



Limestone jewel: A new colourful karst-dwelling pitviper (Serpentes: Viperidae: *Trimeresurus*) from the poorly explored borderlands of southern peninsular Thailand

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Abstract

We describe a new species of pitvipers from Trang Province of Thailand, near the Thailand–Malaysian border, based on morphological and molecular (2427 bp from *cyt b*, ND4, and 16S rRNA mitochondrial DNA genes) lines of evidence. Morphologically, *Trimeresurus ciliaris* **sp. nov.** is distinguished from its congeners by the following combination of morphological characters: a long papillose hemipenis; first supralabial and nasal scale fused; three to four small supraocular scales; internasals not in contact; small scale between nasal and the scale formed by the fused second supralabial and loreal present; dorsal scales in 17–17–15 rows across the body; ventral scales 172–175 in males, 171 in female; subcaudal scales 59–63 in males, 61 in female, all paired; in life an emerald-green dorsum with reddish-brown bands; creamy-white venter lacking dark dots or stripes on the lateral sides of the ventrals; white vertebral spots present in both sexes on every two or three dorsal scales; dark brown spots forming discontinuous pattern present on 1–3 lateral dorsal scale rows; males with reddish-brown postocular stripe. The new species forms a distinct clade on the phylogenetic tree of the genus *Trimeresurus* and differs from the morphologically similar species *T. venustus* by a significant divergence in cytochrome *b* mitochondrial DNA gene sequences ($p = 12.5\%$). The new species is currently known from a small karstic area in the Nakawan Range spanning the border of Thailand and Malaysia, in particular in limestone forests in Trang and Satun provinces (Thailand); it likely also occurs in the adjacent parts of Perlis State (Malaysia). Our study also suggests that the taxonomy of *T. kanburiensis* species complex requires further studies; in particular our study suggests that the status of populations from Chumphon Province of Thailand and Pulau Langkawi Island of Malaysia should be re-assessed.

Keywords

Biodiversity, Malaysia, morphology, phylogenetics, Reptilia, taxonomy, Thai-Malay Peninsula, *Trimeresurus ciliaris* **sp. nov.**, *Trimeresurus kanburiensis* species complex

Introduction

The Asian pitviper genus *Trimeresurus* Lacépède, 1804 is a complex and species-rich group of venomous snakes that currently comprises 46 nominal species of terrestrial to arboreal species distributed across South and Southeast Asia (Mirza et al. 2023; Uetz et al. 2023). The genus is known to contain numerous cryptic species which are very similar morphologically and are often hardly distinguishable in the wild. Recently, molecular methods have helped to resolve some particularly problematic taxonomic issues among such complexes of cryptic pitviper species, with new taxa being described every year such as in *T. albolabris* complex (e.g., Chen et al. 2020, 2021; Mirza et al. 2020), *T. kanburiensis* complex (e.g., Malhotra et al. 2011; Sumontha et al. 2021), *T. popeiorum* complex (e.g., Mirza et al. 2023), and *T. stejnegeri* complex (e.g., Rathee et al. 2022).

Subgeneric taxonomy of the genus *Trimeresurus* is a subject of a long-lasting debate. Following a pioneering attempt to reconstruct a molecular phylogeny of the snakes of the *Trimeresurus* radiation, Malhotra and Thorpe (2004a) split the genus *Trimeresurus* into eight full genera: *Trimeresurus* Lacépède, 1804, *Cryptelytrops* Cope, 1860, *Himalayophis* Malhotra & Thorpe, 2004, *Parias* Gray, 1849, *Peltopelor* Günther, 1864, *Popeia* Malhotra & Thorpe, 2004, *Sinovipera* Guo & Wang 2011, and *Viridovipera* Malhotra & Thorpe, 2004. Subsequently, David et al. (2011) demonstrated that the type-species of *Trimeresurus* Lacépède, 1804 is a member of the genus *Cryptelytrops*. Consequently, *Cryptelytrops* is a junior synonym of *Trimeresurus* sensu stricto, which thus includes only species related to *Trimeresurus albolabris* Gray, 1842. David et al. (2011) referred species placed by Malhotra and Thorpe (2004a) in their genus *Trimeresurus*, namely a group of Indian and Indo-Malayan species, to the next available generic nomen, *Craspedocephalus* Kuhl & van Hasselt, 1822. Lastly, David et al. (2011) recognized at subgeneric level the genera erected or recognized by Malhotra and Thorpe (2004a).

Recently, the peninsular Indian and Sri-Lankan taxon *Peltopelor* and the Southeast-Asian taxon *Craspedocephalus* were regarded as a distinct genus *Craspedocephalus*, following indirectly the conclusions of Malhotra and Thorpe (2004a), with two respective subgenera (e.g., Mallik et al. 2021). However, more recently Mirza et al. (2023), based on an extensive sampling and mitochondrial DNA (hereafter mtDNA)-based genealogy, suggested that these two taxa should be better treated as separate genera due to their deep divergence, along with biogeographic and morphological distinctiveness; this taxonomy was not followed by the Reptile Database (Uetz et al. 2023). Recognizing the limitations of the mtDNA-markers in reconstruction of phylogenetic relationships (see, e.g., Hillis et al. 2021; Gallego-Garcia et al. 2023), we herein treat *Peltopelor* and *Craspedocephalus* as subgenera of the genus *Craspedocephalus*, and recognize the genus *Trimeresurus* sensu lato with six subgenera pend-

ing subsequent multilocus and genome-level studies on evolutionary relationships of this group.

The subgenus *Trimeresurus* is diagnosed from other members of the genus *Trimeresurus* by the combination of a long papillose or calyculate hemipenis and a (partially) fused first supralabial and nasal scales (Malhotra and Thorpe 2004a; Sumontha et al. 2021). Currently, this subgenus includes 21 species namely: *Trimeresurus albolabris*, *T. andersonii* Theobald, 1868, *T. cantori* (Blyth, 1846), *T. cardamomensis* (Malhotra, Thorpe, Mrinalini & Stuart, 2011), *T. caudornatus* Chen, Ding, Vogel & Shi, 2020, *T. davidi* Chandramouli, Campbell & Vogel, 2020, *T. erythrurus* (Cantor, 1839), *T. fasciatus* (Boulenger, 1896), *T. guoi* Chen, Shi, Vogel & Ding, 2021, *T. honsonensis* (Grismer, Ngo & Grismer, 2008), *T. insularis* Kramer, 1977, *T. kanburiensis* Smith, 1943, *T. kuiburi* Sumontha, Suntrarachun, Pauwels, Pawangkhanant, Chomngam, Iamwiriyaikul & Chanhom, 2021, *T. labialis* Fitzinger in Steindachner, 1867, *T. macrops* Kramer, 1977, *T. mutabilis* Stoliczka, 1870, *T. purpureomaculatus* (Gray, 1832), *T. rubeus* (Malhotra, Thorpe, Mrinalini & Stuart, 2011), *T. salazar* Mirza, Bhosale, Phansalkar, Sawant, Gowande & Patel, 2020, *T. septentrionalis* Kramer, 1977, and *T. venustus* Vogel, 1991 (David et al. 2004; Malhotra and Thorpe 2004a, 2004b; Malhotra et al. 2011; Mirza et al. 2020, 2023; Sumontha et al. 2021; Poyarkov et al. 2023; Uetz et al. 2023).

The *Trimeresurus kanburiensis* species complex (previously also often referred to as ‘*T. venustus* species complex’, see David et al. 2004; Sumontha et al. 2021; however as *T. kanburiensis* represents the earliest described taxon of this group, the name ‘*T. kanburiensis* species complex’ is more appropriate) encompasses karst-dwelling pitvipers distributed across the Thai-Malay Peninsula and currently includes three nominal species: *T. kanburiensis* (northern Tenasserim mountains in Kanchanaburi Province of Thailand), *T. kuiburi* (central Tenasserim mountains in Prachuap Khiri Khan Province of Thailand), and *T. venustus* (currently known to occupy the southern part of Thai-Malay Peninsula from the Isthmus of Kra in Chumphon Province of Thailand in the north to northernmost Peninsular Malaysia in the south, Sumontha et al. 2021) (see Fig. 1). For a long time *T. kanburiensis* and *T. venustus* were considered to be synonyms (Viravan et al. 1992; Warrell et al. 1992), until 2004, when both were recognized as distinct species (David et al. 2004; Malhotra and Thorpe 2004b).

Among other members of the *T. kanburiensis* species complex, the Beautiful Pitviper, *T. venustus* apparently has the widest distribution and is reported from Thailand (Surat Thani, Phang-Nga, Trang, Nakhon Si Thammarat, Krabi, and Chumphon provinces), and northern Peninsular Malaysia (Kedah State) (Grismer et al. 2006; Chanhom et al. 2011; Chan-ard et al. 2015; Sumontha et al. 2021; see Fig. 1 for details). However, recently Sumontha et al. (2021) demonstrated some distinctive features of the population from Chumphon and reported that this population differs in coloration and pattern from typical *T. venustus* s. str., and its taxonomic status requires further investigations.



Figure 1. Distribution of the *Trimeresurus kanburiensis* species complex in Thai-Malay Peninsula: Localities. *T. kanburiensis* (yellow): 1 Kanchanaburi Prov., Sai Yok Dist., Wat Tham, Phom Lo Khao Yai; *T. kuiburi* (blue): 2 Prachuap Khiri Khan Prov., Kuiburi Dist., Wat Khao Daeng; 3 Prachuap Khiri Khan Prov., Kuiburi Dist., Khao Daeng Beach; 4 Prachuap Khiri Khan Prov., Kuiburi Dist., Khao Daeng, near Ban Thung Noi; *T. cf. venustus* (1) (green): 5 Chumphon Prov., Wat Tham Sanook; *T. venustus* s. str. (pink): 6 Krabi Prov., Mueang Krabi Dist., Tiger Cave viewpoint; 7 Thailand, Nakhon Si Thammarat Prov., Khao Luang; 8 Nakhon Si Thammarat Prov., Thung Song; 9 Surat Thani Prov.; *Trimeresurus ciliaris* sp. nov. (red): 10 Trang Prov., Palian Dist., Thum Khao Ting; 11 Tha Le Ban NP., Khuan Don Dist., Satun Prov.; 12 Perlis State National Park, Perlis State; *T. cf. venustus* (2) (purple): 13 Pulau Langkawi Island, Kedah State. Remark: stars denote type localities (the type locality is not shown for *T. kanburiensis* as it was not sampled). Abbreviations: MY – Myanmar, MA – Malaysia, Prov. – Province, Dist. – District.

