Tropical Natural History 20(2): 144–161, August 2020

A New Species of *Tylototriton* (Urodela: Salamandridae) from Nan Province, Northern Thailand

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Received: 27 February 2020; Accepted: 6 June 2020

ABSTRACT.– A new species of the genus *Tylototriton*, obtained from Doi Phu Kha National Park, Nan Province, northern Thailand, is described based on molecular and morphological evidence and named herein as *Tylototriton phukhaensis* sp. nov. The new species is morphologically distinct from the four known Thai *Tylototriton* species (*T. panhai*, *T. uyenoi*, *T. anguliceps* and *T. verrucosus*), in having a prominent, narrow, and straight sagittal ridge on the head that distinguishes it from the other Thai species. The molecular analysis also indicated that the new species is a distinct lineage and sister to *T. anguliceps* and *T. uyenoi*. The knowledge obtained in this study will greatly contribute to conducting the future conservation of Thai *Tylototriton*.

KEY WORDS: molecular phylogeny, morphology, new species, Thailand, *Tylototriton*

INTRODUCTION

The salamandrid genus *Tylototriton* was established in 1871 following the discovery of the first species of the crocodile newt, *Tylototriton verrucosus*, in a flooded rice paddy near Nantin high up between the Momien and Husa valleys, Yunnan Province, southwestern China (Anderson, 1871). In the following almost 150 years this species and genus has been found to have a wide distribution range in the hills and mountains of the Himalayan region from northeastern India, Bhutan, eastern Nepal, southern China through northern Myanmar, northern Thailand to northern Vietnam (Wang et al., 2018). The genus has been subdivided into the two subgenera of *Tylototriton* and *Yaotriton* (e.g., Nishikawa et al., 2013a, 2013b; Phimmachak et al., 2015; Wang et al., 2018). From the above records, Thailand is located at the lowest latitude for this genus.

In Thailand, Pomchote et al. (2008) recognized two different phenotypes of *T. verrucosus* that had an allopatric distribution and showed morphological differences. Nishikawa et al. (2013a) analyzed the phylogenetic and morphological characteristics of these populations and described them as new species (*T. uyenoi* and *T. panhai*) and also recognized another cryptic lineage, later described as *T. anguliceps* (Le et al., 2015). At present, four *Tylototriton* species (*T.

panhai, *T. uyenoi*, *T. anguliceps,* and *T. verrucosus*) have been confirmed in Thailand (Pomchote et al., 2020), and includes the southernmost population of the whole genus in Asia, *T. panhai* (Hernandez et al., 2019). The distribution of urodeles is restricted to mainly the northern hemisphere because, in general, they favor cool and moist habitats (Webb et al., 1981). In their southernmost distribution, such as in Southeast Asia, *Tylototriton* tends to be restricted to middle to high elevations in moist and forested mountains (Hernandez et al., 2018). In Thailand, *Tylototriton* species are distributed allopatrically in high mountainous areas at an altitude of more than 1,000 m above mean sea level (amsl) throughout the northern and northeastern parts (Pomchote et al., 2020). However, the phylogeographic survey on the Thai species is not complete yet, which has been basically been reliant on Pomchote et al. (2008) without surveying various high and non-continuous mountain populations in Thailand. From the aforementioned data, it is probable that more unknown newts are existent in Thailand.

From our field survey in Doi Phu Kha National Park (DPKNP), Nan Province, which is located in the northern part of Thailand, we discovered the easternmost population of Thai *Tylototriton* sp. The color pattern of the newts found in DPKNP was rather similar to *T. anguliceps*, *T. uyenoi*, and *T. verrucosus* but with some differences in external morphology. Therefore, we used mitochondrial (mt) DNA sequence data and morphological characters to clarify the taxonomic status of the *Tylototriton* sp. from DPKNP. Based on the molecular and morphological analyses, the specimens from DPKNP belong to a lineage distinct from known species of *Tylototriton*, and so are named and described as *Tylototriton phukhaensis* sp. nov. herein.

MATERIALS AND METHODS

Sampling

Field surveys were conducted on 12 June 2019 at Doi Dong Ya Wai Mountain, DPKNP, Nan Province, northern Thailand (Fig. 1) using the visual encounter survey method. Five specimens were found in an isolated temporary swamp located on the mountain at an elevation of 1,795 m amsl. They were caught by hand and kept in plastic boxes for examination.

All specimens were checked for sex and maturity using the cloacal characters (Pomchote et al., 2008), and were ascribed as three adult males and two adult females. All five samples were used for molecular studies (Table 1), while one of the males and both females were used for the morphological analysis.

Following previous studies (Pomchote et al., 2020), live specimens were euthanized by immersion in a solution of tricaine methane sulfonate (MS-222; 5 g/L) for about 5 min, measured for morphometrics and body weight (BW), as detailed below, and tissue samples of each individual were obtained by removing the tail tip, and then preserved in 95% (v/v) ethanol for molecular analysis. An antiseptic liquid was used to clean the wound for the samples released back in the habitat, whilst those for voucher specimens and morphological analysis were preserved in 70% (v/v) ethanol and temporarily deposited at The Chulalongkorn University Museum of Natural History (CUMZ).

Molecular Study

After removal, the tail-tip tissue samples were used for molecular analysis. Genomic DNA extraction, PCR amplification and sequencing of the mtNADH dehydrogenase subunit 2 region (ND2) gene were performed as previously reported (Pomchote et al., 2020).

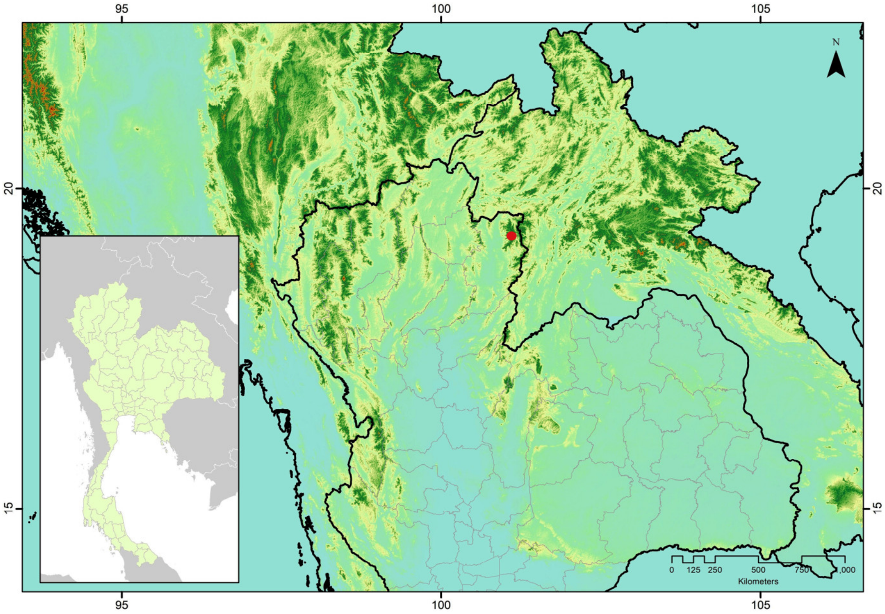

FIGURE 1. Current known distribution of *Tylototriton phukhaensis* sp. nov. from DPKNP, Nan Province (red circle = type locality). The map was modified by Praphatsorn Punsompong

