

**A NEW SPECIES OF *TYLOTOTRITON* (AMPHIBIA: URODELA: SALAMANDRIDAE) FROM CENTRAL MYANMAR**

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ABSTRACT

We describe a new species of *Tylototriton* from Taunggyi, Shan State, central Myanmar, based on molecular and morphological evidence. The new species, *Tylototriton shanorum*, is classified as a member of the subgenus *Tylototriton*. The species differs morphologically from all known congeners by having the combination of dull-colored markings, weakly protruding dorsolateral bony ridges on head, narrow and weakly segmented vertebral ridge, and narrow vomerine tooth series. The taxonomic relationship between *T. verrucosus* and *T. shanjing* is briefly discussed based on the molecular phylogeny obtained in this study.

Key words: *Tylototriton*, morphology, new species, Myanmar

INTRODUCTION

The salamandrid genus *Tylototriton* is widely distributed from eastern Himalaya, Indochina, to central and southern China, and currently includes a total of 19 species/subspecies: *T. asperrimus*, *T. broadoridgus*, *T. daweishanensis*, *T. hainanensis*, *T. kweichowensis*, *T. lizhengchangi*, *T. notialis*, *T. panhai*, *T. pseudoverrucosus*, *T. shanjing*, *T. taliangensis*, *T. uyenoi*, *T. vietnamensis*, *T. verrucosus pulcherrima*, *T. verrucosus verrucosus*, *T. wenxianensis dabienicus*, *T. wenxianensis wenxianensis*, *T. yangi*, and *T. zieglerei* (after NISHIKAWA ET AL., 2013a).

The species of *Tylototriton* are divided into two subgenera, *Tylototriton* DUBOIS & RAFFAELLI, 2009 (= the *T. verrucosus* group [FEI ET AL., 2005]) and *Yaotriton* DUBOIS & RAFFAELLI, 2009 (= the *T. asperrimus* group [FEI ET AL., 2005]). The subgenus *Tylototriton* includes *T. daweishanensis*, *T. kweichowensis*, *T. pseudoverrucosus*, *T. shanjing*, *T. taliangensis*, *T. uyenoi*, *T. verrucosus* (also v. *pulcherrima*), and *T. yangi*, while *Yaotriton* includes *T. asperrimus*, *T. broadoridgus*, *T. hainanensis*, *T. lizhengchangi*, *T. notialis*, *T. panhai*, *T. vietnamensis*, *T. wenxianensis* (also w. *dabienicus*), and *T. zieglerei* (after NISHIKAWA ET AL., 2013a).

Recently, NISHIKAWA ET AL. (2013a) described two new species from Thailand: one from the subgenus *Tylototriton* and the other from the subgenus *Yaotriton*. In that paper, they noted the presence of two more lineages in the subgenus *Tylototriton* based on results of molecular phylogenetic analyses. These two lineages, one from central Myanmar and the

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Received 17 February 2014; accepted 8 May 2014.

other from Nepal, were tentatively identified as *T. verrucosus* and their taxonomic status has been undetermined.

In the present paper, we examined morphology and molecular characteristics of one of them, the lineage from central Myanmar, and describe it as a new species.

## MATERIALS AND METHODS

### DNA Sequence

We obtained sequence data of NADH dehydrogenase subunit 2 region (ND2) of mitochondrial DNA (mtDNA) from tissue samples preserved in 99 % ethanol. The present ND2 region of mtDNA has been proven to be useful for delineating species in the genus *Tylototriton* (NISHIKAWA *ET AL.*, 2013a, b). Methods for DNA extraction and amplification and sequencing of the DNA fragment are the same as those reported by NISHIKAWA *ET AL.* (2013a, b).

We constructed phylogenetic trees using our own new sequences of six specimens of *Tylototriton* from Shan, Myanmar, and Yunnan, China. For comparisons, 12 DNA sequences of *Tylototriton* and one sequence each of *Echinotriton andersoni*, *Pleurodeles waltl*, and *Notophthalmus viridescens* were obtained from GenBank (Table 1, Fig. 1).

The optimum substitution models were selected by Kakusan 4 (TANABE, 2011). We then constructed phylogenetic trees by Bayesian inference (BI) and maximum likelihood (ML) methods using MrBayes v3.1.2 (HUELSENBECK & RONQUIST, 2001) and TREEFINDER ver. Mar. 2011 (JOB, 2011), respectively. The criterion used for model selection was AIC.

The best substitution models for BI and ML were the general time reversible model (GTR: TAVARÉ, 1986) + G, and the J3 (JOB, 2011) with gamma shape parameter (G), respectively. Two independent runs of four Markov chains were conducted for ten million generations. We sampled one tree every 100 generations and calculated a consensus topology for 70,000 trees after discarding the first 30,001 trees (burn-in = 3,000,000).

For the Bayesian analysis, we considered posterior probabilities (bpp) 95 % or greater as significant support (LEACHÉ & REEDER, 2002). The robustness of the ML tree was tested using bootstrap analysis (FELSENSTEIN, 1985) with 1000 replicates. We regarded tree topologies with bootstrap values (bs) of 70 % or greater as sufficiently supported (HUELSENBECK & HILLIS, 1993). Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated using MEGA5 (TAMURA *ET AL.*, 2011).

