RAFFLES BULLETIN OF ZOOLOGY **68**: 214–219
Date of publication: 17 April 2020
DOI: 10.26107/RBZ-2020-0018
http://zoobank.org/urn:lsid:zoobank.org:pub:1B0B88BF-35A2-4520-BDE1-82669226954C

Second specimen of the rare Bornean snake *Xenophidion acanthognathus* (Xenophidiidae, Serpentes, Reptilia) and confirmation as a distinct species from *X. schaeferi*

Ibuki Fukuyama[1*], Mohamad Yazid Hossman[2] & Kanto Nishikawa[1,3]

Abstract. We report a second specimen of *Xenophidion acanthognathus*, collected from Lambir Hills National Park, Malaysian Borneo. We investigate the taxonomic status and relationship between this species and its only other congener *X. schaeferi*, as possible synonymy was suggested. Morphological and genetic analysis confirmed that *X. acanthognathus* and *X. schaeferi* are heterospecific. Here, we provide a detailed morphological description and novel natural history observations of this rare species.

Key words. cyt b, Southeast Asia, Squamata, Sundaland, taxonomy, *Xenophidion*

INTRODUCTION

Snakes of the family Xenophidiidae consist of one genus, *Xenophidion* Günther & Manthey, 1995, which includes two species: *X. acanthognathus* Günther & Manthey, 1995, and *X. schaeferi* Günther & Manthey, 1995, both of which are rare and enigmatic snakes. The former is endemic to Borneo and is only known from the holotype collected in Sipitang District, Mendolong, Sabah, Malaysia, with a recent photographic record of a specimen from Lambir Hills National Park, Miri, Sarawak, Malaysia (no voucher specimen) (Günther & Manthey, 1995; Rowntree et al., 2017). The latter is endemic to the Malay Peninsula and only three specimens have been documented: the type specimen collected in Templer's Park, Selangor, Malaysia (Günther & Manthey, 1995); and two recently collected specimens from Lata Kijang, Jelebu, Negeri Sembilan, Peninsular Malaysia, and Semenyih, Selangor, Peninsular Malaysia (Quah et al., 2018). In addition to those specimens, one unidentified specimen of the genus was reported from Sumatra (Quah et al., 2018). Ecological and behavioural information of the genus is also extremely limited. Günther & Manthey (1995) suggested that this genus lives mainly in the leaf litter and under moss. Quah et al. (2018) mentioned that they may be semiaquatic instead of fossorial, while *X. acanthognathus* may be semi-scansorial. Additionally, *X. schaeferi* was observed to spring forward repeatedly to escape (Quah et al., 2018). The only known food item of the genus is a skink (Wallach & Günther, 1998), and Stuebing et al. (2014) suggested that their large stout teeth are an adaptation for preying on small vertebrates capable of resisting capture.

Quah et al. (2018) collected specimens of *X. schaeferi* and suggested the possible conspecific status of the two species based on morphological examination. However, they could not make a definitive decision because of the lack of specimens and molecular data from *X. acanthognathus*. Thus, additional data from *X. acanthognathus* are crucial to validate the taxonomic status of the two species. Here we report a newly collected specimen of *X. acanthognathus*, from Lambir Hills National Park, Miri, Sarawak, Malaysia. This individual is the second known specimen of the species and only the fifth for the family. We describe the specimen and investigate the taxonomic relationship between the two species based on morphological and genetic information.

MATERIAL AND METHODS

Material examined. Juvenile female (Sarawak Research Collection, Sarawak Forest Department [SRC] 00961) collected by Ibuki Fukuyama at 2117 h on 20 January 2019 at Lambir Hills National Park, Miri, Sarawak, Malaysia (4.1986°N, 114.0386°E: 123 m a.s.l.).

