taxonomy remains challenging, primarily stemming from insufficient morphological resolution and limited molecular data (Nishikawa et al., 2012; Nussbaum & Wilkinson, 1989; San Mauro et al., 2014; Wang et al., 2015; Wilkinson et al., 2011; Zhang & Wake, 2009). The genus *Ichthyophis* Fitzinger, 1826, comprising 49 recognized species, is widely distributed across southern Asia, the western Indo-Australian Archipelago, and the Philippines (AmphibiaChina, 2025; Fei et al., 2006; Frost, 2024). Despite being the most speciose genus of caecilians, ongoing discoveries suggest that species diversity is still significantly underestimated (Nishikawa et al., 2012; Rao et al., 2024; Wang et al., 2015; Wilkinson et al., 2014). For over five decades, *I. kohtaoensis* was the only caecilian species recorded from China. However, the recent description of *Ichthyophis yangi* Rao, Zhou, Mo, Yu, Li, and Liu, 2024 from Yunnan highlights the growing recognition of previously undocumented caecilian diversity within the country (Rao et al., 2024).

In 2024, during targeted herpetofaunal surveys of the Gaoligong Mountain region, five caecilian specimens were collected from Yingjiang County in southwestern Yunnan Province, China, located near the international boundary with Myanmar (Figure 1). Through integrative taxonomic assessment, combining morphological examination and molecular phylogenetic analysis, these specimens were identified as *Ichthyophis moustakius* Kamei, Wilkinson, Gower, and Biju, 2009. This constitutes the first confirmed record of *I. moustakius* in China, supported by voucher specimens. The present study provides a comprehensive morphological description of the Chinese population, thereby contributing new insights into the regional diversity and distribution of *Ichthyophis*.

MATERIALS AND METHODS

Sampling

Fieldwork was conducted in Tongbiguan Town, Yingjiang County, Yunnan Province, China, with permission from the Yunnan Forestry and Grassland Bureau and Tongbiguan Provincial Nature Reserve. Specimens were collected during daylight hours from beneath stones embedded in gravel and crushed rock adjacent to streams. Following photographic documentation, individuals were humanely euthanized using 20% benzocaine for 20 min. Liver tissues were preserved in 95% ethanol and stored at –80℃ for molecular analyses. Specimens were fixed in 10% buffered formalin solution for two days and then transferred to 75% ethanol for long-term storage. All newly collected specimens were deposited at the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). All research protocols were approved by the Ethics Committee of the Kunming Institute of Zoology, CAS (IACUC-OE-2021-07-001).

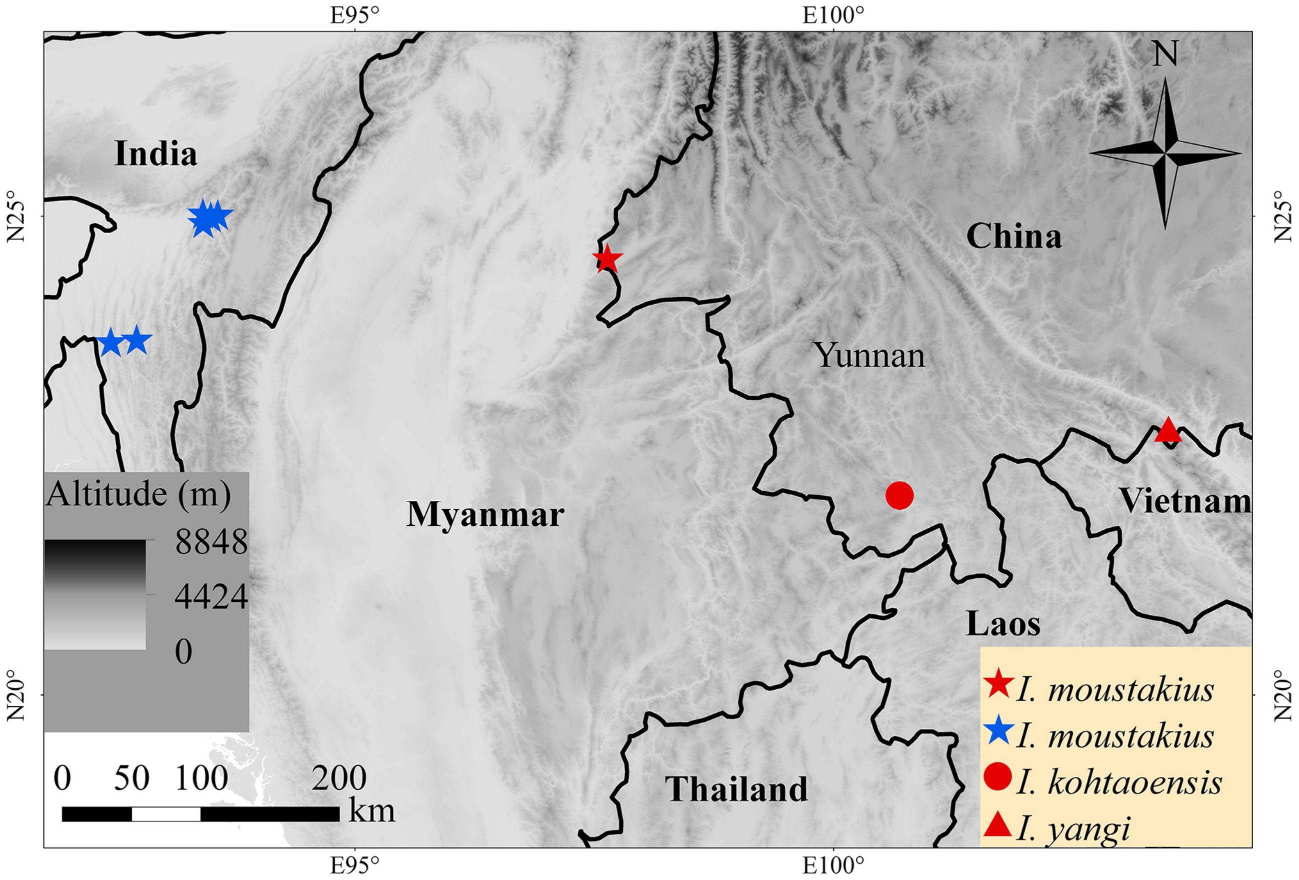

Figure 1 Distribution map of *Ichthyophis moustakius*, *Ichthyophis kohtaoensis*, and *Ichthyophis yangi*

