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Description of a new species of the Asian newt genus *Tylototriton* (Amphibia, Urodela, Salamandridae) from Hunan Province, China

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Abstract

The newt genus *Tylototriton* is widely distributed in east, southeast, and south Asia. Previous studies have shown that basic surveys of this genus are far from comprehensive, and unknown species still exist within the group. In this study, we described a new species of this genus, *Tylototriton gaowangjienensis* **sp. nov.**, from Gaowangjie National Nature Reserve in Hunan Province, China, based on evidence from molecular identification and morphological comparisons. Additionally, we conducted phylogenetic analysis of the genus to elucidate the taxonomic status of the new species. Based on combined mitochondrial 16S rRNA and ND2 gene sequences, the phylogenetic tree supported the new species as a member of the *T. wenxianensis* species subclade in the subgenus *Yaotriton*. It can be distinguished from its congeners by the combination of the following characteristics: (1) medium-sized body; (2) finger tips extending beyond the snout while forelimbs stretched forward; (3) tips of forelimbs and hindlimbs reaching and overlapping when folded towards the body; (4) snout edge round; (5) absence of small papillae in the male cloacal fissure; (6) dorsal ridges narrow, with width smaller than the eye diameter; (7) head length (HDL) greater than head width (HDW) (HDL/HDW = 1.04~1.22); (8) orange markings absence on the parotoid; (9) nodule-like warts on body sides continuous; (10) vomerine teeth not connected at the tip, extending to the occipital bone at the posterior end.

Key Words

morphology, new species, phylogeny, taxonomy

Introduction

The genus *Tylototriton*, within the family Salamandridae, is widely distributed in the eastern, southern, and southeastern regions of Asia (Fei and Ye 2016; Frost, 2020). It was established in 1871 based on the species known as *Tylototriton verrucosus*, collected from Longchuan County, Yunnan Province, China (Anderson 1871). Many phylogenetic studies have suggested that the genus *Tylototriton* is a sister group of *Echinotriton* Nussbaum & Brodie, 1982, and *Tylototriton* is monophyletic, while the previously recognized *Yaotriton* Dubois & Raffaëlli,

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2009, is considered a subgenus within it (Nishikawa et al. 2013a, 2013b; Phimmachak et al. 2015; Wang et al. 2018; Dufresnes and Hernandez 2023). The species of *Tylototriton* have been usually divided into two groups, the *T. verrucosus* and *T. asperrimus* species group (Fei et al. 2005), corresponding to the two subgenera, *Tylototriton* and *Yaotriton*, respectively (Dubois and Raffaëlli 2009).

With the increase in field investigations and the advancement of molecular technology, more and more species of *Tylototriton* have been discovered in recent years, indicating that the species diversity of this genus was previously underestimated. Currently, there are 40 known species of *Tylototriton* worldwide, with 22 species recorded in China. These species distributed in China include: *T. anhuiensis* Qian, Sun, Li, Guo, Pan, Kang, Wang, Jiang, Wu & Zhang, 2017, *T. asperrimus* Unterstein, 1930, *T. broadoridgus* Shen, Jiang & Mo, 2012, *T. dabienicus* Chen, Wang & Tao, 2010, *T. daloushanensis* Zhou, Xiao & Luo, 2022, *T. hainanensis* Fei, Ye & Yang, 1984, *T. joe* Rao, Zeng, Zhu & Ma, 2022, *T. kweichowensis* Fang & Chang, 1932, *T. liuyangensis* Yang, Jiang, Shen & Fei, 2014, *T. lizhenchangi* Hou, Zhang, Jiang, Li & Lu, 2012, *T. maolanensis* Li, Wei, Cheng, Zhang & Wang, 2020, *T. panwaensis* Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019, *T. pseudoverrucosus* Hou, Gu, Zhang, Zeng & Lu, 2012, *T. pulcherrimus* Hou, Zhang, Li & Lu, 2012, *T. shanjing* Nussbaum, Brodie & Yang, 1995, *T. sini* Lyu, Wang, Zeng, Zhou, Qi, Wan & Wang, 2021, *T. taliangensis* Liu, 1950, *T. tongziensis* Li, Liu, Shi, Wei & Wang, 2022, *T. verrucosus* Anderson, 1871, *T. wenxianensis* Fei, Ye & Yang, 1984, *T. yangi* Hou, Zhang, Zhou, Li & Lu, 2012, and *T. ziegleri* Nishikawa, Matsui & Nguyen, 2013. According to the phylogenetic relationships of previous studies, *T. taliangensis*, *T. shanjing*, *T. kweichowensis*, *T. verrucosus*, and *T. yangi* belong to the *T. verrucosus* species group (Yuan et al. 2011; Phimmachak et al. 2015), while *T. asperrimus*, *T. broadoridgus*, *T. dabienicus*, *T. hainanensis*, *T. liuyangensis*, *T. lizhenchangi*, and *T. wenxianensis* belong to the *T. asperrimus* species group (Yuan et al. 2011; Shen et al. 2012; Phimmachak et al. 2015).

In addition to the high species diversity, recent molecular studies have indicated that our understanding of *Tylototriton* taxonomy is far from complete. For instance, Grismer et al. (2018) revealed several mysterious lineages that may represent undescribed species (Grismer et al. 2018). Wang et al. (2018) showed the existence of three cryptic species within *T. wenxianensis*, distributing in the Dabie Mountains in Anhui, Wufeng County in Hubei, and Libo County in Guizhou Province, respectively (Wang et al. 2018). Currently, putative cryptic species from the Dabie Mountains and Libo County have been identified and described as two new species, *T. anhuiensis* and *T. maolanensis*, respectively (Qian et al. 2017; Li et al. 2020). However, individuals from Wufeng County, Hubei Province, have not been thoroughly investigated and further examined. In addition to taxonomic aspects, scientists are also concerned with the biology, characteristics, distribution, reproduction, and conservation of *Tylototriton* in various studies (Sparreboom et al. 2014; Hernandez et al. 2016; Wang et al. 2017).

During an amphibian survey and monitoring project, several *Tylototriton* specimens were collected in the Hunan Gaowangjie National Natural Reserve, an area previously not known to harbor any salamander species. In this study, we evaluated the taxonomic status of these specimens using morphological and molecular methods and described the specimens herein as a new species, *Tylototriton gaowangjienensis* sp. nov.. Based on field observations, we also discussed the biological and ecological characteristics of the new species. Additionally, a mitochondrial genome analysis involving this species has been already conducted in another study (Wang et al. 2022).

Materials and methods

Sampling

In May 2021, thirteen individuals of the newt species were collected in Hunan Gaowangjie National Nature Reserve (Fig. 1). Of these, six were deposited as type specimens, and one was used for making a skeleton specimen, remaining individuals were measured for morphological data and molecular samples were collected before release. Additionally, three fertilized females were supplementary collected for reproduction and juvenile observations. All type specimens were fixed in formalin and then preserved in 80% alcohol, and deposited at the animal museum in Jishou University. The permissions for field surveys for scientific purposes were approved by the local Bureau of the National Nature Reserve, and the sample collections and experiment protocols were approved by the Biomedical Ethics Committee of Jishou University (No: JSDX-2024-0083) adhered to the relevant laws and guidelines of China.