Molecular analysis. Total DNA was extracted from tissue preserved in 99% ethanol using Qiagen DNeasy Blood and Tissue Kit (Valencia, CA, USA) following the manufacturer's protocol. A partial sequence of the mitochondrial cytochrome b gene (716 bp) was amplified using the primers HI4910 and H15720 (Burbrink et al., 2000). PCR amplification conditions included initial denaturation at 94°C for 2 min, followed by

Accepted by: Chan Kin Onn

[1]Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Nihonmatsu, Sakyo, Kyoto 606-8501, Japan; Email: kawashibi@gmail.com (*corresponding author)

[2]Research, Development and Innovation Division, Sarawak Forest Department, Kuching 93250, Sarawak, Malaysia

[3]Graduate School of Global Environmental Studies, Kyoto University, Yoshida-Honmachi, Sakyo, Kyoto 606-8501, Japan

ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

35 cycles at 94°C for 30 s, 53°C for 30 s, and 72°C for 45 s, and was completed by a final extension at 72°C for 5 min. Amplification was performed in a 10-µl volume reaction, with Blend Taq (TOYOBO). Amplified PCR products were electrophoresed on 2.0% agarose gel and viewed under UV light to check for correct fragment size. PCR products were subsequently purified using 13% polyethylene glycol (PEG) purification procedures. The PCR products were sequenced with the PCR primers and BigDye v3.1, using ABI 3130xl Genetic Analyzer, and the obtained sequences were deposited in the DDBJ/EMBL/GenBank database with the accession number LC523630. In addition to the newly sequenced data of *X. acanthognathus* (SRC 00961), we used the sequence data of *X. schaeferi* provided by Lawson et al. (2004) and Quah et al. (2018) for comparisons. Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA7 (Kumar et al., 2016) with default parameters, and the alignment was checked and unaligned parts were manually revised. Uncorrected *p*-distances among sequences were calculated using MEGA7.

Morphological analysis. Information on morphological characters of the other four specimens of the genus were obtained from Günther & Manthey (1995), Wallach & Günther (1998), and Quah et al. (2018), and we followed their measurement and scale count methods. We also basically followed morphological characters examined by Günther & Manthey (1995) and Quah et al. (2018) that include snout-vent length (SVL); total length; tail length; presence or absence of internasal and loreal; number of preoculars, supraoculars, postoculars, supralabials, infralabials, dorsal scale rows, ventrals, and subcaudals; cloacal plate undivided or divided. All measurements, except snout-vent length and tail length, were taken with a Mitutoyo caliper to the nearest 0.1 mm. Ventral counts followed that of Dowling (1951). The terminal scute was not included in the number of subcaudals. Dorsal scale row counts were taken at one head length behind the posterior end of head, at midbody, and at one head length before vent. Values for paired head characters were given in left/right order. The sex was determined by presence or absence of hemipenis. Institutional abbreviations are as follows: FMNH—Field Museum of Natural History, Chicago, USA; LSUHC—La Sierra University Herpetological Collection, La Sierra University, Riverside, California, USA; USMHC—Universiti Sains Malaysia Herpetological Collection, Malaysia; ZMB—Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany.

RESULTS

Molecular analysis. The uncorrected *p*-distance for the cyt b gene fragment was 9.9% between *X. acanthognathus* from Lambir NP, Sarawak (SRC 00961) and the holotype of *X. schaeferi* from Templer's Park, Selangor (ZMB 50534), and 10.1% when compared to *X. schaeferi* from Lata Kijiang, Negeri Sembilan (LSUHC 13481). These values are much higher than the maximum of intraspecific variation in cyt b gene observed in other genera of snakes in Southeast Asia (Supikamolseni et al., 2015).

