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# A new species of the genus *Tylototriton* (Caudata, Salamandridae) from Guangdong, southern China, with discussion on the subgenera and species groups within the genus

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## Abstract

In this work, a new species of the genus *Tylototriton* is described from Guangdong, southern China. *Tylototriton sini* sp. nov. was recorded as *T. asperrimus* for decades, and was indicated to represent an independent lineage based on recent molecular phylogenetic analyses. After detailed molecular analysis and morphological comparisons, *Tylototriton sini* sp. nov. is recognized as a distinct species which can be clearly distinguished from all known congeners by a combination of morphological characteristics and the significant divergence in the mitochondrial gene. Because the genus *Tylototriton* is of high conservation concern and all formally described members are protected by law, we also provide first data on the conservation status and recommendations for IUCN categorization for *Tylototriton sini* sp. nov. A suggestion on the species groups division of the genus *Tylototriton* is also provided based on their morphological differences and phylogenetic relationships.

## Key words

Chresonymy, conservation, morphology, phylogeny, *Tylototriton sini* sp. nov., Yunkai Mountains

# Introduction

The newt genus *Tylototriton* Anderson, 1871 contains 32 known species distributed in the mountain areas of southern and eastern Himalaya, southern and central China, and northern Indochina Peninsula (Frost 2021). *T. asperrimus* Unterstein, 1930, the second described species within this genus, was originally nominated based on two specimens collected by Prof. Shu-Szi Sin (= Shu-Zhi Xin) from Loshiang (= Luoxiang Town), Yao Shan (= Mt Dayao, Jiuxiu Yao Autonomous County), Kwangsi (= Guangxi Zhuang Autonomous Region), China (Unterstein 1930; Fan 1931; Bauer et al. 1993). Subsequently, this species was widely recorded from multiple localities of China and Vietnam (Liu et al. 1973; Zhao and Adler 1993; Bain and Nguyen 2004; Fei et al. 1990, 2006, 2012; Nguyen et al. 2009; Fei and Ye 2016).

Liu et al. (1973) firstly noticed the morphological variations among different recorded populations of *T. asperrimus* in China, and suggested that detailed studies on this wide-spreading species are required. Afterward, based on the morphological differences, the populations in Hainan Island were proposed as an identical species *T. hainanensis* Fei, Ye & Yang, 1984, the populations in central China (Gansu, Sichuan, Guizhou, Hunan, and Anhui provinces) were assigned to *T. wenxianensis* Fei, Ye & Yang, 1984, and the populations in southern China (Guangxi Zhuang Autonomous Region, and Guangdong and Guizhou provinces) and northern Vietnam were kept as *T. asperrimus* (Fei et al. 1984, 1990, 2006). In recent years, the approach of integrative taxonomy combining morphological and molecular data has revealed that the recognition of *T. asperrimus* should be a species complex with multiple paraphyletic lineages, and the populations from northern Vietnam have been described as different new species, *T. ziegleri* Nishikawa, Matsui & Nguyen, 2013, *T. pasmansi* Bernardes, Le, Nguyen, Pham, Pham, Nguyen & Ziegler, 2020, and *T. sparreboomi* Bernardes, Le, Nguyen, Pham, Pham, Nguyen & Ziegler, 2020, respectively (Nishikawa et al. 2013a; Wang et al. 2018; Bernardes et al. 2020). After these taxonomic revisions, *T. asperrimus* is currently known only from southern China (Frost 2021). Nonetheless, the population in Mt Yunkai, Xinyi, Guangdong has been suggested to represent an independent lineage based on phylogenetic analyses using multi-locus of mitochondrial and nuclear data but without morphological comparisons (Wang et al. 2018; Poyarkov et al. 2021). Besides, this population was surprisingly reported as *T. ziegleri* after a rough phylogenetic analysis without including any data of *T. hainanensis* and other recently-described congeners from Vietnam (Li et al. 2020).

In this work, we performed detailed morphological comparisons and molecular analyses on the "*T. asperrimus*" population from Mt Yunkai, Xinyi, Guangdong, China (Fig. 1, site 1), to clarify its exact taxonomic status. The results substantiate that the *Tylototriton* population from Guangdong should be a distinct species and can be distinguished reliably from all known congeners in morphology and phylogeny, especially from *T. asperrimus* from Guangxi, China (Fig. 1, sites 2–4) and *T. ziegleri* from northern Vietnam (Fig. 1, sites 5–6). Therefore, we describe this *Tylototriton* population from Guangdong as a new species below.

# Materials and methods

## Specimens and morphological analyses

Four specimens of the genus *Tylototriton* were collected from Mt Yunkai, Xinyi, Guangdong. All specimens were fixed in 10% buffered formalin, later transferred to 70% ethanol, and deposited in the Museum of Biology, Sun Yat-sen University (**SYS**) and Chengdu Institute of Biology, the Chinese Academy of Sciences (**CIB**), PR China. External measurements were made for the unnamed specimens with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper) to the nearest 0.1 mm. These measurements are as follows: total length (**TOL**) from tip of snout to tip of tail; snout–vent length (**SVL**) from tip of snout to posterior edge of vent; head length (**HL**) from jugular fold to snout tip; maximum head width (**HW**); eye diameter (**ED**) from the anterior corner to the posterior corner of the eye; snout length (**SL**) from tip of snout to the anterior corner of eye; minimum interorbital (**IOD**) distance between the eyes; minimum internasal distance (**IND**) between the external nares; trunk length (**TRL**) from gular fold of throat to anterior tip of vent; tail length (**TAL**) from posterior edge of vent to tip of tail; maximum tail height (**TH**); lower arm length (**LLA**) from elbow to wrist; hand length (**HL**) from elbow to the tip of finger III; the third finger length (**F3L**) from base to tip of finger III; thigh length (**TLH**) from groin to knee; tibia length (**TIB**) from knee to tarsi; the third toe length (**T3L**) from base to tip of toe III.

The morphological comparisons for recognized congeners were attained from their original descriptions and latest revisions based on topotypic specimens (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; Nishikawa et al. 2013a, b, 2014; Yang et al. 2014; Le et al. 2015; Phimmachak et al. 2015; Fei and Ye 2016; Qian et al. 2017; Grismer et al. 2018, 2019; Zaw et al. 2019; Bernardes et al. 2020; Li et al. 2020; Pomchote et al. 2020; Poyarkov et al. 2021) and from the examination of museum specimens listed in Appendix.

## Phylogenetic sampling and analyses

Totally 11 liver samples of the genus *Tylototriton* were used in this study, encompassing four samples of the undescribed *Tylototriton* specimens from Guangdong, four

