Protobothrops kelomohy sp. nov. (Squamata: Viperidae), the Second Known Species of Lance-Headed Pit Viper from Thailand

MONTRI SUMONTHA^{1*}, TAKSA VASARUCHAPONG^{2*}, NIRUTH CHOMNGAM³, SUNUTCHA SUNTRARACHUN², PARINYA PAWANGKHANANT⁴, WEERASAK SOMPAN⁵, TON SMITS⁶, KIRATI KUNYA⁷ AND LAWAN CHANHOME²

 ¹Ranong Marine Fisheries Research and Development Station, 157 M.1 Chaloem Phra Kiat Rd., Paknam, Muang, Ranong 85000, THAILAND
²Queen Saovabha Memorial Institute, The Thai Red Cross Society, 1871 Rama 4 Rd., Patumwan, Bangkok 10330, THAILAND
³54 M.2 Tha Pha, Ban Pong, Ratchaburi 70110, THAILAND
⁴ Bansomdejchaopraya Rajabhat University, 1061 Soi 15, Itsaraphab Rd., Hiran Ruchi, Thon Buri, Bangkok 10600, THAILAND
⁵Omkoi District Non-Formal and Informal Education Centre "Mae Fa Luang", Baan Tee Tor Ta M.1 Sop Khong, Omkoi, Chiang Mai 50310, THAILAND
⁶105 M.4 Kaeng Krachan, Phetchaburi 76170, THAILAND
⁷Nakhonratchasima Zoo, 111 M. 1, Ratchasima-Pak Tongchai Rd., Chaimongkol, Muang, Nakhon Ratchasima 30000, THAILAND
* Corresponding authors. Montri Sumontha (montri.sumontha@gmail.com) and Taksa Vasaruchapong (taksa.v@gmail.com)
Received: 8 August 2019; Accepted: 3 December 2019

ABSTRACT.– *Protobothrops kelomohy* **sp. nov.** is described from dry evergreen forest in Chiang Mai Province, northern Thailand, based on morphology and a molecular phylogeny. It differs from congeners by the following combination of characters: 1) relatively large body size (total length up to 1,310 mm); 2) dorsal scale rows 23–23–17, all keeled; 3) ventral scales 231–234; 4) subcaudal scales 80–84, paired; 5) supralabials 8–9; 6) infralabials 12–13; 7) typical lance-pattern on upper head surface; 8) three bold vertical facial stripes; and 9) dorsal and tail brown, each scale with dark reddish-brown transverse blotches, edged in black, somewhat fused together as a median zigzag line on dorsum, a row of large ventrolateral blotches on each side, each darker marking edged by a narrow yellow line. The new discovery and other recent findings suggest that further herpetological research is required in the montane forest of northern Thailand and adjacent Myanmar and Laos. Data on the natural history of the new species and an updated key to the species of *Protobothrops* are provided. This is the second species of Lance-headed Pit Viper recorded from Thailand after *P. mucrosquamatus*.

KEY WORDS: Protobothrops, Crotalinae, new species, montane forest, Thailand

INTRODUCTION

Asian Lance-headed Pit Vipers of the genus *Protobothrops* (Hoge and Romano Hoge, 1980/1981) are medically important venomous snakes that have a wide distribution in Asia (Uetz et al., 2018). Currently 14 recognized species of *Protobothrops* have been described, based on both morphological and molecular data (Malhotra and Thorpe, 2004; Wüster et al., 2008; Guo et al., 2006, 2009, 2011; Yang et al., 2011; Liu et al., 2012; Guo et al., 2016). Among these, *P. mucrosquamatus* (Cantor) and *P. flavoviridis* (Hallowell) are considered to be species of high medical importance in China (both the mainland and on Taiwan) and Japan respectively, while others are of

lesser concern (WHO, 2016). Previously, Protobothrops had not been recorded from Thailand and did not constitute a medical concern in this country. The occurrence of species of Protobothrops has been reported from some neighboring countries in which they are considered to be of secondary medical importance. including Р. mucrosquamatus (Laos and Myanmar), P. jerdonii (Günther) and P. kaulbacki (Smith) (Myanmar) (WHO, 2016). The occurrence of Protobothrops in Thailand was not known until the report of P. mucrosquamatus from Nan Province, northern Thailand, based on its unambiguous morphology (Vasaruchapong et al., 2017). Subsequently, three specimens (two adults and one juvenile) of another Lance-headed Pit Viper were collected and photographed in life in Chiang Mai Province, northern Thailand. They could not be assigned to any currently recognized species in this genus, and consequently, we describe it herein as a new species.

MATERIALS AND METHODS

Sampling

Two adult and one juvenile specimens were collected by Weerasak Sompun who resides in the locality that the specimens were found during September - October 2017. Specimens were transferred to the Snake Farm, Queen Saovabha Memorial Institute, The Thai Red Cross Society, Bangkok, (QSMI) for further study on their venom property and function. Specimens were kept separately in escape-proof plastic boxes with a hiding place to minimize stress and free access to water. Blood samples for genetic analysis of the two adult specimens were collected from the ventral tail vein before being euthanized by isoflurane. The specimens were then fixed in 95% ethanol

and subsequently stored in 70% ethanol. All procedures with venomous snakes in this study were performed by the authorized snake handlers and veterinarians under the Safety Protocol for Working with Venomous Snake (Doc. No. SN 001). controlled by the Safety Committee of OSMI. This study was conducted under the Ethic Committee of OSMI (OSMI-ACUC-02-2018). Specimens were deposited in the herpetological collections of QSMI under numbers OSMI 1557 and OSMI 1558. The additional juvenile specimen (PMU04) has been maintained alive for further study.

DNA extraction, amplification and sequencing

Blood samples of the two adult specimens (QSMI 1557 and QSMI 1558), and shedded skin of the juvenile specimen (PMU04), were extracted using the Genomic DNA extraction kit (Blood/Bacteria/Cultured cells) (RBC Bioscience, Taipei, Taiwan).