We combined the new sequences of the DPKNP samples with those of the other species available from GenBank (Table 1). The optimum substitution models were selected by Kakusan 4 (Tanabe, 2011). We applied a non-partition model to our dataset, then constructed phylogenetic trees by maximum likelihood (ML) and Bayesian inference (BI) methods. The ML tree was searched using RAxML (Stamatakis, 2014) using the gamma model of rate heterogeneity option, and the BI tree was searched with MrBayes v3.2.6 (Ronquist et al., 2012).

The robustness of the ML tree was tested using bootstrap analysis (Felsenstein, 1985) with 2,000 replicates, and we regarded tree topologies with bootstrap values (bs) of 70% or more to be significantly supported (Huelsenbeck and Hillis, 1993). For BI, two independent runs of four Markov chains were conducted for 10 million generations. The first three million generations were discarded as burn-in, and we considered topologies with posterior probabilities (bpp) of 0.95 or greater as significant support (Leaché and Reeder, 2002). Pairwise comparisons of uncorrected sequence divergences (p-distance by 988 bps) were calculated using MEGA7 (Kumar et al., 2016).

Morphological Study

We compared the morphometric characters of the DPKNP newts with those of three of the four known *Tylototriton* species found in Thailand (*T. anguliceps*, *T. uyenoi*, and *T. verrucosus*) using data from Pomchote et al. (2020) because their color pattern is rather similar and judged as the *T. verrucosus* group (Nishikawa et al., 2013a). Furthermore, the DPKNP population has previously been identified as *T. verrucosus* (Nabhitabhata and Chan-ard, 2005), *T. uyenoi* (Hernandez, 2016), or *T. anguliceps*

TABLE 1. Samples of *Tylototriton* species and related species used for molecular analyses. CAS = California Academy of Sciences; CIB = Chengdu Institute of Biology; CUMZ (A) = Natural History Museum of Chulalongkorn University Section Amphibians; KIZ = Kunming Institute of Zoology; KUHE = Graduate School of Human and Environmental Studies, Kyoto University; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; NMNS = National Museum of Natural Science, Taiwan; VNMN = Vietnam National Museum of Nature; ZMMU = Zoological Museum of Moscow State University. *Topotype.

No.	Species	Voucher number	Locality	GenBank no.	Source
1	*T. phukhaensis* sp. nov.	CUMZ-A-7717	DPKNP, Nan, Thailand	MN912573	This study
2	*T. phukhaensis* sp. nov.	CUMZ-A-7718	DPKNP, Nan, Thailand	MN912574	This study
3	*T. phukhaensis* sp. nov.	CUMZ-A-7719	DPKNP, Nan, Thailand	MN912575	This study
4	*T. phukhaensis* sp. nov.	CUMZ-A-7720	DPKNP, Nan, Thailand	MN912576	This study
5	*T. phukhaensis* sp. nov.	CUMZ-A-7721	DPKNP, Nan, Thailand	MN912577	This study
6	*T. verucossus**	KIZ 201306055	Husa, Yunnan, China	AB922818	Nishikawa et al. (2014)
7	*T. verucossus*	No voucher	Doi Chang, Chiang Rai, Thailand	LC505605	Pomchote et al. (2020)
8	*T. anguliceps**	VNMN A.2014.3	Muong Nhe, Dien Bien, Vietnam	LC017832	Le et al. (2015)
9	*T. uyenoi**	KUHE 19147	Doi Suthep, Chiang Mai, Thailand	AB830733	Nishikawa et al. (2013a)
10	*T. panhai**	No voucher	Phu Luang WS, Loei, Thailand	AB830736	Nishikawa et al. (2013a)
11	*T. shanjing**	NMNS 3682	Jingdong, Yunnan, China	AB830721	Nishikawa et al. (2013a)
12	*T. pulcherrima*	KUHE 46406	Yunnan, China (Pet trade)	AB830738	Nishikawa et al. (2013a)
13	*T. podichthys*	KUHE 34399	Xam Neua, Houa Phan, Laos	AB830727	Nishikawa et al. (2013a)
14	*T. panwaensis**	CAS 245418	Panwa, Myitkyina, Myanmar	KT304279	Grismer et al. (2018)
15	*T. yangi*	KUHE 42282	China (Pet trade)	AB769546	Nishikawa et al. (2013b)
16	*T. shanorum**	CAS 230940	Taunggyi, Shan, Myanmar	AB922823	Nishikawa et al. (2014)
17	*T. himalayanus*	MVZ no number	Nepal	DQ517854	Weisrock et al. (2006)
18	*T. kachinorum**	ZMMU A5953	Indawgyi, Kachin, Myanmar	MK097273	Zaw et al. (2019)
19	*T. kweichowensis*	MVZ 230371	Daguan, Yunnan, China	DQ517851	Weisrock et al. (2006)
20	*T. taliangensis*	KUHE 43361	Unknown (Pet trade)	AB769543	Nishikawa et al. (2013b)
21	*T. asperrimus**	CIB 200807055	Jinxiu, Guangxi, China	KC147815	Shen et al. (2012)
22	*Echinotriton andersoni**	KUHE no number	Nago, Okinawa, Japan	AB769545	Nishikawa et al. (2013b)
23	*Pleurodeles waltl*	MVZ 162384	Rabat, Morocco	DQ517813	Weisrock et al. (2006)
24	*Notophthalmus viridescens*	MVZ 230959	St. Charles, Missouri, USA	DQ517795	Weisrock et al. (2006)

(Thai National Parks, 2020). Note that *T. panhai* (the fourth *Tylototriton* species found in Thailand) was not included in this study because its color pattern was different from those related to the other Thai species, and *T. panhai* is a member of another lineage, the *T. asperrimus* group (Nishikawa et al., 2013a).

A total of 23 adults, comprised of three *T. anguliceps* from Khun Chae NP, seven *T. verrucosus* from Doi Chang, and 10 *T. uyenoi* from Namtok Mae Surin NP [using data from Pomchote et al. (2020)] and three *Tylototriton* sp. from DPKNP (two females and one male) were used for the morphometric comparison.

