

# A new cryptic species of *Tylototriton* (Amphibia, Caudata, Salamandridae) from mysterious mountain lakes in Manipur, north-eastern India

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# Abstract

An integrative taxonomic analysis combining molecular and morphological lines of evidence revealed a new cryptic species in the *Tylototriton verrucosus* species group from Manipur, north-eastern India. The new species was previously confused with *T. hima-layanus* and *T. verrucosus*. *Tylototriton zaimeng* **sp. nov.** can be distinguished from its congeners by medium body size, head massive and wide with rounded snout and very wide and protruding supratemporal bony ridges and a well-developed sagittal ridge, short limbs not overlapping when adpressed along body, wide and not segmented vertebral ridge distinct, 13–14 pairs of rib nodules, brown colouration with dull orange to yellowish-brown markings on head, vertebral ridge, rib nodules, palms, soles, vent and ventral tail ridge and by vomerine teeth organised in two distinctly curved bell-shaped series. Phylogenetic analysis of the ND2 and 16S rRNA mtDNA genes confirmed the placement of the new species to the Clade I of the subgenus *Tylototriton* and suggested it is a sister species of *T. panwaensis* and *T. houi* (*p*-distance 3.0% in ND2 gene). The range of the new species is restricted to the Khongtheng Mountain Range and is isolated from the range of *T. panwaensis* and *T. houi* in northern Myanmar and southern China, respectively. We suggest the new species to be considered as Vulnerable (VU) in the IUCN Red List.

# Key Words

16S rRNA, endemism, Khongtheng Mountain Range, mtDNA genealogy, ND2, taxonomy, Tylototriton zaimeng sp. nov.

# Introduction

The salamandrid genus *Tylototriton* Anderson, 1871 (colloquially known as Crocodile newts) represents an ancient salamander lineage which currently includes 39 recognised species, inhabiting montane forest areas throughout the Asian monsoon climate zone from the southern and eastern Himalaya, southern and central China, to the northern part of Indochina (Poyarkov et

al. 2021a; Dufresnes and Hernandez 2022; Frost et al. 2023). The genus has been traditionally subdivided into the two subgenera: the subgenus *Tylototriton* Anderson, 1871 is characterised by the presence of bright orange or red markings on the head, dorsum, tail or sides of the body, while the subgenus *Yaotriton* Dubois & Raffaëlli, 2009 is characterised by generally much darker almost blackish colouration (Yang et al. 2014; Wang et al. 2018; Li et al. 2020; Poyarkov et al. 2021b; Luy et al. 2021;

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Dufresnes and Hernandez 2022). Many new species of crocodile newts have been described recently, based on a combination of morphological and molecular investigations (e.g. Nishikawa et al. (2013, 2014); Le et al. (2015); Phimmachak et al. (2015); Khatiwada et al. (2015); Grismer et al. (2018, 2019); Zaw et al. (2019); Bernardes et al. (2020); Li et al. (2020); Pomchote et al. (2020a, 2021); Poyarkov et al. (2021b); Dufresnes and Hernandez (2022); Phung et al. (2023)).

In India, salamanders of the genus Tylototriton were reported from north-eastern part of the country and were traditionally identified as Tylotriton verrucosus Anderson, 1871 (Seglie et al. 2003; Ahmed et al. 2009; Mathew and Sen 2010; Singh and Devi 2011). Salamanders of the genus Tylototriton were recorded from the eastern Himalayas (from Nepal to West Bengal, Sikkim, Bhutan and the westernmost Arunachal Pradesh), but were also recorded from montane areas of Meghalaya (Das 1984) and Manipur (Selim 2001; Devi and Shamungou 2006). In the latest reviews on distribution of Tylototriton salamanders (Hernandez 2016; Hernandez et al. 2018), these populations were all tentatively assigned to T. himalayanus Khatiwada et al., 2015, a species described from the Himalaya in eastern Nepal (Khatiwada et al. 2015). Hernandez et al. (2018) noted that the region between the eastern Himalayas and the Irrawaddy River valley is still unclear and might represent a contact area between the two species of crocodile newts, T. himalayanus and T. verrucosus. They suggested that this region may be home to some cryptic, undescribed taxa. However, to date, no integrative analysis of the taxonomic status of Manipur Tylototriton populations has been conducted and these populations were not included in the most recent phylogenetic assessment of the genus (Dufresnes and Hernandez 2022).

In the present study, we apply an integrative taxonomic approach comparing the results of morphological analyses with those of mitochondrial DNA (mtDNA) genealogy, based on the ND2 and 16S rRNA genes to assess the taxonomic status of *Tylototriton* sp. populations from highland montane forests of Manipur, north-eastern India. Our examination of the morphological differentiation of the newly-collected material from Manipur and northern Myanmar resulted in the discovery of stable character state differences in colouration and external morphology between the two populations which are concordant with the results of the molecular phylogenetic analyses. Herein, we describe the population of *Tylototriton* sp. from Manipur State of India as a new, previously overlooked, cryptic species of *Tylototriton*.

### Materials and methods

#### Sample collection

Specimens of *Tylototriton* sp. were collected from three localities within Manipur State of north-eastern India during several field surveys in 2022. Specimens of *Tylototriton* sp. were collected by hand in lakes or 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 euthanised by 20% benzocaine and tissue samples (liver) for genetic analysis were taken and stored in 96% ethanol prior to preservation. Specimens were subsequently fixed in 4% formalin, transferred to 70% ethanol and deposited in the herpetological collection of the Departmental Museum of Zoology, Mizoram University (**MZMU**) in Aizawl, India. Additional specimens examined are listed in Appendix 1.

#### 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 Poyarkov et al. (2021b) and included the following 23 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 (fore-limb length); (11) HLL (hind-limb length); (12) VTW (vomerine tooth series width): greatest width of vomerine tooth series; (13) LJL (lower jaw length from tip of lower jaw to articulation of upper and lower jaws); (14) SL (snout length from tip of snout to anterior tip of upper eyelid); (15) IOD (minimum interorbital distance); (16) UEW (maximum upper eyelid width); (17) UEL (upper eyelid length, distance between anterior and posterior angles); (18) OL (orbit length); (19) BTAW (basal tail width at level of anterior tip of cloaca); (20) MTAW (tail width at mid-level of tail); (21) MXTAH (maximum tail height); (22) MTAH (tail height at mid-level of tail); and (23) ON (orbitonarial distance). For holotype description, we additionally examined the following 12 morphometric characters following Poyarkov et al. (2012, 2021b): (24) ICD (intercanthal distance); (25) CW (chest width); (26) NSD (nostril-snout distance); (27) 1FL (first finger length from base to tip); (28) 2FL (second finger length from base to tip); (29) **3FL** (third finger length from base to tip); (30) 4FL (fourth finger length from base to tip); (31) 1TL (first toe length from base to tip); (32) 2TL (second toe length from base to tip); (33) **3TL** (third toe length from base to tip); (34) **4TL** (fourth toe length from base to tip); (35) 5TL (fifth toe length from base to tip). We also examined the following meristic characters: (36) DLWN (dorsolateral wart number, the number of rib nodules on

right/left sides of the dorsum); (37) UJTN (number of teeth on the upper jaw); (38) LJTN (number of teeth on the lower jaw); (39) VTN (number of teeth on vomer).

An analysis of variance (ANOVA) was performed to ascertain if statistically significant mean differences amongst morphomeristic characters (p < 0.05) existed amongst the discrete populations delimited in the phylogenetic analyses. ANOVAs having a p-value less than 0.05, indicating that statistical differences existed, were subjected to a Tukey HSD test to ascertain which population pairs differed significantly (p < 0.05) from each other. We used the Principal Component Analysis (PCA) to determine if populations from different localities occupied unique positions in morphospace, as well as the degree to which their variation coincided with potential species boundaries as predicted by the molecular phylogeny and univariate analyses. Characters used in the PCA included continuous mensural data. In order to normalise the PCA data distribution and to transform meristic and mensural data into comparable units for analysis, we natural log-transformed all PCA data prior to analysis and scaled it to their standard deviation. To exclude possible overweighting effects, when we found a high correlation between certain pairs of characters, we omitted one of them from the analyses. Statistical analyses were carried out using Statistica 8.0 (Version 8.0; StatSoft, Tulsa, OK, USA).

The diagnosis of the genus *Tylototriton* and morphological characters for comparison were taken from original descriptions and taxonomic reviews of the genus: Anderson (1871); Unterstein (1930); Fang and Chang (1932); Liu (1950); Fei et al. (1984); Nussbaum et al. (1995); Böhme et al. (2005); Chen et al. (2010); Stuart et al. (2010); Hou et al. (2012); Shen et al. (2012); Nishikawa et al. (2013; 2014); Yang et al. (2014); Le et al. (2015); Phimmachak et al. (2015); Hernandez (2016); Khatiwada et al. (2016); Qian et al. (2017); Grismer et al. (2018, 2019); Than Zaw et al. (2019, 2020); Hernandez and Pomchote (2020); Onishi et al. (2020); Pomchote et al. (2020a, 2021); Dufresnes and Hernandez (2022); and Phung et al. (2023).

#### Laboratory methods

Total genomic DNA was extracted from 95% ethanol-preserved liver 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 informative in studies of *Tylototriton* phylogeny and taxonomy (Wang et al. 2018; Than Zaw et al. 2019; Poyarkov et al. 2021b). We used the 16L-1 (forward) (5'-CTGACCGTGCAAA GGTAGCG-TAATCACT-3') and 16H-1 (reverse) (5'-CTCCGG TCT-GAACTCAGATCACGTAGG-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'-TTAAAGTGTCTGGGTTGCAT-TCAG-3') primers of Wang et al. (2018). Polymerase chain reaction (PCR) conditions followed Poyarkovet al. (2021b). PCR was performed in 20 µl using 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl<sub>2</sub>, Taq PCR buffer (10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.1 mmol/l MgCl, 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 visualised by agarose gel electrophoresis in the presence of ethidium bromide and consequently purified using 2 µl from a 1:4 dilution of ExoSAP-IT (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 Barcode Bioscience, Bangalore (India). The newly-obtained sequences from seven specimens of Tylototriton sp. from Manipur were aligned and deposited in GenBank under the accession numbers OQ996133-OQ996139 and OR039162-OR039168 (Table 1). Sequences of 39 other Tylototriton species used for comparisons along with two sequences of Echinotriton Nussbaum & Brodie, 1982 spp. which were used as outgroup taxa were obtained from GenBank (see Table 1).

