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Taxonomic relationship between *Tylototriton daweishanensis* Zhao, Rao, Liu, Li and Yuan, 2012 and *T. yangi* Hou, Li and Lu, 2012 (Amphibia: Urodela: Salamandridae)

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Abstract: We assessed taxonomic relationship of *Tylototriton daweishanensis* Zhao, Rao, Liu, Li and Yuan, 2012 and *T. yangi* Hou, Li and Lu, 2012 using mitochondrial DNA sequence data and found them to be as closely related as to be regarded as conspecific. This result, together with available morphological information, strongly indicates that *T. daweishanensis* is a junior synonym of *T. yangi*.

Key words: Specific status; Synonymy; *Tylototriton*; Molecular phylogeny; China

INTRODUCTION

Tylototriton daweishanensis was described in October 2012 from Mt. Dawei, Pingbian Miao Autonomous County, southern Yunnan Province, China (Zhao et al., 2012). This species has no orange or yellow markings except for ventral edge of the tail, cloacal region, and finger and toe; thus, it looks superficially similar to the species belonging to the subgenus *Yaotriton* (Dubois and Raffaëlli, 2009). However, according to the molecular phylogeny constructed by Zhao et al. (2012), the species belongs to another subgenus, *Tylototriton* (Dubois and Raffaëlli, 2009), which is charac-

terized by having orange or yellow markings on the head, body, and/or tail. In June 2012, four months before the description of *T. daweishanensis*, another species, *T. yangi*, had been described from Gejiu City, southern Yunnan Province, China (Hou et al., 2012), about 50 km northwest of the type locality of *T. daweishanensis*. This species was also confirmed to be a member of the subgenus *Tylototriton* by molecular analysis (Nishikawa et al., 2013a). Furthermore, we recently found a museum specimen that matches the description of *T. yangi* but was collected from the type locality of *T. daweishanensis*. These lines of information suggest the two species may be conspecific. However, no one has yet examined the taxonomic relationship of *T. daweishanensis* and *T. yangi*. We, thus, compared the two species by the use of mitochondrial DNA

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sequences and external morphology in order to clarify their taxonomic relationship.

MATERIALS AND METHODS

Unfortunately, we could not examine any type or topotypic specimens of *T. yangi*. Instead, we used specimens collected from several localities on Mt. Dawei (type locality of *T. daweishanensis*) and from an unknown locality in China (bought from the pet trade). We identified these specimens as *T. yangi* because they had laterally protruding quadrate regions; isolated dorsolateral nobs on body; and reddish-orange markings on posterior end of dorsolateral ridge on head, dorsal ridge of head, posterior half of parotoid, jaw angle, dorsal ridge, dorsolateral nobs on body, ventrolateral sides of trunk, cloaca region, tail, and fingers and toes, but lacked marking on the limbs (Figs. 1 and 2, see Hou et al., 2012; Fei et al., 2012; Le et al., 2015). For comparison, we used measurement and sequence data of type specimens of *T. daweishanensis* in Zhao et al. (2012).

We sequenced 988 bp of the mitochondrial NADH dehydrogenase subunit 2 region

(ND2) for molecular analyses. Sequencing method and primers are as reported by previous studies (Nishikawa et al., 2013a, b). Sequence data of the type specimens of *T. daweishanensis* and one new sequence of *T. yangi* were deposited in GenBank (accession numbers are shown in Table 1). We constructed phylogenetic trees using 12 specimens of *Tylostotriton* and one sequence each of *Echinotriton andersoni*, *Pleurodeles waltl*, and *Notophthalmus viridescens* (Table 1).

Optimum substitution models were selected by Kakusan4 (Tanabe, 2011). Following previous studies (Nishikawa et al., 2013a, b), we applied a non-partition model to our dataset. We then constructed phylogenetic trees by maximum likelihood (ML) and Bayesian inference (BI) methods. The ML tree was searched using TREEFINDER ver. Mar. 2011 (Jobb, 2011) and Phylogears2 (Tanabe, 2008), with 100 trials of the likelihood ratchet method (Vos, 2003), and the Bayesian analysis was conducted with MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001).

