

# Phylogenetic Relationships of the Genus *Paramesotriton* (Caudata: Salamandridae) with the Description of a New Species from Qixiling Nature Reserve, Jiangxi, Southeastern China and a Key to the species

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**Abstract** The matrilineal genealogy of the genus *Paramesotriton* is hypothesized based on DNA sequences from mitochondrial NADH subunit two (*ND2*) and its flanking tRNAs (tRNA<sup>Trp</sup> and a partial tRNA<sup>Ala</sup>). The genealogy identifies a highly divergent, unnamed lineage from Qixiling Nature Reserve, Jiangxi, China and places it as the sister taxon of *P. chinensis*. The newly discovered population differs from other congeners by several features of external morphology including having large clusters of dark brown conical warts on the dorsum of the head, lateral surface of the body and dorsolateral ridges. Its intermittent dorsal vertebral ridge is the same color as other parts of the dorsum and tail narrows gradually from the base to the tip. Further, the new population differs from all congeners by an uncorrected *P*-distance of more than 9.38% in *ND2*. Consequently, we describe the new species of Asian warty newt (Salamandridae) as *Paramesotriton qixilingensis* sp. nov.

**Keywords** Matrilineal genealogy, amphibians, cryptic species, *Paramesotriton qixilingensis* sp. nov.

## 1. Introduction

The genus *Paramesotriton* (Family Salamandridae), commonly known as Asian warty newts, is a member of the monophyletic modern Asian salamandrids, which also includes the genera *Pachytriton*, *Laotriton* and *Cynops* (AmphibiaWeb, 2013; Frost, 2013; Zhang *et al.*, 2008). Currently, *Paramesotriton* contains 12 species, near half of which were described in the last decade (Gu *et*

*al.*, 2012a; Li *et al.*, 2008a, 2008b; Wang *et al.*, 2013; Wu *et al.*, 2009, 2010a; Zhao *et al.*, 2008). The recent discovery of many new species suggests that the true richness remains grossly underestimated, especially in poorly surveyed, remote montane regions of southern China. One species, *P. deloustali* Bourret, is endemic to the northern Vietnam and the other species occurring in China as follows: *P. caudopunctatus* Liu and Hu; *P. chinensis* Gray; *P. fuzhongensis* Wen; *P. guangxiensis* Huang, Tang and Tang; *P. hongkongensis* Myers and Leviton; *P. labitatus* Wu, Rovito, Papenfuss and Hanken; *P. longliensis* Li, Tian, Gu and Xiong; *P. maolanensis* Gu, Chen, Tian, Li and Ran; *P. yunwuensis* Wu, Jiang and Hanken; *P. wulingensis* Wang, Tian and Gu, and *P. zhijinensis* Li, Tian and Gu.

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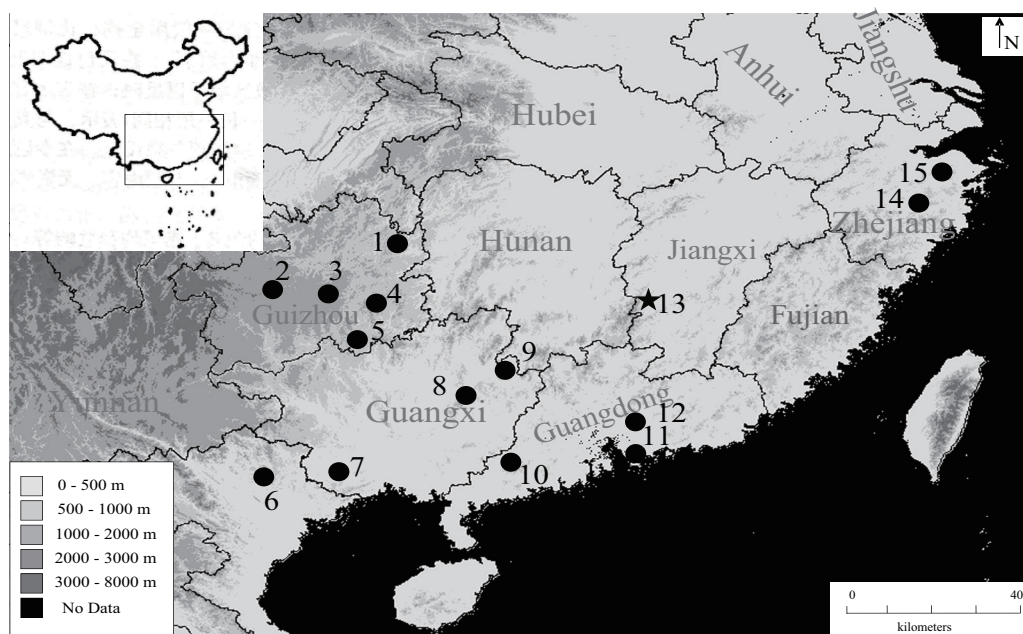
*Paramesotriton* (Wu *et al.*, 2010a; Zhang *et al.*, 2008) contains two species groups: the *P. caudopunctatus* and *P. chinensis* species groups, which sometimes correspond to the subgenera *Allomesotriton* and *Paramesotriton*, respectively (Dubois and Raffaëlli, 2012; Gu *et al.*, 2012a, 2012b). The former species group contains *P. caudopunctatus*, *P. longliensis*, *P. maolanensis*, *P. wulingensis* and *P. zhijinensis*. The remaining seven species belong to the *P. chinensis* species group. Several studies attempted to resolve the phylogenetic relationships within the genus (e.g., Lu *et al.*, 2004; Gu *et al.*, 2012b; Wu *et al.*, 2010a), yet none has included all species of *Paramesotriton*. Thus, the relationships among the species remain unclear, especially in the *P. chinensis* species group.