The following 27 measurements were taken for morphometric comparisons, where the character definitions are as described in Nishikawa et al. (2011): SVL (snout-vent length); HL (head length); HW (head width); MXHW (maximum head width); SL (snout length); LJL (lower jaw length); ENL (eyelid-nostril length); IND (internarial distance); IOD (interorbital distance); UEW (upper eyelid width); UEL (upper eyelid length); OL (orbit length); AGD (axilla-groin distance); TRL (trunk length); TAL (tail length); VL (vent length); BTAW (basal tail width); MTAW (medial tail width); BTAH (basal tail height); MXTAH (maximum tail height); MTAH (medial tail height); FLL (forelimb length); HLL (hindlimb length); 2FL (second finger length); 3FL (third finger length); 3TL (third toe length); and 5TL (fifth toe length). All measurements were taken to the nearest 0.01 mm with a digital sliding caliper. Each measurement was taken three times and the average was used for further analyses. After euthanasia, their body weights (BW) were recorded using a digital weighing scale to the nearest 0.1 gm.

We compared the SVL, BW, and the other 25 measurements above as standardized to the SVL (presented as %SVL) among the four Thai species. Differences in morphological characters between the *Tylototriton* sp. from DPKNP and (i) *T. anguliceps*, (ii) *T. uyenoi*, and (iii) *T. verrucosus* were analyzed by the Mann-Whitney U test. Principal component analysis (PCA) was used to examine the relationships among specimens by all the morphometric characters. The vent length of the two DPKNP females were excluded from the morphological comparison because this parameter showed that males were much longer than females [e.g., male vs. female(s): 9.3 vs. 3.4 and 3.5 in *T. shanorum*; 7.0 vs. 2.9 in *T. verrucosus verrucosus*; 7.4 vs. 1.7 and 1.9 in *T. uyenoi,* data from Nishikawa et al. (2014); 10.5 vs. 4.0 and 3.4 in the DPKNP population from this study). All statistical analyses were performed using SPSS version 22 for Windows. Statistical significance was accepted at the $P < 0.05$ level.

RESULTS

Molecular Study

We analyzed 452–1,035 bp sequences of the partial ND2 region for a total of 24 samples, comprised of the five samples from DPKNP obtained in this study and those from other species, including the outgroup obtained from GenBank (Table 1). Of the 1,090 nucleotide sites of the ND2 region newly sequenced in this study, 299 were variable and 167 were parsimony informative (sequence statistics available upon request from the senior author). The mean likelihood score of the Bayesian analyses for all trees sampled at stationarity was -5463.51, while that for the ML tree was -5713.51.

Phylogenetic analyses employing two different optimality criteria (Ml and BI) yielded nearly identical topologies. We, therefore, present only the ML tree in Fig. 2 and significant bpp support from the BI tree is also presented on each node along with the bs support. Monophyly of *Tylototriton* was not supported in the ML (bs = 65%) or BI (bpp = 0.88) trees. In the *Tylototriton* group, the clade of *T. panhai* and *T. asperrimus* and the other clade of the remaining species were recognized. In the latter, the lineage of *T. taliangensis* and the

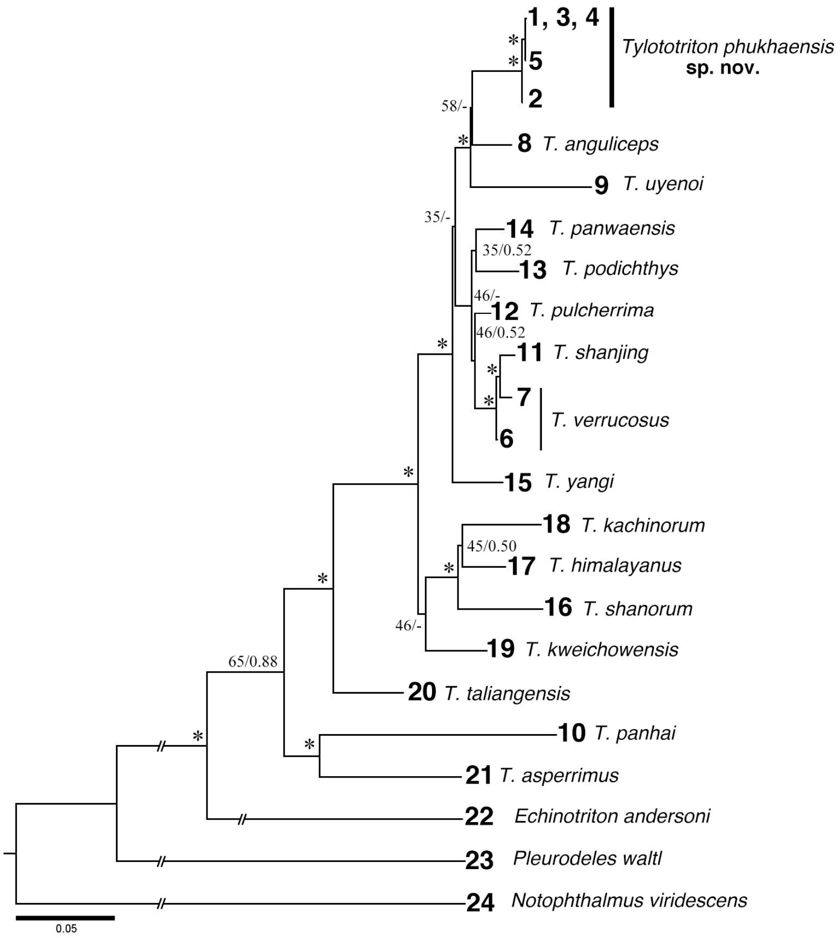

FIGURE 2. Maximum likelihood phylogenetic tree based on the partial ND2 gene for *Tylototriton* and related species (see Table 1). Numbers on branches of the tree show bs and bpp support for the ML and BI trees. Asterisks indicate nodes with a bs of ≥ 70% and bpp ≥ 95%. Numbers at the branch tips are sample numbers shown in Table 1. Scale bar = 0.05 substitutions/site

clade of the remaining species were found. This clade of the remaining species corresponds to the subgenus *Tylototriton* and the *T. verrucosus* group. We found *T. kachinorum*, *T. himalayanus*, *T. shanorum* and *T. kweichowensis* were grouped together separating from the rest. We also found that *T. yangi* was separated from the *T. verrucosus* group. Among the group, we found *T. panwaensis*, *T. podichthys*, *T. pucherrima*, *T. shanjing* and *T. verrucosus* were grouped together. The other group, the samples from DPKNP were included in the clade with *T. anguliceps* and *T. uyenoi*, but those relationships were not resolved with any significance in both trees. However, the samples from DPKNP were not grouped with any others, thus we recognize it as a distinct lineage.

