

A New Species of the Genus *Trimeresurus* from Southwest China (Squamata: Viperidae)

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Abstract Species from the *Trimeresurus popeiorum* complex (Subgenus: *Popeia*) is a very complex group. *T. popeiorum* is the only *Popeia* species known from China. During the past two years, five adult *Popeia* specimens (4 males, 1 female) were collected from Yingjiang County, Southern Yunnan, China. Molecular, morphological and ecological data show distinct differences from known species, herein we describe these specimens as a new species *Trimeresurus yingjiangensis* sp. nov. Morphologically, the new species distinct from other *Popeia* species by a combination of following characters: (1) dorsal body olive drab, without cross bands on the scales; (2) a conspicuous bicolor ventrolateral stripe present on each side of males, first row of dorsal scales firebrick with a white ellipse dot on posterior upper part in male, these strips absent in females; (3) eyes firebrick in both gender; (4) suboculars separated from 3rd upper labial by one scale on each side; (5) ventrals 164–168 ($n = 5$); (6) MSR 21.

Keywords *Popeia*, morphology, phylogenetics, geographical isolation, *Trimeresurus yingjiangensis* sp. nov.

1. Introduction

Asian green pitvipers (*Trimeresurus*) is a very complex group, generally with a green throughout body, tail red on back, and most with lateral lines and postocular stripes in males, while in females these lateral lines and postocular stripes are often absent (Creer *et al.*, 2004; Gumprecht *et al.*, 2004; Malhotra and Thorpe, 2004; Vogel *et al.*, 2004; Guo *et al.*, 2015). There are 51 species in the genus *Trimeresurus* recorded to date in Asia, and the diversity of this genus may still be underestimated (Uetz *et al.*, 2018;

Wostl *et al.*, 2016). Due to their similar body pattern and scales among species and widespread distribution of few species, the species in this genus is difficult to be identified. Based on morphological and molecular characters, genus *Popeia* was split from *Trimeresurus* by Malhotra and Thorpe (2004). Subsequently David *et al.* (2009) and Sumontha *et al.* (2011) relegated *Popeia* as the status of a subgenus, respectively. However, broad scale phylogenetic analysis places them in a single monophyletic clade and are considered as *Trimeresurus* (Figueroa *et al.*, 2016; Pyron *et al.*, 2013). For the simplicity purpose, we will use *Trimeresurus* instead of *Trimeresurus (Popeia)* when referring the species of subgenus *Popeia* in following text. As well as for the subspecies of *T. sabahi*, we will omit “*sabahi*” at the middle.

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Vogel *et al.* (2004) made a new comb of the *T. popeiorum* complex based on morphological characters, and described two new species: *T. fucatus* and *T. nebularis*. Grismer *et al.* (2006) reported *T. buniana* as a new species referred to this subgenus. Then David *et al.* (2009) described *T. toba* from Indonesia. Thus, subgenus *Popeia* was consisted of six species: *T. popeiorum*, *T. fucatus*, *T. nebularis*, *T. sabahi*, *T. barati* and *T. toba*.

A recent research argued that *T. fucatus*, *T. nebularis*, *T. sabahi*, *T. barati* and *T. toba* might be barely a single species, *T. sabahi* (Wostl *et al.*, 2016), because differences in the morphological characters between species could be eliminated when adding new specimens with medium measures. In addition, their molecular characters could not be discriminated. However, considering the morphological distinctiveness, genetic diversity and ecologically distinct, Mulcahy *et al.* (2017) believed that *T. popeiorum*, *T. nebularis*, *T. sabahi* should be consider as distinct species, as well as the specimens from Phangt-nga, Thailand (B467) should be recognize as *T. phuketensis* and the specimens from Phetburi, Thailand (B52 and B34) and Tanintharyi Div.; Kawthaung Dist. Myanmar should be recognized as a new species *T. sp* (Mulcahy *et al.*, 2017). Additionally, those remaining controversial and closely related populations of *T. sabahi* (*T. sabahi fucatus*, *T. sabahi barati*, *T. sabahi buniana* and *T. sabahi toba*) should be recognized as subspecies of *T. sabahi*. Indeed, the concept of subspecies is very helpful in solving the current chaotic situation, but this seems to be a compromise for the controversial situation. Therefore, the taxonomy within this genus remains controversial.

So far, although *Trimeresurus* is widespread in many places of Asia (Davie *et al.*, 2009; Malhotra and Thorpe, 2004; Mulcahy *et al.*, 2017; Vogel *et al.*, 2004; Wostl *et al.*, 2016), *T. popeiorum* is the only *Popeia* species distributed in China (Guo *et al.*, 2015). However, during our animal surveys in Yunnan Province in 2017, we collected five specimens of subgenus *Popeia*, they are similar in morphology to *T. popeiorum*, but can be distinguished from *T. popeiorum* by the absence of postocular streaks, differences in scales and body patterns. Herein, based on the molecular and morphological data by including all available specimens available in GenBank, we proposed it as a new species.

2. Materials and Methods

2.1. Morphological analysis We examined three male specimens preserved in the museum of Chengdu Institute

of Biology (CIB), Chinese Academy of Sciences, one male specimen preserved in the museum of Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences and one female specimen preserved in the museum of Guangdong Institute of Applied Biological Resources (GIABR).

Body and tail length were measured with a tape ruler to the nearest 1 mm: total length (TL) from the tip of snout to the tip of tail; snout-vent length (SVL) from the tip of snout to anterior margin of cloaca; tail length (TaL) from posterior margin of cloaca to the tip of tail. Other measurements were conducted with a digital caliper to the nearest 0.1 mm: head length (HL) from the snout tip to the posterior margin of the mandible; head width (HW) at the maximal widest part of the head; head height (HH) at the maximal highest part of the head; the eye horizontal diameter (ED); Ratio of tail length to total length (TaL/TL). The same observer to avoid inter-observer bias collected all data. The following comparative scales and maxillary teeth were counted: the dorsal scale rows (DSR); the ventrals (VEN); the subcaudals (SC); the supralabials (SL); the infralabials (INF) and Cephalic scales (scales on a line between the middle of supraoculars, Cep); the eye vertical diameter (VED); distance lower eye margin–edge of the lip (DEL). Morphological measurements are listed in Table 1. Information on some morphological characters were also obtained from written accounts from Pope and Pope (1933), Taylor *et al.* (1958), Vogel *et al.* (2004), Grismer *et al.* (2006), Sanders *et al.* (2006), David *et al.* (2009), Wostl *et al.* (2016). Color description reference wiki color-coding.

