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A NEW SPECIES OF THE GENUS *Tylototriton* (AMPHIBIA, CAUDATA, SALAMANDRIDAE) FROM CENTRAL VIETNAM

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Abstract
We describe a new species of the genus *Tylototriton* from Pu Hoat Nature Reserve, Nghe An Province, in the northern part of Central Vietnam, based on morphological and molecular evidence. The new species was previously confused with *T. vietnamensis* from northern Vietnam and *T. notialis* from Laos. The new species can be distinguished from *T. notialis* by the absence of orange coloration on posterior end of parotids (*vs* presence); an indistinct brown coloration on rib nodules (*vs* bright orange); a broader and slightly rounded head (*vs* narrower and angular); comparatively shorter limbs (*vs* longer); slightly fewer number, smaller size and irregular arrangement of rib nodules (*vs* larger rib nodules arranged in two dorsolateral series). Phylogenetic analysis of the ND2 and 16S rRNA mtDNA genes confirmed the placement of the new species to Clade I of the subgenus *Yaotriton*, and suggests it is a sister species of *T. notialis* (*p*-distance 3.0% in ND2 gene). The range of the new species is restricted to the Pu Hoat Mountain Range and is isolated from the range of *T. notialis*, which inhabits Northern Annamites (Truong Son) Mountains by the valley of the Ca River, an important biogeographic barrier. The new species is currently known only from the montane forests of Pu Hoat Nature Reserve (at elevations from 700 to 1000 m a.s.l.). We suggest the new species be classified as Endangered (EN) on the IUCN Red List.

*Key words***:** *Tylototriton thaiorum* sp. nov., mtDNA genealogy, Pu Hoat Nature Reserve, Ca River

Introduction
The salamandrid genus *Tylototriton* Anderson, 1871 (colloquially known as crocodile newts) currently includes 31 recognized species, inhabiting montane forest areas throughout the Asian monsoon climate zone from eastern Himalaya, southern and central China including Hainan Island, to northern Indochina including northern Vietnam, Laos, northern Thailand, and Myanmar (Wang *et al*. 2018, Bernardes *et al*. 2020, Frost 2021). The genus is traditionally partitioned into two subgenera: *Tylototriton sensu stricto* is characterized by the presence of orange spots on the head, dorsum, tail, or sides

of the body, while the subgenus *Yaotriton* is characterized by generally much darker almost blackish coloration (Yang *et al*. 2014, Li *et al*. 2020), though the validity of this subgeneric taxonomy has been questioned in a number of studies (e.g. Wang *et al*. 2018, Than Zaw *et al*. 2019). Several recent phylogenetic studies also demonstrated that our knowledge on taxonomic diversity of the genus *Tylototriton* is still far from complete, indicating the presence of numerous undescribed morphologically cryptic lineages, which actually might represent independent species (Wang *et al*. 2018, Than Zaw *et al*. 2019). The amount of undescribed diversity appears to be especially high in the *T. asperrimus* group of the subgenus *Yaotriton* (Bernardes *et al*. 2020, Li *et al*. 2020). The number of species of *Tylototriton* in Vietnam is high, with seven species of this genus having been recorded so far for the country, namely *T. anguliceps* Le, Nguyen, Nishikawa *et al*. 2015, *T. notialis* Stuart, Phimmachak, Sivongxay & Robichaud, 2010, *T. pasmansi* Bernardes, Le, Nguyen *et al*., 2020, *T*. cf. *pulcherrimus* Hou, Zhang, Li & Lu, 2012, *T. sparreboomi* Bernardes, Le, Nguyen *et al*., 2020, *T. vietnamensis* Böhme, Schöttler, Nguyen & Köhler, 2005, and *T. ziegleri* Nishikawa, Matsui & Nguyen, 2013a (Böhme *et al*. 2005, Nishikawa *et al*. 2013b, Le *et al*. 2015, Fei & Ye 2016, Bernardes *et al*. 2020). It is noteworthy that six of the seven *Tylototriton* species recorded from Vietnam were described during the last ten years. Three other species, namely *T. asperrimus* Unterstein, 1930 (presently restricted to southeastern China), *T. verrucosus* Anderson, 1871 (presently restricted to Yunnan Province of China, northern Myanmar and Thailand), and *T. shanjing* Nussbaum, Brodie & Yang, 1995 (presently restricted to Yunnan Province of China) also have been reported from Vietnam (Nguyen *et al*. 2009, Hernandez 2016). However, recent phylogenetic studies suggest that these records were likely based on misidentifications of *T. pasmansi* or *T. sparreboomi*, and *T. anguliceps* complex members, respectively (see Le *et al*. 2015, Bernardes *et al*. 2020).

Originally, the occurrence of *Tylototriton* in Pu Hoat Forest, Dong Van Commune, Que Phong District, Nghe An Province of Vietnam (Fig. 1, locality 1) was reported by Böhme *et al*. (2005) based on personal observations of T. Schöttler. Böhme *et al*. (2005) provisionally assigned this population to *T. vietnamensis*,

without providing any information on voucher specimens or any other justification for this taxonomy. Soon afterwards, Stuart *et al*. (2010) described a new species *T. notialis* based on three adult specimens from Nakai-Nam Theun National Protected Area, Khammouan Province of Laos (Fig. 1, locality 2). Nishikawa *et al*. (2013b) briefly mentioned the specimens from Pu Hoat and assigned them to *T. notialis*, providing further evidence for the full species status of this taxon as distinct from *T. asperrimus*. At the same time, Nishikawa *et al*. (2013b) noted differences in coloration between the true *T. notialis* from Laos, which has orange coloration on the posterior ends of the parotoid glands, and the Pu Hoat population, which lacks orange coloration on the parotoids. Nevertheless owing to small genetic distance between *T. notialis sensu stricto* and the Pu Hoat population, Nishikawa *et al*. (2013b) concluded that both populations likely belong to the same species. More recently Nishikawa *et al*. (2020) reported on a new locality of *T. notialis sensu stricto* in the close vicinity of the type locality of this species in Khammouan Province of Laos, and stated that "it seems that the only morphological difference between the populations [of this species] from Laos and Vietnam is the presence or absence of the colorful markings" (Nishikawa *et al*. 2020: 135). However, the limited number of specimens examined by Stuart *et al*. (2010) and Nishikawa *et al*. (2013b, 2020) leaves open the question as to the correct identification of morphological differences between these two populations.

In May 2019, during fieldwork in the montane forests of Pu Hoat Nature Reserve in Nghe An Province of Vietnam (Fig. 1, locality 1), we collected a series of six specimens of a newt, which was tentatively identified as *Tylototriton* sp. In the present study, based on this new material we reexamine and compare the morphological and molecular characters of the Pu Hoat *Tylototriton* population with *T. notialis sensu stricto* from Laos, and demonstrate that the concordant differences between them in mtDNA, external morphology and coloration require the recognition of the Pu Hoat population as a distinct species.

Material and methods
Sample collection: Fieldwork was carried out in Pu Hoat Nature Reserve in the environs of Muong Dan Village, Hanh Dich Commune, Que Phong District, Nghe An Province, northern part

of Central Vietnam (Fig. 1, locality 1), from 06–15 May 2018 and from 15–30 May 2019. Specimens of *Tylototriton* sp. were collected by hand in swamps in forest clearings surrounded by montane evergreen tropical forests. Geographic coordinates and altitude were obtained using a Garmin GPSMAP 60CSx GPS receiver (Garmin Ltd., USA) and recorded in datum WGS 84. Specimens were euthanized by 20% benzocaine and tissue samples (liver) for genetic analysis were taken and stored in 96% ethanol prior to preservation. Specimens were subsequently preserved in 70% ethanol and deposited in the herpetological collection of the Zoological Museum of Moscow State University (ZMMU) in Moscow, Russia.
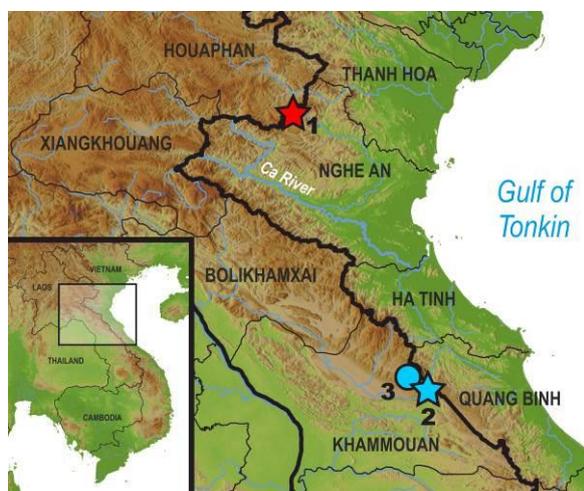

Figure 1. Distribution of *Tylototriton notialis sensu stricto* (blue) and the new species (red) in Central Vietnam and Laos; inset shows the location of this area on the map of Indochina. **1**, Pu Hoat Nature Reserve, Nghe An Province, Vietnam; **2**, Nam On River, Nakai-Nam Theun National Protected Area, Khammouan Province, Laos; **3**, Mt. Jeung, Khammouan Province, Laos. Stars denote the type localities of *T. notialis* and the new species.

