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A New Unstriped *Ichthyophis* (Amphibia: Gymnophiona: Ichthyophiidae) from Mt. Kinabalu, Sabah, Malaysia

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Abstract: A new unstriped *Ichthyophis* is described based on one adult male and five larval specimens collected from the northwestern slope of Mt. Kinabalu, Sabah, Malaysia. The new species is distinguished from all other unstriped congeners by a combination of characters that includes position of tentacles and number of annuli, scale rows, splenial teeth, and vertebrae. The anterior phallodeum morphology is described for the new species. The evolution of large larvae of unstriped *Ichthyophis* is discussed briefly.

Key words: Caecilian; *Ichthyophis*; Taxonomy; New species; Borneo

INTRODUCTION

From the region of Southeast Asia, only one family of caecilians, the Ichthyophiidae, has been recorded. This family has been regarded as consisting of three genera, *Caudacaecilia* Taylor, 1968, *Ichthyophis* Fitzinger, 1826, and *Uraeotyphlus* Peters, 1880 (Frost, 2011). However, based on molecular phylogenetic analyses, Nishikawa et al. (2012a) recently showed that *Caudacaecilia* and *Ichthyophis* form a clade and the species of the two genera are mutually paraphyletic. Therefore, *Caudacaecilia* should be relegated to a junior synonym of *Ichthyophis*. Taylor (1968) designated *Caudacaecilia* solely on the absence of the splenial teeth in adults, in contrast to *Ichthyophis*, which retains the teeth in adults.

Although the presence or absence of splenial teeth proved to be invalid for dividing the genera, these characteristics are still useful for species identification in *Ichthyophis*.

After synonymizing *Caudacaecilia* with *Ichthyophis*, the genus *Ichthyophis* includes 44 species, of which 27 are known from Southeast Asia (Frost, 2011; Nishikawa et al., 2012b). The species of *Ichthyophis* can be divided into two color types: one has a pair of yellow or cream-colored lateral stripes (striped type); the other lacks such stripes (unstriped type; Nishikawa et al., 2012a). Division of these two color types is not supported by the results of molecular phylogenetic analyses (Gower et al., 2002; Nishikawa et al., 2012a), but the presence or absence of stripes is extremely useful for species identification (Taylor, 1968; Wilkinson et al., 2007).

In 2005 and 2006, we collected specimens of an unstriped species of *Ichthyophis* with splenial teeth from Sayap station of Kinabalu

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National Park, Sabah, Malaysian part of Borneo Island. In Sabah and its vicinity, four unstriped *Ichthyophis* with splenial teeth are known: *I. dulitensis* Taylor, 1960 from Sarawak, Malaysia; *I. glandulosus* Taylor, 1923 from Basilan and Mindanao Islands, the Philippines; *I. mindanaoensis* Taylor, 1960 from Mindanao Island, the Philippines; and *I. monochrous* (Bleeker, 1858) from Sarawak of Malaysia, Kalimantan of Indonesia, and Brunei. The present *Ichthyophis* from Sabah has a distinctive combination of morphological characteristics that distinguishes it from the four known species, as well as all the other unstriped congeners with splenial teeth. We therefore describe this caecilian as a new species.

MATERIALS AND METHODS

Field surveys were made at the Sayap station of Kinabalu National Park, Kota Belud district, Sabah, Malaysia in 2005 and 2006. Of the specimens collected, one adult was sexed by direct observation of the phallodeum, and larvae were ascertained by the presence of gills and tail fin. The larval digestive organs were partly dissected in order to examine prey items. After tissues were taken for genetic analysis, specimens were fixed in 10% formalin, transferred to 70% ethanol, and identified by measuring and counting morphological characters. The voucher specimens are stored at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), and the Institute for Tropical Biology and Conservation, University Malaysia Sabah (BORN: BORNEENSIS).