Oligonucleotide primers for cytochrome b and 16S ribosomal RNA were designed using nucleotide sequences in GenBank (Table 1). DNA was amplified using the Polymerase Chain Reaction (PCR) in 50 ul reactions containing 10xbuffer, 100mM of each dNTP, 25 mM MgCl₂, 50 pmol/µl of forward and reverse primers, Taq DNA polymerase and 10 µl of DNA template. The PCR was performed using a thermocycler (MWG Biotech, USA) at 94°C for 3 minutes, followed by 40 cycles of 94°C, 56°C, and 72°C for one minute each, and a final extension of 72°C for 7 minutes. PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide in 1xTAE buffer along with appropriate molecular size markers. The gel fragment containing the amplified product was excised and extracted using the Gel/PCR DNA fragments extraction kit (RBC Bioscience, Taipei, Taiwan). DNA sequencing

TABLE 1. Oligonucleotide primers used for PCR and sequencing in this study. Primers were designed from GenBank sequences of *Laticauda laticaudata* (FJ 587153.1), *Naja atra* (NC011389.1), *Ophiophagus hannah* (NC011394.1), *Bungarus fasciatus* (NC011393.1), and *Daboia russelii* (NC011391.1).

Gene	Primers	Nucleotides (5'→ 3')	Product size (bp)
16S	1212-F	5' GCAATGAAGTGCGCACACACCGCC 3'	538
	1212-R	5' AGCCAGCTATCTCCAGATTC 3'	
	1216-F	5' AAAGGAATCTAAGTTCCACT 3'	498
	1216-R	5' CTAAAGGTTATGTTTTTGTT 3'	
	1616-F	5' AAAGGCAACGCCTGCCCAGT 3'	509
	1616-R	5' CGGTCTGAACTCAGATCACGT 3'	
Cytochrome b	Cytb-F	5' GCCTGAAAAACCACCGTTGT 3'	1,114
-	Cytb-R	5' CCGTCTTTGGTTTACAAGAAC 3'	

TABLE 2. Mitochondrial cytochrome b and 16S ribosomal RNA sequences of *Protobothrops* species used in this study.

Protobothrops species	GenBank No. Cytochrome b	GenBank No. 16S rRNA		
P. maolanensis	KF039900	NC026051		
P. dabieshanensis	KF003004	NC022473		
P. mucrosquamatus	KC438281	NC021412		
P. cornutus	KF110978	NC022695		
P. jerdonii	KC112560	KC112560		
P. mangshanensis	KF039901	KT963029		
P. kaulbacki	KJ689382	KJ689382		
P. himalayanus	KJ689381	KJ689381		
P. elegans	LC073748	LC073748		
P. flavoviridis	LC073746	LC073746		
P. kelomohy (QSMI 1557)	MK834284	MK830671		
C. rhodostoma (Outgroup)	AF292569	AF057237		

was carried out using the amplification primers by 1^{st} BASE sequencing (Malaysiahttp://www.base-asia.com). Newly generated sequences of holotype (QSMI 1557) were deposited in GenBank under the accession numbers MK834284 for Cytochorme *b* and MK830671 for 16S rRNA (Table 2).

Sequence divergence and phylogenetic cluster analysis

Authenticity of DNA sequences were verified using NCBI Nucleotide BLAST (www.ncbi.nlm.nih.gov). Newly-generated sequences and homologous sequences of related species of *Protobothrops* downloaded from GenBank (Table 2) were aligned using the default parameters in MEGA 7: Molecular Evolutionary Genetics Analysis Version 7.0 (Kumar et al., 2016). Calloselasma rhodostoma (Table 2) was selected as the outgroup based on its phylogenetic relationship to the genus Protobothrops (Malhotra and Thorpe. 2000). The aligned datasets of cytochrome bribosomal and 16S RNA fragments contained 1.114 and 1.347 characters. respectively. The level of sequence divergence within and between species was estimated using the uncorrected pairwise distance (p-distance) model in MEGA 7. The best-fit model of DNA substitution was determined for each gene using the program Kakusan 4 and the optimal model for nucleotide evolution was set to GTR+I+G (Tanabe, 2011). The two data sets were

concantenated for phylogenetic analysis using Bayesian Inference (BI). The BI analysis was implemented in the program MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001).

Measurements and meristic counts

Measurements and merestic counts were taken following David et al. (2008). All measurements were taken with a digital slide-calliper to the nearest 0.05 mm. Ventral scales and scale reductions were counted accorded to Dowling (1951 a, b). The terminal scute was not included in the number of subcaudals. Dorsal scale row counts were given at one head length behind head, at midbody (at the level of half of the snout-vent length value), and at one head meristic length before vent. Paired characters are given in left/right order. Characters taken are as follows: Morphometry: ED, eve diameter (horizontal): HD, maximum head depth; HL, head length (from the tip of rostral to the posterior end of the jaw); HW, maximum head width; SnL, snout length (from the tip of rostral to the anterior eye margins); EDNa, distance from posterior margin of nasal to the anterior eye margin; SVL, snout-vent length; TaL, tail length; TL, total length. Scalation and others: ASR, number of dorsal scale rows at neck (at one HL behind head); MSR, number of dorsal scale rows at midbody (at position of SVL/2); PSR, number of dorsal scale rows at one HL before anal plate position; IL, infralabial loreal scale(s): LOR. scale: PosOc. postocular scale(s); PreOc. preocular scale(s): SubOc. subocular scale(s): PreV. preventral(s) (directly preceding the ventrals, unpaired, wider than long but not in contact with the 1st dorsal scale row); SC, subcaudal scale(s); SL, supralabial scale(s); V, ventral scale(s) and A, cloacal plate.

Comparative data for other species of *Protobothrops* were obtained from the literature (Maki, 1931; Ziegler et al., 2000; Gumprecht et al., 2004; Zhao, 2006; David et al., 2008; Orlov et al., 2009; Yang et al., 2011; Huang et al., 2012; Pan et al., 2013; Luu et al., 2015) and literature cited therein.

RESULTS

Molecular phylogeny

The concatenated analysis of two genes, cytochrome *b* and 16S rRNA (Table 3), revealed that mean interspecific *p*-distances between the Chiang Mai specimens and related *Protobothrops* species ranged from 7.2% to 11.6%, with the minimum of 7.2% \pm 0.7% being to *P. kaulbacki* (Smith) and the maximum of 11.6% \pm 0.8% being to *P. elegans* (Gray). Bayesian phylogenetic

TABLE 3. Interspecific, uncorrected (p) sequence divergences (%) between *Protobothrops kelomohy* **sp. nov.** and related *Protobothrops* species using two concatenated genes, cytochrome b and 16S rRNA

Species	Divergences (%)
Protobothrops maolanensis	10.5
Protobothrops dabieshanensis	10.4
Protobothrops mucrosquamatus	10.9
Protobothrops cornutus	11.1
Protobothrops jerdonii	10.1
Protobothrops mangshanensis	9.3
Protobothrops kaulbacki	7.2
Protobothrops himalayanus	7.3
Protobothrops elegans	11.6
Protobothrops flavoviridis	9.9

inference recovered the Chiang Mai specimens to be sister to a clade containing *P. kaulbacki* and *P. himalayanus* in analyses of both genes (Fig. 1).