Morphological description: Specimens of *Tylototriton* sp. were photographed in life and after preservation. The sex and maturity of the specimens were checked by minor dissections. Measurements were taken using a digital caliper to the nearest 0.01 mm, subsequently rounded to 0.1 mm. We used a stereoscopic light binocular microscope when necessary. Morphometrics followed Okamiya *et al*. (2018) and Than Zaw *et al*. (2019) and included the following 24 measurements taken for the type series and used in inter-specific comparisons: (1) SVL (snout-vent length) from tip of snout to anterior tip of vent; (2) HL (head length); (3) HW (head

width); (4) MXHW (maximum head width); (5) IND (internarial distance); (6) AGD (axilla-groin distance); (7) TRL (trunk length); (8) TAL (tail length) from anterior tip of vent to tail tip; (9) VL (vent length); (10) FLL (forelimb length); (11) HLL (hindlimb length); (12) VTW (vomerine tooth series width): greatest width of vomerine tooth series; (13) VTL (vomerine tooth series length): greatest length of vomerine tooth series; (14) LJL (lower jaw length from tip of lower jaw to articulation of upper and lower jaws); (15) SL (snout length from tip of snout to anterior tip of upper eyelid); (16) IOD (minimum interorbital distance); (17) UEW (maximum upper eyelid width); (18) UEL (upper eyelid length, distance between anterior and posterior angles); (19) OL (orbit length); (20) BTAW (basal tail width at level of anterior tip of cloaca); (21) MTAW (tail width at mid-level of tail); (22) MXTAH (maximum tail height); (23) MTAH (tail height at mid-level of tail); and (24) ON (orbitonarial distance). For holotype description, we additionally examined the following 12 morphometric characters following Poyarkov *et al*. (2012) and Than Zaw *et al*. 2019: (25) ICD (intercanthal distance); (26) CW (chest width); (27) NSD (nostril-snout distance); (28) 1FL (first finger length from base to tip); (29) 2FL (second finger length from base to tip); (30) 3FL (third finger length from base to tip); (31) 4FL (fourth finger length from base to tip); (32) 1TL (first toe length from base to tip); (33) 2TL (second toe length from base to tip); (34) 3TL (third toe length from base to tip); (35) 4TL (fourth toe length from base to tip); and (36) 5TL (fifth toe length from base to tip).

The diagnosis of the subgenus *Yaotriton* and morphological characters for comparison were taken from original descriptions and taxonomic reviews of the genus: Bernardes *et al*. (2020), Böhme *et al*. (2005), Chen *et al*. (2010), Fei & Ye (2016), Hou *et al*. (2012), Li *et al*. (2020), Nishikawa *et al*. (2013a, 2013b, 2020), Phimmachak *et al*. (2015), Qian *et al*. (2017), Shen *et al*. (2012), Stuart *et al*. (2010), Than Zaw *et al*. (2019), and Yang *et al*. (2014).

Laboratory methods: Total genomic DNA was extracted from 95% ethanol-preserved muscle tissues using standard phenol-chloroform extraction protocols (Hillis *et al*. 1996). Total DNA concentration was estimated in 1 μL using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and consequently adjusted to 100 ng DNA/μL. We amplified two mtDNA fragments including the partial sequences of the

ND2 and 16S rRNA mtDNA genes. These markers were chosen as they were proven to be useful in studies of *Tylototriton* phylogeny and taxonomy (Nishikawa *et al*. 2013a, 2013b, 2014, Wang *et al*. 2018, Than Zaw *et al*. 2019, and references therein). We used the 16L-1 (forward) (5'-CTGACCGTGCAAA GGTAGCG TAATCACT-3') and 16H-1 (reverse) (5'-CTCCG GTCTGAACTCAGATCACGTAGG-3') primers to amplify the 16S rRNA fragments following Hedges (1994). For amplification and sequencing of the ND2 gene, we used the SL-1 (forward) (5'-ATAGAGGTTCAAACCCTCTC-3') and SL-2 (reverse) (5'- TTAAAGTGTCTGGGTT GCATTCAG-3') primers of Wang *et al*. (2018). Polymerase chain reaction (PCR) conditions followed Than Zaw *et al*. (2019). PCR was performed in 20 µL using 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl2, Taq PCR buffer (10 mmol / L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.1 mmol/L MgCl2, and 0.01% gelatin), and 1 U of Taq DNA polymerase. PCR cycles included an initial denaturation step of 4 min at 94 °C and 35 cycles of denaturation for 30 s at 94 °C, primer annealing for 30 s at 48–58 °C, and extension for 1 min 30 s at 72 °C. PCR products were visualized by agarose gel electrophoresis in the presence of ethidium bromide and consequently purified using 2 µL from a 1: 4 dilution of ExoSapIt (Amersham, UK) per 5 µL of PCR product prior to cycle sequencing. Sequencing was performed in both directions using the same primers as used in PCR on an ABI3730xl automated sequencer (Applied Biosystems, USA) at Evrogen Inc., Moscow (Russia). The newly obtained sequences were aligned and deposited in GenBank under the accession numbers MW883478–MW883485 (Table 1). Sequences of 31 other *Tylototriton* species used for comparisons along with three sequences of outgroup taxa were obtained from GenBank (see Table 1).

Phylogenetic analyses: Sequences of partial fragments of ND2 and 16S rRNA mtDNA for 51 Salamandridae specimens, including 48 representatives of *Tylototriton* (36 taxa) and three sequences of outgroup members of Salamandridae [*Echinotriton andersoni* (Boulenger, 1892), *E. chinhaiensis* (Chang, 1932), and *Pleurodeles waltl* Michahelles, 1830] were included in the final alignment with a total length of up to 1665 bp. Information on voucher specimens and GenBank accession numbers used in phylogenetic analyses is summarized in Table 1. Nucleotide sequences were initially aligned in MAFFT v.6 (Katoh *et al*. 2002) with default parameters, and then checked by eye and slightly adjusted in BioEdit 7.0.5.2 (Hall 1999). The dataset was divided into four partitions: three codon partitions for the ND2 gene and a single partition for 16S rRNA, with the optimal evolutionary models for each estimated using MODELTEST v. 3.06 (Posada & Crandall 1998). According to the Akaike information criterion (AIC), for the ND2 gene the HKY+G model was selected as the best fit for the first and second codon partitions, and the J2+G model was considered the best fit for the third codon partition; while the TVM+G model was the best fit for the 16S rRNA partition. Mean uncorrected genetic distances (*p*-distances) between sequences were calculated in MEGA 7.0 with missing data and gaps excluded from the analysis (Kumar *et al*. 2016). The matrilineal genealogy was inferred using Bayesian inference (BI) and maximum likelihood (ML) algorithms. BI analyses were conducted in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for twenty million generations and sampled every 2000 generations. Five independent MCMCMC runs were performed, the MCMC simulations ran for 100 million generations, were sampled every 10,000 generations, and the first 10% of the trees from each run from each data set were discarded as burn-in. We checked the convergence of the runs and that the effective sample sizes (ESS) were all above 200 by exploring the likelihood plots using TRACER v1.6 (Rambaut *et al*. 2014). Confidence in tree topology was tested by posterior probability (BI PP) for the BI trees (Huelsenbeck & Ronquist 2001). Nodes with PP values over 0.95 were *a-priori* regarded as sufficiently resolved, those between 0.95 and 0.90 were regarded as tendencies, and values below 0.90 were considered to not be supported. We conducted ML analysis in the IQ-TREE webserver. One-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (UFBS; Hoang *et al*. 2018) approximation algorithm were employed and nodes having ML UFBS values of 95 and above were *a-priori* considered highly supported, while the nodes with values of 90–94 were considered well-supported, and the nodes with values of 70–89 were considered as tendencies (Bui *et al*. 2013).

Table 1. Sequences and voucher specimens of *Tylototriton* and outgroup taxa (*Echinotriton chinhaiensis*, *E. andersoni*, *Pleurodeles waltl*) used in this study; L, lineage; NP, National Park; NR, Nature Reserve; Co., County; Dist., District; Mt. Mountain; H, holotype; — not available.

