

Taxonomy & Inventories

Confirmation of *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen, 2013 in China, with discussion on the relationship between *T. verrucosus* Anderson, 1871 and *T. panwaensis* Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019 (Caudata, Salamandridae)

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Abstract

Background

The distribution of the Ziegler's Crocodile Newt *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen, 2013 in China has been controversial. This species was originally recorded uncertainly from Guangxi Autonomous Region, China. Subsequently, this species was recorded from Yunnan and Guangdong provinces, China. Thereafter, the record from Guangdong was denied and the record from Yunnan was questioned.

New information

Two specimens of *Tylototriton* Anderson, 1871 were collected from Wenshan Prefecture, Yunnan Province, China, in 2020. Phylogenetically, the sequences of these two specimens clustered with the sequences of *T. ziegleri* (including the holotype) from its type locality with strong support, and morphologically agree well with the original description of *T. ziegleri*. We confirm the record of *T. ziegleri* in China and present detailed collection site and morphological description of the specimens from China. In addition, we found that *T. panwaensis* Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019 may be the synonym of *T. verrucosus* Anderson, 1871. We discussed the relationship between *T. verrucosus* and *T. panwaensis*.

Keywords

morphology, ND2, newt, phylogeny, Yunnan

Introduction

Tylototriton ziegleri Nishikawa, Matsui & Nguyen, 2013 is a member of the *T. asperrimus* species group, that was described from northern Vietnam (Nishikawa et al. 2013). This species was previously reported in China by Hernandez (2016) from Jingxi County, Guangxi Autonomous Region; however, he also mentioned that the population in Jingxi may not be the true *T. ziegleri*. Subsequently, this species was reported from Malipo County, Yunnan Province, based on only molecular data of a single specimen without morphological description and detailed collection information (Ye et al. 2017). Thereafter, this species was reported from Guangdong Province, China by Li et al. 2020. Lyu et al. (2021) found that the record of *T. ziegleri* from Guangdong by Li et al. (2020) actually represented a new species at that time and described it as *T. sini* Lyu, Wang, Zeng, Zhou, Qi, Wan & Wang, 2021. Therefore, Lyu et al. (2021) removed the record of *T. ziegleri* from the herpetofauna of Guangdong. In addition, Lyu et al. (2021) questioned the record of *T. ziegleri* from Yunnan.

Tylototriton verrucosus Anderson, 1871 is the type species of the genus *Tylototriton*. However, the current taxonomy of the true *T. verrucosus* is problematic because Anderson (1871) neither specified an exact type locality nor mentioned which specimens the original description of *T. verrucosus* was based on. *Tylototriton panwaensis* Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019 is another species of *Tylototriton* closely resembling *T. verrucosus* and this species was described from Kachin State, north-eastern Myanmar, a location close to the border between Myanmar and western Yunnan of China.

During our field surveys in south-eastern Yunnan, China, in 2020, two specimens of *Tylototriton* were collected from Malipo County, Wenshan Prefecture. Detailed morphological comparisons and molecular analysis indicated that these specimens belong to *T. ziegleri*. Therefore, we confirm the distribution of *T. ziegleri* in China and provide a

detailed description of these specimens herein. In addition, we collected some specimens of *T. panwaensis* in 2019 from western Yunnan, China, which is probably the type locality of *T. verrucosus*. Therefore, we also discuss the relationship between *T. verrucosus* and *T. panwaensis* herein.

Materials and methods

Sampling

Specimens were collected, humanely euthanised and then fixed in 75% ethanol for permanent storage. Photographs were taken to document the colour pattern in life prior to euthanasia. Liver tissue samples were preserved in 99% ethanol for molecular analysis. All specimens were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Morphological characteristics

