

# Occurrence of *Tylototriton* species in Kachin State with Emphasis on their Morphology and Molecular Analysis

Than Zaw<sup>1</sup>, Sandar Yin<sup>2</sup>, May Thu Chit<sup>3</sup> and Khin Saw Hmwe<sup>4</sup>

## Abstract

Fieldwork was carried out in Kachin State Northern Myanmar, collected specimens were (9) in Sadung (25° 23' 36.1" N, 97° 53' 55.7"E; 3599ft a.s.l), (11) in Sinlum (24° 15' 38.13" N, 97° 32' 0.07"E; 5589 ft a.s.l) and (8) specimens in Ingyin mountain region (24° 58' 0.62" N, 96° 21' 50.08"E; 3048ft a.s.l), Indawgyi region respectively from 22-30 July 2019. Altogether 19 morphometric and meristic characters were taken into consideration in analyzing and evaluation of the specimens involved in this study. Altogether 19 morphometric parameters, between three different regions and localities of distribution, revealed that among them five parameters were significantly different from respective region ( $p < 0.05$ ). The different color morph of studied species in different areas were found as Orange brown in Sadung, dark brown in Sinlum and brown in Ingyin mountain., Indawgyi region, Kachin State Northern Myanmar were respectively. Morphology data were analyzed by R software (version 3.6.1) by using One-way ANOVA (Tukeys HSD method) and molecular level by DNA sequencing using Sanger method sequence were analyzed by MEGA\_X Software (version-10.0.5).

Keywords: parameters, morphology, molecular, sequencing, salamander

## Introduction

The global amphibian decline is a major conservation crisis, and approximately one-third of amphibian species are at risk of extinction. Within amphibians, the Order Caudata (Salamanders) has the highest proportion of threatened species (Stuarts *et al.*, 2004).

Besides habitat loss, species are harvested from the wild as food source, for use traditional medicine and to supply the international pet trade. The usually predictable high concentration of individual in small breeding sites during the reproductive season makes most *Tylototriton* species.

The genus *Tylototriton* ("Knobby-Newts") has a wide geographic distribution in mountain ranges from eastern Himalaya, through Indochina, to southern and central China, including: Nepal, India, Bhutan, Myanmar, Thailand, Laos, Viet Nam and China at elevations from 181 to 2,679m above sea level (Nishikawa *et al.*, 2014, Sparreboom 2014). The salamandrid genus *Tylototriton* contains 25 nominal species (Grismer *et al.*, 2018a), but up to now totally 26 species including latest *T. panwaensis* (Grismer *et al.*, 2019). In Myanmar, five species of *Tylototriton* were found, *T. verrucosus*, *T. shanorum*, *T. ngasuensis*, *T. kachinorum* and *T. panwaensis*.

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Although the Myanmar is largest country of Southeast Asia, the herpetofauna of country remains one of the least explored in the region (Grismer *et al.*, 2018a; Than Zaw *et al.*, 2019). The genus *Tylototriton* and its members have long been recorded from northern and eastern parts of Myanmar and traditionally classified as *T. verrucosus* Anderson, 1871 (Gyi, 1969). Nishikawa *et al.*, (2014), based on the examination of specimens assigned to *T. verrucosus* collected from the Shan Plateau in eastern Myanmar and pet-trade animals assumed to originate from Myanmar, recently described a new species, *T. shanorum* Nishikawa, Matsui & Rao, 2014. Soon after, Phimmachak *et al.*, (2015) published sequence data for specimens collected from the Sagaing Region and Kachin State in northern Myanmar, which were reported as *T. verrucosus*. More recently, Grismer *et al.*, (2018a) demonstrated the presence of two morphologically and genetically distinct lineages of *Tylototriton* in the Shan Plateau and described a new species from its north-western edge, *T. ngarsuensis* Grismer, 2018. Recent work also indicated the presence of *T. himalayanus* Khatiwada, Wang, Ghimire, Vasudevan, Paudel & Jiang, 2015 (a species described from Nepalese Himalaya) in northern Myanmar, but without providing voucher specimen information or any other justification for this identification (Hernandez, 2016; Hernandez *et al.*, 2018). The recent monographic review of the genus *Tylototriton* by Hernandez (2016) also indicated the possibility of the occurrence of *T. uyenoi* Nishikawa, Khonsue, Pomchote & Matsui, 2013 and *T. shanjing* Nussbaum, Brodie & Yang, 1995 in parts of Myanmar adjacent to northern Thailand and the southwestern Yunnan Province of China; however, these records are not supported by voucher specimens. Thus, our knowledge on the taxonomic composition and diversity of the genus *Tylototriton* in Myanmar is still far from complete.

The field survey was carried out to assess the new finding of salamanders in Kachin State, three species of *Tylototriton* were found, *T. verrucosus*, *T. kachinorum* and *T. panwaensis* according to the present work among these *T. kachinorum* was first erected as a new species of Myanma Salamander by Than Zaw *et al.*, (2019) as a first local author.