In addition, the average p-distances (Table 2), obtained from the ND2 region used for the ML and BI analyses, among the samples from DPKNP and *T. anguliceps* and *T. uyenoi* were 4.6% (range from 4.4–4.7) and 8.1% (range 8.1–8.2), respectively, which were greater than the smallest heterospecific distances in this study of 2.1% (between *T. verrucosus* and *T. pulcherrima*), 2.3% (between *T. verrucosus* and *T. panwaensis*), and 2.6% (between *T. shanjing* and *T. pulcherrima*) (Table 2). Therefore, the genetic distance supports a distinct lineage for the DPKNP samples, and so a potential new species.

Morphological Study

Morphometric comparisons and morphological differences between the DPKNP population and the closely-related Thai species of *Tylototriton* are presented in Tables 3 and 4, respectively.

(i) The DPKNP population vs. T. anguliceps

The newts from DPKNP showed no significant morphometric differences from *T. anguliceps* (Table 3), but there were some distinct morphological differences. The sagittal ridge on head of the DPKNP population was straight, narrower, and rather longer than that for *T. anguliceps*. The dorsolateral granules of *T. anguliceps* were larger and denser compared to those of the DPKNP newts that were small and sparse (Table 4). Furthermore, the dorsal and ventral head, parotoids, vertebral ridge, rib nodules, limbs, vent, and tail were dull reddish-orange to light brown, although *T. anguliceps* had bright to dark orange markings, particularly on part of the ventral trunk (Le et al., 2015).

(ii) The DPKNP population vs. T. verrucosus

The DPKNP population had significantly smaller RHL, RMXHW, RIOD, RMTAW, RMXTAH, RMTAH, and RHLL %SVL measurements, but significantly larger RTRL and RBTAW measurements than *T. verrucosus* (Table 3). In addition, the DPKNP population could be separated from *T. verrucosus* by the shape and surface of the dorsolateral bony ridges on the head, the shape of the sagittal ridge on the head, the vertebral ridge, number of rib nodules, and the characteristic of granules [Table 4 in this study; Pomchote et al. (2020)].

(iii) The DPKNP population vs. T. uyenoi

With respect to the SVL, the DPKNP population was significantly smaller than *T. uyenoi*. For SVL-standardized characters (%SVL), the DPKNP population had significantly smaller RMXHW, RIND, RIOD, ROL, RMTAW, RBTAH, RMXTAH, RMTAH, RFLL, and RHLL, but, in contrast, a significantly larger RAGD, RTRL, and RBTAW than *T. uyenoi* (Table 3). Morphological differences were also present in the shape of the head, dorsolateral bony ridges, sagittal ridge, vertebral ridge, number of rib nodules, and texture of skin [Table 4 of this study; Pomchote et al. (2020)].

The overall morphological differences were examined using PCA for the DPKNP population and its closely-related Thai congeners. The first two principal components (PCs) explained 51.5% of the total variation. The two-dimensional PC1 vs. PC2 plot showed that the DPKNP population was completely separated from all four *Tylototriton* species (Fig. 3).

Based on the molecular and morphological results, the *Tylototriton* sp. from DPKNP, Nan Province, northern Thailand is confirmed as an undescribed species. Thus, we herewith described the *Tylototriton* sp. from DPKNP, Nan Province, northern Thailand as a new species, named as *Tylototriton phukhaensis* sp. nov.

TABLE 2. Genetic uncorrected p-distance (%) of the ND2 region (988 bp for all samples except for sample 18 at 452 bp) between samples examined in this study.

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	-																						
2	0.2	-																					
3	0.0	0.2	-																				
4	0.0	0.2	0.0	-																			
5	0.1	0.3	0.1	0.1	-																		
6	5.2	5.0	5.2	5.2	5.3	-																	
7	6.0	5.8	6.0	6.0	6.1	1.1	-																
8	4.6	4.4	4.6	4.6	4.7	4.6	5.2	-															
9	8.1	8.1	8.1	8.1	8.2	7.9	8.3	8.2	-														
10	15.2	15.5	15.2	15.2	15.1	14.8	15.9	15.7	17.6	-													
11	5.8	5.5	5.8	5.8	5.9	0.9	1.4	4.7	8.3	15.2	-												
12	4.7	4.5	4.7	4.7	4.8	2.1	2.8	4.4	7.6	14.0	2.6	-											
13	6.4	6.2	6.4	6.4	6.5	3.6	4.4	5.3	9.7	14.8	4.2	3.4	-										
14	6.1	5.9	6.1	6.1	6.2	2.3	3.3	4.8	8.5	15.0	3.3	2.7	3.6	-									
15	6.0	5.8	6.0	6.0	6.1	3.9	4.5	4.4	8.5	14.9	4.7	3.9	5.3	4.1	-								
16	9.0	9.0	9.0	9.0	9.1	6.9	7.5	7.7	11.0	14.8	7.2	7.4	8.4	7.2	7.6	-							
17	7.8	7.8	7.8	7.8	7.9	6.7	6.8	7.5	10.1	14.3	6.6	6.8	7.3	7.1	7.2	5.5	-						
18	9.6	9.6	9.6	9.6	9.9	7.6	8.7	8.5	14	16	8.7	8.5	8.8	6.9	9	8.5	5.6	-					
19	6.6	6.8	6.6	6.6	6.7	5.7	6.7	6.5	9.2	12.0	6.0	5.5	6.0	5.9	6.5	6.7	5.6	6.8	-				
20	9.3	9.0	9.3	9.3	9.4	7.6	8.8	9.1	11.3	11.7	7.8	7.6	8.4	8.1	8.4	9.0	8.0	8.0	6.6	-			
21	14.6	14.3	14.6	14.6	14.7	13.6	14.1	13.7	16.4	13.1	13.8	13.0	12.8	12.9	12.0	13.8	13.9	15.8	11.6	10.2	-		
22	22.4	22.8	22.4	22.4	22.6	20.8	21.4	22.2	25.5	20.4	21.1	20.7	21.9	19.4	21.3	21.1	20.6	26.2	19.7	18.7	19.0	-	
23	32.4	32.8	32.4	32.4	32.4	32.5	32.3	34.2	33.3	35.0	31.8	33.1	33.4	33.5	35.1	33.5	32.9	38.1	30.9	29.8	34.4	40.4	-
24	39.4	38.8	39.4	39.4	39.1	35.8	35.7	38.8	43.2	38.1	35.5	37.2	37.7	36.0	37.4	36.4	37.4	48.7	36.1	34.1	36.4	34.0	41.1

TABLE 3. Measurement of the examined specimens of *Tylototriton* [mean ± SD of SVL (in mm), mean ± SD of BW (g), and median of ratios of characters (R: %SVL), with range in parentheses]. Data for *T. anguliceps*, *T. verrucosus*, and *T. uyenoi* are derived from Pomchote et al. (2020). For character abbreviations, refer to the text.