Morphological examination

Morphological studies were conducted by descriptions and measurements following the methods outlined by Fei et al. (2006), Shen (2014), and Li et al. (2020). Measurements were taken point-to-point with a vernier caliper by a single measurer (to the nearest 0.01 mm). Abbreviations of morphological characteristics used in this study are shown in Table 1. The methods for skull specimens followed the procedures of Tang et al. (1985). For several morphometric characters, Kruskal-Wallis test were conducted to test the significance of differences between various species in the *T. asperrimus* species group (at a significance level of P = 0.05, see more in Suppl. material 1: table S1) (Li et al. 2020). Furthermore, principal component analyses (PCA) were conducted to determine whether the different species were separated in morphometric combinations (see more in Suppl. material 1: table S2), using the R software (R Development Core Team).

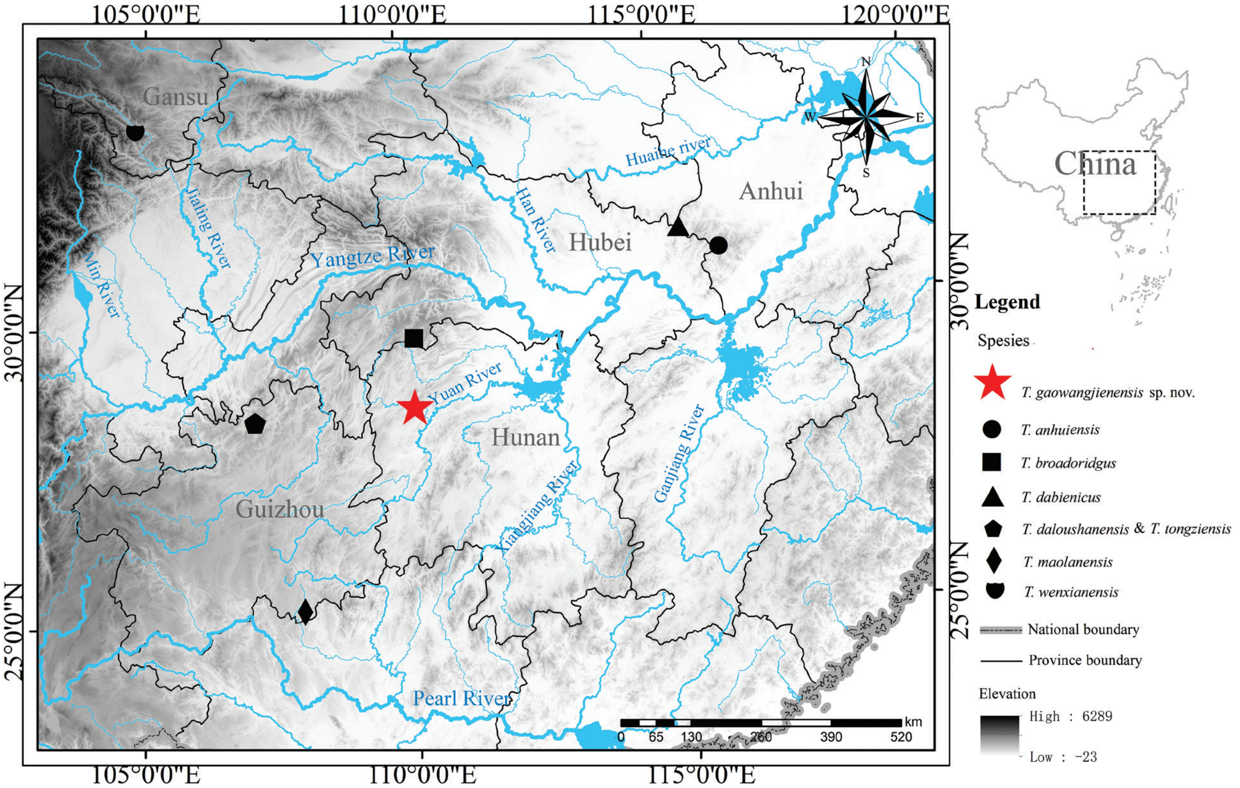

Figure 1. Type locality of species of *T. wenxianensis* species group, Red star: *T. gaowangjienensis* sp. nov.: Gaowangjie National Nature Reserve, Hunan Province, China; Black circle: *T. anhuiensis*: Yaoluoping National Nature Reserve, Anhui Province, China; Black square: *T. broadoridgus*: Badagongshan National Nature Reserve, Hunan Province, China; Black triangle: *T. dabienicus*: Dabie Mountain National Nature Reserve, Hubei Province, China; Black pentagon: *T. daloushanensis*: Huoqiuba Nature Reserve, Guizhou Province, China and *T. tongziensis*: Huanglian Nature Reserve, Guizhou Province, China; Black diamond: *T. maolanensis*: Maolan National Nature Reserve, Guizhou Province, China; Black peltate: *T. wenxianensis*: Wenxian County, Gansu Province, China.

Table 1. Abbreviations for morphological characteristics.

Abbreviation	Morphological characteristics	Explanation
TOL	Total length	Distance from tip of snout to tip of tail
SVL	Snout-vent length	Distance from tip of snout to posterior edge of vent
HDL	Head length	Distance from jugular fold to snout tip
HDW	Head width	Maximum head width
SL	Snout length	Distance from tip of snout to the anterior corner of eye
ED	Eye diameter	Distance from the anterior corner to the posterior corner of the eye
INS	Internasal space	Minimum distance between the external nares
TAL	Tail length	From anterior tip of cloaca to tip of tail
TH	Tail height	Maximum tail height
TRL	Trunk length	From gular fold of throat to anterior tip of vent
IOD	Interorbital distance	Minimum distance between the eyes
HL	Hand length	Wrist from elbow to longest finger
LLA	Length of lower arm	Distance from elbow to tip of wrist
FIIIL	The third finger length	Distance from base to tip of finger III
TLH	Thigh length	Distance from groin to knee
TL	Tibia length	Distance from knee to tarsus
IND	Internasal distance	Minimum distance between the external nares
TIIIL	The third toe length	Distance from base to tip of toe III

After preliminary diagnosis, the species in Gaowangjie National Nature Reserve showed similar features to the *T. asperrimus* species group. Therefore, *T. wenxianensis*, *T. dabienicus*, *T. broadoridgus*, *T. anhuiensis*, *T. maolanensis*, *T. daloushanensis*, and *T. tongziensis*, which belong to the *T. asperrimus* species group in the surrounding areas, were selected for in-depth morphological comparison and statistical analysis. The data of other species of *Tylototriton* were obtained from available literature (Anderson 1871; Fei et al. 1984; Liu et al. 1950; Nussbaum et al. 1995; Böhme et al. 2005; Fei et al. 2006; Chen et al. 2010; Stuart et al. 2010; Hou et al. 2012; Shen et al. 2012; Nishikawa et al. 2013a, 2013b, 2014; Yang et al. 2014; Khatiwada et al. 2015; 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; Pomchote et al. 2020; Lyu et al. 2021; Pomchote et al. 2021; Poyarkov et al. 2021; Luo et al. 2022).

Phylogenetic analysis

Five tissue samples were utilized for molecular phylogenetic analysis. DNA extraction was carried out using the Animal Genome DNA Extraction Kit (Sangon Biotech (Shanghai) Co., Ltd.). The mitochondrial 16S rRNA and NADH dehydrogenase subunit 2 (ND2) genes were amplified using the primer pairs P7 (5'- CGC CTG TTT ACC AAA AAC AT - 3') and P8 (5' - CCG GT CTG AAC TCA GAT CAC GT - 3'), and ND2-4F (5' - TAT GAG TAC GAG CAT CAT ACC C - 3') and ND2-4R (5' - CTT CTG CTT AAG ACT TTG AAG GTC - 3'), respectively. The PCR experiments were conducted following the protocols outlined by Li et al. (2020) and Kyu et al. (2021), respectively. Subsequently, the PCR products were detected by 1% agarose gel electrophoresis and then sequenced. All sequences obtained have been deposited in GenBank (Suppl. material 1: table S3).

