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A new species of striped *Ichthyophis* Fitzinger, 1826 (Amphibia: Gymnophiona: Ichthyophiidae) from Myanmar

MARK WILKINSON[1,5], BRONWEN PRESSWELL[1,2], EMMA SHERRATT[1,3], ANNA PAPADOPOULOU[1,4] & DAVID J. GOWER[1]

[1]*Department of Zoology[!], The Natural History Museum, London SW7 5BD, UK*
[2]*Department of Zoology, University of Otago, PO Box 56, Dunedin New Zealand*
[3]*Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, 26 Oxford St., Cambridge, MA 02138, USA*
[4]*Department of Ecology and Evolutionary Biology, The University of Michigan, Ann Arbor MI 41809, USA*
[5]*Corresponding author. E-mail: m.wilkinson@nhm.ac.uk*
[!] *Currently the Department of Life Sciences*

Abstract

A new species of striped ichthyophiid caecilian, *Ichthyophis multicolor* **sp. nov.**, is described on the basis of morphological and molecular data from a sample of 14 specimens from Ayeyarwady Region, Myanmar. The new species resembles superficially the Indian *I. tricolor* Annandale, 1909 in having both a pale lateral stripe and an adjacent dark ventrolateral stripe contrasting with a paler venter. It differs from *I. tricolor* in having many more annuli, and in many details of cranial osteology, and molecular data indicate that it is more closely related to other Southeast Asian *Ichthyophis* than to those of South Asia. The caecilian fauna of Myanmar is exceptionally poorly known but is likely to include chikilids as well as multiple species of *Ichthyophis*.

Key words: Burma, caecilians, conservation, ichthyophiids, out-of-India, systematics, taxonomy

Introduction

With forty-seven (almost one in four of the approximately two hundred) currently recognised species, *Ichthyophis* Fitzinger, 1826 is the most speciose genus of caecilian amphibians. *Ichthyophis* also has one of the largest geographical distributions of any caecilian genus, occurring in Sri Lanka and India through mainland Indochina, Sundaland and islands (including the Philippines) west of Wallace's Line (Taylor 1968; Gower *et al.* 2002), and with recent addition of *Caudacaecilia* Taylor, 1968 to its synonymy (Nishikawa *et al.* 2012) it is the only caecilian genus known from Southeast Asia. Although multiple species and specimens of *Ichthyophis* have been documented from Thailand and from Northeast India, including some recently described species (e.g., Kamei *et al.* 2009; Mathew & Sen 2009), there are only a few old literature records of any caecilians from Myanmar (Boulenger 1882; 1887; Smith 1940) and the caecilian fauna of that country must be considered essentially unexplored and unknown. A California Academy of Sciences (CAS) expedition in 2000 obtained a good sample (14 specimens) of a striped caecilian from a single locality in Ayeyarwady Region. The specimens can be referred to *Ichthyophis* on the basis of their having a combination of a tertiary annular system and tentacular apertures distant from the eye (e.g. Wilkinson & Nussbaum 2006). The species is unusual among *Ichthyophis* in having a dark ventrolateral stripe (adjacent to and below the lateral pale stripes that are typical of many *Ichthyophis*) bordering a much paler ventral colouration, a feature found elsewhere only in *I. tricolor* from peninsular India. Numerous other features distinguish the Myanmar population from *I. tricolor,* indicating the former to be a new species that we describe below.

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Material and methods

Morphology. Measurements were taken to the nearest 0.1 mm using vernier calipers, except total length and circumference, which were measured to the nearest mm using ruler and thread. Sex was determined by examination of gonads.

We follow Kamei *et al.* (2009, 2013), Wilkinson & Kok (2010) Kotharambath *et al.* (2012a,b) and Wilkinson *et al.* (2013) in using the format 'X'–'Y' to designate the shortest distance between features 'X' and 'Y', in denoting scale pockets as very shallow if they are less than 0.75 annular lengths, and as deep or very deep if they are equal to or more than 1.25 or 1.75 annular lengths respectively, and in using the following abbreviations for anatomical features and ratios: AG = annular groove; AM = anteriormost 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. We use first and last to denote the anteriormost and posteriormost units of serial homologues. Additionally, we use AV = anterior limit of vent; PV = posterior limit of vent; and in measures we abbreviate naris to N, and eye to E.

Of the twenty-nine previously described and currently recognized species of striped *Ichthyophis* we have seen type specimens of all but nine (*I. alfredii* Mathew & Sen 2009; *I. daribokensis* Mathew & Sen 2009; *I. davidi* Bhatta, Dinesh, Prashanth, Kulkarni & Radhakrishnan 2011; *I. humphreyi* Taylor 1973; *I. nguyenorum* Nishikawa, Matsui & Orlov 2012; *I. nigroflavus* Taylor 1960; *I. nokrekensis* Mathew & Sen 2009; *I. paucidentulus* Taylor 1960; and *I. pauli* Nishikawa, Matsui, Sudin & Wong 2013), and we have relied entirely upon their original descriptions for comparative information on these latter species. Kamei *et al.* (2009) listed many features that did not vary across, and which were not repeated for each of the *Ichthyophis* species they described. Unless noted otherwise, these features pertain also to the new Myanmar specimens considered here and are not repeated in the description.

Vertebrae were counted from radiographs. Skulls and mandibles were visualised with high-resolution x-ray computed tomography (HRXCT), a non-destructive process enabling examination of bone *in situ*. The heads of whole, spirit-preserved specimens were scanned using a Metris X-Tek HMX ST 225 System. Scans and reconstructions were undertaken using the methods reported by Gower *et al.* (2010). Using a molybdenum target, scan data were collected at 500ms exposure rate over 3142 projections in 360˚ (scan-specific parameters are given in figure legends). Reconstructed HRXCT slices in the transverse plane were rendered as a three-dimensional volume and the skull and mandibles were digitally dissected using VGStudio MAX v2.2 (Volume Graphics, http://www.volumegraphics.com).

Molecules. Sequence data were generated for eight of the new Myanmar specimens. Details of voucher specimens and GenBank accession numbers are given in Appendix 1. Genomic DNA was extracted from liver samples using commercial kits (Qiagen) and three partial mitochondrial (mt) genes were amplified and sequenced: 12S ribosomal RNA (*12s*), 16S ribosomal RNA (*12s*) and cytochrome b (*cytb*). The *12s* and *16s* primers were as reported by Gower *et al.* (2002). For *cytb*, the latter were prone to amplifying a probable pseudogene (unexpected stop codons in translated amino acid sequences) and so San Mauro *et al.*'s (2004) MNCN-Glu F and Amp-P10 R primers were used instead.