Taxonomy

Protobothrops kelomohy sp. nov.

(Figs. 2–4 and Table 4)

Holotype. – QSMI 1557, adult male (Figs. 2, 3) from Sop Khong Subdistrict, Omkoi District, Chiang Mai Province, northern Thailand (600 m a.s.l.) collected by Weerasak Sompan on 28 September 2017.

Paratype. – QSMI 1558, adult female from the same locality and collector as holotype, collected on 1 October 2017.

Additional material. - A juvenile (PMU04,

Fig. 4A) from the same locality and collector as holotype, collected on 17 September 2017, not preserved but maintained alive at QSMI.

An injured adult from Tha Song Yang District, Tak Province, northern Thailand (689 m a.s.l.) photographed by Ton Smits on 23 September 2018, but not examined due to its injuries.

A live juvenile, sex unknown, from Tha Song Yang District, Tak Province, northern Thailand (701 m a.s.l.) photographed by Ton Smits on 28 October 2018, was not collected for further examination (Fig. 4B).

Diagnosis. – *Protobothrops kelomohy* **sp. nov.** differs from other species of the genus

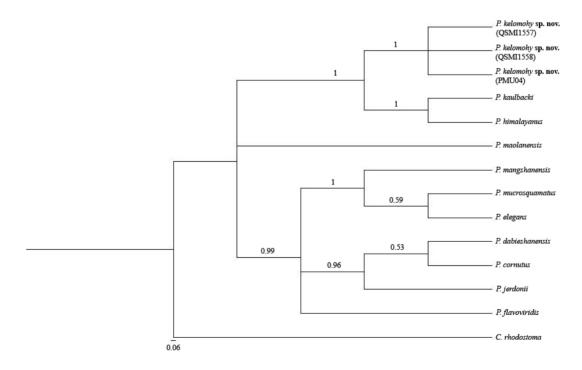


FIGURE 1. Phylogenetic relationships of *Protobothrops kelomohy* **sp. nov.** samples and related *Protobothrops* species based on the cytochrome b and 16S rRNA genes using the Bayesian Inference (BI) analysis implemented under the program MrBayes v3.0b4. Branch labels display bootstrap values and the scale bar represents 0.06 substitutions per site.



FIGURE 2. Holotype male of Protobothrops kelomohy sp. nov. (QSMI 1557) in life (photo by M. Sumontha).

by the combination of the following characters: 1) relative larger body size (TL up to 1310 mm); 2) dorsal scale rows 23-23-17, all keeled; 3) ventral scales 231-234; 4) high number of subcaudals (80-84 pairs); 5) 8-9 supralabials; 6) 12-13 infralabials; 7) typical lance-head pattern on upper head surface; 8) three bold vertical facial stripes, (first from internasal, second from facial pit, third from eye) and a bold postocular stripe extending obliquely downwards on temporal region then curved down immediately at the end of the last supralabial; 9) dorsal body and tail reddish-brown, each scale with black edges; transverse blotches, somewhat fused together as a zigzag median line on dorsum and a row of large ventrolateral blotches on each side, each darker marking edged with a narrow yellow line.

Etymology. – The specific epithet "*kelomohy*" is taken from the romanized nouns "kolo" meaning "fire or thunder" and "môhi" meaning "mothers that lay eggs and stays and look after them during incubation time until hatching". The first noun is drawn from the vernacular name of the new species in the Northern Pwo Karen language, a language of Karen subfamily, Sino-Tibetan family, and probably refers to the snake bite's inducing an immediate sharp, burning pain. The second noun refers to the maternal reproductive behavior of the genus Protobothrops, assumed to be the same for the new species. Suggested common names Lance-headed Omkoi are Pit Viper (English), Ngoo Kelo Omkoi (Thai), and Yum Kelo Mo Hy (Karen).



FIGURE 3. Holotype male of *Protobothrops kelomohy* **sp. nov.** (QSMI 1557) in preservative: A) general dorsal view; B) general ventral view; C) lateral head view (photos by T. Vasaruchapong).

Description of the holotype. – An adult male; body large (SVL 918 mm, TaL 200 elongate, thin and mm), slightly compressed; head triangular and elongated, 0.63 times wider than long, and distinct from neck, covered with small, convex and irregular shaped scales; upper head scales smooth anteriorly, keeled on occipital region; 7 scales in transverse line between supraoculars; snout elongated, SnL/HL ratio 0.29, greater than twice the eye diameter (ED/SnL ratio 0.46), eye convex, pupil vertical.

Rostral triangular, 1.2 times wider than high, slightly visible from above, bordered posteriorly with two apical scales, bordered laterally by triangular internasals; internasals not in contact with rostral and separated from each other by two small scales; nasal trapezoid, undivided, round nostril opening in its middle; anterior part of nasal large, invisible from above; 2 canthal scales between supraocular and internasal on each side, distinctly larger than adjacent scales, approximately of equal size, bordering a sharp, raised *canthus rostralis*; 1/1 loreal; 1/1 supraocular large and elongate, wider

50



FIGURE 4. A) Living juvenile of *Protobothrops kelomohy* **sp. nov.** *in situ* (PMU04), not preserved (photo by M. Sumontha); B) Living juvenile of *Protobothrops kelomohy* **sp. nov.** *in situ*, from Tha Song Yang district, Tak Province (photo by T. Smits)

than adjacent head scales; 2/2 elongate upper preoculars above loreal pit in contact with loreal, lower one longer than upper and forming upper margin of loreal pit; one elongate lower preocular, forming lower margin of loreal pit; 3/3 small postoculars; crescent-shaped 2/2elongate, thin. subocular, separated from lower preocular by one scale; temporal numerous, upper ones keeled, lower ones smooth, those bordering the supralabials larger than latter; 8/8 supralabials, 2nd forming anterior margin of loreal pit and in contact with nasal. 3rd largest and in contact with subocular, 4th separated from subocular by a scale on both sides; 12/13 infralabials, 1st pair in contact with each other, first three in contact with anterior chin shield; 5 pairs of chin shields, anterior one largest.