During our recent field surveys in Trang Province of Thailand, we encountered several unusual pitviper specimens that were superficially similar to *T. venustus* in overall morphological habitus and body coloration. Closer morphological examination of scalation, pattern, color and morphometric characteristics of this population demonstrated clear morphological differences from *T. venustus* s. str. In the present paper we demonstrate that these differences are concordant with a significant divergence between mtDNA gene sequences among the two

populations. Consequent phylogenetic analyses of three mtDNA genes (*cyt b*, ND4 and 16S) confirmed the placement of the Trang population within *Trimeresurus* s. str. where it forms a deeply divergent lineage, with sister relationships to a clade encompassing all other members of *T. kanburiensis* and *T. macrops* species complexes with exception of *T. rubeus*. Therefore, in the present paper we describe the *Trimeresurus* population from Trang Province as a new species and discuss the taxonomy of *T. kanburiensis* species complex.

Materials and Methods

Sampling

Fieldwork was carried out in Khao Ting Cave, Li Phang Subdistrict, Palian District, Trang Province, Thailand in June 2016 by P. Pawangkhanant and in January 2023 by P. Pawangkhanant, S. Idiatullina, and T. Woranuch (Fig. 1, locality 10). Geographic coordinates and altitude were obtained using a Garmin GPSMAP 60CSx GPS receiver (USA) and recorded in datum WGS 84. Specimens were collected by means of snake hooks in the field, photographed in life, and euthanized using MS-222 solution within 24 h after capture following Hepps Keeney and Harrison (2022). Specimens were fixated in 4% buffered formalin for 24 h and later stored in 70% ethanol. Specimens were subsequently deposited in the herpetological collection of the School of Agriculture and Natural Resources, University of Phayao (AUP, Phayao, Thailand), the Queen Saovabha Memorial Institute (QSMI, Bangkok, Thailand), and the Zoological Museum of Moscow State University (ZMMU, Moscow, Russia). Tissues for genetic analyses were taken from liver or heart prior to preservation of specimens and stored in 96% ethanol.

Specimen collection and animal use protocols were approved by the Institutional Ethical Committee of Animal Experimentation of the University of Phayao, Phayao, Thailand (certificate number UP-AE64-02-04-005, issued to Chatmongkon Suwannapoom) and were strictly compliant with the ethical conditions specified in the Thailand Animal Welfare Act. Field work, including collection of animals in the field, was authorized by the Institute of Animals for Scientific Purpose Development (IAD), Bangkok, Thailand (permit numbers U1-01205-2558 and UP-AE59-01-04-0022, issued to Chatmongkon Suwannapoom).

DNA isolation and sequencing

Total genomic DNA was extracted from ethanol-preserved muscle or liver tissues using standard phenol-chloroform extraction procedures (Sambrook et al. 1989) followed with isopropanol precipitation. We used the polymerase chain reaction (PCR) to amplify three mtDNA fragments: complete sequences of cytochrome *b* (cyt *b*) and NADH dehydrogenase subunit 4 (ND4) genes and a fragment of 16S rRNA gene. Primers used for both PCR and sequencing are summarized in Table 1.

PCR conditions for cyt *b* gene followed the protocol of Dahn et al. (2018) with a touchdown, and included the following steps: (1) initial denaturation step at 94°C for 5 min; (2) 10 cycles of denaturation at 94°C for 1 min, annealing for 1 min with temperature decreasing from 50°C to 45°C with cool-down at 0.5°C per each cycle, and extension at 72°C for 1 min; (3) 24 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 1 min; (4) final extension at 72°C for 10 min; and (5) cooling step at 4°C for storage. For ND4 gene PCR conditions followed the protocol from Salvi et al. (2013): (1) initial denaturation step at 92°C for 3 minutes; (2) followed by 16 touchdown cycles with 30 seconds at 92°C, annealing temperature decreasing 0.5°C per cycle from 60°C to 52°C (30 seconds) and extension for 1 minute at 72°C; (3) followed with 20 more cycles similar to the latter but with annealing temperatures stable at 52°C; (4) a final extension at 72°C for 15 minutes; and (5) cooling step at 4°C for storage. For 16S rRNA, we used the PCR protocol that followed Green et al. (2010): (1) initial denaturation step at 94°C for 5 min; (2) 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min; (3) final extension at 72°C for 10 min; and (4) cooling step at 4°C for storage.

All amplifications were run using an iCycler Thermal Cycler (Bio-Rad). PCR products were loaded onto 1% agarose gels in the presence of ethidium bromide and visualized in electrophoresis. The successful targeted PCR products were purified by Diatom DNA PCR Clean-Up kit and outsourced to Evrogen® (Moscow, Russia) for sequencing; sequence data collection and visualization was performed on an ABI 3730xl Automated Sequencer (Applied Biosystems).

Molecular phylogeny

To estimate the phylogenetic relationships of the genus *Trimeresurus*, we aligned the newly obtained cyt *b*, ND4 and 16S rRNA sequences together with all available previously published sequences of *T. venustus*, *T. kanburiensis* and *T. kuiburi*, as well as the sequences of 33 species of *Trimeresurus* species representing all major groups within the genus, five species of *Craspedocephalus*; we used the sequence of *Azemiops feae* to root the tree (GenBank accession numbers, voucher and locality information are summarized in Table 2).

We initially aligned the nucleotide sequences in the MAFFT v.6 online webserver (Katoh et al. 2019) with

Table 1. Primers used in this study. F – forward primer, R – reverse primer.

Gene	Primer name	Sequence	Reference
cyt <i>b</i>	H14910 (F)	5'-GACCTGTGATMTGAAAAACAYCGTT-3'	Dahn et al. (2018)
	THRSN2 (R)	5'-CTTTGGTTTACAAGAACAATGCTTTA-3'	
ND4	Trim-ND4F (F)	5'-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3'	Arevalo et al. (1994)
	Trim-ND4LEUR (R)	5'-CATTACTTTTACTTGGATTTCACCA-3'	
16S rRNA	16S1LM (F)	5'-CCGACTGTTGACCAAAAACAT-3'	Green et al. (2010)
	16SH1 (R)	5'-TCCGGTCTGAACTCAGATCACGTAGG-3'	

Table 2. Sequences and voucher specimens of the genus *Trimeresurus* and outgroup taxa used in this study.

No	Sample ID	GenBank accession number				Species	Country	Locality	References
		cyt b	ND4	16S					
1	ZMMU Re-17661	OR470557	OR470538	OR471621	<i>Trimeresurus ciliaris</i> sp. nov.	Thailand	Trang, Palian, Thum Khao Ting	This study	
2	AUP-02011	OR470558	OR470539	OR471623	<i>Trimeresurus ciliaris</i> sp. nov.	Thailand	Trang, Palian, Thum Khao Ting	This study	
3	ZMMU Re-17662	OR470559	OR470540	OR471624	<i>Trimeresurus ciliaris</i> sp. nov.	Thailand	Trang, Palian, Thum Khao Ting	This study	
4	ZMMU Re-17663	OR470560	OR470541	OR471622	<i>Trimeresurus ciliaris</i> sp. nov.	Thailand	Trang, Palian, Thum Khao Ting	This study	
5	ZMMU Re-17231	OR470575	OR470545	OR471625	<i>Trimeresurus kuiburi</i>	Thailand	Prachuap Khiri Khan, Kuiburi, Wat Khao Daeng	This study	
6	ZMMU Re-17095	OR470576	—	OR471626	<i>Trimeresurus kuiburi</i>	Thailand	Prachuap Khiri Khan, Kuiburi, Khao Daeng Beach	This study	
7	QSMI 1500	MW806923	—	MW699849	<i>Trimeresurus kuiburi</i>	Thailand	Prachuap Khiri Khan, Kui Buri, Khao Daeng	Sumontha et al. (2021)	
8	ZMMU Re-17212	OR470570	OR470547	OR471632	<i>Trimeresurus venustus</i>	Thailand	Krabi, Mueang Krabi, Tiger Cave viewpoint	This study	
9	ZMMU Re-17213	OR470565	OR470548	OR471633	<i>Trimeresurus venustus</i>	Thailand	Krabi, Mueang Krabi, Tiger Cave viewpoint	This study	
10	ZMMU Re-17214	OR470566	OR470549	OR471631	<i>Trimeresurus venustus</i>	Thailand	Krabi, Mueang Krabi, Tiger Cave viewpoint	This study	
11	QSMI TV07	OR470563	OR470537	OR471630	<i>Trimeresurus venustus</i>	Thailand	Krabi	This study	
12	A74	AY289224	AY289230	—	<i>Trimeresurus venustus</i>	Thailand	Nakhon Si Thammarat, Khao Luang	Malhotra and Thorpe (2004b)	
13	A75	AY289223	AY289229	—	<i>Trimeresurus venustus</i>	Thailand	Nakhon Si Thammarat, Khao Luang	Malhotra and Thorpe (2004b)	
14	A249	AY289234	AY289233	—	<i>Trimeresurus venustus</i>	Thailand	Nakhon Si Thammarat, Khao Luang	Malhotra and Thorpe (2004b)	
15	A237	AY289222	AY289228	—	<i>Trimeresurus venustus</i>	Thailand	Nakhon Si Thammarat, Thung Song	Malhotra and Thorpe (2004b)	
16	A241	AF171914	AY293930	AY352723	<i>Trimeresurus venustus</i>	Thailand	Nakhon Si Thammarat, Thung Song	Malhotra and Thorpe (2004b)	
17	B29	—	KR021049	—	<i>Trimeresurus venustus</i>	Thailand	Surat Thani	Mrinalini et al. (2015)	
18	ZMMU Re-17665	OR470569	OR470551	OR471628	<i>Trimeresurus</i> cf. <i>venustus</i> (1)	Thailand	Chumphon, Wat Tham Sanook temple	This study	
19	ZMMU Re-17666	OR470568	OR470552	OR471629	<i>Trimeresurus</i> cf. <i>venustus</i> (1)	Thailand	Chumphon, Wat Tham Sanook temple	This study	
20	ZMMU Re-17664	OR470567	OR470535	OR471627	<i>Trimeresurus</i> cf. <i>venustus</i> (1)	Thailand	Chumphon, Wat Tham Sanook temple	This study	
21	ZMMU Re-17667	OR470579	OR470553	OR471634	<i>Trimeresurus kanburiensis</i>	Thailand	Kanchanaburi, Sai Yok, Khao Yai NP.	This study	
22	QSMI TA091164	OR470562	OR470536	OR471641	<i>Trimeresurus albolabris</i>	Thailand	Bangkok	This study	
23	ZMMU NAP-03760	OR470577	OR470543	OR471635	<i>Trimeresurus cardamomensis</i>	Vietnam	Kien Giang, Phu Quoc	This study	
24	ZMMU NAP-06685	OR470572	OR470544	OR471638	<i>Trimeresurus fucatus</i>	Malaysia	Pahang, Raub, Fraser's Hil	This study	
25	ZMMU NAP-11772	OR470580	OR470554	OR471643	<i>Trimeresurus guoi</i>	Thailand	Nan, Doi Phu Kha Mt.	This study	
26	ZMMU ISS-074	OR470564	KX660619	KX660222	<i>Trimeresurus honsonensis</i>	Vietnam	Hon Son Island, Kien Giang	This study; Figueroa et al. (2016)	
27	ZMMU NAP-09445	OR470573	OR470546	OR471639	<i>Trimeresurus popeiorum</i>	Myanmar	Kachin, Indawgyi NP., Inn Gyiin Taung Mt.	This study	
28	ZMMU Re-17668	OR470574	OR470550	OR471636	<i>Trimeresurus</i> cf. <i>popeiorum</i>	Thailand	Ratchaburi, Suan Phueng, Lamtarn Song	This study	
29	AUP-00061	OR470571	OR470534	OR471637	<i>Trimeresurus</i> cf. <i>popeiorum</i>	Thailand	Chiang Mai, Chom Thong, Doi Inthanon NP.	This study	
30	QSMI Tpuw74	OR470556	AY352772	OR471642	<i>Trimeresurus</i> cf. <i>purpureomaculatus</i>	Myanmar	Ayeyarwade, Mwe Hauk	Malhotra and Thorpe (2004a); this study	
31	ZMMU NAP-02776	OR470578	OR470542	OR471640	<i>Trimeresurus rubens</i>	Vietnam	Lam Dong, Bao Loc	This study	
32	APF/SFRL-1871	MK720609	—	MK722155	<i>Trimeresurus arunachalensis</i>	India	Arunachal Pradesh	Captain et al. (2019)	
33	AM A85	AF171899	U41891	AY352741	<i>Trimeresurus cantori</i>	India	Nicobar	Malhotra and Thorpe (2004a); Kraus et al. (1996)	
34	CAS243566	ON804490	ON804505	—	<i>Trimeresurus erythrurus</i>	Myanmar	Rangoon	Chan et al. (2022)	