Morphological analysis and morphometrics

Morphological measurements were obtained using a digital caliper to the nearest 0.1 mm (Table 1), following the terminology and protocols of Fei et al. (2006) and Kamei et al. (2009). A total of 20 morphometric parameters were recorded, including snout-vent length (SVL), total length (TOL), tail length (TL), head width (HW), head length (HL), snout length (SL), interorbital distance (IOD), internarial distance (IND), distance from center of nostril to corner of eye (NED), distance between tentacle and naris (TN), distance between tentacle and eye (TE), body width one head length behind head (BW1), mid-body width (BW2), body width one head length anterior to vent (BW3), snout-tip to second groove (STSG), length of first collar behind mouth corner (C1), length of second collar behind mouth corner (C2), number of annular grooves in dorsal view (AGs), and number of annular grooves in ventral view of trunk, anus, and tail (AGs-TAT).

Radiography

Radiographic imaging was performed using a Faxitron SR MX-20 system at the Germplasm Bank of Wild Species, Kunming Institute of Botany (KIB), CAS. The imaging parameters were set to an exposure time of 4.8 s and a voltage of 26 kV. Flat-field exposures matched the camera settings. Imaging commenced following system calibration, and window width and level were adjusted to optimize vertebral clarity. Radiographs were saved in both JPEG and dcm format for vertebral counts.

Molecular data and phylogenetic analyses

Total genomic DNA was extracted from liver tissues using standard phenol-chloroform extraction (Sambrook et al., 1989). To confirm species identity and perform preliminary phylogenetic reconstruction, a fragment of the mitochondrial 16S rRNA gene was amplified and sequenced from all samples using primer pairs 16SAR (5’–CGCCTGTTTAYC AAAAACAT–3’) and 16SBR (5’–CCGGTYTGAACTCAGAT CAYGT–3’) (Kocher et al., 1989). Amplifications were performed in a 25 μL reaction volume with the following thermal cycling conditions: initial denaturing step at 94°C for 5 min, 35 cycles of denaturing at 94°C for 45 s, annealing at 46°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. Amplified polymerase chain reaction (PCR) products were sequenced using an ABI 3730 automated sequencer. Raw sequences were assembled using SeqMan in Lasergene v.7.1 (Burland, 2000). All newly generated sequences were deposited in GenBank (accession numbers provided in Table 2).

Table 1 Measurement (in mm) and proportions of the *Ichthyophis moustakius* and *Ichthyophis* cf. *kohtaoensis* from Yingjiang county

Species	*Ichthyophis moustakius*						*Ichthyophis* cf. *kohtaoensis*
NO.	KIZ 059693	KIZ 056482	KIZ 056483	KIZ 056479	KIZ 056481	KIZ 078001	KIZ 035405
sex	♂	♂	♂	Juvenile	Juvenile	Juvenile	Juvenile
total length (TOL)	292.3	175.1	236.3	173.1	140.7	143.6	230.4
snout-vent length (SVL)	285.2	170.6	221.8	167.9	135.5	138.1	225.0
head length (HL)	11.6	8.9	10.4	8.6	6.8	8.0	8.4
TOL/HL	25.2	19.7	22.7	20.1	20.7	18.0	27.4
head width (HW)	8.7	6.6	7.9	6.4	5.3	6.5	7.1
HW/HL	75.0%	74.2%	76.0%	74.4%	77.9%	81.3%	84.5%
snout length (SL)	5.2	3.9	4.7	3.3	2.7	3.1	4.5
SL/HL	44.8%	43.8%	45.2%	38.4%	39.7%	38.8%	53.6%
interorbital distance (IOD)	5.3	3.8	4.9	3.9	3.2	3.6	4.6
SL/IOD	98.1%	102.6%	95.9%	84.6%	84.4%	86.1%	97.8%
internasal distance (IND)	1.7	1.7	1.9	1.9	1.4	1.6	2.4
IND/SL	32.7%	43.6%	40.4%	57.6%	51.9%	51.6%	53.3%
distance from the center of nostril to the corner of the eye (NED)	4.5	3.2	3.7	2.3	1.6	2.7	3.6
tentacle to eye (TE)	1.3	1.1	1.4	0	0	0	1.3
tentacle to nostril (TN)	3.4	2.6	2.8	2.3	1.6	2.7	2.9
TN/TE	261.5%	236.4%	200.0%	-	-	-	223.1%
tail length (TL)	4.3	4.1	4.1	7	3.9	3.8	3.4
TL/SVL	1.5%	2.4%	1.8%	4.2%	2.9%	2.8%	1.5%
body width after-head (BW1)	11.7	6.9	8.2	6.1	5.3	6.5	8.1
mid-body width (BW2)	13.9	7.5	8.9	7.6	5.6	7.5	9.0
body width anterior anal (BW3)	10.9	6.6	8.4	7.1	5.2	5.7	6.4
TOL/BW2	21.0	23.3	26.6	22.8	25.1	19.1	25.6
snout-tip to second groove (STSG)	12.3	10.1	12.7	-	-	-	-
first collar width (C1)	2.4	2.5	2.9	-	-	-	-
second collar width (C2)	2.8	3	3.2	-	-	-	-
C1/C2	85.7%	83.3%	90.6%	-	-	-	-
the number of annular grooves in dorsal view (AGs)	260	282	274	284	279	276	348
the number of annular grooves formula in ventral view of trunk, anus and tail (AGs-TAT)	249+3+6	278+3+7	265+2+7	278+3+7	276+3+7	267+3+6	344+3+6
number vertebra	106	105	109	104	108	108	115