### Morphological Comparisons

We compared morphometric characters of the Myanmar species with those of topotypes of *T. v. verrucosus* and the type series of *T. uyenoii*. The Myanmar species has been identified as the former species (STUART *ET AL.*, 2010; NISHIKAWA *ET AL.*, 2013a). Further, only the latter species has dull-colored markings in the subgenus *Tylototriton*. The remaining taxa of the subgenus have more brightly-colored markings on head, body, and/or tail, except for *T. dawweishanensis* [blackish body like *T. v. verrucosus*]: see below and NISHIKAWA *ET AL.* [2013a]). We also used specimens of the other congeners (16 species/subspecies; see Appendix 1) deposited in the California Academy of Sciences (CAS), Chengdu Institute of Biology, Chinese Academy of Sciences (CIB), Graduate School of Human and Environmental Studies, Kyoto University

Table 1. Samples of *Tylototriton* species and related species used for molecular analyses. CAS = California Academy of Sciences; CIB = Chengdu Institute of Biology; 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.

Sample no.	Species or subspecies	Voucher	Locality	GenBank accession no.	Source
<b>Ingroup</b>					
1	<i>Tylototriton asperrimus</i>	CIB 200807055	Jinxu, Guangxi, China	KC147815	SHEN ET AL. (2012)
2	<i>T. kweichowensis</i>	MVZ 230371	Daguan, Yunnan, China	DQ517851	WEISROCK ET AL. (2006)
3	<i>T. panhai</i>	-	Phu Hin Rong Kla NP, Phitsanulok, Thailand	AB830735	NISHIKAWA ET AL. (2013b)
4	<i>T. shanjing</i>	NMNS 3682	Jingdong, Yunnan, China	AB830721	NISHIKAWA ET AL. (2013b)
5	<i>T. shanjing</i>	KUHE 42348	Myanmar (Pet trade)	AB769544	NISHIKAWA ET AL. (2013a)
6	<i>T. shanjing</i>	KUHE 42842	Myanmar (Pet trade)	AB830722	NISHIKAWA ET AL. (2013b)
7	<i>T. taiiangensis</i>	KUHE 43361	Unknown (Pet trade)	AB769543	NISHIKAWA ET AL. (2013a)
8	<i>T. uyanoi</i>	KUHE 19147	Doi Suthep, Chiang Mai, Thailand	AB830733	NISHIKAWA ET AL. (2013b)
9	<i>T. verrucosus pulcherrima</i>	KUHE 46406	Yunnan, China (Pet trade)	AB830738	NISHIKAWA ET AL. (2013b)
10	<i>T. verrucosus verrucosus</i>	KIZ 201306055	Husa, Yunnan, China	AB922818	This study
11	<i>T. verrucosus verrucosus</i>	KIZ 201306056	Husa, Yunnan, China	AB922819	This study
12	<i>T. verrucosus verrucosus</i>	KIZ 201306058	Husa, Yunnan, China	AB922820	This study
13	<i>T. verrucosus verrucosus</i>	KIZ 201306059	Husa, Yunnan, China	AB922821	This study
14	<i>T. verrucosus verrucosus</i>	CAS 230899	Taunggyi, Shan, Myanmar	HMT70087	STUART ET AL. (2010)
15	<i>T. verrucosus verrucosus</i>	CAS 230933	Taunggyi, Shan, Myanmar	AB922822	This study
16	<i>T. verrucosus verrucosus</i>	CAS 230940	Taunggyi, Shan, Myanmar	AB922823	This study
17	<i>T. verrucosus verrucosus</i>	MVZ no number	Nepal	DQ517854	WEISROCK ET AL. (2006)
18	<i>T. yangi</i>	KUHE 42282	China (Pet trade)	AB769546	NISHIKAWA ET AL. (2013a)
<b>Outgroup</b>					
19	<i>Echinotriton andersoni</i>	KUHE no number	Nago, Okinawa, Japan	AB769545	NISHIKAWA ET AL. (2013a)
20	<i>Pleurodeles waltl</i>	MVZ 162384	Rabat, Morocco	DQ517813	WEISROCK ET AL. (2006)
21	<i>Notophthalmus viridescens</i>	MVZ 230959	St. Charles, Missouri, USA	DQ517795	WEISROCK ET AL. (2006)

