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A new species in the *Tylototriton asperrimus* group (Caudata: Salamandridae) from central Laos

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Abstract

A new species in the morphologically conservative *Tylototriton asperrimus* group is described from Khammouan Province, Laos. Molecular phylogenetic analysis of mitochondrial DNA confirms its placement in the *T. asperrimus* group. *Tylototriton notialis* **sp. nov.** is diagnosable in mitochondrial DNA, nuclear DNA, and morphology from its congeners. The new species represents the first record of the genus from Laos, and is the southernmost known member of the *T. asperrimus* group.

Key words: Caudata, Laos, Southeast Asia, *Tylototriton*

Introduction

The Asian newt genus *Tylototriton* Anderson, 1871 contains eight species (Dubois & Raffaëlli 2009) distributed from Nepal to northern Vietnam. These eight species consist of two clades, the *T. verrucosus* group (= subgenus *Tylototriton* Dubois & Raffaëlli 2009) containing *T. verrucosus* Anderson, 1871, *T. kweichowensis* Fang & Chang, 1932, *T. taliangensis* Liu, 1950, and *T. shanjing* Nussbaum, Brodie & Yang, 1995 and the *T. asperrimus* group (= subgenus *Yaotriton* Dubois & Raffaëlli 2009) containing *T. asperrimus* Unterstein, 1930, *T. hainanensis* Fei, Ye & Yang, 1984, *T. wenxianensis* Fei, Ye & Yang, 1984, and *T. vietnamensis* Böhme, Schöttler, Nguyen & Köhler, 2005. The monophyly of the genus and two subgenera has been demonstrated (Weisrock *et al.* 2006).

The genus *Tylototriton* has not been reported from Laos, although it is expected there based on its occurrence in adjacent parts of Thailand, China, and Vietnam. The only salamandrid known in Laos with certainty is the restricted range and endemic *Laotriton laoensis* (Stuart & Papenfuss 2002). Our fieldwork on the Phou Ak escarpment in Nakai-Nam Theun National Protected Area in Khammouan Province, central Laos, in 2006–2007 resulted in the discovery of a newt population in the *T. asperrimus* group approximately 400 km south of the type locality of *T. vietnamensis* in northeastern Vietnam, the most proximate known species in this group. Here we use mitochondrial DNA, nuclear DNA, and morphology to determine its identity.

Material and methods

Sampling: Specimens were collected by the authors and fixed in 10% buffered formalin after preserving liver in 20% DMSO-salt saturated storage buffer. Adult specimens were later transferred to 70% ethanol.

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Specimens and tissue samples were deposited at the Field Museum of Natural History (FMNH), Chicago, USA. Museum abbreviations follow Leviton *et al.* (1985).

DNA sequencing: Total genomic DNA was extracted from liver or muscle using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A fragment of mitochondrial DNA that encodes part of the 16S rRNA gene, the complete tRNA Leu, NADH dehydrogenase subunit 1, tRNA Ile, tRNA Gln, tRNA Met, NADH dehydrogenase subunit 2, tRNA Trp, tRNA Ala, tRNA Asn, tRNA Cys, and tRNA Tyr genes, and part of the cytochrome oxidase c subunit I gene (mt DNA) was amplified by long PCR (the polymerase chain reaction; one cycle of 94ºC 5 min, 37 cycles of 94ºC 30 s, 50ºC 30 s, 68ºC 9 min, one cycle of 72ºC 20 min) using the primers L3002 and H5934 (Weisrock *et al.* 2001). A fragment of nuclear DNA that encodes part of the proopiomelanocortin (POMC) gene was amplified by standard PCR (one cycle of 94ºC 5 min, 35 cycles of 94ºC 45s, 48ºC 30s, 72ºC 1 min, one cycle of 72ºC 10 min) using the primers POMC_DRV_F3 (5'-ATGAGCCAYTTYCGCTGGAA-3') and POMC_DRV_R1 (Vieites *et al.* 2007). PCR products were cleaned using GELase (Epicentre Technologies) or ExoSAP-IT (USB). Cycle sequencing products were sequenced in both directions on a 3730 DNA Analyzer (Applied Biosystems) using the amplifying primers and Big Dye version 3 chemistry (Perkin Elmer). The internal primers L4437, H4419, L3878, and H4980 (Weisrock *et al.* 2001) were also used in the mt DNA sequencing reactions. Sequences were edited with Sequencher v. 4.1 (Genecodes). New sequences were deposited in GenBank under accession numbers HM462054–HM462079, HM770087–HM770091 (Table 1).

TABLE 1. Samples used in the molecular analyses in this study.