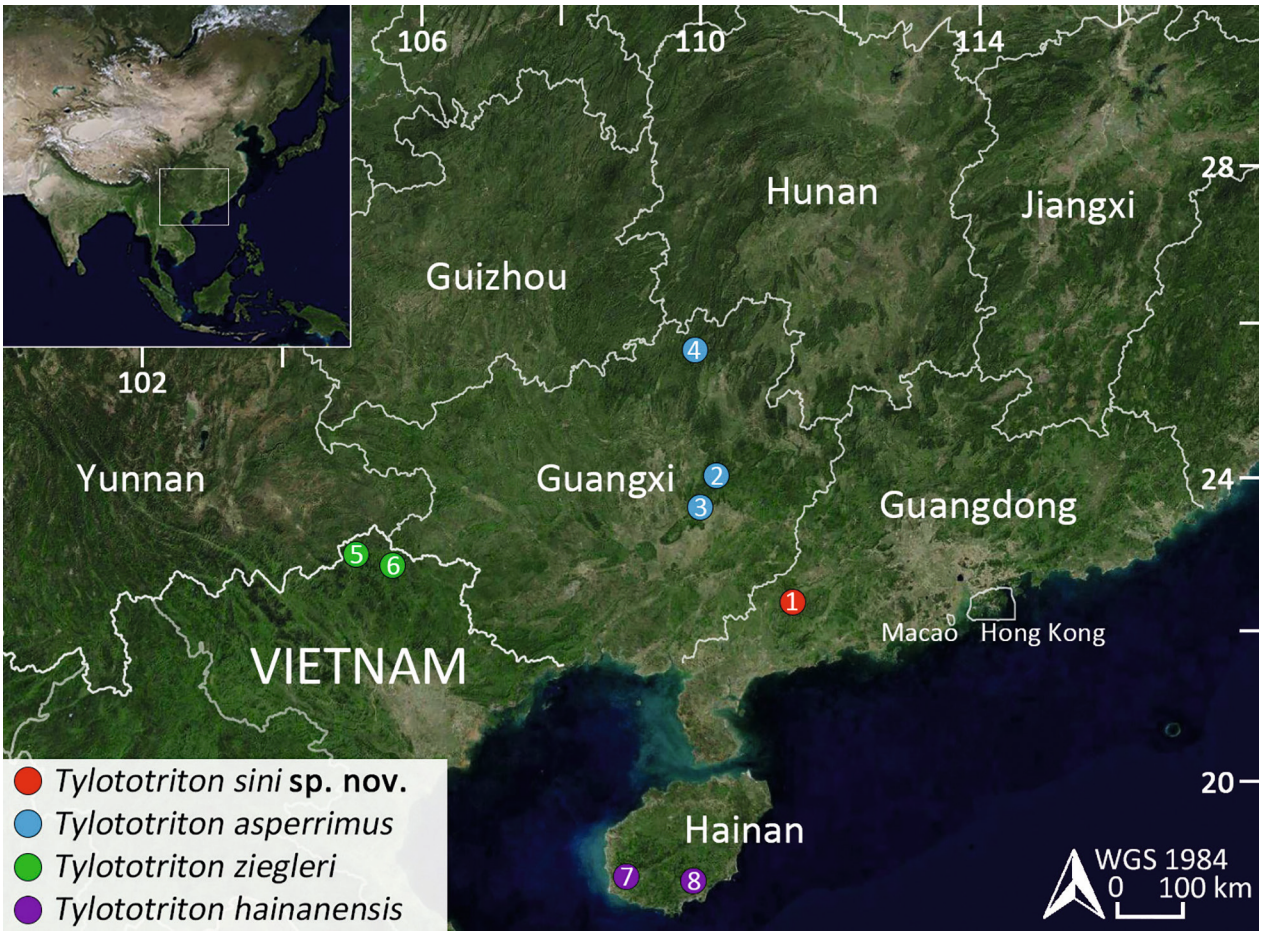


**Figure 1.** Map showing the localities of *Tylototriton sini* sp. nov., *T. asperrimus*, *T. ziegleri*, and *T. hainanensis*. *Tylototriton sini* sp. nov.: **1** Mt Yunkai, Xinyi, Guangdong, China (type locality); *T. asperrimus*: **2** Mt Dayao, Jiuxin, Guangxi, China (type locality) **3** Guiping, Guangxi, China **4** Longsheng, Guangxi, China; *T. ziegleri*: **5** Quan Ba, Ha Giang, Vietnam (type locality) **6** Bao Lac, Cao Bang, Vietnam; *T. hainanensis*: **7** Mt Jianfengling, Hainan, China **8** Mt Diaoluo, Hainan, China.

samples of *T. asperrimus* from Guangxi, one sample of *T. broadoridgus* Shen, Jiang & Mo, 2012, one sample of *T. kweichowensis* Fang & Chang, 1932, and one sample of *T. maolanensis* Li, Wei, Cheng, Zhang & Wang, 2020. All samples were attained from previously anesthetized and subsequently euthanized specimens and then preserved in 95% ethanol and stored at –40 °C.

Genomic DNA was extracted, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. One mitochondrion gene, namely NADH dehydrogenase subunit 2 (ND2), were amplified using the primers ND2-4F (5′-TATGAGTACGAGCATCATACCC-3′) and ND2-4R (5′-CTTCTGCTTAAGACTTTGAAGGTC-3′). PCR amplifications were processed with the cycling conditions that initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 95°C for 40 s, annealing at 53°C for 34 s and extending at 72°C for 60 s, and a final extending step at 72°C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit from Applied Biosystems, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences were deposited in GenBank (Table 1).

For phylogenetic analyses, 35 sequences from additional *Tylototriton* congeners and 2 sequences of the outgroup *Echinotriton* Nussbaum and Brodie, 1982, were obtained from GenBank and incorporated into our dataset. Detailed information is provided in Table 1. DNA sequences were aligned by the Clustal W algorithm with default parameters (Thompson et al. 1997). PartitionFinder2 was used to test the best partitioning scheme and jModelTest v2.1.2 was used to test the best fitting nucleotide substitution model. Sequenced data were analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012), and maximum likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in a BI analysis, each of which was performed for 10,000,000 generations and sampled every 1000 generations with the first 25% samples discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of <0.005. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Genetic distances among samples were calculated in MEGA 6 using the uncorrected *p*-distance model.

**Table 1.** Localities, voucher information, and GenBank accession numbers for all ingroup *Tylototriton* and outgroup *Echinotriton* samples used in this study.

| ID | Species | Locality | Voucher | ND2 |
|---|---|---|---|---|
| 1 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | SYS a004679 | OK539834 |
| 2 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | SYS a008353 | OK539835 |
| 3 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | SYS a008354 | OK539836 |
| 4 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | SYS a008355 | OK539837 |
| 5 | *Tylototriton* **sp. nov.** | China: Guangdong: Xinyi | CIB XZ20091201 | KY800876 |
| 6 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | GIABR 20187231 | MH664279 |
| 7 | *Tylototriton* **sp. nov.** | China: Guangdong: Mt Yunkai | GIABR 20187232 | MH664280 |
| 8 | *T. asperrimus* | China: Guangxi: Guiping | SYS a006890 | OK539838 |
| 9 | *T. asperrimus* | China: Guangxi: Guiping | SYS a006891 | OK539839 |
| 10 | *T. asperrimus* | China: Guangxi: Guiping | SYS a008200 | OK539840 |
| 11 | *T. asperrimus* | China: Guangxi: Guiping | SYS a008201 | OK539841 |
| 12 | *T. asperrimus* | China: Guangxi: Longsheng | CIB 70063 | KC147816 |
| 13 | *T. asperrimus* | China: Guangxi: Jinxiu | CIB 200807055 | KC147815 |
| 14 | *T. asperrimus* | China: Guangxi: Mt. Dayao | KIZ YPX9918 | KT304303 |
| 15 | *T. ziegleri* | Vietnam: Cao Bang: Bao Lac | VNMN 3389 | KY800888 |
| 16 | *T. ziegleri* | Vietnam: Ha Giang: Quan Ba | VNMN 3390 | AB769539 |
| 17 | *T. hainanensis* | China: Hainan: Mt Diaoluo | CIB 20081048 | KC147817 |
| 18 | *T. hainanensis* | China: Hainan: Mt Jianfengling | MVZ 236632 | DQ517850 |
| 19 | *T. anguliceps* | Laos: Luang Namtha: Viengphoukha | NCSM 82952 | KT304300 |
| 20 | *T. anhuiensis* | China: Anhui: Yuexi | AHU-16-EE-007 | KY321413 |
| 21 | *T. broadoridgus* | China: Hunan: Mt Huping | SYS a008391 | OK539842 |
| 22 | *T. dabienicus* | China: Henan: Shangcheng | HNNU10042015 | KC147811 |
| 23 | *T. himalayanus* | Nepal: Mechi: Illam | CIB 201406287 | KT765210 |
| 24 | *T. kachinorum* | Myanmar: Kachin: Indawgyi | ZMMU A5953 | MK097273 |
| 25 | *T. kweichowensis* | China: Guizhou: Shuicheng District | SYS a004967 | OK539843 |
| 26 | *T. liuyangensis* | China: Hunan: Liuyang | CSUFT 20100108 | KJ205598 |
| 27 | *T. lizhengchangi* | China: Hunan: Yizhang | KUHE 42317 | AB769533 |
| 28 | *T. maolanensis* | China: Guizhou: Libo County | SYS a002212 | OK539844 |
| 29 | *T. ngarsuensis* | Myanmar: Shan: Taunggyi | LSUHC 13763 | MH836584 |
| 30 | *T. notialis* | Vietnam: Nghe An: Pu Hoat | VNMN TAO1235 | AB769536 |
| 31 | *T. panhai* | Thailand: Loei: Phu Hin Rong Kla NP | KUHE PH019 | AB830735 |
| 32 | *T. panwaensis* | Myanmar: Kachin: Myitkyina | CAS 245418 | KT304279 |
| 33 | *T. pasmansi* | Vietnam: Phu Tho: Tan Son | IEBR 4467 | MT210167 |
| 34 | *T. phukhaensis* | Thailand: Nan: Doi Phu Kha NP | CUMZ A-7718 | MN912574 |
| 35 | *T. podichthys* | Laos: Luang Phabang: Phoukhoun | NCSM 77725 | KT304295 |
| 36 | *T. pseudoverrucosus* | China: Sichuan: Ningnan | CIB WCG2012003 | KY800861 |
| 37 | *T. pulcherrima* | China: Yunnan: Lyuchun | CIB TY040 | KY800890 |
| 38 | *T. shanjing* | China: Yunnan: Jingdong | MVZ219763 | DQ517852 |
| 39 | *T. shanorum* | Myanmar: Shan: Taunggyi | CAS 230933 | AB922822 |
| 40 | *T. sparreboomi* | Sin Ho, Lai Chau, Vietnam | IEBR 4476 | MT210162 |
| 41 | *T. taliangensis* | China: Sichuan: Liangshan | CAS 195126 | DQ517853 |
| 42 | *T. uyenoi* | Thailand: Chiang Mai: Doi Suthep | KUHE 19147 | AB830733 |
| 43 | *T. verrucosus* | China: Yunnan: Longchuan | CIB TSHS2 | KY800848 |
| 44 | *T. vietnamensis* | Vietnam: Bac Giang: Son Dong | IEBR 3243 | HM770088 |