Morphological terminology followed Nishikawa et al. (2013). Measurements were taken with an electronic vernier caliper to the nearest 0.1 mm. Morphometric characters included: SVL: snout-vent length, from tip of snout to anterior tip of vent; HL: head length, from tip of snout to wrinkle of throat; HW: head width, measured at the angle anterior to the parotid gland; MXHW: maximum head width, measured at widest point; SL: snout length, from tip of snout to anterior tip of upper eyelid; LJL: lower jaw length, from tip of lower jaw to jaw angle; ENL: eyelid-nostril length, minimum distance between eyelid and nostril; IND: internarial distance, minimum distance between the external nares; IOD: interorbital distance, minimum distance between upper eyelids; UEW: upper eyelid width, greatest width of upper eyelid; UEL: upper eyelid length, greatest length of upper eyelid; OL: orbit length, maximum length of orbit; AGD: axilla-groin distance, minimum distance between axilla and groin; TRL: trunk length, from wrinkle of throat to anterior tip of vent; TAL: tail length, from anterior tip of vent to tail tip; VL: vent length, from anterior to posterior tip of vent; BTAW: basal tail width, tail width measured at root of tail; MTAW: medial tail width, tail width measured at middle: BTAH: basal tail height, tail height measured at base of tail; MXTAH: maximum tail height, tail height measured at highest point; MTAH: medial tail height, tail height measured at middle; FLL: fore-limb length, distance from axilla to tip of longest finger; HLL: hind-limb length, 2FL: second finger length; 3FL: third finger length; 3TL: third toe length; and 5TL: fifth toe length.

Molecular analysis

Genomic DNA was extracted from liver tissue samples preserved in 99% ethanol using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). A fragment of the NADH dehydrogenase subunit two (ND2) was amplified for the newly-collected specimens. Methods for amplification and sequencing of the DNA fragment are the same as Lyu et al. (2021). All new sequences were deposited in GenBank. Homologous and outgroup sequences were obtained from GenBank (Table 1).

Table 1.

Localities, voucher information and GenBank accession numbers for all samples used in this study.

Таха	Locality	Voucher	Accession
Tylototriton anguliceps	Vietnam: Dien Bien: Muong Nhe	VNMN A20143	LC017832
Tylototriton anhuiensis	China: Anhui: Yuexi	AHU-13-EE-006	<u>KY321413</u>
Tylototriton asperrimus	China: Guangxi: Jinxiu	CIB GX20080714	<u>KY800819</u>
Tylototriton broadoridgus	China: Hunan: Sangzhi	CIB200084	KY800837
Tylototriton dabienicus	China: Anhui: Shangcheng	HNNU 1004-026	<u>KY800869</u>
Tylototriton daloushanensis	China: Guizhou: Suiyang	CIBWG200600019	<u>KY800817</u>
Tylototriton hainanensis	China: Hainan: Mt Diaoluo	CIB 20081048	KC147817
Tylototriton himalayanus	Nepal: Mechi: Illam	CIB 201406287	<u>KT765210</u>
Tylototriton kachinorum	Myanmar: Kachin: Indawgyi	ZMMU A5953	<u>MK097273</u>
Tylototriton kweichowensis	China: Guizhou: Shuicheng	SYS a004967	<u>OK539843</u>
Tylototriton liuyangensis	China: Hunan: Liuyang	CSUFT 20100108	KJ205598
Tylototriton lizhengchangi	China: Hunan: Yizhang	KUHE 42317	AB769533
Tylototriton maolanensis	China: Guizhou: Libo	CIBML20180427001	<u>MK820699</u>
Tylototriton ngarsuensis	Myanmar: Shan: Taunggyi	LSUHC 13763	<u>MH836584</u>
Tylototriton notialis	Laos: Khammouan: Boualapha	FMNH HERP 271120	HM462061
Tylototriton panhai	Thailand: Loei: Phu Luang WS	PL009	AB830736
Tylototriton panwaensis	Myanmar: Kachin: Myitkyina	CAS 245418	<u>KT304279</u>
Tylototriton panwaensis	China: Yunnan: Tengchong	KIZ 040240	ON159332
Tylototriton panwaensis	China: Yunnan: Tengchong	KIZ 040242	ON159331
Tylototriton panwaensis	China: Yunnan: Tengchong	KIZ 040243	<u>ON159330</u>
Tylototriton panwaensis	China: Yunnan: Tengchong	KIZ 040246	<u>ON159329</u>
Tylototriton panwaensis	China: Yunnan: Tengchong	KIZ 040247	<u>ON159328</u>
Tylototriton panwaensis	China: Yunnan: Yingjiang	KIZ20190701	<u>ON159327</u>
Tylototriton panwaensis	China: Yunnan: Yingjiang	KIZ20190702	<u>ON159326</u>
Tylototriton panwaensis	China: Yunnan: Yingjiang	KIZ20190703	<u>ON159325</u>
Tylototriton panwaensis	China: Yunnan: Yingjiang	KIZ20190704	<u>ON159324</u>
Tylototriton pasmansi	Vietnam: Phu Tho: Tan Son	IEBR 4467	MT210167
Tylototriton phukhaensis	Thailand: Nan: Doi Phu Kha NP	CUMZ A-7718	<u>MN912574</u>
Tylototriton podichthys	Laos: Luang Phabang: Phoukhoun	NCSM 77725	KT304295
Tylototriton pseudoverrucosus	China: Sichuan: Ningnan	CIB WCG2012003	KY800861
Tylototriton pulcherrima	China: Yunnan: Lüchun	CIB TY040	<u>KY800890</u>
Tylototriton shanjing	China: Yunnan: Jingdong	KIZ 201306102	KY800858
Tylototriton shanorum	Myanmar: Shan: Taunggyi	CAS 230933	AB922822
Tylototriton sini	China: Guangdong: Mt Yunkai	SYS a008354	OK539836