No	Sample ID	GenBank accession number				Species	Country	Locality	References
		cyt <i>b</i>	ND4	16S					
35	AM B4	AY352764	AY352830	AY059551	<i>Trimeresurus flavomaculatus</i>	Philippines			Malhotra and Thorpe (2004a)
36	AUP-01988	OR470561	OR470555	AF517181	<i>Trimeresurus gumprechtii</i>	Thailand	Nan, Doi Phu Kha NP.		This study; Creer et al. (2003)
37	AM B33	AY059567	AY059585	AY059552	<i>Trimeresurus hageni</i>	Thailand	Songkhla		Malhotra and Thorpe (2004a)
38	GP1474	KP999371	AY352808	AF517176	<i>Trimeresurus macrops</i>	Laos	Khammouane		Malhotra and Thorpe (2004a); Zhu et al. (2016); Creer et al. (2003)
39	AM B349	AY371832	—	AY371793	<i>Trimeresurus malcolmi</i>	Malaysia	Boneo, Sabah, Kinabalu		Sanders et al. (2004)
40	B416	AY352765	AY352831	AY352735	<i>Trimeresurus medoensis</i>	Myanmar	Kachin		Malhotra and Thorpe (2004a)
41	LSUHC 10268	KX660506	KX660634	KX660236	<i>Trimeresurus nebularis</i>	Malaysia	Pahang		Figueroa et al. (2016)
42	B467	MW806924	—	MW694483	<i>Trimeresurus phuketensis</i>	Thailand	Phuket		Sumontha et al. (2021)
43	AM B210	AY352756	AY352819	AY352725	<i>Trimeresurus schultzei</i>	Philippines	Palawan		Malhotra and Thorpe (2004a)
44	AM B487	AY352755	AY352818	AY352724	<i>Trimeresurus septentrionalis</i>	Nepal	Kathmandu		Malhotra and Thorpe (2004a)
45	GP 07	HQ850448	HQ850449	HQ850446	<i>Trimeresurus sichuanensis</i>	China	Sichuan		Guo and Peng (2011)
46	B367	AY371824	—	AY371792	<i>Trimeresurus sumatranus</i>	Indonesia	Bengkulu, Sumatra		Sanders et al. (2004)
47	ZMB 65641	AY352749	AY352810	AY352715	<i>Trimeresurus tibetanus</i>	Nepal	Helambu		Malhotra and Thorpe (2004a)
48	AM B97	AY059574	AF517225	AF517183	<i>Trimeresurus vogeli</i>	Thailand	Nakhon Ratchasima		Malhotra and Thorpe (2004c); Creer et al. (2003)
49	ROM 30791	AF171903	U41892	AY059562	<i>Trimeresurus cf. vogeli</i>	Vietnam	Gia Lai		Malhotra and Thorpe (2004a); Kraus et al. (1996)
50	SCUM 035045	EF597522	EF597528	EU443812	<i>Trimeresurus cf. yunnanensis</i>	China	Huili, Sichuan		Dawson et al. (2008)
Outgroups									
51	B301	AY352754	AY352817	AY352722	<i>Craspedocephalus borneensis</i>	Malaysia	Borneo		Malhotra and Thorpe (2004a)
52	B392	AY352757	AY352820	AF517177	<i>Craspedocephalus puniceus</i>	Indonesia	—		Creer et al. (2003)
53	AM B261	AY352762	AY352828	AY352732	<i>Craspedocephalus gramineus</i>	India	—		Malhotra and Thorpe (2004a)
54	A218	AY059569	AY352829	AY059564	<i>Craspedocephalus malabaricus</i>	India	Taminadu		Malhotra and Thorpe (2004a)
55	RAP 0453	KC347479	AY059597	AY059565	<i>Craspedocephalus trigonocephalus</i>	Sri Lanka	—		Malhotra and Thorpe (2004a); Pyron et al. (2013)
56	B499	AY352747	AY352808	AY352713	<i>Azeniope feae</i>	China	—		Malhotra and Thorpe (2004a)

default parameters, and subsequently checked them by eye in BioEdit 7.0.5.2 (Hall 1999) and slightly adjusted when required. The mean uncorrected genetic p -distances between sequences were calculated with MEGA 6.0. (Tamura et al. 2013) based on *cyt b* sequences of pitviper species of the subgenus *Trimeresurus*, and missing data or gaps were subjected to the pairwise deletion option. The best-fit substitution models for the data set were selected for genes and codon positions in Partitionfinder 2.1.1 (Lanfear et al. 2012) using the Akaike information criterion (AIC). When the same model was proposed for different codon partitions of a given gene, they were treated as a single partition.

Phylogenetic trees were estimated for the combined mitochondrial DNA fragments (*cyt b*, ND4 and 16S rRNA) data set. We inferred the matrilineal genealogy of *Trimeresurus* using Bayesian inference (BI) and maximum likelihood (ML) approaches. We used the IQ-TREE webserver (Nguyen et al. 2015) to generate the ML-tree and assessed the confidence in tree topology by 1000 ultrafast-bootstrap replications (UFBS). We conducted BI in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Metropolis-coupled Markov chain Monte Carlo (MCMC) analyses were run with one cold chain and three heated chains for one million generations and sampled every 1000 generations. The run was checked to ensure the effective sample sizes (ESS) were all above 200 by exploring the likelihood plots using TRACER v. 1.7 (Rambaut et al. 2018). We discarded the initial 1000 trees as burn-in. For BI-analysis we assessed the confidence in tree topology by the posterior probability (PP) (Huelsenbeck and Ronquist 2001). We a priori regarded the tree nodes with UFBS values of 95% or higher and PP values over 0.95 as strongly supported; UFBS values between 95% and 90% and PP values between 0.95 and 0.90 were regarded as moderate support, and lower values were regarded as a lack of node support (Huelsenbeck and Hillis 1993).

Morphological differentiation

Measurements and meristic counts followed Sumontha et al. (2021). Paired meristic characters were given in the left/right order. The following measurements were taken with a Mitutoyo digital caliper to the nearest 0.1 mm: ED, horizontal eye diameter; 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 margin); SOL, supraocular length; SOW, supraocular width; SVL, snout-vent length; TaL, tail length; TL, total length; VED, vertical eye diameter. Examined meristic characters include: CP, cloacal plate(s) (divided or single); ASR, anterior number of dorsal scale rows (at one HL behind the head); IL, number of infralabial scales; IOS, interorbital scales, counted along a row between the middle of supraocular scales; MSR, number of dorsal scale rows at mid-body (at the level of the ventral plate corresponding to the half of the total number of ventrals); PosOc, number of

postocular scale(s); PSR, posterior number of dorsal scale rows (at one HL before the cloacal plate); PreV, number of prefrontals (scales directly preceding the ventrals, unpaired, wider than long but not in contact on each side with the 1st dorsal scale row); SC, number of subcaudal scales, not including the terminal pointed scute; SL, number of supralabial scales; SRR, dorsal scale rows reduction (counted following Dowling 1951a); VEN, number of ventral scales (counted following Dowling 1951b). We also counted the number of teeth on upper and lower jaws for each specimen. Sex was determined by examination of the hemipenes that were forcedly everted by using water injection in tail base prior to the preservation of the specimen.

Results

Sequence characteristics

A total of 2,427 aligned base pairs were obtained from the three examined mtDNA fragments, including: 1,104 bp from *cyt b*, 803 bp from ND4 and 520 bp from 16S rRNA. Protein-coding sequences were translated into amino acids in order to confirm that no pseudogenes have been amplified. We deposited the newly obtained sequences in GenBank under the accession numbers OR470534–OR470580 and OR471621–OR471643 (see Table 2).

PartitionFinder 2.1.1 proposed the following partition schemes and substitution models: GTR+I+G model for 16S rRNA and the second codon position of ND4, GTR+G model for the first codon position of *cyt b*, HKY+I+G model for the second codon position of *cyt b* and the first and the third codon positions of ND4, and HKY+I model for the third codon position of *cyt b*, which resulted in four partitions in total.