For the ML and BI analyses, the general time-reversible (GTR; Tavaré, 1986) model with a gamma shape parameter (0.485 in ML;

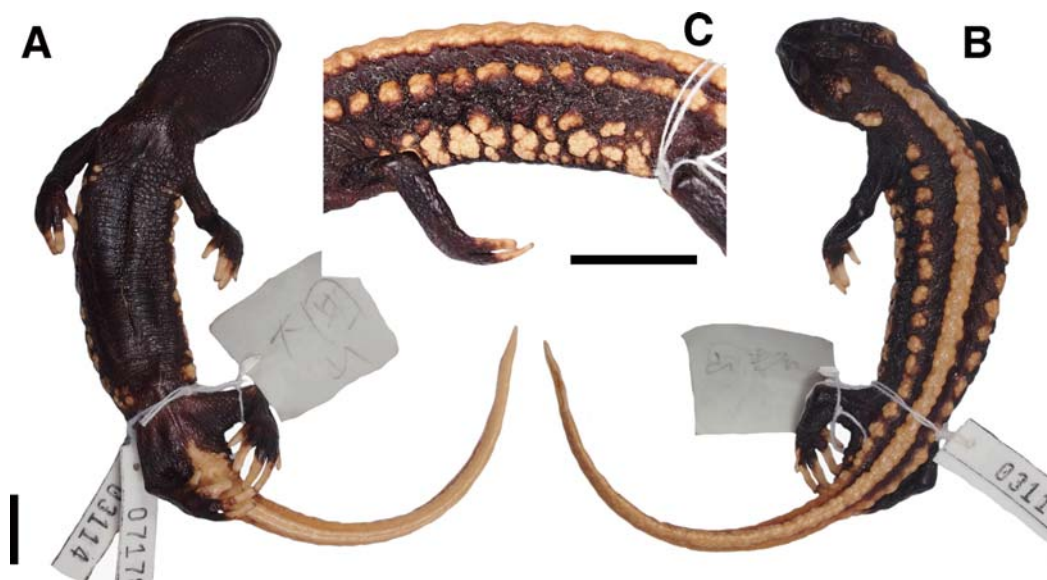


FIG. 1. A female specimen of *T. yangi* from Mt. Dawei, Pingbian Miao Autonomous County, Yunnan Province, China (NMNS 3114): ventral (A) and dorsal body (B), and lateral trunk (C). Scale bar=10 mm.



FIG. 2. Dorsal view of head of a female specimen of *T. yangi* from Mt. Dawei, Pingbian Miao Autonomous County, Yunnan Province, China (NMNS 3114). Scale bar=5 mm.

0.295 in BI) was selected by Kakusan4 as the optimal model. In BI analysis, two independent runs of four Markov chains were conducted for 10 million generations. We sampled one tree every 100 generations and calculated a consensus topology for 70,000 trees after discarding the first 30,001 trees (burn-in=3,000,000 generations).

The robustness of the ML tree was tested using bootstrap analysis (Felsenstein, 1985) with 1000 replicates. We regarded tree topologies with bootstrap values (bs) 70% or greater as well supported (Huelsenbeck and Hillis, 1993). For the Bayesian tree, we considered posterior probabilities (bpp) 0.95 or greater as significant support (Leaché and Reeder, 2002). Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated using MEGA6 (Tamura et al., 2013).

We compared coloration and body shape of *T. daweishanensis* and *T. yangi* based on observations and measurements made by ourselves, and data from Zhao et al. (2012). The following 10 measurements were taken for morphometric comparisons (character

TABLE 1. Samples of *Tylototriton* species and related species used for molecular analyses. CAS=California Academy of Sciences; CIB=Chengdu Institute of Biology; HNUE=Hanoi National University of Education; KUHE=Graduate School of Human and Environmental Studies, Kyoto University; MVZ=Museum of Vertebrate Zoology, University of California, Berkeley; NMNS=National Museum of Natural Science, Taiwan; RDQ=Rao Dingqi's private collection.