| ID | Species | Locality | Voucher | ND2 |
|---|---|---|---|---|
| 45 | *T. wenxianensis* | China: Gansu: Wenxian | CIB 20090527 | KC147813 |
| 46 | *T. yangi* | China: Yunnan: Pingbian | KIZ RDQ201203001 | LC017829 |
| 47 | *E. chinhaiensis* | China: Zhejiang: Ningbo | TP26195 | EU880315 |
| 48 | *E. maxiquadratus* | China | SYNU SY20131101ENT | KM926344 |

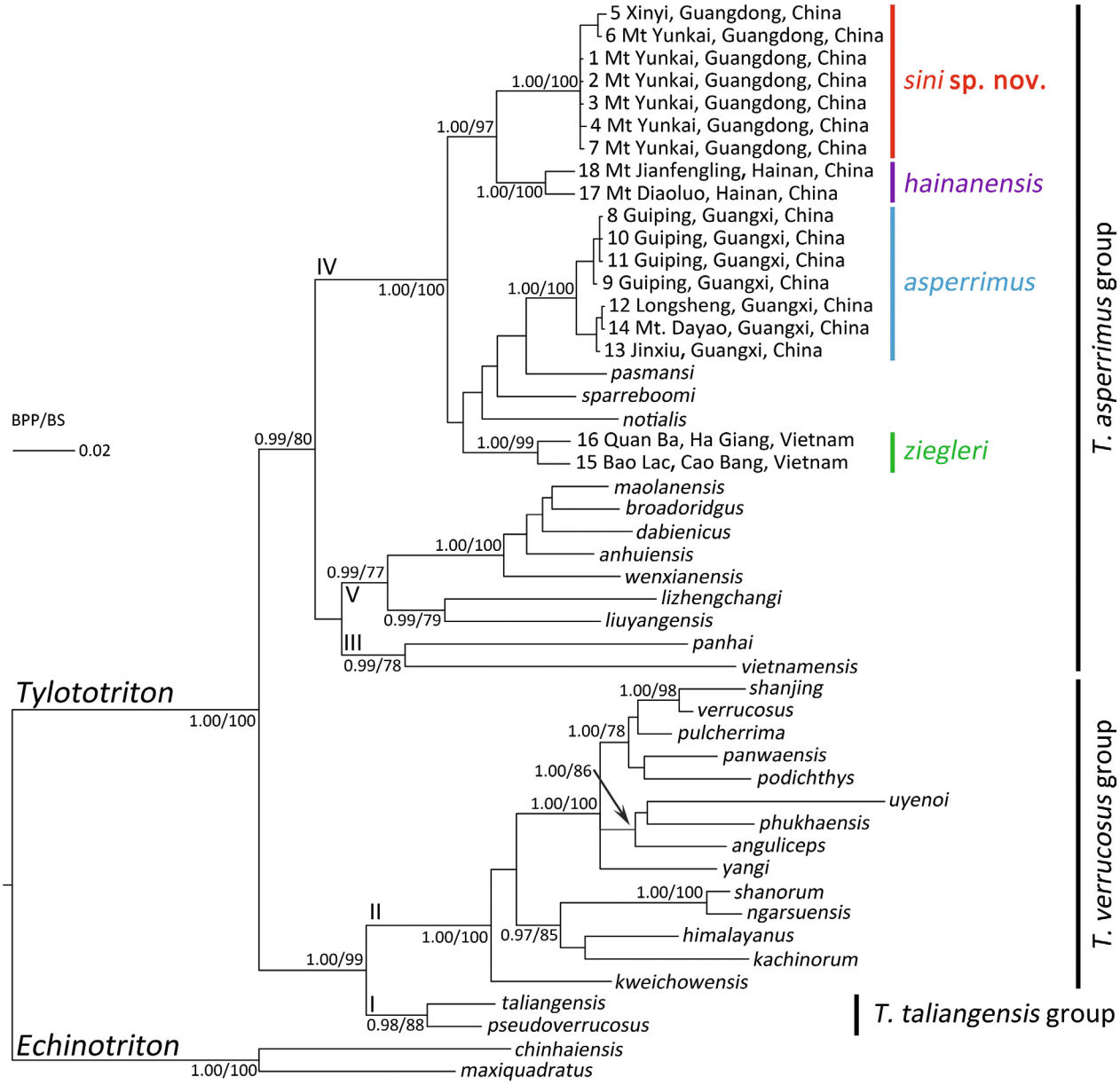


**Figure 2.** Bayesian inference and maximum-likelihood phylogenies based on mitochondrial ND2 gene. Bayesian posterior probabilities (BPP) > 0.95 and the bootstrap supports (BS) > 70 are shown. For the species with more than one samples used, number at the terminal of the lineage corresponds to the ID in Table 1 and the collecting locality is labelled.

## Results

The BI and ML analyses resulted in identical topologies (Fig. 2). Most nodes were well supported with the Bayesian posterior probabilities (BPP) > 0.95 and the bootstrap supports (BS) > 70. The *p*-distances based on the ND2 gene among all samples used in this study are presented in Supplementary Table S1. As shown in the tree, five clades of *Tylototriton* were revealed. Within the clade IV (BPP 1.00, BS 100), the samples from Mt Yunkai, Xinyi, Guangdong, China clustered into a monophyletic lineage with small divergence (*p*-distances 0.0–0.7%) and strong support (BPP 1.00, BS 100). This lineage is phylogenet-

ically distant from *T. asperrimus* from Guangxi, China and *T. ziegleri* from northern Vietnam, but forms the sister taxon to *T. hainanensis* from Hainan, China (BPP 1.00, BS 97).

Morphologically, the *Tylototriton* specimens from this unnamed lineage can be distinguished from all known congeners reliably (details in the Taxonomic account below). Combining the results of the morphological examination presented below and the phylogenetic analyses in this and previous studies, the *Tylototriton* population from Mt Yunkai, Xinyi, Guangdong is regarded as a new species and described herein.