Phylogenetic relationships

The ML and BI analyses recovered trees with very similar topologies, with minor topological differences associated with only a few deeper nodes with insufficient nodal support (Fig. 2). Our mtDNA-genealogy recovers the genus *Trimeresurus* as monophyletic (100/1.0, hereafter node support values are given for ML UFBS/BI PP, respectively) with respect to its sister genus *Craspedocephalus*, however, the genealogical relationships within *Trimeresurus* remain insufficiently resolved. Monophyly of the genus *Craspedocephalus* is not supported in our tree: it consists of two deeply-divergent mtDNA clades corresponding to the Southeast-Asian subgenus *Craspedocephalus* (95/1.0), and to the Indian and Sri-Lankan subgenus *Peltopeltor* (100/1.0), however this topology lacks significant nodal support (56/0.74; see Fig. 2). Monophyly of the subgenus *Trimeresurus* is also not supported in our analysis with *T. albolabris* species group (100/1.0) recovered as genetically distinct in mtDNA se-

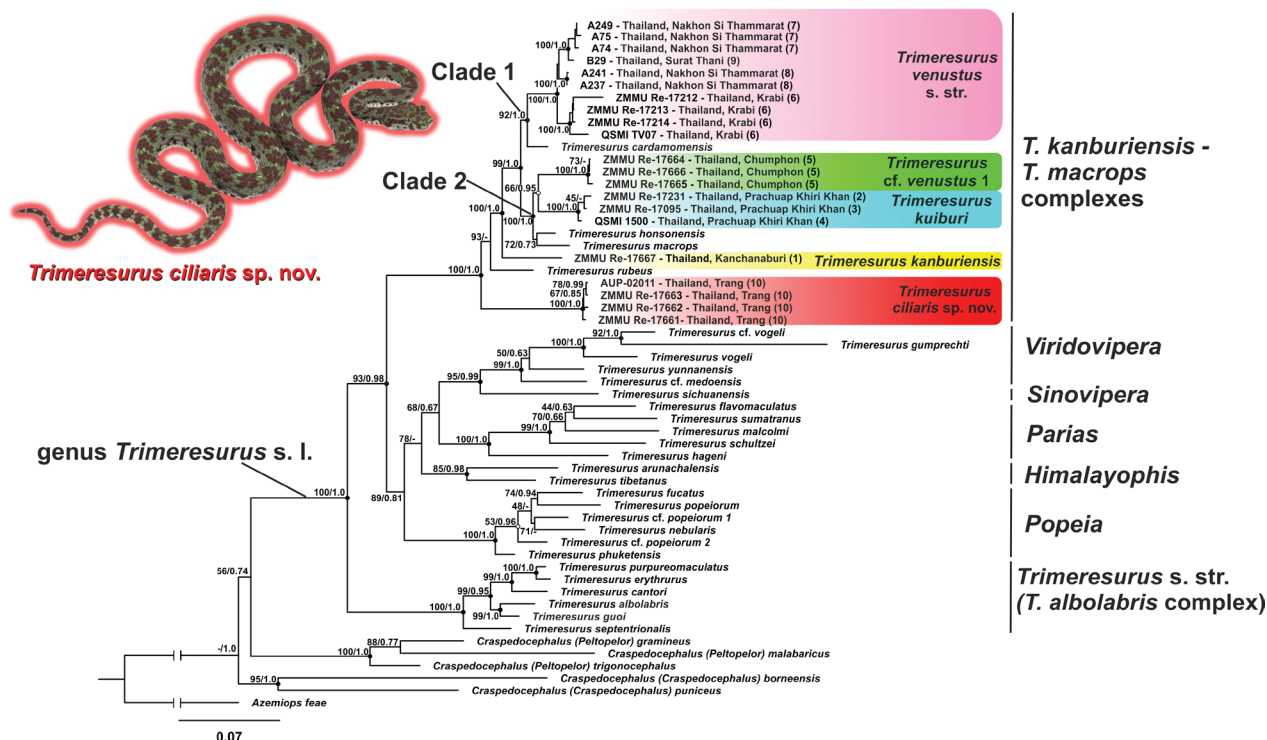


Figure 2. Maximum Likelihood (ML) tree of *Trimeresurus* derived from the analysis of 2427 bp of *cyt b*, ND4 and 16S rRNA mitochondrial DNA gene sequences. For voucher specimen information and GenBank accession numbers see Table 2. Numbers at tree nodes correspond to ML UFBS/BI PP support values, respectively. Colors of clades and locality numbers correspond to those on the map in Figure 1. Photograph by P. Pawangkhanant.

quences from other members of the subgenus (93/0.98, Fig. 2). The latter include the members of *T. macrops* species complex and *T. kanburiensis* species complex which form a strongly supported clade (100/1.0) (Fig. 2).

The genealogical relationships within the *macrops* – *kanburiensis* clade are generally sufficiently resolved and suggest that *Trimeresurus* sp. from Trang Province forms a lineage with sister relationships to all other taxa of this group (this topology received significant support in ML analysis, but was not supported in BI analysis, 93/-; see Fig. 2). The phylogenetic position of *T. rubeus* from Vietnam remains sufficiently unresolved in our tree. The *T. macrops* and *T. kanburiensis* species complexes were recovered as not monophyletic, with their members grouped into two major clades. The first clade (Clade 1) includes *T. venustus* s. str. (from Nakhon Si Thammarat, Krabi, and Surat Thani provinces, 100/1.0) and *T. cardamomensis* (from eastern Thailand, Cambodia, and southern Vietnam); monophyly of this group got significant nodal support (92/1.0, Fig. 2). Clade 2 (100/1.0) includes specimens of *T. kuiburi* (from Prachuap Khiri Khan Province, 100/1.0), *T. cf. venustus* 1 (from Chumphon Province, 100/1.0), *T. honsonensis*, and *T. macrops* (Fig. 2), with *T. kuiburi* + *T. cf. venustus* 1 forming a monophyletic group which was not supported by ML analysis, but got a significant support in BI analysis (66/0.95). *Trimeresurus kanburiensis* was strongly suggested as a sister taxon to a group including Clades 1 and 2 (99/1.0, Fig. 2). In summary, our analyses demonstrate that *Trimeresurus* sp. from Trang Province (100/1.0) represents a new highly divergent and previously unknown lineage of pitvipers,

which does not fall into radiation of *T. venustus* sensu lato, and is suggested as a sister lineage to all remaining members of the *T. macrops* and *T. kanburiensis* species complexes (Fig. 2).

Genetic distances

The uncorrected *p*-distances for the *cyt b* gene fragment among examined members of the subgenus *Trimeresurus* are presented in Table 3. Interspecific distances among the members of the subgenus *Trimeresurus* varied from *p* = 3.0% (between *T. venustus* and *T. honsonensis*) to *p* = 15.1% (between *T. kuiburi*, *T. erythrus* and *T. albolabris*). The newly discovered *Trimeresurus* sp. lineage from Trang is highly divergent from other congeners with the minimal genetic distance of *p* = 7.7% (with *T. honsonensis* and *T. cardamomensis*) and the maximal distance of *p* = 17.3% (with *T. albolabris*) of sequence divergence in *cyt b* mtDNA gene.

Taxonomy

Our molecular results are further corroborated by the morphological analysis, which recovered a number of important diagnostic characters which allow distinguishing the population of *Trimeresurus* sp. from Trang from *T. venustus* s. str. and all other congeners (summarized

Table 3. Uncorrected *p*-distances (percentage) between the sequences of cyt *b* mtDNA gene of species of the subgenus *Trimeresurus* included in the phylogenetic analyses.

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Trimeresurus ciliaris</i> sp. nov.														
2	<i>T. venustus</i> s. str.	12.5													
3	<i>T. cf. venustus</i> (1)	12.2	5.9												
4	<i>T. kuiburi</i>	12.1	9.5	6.6											
5	<i>T. kanburiensis</i>	8.8	6.9	7.2	7.2										
6	<i>T. honsonensis</i>	7.7	3.0	4.1	7.4	8.7									
7	<i>T. macrops</i>	10.9	5.6	5.9	5.3	6.4	4.8								
8	<i>T. cantori</i>	13.4	12.9	13.0	13.4	13.2	—	12.4							
9	<i>T. erythrurus</i>	17.0	17.4	14.0	15.1	15.1	13.7	13.9	5.2						
10	<i>T. septentrionalis</i>	14.1	17.9	13.3	14.9	15.4	4.8	13.1	7.6	8.1					
11	<i>T. purpureomaculatus</i>	14.0	18.2	13.3	14.0	14.4	5.6	12.6	5.0	1.7	8.1				
12	<i>T. albolabris</i>	17.3	14.0	13.9	15.1	14.9	13.3	14.1	7.0	6.4	8.0	7.2			
13	<i>T. cardamomensis</i>	7.7	9.3	6.0	5.6	5.0	—	4.9	10.8	13.5	13.2	12.9	12.4		
14	<i>T. rubeus</i>	8.1	12.7	8.0	8.0	7.7	—	6.7	12.6	13.7	14.4	12.5	14.1	6.7	
15	<i>T. guoi</i>	12.8	18.0	12.3	13.4	13.4	—	11.2	4.2	5.0	7.0	4.9	3.9	11.9	12.4

below). These results support our hypothesis that this recently discovered lineage of *Trimeresurus* sp. from Trang Province represents a previously unknown species, which we formally describe below.

Trimeresurus ciliaris sp. nov.

<https://zoobank.org/898F9CF2-0457-4F77-B53A-BB8D1E-8AB3F2>

Figures 3, 4, 5 (A), 6 (A, B), 7 (B–E), Table 4

Chresonymy. *Cryptelytrops* cf. *venustus* (partim): Chan et al. (2011: 258).

Holotype. ZMMU Re-17661, adult male collected by S. Idiatullina, P. Pawangkhanant and T. Woranuch on 23 January 2023 near Thum Khao Ting, Palian District, Trang Province, Thailand (07°09.943N, 99°48.142E; elevation 28 m a.s.l).

Paratypes (n = 4). QSMI 1538, adult female collected by P. Pawangkhanant on 18 June 2016 from the same locality as the holotype; AUP-02011, ZMMU Re-17662, ZMMU Re-17663, three adult males collected on 23 January 2023 from the same locality and by the same collectors as for the holotype.

Diagnosis. A species of the genus *Trimeresurus* which is assigned to the subgenus *Trimeresurus* based on the following morphological attributes: a long papillose or calyculate hemipenis and partially fused first supralabial and nasal scales (Malhotra and Thorpe 2004a; David et al. 2011). The new species *Trimeresurus ciliaris* sp. nov. can be distinguished from all other congeneric species by the following combination of morphological characters: three or four small convex supraocular scales; internasals not in contact; small scale between nasal and the

scale formed by the fused second supralabial and loreal present; dorsal scales in 17–17–15 rows; ventral scales 172–175 in males, 171 in a single female; subcaudal scales 59–63 in males, 52 in a single female, all paired; in life an emerald-green dorsum with reddish-brown cross-bands; creamy-white venter lacking dark dots or stripes on the lateral sides of the ventrals; iris olive-green with faded-brown horizontal stripe; tail dark brown mottled with rusty spots; white vertebral spots present in both sexes located approximately every two or three dorsal scales; dark brown spots forming discontinuous pattern present on 1–3 lateral dorsal scale rows; males having a reddish-brown postocular stripe.