2.2. Molecular Phylogenetic analysis Genomic DNA was extracted from the collected tissue samples at the CIB. Four specimens from Yingjiang County, Yunnan Province were used for DNA extraction. Four fragments of the mitochondrial were specifically amplified, including *cytochrome b* and one nuclear gene fragment (*cyt b*, L14910/H16064, 1100bp, Burbrink, *et al.* 2000), *NADH dehydrogenase subunit 4* (ND4, ND4/Leu, 670bp, Parkinson *et al.*, 2000; Shi *et al.*, 2017), *12S ribosomal RNA* (12S, 12SFPh/RVal, 659 bp, Knight and Mindell, 1993) and *16S ribosomal RNA* (16S, 16sF-L/16sR-H, 500bp, Parkinson *et al.*, 2000), *Oocyte maturation factor Mos gene* (C-mos, S77/S78, 587bp, Pyron *et al.*, 2009).

PCR amplifications were performed in 25 μ l reactions by using the following cycling conditions: initial denaturation for 2min at 95 °C, followed by 35 cycles: denaturation at 94 °C for 40 s, annealing at different temperatures (48.5 °C for *cyt b*, 56 °C for ND4 and

Table 1 Measurements (0.1 mm) of *Trimeresurus yingjiangensis* sp. nov. Missing data are denoted with “-”

Taxa	Voucher	Preserve	Locality	Sex	DOR	VEN	SC	SL	INF	Cep	SVL	TaL	TL	TaL/TL	HL	HW	HH	ED
<i>Trimeresurus yingjiangensis</i>	DL2017070101	CIB	Yingjiang, Yunnan	M	21-21-15	164	73	10/10	12/12	11	688	176	864	0.204	31.5	22.2	11.7	13.4
<i>T. yingjiangensis</i>	ZLtspynglg201801	GIABR	Yingjiang, Yunnan	F	21-21-15	168	60	10/10	12/12	11	710	139	849	0.163	-	-	-	-
<i>T. yingjiangensis</i>	DL201070102	CIB	Yingjiang, Yunnan	M	21-21-15	164	76	10/11	12/12	11	594	148	742	0.199	29.8	18.1	9.5	12.9
<i>T. yingjiangensis</i>	DL201070103	CIB	Yingjiang, Yunnan	M	21-21-15	167	72	10/10	12/12	11	675	178	853	0.209	31.4	19.7	9.8	13.2
<i>T. yingjiangensis</i>	OY2671	IVPP	Yingjiang, Yunnan	M	21-21-15	166	71	9/10	12/12	12	692	194	886	0.219	33.6	20.8	11.7	14.2

C-mos, 52 °C for 12S and 16S) for 25 s, elongation at 72 °C for 15 s, and then finalized with elongation step of 2min at 72 °C, with the PTC-100 thermal cycler (BioRad, USA). The products were purified by using the DNA Agarose Gel Extraction Kit (Omega, USA) according to the manufacturer’s instructions. Purified PCR products were sequenced using the same PCR primers. Sequencing was completed by Beijing Qingke New Industry Biotechnology Co., Ltd. Sequence data were uploaded to GenBank, available accession numbers showed in Table S1.

All sequences were aligned with other retrieved sequences on the same gene loci, respectively, by using software MEGA 7 (Kumar *et al.*, 2016), after converting the file format with the software BioEdit (Alzohairy, 2011). Our dataset totally contained 38 specimens, each with 2 567 base pairs. Partition finder 2.1.1 under BIC (Lanfear *et al.*, 2012) identified the optimal models of sequence evolution for each partition. Therefore, dataset was combined into three partitions. The evolution models of each partition combination were as follows: partition 1: 16S 1, 12S 3, 12S 1, 16S 3, 16S 2, 12S 2, *cyt b* 1, ND4, 3, HKY+I+G, 1424 bp; partition 2: ND4 1, *cyt b* 2, HKY+I, 572bp; partition 3: *cyt b* 3, ND4 2, GTR+G, 571bp.

General time reversible (GTR+I+G) model, the most probable substitution model for the uncorrected *cyt b p-distance* matrix, was applied on MEGA 7 (Kumar *et al.*, 2016). Phylogenetic relationships derived from the combined gene fragments were performed based on Bayesian Inference (BI), which was conducted by using MrBayes 3.2 (Ronquist *et al.*, 2012), and all searches consist of three heated chains and a single cold chain. Three independent iterations with each comprising two runs of 20 000 000 generations are applied, sampling every 1 000 generations. The parameter estimates were plotted against the generation. The first 25 percent of the samples were discarded as burning. ML trees were constructed by using the program RaxML v7.2.6 (Stamatakis, 2006) in GTRGAMMA model with 1 000 fast bootstrap repeats. Outgroups were chosen based on close phylogenetic relationship between taxa (Mulcahy *et al.*, 2017).

3. Result

Taxonomic position

Class: Reptilia, Order: Squamata, Suborder: Serpentes, Family: Viperidae

Trimeresurus yingjiangensis sp. nov. Chen, Ding, Shi and Zhang, 2018.

ZooBank accession: BD3E2F08-1C1E-40E7-AEBA-E37380268F06

Etymology. The specific name *yingjiangensis* refers to the location of type specimens, Yingjiang Country, Yunnan Province, China. The common name is suggested as “Yingjiang green pitviper” in English and “Ying jiāng zhú yè qīng (盈江竹叶青)” in Chinese.

Holotype. DL2017070101 (CIB), adult male, collected from the Heihe Village, Kachang Town, Yingjiang County, Yunnan Province (24.782° N, 97.878° E, 1 112 m a.s.l) by Li DING on 19 July 2017 (Figure 1).