For phylogenetic analysis, 94 sequences of 53 *Tylototriton* species (including the new species) were downloaded and incorporated as ingroup datasets, while 6 sequences of 3 species in Salamandridae (*Echinotriton chinhaiensis* Chang 1932, *Pachytriton granulosus* Chang, 1933, and *Pleurodeles waltl* Michahelles, 1830) were selected and downloaded as outgroups (Suppl. material 1: table S3). Both the 16S rRNA and ND2 genes were aligned using MAFFT and manually checked for accuracy. Subsequently, the aligned sequences were combined to construct a concatenated sequence. The uncorrected pairwise distances (p-distance) were calculated using MEGA11.

IQ-TREE 1.6.12 was utilized to conduct the maximum likelihood (ML) analysis (Nguyen et al. 2015), under the best-fit model TN+F+I+G4 that was selected by Modelfinder according to the Bayesian Information Criterion (BIC) (Kalyaanamoorthy et al. 2017). Ultrafast bootstrap approximation (UFB) was assessed using 20,000 ultrafast bootstrap replicates, and nodes with UFB values ≥ 95 were considered significantly supported (Hoang et al. 2018). For Bayesian inference (BI) analysis, the best-fitting model GTR+F+G4 was selected by Modelfinder according to the BIC (Kalyaanamoorthy et al. 2017). The Markov Chain Monte Carlo (MCMC) method was applied in MRBAYES 3.2.2., with four chains run for 20 million generations, sampled every 1000 generations, and the first 25% of samples discarded as burn-in. Nodes with Bayesian posterior probabilities (BPP) ≥ 0.95 were considered well supported.

Results

Taxonomic description

Tylototriton gaowangjienensis J. Huang, Y. Xiang, Y.-X. Zhang, T. Wu & W.-S. Jiang, sp. nov.

https://zoobank.org/15A7EEED-B7DA-4C27-BA9F-CE0C53491BA2
Fig. 2

Type materials. ***Holotype*** • 2021051701, adult, male; China, Hunan Province, Xiangxi Tujia and Miao Autonomous Prefecture, Guzhang County, Gaowangjie National Nature Reserve; 28°40'39.80"N, 110°07'8.54"E, alt. 677 m; collected on 17 May, 2023 by You-Xiang Zhang. ***Allotype*** • 2021051702, adult, female; same locality and date as holotype. ***Paratypes*** • 2021051703~2021051704, adult, male, 2021051705~2021051706, adult, female; same locality and date as holotype.

Etymology. The new species is named after its type locality. We suggest the Chinese name “高望界疣螈” (pinyin: Gāo Wàng Jiè Yóu Yuán), and the English name Gaowangjie crocodile newt.

Diagnosis. The new species can be distinguished from other members of *Tylototriton* by the following combination of characteristics: (1) medium-sized body; (2) finger tips extending beyond the snout while forelimbs stretched forward; (3) tips of forelimbs and hindlimbs reaching and overlapping when folded towards the body; (4) snout edge round; (5) absence of small papillae in the male cloacal fissure; (6) dorsal ridges narrow, with width smaller than the eye diameter; (7) head length (HDL) greater than head width (HDW) (HDL/HDW=1.04~1.22); (8) orange markings absence on the parotoid; (9) nodule-like warts on body sides continuous; (10) vomerine teeth not connected at the tip, extending to the occipital bone at the posterior end.

Description of holotype (Table 2, Figs 2, 3). Adult male, medium-sized, measures 123.69 mm TL, 67.20 mm SVL, and 56.06 mm TAL. Head length greater than head width (HDL/HDW = 1.13). Snout short and blunt, longer than

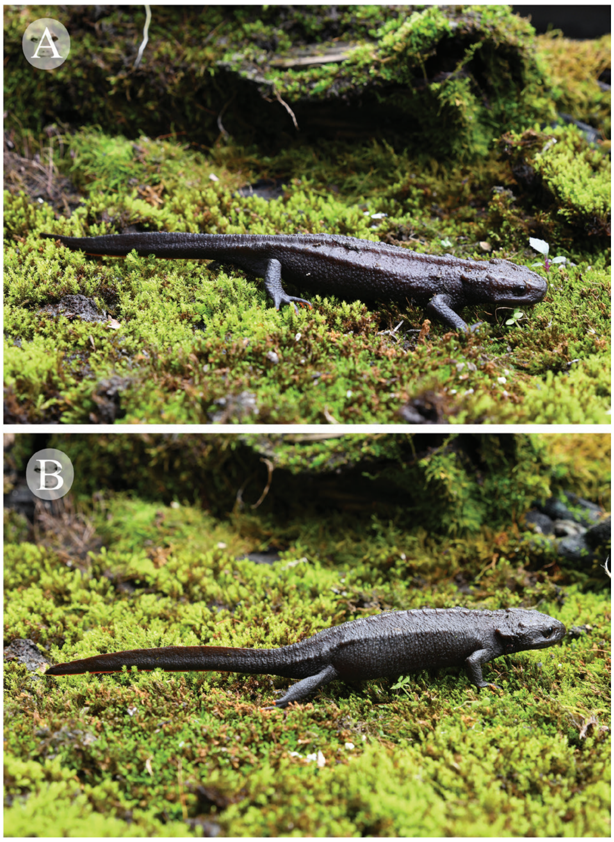

Figure 2. Photos of *T. gaowangjienensis* sp. nov. in live specimens. **A.** Female (2021051702); **B.** Male (2021051701). Photos by You-Xiang Zhang.

eye diameter, extends beyond lower lip from dorsal view. Eyes relatively large (3.8 mm), laterally oriented but visible in dorsal view, with nearly round eyelids. Mouth crack straight, exceeds half head, extending to posterior eyelids; jawbone with teeth. Vomerine teeth arranged linearly in "∧" shape, separated at anterior end, located between choanas, extending to posterior edge of orbit. Tongue oval-shaped, slightly concave in center, with bottom fixed and side edges free. Head ridges prominent, extending from snout to back head, forming longitudinal approximation "п" shape. Bone ridges form weak "v" shape at head; edge relatively smooth, and basically connected to middle ridge.

Table 2 Description of the morphology associated with the holotype, allotype and paratypes.