The main objective of present work was to record and identify *Tylototriton* species in Kachin State and the **Hypothesis:**

H<sub>0</sub>: The species occurrence of *Tylototriton* are the same within Kachin State

H<sub>1</sub>: The species occurrence of *Tylototriton* are differ within Kachin State

## Materials and Methods

### Study areas

The specimen collection areas were in Sadung (25° 23' 36.1" N, 97° 53' 55.7"E; 3599ft a.s.l), Sinlum (24° 15' 38.13" N, 97° 32' 0.07"E; 5589 ft a.s.l) and Indawgyi region (24° 58' 0.62" N, 96° 21' 50.08"E; 3048ft a.s.l) in the Northern part of Myanmar, Kachin State (Fig 1. Table 1, Plate I & II).

### Observation and specimen collection

Fieldwork was carried out in Sadung, Myitkyina environ, Sinlum mountain, Momauk Township, Banmaw District, and Ingyin mountain, Indawgyi, Mohnyin Township, Kachin State, Northern Myanmar, from 22 to 30 July 2019. Specimens of *Tylototriton* sp. were collected by hand in farm land, small ponds, and swamps in forest clearings surrounded by montane evergreen tropical forests. Specimens were euthanized by 20% benzocaine and tissue

samples for DNA analysis were taken and immersed in 95% ethanol (femoral muscles and heart) before preserving. The whole mount specimens were preserved in 70% ethanol and placed at Museum of Zoology Department, Mandalay University.

### Morphometric and Meristic Data

Altogether 19 morphometric and meristic characters were taken into consideration in analyzing and evaluation of the specimens. These included: the snout vent length (SVL), tail length (TL), head length (HL), eye-naris distance (END); internarial distance (IND); head width (HW), interorbital distance (IOD), length of cranial crest (CCL), parotoid gland length (PGL); width of cranial crest (CCW), boss length (BL), width of the middorsal crest or vertebral ridge (MCW), number of body warts (NBW), length of extended right fore limb (LFL) and right hind limb (LHL),

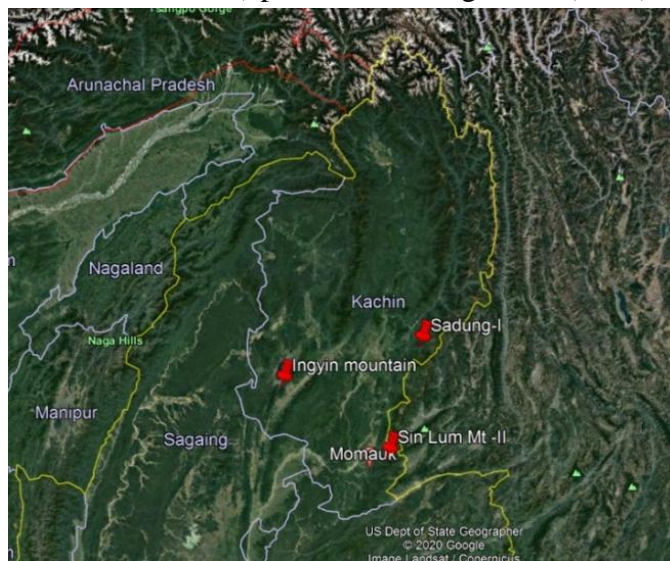
A part from these 16 parameters, which were the same parameters involved in the evaluation of different color morphs *Tylotriton verrucosus* specimens in China (Nussbaum et al., 1995), three additional characters of the head, namely the height of the triangle (TRH), the base of the triangle (TRB), and the area of the triangle (TRA), calculated by the formula ( $\frac{1}{2} \times \text{base TRB} \times \text{height TRH}$ ) were used according to Than Zaw (2004). Respective measurements were taken with a Helios digital caliper (Fig 2).

### Data Analysis

Data were statistically analyzed by R software (version 3.6.1) by using One-way ANOVA (Tukeys HSD method), DNA analyze by Sanger sequencing method and MEGA\_X software (version 10.0.5).

### DNA isolation, PCR, and sequencing

The DNA analysis processes were carried out by aid of Nikolay Poyarkov. A, Associate Professor, Department of Vertebrate Zoology, Biological Faculty, Lomonosov Moscow State University, Moscow 119234, Russia. We used the primers 16L-1 for forward (5'-CTGACCGTGCAAAGGTAGCGTAATCACT-3') and 16H-1 for reverse (5'-CTCCGGTCTGAACTCAGATCACGTAGG-3') to amplify the DNA. For amplification and sequencing of the *ND2* gene, we used the SL-1 for forward (5'-ATAGAGGTTCAAACCTCTC-3') and SL-2 for reverse (5'-TTAAAGTGTCTGGGTTGCATTTCAG-3') primers of Wang *et al.*, (2018).