Species	*T. phukhaensis* sp. nov.		*T. anguliceps*	*T. verrucosus*	*T. uyenoi*
Number and sex	1 male	2 females	3 males	7 males	10 males
SVL	64.3	68.9, 70.2	64.8 ± 3.5 (60.9–67.4)	66.6 ± 2.7 (62.6–70.3)	71.8 ± 2.4^{+} (68.9–75.8)
BW	8.2	9.6, 13.9	14.0 ± 2.3 (12.2-16.6)	10.7 ± 0.6 (10.2-12.0)	14.8 ± 1.8 (11.2-17.0)
RHL	25.0	23.1, 24.8	26.1 (25.7–26.4)	26.4^{+} (24.9–27.5)	25.1 (24.2–26.3)
RHW	19.2	18.7, 17.7	19.6 (19.5–20.3)	17.6 (17.5–18.4)	18.8 (17.5–19.3)
RMXHW	22.1	22.0, 22.4	24.4 (24.4)	24.1^{+} (23.2–25.1)	25.8^{+} (24.5–26.4)
RSL	8.8	8.2, 9.1	7.4 (7.1–8.2)	8.1 (7.6–9.0)	8.8 (8.1–9.4)
RLJL	22.7	21.3, 22.2	20.5 (20.1–21.4)	20.7 (19.7–21.5)	22.0 (20.7–22.5)
RENL	6.3	5.5, 6.4	6.2 (6.1–6.3)	6.2 (5.4–7.9)	6.8 (6.0–7.5)
RIND	5.6	6.0, 5.9	7.8 (6.8–7.9)	6.2 (5.3–6.7)	6.8^{+} (5.6–7.5)
RIOD	11.0	10.7, 11.3	12.3 (12.1–12.4)	13.3^{+} (12.1–13.5)	13.0^{+} (12.6–14.4)
RUEW	2.9	2.5, 2.3	3.2 (2.7–3.4)	2.6 (2.2–3.1)	3.1 (2.2–3.8)
RUEL	6.2	5.3, 5.8	7.3 (6.8–3)	6.2 (5.6–7.4)	6.4 (5.8–7.1)
ROL	3.8	3.1, 3.2	4.2 (4.2–4.4)	4.4 (3.6–5.7)	4.2^{+} (3.5–4.8)
RAGD	56.6	54.7, 56.6	52.3 (51.1–52.4)	55.1 (53.9–56.4)	49.9^{+} (45.7–52.3)
RTRL	76.4	76.5, 76.8	71.2 (70.8–71.5)	73.6^{+} (72.1–74.5)	75.0^{+} (71.8–98.0)
RTAL	108.7	88.1, 87.0	87.3 (87.2–87.8)	94.3 (93.5–110.4)	98.0 (88.8–110.4)
RVL	10.5	4.0, 3.4	11.4 (9.6–11.4)	10.1 (9.7–11.9)	12.4 (7.4–15.3)
RBTAW	10.9	9.2, 8.4	7.5 (7.3–7.7)	5.9^{+} (5.1–7.0)	5.8^{+} (4.4–6.2)
RMTAW	1.7	2.0, 2.1	4.6 (3.8–4.7)	3.7^{+} (3.0–4.0)	3.7^{+} (2.9–4.3)
RBTAH	11.0	9.0, 7.9	13.1 (12.7–14.1)	9.8 (8.6–11.0)	12.1^{+} (11.5–12.9)
RMXTAH	8.3	8.9, 8.4	14.4 (13.5–14.6)	10.0^{+} (9.8–11.2)	12.7^{+} (11.0–14.2)
RMTAH	8.0	7.5, 6.9	14.2 (13.5–14.3)	8.6^{+} (8.0–10.3)	11.8^{+} (11.0–13.3)
RFLL	38.4	31.8, 31.2	40.3 (39.8–41.2)	38.6 (37.6–39.1)	43.7^{+} (42.6–44.6)
RHLL	36.7	30.1, 34.5	44.2 (43.9–45.4)	41.4^{+} (40.2–42.5)	44.8^{+} (42.3–48.1)
R2FL	6.4	5.8, 5.5	6.1 (5.6–6.8)	6.0 (5.6–6.8)	5.5 (4.5–6.8)
R3FL	7.5	6.4, 6.4	7.6 (7.1–7.9)	7.3 (5.9–8.4)	6.8 (5.5–7.4)
R3TL	9.5	7.8, 8.4	9.6 (9.6–11.1)	8.6 (7.7–9.5)	8.3 (7.0–9.2)
R5TL	4.4	3.7, 3.5	4.8 (4.3–.9)	4.2 (3.7–4.7)	4.1 (2.8–6.1)

$^{+}P < 0.05$ compared to *T. phukhaensis* sp. nov. (Mann-Whitney U test)

TABLE 4. Morphological difference between the DPKNP population from this study, and three *Tylototriton* species from Thailand. Data for *T. anguliceps*, *T. verrucosus*, and *T. uyenoi* are derived from Pomchote et al. (2020).

Character	*T. phukhaensis* sp. nov.	*T. anguliceps*	*T. verrucosus*	*T. uyenoi*
Number and sex	1 male, 2 females	3 males	7 males	10 males
Head	Snout truncate; dorsolateral bony ridges narrow, distinctly curved medially at posterior end; surface of dorsolateral bony ridges rough; sagittal ridge narrow but clearly distinct	Snout is truncate; dorsolateral bony ridges narrow, distinctly curved medially at posterior end; surface of dorsolateral bony ridges rough; sagittal ridge clearly distinct	Snout truncate; dorsolateral bony ridges narrow, weakly curved medially at posterior end; surface of dorsolateral bony ridges rather smooth; sagittal ridge rather distinct	Snout rounded to blunt; dorsolateral bony ridges wide, weakly or rather curved medially at posterior end; surface of dorsolateral bony ridges rough; sagittal ridge rather distinct
Vertebral ridge	Narrow, weakly segmented	Narrow, weakly segmented	Narrow, segmented	Wide, segmented
Rib nodules	Isolated but posterior nodules connected, rounded, 14–15	Isolated, rounded, 14–15	Isolated but few individuals connected, rounded but few individuals oval, 12–16	Isolated, rounded, 12–16
Glandular skin	Small granules, sparse	Large granules, dense	Small granules, sparse	Large granules, dense