Mean nucleotide p-distances among the eight samples were calculated using MEGA 4 (Tamura *et al.* 2007). In order to assess phylogenetic relationships, the newly generated sequences of one specimen (CAS 212254) were added to Gower *et al.*'s (2002) dataset, comprising mt sequences for two unstriped and thirteen striped individuals of *Ichthyophis*, three *Uraeotyphlus* (India) and two South American rhinatrematids as outgroups. See Gower *et al.* (2002) for details of the other voucher specimens.

The *12s* and *16s* sequences were aligned using ClustalX version 2.0.12 (Larkin *et al.* 2007) with default parameters. Ambiguously aligned positions were filtered out using Gblocks v. 0.91b (Castresana 2000), allowing positions with a gap in fewer than 50% of the sequences, and default values for all other block parameters. *Cytb* sequences were aligned by eye, and their reading frames were checked to ensure that they could be translated to continuous amino acid sequences. The alignment is available from TreeBASE (http://purl.org/phylo/treebase/

phylows/study/TB2:S15332). Saturation of third codon positions was assessed by plotting the number of transitions and transversions against overall ML pairwise distances, as calculated by PAUP* 4.0b10 (Swofford 2002).

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI), under two alternative partitioning schemes (data divided either by gene or by gene and codon position for *cytb*), which were compared using the Akaike information criterion (AIC). ML searches were conducted with RAxML 7.2.6 (Stamatakis 2006) with 100 replicates, applying a separate GTR+Γ model to each partition. Clade support was evaluated using non-parametric bootstrapping with 1000 pseudoreplicates. BI was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), applying a separate best-fit model, as determined by AIC in jModeltest (Posada 2008), to each partition. Two independent runs were performed of 10 million generations each, using default parameters for the MCMC and sampling every 1000 generations. Posterior probabilities were calculated after checking convergence diagnostics and discarding the first one million generations as burn-in. All analyses were repeated after removing the third codon positions of *cytb*.

Ichthyophis multicolor sp. nov.

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Figs. 1–3; Tables 1–2

Holotype. CAS 212264, an adult female, collected by villagers for Jens V. Vindum on 25 April 2000 from the vicinity of Mwe Hauk Village (26°16'39.2"N, 94°45'32.5"E; c. 10 m asl), Ayeyarwady Region, Myanmar.

Paratypes (n = 13). CAS 212254–63 and 212265–67 (eleven females and two males) collected along with holotype.

Diagnosis. A striped *Ichthyophis* that differs from all other striped congeners except *I. tricolor* in having a pale venter, typically with whitish markings mid-ventrally, bordered by a darker ventrolateral longitudinal stripe immediately below the pale yellowish lateral stripe on each side. Differs from *I. tricolor* in having many more AGs (> 325) and vertebrae (> 120) and TAs nearly or more than twice as far from nares than from eyes (N–TA/E–TA > 1.8).

Description of holotype. Some morphometric and meristic data are given in Table 1. Mostly good condition, damaged skin dorsally c. 55 mm anterior to terminus, small depressed scar on right about eight annuli anterior to the vent, several scale pockets opened, small (< 15 mm) midventral longitudinal incision 125 mm behind ST with some viscera protruding including bean-shaped eggs (9 x 6.5 mm). Head, nuchal region and trunk dorsoventrally compressed. Girth maximal throughout midbody, increasing very gradually over first 60 mm, decreasing less gradually over last c. 30 mm, L/W = c. 25. Tail upturned towards tip, short (about as long as ST–TA). Head somewhat more V- than U-shaped in dorsal view. In lateral view, distance of CM from top of head a little greater than distance from bottom of head. In ventral view, lower jaws inset from upper jaws, not notably more so anteriorly than at level of TAs. Eye equidistant from lip and top of head in lateral view, not surrounded by narrow whitish ring, eye diameter (c. 0.7 mm) distinctly larger than that of naris and TA, about equal to TP. TAs more than twice as distant from nares as from eyes, fairly close to lips (0.6 mm), on imaginary lines between nares and CMs. Naris approximately level with AM; in lateral view equidistant from top and bottom of head and ST; close to but inset from side of head in dorsal view. Teeth slender, strongly recurved, OM series extends approximately one quarter to one third the length of IM series (with three to five OMs on each side) behind posteriormost IMs. Bluntly tipped tongue strongly plicate (with approximately a dozen longitudinal ridges and grooves) posteriorly, lacking a distinct longitudinal medial groove, margin overlying all except anteriormost IMs. Choanae very narrow, distance between them six or seven times each of their greatest widths. Collar region more massive than adjacent head and body, delimited by strong constrictions. C2 slightly longer than C1 measured laterally. NG1 incomplete dorsally, not pale ventrally. NG3 with gentle middorsal anterior flexure. Four anteromedially flexed, evenly spaced TGs on C2 dorsally, first two short and third poorly developed on right, last longer, crossing midline, not extending as far as lateral stripes.

The first three AGs widely incomplete on venter, otherwise mostly complete midventrally with five (on left) or six (on right) widely incomplete near (i.e. interrupted by) the vent, and five more narrowly incomplete on tail. Dorsally, AGs are mostly complete, those on first two thirds of the body and last eight curve anteromedially, the

former gently, the latter very strongly angulate and narrowly incomplete (giving the appearance of a notch) at midline. Ventrally, anterior AGs angulate, degree of posteromedial curvature increasing from two to 2.5 times the length of an annulus at midbody, decreasing posteriorly, orthoplicate by 36th AG anterior to vent. The last few annuli shorter. The terminus ends in a short (1.3 mm) cap, approximately the length of the three preceding tail annuli or two body annuli.