#### Phylogenetic analyses

Sequences of partial fragments of ND2 and 16S rRNA mtDNA for 53 Salamandridae specimens, including 51 representatives of Tylototriton (representing 39 taxa) and two sequences of outgroup members of Salamandridae Echinotriton raffaellii Hernandez & Dufresnes, 2022 and Echinotriton chinhaiensis (Chang, 1932) were included in the final alignment with a total length of up to 1665 bp. For comparison purposes, we selectively picked one individual per each nominal species/lineage of Tylototriton; therefore, the resulting phylogenetic hypothesis is not representative of the entire variation of Tylototriton at 16S rRNA and ND2 mtDNA genes. Information on voucher specimens and GenBank accession numbers used in phylogenetic analyses are summarised in Table 1. Nucleotide sequences were initially aligned in MAFFT v.6 (Katohet 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 and Crandall 1998). According to the Akaike Information Criterion (AIC), the HKY+G model was selected for the ND2 gene as the best fit for the first and second codon partitions

No.	Species name	Voucher number	Locality	16S rRNA	ND2
	Ingroup:				
1	T. anguliceps	TBU PAE671	Thuan Chau, Son La, Vietnam	_	LC017833
2	T. anhuiensis	CIB 08042905-2	Yuexi Co. Anhui, China	KY800587	KY800853
3	T. asperrimus	CIB 70063	Longsheng Co., Guangxi, China	KY800549	KC147816
4	T. broadoridgus	CIB 200084	Sangzhi Co., Hunan, China	KY800570	KY800837
5	T. dabienicus	HNNU 1004-015	Shangcheng Co., Anhui, China	KY800607	KC147811
6	T. daloushanensis	CIB WG200600019	Suiyang Co., Zunyi, Guizhou, China	KY800544	KY800817
7	T. hainanensis	CIB 20081048	Mt. Diaoluo, Hainan, China	KY800553	KC147817
8	T. gaowangjienensis	JWS20210100	Guzhang Co., Hunan, China	ON764431	ON764431
9	T. himalayanus	CIB 201406246	Mai Pokhari, Illam, Mechi, Nepal (1)	KY800590	KT765173
10	T. houi	MZL46960	Yulong, Lijiang, Yunnan, China (13)	ON008211	_
11	T. zaimeng sp. nov.	MZMU-2947	Zaimeng Lake, Kangpokpi Dist., Manipur, India (2)	OQ996133	OR039162
12	T. zaimeng sp. nov.	MZMU-2948	Zaimeng Lake, Kangpokpi Dist., Manipur, India (2)	OQ996133	OR039162
13	T. zaimeng sp. nov.	MZMU-3035	Phungyar, Kamjong Dist., Manipur, India (4)	OQ996134	OR039163
14	T. zaimeng sp. nov.	MZMU-3036	Phungyar, Kamjong Dist., Manipur, India (4)	OQ996135	OR039164
15	T. zaimeng sp. nov.	MZMU-3037	Chingjaroi Ngachaphung, Ukhrul Dist., Manipur, India (6)	OQ996136	OR039165
16	T. zaimeng sp. nov.	MZMU-3038	Chingjaroi Ngachaphung, Ukhrul Dist., Manipur, India (6)	OQ996137	OR039166
17	T. zaimeng sp. nov.	MZMU-3041	Chingjaroi Ngachaphung, Ukhrul Dist., Manipur, India (6)	OQ996138	OR039167
18	T. kachinorum	ZMMU A5953	In Gyi Taung Mt., Indawgyi, Kachin, Myanmar (8)	MK095618	MK097273
19	T. kweichowensis	CIB Wg20080818014	Bijie City, Guizhou, China	KY800551	KY800823
20	T. liuyangensis	CSUFT 20100108	Liuyang City, Hunan, China	KY800606	KJ205598
21	T. lizhenchangi	KUHE 42316	Yizhang Co., Hunan, China	KY800621	KY800881
22	T. maolanensis	CIB Wg20090730001	Libo Co., Guizhou, China	KY800575	KY800842
23	T. ngarsuensis	LSUHC 13763	Ngar Su, Taunggyi Dist., Shan, Myanmar (14)	_	MH836585
24	T. notialis	FMNH 271120	Boualapha Dist., Khammouan, Laos	_	HM462061
25	T. panhai	PH019	Phu Hin Rong Kla NP, Phitsanulok, Thailand	_	AB830735
26	T. panwaensis	CAS 245418	Panwa, Myitkyina Dist., Kachin, Myanmar (10)	_	KT304279
27	T. obsti	IEBR 4471	Xuan Nha NR, Van Ho, Son La, Vietnam	_	MT210168
28	T. pasmansi	IEBR 4466	Phu Canh NR, Da Bac, Hoa Binh, Vietnam	_	MT201166
29	T. phukhaensis	CUMZ A-7717	Doi Phu Kha NP, Nan, Thailand	_	MN912573
30	T. podichthys	IEBR A.2014.1	Xam Neua, Huaphanh, Laos	_	LC017835
31	T. pseudoverrucosus	CIB WCG2012012	Ningnan Co., Liangshanyizu, Sichuan, China	KY800599	KY800860
32	T. pulcherrimus	CIB TY040	Lüchun Co., Yunnan, China	KY800626	KY800890
33	T. shanjing	KIZ 201306081	Yongde Co., Yunnan, China	KY800593	KY800856
34	T. shanorum (lineage 1)	CAS 230940	Taunggyi Township, Shan, Myanmar (16)	_	AB922823
35	T. shanorum (lineage 2)	KUHE 42348	Pinluang Township, Shan, Myanmar (17)	_	AB769544
36	T. sini	CIB XZ20091201	Xinyi City, Guangdong, China	KY800616	KY800876
37	T. sparreboomi	IEBR 4476	Sin Ho, Lai Chau, Vietnam	_	MT210162
38	T. taliangensis	CIB GG200110183	Shimian Co., Yan'an City, Sichuan, China	KY800559	KC147819
39	T. thaiorum	ZMMU A-7577	Pu Hoat NR, Nghe An, Vietnam	MW883482	MW883478
40	T. tongziensis	CIB WH10001	Wufeng Co., Hubei, China	KY800600	KY800863
41	T. uyenoi	KUHE 19037	Doi Inthanon, Chiang Mai, Thailand	_	AB830730
42	T. verrucosus	CIB TSHS1	Longchuan Co., Dehong, Yunnan, China (12)	KY800581	KY800847
43	T. vietnamensis	IEBR A.3674	Tay Yen Tu, Bac Giang, Vietnam	KY800614	KY800874
44	T. wenxianensis (lineage 1)	CIB 20090527	Wenxian Co., Gansu, China	KY800579	KC147813
45	T. wenxianensis (lineage 2)	CIB 20070638	Qingchuan Co., Sichuan, China	KY800543	KY800816
46	T. wenxianensis (lineage 3)	CIB 20090601	Wangcang Co., Sichuan, China	KY800571	KY800838
47	T. wenxianensis (lineage 4)	CIB 20080002	Yunyang Co., Chongqing, China	KY800540	KY800813
48	T. wenxianensis (lineage 5)	CIB 20080003	Yunyang Co., Chongqing, China	KY800541	KY800814
49	T. yangi	KUHE 42282	Pingbian Co., Yunnan, China	KY800624	KY800887
50	T. ziegleri	KUHE 55078	Quan Ba, Ha Giang, Vietnam	_	AB769541
51	T. umphangensis	CUMZ-A-8243	Umphang WS., Tak, Thailand	_	OK092618
	Outgroups:				
52	Echinotriton chinhaiensis	CIB ZHJY1	Zhenhai Co., Zhejiang, China	KY800627	KY800891
53	Echinotriton raffaellii	MVZ 232187	Tokunoshima, Kagoshima, Japan	EU880314	EU880314

**Table 1.** Sequences and voucher specimens of *Tylototriton* and outgroup taxa used in this study. For institutional abbreviations, see below. Numbers in brackets after the locality name correspond to those shown in Fig. 1.

and the J2+G model was considered the best fit for the third codon partition; 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 analysis was

conducted in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Metropolis coupled Markov Chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for 20,000,000 generations and sampled every 2,000 generations. Five independent MCMCMC runs were performed, the MCMC simulations ran for 100,000,000 generations, were sampled every 10,000 generations and the first 10% of the trees from each run 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 v.1.6 (Rambaut et al. 2014). Confidence in tree topology was tested by posterior probability (BI/PP) for the BI trees (Huelsenbeck and Ronquist 2001). Nodes with PP values over 0.95 were a priori regarded as sufficiently resolved, those between 0.90 and 0.95 were regarded as tendencies and values below 0.90 were considered as not 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).

## Results

#### Sequence variation

The final alignment of the ND2 gene contained 1157 aligned nucleotides, of which 716 sites were conserved and 441 sites were variable, of which 292 were parsimony-informative. The transition-transversion bias (R) was estimated at 4.45. Nucleotide frequencies were 37.49% (A), 23.82% (T), 28.10% (C) and 10.59% (G). The final alignment of the 16S rRNA gene contained 508 aligned characters, of which, 422 sites were conserved and 84 sites were variable, of which 51 were suggested as parsimony-informative. The transition-transversion bias (R) was estimated at 6.16. Nucleotide frequencies were 36.87% (A), 24.85% (T), 20.27% (C) and 18.01% (G) (all data given for ingroup only).