Species	ID	Voucher	Locality	GenBank Accession Nos. Sequence Source	
				mt DNA	POMC
Echinotriton andersoni	1	MVZ 232187	Kagoshima Pref., Kyushu, Japan, 27°46’18”N 128°58’09”E	DQ517774 Weisrock *et al.* (2006)	HM462065 This study
Echinotriton chinhaiensis	2	MVZ 230536	Ningho, Zhejiang Prov., China	DQ517775 Weisrock *et al.* (2006)	-
Lyciasalamandra atifi	3	-	Turkey	NC_002756 Zardoya *et al.* (2003)	-
Notophthalmus viridescens	4	MVZ 230959	St. Charles Co., Missouri, USA	DQ517795 Weisrock *et al.* (2006)	-
Pleurodeles poireti	5	MVZ 235673	Jundubah Governorate, Tunisia, 36°40.38’N 08°42.12’E	-	EU275820 Vieites *et al.* (2007)
Pleurodeles waltl	6	MVZ 162384	Rabat Prov., Morocco, 34°01’31”N 06°50’10”W	DQ517813 Weisrock *et al.* (2006)	-
Taricha granulosa	7	MVZ 225502	Annette Island, Alaska, USA, 55°08’26”N 131°28’02”W	-	HM462064 This study
Tylototriton asperrimus	8	MVZ 237103	Guizhou Prov., China,	DQ517849 Weisrock *et al.* (2006)	HM462069 This study
Tylototriton hainanensis	9	MVZ 230352	Hainan Prov., China, 18.77666°N 108.70289°E	DQ517850 Weisrock *et al.* (2006)	HM462070 This study
Tylototriton kweichowensis	10	MVZ 230371	Daquan Co., Yunnan Prov., China, 27°50’N 103°55’E	DQ517851 Weisrock *et al.* (2006)	-
Tylototriton notialis **sp. nov.**	11	FMNH 271120	Boualapha Dist., Khammouan Prov., Laos, 17°38’39.6”N 105°44’12.3”E	HM462061 This study	HM462077 This study
Tylototriton notialis **sp. nov.**	12	FMNH 271121	Boualapha Dist., Khammouan Prov., Laos, 17°38’39.6”N 105°44’12.3”E	HM462062 This study	HM462078 This study

continued next page

TABLE 1. (continued)

Species	ID	Voucher	Locality	GenBank Accession Nos. Sequence Source	
				mt DNA	POMC
Tylototriton notialis **sp. nov.**	13	FMNH 271122	Boualapha Dist., Khammouan Prov., Laos, 17°39'03.4"N 105°44'25.2"E	HM462063 This study	HM462079 This study
Tylototriton shanjing	14	MVZ 219763	Jingdong Co., Yunnan Prov., China, 24.46667°N 100.90000°E	DQ517852 Weisrock *et al.* (2006)	-
Tylototriton shanjing	15	CAS 215071	Nu Jiang Pref., Yunnan Prov., China, 26°05'31.5"N 98°35'05.2"E	HM462054 This study	HM462066 This study
Tylototriton shanjing	16	CAS 234479	Nu Jiang Pref., Yunnan Prov., China, 26°05'08.8"N 98°35'16.3"E	HM462055 This study	HM462068 This study
Tylototriton taliangensis	17	CAS 195126	Liangsha Yizu Auton. Pref., Sichuan Prov., China, 28°49'N 102°17'E	DQ517853 Weisrock *et al.* (2006)	-
Tylototriton verrucosus	18	-	Nepal	DQ517854 Weisrock *et al.* (2006)	-
Tylototriton verrucosus	19	CAS 230899	Taunggyi Township, Shan State, Myanmar, 20°45'20.5"N 97°03'0.6"E	HM770087 This study	HM462067 This study
Tylototriton vietnamensis	20	NCSM 77330	Son Dong Dist., Bac Giang Prov., Vietnam, 21°12'N 106°40'E	HM770088 This study	HM770090 This study
Tylototriton vietnamensis	21	NCSM 77331	Son Dong Dist., Bac Giang Prov., Vietnam, 21°12'N 106°40'E	HM770089 This study	HM770091 This study
Tylototriton cf. *vietnamensis*	22	ROM 35330	Quang Thanh, Cao Bang Prov., Vietnam, 22°37'43"N 105°54'46"E	DQ517856 Weisrock *et al.* (2006)	-
Tylototriton cf. *vietnamensis*	23	ROM 35364	Quang Thanh, Cao Bang Prov., Vietnam, 22°37'43"N 105°54'46"E	HM462056 This study	HM462072 This study
Tylototriton wenxianensis	24	MVZ 236632	Pingure Co., Sichuan Prov., China, 32.16°N 104.49°E	DQ517855 Weisrock *et al.* (2006)	HM462071 This study

Molecular analyses: The mt DNA and POMC datasets were supplemented with sequences downloaded from GenBank (Table 1) to include every species of *Tylototriton* recognized by Dubois & Raffaëlli (2009) in the molecular analyses. Sequence data were aligned using MUSCLE (Edgar 2004). Pairwise distances of sequences were calculated using PAUP* 4.0b10 (Swofford 2002).