Table 2 Localities, voucher information, and Genbank accession numbers for all specimens used in 16S rRNA analyses of this study

Species name	Voucher	Locality	Accession No.	References
Ingroup				
Ichthyophis kohtaoensis	UMMZ 189122	China, Yunnan, Longlin	AY101235	Gower et al., 2002
Ichthyophis kohtaoensis	VUB 0697	Vietnam	EF107165	Roelants et al., 2007
Ichthyophis kohtaoensis	—	—	AY458594	Zhang et al., 2005
Ichthyophis kohtaoensis	—	—	Y10949	Feller and Hedges 1998
Ichthyophis cf. *hypocyaneus*	MZB:Amp:12891	Indonesia, Central Java, Pekalongan	AB686166	Nishikawa et al., 2012
Ichthyophis cf. *beddomei*	MW 283	India, Kerala, Wayanad District, near Periy	AY101230	Gower et al., 2002
Ichthyophis cf. *mindanaoensis*	KUHE 33345	Philippine, Mindanao	AB686120	Nishikawa et al., 2012
Ichthyophis cf. *supachaii*	UKMHC 877	Malaysia, Terengganu, Hulu Terengganu	AB686168	Nishikawa et al., 2012
Ichthyophis cf. *tricolor*	MW 322	India, Kerala, Idukki District, near Vandiperiy	AY101228	Gower et al., 2002
Ichthyophis glutinosus	MW 1733	Sri Lanka, Central Province, near Peradeniy	AY101234	Gower et al., 2002
Ichthyophis longicephalus	—	—	JQ040048	Unpublished
Ichthyophis multicolor	CAS 212263	Myanmar, Ayeyarwady Region, Mwe Hauk Village	FR716010	Wilkilson et al., 2014
Ichthyophis multicolor	CAS 212266	Myanmar, Ayeyarwady Region, Mwe Hauk Village	FR716013	Wilkilson et al., 2014
Ichthyophis orthoplicatus	MW 1722	Sri Lanka, Province of Uva, near Passar	AY101233	Gower et al., 2002
Ichthyophis benjii	MZMU 2458	—	OM501568	Unpublished
Ichthyophis benjii	MZMU 2025	India, Mizoram, Aizawl, Lalengzuala Tochhawng	MZ153116	Unpublished
Ichthyophis khumhzi	MZMU 1796	—	OQ780535	Unpublished
Ichthyophis khumhzi	MZMU 1460	—	OQ780536	Unpublished
Ichthyophis moustakius	MZMU 1758	India, Mizoram, Mamit District, Dampa Tiger Reserve	MZ098158	Unpublished
Ichthyophis moustakius	MZMU 1847	India, Mizoram, Aizawl District, Thakthing	MZ098159	Unpublished
Ichthyophis moustakius	KIZ 056478	China, Yunnan, Yingjiang, Tongbiguan Provincial Nature Reserve	PX090926	This study
Ichthyophis moustakius	KIZ 056479	China, Yunnan, Yingjiang, Tongbiguan Provincial Nature Reserve	PX090927	This study
Ichthyophis moustakius	KIZ 056481	China, Yunnan, Yingjiang, Tongbiguan Provincial Nature Reserve	PX090928	This study
Ichthyophis moustakius	KIZ 056482	China, Yunnan, Yingjiang, Tongbiguan Provincial Nature Reserve	PX090929	This study
Ichthyophis moustakius	KIZ 056483	China, Yunnan, Yingjiang, Tongbiguan Provincial Nature Reserve	PX090930	This study
Outgroup				
Uraeotyphlus cf. oxyurus	MW 212	India, Kerala, Kannur District, near Payyanu	AY101223	Gower et al., 2002
Uraeotyphlus narayani	MW 1417	India, Kerala, Kottayam District, Kannam	AY101222	Gower et al., 2002

To further clarify species distributions, phylogenetic analysis based on the more extensively sampled cyt *b* gene was conducted using additional sequences from GenBank that span a broader range of taxa and localities. Amplification of the cyt *b* fragment employed primers L14014 (5’-GGATCT AACCAAGACTAATGGTC-3’) and H15198 (5’-GCTAATGC TTTCTGATAAGCTAC-3’) (Li et al., 2010), with PCR amplification, sequencing, and assembly protocols identical to those used for 16S rRNA, except for an annealing temperature of 52°C.

Phylogenetic inference based on 16S rRNA was conducted using maximum-likelihood (ML) and Bayesian inference (BI) approaches. In addition to the newly generated sequences, 29 homologous *Ichthyophis* sequences and two outgroup taxa (*Uraeotyphlus narayani* and *Uraeotyphlus*. cf. *oxyurus*) were downloaded from GenBank (Table 2). Sequence alignment was performed using MUSCLE v.3.8 with default parameters (Edgar, 2004), followed by manual refinement in MEGA v.6.0 (Tamura et al., 2013) to remove ambiguous regions and reduce missing data. BI and ML analyses were performed on the CIPRES web server (Miller et al., 2010). BI was performed using MrBayes v.3.2.4 (Ronquist et al., 2012) under the GTR+G model selected as the best-fit substitution model based on the Bayesian Information Criterion (BIC; Posada, 2008) in jModelTest v.2 (Darriba et al., 2012). Two independent runs of 10 million generations were sampled every 1 000 generations, with a burn-in of 25%. Convergence was assessed in Tracer v.1.6. (Rambaut et al., 2014) based on stationarity and effective sample size values greater than 200. ML was performed in RAxML-HPC BlackBox v.8.2.10 (Stamatakis, 2014) under the GTR+gamma nucleotide substitution model with 1 000 bootstrap replicates and a standard bootstrap search (random seed value 12 345). Genetic distances for the 16S rRNA dataset were calculated using the *p*-distance model in MEGA v.6.0 with pairwise deletion of missing data and gaps (Tamura et al., 2013).