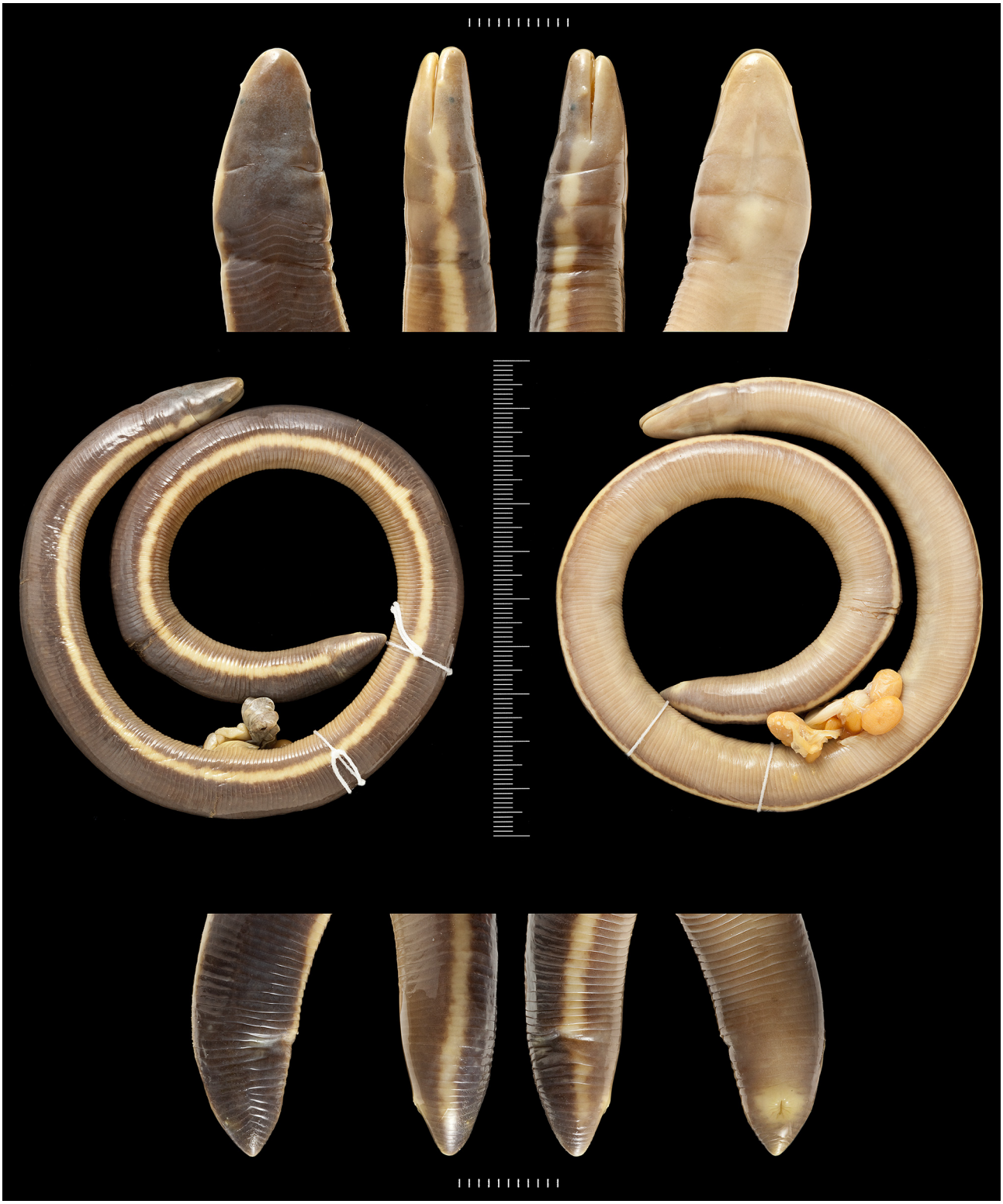

FIGURE 1. Preserved holotype (CAS 212264) of *Ichthyophis multicolor* **sp. nov.** Photos by Harry Taylor (The Natural History Museum, London). Scale gradations = 1 mm.