Species name	Voucher Number	Locality	16S rRNA	ND2
Ingroup:				
T. anguliceps	TBU PAE671	Copia NR, Thuan Chau, Son La, Vietnam	—	LC017833
T. anhuiensis	CIB 08042905-2	Yuexi Co., Anhui, China	KY800587	KY800853
T. asperrimus	CIB 70063	Longsheng Co., Guangxi, China	KY800549	KC147816
T. broadoridgus	CIB 200084	Sangzhi Co., Hunan, China	KY800570	KY800837
T. dabienicus	HNNU 1004-015	Shangcheng Co., Anhui, China	KY800607	KC147811
T. hainanensis	CIB 20081048	Mt. Diaoluo, Hainan, China	KY800553	KC147817
T. himalayanus	CIB 201406246	Mai Pokhari, Illam, Mechi, Nepal	KY800590	KT765173
T. kachinorum	ZMMU A5953	In Gyi Taung Mt., Kachin, Myanmar	MK095618	MK097273
T. kweichowensis	CIBW g20080818014	Bijie City, Guizhou, China	KY800551	KY800823
T. liuyangensis	CSUFT20100108	Liuyang City, Hunan, China	KY800606	KJ205598
T. lizhenchangi	KUHE 42316	Yizhang Co., Hunan, China	KY800621	KY800881
T. maolanensis	CIBW g 20090730001	Libo Co., Guizhou, China	KY800575	KY800842
T. ngarsuensis	LSUHC 13763	Ngar Su, Taunggyi Dist., Shan, Myanmar	—	MH836585
T. notialis	FMNH HERP 271120	Boualapha Dist., Khammouan, Laos	—	HM462061
T. notialis	FMNH HERP 271121	Boualapha Dist., Khammouan, Laos	—	HM462062
T. notialis	FMNH HERP 271122	Boualapha Dist., Khammouan, Laos	—	HM462063
T. notialis	FES RA.18.001	Mt. Jeung, Boualapha, Khammouan, Laos	—	LC522466
T. panhai	PH019	Phu Hin Rong Kla NP, Thailand	—	AB830735
T. panwaensis	CAS 245418	Panwa, Myitkyina Dist., Kachin, Myanmar	—	KT304279
T. pasmansi obsti	IEBR 4471	Xuan Nha NR, Van Ho, Son La, Vietnam	—	MT210168
T. p. pasmansi	IEBR 4466	Phu Canh NR, Da Bac, Hoa Binh, Vietnam	—	MT201166
T. phukhaensis	CUMZ A-7717	Doi Phu Kha NP, Nan, Thailand	—	MN912573
T. podichthys	IEBR A2014-1	Xam Neua, Huaphanh, Laos	—	LC017835
T. pseudoverrucosus	CIB WCG2012012	Ningnan Co., Liangshanyizu, China	KY800599	KY800860
T. pulcherrimus	CIB TY040	Lüchun Co., Yunnan, China	KY800626	KY800890
T. shanjing	KIZ 201306081	Yongde Co., Yunnan, China	KY800593	KY800856
T. shanorum	CAS 230940	Taunggyi Township, Shan, Myanmar	—	AB922823
T. sparreboomi	IEBR 4476	Sin Ho, Lai Chau, Vietnam	—	MT210162
T. taliangensis	CIB GG200110183	Shimian Co., Yan'an City, Sichuan, China	KY800559	KC147819
T. thaiorum sp. nov.	ZMMU A-7577[H]	Pu Hoat NR, Nghe An, Vietnam	MW883482	MW883478
T. thaiorum sp. nov.	ZMMU A-7578	Pu Hoat NR, Nghe An, Vietnam	MW883483	MW883479
T. thaiorum sp. nov.	ZMMU A-7579	Pu Hoat NR, Nghe An, Vietnam	MW883484	MW883480
T. thaiorum sp. nov.	ZMMU A-7580	Pu Hoat NR, Nghe An, Vietnam	MW883485	MW883481
T. thaiorum sp. nov.	VNMN TAO1229	Pu Hoat NR, Nghe An, Vietnam	—	KY800883
T. thaiorum sp. nov.	VNMN TAO1235	Pu Hoat NR, Nghe An, Vietnam	—	KY800884
T. uyenoi	KUHE 19037	Doi Inthanon, Chiang Mai, Thailand	—	AB830730
T. verrucosus	CIB TSHS1	Longchuan Co., Dehong, Yunnan, China	KY800581	KY800847
T. vietnamensis	IEBR A.3674	Tay Yen Tu NR, Bac Giang, Vietnam	KY800614	KY800874
T. wenxianensis L1	CIB 20090527	Wenxian Co., Gansu, China	KY800579	KC147813
T. wenxianensis L2	CIB 20070638	Qingchuan Co., Sichuan, China	KY800543	KY800816
T. wenxianensis L3	CIB 20090601	Wangcang Co., Sichuan, China	KY800571	KY800838
T. wenxianensis L4	CIB 20080002	Yunyang Co., Chongqing, China	KY800540	KY800813
T. wenxianensis L5	CIB 20080003	Yunyang Co., Chongqing, China	KY800541	KY800814
T. yangi	KUHE 42282	Pingbian Co., Yunnan, China	KY800624	KY800887
T. ziegleri	VNMN 3390	Quan Ba, Ha Giang, Vietnam	KY800625	KY800889
Tylototriton sp. 1	CIB WG200600019	Suiyang Co., Zunyi, Guizhou, China	KY800544	KY800817
Tylototriton sp. 2	CIB WH10001	Wufeng Co., Hubei, China	KY800600	KY800863
Tylototriton sp. 3	CIB XZ20091201	Xinyi City, Guangdong, China	KY800616	KY800876
Outgroup:				
E. chinhaiensis	CIB ZHJY1	Zhenhai Co., Zhejiang, China	KY800627	KY800891
E. andersoni	MVZ 232187	Tokunoshima, Kagoshima, Japan	EU880314	EU880314
P. waltl	MVZ 231894	Cadiz, Andalusia, Spain	EU880330	EU880330

Results

Sequence variation. The final alignment of the ND2 gene contained 1067 aligned nucleotides, of which, 645 sites were conserved and 422 sites were variable, of which 276 were parsimony-informative. The transition–transversion bias (R) was estimated as 5.17. Nucleotide frequencies were 37.54% (A), 23.81% (T), 28.23% (C), and 10.42% (G). The final alignment of the 16S rRNA gene contained 508 aligned characters, of which, 424 sites were conserved and 82 sites were variable, of which 53 were suggested as parsimony-informative. The transition–transversion bias (R) was estimated as 7.45. Nucleotide frequencies were 36.97% (A), 24.81% (T), 20.29% (C), and 17.93% (G) (all data given for ingroup only).

MtDNA genealogy: BI and ML phylogenetic analyses resulted in almost identical topologies (Fig. 2). The topology of the matrilineal genealogies was largely consistent with the phylogeny of *Tylototriton* presented by Wang *et al*. (2018) and Than Zaw *et al*. (2019) and recovered five main clades grouped into two major reciprocally monophyletic groups, corresponding to the subgenera *Tylototriton sensu stricto* and *Yaotriton* (Fig. 2). Monophyly of the subgenus *Yaotriton* was well-supported by ML analysis, and got no significant support in BI analysis (Fig. 2, 91/0.78, hereafter node values given for UFBS/BI PP, respectively); this group included three highly supported main clades:

(1) Clade 1 includes the members of the *Tylototriton asperrimus* species group from China, northern Vietnam and Laos, and included six nominal *Tylototriton* species: *T. asperrimus*, *T. hainanensis* Fei, Ye & Yang, 1984, *T. notialis*, *T. pasmansi* (with two subspecies *T. pasmansi pasmansi* and *T. pasmansi obsti*), *T. sparreboomi*, and *T. ziegleri*, as well as one undescribed lineage *Tylototriton* sp. 3 from Guangdong Province of China previously identified as *T. asperrimus* (see Wang *et al*. 2018), and the population of *Tylototriton* sp. from Pu Hoat Nature Reserve.

(2) Clade 2 joined the members of the *Tylototriton wenxianen*sis species group from China, and included seven species of *Tylototriton*: *T. anhuiensis* Qian, Sun, Li *et al*., 2017, *T. broadoridgus* Shen, Jiang & Mo, 2012, *T. dabienicus* Chen, Wang & Tao, 2010, *T. liuyangensis* Yang, Jiang, Shen &

Fei, 2014, *T. lizhenchangi* Hou, Zhang, Jiang *et al*., 2012, *T. maolanensis* Li, Wei, Cheng *et al*., 2020, and *T. wenxianensis* Fei, Ye & Yang, 1984, as well as two undescribed lineages *Tylototriton* sp. 1 from Guizhou and *Tylototriton* sp. 2 from Hubei provinces of China.

(3) Clade 3, which we refer to here as the *T. vietnamensis* species group, included two peculiar species of *Tylototriton* from northern Vietnam and north-eastern Thailand and adjacent Laos: *T. panhai* Nishikawa, Khonsue, Pomchote & Matsui, 2013 and *T. vietnamensis*.

Monophyly of the subgenus *Tylototriton sensu stricto* received strong support both in ML and BI analyses (Fig. 2, 98/1.0); this group included two strongly supported clades:

(4) Clade 4 joined the members of the *T. verrucosus* species group from Nepal, Myanmar, China and northern Indochina, and included 14 nominal species of *Tylototriton*: *T. anguliceps*, *T. himalayanus* Khatiwada, Wang, Ghimire *et al*., 2015, *T. kachinorum* Zaw, Lay, Pawangkhanant *et al*., 2019, *T. kweichowensis* Fang & Chang, 1932, *T. ngarsuensis* Grismer, Wood, Quah *et al*., 2018, *T. panwaensis* Grismer, Wood, Quah *et al*., 2019, *T. phukaensis* Pomchote, Khonsue, Thammachoti *et al*., 2020, *T. podichthys* Phimmachak, Aowphol & Stuart, 2015, *T. pulcherrimus*, *T. shanjing*, *T. shanorum* Nishikawa, Matsui & Rao, 2014, *T. uyenoi* Nishikawa, Khonsue, Pomchote & Matsui, 2013, *T. verrucosus*, and *T. yangi* Hou, Zhang, Zhou *et al*., 2012.

(5) Clade 5, which we refer to here as the *T. taliangensis* species group, included two species from China, namely *T. pseudoverrucosus* Hou, Gu, Zhang *et al*., 2012 and *T. taliangensis* Liu, 1950.

Following the results of earlier studies (Wang *et al*. 2018, Than Zaw *et al*. 2019, Bernardes *et al*. 2020, Li *et al*. 2020), our analysis indicated the presence of several divergent lineages of *Tylototriton*, which might correspond to species that are yet to be described, referred to here as *Tylototriton* spp. 1–3 (see Table 1, Fig. 2). All these lineages belong to the subgenus *Yaotriton* and were until recently confused with either *T. asperrimus* or *T.*

wenxianensis. Our analysis also indicated deep phylogenetic structuring within *T. wenxianensis* which consists of five mtDNA lineages, forming a well-supported clade (Fig. 2, 96/0.98). Overall, these results suggest that the taxonomy of this group remains incomplete and further taxonomic and phylogenetic research is needed.

The population of *Tylototriton* sp. from Pu Hoat belongs to clade 1 (Fig. 2), where it is reconstructed as a sister lineage of *T. notialis* from Laos with high levels of node support (99/1.0). The group formed by these two species is suggested to be a sister clade of the group including the members of the *T. asperrimus* species complex from Guangxi Province of China and northern Vietnam (*T. asperrimus*, *T. pasmansi*, and *T. sparreboomi*), although the phylogenetic position of the latter species is poorly supported; 85/0.60).

***Genetic distances*:** The interspecific uncorrected genetic *p*-distances between the sequences of the ND2 mtDNA gene of *Tylototriton* sp. from Pu Hoat and other congeners varied from 3.0% (between *Tylototriton* sp. and its sister species *T. notialis*) to 13.5% (between *Tylototriton* sp. and *T. ngarsuensis*) (see Table 2). There was no intraspecific variation in ND2 gene sequences for *Tylototriton* sp. from Pu Hoat; for *T. notialis* it comprised 0.2% of substitutions (Table 2). The mtDNA lineages within *T. wenxianensis* were moderately divergent in ND2 gene sequences (*p*=2.3%, Table 2).