Paratypes (four specimens). ZL-tspynglg-2018-01 (allotype, GIABR) adult female, collected from Heping Village, Tongbiguan Town, Yingjiang County, Yunnan Province (24.584° N, 97.738° E, 1 200 m) by Jian XU (Figure 1 and Figure 2). DL201070102 (CIB), adult male, and DL201070103 (CIB), adult male, collected by Li DING at the same time as Holotype. OV2671 (IVPP), collected from the Yingjiang County (24.734° N, 97.843°

E, 1 074 m) by Jingsong SHI on 6 September 2017 (Figure 2).

Diagnosis. *Trimeresurus yingjiangensis* sp. nov. is assigned to *Popeia* group by hemipenes morphology (Malhotra and Thorpe, 2004), differ with its congeners by a combination of following characters: (1) dorsal body olive drab, without cross bands on the scales; (2) a conspicuous bicolor ventrolateral stripe present on each side of males, first row of dorsal scales firebrick with a white ellipse dot on posterior upper part in male, these strips absent in females; (3) the eyes firebrick in both gender; tail red, mottled with green laterally, and the ventrolateral stripes discontinuous on the tail; (4) hemipenes long, reaching 23rd to 25th SC, forked opposite 5-6th SC ($n = 4$), bifurcated near the base and the sulcus spermaticus split from the apex to basal without spines; (5) 21 DSR at middle body, moderately keeled; VEN = 164–168 ($n = 5$), SC = 60–76 ($n = 5$); Sexual dimorphism, the female has more ventrals and fewer subcaudals than males; (6) tail long, with ratios of TaL/TL between 0.199



Figure 1 *Trimeresurus yingjiangensis* sp. nov. in life: A and B, holotype, male: DL2017070101, photo by Li DING; C allotype, female: ZL-tspynglg-2018-01, photo by Liang ZHANG; D, paratype, male: DL201070102, has a weak and short post ocular streak, photographed by Li DING.



Figure 2 Three of the paratypes of *T. yingjiangensis* sp. nov.: DL201070102 (A), picture took after the specimen euthanized by freezing, body color start to change from olive drab to green yellow (photographed by Shengchao SHI); DL201070103 (B), picture took after the specimen injecting alcohol, body color start to change from olive drab to olive (photographed by Shengchao SHI); OV2671 (C), picture took after the specimen conserved in 80% ethanol after seven months, body color becomes olive (photographed by Jingsong SHI). The red arrow indicates the color change. Scale: 25 mm

and 0.219 in male.

Comparison with other species. *T. yingjiangensis* sp. nov. is distinct from *T. popeiorum* (Lectotype, Pope and Pope, 1933) by the following characters: (1) First row of dorsal scales on each side firebrick with a white ellipse dot on posterior upper part in males vs. “brown with yellow tip” in males; (2) lowest quarter of second row white in males vs. “yellow below keel”; (3) suboculars separated from 3rd upper labial by one scale on each side vs. “two scales on each side”; (4) upper part of second upper labial separated from nasal by two small scales smaller than nostril on either side vs. “separated from nasal by two large scales on either side”.

T. yingjiangensis sp. nov. is distinct from *T. nebularis* by the following characters: (1) body olive drab, upper lips green with white tip, vs. body bright green with blue tones and blue upper lips; (2) eyes firebrick vs. pale green; (3) ventrolateral stripes bicolor in males vs. uniformly white or blue; (4) in males: ventrals 164–167 ($n = 4$), vs. 149–153 ($n = 8$), subcaudals 71–76 ($n = 4$) vs. 61–65 ($n = 8$).

T. yingjiangensis sp. nov. is different from *T. sabahi*, *T. cf. sabahi* (from Sumatra), *T. fucatus*, *T. barati* and *T. toba* by following characters: (1) MSR 21 (vs. MSR 19 in *T.*

barati); (2) dorsal color was olive drab with conspicuous bicolor ventrolateral stripes and no crossbars (vs. dorsal color was green with irregular rusty or reddish-brown dorsal crossbands and white dots on the vertebral scales in *T. fucatus*); (3) the ventrolateral stripe bicolor in males (vs. white in males of *T. cf. sabahi* from Sumatra, and white in males of *T. toba*); (4) the temporal scales smaller than those in *T. toba*; (5) no ventrolateral stripe present on females (vs. white or yellow ventrolateral stripe present on females of *T. sabahi*); (6) the eyes firebrick (vs. yellow in *T. sabahi*, and orange in *T. barati*); (7) VEN 164–168, $n = 5$ (vs. VEN 142–158, $n = 17$ in *T. barati*, 147–157, $n = 14$, in *T. sabahi*, and 153–155, $n = 3$, in Sumatra *T. cf. sabahi*). The comparisons of main morphological characters are summarized in Table 2.

Description of the holotype. Body elongated; head triangular, wide at base, length 1.4 times as long as width, clearly distinct from neck; snout moderate, accounting for 23.4% of HL, 1.7 times as long as ED, eyes large, VED/DEL ratio 0.8, pupil vertical in life.

SVL: 688 mm; TaL: 176 mm; TL: 864 mm; TaL/TL: 0.204.

VEN: 164; SC: 73, paired, plus one terminal scale; anal shield entire.

DSR: 21–21–15 scales, moderately keeled, first row smooth.

Hemipenes long, reaching to 23–24 SC, forked opposite 5–6th plate, no spines (Figure 3F)

Rostral overall trapezoidal, lower margin of rostral nearly 3 times wider than upper margin, height two third of width at base, obliquely truncated when seen from lateral side; nasal large, undivided, dorsal margin forming part of canthus rostralis; internasals distinct, elongate about 2.3 times as long as wide, separated by a scale of one-half size of each internasal; 4/4 canthal scales bordering the *canthus rostralis* between the internasal and corresponding supraocular, slightly larger than adjacent snout scales; 1 elongated loreal between upper preocular and nasal; 2 upper preoculars above the loreal pit; elongated and in contact with the loreal; 2/1 postoculars; 1 entire, long and elongated supraocular on left side, about 3.9 times as long as wide, about 1.2 times as wide as the internasals, supraocular on right side broken into 2 scales, the former scale twice larger than the rear scale; supraocular indented on their inner margin by the upper head scales; scales on upper snout surface smooth, juxtaposed, irregular in shape, enlarged, with 6 snout scales on a line between the scale separating the internasals and a line connecting the anterior margins of eyes; cephalic scales small, irregular, smooth, 11 Cep in a line between supraoculars; occipital scales flat and feebly keeled; temporals small, subequal, in 4/4 rows, smooth; 1 thin, elongated, subocular crescent-like, surrounded by 12/11 scales; 10 supralabials on both sides; the 1st supralabial triangular; the 2nd, high, 1.8 times as high as wide, upper part concave, forming the anterior border of loreal pit, separated from nasal by two small scales on either side of the size about or smaller than nostrils, the upper of the two scales inverted trapezoidal while the lower scale long rectangular and smaller; the 3rd widest, 1.2 times as wide as high, separated from subocular by one small scale on either side; the 4th irregularly quadrangular/pentagonal, smaller than the 3rd, right below the pupil and separated from subocular at least by one scale on both sides. the 5th smaller than the 4th, separated from subocular by at least 2/1 scales, others in contact with the first row of temporals; 12 infralabials on both sides, the first pair of infralabials in contact with each other and the first three pairs in contact with the chin shields.