Item (mm)	Holotype (♂)	%	Allotype (♀)	%	Holotype+Paratype (♂) *2		%	Allotype (♀)+Paratype (♀) *2		%
					Means ± SD	**Range**		**Means ± SD**	**Range**	
SVL	67.20	100%	78.50	100%	69.15 ± 1.955	67.20~71.11	100%	74.01 ± 4.122	70.40~78.50	100%
TOL	123.69	184.06%	139.62	177.86%	134.2 ± 9.672	123.69~142.71	194.07%	135.5 ± 3.715	132.48~139.62	183.08%
HDL	15.93	23.71%	18.94	24.13%	18.26 ± 2.825	15.93~21.40	26.41%	17.09 ± 2.08	14.84~18.94	23.09%
HDW	14.06	20.92%	15.98	20.36%	14.91 ± 0.7903	14.06~15.62	21.56%	16.39 ± 1.066	15.59~17.6	22.15%
SL	4.38	6.52%	4.93	6.28%	5.013 ± 0.5594	4.38~5.44	7.25%	5.727 ± 0.69	4.93~6.13	7.74%
ED	3.80	5.65%	4.05	5.16%	4.14 ± 0.3027	3.80~4.38	5.99%	4.113 ± 0.5478	3.60~4.69	5.56%
TAL	56.06	83.42%	59.97	76.39%	61.57 ± 4.778	56.06~64.61	89.04%	61.4 ± 1.24	59.97~62.18	82.96%
TH	6.99	10.40%	6.59	8.39%	7.85 ± 0.7454	6.99~8.31	11.35%	7.52 ± 0.8843	6.59~8.35	10.16%
TRL	40.39	60.10%	52.2	66.50%	47.63 ± 7.039	40.39~54.45	68.88%	46.45 ± 5.082	42.57~52.2	62.76%
IOD	7.93	11.80%	8.96	11.41%	8.41 ± 0.4176	7.93~8.69	12.16%	8.197 ± 0.6643	7.75~8.96	11.08%
HL	8.50	12.65%	8.12	10.34%	8.343 ± 0.3066	7.99~8.54	12.07%	7.673 ± 0.395	7.37~8.12	10.37%
LLA	8.69	12.93%	7.57	9.64%	8.137 ± 1.339	6.61~9.11	11.77%	7.33 ± 0.2227	7.13~7.57	9.90%
FIIIL	3.93	5.85%	4.46	5.68%	4.51 ± 1.022	3.91~5.69	6.52%	4.04 ± 0.3897	3.69~4.46	5.46%
TLH	7.12	10.60%	8.25	10.51%	7.76 ± 1.358	6.84~9.32	11.22%	7.447 ± 0.6962	7.02~8.25	10.06%
TL	6.55	9.75%	5.98	7.62%	5.883 ± 0.6178	5.33~6.55	8.51%	6.67 ± 0.6951	5.98~7.37	9.01%
IND	4.34	6.46%	6.22	7.92%	4.9 ± 0.8931	4.34~5.93	7.09%	6.08 ± 0.1217	6~6.22	8.22%
TIIIL	5.91	8.79%	4.46	5.68%	5.957 ± 0.5615	5.42~6.54	8.61%	5.347 ± 0.7679	4.46~5.8	7.22%

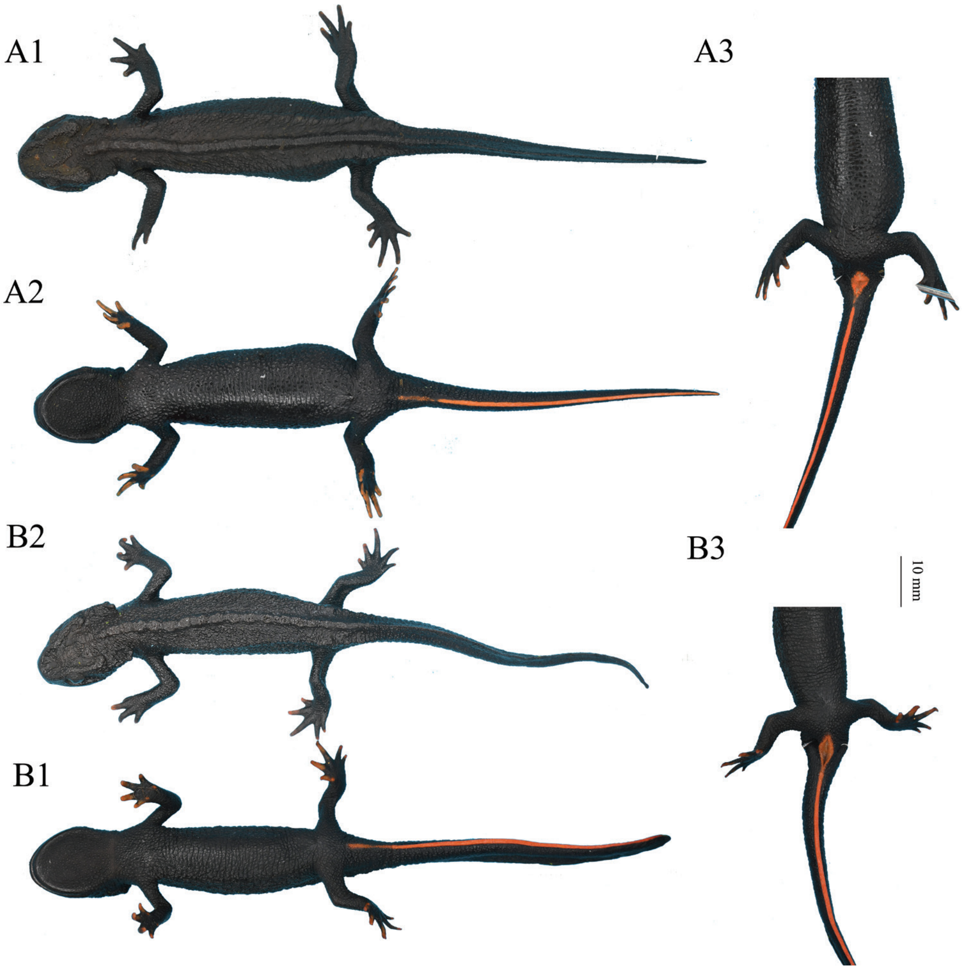

Figure 3. Photos showing color variation in *T. gaowangjienensis* sp. nov. in live specimens. **A.** Female (2021051702); **B.** Male (2021051701). Photos by You-Xiang Zhang.

Skin rough, small granules present on dorsal surfaces of head and dorsum, lateral sides of body and tail. On both sides of the trunk, a row of rib nodules composed of numerous slightly thick warts, slightly protruding outward but relatively flat; the labial margin, distal limbs, ventral limbs and the ventral edge of the tail smooth. Limbs relatively slender, with the hind limbs slightly longer than the forelimbs; tips of forelimbs and hindlimbs reached and overlapped when folding towards; finger tips extending beyond the snout while forelimbs stretched forward. Four fingers, comparative finger length: III > II > IV > I; five toes, comparative toes length: III > IV > II > V > I; all fingers and toes slightly flattened, with blunt ends and no fins.

The tail height exceeds the width at the tail base, with thin and lower dorsal fin folds. Caudal dorsal fin originates from the upper middle section of the tail, connected to the middle ridge of the back, without obvious segmentation. Ventral tail fin folds short and thick, starting from the tail base, with a small gap from the cloacal fissure's posterior edge. Cloacal region notably swollen, vent as longitudinal slit. Skin mostly black or dark gray. Ventral surface, distal ends of digits, peripheral area of cloaca, and ventral edge of tail orange-red; the orange-red region between tail's ventral edge and cloaca's periphery connected. After alcohol preservation, orange-red fades to milky white.

Description of skull (Fig. 4). Back of the skull rough, covered with numerous sculpture-shaped ridges. Skull wider than length, with obvious globular protrusions at the lateral posterior side of the squamosal. Square protrusions evident on the posterior lateral side of squamosal, extending to the base of the forelimbs. Ventral surface of the skull "∧" shaped. Left and right dentitions extend posteriorly along the inner wall of the eye socket to the base of the orbit and reach the occipital bone.

Secondary sexual characteristics and reproduction (Figs 3, 6). There was a remarkable difference in appearance between sexually mature females and males during the reproductive period: females exhibited a relatively thicker body compared to males, with a particularly prominent abdomen. In females, the cloacal fissure in the cloaca was short but significantly expanded outward, and the grooves on the inner wall protruded radially from the center to the surrounding areas. In contrast, males had a relatively long cloacal fissure but with moderate protrusion, smooth folds, and no grooves, which were less remarkable than those in females. However, both males and females showed no papilla within their cloacal fissures during the reproductive period.