We measured the following morphometric characters to the nearest 0.1 mm with a dial caliper following Nishikawa et al. (2012b): total length (TL); head length (HL) from tip of snout to first collar groove, measured dorsally; trunk length (TRL) from first collar groove to posterior end of vent; tail length (TAL) from posterior end of vent to tail tip; vent length (VL); snout length (SL) from tip of snout to jaw angle; lower jaw length (LJL) from tip of

lower jaw to jaw angle; snout-2nd collar groove length (S2CL), measured ventrally; snout-3rd collar groove length (S3CL), measured dorsally; 1st collar length (1CL), measured laterally; 2nd collar length (2CL), measured laterally; head width (HW) at jaw angle; maximum head width (MXHW); body width at middle (BWM); tail width at posterior vent (TAW); interorbital distance (IOD); intertentacle distance (ITD); internarial distance (IND); eye-nostril distance (END); eye-tentacle distance (ETD); tentacle-nostril distance (TND); and eye-jaw angle distance (EJD).

The following meristic characters were counted: total annuli (TA); annuli interrupted by vent (VA); post-vent annuli (PVA); dorsal transverse grooves on 2nd collar (DTG); premaxillary-maxillary teeth (PMM); vomeropalatine teeth (VP); dentary teeth (DE); splenial teeth (SP); and vertebrae (VER). The number of vertebrae was counted from a soft X-ray photograph using Fuji Medical X-Ray Film (RX-U).

Since the single adult proved to be a male, its phallodeum was everted after anesthetization and fixed by 10% formalin injection. For describing the everted morphology of the anterior phallodeum, we followed the terminology of Gower and Wilkinson (2002) and Kupfer and Müller (2004).

We examined the mitochondrial DNA genes of 12S rRNA and 16S rRNA and complete cytochrome b from the male holotype together with three congeneric species from Borneo and the Philippines. Methods of DNA preparation and data analyses are as reported in Nishikawa et al. (2012a).

SYSTEMATICS

Ichthyophis lakimi sp. nov.

Figs. 1–4

Ichthyophis monochrous: Inger, 1964, p. 46; 1966, p. 355 (part); Malkmus et al., 2002, p. 219.

Ichthyophis sp. 3: Nishikawa et al., 2012a, p. 716.

FIG. 1. Holotype of *Ichthyophis lakimi* sp. nov. (KUHE 38275) in dorsal (top), ventral (middle), and lateral (bottom) views. Scale=20 mm.

Diagnosis

Ichthyophis without lateral stripe and with splenial teeth; body uniformly slate dorsally, slightly paler ventrally; total length of the single male 292.5 mm; TL/BWM ratio=30.2; 349 annuli, six of which in tail; 48 premaxillary-maxillopalatine, 45 vomeropalatine, 41 dentary, 14 splenial teeth; TND/ETD ratio=1.9; scales from anteriormost 30th annuli to end of body; scales in one row except for two rows in posteriormost 150 annuli.

Holotype

KUHE 38275, an adult male (Figs. 1–4) from Sayap station of Kinabalu National

Park, about 30 km southeast from the city of Kota Belud, Kota Belud District, State of Sabah, Malaysia (6°03'N, 116°33'E, 960 m asl), collected by Kanto Nishikawa and Paul Yambun, at 14:59 h on 29 November 2006. The GenBank accession numbers of mtDNA sequences are AB686159 (12S rRNA and 16S rRNA) and AB686094 (cytochrome b).

Paratypes

KUHE 38276 (one larva), same collection date and locality as the holotype; BORN 23334–23337 (four larvae), collected by Kanto Nishikawa at the same locality as the holotype on 13 August 2005.

FIG. 2. Holotype of *Ichthyophis lakimi* sp. nov. (KUHE 38275). From top to bottom: head and anterior body in dorsal, ventral, and lateral views; posterior body in ventral view. Scale=10 mm.

Description of holotype (measurements in mm)

Moderate size (TL 292.5); body subcylindrical, slightly depressed dorsoventrally, tapering posteriorly, more abruptly at about one-fifth of body, ending in blunt tail tip, lacking nipple-like terminal cap; head widened slightly around

jaw angle and narrowing anteriorly; snout rounded anterior to tentacles, slightly longer (7.8) than lower jaw (6.8); intertentacle distance (6.1) larger than interorbital distance (5.4), which in turn is much larger than internarial distance (2.9); eyes slightly protruding, almost midway between top of head and edge of mouth in lateral view, slightly inset from edge of head in dorsal view; tentacles nearly twice as far from nostril than from eyes ($TND/ETD=1.9$); eye-jaw angle distance (3.0) larger than eye-tentacle distance (1.3) and tentacle-nostril distance (2.5); tentacles very close to edge of mouth, long and thin in life, tip slightly protruding from tentacular sheath in preservative; second collar (3.2) longer than first collar (2.7); nostrils round, positioned closely at anterior margin of mouth in dorsal and lateral views, invisible in ventral view.