NP: National Park

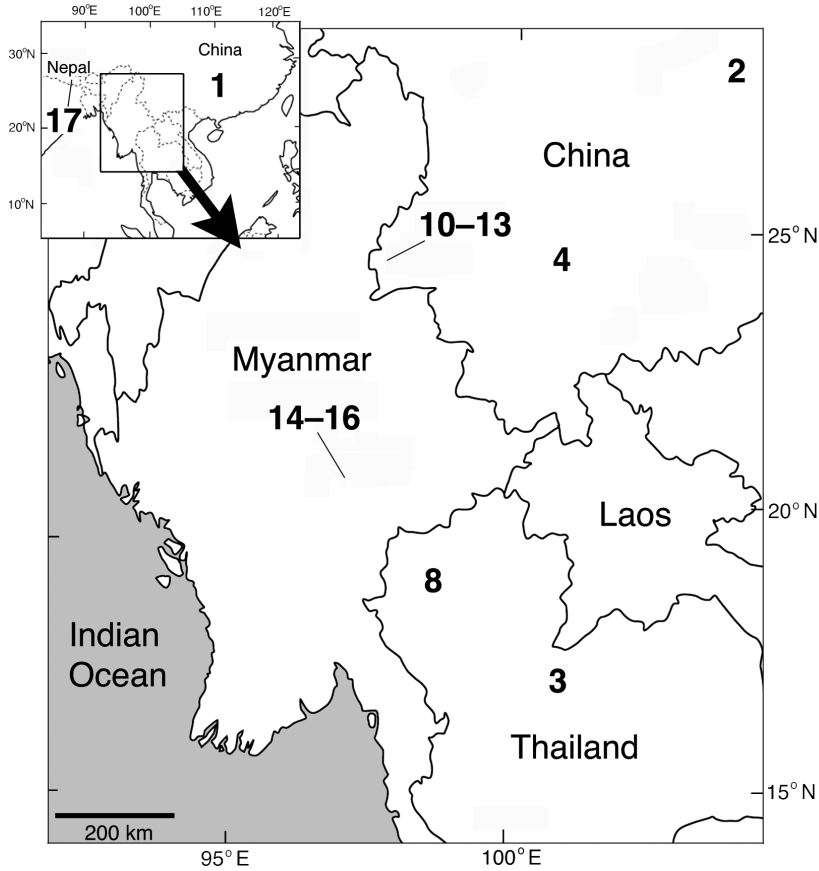


Figure 1. A map showing localities of the samples of *Tylototriton*. For sample number, refer to Table 1. Samples of 5–7, 9, and 18 obtained from pet traders are not shown.

(KUHE), Herpetological collection of the National Museum of Nature and Science, Tokyo (NSMT-H), National Museum of Natural Science, Taiwan (NMNS), Thailand Natural History Museum (THNHM), and Vietnam National Museum of Nature (VNMN) for comparisons (Appendix 1).

The following 13 measurements were taken for morphometric comparisons (character definitions not mentioned below are given in NISHIKAWA *ET AL.* [2011]): SVL (snout-vent length) from tip of snout to anterior tip of vent; HL (head length); HW (head width); MXHW (maximum head width); IND (internarial distance); AGD (axilla-groin distance); TRL (trunk length); TAL (tail length) from anterior tip of vent to tail tip; VL (vent length); FLL (forelimb length); HLL (hindlimb length); VTW (vomerine tooth series width): the greatest width of vomerine tooth series; 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, a total of 12 ratio values to SVL (R, %), and VTL/VTW ratio value among the Myanmar species, and topotypic specimens of *T. v. verrucosus*, and type specimens of *T. uyenoi*, because the Myanmar species has been identified as *T. v. verrucosus* (STUART ET AL., 2010, NISHIKAWA ET AL., 2013a) and some specimens with dark markings look like *T. uyenoi* that is distributed closest to the Myanmar species among the congeners (Fig. 1). We also examined skull morphology and counted the number of trunk vertebrae of specimens from central Myanmar. These characters were examined in soft X-ray photographs using Fuji Medical X-Ray Film.

## RESULTS

### Genetic Divergence

We obtained 1035 bp sequences of partial ND2 region for 21 specimens, including outgroup (Table 1). Of 1035 nucleotide sites, 330 were variable and 171 were parsimony informative within the ingroup. The mean likelihood score of the Bayesian analyses for all trees sampled at stationarity was  $-5315.361$ , and the likelihood value of the ML tree was  $-5284.73$ .

Phylogenetic analyses employing two different optimality criteria (BI and ML) yielded nearly identical topologies. We therefore present only the ML tree in Fig. 2. Monophyly of *Tylostotriton* was supported in the ML tree, but not in BI tree (bs = 74 % and bpp = 91 %). Within *Tylostotriton*, two major groups were recognized: one included species of the subgenus *Tylostotriton* and the others included the subgenus *Yaotriton*. Monophyly of each of these two subgenera was significantly supported (*Tylostotriton*: bs = 96 % and bpp = 100 %; *Yaotriton*: bs = 78 % and bpp = 99 %).

The Myanmar species (Samples 5, 6, 14 and 15) were included in the subgenus *Tylostotriton* group. In this group, the Myanmar species formed a clade which grouped with *T. v. verrucosus* from Nepal (17). Topotypes of *T. v. verrucosus* (10–13) and *T. shanjing* (4) were grouped together with *T. v. pulcherrima* (9) and formed one clade (hereafter, the *T. shanjing* complex, which corresponds to the group with the same name recognized in NISHIKAWA ET AL. [2013a]).

The p-distances obtained were almost the same as those obtained by NISHIKAWA ET AL. (2013b); thus we refer only the distances obtained in the present study. The mean distance of the Myanmar species was 6.2 % (5.9–6.4) from topotypic *T. v. verrucosus*, 9.4 % (9.2–9.6) from *T. uyenoi*, and 5.0 % (4.8–5.3) from *T. v. verrucosus* from Nepal. These distances were much larger than the minimum values already reported between species of *Tylostotriton* (sensu lato) ( $\geq 3.3$  %: NISHIKAWA ET AL., 2013b; STUART ET AL., 2010; SHEN ET AL., 2012) and were also larger than and/or similar to values in some Asian salamandrids ( $\geq 4.0$  % in *Paramesotriton*: WU ET AL., 2010a;  $\geq 6.6$  % in *Cynops*: WU ET AL., 2010b). The mean distance among *T. v. pulcherrima* and topotypic *T. v. verrucosus* and *T. shanjing* (= the *T. shanjing* complex) was 1.6 % (range 0.9–2.5 %), which is much smaller than any distances noted above.