## Taxonomic account

### *Tylototriton sini* sp. nov. Lyu, Wang, Zeng, Zhou, Qi, Wan & Wang

*Tylototriton asperrimus* — Fei et al. 1990 (Xinyi, Guangdong); Fei et al. 2006 (Xinyi, Guangdong); Li et al. 2011 (Mt Yunkai, Xinyi, Guangdong)

*Echinotriton asperrimus asperrimus* — Zhao and Adler 1993 (Guangdong)

*Yaotriton asperrimus* — Fei et al. 2012 (Xinyi, Guangdong); Fei and Ye 2016 (Xinyi, Guangdong)

*Tylototriton asperrimus* lineage 2 — Wang et al. 2018 (Xinyi, Guangdong)

*Tylototriton ziegleri* — Li et al. 2020 (Mt Yunkai, Xinyi, Guangdong)

*Tylototriton* sp. 3 — Poyarkov et al. 2021 (Xinyi, Guangdong)

**Holotype.** SYS a008354 (Figs. 3, 4A), adult male, collected by Jian Wang, Shuo Qi, and Hong-Hui Chen on 14 June 2020 from Mt Yunkai (22°16’32.90”N, 111°11’42.87”E; ca 1500 m a.s.l.), Xinyi, Guangdong, China.

**Paratypes.** Two adult males and one adult female (Figs. 4B, C, D, 5A, B). Male SYS a008353 and female SYS a008355, the same collection data as the holotype; male SYS a004679 /CIB 116083, collected by Jian Wang, Zhi-Tong Lyu, and Zhao-Chi Zeng on 16 April 2016 from the same locality as the holotype.

**Etymology.** The specific name *sini* refers to the outstanding biologist Prof. Shu-Szi Sin (= Shu-Zhi Xin, 辛树帜, 1894–1977). During his position at Sun Yat-sen University (1927–1931), Prof. Shu-Szi Sin organized repeated biology surveys throughout Guangxi, Guangdong, Guizhou, Hunan, and Hainan in southern China, pochally promoting the developments of zoological and botanic studies in this region. He collected specimens of *T. asperrimus* for the first time, as well as other amphibians and reptiles such as *Quasipaa shini* (Ahl, 1930) and the famous *Shinisaurus crocodilurus* Ahl, 1930. His family name “Sin” was mispronounced as “Shin” by the German researchers (Beolens et al. 2011), and we decided to use the correct spelling for this new species as *Tylototriton sini* sp. nov. in honor of Prof. Sin and his contributions.

**Common name.** Sin’s Knobby Newt (in English) / xīn shì yóu yuán **(辛氏疣螈** in Chinese).

**Diagnosis.** (1) Dorsolateral bony ridges on head low; (2) quadrate spines absent; (3) medium body size, TOL 118.4–124.5 mm in males, 144.5 mm in a single female; (3) snout obtusely rounded in dorsal view and rounded in lateral profile; (4) head longer than wide, HW/HL ratio 0.87–0.95; (5) supratemporal bony ridges and the sagittal ridge on head distinctly visible; (6) limbs slender, tips of forelimb and hindlimb overlapping when adpressed along the body; (7) vertebral ridge distinct, relatively smooth, not segmented; (8) rib nodules 12–13, relatively small, distinctly isolated from each other; (9) ground coloration dark brown; (10) digits orange with irregular dark brown mottling; (11) in breeding season, rib nodules mottled with orange coloration, much brighter in the first two rib nodules; (12) in breeding season, lateral tail dark brown, fin with dorsal orange margin, ventral tail ridge orange.

**Description of the holotype.** SYS a008354 (Figs. 3, 4A), adult male with a stout body, medium in size (SVL 62.0 mm, TAL 56.4 mm). Head longer than wide (HW/HL ratio 0.93); maximum head width slightly larger than the maximum trunk width; head nearly rounded hexagonal in shape in dorsal view, depressed, gently sloping in profile. Snout obtusely rounded in dorsal view, rounded in profile view, projecting beyond lower jaw. Nostril on anterior margin of snout, located notably closer to snout tip than to eye, with anterolateral orientation, not visible from dorsal view. Tongue oval, not notched distally; 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, distinctly protruding, beginning at the anterior corner of orbit continuing to anterior end of parotoid, posterior ends slightly curved inside; sagittal bony ridge on head strong.

Vertebral middorsal ridge distinct, wide, not segmented, running from occiput region to sacrum and the base of tail. Rib nodules distinct, relatively small, distinctly isolated from each other but arranged in two longitudinal series on dorsolateral surfaces of dorsum from shoulder to base of tail, counting 13 nodules on each side of body.

Limbs slender, forelimb and hindlimb overlapping when adpressed towards each other along body; fingers and toes well developed, lacking webbing or fringes; relative finger lengths I < IV < III = II, relative toe lengths I = V < II < III = IV. Tail long, TAL/SVL ratio 0.91; laterally compressed along entire length, tapering posteriorly, lateral grooves on tail distinctly visible in dorsal view.

Skin of dorsum, flanks, and lateral sides of tail very rough with small granules and larger warts. Skin of head ridges and middorsal vertebral ridge relatively smooth. Skin of limbs with numerous tiny tubercles. Ventral surfaces relatively smoother, corrugated, with smaller granules arranged in transverse striations; throat with numerous tiny flat tubercles; weak gular fold present. Cloacal region slightly swollen, vent as a longitudinal slit, vent edges with numerous small transverse folds.

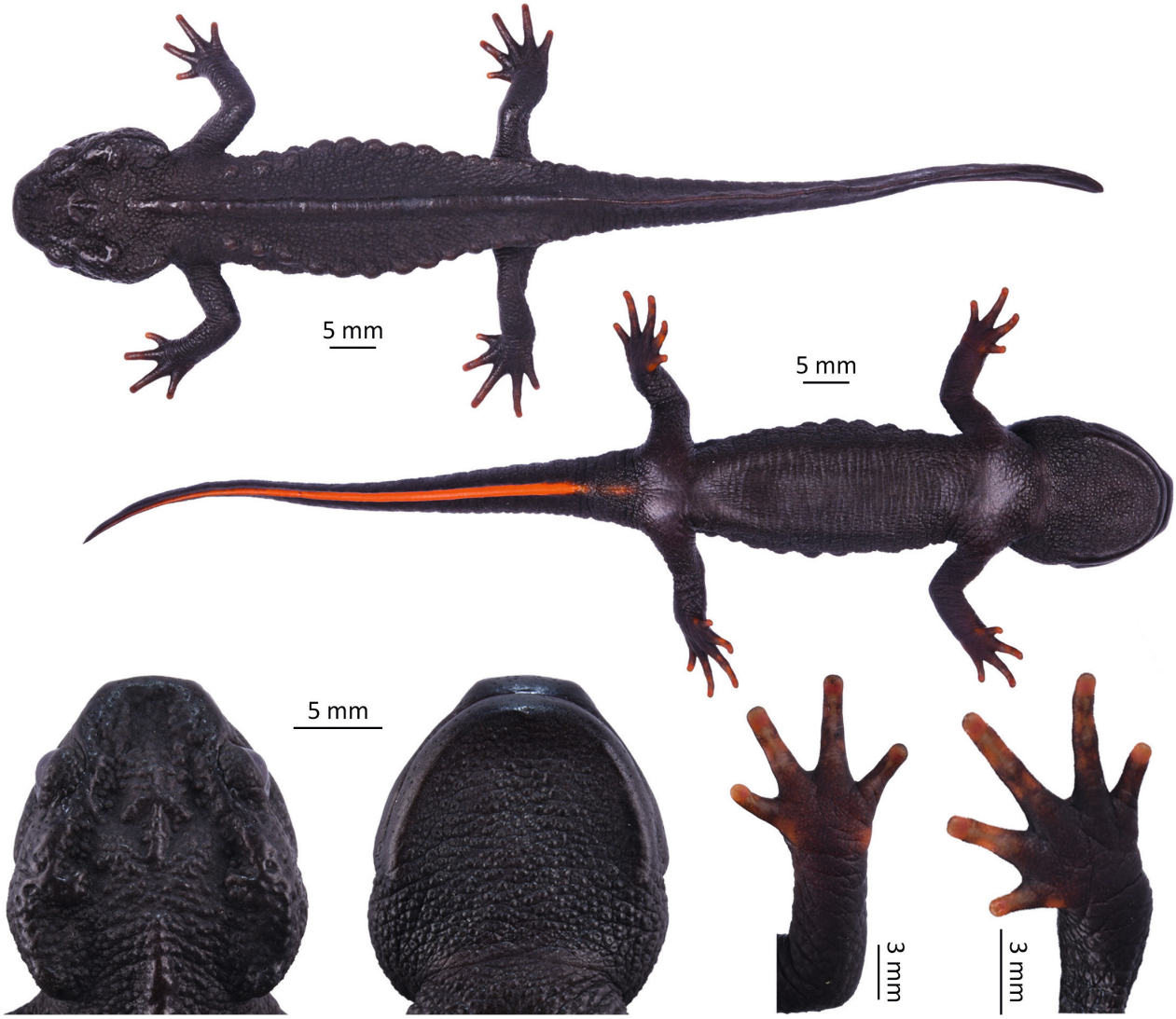


**Figure 3.** Holotype SYS a008354 of *Tylototriton sini* sp. nov. in life., showing the features in dorsal full body, ventral full body, dorsal head, ventral head, ventral left hand, and ventral left foot.