Collar region slightly wider than head and anterior body in dorsal and ventral views, not higher than head and anterior body in lateral view; first collar groove evident as constriction separating head and trunk, curving slightly anteriorly towards dorsal midline, where groove becomes incomplete and tips separated; second collar groove evident ventrally but not apparent dorsally, parallel to first groove laterally, fading out near lateral midline; third collar groove not clearly differentiated from anteriormost annular grooves of trunk and dorsal transverse groove on second collar, but recognized as first groove crossing lateral to ventral body; third groove dorsally complete, curving slightly anteriorly towards dorsal midline, but tips separated midventrally; one dorsal transverse groove on left side of second collar.

Annular count 349; annular grooves complete dorsally, but narrowly separate ventrally except for posterior three-fifths of body; dorsally, annular grooves curving slightly anteriorly towards midline on anterior one-fourth of body and nearly orthoplicate on posterior three-fourths of body; ventrally, grooves strongly angulate posteriorly towards midline, degree of curvature decreasing posteriorly to becoming orthoplicate at ca. one-tenth of

body; scales found on posterior five-sixths of body, increasing in size posteriorly, where number of rows is one in the anterior half and two in the posterior half.

Longitudinal vent surrounded by small and whitish subcircular disc; denticulations on vent unclear because of everted phallodeum; no papillae on disc; six annuli each in vent slit including denticulations, and in tail.

Teeth on premaxillary-maxillopalatine 48, vomeropalatine 45, dentary 41, splenial 14; choanae elongated, about three times as long as broad, obliquely extending posterolaterally, without a flap on edge.

Anterior phallodeum with a pair of blind sacs laterally and longitudinal ridges dorsolaterally, laterally, and ventrolaterally (Fig. 3); ventrolateral longitudinal ridges smaller than

FIG. 3. Anterior phallodeum of holotype of *Ichthyophis lakimi* sp. nov. (KUHE 38275) in dorsal (top left), ventral (top right), left lateral (bottom left), right lateral (bottom right), and distal views (center). ebs: entrance of blind sac; eu: entrance of urodeum; l.dl: left dorsolateral longitudinal ridge; l.mdk: left mid-dorsal knob; l.vl: left ventrolateral longitudinal ridge; p.dl: posterior tuberosity of l.dl; p.lvl: posterior tuberosity of l.vl; p.rl: posterior tuberosity of right lateral longitudinal ridge; p.rdl: posterior tuberosity of right dorsolateral longitudinal ridge; p.rvl: posterior tuberosity of right ventrolateral longitudinal ridge; r.dl: right dorsolateral longitudinal ridge; r.l: right lateral longitudinal ridge; r.vl: right ventrolateral longitudinal ridge. Scale=10 mm.

FIG. 4. Holotype of *Ichthyophis lakimi* sp. nov. (KUHE 38275) in life.

dorsolateral longitudinal ridges, but larger than right lateral longitudinal ridge; left lateral longitudinal ridge lacking; each longitudinal ridge having a posterior tuberosity but lacking distinct anterior tuberosity; no mid-dorsal longitudinal ridge; mid-dorsal knob present only on left side.

Color

In life, ground color of dorsum uniform slate; slightly paler ventrally (Fig. 4); eye surrounded by narrow whitish ring; tentacles and surrounding tentacle aperture whitish; vent disc whitish. In preservative, color faded but not obviously changed.

Variation

The larval paratypes, lacking tentacles and possessing gills and a tail fin, are similar to the metamorph (holotype) in shape and color, but are smaller in TL (121.6–245.0 mm) and TL/BWM (20.6–24.7), and have a larger number of TA (354–374) than the holotype (292.5 mm, 30.2, and 349, respectively). The larval paratypes form three groups in TL: 240.8–245.0 mm (KUHE 38276 and BORN 23334), 195.1–198.0 mm (BORN 23335 and 23336), and 121.6 mm (BORN 23337) (Table 1). Tail fins are greatly reduced in the large- and medium-sized groups. Collars are absent in the medium and small-sized groups. First collar groove is indistinct in the small-sized group.