**Fig 1.** Three study sites of map source from Google Earth



(a) Sadung paddy field



(a) Sadung specimen



(b) Small pond on top of the Sinlum mountain



(b) Sinlum specimen



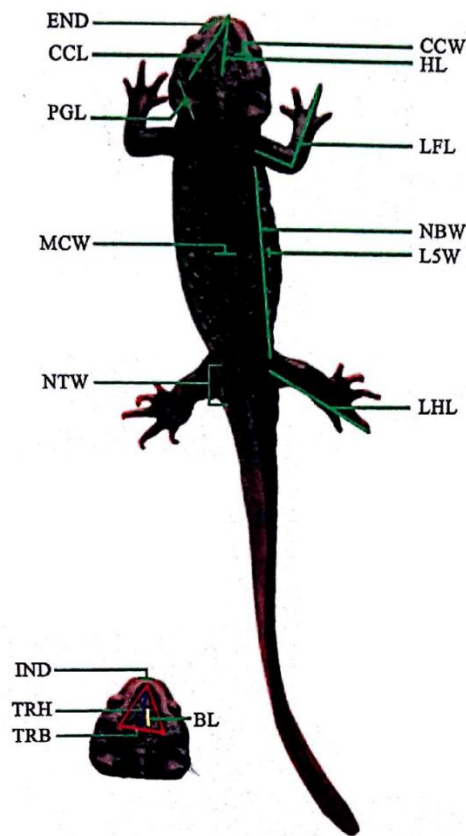
(c) Summit of Ingyin mountain



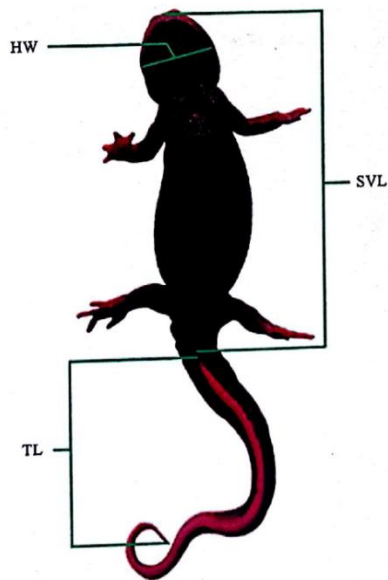
(c) Ingyin specimen

**Plate I.** Habitats of studied species

**Plate II.** Specimen from respective study site



(a). dorsal view



(b). ventral view

**Fig 2.** Meristic and Parametric measurement taken on salamander specimen

## Results

Data were analyzed by using One Way ANOVA (Tukey's HSD method) on altogether 19 morphometric parameters, between three different regions and localities of distribution, revealed that among them five parameters namely: SVL, TL, HL, HW and HA were significantly different from respective region ( $p < 0.05$ ) (Table 2 & Fig 3-12) .

Multiple comparison on the snout vent length (SVL) of Sadung specimens and Ingyin mountain specimens were significantly different, similarly between specimens of Sinlum mountain and Sadung were significant ( $p < 0.05$ ) but not in between Sinlum mountain and Ingyin (Fig 3&4). Taken into consideration of tail length (TL) of Ingyin mountain specimens was significantly different ( $p < 0.05$ ) from that of both Sadung and Sinlum specimens but Sinlum and Sadung was not significant (Fig 5&6).

Although the head length of Sadung specimens with both Ingyin mountain and Sinlum mountain individuals was not significant, the head length of Sinlum vs Sadung individuals was significantly different ( $p < 0.05$ ) (Fig 7-8).

In head width, the individual of Sinlum and Ingyin was not significant, but the specimens of Sadung head width was significantly different ( $p < 0.05$ ) to that of both Sinlum and Ingyin mountain specimens. (Fig 9-10).

Although the head area of Ingyin specimens with both Sadung mountain and Sinlum mountain individuals was not significant, the head area of Sinlum vs Ingyin mountain individuals was significantly different ( $p < 0.05$ ) (Fig 11-12). The different color morph of studied species in different areas were found as Orange brown in Sadung, dark brown in Sinlum and brown in Ingyin Mt., Indawgyi region, Kachin State Northern Myanmar were respectively (Plate II).

According to analysis by Sanger sequencing and MEGAX software, the phylogenetic tree of twenty six *Tylototriton* species were presented, suggesting that the species were dispersed within four clades. We analyzed the FASTA file type by MEGA\_X revealed that the uncorrelated 'p' distance value for pair wise 26 species (Table 4). Among 26 species, 5 species were found in Myanmar described as red color font, *T. panwaensis* in Clade-I and *T. verrucosus* in Clade-II, *T. shanorum* and *T. kachinorum* were in Clade III and *T. ngarsuensis* in Clade-IV respectively, two blue stars are represent the new finding for present work (Fig. 13).

Table.1. Number of collected specimens, locations, elevation and habitat types on the study sites.

site	state/ division	Longitude and Latitude		collection sites	nature of habitat	month of trips taken	sex	
		(a.s.l ft)					male	female
Sadung	Myit kyina	25°23'36.1" N, 97° 53' 55.7"E	3599ft	Farm land, Paddy field	Paddy field	July 22- 24,2019	4	5
Sinlum	Mo mauk,	24°15'38.13" N, 97° 32' 0.07"E	5589 ft	Sinlum village	Mountain forest	July 25- 27,2019	5	6
Indaw gyi	Indaw gyi,	24°58'0.62" N, 96° 21' 50.08"E	3048ft	Ingyin mountain	Mountain forest	July28- 30 ,2019	5	3

Table 2. Significantly different parameter of studied species from three study areas