Dorsal scales: 23-23-17, narrow, pointed, strongly keeled throughout, including the first row; two preventral scales; 234 ventral scales; cloacal scale entire; 84 pairs of subcaudals. Dorsal scale reduction formula:

3+4 → 3[128]	4+5 → 4[140]	4+5 → 3[165]
23	▶21 → 19-	▶ 17
3+4 → 3[128]	4+5 → 4[140]	3+4 → 3[161]

Coloration in life. - Dorsal body and tail background colour brown; dorsum with 53/51 dark reddish-brown, black edged blotches. somewhat transverse fused together as a zigzag median line on dorsum; a row of large ventrolateral blotches on each side, each dark edged by a very narrow vellow line; tail with 16/15 dark bands. Venter whitish-brown with irregular series of brown blotches. Dorsal surface of head reddish-brown with a distinctly symmetrical dark pattern: a pair of dark brown, parallel, elongate blotches on forehead, between posterior edge of internasals and prefrontal region, followed on the central and posterior regions of the head by a complex pattern made of symmetrical, dark brown areas and blotches separated by pale reddish-brown. There are three bold dark reddish-brown facial stripes with continuous pale yellow edges. The first from internasal vertically; the second from facial pit vertically; and the third form the facial pit. A bold postocular strip starting from posterior margin of eye downward obliquely on to the temporal region then curved down immediately at the end of last supralabials.

Hemipenis. – The everted left hemipenis is long, robust and forked, spinous proximally

	QSMI 1557	QSMI 1558		
Sex	Holotype male	Paratype female		
TL (mm)	1118	1310		
SVL	918	1107		
TaL	200	203		
HL	36.86	47.02		
HW	23.21	27.71		
HD	13.19	13.65		
SnL	10.75	13.37		
ED	4.97	4.84		
EDNa	8.35	10.94		
TaL/SVL	0.22	0.18		
HW/HL	0.63	0.59		
SnL/HL	0.29	0.28		
ED/HL	0.13	0.10		
ED/SnL	0.46	0.36		
ASR	23	23		
MSR	23	23		
PSR	17	17		
PreV	2	2		
V	234	231		
A	1	1		
SC	84	80		
LOR	1	1		
PreO	3	3		
SubO	2	2		
PosO	3	3		
SL [SL which touch orbit]	8[3rd-4th]/8[3rd-4th]	8[3rd-4th]/9[3rd-4th]		
IL	12/13	13/13		
1st dorsal scale row	Keeled	keeled		
Body bands	51/53	43/44		
Tail bands	16/15	19/19		

TABLE 4. Measurements and scale characters of the type specimens of Protobothrops kelomohy sp. nov.

and calyculate distally; it bears two lobes covered with spines and microspines, extending to the 7th subcaudal. Sulcus spermaticus forked at the truncus 6 mm from the base of the hemipenis. Strong spines on asulcate side at the base part of each lobe, followed by smaller spines more distally: sulcate side covered with microspines. The hemipenis of Protobothrops kelomohy sp. nov. closely resembles the Type 3 spinose hemipenis of Malhotra and Thorpe (2004).

Variation and sexual dimorphism. – Measurements and scalations of type specimens are listed in Table 4. No significant sexual dimorphism occurs, other than the female having slightly fewer ventrals (231 vs 234 in male) and subcaudals (80 vs 84 in male). The coloration and pattern of the juvenile (PMU04) resembled those of the adults (Fig. 4A).

Distribution and natural history. - All specimens of Protobothrops kelomohy sp. nov. in this report were found in Sop Khong Subdistrict, Omkoi District, Chiangmai Province, Thailand, a mountainous area that has an estimated elevation range between 600-1,200 m a.s.l. (red solid circle in Fig. 5). The holotype male (QSMI 1557) and referred juvenile (PMU04) (Fig. 4A) were found on the ground near a rock and a shrub respectively at night in dry evergreen forest, in the vicinity of human dwellings (Fig. 6). This snake is also known by locals from adjacent mountainous area for which we found evidence through two other specimens, a juvenile and an adult, that were

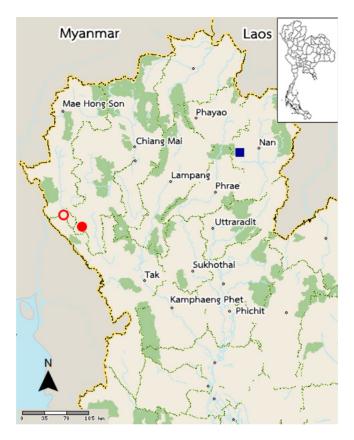


FIGURE 5. Map of Thailand showing the type locality of *Protobothrops kelomohy* **sp. nov.** at Omkoi District, Chiangmai Province (red solid circle); Tha Song Yang District, Tak Province (red hollow circle); the locality of *Protobothrops mucrosquamatus*. Ban Luang District, Nan province. (Dark blue square) (map by Wachira Sodob)

found in Tha Song Yang District, Tak Province, Thailand (red hollow circle in Fig. 5). The adult, from Tha Song Yang, was found on a road late at night, 2:30 AM, still alive, but fatally injured by a vehicle. The juvenile, from Tha Song Yang, was found nearby at night in ambush position on a limestone rock on a forested slope (Fig. 4B). The Tha Song Yang specimens were not examined as referred specimens, because the adult specimen carcass was badly damaged by a vehicle and the juvenile specimen was photographed, but not collected because it was found near a

wildlife protection area. Other snakes found in the same habitat at the type locality survey included Ovophis during the monticola (Günther), Trimeresurus [Popeia] popeiorum (Smith) (Viperidae), Pareas hamptoni (Boulenger), P. margaritophorus (Jan) (Pareatidae), Rhabdophis chrysargos (Schlegel), *Xenochrophis* piscator (Schneider) (Natricidae), Boiga cyanea (Duméril. Bibron and Duméril). В. multomaculata (Boie), Lycodon laoensis (Günther), L. septentrionalis (Günther), Oligodon cf. fasciolatus (Günther), Ptyas korros (Schlegel) (Colubridae), and lizards



FIGURE 6. Biotope of Protobothrops kelomohy sp. nov. (photo by M. Sumontha)

included *Cyrtodactylus inthanon* (Gekkonidae), *Acanthosaura* sp., *Calotes emma alticristata* (Schmidt), *Draco blanfordii* (Blanford) and *D. taeniopterus* (Günther) (Agamidae).

