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Author(s): Dzung Trung Le, Tao Thien Nguyen, Kanto Nishikawa, Son Lan Hung Nguyen, Anh Van Pham, Masafumi Matsui, Marta Bernardes and Truong Quang Nguyen

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A New Species of *Tylototriton* Anderson, 1871 (Amphibia: Salamandridae) from Northern Indochina

Dzung Trung LE[1], Tao Thien NGUYEN[2], Kanto NISHIKAWA[3]*, Son Lan Hung NGUYEN[1], Anh Van PHAM[1,4], Masafumi MATSUI[3], Marta BERNARDES[5], and Truong Quang NGUYEN[6]

[1]*Hanoi National University of Education, 136 Xuan Thuy, Cau Giay, Hanoi, VIETNAM*
[2]*Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, VIETNAM*
[3]*Graduate School of Human and Environmental Studies, Kyoto University, Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606–8501, JAPAN*
[4]*Faculty of Biology and Chemistry, Tay Bac University, Quyet Tam Ward, Son La City, Son La Province, VIETNAM*
[5]*Department of Terrestrial Ecology, Zoological Institute, University of Cologne, Zülpicher Strasse 47b, 50674 Cologne, GERMANY*
[6]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, VIETNAM*

Abstract: We describe a new species of *Tylototriton* from northwestern Vietnam and northern Thailand based on morphological and molecular evidence. *Tylototriton anguliceps* sp. nov. is distinguishable from all the other congeners by the bright to dark orange markings on the head, body, and tail, prominent dorsal and dorsolateral ridges (crests) on the head, skeletal connection between maxillary and pterygoid, and unique mitochondrial and nuclear DNA sequences. Our molecular data show that the new species is nested within the clade comprising *T. uyenoi*, *T. shanjing*, *T. verrucosus*, and *T. yangi*. The new species is expected to be recorded from other countries in the Indochina region such as southern China, western Myanmar and northern Laos in the future.

Key words: *Tylototriton*; Molecular phylogeny; Morphology; New species; Vietnam; Thailand

Introduction

Recently, several new species have been described in the genus *Tylototriton*, but some lineages identified in previous molecular phylogenies have yet to be formally described (Nishikawa et al., 2013a, 2014). Nishikawa et al. (2013a) recognized three new lineages from Thailand and described two of them as new species, but could not describe the remaining one ("Group 1 of *T. shanjing*" from Doi Lahnga, Chiang Rai, Thailand) because there existed

* Corresponding author. Tel: +81–75–753–6348; Fax: +81–75–753–2891;
E-mail address: hynobius@zoo.zool.kyoto-u.ac.jp

no preserved voucher specimen for the tissue used in the phylogenetic analysis of DNA. Rather, the only identifying information for this individual was a photograph.

During our recent fieldwork in northwestern Vietnam, specimens of *Tylototriton* sp. were collected from Dien Bien and Son La provinces. Subsequent molecular analyses revealed these specimens to form a clade with the undescribed taxon from northern Thailand mentioned in Nishikawa et al. (2013a). Our further morphological examination showed that the populations from both countries belong to the same taxon which is distinguished from all other known members of the genus. On the basis of morphological and molecular distinctions from the known congeners, we herein describe the populations from northwestern Vietnam and northern Thailand as a new species.

Materials and Methods

Sampling

The fieldwork was conducted from 14 to 21 December 2012, from 12 to 21 February 2013, and 12 June to 20 July 2013 in Muong Nhe, Dien Bien Province and Thuan Chau and Song Ma, Son La Province, Vietnam. Nine specimens were collected by D. T. Le and A. V. Pham. Specimens were fixed in 80% ethanol, later transferred to 70% ethanol for permanent storage, and deposited in the Institute of Ecology and Biological Resources, Vietnam (IEBR), the Museum of Biology, Hanoi National University of Education (HNUE), the Tay Bac University (TBU), and the Vietnam National Museum of Nature (VNMN). Four specimens (two from Dien Bien and two from Son La: see Table 1) were used for molecular analyses and eight (HNUE A.I.1.109 [male], HNUE

Table 1. Samples of *Tylototriton* species and related species used for molecular analyses. CAS=California Academy of Sciences; CIB=Chengdu Institute of Biology; HNUE=Hanoi National University of Education; IEBR=Institute of Ecology and Biological Resources, Hanoi; 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; TBU=Tay Bac University, Son La; VNMN=Vietnam National Museum of Nature, Hanoi.