**Coloration of holotype.** In life (Fig. 3), ground color of head, dorsum, lateral tail, limbs, and venter uniform dark brown. Rib nodules mottled with orange coloration, the coloration of the first two rib nodules much brighter. Dorsal skin of hands and feet excluding the digits dark brown. Digits orange, with irregular dark brown mottling. Tail fin with dorsal orange margin, ventral tail ridge orange.

In preservative (Fig. 4A), ground color of head, dorsum, tail, limbs, and venter uniform dark. Orange coloration on all rib nodules fade. Orange coloration on the digits change into light brown. Ventral tail ridge pale yellow. Vent region pale yellow.

**Variations.** Measurements of the type series are given in Table 2. The single female paratype SYS a008355 (Figs. 4D) possesses a larger and more robust body, however, its tail is relatively shorter, TAL/SVL ratio 0.82 vs 0.87–0.91 in males. Both the male holotype SYS a008354 (Figs. 3, 4A) and the male paratype SYS a004679 (Figs. 4B, 5A) bear 13 rib nodules on each side of body, while only 12 rib nodules in the male paratype SYS a008353 (Figs. 4C, 5B) and the female paratype SYS a008355 (Figs. 4D). In life, all rib nodules mottled with orange coloration, and much brighter in the first two rib nodules in several individuals (Figs. 3, 5A, 5C).

**Comparisons.** *Tylototriton sini* sp. nov. was recorded as *T. asperrimus* for a long time, but can be distinguished by the head longer than wide (vs head wider than long in *T. asperrimus*), the snout obtusely rounded in dorsal view (vs truncate), the distal tip of limbs greatly overlapping when the fore and hind limbs pressed along the trunk (vs slightly overlapping or just meeting), rib nodules small and distinctly isolated from each other (vs rib nodules large, knob-like, and nearly in contact with each other), rib nodules with orange coloration (vs rib nodules completely black or brown), and tail fin with dorsal orange margin (vs completely brownish black).

*Tylototriton sini* sp. nov. further differs from other congeners within the clade IV (Fig. 2) by the head longer than wide (vs head wider than long in *T. hainanensis*, *T. thaiorum* Poyarkov, Nguyen & Arkhipov, 2021, and *T. ziegleri*), snout obtusely rounded in dorsal view (vs truncate in *T. pasmansi*, *T. sparreboomi*, *T. notialis*

**Table 2.** Measurements (in mm) of *Tylototriton sini* sp. nov., * for the holotype.

| | SYS a008354 * | SYS a004679 /CIB 116083 | SYS a008353 | SYS a008355 |
|---|---|---|---|---|
| Sex | M | M | M | F |
| TOL | 118.4 | 124.5 | 118.6 | 144.5 |
| SVL | 62.0 | 66.5 | 63.2 | 79.3 |
| HL | 18.1 | 18.5 | 19.1 | 20.1 |
| HW | 16.8 | 16.8 | 16.7 | 19.0 |
| ED | 4.0 | 4.1 | 4.1 | 4.6 |
| SL | 5.8 | 5.7 | 5.9 | 6.5 |
| IOD | 7.3 | 7.6 | 7.1 | 6.7 |
| IND | 5.1 | 5.5 | 5.4 | 6.4 |
| TRL | 42.5 | 46.4 | 44.3 | 57.0 |
| TAL | 56.4 | 58.0 | 55.4 | 65.2 |
| TH | 8.1 | 8 | 7.1 | 7 |
| LLA | 7.3 | 7.3 | 6.9 | 8.9 |
| HL | 6.8 | 7.7 | 7.1 | 8.6 |
| F3L | 4.4 | 4.5 | 4.5 | 5.3 |
| TLH | 4.7 | 5.8 | 5.8 | 6.7 |
| TIB | 4.9 | 5.6 | 5.8 | 7.1 |
| T3L | 4.6 | 5.1 | 5.1 | 6.3 |

Stuart, Phimmachak, Sivongxay & Robichaud, 2010, and *T. ziegleri*), snout rounded in profile view (vs slightly angular in *T. pasmansi* and *T. notialis*), sagittal bony ridge on head strong (vs obscure in *T. hainanensis*), vertebral ridge not segmented (vs segmented, forming a row of tubercles in *T. ziegleri*), absence of orange markings on the parotoid (vs present in *T. pasmansi* and *T. notialis*), and the presence of orange coloration on all rib nodules (vs absent in *T. hainanensis*, *T. pasmansi*, *T. sparreboomi*, *T. thaiorum*, and *T. ziegleri*).

For the species within clades III and IV (Fig. 2), *Tylototriton sini* sp. nov. can be distinguished from *T. panhai* Nishikawa, Khonsue, Pomchote & Matsui, 2013 by the absence of orange markings on the parotoid (vs present); from *T. vietnamensis* Böhme, Schöttler, Nguyen & Köhler, 2005 by the presence of gular fold (vs absent); from *T. maolanensis* by the smaller body size with TOL 118.4–124.5 mm in adult males (vs larger body size with TOL 151.0–172.0 mm in adult males); and from *T. anhuiensis* Qian, Sun, Li, Guo, Pan, Kang, Wang, Jiang, Wu & Zhang, 2017, *T. broadoridgus*, *T. dabienicus* Chen, Wang & Tao, 2010, *T. liuyangensis* Yang, Jiang, Shen & Fei, 2014, *T. lizhengchangi* Hou, Zhang, Jiang, Li & Lu, 2012, and *T. wenxianensis* by the rib nodules swollen and distinctly isolated from each other (vs rib nodules not separated, almost in continuous longitudinal rows).

For the remaining species within clades I and II (Fig. 2), *Tylototriton sini* sp. nov. can be distinguished from *T. taliangensis* Liu, 1950 by the absence of orange markings on the parotoid (vs present); from *T. pseudoverrucosus* Hou, Gu, Zhang, Zeng & Lu, 2012, *T. anguliceps* Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes & Nguyen, 2015, *T. himalayanus* Khatiwada, Wang, Ghimire, Vasudevan, Paudel & Jiang, 2015, *T. kachinorum* Zaw, Lay, Pawangkhanant, Gorin & Poyarkov, 2019, *T. ngarsuensis* Grismer, Wood, Quah, Thura, Espinoza, Grismer, Murdoch & Lin, 2018, *T. panwaensis* Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019, *T. phukhaensis* Pomchote, Khonsue, Thammachoti, Hernandez, Peerachidacho, Suwannapoom, Onishi & Nishikawa, 2020, *T. podichthys* Phimmachak, Aowphol & Stuart, 2015, *T. pulcherrima* Hou, Zhang, Li & Lu, 2012, *T. shangjing* Nussbaum, Brodie & Yang, 1995, *T. shanorum* Nishikawa, Matsui & Rao, 2014, *T. uyenoi* Nishikawa, Khonsue, Pomchote & Matsui, 2013, and *T. verrucosus* Anderson, 1871 by its limbs dark brown except for the orange digits, palms, and soles (vs limbs uniformly orange or light brown); and from *T. kweichowensis* and *T. yangi* Hou, Zhang, Zhou, Li & Lu, 2012 by its tail dark brown, except fin with dorsal orange margin and ventral tail ridge orange (vs tail uniformly orange).