In life, the dorsal color was olive drab with conspicuous bicolor ventrolateral stripes and no crossbars; first row of DSR on each side firebrick with an white ellipse dots on posterior upper part; lowest

Table 2 Comparisons of main morphological characters among *Trimeresurus (Popeia)* species (data based from Vogel *et al.*, 2004; Grismer *et al.*, 2006; Sanders *et al.*, 2006; David *et al.*, 2009; Wostl *et al.*, 2016) TaL/TL = Tail length/Total length, VEN = ventral scales, SC = subcaudal scales, MSR = dorsal scale rows at midbody, POS = postocular stripe, VES = ventrolateral stripe, M = males, F = females. Bold text indicated the different characters.

Taxa	TaL/TL		VEN		SC		MSR		POS		VES		Eye color		
	M	F	M	F	M	F	Both sex	M	F	M	F	M	F	M	F
<i>T. yingjiangensis</i> (n = 5)	0.20–0.22	–	164–167	168	71–76	60	21	Absent	Absent	Bicolor	Absent	Firebrick	Firebrick	Firebrick	F
<i>T. popeiorum</i> (n = 32)	0.18–0.21	0.15–0.17	151–166	154–168	59–75	56–64	21	Bicolor, wide	White, wide or absent	Bicolor, wide	White	Deep red	Deep red	Deep red	
<i>T. sabahi</i> (n = 14)	0.19–0.24	0.17–0.18	147–157	148–156	69–76	59–65	21	Absent	Absent	Bicolor	White or yellow	Deep red or orange	Deep red or orange	Deep red or orange	
<i>T. barati</i> (n = 17)	0.19–0.23	0.15–0.18	142–153	170	62–73	55–63	17–19	Absent	Absent	White or bicolor	Absent	Orange	Orange	Orange or yellow	
<i>T. buaniana</i> (n = 4)	0.22–0.23	0.22	170–174	170	76–78	61	21	Present	Absent	Bicolor	White or bicolor	Gold	Turquoise with maroon center	Turquoise with maroon center	
<i>T. fucatus</i> (n = 115)	0.20–0.24	0.16–0.19	156–171	151–170	69–84	59–73	19–21	White, bicolor	Absent	Bicolor	White	Yellow-green, gold or copper	Yellow-green, gold or copper	Yellow-green, gold or copper	
<i>T. toba</i> (n = 6)	0.20–0.23	0.15–0.18	153–159	147–156	73–77	57–64	19–21	Absent	Absent	Bicolor, thin	Bicolor, thin or faint, or absent	Deep orange	Deep orange	Deep orange	
<i>T. nebularis</i> (n = 8)	0.19–0.20	0.17–0.17	149–153	147–153	61–65	50–60	21	Absent	Absent	White or blue	Absent	Pale green	Pale green	Pale green	
<i>T. cf. sabahi</i> (n = 3)	0.212–0.23	0.157	153–155	153	73	58	21	Absent	Absent	White	Absent	unknown	unknown	unknown	

quarter of second row white, from neck to over half of tail; gradually disconnected on tail; upper half of 2nd row to 11th row of DSR edged with sky blue and mottled with irregular sky blue dots. Ventral surface was yellow green, ventrals start from 101th to anal scale edged with firebrick on both sides. Dorsal tail heavily mottled with dark red blotches, the dark red blotches contiguous posteriorly; tip of tail Indian red on dorsal while dark salmon on ventral. Head olive drab, without postocular streak; eyes firebrick, with a narrow golden edge around pupil; upper lips green yellow, supralabials and infralabials green yellow edged with white and molted with light cyan.

Intraspecific morphological variation. All five specimens were collected from the same location with similar body coloration and body shape with only one sample (DL201070102, Figure 1) with a short postocular streak posterior to eyes; 10 supralabials in general but one sample (OV2671) with only 9 and 11 supralabials on two sides, respectively; ventrals 164–167 in males, while

168 in females; subcaudals 71–76 in males, while 60 in females.

Molecular phylogeny. Considering that there are very little nuclear gene data about genus *Trimeresurus* in NCBI, and the sequence is rarely from the same specimen, we only listed the C-mos gene accession numbers for future research, but did not use this gene fragments when building the gene tree. Result showed that the two consistent phylogenetic trees were achieved by using Bayesian Inference (BI) and Maximum likelihood (ML) methods (Figure 4), respectively. The topological structure of the phylogenetic trees are approximately identical with those of earlier studies (Guo *et al.*, 2015; Kate *et al.*, 2004; Malhotra *et al.*, 2000; Malhotra *et al.*, 2004; Mulcahy *et al.*, 2017). All subgenus species should be consider as six clades including *T. sabahi* complex, *T. yingjiangensis* sp. nov., *T. popeiorum*, *T. nebularis*, *T. phuketensis* and *T. sp.* Additionally, all of the four specimens of *T. yingjiangensis* sp. nov. performs as a