Sample no.	Species or subspecies	Voucher numbers	Field numbers	Locality	GenBank accession no.	Source
Ingroup						
1	*Tylototriton asperrimus*	CIB 200807055	NA	Jinxiu, Guangxi, China	KC147815	Shen et al. (2012)
2	*T. kweichowensis*	MVZ 230371	NA	Daguan, Yunnan, China	DQ517851	Weisrock et al. (2006)
3	*T. shanjing*	NMNS 3682	NA	Jingdong, Yunnan, China	AB830721	Nishikawa et al. (2013b)
4	*T. shanorum*	CAS 230940	NA	Taunggyi, Shan, Myanmar	AB922823	Nishikawa et al. (2014)
5	*Tylototriton* sp.	HNUE A.I.1.111	VNMN MNA2012-0111	Muong Nhe, Dien Bien, Vietnam	LC017836	This study
6	*Tylototriton* sp.	VNMN A.2014.3	VNMN MN2013.2	Muong Nhe, Dien Bien, Vietnam	LC017832	This study
7	*Tylototriton* sp.	TBU PAE.671	VNMN 20130914-1	Thuan Chau, Son La, Vietnam	LC017833	This study
8	*Tylototriton* sp.	IEBR A.2014.10	VNMN 20130914-3	Thuan Chau, Son La, Vietnam	LC017834	This study
9	*Tylototriton* sp.	No voucher	NA	Doi Lahnga, Chiang Rai, Thailand	AB830728	Nishikawa et al. (2013b)
10	*T. taliangensis*	KUHE 43361	NA	Sichuan, China (Pet trade)	AB769543	Nishikawa et al. (2013a)
11	*T. uyenoi*	No voucher	NA	Doi Ang Khang, Chiang Mai, Thailand	AB830729	Nishikawa et al. (2013b)
12	*T. uyenoi*	KUHE 19037	NA	Doi Inthanon, Chiang Mai, Thailand	AB830730	Nishikawa et al. (2013b)
13	*T. uyenoi*	KUHE 19147	NA	Doi Suthep, Chiang Mai, Thailand	AB830733	Nishikawa et al. (2013b)
14	*T. verrucosus pulcherrima*	KUHE 46406	NA	Yunnan, China (Pet trade)	AB830738	Nishikawa et al. (2013b)
15	*T. verrucosus verrucosus*	KIZ 201306055	NA	Husa, Yunnan, China	AB922818	Nishikawa et al. (2014)
16	*T. verrucosus verrucosus*	IEBR A.2014.11	VNMN AT2013.3	Xam Neua, Houa Phan, Laos	LC017835	This study
17	*T. verrucosus verrucosus*	KUHE 34399	NA	Xam Neua, Houa Phan, Laos	AB830727	Nishikawa et al. (2013b)
18	*T. verrucosus verrucosus*	MVZ no number	NA	Nepal	DQ517854	Weisrock et al. (2006)
19	*T. yangi*	KUHE 42282	NA	China (Pet trade)	AB769546	Nishikawa et al. (2013a)
Outgroup						
20	*Echinotriton andersoni*	KUHE no number	NA	Nago, Okinawa, Japan	AB769545	Nishikawa et al. (2013a)
21	*Pleurodeles waltl*	MVZ 162384	NA	Rabat, Morocco	DQ517813	Weisrock et al. (2006)
22	*Notophthalmus viridescens*	MVZ 230959	NA	St.Charles, Missouri, USA	DQ517795	Weisrock et al. (2006)

A.I.1.110 [male], and HNUE A.I.1.111 [female] from Dien Bien, and TBU PAE.358, 486, and 671 and IEBR A.2014.10 [four females] and TBU PAE 359 [juvenile] from Son La) were used for morphological comparisons.

Molecular phylogenetic analyses

We obtained sequence data of NADH dehydrogenase subunit 2 region (ND2) of mitochondrial DNA (mtDNA) from tissue samples preserved in 99% ethanol. Methods for DNA extraction and amplification and sequencing of the DNA fragment are the same as those reported by Nishikawa et al. (2013a). The PCR primers were Sal_ND2_F1 (forward: 5'-AAGCTTTTGGGCCCATACC-3') and Sal_ND2_R2 (reverse: 5'-GGTTGCATTCAGAAGATGTG-3'), and the cycle sequencing primers, Sal_ND2_R1 (reverse: 5'-GTTATAAATATGGAKGARGTTA-3') and Sal_ND2_F2 (forward: 5'-ATAGCATAYTCRTCYATTGC-3') were designed in the middle of the ND2 region, in addition to the two PCR primers used in Nishikawa et al. (2013a). The ND2 region of mtDNA has been proven to be useful for delineating species in the genus *Tylototriton* (Nishikawa et al., 2013a, b; 2014).

We reconstructed phylogenetic trees using our own samples of five specimens of *Tylototriton* including samples from Vietnam and Laos. For comparisons, 14 DNA sequences of *Tylototriton* and one sequence each of *Echinotriton andersoni*, *Pleurodeles waltl*, and *Notophthalmus viridescens* were obtained from GenBank (Table 1, Fig. 1). Outgroup species (Sample 20–22 in Table 1) were selected based on Weisrock et al. (2006).

The optimum nucleotide substitution models were selected by Kakusan4 (Tanabe, 2011). We then constructed phylogenetic trees by Bayesian inference (BI) and maximum likelihood (ML) methods using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) and TREEFINDER ver. Mar. 2011 (Jobb, 2011), respectively. The criterion used for model selection was AIC.

The best substitution model for both BI and ML was the general time reversible model

FIG. 1. Map showing localities of the samples of *Tylototriton* and *Echinotriton* used for molecular analyses. For localities, refer to the sample numbers in Table 1. Samples obtained from the pet trade are positioned at the supposed localities.

(GTR: Tavaré, 1986) with gamma shape parameter (G: 0.304 in BI and 0.266 in ML). Two independent runs of four Markov chains were conducted for 10 million each. We sampled one tree every 100 generations and calculated a consensus topology for 90,000 trees after discarding the first 10,001 trees as burn-in.

For the Bayesian analysis, we considered posterior probabilities (bpp) of 0.95 or greater as significant support (Leaché and Reeder, 2002). The robustness of the ML tree was tested using bootstrap analysis (Felsenstein 1985) with 1,000 pseudoreplicates. We regarded tree topologies with bootstrap values (bs) of 70% or greater as strongly supported (Huelsenbeck and Hillis, 1993). Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated using MEGA5 (Tamura et al., 2011).

Morphological comparisons

We compared the morphology of the *Tylototriton* sp. populations from Dien Bien and Son La, Vietnam, and the topotypic specimens of *T. shanjing* (deposited in the National Museum of Natural Science, Taiwan [NMNS]) and the type series of *T. uyenoi* (in the Graduate School of Human and Environmental Studies, Kyoto University [KUHE] and the herpetological collection of the National Museum of Nature and Science, Tokyo [NSMT-H]), both of which are most similar to *Tylototriton* sp. from Vietnam.

The following 29 measurements were taken for morphological comparisons (character definitions not mentioned below are given in Nishikawa et al. [2011]): SVL (snout-vent length) from tip of snout to anterior margin of vent; 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, from anterior margin of vent to tail tip); 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); 5TL (fifth toe length); VTW (vomerine tooth series width, the greatest width of vomerine tooth series); and VTL (vomerine tooth series length, the greatest length of vomerine tooth series). All measurements were taken to the nearest 0.1 mm with a dial caliper. We used a stereoscopic binocular microscope when necessary. The sex and maturity of the specimens and number of eggs were checked and counted by minor dissections.