Mixed-model Bayesian phylogenetic inference was performed separately on the mt DNA and POMC data using MrBayes 3.1 (Ronquist & Huelsenbeck 2003). Data were partitioned by rRNA, tRNA, ND1 codon position, ND2+COXI codon position, and POMC codon position. COXI was combined with ND2 because it contained fewer than 10 characters for each codon position partition. The model of sequence evolution that best described each of the data partitions was inferred using the Akaike Information Criterion as implemented in MrModeltest 2.3 (Nylander 2004). These were GTR+I for rRNA, ND1 second codon position, and ND2+COXI second codon position; GTR+G for ND1 first codon position and ND2+COXI first codon position; GTR+I+G for tRNA, ND1 third codon position, and ND2+COXI third codon positions; F81 for POMC first and second codon positions; and GTR for POMC third codon position. Four independent Bayesian analyses were performed on each data set. In each analysis, four chains were run for 20,000,000 generations using the default priors, trees were sampled every 4,000 generations, and the first 25% of trees

were discarded as 'burn-in.' A 50% majority rule consensus of the sampled trees was constructed to calculate the posterior probabilities of the tree nodes.

Morphology: Examined specimens are listed in the Appendix. Data were also obtained from original descriptions (Unterstein 1930; Fei & Yang 1984; Böhme *et al*. 2005) and photographs in life (Raffaëlli 2007; Stuart *et al.* 2008; Ziegler & Nguyen 2008; Nguyen *et al.* 2009a,b). Measurements were taken to the nearest 0.1 mm with dial calipers: SVLA = snout-vent length from tip of snout to anterior edge of vent; SVLP = snout-vent length from tip of snout to posterior edge of vent; AXGR = axilla to groin; TTL = total length; TAL = tail length from posterior edge of vent to tail tip; TAD = maximum tail depth; HL = head length from posterior edge of parotoid to snout tip; HW = maximum head width; EN = eye-nostril from anterior corner of eye to nostril; IN = internostril distance; AL = anterior limb length from axilla to tip of longest toe; PL = posterior limb length from groin to tip of longest toe.

Results

Molecular analyses: The aligned datasets contained 2,930 mt DNA characters (1,167 variable) and 475 POMC characters (48 variable). The standard deviation of split frequencies among the four Bayesian runs was 0.000993 for the mt DNA data and 0.004551 for the POMC data, and trace plots of clade probabilities viewed using AWTY (Wilgenbusch *et al.* 2004) were relatively stationary. This suggests that the four runs in each analysis had sufficiently converged and that topologies were sampled in proportion to their true posterior probability distribution.

In the mt DNA tree, the genus *Tylototriton* formed a strongly supported monophyletic group and was the sister taxon to *Echinotriton* (Fig. 1). The *T. asperrimus* and *T. verrucosus* groups were recovered with strong support. The *T. asperrimus* group contained three strongly supported major clades, one containing *T. vietnamensis*, one containing *T. asperrimus* and *T. wenxianensis*, and one containing *T. hainanensis*, *T.* cf. *vietnamensis* (Cao Bang Province, Vietnam), and the Laos population. *Tylototriton vietnamensis*, *T.* cf. *vietnamensis* and the Laos population were represented by at least two samples and were each monophyletic with strong support. Relationships among the three clades within the *T. asperrimus* group were not resolved (Fig. 1). Uncorrected pairwise distances of the mt DNA data were 3.44–9.77% between species in the *T. asperrimus* group, and 3.59–9.18% between the Laos population and species in the *T. asperrimus* group (Table 2).

TABLE 2. Uncorrected pairwise distances (%) of the mt DNA sequences used in this study within (diagonal) and between (below diagonal) members of the *Tylototriton asperrimus* group.

	T. asperrimus	*T. wenxianensis*	*T. hainanensis*	*T. vietnamensis*	*T.* cf. *vietnamensis*	*T. notialis* **sp. nov.**
T. asperrimus (*n* = 1)	-					
T. wenxianensis (*n* = 1)	3.44	-				
T. hainanensis (*n* = 1)	7.51	7.29	-			
T. vietnamensis (*n* = 2)	9.77	9.38	9.26	0.00		
T. cf. *vietnamensis* (*n* = 2)	7.77–7.86	7.36–7.43	3.91–3.99	9.25–9.66	0.15	
T. notialis **sp. nov.** (*n* = 3)	7.54–7.55	7.14–7.18	3.72–3.76	9.11–9.18	3.59–3.94	0.00–0.10

In the POMC tree, the *T. asperrimus* and *T. verrucosus* groups were not recovered, and posterior probabilities were low in the ingroup (Fig. 2). The samples from Laos were recovered as monophyletic with

strong support (Fig. 2) and had a unique C at nucleotide position 356 in the POMC dataset (versus a G in all other studied taxa).