**Distribution.** *Tylototriton sini* sp. nov. is currently known only from its type locality Mt Yunkai and the neighboring Mt Ehuangzhang (this study; Hernandez 2018), both situated in the Yunkai Mountains of western Guangdong.

**Natural history.** This newt is terrestrial and inhabits leaf litters in well-preserved montane evergreen broadleaf forest. During its breeding season from April to July, adult individuals can be observed in small ponds with muddy bottoms, small marshes, and vernal pools. Larvae can be found from June to August. On 15 August 2017, different stages of larvae were observed in the same vernal pool near the road (ca 2 m long and ca 3 m wide of the pool with water depth ca 4 cm), without adults observed (Fig. 6).

**Conservation recommendation.** The extent of occurrence of *Tylototriton sini* sp. nov. is estimated to be less than 100 km$^2$, and the area of occupancy is estimated to be less than 10 km$^2$. Habitat degradation due to tourism development and illegal capture are the major threats. We recommend *Tylototriton sini* sp. nov. to be listed

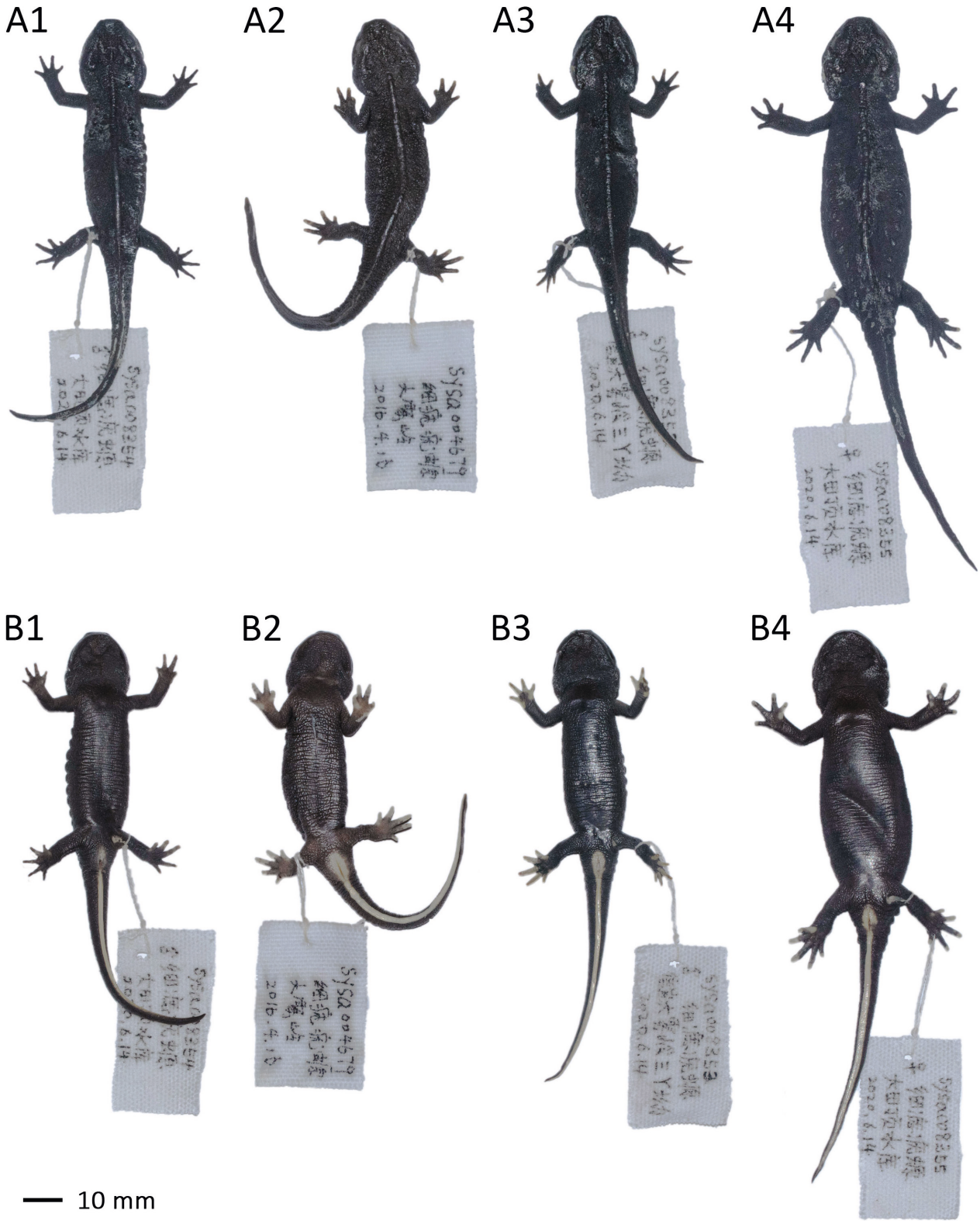


**Figure 4.** Type series of *Tylototriton sini* sp. nov. in preservative. **A** dorsal view **B** ventral view **1** male holotype SYS a008354 **2** male paratype SYS a004679 **3** male paratype SYS a008353 **4** female paratype SYS a008355.

as Critically Endangered (CR) [IUCN Red List criteria B1ab(iii)+2ab(iii)]. This species must be added in the Appendix II of CITES, as the *Tylototriton* spp. are collectively included (CITES 2021). National Forestry and Grassland Administration of China (2021) has declared that the species *T. asperrimus* (as *Y. asperrimus*) is one of the Class II protected species of China, which was before the description of *Tylototriton sini* sp. nov. in this work. Therefore, the "*T. asperrimus* (as *Y. asperrimus*)" being listed as protected species of China should include the Guangdong population (now *Tylototriton sini* sp. nov.). Thus, we suggest *Tylototriton sini* sp. nov. should be also regarded as protected species of China with at least Class II.


**Figure 5.** *Tylototriton sini* sp. nov. in life. **A** male paratype SYS a004679 **B** male paratype SYS a008353 in situ **C** an uncaptured individual in situ.

## Discussion

The phylogeny of genus *Tylototriton* has been well studied on the basis of multi-locus of mitochondrial and nuclear data (Wang et al. 2018). Subsequently, Poyarkov et al. (2021) employed two mitochondrial segments and got a unanimous result. In this study, our phylogenetic result from the sole mitochondrial ND2 segment is consistent with the topology revealed in the above studies, suggesting that the ND2 gene is adequate for settling the phylogeny in the genus *Tylototriton*.

After the taxonomic revisions in this work and previous studies (Nishikawa et al. 2013a; Wang et al. 2018; Bernardes et al. 2020), the species *T. asperrimus* can be confirmed to be present in Jiuxiu and Longsheng counties in northeastern Guangxi based on molecular data, while the taxonomic status for the populations from other localities such as northern and southwestern Guangxi (Mo et al. 2014) remain unknown and further studies are required. As the *Tylototriton* population from Guangdong is substantiated to be a new species *Tylototriton sini* sp. nov., the records of *T. asperrimus* and *T. ziegleri* should be removed from the herpetofauna of Guangdong. Given our recent findings, another record of *T. ziegleri* from China (Malipo County, Yunnan), recently published by Ye et al. (2017), should be re-examined whether it in fact represents the species that originally was described from northern Vietnam.

**Figure 6.** Different stages of larvae were observed in the same vernal pool without adults observed on 15 August 2017 from the type locality of *Tylototriton sini* sp. nov. in Mt Yunkai.