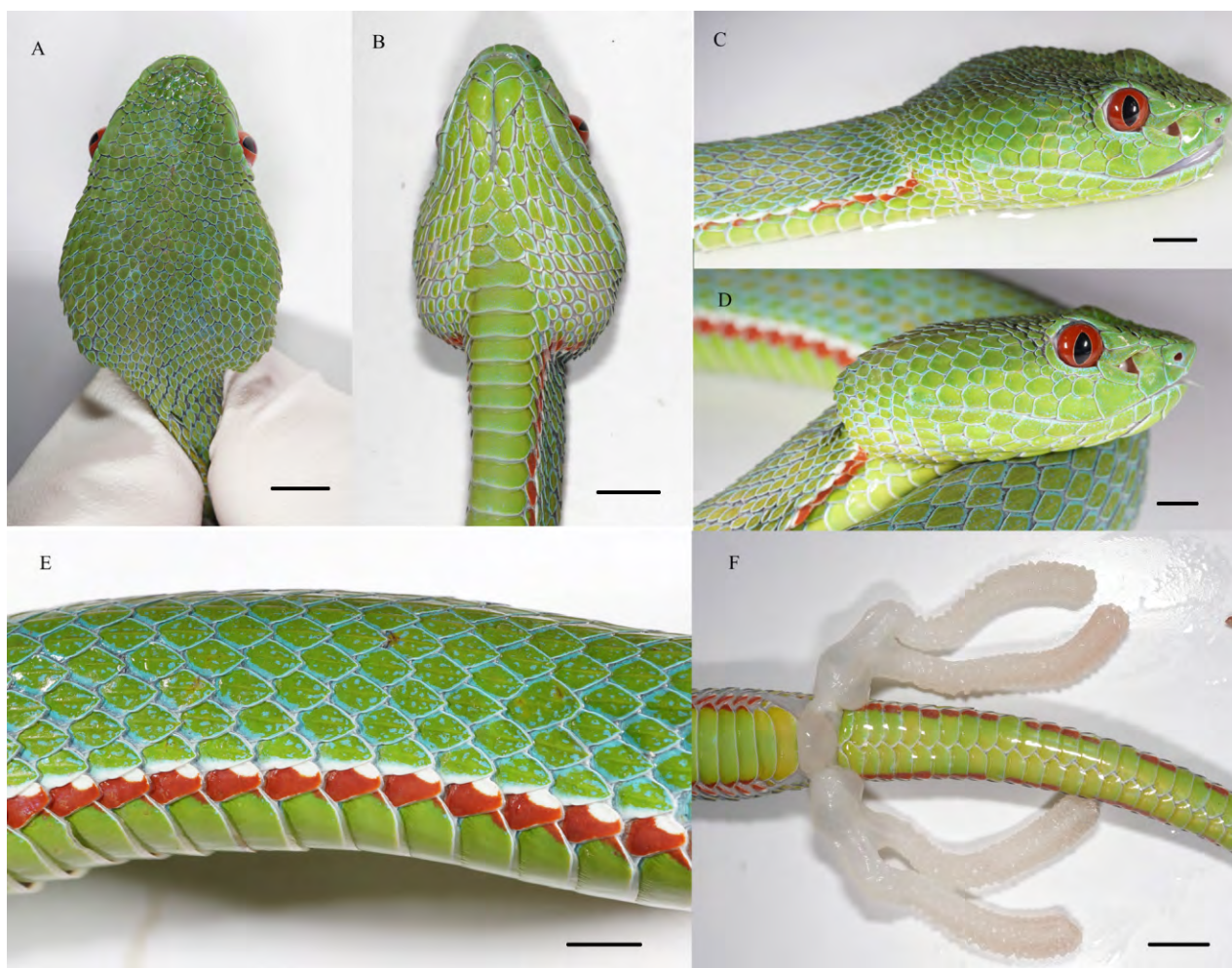


Figure 3 *Trimeresurus yingjiangensis* sp. nov. holotype: DL201070101. Head of view: A, dorsal; B, ventral; C and D, latera (left and right); E, lateral view of the body (left side); F, hemipenes (photographed by Shengchao SHI). Scale: 5 mm

strongly supported monophyletic group and then shares a common ancestor with *T. sabahi* and other *Popeia* species. *T. yingjiangensis* sp. nov. was placed sibling to *T. sabahi* complex with high support (100%).

We support the newest classification by Mulcahy *et al.* (2017), the species from west Thai (AB52, B34; clade F) as well as *T. phuketensis* and *T. nebularis* should be considered as distinct species, but the specimens of *T. sp* (clade F) performs as a weak supported (27%, indicated by red in the phylogenetic trees). This is different from the previous research by Mulcahy *et al.* (2017), in their study, the popularity is 60%. This may be due to the different data we use. Additionally, in the clade A, the support rates between the subspecies are generally low (indicated by red in the phylogenetic trees), especially the *T. barati* and *T. fucatus* do not performs as a monophyletic group, the phylogenetic relation between *T. barati* and

T. buniana could not be discriminated. Same as this the *p*-distances in the clade A are low too, ranging from 0.011 to 0.031 (Table 3). The greatest disparity is between a specimen of *T. fucata* (A203) and a specimen of *T. barati* (UTA-R61639). In comparison, the uncorrected *p*-distances within a samples of *T. yingjiangensis* sp. nov. and a samples of *T. sp* from Myanmar (CAS247754) are greater than those between other congeneric species (0.059 for *cyt b*, Table 3). Therefore, our results confirm clearly that the individuals from the Yingjiang Country, Yunnan Province as a distinct species.

Distribution and habitat. *Trimeresurus yingjiangensis* sp. nov. was found in Yingjiang Country, Yunnan Province. At its typical locality, this species prefers to inhabit in streams about 1 000 meters elevation, and perch on branches waiting for prey (Figure 5).