Parameter	Area	Min	Max	Mean	SD
SVL	Sadung	79.06	91.4	86.12	3.9176
	Sinlum	65.77	87.35	79.01	6.3478
	Ingyin	79.23	98.59	87.34	6.5635
TL	Sadung	76.81	101.08	86.56	7.0352
	Sinlum	69.06	90.87	83.54	6.7074
	Ingyin	90.19	101.35	95.32	4.2735
HL	Sadung	14.57	19.17	16.85	1.4679
	Sinlum	13.05	16.69	15.68	1.0256
	Ingyin	12.9	16.38	14.91	1.1432
HW	Sadung	13.86	19.41	17.89	1.7265
	Sinlum	17.92	20.37	19.50	0.6761
	Ingyin	14.38	18.87	16.56	1.7545
HA	Sadung	48.76	65.98	58.49	5.2236
	Sinlum	55.34	69.37	64.42	4.2512
	Ingyin	53.26	68.00	60.12	5.1185

Table 3. Voucher specimens and GenBank Sequences of *Tylototriton* with out-group taxa

No.	Species name	Voucher number	GenBank: Accession No.
1	<i>kachinorum</i>	ZMMU A5953	GenBank: MK095618.1
2	<i>taliangensis</i> .	<NA>	GenBank: KP979646.1
3	<i>pseudoverrucosus</i>	CIB CG2012001	GenBank: KU255458.1
4	<i>kweichowensis</i> .	<NA>	GenBank: KU320632.1
5	<i>shanorum</i>	CAS 230933	GenBank: KY225575.1
6	<i>ngarsuensis</i>	LSUHC 13762	GenBank: MH836585.1
7	<i>Tylototriton</i> _sp._1.	MH-2011 haplotype	GenBank: JF827270.1
8	<i>himalayanus</i>	CIB 201406285	GenBank: KY800772.1
9	<i>yangi</i>	isolate 20120231	GenBank: KY225578.1
10	<i>uyenoi</i>	isolate DOI 070960	GenBank: MG018994.1
11	<i>anguliceps</i>	NCSM 82953	GenBank: KT304297.1
12	<i>podichthys</i>	NCSM 77722	GenBank: KT304296.1
13	<i>dabienicus</i>	HNNU:1004-026	GenBank: KY800869.1
14	<i>verrucosus</i>	CAS 230940	GenBank: JX444703.1
15	<i>shanjing</i>	haplotype H47 D-loop	GenBank: KC507867.1
16	<i>vietnamensis</i>	IEBRA. 0701	GenBank: KY800873.1
17	<i>ziegleri</i>	isolate 20187231	GenBank: MH664291.1
18	<i>hainanensis</i>	CIB20081052	GenBank: KY800826.1
19	<i>asperrimus</i>	TP26278	GenBank: DQ517849.2
20	<i>notialis</i>	VNMNTAO 1229	GenBank: KY800883.1
20	<i>liuyangensis</i>	CSUFT20100108	GenBank: KY800695.1
21	<i>lizhanchangii</i>	KUHE:42316	GenBank: KY800881.1
23	<i>broadoridgus</i>	CIB200085	GenBank: KY800749.1
24	<i>wenxianensis</i>	CIB2010123102	GenBank: KY800868.1
25	<i>panwarensis</i>	CAS 245418	GenBank: KT304279.1
26	<i>pulcherrimus</i>	CIBTY040	GenBank: KY800890.1

Table 4. Uncorrelated 'p' distance of twenty six species of genus *Tylototriton*.