### Morphological Comparisons

A total of 13 adult males and five adult females of the Myanmar species, topotypes of *T. v. verrucosus*, and the type series of *T. uyenoi* were used for morphometric comparisons (Table 2, Appendix 1). Because of the paucity of specimens, we could not run statistical tests.

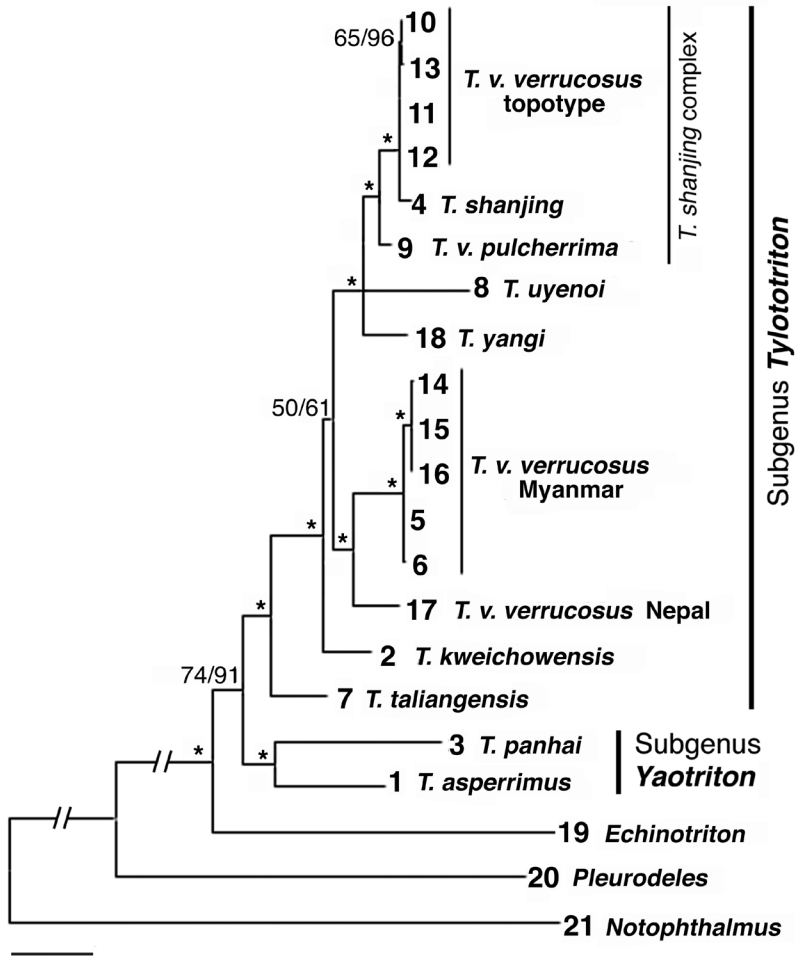


Figure 2. Maximum likelihood tree based on the partial ND2 gene for *Tylototriton* and related species (see Table 1). Numbers above branches represent bootstrap supports for ML inference and Bayesian posterior probability (bs/bpp). Asterisks indicate nodes with bs ≥ 70 % and bpp ≥ 95 %. Scale bar = 0.05 substitutions/site.

In SVL, the Myanmar species tended to have larger values than *T. v. verrucosus* and *T. uyenoii* in both sexes. In RHW and RMXHW, the Myanmar species had larger values than *T. v. verrucosus*, but the values widely overlapped with *T. uyenoii*. In the shape of head, the Myanmar species and *T. v. verrucosus* had a truncate snout while *T. uyenoii* had a rounded one (Figs. 3, 4). In RVTW, the Myanmar species tended to have smaller values than *T. v. verrucosus* and male *T. uyenoii*, and this trend was also seen in VTW/VTL values.

Color pattern varies among the Myanmar species, *T. v. verrucosus*, and *T. uyenoii* (Fig. 3). *Tylototriton v. verrucosus* have mostly blackish ground color with orange markings only on