The taxonomy for interspecific relationships in the genus *Tylototriton* is controversial for decades (Zhao and Adler 1993; Dubois and Raffaëlli 2009; Fei et al. 2012; Nishikawa et al. 2013a; Fei and Ye 2016; Dubois et al. 2021; Poyarkov et al. 2021), and several nomenclatures were proposed to accommodate different species, such as *Yaotriton* Dubois & Raffaëlli, 2009, *Qiantriton* Fei, Ye & Jiang, 2012, and *Liangshantriton* Fei, Ye & Jiang, 2012. In the nearest study, the genus *Tylototriton* was partitioned into two subgenera *Tylototriton* and *Yaotriton*, and further into five species groups, based on the phylogenetic topology (Poyarkov et al. 2021). Despite the phylogenetic separation of *Tylototriton* and *Yaotriton*, the morphological characters for these two subgenera/genera remain unclear (Nishikawa et al. 2013a). The five species groups are corresponding to the five robust clades in phylogeny (Wang et al. 2018; Poyarkov et al. 2021; this work), while the morphological definitions for these five groups were not yet provided. Indeed, the morphological differences are unclear among some of these groups, for instance, *T. maolanensis* is morphologically similar to *T. asperrimus* but phylogenetically close to *T. wenxianensis*, and *T. pasmansi*, *T. thaiorum*, and *T. ziegleri* used to be morphologically identified as *T. vietnamensis* but phylogenetically close to *T. asperrimus*. Furthermore, as a genus including 33 recognized species, we consider it is too overstaffed to partition *Tylototriton* into both subgenera and species groups.

Thus, after a comprehensive review on these species, we suggest to divide the genus *Tylototriton* into three species groups, which is most reasonable with their morphological differences and phylogenetic relationships. Below we provide a key for the three species groups with their morphological definitions. We further provide a key for the *T. asperrimus* group recognized in this work, which includes the former *T. asperrimus*, *T. wenxianensis*, and *T. vietnamensis* groups in Poyarkov et al. (2021).

## Key to species groups within genus *Tylototriton*

1a Body relatively robust, TAL smaller, equal, or slightly larger than SVL, TAL/SVL < 120% in adult males .....**2**

1b Body slender, TAL distinctly larger than SVL, TAL/SVL > 125% in adult males..... ..... ***T. taliangensis* group (2 species)**

2a Tail with different colorations, the lateral tail with the same color as the ground coloration of body, the dorsal tail fin or/and ventral tail ridge with orange margin ..... ***T. asperrimus* group (17 species)**

2b Tail with uniform coloration, orange or light brown, much brighter and lighter than the ground coloration of body and head ..... ***T. verrucosus* groups (14 species)**

## Key to species within *Tylototriton asperrimus* group

1a Vertebral ridge segmented, tuberculate ..... ***T. ziegleri***
1b Vertebral ridge not segmented ..... **2**
2a Rib nodules flatten, indistinct, not separated, and almost in continuous longitudinal rows forming a dorsolateral fold ..... **3**
2b Rib nodules swollen, distinct, clearly separated or slightly contacted at the base ..... **8**
3a Orange markings on the parotoid present in males ..... ***T. lizhengchangi***
3b Orange markings on the parotoid absent in males ..... **4**
4a Head as wide as long ..... **5**
4b Head longer than wide ..... **6**
5a Peripheral area of cloaca brownish black ..... ***T. wenxianensis***
5b Peripheral area of cloaca orange ..... ***T. liuyangensis***
6a Vertebral ridge rather wide, its width approximately equal to the eye diameter ..... ***T. broadoridgus***
6b Vertebral ridge narrow, its width smaller than the eye diameter ..... **7**
7a The orange coloration at the ventral edge of tail contacted with the orange coloration around the cloaca ..... ***T. anhuiensis***
7b The orange coloration at the ventral edge of tail isolated from the orange coloration around the cloaca ..... ***T. dabienicus***
8a Orange markings on the parotoid present ..... **9**
8b Orange markings on the parotoid absent ..... **10**
9a Anterior half of head and vertebral ridge yellow to reddish brown ..... ***T. panhai***
9b Anterior half of head and vertebral ridge dark brown, with the same color as the ground coloration of body ..... ***T. notialis***
10a Head wider than long ..... **11**
10b Head longer than wide ..... **12**
11a Sagittal bony ridge on head strong ..... ***T. asperrimus***
11b Sagittal bony ridge on head obscure ..... ***T. hainanensis***
12a Orange coloration of the rib nodules present ..... **13**
12b Orange coloration of the rib nodules absent ..... **14**
13a Smaller body size with TOL 118.4–124.5 mm in adult males ..... ***T. sini* sp. nov.**
13b Larger body size with TOL 151.0–172.0 mm in adult males ..... ***T. maolanensis***
14a Gular fold absent ..... ***T. vietnamensis***
14b Gular fold present ..... **15**
15a Snout obtusely rounded in dorsal view; rib nodules light brown ..... ***T. thaiorum***
15b Snout truncate in dorsal view; rib nodules uniformly black ..... **16**
16a Tips of fingers reaching nostril, rib nodules slightly enlarged round-like ..... ***T. sparreboomi***
16b Tips of fingers reaching eye, rib nodules slightly smaller, pointy to rounded ..... ***T. pasmansi***

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## References

Anderson J (1871) Description of a new genus of newts from western Yunnan. Proceedings of the Zoological Society of London 1871: 423–425.

Bain RH, Nguyen TQ (2004) Herpetofauna diversity of Ha Giang Province in northeastern Vietnam, with descriptions of two new species. American Museum Novitates 3453: 1–42.

Bauer AM, Good DA, Günther R (1993) An annotated type catalogue of the caecilians and salamanders (Amphibia: Gymnophiona and Caudata) in the Zoological Museum, Berlin. Mitteilungen aus dem Zoologischen Museum in Berlin 69: 285–306.

Beolens B, Watkins M, Grayson M (2011) The Eponym Dictionary of Reptiles. The Johns Hopkins University Press, Baltimore, Maryland.

Bernardes M, Le MD, Nguyen TQ, Pham CT, Pham AV, Nguyen TT, Rödder D, Bonkowski M, Ziegler T (2020) Integrative taxonomy reveals three new taxa within the *Tylototriton asperrimus* complex (Caudata, Salamandridae) from Vietnam. ZooKeys 935: 121–164. https://doi.org/10.3897/zookeys.935.37138

Böhme W, Schöttler T, Nguyen TQ, Köhler J (2005) A new species of salamander, genus *Tylototriton* (Urodela: Salamandridae) from northern Vietnam. Salamandra 41: 215–220.

Chen XH, Wang XW, Tao J (2010) A new subspecies of genus *Tylototriton* from China (Caudata, Salamandridae). Acta Zootaxonomica Sinica 35: 666–670.

CITES (2021) Convention on international trade in endangered species of wild fauna and flora Appendices I, II and III valid from 22 June 2021. https://cites.org/eng/app/appendices.php

Dubois A, Raffaëlli J (2009) A new ergotaxonomy of the family Salamandridae Goldfuss, 1820 (Amphibia, Urodela). Alytes 26: 1–85.

Dubois A, Ohler A, Pyron RA (2021) New concepts and methods for phylogenetic taxonomy and nomenclature in zoology, exemplified by a new ranked cladonomy of recent amphibians (Lissamphibia). Megataxa 5: 1–738. https://doi.org/10.11646/megataxa.5.1.1

Fan TH (1931) Preliminary report of reptiles from Yaoshan, Kwangsi, China. Bulletin of the Biological Department, College of Science, Sun Yat-sen University 11: 1–154.

Fang PW, Chang MLY (1932) Notes on *Tylototriton kweichowensis* sp nov. and *asperrimus* Unterstein. Sinensia 2: 111–122.

Fei L, Hu SQ, Ye CY, Huang YZ (2006) Fauna Sinica. Amphibia. Volume 1. General Accounts of Gymnophiona and Urodela. Science Press, Beijing.

Fei L, Ye CY (2016) Amphibians of China (Vol. 1). Science Press, Beijing.

Fei L, Ye CY, Huang YZ (1990) Key to Chinese Amphibians. Publishing House for Scientific and Technological Literature, Chongqing.

Fei L, Ye CY, Jiang JP (2012) Colored Atlas of Chinese Amphibians and Their Distributions. Sichuan Publishing House of Science & Technology, Chengdu.

Fei L, Ye CY, Yang RS (1984) A new species and subspecies of the genus *Tylototriton* (Caudata: Salamandridae). Acta Zoologica Sinica 30: 85–91.

Frost DR (2021) Amphibian Species of the World 6.1, an Online Reference. American Museum of Natural History, New York. https://amphibiansoftheworld.amnh.org [accessed 20 August 2021]

Grismer LL, Wood PL Jr, Quah ESH, Thura MK, Espinoza RE, Grismer MS, Murdoch ML, Lin A (2018) A new species of Crocodile Newt *Tylototriton*(Caudata: Salamandridae) from Shan State, Myanmar (Burma). Zootaxa 4500: 442–573.