Phylogenetic reconstruction of cyt *b* was also performed using ML and BI methods. In addition to the newly generated sequence, 81 homologous sequences of *Ichthyophis* and two outgroup taxa (*Uraeotyphlus bombayensis* and *U.* cf. *oxyurus*) were downloaded from GenBank (Supplementary Table S1). The HKY+G model was selected as the best-fit substitution model by jModelTest v.2 (Darriba et al., 2012) for BI analyses. The methods applied for phylogenetic reconstruction were the same as those used for the 16S gene.

Alignment files and intermediate outputs from all phylogenetic analyses were deposited at https://github.com/CheLab-KIZ/Paper-codes/tree/main/CJPA202501. All remaining data are available within the manuscript and/or Supplementary Material.

RESULTS

The aligned 16S rRNA sequence matrix comprised 557 nucleotide positions, including 165 variable and 128 parsimony-informative sites (with outgroup taxa). The GTR+G and HKY+G models were selected as the best-fit substitution models for the 16S and cyt *b* datasets, based on BIC in jModelTest v.2. The ML and BI analyses produced congruent topologies, with most terminal nodes exhibiting relatively high support, except for some internal branches within species that lacked resolution or collapsed due to weak support. The phylogenetic tree shown in Figure 2 presents the BI topology, with associated nodal support values from both BI and ML analyses. Specimens collected from Tongbiguan Township, Yingjiang County, Yunnan Province, China, clustered robustly with Indian *I. moustakius* specimens (Bayesian posterior probability (BPP)=1.00, bootstrap support (BS)=93), confirming their identity as *I. moustakius* (Figure 2). This clade was resolved as sister to a lineage comprising Sri Lankan taxa *Ichthyophis glutinosus* and *Ichthyophis orthoplicatus*. Uncorrected pairwise genetic distances (*p*-distance) in the 16S rRNA gene between Chinese and Indian *I. moustakius* specimens ranged from 1.2% to 2.6% (Table 3). Morphological congruence further confirmed this identification, including the presence of lateral yellow stripes extending from the anterior tail to the lower jaw near mandibular tip, reduced annular groove counts (260–284 dorsally; 258–288 ventrally), short head proportions (TOL/HL 18.0–25.2), and tentacular apertures positioned approximately twice as far from the nares as from the eyes (TN/TE 200.0%–261.5%). These diagnostic characters collectively distinguish *I. moustakius* from all other *Ichthyophis* species known from China. Based on both phylogenetic evidence and detailed morphological comparison, the specimens from Tongbiguan Nature Reserve are confirmed as *I. moustakius*, and a comprehensive description of these Chinese specimens is provided below.

An additional phylogenetic reconstruction based on the cyt *b* gene supported the same conclusion. The Tongbiguan specimens formed a distinct clade as the sister lineage to *Ichthyophis glutinosus*, with both lineages positioned at the base of the *Ichthyophis* genus (Figure 3). This result clearly separated *I. moustakius* from other *Ichthyophis* taxa recorded in China and was consistent with the topology recovered from the 16S rRNA dataset (Figure 2).

Taxonomic account

Ichthyophis moustakius Kamei, Wilkinson, Gower, and Biju, 2009 (Figure 4; Figure 5)

Chinese name: The newly recorded species was discovered in western Yunnan Province. Thus, “diān xī yú yuán (滇西鱼螈)” is suggested as its Chinese common name.

Chresonymy: *Ichthyophis bannanica* (Fei et al., 2006; Yang, 1991; Yang & Rao, 2008); *Ichthyophis kohtaoensis* (Wang et al., 2024; Yuan et al., 2022).

Specimens examined: Three adult males (KIZ 056482–483, KIZ 059693) and two larvae (KIZ 056479, KIZ 056481). Two adult males and two larvae were collected on 27 February 2024 by Zhengpan Duan from Tongbiguan Township, Yingjiang County, Yunnan Province, China. Another adult male was collected on 24 August 2024 by Shenpin Yang,