During fieldwork in Qixiling Nature Reserve (Jiangxi) in August 2012, we collected a large, warty newt. Specimens differed from other congeners by several morphological characters, including the occurrence of remarkably large warts and a tail that was distinct in attenuating from its base to the tip. Given the morphological distinctiveness, we explored the matrilineal relationships of the newt and took the opportunity to hypothesize its relationships to all species of *Paramesotriton*. Our results indicated that the newly discovered newts constituted an undescribed species.

## 2. Materials and Methods

**2.1 Sampling** Six specimens of the salamander collected in the Qixiling Nature Reserve were euthanized using chlorobutanol following an approved Animal Use Protocol. Tissue samples were biopsied immediately after euthanization and preserved in 95% ethanol. Subsequently the biopsies were maintained at  $-80^{\circ}\text{C}$ . Voucher specimens were fixed with 10% buffered formalin for several days, washed in water, and then stored in 70% ethanol. All specimens were deposited the herpetological collection of the Museum of the Kunming Institute of Zoology (KIZ), the Chinese Academy of Sciences. Locality data were recorded using a handheld GPS unit. The maternal relationships of 41 newts including four individuals from Qixiling Nature Reserve were reconstructed (Table 1). All known species of *Paramesotriton* from or near their respective type localities were included (Figure 1, Table 1). *Cynops cyanurus*, *Pachytriton granulosus* and *Pac. brevipes* were chosen as the outgroup taxa based on the studies of Weisrock *et al.* (2006) and Zhang *et al.* (2008).

**2.2 Morphological examinations** Fifteen morphological characters (Table 2) widely used in salamander taxonomy and species diagnosis (García-París and Wake, 2000; Poyarkov *et al.*, 2012) were taken with digital calipers



**Figure 1** Sampling localities used in this study of *Paramesotriton*. Locality numbers refer to Table 1 and Figure 2; asterisk denotes the type locality of *P. qixilingensis* sp. nov.

**Table 1** Voucher specimens, localities (codes shown in Figure 1, 2), and GenBank accession numbers for species included in the molecular genealogical analysis. CIB = Chengdu Institute of Biology; GZNU = Guizhou Normal University; KIZ = Kunming Institute of Zoology; MVZ = Museum of Vertebrate Zoology (Berkeley, USA); \* = type locality.