We compared SVL, 28 ratio values to SVL (R, %), and VTL/VTW ratio value among male specimens of *Tylototriton* sp., topotypic specimens of *T. shanjing*, and the type series of *T. uyenoi*. Because of low sample size, we could not examine statistical significance of morphological differences. We conducted multivariate analysis to examine overall morphological variation among male samples of *Tylototriton* sp., topotypic specimens of *T. shanjing*, and the type series of *T. uyenoi*. Using a total of 29 ln-transformed values of the metric characters noted above, we conducted Principal Component Analysis (PCA) by SAS (1990).

We also examined the skull morphology and counted the number of the trunk vertebrae of all examined voucher specimens (see Appendix 1). These characters were examined in soft X-ray photographs using Fuji Medical X-Ray Film. For each specimen of *Tylototriton* sp., topotypic specimens of *T. shanjing*, and the type series of *T. uyenoi* (see Appendix 1), we employed a Comscan ScanXmate-A080S to examine the detailed structure of the skulls using X-ray microtomography (micro CT; 80 kV and 100 A, isotropic voxel size: 0.052 mm, 900 projections at 360º). The 3D reconstruction was performed using the ImageJ (http://rsb.info.nih.gov/ij). The 3D images were produced in 16-bit and then converted into 8-bit voxel for visualization.

Results

Phylogenetic relationships and genetic divergence

We obtained 988 bp sequences of the partial ND2 mtDNA region for 22 specimens, including outgroups (Table 1). Of these sequenced nucleotides, 316 were variable and 187 were parsimony informative. The mean likelihood score of the Bayesian analyses for all trees sampled at stationarity was -5347.565. The likelihood value of the ML tree was -5314.038.

Phylogenetic analyses employing two different phylogenetic methods (BI and ML) yielded nearly identical topologies. We therefore present only the ML tree in Fig. 2. Monophyly of *Tylototriton* was not supported in either ML or BI trees (bs=63% and bpp=0.86. Within *Tylototriton*, *T. asperrimus* was first separated from the group including all the remaining species examined. The former belongs to the subgenus *Yaotriton*, and the latter to the subgenus *Tylototriton* (according

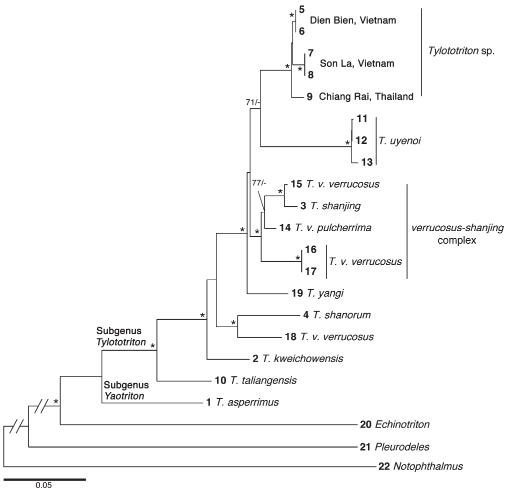

FIG. 2. Maximum likelihood tree based on partial ND2 mtDNA gene for *Tylototriton* and related species (see Table 1 and Fig. 1). Numbers at the end of branches represent sample numbers shown in Table 1. Numbers above branches represent bootstrap supports for ML inference and Bayesian posterior probability (bs/bpp). Asterisks indicate nodes with bs≥70% and bpp≥0.95. Scale bar=0.02 substitutions/site.

to Dubois and Raffaëlli [2009]). *Tylototriton* sp. from Vietnam and Thailand (Samples 5–9) formed one clade (bs=99% and bpp=1.00) and were included in the subgenus *Tylototriton*. This clade was grouped with *T. uyenoi* (11–13) significantly in the ML but not in the BI tree (bs=71% and bpp=0.85). *Tylototriton shanjing* (3), *T. verrucosus pulcherrima* (14), *T. v. verrucosus* from the type locality (15), and *T. v. verrucosus* from Laos (16 and 17) formed a clade (hereafter, the *verrucosus-shanjing* complex). *Tylototriton* sp., *T. uyenoi*, the *verrucosus-shanjing* complex, and *T. yangi* (19) formed a clade but their relationships were unresolved. *Tylototriton v. verrucosus* from Nepal (18) was not grouped with the *verrucosus-shanjing* complex but with *T. shanorum* (4).

The p-distances among the samples are shown in Table 2. The distances between *Tylototriton* sp. and its closest relatives were 4.1–4.6% (*T. yangi* and *T. v. pulcherrima*), 4.4–4.8% (*T. v. verrucosus* from Husa), and 4.5–5.1% (*T. shanjing*). These values were greater than the minimum values between other congeners (≥3.3%: Nishikawa et al.

TABLE 2. Uncorrected p-distance (%) between samples examined in this study.