Morphological comparison. The present specimen (SRC 00961) was identified as belonging to the genus *Xenophidion* based on the following characteristics: a small snake with laterally compressed body and a short tail; nasal undivided; internasals absent; prefrontals greatly enlarged; loreals and suboculars absent; undivided subcaudals; irregular dark zig-zag vertebral stripe bordered by whitish zig-zag stripes running along the length of the body. Furthermore, this specimen was collected in northern Borneo (locality of *X. acanthognathus*) and the following characteristics are closer to *X. acanthognathus* than *X. schaeferi*: 185 ventrals; 55 subcaudal scales; relatively short tail (SVL/tail length = 4.20) (Günther & Manthey, 1995; Quah et al., 2018). Thus, this specimen is most probably *X. acanthognathus*. Although dentition characters are known to be diagnostic for this genus (Günther & Manthey, 1995), we were unable to examine those characters due to the small size of our specimen. Examination via CT scanning is planned for future studies.

Intraspecific variation (Table 1). Our specimen (SRC 00961) is generally similar in pholidosis to the holotype of the species (FMNH 235170) reported by Günther & Manthey (1995). Differences between SRC 00961 and the holotype were the number of ventrals (185 vs. 181); number of subcaudals (55 vs. 51); number of dorsal scales rows (22/24/20 vs. 19/23/19); morphology of body scales (slightly keeled only posteriorly vs. strongly keeled); position of the largest supralabial (2nd vs. 1st and 2nd); number of postoculars (3/2 vs. 2/2); SVL/tail length ratio (4.20 vs. 4.52); condition of yellowish-white patch on dorsum behind neck (small and unclear vs. large and clear). Difference in the yellowish-white patch between our specimen and the holotype may be ontogenetic because our specimen is a small juvenile and it is common for some snakes to exhibit change of colour pattern during development (Lillywhite, 2014).

Interspecific comparisons (Table 1). *Xenophidion acanthognathus* can be differentiated from its sister species, *X. schaeferi* by the following characteristics: larger number of ventrals (181–185 vs. 176–178); larger number of subcaudals (51–55 vs. 43–45); and shorter tail (SVL/tail length = 4.20–4.52 vs. 4.69–4.84).

Description of SRC 00961 (Figs. 1–4, Table 1). Juvenile female; total length 156 mm; tail length 30 mm; head length 7.1 mm from anterior edge of rostral to posterior end of mandible; head width 3.9 mm at broadest point; head distinct from neck; eyes small, pupils round; snout short and rounded in dorsal profile; rostral scale triangular, broader than tall, visible from above; nasals undivided, extending from middle of snout above to nearly the mouth opening below, nares in the distal corners; internasal absent (probably fused with prefrontals or nasals); loreal absent; preocular 1/1, large, extending dorsally on each side contacting prefrontal, frontal, supraocular, one head scale, 2nd and 3rd supralabials and eye; supraocular 1/1, small; postoculars 3/2, upper smaller than lower; prefrontals two, very large, longer than wide, in broad contact with each other, frontal, preoculars, posterior tip of nasals, and 1st and 2nd supralabials; frontal hexagonal, small, less than 0.5 times of preoculars and less than 0.25 times

Table 1. Morphological information of two species of *Xenophidion*. *: Data from Günther & Manthey (1995) and Wallach and Günther (1998) – no data available. **: Data from Quah et al., 2018.

Characteristics	***Xenophidion acanthognathus** (FMNH 235170, holotype)**	***Xenophidion acanthognathus* (SRC 00961)**	***Xenophidion schaeferi** (ZMB 50534, holotype)**	***Xenophidion schaeferi*** (LSUHC 13481)**	***Xenophidion schaeferi*** (USMHC 2389)**
Sex	Female	Female (Juvenile)	Female	Male	Male
SVL	276 mm	126 mm	218 mm	239 mm	211 mm
Tail length	61 mm	30 mm	45 mm	51 mm	44 mm
SVL/tail length	4.52	4.20	4.84	4.69	4.79
Supralabials	8/8	8/8	8/8	8/9	8/8
Largest supralabials	1–2nd/1–2nd	2nd/2nd	1–2nd/1–2nd	3rd/2nd	2nd/2nd
Supralabials touching the eye	3–4th/3–4th	3–4th/3–4th	3–4th/3–4th	3–4th/3–5th	3–4th/3–4th
Infralabials	8 or 9	9/9	8/8	9/9	8/8
Postoculars	2/2	3/2	2/–	2/2	2/2
Dorsal scales rows	19/23/19	22/24/20	–/23/–	20/21/18	21/23/20
Ventrals	181	185	178	176	176
Subcaudals	51	55	43	45	45
Body scales	Strongly keeled	Slightly keeled only posteriorly	Weakly keeled	Keeled	Keeled
Yellowish-white patch on dorsum behind neck	Present	Present (faded)	Absent	Present	Present