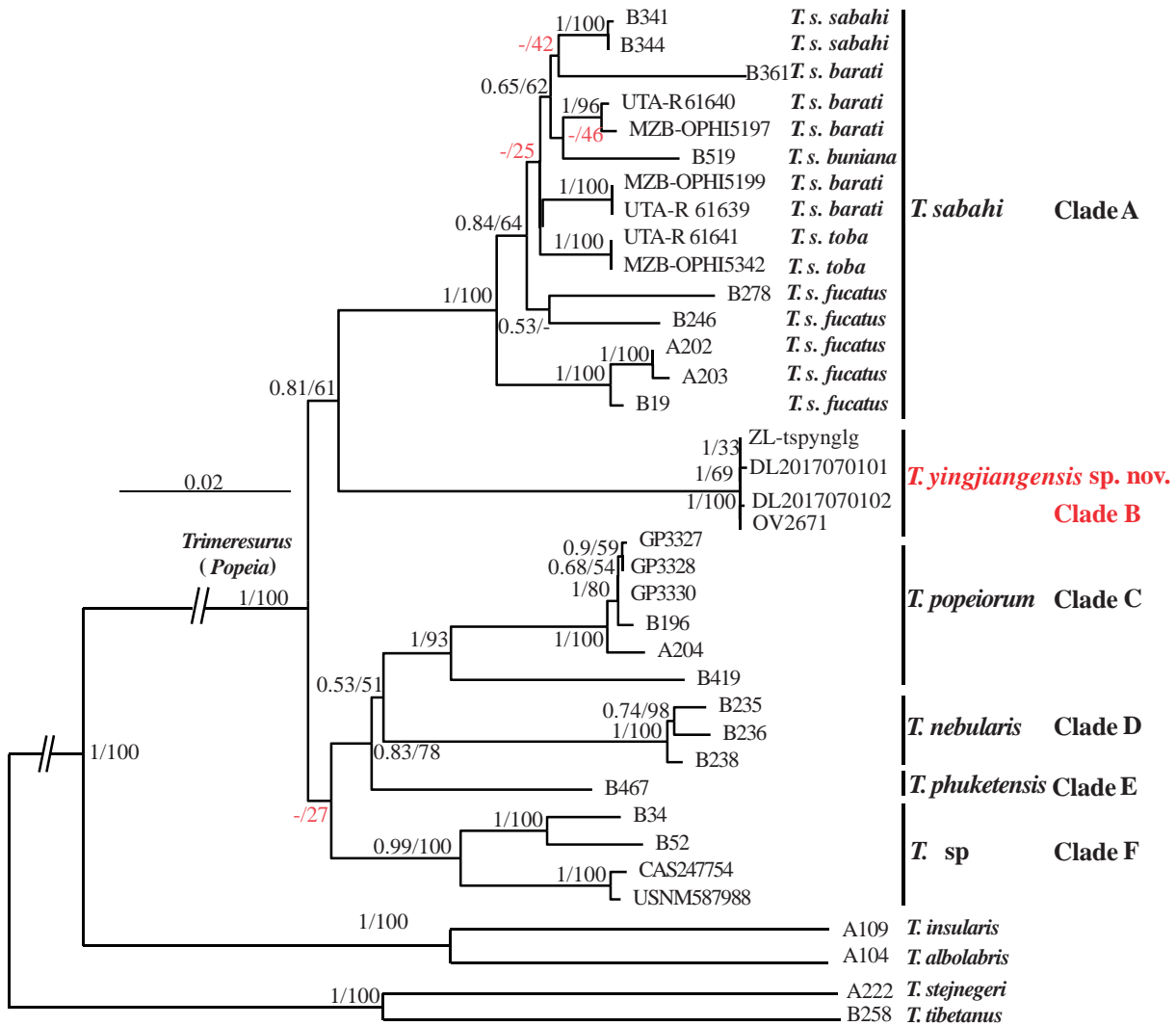


Figure 4 Bayesian phylogenetic tree of the genus *Trimeresurus* based on *cytb*, *ND4*, *12S* and *16S*. The major cladogenetic events ML/BI bootstrap and posterior probabilities values were presented (the ones lower than 50% are displayed as “-”). The clade of new species was marked in red. *T. tibetanus* and *T. stejnegeri* were used as outgroup.

4. Discussion

Being in very complex genus, many species of Asian green pitvipers (*Trimeresurus*) shared similar morphological characteristics (Gumprecht *et al.*, 2004;

Guo *et al.*, 2015; Malhotra and Thorpe, 2004; Mulcahy *et al.*, 2017; Vogel *et al.*, 2004), so that some validated species were often diagnosed as one species. For example, *T. gumprechtii* and *T. popeiorum* distributed in Yunnan, China were recognized as *T. stejnegeri* before Guo (2010,

Table 3 Uncorrected *p*-distances between *Trimeresurus (Popeia)* sp. based on 731 base pairs from the mitochondrial genes *cyt b*. Specimens *T. yingjiangensis* sp. nov. are in bold font.

ID	Species	Voucher Number	Locality	1	2	3	4	5	6	7	8	9	10
1	<i>T. popeiorum</i>	GP3330	Mengla, Yunnan, China										
2	<i>T. phuketensis</i>	B467	Phangt-nga, Thailand	0.040									
3	<i>T. buniana</i>	B519	Tioman, peninsular Malaysia	0.037	0.054								
4	<i>T. fucatus</i>	A203	Nakhon si Thammarat, Thailand	0.048	0.065	0.031							
5	<i>T. sabahi</i>	B341	Borneo (East Malaysia)	0.042	0.054	0.011	0.025						
6	<i>T. barati</i>	B361	Bengkulu, Sumatra	0.048	0.059	0.017	0.031	0.017					
7	<i>T. nebularis</i>	B236	Cameron Highlands	0.042	0.048	0.040	0.051	0.045	0.045				
8	<i>T. toba</i>	MZB-OPHI5342	Sumatra Utara Prov. Indonesia	0.048	0.059	0.023	0.028	0.023	0.023	0.045			
9	<i>T. sp</i>	B52	Phetburi, Thailand	0.034	0.045	0.042	0.054	0.048	0.054	0.048	0.048		
10	<i>T. sp</i>	CAS247754	Tanintharyi Div. Myanmar	0.048	0.042	0.045	0.048	0.045	0.045	0.045	0.045	0.037	
11	<i>T. yingjiangensis</i> sp. nov.	DL2017070101	Yingjiang, Yunnan, China	0.071	0.076	0.068	0.071	0.068	0.071	0.071	0.076	0.065	0.059



Figure 5 Habitat of *Trimeresurus yingjiangensis* sp. nov., Heping Village, Tongbiguan Town, Yingjiang County, Yunnan Province (24.584° N, 97.738° E, 1 200 m a.s.l.), photographed by Jian XU.