Sample no.	Species or subspecies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*Tylototriton asperrimus*	—																				
2	*T. kweichowensis*	9.9	—																			
3	*T. shanjing*	11.4	5.6	—																		
4	*T. shanorum*	11.4	6.2	6.6	—																	
5	*Tylototriton* sp.	11.3	6.0	4.5	7.0	—																
6	*Tylototriton* sp.	11.3	6.0	4.5	7.0	0.0	—															
7	*Tylototriton* sp.	11.8	6.3	5.1	7.5	1.0	1.0	—														
8	*Tylototriton* sp.	11.8	6.3	5.1	7.5	1.0	1.0	0.0	—													
9	*Tylototriton* sp.	11.3	6.2	5.0	7.2	0.9	0.9	1.5	1.5	—												
10	*T. taliangensis*	8.9	6.1	7.0	8.0	8.1	8.1	8.8	8.8	8.6	—											
11	*T. uyenoi*	13.3	8.1	7.3	9.4	7.2	7.2	7.6	7.6	7.1	9.8	—										
12	*T. uyenoi*	13.2	8.0	7.2	9.3	7.1	7.1	7.5	7.5	7.0	9.7	0.1	—									
13	*T. uyenoi*	13.2	8.2	7.5	9.5	7.4	7.4	7.8	7.8	7.3	9.7	0.6	0.5	—								
14	*T. verrucosus pulcherrima*	10.9	5.2	2.5	6.7	4.1	4.1	4.6	4.6	4.5	6.9	6.7	6.6	6.9	—							
15	*T. verrucosus verrucosus*	11.3	5.3	0.9	6.3	4.4	4.4	4.8	4.8	4.7	6.9	7.0	6.9	7.2	2.0	—						
16	*T. verrucosus verrucosus*	10.7	5.6	0.4	7.5	5.0	5.0	5.8	5.8	5.5	7.5	8.4	8.3	8.6	3.2	3.4	—					
17	*T. verrucosus verrucosus*	10.7	5.6	0.4	7.5	5.0	5.0	5.8	5.8	5.5	7.5	8.4	8.3	8.6	3.2	3.4	0.0	—				
18	*T. verrucosus verrucosus*	11.5	5.3	6.1	5.2	6.8	6.8	7.3	7.3	6.8	7.2	8.8	8.7	8.9	6.3	6.2	6.7	6.7	—			
19	*T. yangi*	10.1	6.0	4.5	6.9	4.1	4.1	4.6	4.6	4.6	7.5	7.5	7.4	7.7	3.7	3.7	5.0	5.0	6.6	—		
20	*Echinotriton andersoni*	14.8	15.2	16.1	15.9	16.6	16.6	16.6	16.6	16.0	14.7	18.1	18.0	18.2	15.8	15.9	16.4	16.4	15.7	16.1	—	
21	*Pleurodeles waltl*	21.6	20.2	20.5	21.3	21.5	21.5	21.8	21.8	21.6	19.7	21.3	21.2	20.9	21.1	20.9	21.2	21.2	21.1	21.9	23.5	—
22	*Notophthalmus viridescens*	22.3	22.0	21.5	21.9	22.6	22.6	23.2	23.2	22.9	21.4	24.0	23.9	23.9	22.1	21.6	22.1	22.1	22.3	22.2	21.2	23.6

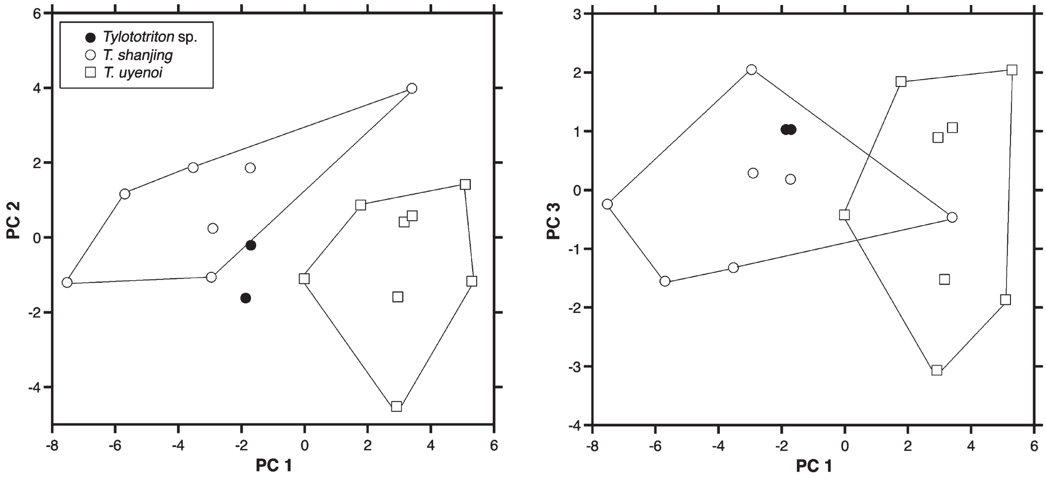

FIG. 3. Two dimensional plots of the first principal component (PC 1) versus the second (PC 2) (left), and PC 1 versus the third (PC 3) (right) of the male specimens of *Tylototriton* sp., *T. shanjing*, and *T. uyenoi*.

TABLE 3. Measurements of specimens of *Tylototriton* examined (mean ± SD of SVL [in mm] and median of ratios of characters [R: %SVL] and VTW/VTL, with range in parentheses). H: holotype, P: paratype, T: topotype. M: male; F: female. For character abbreviations, refer to text.