Comparisons. – A comparison of Protobothrops kelomohy sp. nov. with the other species of genus Protobothrops is shown in Table 5. The absence of a hornlike supraocular in Protobothrops kelomohy sp. nov. easily distinguishes it from P. cornutus and P. sieversorum; by its high number of ventrals, greater than 200, Protobothrops kelomohy sp. nov. (231-234) differs from P. dabieshanensis (187), P. elegans (179-196), P. jerdonii jerdonii (160-173), P. jerdonii bourreti (189-192), P. xanthomelas (176-188), jerdonii Р. mangshanensis (187-198), P. maolanensis (186-193), P. trungkhanhensis (188-194) and P. xiangchengensis (175-194); by its lesser number of mid-body scale rows, Protobothrops kelomohy sp. nov. (23) differs from P. flavoviridis (33-39), P.

himalayanus (25), P. kaulbacki (25) and P. tokarensis (31-33). Protobothrops kelomohy **sp. nov.** differs from *P. mucrosquamatus* by the number of mid-body dorsal scale rows, 23, all keeled distinctly vs 25 - 27 (rarely 23) and keeled, except the 1st row smooth in P. mucrosquamatus, one loreal (vs two) and the presence of vertical stripes from eyes (vs absent). Furthermore, **Protobothrops** kelomohy sp. nov. is slightly longer, has higher numbers of ventrals, supralabials and infralabials than P. mucrosquamatus (TL max 1,310 mm vs 1,280 mm, V 231 - 234 vs 194 – 233, SL 8 – 9 vs 7 – 8, IL 12 – 13 vs 11 – 12) (Table 5).

New species registration

The new name was registered in ZooBank with the following information: Publication LSID: unr:lsid:zoobank.org:pub: D57EBD57-AC85-4416-814F-7F28C21762AC; New species registration: urn:lsid:zoobank. org:act: ACC5F74A-E445-4B20-A444-422 8DDE24FA6

Key to the species of *Protobothrops*.

The key of Yang et al., (2011) is modified and updated using data from later publications (Gong et al., 2011; Huang et al., 2012; Pan et al., 2013; Luu et al., 2015) and this study, as follows.

1	Horn-like supraocular present
-	Horn-like supraocular absent
2	V 228 – 235 and SC 79 – 82 pairs of subcaudals <i>P. sieversorum</i>
-	V = 187 - 202 and SC 71 - 78 pairs of subcaudals <i>P. cornutus</i>
3	$MSR \ge 31.$
-	$MSR \le 27$
- 4	V 99 - 210 and ASR 31 - 33 P. tokarensis
4	V = 210 and ASR $S1 = 55$
-	More or less distinct darker marking on supralabial region
5	Uniform supralabial region
-	
6	Postocular stripe absent; body and head entirely blackish-brown marked with minute
	yellowish-green or rusty spots giving a reticulate pattern
-	Postocular stripe present
7	V > 197
-	V < 193
8	V 231 – 234, SC 80 – 84, MSR 23, 1 st dorsal scale row keeled, vertical stripe from eyes
	present
-	V 198 – 205, SC 71 – 75, MSR 25, 1^{st} dorsal scale row smooth, present of faint orange
	marking of the supralabials <i>P. himalayanus</i>
9	V 160 – 173 and SC 44 – 57 pairs P. jerdonii jerdonii
-	V 176 – 188 and SC 54 – 67 pairs P. jerdonii xanthomelas
-	V 189 – 192 and SC 65 – 72 pairs P. jerdonii bourreti
10	LOR 211
-	LOR 114
11	MSR $19 - 21$, 1^{st} dorsal scale row keeled
-	MSR 23 – 27, 1 st dorsal scale row smooth
12	Dorsal scale formula 19 – 19 – 17 P. trungkhanhensis
	Dorsal scale formula 25 (rarely 23) – 19 (21) – 17 P. maolanensis
13	V 194 – 233, SL 9 – 11, SC 44 – 66 pairs, $10 - 12$ scales between supraocular
	P xiangchengensis
-	V 175 – 194, SL 7 – 8, SC 70 – 100 pairs, $14 – 18$ scales between supraocular
	<i>P. mucrosquamatus</i>
14	• V 201 – 212, SC 66 – 78 pairs, dorsal scale formula 25 – 25 – 17
	P. kaulbacki
	V < 200
	MSR 23 – 25, PSR 19, SC 63 – 90 pairs, bold postocular strip and dorsum with yellow or
15	red-brown background color
-	MSR 21, PSR 15, SC 58 pairs, narrow post ocular stripe and the tip of tail orange,
-	differing from body color distinctly
	differing from body color distinctly F. audiesnanensis

DISCUSSION

We hypothesize that *P. kelomohy* **sp. nov.** is a distinct species based on obvious morphological character differences and interspecific mitochondrial DNA sequence divergences (Table 3-5 and Fig. 1), Moreover, phylogenetic cluster showed that *P. kelomohy* was most closely related to *P. kaulbacki* and *P. himalayanus*.

Protobothrops kelomohy sp. nov. is the second species of its genus known from Thailand. All individuals were found in remote montane areas of northern Thailand. The reddish-brown coloration of the new species appears to he an efficient camouflage in its habitat, especially with dry fallen leaves on the ground and shrub. According to local residents, the new species used to be common around the type locality, but now may be uncommon

because of expanding habitat destruction around agricultural areas and persecution by humans. Previously, all specimens found in the area were killed due to their venom considered to be potentially dangerous. Therefore, public knowledge and medical information about this snake should be made known to local people and healthcare providers to reduce conflict between snake and people. The herpetological diversity of montane forest is high. Herpetological surveys in the montane forests in northern Thailand during the last decade have led to numerous discoveries of species of amphibians and reptiles, including the first record of snakes, Plagiopholis blakewayi Boulenger (Tillack et al., 2006), Sinonatrix vunnanensis Rao and Yang (Pauwels et al., 2009), Ptvas nigromarginata (Vogel and Hauser, 2013), Parafimbrios laos Teynié et al. (Tevnié and Hauser, 2017). Protobothrops

TABLE 5. Comparison of morphological data among species in the genus Protobothrops