Description of the holotype. Adult male (Fig. 3), specimen in a good state of preservation. Body cylindrical, long and thin (SVL 345 mm, TaL 72 mm, TL 417 mm, TaL/TL 0.17) (Fig. 3A,B). Head triangular in dorsal view (Fig. 3C), elongate, clearly distinct from the neck (HL 16.5 mm, HW 11.4 mm, HW/HL 0.69). Snout elongate, flattened and rounded in dorsal view (Fig. 3C), rather rectangular in lateral view (Fig. 3E,F), with a very distinct and sharp canthus rostralis (SnL/HL 0.30, SnL/ED 1.92). Distance between eye and nostril 3.9 mm on both sides. Rostral slightly visible in dorsal aspect, triangular (Fig. 3C). Pupil vertically elliptical, loreal pit present, triangular in shape (Fig. 3E,F). Nostril completely enclosed in nasal scale; nasal scale partially fused with first supralabial (Fig. 3E). Shield bordering the anterior edge of loreal pit fused with second supralabial. Anterior subocular long, thin, and crescent-like, separated from the 4th and 5th supralabials by one row of scales; posterior subocular ovoid, separated from the 6th supralabial by 2/1 scales (Fig. 3E, F). Three preoculars on each side of the head; two upper preoculars located above the loreal pit, elongated, in contact with the single loreal which separates them from the nasal; lower preocular forming the lower margin of the loreal pit, lower preocular in contact with third supralabial (Fig. 3E, F). A small scale between the

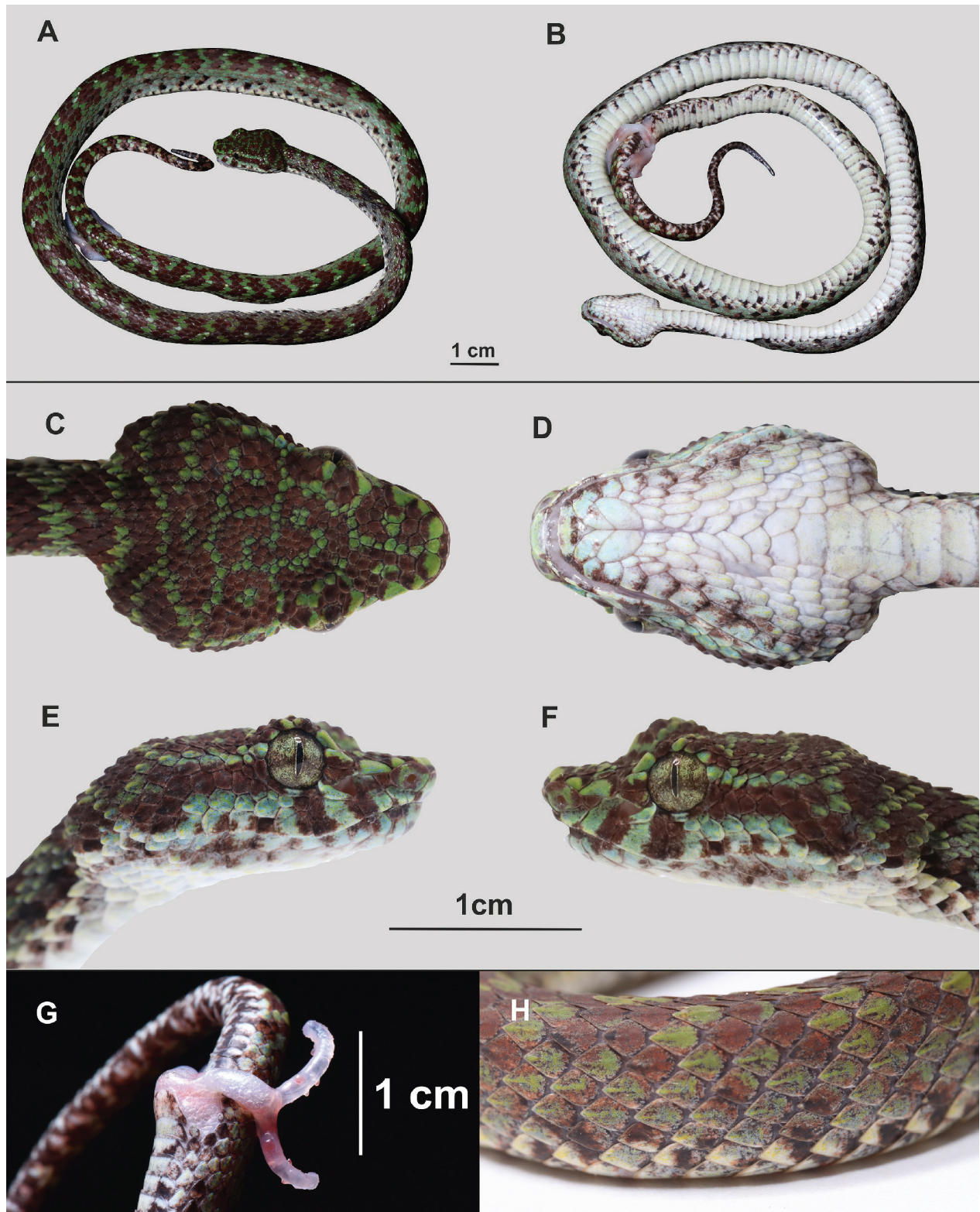


Figure 3. The holotype of *Trimeresurus ciliaris* **sp. nov.** in life (ZMMU Re-17661, adult male) from Thum Khao Ting, Palian District, Trang Province, Thailand. **A** dorsolateral and **B** ventral view of body; **C** head in dorsal, **D** in ventral, **E** in lateral right, and **F** in lateral left aspects; **G** right hemipenis, partially everted; **H** close-up of dorsal scales. Photographs by P. Pawangkhanant.

nasal and the scale formed by the fused second supralabial and loreal; 2/2 postoculars; 9/9 supralabials, third the largest (Fig. 3E,F); 11/10 infralabials, those of the first pair in contact with each other behind the mental; the first three pairs of infralabials in contact with the single pair of chin shields (Fig. 3D). Six pairs of gulars aligned be-

tween the chin shields and the first preventral (Fig. 3D). One large pair of enlarged internasals, separated by three small scales. Three small slightly convex supraoculars, same size as or smaller than preoculars and postoculars (Fig. 3C). Scales on snout and in the interorbital region smooth, irregular, subimbricate; temporal and occipital

Table 4. Morphological data on the type series of *Trimeresurus ciliaris* **sp. nov.** Notes: For abbreviations see Materials and methods section.

No.	ZMMU Re-17661	AUP-02011	ZMMU Re-17662	ZMMU Re-17663	QSMI 1538
Type status	Holotype	Paratype	Paratype	Paratype	Paratype
Sex	Male	Male	Male	Male	Female
SVL (mm)	345	357	360	352	333
TaL (mm)	72	73	72	71	51
TL (mm)	417	430	432	423	384
HL (mm)	16.5	16.9	17.1	16.8	16
HW (mm)	11.4	12.2	12.7	11.3	11.3
HD (mm)	5	5.7	6.1	5.6	5.7
SnL (mm)	5	5.1	5.3	4.9	5.6
ED (mm)	2.6	2.8	3	2.5	2.7
VED (mm)	2.3	2.1	2.5	2.1	2.2
SOL (mm)	2.8	3	2.8	2.7	3.4
SOW (mm)	0.7	0.6	0.7	0.7	0.8
Internasals in contact	no	no	no	no	no
Contact nasal-1st SL	partially fused	partially fused	partially fused	partially fused	partially fused
SupOc	3	3	4	4	3
PosOc	2/2	2/2	2/2	2/2	2/2
IOS	11	14	11	11	12
SL	9/9	9/8	9/9	9/9	9/9
IL	11/10	10/10	10/10	11/10	10/11
ASR	17	17	17	17	17
MSR	17	17	17	17	17
PSR	15	15	15	15	15
SRR 17 to 15 (V)	120–121	123–124	123–124	125–126	123
PreVEN	2	2	2	2	2
VEN	172	173	175	172	171
CP	1	1	1	1	1
SC	61	59	63	61	52
Postocular stripe	reddish-brown	reddish-brown	reddish-brown	reddish-brown	Faint, reddish-brown
Number of red bands on dorsum	74	81	73	70	80
White vertebral dots	every 2–3 scales	every 2–3 scales	every 2–3 scales	every 2–3 scales	every 2–3 scales

scales moderately keeled (Fig. 3C,E,F). Dorsal scales in 17–17–15 rows. Dorsal scales all strongly keeled, except the first row, which is smooth (Fig. 3H). One preventral + 172 ventrals. Cloacal plate single; 61 subcaudals, all divided. Hemipenes long, papillose, and deeply forked with small, soft, basal spines (Fig. 3G). Each maxilla bearing a single large venom tooth; three teeth on palatine, 11 on pterygoid, and 12 on dentary.

Coloration in life (Figs 3–5). Dorsal surface of the head emerald-green with many scales partly or entirely dark red, especially on the snout, and on the interorbital, temporal and occipital regions (Fig. 3C). A reddish-brown postocular stripe extends from postocular scales to the neck (Fig. 3E,F). Black vertical pupil; iris olive-green with faded brown horizontal stripe (Fig. 3E,F). The background color of the dorsum emerald-green, similar to that of the head, with about 75 irregular, dark red cross-bands (Fig. 3A). These red bands are about two dorsal scales long mediodorsally, but getting narrower on the lower flanks where they are about 1–2 dorsal scale in length. At the level of the vertebral row, the red bands are generally

separated by one dorsal scale. White vertebral spots present along the vertebral scale row, approximately every two or three scales (Fig. 3A). Ventrolateral stripe absent. Dark brown spots forming discontinuous pattern present on first to third lateral dorsal scale rows (Fig. 3H). Tail dark brown mottled with rusty spots. Ventral surface of the tail dark brown with white irregular bands, tail tip completely black. Infralabials greenish-white with faint brown spots (Fig. 3E,F); the ventral surfaces of the head creamy white with bluish tint (Fig. 3D); the ventral surfaces of body creamy white (Fig. 3B).

In preservative the background dorsal color faded to greyish-brown, with less contrasting dark marks on the head and bands on the dorsum; the ventral color became whitish grey.