Morphology: The Laos population is distinctive among members of the *T. asperrimus* group in the combination of the condition of the rib nodules (dorsolateral row of enlarged glands), dorsal and ventral skin texture, and dorsal coloration (below).

Taxonomic conclusion: The Laos population is diagnosable in mitochondrial DNA, nuclear DNA, and morphology from all other species in the *T. asperrimus* group. These three independent data sets provide a corroborated hypothesis that this population represents a distinct species, described herein as new.

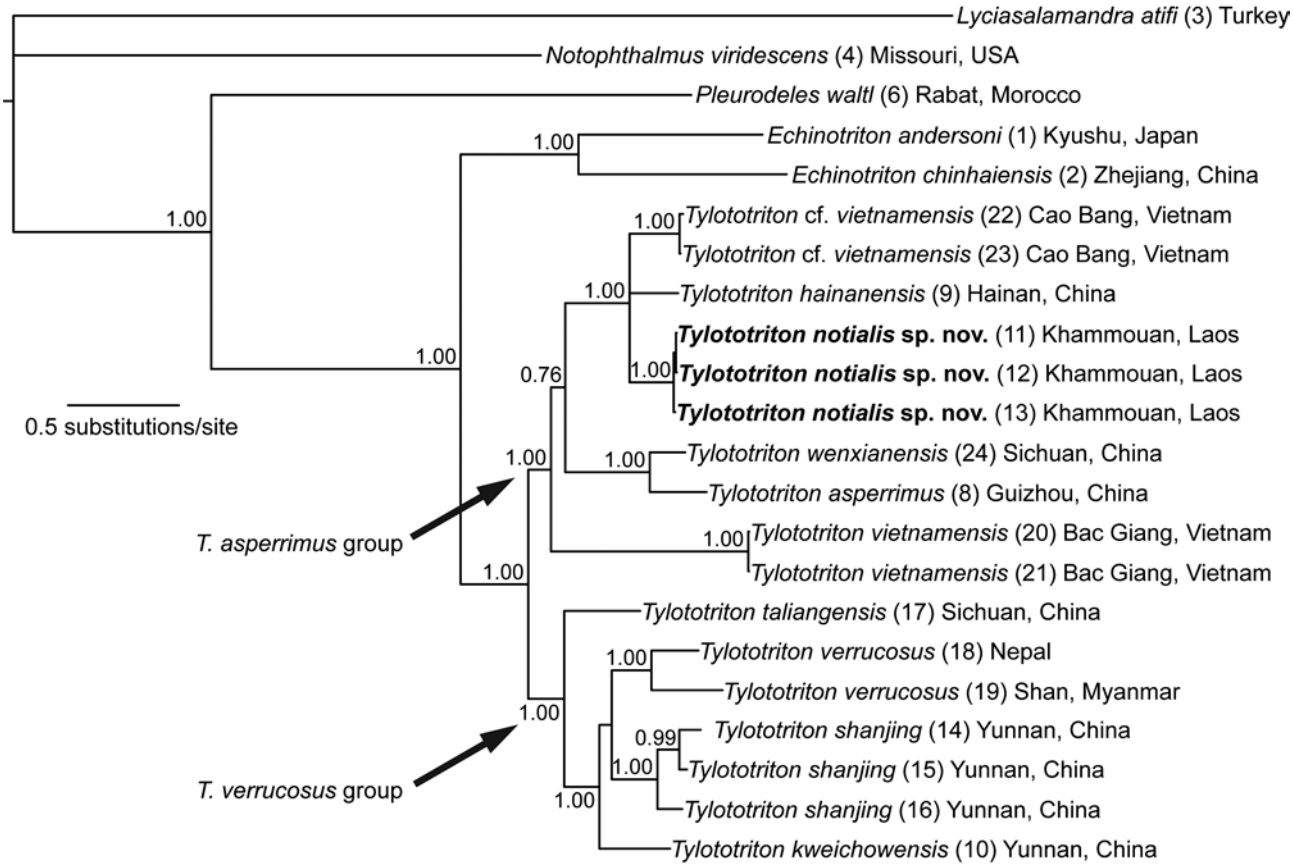

FIGURE 1. Fifty percent majority-rule consensus phylogram resulting from mixed-model Bayesian analysis of mitochondrial DNA from salamandrids. Numbers at nodes are Bayesian posterior probabilities. Numbers in parentheses refer to ID in Table 1.

***Tylototriton notialis* sp. nov.**

Holotype: FMNH 271121 (field tag BLS 10775), adult male (Fig. 3), Laos, Khammouan Province, Boualapha District, Nakai-Nam Theun National Protected Area, Nam On River catchment, Phou Ak escarpment, 17°38'39.6"N 105°44'12.3"E (Fig. 4), 980 m elev., coll. 27 May 2007 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay.