No.	species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	<i>kachinorum</i>																										
2	<i>taliangensis.</i>	8.1																									
3	<i>pseudoverrucosus</i>	7.0	12.7																								
4	<i>kweichowensis.</i>	7.7	8.0	12.0																							
5	<i>shanorum</i>	6.6	7.4	7.7	10.9																						
6	<i>ngarsuensis</i>	10.1	6.0	11.5	7.1	7.8																					
7	<i>Tylototriton_sp_1.</i>	10.7	10.9	5.2	11.6	12.4	10.2																				
8	<i>himalayanus</i>	5.6	11.8	11.0	7.4	7.0	11.4	12.8																			
9	<i>yangi</i>	6.4	7.1	11.6	7.4	6.3	5.9	10.8	7.7																		
10	<i>uyenoi</i>	10.8	9.8	11.0	8.3	7.6	10.3	7.6	12.2	11.2																	
11	<i>anguliceps</i>	12.6	12.0	6.8	7.4	12.9	10.4	11.9	9.8	11.4	7.5																
12	<i>podichthys</i>	8.0	10.3	6.7	11.8	10.5	12.9	9.8	15.5	12.7	6.5	6.3															
13	<i>dabienicus</i>	9.6	9.9	6.7	11.8	10.8	12.8	10.4	15.8	12.5	6.5	6.5	0.1														
14	<i>verrucosus</i>	10.6	10.8	5.3	11.5	12.5	10.4	0.0	12.7	10.6	7.8	11.9	9.9	10.6													
15	<i>shanjing</i>	7.1	7.6	8.1	10.2	7.8	8.2	7.8	8.3	7.3	7.5	11.3	8.1	8.2	7.7												
16	<i>vietnamensis</i>	9.9	9.6	7.6	11.6	11.1	12.9	10.4	13.2	12.7	6.8	6.0	0.2	0.1	10.5	9.7											
17	<i>ziegleri</i>	8.2	6.9	8.0	8.1	11.5	10.5	11.8	12.3	7.9	8.1	12.6	11.9	12.0	11.9	6.4	11.6										
18	<i>hainanensis</i>	10.1	10.1	6.8	11.9	10.6	12.7	10.0	15.6	12.8	6.1	6.4	0.1	0.1	10.1	8.1	0.1	12.0									
19	<i>asperrimus</i>	9.5	9.9	6.7	11.8	10.8	12.6	10.4	13.2	12.3	6.8	6.5	0.1	0.0	10.6	8.2	0.1	12.0	0.1								
20	<i>notialis</i>	9.9	10.0	6.7	12.0	10.8	12.8	9.8	15.5	12.7	6.0	6.3	0.1	0.1	9.9	8.2	0.1	11.9	0.0	0.1							
21	<i>liuyangensis</i>	11.7	12.1	7.5	8.5	7.9	11.5	7.8	6.6	10.0	11.6	8.3	10.6	10.6	7.6	12.3	11.4	10.2	11.1	10.6	11.2						
22	<i>lizhenchangi</i>	8.1	9.8	6.5	11.5	10.9	13.0	10.0	13.1	12.8	6.4	6.4	0.1	0.1	10.1	9.6	0.1	12.0	0.1	0.1	0.1	10.7					
23	<i>broadoridgus</i>	5.6	11.8	11.0	7.4	7.0	11.4	12.8	0.0	7.7	12.2	9.8	15.5	15.8	12.7	8.3	13.2	12.3	15.6	13.2	15.5	6.6	13.1				
24	<i>wenxianensis</i>	9.9	9.9	6.7	11.6	10.8	12.8	10.1	15.7	12.5	6.8	6.3	0.1	0.1	10.2	8.2	0.1	12.2	0.1	0.0	0.1	10.6	0.1	15.7			
25	<i>panwarensis</i>	8.0	10.3	6.4	11.8	10.5	13.1	8.2	15.5	12.8	6.3	6.5	0.0	0.1	9.6	9.7	0.1	11.9	0.1	0.1	0.1	10.4	0.1	15.5	0.1		
26	<i>pulcherrimus</i>	8.1	10.1	6.5	11.9	10.3	13.0	8.3	15.4	12.8	6.4	6.4	0.0	0.1	9.8	8.2	0.1	11.8	0.1	0.1	0.1	10.5	0.1	15.4	0.1	0.0	



Sequences of *Tylototriton verrucosus* from Sinlum specimen <1040 bp>

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tgaaccgcta tgcactatca atcctgttgt caagcctagc agtaggaaca attacaactc tctcaagctc
ccattgattt ctggcatgag taggactaga aattaataca ctagcaataa tcccgcataa aacaaaaata
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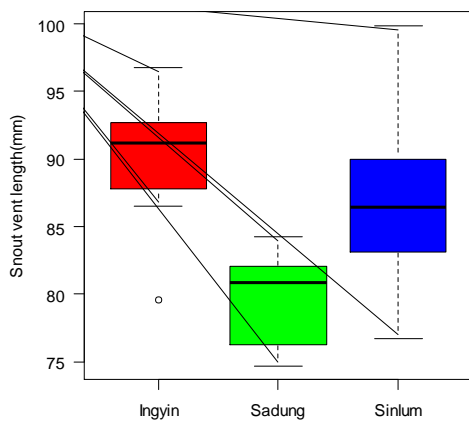


Fig 3. Snout Vent Length mean of studied species from three areas

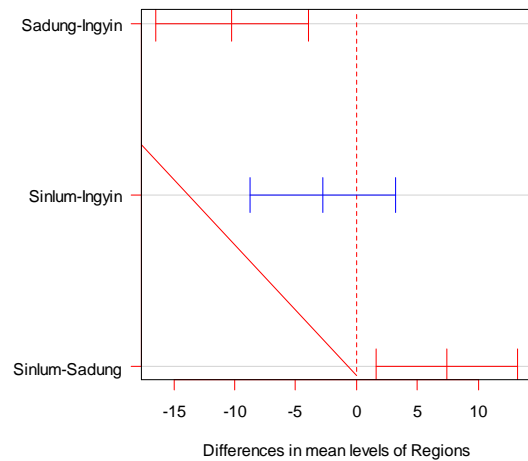


Fig 4. Multiple comparison of difference in mean levels of snout vent length of studied species from three areas

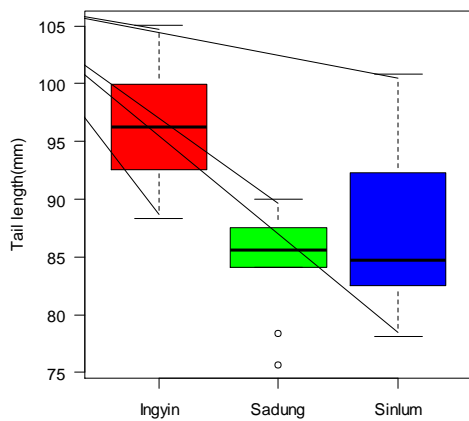


Fig 5. Tail length mean of studied species from three areas

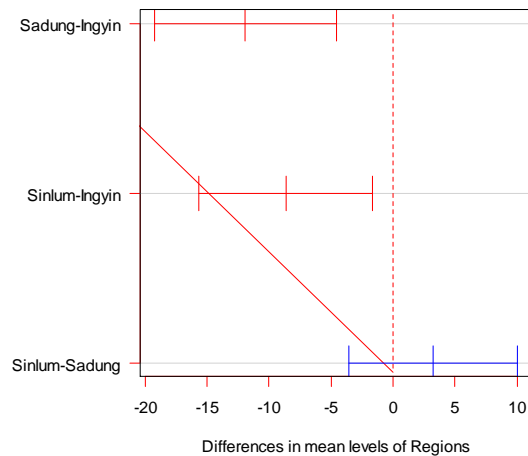


Fig 6. Multiple comparison of difference in mean levels of tail length of studied species from three areas