#### Molecular phylogenetic analysis

BI and ML phylogenetic analyses resulted in almost identical topologies (Fig. 2). The topology of the matrilineal genealogy was largely consistent with the phylogeny of *Tylototriton* presented by Wang et al. (2018), Than Zaw et al. (2019), Poyarkov et al. (2021b), Luy et al. (2021) and Dufresnes and Hernandez (2022) and recovered five main clades grouped into two major reciprocally monophyletic groups, corresponding to the subgenera *Tylototriton* (clades 1–2; Fig. 2) and *Yaotriton* (clades 3–4; Fig. 2). Monophyly of the subgenus *Tylototriton* received strong support both in ML and BI analyses (100/1.0; Fig. 2); this group included two strongly supported clades:

- 1) Clade 1 joined the members of T. verrucosus species group from India, Nepal, Myanmar, China and northern Indochina (100/1.0) and included 16 nominal species of Tylototriton: T. anguliceps Le et al., 2015, T. himalayanus, T. houi Hernandez & Dufresnes, 2022, T. kachinorum Zaw et al., 2019, T. kweichowensis Fang & Chang, 1932,, T. ngarsuensis Grismer et al., 2018, T. panwaensis Grismer et al., 2019, T. phukhaensis Pomchote et al., 2020, T. podichthys Phimmachak et al., 2015, T. pulcherrimus Hou et al., 2012, T. shanjing Nussbaum et al., 1995, T. shanorum Nishikawa et al., 2014, T. umphangensis Pomchote et al., 2021, T. uyenoi Nishikawa et al., 2013, T. verrucosus Anderson, 1871 and T. yangi Hou et al., 2012 and a clade, including seven specimens of Tylototriton sp. from Manipur, India.
- Clade 2, which herein is referred to as *T. talian-gensis* species group (99/1.0), included two species from western China, namely *T. pseudoverrucosus* Hou et al., 2012 and *T. taliangensis* Liu, 1950.

Monophyly of the subgenus *Yaotriton* was poorly supported in ML analysis, but had significant support in BI analysis (86/1.0, hereafter node values given for ML UFBS/BI PP, respectively; Fig. 2). Subgenus *Yaotriton* included three highly-supported main clades with essentially unresolved phylogenetic relationships amongst them:

- 3) Clade 3 joined the members of *T. wenxianensis* is species group from China (98/1.0) and included ten species of *Tylototriton: T. anhuiensis* Qian et al., 2017, *T. broadoridgus* Shen, Jiang & Mo, *T. dabienicus* Chen et al., 2010, *T. daloushanensis* Zhou et al., 2022, *T. gaowangjienensis* Wang et al., 2022, *T. liuyangensis* Yang et al., 2014, *T. lizhenchangi* Hou et al., 2012, *T. maolanensis* Li et al., 2020, *T. tongziensis* Li et al., 2022 and *T. wenxianensis* Fei et al., 1984.
- 4) Clade 4 joined the members of *T. asperrimus* species group from China, northern Vietnam and Laos (100/1.0) and included nine nominal *Tylototriton* species: *T. asperrimus* Unterstein, 1930, *T. hainanensis* Fei et al., 1984, *T. notialis* Stuart et al., 2010, *T. pasmansi* Bernardes et al., 2020, *T. obsti* Bernardes et al., 2020, *T. sini* Lyu et al., 2021, *T. sparreboomi* Bernardes et al., 2020, *T. thaiorum* Poyarkov et al., 2021 and *T. ziegleri* Nishikawa et al., 2013.
- 5) Clade 5, which herein is referred to as *T. vietnam-ensis* species group (92/1.0), included two peculiar species of *Tylototriton* from northern Vietnam and north-eastern Thailand and adjacent Laos: *T. panhai* Nishikawa et al., 2013 and *T. vietnamensis* Böhme et al., 2005.

The population of *Tylototriton* sp. from Manipur, India, belongs to clade 1 (Fig. 2), where it is reconstructed as a member of a clade also including *T. panwaensis* from Myanmar and *T. houi* from Yunnan, China. Although monophyly of this clade had strong node support (98/1.0; Fig. 2), genealogical relationships amongst the three included lineages appear to be insufficiently resolved, with *T. houi* and *T. panwaensis* forming a clade which had strong support in BI-analysis, but was poorly supported in ML-analysis (76/1.0; Fig. 2).

#### **Genetic distances**

The interspecific uncorrected genetic *p*-distances between the sequences of ND2 mtDNA gene of *Tylototriton* sp. from Manipur, India and other members of *T. verrucosus* species group varied from 3.0% (between *Tylototriton* sp. and its sister species *T. panwaensis*) to 8.6% (between *Tylototriton* sp. and *T. uyenoi*) (see Table 2). Genetic *p*-distances in the 16S rRNA gene were much lower and varied from 0.7% (between *Tylototriton* sp. and its sister species *T. houi*) to 3.3% (between *Tylototriton* sp. and *T. himalayanus*) (see Table 2). There was no intraspecific variation in ND2 and 16S rRNA gene sequences recorded for *Tylototriton* sp. from Manipur (Table 2).

#### Morphological analysis

Our phylogenetic analyses have unambiguously placed the Manipur population of Tylototriton sp. in the T. verrucosus species group as a sister lineage to a clade including T. panwaensis from Kachin State in northern Myanmar and T. houi from northern Yunnan, China. The detailed morphometric data are not available for T. houi which was described, based on a single partially decomposed subadult specimen (Dufresnes and Hernandez 2022); however, this species can be unambiguously diagnosed from the Manipur population of Tylototriton sp. by a different colouration pattern (black background pattern with bright orange-red markings on parotoids, vertebral ridge, rib nodules, vent, chest, light mid-ventral line, whole limbs and tail; see "Comparisons" section below) and is geographically isolated from north-eastern India.



**Figure 1.** Distribution of *Tylototriton* ssp. in India and adjacent areas and sampling localities examined in this study. Colours of icons correspond to species marked in Fig. 2; a dot in a centre of an icon indicates the type locality. For locality info, see Table 1. *Tylototriton himalayanus*: 1 – Mai Pokhari, Illam, Mechi, Nepal; *T. zaimeng* sp. nov.: 2 – Zaimeng Lake, Koubru Forest Division, Kangpokpi, Manipur, India; 3 – Zeliad, Manipur, India; 4 – Phungyar, Kamjong, Manipur, India; 5 – Shiroy Lily Hills, Manipur, India; 6 – Chingjaroi Ngachaphung, Ukhrul, Manipur, India; *Tylototriton* sp.: 7 – Lahe, Khandi, Sagaing, Myanmar; *T. kachinorum*: 8 – In Gyi Taung Mt., Indawgyi, Kachin, Myanmar; *T. panwaensis*: 9 – Sadung, Myitkyina, Kachin, Myanmar; 10 – Panwa, Myitkyina, Kachin, Myanmar; *T. verrucosus*: 11 – Sinlum, Momauk, Banmaw, Kachin, Myanmar; 12 – Nantin, Momien and Hotha valleys, Yunnan, China; *T. houi*: 13 – Yulong, Lijiang, Yunnan, China; *T. ngarsuensis*: 14 – Ngar Su, Taunggyi, Shan, Myanmar; *T. shanorum*: 15 – Pindaya, Heho and Aungpan, Kalaw, Shan, Myanmar; 16 – Taunggyi, Shan, Myanmar; 17 – Pinlaung, Shan, Myanmar.



**Figure 2.** Maximum Likelihood inference consensus tree of genus *Tylototriton* derived from analysis of 1,157 bp ND2 and 508 bp 16S rRNA gene fragments. For voucher specimen information, geographic localities and GenBank accession numbers, see Table 1. Distribution of the species of *Tylototriton* highlighted in colour is shown in Fig. 1. Numbers at tree nodes correspond to ML UFBS and BI PP support values, respectively. Photograph by Andrey M. Bragin.

						-					10			- 12			4.6	
	Taxon	1	2	3	4	5	6	7	8	9	10	Ш	12	13	14	15	16	17
1	T. zaimeng sp. nov.	0.0\0.0	0.9	0.7	_	1.3	1.3	1.3	_	1.8	2.9	_	1.3	2.2	-	3.3	3.1	2.4
2	T. panwaensis	3.0	-	0.7	-	0.9	1.3	1.3	-	1.8	2.9	-	1.8	2.7	-	3.6	3.3	2.5
3	T. houi	_	_	-	_	1.1	1.1	1.1	_	1.5	2.6	_	1.5	2.4	_	3.3	3.1	2.6
4	T. podichthys	4.4	3.3	-	_	_	_	_	_	_	_	_	_	_	_	_	_	_
5	T. verrucosus	3.3	2.5	_	3.6	_	0.9	1.8	_	2.2	2.9	_	2.2	3.5	_	4.0	3.7	2.9
6	T. shanjing	3.8	3.3	_	4.1	1.3	_	0.9	_	1.8	2.9	_	1.8	3.5	_	4.0	3.7	3.3
7	T. pulcherrimus	3.9	2.7	_	3.2	2.2	2.9	_	_	1.8	2.9	_	1.8	3.5	_	4.0	3.7	3.3
8	T. phukhaensis	6.5	5.6	_	5.8	4.9	5.2	4.3	_	_	_	_	_	_	_	_	_	_
9	T. anguliceps	5.9	4.9	_	5.7	4.8	5.1	4.4	4.6	_	2.4	_	1.3	3.1	_	3.5	3.3	2.9
10	T. uyenoi	8.6	7.6	_	8.1	7.2	7.3	6.9	7.0	7.7	_	_	2.4	4.6	_	4.6	4.8	4.4
11	T. umphangensis	6.5	5.5	_	7.0	5.0	5.1	5.6	6.0	5.4	5.1	_	_	_	_	_	_	_
12	T. yangi	5.3	4.0	_	5.0	3.9	4.6	3.7	5.5	4.5	7.8	5.6	_	2.2	_	2.4	3.1	2.4
13	T. shanorum	7.3	6.3	_	7.2	6.2	6.5	6.3	7.7	7.2	9.0	8.1	6.6	0.6\0.1	_	1.5	1.8	3.1
14	T. ngarsuensis	6.8	6.3	_	7.2	6.5	6.8	6.5	8.3	7.2	8.5	8.4	6.4	1.1	_	_	_	_
15	T. himalayanus	7.6	6.6	_	6.8	6.4	6.1	6.3	7.1	7.3	8.7	8.0	6.6	5.1	5.9	_	2.0	3.7
16	T. kachinorum	7.3	6.4	_	8.0	7.3	8.0	7.7	8.6	8.6	11.3	8.9	8.2	7.4	7.7	5.3	_	2.6
17	T. kweichowensis	6.5	5.7	_	5.8	5.6	5.9	5.3	6.2	6.4	7.9	7.8	6.2	5.9	6.4	5.4	6.6	_

**Table 2.** Uncorrected *p*-distance (percentage) between the sequences of 1157 bp fragment of ND2 gene (below the diagonal) and 508 bp fragment of 16S rRNA gene (above the diagonal) of *Tylototriton verrucosus* species group members included in the phylogenetic analyses. Intraspecific genetic distances are shown on the diagonal.