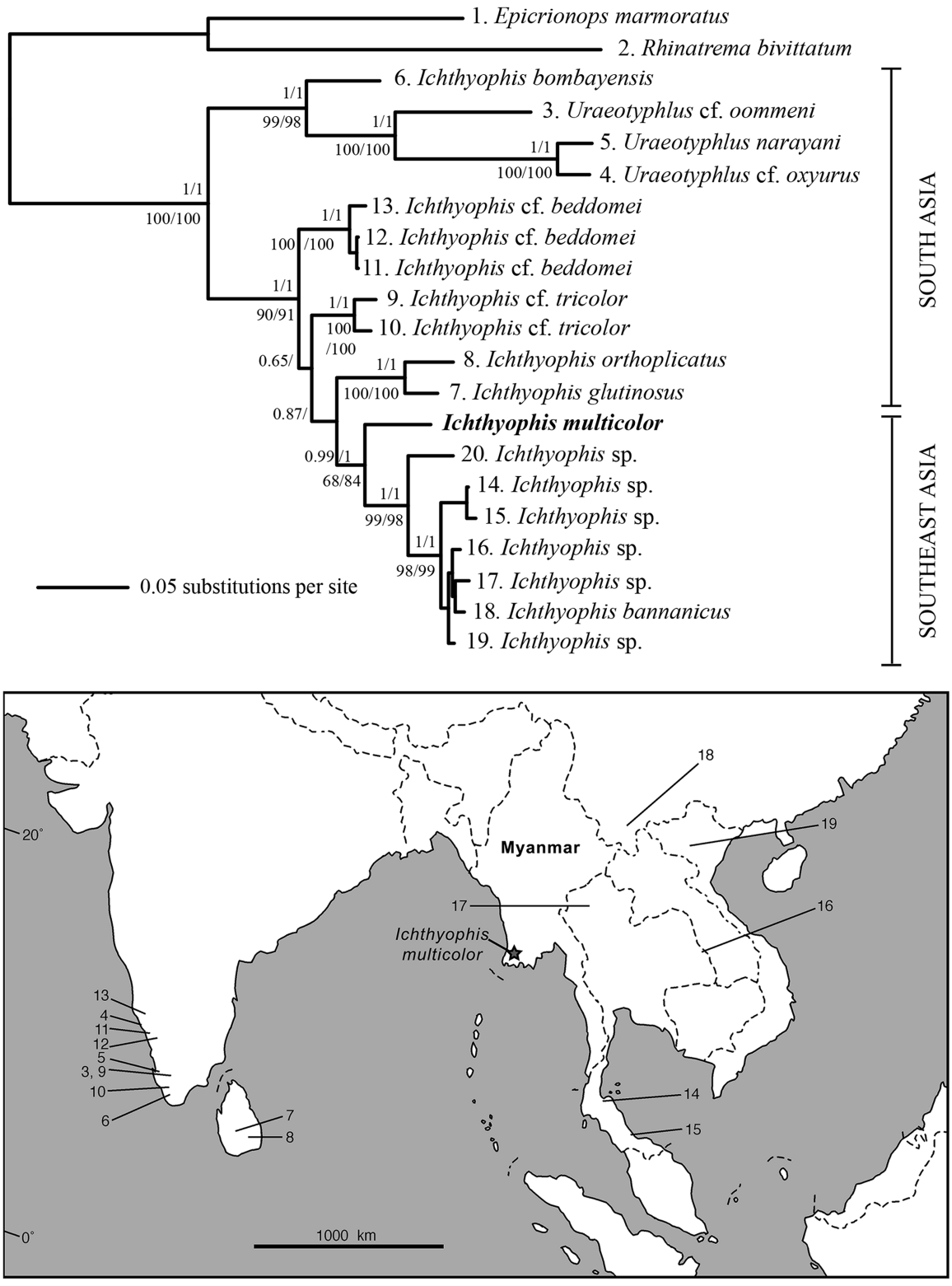

FIGURE 2. Inferred phylogenetic relationships and map showing provenance of included samples. The tree is a Bayesian majority rule consensus (of post-burn-in trees with compatible minority partitions and mean branch lengths), from analysis without third codon positions of *cytb,* and data partitioned by gene and codon position. Bayesian posterior probabilities and ML bootstrap support are shown for each clade (when > 50%). Values above branches: posterior probabilities from the analyses without third codon positions/with all positions. Values below branches: bootstrap values from analyses without third codon positions/with all positions. Numbers associated with leaves correspond to localities shown on the map.

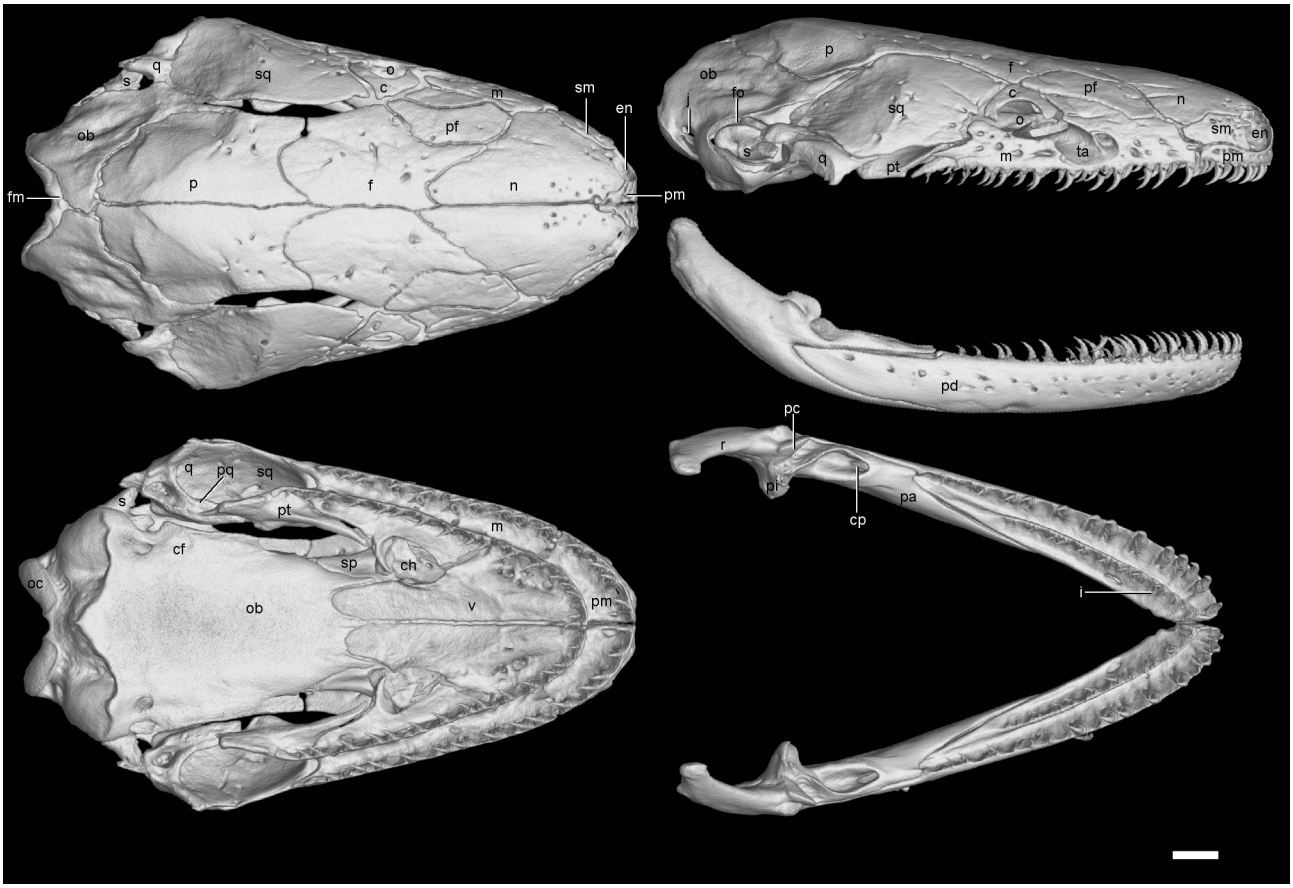

FIGURE 3. CT scan skull of paratype of *Ichthyophis multicolor* **sp. nov.** (CAS 212266). Left side, from top to bottom: cranium in dorsal and palatal view. Right side, from top to bottom: cranium in lateral view, mandible in lateral and dorsal view. Scale bar = 1mm. Abbreviations as in Appendix 2. Scan parameters: a molybdenum target set at 98–100kV and 190µA, with a reconstructed voxel size of 11µm.

Small (0.6 x 0.4 mm) scattered scales present in a single row in very shallow pocket of first AG on dorsum; posteriorly, four to five rows of large (1.7 x 1.8 mm) scales present in very deep pockets on dorsum, reducing to deep pockets with three to four scale rows on the venter. Margins of vent slightly elevated, formed of twelve main denticulations, six on each side, with some irregular subdivisions. No associated papillae.

In preservation, dorsum brownish grey, more grey on head. A distinct pale spot, wider than long (4.5 x 4 mm), forming a disc around the vent. Narrow, irregular, pale yellowish bilateral stripes, extend unbroken from just in front of CMs to the fifth or sixth last annulus, fading gradually on first annulus behind the vent, almost broken on C2, not visible ventrally on collars, not connecting with or curving towards disc. Immediately below each lateral stripe, running from C2 to the level of the vent, a mostly slightly narrower brown line, a little paler than the dorsal colour, with a gradual but rapid transition to a much paler tan colour that predominates on the venter but which is interrupted by small whitish patches midventrally. Narrow, pale lines along upper margins of lower jaw. TPs and ST pale. Pale midventral line extends across nuchal region to the level of CMs. Except where crossing pale lateral stripes, AGs appear mostly paler than adjacent skin, especially on venter.