Species	Voucher	Locality	GenBank Acc. No.		Source
			ND2	COI	
<b>Ingroup</b>					
<i>Paramesotriton caudopunctatus</i>	MVZ 236250	Leishan, Guizhou, China*	DQ517799	—	Weisrock <i>et al.</i> (2006)
	GZNU2009042501	Leishan, Guizhou, China*	JF438983	—	Gu <i>et al.</i> (2012)
<i>Paramesotriton chinensis</i>	CIB 95899	Ningbo, Zhejiang, China*	FJ744603	—	Wu <i>et al.</i> (2009)
	CIB 95911	Jinhua, Zhejiang, China	FJ744604	—	Wu <i>et al.</i> (2009)
	CIB 95910	Jinhua, Zhejiang, China	FJ744605	—	Wu <i>et al.</i> (2009)
<i>Paramesotriton deloustali</i>	MVZ 223627	Tam Dao, Vinh Phu, Vietnam*	DQ517802	—	Weisrock <i>et al.</i> (2006)
	MVZ 223628	Tam Dao, Vinh Phu, Vietnam*	FJ744599	—	Wu <i>et al.</i> (2009)
	MVZ 223629	Tam Dao, Vinh Phu, Vietnam*	FJ744600	—	Wu <i>et al.</i> (2009)
<i>Paramesotriton fuzhongensis</i>	MVZ 230363	Xiling, Guangxi, China	DQ517803	—	Weisrock <i>et al.</i> (2006)
	GZNU2007052001	Fuchuan, Guangxi, China	FJ169605	—	Gu <i>et al.</i> (2012)
	GZNU2007052002	Fuchuan, Guangxi, China	JF438981	—	Gu <i>et al.</i> (2012)
<i>Paramesotriton guangxiensis</i>	MVZ 220905	Ningming, Guangxi, China*	DQ517804	—	Weisrock <i>et al.</i> (2006)
	GZNU2006001	Ningming, Guangxi, China*	FJ169604	—	Gu <i>et al.</i> (2012)
<i>Paramesotriton hongkongensis</i>	MVZ 230366	Hong Kong, China*	GU980581	—	Wu <i>et al.</i> (2010)
	MVZ 230368	Hong Kong, China*	GU980582	—	Wu <i>et al.</i> (2010)
	CIB 97898	Huizhou, Guangdong, China	GU980580	—	Wu <i>et al.</i> (2010)
<i>Paramesotriton labitatus</i>	CIB 88140	Jinxiu, Guangxi, China*	FJ744602	—	Wu <i>et al.</i> (2009)
	CIB 88141	Jinxiu, Guangxi, China*	FJ744601	—	Wu <i>et al.</i> (2009)
	MVZ 230616	Jinxiu, Guangxi, China*	DQ517801	—	Weisrock <i>et al.</i> (2006)
<i>Paramesotriton longliensis</i>	KIZ-GZH 081025	Longli, Guizhou, China*	GU980576	—	Wu <i>et al.</i> (2010)
	GZNU20070421001	Longli, Guizhou, China*	FJ169608	—	Gu <i>et al.</i> (2012)
	GZNU20070421002	Longli, Guizhou, China*	JF438973	—	Gu <i>et al.</i> (2012)
<i>Paramesotriton maolanensis</i>	GZNU2006030003	Maolan, Lobi, Guizhou, China*	FJ169607	—	Gu <i>et al.</i> (2012)
	GZNU2006030006	Maolan, Lobi, Guizhou, China*	JF438972	—	Gu <i>et al.</i> (2012)
	GZNU2006030004	Maolan, Lobi, Guizhou, China*	JF438993	—	Gu <i>et al.</i> (2012)
	GZNU2006030005	Maolan, Lobi, Guizhou, China*	JF438994	—	Gu <i>et al.</i> (2012)
<i>Paramesotriton qixilingensis</i>	KIZ 022289	Qixiling, Yongxin, Jiangxi, China	KJ650051	KJ650045	this study
	KIZ 2012070605	Qixiling, Yongxin, Jiangxi, China	KJ650052	KJ650046	this study
	KIZ 2012070606	Qixiling, Yongxin, Jiangxi, China	KJ650053	KJ650047	this study
	KIZ 2012070607	Qixiling, Yongxin, Jiangxi, China	KJ650054	KJ650048	this study
<i>Paramesotriton wulingensis</i>	KIZ 21898	Fanjingshan, Guizhou, China*	KJ650055	KJ650049	this study
	KIZ 21899	Fanjingshan, Guizhou, China*	KJ650056	KJ650050	this study
<i>Paramesotriton yunwuensis</i>	CIB 97854	Luoding, Guangdong, China*	GU980579	—	Wu <i>et al.</i> (2010)
	CIB 97855	Luoding, Guangdong, China*	GU980577	—	Wu <i>et al.</i> (2010)
	CIB 97856	Luoding, Guangdong, China*	GU980578	—	Wu <i>et al.</i> (2010)
<i>Paramesotriton zhijinensis</i>	KIZ-GZH 081026	Zhijin, Guizhou, China*	GU980575	—	Wu <i>et al.</i> (2010)
	GZNU20070415001	Zhijin, Guizhou, China*	FJ169609	—	Gu <i>et al.</i> (2012)
	GZNU20070415002	Zhijin, Guizhou, China*	JF438976	—	Gu <i>et al.</i> (2012)
<b>Outgroup</b>					
<i>Cynops cyanurus</i>	TP-MVZ 02	Chuxiong, Yunnan, China	EU880309	—	Zhang <i>et al.</i> (2008)
<i>Pachytriton brevipes</i>	CIB95926	Mt. Junfeng, Nanfeng, Jiangxi, China	GQ303626	—	Wu <i>et al.</i> (2010)
<i>Pachytriton granulosis</i>	CIB88145	Mt. Xitianmu, Lin'an, Zhejiang, China	GQ303606	—	Wu <i>et al.</i> (2010)