Paratypes: FMNH 271120, adult male (Fig. 3), same data as holotype except coll. 26 May 2007. FMNH 271122, adult female, same data as holotype except 17°39'03.4"N 105°44'25.2"E, ca. 1,000 m elev., coll. 22 May 2006 by William G. Robichaud.

Referred material: FMNH 271125 (one larva; Fig. 5), same data as paratype male. FMNH 271129 (nine larvae), same data as paratype male except coll. 29 May 2007.

Subgenus: The new species is assigned to the subgenus *Yaotriton* Dubois & Raffaëlli 2009 (= *T. asperrimus* species group of Fei *et al.* 2005) based on its phylogenetic position (Fig. 1) and mostly dark dorsal and ventral coloration (Dubois & Raffaëlli 2009).

Etymology: The specific epithet taken from *notialis* L. for southern, in reference to the new species having the southernmost known locality of any member in the *T. asperrimus* group.

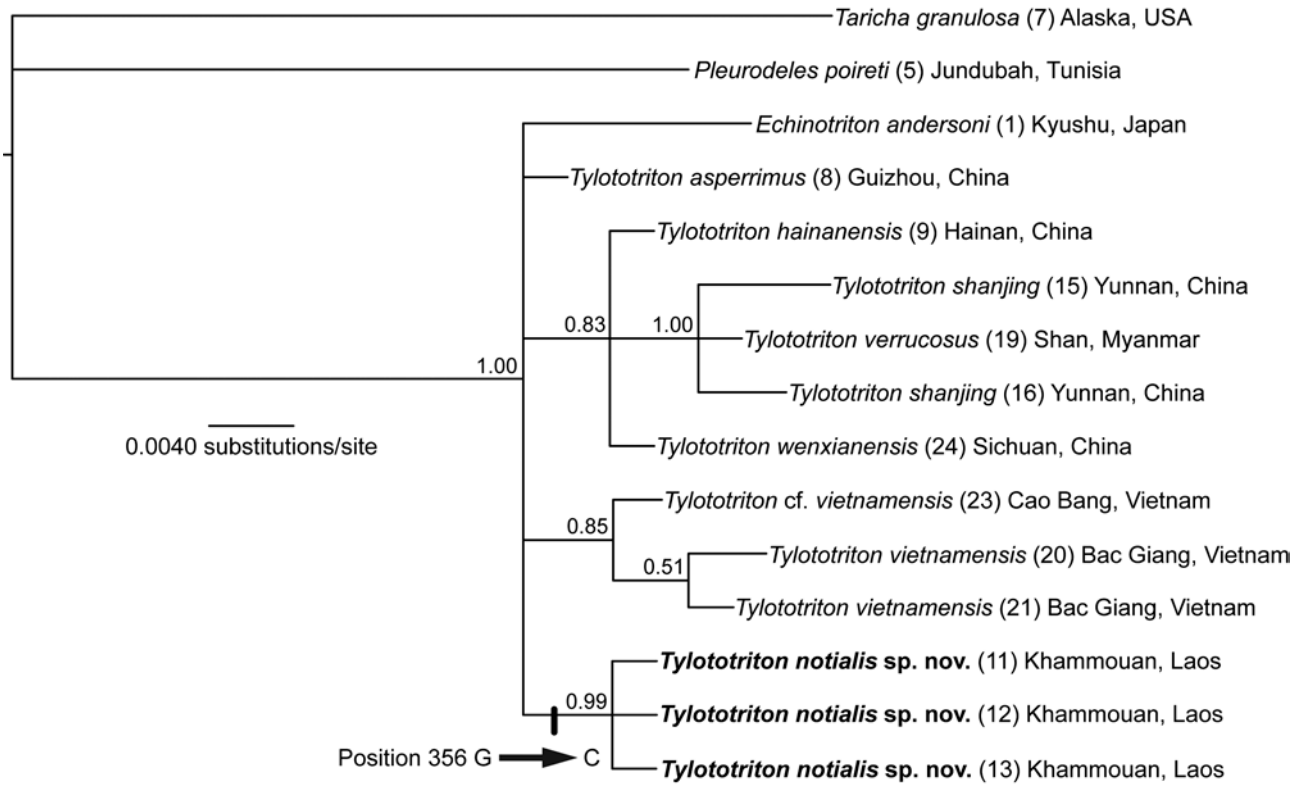

FIGURE 2. Fifty percent majority-rule consensus phylogram resulting from mixed-model Bayesian analysis of nuclear DNA (part of the POMC gene) from salamandrids. Numbers at nodes are Bayesian posterior probabilities. Numbers in parentheses refer to ID in Table 1.

Diagnosis: *Tylototriton notialis* is a *Tylototriton* (*Yaotriton*) having the combination of distinct, knob-like rib nodules (dorsolateral row of glandular warts); glandular warts on most of the remaining dorsal and ventral surfaces; very dark brown to black coloration on dorsum and venter; and bright orange coloration on rib nodules and posterior end of parotoid.