Variation. Morphometric and meristic data for the type series are given in Table 1. The sample is trimodal in total length, with eight specimens less than 200 mm, five greater than 350 mm and one at 256 mm. Relative overall proportions (L/W = 25.1 to 28.4) and some features of the head (N–TA/E–TA = 1.9 to 2.6; E–ST/E–E = 1.0 to 1.1) show no correlation with total length and are seemingly unaffected by growth. In contrast, there are some substantial differences related to size. For example, W/S ranges from 4.4 to 5.4 in the eight smallest specimens and from 6.1 to 7.2 in larger specimens, indicating that the width of the stripe does not grow as fast as the width of the body. Similar allometries are apparent in the relative lengths of the head (L/H) and of the tail (L/T), and the tail appears to lengthen more slowly than the head. The sample includes only two males (both small), and we can reject the hypothesis that it is an unbiased sample from a population with an even sex ratio (p = 0.0129, two-tailed

binomial test). The five largest females all have large yolky eggs and all other females have small, non-yolky eggs (< 0.5 mm) and might therefore be considered subadult.

TABLE 1. Some morphometric (in mm) and meristic data for the type series of *Ichthyophis multicolor*. See Materials and Methods for explanation of abbreviations. * denotes holotype, / separates different counts made on left and right. Measures are at midbody unless otherwise indicated. Specimen numbers are last two digits of CAS numbers beginning 2122.

Specimen	59	60	55	58	61	56	57	54	63	62	64*	65	66	67
Sex	f	f	f	m	m	f	f	f	f	f	f	f	f	f
Length	168	171	175	180	181	186	188	190	256	358	367	368	395	402
Width	6.7	7	6.5	6.7	7.2	7.4	7.5	6.7	10.2	13.9	14.5	13.4	15.1	15.8
Circumference	21	21	20	23	25	24	23	24	33	42	46	44	45	49
Width of stripe	1.3	1.3	1.4	1.5	1.4	1.7	1.5	1.1	1.5	2.2	2.1	2	2.1	2.2
Width at AV	3.6	3.3	3.6	3.9	3.2	3.5	4	3.9	5.7	6.8	7	7.2	7.9	7.7
NG1–ST	8.4	8.3	7.9	8.6	9	8.5	9.1	8.9	10.6	13.2	13.3	12.6	14.3	13.3
Length of C1	2.0	1.9	2.0	1.8	2.3	1.9	2.3	2.3	2.4	2.7	3.3	3.0	3.4	3.3
Length of C2	2.3	2.3	2.5	2.1	2.5	2.3	2.4	2.4	2.8	3.8	3.8	4.0	4.2	4.5
Width at CM	5.5	5.7	5.6	6	5.8	6.5	6.3	6	7.5	8.7	9.1	8.8	9.9	9.2
Depth behind CM	5.0	4.8	4.9	4.8	5.1	5.0	5.2	5.2	6.6	8.0	6.8	7.5	7.1	8.6
Width at NG1	6	6.2	6.4	6.6	6.4	7.2	6.8	6.5	8.6	9.7	9.5	10.2	10	10.2
E–E	3.7	3.9	3.9	4	4.2	4.1	4.1	4.2	5.3	5.9	6.3	5.9	6.2	6.7
N–N	1.7	1.7	1.6	1.7	1.8	1.8	1.8	1.7	1.9	2.2	2.4	2	2.4	-
E–N	3	3	3	3.2	3.4	3.2	3.2	3.3	4.4	5.2	5	4.8	5.3	5.2
TA–TA	4.4	4.2	4.3	4.4	4.4	4.7	4.5	4.5	5.3	6.3	6.7	6.3	6.8	7.3
E–TA	1.1	1	1	1.2	1.1	1.2	1.2	1	1.5	1.6	1.6	1.8	2	1.8
N–TA	2.4	2.1	2.2	2.3	2.6	2.4	2.4	2.6	3	4	3.7	3.6	4	4
E–ST	3.7	3.9	3.9	4.2	4.2	4.3	4.3	4.3	5.3	6	6.3	6.1	6.5	6.6
AM–ST	0.8	0.8	0.8	0.7	0.9	0.9	0.8	0.7	0.8	1	1.1	1.1	1	1.1
N–L	0.8	0.7	0.8	0.8	0.8	0.9	0.8	0.7	0.8	0.9	1.1	1	1.1	1.3
E–L	0.8	0.8	0.8	0.8	0.9	0.9	0.8	0.8	1	1.1	1.5	1.3	1.4	1.5
AV–TT	3.8	2.8	3.8	2.8	3.8	2.8	3.8	2.8	3.8	2.8	2.8	3.8	2.8	3.8
PV–TT	3.3	2.5	3.3	2.5	3.3	2.5	3.3	2.5	3.3	2.5	2.5	3.3	2.5	3.3
AGs	358	352	363	372	363	358	368	347	346	378	365	369	377	386
Vertebrae	127	131	130	126	126	128	129	128	127	131	129	131	129	132
TGs	3	4	2	3	3	2	2	2	3	3	4	3	2	3
AGs behind vent	4/3	3/4	3	6/4	4	5	5	4	3	4/5	4	4/5	3	4
AGs interrupted in vent region	6/5	7/8	7/8	7	5	6/7	5/6	7/8	7/6	6	6/5	6	6/5	6/7
PMs	38	36	37	36	38	40	39	40	51	47	42	50	50	49
VPs			36	35	39	38	42	38	48	46	42	46	47	48
OMs	32	30	33	34	33	35	30	31	38	38	39	43	41	42
IMs			26	26	28	31	26	22	29	34	33	34	36	37
L/W	25.1	24.4	26.9	26.9	25.1	25.1	25.1	28.4	25.1	25.8	25.3	27.5	26.2	25.4
L/H	28.0	27.6	27.3	27.3	28.3	25.8	27.6	29.2	29.8	36.9	38.6	36.1	39.5	39.4
L/T	50.9	68.4	53.0	72.0	54.8	74.4	57.0	76.0	77.6	143.2	146.8	111.5	158.0	121.8
W/S	5.2	5.4	4.6	4.5	5.1	4.4	5.0	6.1	6.8	6.3	6.9	6.7	7.2	7.2
N–TA/E–TA	2.2	2.1	2.2	1.9	2.4	2.0	2.0	2.6	2.0	2.5	2.3	2.0	2.0	2.2
E–ST/E–E	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