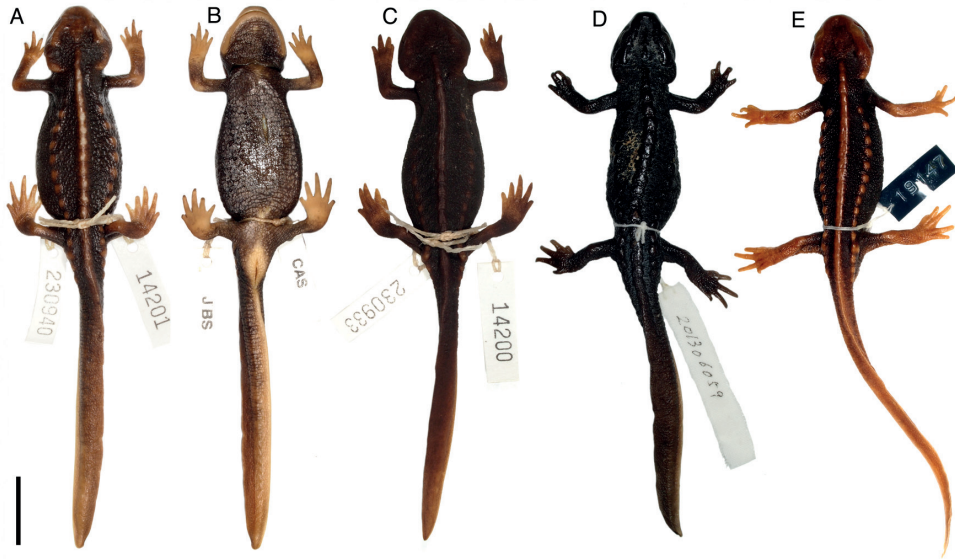


Figure 3. Photograph of specimens of *Tylototriton verrucosus verrucosus* from Myanmar (A and B: dorsal and ventral views of CAS 230940 [male]; C: dorsal view of CAS 230933 [female]), dorsal view of male topotype of *T. verrucosus verrucosus* (D), and dorsal view of male holotype of *T. uyenoi* (E). Scale bar = 20 mm.

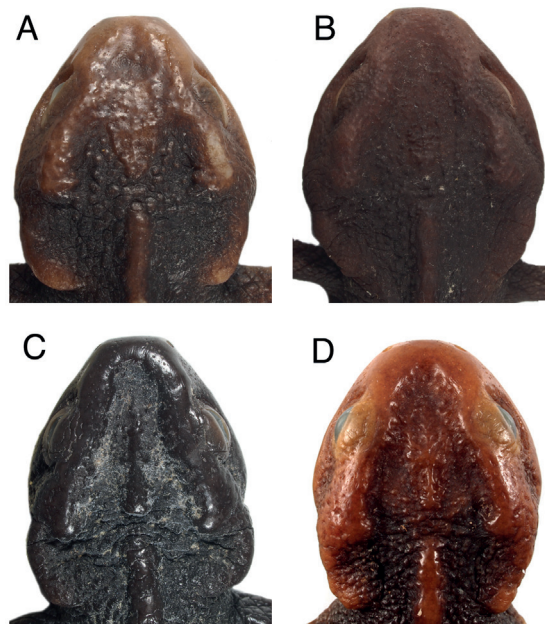


Figure 4. Photograph of dorsal head of specimens of *Tylototriton verrucosus verrucosus* from Myanmar (A: CAS 230940 [male]; B: CAS 230933 [female]), male topotype of *T. v. verrucosus* (C), and male holotype of *T. uyenoi* (D). Scale bar = 10 mm.

palm and sole, vent region, and ventral ridge of tail (consistent with the original description by ANDERSON [1871]), and *T. uyenoi* has dark brown to black ground color with orange to reddish brown markings on head, vertebral ridge, rib nodules, whole limbs, vent region, and the whole tail. The Myanmar species has a color pattern similar to *T. uyenoi*, but the coloration of markings is much duller than the latter.

The snout was truncate in the Myanmar species and *T. v. verrucosus*, but tended to be more rounded in *T. uyenoi*. Bony ridges on the head were less steep and narrow in the Myanmar species than *T. v. verrucosus* and *T. uyenoi*. The surface of the bony ridges tended to be rougher in the Myanmar species than in *T. v. verrucosus* and *T. uyenoi* (Fig. 4). The Myanmar species, together with *T. uyenoi*, had less prominent rib nodules and less segmented vertebral ridge than *T. v. verrucosus* (Fig. 3).

The Myanmar species was distinguishable from the remaining species of *Tylostotriton* in both molecular and morphological properties; thus we describe it as below.

## SYSTEMATICS

### *Tylostotriton shanorum* sp. nov.

(Figs. 3A–C, 4A, B)

*Tylostotriton verrucosus*: STUART ET AL. (2010): 21.

*Tylostotriton verrucosus verrucosus* Lineage 1: NISHIKAWA ET AL. (2013a): 266.

**Holotype.**—CAS 230940, an adult male from Taunggyi Township, Shan State, Myanmar (20°48'28.5" N, 97°02'45.1" E, 1457 m asl), collected on 6 August 2002 by K. S. Lwin.

**Paratypes.**—A total of two adult females: CAS 201814 from Nyaung Shwe Township, Shan State, Myanmar (20°47'06" N, 96°59'13" E), collected on 15 November 1996 by C. J. Ferraris; CAS 230933 from Nyo Mi Village, Taunggyi Township, Shan State, Myanmar (20°42'31.9" N, 97°04'06.0" E, 1393 m asl), collected on 6 August 2002 by H. Win and K. S. Lwin.

**Referred specimens.**—KUHE 42348 and 42842, two pet-traded juveniles raised from eggs in captivity. The parents of these juveniles are originally collected from unknown locality in Myanmar.

**Etymology.**—The specific epithet is dedicated to the people of the Shan State where the new species was found.

**Diagnosis.**—A medium to large-sized newt of *Tylostotriton* (subgenus *Tylostotriton* DUBOIS & RAFFAELLI, 2009); head wide; limbs long and thin; tips of forelimb and hindlimb greatly overlapping when adpressed along body; tail thin; skin rough with fine granules; dorsolateral bony ridges on head not prominent and wide; vertebral ridge narrow and weakly segmented; rib nodules moderately prominent; dorsal ground color dark brown to black; anterior head, parotoid, vertebral ridge, rib nodules, limbs, and lateral side of tail dull reddish brown; upper and lower lips, palm and sole, vent region, and ventral side of tail dark yellow.