Description of holotype: Habitus moderately stout. Head broader than body, longer than wide, slightly sloping in profile. Snout short, truncate in dorsal view, rounded in profile, exceeding beyond lower jaw. Nostrils close to snout tip, right nostril slightly visible from above. Vomeropalatine teeth in two rows, anteriorly in contact and beginning posterior to the anterior margin of the choanae, converging into two parallel rows briefly, then diverging from one another. Glandular ridge on midline of crown from above anterior edge of eye to middle of head. Glandular ridge on outer margin of crown from above eye to base of parotoid. Parotoids enlarged, projecting backward. Glandular patch of skin on nape. Distinct vertebral tubercular ridge from posterior end of crown to base of tail, separated from ridge on midline of crown. Dorsolateral row of approximately 14 large glandular warts (rib nodules) on each side from level of axilla to base of tail, distinctly knob-like anteriorly, becoming smaller posteriorly, merging at level of groin. Smaller, glandular warts on most of remaining dorsal and ventral surfaces, warts on crown, nape, and back with clusters of glands and sometimes conical, those on throat granular and widely spaced, those on belly arranged in striations perpendicular to body axis, with a smooth, glandular, ovoid patch of skin on chest. Weak gular fold present. Four fingers, five toes, all without webbing. Tail laterally compressed, narrow dorsal fin, smooth ventral ridge, tip acuminate in profile.

Color of holotype in life: Body very dark brown. Margin of upper and lower lip, posterior end of parotoid, dorsolateral glands (rib nodules), dorsal margin of tail, outer margin of upper surface of hand and finger tips, lower surface of hand except center of palm, dorsal surface of toe tips, ventral surface of toes and extending from fifth toe to base of foot, cloacal region continuing to ventral ridge of tail bright orange.

Anterior surface of forelimb from axilla to base of first finger with narrow, broken, yellow stripe. Anterior and ventral surfaces of hindlimbs with small, scattered yellow spots. Iris dark brown, pupil black.

FIGURE 3. *Tylototriton notialis* **sp. nov.** in life. From top to bottom: dorsal view of holotype (FMNH 271121), dorsal view of paratype (FMNH 271120), ventral view of holotype (FMNH 271121), ventral view of paratype (FMNH 271120).

Color of holotype in preservative: Body faded to a lighter shade of brown. Orange markings faded to yellow or creamy-yellow.

Measurements of holotype: SVLA 60.2; SVLP 69.1; AXGR 30.5; TTL 130.4; TAL 63.1; TAD 6.5; HL 19.6; HW 15.9; EN 4.0; IN 4.9; AL 23.7; PL 23.0.

Variation: The body coloration of the paratype male (collected on land) was black rather than very dark brown as in the holotype (collected in water) and paratype female (collected on land). Böhme *et al.* (2005) reported that *T. vietnamensis* taken on land were darker in coloration than those living in water. Measurements are summarized in Table 3.

TABLE 3. Measurements (mm) of *Tylototriton notialis* **sp. nov.**, *T. vietnamensis*, and *T.* cf. *vietnamensis*. Abbreviations defined in the text.

Measurement	***T. notialis* sp. nov.** Khammouan, Laos		*T. vietnamensis* types[a] Bac Giang, Vietnam	*T. vietnamensis* topotypes Bac Giang, Vietnam	*T.* cf. *vietnamensis* Cao Bang, Vietnam
	Males (*n*=2) Range	Female (*n*=1)	Males (*n*=3) Range; Mean ± SD	Males (*n*=2) Range	Males (*n*=30) Range; Mean ± SD
SVLA	56.5–60.2	73.4	48.3–53.6; 50.7 ± 2.7	61.3–62.8	56.1–68.4; 61.7 ± 3.2
SVLP	64.5–69.1	78.0	-	66.0–69.8	61.5–74.3; 67.7 ± 3.5
AXGR	27.1–30.5	39.3	-	30.6–33.7	28.4–39.4; 34.0 ± 2.4
TTL	109.1–130.4[b]	141.8	113.9–121.8; 118.4 ± 4.1	125.3–125.4	109.6–142.3; 126.1 ± 7.5
TAL	45.3–63.1[2]	65.2	62.4–63.9; 63.1 ± 0.8	55.6–59.4	44.7–68.5; 58.4 ± 5.4
TAD	6.5–7.1	7.4	6.6–8.0; 7.4 ± 0.7	8.0–8.1	6.4–9.2; 7.6 ± 0.7
HL	19.4–19.6	21.5	15.3–18.6; 17.2 ± 1.7	18.3–18.4	17.5–21.2; 19.3 ± 0.9
HW	15.8–15.9	19.4	15.7–17.0; 16.5 ± 0.7	16.8	15.7–18.9; 17.2 ± 0.9
EN	3.5–4.0	4.7	4.0–4.4; 4.1 ± 0.2	3.8–4.4	3.2–4.3; 3.7 ± 0.3
IN	4.9–5.0	5.8	5.4–6.1; 5.7 ± 0.4	5.6–5.7	4.3–6.2; 5.4 ± 0.4
AL	22.9–23.7	26.6	16.0–19.8; 18.2 ± 2.0	20.9–22.4	18.5–25.9; 22.2 ± 1.6
PL	22.7–23.0	28.0	20.1–21.3; 20.7 ± 0.6	22.2–23.5	18.6–24.1; 21.5 ± 1.6