**Table 2** Linear measurements (in mm) of *Paramesotriton qixilingensis* sp. nov. Abbreviations provided in the text. The holotype is included in the summary statistics for females.

Measurements	Holotype (KIZ 09791)	Males (n = 2)		Females (n = 4)	
		Range	Mean ± SE	Range	Mean ± SE
TTL	139.86	139.86–140.76	139.18 ± 1.59	13.89–155.10	148.78 ± 3.29
SVL	66.80	65.86–67.65	66.76 ± 0.90	66.83–74.44	71.78 ± 1.69
TAL	73.06	71.73–73.11	72.42 ± 0.69	73.06–80.66	76.99 ± 1.72
HDL	17.35	19.12–20.39	19.76 ± 0.64	17.35–23.22	19.84 ± 1.29
HDW	17.06	16.64–16.87	16.76 ± 0.12	16.93–18.49	17.61 ± 0.37
OND	4.79	5.37–6.23	5.80 ± 0.43	4.79–5.71	5.15 ± 0.21
IND	4.30	3.42–4.74	4.08 ± 0.66	4.19–4.44	4.33 ± 0.05
GAD	32.42	27.89–31.17	29.53 ± 1.64	32.42–36.83	35.05 ± 0.95
TAD	9.70	7.09–11.98	9.54 ± 2.45	9.70–12.23	10.83 ± 0.52
FLL	24.82	26.8–28.15	27.48 ± 0.68	24.82–28.27	26.80 ± 0.83
HLL	25.37	26.72–26.89	26.81 ± 0.09	25.37–29.05	26.86 ± 0.78
CHW	12.90	11.93–14.55	13.24 ± 1.31	12.90–14.25	13.56 ± 0.29
ICD	8.77	8.44–9.95	9.20 ± 0.76	8.77–10.37	9.48 ± 0.34
EYL	4.07	4.03–4.98	4.51 ± 0.48	4.07–5.32	4.62 ± 0.26
ORD	7.28	7.86–8.01	7.94 ± 0.08	7.28–8.09	7.79 ± 0.18

to the nearest 0.01 mm from each salamander by one person. All specimens from Qixiling Nature Reserve were examined. Abbreviations were as follows: TTL (total length); SVL (snout-vent length, from snout to the anterior end of the vent); TAL (tail length, from the tip of the tail to the anterior end of the vent); HDL (head length, from the tip of the snout to the gular fold); HDW (head width); OND (orbitonarial distance, distance between external nares and the anterior corner of the eye on the same side of the head); IND (internarial distance, distance between the external nares); GAD (gleno-acetabular distance, distance between axilla and groin); TAD (maximum tail depth); FLL (forelimb length, from axilla to tip of the longest finger of forelimb); HLL (hindlimb length, from groin to tip of the longest toe of hindlimb); CHW (chest width, distance between left and right axillae); ICD (intercanthal distance, distance between anterior corners of the eyes); EYL (eye length, distance from the anterior corner of the eye to the posterior corner of the eye); and ORD (orbitorostral distance, snout length, distance from tip of snout to the anterior corner of the eye).

Morphological comparisons were based on specimens in KIZ as follows: *P. caudopunctatus* (KIZ 03903–03909 and 03629, from Guizhou, China), *P. chinensis* (KIZ 012961–012971, from Zhejiang, China), *P. deloustali* (KIZ 048729 and 048734, from Tam Dao, Vietnam), *P. fuzhongensis* (KIZ 08532–08540, from Guangxi Zhuang Autonomous Region, China), *P. guangxiensis*

(KIZ 09284–09298, from Guangxi Zhuang Autonomous Region, China), *P. hongkongensis* (KIZ 013508–013520, from Shenzheng, China), *P. labitatus* (KIZ 08766–08779, from Guangxi Zhuang Autonomous Region, China), *P. longliensis* (KIZ 08439–08448, from Longli, Guizhou, China), *P. wulingensis* (KIZ 021898–KIZ 021899, from Fanjinshan, Guizhou, China), *P. yunwuensis* (KIZ 09730–09742, from Luoding, Guangdong, China) and *P. zhijinensis* (KIZ 04292–04296, from Guizhou, China). We also incorporated data from the original descriptions of the species.