The breeding season of *T. gaowangjienensis* lasted from April to June, with a peak period from late April to early May. In late March, the population gradually ended hibernation, and in early April, the males began to migrate to the breeding ground, usually pools or shallow waters, to await breeding opportunities. The females then entered the breeding ground about a week later. In late May, the females usually left the breeding ground after laying eggs, showing no protective behavior. After the females laid eggs and left, the males still stayed in the breeding ground for a period of time, and then left in mid to early June. With that, the breeding season ended, and the newts returned to a terrestrial preferred life in the forest.

Description of larva (Fig. 5). The juvenile's body is slender. Head length exceeds width. Eyes are large, rounded, laterally oriented but still visible in dorsal view. The front end of the three outer gills is obtuse. Skin is smooth, fingers and toes are light yellow, body color ranges from light black to brown. Dorsal fin folds are relatively high, occurring from about 35% of the total body length. Ventral fin folds are short and thick, extending from the cloaca to the end of the tail.

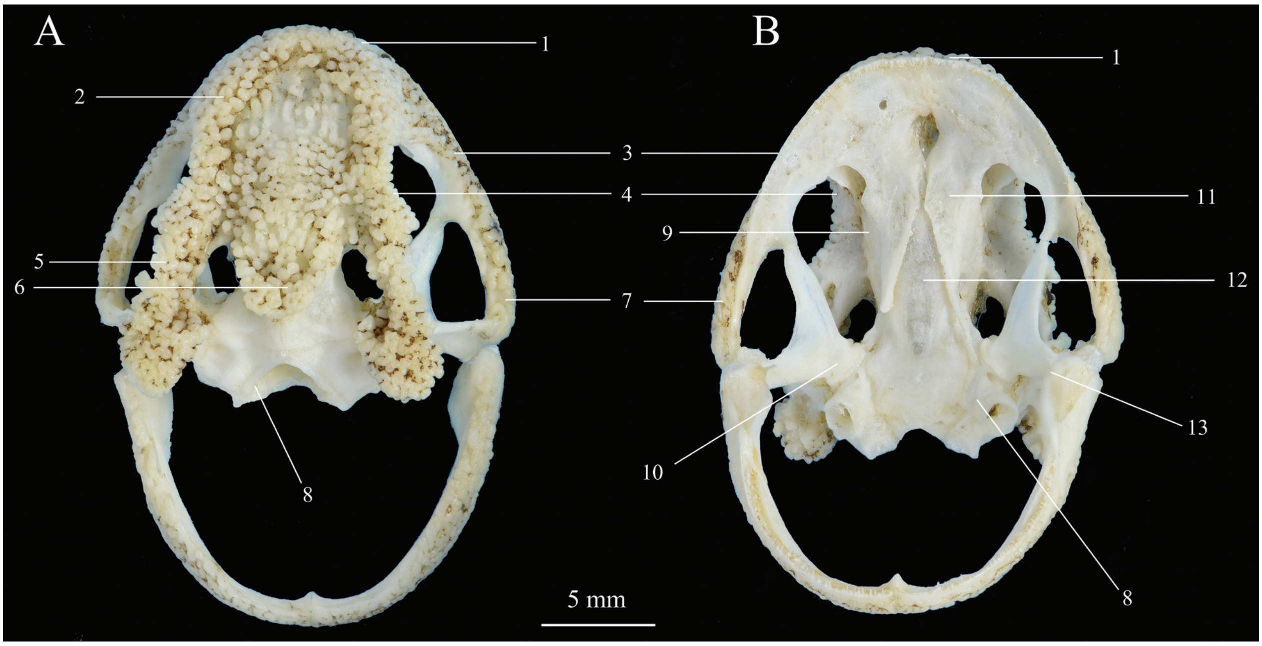

Figure 4. Skull specimen (JSUYY01) of *T. gaowangjienensis* sp. nov. **A.** Dorsal view; **B.** Ventral view. (1) premaxilla; (2) nasal; (3) maxilla; (4) prefrontal ; (5) squamosal; (6) parietal; (7) quadrate frontal; (8) prootic-exoccipital complex; (9) orbitosphenoid; (10) pterygoid ceratohyal; (11) vomer; (12) parasphenoid; (13) prearticular-angular. Photos by You-Xiang Zhang.

Distribution and habits (Figs 1, 6). *T. gaowangjienensis* is only known from Gaowangjie National Nature Reserve, Hunan Province, China. It was primarily distributed in natural broad-leaved or coniferous broad-leaved mixed forests at altitudes of 500–700 meters. During the breeding season, the habitats featured water and could be categorized into three types: seasonal roadside ponds, small creek ponds, and rice fields. The main vegetation types in the breeding areas were evergreen broad-leaved forests, evergreen coniferous broad-leaved mixed forests, and bamboo forests. The environment was lush with high humidity, and the ground was covered with a thick layer of fallen leaves and humus.

Figure 5. Photos of larva (JSUYY02) of *T. gaowangjienensis* sp. nov. in live specimens. **A.** Lateral view; **B.** Dorsal view. Photos by You-Xiang Zhang.

Figure 6. Breeding ground of *T. gaowangjienensis* sp. nov. in the Gaowangjie National Nature Reserve. Photos by You-Xiang Zhang.

Morphological comparisons

In PCA, the total variations of the first principal component was 67.7% and the second was 15.8% which, when combined, explained how the variations exceed 80%. The two-dimensional plots of PC1 vs. PC2 showed that the new species could be distinguished from its closely related species *T. dabienicus* and *T. tongziensis* (Fig. 7). Results from Kruskal-Wallis test also indicated that the new species was significantly different from *T. tongziensis*, *T. maolanensis*, *T. daloushanensis*, *T. anhuiensis*, *T. dabienicus*, and *T. wenxianensis* in specific morphometric characters, especially in males (P-values < 0.05, see more in Suppl. material 1: table S4). These findings demonstrated that the new species is morphologically distinct from all other known congeners.

Molecular analysis

The final alignment of the combined 16S rRNA and ND2 sequence dataset was 1925 bp in length. Both the ML and BI analyses yielded essentially identical topologies and ML tree was presented as Fig. 8. The monophyly of *Tylototriton* was strongly supported, with the 53 nominal species (or populations) mainly divided into two clades, corresponding to the subgenus *Tylototriton* (BPP/UFB = 1.00/99) and *Yaotriton* (BPP/UFB = 1.00/84), respectively. The subgenus *Yaotriton* further divided into two subclades, namely, the *T. wenxianensis* species subclade (BPP/UFB = 1.00/84) and the *T. asperrimus* species subclade (BPP/UFB = 1.00/77). The four individuals of our new species formed a monophyletic group, showing as a sister species to *T. dabienicus* (BPP/UFB = 1.00/96).

The p-distance values of 16S rRNA between the new species and other congeners ranged from 1.57% to 13.55% (see more in Suppl. material 1: table S5). The lowest value (1.57%) was observed between it and *T. dabienicus*, while the values between it and other species in the *T. asperrimus* species group were 2.69%, 3.65%, 3.85%, 3.85%, 3.85% and 4.81% relative to *T. tongziensis*, *T. anhuiensis*, *T. broadoridgus*, *T. maolanensis*, *T. daloushanensis*, and *T. wenxianensis* respectively. The p-distance values of ND2 sequence between the new species and other congeners ranged from 1.7% to 13.27% (see more in Suppl. material 1: table S6). The lowest value (1.7%) was still observed between it and *T. dabienicus*. The p-distance values indicated the degrees of differentiation between the new species and its sister species *T. dabienicus* were larger than some other known sister species pairs, such as these p-distances between *T. sparreboomi* and *T. pasmansi* (0.48 in 16S rRNA and 0.76 in ND2 gene) and between *T. verrucosus* and *T. shanjing* (0.98 in 16S rRNA and 0.92 in ND2 gene). It is of similar differentiations to that between *T. shanorum* and *T. ngarsuensis* (1.76 in 16S rRNA and 1.83 in ND2 gene).