Таха	Locality	Voucher	Accession
Tylototriton sparreboomi	Sin Ho, Lai Chau, Vietnam	IEBR 4476	MT210162
Tylototriton taliangensis	China: Sichuan: Liangshan	CAS 195126	DQ517853
Tylototriton thaiorum	Vietnam: Nghe An: Pu Hoat NR	ZMMU A-7577	<u>MW883478</u>
Tylototriton umphangensis	Thailand: Tak: Umphang WS	CUMZ-A-8243	<u>OK092618</u>
Tylototriton uyenoi	Thailand: Chiang Mai: Doi Suthep	KUHE 19147	<u>AB830733</u>
Tylototriton verrucosus	China: Yunnan: Longchuan	CIB TSHS1	<u>KY800847</u>
Tylototriton vietnamensis	Vietnam: Bac Giang: Son Dong	IEBR 3243	<u>HM770088</u>
Tylototriton wenxianensis	China: Gansu: Wenxian	CIB 20090527	KC147813
Tylototriton yangi	China: Yunnan: Pingbian	KUHE 42282	KY800887
Tylototriton ziegleri	Vietnam: Ha Giang: Quan Ba	VNMN 3390	<u>AB769539</u>
Tylototriton ziegleri	Vietnam: Ha Giang: Quan Ba	KUHE 55077	<u>AB769540</u>
Tylototriton ziegleri	Vietnam: Ha Giang: Quan Ba	KUHE 55078	<u>AB769541</u>
Tylototriton ziegleri	Vietnam: Ha Giang: Quan Ba	VNUH HG.081	<u>KY800871</u>
Tylototriton ziegleri	Vietnam: Ha Giang: Quan Ba	VNUH HG.082	<u>KY800870</u>
Tylototriton ziegleri	China: Yunnan: Malipo	KIZ20210504	<u>ON159334</u>
Tylototriton ziegleri	China: Yunnan: Malipo	KIZ20210505	<u>ON159333</u>
Tylototriton cf. ziegleri	Vietnam: Cao Bang: Bao Lac	VNMN 3389	<u>AB769542</u>
Tylototriton cf. ziegleri	Vietnam: Cao Bang: Quang Thanh	ROM 35330	DQ517856
Tylototriton cf. ziegleri	Vietnam: Cao Bang: Quang Thanh	ROM 35364	HM462056
Echinotriton chinhaiensis	China: Zhejiang: Ningbo	TP26195	EU880315
Echinotriton andersoni	Japan: Kagoshima: Tokunoshima	MVZ 232187	EU880314

Phylogenetic analyses

Sequences were aligned using MUSCLE 3.6 (Edgar 2004) with default parameters. Average genetic distances were calculated in MEGA 11 (Tamura et al. 2021) using the uncorrected p-distance model. The best substitution model GTR+F+I+G4 was selected using the Akaike Information Criterion (AIC) in ModelFinder (Kalyaanamoorthy et al. 2017). Bayesian Inference was performed in MrBayes 3.2.7 (Ronquist et al. 2012). Two runs were performed simultaneously with four Markov chains, the chains were run for 10,000,000 generations and sampled every 1,000 generations, the first 25% of the initial samples was discarded as burn-in after the standard deviation of split frequencies of the two runs was less than a value of 0.01. Maximum Likelihood analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015). The ultrafast bootstrap approximation algorithm was used via 1,000 bootstrap pseudoreplicates.