Sample no.	Species or subspecies	Voucher numbers	Locality	GenBank accession no.	Source
Ingroup					
1	<i>Tylototriton anguliceps</i>	HNUE A.I.1.111	Muong Nhe, Dien Bien, Vietnam	LC017836	Le et al. (2015)
2	<i>T. asperrimus</i>	CIB 200807055	Jinxiu, Guangxi, China	KC147815	Shen et al. (2012)
3	<i>T. daweishanensis</i>	RDQ 201203001	Mt. Dawei, Pingbian, Yunnan, China	LC017829	This study
4	<i>T. daweishanensis</i>	RDQ 201203002	Mt. Dawei, Pingbian, Yunnan, China	LC017830	This study
5	<i>T. kweichowensis</i>	MVZ 230371	Daguan, Yunnan, China	DQ517851	Weisrock et al. (2006)
6	<i>T. shanjing</i>	NMNS 3682	Jingdong, Yunnan, China	AB830721	Nishikawa et al. (2013a)
7	<i>T. shanorum</i>	CAS 230940	Taunggyi, Shan, Myanmar	AB922823	Nishikawa et al. (2014)
8	<i>T. taliangensis</i>	KUHE 43361	Sichuan, China (Pet trade)	AB769543	Nishikawa et al. (2013b)
9	<i>T. uyenoi</i>	KUHE 19147	Doi Suthep, Chiang Mai, Thailand	AB830733	Nishikawa et al. (2013a)
10	<i>T. verrucosus</i>	KIZ 201306055	Husa, Yunnan, China	AB922818	Nishikawa et al. (2014)
11	<i>T. yangi</i>	KUHE 42282	China (Pet trade)	AB769546	Nishikawa et al. (2013b)
12	<i>T. yangi</i>	NMNS 3114	Mt. Dawei, Pingbian, Yunnan, China	LC017831	This study
Outgroup					
13	<i>Echinotriton andersoni</i>	KUHE no number	Nago, Okinawa, Japan	AB769545	Nishikawa et al. (2013b)
14	<i>Pleurodeles walil</i>	MVZ 162384	Rabat, Morocco	DQ517813	Weisrock et al. (2006)
15	<i>Notophthalmus viridescens</i>	MVZ 230959	St. Charles, Missouri, USA	DQ517795	Weisrock et al. (2006)

definitions not mentioned below are given in Nishikawa et al. [2011]): SVL (snout-vent length) from tip of snout to posterior tip of vent; HL (head length); MXHW (maximum head width); SL (snout length); IOD (interorbital distance); OL (orbit length); AGD (axilla-groin distance); TAL (tail length) from posterior tip of vent to tail tip; MTAW (tail width at middle); MXTAH (tail height at maximum). All measurements were taken to the nearest 0.1 mm with dial calipers. We used a stereoscopic binocular microscope when necessary. Sex and maturity of specimens and number of eggs were checked and counted by minor dissections.

We compared SVL and a total of nine ratio

values to SVL (R, %) between *T. daweishanensis* and *T. yangi*, although we could not conduct statistical analyses because of the paucity of sufficient specimens.

RESULTS

We obtained 988 bp of ND2 sequence from 15 specimens including the outgroups (Table 1). Of the 988 nucleotide sites, 209 sites were variable and 106 sites were parsimony informative for the ingroup (sequence statistics available upon request from the senior author). The likelihood value of the ML tree was -4440.538. The mean likelihood score of the Bayesian analyses for all trees sampled at