Larvae (measurements in mm)

Large sized (TL=121.6–245.0 mm), body subcylindrical, slightly depressed dorsoventrally, tapering posteriorly, ending in short tail fin (tail fin length: from beginning of dorsal fin to tip of tail 3.3–3.7, mean=3.5); head more flattened than in metamorph, widened slightly around jaw angle and narrowing anteriorly; snout rounded, slightly longer (4.0–6.7, mean=6.1) than lower jaw (3.1–6.0, mean=5.3); labial fold evident, especially in posterior two-thirds of upper jaw; interorbital distance (2.4–4.9, mean=3.9) much larger than internarial distance (1.7–3.2, mean=2.7); eyes slightly protruding, closer to top of head than to edge of mouth in lateral view, slightly inset from edge of head in dorsal view; collar indistinct, absent in smaller individuals; nostril elongated, obliquely extending dorsolaterally in frontal view, positioned at anterior margin of mouth in ventral view, slightly apart from margin in lateral view, invisible in dorsal view.

Annular count 354–374 (mean=362.0), tending to be indistinct in smaller individual; annular grooves complete dorsally, but narrowly separate ventrally in several anterior-most grooves; dorsally, annual grooves curving slightly anteriorly towards midline on anterior one-tenth of body and nearly orthoplicate on posterior nine-tenths of body; ventrally, grooves strongly angulate posteriorly towards midline, degree of curvature decreasing posteriorly to becoming orthoplicate at ca. one-fifth of body.

Locality	<i>Ichthyophis lakini</i> sp. nov.										<i>I. glandulosus</i>				<i>I. mindanaensis</i>										<i>I. monochrous</i>						
	Sayap, Kota Belud, Sabah					Mt. Dulit, Sarawak					Abung, Abung, Basilan					Todaya, Mindanao					Todaya, Mindanao					Sinkawang, Lawas, NP Sarawak					
	Paratype					Holotype					Holotype					Holotype					Holotype					Holotype					
	KUHE	BORN	BORN	BORN	BORN	BORN	BMNH	CAS	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	FMNH	
Specimen No.	38275	38276	23334	23335	23336	23337	926.63.23*	60073*	50958*	50957	50960	50961	50963	50964	50968	50969	50970	50971	50972	50976	63.12.43*	67349	P0046								
Sex/Age	Male	Male	Male	Male	Male	Male	Unknown	Male	Unknown	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Unknown	Juvenile	Male								
Morphometric																															
TTL	292.5	245.0	240.8	198.0	195.1	121.6	235.0	250.0	276.0	316.0	207.5	180.1	212.0	216.5	143.6	141.4	203.8	220.5	228.0	223.0	226.0	215.0	251.0								
HL	12.6	8.3	9.2	9.7	9.3	—	11.0	12.5	12.0	13.0	8.9	7.7	9.1	9.2	—	—	8.8	9.5	9.8	9.4	9.0	9.4	12.1								
TRL	275.8	232.3	227.7	184.6	182.5	—	218.4	231.3	257.6	297.7	193.4	167.2	199.0	201.0	—	—	189.6	204.3	215.4	210.1	213.2	203.6	236.8								
TAL	4.1	4.4	3.9	3.7	3.3	2.7	5.6	6.2	6.4	5.3	5.2	5.2	3.9	6.3	3.9	5.2	5.4	6.7	2.8	3.5	3.8	2.0	2.1								
VL	2.0	2.3	2.6	2.0	1.5	1.4	NA	NA	NA	2.2	2.0	1.8	1.9	1.9	1.4	1.4	1.6	1.4	2.1	2.2	NA	1.4	1.6								
SL	7.8	6.7	6.7	6.6	6.5	4.0	NA	NA	NA	8.1	6.5	4.8	7.1	7.0	4.4	3.5	5.8	7.2	7.5	7.1	NA	7.2	6.9								
LJL	6.8	5.8	6.0	5.9	5.8	3.1	NA	NA	NA	7.2	3.8	3.8	5.7	4.9	4.0	2.9	4.6	6.3	6.0	6.2	NA	7.0	6.0								
S2CL	13.3	8.9	11.1	—	—	—	13.2	NA	15.0	15.7	10.8	9.3	—	11.0	—	—	11.8	12.1	12.9	13.5	NA	12.1	13.0								
S3CL	17.5	14.0	15.6	14.7	14.1	9.4	17.0	NA	20.0	21.8	14.5	12.3	17.6	15.6	11.4	11.7	15.4	16.0	16.8	17.0	NA	14.8	17.2								
ICL	2.7	2.7	2.6	—	—	—	NA	NA	NA	2.9	3.4	3.7	—	2.9	—	—	3														