Discussion

The new species described here, *T. gaowangjienensis* sp. nov., represents a distinct species based on both morphological and molecular analyses in this study. Based on the phylogenetic tree (Fig. 8) , *T. gaowangjienensis* sp. nov. is a new member in the *T. wenxianensis* species subclade that in the *T. asperrimus* species group (equals to the subgenus *Yaotriton*). The new species can be distinguished from other congeners in the genus *Tylototriton* by the following specific morphological characteristics (see more in Suppl. material 1: table S4).

T. gaowangjienensis sp. nov. can be distinguished from its sister group *T. dabienicus* by having the tip of the vom-

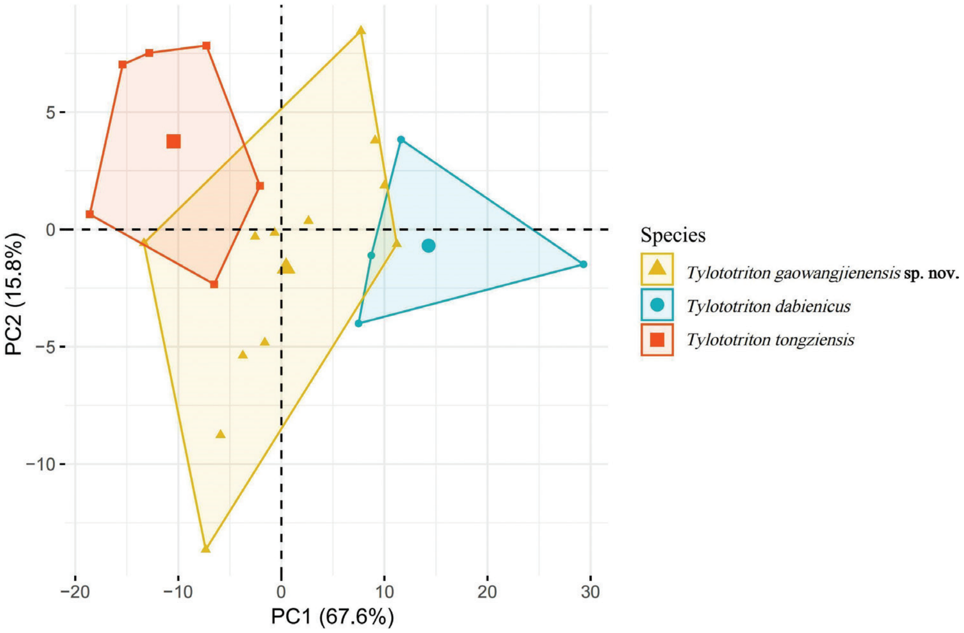

Figure 7. Plots of principal component analyses for *T. gaowangjienensis* sp. nov. and its closely related species according to morphological characteristics. PC1, the first principal component; PC2, the second principal component.

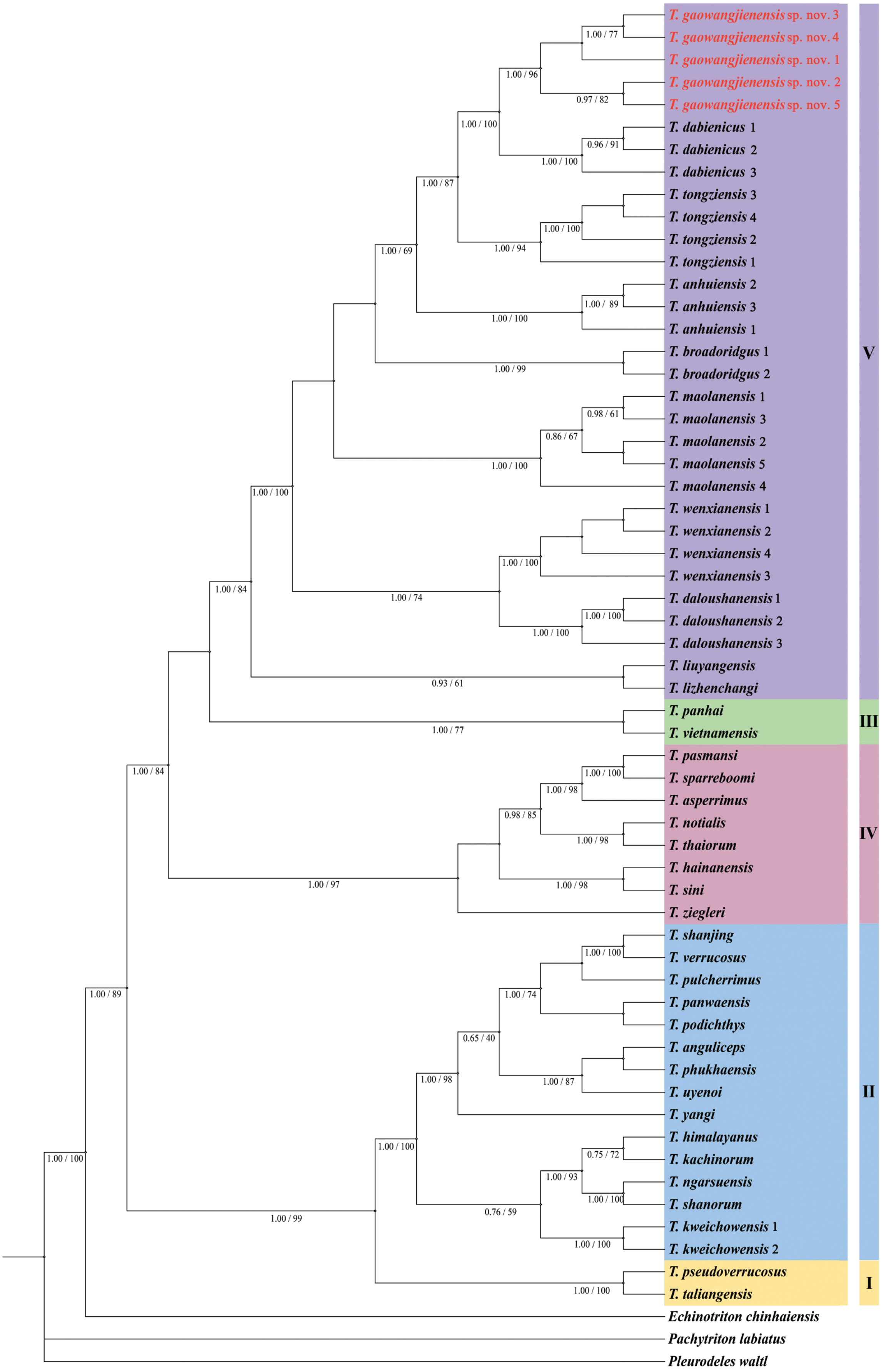

Figure 8. Phylogenetic tree of the genus *Tylototriton* reconstructed based on the mitochondrial 16S and ND2 gene sequences. I: *T. taliangensis* species group; II: *T. verrucosus* species group; III: *T. vietnamensis* species group; IV: *T. asperrimus* species group; V: *T. wenxianensis* species group.

erine teeth separated (vs. connected), and the fact that the posterior end extends to the occipital bone (vs. behind the eye socket); finger tips of forelimbs extending beyond the snout while forelimbs stretched forward (vs. to anterior corner of eyes); tips of forelimbs and hindlimbs reached and overlapped (vs. only slightly reached) when folding towards. In addition, the Kruskal-Wallis test showed that the two species were significantly different in the characters of TL and TAL/SVL of males, and significantly different in the characters of FIIIL and TL of females.