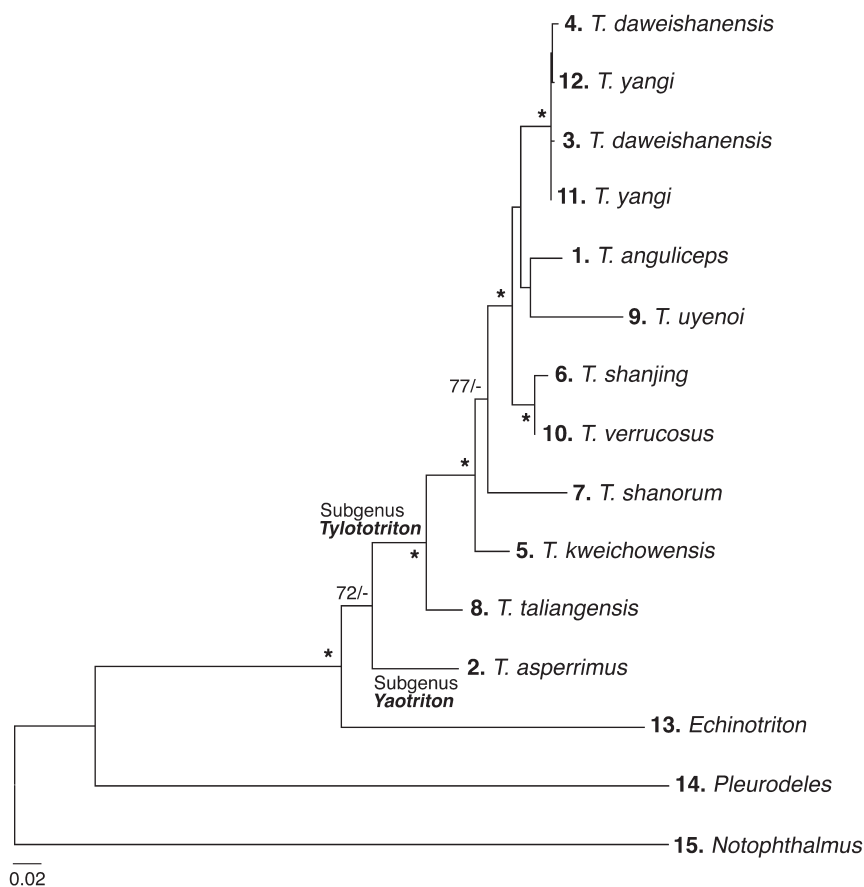


FIG. 3. Maximum likelihood tree based on ND2 sequence data for *Tylostotriton* and outgroup species. Numbers above branches represent bootstrap support values for ML inference and Bayesian posterior probability (bs/bpp). Asterisks indicate nodes with bs $\geq 70\%$ and bpp ≥ 0.95 .

TABLE 2. Uncorrected p-distances (%) between samples examined in this study.

Sam- ple no.		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Tylototriton anguliceps</i>	—													
2	<i>T. asperrimus</i>	11.3	—												
3	<i>T. daweishanensis</i>	4.3	10.0	—											
4	<i>T. daweishanensis</i>	4.6	9.8	0.5	—										
5	<i>T. kweichowensis</i>	6.0	9.9	6.2	5.9	—									
6	<i>T. shanjing</i>	4.5	11.4	4.7	5.0	5.6	—								
7	<i>T. shanorum</i>	7.0	11.4	7.1	7.3	6.2	6.6	—							
8	<i>T. taliangensis</i>	8.1	8.9	7.5	7.4	6.1	7.0	8.0	—						
9	<i>T. uyenoii</i>	7.4	13.2	7.7	7.6	8.2	7.5	9.5	9.7	—					
10	<i>T. verrucosus</i>	4.4	11.3	3.9	4.3	5.3	0.9	6.3	6.9	7.2	—				
11	<i>T. yangi</i>	4.1	10.1	0.2	0.5	6.0	4.5	6.9	7.5	7.7	3.7	—			
12	<i>T. yangi</i>	4.4	10.1	0.4	0.5	6.2	4.7	7.1	7.7	7.9	3.9	0.2	—		
13	<i>Echinotriton andersoni</i>	16.6	14.8	16.1	16.6	15.2	16.1	15.9	14.7	18.2	15.9	16.1	16.1	—	
14	<i>Pleurodeles waltl</i>	22.6	22.3	22.2	22.3	22.0	21.5	21.9	21.4	23.9	21.6	22.2	22.0	21.2	—
15	<i>Notophthalmus viridescens</i>	21.5	21.6	21.8	22.0	20.2	20.5	21.3	19.7	20.9	20.9	21.9	22.1	23.5	23.6

stationarity was -4472.260.

Phylogenetic analyses employing two different optimality criteria (ML and BI) yielded identical topologies except for relationships among specimens of *T. daweishanensis* and *T. yangi*. We therefore present only the ML tree in Fig. 3. Monophyly of *Tylototriton* was supported in ML (bs=72%), but not in BI (bpp=0.85). Within *Tylototriton*, *T. asperrimus* (subgenus *Yaotriton*) was first separated and the remaining species formed a clade (subgenus *Tylototriton*; bs=93%, bpp=1.00). Neither *T. daweishanensis* nor *T. yangi* formed a clade with significant support in either tree, but the specimens of the two species formed a clade (bs=99%, bpp=1.00) with extremely short branches that was grouped into the subgenus *Tylototriton* clade.

Genetic distance between *T. daweishanensis* and *T. yangi* was extremely small (mean 0.4%, range 0.2–0.5%; Table 2) and nearly identical with values within *T. daweishanensis* (0.5%) and *T. yangi* (0.2%). These values were much smaller than those between any pair of the remaining congeners examined in this study (smallest: 0.9% between *T. shanjing* and *T. verrucosus*, which were suggested to be conspecific by Zhang et al. [2007]).

In SVL and most of the character ratios examined, clear difference was not found

between the two species (Table 3). The degree of development of the dorsolateral ridge and dorsal ridge on the head was similar in the two species. Sparse granules and smooth skin on the dorsal head (also partly on the dorsal body) and laterally protruding quadrate region are shared in the two species. Dorsolateral nobs on the body were well developed, and each nob was isolated in both the species. Differences in external morphology could be recognized only for coloration. *Tylototriton daweishanensis* had orange markings on the ventrolateral sides of the trunk, cloacal region, ventral edge of tail, and fingers and toes, whereas *T. yangi* had markings not only on the areas noted above but also on the posterior end of the dorsolateral ridge on head, dorsal ridge on head, posterior half of parotoid, jaw angle, dorsal ridge, dorsolateral nobs on body, and dorsal ridge and side of tail.