Scales found on posterior two-thirds of body, increasing in size posteriorly, with number of rows one in the anterior half and two in the posterior half, absent in the smallest larva.

Longitudinal vent surrounded by small and whitish subcircular disc; denticulations on vent unclear, one or two on posterior end; no papillae on disc; five to six annuli in vent slit including denticulations, and four to seven in tail.

Teeth on premaxillary-maxillopalatine 32–38 (mean=35.0), vomeropalatine 34–41 (37.4), dentary 37–39 (38.2), splenial 11–14 (13.4). Choanae elongated, about two and a half to three times as long as broad, obliquely extending posterolaterally, with a flap on proximal edge.

In life, ground color of dorsum uniform slate; slightly paler ventrally, except for the smallest specimen showing pale slate dorsally and pale brown ventrally; eyes surrounded by wider whitish ring than in metamorph; vent disc and tail fin whitish. In preservative, color faded but not obviously changed.

Comparisons

Ichthyophis lakimi differs from other unstriped *Ichthyophis* with splenial teeth from Borneo and the Philippines in the following way (data from Bleeker, 1858; Taylor, 1923, 1960, 1968): from *I. dulitensis* by having a uniformly slate head (head lighter than body in *I. dulitensis*), larger number of splenial teeth (SP=14 vs. eight in *I. dulitensis*), and smaller number of vertebrae (VER=110–112 vs. 114 in *I. dulitensis*); from *I. glandulosus* by lacking a lateral ridge (strong lateral ridge in *I. glandulosus*); from *I. glandulosus* by having the tentacles closer to the eyes (TND/ETD ratio=1.9 vs. 2.4 in *I. glandulosus*); from *I. dulitensis*, *I. glandulosus*, *I. mindanaoensis*, and *I. monochrous* by having a larger number of TA (349–374) and a smaller number of scale rows (two) (TA≤336 and scale rows≥three in *I. dulitensis*, *I. glandulosus*, *I. mindanaoensis*, and *I. monochrous*).

The new species differs from other unstriped *Ichthyophis* with splenial teeth in the following way (data from Taylor, 1960, 1965, 1968, 1969; Pillai and Ravichandran, 1999): from *I.*

acuminatus, *I. husaini*, *I. javanicus*, *I. laosensis*, *I. sikkimensis*, *I. sumatranus*, and *I. youngorum* by having a smaller number of splenial teeth (SP=14 vs. 44 in *I. acuminatus*, 33 in *I. husaini*, 24 in *I. javanicus*, 32 in *I. laosensis*, 20 in *I. sikkimensis*, 26 in *I. sumatranus*, and 24 in *I. youngorum*); from *I. billitonensis* and *I. singaporensis* by having a larger number of splenial teeth (14 vs. two in *I. billitonensis*, and six in *I. singaporensis*); from *I. bombayensis* by having a smaller number of vertebrae (VER=111 vs. 121 in *I. bombayensis*); and from *I. orthoplicatus* by having angulate grooves on the ventral body (orthoplicate in *I. orthoplicatus*).

Finally, the uncorrected pairwise sequence divergences in 2700 bp of mitochondrial genes of 12S rRNA and 16S rRNA and 1140 bp of complete cytochrome b between *I. lakimi* and the three congeners (*I. biangularis*, *I. cf. mindanaoensis*, and *Ichthyophis* sp. from Borneo and the Philippines) are as large as 7.1–7.4% in 12S rRNA and 16S rRNA and 8.5–11.8% in cytochrome b genes.

Range

Known only from the type locality at a low elevation on Mt. Kinabalu.