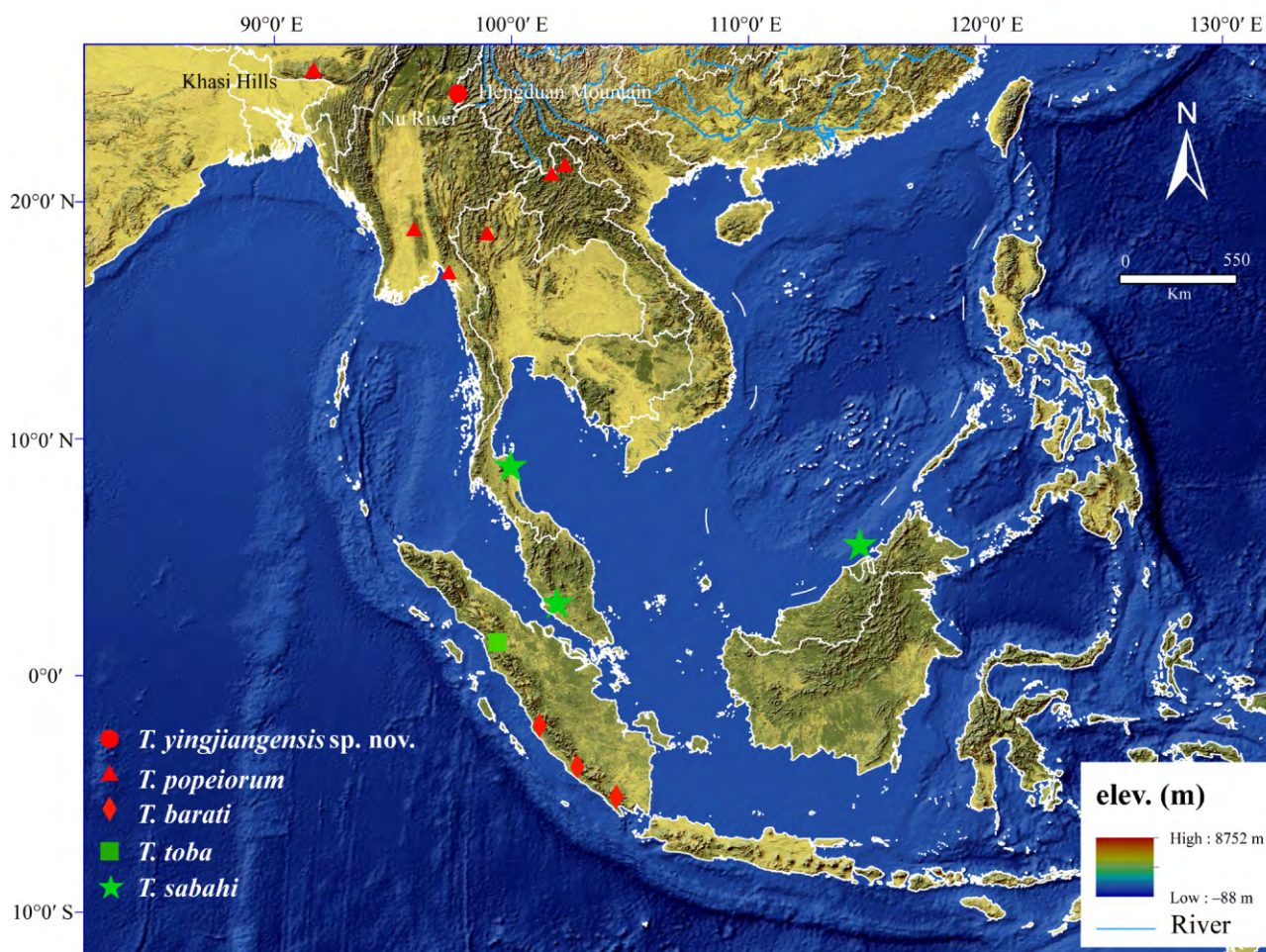


Figure 6 Distribution map of the *T. yingjiangensis* sp. nov., *T. popeiorum*, *T. barati*, *T. toba* and *T. sabahi*. The samples which are included in this study are labeled with the codes (constructed by ArcMap 10.3)

2015). Therefore, combined morphological and molecular characters and even ecological traits could be helpful to solve the classification problem of *Trimeresurus* (Figuroa *et al.*, 2016; Malhotra, 2004; Mulcahy *et al.*, 2017; Sanders *et al.*, 2006; Pyron *et al.*, 2013; Wostl *et al.*, 2016).

T. yingjiangensis sp. nov. inhabits in Yingjiang County, Southern Yunnan Province which locates geographically in the southeastern-most corner of the Tibetan Plateau and the southern-most tip of the Hengduan Mountains. There are numerous mountains and rivers in this area, which restrict gene flows, and lead to regional divergences of species and generate biodiversity (Huang *et al.*, 2009; Peng *et al.*, 2015). Although 31 species of snakes have been recorded in this region to date, the local biodiversity of reptiles may be largely underestimated (Vogel and Luo, 2011; Peng *et al.*, 2015). *T. yingjiangensis* sp. nov. is the only species of *Popeia* was found in this area, and, nearby, *T. popeiorum* is distributed in Mengla County,

the vicinity of Yingjiang County. Due to the geographical isolations between two closed counties (e.g. Lancang River, Nu River and Gaoligong Mountain, Figure 6), there are evident differences in both morphological characters and mtDNA sequences between two species. The same situation also can be found when comparing *T. yingjiangensis* sp. nov. with the other species. Thus, we believed that *T. yingjiangensis* sp. nov. should be considered as a distinct species.