Tylototriton panwaensis, a species recently described from Kachin State of northern Myanmar (Grismer et al. 2019), superficially more closely resembles the Manipur Tylototriton sp. population (Fig. 3A). We have compared external morphology of *Tylototriton* sp. from Manipur with a series of five adult T. panwaensis males from Sadung, Kachin, Myanmar (ZMMU NAP-09477, NAP-09479, NAP-09482, NAP-09484-09485; measurements taken by one of us (AMB), species identification confirmed by Than Zaw et al. (2020)). With respect to the SVL, our analysis revealed that males from Manipur have significantly smaller body size (65.5  $\pm$  2.4 mm, 61.4–67.5 mm) than T. panwaensis (72.9  $\pm$ 4.3 mm, 68.9-79.3 mm). For SVL-standardised characters (%SVL), the males from Manipur population had significantly larger RHW, RMXHW RVL, RFLL, RHLL, RLJL, RSL, RIOD, RBTAW, RMXTAH and RON, but, in contrast, a significantly smaller RUEW, RUEL, ROL and DLWN than T. panwaensis (Table 3). The overall morphological differences were examined using PCA for the Manipur population of Tylototriton sp. and T. panwaensis. The first two principal components (PCs) explained 66.38% of the total variation in the dataset. Despite combining the sexes for the Manipur population, the two-dimensional PC1 vs. PC2 plot showed that the Manipur Tylototriton sp. population was completely separated from T. panwaensis and the two species occupy completely separate positions in morphospace (Fig. 3B). Morphological differences between the two populations were also present in the shape of the head, dorsolateral bony ridges, sagittal ridge, vertebral ridge, number of rib nodules, texture of skin and colouration (see "Comparisons" section below, Fig. 3A).

Finally, all specimens of *Tylototriton* sp. from Manipur examined had a peculiar shape of the vomerine tooth series, which was until now, to the best of our knowledge, not yet reported for any other *Tylototriton* species.

In Manipur population of *Tylototriton* sp., vomerine teeth were arranged in two strongly-curved series, touching choanae, which start to widen in the anterior one third of series length, extends as two almost parallel lines in the middle of their length and, finally, widen again in the posterior one third of their length; thus, the two series form a curved shape somewhat resembling a bell or a bottle (Fig. 4A). In contrast to this, in all other *Tylototriton* species for which the shape of vomerine tooth series was reported, it is described as an inverted V-shape and it seems that the branches of the series are much less curved. This is also true in the case of *T. panwaensis*, where the vomerine teeth in five male specimens examined had a simple inverted V-shape with the anterior portion of the branches forming almost straight lines (Fig. 4B).

## Systematic account

Despite the significant differences amongst Tylototriton sp. from Manipur, T. panwaensis and T. houi in external morphology, vomerine teeth arrangement and colouration, the genetic differentiation between these populations is not deep. However, the observed degree of pairwise divergence in ND2 mtDNA gene between these populations (p = 3.0%) is comparable or greater than the level of genetic divergence observed between many recognised species of Tylototriton, including members of the subgenus Tylototriton: T. verrucosus and T. shanjing (1.3%, though the latter was recently suggested as a subspecies or an ecological morph of T. verrucosus, see Dufresnes and Hernandez (2022)); T. verrucosus and T. pulcherrimus (2.2%); T. verrucosus and T. panwaensis (2.5%); T. pulcherrimus and T. panwaensis (2.7%); T. pulcherrimus and T. shanjing (2.9%); T. taliangensis and T. pseudoverrucosus (2.4%); and T. ngarsuensis and T. shanorum (1.1%) (see Table 2). This and the phylogeny

raise the question as to whether or not the Manipur population of *Tylototriton* sp. may warrant taxonomic recognition as an independent taxon, as an integrative taxonomic approach might reveal.

In this study, based on newly-collected material of *Tylototriton* sp. from Manipur, north-eastern India, we provide a detailed examination of the external morphology and colouration of this population and report on a number of diagnostically important characters readily distinguishing this population of *Tylototriton* from all other congeners (summarised in "Comparisons" section below). Moreover, the presently known range of *Tylototriton* sp. is restricted to montane forests of Khongtheng Mountain Range in Manipur and is apparently isolated from the distribution of *Tylototriton* in Himalaya and north-eastern Myanmar (see Fig. 1). These arguments together support our hypothesis that the Manipur population of *Tylototriton* sp. represents a previously unknown species, which we formally describe below.

#### Family Salamandridae Goldfuss, 1820 Subfamily Pleurodelinae Tschudi, 1838 Genus *Tylototriton* Anderson, 1871 Subgenus *Tylototriton* Anderson, 1871

#### Tylototriton zaimeng sp. nov.

https://zoobank.org/2A07583B-5CEF-42DD-99E4-38F366575304 Tables 3, 4; Figs 2–9 Proposed English name: Zaimeng Lake Crocodile Newt

**Type material.** *Holotype*. MZMU3041, an adult female from a swamp on forest clearing surrounded by montane evergreen tropical forest in Chingjaroi Ngachaphung, Ukhrul District, Manipur State, north-eastern India (coordinates 25.385°N, 94.458°E; elevation 1,630 m a.s.l.; datum = WGS84), collected on 20 November 2022 at 18:00 h by Ht. Decemson.

Paratypes. MZMU3035-3036, two adult males from a forest lake in Phungyar (environs of Tangkhul Hungdung), Kamjong District, Manipur State, north-eastern India (coordinates 24.811°N, 94.245°E; elevation 1,184 m a.s.l.), collected in July 2022 by Ht. Decemson and Shamungou; MZMU-2942-2947, six adult females from the Zaimeng Lake, Koubru Forest Division, environs of Chawangkining Village, Kangpokpi District, Manipur State, North-eastern India (coordinates 25.238°N, 93.944°E; elevation 2,212 m a.s.l.), collected on 18 July 2022 at 18:00 h by Ht. Decemson; MZMU-2948-2950, three adult males from the Zaimeng Lake, Koubru Forest Division, environs of Chawangkining Village, Kangpokpi District, Manipur State, north-eastern India (coordinates 25.238°N, 93.944°E; elevation 2,212 m a.s.l.; datum = WGS84), collected on 18 July 2022 at 18:00 h by Ht.Decemson.

**Referred specimens.** MZMU-3037–3040, four larvae premetamorphic stage 45 (Bernardes et al. 2017) from the same locality and with the same collection information as the holotype.

Diagnosis. The new species is assigned to the genus Tylototriton by having the following combination of morphological attributes: (1) the presence of dorsal granules, (2) dorsolateral bony ridges on the head, (3) the presence of dorsolateral series of rib nodules (knob-like warts); and (4) the absence of a quadrate spine and molecular data (see Fig. 2). Tylototriton zaimeng sp. nov. is distinguished from all other congeners by a combination of the following morphological attributes: (1) medium body size, adult SVL 61.4-67.5 mm in males, 61.6-68.7 mm in females; (2) tail comparatively short, subequal or slightly longer than body in both sexes, lacking lateral grooves; (3) skin roughly granulated; (4) head massive and wide, relative maximal head width comprising 24.3-27.9% of SVL; (5) snout rounded in dorsal view; (6) supratemporal bony ridges on head very wide, protruding, with rough surface, beginning at the snout; (7) sagittal ridge on head well-distinct, very wide and protruding; (8) limbs comparatively short, tips of fore-limb and hind-limb not overlapping when adpressed along body; (9) vertebral ridge distinct, wide and not segmented; (10) rib nodules distinct, 13–14 along each side of body; (11) background colouration brown; (12) head, vertebral ridge, rib nodules, palms, soles, vent and ventral tail ridge with dull orange to yellowish-brown markings; (13) vomerine teeth organised in two distinctly curved bell-shaped widening anteriorly series, with 81-113 teeth.

Comparisons. The new species can be easily distinguished from members of the subgenus Yaotriton (clades 3–5 in Fig. 2) by having light colour markings on head, vertebral ridge, rib nodules, palms, soles, vent and ventral tail ridge (vs. dark body colouration, except for palms and soles, vent region and ventral ridge of tail in most members of the subgenus Yaotriton with the exception of T. panhai). The new species can be further distinguished from T. panhai by having light colour markings on entire limbs (vs. distinct light markings only on palms, soles and fingers in T. panhai). Tylototriton zaimeng sp. nov. can be distinguished from T. taliangensis (member of clade 2, Fig. 2), by having light markings on distinct rib nodules, lips and parotoids (vs. lacking distinct rib nodules, generally dark charcoal-black body colouration with light orange to red markings only on the posterior part of parotoids, digits, palms, soles, vent and ventral tail ridge in T. taliangensis). Tylototriton zaimeng sp. nov. can be distinguished from T. pseudoverrucosus (clade 2, Fig. 2) and T. kweichowensis (clade 1, Fig. 2) by having isolated light markings on rib nodules (vs. connected markings forming light dorsolateral lines in T. pseudoverrucosus and T. kweichowensis).

Based the results of phylogenetic analyses (Fig. 2), *Tylototriton zaimeng* sp. nov. falls into clade 1 of the subgenus *Tylototriton* s. str. and, hence, morphological comparisons with members of the *T. verrucosus* species group members appear to be the most pertinent. Morphological comparisons on several diagnostic characters between *Tylototriton zaimeng* sp. nov. and the closely-related members of *T. verrucosus* species group are summarised



**Figure 3.** Morphological comparison between *Tylototriton zaimeng* sp. nov. and *T. panwaensis*. **A.** *Tylototriton panwaensis* (adult male ZMMU NAP-09477, from Sadung, Myitkyina, Kachin, Myanmar; photograph by Nikolay A. Poyarkov) and *Tylototriton zaimeng* sp. nov. (adult female MZMU3041, from Chingjaroi Ngachaphung, Ukhrul, Manipur, India; photograph by Andrey M. Bragin) in life; **B.** PCA plots of PCA1 versus PCA2 for morphological parameters of the two *Tylototriton* species.