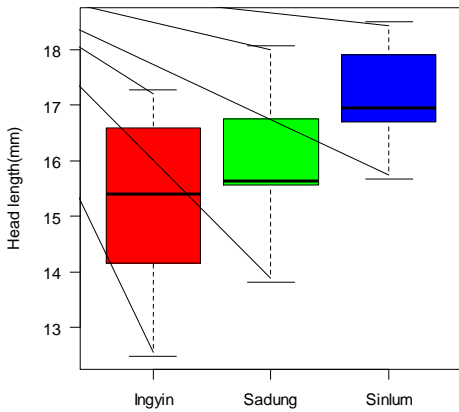


Fig 7. Head Length mean of studied species from three areas

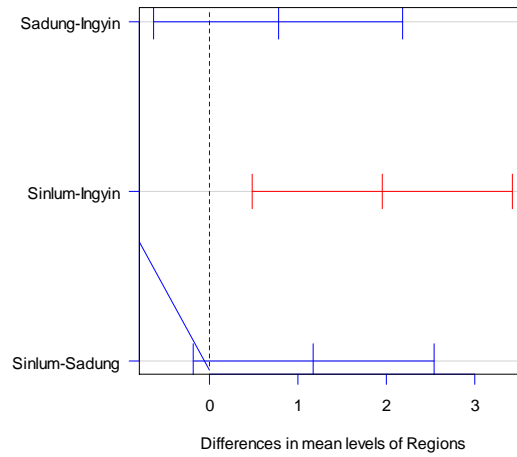


Fig 8. Multiple comparison of difference in mean levels of head length of studied species from three areas

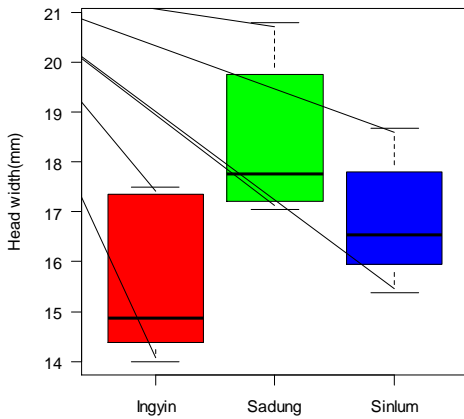


Fig 9. Head width mean of studied species from three areas

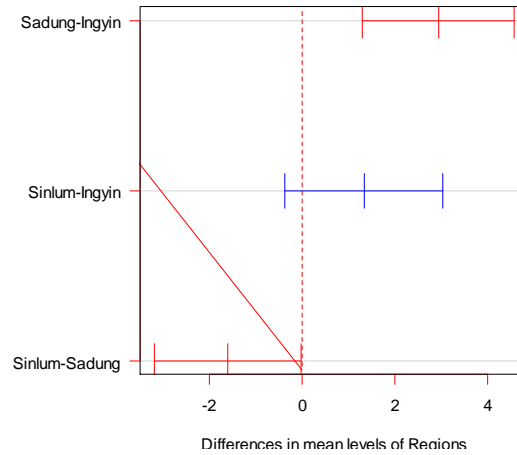


Fig 10. Multiple comparison of difference in mean levels of head width of studied species from three areas

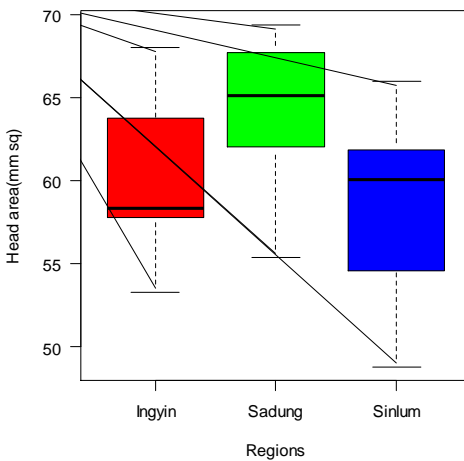


Fig 11. Head area mean of studied species from three areas

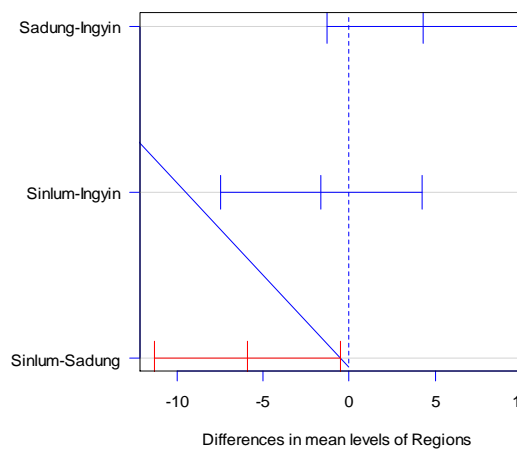


Fig 12. Multiple comparison of difference in mean levels of head area of studied species from three areas