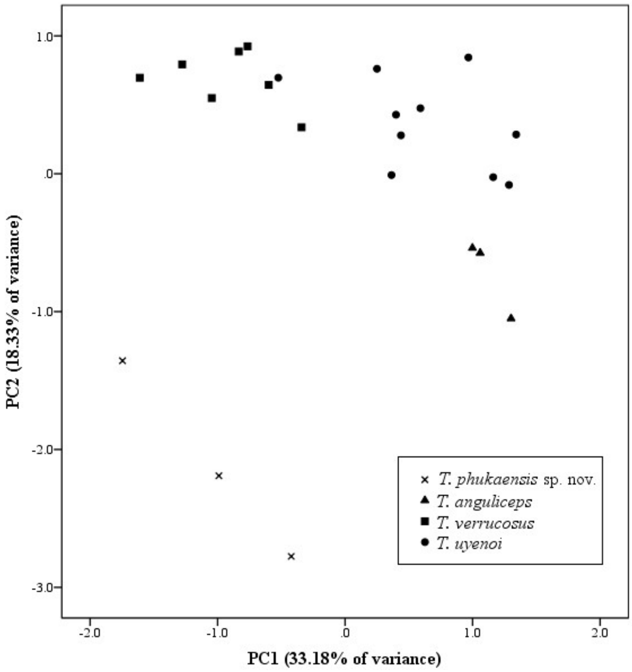

FIGURE 3. The PCA plots of PC1 versus PC2 for morphological parameters of the four *Tylototriton* species.

SYSTEMATICS

Tylototriton phukhaensis Pomchote, Khonsue, Thammachoti, Hernandez, Suwannapoom et Nishikawa sp. nov.

(Thai name: Kra Tang Nam Doi Phu Kha)
(English name: Doi Phu Kha newt)
(Figs. 4–6)

Tylototriton verrucosus: Nabhitabhata and Chan-ard (2005): 181.
Tylototriton uyenoi: Hernandez (2016): 168.

Holotype.– CUMZ-A-7719, an adult male from Doi Dong Ya Wai Mountain, DPKNP, Nan Province, Thailand (approximate coordinate 19˚ 11' N, 101˚ 6' E; 1,795 m amsl), collected on 12 June 2019 by Porrawee Pomchote, Wichase Khonsue, and Panupong Thammachoti.

Paratypes.– A total of two adult females: CUMZ-A-7717 and CUMZ-A-7718 (data same as the holotype).

Etymology.– The specific epithet *phukhaensis* is derived from the name of DPKNP, where the species occurs.

Diagnosis.– The new species is placed in the genus *Tylototriton* by having the combination of dorsal granules present; dorsolateral bony ridges on head present; knob-like warts (rib nodules) on dorsolateral body present; quadrate spine absent. *Tylototriton phukhaensis* sp. nov. differs from other congeners by having the following morphological characters: medium size, adult SVL 64.3 mm in male and 68.9–70.2 mm in females; skin rough with fine granules; sagittal ridge on head narrow, long, and distinct; vertebral ridge distinct and weakly segmented; rib nodules

FIGURE 4. Male *Tylototriton phukhaensis* sp. nov. in its habitat. Photo by Porrawee Pomchote

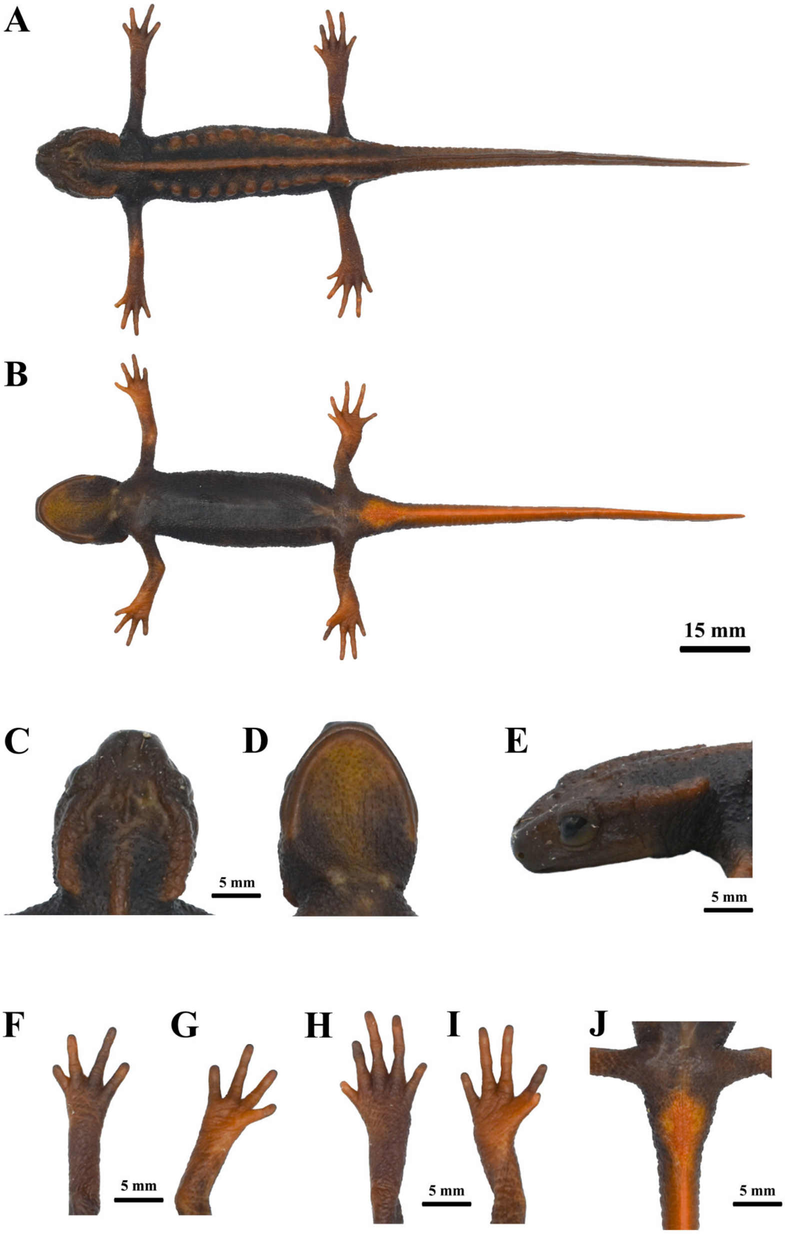

FIGURE 5. Holotype of *Tylototriton phukhaensis* sp. nov. (CUMZ-A-7719) before preservation. Dorsal (A), ventral (B), dorsal head (C), ventral head (D), lateral head (E), dorsal right hand (F), ventral right hand (G), dorsal right foot (H), ventral right foot (I), and cloacal area (J) views. Photos by Parada Peerachidacho

prominent; limbs long and thin; tips of forelimb and hindlimb overlapping when adpressed along body; tail thin.