Character		Tylototriton panwaensis								
	MZMU-3041, female	5 n	nales	6 fe	males	5 males				
	holotype		parat	ypes		-				
		Mean	Min-Max	Mean	Min-Max	Mean	Min-Max			
SVL*	76.09	65.5±2.4	(61.4–67.5)	64.7±2.6	(61.6–68.7)	72.9±4.3	(68.9–79.3)			
HL	17.68	15.6±1.4	(13.8–17.1)	14.5±0.3	(14.3–15.1)	$15.52{\pm}1.0$	(13.7–16.3)			
HW*	16.4	$14.5 \pm 1.0$	(13.2–15.8)	$14.0\pm0.6$	(12.9–14.7)	$13.46 \pm 0.6$	(13.0–14.2)			
MXHW*	19.4	$17.2 \pm 1.6$	(14.9–18.8)	$16.4 \pm 0.5$	(15.7–17.3)	$16.4 \pm 0.4$	(16.1 - 17.0)			
IND	5.08	$3.8 {\pm} 0.5$	(3.1–4.3)	3.7±0.4	(3.3-4.1)	$4.64 \pm 0.3$	(4.3 - 5.0)			
AGD	42.97	34.6±2.7	(30.4–37.5)	32.2±2.4	(28.2–34.8)	$38.8 {\pm} 1.5$	(36.9-41.0)			
TRL	63.12	55.2±1.5	(53.6–56.9)	54.9±1.7	(53.4–57.9)	$59.44 \pm 2.0$	(57.6-62.2)			
TAL	73.8	72.5±3.3	(68.1–76.3)	$69.0{\pm}4.4$	(63.7–77.1)	71.46±12.5	(52.3-83.1)			
VL*	4.08	$6.4{\pm}0.4$	(6.1 - 7.0)	$5.0{\pm}0.7$	(4.2–5.9)	$4.1 \pm 0.9$	(3.5 - 5.6)			
FLL*	26.74	22.7±1.0	(21.3–24.1)	23.4±0.8	(22.2–24.5)	23.26±1.1	(21.9–24.9)			
HLL*	28.6	$23.8 \pm 0.8$	(22.8–24.8)	24.7±1.9	(22.1–27.6)	25.4±0.9	(24.2-26.6)			
LJL*	16.27	$14.3 \pm 1.1$	(13.5–15.9)	13.1±0.8	(11.7–13.9)	$14.24 \pm 0.3$	(14.0-14.6)			
SL*	7.02	$6.7 \pm 0.5$	(6.1–7.5)	6.3±0.3	(5.8-6.7)	$6.72 \pm 0.1$	(6.5–6.9)			
IOD*	9.8	$8.9{\pm}0.6$	(8.5–9.6)	8.6±0.3	(8.1-8.9)	$7.36 \pm 0.4$	(6.8–7.9)			
UEW*	2.57	$1.5 \pm 0.3$	(1.0-1.8)	$1.8\pm0.2$	(1.6–2.1)	$2.46{\pm}0.1$	(2.4–2.5)			
UEL*	4.1	3.6±0.2	(3.3–3.9)	3.9±0.3	(3.6–4.3)	$4.76 \pm 0.2$	(4.5 - 5.0)			
OL*	2.6	$2.6{\pm}0.5$	(2.3–3.5)	2.6±0.3	(2.2 - 3.0)	$4.54 \pm 0.2$	(4.3–4.7)			
BTAW*	8.72	$8.8 {\pm} 0.9$	(8.0-10.2)	7.3±0.7	(6.9-8.7)	$6.36 \pm 0.2$	(6.0-6.6)			
MTAW	5.47	$3.0{\pm}0.5$	(2.6–3.7)	$2.6{\pm}0.2$	(2.4–2.9)	$2.92 \pm 0.4$	(2.3–3.3)			
MXTAH*	10.17	$9.4{\pm}0.7$	(8.6–10.4)	$8.2{\pm}0.7$	(7.4–9.3)	$7.0{\pm}0.6$	(6.3–7.7)			
MTAH	9.1	7.4±1.5	(5.7–9.5)	$5.4{\pm}0.9$	(4.6 - 7.0)	$6.34 \pm 0.6$	(5.9–7.4)			
ON*	4.39	$4.4 \pm 0.4$	(3.9–5.0)	4.3±0.3	(4.0-4.8)	$3.82 \pm 0.6$	(2.8-4.4)			
DLWN*	28	$27.6 \pm 0.9$	(27.0-29.0)	$27.7 \pm 0.8$	(27.0-29.0)	$29.8 \pm 0.4$	(29.0-30.0)			

**Table 3.** Measurements (mm) of adult *Tylototriton zaimeng* sp. nov. and *T. panwaensis*. Abbreviations defined in the text. Asterisk (\*) indicates significant differences between relative character values in males (p < 0.05).

in Table 4. An important morphological difference which distinguishes Tylototriton zaimeng sp. nov. from all other members of T. verrucosus species group members is the shape of vomerine tooth series (VTS), which are distinctly curved and bell-shaped in the new species (Fig. 4A): VTS are distinctly widening in the anterior one-third of their length, further gradually widening posteriorly, reaching maximal width in the posterior one third of VTS length; this shape of VTS appears to be quite stable and was observed in all examined adult specimens of the new species. In contrary, in all members of T. verrucosus species group for which the shape of VTS was reported (in T. houi, T. panwaensis, T. kachinorum, T. verrucosus, T. shanjing, T. uyenoi, T. anguliceps, T. podichthys and T. yangi), it was described as inverted V-shape with VTS branches being comparatively straight in the anterior half of their length (see Fig. 4B).

Tylototriton zaimeng sp. nov. can be further distinguished from T. uyenoi, T. pulcherrimus, T. shanjing, T. houi and T. yangi by having dull orange-brown to yellowish-brown light markings (vs. much brighter orange to bright-yellow light markings in T. uyenoi, T. pulcherrimus, T. shanjing and T. yangi and vs. bright orange-red markings in T. houi). In particular, T. houi has bright orange-red markings on ventral surfaces including vent, chest, light mid-ventral line (vs. absent in the new species); T. pulcherrimus has a series of bright-orange glandular spots located ventro-laterally and on flanks (vs. flanks lacking light spots in the new species); while T. yangi has contrasting charcoal-black colouration of head and lips with only posteriormost part of parotoid colored bright orange and no light ventral markings on body and tail (vs. all head dull orange-brown, light markings present on ventral tail ridge and vent in the new species). Tylototriton zaimeng sp. nov. has relatively narrower head in both sexes (RHW 22.2 vs. 25.0 in males; 21.6 vs. 23.1-24.0 in females); shorter internarial distance (RIND 5.7 for males, 5.8 for females vs. 7.0-7.1 for both sexes); and very wide, protruding, and glandular dorsolateral and sagittal head ridges (vs. narrow steep ridges) than in T. uyenoi (see Table 4). Males of the new species can be further diagnosed from males of T. umphangensis by having shorter limbs which do not overlap when adpressed along the body (vs. overlap), by comparatively longer trunk (RTRL84.4 vs. 76.8), longer tail (RTAL 110.6 vs. 104.7), by having snout rounded in dorsal aspect (vs. truncate) and by having a non-segmented vertebral ridge (vs. distinctly segmented) (see Table 4). Males of the new species can be further diagnosed from males of T. shanjing by having comparatively shorter head (RHL 23.8 vs. 26.6), longer tail (RTAL 110.6 vs. 104.4), by shorter internarial distance (RIND 5.7 vs. 7.1) and by having a non-segmented vertebral ridge (vs. well-segmented) and brown to dark-brown background body colour (vs. blackish) (see Table 4). Tylototriton zaimeng sp. nov. can be distinguished from T. verrucosus by having light ventral markings on body and tail (vs. no light markings on body and tail). The new species can be further differentiated from *T. verrucosus* by having comparatively shorter internarial distance in both sexes (RIND 4.8–6.7 vs. 6.2–7.0) and longer tail in both sexes (RTAL 110.6 vs. 104.9 for males; 105.3 vs. 102.5 for females); the new species also has a non-segmented vertebral ridge (vs. well-segmented) and brown background colouration of body (vs. blackish) (see Table 4).

Tylototriton zaimeng sp. nov. can be distinguished from T. podichthys by having comparatively shorter head in both sexes (RHL 21.8-25.6 vs. 28.1-34.3), slightly longer tail in both sexes (RTAL in males 108.1-114.1 vs. 80.2-104.8; in females 96.6-112.2 vs. 79.2-81.4), in having 13–14 rib nodules (vs. 15–16 rib nodules), by comparatively shorter limbs which do not overlap when adpressed to body (vs. digit tips touching when limbs are adpressed to body) and by having duller colouration with orange to yellowish-brown light markings and brown background (vs. orange to dark-red light markings and blackish background) (see Table 4). The new species can be distinguished from T. phukhaensis by having very wide, protruding and glandular head ridges (vs. narrow steep head ridges), by having snout rounded in dorsal aspect (vs. truncate), by comparatively shorter limbs which do not overlap when adpressed to body (vs. digit tips touching when limbs are adpressed to body), by having comparatively wider head in both sexes (RHW 19.6-23.4 vs. 17.7-19.2), by having comparatively longer trunk in both species (RTRL 79.5-88.7 vs. 76.4-76.8) and by a having wide non-segmented vertebral ridge (vs. narrow, segmented) (see Table 4).

Tylototriton zaimeng sp. nov. can be distinguished from T. anguliceps by having a comparatively shorter head in males (RHL 22.5-25.6 vs. 26.2-29.5), by shorter internarial distance in both sexes (RIND 4.8-6.7 vs. 6.6-7.4), by having a wide non-segmented vertebral ridge (vs. weakly segmented), by having snout rounded in dorsal aspect (vs. truncate), by having very wide, protruding and glandular head ridges (vs. narrow steep head ridges, including narrow and long sagittal ridge), by having 13–14 rib nodules (vs. not less than 15 rib nodules), by comparatively shorter limbs which do not overlap when adpressed to body (vs. digit tips touching when limbs are adpressed to body) and by having duller colouration with orange to yellowish-brown light markings and brown background (vs. bright-orange markings and blackish background) (see Table 4).