Systematics. Our phylogenetic analysis has unambiguously placed the Pu Hoat population of *Tylototriton* sp. as a sister lineage of *T. notialis*, in agreement with earlier phylogenies of the group (Wang *et al.* 2018, Than Zaw *et al.* 2019). Additionally, the degree of pairwise divergence in the ND2 mtDNA gene of this population is comparable or greater than the level of genetic divergence observed between many recognized species of *Tylototriton*, within both the subgenus *Tylototriton* [*T. verrucosus* and *T. shanjing* (1.5%), *T. verrucosus* and *T. pulcherrimus* (2.6%), *T. verrucosus* and *T. panwaensis* (2.2%), *T. pulcherrimus* and *T. panwaensis* (1.8%), *T. taliangensis* and *T. pseudoverrucosus* (2.4%), *T. ngarsuensis* and *T. shanorum* (0.5%), *T. panwaensis* and *T. podichthys* (2.9%)] and the subgenus *Yaotriton* [*T. anhuiensis* and *T. broadoridgus* (2.6%), *T. anhuiensis* and *T. maolanensis* (2.6%), *T. dabienicus* and *Tylototriton* sp. 2 (2.6%), *T. anhuiensis* and *Tylototriton* sp. 2 (2.6%), *T. broadoridgus* and *Tylototriton* sp. 2 (2.2%)] (see Table 2). This and the phylogeny raise the question as to whether the Pu Hoat population of *Tylototriton* sp. is conspecific with *T. notialis*, as was suggested earlier (Nishikawa *et al.* 2013b, 2020), or whether it warrants taxonomic recognition as an independent taxon, which an integrative taxonomic approach might reveal?

Earlier works have reported morphological data only for a single male specimen of *Tylototriton* sp. from Pu Hoat Nature Reserve (Nishikawa *et al.* 2013b). Due to this limited sampling, only characters of body coloration were assessed. Nishikawa *et al.* (2013b) noted significant differences in coloration between the Pu Hoat *Tylototriton* sp. and *T. notialis* but interpreted these differences as intraspecific variation and concluded that "the presence of orange coloration on the parotoid cannot be used as a diagnostic characteristic for *T. notialis*" (Nishikawa *et al.* 2013b: 46). Recently, examining newly collected material on *T. notialis* from Laos, Nishikawa *et al.* (2020) argued that the only morphological difference between the populations from Laos and Vietnam is the presence or absence of the colorful markings (Nishikawa *et al.* 2020: 136). However, a detailed morphological examination of the Pu Hoat population of *Tylototriton* was never completed.

Here, based on newly collected material of *Tylototriton* sp. from Pu Hoat, we examine the external morphology and coloration of this population, and report on a number of diagnostically important characters readily distinguishing this population from *T. notialis* and all other congeners. These include not only the coloration of parotids and rib nodules, but also the shape and width of the head, relative length of limbs, size and arrangement of rib nodules. Moreover, the presently known ranges of *T. notialis* and the Pu Hoat populations are separated by the Ca River valley, which is considered an important biogeographic barrier, separating the subtropical montane forests of northern Vietnam from the tropical montane forests of the Northern Annamites (Truong Son Range) (Abramov & Tran 2017; Poyarkov *et al.* 2021 *in press*). These arguments together support our hypothesis that the Pu Hoat population of *Tylototriton* represents a previously unknown species, which we describe below.

A NEW *Tylototriton* SPECIES FROM CENTRAL VIETNAM

Table 2. Uncorrected *p*-distance (percentage) between the sequences of ND2 mtDNA gene (below the diagonal), and intraspecific genetic p-distance (on the diagonal) of *Tylototriton* species included in phylogenetic analyses.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 *T. thaiorum* sp.nov.	**0.0**																	
2 *T. notialis*	3.0	**0.2**																
3 *T. p. pasmansi*	5.7	6.1	—															
4 *T. pasmansi obsti*	5.7	5.2	4.2	—														
5 *T. asperrimus*	6.6	6.0	5.1	5.5	—													
6 *T. sparreboomi*	5.1	5.2	5.1	4.9	4.6	—												
7 *T. hainanensis*	4.6	5.2	6.4	6.2	6.0	4.4	—											
8 *Tylototriton* sp. 3	7.3	7.4	7.1	7.9	6.8	6.2	3.5	—										
9 *T. ziegleri*	5.7	5.8	5.7	5.1	4.9	4.2	4.2	6.8	—									
10 *T. wenxianensis*	9.7	10.0	10.6	10.3	10.3	10.6	9.9	11.6	10.5	**2.3**								
11 *Tylototriton* sp. 1	9.5	9.8	10.1	10.4	10.6	10.4	9.7	10.6	10.6	3.6	—							
12 *T. dabienicus*	9.0	9.4	9.9	9.7	9.7	9.5	9.3	11.0	9.7	4.4	4.6	—						
13 *Tylototriton* sp. 2	7.7	8.0	8.6	8.8	9.3	8.6	7.9	9.5	8.2	4.2	3.7	2.6	—					
14 *T. anhuiensis*	8.4	8.7	8.8	9.0	9.5	9.3	8.4	10.1	9.0	3.8	3.7	3.1	2.6	—				
15 *T. broadoridgus*	8.2	8.5	8.8	9.0	9.7	9.0	8.4	10.6	8.6	4.3	4.2	3.1	2.2	2.6	—			
16 *T. maolanensis*	8.6	8.9	10.1	9.9	9.9	10.1	9.5	11.2	9.9	4.1	4.6	3.1	3.1	2.6	3.1	—		
17 *T. liuyangensis*	8.8	9.1	9.0	9.3	9.7	9.5	8.4	10.6	9.3	6.9	7.1	6.0	6.0	6.0	6.2	6.4	—	
18 *T. lizhenchangi*	11.2	11.8	12.1	13.2	13.2	12.6	11.0	12.3	12.6	9.6	9.9	9.3	7.9	9.3	8.6	9.3	7.3	—
19 *T. panhai*	11.0	11.6	12.8	12.1	13.0	11.0	9.7	12.3	11.2	11.3	10.6	10.1	9.5	10.1	10.1	11.0	9.9	9.9
20 *T. vietnamensis*	12.8	11.9	13.0	11.5	12.3	10.4	11.0	13.2	11.5	11.5	10.8	11.2	10.8	10.8	11.5	11.2	10.8	12.8
21 *T. verrucosus*	11.5	11.2	12.8	12.6	13.0	11.9	11.0	12.8	11.9	9.5	9.5	9.3	8.4	9.3	9.0	9.5	9.9	11.9
22 *T. shanjing*	11.7	11.5	12.6	12.3	13.2	12.1	11.2	13.0	12.1	9.7	9.7	9.5	8.6	9.5	9.3	9.7	10.1	12.1
23 *T. pulcherrimus*	10.1	10.5	11.9	11.7	12.3	11.0	9.7	11.9	10.6	8.1	8.2	8.4	7.5	7.9	8.6	8.6	8.4	10.6
24 *T. panwaensis*	11.0	10.9	12.1	11.9	12.3	11.0	10.6	11.9	10.6	9.3	9.0	8.8	8.4	8.8	9.0	9.5	9.5	11.7
25 *T. podichthys*	11.7	11.6	12.8	12.8	12.6	11.7	11.2	12.1	11.9	10.3	9.5	10.1	9.3	9.7	9.9	10.4	10.1	12.8
26 *T. anguliceps*	11.0	10.9	11.9	12.3	12.6	10.8	11.0	11.5	11.2	11.2	10.4	10.4	9.9	10.1	9.9	10.6	10.1	13.7
27 *T. phukaensis*	11.2	11.5	13.4	13.0	13.0	11.5	11.5	12.3	11.5	11.7	11.5	11.0	10.6	10.8	11.0	11.2	9.0	13.4
28 *T. yangi*	11.2	11.1	11.0	11.0	11.2	10.4	10.6	11.5	10.4	10.2	9.5	9.5	8.6	9.3	9.9	9.9	9.5	12.1
29 *T. uyenoi*	12.6	12.8	14.4	14.4	14.2	13.9	13.3	14.6	13.7	12.8	12.4	12.2	11.7	12.2	12.4	12.2	12.6	13.7
30 *T. ngarsuensis*	13.5	10.7	15.1	13.0	13.0	13.0	10.8	12.4	12.4	9.6	8.7	9.7	8.7	8.7	9.2	10.8	10.8	12.4
31 *T. shanorum*	11.0	10.6	12.6	12.3	12.6	11.2	11.2	12.6	11.7	10.4	10.1	9.3	8.8	9.5	9.7	9.7	9.7	12.3
32 *T. himalayanus*	10.6	10.6	12.1	11.9	12.6	10.6	10.6	11.7	11.5	10.2	9.9	9.5	8.6	9.3	9.5	9.9	9.7	12.1
33 *T. kachinorum*	12.8	12.0	13.5	13.3	13.3	11.7	12.0	12.8	11.7	12.0	11.7	11.5	10.6	10.6	11.3	11.7	12.4	14.6
34 *T. kweichowensis*	10.6	10.6	10.1	11.0	10.8	10.1	9.7	11.0	9.3	10.4	10.1	9.7	8.6	9.5	8.6	10.1	9.5	11.7
35 *T. taliangensis*	10.4	10.8	11.9	11.9	11.2	11.2	9.7	11.9	10.8	9.3	9.3	8.8	7.9	8.4	8.4	9.3	8.6	11.0
36 *T. pseudoverrucosus*	9.5	10.0	11.0	11.0	11.5	10.4	9.0	11.2	9.9	9.3	9.0	8.6	7.7	8.4	8.2	9.3	8.4	11.0

Table 2 continued. Uncorrected *p*-distance (percentage) between the sequences of ND2 mtDNA gene (below the diagonal), and intraspecific genetic p-distance (on the diagonal) of *Tylototriton* species included in phylogenetic analyses.