Description of holotype.– Body rather slim; skin rough; fine granules dense on dorsum, ventral side of head, sides of body

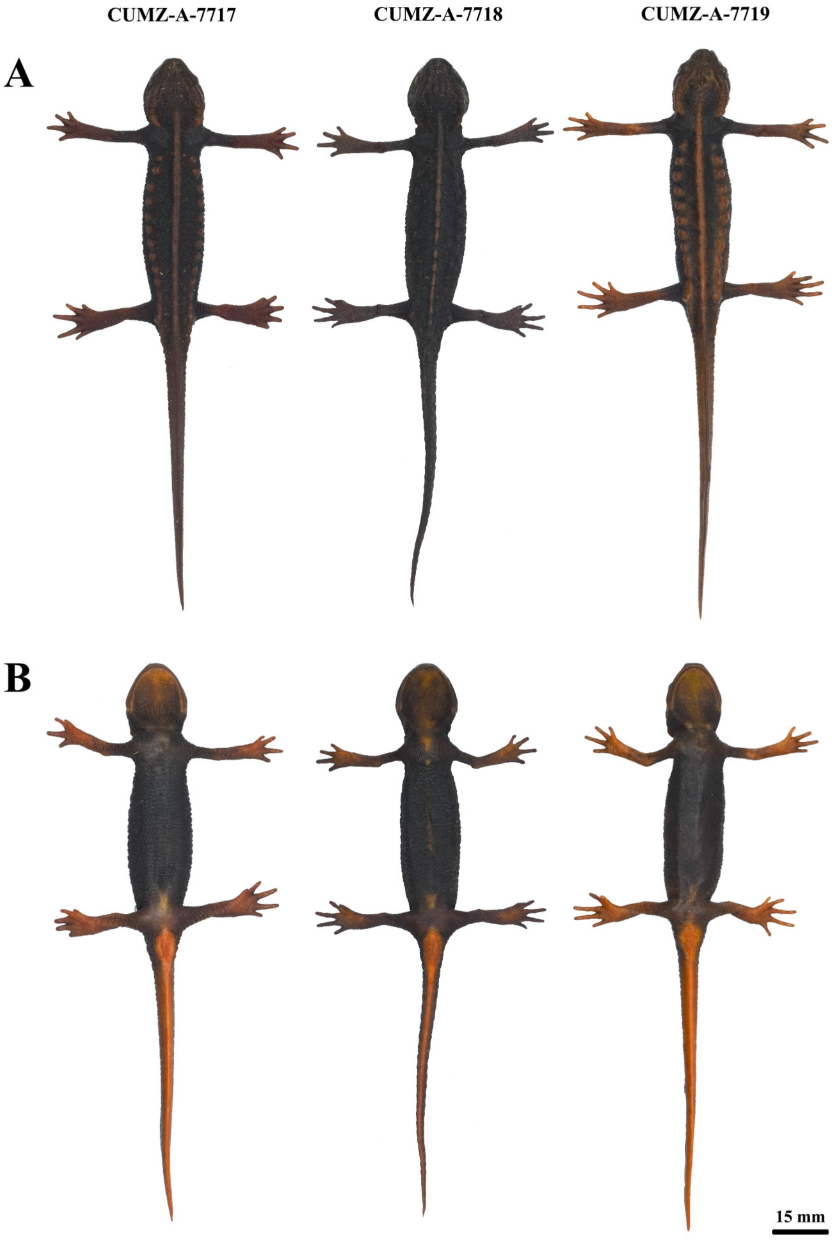

FIGURE 6. Holotype (CUMZ-A-7719) and paratypes (CUMZ-A-7717 and CUMZ-A-7718) of *Tylototriton phukhaensis* sp. nov. before preservation. Dorsal (A) and ventral (B) views. Photos by Parada Peerachidacho

and tail, arranged in transverse striations on mid ventrum, and small and sparse on throat; head longer (25.0%SVL) than wide (19.2%SVL), hexagonal in shape, depressed and slightly oblique in profile; snout short (8.8%SVL), truncate, slightly beyond lower jaw; nostril close to snout tip; dorsolateral bony ridges on head prominent, narrow, protruding, and slightly rough, from above eye to above anterior end of parotoid, posterior ends scrolled proximally; sagittal ridge on head narrow, long, and prominent; labial fold absent; parotoid distinct and projecting posteriorly; gular fold present; costal folds absent; vertebral ridge prominent and weakly segmented, from neck to base of tail, separated from sagittal ridge on head; two low and flat bony ridges on the dorsal head surface forming a "V" shape, connected with the anterior end of vertebral ridge; rib nodules distinct, forming knob-like warts, 15 on both sides of body from axilla to base of tail; rib nodules slightly increasing in size from most anterior to fifth nodule, then decreasing posteriorly; forelimbs (38.4%SVL) slightly longer than hindlimbs (36.7%SVL); tips of fore- and hindlimbs greatly overlapping when adpressed along body; fingers and toes free of webbing; comparative finger lengths 3 > 2 > 4 > 1; comparative toe lengths 4 > 3 > 2 > 5 > 1; tail laterally compressed, dorsal fin more distinct posteriorly, ventral edge smooth, tip pointed; and tail long (108.7%SVL).

Color of holotype.– In life, dorsal ground coloration is dark-brown, while the ventral color is little lighter. Dorsal and ventral head, parotoids, vertebral ridge, rib nodules, limbs, vent region, and whole tail are orange to light-brown. Ventral side of head, part of pectoral and pubic region, limbs, and tail slightly lighter than dorsum. The lighter region between the ventral edge of the tail and the area of the vent is connected. In preservation, the color pattern is rather similar to that in life but faded to lighter brown.

Measurement of holotype (in mm) .– SVL 64.3; HL 16.1; HW 12.3; MXHW 14.2; SL 5.7; LJL 14.6; ENL 4.1; IND 3.6; IOD 7.1; UEW 1.9; UEL 4.0; OL 2.4; AGD 36.3; TRL 49.1; TAL 69.8; VL 6.7; BTAW 7.0; MTAW 1.1; BTAH 7.1; MXTAH 5.3; MTAH 5.2; FLL 24.7; HLL 23.6; 2FL 4.1; 3FL 4.8; 3TL 6.1; and 5TL 2.8.