Fig. 1. The juvenile *Xenophidion acanthognathus* (SRC 00961) trying to escape under the bark in situ.

Fig. 2. The live juvenile of *Xenophidion acanthognathus* (SRC 00961).

of prefrontals; supralabials 8/8, largest supralabials 2/2, 3rd and 4th supralabials entering orbit; mental groove present; infralabials 9/9, first two in contact with anterior pair of chin shields; body laterally compressed, gradually increasing in circumference from behind neck, reaching its maximum circumference at midbody; dorsal scales smooth anteriorly, slightly keeled posteriorly, imbricate, anterior dorsal scale rows 22, dorsal scale rows at midbody 24, posterior dorsal

Fig. 3. Ventral colouration of *Xenophidion acanthognathus* (SRC 00961) in life. Scale bar = 10 mm.

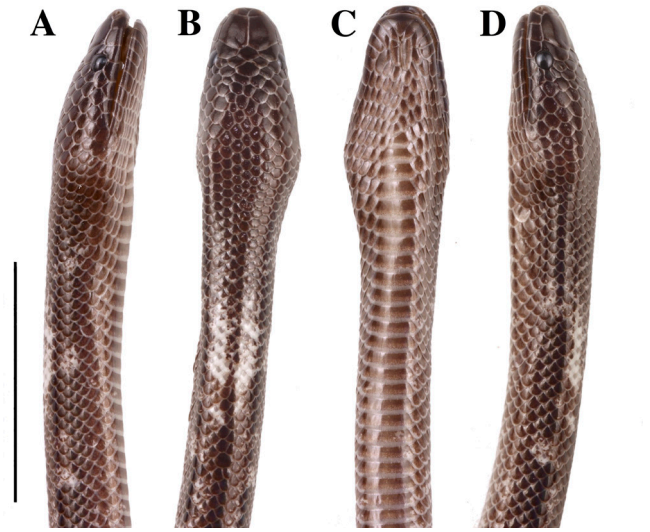

Fig. 4. A, right-lateral; B, dorsal; C, ventral; and D, left-lateral aspects of the head of *Xenophidion acanthognathus* (SRC 00961). Scale bar = 10 mm.

scale rows 20; 185 ventrals; cloacal plate undivided; and 55 unpaired subcaudal scales.

Colouration in life (Figs. 2, 3). The head is uniform black with white tiny markings on the scales on posterior part of the head and nape. The iris is black. The white patch on the neck is faded and inconspicuous, located between the 9th and 14th dorsal scale rows, and is approximately the same length as the head. The dorsum is black and slightly lighter along the flanks. An irregular dark zig-zag stripe runs along the vertebral column starting from the anterior end of the white neck patch and through centre line of the patch to the middle of the tail and it breaks into blotches along the posterior body and tail. Running alongside the dark, zig-zag vertebral stripe are two whitish, irregularly shaped, faded zig-zag stripes that extend from the posterior end of the white neck patch to the middle of the tail and break into small spots on the tail. Along the lower flanks from the middle to the posterior of the body is a row of whitish, irregularly shaped, faded blotches that form a chequered pattern along the lower flanks. The infralabials and chin are black and throat is dark-gray. The ventrals and subcaudals are dark-grey and marked with white spots that are one or two ventral scales in width along the edges of the scales from the middle of the body to the tail. The white ventral markings extend onto the 1st to 3rd row of dorsal scales and may connect with the whitish faded blotches on the lower flanks.