Taxon treatment

Tylototriton ziegleri Nishikawa, Matsui & Nguyen, 2013

Materials

- a. scientificName: *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen, 2013; family: Salamandridae; country: China; stateProvince: Yunnan; locality: Zhongzhai Village, Xiajinchang Township, Malipo County, Wenshan Prefecture; verbatimElevation: 1750 m; verbatimCoordinates: 23°7′8″N 104°50′6″E; eventRemarks: collected by Shuo Liu on 10 May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ20210504; basisOfRecord: preserved specimen
- scientificName: *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen, 2013; family: Salamandridae; country: China; stateProvince: Yunnan; locality: Zhongzhai Village, Xiajinchang Township, Malipo County, Wenshan Prefecture; verbatimElevation: 1750 m; verbatimCoordinates: 23°7'8'N 104°50'6"E; eventRemarks: collected by Shuo Liu on 10 May 2020; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ20210505; basisOfRecord: preserved specimen

Description the specimens from China: Morphometric data are provided in Table 2. Body moderately stout, medium in size (SVL 58.2-60.8 mm, TAL 68.0-69.7 mm). Head width almost equal to head length (HW/HL 0.96-1.05); head nearly hexagonal in shape in dorsal view, depressed, gently sloping in profile. Snout short, truncate, slightly beyond lower jaw. Nostril on anterior margin of snout, located notably closer to snout tip than to eye. Tongue oval, not notched distally, attached to mouth floor, but free laterally; vomerine tooth series in an inverted V-shape, converging anteriorly, but not reaching choanae. Labial fold absent; gular fold present, but weak; parotoids distinct, projecting posteriorly; costal folds absent. Dorsolateral supratemporal bony ridges on head protruding, beginning at the anterior corner of orbit continuing to anterior end of parotoid, posterior ends curved inside; mid-dorsal bony ridge on head short. Vertebral mid-dorsal ridge distinctly protruding, segmented, forming a row of tubercles, running from occiput region to the base of tail, separated from mid-dorsal bony ridge on head by a small gap. Rib nodules distinct, forming knob-like warts, relatively small, arranged in two longitudinal series on dorsolateral surfaces of dorsum from axilla to base of tail, counting 15-17 nodules on each side of body; rib nodules in the middle largest and decreasing anteriorly and posteriorly (Fig. 1).

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Ta	ble	2.

Measurements (in mm) of the specimens of Tylototriton ziegleri from China.

	KIZ20210504	KIZ20210505
SVL	58.2	60.8
HL	15.3	15.7
HW	16.0	15.1
MXHW	16.0	15.2

	KIZ20210504	KIZ20210505
SL	5.8	6.3
LJL	12.7	12.6
ENL	3.7	3.8
IND	5.4	6.1
IOD	8.6	8.6
UEW	1.6	1.6
UEL	3.3	3.2
OL	4.2	4.2
AGD	28.9	31.6
TRL	43.2	45.8
TAL	69.7	68.0
VL	7.1	5.9
BTAW	5.5	6.0
MTAW	2.7	3.0
BTAH	7.4	7.2
MXTAH	7.4	7.3
МТАН	6.9	7.0
FLL	19.5	21.8
HLL	22.5	24.0
2FL	3.7	4.2
3FL	4.0	4.5
3TL	5.5	5.3
5TL	2.3	2.4

Limbs slender, tips of fore-limbs and hind-limbs overlapping when adpressed towards each other along body; fingers and toes free of webbing; relative finger lengths III > II > I > I > I > IV, relative toe lengths III > IV > II > I > V. Tail long, TAL/SVL 1.12–1.20; laterally compressed along entire length, tapering posteriorly, tip pointed, dorsal fin more distinct posteriorly, ventral ridge smooth.