Species	*Tylototriton* sp. (***anguliceps* sp. nov.**)		*T. shanjing*	*T. uyenoi*	Species	*Tylototriton* sp. (***anguliceps* sp. nov.**)		*T. shanjing*	*T. uyenoi*
Types	H, P	P	T	H, P	Types	H, P	P	T	H, P
n and sex	2M	5F	7M	9M	n and sex	2M	5F	7M	9M
SVL	61.1, 62.5	70.6±3.4 (65.4–74.1)	62.6±6.2 (54.5–74.6)	68.1±3.8 (63.9–74.9)	RVL	7.2, 7.2	2.5 (2.1–3.6)	8.3 (5.7–10.1)	7.4 (6.0–9.0)
RHL	26.2, 29.5	23.4 (22.3–25.1)	26.6 (22.5–29.4)	24.7 (23.0–28.0)	RBTAW	8.8, 8.8	7.8 (6.7–8.6)	9.1 (6.6–10.3)	8.5 (8.0–9.9)
RHW	22.7, 23.4	22.7 (21.0–23.2)	22.2 (21.0–24.4)	25.0 (22.2–26.5)	RMTAW	5.1, 5.4	5.0 (4.4–6.0)	5.8 (5.0–6.6)	4.9 (4.5–5.2)
RMXHW	23.7, 24.1	23.3 (21.6–23.9)	23.2 (21.6–24.7)	25.9 (23.6–26.8)	RBTAH	11.0, 12.6	10.1 (9.5–10.5)	10.9 (9.9–11.7)	12.4 (10.0–14.5)
RSL	9.7, 9.8	8.6 (8.2–9.7)	9.8 (8.8–10.5)	9.7 (8.5–10.6)	RMXTAH	11.7, 14.4	10.1 (9.5–12.2)	10.9 (9.9–13.9)	13.1 (10.9–15.0)
RLJL	20.2, 20.5	19.3 (17.0–19.7)	18.6 (17.9–19.7)	19.8 (17.5–20.5)	RMTAH	11.0, 13.7	9.4 (8.8–12.1)	10.4 (8.1–12.1)	10.9 (9.2–13.3)
RENL	6.1, 6.2	5.7 (5.1–6.7)	6.4 (5.8–6.7)	6.7 (5.7–7.2)	RFLL	32.7, 34.9	30.2 (29.4–30.6)	31.3 (30.3–33.2)	34.3 (29.3–36.3)
RIND	6.6, 7.4	6.5 (5.8–6.9)	7.1 (6.1–7.6)	7.0 (6.2–7.6)	RHLL	36.8, 37.3	33.3 (31.6–35.1)	36.4 (35.4–37.1)	37.5 (35.9–42.1)
RIOD	10.7, 11.1	10.4 (10.1–11.4)	10.8 (7.6–11.2)	11.0 (9.5–12.2)	R2FL	6.1, 7.2	5.2 (5.0–6.6)	6.7 (5.5–7.1)	6.3 (1.8–6.9)
RUEW	3.3, 3.7	2.7 (2.1–3.1)	3.5 (3.0–4.0)	3.8 (3.3–4.3)	R3FL	7.4, 7.5	6.4 (5.8–6.9)	7.3 (5.9–7.6)	6.9 (2.5–11.9)
RUEL	7.0, 7.4	6.0 (4.9–7.0)	7.0 (6.2–8.3)	7.6 (6.7–8.4)	R3TL	9.0, 9.3	9.0 (7.2–9.5)	8.8 (7.8–9.9)	9.4 (7.1–11.3)
ROL	5.4, 5.8	4.4 (3.8–5.2)	5.0 (3.6–5.2)	5.0 (4.5–5.9)	R5TL	3.1, 4.3	3.3 (2.7–3.8)	3.7 (2.7–4.7)	2.9 (2.0–5.0)
RAGD	47.2, 47.8	52.8 (51.6–54.0)	47.9 (45.3–53.4)	48.8 (46.7–51.5)	RVTW	6.4, 7.8	6.9 (5.5–7.5)	6.7 (5.6–7.4)	7.6 (6.5–9.1)
RTRL	70.5, 73.8	76.6 (74.9–77.7)	77.5 (70.6–77.5)	75.3 (72.0–77.0)	RVTL	9.9, 10.8	9.2 (9.0–9.6)	8.8 (6.3–10.0)	10.3 (9.3–11.9)
RTAL	97.1, 102.3	91.2 (87.2–101.4)	104.4 (74.4–114.4)	115.0 [n=8] (101.4–127.7)	VTW/VTL	0.6, 0.8	0.8 (0.6–0.8)	0.8 (0.6–1.0)	0.7 (0.7–0.9)

2013a; Stuart et al. 2010; Shen et al. 2012).

Morphological comparisons

Two adult males and five adult females of *Tylototriton* sp., seven adult males of the topotypic specimens of *T. shanjing*, and nine adult males of the type series of *T. uyenoi* were used for morphometric comparisons (Table 3, Appendix 1). In male SVL, *Tylototriton* sp. was smaller than *T. uyenoi*, but similar to *T. shanjing*. In character ratios of males, *Tylototriton* sp. was larger than *T. shanjing* in RLJL and ROL, but overlapped with *T. shanjing* and *T. uyenoi* in the ranges of the remaining ratios. In PCA (Fig. 3), two males of *Tylototriton* sp., seven males of topotypic *T. shanjing*, and eight males (one specimen with injured tail was excluded from the analysis) of *T. uyenoi* were used. The first three principal components accounted for 71.1% of the total variation. On the two-dimensional plot of PC 2 versus PC 1 (Fig. 3, left), *Tylototriton* sp. was separated from *T. uyenoi* but overlapped with *T. shanjing*, and a similar tendency was recognized in the plot of PC 3 versus PC 1 (Fig. 3, right).

The color pattern was almost the same among *Tylototriton* sp., *T. shanjing*, and *T. uyenoi*, although orange marking was much darker in *T. uyenoi* than in other species (Fig. 4). In contrast, the ground color of the head and body was darkest in *Tylototriton* sp., which made a most distinct contrast to the orange markings among the species compared.

The dorsal granules tended to be more prominent and denser in *Tylototriton* sp. than in *T. shanjing* and *T. uyenoi*. Rib nodules were similar in size, and the vertebral ridge was weakly tubercular in the three species.

The middorsal crest on the skull was more prominent in *Tylototriton* sp. and *T. shanjing* than in *T. uyenoi*. Dorsolateral crests on the skull were steeper in *Tylototriton* sp. than in *T.*

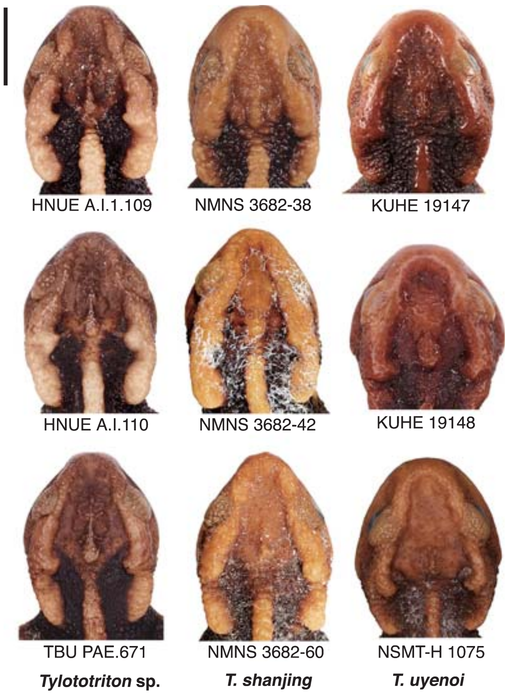

FIG. 4. Dorsal view of the heads of *Tylototriton* sp. (left), *T. shanjing* (center), and *T. uyenoi* (right). The scale shows 10 mm.