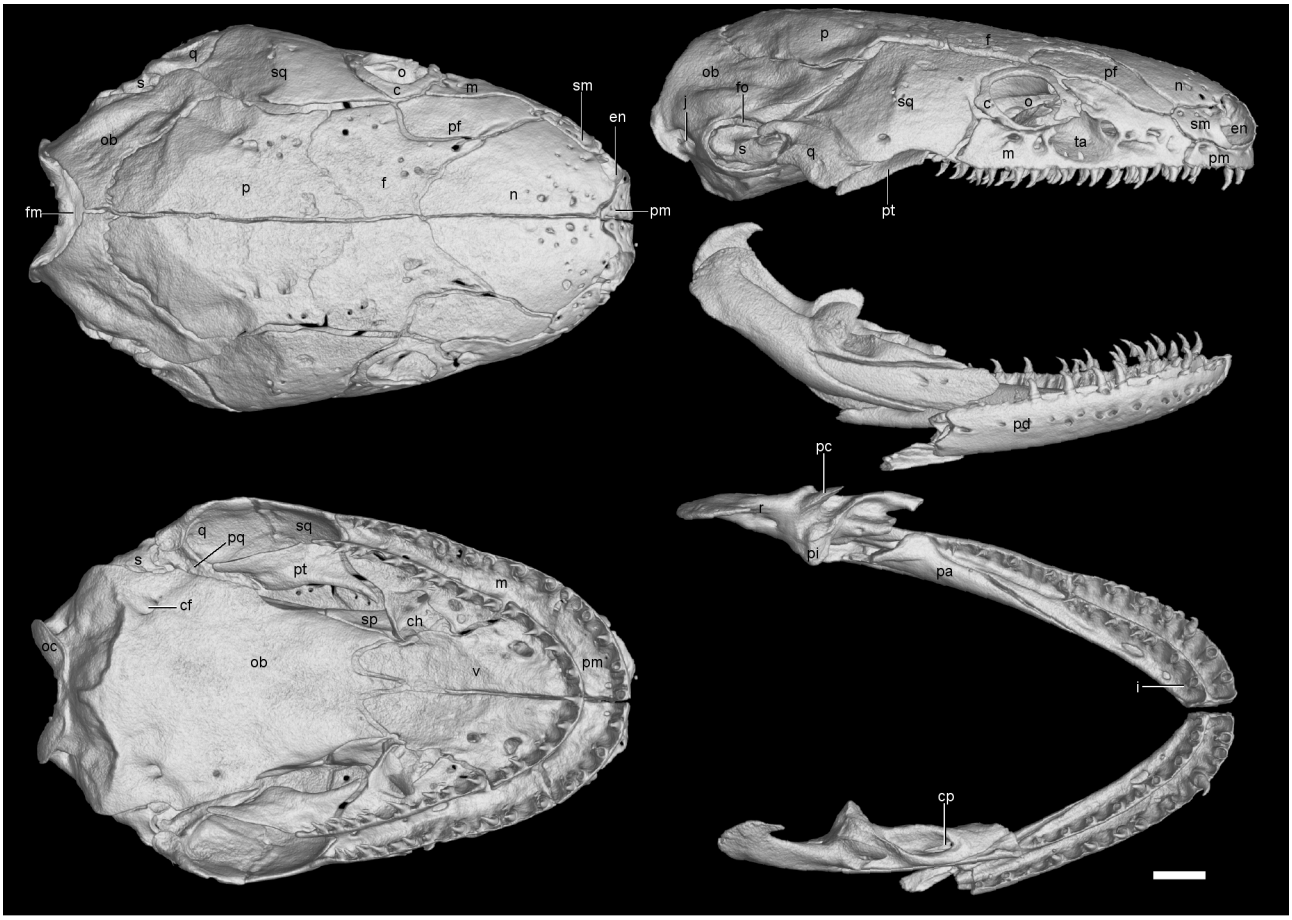

FIGURE 4. CT scan skull of the holotype of *Ichthyophis kohtaoensis* Taylor, 1960. Smithsonian (USNM 72293). The lower jaw was cut previously, so the pseudangular is broken on both sides. Views as in Fig. 2. Scale bar = 1mm. Abbreviations as in Appendix 2. Scan parameters: a molybdenum target set at 145kV and 100µA, with a reconstructed voxel size of 11µm.

The tail is upturned only in the holotype. All paratypes have the stripe beginning closer to the eye than in the holotype, with some slight downturn of the stripe near the vent, and pale markings on the throat are not apparent in CAS 212255. Ventral colour varies substantially. All specimens have dark stripes immediately below the pale lateral stripes that give way to a paler tan ground colour on the venter, and all but one (CAS 212257) have some white mid-ventrally, either as patches or forming an irregular but unbroken stripe that may be very broad (e.g. CAS 212267). In general, the colours are a little more intense in the smaller specimens. Scales are less well developed in the smallest specimens, with no scales in anteriormost annuli and only a single scale row in shallow pockets at midbody and posteriorly in the smallest paratype (CAS 212259). The right naris of the largest paratype (CAS 212267) is malformed and especially large. Larger specimens have more teeth in all series but the relative numbers between series does not vary much with size.

Molecular systematics. There is very little variation among the eight specimens of *Ichthyophis multicolor* sequenced for the three mt gene fragments (mean nucleotide p-distance *16s*: 0.0008, cytb: 0.0010; no variation in *12s*), consistent with the interpretation that they are conspecific. Visual inspection of transition and transversion plots (not shown) indicated substantial saturation of *cytb* third codon positions across the multiple alignment. Partitioning by gene and codon position was favoured over partitioning by gene only in both ML and BI analyses, but the different partitioning schemes had little effect on the phylogenetic results (Fig. 2) which are very similar to those reported by Gower *et al.* (2002) in that sampled South Asian *Ichthyophis* are paraphyletic with respect to the monophyletic Southeast Asian *Ichthyophis* and the relationships among sampled peninsular Indian (*I. beddomei* Peters 1879*, I. tricolor*) and Sri Lankan (*I. glutinosus* L*, I. orthoplicatus* Taylor 1960) *Ichthyophis* are not particularly well-supported. *I. multicolor* is distinct genetically from other sampled species, and is recovered as sister to all other sampled Southeast Asian *Ichthyophis*.