Skin rough with fine granules, dense on dorsum and ventrum, but small and sparse on throat. Cloacal region slightly swollen, vent as a longitudinal slit, vent edges with numerous small transverse folds.

Colouration in life: Dorsum almost uniformly black; venter slightly lighter than dorsum; bony ridges on head and vertebral ridge black, rib nodules black or reddish,

only tips or most regions of fingers and toes, vent and ventral ridge of tail orange (Fig. 2).



Figure 1. doi

Tylototriton ziegleri from China in preservative. Dorsal view (left) and ventral view (right).





Tylototriton ziegleri from China in life: **A-C** the adult male KIZ20210504; **D-F** the adult male KIZ20210505.

Ecological notes: The specimens were collected in a small stream in the forest at night, the water in the stream was shallow, and both sides of the stream were covered with vegetation. No eggs or larvae were found.

Distribution: This species was recorded from Ha Giang and Cao Bang provinces, northern Vietnam; Malipo County, Yunnan Province and Jingxi County, Guangxi Autonomous Region, China (Fig. 3). However, the populations in Cao Bang and Jingxi may not represent the true *Tylototriton ziegleri* (see Discussion section).

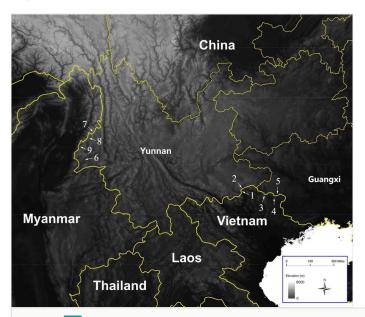


Figure 3. doi

Map showing the type locality of *Tylototriton ziegleri* in Ha Giang, Vietnam (1); the collection site of *T. ziegleri* in Malipo, Yunnan, China (2); the distributions of *T. cf. ziegleri* in Cao Bang, Vietnam (3 and 4); the distribution of *T. cf. ziegleri* in Jingxi, Guangxi, China (5); the type locality of *T. verrucosus* designated by Nussbaum et al. (1995) in Longchuan, Yunnan, China (6); the type locality of *T. panwaensis* in Kachin, Myanmar (7); the collection site of *T. panwaensis* in Yingjiang, Yunnan, China (9).

Analysis

Morphologically, the specimens from Malipo County, Wenshan Prefecture, Yunnan, China, agree well with the original description of *Tylototriton ziegleri*: medium body size; skin rough with fine granules; bony ridges on head distinct; vertebral ridge prominent and segmented; rib nodules prominent; tips of fore-limbs and hind-limbs overlapping when adpressed along body; tail thin; dorsum uniform blackish; finger and toe tips, vent and ventral ridge of tail orange.

The obtained sequence alignment is 1044 bp in length. BI and ML analyses showed basically consistent topology (Fig. 4). The two specimens collected from Malipo County, Wenshan Prefecture, Yunnan, China, were homogeneous and clustered with *Tylototriton ziegleri* (including the holotype) from Ha Giang Province, Vietnam, with strong support. The genetic distance (uncorrected p-distance) between the specimens from China and *T. ziegleri* (including the holotype) from Ha Giang, Vietnam was only 0.1% (Suppl. material 1).

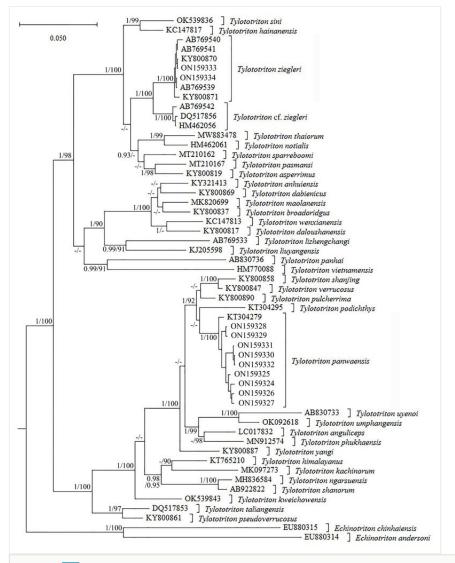


Figure 4. doi

Bayesian Inference tree, based on mitochondrial ND2 sequences. Numbers before slashes indicate Bayesian posterior probabilities (values below 0.9 are not shown) and numbers after slashes indicate bootstrap support for Maximum Likelihood analyses (values below 90 are not shown).