Variation. The main meristic and morphometric characters of the type series of *Trimeresurus ciliaris* **sp. nov.** are summarized in Table 4; color variation of the type series is presented in Figures 4, 5A, 6A–B, 7B–E. All members of the type series, similarly to the holotype, show a nasal scale partially fused with the first supralabial. Head

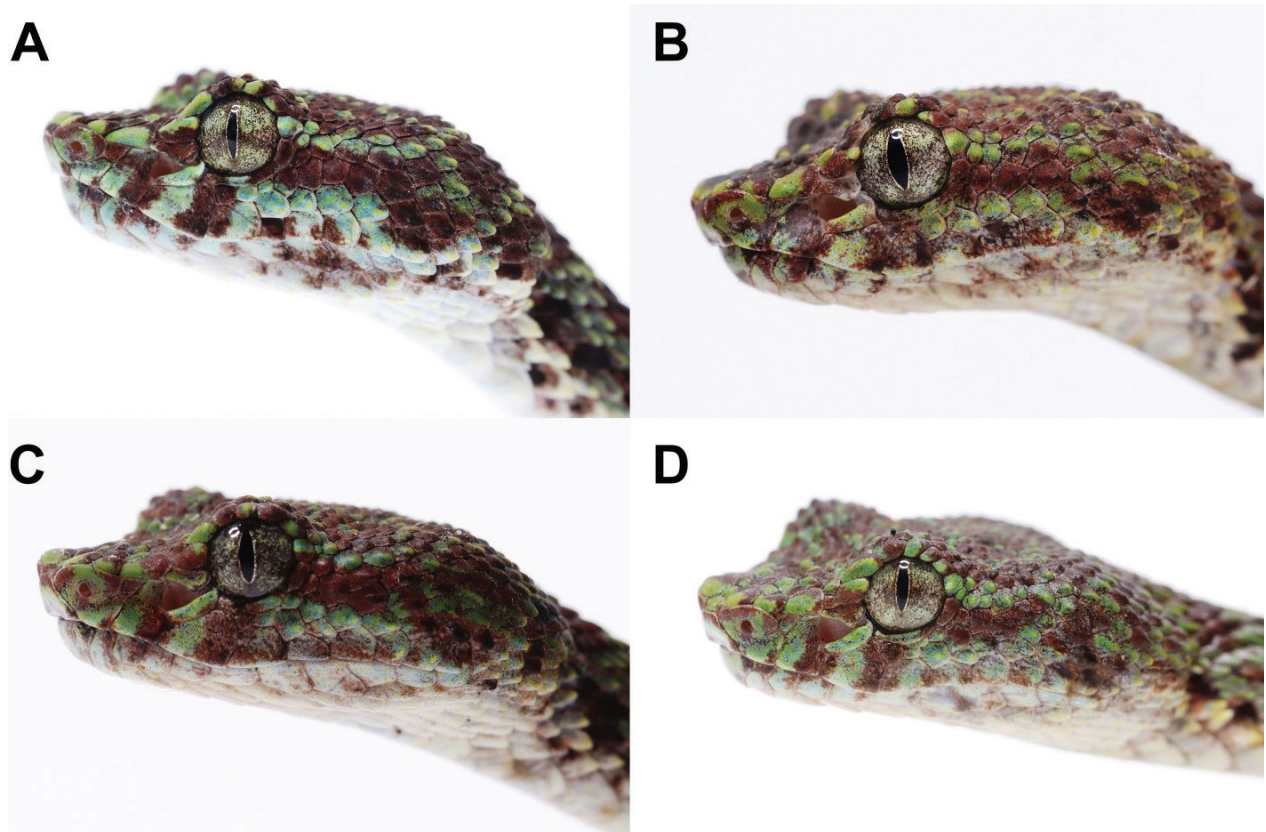


Figure 4. Variation in head scalation and coloration in male paratypes of *Trimeresurus ciliaris* **sp. nov.**: **A** ZMMU Re-17661; **B** ZMMU Re-17662; **C** AUP-02011; and **D** ZMMU Re-17663. See Table 4 for details. Photographs by P. Pawangkhanant.

scalation of the paratypes generally agrees with that of the holotype, however male ZMMU Re-17662 has a suboculars fused in a single long crescent-shaped scale (Fig. 4B), while the two males AUP-02011 (Fig. 4C) and ZMMU Re-17663 (Fig. 4D) have four small supraoculars on each side of the head instead of three as in the holotype. Coloration of paratypes is very similar to that of the holotype; all specimens have a creamy-white belly and lack ventrolateral stripe. Female specimen QSMI 1538 has slightly lower number of subcaudals than in males (SC 52 vs. 59–63). The white vertebral spots are present both in males and in females; they are regularly spaced by two or three vertebral scales. The number of dark bands on dorsum varies from 70 to 81 among the members of the type series, without apparent sexual dimorphism. Coloration of female specimen QSMI 1538 largely faded due to preservation in formalin for seven years.

Distribution and natural history. Currently, *Trimeresurus ciliaris* **sp. nov.** is known only from a narrow limestone area in the Nakawan Range spanning the border of Thailand and Malaysia, in particular in limestone forests in Trang (Palian District) and Satun (Tha Le Ban National Park) provinces, Thailand (Fig. 1). It is highly likely that the new species also inhabits the northernmost part of Perlis State of Peninsular Malaysia (Fig. 1, see Discussion for details). The new species occurs in lowland dipterocarp forest on limestone rocks and appears to be strongly associated with a karst landscape. The new species was recorded for the first time in limestone

formations within the Tha Le Ban National Park, Satun Province of Thailand, in June 2016 (P. Pawangkhanant pers. obser.), where it was observed in a lowland dipterocarp forest with numerous large karst rocks. Almost all specimens of *Trimeresurus ciliaris* **sp. nov.** were collected during daytime (except ZMMU Re-17663 that was collected at night around 20h50). All specimens were found near large boulders of karst rocks covered with dense vegetation and leaf litter (Fig. 7A); one specimen was found after heavy rain around 18h00 while perching vertically on a limestone rock surface ca. 5 m above the ground.

In captivity, due to its small size, this species of pitviper mostly feeds on small geckos and microhylid frogs [*Microhyla butleri* Boulenger, *M. cf. heymonsi* Vogt, and *Micryletta cf. lineata* (Taylor)]. Nothing is known about the diet of the new species in the wild, but in one specimen from Mueang District, Satun Province, caudal luring behavior was observed: the male specimen was wiggling its tail tip when an adult gecko *Cnemaspis biocellata* Grismer, Chan, Nasir & Sumontha approached its shelter. Other amphibian and reptile species recorded in syntopy with the new species include: *Ingerophrynus parvus* (Boulenger), *Micryletta cf. lineata*, *Microhyla butleri*, *Kaloula latidisca* (Chan, Grismer & Brown), *Cnemaspis niyomwanae* Grismer, Sumontha, Cota, Grismer, Wood, Pauwels & Kunya, *C. biocellata*, *Gekko gecko* (Linnaeus), *Cyrtodactylus astrum* Grismer, Wood, Quah, Anuar, Muin, Sumontha, Ahmad, Bauer, Wangkulangkul, Grismer & Pauwels, *C. quadrivirgatus* Taylor, *Cyrtodactylus*

Table 5. Comparison of morphological characters of *Trimeresurus ciliaris* **sp. nov.** with *T. kanburiensis*, *T. kuiburi*, and *T. venustus* s. str. Diagnostic differences from the new species are marked in bold.

Character	<i>T. ciliaris</i> sp. nov.	<i>T. kanburiensis</i>	<i>T. kuiburi</i>	<i>T. venustus</i> s. str.
Max SVL (males, mm)	360	412	376	490
Max SVL (females, mm)	333	572	451	456
IOS	11–14	7–9	9–11	8–12
Internasals in contact	0%	0%	100%	45%
ASR	17	23 (21 or 22)	21 (23)	23 (21 or 25)
MSR	17	19	19	21 (19)
PSR	15	15 (16)	15	15
VEN males	172–175	172	164–166	166–177
VEN females	171	170–178	164–171	172–181
SC males	59–63	59	63–65	63–72
SC females	52	41–51	51–53	50–66
Small scale between nasal and the scale formed by the fused second supralabial and loreal	present	present/absent	absent	absent
Dorsum color in life	Reddish-brown bands on emerald-green background	Dark olive-brown bands on olive-grayish background	Red/purple bands on bottle green background	Red/purple bands on dark/bottle green background
White vertebral dots (in males)	Present, every 2–3 scales	Present, every 3–5 scales	Present, every 5–6 scales	Absent
White vertebral dots (in females)	Present, every 2–3 scales	Absent	Absent	Absent
Belly background color in life	creamy white	creamy white	pale green	pale green
Lateral dark stripe on ventrals	Absent	Always present, discontinuous, olive-brown	Absent	Always present, continuous or discontinuous, red
Supraocular scale	Three or four, small	One, large	One, large	One, large
Iris color	Olive-green with faded brown horizontal stripe	brown, slightly golden	copper	yellowish-brown/gold
Tail color	dark brown mottled with rusty	brownish-gray with olive-brown blotches	red with some thin lighter bands	brown with dark purplish-brown crossbars
Distribution	Thailand (Trang, Satun), Malaysia (Perlis)	Thailand (Kanchanaburi)	Thailand (Prachuap Khiri Khan)	Thailand (Surat Thani, Phang-Nga, Trang, Nakhon Si Thammarat, Surat Thani, Krabi, Chumphon?), Malaysia (Kedah?)
Sources	our data	Smith (1943); David et al. (2004); our data	Sumontha et al. (2021); our data	Vogel (1991); David et al. (2004); Malhotra and Thorpe (2004b); our data

lus sp., *Elaphe taeniura ridleyi* (Cope), and *Tropidolaemus wagleri* (Boie).

Etymology. The species name “*ciliaris*” is a Latin adjective in the nominative singular, masculine gender, derived from Latin word “*cilium*” meaning “an eyelash”, and is given in reference to the characteristic small and distinct supraocular scales in the new species, which resemble eyelashes or eyebrows in lateral view. We suggest the following common names for the new species: “Ngu Hang Mhai Khao Hin Poon” (งูหางหมัดหินปูน) (in Thai), “Limestone Eyelash Pitviper” (in English), and “*Resnitchataya ukrashennaya kufiya*” (in Russian).

Comparisons. The new species is morphologically and phylogenetically placed within the subgenus *Trimeresurus* (Malhotra and Thorpe 2004a; David et al. 2011) and morphologically is overall most similar to other limestone-dwelling pitviper species, including *T. kanburiensis*, *T. kuiburi*, and *T. venustus* s. str., so the comparisons with these three species appear to be the most pertinent. *Trimeresurus ciliaris* **sp. nov.** can be easily distinguished from other congeners by having: 17 midbody scale rows, three or four small supraocular scales, and a small scale between the nasal and the scale formed by the fused second supralabial and loreal. The main diagnostic characters separating *Trimeresurus ciliaris* **sp. nov.** from these three species are summarized in Table 5. The comparison of body coloration and head scalation of these species is presented in Figures 5 and 6, respectively. From the superficially similar *Trimeresurus truongsongensis* Orlov, Ryabov, Vu & Ho, a member of the subgenus *Viridovipera*, the new species can be easily distinguished by having a long papillose and deeply forked hemipenis (vs. short spinose hemipenis) and partially fused first supralabial and nasal scales (vs. separate).