The new species can be readily distinguished from *T. shanorum* by having smaller body size in both sexes (SVL 61.4–76.1 mm vs. 76.0–87.9 mm), by having comparatively narrower head in both sexes (RHW 19.6–23.4 vs. 24.8–26.3), by having comparatively longer trunk in both sexes (RTRL 79.5–88.7 vs. 74.3–77.6), by having snout rounded in dorsal aspect (vs. blunt to truncate), by comparatively shorter limbs which do not overlap when adpressed to body (vs. limbs overlapping when limbs are adpressed to body) and by having a wide non-segmented vertebral ridge (vs. weakly segmented) (see Table 4). *Tylototriton zaimeng* sp. nov. can be easily distinguished from



Figure 4. Vomerine tooth series of A. *Tylototriton zaimeng* sp. nov. (MZMU3041) and B. *T. panwaensis* (ZMMU NAP-09477). Drawing by Andrey M. Bragin.

Table 4. Morphological comparison between	Tylototriton verrucosus	species group men	nbers found in India a	nd adjacent territories
(Continued on next page).				

Species	T. ngarsuensis		T. pan	waensis	T. phu	khaensis	T. podichthys		
	М	F	М		М	F	М	F	
Character	2	1		5	1	2	2	2	
SVL (in mm)	84.5	74.9–102.3	72.9 68.9–79.3		64.3	68.9–70.2	56.5-60.2	73.4–78.3	
RHL	24.0	22.0-26.0	21.4 17.3–23.4		25.0	23.1–24.8	32.6-34.3	28.1-29.3	
RHW	26.3	24.0-28.0	18.5	16.4-20.6	19.2	17.7–18.7	26.4-28.0	24.8-25.9	
RIND	8.2	7.7–8.8	6.4	6.0–6.7	5.6	6.0–5.9	8.3-8.7	7.5–7.9	
RAGD	49.7	48.0-51.0	53.4	46.5-55.9	56.6	54.7-56.6	48.0-50.7	52.1-53.5	
RTRL	76.0	74.0-78.0	81.8	74.1-87.0	76.4	76.5–76.8	65.7–67.4	70.7–71.9	
RTAL	102.3	98.0-105.0	98.7 66.0–116.5		108.7	88.1-87.0	80.2-104.8	79.2-81.4	
RVL	10.3	8.0-12.0	5.6	4.8–7.9	10.5	4.0-3.4	14.2–14.8	6.3–7.7	
RFLL	38.3	35.0-40.0	32.0	28.6-34.9	38.4	31.8–31.2	39.4-40.5	34.0-36.2	
RHLL	42.7	39.0-47.0	34.9	33.5-36.2	36.7	30.1-34.5	38.2-40.2	35.8-36.1	
RMXHW	_	_	22.6	20.4–24.4	22.1	22.0-22.4	_	_	
Snout	trui	ncate	tru	ncate	truncate		rounded		
Dorsolateral head ridges	very wide, protruding		narro	w, steep	narrow,	very steep	very wide,	protruding	
Sagittal ridge	very weal	c, indistinct	narro	ow, low	narrow	, distinct	weak, glandular		
Surface of head ridges	ro	ugh	rc	ough	ro	ugh	very	rough	
Adpressed limbs	Adpressed limbs ove		overlap		overlap		touch		
Vertebral ridge	not seg	gmented	weakly segmented		narrow, wea	kly segmented	not segmented		
Rib nodules	distii	nct, 15	distinct, 15		prominent, 14-15		large, prominent, 15-16		
Ground colour	very da	rk brown	dark reddish-brown		dark brown		blackish		
Colour of light markings	our of light markings light brown		reddish-brown		orange to	light-brown	orange to dark-red		
Location of light markings	ocation of light markings parotoids, palms, soles,		parotoids, vertebral ridge,		head, paroto	oids, vertebral	head, vertebral ridge, rib		
	vent, venti	vent, ventral tail ridge		rib nodules, vent, whole		odules, limbs,	nodules, vent, dorsal surface		
			limbs	and tail	vent region,	and whole tail	limbs, vent	ral tail ridge	
Lateral grooves on tail	ab	sent	W	veak	ab	sent	absent		
hape of VTS ?		inverte	d V-shape	invertee	l V-shape	inverted V-shape			

*T. ngarsuensis* by having generally smaller body size in males (SVL 61.4–67.5 mm vs. 74.9–76.4 mm) and in females (SVL 61.6–76.1 mm vs. 102.3 mm), comparatively much narrower head in both sexes (RHW19.6–23.4 vs. 24.0–28.0), by notably more narrow internarial distance in both sexes (RIND 4.8–6.7 vs. 7.7–8.8), by having comparatively longer trunk in both sexes (RTRL 79.5–88.7 vs. 74.0–78.0), by having snout rounded in dorsal aspect (vs. truncate). The new species can be further distinguished

from *T. ngarsuensis* by having dorsolateral head ridges starting at the snout (vs. posterior to orbit), by having a non-segmented vertebral ridge (vs. weakly segmented) and by having 13–14 rib nodules (vs. 15 rib nodules). *Ty-lototriton zaimeng* sp. nov. has much lighter and duller co-louration than *T. ngarsuensis*: background colour brown (vs. nearly black) with light orange-brown markings on rib nodules and parotoids and limbs (vs. no light markings on rib nodules and parotoids) (see Table 4).

Tylototriton zaimeng sp. nov. can be easily distinguished from T. himalayanus from Nepal by the following morphological attributes: by notably narrower internarial distance in both sexes (RIND 4.8-6.7 vs. 8.2-8.4), by having generally longer trunk in both sexes (RTRL 79.5-88.7 vs. 77.1-79.9), by having longer tail in males (RTAL 108.1–114.1 vs. 98.0), by having snout rounded in dorsal aspect (vs. blunt), by comparatively shorter limbs which do not overlap when adpressed to body (vs. limbs overlapping when limbs are adpressed to body), by having 13-14 rib nodules (vs. 16 rib nodules) and by lacking lateral transverse grooves on tail (vs. clearly distinct) (see Table 4). The new species can be further distinguished from T. kachinorum from Kachin State of Myanmar by shorter head in both sexes (RHL 22.5-25.6 vs. 27.6 in males, 21.8-23.6 vs. 23.9-24.9 in females), by shorter tail in both males (RTAL 108.1-114.1 vs. 120.5), by notably narrower internarial distance in both sexes (RIND 4.8-6.7 vs. 7.7–8.0), by having snout rounded in dorsal aspect (vs. truncate) and by comparatively shorter limbs which do not overlap when adpressed to body (vs. limbs overlapping when limbs are adpressed to body) (see Table 4).

Phylogenetically and morphologically, *Tylototriton zaimeng* sp. nov. is most closely related to two species of *Tylototriton* inhabiting northern Myanmar and Yunnan Province of China – *T. panwaensis* and *T. houi* (see Fig. 2, Table 4). From *T. houi*, the new species can be easily distinguished by its much duller colouration (see above and Table 4) and can be further distinguished by having snout rounded in dorsal aspect (vs. truncate), by having very wide, protruding and glandular head ridges with rough surface (vs. narrow steep head ridges with smooth surface, including short sagittal ridge) and by having 13–14 rib nodules (vs. 16 rib nodules).

From its sister species T. panwaensis, Tylototriton zaimeng sp. nov. can be distinguished by having snout rounded in dorsal aspect (vs. truncate), by having very wide, protruding and glandular head ridges including prominent sagittal ridge (vs. narrow steep head ridges with smooth surface, including very weak and low sagittal ridge), by having 13–14 rib nodules (vs. 15 rib nodules), by having a very wide non-segmented vertebral ridge (vs. narrow, weakly segmented), by lacking lateral transverse grooves on tail (vs. weak grooves present) and by comparatively shorter limbs which do not overlap when adpressed to body (vs. limbs overlapping when limbs are adpressed to body). Tylototriton zaimeng sp. nov. has generally lighter colouration than T. panwaensis: brownish-ground colour with orange-brown light markings (vs. more contrasting dark reddish-brown to black background colour with reddish-brown light markings in T. panwaensis) (see Table 4). Moreover, males of the new species are different from males of T. panwaensis in a number of morphometric characters. Males of Tylototriton zaimeng sp. nov. have greater maximal head width (RMXHW 24.3-27.9 vs. 20.4-24.4), generally longer snout (RSL 9.2-11.2 vs. 8.7-9.6), wider interorbital distance (RIOD 12.3-14.3 vs. 9.9-10.7), smaller eyes (ROL 3.3-5.2 vs. 5.9-6.4) and wider tail base (RBTAW 10.4–15.2 vs. 7.9–9.5) than in *T. panwaensis* (see Table 3).