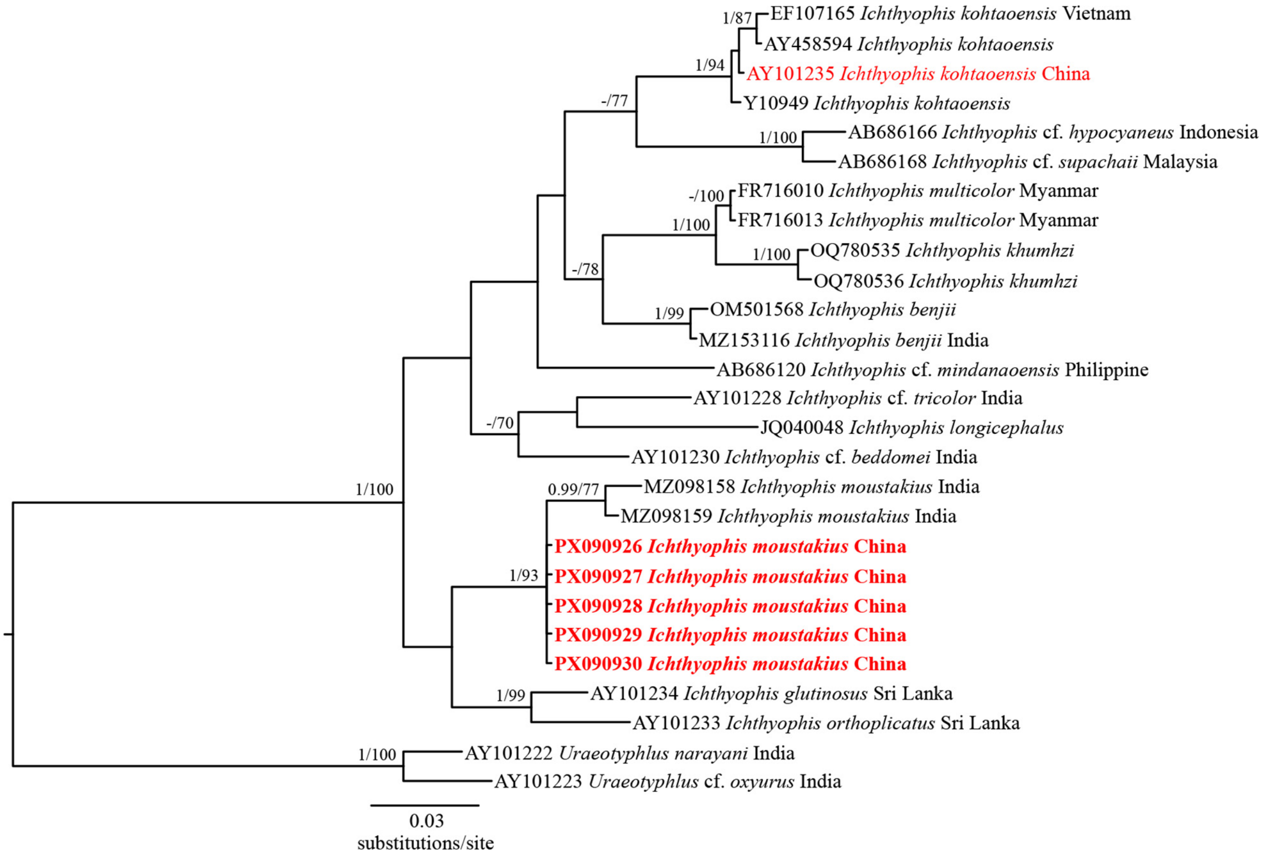

Figure 2 Bayesian inference (BI) and maximum-likelihood (ML) analyses of the genus *Ichthyophis* based on partial DNA sequences of the mitochondrial 16S rRNA gene

Ichthyophis moustakius and *Ichthyophis kohtaoensis* individuals from China are highlighted in red. Node values with Bayesian posterior probabilities (BPP)<0.95/bootstrap support (BS)<70 are not shown. A “–” denotes BPP<0.95 or BS<70.

Table 3 Uncorrected *p*-distance (%) among *Ichthyophis* species calculated from 16S rRNA gene sequences (below the diagonal) and standard error estimates (above the diagonal)