Species	TL max (mm)	v	SC	ASR	MSR	PSR	SL	IL	1 st DSR	SupO	Vertical stripe from eye
P. kelomohy sp. nov.	1310	231-234	80-84	23	23	17	8-9	12-13	Keeled	Smooth	Present
P. dabieshanensis	836	187	58	21	21	15	8	11	Smooth	Smooth	Absent
P. cornutus	680	189-202	71-78	19-23	19-21	15-17	9	12-14	Smooth	Horn-like	Absent
P. elegans	1287	179-196	63-90	23-25	23-25	19	7-8	10-12	Smooth	Smooth	Absent
P. flavoviridis	2315	217-239	72-95	35-39	33-37(39)	23-25	7-9	14-17	Smooth	Smooth	Absent
P. j. jerdonii	1090	160-173	44-57	(21)23-25	21(23)	15-17	7-8	11-12	Smooth	Smooth	Present/Absent
P. j. bourreti		189-192	65-72	21-23	21-23	15-17	7-8	11-12	Smooth	Smooth	Present/Absent
P. j. xanthomelas		176-188	54-67	(21)23-25	21-23	15-17	7-8	11-12	Smooth	Smooth	Present/Absent
P. himalayanus	1510	198-205	71-75	25	25	19	7-8	11-13	Smooth	Smooth	Absent
P. kaulbacki	1447	201-212	66-78	25(23)	25	19(17)	7-8	12-14	Smooth	Smooth	Absent
P. mangshanensis	2030	187-198	60-67	25	25	17	7-8	14-16	Smooth	Smooth	Present
P. maolanensis	805	186-193	74-85	23(25)	21(19)	17(15)	7-8	11-12	Keeled	Smooth	Absent
P. mucrosquamatus	1280	194-233	70-100	23-29	(23)25-27	17-23	9-11	11-17	Smooth	Smooth	Absent
P. tokarensis	1500	199-210	72-84	31-33	31(32-33)	23-25	7-9	12-16	Smooth	Smooth	Absent
P. trungkhanhensis	733	188-194	75-76	19	19	17	8-9	11-12	Keeled	Smooth	Absent
P. xiangchengensis	1150	175-194	44-66	25	25(23-24)	17	7-8	11-13	Smooth	Smooth	Absent
P. sieversorum	1257	228-235	79-82	23-24	21-23	17	8-10	13-14	Smooth	Horn-like	Present

mucrosquamatus (Cantor) (Vasaruchapong et al., 2017), Dendrelaphis nigroserratus Vogel et al., (2012), Liopeltis frenatus (Günther) and *Ptvas multicincta* (Roux) (Figueroa et al., 2016; Hauser, 2018); rare rediscovered. species were such as Opisthotropis spenceri Smith (Chuaynkern et al., 2014) and Paratapinophis praemaxillaris Angel (Murphy et al., 2008); new species and new country records were also recorded Diploderma of lizards. vunnanense (Anderson) (Manthey and Denzer, 2012; Wang et.al., 2018), Hemiphyllodactylus chiangmaiensis Grismer et al. (2014), Cvrtodactvlus doisuthep Kunya et al. (2014), C. inthanon Kunya et al. (2015) and Pseudocalotes kakhienensis (Anderson) from Doi Pui. Doi Inthanon and Omkoi District, Chiangmai Province (Montri Sumontha and Kirati Kunya observed data); new species of amphibians include Limnonectes tavlori Matsui et al. (2010), Leptolalax zhangvapingi Jiang et al. (2013), Tylototriton anguliceps Le et al. (2015), T. uvenoi Nishikawa et al. (2013), Gracixalus seesom Matsui et al. (2015), Minervarya (Suwannapoom et al.) chiangmaiensis (Suwannapoom et al., 2016; Niyomwan et al., 2019). We suggest that the further research in montane forests in northern Thailand and adjacent Myanmar and Laos requires additional attention.

Materials examined:

Protobothrops mucrosquamatus [QSMI 1525] Ban Luang district, Nan Province, northern Thailand.

Protobothrops sieversorum [QSMI 1556] Khammouane Subdivision, Laos PDR.

ACKNOWLEDGEMENTS

This study supported bv was Chonkolneenithi Foundation and Center of Excellence on Biodiversity (BDC), Office of Higher Education Commission (BDC-PG4-161009). We would like to thank Suthipong Thamawut and Sutee Ruangroj from TV Burapa for financially supporting the field survey, Borvorn Chaiyot, Dusit Tongsri, Surasak Putti, Paramet Loeschirarak, Siri Kantasing, Suwapich Kongsabuv for their assistance in the field survey, We also thank Panithi Laoungbua and Tanapong Tawan for their good caretaking of living specimens, Praphanth Iamwiriyakul receives our gratitude for latinizing the specific epithet from the Karen language as does, Watchira Sodob for providing the map, and Nararat Laopichienpong for suggestions on bioinformatic analysis. We also thank Patrick David. Merel J. Cox and Mark F. Hoover for their review and suggestions on this manuscript.

LITERATURE CITED

- Chuaynkern, Y., Duengkae, P., Pongcharoen, C., Chuaynkern, C. and Horsin, L. 2014. *Opisthotropis spenceri* Smith, 1918 (Serpentes: Natricidae): the third and fourth specimens. Journal of Wildlife Thailand, 21(1): 1-14.
- David, P., Tong, H., Vogel, G. and Tian, M. 2008. On the status of the Chinese pitviper *Ceratrimeresurus shenlii* Liang and Liu *in* Liang, 2003 (Serpentes, Viperidae), with the addition of *Protobothrops cornutus* (Smith, 1930) to the Chinese snake fauna. Asiatic Herpetological Research, 11: 17-23.
- Dowling, H.G. 1951a. A proposed standard system of counting ventrals in snakes. British Journal of Herpetology, 1: 97-99.
- Dowling H. G. 1951b. A Proposed Method of Expressing Scale Reductions in Snakes. Copeia, 2: 131-134.