Description of holotype. A medium-sized specimen in a good state of preservation (Figs 5, 6). Head. Head slightly longer than wide (HW/HL ratio 92.8%) (Fig. 5C-E), head slightly wider than body; pentagonal in shape in dorsal view, flattened in profile (Fig. 5E); snout long, about three times longer than eye (UEW/SL ratio 36.6%), gently rounded in dorsal view (Fig. 5C), rounded in lateral view (Fig. 5E), notably projecting beyond lower jaw; nostrils on anterior margin of snout located notably closer to snout tip than to eye (NSD/ON ratio 39.9%), nostrils with antero-lateral orientation, not visible from dorsal view; eyes small, not projecting in lateral view (Fig. 5E), slightly projecting in dorsal view (Fig. 5C); labial folds absent; tongue oval, attached to anterior floor of mouth cavity, laterally and posteriorly free; vomerine teeth arranged in a bell-shaped distinctly curved series (Fig. 4A), distinctly widening in the anterior one-third of vomerine tooth series length, further gradually widening posteriorly, reaching maximal width in the posterior one third of vomerine tooth series length; vomerine tooth series notably longer than wide (VTW/VTL ratio 40.4%), anteriorly reaching beyond the level of choanae, numbers of vomerine teeth 113 (62/51 in right and left branches, respectively), upper jaw teeth 72 and lower jaw teeth 90; parotoids distinct, large and protruding, bean-shaped, slightly projecting posteriorly (Fig. 5E); dorsolateral supratemporal bony ridges on head very wide, with rough surface, notably protruding, continuing from the snout tip to the anterior end of parotoid, becoming wider towards the posterior end (Fig. 5C); sagittal bony ridge on head very wide and protruding, becoming higher and wider posteriorly (Fig. 5C); gular fold indistinct (Fig. 5D). Body. Body habitus stout (Fig. 5A); costal folds absent; vertebral mid-dorsal ridge very wide, not segmented, beginning at the occiput region and continuing to the anterior one fourth of tail length, separated from sagittal head ridge with gap subequal to eye in length (Fig. 5C); rib nodules prominent, distinct, forming knob-like glandular warts, 14 on both sides of body, arranged in two longitudinal lines on dorsolateral surfaces of dorsum, running from area posterior to axilla to tail base (Fig. 5A); on body, rib nodules almost of the same size, rounded, decreasing in size posteriorly on sacrum and tail basis. Limbs. Limbs comparatively short and slender (Fig. 5A); forelimbs slightly shorter than hind-limbs; relative length of fore-limb FLL/SVL ratio 35.1%, relative length of hindlimb ratio 37.6%; fore- and hind-limbs not overlapping when adpressed towards each other along sides of the body; fingers and toes well developed (Fig. 5F-I), free of webbing; fingers four, comparative finger lengths: 1FL < 4FL < 2FL < 3FL; toes five, comparative toe lengths: 1TL < 2TL < 5TL < 4TL < 3TL. *Tail.* Tail comparatively short and thick, slightly shorter than body length (TAL/ SVL ratio 97.0%); tail laterally compressed on all of its length, gently tapering posteriorly, lateral grooves on tail absent; dorsal tail fin starting at the anterior one fourth



Figure 5. Holotype of *Tylototriton zaimeng* sp. nov. (MZMU3041, female) in life. A. Dorsal view; B. Ventral view; C. Head, dorsal view; D. Head, ventral view; E. Head, lateral view; F. Opisthenar view of right hand; G. Volar view of right hand; H. Opisthenar view of right foot; I. Plantar view of right foot; J. Ventral view of cloacal area. Photographs by Andrey M. Bragin.

of tail length, becoming more distinct posteriorly, with maximal tail height at posterior two thirds of tail length, dorsal and ventral tail fins smooth; tail tip pointed. *Skin texture and skin glands.* Skin dorsally very rough, with numerous small granules present on dorsal surfaces of

head and dorsum (Fig. 5A, C), lateral sides of body and tail; ventral surfaces more smooth with smaller granules arranged in transverse striations (Fig. 5B); small granules regularly arranged on throat (Fig. 5D); head ridges and parotoids with rough surface; skin on dorsal surfaces of



Figure 6. Holotype of Tylototriton zaimeng sp. nov. (MZMU-3041, female) in situ. Photograph by Andrey M. Bragin and Ht. Decemson.

limbs granular, on volar and plantar surfaces of hands (Fig. 5G) and feet (Fig. 5I) with tiny grooves forming a reticulated pattern; metacarpal or metatarsal tubercles absent. Cloacal region slightly swollen, vent as a longitudinal slit (Fig. 5J), vent edges with small transverse folds.

**Colour of holotype in life.** Ground colour of dorsal surfaces of head and trunk dark brown (Figs 5A, 6); dorsal surfaces of limbs and lateral surfaces of tail yellowish-brown to light orange (Fig. 5A); iris dark-brown with copper speckles along its outer margins (Fig. 5E); throat, belly and ventral surfaces of limbs light brown (Fig. 5B); anterior parts of head, including snout, light orange to yellowish-brown; rib nodules and vertebral ridge light orange-brown, notably discernible from dark brown trunk colouration; upper and lower lips, head bony ridges and parotoids, palms and soles light-orange.

**Colour of holotype in preservative.** After preservation in ethanol for six months, the colouration pattern of the holotype resembles that observed in life; however, yellowish and orange tints faded turning light brownish-grey.

**Measurements and counts of the holotype.** Measurements of the holotype are presented in Table 3. Additional morphometric characters (all in mm) include: ICD 9.1; CW 13.0; NSD 1.8; 1FL 3.8; 2FL 5.7; 3FL 6.7; 4FL 4.1; 1TL 3.1; 2TL 4.7; 3TL 8.6; 4TL 8.4; 5TL 4.8. Meristic characters: UJTN 72; LJTN 90; VTN 62/51 (right/left); DLWN: 14/14 (right/left). Body weight (when alive): 13.5 g.

Variation. All individuals in the type series are generally similar in morphology and agree well with the description of holotype in body proportions and colouration; variation of morphometric characters within the type series is shown in Table 3. Variation of the dorsal colouration in eleven paratypes in preservative is presented in Fig. 7. The state of preservation of nine paratypes MZMU-2942–2950 is much worse than that of the holotype and the paratypes MZMU-3035–3036: they are desiccated what caused partial discolouration and skin damage (Fig. 7). Colouration of the paratypes MZMU-3035–3036 closely agrees with that described for the holotype. In general, males have more slender bodies than females. Males and females do not differ in body size (SVL 61.4–67.5 mm in males vs. SVL 61.6–68.7 mm in females) (Table 3).

Larval morphology. Description of larval morphology is based on four premetamorphic larval specimens (MZMU-3037–3040, Bernardes et al. (2017) stage 45) (see Referred specimens for details).

Larval measurements (n = 4; in mm). SVL 35.1  $\pm$  2.5; HL 10.2  $\pm$  0.7; HW 10.3  $\pm$  0.7; OL 2.7  $\pm$  0.2; AGD 21.1  $\pm$  1.5; TAL 35.3  $\pm$  2.5; FLL 11.9  $\pm$  0.8; HLL 12.1  $\pm$  0.8; MXTAH 5.2  $\pm$  0.4.

Larval external morphology. Body elongated, as high as wide (Fig. 8). Head large, ovoid in dorsal view, wide and slightly depressed with a short and rounded snout, gently sloping in lateral view, slightly wider than body in dorsal view. Snout rounded in dorsal view (Fig. 8B), as well as in lateral view (Fig. 8A). Tail equal to body length comprising 100.4% of SVL; myotomes on body and tail not discernible in lateral view. Nostrils rounded, small, orientated anterolaterally, located much closer to snout tip



**Figure 7.** Variation of dorsal colouration in paratypes of *Tylototriton zaimeng* sp. nov. Scale bar: 10 mm. Photographs by Andrey M. Bragin.

than to eye (Fig. 8A). Eyes large, rounded, orientated dorsolaterally, well visible in dorsal view (Fig. 8B). Limbs short, fore-limbs subequal to hind-limbs, FLL/HLL ratio 98.5%. Fore-limbs with four well-developed elongated fingers; relative finger lengths: 4FL < 3FL < 1FL < 2FL. Hind-limbs with five well-developed toes; relative toe lengths: 5TL < 1TL < 2TL < 4TL < 3TL. Orbit diameter (OL) 7.7% of SVL. Vent a short longitudinal slit. Tail fins reduced; maximum height of dorsal tail fin ca. 10% of maximum tail height. Ventral tail fin almost completely

reduced. Dorsal tail fin starts roughly above the cloaca, though it remains still visible as a thin mid-dorsal line starting at the level of axilla (Fig. 8B). Tail tip pointed in lateral view (Fig. 8A). Skin completely smooth; lateral line organs visible on dorsal surface of head; three pairs of gills partially reduced, not reaching the level of axilla.

Larval colouration in life. In life, larval background colour ochre to light brown dorsally (Fig. 8A, B), ventral surfaces of body off-white to pinkish, translucent, ventral surface of tail and vent bright yellow (Fig. 8C). Dorsal surfaces of body, tail and head with indistinct dark-grey marbling. Eyes, except for pupil, fully pigmented, iris copper-coloured (Fig. 8A).

**Position in mtDNA genealogy and sequence divergence.** According to our mtDNA genealogy, *Tylototriton zaimeng* sp. nov. belongs to clade 1 of the subgenus *Tylototriton* s. str., corresponding to the *T. verrucosus* species group (see Fig. 2). The new species is grouped together with *Tylototriton* species from northern Myanmar (*T. panwaensis*), northern Indochina (*T. podichthys*) and Yunnan Province of China (*T. verrucosus*, *T. shanjing*, *T. houi* and *T. pulcherrimus*), forming clade with *T. panwaensis* and *T. houi*. Uncorrected genetic *p*-distances between *Tylototriton zaimeng* sp. nov. ND2 sequences and all homologous sequences of other members of *T. verrucosus* species group included in our analyses varied from 3.0% (with its sister species *T. panwaensis*) to 8.6% (with *T. uyenoi*) (see Table 2).

Distribution and Natural history. To date Tylototriton zaimeng sp. nov. is known from five localities in montane areas of Manipur State, north-eastern India (see Fig. 1: localities 2–6) on elevations from 1,180 to 2,210 m a.s.l. The actual extent of distribution of the new species remains unknown; it is likely that Tylototriton zaimeng sp. nov. occurs further northwards along the Khongtheng Mountain Range and other heavily forested highlands in the Nagaland State of India and even may penetrate to the easternmost parts of Arunachal Pradesh State of India and Sagaing Division of Myanmar. The taxonomic status of the existing record of Tylototriton sp. from Sagaing, Myanmar (Fig. 1: locality 7), tentatively identified as T. cf. panwaensis by Grismer et al. (2019), requires further studies as it may represent a lineage closely related to Tylototriton zaimeng sp. nov.

Our knowledge on biology of *Tylototriton zaimeng* sp. nov. is incomplete. Adult animals were encountered both at night and during the day-time on the shallow parts of the lake (Fig. 9). The Zaimeng Lake, where the new species was for the first time encountered, is situated on top of Khongtheng Mountain Range at Thonglang Village (Bena Tababang); it is located on an elevation of ca. 2,212 metres above sea level and is one of the highest lakes in Manipur (Fig. 9A). The lake total area measures about 90,580.46 m<sup>2</sup> and is surrounded by evergreen montane forest composed of *Michelia champacca*, *Phoebe hainensenia*, *Magnolia* sp. and *Quercus* sp. with dense *Arundinaria munro* bamboo undergrowth (Sebastian 2015; Singh et al. 2018). Adult male and female newts were observed

slowly moving along the muddy bottom in clear water. *Tylototriton zaimeng* sp. nov. is locally abundant: in July,

*Tylototriton zaimeng* sp. nov. is locally abundant: in July, hundreds of adult newts could be seen on the bottom of Zaimeng Lake (Fig. 9B, C). The new species is known to local Liangmei people as "*Takope*", or "*Tadui taku*" in Liangmei dialect (Singh et al. 2018), "Lengva" in Tangkhul and "Hangoi mamei panba" in Manipuri meaning "Tailed amphibian" (Selim 2001). Other species of amphibians recorded syntopically with the new species at the type locality include *Polypedates* sp. and *Zhangixalus* sp.