ID	Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	*Ichthyophis kohtaoensis* AY101235		0.3	0.3	0.2	1.0	1.1	1.1	1.1	1.0	1.2	1.2	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.3	1.4	1.2	1.2	1.2	1.2	1.2
2	*Ichthyophis kohtaoensis* EF107165	0.6		0.2	0.4	1.1	1.1	1.1	1.1	1.1	1.2	1.2	1.0	1.0	1.2	1.1	1.1	1.1	1.1	1.3	1.4	1.2	1.2	1.2	1.2	1.2
3	*Ichthyophis kohtaoensis* AY458594	0.4	0.2		0.3	1.0	1.1	1.1	1.1	1.1	1.2	1.2	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.3	1.4	1.2	1.2	1.2	1.2	1.2
4	*Ichthyophis kohtaoensis* Y10949	0.2	0.8	0.6		1.0	1.1	1.1	1.1	1.0	1.1	1.2	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.3	1.4	1.2	1.1	1.2	1.2	1.2
5	*Ichthyophis* cf. *hypocyaneus* AB686166	6.2	6.5	6.4	6.4		1.1	1.2	0.5	1.2	1.2	1.3	1.1	1.1	1.2	1.1	1.1	1.2	1.2	1.2	1.3	1.2	1.1	1.2	1.2	1.2
6	*Ichthyophis* cf. *beddomei* AY101230	7.1	7.9	7.5	7.1	9.3		1.1	1.1	0.9	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.3	1.3	1.2	1.3	1.1	1.0	1.1	1.1	1.1
7	*Ichthyophis* cf. *mindanaoensis* AB686120	7.1	7.9	7.5	7.1	9.2	7.3		1.2	1.1	1.1	1.3	1.1	1.1	1.1	1.1	1.1	1.2	1.2	1.3	1.3	1.2	1.1	1.2	1.2	1.2
8	*Ichthyophis* cf. *supachaii* AB686168	6.6	7.1	7.0	6.8	1.9	9.2	8.6		1.2	1.1	1.3	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.2	1.3	1.1	1.0	1.1	1.1	1.1
9	*Ichthyophis* cf. *tricolor* AY101228	7.5	8.3	7.9	7.3	9.9	5.4	7.8	9.4		1.2	1.0	1.2	1.2	1.1	1.2	1.2	1.3	1.3	1.3	1.3	1.2	1.1	1.2	1.2	1.2
10	*Ichthyophis glutinosus* AY101234	8.3	9.1	8.7	8.1	9.0	7.9	7.9	7.9	8.5		1.2	1.2	1.2	0.8	1.1	1.1	1.2	1.2	1.2	1.2	1.0	1.0	1.0	1.0	1.0
11	*Ichthyophis longicephalus* JQ040048	8.0	8.4	8.4	7.8	10.8	7.4	9.2	10.2	6.4	9.2		1.2	1.2	1.2	1.1	1.1	1.2	1.2	1.3	1.3	1.2	1.2	1.2	1.2	1.2
12	*Ichthyophis multicolor* FR716010	5.6	6.1	5.8	5.8	8.6	8.2	7.3	8.4	8.0	7.7	8.1		0.0	1.2	0.9	0.9	0.6	0.6	1.3	1.4	1.3	1.2	1.3	1.3	1.3
13	*Ichthyophis multicolor* FR716013	5.6	6.1	5.8	5.8	8.6	8.2	7.3	8.4	8.0	7.7	8.1	0.0		1.2	0.9	0.9	0.6	0.6	1.3	1.4	1.3	1.2	1.3	1.3	1.3
14	*Ichthyophis orthoplicatus* AY101233	8.5	9.2	8.9	8.5	9.4	7.9	8.3	8.6	8.3	3.7	9.8	9.0	9.0		1.2	1.2	1.3	1.3	1.2	1.3	1.1	1.0	1.1	1.1	1.1
15	*Ichthyophis benjii* OM501568	7.1	7.3	7.3	6.9	8.1	8.0	7.3	7.9	8.4	6.9	7.3	5.1	5.1	8.1		0.3	0.9	0.9	1.3	1.4	1.2	1.2	1.2	1.3	1.2
16	*Ichthyophis benjii* MZ153116	6.8	7.2	7.2	6.6	8.1	7.7	6.9	7.6	8.1	6.6	6.8	5.1	5.1	7.8	0.4		0.9	0.9	1.3	1.4	1.2	1.2	1.2	1.3	1.2
17	*Ichthyophis khumhzi* OQ780535	7.2	7.3	7.6	7.4	9.7	10.0	8.9	8.7	9.6	8.9	8.1	2.5	2.5	9.9	5.6	5.3		0.2	1.3	1.4	1.3	1.3	1.3	1.3	1.3
18	*Ichthyophis khumhzi* OQ780536	7.2	7.3	7.6	7.4	9.7	10.0	8.9	8.7	9.6	8.9	8.1	2.5	2.5	9.9	5.6	5.3	0.4		1.3	1.4	1.3	1.3	1.3	1.3	1.3
19	*Ichthyophis moustakius* MZ098158	9.2	9.4	9.4	9.0	9.2	8.1	8.9	8.6	8.8	6.5	9.2	8.7	8.7	8.1	8.5	8.5	9.1	9.1		0.4	0.7	0.7	0.7	0.7	0.7
20	*Ichthyophis moustakius* MZ098159	9.3	9.5	9.5	9.0	9.2	8.2	9.2	8.5	9.0	6.3	9.0	8.7	8.7	8.2	8.7	8.7	9.2	9.2	0.7		0.5	0.5	0.5	0.5	0.5
21	*Ichthyophis moustakius* PX090926	8.3	8.5	8.5	8.1	8.5	7.2	8.9	7.7	8.2	5.0	9.0	8.6	8.6	6.6	8.5	8.6	9.0	9.0	2.6	1.3		0.0	0.0	0.0	0.0
22	*Ichthyophis moustakius* PX090927	7.9	8.3	8.1	7.7	8.0	7.1	8.4	7.3	8.0	5.3	8.7	8.2	8.2	6.6	8.1	8.1	9.6	9.6	2.5	1.2	0.0		0.0	0.0	0.0
23	*Ichthyophis moustakius* PX090928	8.2	8.4	8.4	8.0	8.4	7.2	8.8	7.6	8.2	4.9	9.0	8.6	8.6	6.6	8.5	8.5	9.0	9.0	2.6	1.3	0.0	0.0		0.0	0.0
24	*Ichthyophis moustakius* PX090929	8.3	8.5	8.5	8.1	8.4	7.2	8.8	7.6	8.2	5.0	9.0	8.6	8.6	6.6	8.5	8.6	9.0	9.0	2.6	1.3	0.0	0.0	0.0		0.0
25	*Ichthyophis moustakius* PX090930	8.3	8.5	8.5	8.1	8.4	7.2	8.8	7.6	8.2	4.9	9.0	8.6	8.6	6.6	8.5	8.6	9.0	9.0	2.6	1.3	0.0	0.0	0.0	0.0	

Jiasheng Liu, and Shenquan Liu from Tongbiguan Township, Yingjiang County, Yunnan Province, China (N24.56267°, E97.63946°, 1 478 m above sea level (a.s.l.).

Morphological description: Three adult males (KIZ 056482–483, KIZ 059693), body subcylindrical (SVL 170.6–285.2 mm; TOL 175.1–292.3 mm); body surface smooth with abundant secretory glands; head, nuchal region, trunk, and tail slightly flattened; head longer than wide (HW/HL 74.2%–76.0%; TOL/HL 19.7–25.2); snout blunt, round, obtuse beyond lower jaw in ventral view; snout length significantly less than half of head length, almost equal to interorbital distance (SL/HL 43.8%–45.2%; SL/IOD 95.9%–102.6%); nostril oval, closer to top of snout, internarial distance less than half of snout length (IND/SL 32.7%–43.6%); tentacles short, thin, located at edge of upper lip in front of eye, distance between tentacle and eye less than distance between tentacle and snout (TN/TE 200.0%–261.5%); collar region slightly more massive than adjacent head and body, delimited by weak annular constrictions, lacking transverse grooves in dorsal view, second nuchal groove more prominently visible than first in ventral view; width of first collar less than second (C1/C2 85.7%–90.6%); girth maximal near midbody (TOL/BW2 21.0–26.6); tail short (TL/SVL 1.5%–2.4%), slightly down-curved (Figure 4; Figure 5).

Annular grooves ventrally incomplete on anterior one-fifth, 2–3 interrupted in disc region, 6–7 ventrally complete on tail. Ventral annular groove formula on trunk, anus region, and tail 249+3+6, 265+2+7, and 278+3+7, respectively. Dorsal annular groove count 260–282. Tiny scales present in anterior ventral grooves; 4–5 rows at mid-body; three rows anterior to anus.