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References

Alencar L. R., Quental T. B., Grazziotin F. G., Alfaro M. L., Martins M., Venzon M. 2016. Diversification in vipers:

- phylogenetic relationships, time of divergence and shifts in speciation rates. *Mol Phylogenet Evol*, 105, 50–62
- Alzohairy A. M.** 2011. Bioedit: an important software for molecular biology. *GERF Bull Biosci*, 2(1), 60–61
- Burbrink, F. T., Lawson R., Slowinski J. B.** 2000. Mitochondrial dna phylogeography of the polytypic north american rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution*, 54(6), 2107–2118
- Creer S., Malhotra A., Thorpe R. S., Chou W. H.** 2001. Multiple causation of phylogeographical pattern as revealed by nested clade analysis of the bamboo viper (*Trimeresurus stejnegeri*) within Taiwan. *Mol Ecol*, 10(8), 1967–1981
- David P., Petri M., Vogel G., Doria G.** 2009. A new species of pitviper of the genus *Trimeresurus* (*popeia*) from northern Sumatra (reptilia, squamata, viperidae). *Annali Dellistituto E Museo Di Storia Della Scienza Di Firenze*, 323–346
- Figueroa A., Mckelvy A. D., Grismer L. L., Bell C. D., Lailvaux S. P.** 2016. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. *Plos One*, 11(9), 1–31
- Grismer L. L., Grismer J. L., McGuire J. A.** 2006. A new species of pitviper of the genus *Popeia* (Squamata: Viperidae) from Pulau Tioman, Pahang, West Malaysia. *Zootaxa*, 1305, 1–19
- Gumprecht A., Tillack F., Orlov N. L., Captain A., Ryabov S.** 2004. *Asian Pitvipers*. Berlin: Geitje Books
- Guo P., Jadin R. C., Malhotra A., Li C.** 2010. An investigation of the cranial evolution of Asian pitvipers (Serpentes: Crotalinae), with comments on the phylogenetic position of *Peltopelorus macrolepis*. *Acta Zoologica*, 91(4), 402–407
- Guo P., Qin L., Zhong G. H., Zhu F., Yan F., Tang T., Xiao R., Fang M., Wang P., Fu X.** 2015. Cryptic diversity of green pitvipers in Yunnan, South-west China (Squamata, Viperidae). *Amphibia-Reptilia* 36 (3): 265–276
- Huang S., Liu S. Y., Guo P., Zhang, Y. P., Zhao E. M.** 2009. What are the closest relatives of the hot-spring snakes (Colubridae, Thermophis), the relict species endemic to the Tibetan plateau? *Mol Phylogenet Evol*, 51(3), 438–446
- Knight A., Mindell D. P.**, 1993. Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Feas viper. *Syst. Biol*, 42, 18–31
- Kumar S, Stecher G, Tamura K.** 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Bio Evol*, 33(7):1870
- Lanfear R., Calcott B., Ho S. Y. W., Guindon S.** 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Phylogenet Evol*, 29(6), 1695–1701
- Peng L. F., Liu C. H., Huang S.** 2014. A new species of the genus *Thermophis* (Serpentes: Colubridae) from Shangri-La, northern Yunnan, China, with a proposal for an eclectic rule for species delimitation. *Asian Herpetol Res*, 5(4), 228–239
- Malhotra A., Thorpe R. S.** 2000. A phylogeny of the *Trimeresurus* group of pit vipers: new evidence from a mitochondrial gene tree. *Mol Phylogenet Evol*, 16(2), 199–211
- Malhotra A., Thorpe R. S.** 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). *Mol Phylogenet Evol*, 32(1), 83–100
- Mulcahy D. G., Lee J. L., Miller A. H., Zug G. R.** 2017. Troublesome Trimes: Potential cryptic speciation of the *Trimeresurus* (*Popeia*) *popeiorum* complex (Serpentes: Crotalidae) around the Isthmus of Kra (Myanmar and Thailand). *Zootaxa*, 4347(2), 301–315
- Parkinson C. L., Zamudio K. R., Greene H. W.**, 2000. Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, Species status, and conservation of cantils. *Mol Ecol*, 9, 411–420
- Pope C. H., Pope S. H.** 1933. A study of the green pit-vipers of southeastern Asia and Malaysia, commonly identified as *Trimeresurus gramineus* (Shaw): with description of a new species from peninsular India. *N Y Amer Mus Nat Hist*, 620(5): 1–12
- Pyron R. A., Burbrink F. T.** 2009. Neogene diversification and taxonomic stability in the snake tribe Lampropeltini (Serpentes: Colubridae). *Mol Phylogenet Evol*, 52(2), 524–529
- Pyron R. A., Kandambi H. K., Hendry C. R., Pushpamal V., Burbrink F. T., Somaweera R.** 2013. Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. *Mol Phylogenet Evol*, 66(3), 969–978
- Ronquist F., Teslenko M., Mark P. V. D., Ayres D., Darling A., Höhna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P.** 2012. Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*, 61(3), 539–542
- Sanders K. L., Malhotra A., Thorpe R. S.** 2006. Combining molecular, morphological and ecological data to infer species boundaries in a cryptic tropical pitviper. *Biol J Linn Soc*, 87 (3), 343–364
- Shi J., Wang G., Chen X., Fang Y., Ding L., Huang S., Hou M., Liu J., Li P.** 2017. A new moth-preying alpine pit viper species from Qinghai-Tibetan Plateau (Viperidae, Crotalinae). *Amp Rep*, 38(4), 517-532
- Stamatakis A.** 2006. Raxml-vi-hpc: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21), 2688
- Sumontha M., Kunya K., Pauwels O. S. G., Nitikul A., Punnadee S.** 2011. *Trimeresurus* (*Popeia*) *phuketensis*, a new pitviper (Squamata: Viperidae) from Phuket island, southwestern Thailand, *Russ J Her*, 18 (3), 185–194
- Taylor E. H., Elbel R. E.** 1958. Contribution to the Herpetology of Thailand. *Univ Kansas sci bulletin*, 38 (13): 1033–1189
- Vogel G., David P., Pauwels O. S. G.** 2004. A review of morphological variation in *Trimeresurus popeiorum* (Serpentes: Viperidae: Crotalinae), with the description of two new species. *Zootaxa*, 727(727), 1–61
- Vogel G., Luo J.** 2011. A new species of the genus *Lycodon* (Boie, 1826) from the southwestern mountains of China (Squamata: Colubridae). *Zootaxa* (2807), 29–40
- Wostl E., Sidik I., Trilaksono, W., Shaney K. J., Kurniawan N., Smith E. N.** 2016. Taxonomic Status of the Sumatran Pitviper *Trimeresurus* (*Popeia*) *toba* David, Petri, Vogel & Doria, 2009 (Squamata: Viperidae) and Other Sunda Shelf Species of the Subgenus *Popeia*. *J Herpetol*, 50 (4), 633–641