In particular, *Trimeresurus ciliaris* **sp. nov.** differs from *T. kanburiensis* by having: smaller maximal SVL

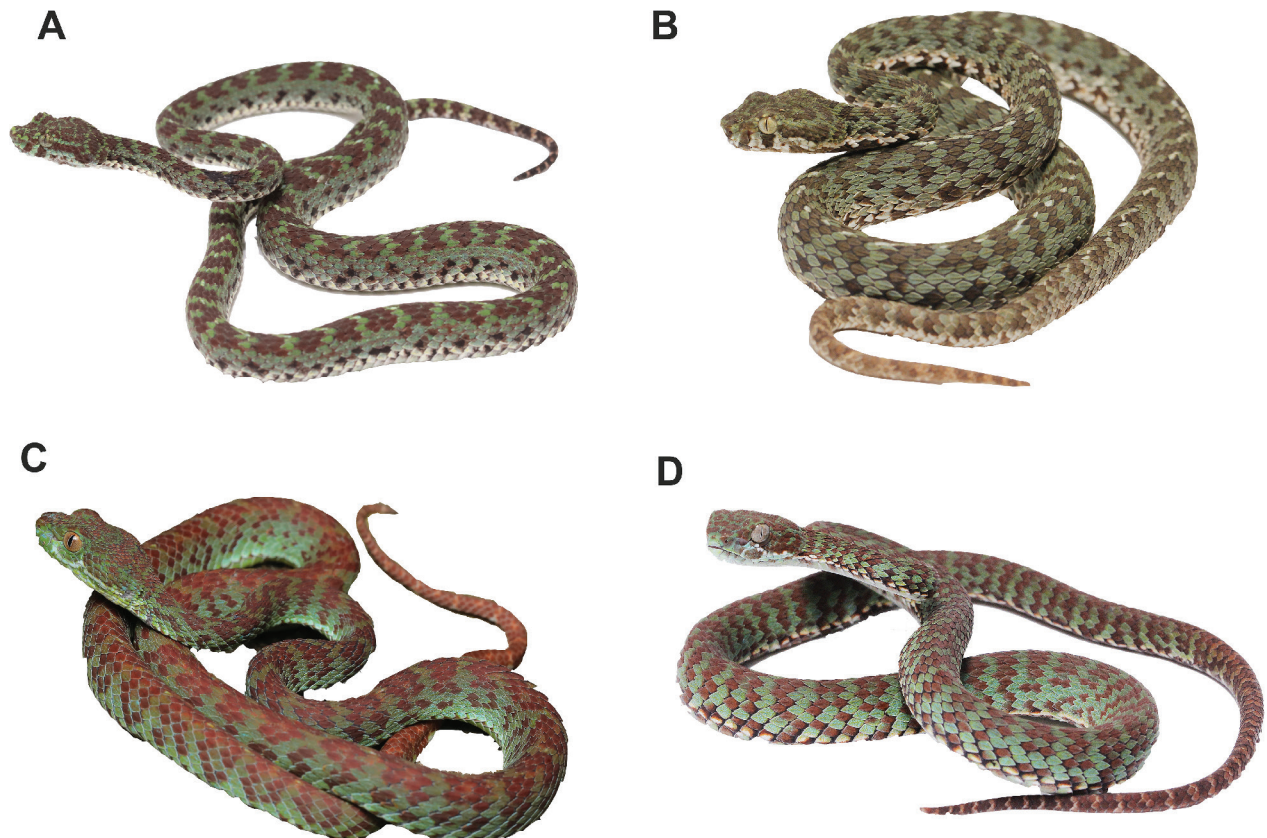


Figure 5. Comparison of body coloration between males of **A** *Trimeresurus ciliaris* **sp. nov.**; **B** *T. kanburiensis*; **C** *T. kuiburi*; and **D** *T. venustus* s. str. Photographs by P. Pawangkhanant (A, B, D) and T. Woranuch (C).

(360 mm in male, 333 mm in female vs. 412 mm in male, 572 mm in female); lower anterior number of dorsal scale rows (17 vs. 23, rarely 21 or 22); lower number of midbody scale rows (17 vs. 19); absence of dark lateral stripe on ventrals, Fig. 5A (vs. dark lateral stripe on ventrals always present, discontinuous, olive-brown, Fig. 5B); three or four small supraocular scales, Fig. 6B (vs. single large supraocular scale, Fig. 6D); body color in life (reddish-brown bands on an emerald-green background [Fig. 5A] vs. dark olive-brown bands on olive-greyish background [Fig. 5B]); white vertebral spots present in both sexes separated by 2–3 dorsal scales vs. white vertebral spots present only in males and are separated by 3–5 dorsal scales; iris color (olive-green with faded brown horizontal stripe [Fig. 6A] vs. brown, slightly golden [Fig. 6C]); and tail color (dark brown mottled with rusty spots vs. brownish-gray with olive-brown blotches).

Trimeresurus ciliaris **sp. nov.** further differs from *T. kuiburi* by having: smaller maximal SVL in female (333 mm vs. 451 mm); internasals separated by two or three scales (vs. always in contact); lower anterior number of dorsal scale rows (17 vs. 21 rarely 23); lower number midbody scale rows (17 vs. 19); higher number of ventrals in females (172–175 vs. 164–166); ventral surfaces of body creamy-white (vs. pale-green); three or four small supraocular scales, Fig. 6A (vs. one large supraocular scale, Fig. 6F); small scale between nasal and second supralabial present vs. absent; body color in life (reddish-brown bands on emerald-green background

[Fig. 5A] vs. red to purple bands on bottle-green background [Fig. 5C]); white vertebral spots present in both sexes separated by 2–3 dorsal scales vs. white vertebral dots present only in males and are separated by 5–6 dorsal scales; iris color (olive-green with faded brown horizontal stripe [Fig. 6A] vs. copper [Fig. 6E]); and tail color (dark brown mottled with rusty spots vs. red with some thin lighter bands).

Trimeresurus ciliaris **sp. nov.** can be further differentiated from *T. venustus* s. str. by having: smaller maximal SVL (360 mm in male, 333 mm in female vs. 490 mm in male, 456 mm in female); lower anterior number of dorsal scale rows (17 vs. 23, rarely 21 or 25); lower number midbody scale rows (17 vs. 21, rarely 19); white vertebral spots present in both sexes separated by 2–3 dorsal scales (vs. absent); ventral surfaces creamy-white (vs. pale-green); lateral dark stripe on ventrals absent (vs. lateral dark stripe on ventrals always present, discontinuous, red); three or four small supraocular scales, Fig. 6A (vs. one large supraocular scale, Fig. 6H); small scale between nasal and second supralabial present vs. absent; body color in life (reddish-brown bands on emerald-green background [Fig. 5A] vs. red to purple bands on dark bottle-green background [Fig. 5D]); white vertebral spots present in both sexes separated by 2–3 dorsal scales vs. white vertebral dots absent in both sexes; iris color (olive-green with faded brown horizontal stripe [Fig. 6A] vs. yellowish-brown to gold [Fig. 6G]); and tail coloration (dark brown mottled with rusty spots vs. brown with dark purplish-brown crossbars).

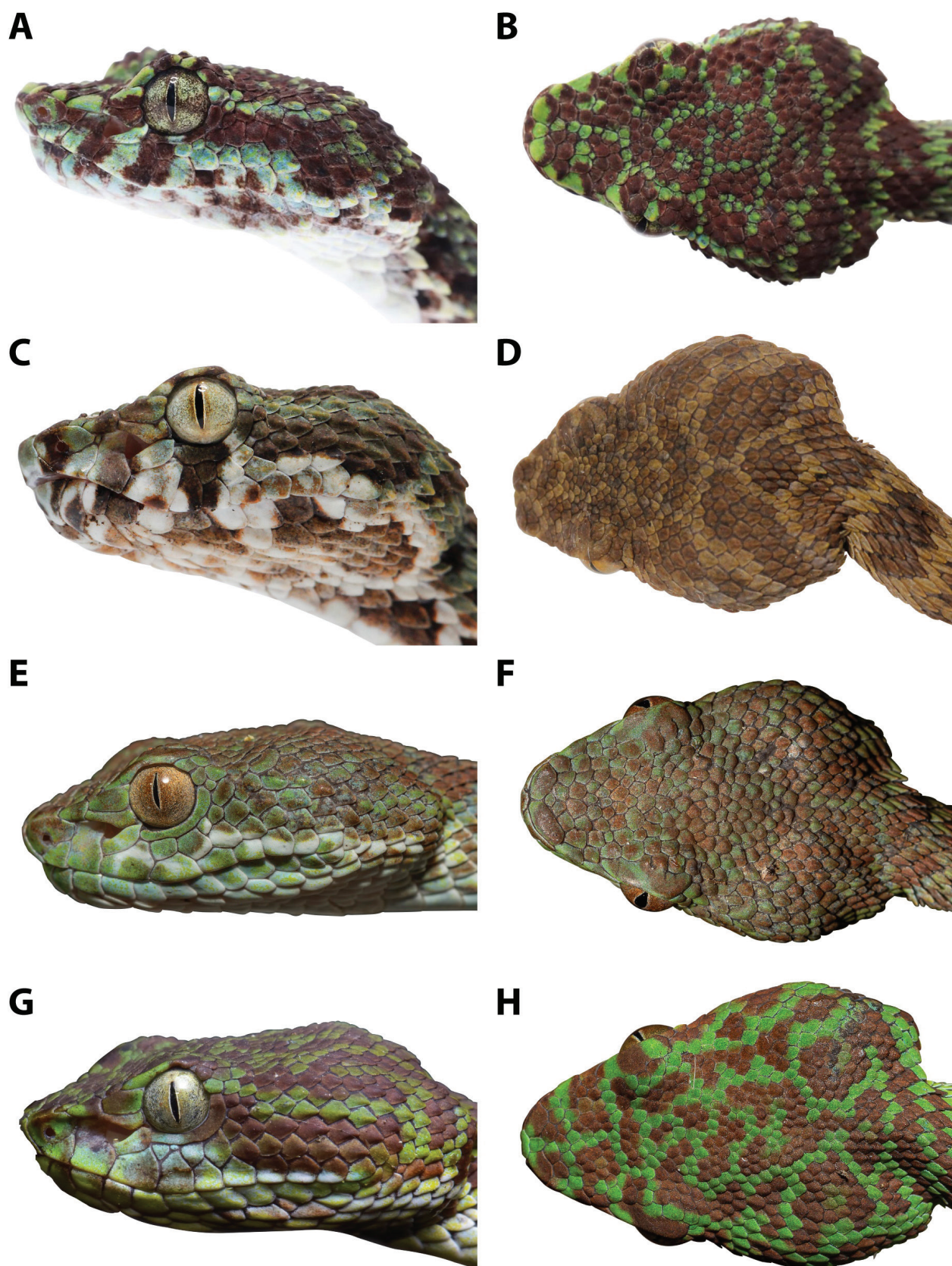


Figure 6. Comparison of head coloration (left profile and dorsal view of the head) between males of **A–B** *Trimeresurus ciliaris* **sp. nov.**; **C–D** *T. kanburiensis*; **E–F** *T. kuiburi*; and **G–H** *T. venustus* s. str. Photographs by P. Pawangkhanant (A–D), A. Kaosung (E–F, H) and M. Naiduangchan (G).

Among the other species in the subgenus *Trimeresurus*, the new species can be readily distinguished from *T. albolabris*, *T. andersonii*, *T. cantori*, *T. cardamomensis*, *T.*

caudornatus, *T. davidi*, *T. erythrurus*, *T. fasciatus*, *T. guoi*, *T. honsonensis*, *T. insularis*, *T. labialis*, *T. macrops*, *T. mutabilis*, *T. purpureomaculatus*, *T. rubeus*, *T. salazar*, and



Figure 7. Habitat of *Trimeresurus ciliaris* **sp. nov.** **A** microhabitat of the new species near the Thum Khao Ting Cave, Palian District, Trang Province, Thailand; **B** holotype ZMMU Re-17661, adult male, in life in situ; **C** paratype QSMI 1538, adult female, in life in situ; **D–E** male specimen from Wang Kelian, Perlis State, Malaysia (not collected). Photographs by P. Pawangkhanant (A–C) and Azrul Azrizal Azmy (D–E).