a. Data from Böhme *et al.* (2005)

b. Tail tip missing in paratype male

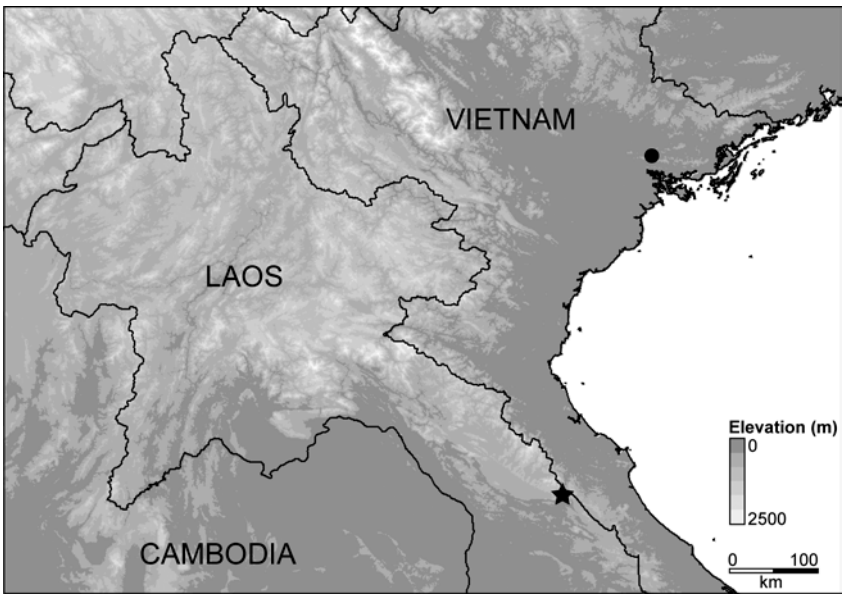

FIGURE 4. Map illustrating the type localities of *Tylototriton notialis* **sp. nov.** (star) and *T. vietnamensis* (circle).

Larvae: Two size classes of larvae were obtained in syntopy with the holotype: FMNH 271125 (one larva) with TTL 33.1, and FMNH 271129 (nine larvae) with TTL 17.8–20.9 (mean 19.4 ± S.D. 0.9).

Distribution and natural history: The new species is currently known only from the type locality, although it likely also occurs in adjacent Vietnam given its proximity to the border. All were taken in

evergreen mixed with deciduous and pine forest (Fig. 6). The holotype and larvae were collected at 1940–2042 h on the stream bottom under 10–30 cm of water (pH 5.0, temperature 19.5ºC) in a 3 m wide stream with slow current and sand and bedrock substrate, including leaf litter-filled potholes, just upstream from a 3 m high chute over bedrock. The paratype male was found at 1030 h, 30 cm above the ground on a 20 cm diameter log covered by another log, approximately 20 m from a steep escarpment. The type locality lies near the former Ho Chi Minh Trail, and a large, unexploded bomb dropped by American forces during the war was embedded in the ground approximately 3 m from the paratype male (Fig. 7). The paratype female was found during the day on the damp forest floor.

FIGURE 5. Dorsal and lateral view of larva (FMNH 271129) of *Tylototriton notialis* in preservative (total length 20.9 mm).

The testes of the holotype and paratype males are mature but not enlarged, suggesting these males were in their first reproductive season. The paratype female has enlarged oviducts, indicating that she had recently laid eggs. Eggs of the new species were not found.

Conservation: Overharvesting for traditional medicine and the international pet trade has been identified as a major threat to Asian salamandrids (Rowley *et al.* 2010). The formal description of *Laotriton laoensis* (Stuart & Papenfuss 2002), the first salamandrid known from Laos, inadvertently led to its exploitation for the international pet trade (Stuart *et al.* 2006). That species is now protected from commercial collecting by national legislation in Laos. It is hoped that the muted coloration and superficial similarity of *T. notialis* to other members of the *T. asperrimus* group will minimize its demand in the international pet trade. The new species' occurrence in a remote area within Nakai-Nam Theun National Protected Area, which is one of Laos' largest and best-funded national protected areas (WMPA 2005), should also afford it protection.