|	Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
19	*T. panhai*	—																	
20	*T. vietnamensis*	10.4	—																
21	*T. verrucosus*	12.8	11.2	—															
22	*T. shanjing*	13.4	11.9	1.5	—														
23	*T. pulcherrimus*	11.5	11.5	2.6	3.3	—													
24	*T. panwaensis*	12.1	11.2	2.2	3.3	1.8	—												
25	*T. podichthys*	12.6	12.1	4.2	5.3	3.7	2.9	—											
26	*T. anguliceps*	13.9	13.9	6.4	6.6	5.1	5.1	6.6	—										
27	*T. phukaensis*	13.4	13.4	6.0	6.2	5.5	6.0	6.8	4.9	—									
28	*T. yangi*	13.0	11.9	4.0	4.6	3.1	3.1	4.9	4.6	6.0	—								
29	*T. uyenoi*	14.6	15.3	7.5	7.3	7.3	8.2	9.7	9.1	9.1	7.7	—							
30	*T. ngarsuensis*	10.3	13.0	5.4	6.5	4.9	6.0	7.6	6.5	8.1	5.4	7.7	—						
31	*T. shanorum*	11.9	12.3	6.2	6.8	6.0	6.4	7.9	7.7	7.7	7.1	9.5	0.5	—					
32	*T. himalayanus*	11.5	11.5	5.7	5.5	5.7	5.7	6.4	6.4	6.2	6.4	8.4	5.4	5.1	—				
33	*T. kachinorum*	13.3	12.8	7.3	8.0	7.7	6.4	8.0	8.6	8.6	8.2	11.7	7.7	7.7	5.3	—			
34	*T. kweichowensis*	11.2	10.8	5.7	6.4	5.7	5.7	6.8	6.0	6.8	6.0	9.1	6.0	6.4	5.3	6.6	—		
35	*T. taliangensis*	10.4	11.0	6.0	6.2	5.7	6.2	7.1	8.2	7.1	7.3	8.9	6.0	6.6	5.5	7.3	4.4	—	
36	*T. pseudoverrucosus*	9.9	10.8	6.0	6.6	5.1	5.5	6.4	7.5	7.1	6.6	9.5	6.5	6.4	5.5	6.9	4.0	2.4	—
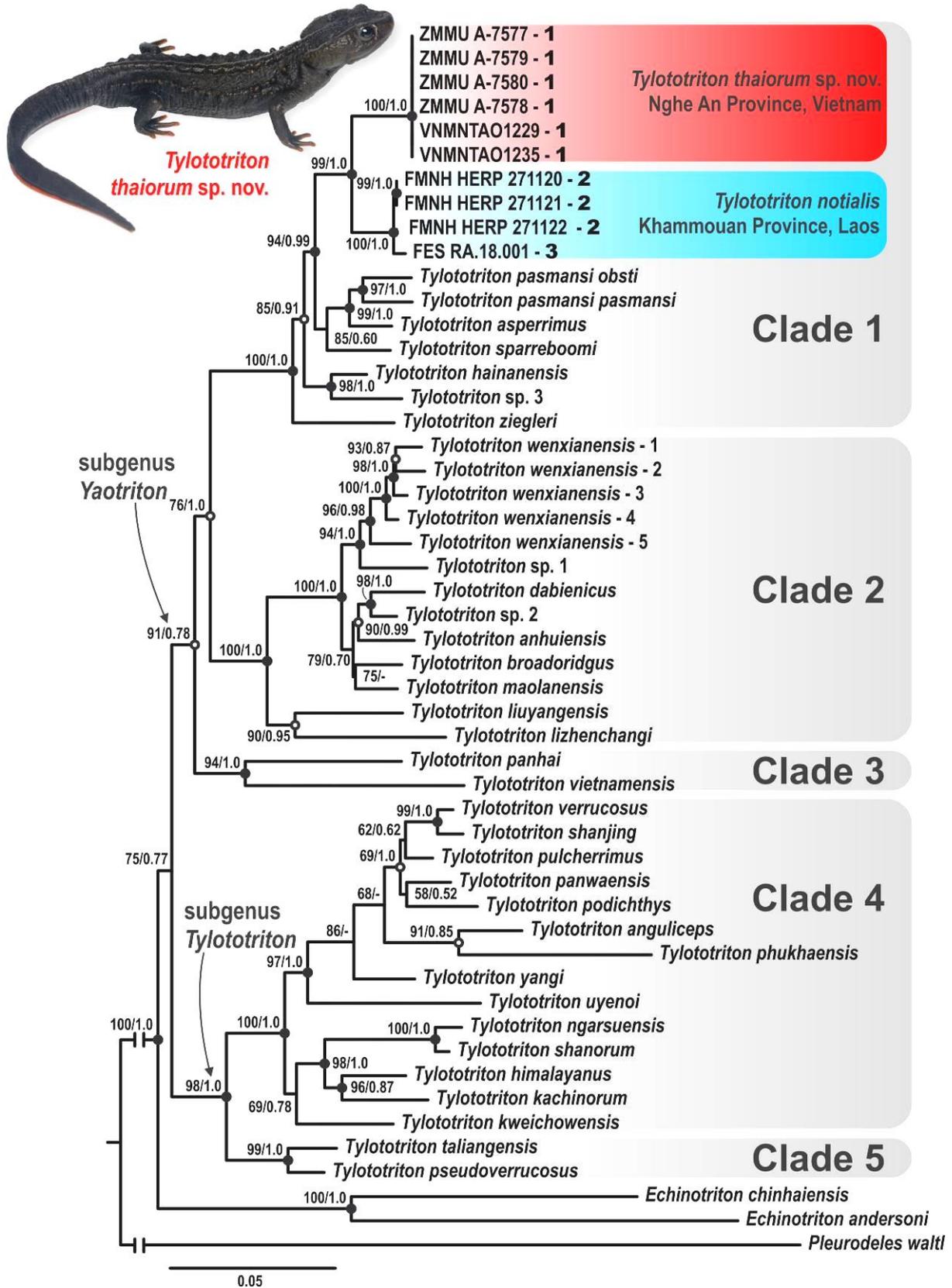
Figure 2. Maximum Likelihood tree of *Tylototriton* derived from the analysis of 1665 bp of alignment including ND2 and 16S rRNA gene fragments. For voucher specimen information and GenBank accession numbers see Table 1. Red and blue colours denote the new species and *T. notialis sensu stricto*, respectively; numbers in bold after the specimen IDs correspond to the locality numbers in Fig. 1. Numbers at tree nodes correspond to UFBS/PP support values, respectively; well-supported nodes are marked with solid circle, moderate-support is indicated with empty circle, no circle means no-support. © Photo: Nikolay A. Poyarkov.

Salamandridae Goldfuss, 1820
Tylototriton Anderson, 1871

Tylototriton thaiorum sp. nov.
[urn:lsid:zoobank.org:act:D7E7A23D-72FE-416E-BFE7-
1D14B03612FF]
(Figs. 3–5; Tables 3–4)
Tylototriton vietnamensis — Böhme *et al.* 2005
[*partim*]
Tylototriton notialis — Nishikawa *et al.* 2013b
[*partim*], Phimmachak *et al.* 2015 [*partim*],
Yang *et al.* 2018 [*partim*], Than Zaw *et al.* 2019
[*partim*], Bernardes *et al.* 2020 [*partim*]

Holotype. Adult male, ZMMU A-7577 (field number NAP-08957), collected from an ephemeral swamp in a forest clearing surrounded by montane evergreen tropical forest in Pu Hoat Nature Reserve, environs of Muong Dan Village, Hanh Dich Commune, Que Phong District, Nghe An Province, Central Vietnam (19.71715°N, 104.83791°E; 709 m a.s.l.), by Nikolay A. Poyarkov on 20th of May, 2019.

Paratypes (*n*=5). All adult males [ZMMU A-7578 (field number NAP-08958), and ZMMU A-7579–7682 (field numbers NAP-09049–09052)], all from the same locality and with the same collection information as the holotype.

Diagnosis. The new species is assigned to the genus *Tylototriton* and the subgenus *Yaotriton* based on the results of the molecular phylogenetic analyses and the following combination of morphological attributes: presence of dorsal granules, dorsolateral bony ridges on head, presence of dorsolateral series of knob-like warts (rib nodules), and absence of quadrate spine (Figs. 3–5). *Tylototriton thaiorum* sp. nov. is differentiated from all other members of the subgenus *Yaotrion* by the following combination of morphological characters: medium body size (SVL 59.3–68.6 mm in males); snout obtusely rounded in dorsal view and in lateral profile; skin rough with fine granules; supratemporal bony ridges on head wide, slightly protruding, beginning at the anterior corner of orbit; sagittal ridge on head low and almost indistinct; limbs thin, tips of forelimb and hindlimb overlapping when adpressed along body; vertebral ridge distinct, rough, not segmented; rib nodules 10–14, small, poorly distinct, arranged in two irregular dorsolateral series intermittent with smaller warts; dorsal background coloration dark-brown, ventral coloration greyish brown, limbs blackish; fingers and toe tips, parts of soles and palms, and vent bright orange, orange stripe

continuing along the ventral edge of tail to the tail tip.

Description of holotype. A medium-sized male specimen in a good state of preservation. Head longer than wide (HW/HL 0.94), head notably wider than body; rounded hexagonal in shape in dorsal view, depressed, gently sloping in profile; snout comparatively long, three times longer than eye (UEW/SL 0.38), obtusely rounded in dorsal view, rounded in lateral profile, projecting beyond lower jaw; nostrils on anterior margin of snout, located notably closer to snout tip than to eye (NSD/ON 0.61), with antero-lateral orientation, not visible from dorsal view; labial folds absent; tongue oval, attached to anterior floor of mouth, free posteriorly and laterally; vomerine tooth series in an inverted V-shape, converging anteriorly but not reaching choanae; parotoids distinct, large, crescent-shaped, slightly projecting posteriorly; dorsolateral supratemporal bony ridges on head wide, slightly protruding, beginning at the anterior corner of orbit continuing to anterior end of parotoid, posterior ends slightly curved inside; sagittal bony ridge on head very weak, almost indistinct; gular fold present.