Fig. 5. Habitat of *Xenophidion acanthognathus* in Lambir Hills National Park, Miri, Sarawak, Malaysia. Photograph courtesy of Sally Kanamori.

Natural history. The new specimen (SRC 00961) was found moving slowly on the trunk of a tree ca. 20 cm above the ground in a lowland dipterocarp forest, at 2117 h (Figs. 1, 5). The air temperature at the site was 24.7°C and it had not rained for several days except for a very light rain during the day, thus the ground was dry. When the first author spotted the snake under the beam of his flashlight, it tried to escape under the bark (Fig. 1). The holotype of the species was collected under moss covering a rock, 10 m from a stream, at 0815 h (Günther & Manthey, 1995). One photographed individual was found lying vertically on a damp, mossy log, at 2015 h (Rowntree et al., 2017). The only known food item of the species is a skink (*Sphenomorphus* sp.) that was found in the gut of the holotype (Wallach & Günther, 1998).

DISCUSSION

The results of our study demonstrate that *Xenophidion acanthognathus* and *X. schaeferi* are sufficiently distinct morphologically and genetically to warrant specific status. With the acquisition of a second specimen of *X. acanthognathus*, this study is the first to examine the intraspecific variation within the species. However, additional specimens of both species are still needed to clarify their interspecific and intraspecific morphological variations. Genetic material of *Xenophidion* from Sumatra is also urgently needed to clarify its taxonomic status and phylogenetic placement. The distribution of the genus that is spread across Borneo, Peninsular Malaysia, and presumably Sumatra reflects the connection between these land masses until 400 kya as indicated by recent biogeographical and geological studies (Husson et al., 2019; Sarr et al., 2019). In addition, *Xenophidion* was shown to be closely related phylogenetically to the family Bolyeridae that is found only in the Mauritius (Lawson et al., 2004; Figueroa et al., 2016). Only a few genera of reptiles show similar relictual distributions in Sundaland like *Xenophidion*, such as the cat gecko, *Aeluroscalabotes*, which is the most basal linage and the only genus distributed in Sundaland in the family Eublepharidae, and the false gharial, *Tomistoma*, which is the sister species of *Gavialis* in South Asia (Willis et al., 2007; Jonniaux & Kumazawa, 2008). Comprehensive molecular phylogenetic analyses of these taxa may provide new insights into the biogeographical history of Sundaic fauna.

Inger & Voris (2001) regarded *X. acanthognathus* as fossorial or secretive, but Quah et al. (2018) discussed the possible ecological niche of the genus and suggested that they may be of semiaquatic or semi-scansorial habit, and not burrowers. Our observation of the new specimen crawling on the trunk of a tree may support the hypothesis of Quah et al. (2018) that they are semi-scansorial by nature. Although many herpetological surveys were conducted in Malay Peninsula and Borneo, this genus has only been documented six times. It is possible that the extremely low encounter rate in the field with members of this genus is related to their low density as suggested by Günther & Manthey (1995), or may be due to specialised ecological habits. Further observations are essential to understand the natural history of the genus.

ACKNOWLEDGEMENTS

The State Government of Sarawak kindly permitted us to conduct the project (Permit No. [185]JHS/NCCD/600-7/2/107 and Park Permit No. WL92/2018), and the RDID provided facilities for conducting research. We are grateful to R. ak S. Pungga, P. ak Meleng, and T. Itioka for their support in getting permission to conduct research and export specimens. We would like to thank Sally Kanamori for providing the photograph of Lambir Hills National Park. We also thank K. O. Chan, E. S. H. Quah, and G. Vogel for providing valuable comments on the earlier version of the manuscript. This study was supported by the Shikata Memorial Trust for Nature Conservation, the Kyoto University Omoro Challenge Scholarship Program 2018 to IF, JSPS Core-to-Core Program B, and the Kondo Grant of the Asahi Glass Foundation and JST/JICA, SATREPS to KN.