- Figueroa, A., McKelvy, A.D., Grismer, L.L., Bell, C.D. and Lailvaux, S.P. 2016. A Species-Level Phylogeny of Extant Snakes with Description of a New Colubrid Subfamily and Genus. PLoS One, 11(9): e0161070. doi:10.1371/journal.pone.016070.
- Grismer, L.L., Wood Jr., P.L. and Cota, M. 2014. A new species of *Hemiphyllodactylus* Bleeker, 1860 (Squamata: Gekkonidae) from northwestern Thailand. Zootaxa, 3760(1): 067-078.
- Gong, S., Hitschfeld, E., Hundsdörfer, A.K., Auer, M., Wang, F., Zhou, L. and Fritz, U. 2011. Is the horned pitviper *Ceratrimeresurus shenlii* Liang and Liu, 2003 from China a valid *Protobothrops*?. Amphibia-Reptilia, 32: 132-135.
- Gumprecht, A., Tillack, F., Orlov, N.L., Captain, A. and Ryabov, S. 2004. Asian Pitvipers. Geitje Books Berlin, 368 pp.
- Guo, P., Pang, J.F., Zhang, Y.P. and Zhao, E.M. 2006. A re-analysis of the phylogeny of the genus *Protobothrops* (Reptilia: Viperidae), with particular reference to the systematic position of *P. xiangchengensis*. Amphibia-Reptilia, 27: 433-439.
- Guo, P., Malhotra, A., Li, C., Creer, S., Pook, C.E. and Wen, T. 2009. Systematics of the *Protobothrops jerdonii* complex (Serpentes, Viperidae, Crotalinae). Herpetological Journal, 19: 85-96.
- Guo, P., Liu, Q., Li, C., Chen, X., Jiang, K., Wang, Y.Z. and Malhotra, A. 2011. Molecular phylogeography of Jerdon's pitviper (*Protobothrops jerdonii*): importance of the uplift of the Tibetan plateau. Journal of Biogeography, 38: 2326–2336. Available from: http://dx.doi.org/ 10.1111/j.1365-2699.2011.02566.x (accessed 1 August 2018)
- Guo, P., Liu, Q., Wen, T., Xiao, R., Fang, M., Zhong, G., Truong, N.Q., Zhu, F., Jadin, R.C. and Li, C. 2016. Multilocus phylogeny of the Asian Lanceheaded pitvipers (Squamata, Viperidae, *Protobothrops*). Zootaxa, 4093(3): 382-390.
- Hauser, S. 2018. Addition of *Liopeltis frenatus* (Günther, 1858) and *Cyclophis multicinctus* (Roux, 1907) to the herpetofauna of Thailand (Squamata: Colubridae). Tropical Natural History, 18(1): 54-67.
- Hoge, A.R. and Romano Hoge, S.A. 1980/1981. Notes on micro and ultrastructure of "Oberhäutschen" in Viperoidea. Memorias do Instituto Butantan 44/45, 81-118.
- Huang, X., Pan, T., Han, D.M., Zhang, L., Hou, Y.X., Yu, L., Zheng, H.M. and Zhang, B.W. 2012. A new species of the genus *Protobothrops* from the

Dabie Mountains, Anhui, China. Asian Herpetological Research, 3(3): 213-218.

- Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: Bayesian Inference of Phylogenetic Trees. Bioinformatics, 17: 754-755.
- Jiang, K., Yan, F., Suwannapoom, C., Chomdej, S. and Che, J. 2013. A new species of the genus *Leptolalax* (Anura: Megophryidae) from northern Thailand. Asian Herpetological Research, 4(2): 100-108.
- Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33(7): 1870-4.
- Kunya, K., Panmongkol, A., Pauwels, O.S.G., Sumontha, M., Meewassana, J., Bunkhwamdi, W. and Dangsri, S. 2014. A new forest-dwelling Bent-toed Gecko (Squamata: Gekkonidae: *Cyrtodactylus*) from Doi Suthep, Chiang Mai Province, northern Thailand. Zootaxa, 3811(2): 251-261.
- Kunya, K., Sumontha, M., Panitvong, N., Dongkumfu, W., Sirisamphan, T. and Pauwels, O.S.G. 2015. A new forest-dwelling Bent-toed Gecko (Squamata: Gekkonidae: *Cyrtodactylus*) from Doi Inthanon, Chiang Mai Province, northern Thailand. Zootaxa, 3905(4): 573-584.
- Le, D.T., Nguyen, T.T., Nishikawa, T., Nguyen, S.L.H., Pham, A.V., Matsui, M., Bernardes, M. and Nguyen, T.Q. 2015. A new species of *Tylototriton* Anderson, 1781 (Amphibia: Saramandridae) from northern Indochina. Current Herpetology, 34(1): 38-50.
- Liu, Q., Myers, E.A., Zhong, G.H., Hu, J., Zhao, H. and Guo, P. 2012. Molecular evidence on the systematic position of the lance-headed pitviper *Protobothrops maolanensis* Yang *et al.*, 2011. Zootaxa, 3178: 57-62.
- Luu, V.Q., Nguyen, T.Q., Lehmann, T., Bonkowski, M. and Ziegler, T. 2015. New records of the Horned Pitviper, *Protobothrops cornutus* (Smith, 1930) (Serpentes: Viperidae), from Vietnam with comments on morphological variation. Herpetology Notes, 8: 149-152.
- Maki, M. 1931. A Monograph of the Snakes of Japan. Dai-ichi Shobo Tokyo, 240 pp.
- Malhotra, A. and Thorpe, R.S. 2000. A phylogeny of the *Trimeresurus* group of pit vipers: new evidence from a mitochondrial gene tree. Molecular Phylogenetics and Evolution, 16: 199-211.
- Malhotra, A. and Thorpe, R.S. 2004. A phylogeny of four mitochondrial gene regions suggests a

revised taxonomy for Asian pit vipers (*Trimeresurus* and *Ovophis*). Molecular Phylogenetics and Evolution, 32: 83-100.