**Etymology.** The specific name "*zaimeng*" is given as a noun in apposition and represents a Latinised version of the Liangmei dialect word "*zaimeng*" literally meaning "Puzzle Lake" or "Mystery Lake". The name is given in reference to the Zaimeng Lake – a high-elevation mountain lake in Koubru Forest Division, the famous location where the crocodile newts were for the first time recorded in Manipur. It is believed that the name of the Lake was given by the Zeliangrong ancestors of Thonglang Village who passed by the Lake, but could not find their way to their destination as they used to return to the same spot again and again and circled the Lake over and over again (Sebastian 2015).

**Recommended vernacular names.** We recommend the following trivial name in English: Zaimeng Lake Crocodile Newt. The vernacular name in Liangmei dialect: Tadui taku; Tangkhul: Lengva; Manipuri: Hangoi mamei panba.

Conservation status. Tylototriton zaimeng sp. nov. is to date known from not more than five localities in montane areas of Manipur State of north-eastern India; the actual extent of range of the new species is unknown (see Fig. 1). The new species is anticipated to inhabit elevations above 1,000 m a.s.l. on mountains of the Khongtheng Mountain Range and could be possibly found in the adjacent parts of Nagaland and perhaps even the Sagaing Division of Myanmar. Further studies are needed to understand the current distribution range, population trends and possible threats to Tylototriton zaimeng sp. nov. The montane forests where the new species occurs are affected by growing forest destruction and anthropogenic pressure. Given this information, we tentatively suggest Tylototriton zaimeng sp. nov. to be considered as a Vulnerable (VU) species, following IUCN's Red List categories (IUCN 2019).

## Discussion

The discovery of *Tylototriton zaimeng* sp. nov. brings the total number of the nominal species of the genus *Tyloto-triton* to 40. Our study also suggests that *T. verrucosus* sensu stricto likely does not occur in India and its range is likely restricted to north-eastern Myanmar, south-western Yunnan Province of China and northernmost Thailand (Pomchote et al. 2020b). It has to be noted, however, that many taxonomic issues regarding Indian *Tylototriton* remain unresolved, even after our publication and are awaiting further research. In particular, the taxonomic



**Figure 8.** Lateral (**A**), dorsal (**B**) and ventral (**C**) views of larval specimen (MZMU3037; Bernardes et al. (2017) stage 45) of *Ty-lototriton zaimeng* sp. nov. in life. Scale bar: 5 mm. Photographs by Andrey M. Bragin.

status of the Tylototriton populations in Indian Eastern Himalaya (West Bengal, Sikkim, Arunachal Pradesh) remains to be assessed: these populations may be conspecific to T. himalayanus originally described from eastern Nepal or may represent new, previously unknown lineages that could be candidates for future taxonomic assessment (Hernandez et al. 2018). The extent of distribution of Tylototriton salamanders in north-eastern India also remains insufficiently understood: the records of Tylototriton from Meghalaya, previously tentatively assigned to T. himalayanus (Hernandez et al. 2018) may be found to be closely related to Tylototriton zaimeng sp. nov. and further integrative taxonomic studies are needed to clarify the taxonomic status of these populations. Finally, the taxonomic status of the existing record of Tylototriton from Sagaing Division of north-western Myanmar (Fig. 1, locality 7) has to be clarified using an integrative taxonomic approach. Previously, this population was listed as T. cf. panwaensis (Grismer et al. 2019); however, as it was recorded in the mountain system connected to Khongtheng Mountain Range where *Tylototriton zaimeng* sp. nov. occurs, these populations may be conspecific.

Our work further emphasises the importance of the integrative taxonomic approach, combining data from multiple lines of evidence, including molecular and morphological differentiation, for assessing the diversity and evolutionary relationships of Tylototriton salamanders. Numerous previous studies have demonstrated that evolutionary independent lineages of Tylototriton may demonstrate only subtle differences between each other in morphological characters (Khatiwada et al. 2015; Phimmachak et al. 2015; Grismer et al. 2018, 2019; Than Zaw et al. 2019; Bernardes et al. 2020; Li et al. 2020; Pomchote et al. 2020a, 2021; Poyarkov et al. 2021b). It has to be noted, however, that species delimitation, based on just two mtDNA markers used in the present study (16S rRNA and ND2 genes), has certain limitations. For example, high mtDNA polymorphism was reported within single geographic populations of several Tylototriton species, such as T. ziegleri and T.



**Figure 9.** Natural habitat of *Tylototriton zaimeng* sp. nov. at Zaimeng Lake, Koubru Forest Division, Kangpokpi, Manipur, India. **A.** Vegetation surrounding the Zaimeng Lake; **B.** Males of *Tylototriton zaimeng* sp. nov. *in situ*; **C.** Breeding habitat of *Tylototriton zaimeng* sp. nov., numerous individuals can be seen on the muddy bottom of the lake. Photographs by Parag Shinde and Ht. Decemson.

*maolanensis* (Dufresnes and Hernandez 2022). Furthermore, matching the review by Dufresnes and Hernandez (2022), in our study the genetic distances in 16S rRNA gene, which was earlier proposed as a 'barcoding' gene for amphibians (Vences et al. 2005a, b; Vieites et al. 2009), did not closely correlate with distances of the more fast-evolving locus (ND2; see Table 2). Therefore, it seems that, at least in *Tylototriton*, estimating taxonomic ranks, based on 16S rRNA thresholds is not reliable and a truly multilocus approach combining data from mtDNA and nuDNA genetic markers using larger sample sizes is required for more accurate species delimitation in this group (Dufresnes and Hernandez 2022).

Despite comparatively shallow divergences amongst *T. panwaensis*, *T. houi* and *Tylototriton zaimeng* sp. nov. in mtDNA sequences, these taxa show unique phenotypic features allowing distinguishing them as independent

species. The peculiar curved bell-shape of the vomerine tooth series observed in Manipur populations of crocodile newts was never previously reported in any other member of T. verrucosus species group; therefore, this feature appears to be an important diagnostic character supporting the full species status of Tylototriton zaimeng sp. nov. Moreover, our study revealed significant morphological differentiation between Tvlototriton zaimeng sp. nov. and its sister species T. panwaensis, which are clearly separated in the morphospace of PCA analysis (Fig. 3). Despite the small sample size for T. panwaensis (five male specimens) which may potentially affect the results of PCA analysis, the complete absence of an overlap between the two species in morphospace, along with diagnostic characters, such as the shape of vomerine tooth series and number of rib nodules, overall support the status of Manipur populations of crocodile newts as a separate species.

Though significant progress was recently achieved in summarising the available information of evolutionary relationships and phylogeography in Tylototriton salamanders (e.g. Dufresnes and Hernandez (2022), many questions still await to be answered. Future survey efforts might focus on insufficiently explored regions where new lineages and species are likely to be found, such as the remaining states of north-eastern India (Nagaland, Arunachal Pradesh, Meghalaya), northern and eastern Myanmar, Bhutan and southern China. Taxonomy of several species complexes within the T. verrucosus species group still remains insufficiently understood. For example, as in case of T. verrucosus - T. shanjing and T. shanorum - T. ngarsuensis species complexes, significant differentiation in morphology and colouration are combined with very shallow divergence in mitochondrial loci examined. Further comprehensive taxonomic studies, including genomic-level analyses and examination of a broader geographic sampling, are required for a better understanding of the diversity and distribution of these iconic amphibians.

The recognition of Manipur populations of *Tylototriton* as a distinct species, *Tylototriton zaimeng* sp. nov., would benefit its conservation, as well as further underline the importance of the montane subtropical forests of north-eastern India as one of the key centres of herpetofaunal diversity in Asia (Selim 2001; Sebastian 2015; Mirza et al. 2022). Further intensified survey efforts are required to document the amphibian fauna of the region and to elaborate the corresponding conservation measures. Mountain forests in north-eastern India are threat-ened to a greater degree than in other parts of India and, hence, immediate efforts to document the biodiversity of the region are imperative to ensure its conservation.

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# Appendix 1

Specimens examined:

- *Tylototriton zaimeng* sp. nov.: Zaimeng Lake, Koubru Forest Division, environs of Chawangkining Village, Kangpokpi District, Manipur State, Northeastern India (MZMU-2942–2950)
- *Tylototriton zaimeng* sp. nov.: Chingjaroi Ngachaphung, Ukhrul District, Manipur State, Northeastern India (MZMU-3037–3041)
- *Tylototriton zaimeng* sp. nov.: Phungyar (environs of Tangkhul Hungdung), Kamjong District, Manipur State, Northeastern India (MZMU-3035–3036)
- *Tylototriton panwaensis*: Myitkyina area, Sadung area, environs of Sadung village, Kachin, Myanmar (ZMMU NAP-09477–09485)

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- *Tylototriton verrucosus*: Bhamo area, Momauk Dist., Lawmun village environs, Sen Lum Mt., Kachin, Myanmar (ZMMU NAP-09486–09493)
- Tylototriton verrucosus: Doi Chang Mt., Chiang Rai, Thailand (ZMMU NAP-11668, ZMMU NAP-12905)
- Tylototriton kachinorum: Ingyin Taung Mountain, Indawgyi Lake area, Mohnyin Township, Kachin State, Myanmar (ZMMU A5953–A5957, ZISP 13721, ZDUM-0101–0105)
- Tylototriton uyenoi: Chiang Mai, Thailand (ZMMU NAP-08220)
- *Tylototriton uyenoi*: Doi Suthep, Chiang Mai Province, Thailand (NSMTH 1073-1077)

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