Combining the results of morphological and molecular analysis, we determined that the specimens from Malipo County, Wenshan Prefecture, Yunnan, China, belong to *Tylototriton ziegleri*.

Discussion

In the phylogenetic analyses in Nishikawa et al. (2013) and in this study, Tylototriton ziegleri formed two strongly-supported lineages, one (including the holotype) from Ha Giang Province, Vietnam and the other from Cao Bang Province, Vietnam and there is a relatively large genetic divergence (2.6%) between the two lineages, more than that (2.1%) between T. verrucosus and T. pulcherrima and close to that (2.7%) between T. taliangensis and T. pseudoverrucosus. Therefore, we regard the lineage containing the holotype as T. ziegleri sensu stricto and the other lineage as Tylototriton cf. ziegleri. The lineage of Tylototriton cf. ziegleri may, therefore, represent a cryptic new species of Tylototriton. Our newly-collected specimens from China clustered with the lineage of T. ziegleri sensu stricto and showed negligible genetic divergence (0.1%) with this lineage, confirming T. ziegleri sensu stricto. Ziegler et al. (2018) reported the longevity of T. ziegleri and extended the diagnosis of this species, based on specimens collected from Cao Bang, Vietnam; according to their result of molecular identification, these specimens from Cao Bang should be assigned to to Tylototriton cf. ziegleri rather than T. ziegleri sensu stricto. As for the population in Jingxi, Guangxi, China, record by Hernandez (2016), judging from the geographical location, we consider that this population should also belong to Tylototriton cf. ziegleri.

In the original description of Tylototriton verrucosus, Anderson (1871) described this species as uniform blackish-brown, but not orange-patterned. Usually, the uniform blackish-brown colour of these salamanders in life remains constant in preservative and the orange colour of these salamanders in life fades to light yellow or white, but the pattern remains distinct in preservative. However, according to Nussbaum et al. (1995) and Chanda et al. (2000), all the syntypes of T. verrucosus are orange-patterned. This has always made people wonder why the syntypes were inconsistent with the original description by Anderson (1871). Anderson (1871) did not specify a precise type locality, but merely mentioned some valleys in western Yunnan, including Nantin, Momien, Hotha, Ponsee and Nampoung. That is to say, any of these valleys may be the type locality of T. verrucosus. "Momien" generally refers to Tengchong City, Yunnan, China, nowadays; "Hotha" generally refers to Husa Township, Longchuan County, Yunnan, China, nowadays; "Ponsee" generally refers to Xueli Village, Yingjiang County, Yunnan, China, nowadays; "Nampoung" generally refers to Nabang Town, Yingjiang County, Yunnan, China, nowadays; and "Nantin" is uncertain. Nussbaum et al. (1995) considered that the syntypes of T. verrucosus are inconsistent with the original description of T. verrucosus and, as the specimens from Husa agree with the original description of T. verrucosus, they designated a neotype (KIZ 74II0061 VI.16) of T. verrucosus from Gongwa Village, Longchuan County, Yunnan Province, China. However, Fei et al. (2006) guestioned the validity of this neotype. Since the syntypes of T. verrucosus are extant (Sclater 1892, Chanda et al. 2000), one of the syntypes should be selected as the lectotype instead of designating a neotype and this designation contravenes the requirements of Art. 74.7 of the Code (ICZN, 1999). Therefore, we agree with Fei et al. (2006) that the designation of the neotype (KIZ 74II0061 VI.16) is unvalid.

We collected some specimens from western Tengchong City and neighbouring northwestern Yingjiang County, Yunnan, China, in 2019; these specimens were uniform blackish-brown or uniform brown in life, that is to say, not only the specimens from Husa agree with the original description of *T. verrucosus*, but also the specimens from western Tengchong and north-western Yingjiang agree with the original description of *T. verrucosus*. Therefore, Tengchong or Yingjiang cannot be ruled out as the type locality of *T. verrucosus*. Interestingly, the black surface skins of the ones who underwent poor preservation fell off and the body colour turned to orange-patterned, while others are still uniform blackish-brown or uniform brown (Fig. 5). The colour of some of these specimens in preservative became inconsistent with that in life; this seems to be similar to the case of the syntypes of *T. verrucosus*, we cannot determine whether the colours of these syntypes have changed. More interestingly, the specimens from western Tengchong and north-western Yingjiang were assigned to *T. panwaensis* in the molecular analysis.