**2.3 DNA extraction and sequencing** Whole genomic DNA was extracted using proteinase K (10 mg/ml) digestion followed by a standard phenol-chloroform method (Sambrook *et al.*, 1989). Two fragments of the mitochondrial sequences are amplified. One fragment covers a 1143 bp region that encodes part of the NADH dehydrogenase subunit two (*ND2*) and its flanking tRNAs (tRNA<sup>Trp</sup> and a partial tRNA<sup>Ala</sup>). Another gene was a 561 bp region that encoding cytochrome oxidase subunit I (*COI*; Entrez *COXI*). Standard polymerase chain reactions (PCR) and sequencing conditions were same as Che *et al.* (2012) and Wu *et al.* (2010c). The sequences of *COI* were not used for the molecular analysis because no *COI* data of this genus in GenBank; these data were used only as part of the international barcoding initiative Cold Code (Murphy *et al.*, 2013) via the BOLD database.

**Table 3** Genetic uncorrected *P*-distances (%) of the mtDNA *ND2* sequences used in this study within (diagonal) and between (below diagonal) members of the *Paramesotriton*. *P. cau* = *P. caudopunctatus*; *P. chi* = *P. chinensis*; *P. del* = *P. deloustali*; *P. fuz* = *P. fuzhongensis*; *P. gua* = *P. guangxiensis*; *P. hon* = *P. hongkongensis*; *P. lab* = *P. labitatus*; *P. lon* = *P. longliensis*; *P. mao* = *P. maolanensis*; *P. qix* = *P. qixilingensis* sp. nov.; *P. wul* = *P. wulingensis*; *P. zhi* = *P. zhijinensis*; *P. yun* = *P. yunwuensis*.

	<i>P. cau</i>	<i>P. chi</i>	<i>P. del</i>	<i>P. fuz</i>	<i>P. gua</i>	<i>P. hon</i>	<i>P. lab</i>	<i>P. lon</i>	<i>P. mao</i>	<b><i>P. qix</i></b>	<i>P. wul</i>	<i>P. yun</i>
<i>P. caudopunctatus</i>												
<i>P. chinensis</i>	0.1303											
<i>P. deloustali</i>	0.1122	0.0861										
<i>P. fuzhongensis</i>	0.1134	0.083	0.0508									
<i>P. guangxiensis</i>	0.1205	0.0929	0.058	0.0422								
<i>P. hongkongensis</i>	0.125	0.0852	0.0852	0.083	0.0961							
<i>P. labitatus</i>	0.1477	0.1151	0.1065	0.102	0.1062	0.1045						
<i>P. longliensis</i>	0.0918	0.1148	0.1136	0.1165	0.1236	0.1207	0.1443					
<i>P. maolanensis</i>	0.0905	0.1143	0.1131	0.1142	0.1232	0.1214	0.1439	0.0058				
<b><i>P. qixilingensis</i> sp. nov.</b>	<b>0.1415</b>	<b>0.0997</b>	<b>0.1014</b>	<b>0.1014</b>	<b>0.1006</b>	<b>0.0938</b>	<b>0.1171</b>	<b>0.1375</b>	<b>0.1353</b>			
<i>P. wulingensis</i>	0.0266	0.1356	0.1185	0.1222	0.1285	0.1297	0.1513	0.0963	0.0949	<b>0.1429</b>		
<i>P. yunwuensis</i>	0.1217	0.0878	0.0559	0.044	0.0438	0.089	0.1029	0.1142	0.1137	<b>0.1041</b>	0.1288	
<i>P. zhijinensis</i>	0.0924	0.1222	0.1148	0.1176	0.123	0.1227	0.1464	0.0183	0.0152	<b>0.1369</b>	0.0969	0.1142

**2.4 Molecular analysis** ClustalX 1.81 (Thompson *et al.*, 1997) was employed for aligning the *ND2* sequences using default parameters. The alignments were subsequently confirmed by eye. MEGA 5.0 (Tamura *et al.*, 2011) was used to compute uncorrected *P*-distances within and between species.