Variation.– Male has a relatively longer tail (RTAL: 108.7%SVL in male vs. 87.0 and 88.1%SVL in females) and vent slit (RVL: 10.5%SVL in male vs. 3.4 and 4.0%SVL in females) than females. Some differences in morphological variation were observed among the three specimens. One paratype, CUMZ-A-7717, has short right toe lengths. The dorsolateral bony ridges on the head of the other paratype, CUMZ-A-7718, are better developed than the holotype and the other paratype. The rib nodules are more prominent in the holotype and CUMZ-A-7717 than the other paratype. Type specimens are generally similar in color pattern, but the coloration of the dorsal and ventral head, parotoids, vertebral ridge, rib nodules, limbs, and lateral side of tail is much lighter in the holotype and CUMZ-A-7717 than the other paratype. Morphological variations between the specimens are shown in Fig. 6.

Eggs and clutch.– Fertilized eggs (single, double and triple) were attached on the tip of grasses and on a big rock covered with some vegetation that was 3–82 cm above the water surface (Fig. 7). The mean diameter of fertilized eggs found in the breeding site ranged from 5.7–12.3 mm (n = 5, mean = 7.8 mm).

Comparisons.– The new species differs from *T. asperrimus* and *T. taliangensis* by having orange to light-brown markings on the head, trunk, limbs, and tail (vs. uniformly black body except for distal

FIGURE 7. Habitat at the type locality of *Tylototriton phukhaensis* sp. nov. in the DPKNP, Nan Province, Thailand. Photo by Porrawee Pomchote

fingers and toes in *T. asperrimus* and *T. taliangensis*, and except for the posterior parotoids in *T. taliangensis*); from *T. kweichowensis* by having separated orange markings on rib nodules (vs. connected orange markings forming continuous dorsolateral lines in *T. kweichowensis*; from *T. shanorum* by having a prominent sagittal ridge and dorsolateral bony ridges on the head (vs. no sagittal ridge and rather flat dorsolateral bony ridges on head in *T. shanorum*); from *T. himalayanus* by having distinct 14–15 rib nodules (vs. 16 rib nodules in *T. himalayanus*) and lacking grooves on either side at the basal tail (vs. present in *T. himalayanus*); from *T. yangi* by having orange dorsolateral bony ridges and parotoids on the head (vs. black coloration except for posterior margin of dorsolateral bony ridges and parotoids in *T. yangi*); from *T. shanjing*, *T. pulcherrima*, and *T. kachinorum* by having light-orange to light-brown pectoral and pubic region (vs. yellowish orange to bright yellow ventral trunk in *T. shanjing* and *T. pulcherrima*, and light yellowish gray ventral surfaces in *T. kachinorum*); from *T. podichthys* by having a narrow, long, and prominent sagittal ridge on the head (vs. indistinct sagittal ridge on head in *T. podichthys*); from *T. panwaensis* by having narrow vertebral ridge (vs. wide in *T. panwaensis*).

Distribution and natural history.– The distribution of *Tylototriton phukhaensis* sp. nov. is currently only known from a single locality in a temporary swamp in the Doi Dong Ya Wai Mountain, DPKNP, Nan Province, northern Thailand at an elevation of 1,795 m amsl (Fig. 7). The Phu Kha Mountain or Doi Phu Kha is one of the mountains located along the Luang Prabang Range that consists of a series of high mountain ranges from Thailand to Laos (Fig. 1). Thus, the range distribution of this species is expected to be wider.

To date, the biological data of *Tylototriton phukhaensis* sp. nov. is scare. All specimens were found between noon and around 13.00 in a temporary swamp during the raining season. The isolated swamp was located in a forest, had clear water, and the bottom was covered with dense vegetation. Surrounding areas were dry evergreen forest. The swamp size was approximately 200 m^2, and 11 cm maximum water depth. The water pH was 6.4 and no fish were observed.

DISCUSSION

Phylogenetic and morphological analyses indicated that the DPKNP specimens were a distinct species, which is herein described as *T. phukhaensis* sp. nov. In Northern Indochina, extraordinary high species diversity of *Tylototriton* has been discovered, which includes *T. anguliceps*, *T. asperrimus*, *T. notialis*, *T. panhai*, *T. pulcherrima*, *T. shanjing*, *T. uyenoi*, *T. verrucosus*, *T. vietnamensis*, *T. yangi*, *T. ziegleri*, and this new species. This number is 48% of the all species of the genus. Wang et al. (2018) discussed that the speciations in the genus were caused by geomorphological changes, which involved climatic changes in Indochina. In northern Thailand, which is located in Northern Indochina, there are still many unexplored high marsh and wetlands that are suitable for *Tylototriton* habitation. These suitable habitats are distinctly separated by complex geomorphology in northern Thailand (Fig. 1) formed by Indian subcontinent collision (Songtham et al., 2003). Our future survey may further discover more new species from the northern region of Thailand.

Although, the color pattern of *T. phukhaensis* is rather similar to *T. anguliceps*, *T. uyenoi*, and *T. verrucosus*, it can be distinguished from the other congeners by the other external morphological characteristics. *Tylototriton phukhaensis* sp. nov. has distinctly large sagittal ridge that differs from the other Thai *Tylototriton*. Even though the morphometric characters examined in this study do not show clearly the difference between *T. phukhaensis* sp. nov. and *T. anguliceps*, the former has a morphometric difference from *T. uyenoi* and *T. verrucosus* in particular tail sizes that may associate with their behavior and/or breeding activities.

According to Thai National Parks, the DPKNP is one of the most important ones that needs urgent attention for conservation. Actually the habitat of *T. phukhaensis* sp. nov. is degraded due to deforestation and human activities. Many villagers who live inside or at the edges of the NP are permitted to use forest resources, which causes more deforestation by various activities. Illegal hunting on animals is also found all over the park. Moreover, there are hundreds or thousands of feral cattle that graze inside the park, resulting in severe damage to the forest. Thus, we propose that this species is threatened and should be protected by law urgently.

ACKNOWLEDGEMENTS

We would express our thanks to Phasin Inkaew and Sakchai Korkerd for kind support in the field work. We wish to thank the Institute of Animal for Scientific Purposes Development (IAD), which issued fieldwork permission (No. 610104022) and the Department of National Parks, Wildlife and Plant Conservation (DNP) for research permission. We would like to thank Unit of Excellence 2020 on Biodiversity and Natural Resources Management, University of Phayao (UoE63005) and Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, University of Phayao (RD61017) to Chatmongkon Suwannapoom. This research was supported by the Kyoto University Foundation in 2008, the Ministry of Education, Science and Culture, Japan (No. 18H03602), and JSPS Core-to-Core program B (to Kanto Nishikawa [coordinator: Masaharu Motokawa]).

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