**Description of holotype.**—Body stout; head wider than long, angularly hexagonal in shape, depressed and slightly oblique in profile; snout short, truncate, slightly beyond lower jaw; nostril close to snout tip; labial fold absent; tongue oval and attached to mouth floor but free



Table 2. Measurements of specimens of *Tylototriton* examined (means  $\pm$  SD of SVL [in mm] and medians 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	<i>Tylototriton shanorum</i> sp. nov.		<i>T. verrucosus verrucosus</i>		<i>T. uyenoii</i>	
	H	P	T	T	H, P	P
<i>n</i> and sex	M	2F	3M	F	9M	2F
SVL	76.0	76.5, 87.9	70.7 $\pm$ 4.7 (63.3–75.7)	83.9	68.1 $\pm$ 3.8 (63.9–74.9)	69.3, 78.3
RHL	22.4	24.3, 25.7	24.3 (22.9–25.7)	21.7	24.7 (23.0–28.0)	25.8, 26.9
RHW	25.4	24.8, 26.3	23.7 (23.4–24.4)	20.5	25.0 (22.2–26.5)	23.1, 24.0
RMXHW	25.9	25.7, 27.1	25.3 (24.0–25.4)	22.3	25.9 (23.6–26.8)	23.6, 25.0
RIND	6.7	6.0, 6.3	7.0 (5.8–7.4)	6.2	7.0 (6.2–7.6)	7.0, 7.1
RAGD	49.6	49.4, 51.9	50.7 (50.0–53.0)	52.6	48.8 (46.7–51.5)	47.9, 50.7
RTRL	77.6	74.3, 75.7	75.7 (74.3–77.1)	78.3	75.3 (72.0–77.0)	73.1, 74.2
RTAL	111.2	97.0, 97.8	104.9 (103.9–105.7)	102.5	115.0 [n=8] (101.4–127.7)	88.0, 97.0
RVL	9.3	3.4, 3.5	7.0 (4.8–7.7)	2.9	7.4 (6.0–9.0)	1.7, 1.9
RFLL	34.7	31.7, 32.5	34.3 (32.8–36.6)	33.8	34.3 (29.3–36.3)	30.6, 32.7
RHLL	37.2	35.6, 37.0	37.3 (36.1–38.6)	34.6	37.5 (35.9–42.1)	37.4, 37.4
RVTW	6.1	6.1, 7.5	7.7 (7.4–9.5)	9.1	7.6 (6.5–9.1)	6.6, 7.0
RVTL	10.7	11.4, 12.0	9.8 (9.5–11.2)	10.7	10.3 (9.3–11.9)	8.8, 10.2
VTW/VTL	0.6	0.5, 0.6	0.8 (0.7–0.8)	0.8	0.7 (0.7–0.9)	0.7, 0.8

laterally and posteriorly; vomerine tooth series in an inverted V-shape, narrow and converging anteriorly and reaching choana; numbers of upper jaw teeth 90, lower jaw teeth 108, and vomerine teeth 100; parotoid distinct but small, and projecting posteriorly; dorsolateral bony ridges on head wide and slightly protruding, from lore to anterior end of parotoid, posterior ends weakly scrolled proximally; middorsal ridge on head very weak; gular fold present; costal folds absent; vertebral ridge narrow and slightly segmented, from neck to base of tail, separated from middorsal ridge on head with a small gap; rib nodules distinct but small, forming knob-like warts, 14 on both sides of body from axilla to base of tail; rib nodules slightly increasing in size from anteriormost to fourth, then decreasing posteriorly; tips of fore- and hindlimbs greatly overlapping when adpressed along body; fingers and toes free of webbing; tail laterally compressed, dorsal fin more distinct posteriorly, ventral edge smooth, tip pointed; skin rough; fine granules dense on dorsum, arranged in transverse striations on mid ventrum, and small and sparse on throat; skull wide and hexagonal, dorsolateral and middorsal crests weak and wide; maxillary connecting with quadrate, but slightly separated from pterygoid; front-squamosal arch robust and wide; number of trunk vertebrae (presacral vertebrae including atlas) 14.

**Measurements of holotype (in mm).**—SVL 76.0; HL 17.0; HW 19.3; MXHW 19.7; SL 7.6; LJL 15.5; ENL 4.9; IND 5.1; IOD 8.7; UEW 2.2; UEL 5.4; OL 3.9; AGD 37.7; TRL 59.0; TAL 84.5; VL 7.1; BTAW 7.0; MTAW 4.4; BTAH 11.1; MXTAH 11.1; MTAH 10.1; FLL 26.4; HLL 28.3; 2FL 3.6; 3FL 5.2; 3TL 8.0; 5TL 3.4; VTW 4.6; VTL 8.1.

**Color in alcohol.**—Dorsal ground color dark brown to black; venter slightly lighter than dorsum; anterior head, parotoid, vertebral ridge, rib nodules, limbs, and lateral side of tail dull reddish brown; upper and lower lips, palm and sole, vent region, and ventral side of tail dark yellow.