T. gaowangjienensis sp. nov. can be distinguished from *T. maolanensis* by having the nose end rounded (vs. truncated); the male cloacal wall has no papilla (vs. with papilla). The Kruskal-Wallis test showed that the males of two species were significantly different in the characters of SVL, TOL, HDW, TRL, SL, TLH, ED, TH, IND, LLA, HDL/SVL, TAL/SVL, and HDL/HDW.

T. gaowangjienensis sp. nov. can be distinguished from *T. broadoridgus* by having the tip of the vomerine teeth separated (vs. connected), and the posterior end extends to the occipital bone (vs. extends behind the eye socket); tips of forelimbs and hindlimbs reached and overlapped when folding towards (vs. touched a little or not); the midline spine of the dorsal is narrower, with a width smaller than the eye diameter (vs. the midline spine is wider, with a width equal to the eye diameter). The Kruskal-Wallis test showed that the males of two species were significantly different in the characters of HDL/SVL.

T. gaowangjienensis sp. nov. can be distinguished from *T. anhuiensis* by having tips of forelimbs and hindlimbs reached and overlapped when folding towards (vs. touched a little or not); the male cloacal wall lacks papilla (vs. with papilla). The Kruskal-Wallis test showed significant differences in TIIIL,TLH, HDL/SVL, TRL/SVL, HDL/HDW, IOD/HDW between the males of *T. gaowangjienensis* sp. nov. and *T. anhuiensis*.

T. gaowangjienensis sp. nov. can be distinguished from *T. wenxianensis* by having the tip of the vomerine teeth separated (vs. connected); tips of forelimbs and hindlimbs reached and overlapped when folding towards (vs touched a little); HL > HW (vs. HL = HW); finger tips extending beyond the snout while forelimbs stretched forward (vs. not reach the snout). The Kruskal-Wallis test showed significant differences in TAL, TL, HDL/SVL, ED/SVL and IOD/HDW between females of *T. gaowangjienensis* sp. nov. and *T. wenxianensis*.

T. gaowangjienensis sp. nov. can be distinguished from *T. tongziensis* by the lengths of TOL in females (TOL 130.2~141.1 mm vs. TOL 123.5–127.6 mm). The male cloacal wall has no small papilla (vs with papilla). The Kruskal-Wallis test showed significant differences in TLH, TRL/SVL, HDL/HDW between males of *T. gaowangjienensis* sp. nov. and *T. tongziensis*.

T. gaowangjienensis sp. nov. can be distinguished from *T. daloushanensis* by having finger tips extending beyond the snout while forelimbs stretched forward (vs. the finger tips are between the eyes and nostrils); the male cloacal wall lacks papilla (vs. with papilla). The Kruskal-Wallis test showed significant differences in HL, LLA, TL and FIIIL between *T. gaowangjienensis* sp. nov. and *T. daloushanensis* in males and females.

T. gaowangjienensis sp. nov. can be distinguished from *T. liuyangensis* by having finger tips extending beyond the snout while forelimbs stretched forward (vs. reaching the level of eye); tips of forelimbs and hindlimbs reached and overlapped when folding towards (vs. not touched).

T. gaowangjienensis sp. nov. can be further distinguished from *T. panhai* by the absence of orange markings on the parotoid (vs. present); from *T. vietnamensis* by the presence of gular fold (vs. absent); from *T. sparreboomi* by finger tips beyond the snout much while forelimbs stretched forward (vs. reaching the level of nostril), and nodule-like warts on body sides continuous (vs. separated); from *T. asperrimus* by nodule-like warts on body sides continuous (vs. separated), and tips of forelimbs and hindlimbs overlapping much when addressed along body (vs. meeting or overlapping); from *T. thaiorum* by nodule-like warts on body sides continuous (vs. separated); from *T. notialis* by nodule-like warts on body sides continuous (vs. separated), and orange markings on the parotoid and the rib nodules absent (vs. present); from *T. sini* by having nodule-like warts on body sides continuous (vs. separated); from *T. hainanensis* by finger tips extending beyond the snout much while forelimbs stretched forward (vs. reaching the level of eye); from *T. ziegleri* by nodule-like warts on body sides continuous (vs. separated), and vertebral ridge not segmented (vs. segmented).

T. gaowangjienensis sp. nov. can be distinguished from species of the *T. verrucosus* species group (equals to the subgenus *Tylototriton*), except for *T. taliangensis* and *T. pseudoverrucosus*, by having a black body except for tips of fingers and toes, margin of vent, and ventral edge of tail (vs. having distinct yellow to reddish brown markings on head, trunk, and tail edges). *T. gaowangjienensis* sp. nov. can be further distinguished from *T. lizhenchangi*, *T. taliangensis* and *T. pseudoverrucosus* by having a shorter body length in males and by having TOL 123.69~142.71 mm (vs. 145.6~173.0 mm, 186.0~220.0 mm, and 156.2~173.0 mm for the other three species in subgenus *Tylototriton*, respectively).

Only three *Tylototriton* species had been reported in Hunan Province prior to this study, all belonging to the subgenus *Yaotriton*. Besides the aforementioned morphological variations among them, they also exhibited distinct distribution patterns. Among these, *T. broadoridgus* is predominantly found in the northern part of Wuling Mountains, whereas *T. liuyangensis* and *T. lizhenchangi* are located at the border of Dawei Mountain and Yizhang County near Guangdong Province, respectively. Despite the relatively short geographic distances between *T. gaowangjienensis* and the other species in Hunan Province, it displayed the closest genetic relationship with *T. dabienicus* in Anhui Province, then followed by *T. tongziensis* in Guizhou and *T. anhuiensis* in Anhui Province. It also showed a phylogenetic affinity with *T. wenxianensis* in Gansu Province, while exhibiting a relatively larger genetic distance from *T. liuyangensis* and *T. lizhenchangi* in Hunan Province. However, all of these species mentioned above were placed in the same subclade as the *T. wenxianensis* species subclade. Wang et al. (2018) have indicated that the origin and primary distribution of the *T. wenxianensis* species subclade were in central and southern China. Through ancestral tracing and divergence time estimation, it was revealed that *T. liuyangensis* diverged earlier than other remaining species, such as *T. wenxianensis*, *T. broadoridgus*, and *T. dabienicus*.

Based on speculation suggesting that the Dabie Mountains to the Huaihe River may serve as a secondary distribution and specialization center for most species (Yao et al. 2018), it is inferred that *Tylototriton* species within the *T. wenxianensis* species subclade primarily spread eastward along this water system. It is speculated that the ancestor of the *T. wenxianensis* species subclade initially dispersed from the southwestern mountains and the eastern Himalayas into the Qinling Mountains along the Hengduan Mountains, traversing along the Jialing River in the upper reaches of the Yangtze River and its tributary, the Bailong River, to their current distribution area in southern China. However, owing to the geographical barrier between the Qinling Mountains and the Dabie Mountains-Huaihe River basin, the species in this secondary distribution and specialization center underwent separation and rapid evolution. This led to one subclade spreading eastward along the rivers to the Dabie Mountains, while another subclade dispersed to the Dongting Lake area southeast of the Qinling Mountains. The abundant water systems in the lake area provided conducive conditions for the dispersal of these newts thereafter. However, as of this study, more lineages and new species have been discovered, including monophyletic groups composed of several lineages such as *T. anhuiensis*, *T. dabienicus*, T. *tongziensis*, and *T. gaowangjienensis*, which are distributed in the Dabie Mountains to Wuling Mountains region. This may be the result of secondary or even multiple dispersal.