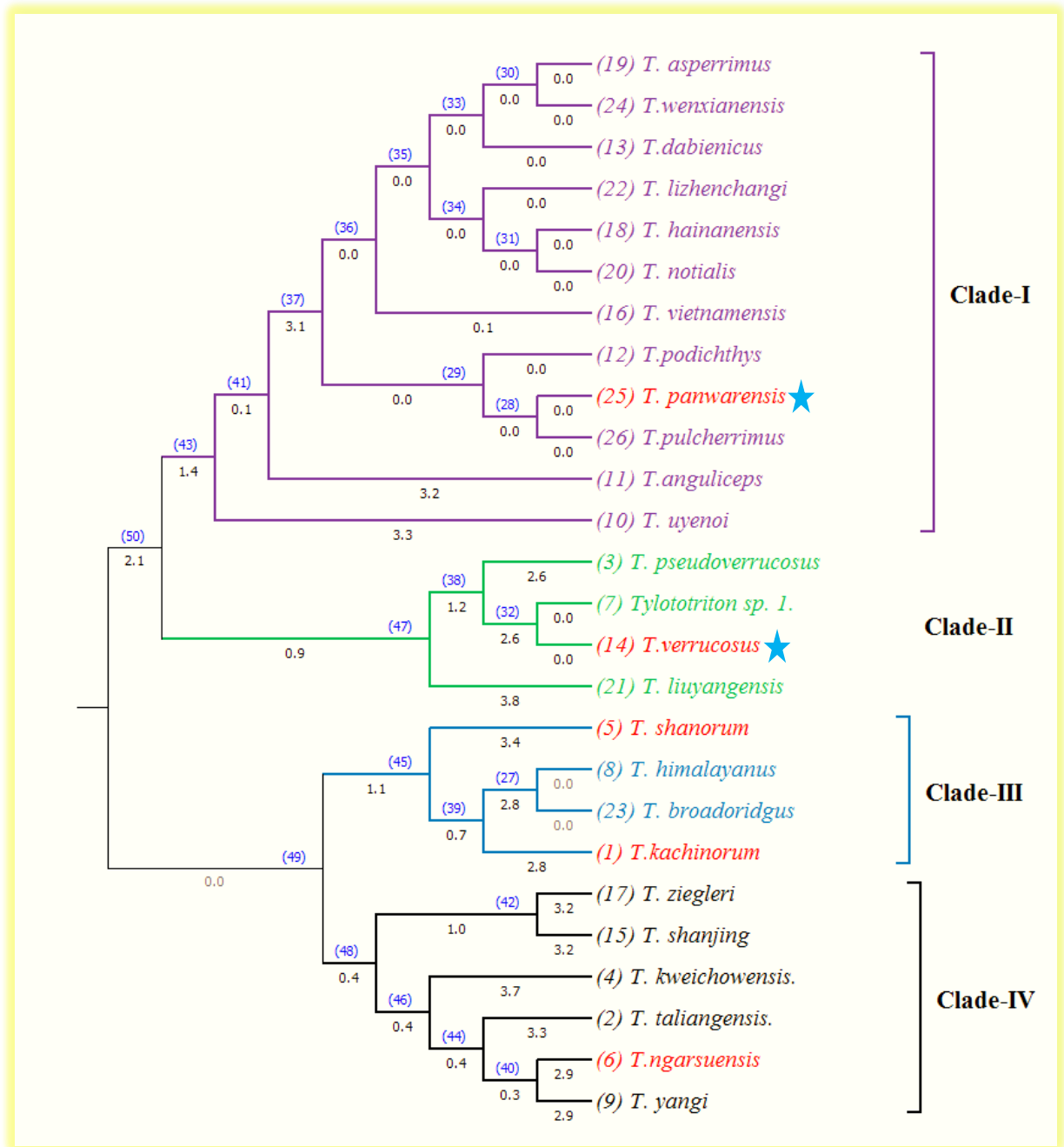


Fig 13. Maximum likelihood phylogenetic tree topology of twenty six *Tylototriton* species show the clades I to IV (red color species found in Myanmar, species mark with blue stars represent new finding of present work)

## Discussion

The present work studied the occurrence of *Tylototriton* species from three study areas namely Sadung environ, Myitkyina Township; Sinlum mountain, Momauk Township, and Ingyin mountain, Indawgyi region, Kachin State, Northern Myanmar.

Members of the genus *Tylototriton* have long been recorded from northern and eastern parts of Myanmar and have been traditionally classified as *T. verrucosus* Anderson, 1871 (Gyi, 1969). Nishikawa *et al.*, (2014), based on the examination of specimens assigned to *T. verrucosus* collected from the Shan Plateau in eastern Myanmar and pet-trade animals assumed to originate from Myanmar. During last six years, Nishikawa, Matsui & Rao, (2014) described a new species from Taunggyi environ, Shan State, so named as *T. shanorum*. Grismer *et al.*, (2018) developed a new species from Phayar ngarsu village, Ywar ngan area, Pindaya Township, Shan State, so named as *T. ngarsuensis*. A species of *T. kachinorum* was erected as new species by Than Zaw *et al.*, (2019) from Ingyin mountain, Indawgyi area, Kachin State, and Grismer *et al.*, (2019) established as new species of *T. panwaensis* from Panwa Town, Kachin State, Northern Myanmar respectively. Therefore currently four species of genus *Tylototriton* were erected as new species in Myanmar.