**Variation.**—Male holotype has more robust body than females. Male has a longer tail (RTAL: 111.2 %SVL in male vs. 97.0 and 97.8%SVL in females) and vent slit (RVL: 9.3%SVL in male vs. 3.4 and 3.5%SVL in females) than females. Female paratypes are basically similar in morphology, but one (CAS 201814: SVL = 87.9 mm) is much larger than the other (CAS 230933: 76.5 mm). The dorsolateral bony ridges on head of the paratypes are less developed than the holotype. The surface of the ridges is rough with fine granules in the paratypes (smooth in the holotype). Type specimens are generally similar in color pattern, but the coloration of upper and lower lips, parotoid, vertebral ridge, rib nodules, limbs, and lateral side of tail is much duller in the paratypes than the holotype.

**Eggs.**—The diameter of ripe ova in ovaries of a paratype (CAS 201814) ranges from 1.5 to 2.0 mm ( $n = 10$ , mean = 1.7 mm). The clutch size is unknown. The animal pole is dark brown and the remaining area is dark cream in color.

**Juveniles.**—The SVL and percentage ratio of MXHW against SVL of two juveniles (KUHE 42348 and 42842) are 34.0 mm and 27.6% and 43.0 mm and 27.2%, respectively.

Dorsal ground color dark brown to light brown; venter slightly lighter than dorsum; dorsal head, upper and lower lips, parotoid, vertebral ridge, rib nodules, limbs, palm and sole, vent region, and whole tail bright yellow.

**Comparisons.**—*Tylototriton shanorum* is a member of the subgenus *Tylototriton* based on the molecular phylogeny. This species differs from members of the subgenus *Yaotriton* by

having color markings on lip, parotoid, vertebral ridge, rib nodules, limbs, and lateral side of tail (the members of *Yaotriton* have blackish body except for palm and sole, vent region, and ventral ridge of tail. Exceptionally *T. panhai* has color markings on head, lip, parotoid, vertebral and dorsal tail ridge, and rib nodules, but lacks markings on limbs and lateral side of tail). The species also differs from members of the subgenus *Tylostotriton* as follows: from *T. daweshanensis* and *T. v. verrucosus* by having ventral markings on body and tail (vs. no ventral markings on body and tail in *T. daweshanensis* and *T. v. verrucosus*), from *T. kweichowensis* and *T. pseudoverrucosus* by having isolated markings on rib nodules (vs. connected markings forming dorsolateral lines in *T. kweichowensis* and *T. pseudoverrucosus* [data taken from HOU ET AL., 2012]), from *T. taliangensis* by having distinct rib nodules and lacking reddish markings on posterior parotoid (vs. lacking distinct rib nodules and having reddish markings on posterior parotoid in *T. taliangensis*), from *T. uyenoi*, *T. v. pulcherrima*, and *T. yangi* by having dull markings (vs. much bright markings in *T. uyenoi*, *T. v. pulcherrima*, and *T. yangi*). Specimens with relatively dark-colored markings look like *T. daweshanensis* and *T. v. verrucosus*, but can be distinguished from them by narrow and weakly segmented dorsal ridge and less developed dorsolateral bony ridges on head (vs. wide and distinctly segmented dorsal ridge and much developed dorsolateral bony ridges on head in *T. daweshanensis* and *T. v. verrucosus*). Specimens with relatively bright-colored markings resemble *T. uyenoi*, but can be distinguished from it by deep vomerine tooth series (vs. shallow in *T. uyenoi* [Table 2]).

**Range.**—Taunggyi, Shan State, Myanmar.

## DISCUSSION

Recently, NISHIKAWA ET AL. (2013a, b) described a total of three new species of *Tylostotriton* from Indochina and the present study added another one. The present study also showed that the specimen from eastern Nepal was genetically differentiated from the nominal species. Unfortunately, the Nepalese specimen remained unidentified because no voucher specimens were available at the moment and the morphological examination of the Nepalese population is strongly required to determine its taxonomic status. The trend of a rapid increase of the species number in *Tylostotriton* is not surprising in view of accelerative accumulation of new amphibian taxa described from the region (e.g. MATSUI, 2013; NGUYEN ET AL., 2013a, b; OHLER ET AL., 2011). Because the species of *Tylostotriton* need lentic water bodies suitable for breeding, that are usually shallow ponds or wetlands, the populations would be easily isolated by the absence of such breeding sites and eventually could be led to speciation. Drastic geographic changes in northern Indochina and adjacent areas after a collision of Indian subcontinent have been suggested (HALL, 1998), and these changes could have produced opportunities for allopatric speciations in the genus and differentiation into the two subgenera, *Tylostotriton* and *Yaotriton*, that are found together only in Indochina. Presently, *Tylostotriton* is the most speciose genus in the family Salamandridae. This diversification may be associated with its range in the Indochina-Himalaya area that has experienced the drastically topographic changes (HUCHON ET AL., 1994).