- Manthey, U. and Denzer, W. 2012. Bemerkungen zur Verbreitung von *Japalura yunnanensis* Anderson, 1878 (Squamata: Agamidae: Draconinae). Sauria, 34(2): 35-40.
- Matsui, M., Panha, S., Khonsue, W. and Kuraishi, N. 2010. Two new species of the "kuhlii" complex of the genus *Limnonectes* from Thailand (Anura: Dicroglossidae). Zootaxa, 2615: 1-22.
- Matsui, M., Khonsue, W., Panha, S. and Eto, K. 2015. A new tree frog of the genus *Gracixalus* from Thailand (Amphibia: Rhacophoridae). Zoological Science, 32(2): 204-210.
- Murphy, J.C., Chan-ard, T., Mekchai, S., Cota, M. and Voris, H.K. 2008. The Rediscovery of Angel's Stream Snake, *Paratapinophis praemaxillaris* Angel, 1929 (Reptilia: Serpentes: Natricidae). The Natural History Journal of Chulalongkorn University, 8(2): 169-183.
- Niyomwan, P., Srisom, P. and Phawangkhanant, P. 2019. Amphibians of Thailand. Parbpim, Bangkok. 478 p. (in Thai)
- Nishikawa, K., Khonsue, W., Pomchote, P. and Matsui, M. 2013. Two new species of *Tylototriton* from Thailand (Amphibia: Urodela: Salamandridae). Zootaxa, 3737(3): 261-279.
- Orlov, N.L., Ryabov, S.A. and Nguyen, T.T. 2009. Two new species of genera *Protobothrops* Hoge et Romano-Hoge, 1983 and *Viridovipera* Malhotra et Thorpe, 2004 (Ophidia: Viperidae: Crotalinae) from karst region in northeastern Vietnam. Part I. Description of a new species of *Protobothrops* Genus. Russian Journal of Herpetology, 16: 69-82.
- Pan, H., Chettri, B., Yang, D., Jiang, K., Wang, K., Zhang, L. and Vogel, G. 2013. A New Species of the Genus *Protobothrops* (Squamata: Viperidae) from Southern Tibet, China and Sikkim, India. Asian Herpetological Research, 4(2): 109-115.
- Pauwels, O.S.G., Kunya, K., David, P. and Sumontha, M. 2009. First record of the Yunnan Keelback *Sinonatrix yunnanensis* Rao and Yang, 1998 (Serpentes: Natricidae) from Thailand. Salamandra, 45(3): 165-169.
- Smith, M.A. 1943. The fauna of British India, Ceylon and Burma. Reptilia and Amphibia, Vol. III -Serpentes. Taylor and Francis London, 583 pp.
- Sumontha, M., Kunya, K., Pauwels, O.S.G., Nitikul, A. and Punnadee, A. 2011. *Trimeresurus (Popeia) phuketensis*, a new pitviper (Squamata: Viperidae)

from Phuket Island, Southwestern Thailand. Russian Journal of Herpetology, 18(3): 185-194.

- Suntrarachun, S., Chanhome, L., Thaweekarn, W, and Sumontha, M. 2014. Molecular identification of venomous snakes in Thailand using PCR-RFLP. International Journal of Pure & Applied Bioscience, 2(1): 133-138.
- Suntrarachun, S., Chanhome, L., and Sumontha, M. 2018. Identification of sea snake meat adulteration in meat products using PCR-RFLP of mitochondrial DNA. Food Science and Human Wellness, 7: 170-174.
- Suwannapoom, C., Yuan, Z.Y., Poyarkov Jr., N.A., Yan, F., Kamtaeja, S., Murphy, R.W. and Che, J. 2016. A new species of genus *Fejervarya* (Anura: Dicroglossidae) from northern Thailand. Zoological Research (Kunming), 37: 227-237.
- Tanabe, A. S. 2011. Kakusan-4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate model for combined molecular phylogenetic analyses of multilocus sequence data. Molecular Ecology Resources, 11: 914-921.
- Teynié, A. and Hauser, S. 2017. First Record of *Parafimbrios lao* Teynié, David, Lottier, Le, Vidal et Nguyen, 2015 (Squamata: Caenophidia: Xenodermatidae) for Thailand. Russian Journal of Herpetology, 24(1): 41-48.
- Tillack, F.B., Scheidt, U.E. and Ihle, T.L. 2006. First record of Blakeway's mountain snake, *Plagiopholis blakewayi* Boulenger, 1893, from Thailand, with remarks on the distribution of *Plagiopholis nuchalis* (Boulenger, 1893) (Reptilia: Squamata: Colubridae, Pseudoxenodontinae). Veröffentlichungen Naturkundemuseum Erfurt, 25: 181–186.
- Uetz, P., Freed, P. and Hošek, J. 2018. The Reptile Database, Available from: http://www.reptiledatabase.org, (accessed on 16 August 2018)
- Vasaruchapong, T., Laoungbua, P., Tangrattanabul, K., Tawan, T. and Chanhome, L. 2017. *Protobothrops mucrosquamatus* (Cantor, 1839), A highly venomous species added to the snake fauna of Thailand (Squamata: Viperidae). Tropical Natural History, 17(2): 111-115.
- Vogel, G. and Hauser, S. 2013. Addition of *Ptyas* nigromarginata (Blyth, 1854) (Squamata: Colubridae) to the Snake Fauna of Thailand with Preliminary Remarks on Its Distribution. Asian Herpetological Research, 4(3): 166-181.
- Vogel, G., Rooijin, J.V. and Hauser, S. 2012. A new species of *Dendrelaphis* Boulenger, 1890

(Squamata: Colubridae) from Thailand and Myanmar. Zootaxa, 3392: 35-46.

- Wang, K., Che, J., Lin, S., Deepak, V., Datta-Roy, A., Jiang, K., Jin, J., Chen, H. and Siler, C.D. 2018. Multilocus phylogeny and revised classification for mountain dragons of the genus *Japalura s.l.* (Reptilia: Agamidae: Draconinae). Asia. Zoological Journal of the Linnean Society, 185(1): 1-22.
- World Health Organization (WHO) 2016. WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins, WHO press, Geneva, 146. Available from: http://www.who.int/biologicals/ECBS_2016_BS2 300_WHO_Guidelines_antivenom_clean1.pdf, (accessed 8 September 2018).
- Wüster, W., Peppin, L., Pook, C.E. and Walker, D.E. 2008. A nesting of vipers: Phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). Molecular Phylogenetics and Evolution, 49: 445-459. http://dx.doi.org/10. 1016/j.ympev.2008.08.019
- Yang, J.H., Orlov, N.L. and Wang, Y.Y. 2011. A new species of pitviper of the genus *Protobothrops* from China (Squamata: Viperidae). Zootaxa, 2936: 59-68.
- Zhao, E.M. 2006. Snakes of China. Anhui Science and Technology Publishing House Hefei, 372 pp.
- Ziegler, T., Herrmann, H.W., David, P., Orlov, N.L. and Pauwels, O.S.G. 2000. *Triceratolepidophis* sieversorum, a new genus and species of pitviper (Reptilia: Serpentes: Viperidae: Crotalinae) from Vietnam. Russian Journal of Herpetology, 7: 199-214.