Cranial and mandibular osteology. A reconstruction of the skull and mandible of one large, female paratype

(CAS 212266) of *Ichthyophis multicolor* is shown in Figure 3 and similar illustrations of *Ichthyophis kohtaoensis* Taylor 1960 (a 'typical', or at least not obviously unusual, Southeast Asian congener) and *I. tricolor* (the most similarly coloured congener) are shown in Figures 4 and 5. There are no differences between these species and the type species of the genus, *I. glutinosus*, in terms of the number of separate skull bones and their general arrangement (Wilkinson *et al.* 2011: fig 5). Comparison of these three reconstructions (Figs. 3–5) immediately suggests several notable differences among the three species. However, some of these differences do not stand scrutiny as interspecific because they are variable among the other specimens examined. Thirteen characters that, based on our sampling, do represent interspecific differences separating one of the three species are reported in Table 2. Several of these differences are substantial and obvious, such as the relative size of teeth in the outer rows, and whether the tentacular canal is open within the maxillopalatine or roofed with bone.

Notable differences among the three specimens illustrated in Figs. 3–5 which vary within species are: the presence or absence of prefrontal-septomaxilla contact; the relative size of the upper temporal fenestra; the relative breadth of the pterygoid in palatal view; the relative width of the skull roof level with the parietal-frontal suture. In a simple count of similarities summarized in Table 2, *Ichthyophis multicolor* and *I. tricolor* are about as similar to each other as are *I. multicolor* and *I. kohtaoensis*, with the least similar pair of species being *I. tricolor* and *I. kohtaoensis*.

Etymology. Named for its having more distinct colours than most other *Ichthyophis*. For nomenclatural purposes the specific epithet is considered to be a noun in apposition.

Suggested English name. Colourful Ichthyophis or Colourful Ich.

Distribution and natural history. This species is known only from the type locality. Specimens were collected on the surface after heavy rain from an area of secondary forest and agriculture. The soil was sandy and hard packed. Local people brought the field team to the locality in response to questions about where ichthyophiids could be found. Although the type series includes no larval specimens it seems very likely that the species, like all other *Ichhyophis* as far as is known, is oviparous with an aquatic larval stage. This life history is also suggested by the large size of the unlaid eggs (e.g. Wilkinson & Nussbaum 1998).

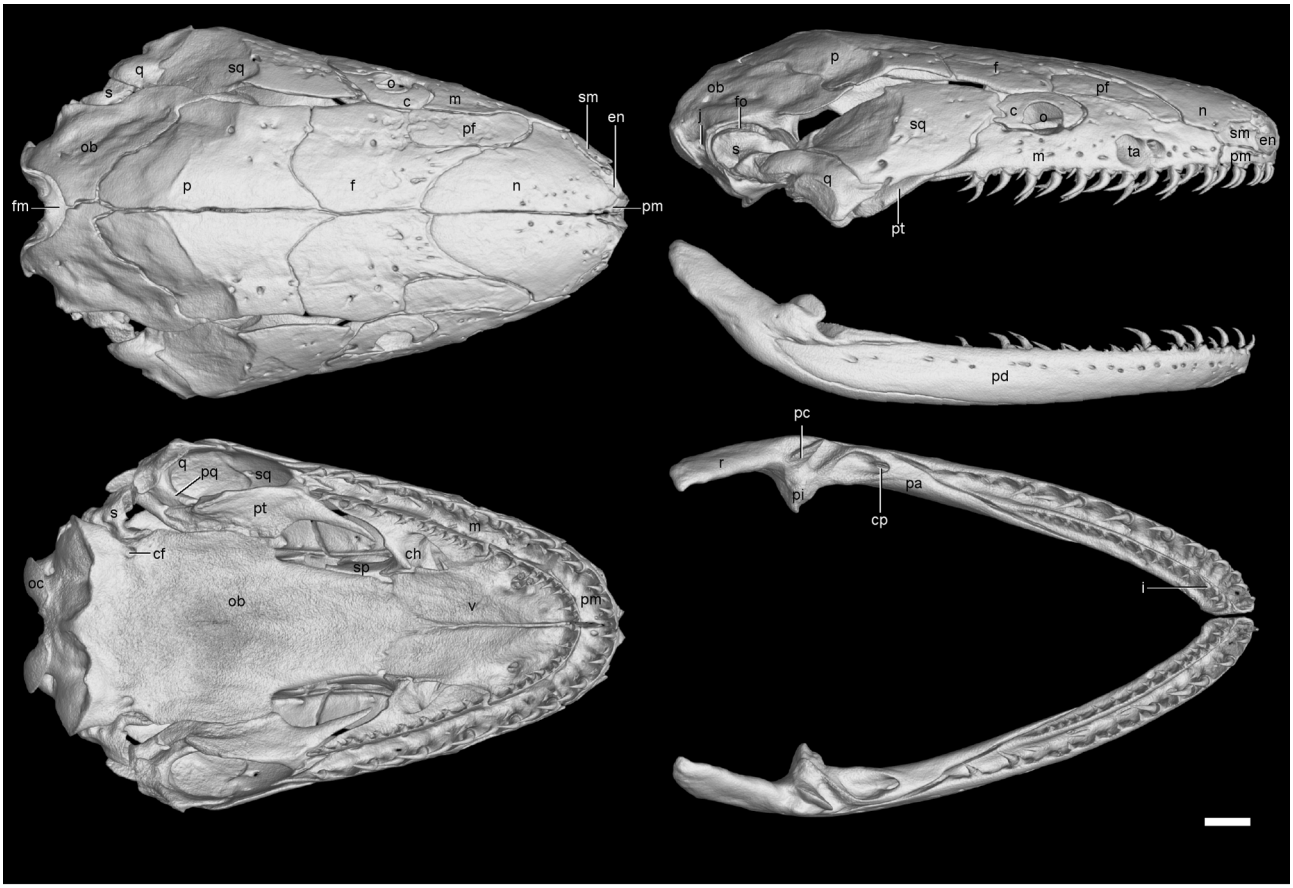

FIGURE 5. CT scan skull of *Ichthyophis tricolor* (University of Kerala MW 747). Views as in Fig. 2. Scale bar = 1mm. Abbreviations as in Appendix 2. Scan parameters: a molybdenum target set at 98kV and 190µA, with a reconstructed voxel size of 11µm.

TABLE 2. Thirteen characters of the skull and mandible that differ among, and do not vary within, the investigated species of *Ichthyophis.* See Figs. 3–5 for skull and mandible reconstructions.