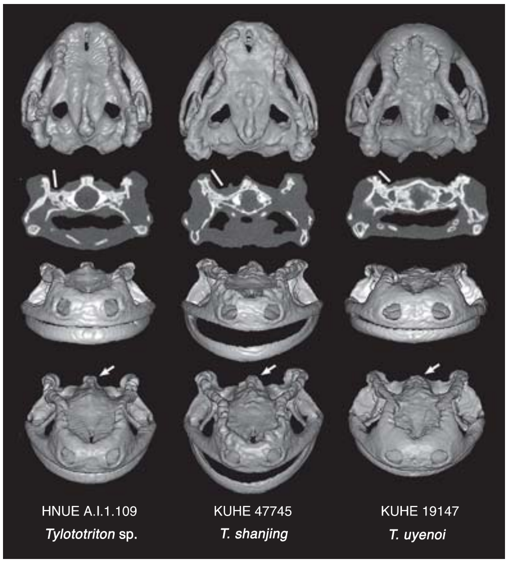

FIG. 5. High-resolution CT scans of the head of male specimens of *Tylototriton* sp. (left), *T. shanjing* (center), and *T. uyenoi* (right). White lines showing slopes of dorsolateral ridges and arrows indicating middorsal ridge. Top: dorsal view of skull; Second from the top: vertical plane of skull at the widest point of head; Second from the bottom: anterior view of skull; Bottom: anteriodorsal view of skull.

shanjing and *T. uyenoi*. The posterior end of the dorsolateral crests did not reach that of exoccipital in *Tylototriton* sp. and *T. uyenoi*, but reached it in *T. shanjing* (Fig. 5).

Tylototriton sp. from northwestern Vietnam and northern Thailand is distinguishable from the remaining species of *Tylototriton* by both mitochondrial DNA and morphological characters. Nishikawa et al. (2013a) indicated that the species formed a sister group with *T. uyenoi* both in nuclear DNA and mitochondrial DNA analysis, but the distance between them was comparable to those between other congeners. From these lines of information, we consider *Tylototriton* sp. to be distinct and describe it as a new species below.

***Tylototriton anguliceps* sp. nov.**
(English name: Angular-headed newt)
Figs. 4–7

Tylototriton shanjing Group 1: Nishikawa et al. (2013a): 262.

Holotype

HNUE A.I.1.109 (Field No. MNA2012-0109), an adult male from Muong Nhe Nature Reserve, Muong Nhe District, Dien Bien Province, Vietnam (22°18.580'N, 102°11.026'E, elevation 1704 m asl), collected on 21 December 2012 by Dzung Trung Le.

Paratypes

One adult male: HNUE A.I.1.110 (Field No. MNA2012-0110) (collection data the same as in the holotype). Five adult females: HNUE A.I.1.111 (Field No. MNA2012.111) (from the type locality by the same collector), collected on 17 February 2013; TBU PAE.671 (Field No. 20130914-1) from Nong Vai Village, Thuan Chau District, Son La Province (21°19.585'N, 103°35.082'E, 1621 m), collected on 20 July 2013 by Anh Van Pham; IEBR A.2014.10 (Field No. 20130914-3) and TBU PAE.358 from forest near Nong Vai Village, Thuan Chau District, Son La Province (21°19.795'N, 103°35.011'E, 1595 m asl), collected on 12 June 2013 by Anh Van Pham; and TBU PAE 486 from Tup Pha B Village, Song Ma District, Son La Province, Vietnam (20°58.204'N, 103°41.985'E, 1778 m), collected on 13 July 2013 by Anh Van Pham. One juvenile: TBU PAE.359 (data same as TBU PAE.358).

Etymology

The specific epithet is from the Latin "angulus" (angular) and "ceps" (head) alluding to steep and narrow bony ridges on the head of the new species.

Diagnosis

The new species is assigned to the genus *Tylototriton* by having the combination of: the presence of dorsal granules, dorsolateral bony ridges on the head, knob-like warts on dorsolateral body; and the absence of a quadrate spine. *Tylototriton anguliceps* differs from its congeners by the following combination of characters: skin rough with fine granules; middorsal ridge on head prominent; maxillary usually connecting with quadrate and pterygoid; dorsolateral bony ridges on head steep and narrow; vertebral ridge distinct and slightly segmented; rib nodules prominent; limbs long and thin; tips of forelimb and hindlimb greatly overlapping when adpressed along body; tail thin; dorsal and ventral head, parotoid, vertebral ridge, rib nodules, limbs, part of ventral trunk, pectral and pubic regions, vent, and whole tail bright to dark orange.

Description of holotype

Body moderately stout; skin rough; fine granules dense on dorsum, arranged in transverse striations on mid ventrum, and small and sparse on throat; head longer (29.5%SVL) than wide (23.4%SVL), hexagonal in shape, depressed and slightly oblique in profile; snout short (9.7%SVL), truncate, slightly beyond lower jaw; nostril close to snout tip; dorsolateral bony ridge on head prominent, narrow, sharply protruding, and slightly rough, from above eye to above anterior end of parotoid, posterior end scrolled proximally; middorsal ridge on head long, prominent; labial fold

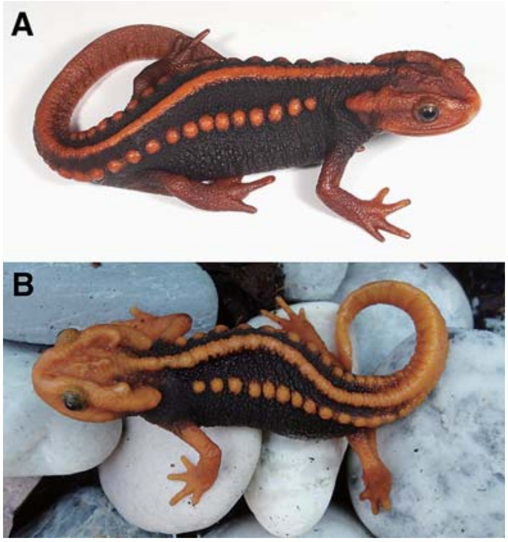

Fig. 6. The female paratype (A: IEBR A.2014.10) and juvenile (B: Sample 9 in Table 1) of *T. anguliceps* in life. The photo of the juvenile was taken at Doi Lahnga, Chiang Rai Province, Thailand, by Porrawee Pomchote.