T. septentrionalis by body coloration and pattern (red-dish-brown bands on emerald-green background in the new species vs. uniform green or green coloration with no pattern, or pattern consisting of small brownish spots or speckles in other species, or straw-yellow background with irregular, dark-brown transverse body bands in *T. honsonensis*). Moreover, by having 17 MSR, the new species can be further separated from *T. albolabris* (21 MSR), *T. andersonii* (21 MSR), *T. cantori* (27, 29 or 31 MSR), *T. cardamomensis* (21 MSR), *T. caudornatus* (21 MSR), *T. davidi* (21 or 23 MSR), *T. erythrurus* (23 rarely 21, 25 MSR), *T. fasciatus* (21 MSR), *T. guoi* (21 MSR), *T. honsonensis* (21 MSR), *T. insularis* (21 MSR), *T. labialis* (21 or 23 MSR), *T. macrops* (21 MSR), *T. mutabilis* (21 MSR), *T. purpureomaculatus* (25 rarely 27, 29 MSR), *T. rubeus* (21 MSR), *T. salazar* (21 MSR), and *T. septentrionalis* (21 MSR) (see Gumprecht et al. 2004; Grismer et al. 2008; Malhotra et al. 2011; Chandramouli et al. 2020; Chen et al. 2020, 2021; Mirza et al. 2020; our data).

Discussion

In the present study we report on a previously unknown lineage of limestone-associated pitvipers of the subgenus *Trimeresurus*. According to our mtDNA-based genealo-

gy, the newly discovered species *Trimeresurus ciliaris* **sp. nov.** forms a highly divergent lineage of pitvipers, which is suggested as the sister lineage to all other members of *T. kanburiensis* and *T. macrops* species complexes (see Fig. 2). The new species is also separated from its congeners by a significant divergence in *cyt b* gene sequences (with $p = 7.7\text{--}17.3\%$), which is higher than the genetic distances between many other recognized species of the group (see Table 3). The mtDNA-based genealogy presented herein is insufficiently resolved in several deeper nodes; however in general it agrees well with the earlier genome-wide analysis by Mrinalini et al. (2015) which demonstrated the non-monophyly of *T. kanburiensis* and *T. macrops* species complexes suggesting complicated evolutionary history of this group. Though the phylogenetic position of the new species is not completely resolved in our phylogenetic tree, it is obvious that *Trimeresurus ciliaris* **sp. nov.** represents a deeply-divergent, previously unknown taxon which warrants taxonomic recognition.

Our study also provides some insights on phylogenetic relationships within the subgenus *Trimeresurus*. This subgenus was originally proposed by Malhotra and Thorpe (2004a) as *Cryptelytrops* Cope, 1860 (type species: *Trimeresurus carinatus* Gray, 1842, subjective junior synonym of *Trigonocephalus purpureomaculatus* Gray, 1832 based on David et al. 2011) based on subtle morphological similarities (form of hemipenial structures

and the fusion of the first supralabial and nasal scales). However, the monophyly of this group was not supported in the phylogenetic analysis of Malhotra and Thorpe (2004a). Later, David et al. (2011) demonstrated that the name *Trimeresurus* Lacépède, 1804 should be applied to this group (type species: *Trimeresurus viridis* Lacépède, 1804, nomen oblitum, a senior synonym of *Trimeresurus albolabris insularis* Kramer, 1977), however, these authors only discussed the nomenclature of the subgenus *Trimeresurus* and did not address its systematics. Our mtDNA-based genealogy suggested the significant differences between the members of *T. albolabris* and *T. kanburiensis* + *T. macrops* species groups included in this taxon. Though only based on limited data from three mtDNA genes, our study strongly suggests that the subgenus *Trimeresurus* sensu lato David et al. (2011) is likely non-monophyletic (Fig. 2). At the same time, the study by Malhotra et al. (2010) which included nuclear intron sequence data recovered the subgenus *Trimeresurus* as monophyletic. In summary, it is highly likely that the clade encompassing the *T. kanburiensis* + *T. macrops* species groups might warrant a taxonomic recognition as a separate subgenus of the genus *Trimeresurus*. Further comprehensive phylogenetic studies, including the analyses of multiple nuclear DNA genetic markers and additional morphological characters, are required to achieve a better resolution of evolutionary relationships within the subgenus *Trimeresurus*.

Our updated mtDNA-based genealogy also provides new data on phylogenetic relationships within the *T. kanburiensis* + *T. macrops* clade. Two morphologically defined species complexes were recognized within this clade: limestone-associated *T. kanburiensis* species group joining species with blotched coloration pattern on the dorsum (including *T. kanburiensis*, *T. venustus*, *T. kuiburi*, and also including *Trimeresurus ciliaris* **sp. nov.**) and lowland forest-associated *T. macrops* species group, which includes species with a uniform green coloration of dorsum (*T. macrops*, *T. cardamomensis*, and *T. rubeus*). *Trimeresurus honsonensis* has also a blotched dorsum coloration pattern and was reported to superficially resemble *T. venustus*, though this species is not associated with karst landscapes and occurs only in secondary forests with large granite boulders on Hon Son Island of southern Vietnam (Grismer et al. 2008). In our mtDNA-based genealogy the members of *T. kanburiensis* and *T. macrops* species complexes did not form reciprocally monophyletic clades, but were mixed with each other: *T. cardamomensis* is suggested as a sister species of *T. venustus* s. str., the phylogenetic position of *T. rubeus* remains unresolved, while *T. macrops*, *T. honsonensis*, *T. kuiburi* and *T. cf. venustus* 1 form a well-supported group (Fig. 2). Apparently, this result suggests that the transition between forest-dwelling and karst-dwelling lifestyle likely occurred multiple times independently within the subgenus *Trimeresurus*, often resulting in strikingly similar morphologies. It is noteworthy, that among other *Trimeresurus* subgenera, limestone-associated species often show coloration similar to that in *T. kanburiensis* group; for example, the karst-dwelling species *Trimeresurus*

(*Viridovipera*) *truongsonensis* from central Vietnam and Laos also has a greenish dorsum with red blotches and superficially resembles *T. venustus* (Orlov et al. 2004). Further research is needed to investigate the consequences of lifestyle and habitat shifts in the evolution of *Trimeresurus* pitvipers (see discussion in Sanders et al. 2006).

Our work also provides new data on the diversity of limestone-associated *Trimeresurus* pitvipers in Thailand. In addition to the description of *Trimeresurus ciliaris* **sp. nov.**, our study further corroborates the results of Sumontha et al. (2021), who suggested that the taxonomic status of *T. venustus* from Chumphon Province of Thailand required further study. Our molecular analyses demonstrated that despite its morphological similarity to *T. venustus*, the Chumphon population represents a distinct lineage of *Trimeresurus*, phylogenetically more closely related to *T. kuiburi*, *T. macrops* and *T. honsonensis* than to *T. venustus* sensu stricto. In the present paper, we tentatively refer to this population as *T. cf. venustus* 1, though we recognize this lineage as a distinct new yet undescribed species, the formal description of which is currently in progress and will be published elsewhere.

Further research is needed to clarify the taxonomic status of the *T. kanburiensis* species complex members occurring in Malaysia. A strange population of pitvipers superficially resembling *T. venustus* was recorded on Pulau Langkawi Island, Kedah State, Malaysia (Fig. 1, locality 13). This record published by Grismer et al. (2006: 65, fig. 18) is based on two specimens (LSUDP 974–75) originally identified as *T. venustus*. However, the coloration pattern of these specimens seem to be quite distinct as compared to other congeners, in particularly to *Trimeresurus ciliaris* **sp. nov.** and *T. venustus* s. str. As the genetic information for these specimens is still lacking, we tentatively refer to the Langkawi population as *T. cf. venustus* 2. Further integrative taxonomic analysis of additional materials from Malaysia, including morphological and molecular data, is required to clarify the status of this population which well may also represent a previously undescribed taxon.

Based on our observations, the newly described species *Trimeresurus ciliaris* **sp. nov.** inhabits not only Trang Province, but is also distributed in Satun Province of Thailand (recorded in Thale Ban National Park, Wang Prachan, Kuan Don District based on P. Pawangkhanant pers. obser.). Moreover, the new species likely also inhabits Perlis State of Malaysia (Fig. 1). Chan et al. (2011) reported on a strange population of *T. cf. venustus* from Perlis State Park, Northern Malaysia. This record was originally published under the name *Cryptelytropis* cf. *venustus*; based on examination of the available photo material it is conspecific with *Trimeresurus ciliaris* **sp. nov.** (Universiti Kebangsaan Malaysia Digital Photograph Collection: UKMDPC 1.0144). Additional records of a similarly-looking pitviper have been recently provided for Wang Kelian View Point, Kaki Bukit, Perlis State (Azrul Azrizal Azmy pers. obser.; see Fig. 6D,E). Morphologically these records are similar to *Trimeresurus ciliaris* **sp. nov.** in body habitus and coloration pattern; moreover the

localities belong to the same limestone massif (Nakawan Range) continuing from Perlis State of Malaysia to Trang Province of Thailand and are located within approximately 90 km from the type locality of the new species. Located on the Malaysia-Thailand border, the Nakawan Range represents Malaysia's largest continuous limestone area and is known as an area with high herpetofaunal diversity (Chan et al. 2009; Grismer et al. 2014). Additional sampling efforts and along with molecular and morphological data on the Perlis population of *Trimeresurus ciliaris* **sp. nov.** are required; we suspect that this locality represents the southern limit of the new species distribution. Toxicological status of the new species remains unknown. In summary, further investigations are required to elucidate the distribution, diet, reproductive biology, population size, population trends and conservation status of the new species.

Our work raises the total number of species of the genus *Trimeresurus* to 47; *Trimeresurus ciliaris* **sp. nov.** represents the fifteenth species of *Trimeresurus* known for Thailand. Despite the recent significant progress, our knowledge of molecular phylogeny, classification and distribution of Asian pitvipers remains incomplete. Last but not least, the medical relevance of *Trimeresurus* pitvipers further underlines the necessity for future studies on the taxonomy of this genus.

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collection and animal use protocols were approved by the Institutional Ethical Committee of Animal Experimentation of the University of Phayao, Phayao, Thailand (certificate number UP-AE64-02-04-005, issued to C. Suwannapoom) and were strictly compliant with the ethical conditions of the Thailand Animal Welfare Act. Field work, including collection of animals in the field, was authorized by the Institute of Animals for Scientific Purpose Development (IAD), Bangkok, Thailand (permit numbers U1-01205-2558 and UP-AE59-01-04-0022, issued to C. Suwannapoom).

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