ZHANG ET AL. (2007) and YU ET AL. (2013) found no significant genetic difference between topotypic specimens of *T. verrucosus* and *T. shanjing* and synonymized the latter with the former. However, ZHANG ET AL. (2007) (and also YU ET AL. [2013]) used only a single and

unvouchered sample of *T. verrucosus* without substantial morphological information. Thus, the subsequent study like STUART *ET AL.* (2010) and NISHIKAWA *ET AL.* (2013a) did not follow taxonomic conclusion of ZHANG *ET AL.* (2007) and kept *T. shanjing* as a valid species. The present study practically first examined phylogenetic relationship between the topotypes of *T. verrucosus* and *T. shanjing*. Both the two species were nested in a clade together with *T. v. pulcherrima* (= the *T. shanjing* complex) with very small genetic distances between them despite their great diversification in body color pattern (*T. v. pulcherrima*: pale reddish brown ground color with light yellow dorsal markings, *T. v. verrucosus*: black body without dorsal markings, *T. shanjing*: black to dark brown ground color with bright yellow dorsal markings). In order to more deeply understand the taxonomic status of the *T. shanjing* complex, further study on morphology and nuclear DNA analyses using more populations, and examination on the type specimens of *T. verrucosus* discovered by CHANDA *ET AL.* (2000) are required.

Extensive poaching for pet-trading and traditional medicine is one of the major threats for the species of *Tylotriton*, most of which are legally protected (NISHIKAWA *ET AL.*, 2013b). Actually, *T. shanorum* has been sold in pet shops in Japan. Effective management for the protection of this new narrow-ranged species must be taken into action urgently. The two juveniles of the new species sold in a pet-shop were raised in captivity, which means that the breeding technique is already established. Regulation on trading wild newts and recommendation on trading captive-bred ones will prohibit not only damaging wild population but also spreading hazardous diseases caused by the newt-specific chytrid fungus (MARTEL *ET AL.*, 2013) to the world.

#### ACKNOWLEDGEMENTS

The authors would like to thank Jens Vindum (CAS), Jianping Jiang and Yuezhao Wang (CIB), Shin-ichiro Kawada (NSMT), Tanya Chan-ard (THNHM), Tao T. Nguyen (VNMN), Wenhao Chou (NMNS) for allowing us to examine specimens under their care, Atsushi Tominaga for field supports, Jishan Wang (KIZ) for facilitating our specimen survey in KIZ, and Koshiro Eto for laboratory assistance. This work was partly supported by grants from The Monbukagakusho through the Japanese Society for the Promotion of Sciences (JSPS: Field Research, Nos. 06041066, 08041144, 20770066, and 23770084, the Kyoto University Foundation, JSPS AA Science Platform Program, JSPS Core-to-Core Program, B. Asia-Africa Science Platforms, and Fellowship for Young International Scientists of the Chinese Academy of Sciences (No. 2013Y1SA0005) to MM and KN, and the National Natural Science Foundation of China (NSFC: No. 31071894) to DR.

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## APPENDIX 1

**Comparative specimens examined**

*Tylototriton asperrimus*: CIB GX200807010–200807016 (seven males) from Mt. Dayao, Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, China.

*Tylototriton dawweishanensis*: KIZ RDQ0000200, 201203001, 201203002 (one male and two females) from Mt. Dawei, Pingbian County, Yunnan, China.

*T. hainanensis*: CIB 83360–83363 (four males) from Jianfengling, Hainan Province, China.

*T. kweichowensis*: KUHE 40314 (one female) from pet trade; KUHE 46534, 46535 (one male and one female) from Shuicheng County, Guizhou Province, China.

*T. lizhenchangi*: KUHE 42316 and 42317 (two males) from Mt. Mang, Yizhang County, Hunan Province, China.

*T. notialis*: VNMN 1194 (one male) from Pu Hoat District, Nghe An Province, Vietnam.

*T. panhai*: THNHM 2800 (one female) from Pu Luang Wildlife Sanctuary, Loei Province, Thailand.

*T. shanjing*: NMNS 3682, 3682-6, 3682-13, 3682-38, 3682-42, 3682-53, 3682-56, 3682-68, 3682-69, 3682-92 (seven males and three females) from Jingdong County, Yunnan Province, China

*T. taliangensis*: KUHE 40180, and 43361–43363 (four males) from pet trade.

*T. uyenoii*: KUHE 19037 and 19038 (two males) from Doi Inthanon, Chiang Mai Province, Thailand, 19146–19150 and NSMT-H 1073, 1075–1077 (seven males and two females) from Doi Suthep, Chiang Mai Province, Thailand.

*T. verrucosus verrucosus*: KIZ 201306055, 201306056, 201306058, 201306059 (three males and one female) from Husa, Yunnan Province, China.

*T. verrucosus pulcherrima*: KUHE 46406 (one female) from pet trade.

*T. vietnamensis*: KUHE 55172, VNMN 3032, 3168, 3363, 3364, 3374 (six males) from Yen Tu District, Bac Giang Province, Vietnam.

*T. wenxianensis wenxianensis*: CIB 750041 (one male) from Pingwu County, Sichuan Province, China.

*T. yangi*: KUHE 42282 and 42283 (two females) from pet trade.

*T. zieglerei*: VNMN 3390 (one male) from Quan Ba District, Ha Giang Province, Vietnam, VNMN 907, 1310–1316 (eight males) from Bac Quang District, Ha Giang Province, Vietnam, VNMN 3389 (one male) from Bao Lac District, Cao Bang Province, Vietnam, NSMT-H 05679–05688 (nine males and one female) from Nguyen Binh District, Cao Bang Province, Vietnam.