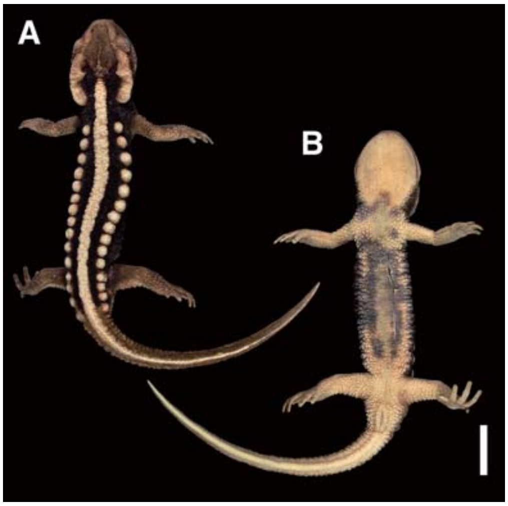

Fig. 7. The male holotype (HNUE A.I.1.109) of *T. anguliceps*. A: dorsal view, B: ventral view. The scale shows 10 mm.

absent; skull wide and hexagonal, dorsolateral crest steep and narrow; posterior end of dorsolateral crest not reaching those of exoccipital; middorsal crest prominent; maxillary connecting with quadrate and pterygoid; front-squamosal arch robust and wide; tongue oval and attached to mouth floor but free laterally and posteriorly; parotoid distinct, and projecting posteriorly; gular fold present; costal folds absent; vertebral ridge prominent and slightly segmented, from neck to base of tail, separated from middorsal ridge on head by a small gap; 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 anteriormost to fifth nodule, then decreasing posteriorly; fore- (32.7%SVL) and hindlimb (36.8%SVL) long; tips of fore- and hindlimbs greatly overlapping when adpressed along body (longest toe reaching at palm); number of trunk vertebrae (presacral vertebrae including atlas) 14; fingers and toes free of webbing; relative length of fingers IV<I<II<III, toes V<I<II<IV<III; tail laterally compressed, dorsal fin more distinct posteriorly, ventral edge smooth, tail long (102.3%SVL); tip pointed; numbers of upper jaw teeth 98, lower jaw teeth 112, and vomerine teeth 66; vomerine tooth series in an inverted V-shape, converging anteriorly and reaching choana.

Measurements of holotype (in mm)

SVL 61.1; HL 18.0; HW 14.3; MXHW 14.7; SL 5.4; LJL 12.5; ENL 3.8; IND 4.5; IOD 6.8; UEW 2.0; UEL 4.0; OL 3.3; AGD 29.2; TRL 43.1; TAL 62.6; VL 4.4; BTAW 5.4; MTAW 3.3; BTAH 7.7; MXTAH 8.8; MTAH 8.4; FLL 20.0; HLL 22.5; 2FL 3.7; 3FL 4.5; 3TL 5.7; 5TL 1.9; VTW 3.9; VTL 6.6.

Color

In life, dorsal ground color dark brown to black; venter slightly lighter than dorsum; dorsal and ventral head, parotoids, vertebral ridge, rib nodules, limbs, part of ventral trunk, pectoral and pubic regions, vent, and whole tail, bright to dark orange. In preservative, dorsal ground coloration tending to fade, becoming light brown and orange markings fading to light cream.

Variation

Males have more robust limbs, but smaller SVL than females (61.1 and 62.5 mm in males vs. 65.4–74.1, mean=70.6 mm, in females). Males have a relatively longer tail (RTAL: 97.1 and 102.3%SVL in males vs. 87.2–101.4, median=91.2%SVL in females) and vent slit (RVL: 7.2%SVL in males vs. 2.1–3.6, median=2.5%SVL in females) than females. Specimens of the type series are basically similar in morphology, but some specimens have more distinctly segmented vertebral ridges than the holotype. Maxillary connects with quadrate and pterygoid in all type series except for HNUE A.I.1.111 (slightly separated); length of vomerine tooth rows is variable among specimens, but reaching/exceeding posterior end of choana in all specimens except for HNUE A.I.1.110. Number of trunk vertebrae is always 14. Specimens are generally uniform in color pattern, but markings vary in color from bright to dark orange.

Egg

The mean diameter of mature ova in ovaries of the paratype (HNUE A.I.1.111) ranged from 2.0 to 2.7 mm (n=10, mean=2.4 mm). The clutch size is unknown. The animal pole is dark brown and the remaining area is dark cream in color.

Larva

A larva (TBU PAE.739) was collected from a shallow stream (ca. 20 cm in depth) in the secondary forest near Nong Vai Village, Thuan Chau District, Son La (21°19.795'N, 103°35.011'E, 1595 m) at night time (2030 h) on 2 August 2013 by Anh Van Pham. It was a fully developed larva with all toes completely developed and the total length 37.5 mm (SVL 21.0 mm, TAL 16.5 mm) and MXTAH 4.4 mm. The date at metamorphosis is unknown.

The following description of the larva is based on this specimen (TBU PAE.739). Head nearly trapezoidal; depressed and sloping in profile; snout short and slightly truncate; labial fold distinct at posterior half of upper jaw; caudal fin higher than head; dorsal fin higher than ventral fin; origin of dorsal fin intermediate between insertion of forelimb and middle of trunk; ventral fin originating from vent; tail tip pointed. In life, dorsum light yellowish brown; venter whitish and transparent; black spots finely scattered on dorsal head and forming irregular dark blotches on dorsal trunk, lateral body, and edge of tail fin; fingers and toes yellow. In preservative, dorsal coloration tending to fade, becoming light cream.