Grismer LL, Wood PL Jr, Quah ESH, Thura MK, Espinoza RE, Murdoch ML (2019) A new species of crocodile newt *Tylototriton* (Caudata: Salamandridae) from northern Myanmar (Burma). Journal of Natural History 53: 475–495. https://doi.org/10.1080/00222933.2019.1587534

Hernandez A (2018) Ecological affinities of *Tylototriton asperrimus* Unterstein, 1930 (Amphibia, Caudata: Salamandridae) at Bainiu, Dayaoshan, Guangxi Province, China with an emphasis on its cryptic diversity. Bulletin de la Société Herpétologique de France 166: 79–82.

Hou M, Li PP, Lü SQ (2012) Morphological research development of genus *Tylototriton* and primary confirmation of the status of four cryptic populations. Journal of Huangshan University 14: 61–65.

Le DT, Nguyen TT, Nishikawa K, Nguyen SHL, Pham AV, Matsui M, Bernardes M, Nguyen TQ (2015) A new species of *Tylototriton* Anderson, 1871 (Amphibia: Salamandridae) from northern Indochina. Current Herpetology 34: 38–50.

Li H, Yang B, Chi H, Lai R, Liu C, Xu Z, Zhu X, Gong S, Chen J (2020) Distribution and Supplementary Description of *Tylototriton ziegleri* in China. Chinese Journal of Wildlife 41: 791–795. https://doi.org/10.19711/j.cnki.issn2310-1490.2020.03.032

Li S, Wei G, Cheng Y, Zhang B, Wang B (2020) Description of a new species of the Asian newt genus *Tylototriton* sensu lato (Amphibia: Urodela: Salamandridae) from southwest China. Asian Herpetological Research 11: 282–296. https://doi.org/10.16373/j.cnki.ahr.200026

Li ZC, Xiao Z, Liu SR (2011) Amphibians and reptiles of Guangdong. Guangdong Science and Technology Press, Guangzhou.

Liu CC (1950) Amphibians of western China. Fieldiana. Zoology Memoires 2: 1–397.

Liu CC, Hu SQ, Fei L, Huang CC (1973) On collections of amphibians from Hainan Island. Acta Zoologica Sinica 19: 385–404.

Mo YM, Wei ZY, Chen WC (2014) Colored atlas of Guangxi amphibians. Guangxi Science & Technology Publishing House, Nanning.

National Forestry and Grassland Administration of China (2021) Official release of the updated List of Wild Animals under Special State Protection in China. https://www.forestry.gov.cn/main/586/20210208/095403793167571.html

Nguyen TQ, Nguyen VS, Ho LT, Le QK, Nguyen TT (2009) Phylogenetic relationships and taxonomic review of the family Salamandridae (Amphibia: Caudata) from Vietnam (in Vietnamese). Vietnam Journal of Biotechnology 7: 325–333.

Nishikawa K, Khonsue W, Pomchote P, Matsui M (2013a) Two new species of *Tylototriton* from Thailand (Amphibia: Urodela: Salamandridae). Zootaxa 3737(3): 261–279. https://doi.org/10.11646/zootaxa.3737.3.5

Nishikawa K, Matsui M, Nguyen TT (2013b) A New Species of *Tylototriton* from northern Vietnam (Amphibia: Urodela: Salamandridae). Current Herpetology 32: 34–49.

Nishikawa K, Matsui M, Rao DQ (2014) A new species of *Tylototriton* (Amphibia: Urodela: Salamandridae) from central Myanmar. Natural History Bulletin of the Siam Society 60: 9–22.

Nussbaum RA, Brodie ED Jr, Yang DT (1995) A taxonomic review of *Tylototriton verrucosus* Anderson (Amphibia: Caudata: Salamandridae). Herpetologica 51: 257–268.

Phimmachak S, Aowphol A, Stuart BL (2015) Morphological and molecular variation in *Tylototriton* (Caudata: Salamandridae) in Laos, with description of a new species. Zootaxa 4006: 285–310.

Pomchote P, Khonsue W, Thammachoti P, Hernandez A, Peerachidacho P, Suwannapoom C, Onishi Y, Nishikawa K (2020) A new species of *Tylototriton* (Urodela: Salamandridae) from Nan Province, northern Thailand. Tropical Natural History 20: 144–161.

Poyarkov NA Jr, Nguyen TV, Arkhipov DV (2021) A new species of the genus *Tylototriton* (Amphibia, Caudata, Salamandridae) from central Vietnam. Taprobanica 10: 4–22.

Qian L, Sun X, Li J, Guo W, Pan T, Kang X, Wang H, Jiang J, Wu J, Zhang BW (2017) A new species of the genus *Tylototriton* (Amphibia: Urodela: Salamandridae) from the southern Dabie Mountains in Anhui Province. Asian Herpetological Research 8: 151–164.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Silvestro D, Michalak I (2012) RaxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stuart BL, Phimmachak S, Sivongxay N, Robichaud WG (2010) A new species in the *Tylototriton asperrimus* group (Caudata: Salamandridae) from central Laos. Zootaxa 2650: 19–32.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.

Nucleic Acids Research 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Unterstein W (1930) Beiträge zur Lurch- und Kriechtierfauna Kwangsi's. 2. Schwanzlurche. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 1930: 313–315.

Wang B, Nishikawa K, Matsui M, Nguyen TQ, Xie F, Li C, Khatiwada JR, Zhang B, Gong D, Mo Y, Wei G, Chen X, Shen Y, Yang D, Xiong R, Jiang J (2018) Phylogenetic surveys on the newt genus *Tylototriton* sensu lato (Salamandridae, Caudata) reveal cryptic diversity and novel diversification promoted by historical climatic shifts. PeerJ 6: e4384. https://doi.org/10.7717/peerj.4384

Yang DD, Jiang JP, Shen YH, Fei DB (2014) A new species of the genus Tylototriton (Urodela: Salamandridae) from northeastern Hunan Province, China. Asian Herpetological Research 5: 1–11.

Ye J, Wei Z, Han F, Ni Q, Yao Y, Xu H, Li Y, Rao D, Zhang M (2017) The complete mitogenome sequence of *Tylototriton ziegleri* (Amphibia: Caudata). Conservation Genetics Resources 9: 503–506. https://doi.org/10.1007/s12686-017-0710-8

Zaw T, Lay P, Pawangkhanant P, Gorin VA, Poyarkov NA Jr (2019) A new species of Crocodile Newt, genus *Tylototriton* (Amphibia, Caudata, Salamandridae) from the mountains of Kachin State, northern Myanmar. Zoological Research 40: 151–174.

Zhao EM, Adler KA (1993) Herpetology of China. Contributions to Herpetology 10. Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

# Appendix 1

## Examined specimens

*Tylototriton asperrimus* (*N*=4): **China: Guangxi:** Guiping: SYS a006890–6891, 8200–8201.
*Tylototriton kweichowensis* (*N*=6): **China: Guizhou:** Shuicheng: SYS a004967; Qixingguan: SYS a007119–7120, 7287, 7307–7308.
*Tylototriton maolansis* (*N*=5): **China: Guizhou:** Libo: SYS a000950–0953, 2212.
*Tylototriton pulcherrima* (*N*=3): **China: Yunnan:** Mojiang: SYS a003438–3440.
*Tylototriton shanjing* (*N*=3): **China: Yunnan:** Zhenyuan: SYS a001924–1925, 3371.
*Tylototriton verrucosus* (*N*=3): **China: Yunnan:** Tengchong: SYS a003768–3769; Longyang: SYS a006607.

# Supplementary material 1

## Table S1

**Authors:** Lyu Z-T, Wang J, Zeng Z-C, Zhou J-J, Qi S, Wan H, Li Y-Y, Wang Y-Y (2021)
**Data type:** .xlsx
**Explanation note:** Pairwise distances among all Tylototriton samples used in this study.

**Link:** https://doi.org/10.3897/vz.71.e73563.suppl1