Body shape moderately stout; costal folds absent; vertebral middorsal ridge distinct, wide, not segmented, rough, running from occiput region to sacrum and anterior part of tail. Rib nodules poorly distinct, small, forming knoblike glandular warts, arranged in two irregular longitudinal series on dorsolateral surfaces of dorsum, 12 nodules placed on each side of body from posterior to axilla to level of posterior vent margin (base of tail). Size of rib nodules varies from rounded, distinctly knob-like anteriorly, to smaller and of more irregularly shaped posteriorly; rib nodules intermittent with smaller irregular warts, making dorsolateral series slightly ragged in shape.

Limbs comparatively thin; forelimbs slightly shorter than hindlimbs; relative lengths of limbs: FLL/SVL 0.31, HLL/SVL 0.33; fore- and hindlimbs overlapping when adpressed towards each other along body; fingers and toes well developed, free of webbing or folds; fingers four, comparative finger lengths: 1FL<4FL<2FL=3FL; toes five, comparative toe lengths: 1TL=5TL<2TL<3TL=4TL. Tail long, notably exceeding body length (TAL/SVL 1.12); laterally compressed along entire length, tapering posteriorly, lateral grooves on tail indistinct; dorsal tail fin starting at anterior one fifth of tail length, becoming higher and more

distinct posteriorly, with maximal tail height at the middle of tail length, dorsal tail fin slightly serrated posteriorly; ventral tail ridge smooth; tail tip pointed.

Dorsal skin very rough, small granules and larger warts present on dorsal surfaces of head and dorsum, lateral sides of body and tail; ventral surfaces smoother with smaller granules arranged in transverse striations; throat with numerous tiny flat tubercles; surfaces of head ridges and middorsal vertebral ridge rough; limbs dorsally with numerous tiny tubercles, volar and plantar surfaces of hands and feet with tiny grooves forming reticulated pattern; flattened outer metacarpal and metatarsal tubercles distinct on palms and soles, respectively. Cloacal region slightly swollen, vent as a longitudinal slit, vent edges with numerous small transverse folds.

Colouration. In life, dorsum uniformly dark brown (Fig. 3); venter greyish brown, slightly lighter than dorsum; limbs blackish; rib nodules and vertebral ridge brown; fingers and toes bright reddish orange (ventrally along entire lengths, dorsally only on tips of the digits), parts of soles and palms corresponding to outer metatarsal and metacarpal tubercles reddish-orange, vent and ventral tail ridge bright orange. After two years in preservative, dorsal coloration slightly faded, becoming dark grey, while orange markings faded to cream.

Measurements of holotype. (in mm). SVL 65.2; HL 15.2; HW 14.3; MXHW 18.3; IND 5.4; AGD 30.9; TRL 52.3; TAL 72.8; VL 6.5; FLL 20.5; HLL 21.7; LJL 13.4; SL 6.6; IOD 7.6; UEW 2.5; UEL 4.4; OL 3.8; BTAW 7.8; MTAW 2.8; MXTAH 9.2; MTAH 7.9; ON 3.8; ICD 9.8; CW 12.3; NSD 2.3; FL_I 2.0; FL_{II} 3.7; FL_{III} 3.7; FL_{IV} 2.3; TL_I 2.0; TL_{II} 3.5; TL_{III} 5.3; TL_{IV} 5.3; TL_V 5.3.

Variation. All males were generally similar in morphology and coloration and agreed with the holotype description in body proportions and coloration; variation of morphometric characters within the type series is shown in Table 3. Variation of the dorsal coloration in four male paratypes in life is shown in Fig. 4. Males ZMMU A-7578 and ZMMU A-7582 had slightly lighter brownish coloration of dorsum. The number of rib nodules varies from 10 to 14 (mean 12.1±1.1; n=10). Male ZMMU A-7580 had notably shorter tail than other type specimens (Table 3; Fig. 4) likely due to regeneration of tail tip after damage; this specimen also had short regenerated fingers on

left forelimb and toes on right hindlimb. In male ZMMU A-7578 second finger on left forelimb is regenerated and is notably curved (Fig. 4). Male ZMMU A-7581 lacked forearm on the right forelimb, also due to some sort of damage. The reasons behind the high proportion of regenerated limbs appendages in the new species is not clear, it may be related to possible combat between the males during the courtship season, or may also result from predators.

Etymology. The specific name "*thaiorum*" is a Latin adjective in the genitive plural (masculine gender), derived from the name of the Thai people who inhabit the montane areas of northern Vietnam and adjacent territories, including the type locality of the new species. The people of Muong Dan Village pointed out to us the breeding habitat of the new species. We suggest the following common names: *Thai Crocodile Newt* (English), *Cá cóc thái* (Vietnamese), *Taiskiy krokodilovyi triton* (Russian).

Comparisons. According to the results of our phylogenetic analyses, *Tylototriton thaiorum* sp. nov. falls into clade 1 of the subgenus *Yaotriton* and morphological comparisons with members of this group appear to be the most pertinent. The differences of the new species from all other congeners of the subgenus *Yaotriton* are summarized in Table 4. The new species can be easily distinguished from all members of the subgenus *Tylototriton* (clades 4–5 in Fig. 2) and *T. panhai* by dark body coloration except for bright orange palms and soles, vent region, and ventral ridge of tail (*vs* light color markings present on parotoids, lips, vertebral ridge, rib nodules, limbs, and ventral tail ridge). The new species can be distinguished from *T. anhuiensis*, *T. broadoridgus*, *T. dabienicus*, *T. lizhenchangi*, *T. wenxianensis* and *T. vietnamensis* by having prominent bony ridges on head, denser granules on body, large and small warts among the rib nodules, and distinct interspaces between rib nodules (*vs* less developed ridges on head, smoother skin, slightly flattened to nearly indistinct rib nodules). The new species further differs from *T. vietnamensis* by having dark brown coloration of dorsum (*vs* gray to tan body coloration). The new species can be distinguished from *T. ziegleri* by smaller rib nodules (*vs* enlarged knob-like rib nodules) and vertebral ridge wide, not segmented and rough (*vs* distinctly tubercular). *Tylototriton thaiorum* sp. nov. can be distinguished from *T. asperrimus* and *T.*

Plate 01

Figure 3. Holotype of *Tylototriton thaiorum* sp. nov. male in life (collected, ZMMU A-7577). Photo ©: Nikolay A. Poyarkov.

ZMMU A-7578   ZMMU A-7580   ZMMU A-7581   ZMMU A-7582

Figure 4. Variation of dorsal coloration in four adult male paratypes (ZMMU A-7578, 7580–82) of *Tylototriton thaiorum* sp. nov. (scale: 10 mm). Photos ©: Nikolay A. Poyarkov.
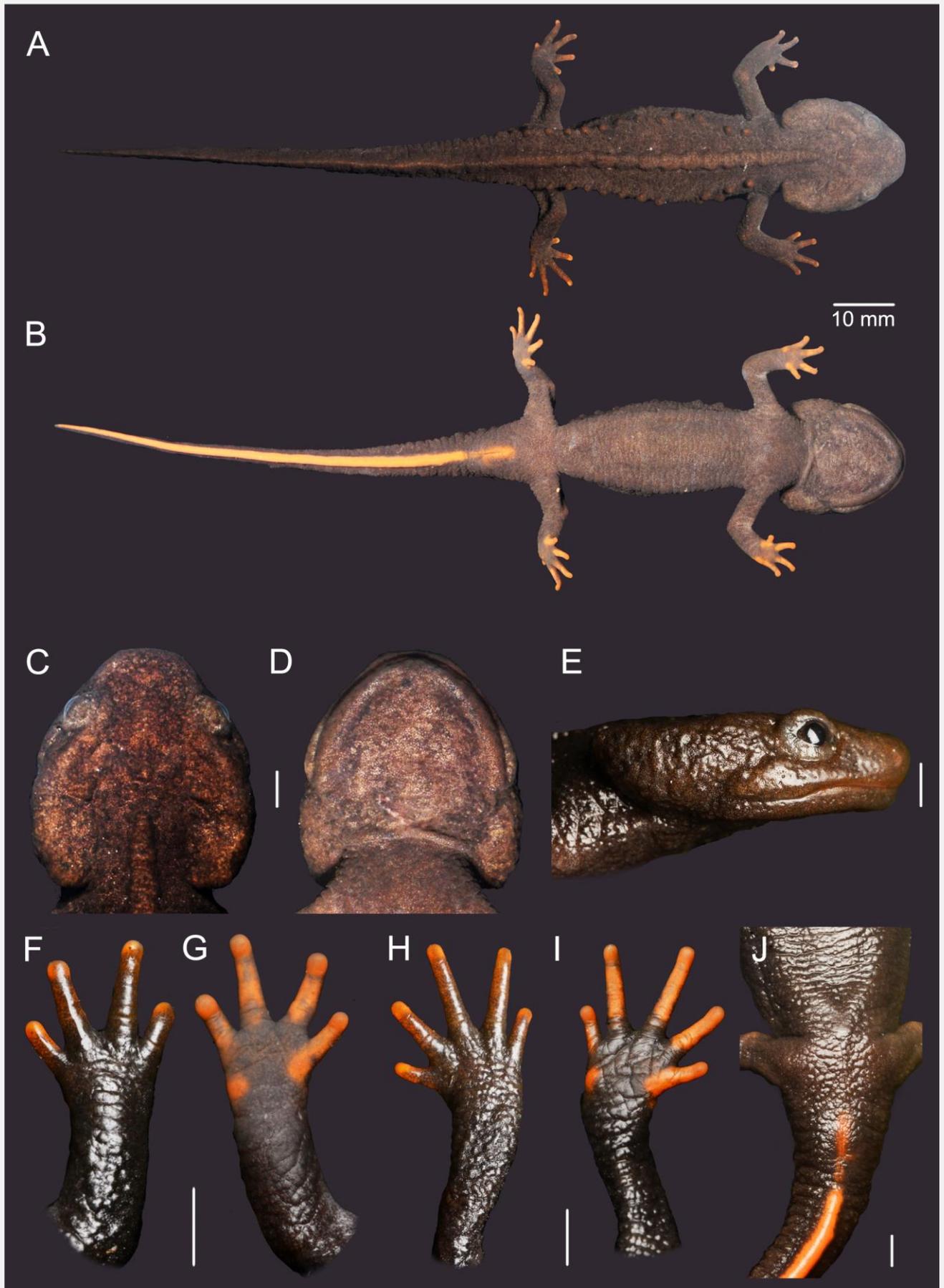
Plate 02