Comparisons: *Tylototriton notialis* differs from all other species in the *T. asperrimus* group by having bright orange coloration on the posterior end of the parotoids (parotoid coloration dark like dorsum in all other species). *Tylototriton notialis* differs from *T. asperrimus*, *T. wenxianensis*, *T. hainanensis*, and *T.* cf. *vietnamensis* from Cao Bang Province, Vietnam, by having bright orange coloration on the rib nodules (uniformly dark dorsum in *T. asperrimus*, *T. wenxianensis*, *T. hainanensis*, and *T.* cf. *vietnamensis*). *Tylototriton notialis* differs from *T. vietnamensis* by having glandular warts on most of the dorsal and ventral surfaces and very dark brown to black body coloration (*T. vietnamensis* with mostly smooth skin and gray to tan body coloration). *Tylototriton notialis* further differs from *T. hainanensis*, *T. vietnamensis*, and *T. wenxianensis* by having knob-like rib nodules (slightly flattened rib nodules in *T. vietnamensis* and *T. hainanensis* and indistinct nodules in *T. wenxianensis*).

FIGURE 6. Habitat of *Tylototriton notialis* at the type locality.

Discussion

Detecting species borders within the *T. asperrimus* group is challenging, as all are superficially very similar species having a dark body with orange to red coloration restricted to the digits, vent, and ventral surface of the tail (and in some, rib nodules and parotoids), and that mostly overlap in body sizes (Table 3). Contrary to Böhme *et al*. (2005), all specimens in the *T. asperrimus* group examined here have the head longer than wide and similar snout shape in dorsal and lateral views. As with the new Laos species, coloration appears to be useful for distinguishing species of *Tylototriton*; for example, Nussbaum *et al.* (1995) separated *T. verrucosus* and *T. shanjing* primarily on this basis (the synonymy of these two species by Zhang *et al*. 2007 is not followed here because only a single, unvouchered sample of *T. verrucosus* was included in their molecular analysis).

An alternative, more conservative taxonomy might propose recognizing only the three major subclades of the *T. asperrimus* group as species (*T. asperrimus*, *T. hainanensis*, and *T. vietnamensis* being the oldest available names for those subclades), but this approach would obscure considerable morphological and genetic variation. Although *T. wenxianensis* was originally described only as a subspecies of *T. asperrimus* (see Fei *et al.* 1984), it is morphologically the most easily recognized member of the group owing to its lack of distinct rib nodules (Fei *et al.* 1984; Fei *et al.* 2005). Thresholds of genetic distances should not be used alone to determine species status (Wake & Schneider 1998; Frost & Hillis 1990), but genetic distances do convey information on evolutionary history and are often useful heuristic measures to help recognize species (Vieites *et al*. 2009). Genetic distances between *T. hainanensis*, *T. notialis*, and *T.* cf. *vietnamensis* are greater than those between *T. asperrimus* and *T. wenxianensis* (Table 2).

FIGURE 7. Microhabitat (log in foreground) of the paratype (FMNH 271120) of *Tylototriton notialis*. The arrow indicates an unexploded bomb dropped by American forces during the war.

Additional species diversity may be hidden within the name *T. vietnamensis*. The original description of *T. vietnamensis* specifically included Cao Bang Province, Vietnam, within the range of the new species (Böhme *et al*. 2005), although without supporting evidence for that occurrence (specimens or photographs). Weisrock *et al*. (2006) were consistent with the original description by referring their sample from Cao Bang to this species. However, Cao Bang specimens (here as *T.* cf. *vietnamensis*) are much darker in coloration and genetically very distinct from topotypes of *T. vietnamensis* from Bac Giang Province, Vietnam (Figs. 1–2, Table 2), and are doubtfully conspecific with them. Nguyen *et al*. (2009a,b) included both *T. vietnamensis* and *T. asperrimus* in the northern Vietnamese fauna, but the Cao Bang samples are also genetically distinct from the Chinese *T. asperrimus* sample used here (Figs. 1–2, Table 2). Further study on species borders within the *T. asperrimus* group is warranted.

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APPENDIX. Specimens of *Tylototriton* examined.

Tylototriton hainanensis: MVZ 230373, China, Hainan Province, Hainan Island, 18.77666°N 108.70289°E (topotype).
Tylototriton vietnamensis: NCSM 77330–31, Vietnam, Bac Giang Province, Son Dong, Tay Yen Tu Nature Reserve, 21°12'N 106°40'E (topotypes).
Tylototriton cf. *vietnamensis*: ROM 35336–65, Vietnam, Cao Bang Province, Quang Thanh, 22°37'43"N 105°54'46"E.
Tylototriton verrucosus: UMMZ 189647–48, UTA A 27588, China, Yunnan Province, Longchuan, Gongwa, 24°11'N 97°48'E (topotypes).
Tylototriton wenxianensis: MVZ 236632-35, 236637, China, Sichuan Province, Pingure County, 32.16°N 104.49°E.