Natural history

The type locality is in primary forest dominated by dipterocarps. The holotype was collected by digging about 50 mm in wet soil close to a small tributary (45–60 cm in width and 5–30 mm in depth) of the Kemantis River. The streambed was sandy and the water was clear. Air, water, and soil temperatures at the time of collection of the holotype were 23.5, 19.5, and 20.5 °C, respectively. Information on breeding, hatchlings, and metamorphosis is lacking. The larvae were found under stones or rotten logs in the shallows of the stream. The larva ate arthropods like larval Ephemeroptera and Trichoptera and earthworms. The larval life may continue more than one year before metamorphosis because several size groups with great difference in body size (the largest individual was more than twice as

large as the smallest) were found at the same time. No predators of this species are known.

Etymology

The species name honors Dr. Maklarin B. Lakim who is in charge of the research division of Sabah Parks and has supported our survey.

DISCUSSION

Mt. Kinabalu, the highest mountain in Southeast Asia, encompasses various amphibian habitats along its extensive altitudinal range up to ca. 4100 m asl. This geographical feature of Mt. Kinabalu produces a rich amphibian fauna with at least 77 known species (Malkmus et al., 2002). The amphibian inventory study at Mt. Kinabalu began in the 1850s and precedes most other regions in Southeast Asia. However, caecilian diversity beyond doubt remains incompletely surveyed even on this well-surveyed mountain. In addition to the new caecilian species described here, Nishikawa et al. (2012a) reported the presence of two more unidentified caecilians from this mountain.

Ichthyophis lakimi is known only from Mt. Kinabalu. *Ichthyophis monochrous* from 914 m asl (Inger, 1964) and Sungei (=River) Kipungit (Malkmus et al., 2002), and a larval specimen of unstriped caecilian from 580 m asl at Sungei Mamut deposited at the Field Museum of Natural History (FMNH 130925, K. Nishikawa personal observation) probably represent this new species. The elevations of these localities are rather high compared with other Bornean records, where all known caecilians were reported from lowlands (Taylor, 1968) except for *I. dulitensis* from 610 m (Taylor, 1960). In order to know the correct distribution range of *I. lakimi*, further field surveys must be conducted in mountain areas adjacent to Mt. Kinabalu, such as the Crocker Range.

Descriptions of phallodeum morphology are rare for *Ichthyophis*, and taxonomic utility of the morphology remains unevaluated (Gower and Wilkinson, 2002; Kupfer and Müller,

2004). In order to assess the utility for species identification in *Ichthyophis*, more information must be gathered for intra- and interspecific variation in phallodeum morphology for many congeners. Asymmetric morphology of the phallodeum found in *I. lakimi* (absence of left lateral longitudinal ridge and right mid-dorsal knob) has never been detected in *I. glutinosus*, *I. paucisulcus*, *I. peninsularis*, or *I. pseudangularis*, whose phallodeum has been described (summarized in Taylor, 1968), or in *I. monochrous* from Mulu National Park, Sarawak, Malaysia (examined in this study: ID P0046 in Table 1).

Larvae of *I. lakimi* grow up to 245 mm, and such large size is known only in larval *I. mindanaoensis* (up to 238 mm: Taylor, 1960) from the montane region in Mindanao Island, the Philippines. Based on molecular data, Matsui et al. (2010a, b) found that montane frog species of the genera *Ansonia* and *Leptobrachium* from Mt. Kinabalu and Mindanao Island to form a clade, and suggested a past geohistorical connection between these two areas. *Ichthyophis lakimi* and *I. mindanaoensis* are similar in terms of their large larval size and unstriped adult body, but this similarity is not ascribed to a phylogenetically close relationship. On the phylogenetic tree from mitochondrial DNA, the two species (also all unstriped species examined) do not form a clade and paraphyly of unstriped species is indicated (Nishikawa et al., 2012a). Consequently, large larval size in *I. lakimi* and *I. mindanaoensis* might have resulted from convergence associated with a possible long larval life in cool mountain streams on Mt. Kinabalu and Mindanao Island. In order to understand their phylogenetic relationship and estimate the evolution of larval life history in Bornean and Philippine caecilians, more samples, especially from the Philippines, are needed.

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