LITERATURE CITED

Burbrink FT, Lawson R & Slowinski JB (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution, 54(6): 2107–2118.

Dowling HG (1951) A proposed standard system of counting ventrals in snakes. British Journal of Herpetology, 1(5): 97–99.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792–1797.

Figueroa A, McKelvy AD, Grismer LL, Bell CD & Lailvaux SP (2016) A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. PLoS One, 11(9): e0161070.

Günther R & Manthey U (1995) *Xenophidion*, a new genus with two new species of snakes from Malaysia (Serpentes, Colubridae). Amphibia-Reptilia, 16(3): 229–240.

Husson L, Boucher FC, Sarr AC, Sepulchre P & Cahyarini SY (2019) Evidence of Sundaland's subsidence requires revisiting its biogeography. Journal of Biogeography, 47(4): 843–853.

Inger RF & Voris HK (2001) The biogeographical relations of the frogs and snakes of Sundaland. Journal of Biogeography, 28(7): 863–891.

Jonniaux P & Kumazawa Y (2008) Molecular phylogenetic and dating analyses using mitochondrial DNA sequences of eyelid geckos (Squamata: Eublepharidae). Gene, 407(1–2): 105–115.

Kumar S, Stecher G & Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874.

Lawson R, Slowinski JB & Burbrink FT (2004) A molecular approach to discerning the phylogenetic placement of the enigmatic snake *Xenophidion schaeferi* among the Alethinophidia. Journal of Zoology, 263(3): 285–294.

Lillywhite HB (2014) How snakes work: structure, function and behavior of the world's snakes. Oxford University Press, Oxford, 256 pp.

Quah ESH, Grismer LL, Jetten T, Wood Jr PL, Miralles A, Shahrul Anuar MS, Guek KHP & Brady ML (2018) The rediscovery of Schaefer's spine-jawed snake (*Xenophidion schaeferi* Günther & Manthey, 1995) (Serpentes, Xenophidiidae) from Peninsular Malaysia with notes on its variation and the first record of the genus from Sumatra, Indonesia. Zootaxa, 4441(2): 366–378.

Rowntree N, Griffiths J & Rowntree P (2017) *Xenophidion acanthognathus* (Bornean Spine-jawed Snake) Geographic distribution. Herpetological Review, 48(1): 132–133.

Sarr AC, Husson L, Sepulchre P, Pastier AM, Pedoja K, Elliot M, Arias-Ruiz C, Solihuddin T, Aribowo S & Susilohadi (2019) Subsiding Sundaland. Geology, 47(2): 119–122.

Stuebing RB, Inger RF & Lardner B (2014) A field guide to the snakes of Borneo. Second Edition. Natural History Publications (Borneo), Kota Kinabalu, 310 pp.

Supikamolseni A, Ngaoburanawit N, Sumontha M, Chanhome L, Suntrarachun S, Peyachoknagul S & Srikulnath K (2015) Molecular barcoding of venomous snakes and species-specific multiplex PCR assay to identify snake groups for which antivenom is available in Thailand. Genetics and Molecular Research, 14(4): 13981–13997.

Wallach V & Günther R (1998) Visceral anatomy of the Malaysian snake genus *Xenophidion*, including a cladistics analysis and allocation to a new family (Serpentes: Xenophidiidae). Amphibia-Reptilia, 19(4): 385–404.

Willis RE, McAliley LR, Neeley ED & Densmore III LD (2007) Evidence for placing the false gharial (*Tomistoma schlegelii*) into the family Gavialidae: inferences from nuclear gene sequences. Molecular Phylogenetics and Evolution, 43(3): 787–794.