Figure 5. Holotype of *Tylototriton thaiorum* sp. nov. male in life (collected, ZMMU A-7577): body **(A)** dorsal and **(B)** ventral views; head **(C)** dorsal, **(D)** ventral, and **(E)** lateral views; right hand **(F)** dorsal and **(G)** ventral views; right foot **(H)** dorsal and **(I)** ventral views; **(J)** cloacal area (scale: 3 mm) Photos ©: Nikolay A. Poyarkov.

hainanensis by lacking orange color markings on posterior parotoids (*vs* present), and light brown coloration on the rib nodules (*vs* uniformly black). The new species can be distinguished from *T. liuyangensis* by having smaller body size in males (male SVL 59.3–68.6 mm *vs* 64.2–82.0 mm), by having light brown coloration on the rib nodules (*vs* uniformly black), and by ventral granules arranged in transverse striations (*vs* no transverse striations on belly). The new species is distinguished from *T. maolanensis* by having smaller body size in males (male SVL 59.3–68.6 mm *vs* 76.8–85.2 mm), by snout obtusely rounded in dorsal view (*vs* truncate), by comparatively narrower vertebral ridge (*vs* very wide vertebral ridge). The new species can be diagnosed from *T. pasmansi* and *T. sparreboomi* by having snout obtusely rounded in dorsal view (*vs* truncate), and by having light brown coloration on the rib nodules (*vs* uniformly black). In general morphology, *Tylototriton thaiorum* sp. nov. superficially most closely resembles its sister species *T. notialis*, however the new species can be readily distinguished from the latter by having the following suite of morphological characters: (1) absence of orange coloration on posterior end of parotid (*vs* presence); (2) light-brown coloration on rib nodules (*vs* bright orange), (3) a comparatively broader head in males: HW/HL 0.92–0.96 (mean 0.94±0.02, *n*=6) *vs* 0.81 (mean 0.81±0.00, *n*=2; data from Stuart *et al.* 2010); (4) obtusely rounded snout in dorsal view (*vs* truncated, slightly angular snout); (5) relatively shorter forelimb length in males: FLL/SVL 0.31–0.34 (mean 0.32±0.01, *n*=6) *vs* 0.39–0.41 (mean 0.40±0.01, *n*=2; data from Stuart *et al.* 2010); (6) relatively shorter forelimb length in males: HLL/SVL 0.33–0.36 (mean 0.35±0.01, *n*=6) *vs* 0.38–0.40 (mean 0.39±0.01, *n*=2; data from Stuart *et al.* 2010); (7) slightly smaller size of rib nodules intermittent with numerous smaller warts and arranged in irregular series, rib nodules notably vary in size and shape (*vs* generally larger rounded rib nodules of approximately uniform size and shape, arranged in regular dorsolateral series lacking intermittent smaller warts in gaps between them); (8) lower number of rib nodules: 10–14, mean 12.1±1.1, *n*=12 (*vs* 14–16, mean 15.2±0.8, *n*=6; data from figure 3 in Stuart *et al.* 2010, and figure 1 in Nishikawa *et al.* 2020).

Distribution and biogeography. To date, *Tylototriton thaiorum* sp. nov. is known only from a restricted area in montane forests of Pu Hoat Nature Reserve, Nghe An Province, northern part of Central Vietnam (Fig. 1) at elevations from 700 to 1000 m a.s.l. This area is located ca. 220 km air distance northwards from the type locality of *T. notialis* in Khammouan Province of Laos. The Pu Hoat Mountain Range is located on the northeastern edge of Xiangkhouang Plateau; it separates the Nghe An Province of Vietnam from the Houaphan Province of Laos. The locality of the new species is ca. 4 km from the national border of Vietnam and Laos, thus the occurrence of *Tylototriton thaiorum* sp. nov. is also anticipated on the north-western slopes of Pu Hoat Mountain Range in the adjacent parts of Houaphan Province of Laos.

Natural history. Our knowledge of the biology of *Tylototriton thaiorum* sp. nov. is scarce. Adult male newts were encountered at night after 1900 h in the water in ephemeral swamps in forest clearings. Surrounding areas were covered by dominant evergreen montane subtropical forest and secondary bamboo forest. Adult males were moving slowly along the muddy bottom in clear water 20–100 cm deep; the temperatures at the time of collection were air 25°C, and water 22°C. Females and larvae of the new species were not observed. We assume that the new species, similar to other members of the subgenus *Yaotriton*, is basically terrestrial and cannot be found in the water except during the breeding season. Other syntopic species of amphibians recorded at the type locality include: *Leptobrachium masatakasatoi* Matsui, 2013, *Sylvirana maosonensis* (Bourret, 1937), *Rhacophorus orlovi* Ziegler & Köhler, 2001, *Polypedates* cf. *mutus* (Smith, 1940), *Limnonectes limborgi* (Sclater, 1892) and *Microhyla pulchra* (Hallowell, 1861).

Conservation status. *Tylototriton thaiorum* sp. nov. is to date known only from a single location in Pu Hoat Nature Reserve, Nghe An Province of Vietnam, and is expected to be recorded in the adjacent parts of the Houphan Province of Laos. Though the actual extent of distribution of the new species remains unknown, it is likely a range-restricted species, restricted to the Pu Hoat Mountain Range. Habitat loss due to intensifying agriculture and forest logging and collection (locally) for traditional medicinal use (for abdominal pain and some parasitic infections) are among the major threats. We thus suggest *Tylototriton thaiorum* sp. nov. to be listed as Endangered (EN) [IUCN Red List criteria B1ab(iii)].

Table 3. Measurements of the type series of *Tylototriton notialis* (data from Stuart *et al.* 2010; ? = not available) and *Tylototriton thaiorum* sp. nov. (all in mm); H, holotype; SD, standard deviation.

Cat. No	SVL	HL	HW	MXHW	IND	AGD	TRL	TAL	VL	FLL	HLL	LJL
T. notialis s. str.												
males												
1. FMNH 271121 [H]	60.2	19.6	15.9	?	?	30.5	?	63.1	?	23.7	23	?
2. FMNH 271120	56.5	19.4	15.8	?	?	27.1	?	?	?	22.9	22.7	?
Range	58.4±2.6	19.5±0.1	15.9±0.1	?	?	28.8±2.4	?	63.1±0.0	?	23.3±0.6	22.9±0.2	?
T. thaiorum sp. nov.												
males												
1. ZMMU A-7577 [H]	65.2	15.2	14.3	18.3	5.4	30.9	52.3	72.8	6.5	20.5	21.7	13.4
2. ZMMU A-7579	68.6	15.6	14.3	18.8	5.3	33.2	53.6	54.5	6.5	22.1	24.8	14.7
3. ZMMU A-7580	59.3	13.5	12.7	17.0	5.5	28.0	47.7	56.8	5.6	19.6	20.8	12.5
4. ZMMU A-7581	63.8	14.4	13.6	17.3	5.1	30.9	51.0	64.0	6.2	21.8	23.0	13.7
5. ZMMU A-7582	60.5	13.7	13.2	18.7	4.9	29.4	48.8	63.4	5.7	19.3	21.0	13.2
6. ZMMU A-7578	63.4	14.3	13.7	17.8	5.1	29.6	51.3	65.2	5.6	20.4	21.2	13.2
Mean±SD	63.5±3.3	14.5±0.8	13.6±0.6	18.0±0.7	5.2±0.2	30.3±1.8	50.8±2.2	62.8±6.5	6.0±0.4	20.6±1.1	22.1±1.5	13.5±0.7
Range	59.3-68.6	13.5-15.6	12.7-14.3	17.0-18.8	4.9-5.5	28.0-33.2	47.7-53.6	54.5-65.2	5.6-6.5	19.3-22.1	20.8-24.8	12.5-14.7

Table 3 continued. Measurements of the type series of *Tylototriton notialis* (data from Stuart *et al.* 2010; ? = not available) and *Tylototriton thaiorum* sp. nov. (all in mm); H, holotype; SD, standard deviation.