Coloration: In life, dorsum surface uniformly dark brown, venter surface purple brown; narrow lateral stripe bright yellow, extending from fourth posterior-most annulus on tail to posterior of eye on lower jaws near mandibular tip; dorsum of head uniformly dark brown, without arched stripe between nostril and tentacle; ventral surface of throat pale purplish-brown; vent disc faint pale pinkish gray (Figure 4). In preservation, body darkened compared to life, dorsal surface of body dark gray, some white secretions on annular grooves; venter surface dark purplish-brown; lateral stripe and vent disc light beige; eyes deeply buried under skin, orbit white, eyes black faintly visible (Figure 5).

Larvae: Two larvae (KIZ 056481, KIZ 056479; TOL 173.1 mm and 140.7 mm respectively), morphological characteristics similar to those of adults except for the following distinctions (Table 1; Figure 6): (1) tentacle close to eye, positioned below front of eye; (2) tail fin fold present; (3) pair of spiracles on lateral apex of first nuchal groove; (4) groove between first and second nuchal collars indistinct; (5) body color lighter than adults, dorsal surface brown, venter surface brown or light brown; (6) dorsal annular groove count 279 (KIZ 056481), ventral annular groove formula 276+3+7; dorsal annular groove count 284 (KIZ 056479), ventral annular groove formula 278+3+7.

Vertebrae: Radiographs revealed 104–109 total vertebrae in *I. moustakius* specimens; single cervical vertebra present, indistinct from adjacent trunk and tail vertebrae.

Specimen comparisons: Two larval specimens collected from Yingjiang City in 2003 (KIZ 035405, KIZ 78001) were examined. Based on the morphological description provided by Kamei et al. (2009) and Lalremsanga et al. (2021), KIZ 78001 was identified as *I. moustakius* based on its annular grooves in ventral view (267+3+6) and dorsal view (276).

Figure 3 Bayesian inference (BI) and maximum-likelihood (ML) analyses of the genus *Ichthyophis* based on partial DNA sequences of the mitochondrial cyt *b* gene

Ichthyophis moustakius, *Ichthyophis yangi*, and *Ichthyophis kohtaoensis* individuals from China are highlighted in red. Node values with Bayesian posterior probabilities (BPP)<0.95/bootstrap support (BS)<70 are not shown. A "–" denotes BPP<0.95 or BS<70.

According to Fei et al. (2006), KIZ 035405 was tentatively identified as *I.* cf. *kohtaoensis* based on its annular grooves in ventral view (344+3+6) and dorsal view (348).

Distribution: Originally thought to be restricted to northeastern India, *I. moustakius* was previously known only from the Tamenglong District in Manipur and Mamit and the Aizawl District in Mizoram (Kamei et al., 2009; Lalremsanga et al., 2021). However, the present study expands its known range to the Dehong Prefecture, southern Yunnan Province, China. Current records now include Dehong Prefecture in southern Yunnan Province and multiple localities in northeastern India. Given the geographical continuity between these areas, occurrence in adjacent regions of northern Myanmar is also likely.

Ecology: The habitat of *I. moustakius* closely resembles that of *I. kohtaoensis*, differing primarily in altitudinal distribution. Specimens were encountered at 1 300–1 500 m a.s.l. in evergreen broad-leaved forest near a small waterfall in Yunnan Tongbiguan Provincial Nature Reserve, compared to the 100–900 m range reported for *I. kohtaoensis*. Individuals were observed at night beneath wet rocks and co-occurred with species including *Amolops afghanus*, *Rhacophorus dulongensis*, *Kurixalus yangi*, *Theloderma pyaukkya*, *Leptobrachella ventripunctata*, and *Xenophrys dehongensis* (Figure 4F).

DISCUSSION

Figure 4 Male *Ichthyophis moustakius* (KIZ 056483) in life

A: Dorsal view; B: Ventral view; C: Dorsal view of head; D: Ventral view of head; E: Ventral view of tail; F: Habitat. Photos by Zhong-Bin Yu.

The absence of the moustache-like arched stripes—previously regarded as a diagnostic character of *I. moustakius*—in the Chinese specimens suggests that this character may exhibit population-level variability and cannot be considered a reliable diagnostic trait. Earlier research has already questioned the consistency of this feature across *Ichthyophis* species (Kamei & Biju, 2016). Therefore, reliance on this character for species identification warrants re-evaluation. In addition, except for the presence of caudal fin folds, a pair of spiracles on the lateral apex of the first nuchal groove serve as diagnostic features distinguishing larvae from adults. Additional larval features included tentacles positioned close to the eye margin and indistinct separation between the first and second nuchal grooves. However, no marked differences in annular groove or vertebral counts were observed between the larval and adult stages.

The Gaoligong Mountain region, straddling the border between western Yunnan Province and northern Myanmar, is recognized for its exceptional amphibian diversity. Southern Gaoligong, particularly Yingjiang County, has yielded various new species and distribution records in recent years. Notable examples include *Polypedates teraiensis* (Yu et al., 2024), *Xenophrys yingjiangensis* (Wu et al., 2024b), *X. dehongensis* (Lyu et al., 2023), and *T. pyaukkya* (Du et al., 2020). Several studies have also revealed the presence of cryptic taxa in this region (e.g., Chen et al., 2017, 2018; Wu et al., 2024b),