The salamandrid genus *Tylototriton* contains 25 nominal species (Frost 2018; Grismer *et al.*, 2018a, 2019; Than Zaw *et al.*, 2019) that range across the Asian monsoon climatic zone from the eastern Himalayas through much of hilly Indochina (Wang *et al.*, 2018) but at present time the nominal species were 26 including latest species *T. panwaensis* developed by Grismer (2019).

In the present study, collected species of *Tylototriton* on the summit of Ingyin Mt., Indawgyi region, Mohnyin District, Kachin State this species must be *T. kachinorum* because it was not so far from the place of species that recently discovered and erected by Than Zaw *et al.*, (2019) as a new species of *T. kachinorum* in that region.

According to the statistical analysis of Sadung specimens they were significantly shorter than that of Ingyin and Sinlum specimens in SVL and TL, and significantly wider than that of Ingyin and Sinlum specimen in HA and HW ( $p < 0.05$ ).

In Sinlum study area, according to the statistical analysis of Sinlum specimens were significantly longer than that of Sadung and Ingyin specimens in SVL and HL, and significantly narrower than that of Sadung specimen in HA ( $p < 0.05$ ).

Taken into consideration of Ingyin specimens, the parameter of HL and HW significantly shorter than that of Sinlum and Sadung specimens but significantly longer than that of Sadung specimens in TL ( $p < 0.05$ ).

With aspect of color morph, Sadung specimens are Orange Brown, Sinlum specimens are dark brown and Ingyin specimens are brown respectively this may be due to the different ecology of their habitats.

According to the DNA analysis, currently new species *Tylototriton panwaensis* (Grismer *et al.*, 2019) is known only from the vicinity of Panwa Town, Panwa Township, Myitkyina District, Kachin State, Myanmar. In the present study stated that a *Tylototriton* species also found in Sadung, Myitkyina District and the sample specimen was sent to Moscow State University, Russia to analyze the DNA sequencing. Now, when we have a sequence of this specimen and analyze it, this species is also *T. panwaensis* described as Grismer from Panwa. Therefore, it was a new record of this species for that area of Sadung, Myitkyina Township.

And also one species of *Tylototriton* collected on the summit of Sinlum mountain, Momauk Township, Banmaw District, Kachin State, Myanmar and the sample specimen also sent to Moscow State University, Russia to analyze the DNA sequencing for evaluate whether may be new or other known species. Now, DNA sequence of this specimen revealed that *Tylototriton verrucosus* species when analyzed by Sanger sequencing method and MEGA\_X software.

Altogether 26 species of this genus from which describe as red font of five species were found in Myanmar, among these five species, two species mark with blue stars of *T. panwaensis* and *T. verrucosus* were new finding from Sadung environ and Sinlum mountain respectively at present work. New finding of these two species were belong to different Clades, *T. panwaensis* in Clade I and *T. verrucosus* in Clade II. The species of *T. shanorum* and *T. kachinorum* were in Clade III and *T. ngarsuensis* was in Clade IV respectively. Of the five species found in Myanmar, the newly found *T. panawaensis* is far distance from *T. ngarsuensis* but closer to *T. kachinorum* according to phylogeny tree. Although the species *T. shanorum* and *T. kachinorum* were within the same clade, *T. kachinorum* is closer to *T. himalayanus* than that of *T. shanorum* because different node with *T. shanorum* and within same node with *T. himalayanus*.

Recently erected newly found species named *T. kachinorum* was discovered by Than Zaw *et al.*, (2019) from Ingyin mountain, Indawgyi region, Kachin State was compared with two new finding species in the present study.

In Myanmar, up to now there are four new species of *Tylototriton* were erected within six years out of totally 26 species found in world from which two species, *T. shanorum* (Nishikawa *et al.*, 2014) and *T. ngarsurensis* (Grismer *et al.*, 2018a) were discovered from Shan State and the latest two species *T. kachinorum* (Than Zaw *et al.*, 2019) and *T. panwaensis* (Grismer *et al.*, 2019) were from Kachin State respectively. In the present study we mentioned that the *T. panwaensis* from Sadung environ as first new finding.

Anderson (1871) erected the genus *Tylototriton* and described the type species *Tylototriton verrucosus* based on specimens from Nantin, Momein and Hotha valleys, in western Yunnan Province, People's Republic of China. However, he neither designated a type series nor deposition of the specimens upon which the description of *T. verrucosus* was based (Nussbaum, Brodie and Datoong 1995). In the present study, *T. verrucosus* was found in Sinlum mountain this is also new finding for Kachin State, Northern Myanmar.

### Conclusion

The present study carried out two regions of Kachin State, Northern Myanmar with the aim of finding Salamander, we founded the two new finding species of *T. panwaensis* from Sadung and *T. verrucosus* from Sinlum mountain by statistical, morphological analysis by R software and molecular DNA analysis by Sanger sequencing method and MEGAX software.

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