Cat. No	SL	IOD	UEW	UEL	OL	BTAW	MTAW	MXTAH	MTAH	ON	HW/HL	FLL/SVL	HLL/SVL
T. notialis s. str.													
males													
1. FMNH 271121 [H]	?	?	?	?	?	?	?	6.5	?	?	0.81	0.39	0.38
2. FMNH 271120	?	?	?	?	?	?	?	7.1	?	?	0.81	0.41	0.40
Mean	?	?	?	?	?	?	?	6.8±0.4	?	?	0.81±0.00	0.40±0.01	0.39±0.01
T. thaiorum sp. nov.													
males													
1. ZMMU A-7577 [H]	6.6	7.6	2.5	4.4	3.8	7.8	2.8	9.2	7.9	3.8	0.94	0.31	0.33
2. ZMMU A-7579	6.9	7.5	2.2	4.1	3.8	8.8	2.8	10.1	8.6	5	0.92	0.32	0.36
3. ZMMU A-7580	6.4	7.8	2.2	4.2	3.7	8	2.4	8.3	7	3.7	0.94	0.33	0.35
4. ZMMU A-7581	6.9	7.4	2	4.3	3.7	6.7	2.5	8.6	7	4.8	0.94	0.34	0.36
5. ZMMU A-7582	6.4	7.2	2	4.2	3.6	8.3	2.4	8.8	7.9	4.4	0.96	0.32	0.35
6. ZMMU A-7578	6.4	7.2	2.5	4.4	3.7	7.8	2.6	9.3	8.2	3.9	0.96	0.32	0.33
Mean±SD	6.6±0.2	7.5±0.2	2.2±0.2	4.3±0.1	3.7±0.1	7.9±0.7	2.6±0.2	9.1±0.6	7.8±0.6	4.3±0.6	0.94±0.0	0.32±0.0	0.35±0.0
Range	6.4-6.9	7.2-7.8	2-2.5	4.1-4.4	3.6-3.8	6.7-8.8	2.4-2.8	8.3-10.1	7.0-8.6	3.7-5.0	0.92-0.96	0.31-0.34	0.33-0.36

Table 4. Morphological comparisons between *Tylototriton thaiorum* sp. nov. and the 17 currently recognized species of the subgenus *Yaotriton*; ? = no data; * = requires further verification.

Species	*T. thaiorum* sp. nov.	*T. anhuiensis*	*T. asperrimus*	*T. broadoridgus*	*T. dabienicus*	*T. hainanensis*	*T. liuyangensis*	*T. lizhengchangi*
SVL (males)	59.3–68.6	59.4–74.8	55.8–63.3	61.1–72.6	70.7±4.7	55.7–57.7	64.2–82.0	?
SVL (females)	?	51.0–96.1	73.5	?	73.6±5.0	?	80.8–88.0	?
Snout in dorsal view	obtusely rounded	rounded	truncate	truncate	rounded	rounded	truncate	truncate
Snout in profile view	rounded	rounded	rounded	rounded	rounded	rounded	slightly angular	slightly angular
Head	wider than long	longer than wide	wider than long	longer than wide	longer than wide	wider than long	equally long and wide	longer than wide
Gular fold	present	present	present	absent	present	present	present	present
Rib nodules shape	knob-like, in irregular series	slightly flattened	knob-like	slightly flattened	slightly flattened	slightly flattened	slightly flattened	slightly flattened
Vertebral ridge	not segmented	not segmented	not segmented	not segmented	not segmented	not segmented	not segmented	not segmented
Ground color	dark brown	blackish	brownish black	brownish black	brown	blackish / dark brown	blackish	blackish
Orange markings on the parotoid	absent	absent	absent	absent	absent	absent	absent	present
Orange coloration of the rib nodules	absent	absent	absent	absent	absent	absent	absent	absent
Distribution	Vietnam (Nghe An)	China (Anhui)	China (Guangxi)	China (Hubei, Hunan)	China (Henan)	China (Hainan)	China (Hunan)	China (Hunan)
Sources	Our data	Qian *et al.* 2017	Fei & Ye 2016; Bernardes *et al.* 2020	Shen *et al.* 2012	Chen *et al.* 2010; Fei & Ye 2016	Fei & Ye 2016	Yang *et al.* 2014	Hou *et al.* 2012; Fei & Ye 2016

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Table 4 continued. Morphological comparisons between *Tylototriton thaiorum* sp. nov. and the 17 currently recognized species of the subgenus *Yaotriton*; ? = no data; * = requires further verification.

Species	*T. maolanensis*	*T. notialis*	*T. panhai*	*T. pasmansi*	*T. sparreboomi*	*T. vietnamensis*	*T. wenxianensis*	*T. ziegleri*
SVL (males)	76.8–85.2	56.5–60.2	?	61.57–70.69	63.20–68.71	48.3–53.6	61.9–77.3	54.4–68.3
SVL (females)	76.3–87.4	73.4–78.3	71.6	76.8	?	?	?	70.8
Snout in dorsal view	truncate	truncate	truncate	truncate	truncate	truncate	rounded	truncate
Snout in profile view	slightly angular	slightly angular	rounded	slightly angular	rounded	slightly angular	rounded	rounded
Head	longer than wide	slightly longer than wide	longer than wide	slightly longer than wide	longer than wide	longer than wide	longer than wide	wider than long
Gular fold	present	present	present	present	present	absent	absent	present
Rib nodules shape	knob-like	knob-like	knob-like	knob-like	knob-like	slightly flattened	slightly flattened	knob-like
Vertebral ridge	not segmented	not segmented	not segmented	not segmented	not segmented	not segmented	not segmented	segmented, tuberculate
Ground color	dark brown	brownish black to black	blackish	blackish	blackish	gray to tan	blackish	blackish
Orange markings on the parotoid	absent	present	present	present	absent	absent	absent	absent
Orange coloration of the rib nodules	absent	present	present	absent	absent	absent	absent	absent
Distribution	China (Guizhou)	Laos (Khammouan)	Thailand (Phetchabun, Loei, Phitsanulok), Laos (Sayabouly)	Vietnam (Son La, Thanh Hoa, Phu Tho, Hoa Binh, Lao Cai)	Vietnam (Lai Chau)	Vietnam (Bac Giang, Lang Son, Phu Tho)	China (Chongqing*, Gansu, Guizhou*, Sichuan*, Hubei*)	Vietnam (Ha Giang, Cao Bang); China (Yunnan)
Sources	Li *et al.* 2020	Stuart *et al.* 2010	Nishikawa *et al.* 2013a; Hernandez 2017	Bernardes *et al.* 2020	Bernardes *et al.* 2020	Böhme *et al.* 2005; Bernardes *et al.* 2020	Fei & Ye 2016; Wang *et al.* 2018	Nishikawa *et al.* 2013b

Discussion

The range of *Tylototriton thaiorum* sp. nov. seems to be restricted to the Pu Hoat Mountain Range located at the southernmost edge of the Northern Indochina subtropical forests ecoregion according to the terrestrial ecoregion classification of Olson *et al.* (2001). Its sister species *T. notialis* is located in the heart of the northern part of the Truong Son Mountain Range, which belongs to the Northern Annamites rain forests ecoregion (Olson *et al.* 2001). These areas differ markedly in the amount of annual rainfall, climate types, and vegetation (e.g. Pham & Phan 1993, Nguyen *et al.* 2000, Averyanov *et al.* 2003), and correspond to different biogeographic subregions of Indochina for herpetofauna (Bain & Hurley 2011, Poyarkov *et al.* 2021 *in press*). These two ecoregions are separated by the valley of the Ca River, which has been shown to be an important biogeographic barrier for vascular plants (Averyanov *et al.* 2003), insectivorous mammals (Abramov & Tran 2017), and amphibians (Bain & Hurley 2011, Poyarkov *et al.* 2021 *in press*). Since the Ca River is the largest river in the northern part of Central Vietnam, its valley may serve as a physical barrier restricting gene flow between *Tylototriton* populations. Further studies, including intensive field surveys in the Annamite part of Nghe An and Ha Tinh provinces of Vietnam, are required to clarify the role of the Ca River valley in divergence between *T. thaiorum* and *T. notialis*.

Our phylogenetic analysis confirmed the earlier results of Wang *et al.* (2018), Than Zaw *et al.* (2019), and Li *et al.* (2020), which suggest that our knowledge on the actual taxonomic diversity of *Tylototriton* still remains incomplete. Based on our mtDNA genealogy, at least two lineages of the *T. wenxianensis* species group (*Tylototriton* sp. 1 from Guizhou Province, China, and *Tylototriton* sp. 2 from Hubei Province, China) and one lineage of the *T. asperrimus* species group (*Tylototriton* sp. 3 from Guangdong Province, China) likely represent undescribed new species. Further integrative taxonomic studies are required to clarify the taxonomic status of these lineages. Moreover, our analysis demonstrated that the level of genetic differentiation between the two subspecies of *T. pasmansi* from Vietnam ($p = 4.2\%$ for ND2 gene) actually corresponds to the species level of divergence in *Tylototriton*. Bernardes *et al.* (2020) have cautiously described *T. p. pasmansi* and *T. pasmansi obsti* as subspecies, largely due to the limited sample size they could examine as well as due to the lack of clear morphological differences between these lineages. We call for additional field surveys and genetic and morphological studies which are required for a comprehensive taxonomic evaluation of these taxa.

Our description of *T. thaiorum* brings the total number of recognized *Tylototriton* species to 32. The previous record of *T. notialis* from Vietnam is now revised as *T. thaiorum* in this study; therefore the total number of *Tylototriton* species (7) known from Vietnam remains unchanged. The occurrence of *T. notialis sensu stricto* in Vietnam is nevertheless expected in the parts of the Northern Annamites adjacent to the Khammouan Province of Laos; further surveys in montane areas of Quang Binh and Ha Tinh provinces of Vietnam may lead to discovery of new populations of this species. China with 17 species and Vietnam with seven species, currently host the largest proportion of the *Tylototriton* newt diversity; four of the seven *Tylototriton* species recorded in Vietnam are endemic to the country. The Vietnamese populations of *Tylototriton* are under constantly growing threat from many pressures such as habitat loss, water pollution, and poaching for the pet trade (Rowley *et al.* 2016, Bernardes *et al.* 2017, 2020, Grismer *et al.* 2018). Hernandez *et al.* (2018) suggested that niche conservatism along with niche differentiation shape the patterns of macro-ecological niche differentiation in *Tylototriton*. Most species are restricted to cooler and more humid high-elevation forested habitats, while lowland and open areas are unsuitable for the *Tylototriton* newts (Hernandez *et al.* 2018). Hence, the unsuitable micro-climatic conditions in the lowland regions of Indochina might limit dispersal and gene flow in *Tylototriton*. Further intensive fieldwork efforts in different isolated mountain massifs of northern Indochina, including central and northern Vietnam and the adjacent parts of Laos are required to obtain a more comprehensive understanding of *Tylototriton* diversity and distribution in the region.

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