Based on field observations, one of the primary breeding areas of *T. gaowangjienensis* identified was that in the rice field, yet this habitat is increasingly facing challenges due to human activities. While it remains unclear whether this habitat selection was actively or passively chosen by the species in response to rapid environmental disturbances, it serves as a reminder to prioritize attention to these special habitats of such amphibians. Therefore, it is imperative to conduct more investigations and monitoring in the future to better understand and protect the species in the face of the ongoing environmental changes.

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References

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

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. [In Chinese]

Dubois A, Raffaëlli J (2009) A new ergotaxonomy of the family Salamandridae Goldfuss, 1820 (Amphibia, Urodela). Alytes 26(1–4): 1–85.

Dufresnes C, Hernandez A (2023) Towards completing the crocodile newts' puzzle with all-inclusive phylogeographic resources. Zoological Journal of the Linnean Society 197: 620–640. https://doi.org/10.1093/zoolinnean/zlac038

Fei L, Hu SQ, Ye CY, Huang YZ (2006) Fauna Sinica. Amphibia, Vol. I. Science Press, Beijing, 133 pp. [In Chinese]

Fei L, Ye CY (2016) Amphibians of China, Vol. Science Press, Beijing, 1060 pp. [In Chinese]

Fei L, Ye CY, Huan YZ, Jiang JP, Xie F (2005) An illustrated key to Chinese amphibians. Sichuan Publishing House of Science and Technology, Chengdu, 340 pp. [In Chinese]

Fei L, Ye CY, Yang RS (1984) A new species and new subspecies of the genus *Tylototriton* (Caudata: Salamandridae). Acta Zootaxonomica Sinica 30(11): 85–89. [In Chinese]

Frost DR (2020) Amphibian species of the world version 6.2. https://amphibiansoftheworld.amnh.org/Amphibia/Caudata/Salamandridae/Pleurodelinae/Tylototriton [accessed 6 January October 2024]

Grismer LL, Wood Jr PL, 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(4): 553–573. https://doi.org/10.11646/zootaxa.4500.4.5

Grismer LL, Wood Jr PL, 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 (2016) Crocodile newts, the primitive Salamandridae of Asia (Genera *Echinotriton* and *Tylototriton*). edn. Chimaira, Frankfurt, 415 pp.

Hoang DT, Chernomor O, Haeseler AV, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Hou M, Li PP, Lyu 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. [in Chinese]

Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler AV, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Khatiwada JR, Wang B, Ghiire S, Vasudmevan K, Paudel S, Jiang JP (2015) A new species of the genus *Tylototriton*(Amphibia: Urodela: Salamandridae) from eastern Himalaya. Asian Herpetological Research 6: 245–256. https://doi.org/10.16373/j.cnki.ahr.140097

Le DT, Nguyen TT, Nishikawa K, Nguyen SLH, 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. https://doi.org/10.5358/hsj.34.38

Li SZ, Wei G, Cheng YL, Zhang BW, 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(4): 282–296. https://doi.org/10.16373/j.cnki.ahr.200026

Liu CC (1950) Amphibians of western China. Fieldiana. Zoology Memoires 2: 1–10. https://doi.org/10.5962/bhl.part.4737

Luo T, Yan SS, Xiao N, Li W, Deng HQ, Zhou J (2022) A new species of the genus *Tylototriton* (Amphibia: Urodela: Salamandridae) from the Eastern Dalou Mountains in Guizhou, China. Zoological Systematics.

Lyu ZT, Wang J, Zeng ZC, Zhou JJ, Qi S, Wan H, Li YY, Wang YY (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 LT, Schmidt HA, Haeseler AV, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

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(1): 34–49. https://doi.org/10.5358/hsj.32.34

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 Jr ED, 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(2): 285–310. https://doi.org/10.11646/zootaxa.4006.2.3

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, Chulalongkorn University, Thailand 20: 144–161.

Poyarkov Jr NA, Nguyen TV, Arkhipov DV (2021) A new species of the genus Tylototriton (Amphibia, Caudata, Salamandridae) from central Vietnam. The Journal of Asian Biodiversity 10: 4–22. https://doi.org/10.47605/tapro.v10i1.244

Qian L, Sun X, Li J, Guo W, Pan T, Kang X, Jiang J, Wu J, Zhang B (2017) A new species of the genus *Tylototriton* (Amphibia: Urodela: Salamandridae) from the southern Dabie Mountains in Anhui Province. Asian Herpetological Research 8(3): 151–164. https://doi.org/10.16373/j.cnki.ahr.170013

Shen YH, Jiang JP, and Mo XY (2012) A new species of the genus *Tylototriton* (Amphibia, Salamandridae) from Hunan, China. Asian Herpetological Research 3(1): 21–30. https://doi.org/10.3724/SP.J.1245.2012.00021

Shen YH (2014) Fauna Hunan: Amphibia. Hunan Science and Technology Press, Hunan, 390 pp.

Sparreboom M (2014) Salamanders of the Old World: The Salamanders of Europe, Asia and Northern Africa. KNNV Publishing, Netherlands. https://doi.org/10.1163/9789004285620

Stuart BL, Phimmachak S, Sivongxay N, Robichaud WG (2010) A new species in the Tylototriton asperrimus group (Caudata: Salamandridae) from central Laos. Zootaxa 2650(1): 19–32. https://doi.org/10.11646/zootaxa.2650.1.2

Tang ZY, Tang ZM, Tang QY (1985) Preparation of Vertebrate Specimens, Fudan University Press, Shanghai, 196 pp.

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

Wang JX, Lan XY, Luo QH, Gu ZR, Zhou Q, Zhang MY, Zhang YX, Jiang WS (2022) Characterization, Comparison of Two New Mitogenomes of Crocodile Newts Tylototriton (Caudata: Salamandridae), and Phylogenetic Implications. Genes 13: 1878. https://doi.org/10.3390/genes13101878

Wang K, Yuan ZY, Zhong G, Li G, and Verrell PA (2017) Reproductive biology of *Tylototriton* yangi (Urodela: Salamandridae), with suggestions on its conservation. Amphibian & Reptile Conservation 11(2): 33–43.

Yao MC, Wei MC, Nie HY (2018) Geographic Distribution Pattern and Dispersal Route of Urodela in China. Chinese Journal of Zoology 53(1): 1–16. [In Chinese with English abstract]

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. https://doi.org/10.3724/SP.J.1245.2014.00001

Yuan ZY, Jiang K, Lyu SQ, Yang JX, Nguyen TQ, Nguyen TT, Jin JQ, Che J (2011) A phylogeny of the Tylototriton asperrimus group (Caudata: Salamandridae) based on a mitochondrial study: suggestions for a taxonomic revision. Zoological Research/Dōngwùxué yánjiū 32: 577–584.

Zaw T, Lay P, Pawangkhanant P, Gorin VA, Poyarkov Jr NA (2019) A new species of Crocodile Newt, genus Tylototriton (Amphibia, Caudata, Salamandridae) from the mountains of Kachin State, northern Myanmar. Zoological Research 40(3): 1–24. https://doi.org/10.24272/j.issn.2095-8137.2019.043

Supplementary material 1

Supplementary information

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