DISCUSSION

Although we recognize coloration differences between *T. daweishanensis* and *T. yangi*, we find no other morphological or molecular differences between them. Furthermore, both species share sparse granules and smooth skin on the dorsal head and have laterally protruding quadrate

TABLE 3. Measurements and ratios (R: %SVL) of examined specimens of *Tylototriton*. For character abbreviations, see text.

Sex	Male			Female				
	<i>daweishanensis</i>	<i>yangi</i>		<i>daweishanensis</i>		<i>yangi</i>		
Specimen No.	RDQ 201203001	RDQ 201307301	RDQ 201307302	RDQ 000020	RDQ 201203002	NMNS 3114	KUHE 42282	KUHE 42283
Type	Paratype			Holotype	Paratype			
Locality	Mt. Dawei	Mt. Dawei	Mt. Dawei	Mt. Dawei	Mt. Dawei	Mt. Dawei	Unknown	Unknown
Measurement								
SVL	76.1	80	76.2	83	72.1	83.7	91.3	88.4
HL	19.7	19.8	19.7	20.3	18.7	20.5	21.0	19.8
MXHW	16.4	17.4	17.9	17.6	15.0	17.9	19.8	18.0
SL	6.0	7.0	6.9	6.4	5.2	8.0	7.8	6.7
IOD	5.1	8.0	7.8	9.0	8.8	8.6	9.2	8.7
OL	3.8	3.4	3.8	5.1	4.2	3.8	3.2	3.0
AGD	26	30.9	31.1	36.8	31.1	34.6	46.8	47.1
TAL	57	62.9	70.0	68.1	62.1	87.9	76.4	73.3
MTAW	3.8	4.3	3.5	5.2	3.7	3.7	4.2	4.1
MXTAH	7.7	8.1	7.7	7.5	6.2	7.2	8.5	9.8
R (% of SVL)								
RHL	25.9	24.8	25.9	24.5	25.9	24.5	23.0	22.4
RMXHW	21.6	21.8	23.5	21.2	20.8	21.4	21.7	20.4
RSL	7.9	8.8	9.1	7.7	7.2	9.6	8.5	7.6
RIOD	6.7	10.0	10.2	10.8	12.2	10.3	10.1	9.8
ROL	5.0	4.3	5.0	6.1	5.8	4.5	3.5	3.4
RAGD	34.2	38.6	40.8	44.3	43.1	41.3	51.3	53.3
RTAL	74.9	78.6	91.9	82.0	86.1	105.0	83.7	82.9
RMTAW	5.0	5.4	4.6	6.3	5.1	4.4	4.6	4.6
RMXTAH	10.1	10.1	10.1	9.0	8.6	8.6	9.3	11.1

regions, which are not known from other species of the genus *Tylototriton* (dense granules and rough skin on dorsal head, and weakly curving quadrate region in the remaining species of the genus *Tylototriton*). Because we could not examine type or topotypic specimens of *T. yangi* in the present study, we cannot completely preclude the possibility of their heterospecific relationship. However, it is almost certain that *T. yangi* varies in body coloration and that blackish individuals were described as *T. daweishanensis*. The description of *T. yangi* is short and uninformative, but the name is available according to the International Code of Zoological Nomenclature. We, thus, conclude that *T. daweishanensis* is a junior synonym of *T. yangi* based on the principle of priority.

A similar example is found with *T. verrucosus*

and *T. shanjing*. Although the two species clearly differ in body coloration (blackish body in the former and orange markings in the latter: Nussbaum et al., 1995), they were suggested to be conspecific by the results of mitochondrial DNA analyses (Zhang et al., 2007). However, as Stuart et al. (2010) cautioned, future reexamination is surely needed to make such a taxonomic conclusion. Following Stuart et al. (2010), we temporarily treat each of those two species as valid. The subgenus *Tylototriton* shows great variation in body coloration among species. The unique color pattern of each species is generally very useful for species identification, but does not apply to *T. yangi* and probably to *T. verrucosus*. The two species exceptionally show greater intraspecific variation in body coloration.

Reasons for such unique pattern in body color variation in these species are unknown, but may include individual and/or ontogenetic variations. Examination of variation in metamorphic individuals raised from one clutch might help clarifying this problem. Bearing this in mind, we need to examine populations from Gejiu City, as well as those from localities between Gejiu City and Mt. Dawei, which we could not examine in the present study, in order to further clarify degree of variation in body coloration within *T. yangi*.

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