Comparisons

Based on the molecular phylogeny, *T. anguliceps* sp. nov. is a member of the subgenus *Tylototriton*. The new species differs from *T. daweishanensis*, *T. taliangensis*, and *T. v. verrucosus*, and members of the subgenus *Yaotriton* except for *T. panhai* (data of *T. daweishanensis* were taken from Zhao et al., 2012 and those of *T. v. verrucosus* were taken from Anderson, 1871), by having bright orange markings on the head, trunk, limbs, and tail (vs. black body except for the ventral edge of tail in *T. daweishanensis*, *T. v. verrucosus*, and the remaining species of the subgenus *Yaotriton* except for *T. panhai*, and black body except for the posterior parotoids in *T. taliangensis*). The new species differs from *T. panhai* by having orange limbs and tail (vs. black limbs and tail except for edges in *T. panhai*), from *T. kweichowensis* and *T. pseudoverrucosus* by having isolated orange markings on the rib nodules (vs. connected markings forming dorsolateral lines in *T. kweichowensis* and *T. pseudoverrucosus*), from *T. shanjing*, *T. shanorum*, and *T. uyenoi* by having prominent middorsal bony ridge and steep and narrow dorsolateral bony ridges on the head (vs. less prominent middorsal bony ridge and gentle and wide dorsolateral bony ridges on the head in *T. shanjing* and *T. uyenoi*, and no middorsal bony ridge and nearly flat dorsolateral bony ridges on head in *T. shanorum*), from *T. v. pulcherrima* by having a black ground color of the body (vs. a light brown ground color in *T. v. pulcherrima*), and from *T. yangi* by having an orange anterior half of head (vs. black in *T. yangi*).

Range

Vietnam: Muong Nhe, Dien Bien Province and Thuan Chau, Son La Province; Thailand: Doi Lahnga, Chiang Rai Province.

Natural history

The holotype and paratype from Muong Nhe, Dien Bien, Vietnam were found between 9:00 and 11:30 h under rotten trees near a dry stream. The air temperature was 21C and humidity was 79% at the collection site. The other paratypes and one juvenile (TBU PAE359, SVL=34.1 mm) were collected under the leaf litter near a small stream. All of the known habitats in Vietnam are found in the evergreen forest. The specimens in Thailand (a tail tip of one of these specimens was used in molecular analysis) were collected in pools and under rocks at 1443 m asl in May. The air temperature was 21.2C and the water temperature was 21.4C at the time of collection.

The adults are terrestrial, and were found under rotten log or leaf litter near breeding water bodies, or on the forest floor after rainfall. Two clutches, four eggs each, were observed on 19 June 2013 in the forest near Nong Vai Village, Thuan Chau District, Son La Province.

Discussion

Tylototriton anguliceps sp. nov. has long been confused with *T. shanjing* and related species due to their morphological similarity (e.g., Bourret, 1942; Orlov et al., 2002; Nguyen et al. 2009). The taxonomy of the *Tylototriton* species is quite difficult because of the absence of clear differences in their external morphology (Stuart et al., 2010). However, recent studies revealed the fine-scale skull morphology as a useful character for diagnosing cryptic *Tylototriton* taxa (Nishikawa et al., 2014; present study). The most prominent diagnostic character of the new species is its well-developed crest on the skull and a skeletal connection between maxillary and pterygoid. The latter skeletal connection is one of the unique characteristics of *Echinotriton* (Nussbaum and Brodie, 1982), the sister genus of *Tylototriton*, which was not previously identified in *Tylototriton* so far. Thus, further investigation on this character in the remaining species of *Tylototriton* is required.

In Thailand, the new species is only known from Doi Lahnga, Chiang Rai Province, which is very close to the range of *T. uyenoi* from Doi Inthanon, Doi Suthep, and Doi Ang Khang, Chiang Mai Province. For identifying species boundaries between *T. anguliceps* and *T. uyenoi*, further field research is needed in northern Thailand.

In Vietnam, Orlov et al. (2002) mentioned records of *T. verrucosus* from Lao Cai and Lai Chau provinces, although Dien Bien Province is now separated from the former Lai Chau Province. The species from Dien Bien probably represents *T. anguliceps*, however, the taxonomic status of the *Tylototriton* from Lao Cai Province requires further research.

Possibly, *T. anguliceps* does not only occur from northwestern Vietnam to northern Thailand, but also might be found in the adjacent and/or intervening countries, like southern China, western Myanmar, and northern Laos. Because one species of the *T. verrucosus-shanjing* complex (samples 16 and 17 in this study) occurs in northern Laos, this species might exist sympatrically with *T. anguliceps* in the area, although sympatric occurrence of any two *Tylototriton* species has not yet been recorded. Information on the specific season and habit of breeding is also required to estimate the historical biogeography of *Tylototriton* species, because such life history traits may affect co-occurrence of multiple *Tylototriton* species.

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Appendix 1

Specimens used for morphological examination and soft X-ray.

Tylototriton anguliceps: HNUE A.I.1.109–111 from Muong Nhe, Dien Bien, Vietnam; TBU PAE358, PAE486, PAE.671, and IEBR A.2014.10 from Thuan Chau, Son La, Vietnam

T. shanjing: NMNS 3682, 3682-38, 3682-42, 3682-56, 3682-68, 3682-69, 3682-92 from Jingdong County, Yunnan Province, China

T. uyenoi: KUHE 19037 and 19038 from Doi Inthanon, Chiang Mai Province, Thailand, 19146–19150 and NSMT-H 1076 and 1077 from Doi Suthep, Chiang Mai Province, Thailand.

Specimens of the species of subgenus *Tylototriton* used for comparisons

Tylototriton kweichowensis: KUHE 40314 from pet trade; KUHE 46534, 46535 from Shuicheng County, Guizhou Province, China

T. pseudoverrucosus: CIB NN20120609003–9 from Ningnan County, Sichuan Province, China

T. taliangensis: KUHE 40180, and 43361–63 from pet trade.

T. verrucosus verrucosus: CAS 234478, 234480, and 234481 from Nu Jiang, Yunnan Province, China

T. verrucosus pulcherrima: KUHE 46406 from pet trade

T. yangi: KUHE 42282 and 42283 from pet trade.

Specimens used for micro CT scanning.

Tylototriton anguliceps: HNUE A.I.1.109, from Muong Nhe, Dien Bien, Vietnam

T. shanjing: KUHE unnumbered, from Jingdong, Yunnan, China

T. uyenoi: KUHE 19147, from Doi Suthep, Chiang Mai, Thailand.

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