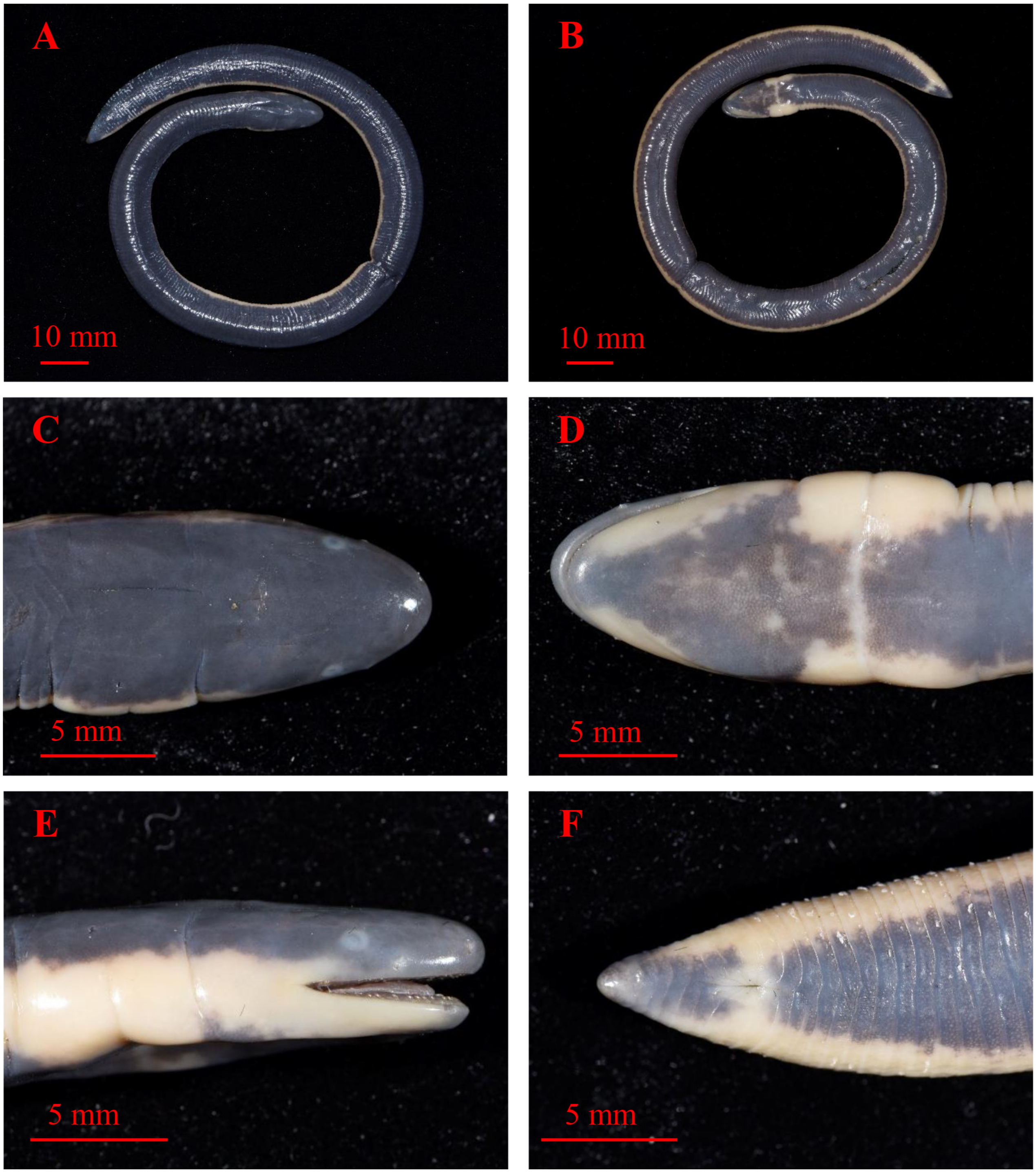

Figure 5 Male *Ichthyophis moustakius* (KIZ 056483) in preservative

A: Dorsal view; B: Ventral view; C: Dorsal view of head; D: Ventral view of head; E: Lateral view of head; F: Ventral view of tail. Photos by Zhong-Bin Yu.

highlighting that current estimates significantly underestimate true amphibian diversity. The discovery of *I. moustakius* in this region further supports this view.

Previously, only two species of *Ichthyophis* were known to occur in China. The present finding increases this number to three (*I. moustakius*, *I. yangi*, and *I. kohtaoensis*), all of which are distributed within Yunnan Province. The current record from western Yunnan lies far from previously reported localities in northeastern India. Given the lack of systematic herpetofaunal surveys in northern Myanmar, which forms a biogeographic corridor between Yunnan Province and northeastern India, it is plausible that additional populations of *I. moustakius* remain undetected in that region. Addressing the biodiversity knowledge gap in northern Myanmar will require strengthened international collaboration and targeted herpetofaunal field investigations. Furthermore, a reassessment of the distribution and conservation status of all three *Ichthyophis* species across their known localities in China is necessary to inform future research and conservation strategies.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Figure 6 ***Ichthyophis moustakius*** **(KIZ 56481) larva in preservative**

A: Dorsal view; B: Ventral view; C: Dorsal view of head; D: Lateral view of head. E: Ventral view of head; F: Lateral view of tail; a: spiracle; b: tentacle; c: fin fold. Photos by Zhong-Bin Yu.

Field survey permission was granted by the Yunnan Provincial Forestry and Grassland Department and Tongbiguan Provincial Nature Reserve.

SUPPLEMENTARY DATA

Supplementary Table S1 Localities, voucher information, and GenBank accession numbers for all specimens used in cyt b analyses.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Z.B.Y., Y.H.W., and K.W. conducted the field surveys, identified the species post-collection, and collected morphological data; Z.B.Y. and F.K.K. prepared the draft; Z.B.Y., Y.H.W., and J.C. founded the research idea and revised the draft with contributions of all other authors; Y.Y.W., H.L., D.Z., H.P.Z., S.P.Y., A.R.Z., Z.P.D., D.C.Z., and Y.T.L. participated in the field surveys and contributed to the revision process. All authors read and approved the final version of the manuscript**.**

ACKNOWLEDGMENTS

We thank the Germplasm Bank of Wild Species and National Wild Plant Germplasm Resource Center for providing radiographic images. We thank Dong An and Xian-Rong Wu for their help in the field. We thank the Tongbiguan Provincial Nature Reserve for their support in facilitating field surveys and specimen collections.

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