	***Ichthyophis multicolor* (n = 4)**	***Ichthyophis tricolor* (n = 6)**	***Ichthyophis kohtaoensis* (n = 1)**
Tentacular canal in maxillopalatine	Open groove	Enclosed channel	Open groove
Skull and mandible shape in dorsal view	More V-shaped	More V-shaped	More U-shaped
Posterior edge of prefrontal in dorsal view	Oblique	Subtransverse	Transverse
Outer rows of teeth	Moderately sized, gently recurved	Larger, strongly recurved	Moderately sized, gently recurved
Palatine tooth row	Extends behind anterior limit of adductor chamber	Terminates anterior to adductor chamber	Extends behind anterior limit of adductor chamber
Snout tip to posterior of vomers	ca. 50% of skull length	ca. 40% of skull length	ca. 50% of skull length
Anterior process of pterygoid	Long	Long	Short
Circumobrital	Widely open ventrally, with short posterior ventral process	Widely open ventrally, with short posterior ventral process	Narrowly open ventrally, with longer posterior ventral process
Squamosal-prefrontal gap (contact between frontal and circumorbital)	Narrow	Wide	Narrow
Ventral limit of pterygoid	Barely below maxillary teeth	Far below maxillary teeth	Below maxillary teeth
Midline contact between frontals	Longer	Longer	Short
Carotid foramina more or less inset from posterolateral edge of os basale in ventral view	Less	Less	More
Pterygoid process of quadrate	Short, blunt	Long, tapering	Long, tapering

Discussion

The distinctive colour of *Ichthyophis multicolor* serves to differentiate it from all other species of *Ichthyophis* with the exception of *I. tricolor*, a species from the southern Western Ghats of India. Other than colour, there are few similarities between *I. multicolor* and *I. tricolor* that suggest an especially close relationship between them. Conversely, there are many substantial differences that suggest that *I. multicolor* and *I. tricolor* are not especially closely related. This is supported by the molecular data, which indicate that *I. multicolor* is more closely related to Southeast Asian *Ichthyophis* than it is to *I. tricolor*.

The "Out of South Asia" (sometimes "Out of India") hypothesis (see Gower *et al.* 2002; Wilkinson *et al.* 2002) postulates that Southeast Asian ichthyophiids radiated into Southeast Asia from South Asia, and paraphyly of the South Asian *Ichthyophis* with respect to the monophyetic group of Southeast Asian ichthyophiids (including *I. multicolor*) supports this. The most likely dispersal route is via northeast India (rather than across the Bay of Bengal), and so it is interesting that among the sampled ichthyophiids, the westernmost Southeast Asian taxon (*I. multicolor*) is sister to all other Southeast Asian taxa. This raises the possibility that signatures of the west to east/ southeast dispersal might be preserved in the phylogenetic relationships among extant ichthyophiids.

About one quarter of the currently recognised species of *Ichthyophis* have been described in the last 15 years from localities throughout South and Southeast Asia, suggesting that species discovery in this group is far from complete. *I. multicolor* is the first species of caecilian to be described from Myanmar and highlights that the caecilian fauna of this country is extremely poorly known. Shaded polygons of the geographic range of *I. kohtaoensis* have included peninsular Myanmar (e.g., IUCN 2013; AmphibiaWeb 2013), but this is probably based on extrapolation of supposed records of this species in both northern and southern Thailand, and not on any verified Myanmar records. Previous records of caecilians from Myanmar are of striped *Ichthyophis* that were identified as *I. glutinosus* prior to the development of the current view (Taylor 1968; Nussbaum and Gans 1981) that *I. glutinosus* is restricted to Sri Lanka. Thus the historical reports of caecilians from Myanmar may well be of as yet undescribed species. In addition to further *Ichthyophis*, known Northeast Indian localities of the recently described teresomatan caecilian family Chikilidae are sufficiently close to the border with Myanmar to render their presence in Myanmar extremely likely (Kamei *et al.* 2013).

We suggest that, as with the majority of nominal caecilian species (Gower & Wilkinson 2005), *Ichthyophis multicolor* be categorized as Data Deficient in the IUCN Red List given that we know very little about its geographic range or environmental requirements and tolerances. That specimens were found in areas of human disturbance gives some hope that they are not immediately threatened, but this depends foremost on a reasonable range size. Myanmar's caecilian fauna is very poorly known. Dedicated fieldwork and systematic research are priorities for a more accurate and precise inventory of the caecilian fauna of Myanmar, and for well-informed assessments of conservation status.

The taxonomy of *Ichthyophis* is challenging, there are many species but few characters that serve to distinguish them. There is a long and continuing tradition of osteological data being used for inferring phylogeny and in higher-level caecilian classification (e.g., Nussbaum 1979; Wilkinson & Nussbaum 1999; Maddin 2012; Wilkinson *et al.* 2011). However, osteology has been little used in lower-level taxonomy, not least because of the limited availability of material that could be prepared. The non-destructive nature of CT scanning facilitates investigations of caecilian osteology even when samples are minimal (e.g., Gower *et al.* 2010), as they are for many described species. Cranial osteology of the few *Ichthyophis* species compared herein, indicates that this could be a very useful source of data both for taxonomy and phylogenetics but that seemingly substantial differences may prove variable within species. Thus sample size is something to be considered carefully when cautiously interpreting osteological differences.

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APPENDIX 1

Voucher and GenBank accession numbers for specimens of *Ichthyophis multicolor* **sp. nov.** for which DNA sequence data were generated. * denotes holotype. GenBank accession numbers given in order: *12s, 16s, cytb*.

CAS 212254: FR715999, FR716007, FR716015
CAS 212255: FR716000, FR716008, FR716016
CAS 212262: FR716001, FR716009, FR716017
CAS 212263: FR716002, FR716010, FR716018
CAS 212264*: FR716003, FR716011, FR716019
CAS 212265: FR716004, FR716012, FR716020
CAS 212266: FR716005, FR716013, FR716021
CAS 212267: FR716006, FR716014, FR716022

APPENDIX 2

Abbreviations of osteological features labelled in Figures 3–5.
af—antorbital fenestra
c—circumorbital bone (= postfrontal of some authors)
cf—carotid foramen
ch—choana
cp—canalis primordialis
en—external naris
f—frontal
fm—foramen magnum
fo—foramen ovalis
i—inner mandibular tooth series
j—jugular foramen
m—maxillopalatine
n—nasal
np—nasopremaxilla
ob—os basale
oc—occipital condyle
o—orbit
p—parietal
pa—pseudangular
pc—processus condyloides of the pseudangular
pd—pseudodentary
pf—prefrontal
pi—processus internus of the pseudangular
pm—premaxilla
pq—pterygoid process of the quadrate
pt—pterygoid
q—quadrate
r—retroarticular process of the pseudangular
s—stapes
sm—septomaxilla
sp—sphenethmoid
sq—squamosal
sm—septomaxilla
ta—tentacular aperture
v—vomer