The specimens of Tylototriton panwaensis from western Yunnan, China, in preservative.

Although the population in Husa and the population in western Tengchong are both in agreement with the original description of *Tylototriton verrucosus*, the two populations

belong to two different species. Since it is impossible to tell whether Hotha or Momien is the type locality of *T. verrucosus*, it is impossible to identify whether the population in Husa or in western Tengchong is the true *T. verrucosus*. If Hotha is the type locality of *T. verrucosus*, then the population in Husa is the true *T. verrucosus* and *T. panwaensis* is a valid species and also distributed in China; and if Momien is the type locality of *T. verrucosus*, then the population in western Tengchong may be the true *T. verrucosus*, *T. panwaensis* may be the synonym of *T. verrucosus* and the population in Husa may remain an unnamed species. We can only make this speculation at present, as more research is needed to solve this problem.

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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.
- Chanda S, Das I, Dubois A (2000) Catalogue of amphibian types in the collection of the Zoological Survey of India. Hamadryad 25 (2): 100-128.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32 (5): 1792-1797. <u>https://doi.org/10.1093/nar/</u> gkh340
- Fei L, Hu S, Ye C, Huang Y (2006) Fauna Sinica. Amphibia. Volume 1. General Accounts of *Gymnophiona* and *Urodela*. Science Press, Beijing, 471 pp. [ISBN 9787030161475]
- Hernandez A (2016) Crocodile Newts: The Primitive Salamandridae of Asia: Genera *Echinotriton* and *Tylototriton*. Edition Chimaira, Frankfurt, 416 pp. [ISBN 978-3-89973-521-5]
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14 (6): 587-589. <u>https://doi.org/10.1038/nmeth.4285</u>
- 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. <u>https://doi.org/10.19711/j.cnki.issn2310-1490.2020.03.032</u>
- Lyu Z, Wang J, Zeng Z, Zhou J, Qi S, Wan H, Li Y, Wang Y (2021) A new species of the genus *Tylototriton* (Caudata, Salamandridae) from Guangdong, southern China, with discussion on the subgenera and species groups within the genus. Vertebrate Zoology 71: 697-710. https://doi.org/10.3897/vz.71.e73563

- Nguyen L, Schmidt H, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32 (1): 268-274. <u>https://doi.org/10.1093/molbev/msu300</u>
- Nishikawa K, Matsui M, Nguyen TT (2013) A new species of *Tylototriton* from Northern Vietnam (Amphibia: Urodela: Salamandridae). Current Herpetology 32 (1): 34-49. <u>https://doi.org/10.5358/hsj.32.34</u>
- Nussbaum R, Brodie JE, Yang D (1995) A taxonomic review of *Tylototriton verrucosus* Anderson (Amphibia: Caudata: Salamandridae). Herpetologica 51: 257-268.
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard M, Huelsenbeck J (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61 (3): 539-542. <u>https://doi.org/10.1093/sysbio/sys029</u>
- Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd Edition. Vol. 1–3. Cold Spring Harbor Laboratory Press, New York, 1626 pp. [ISBN 0-87969-309-6]
- Sclater W (1892) A list of the Batrachia of the Indian Museum. Taylor and Francis, London, 43 pp. URL: <u>https://doi.org/10.5962/bhl.title.9081</u>
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution 38 (7): 3022-3027. <u>https://doi.org/10.1093/molbev/msab120</u>
- 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 (3): 503-506. <u>https://doi.org/10.1007/s12686-017-0710-8</u>
- Ziegler T, Marcec R, Vardukyan D, Nguyen TQ, Le MD, Bernardes M (2018) First record of longevity in Ziegler's crocodile newt, *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen, 2013 (Caudata, Salamandridae). Alytes 36 (1–4): 328-338.

Supplementary material

Suppl. material 1: Mean uncorrected p-distances (%), based on mitochondrial ND2 sequences. doi

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