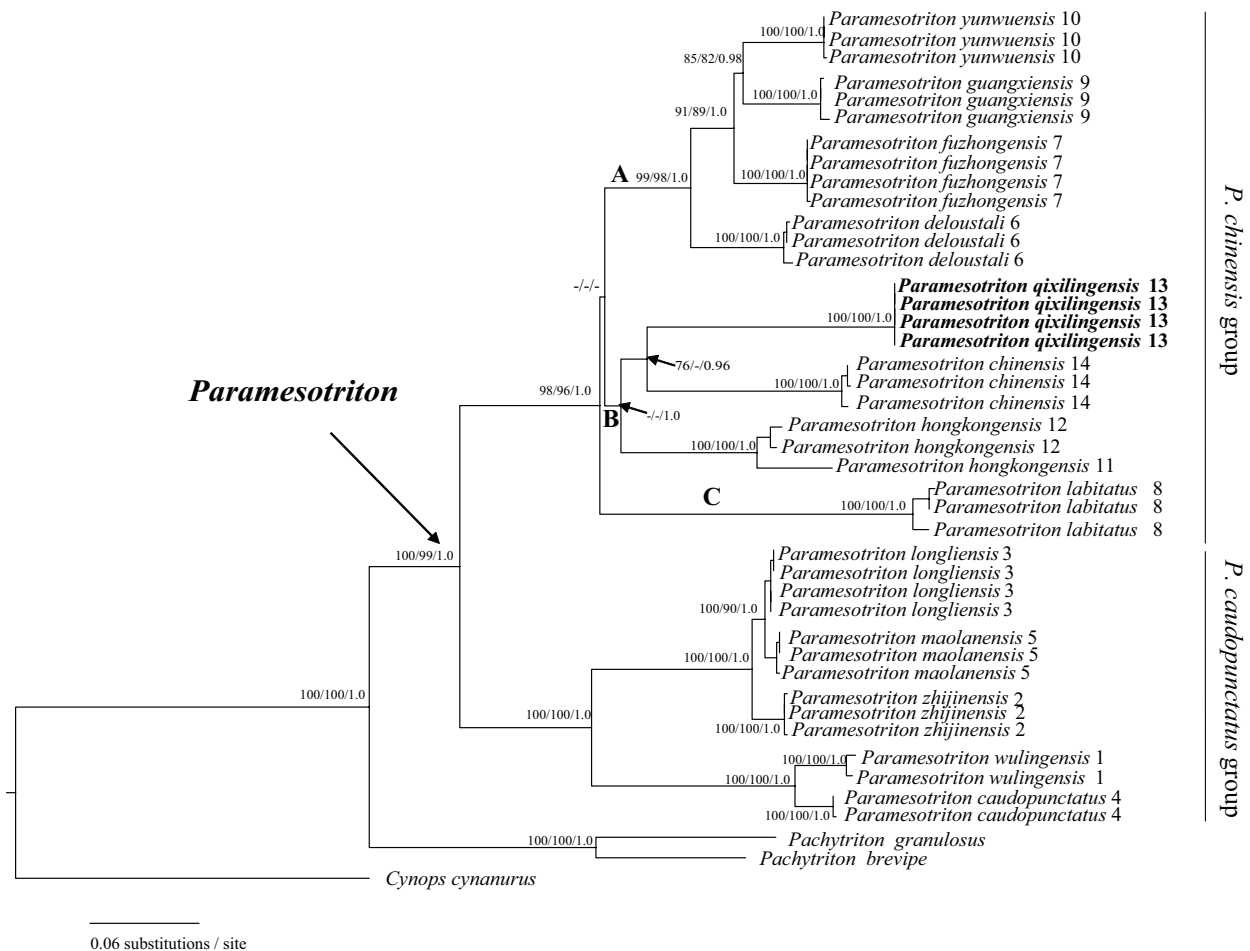
The matrilineal genealogy, which was assumed to reflect the species' phylogenetic relationships, was hypothesized via partitioned Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) analyses. We partitioned the data by codon position and tRNA for ML and BI. The following best-fit models of nucleotide substitution were selected using MrModeltest 2.2 (Nylander, 2004) based on the Akaike Information Criterion: 1<sup>st</sup> codon position, K81uf+I+G; 2<sup>nd</sup> codon position, TrN+I; and for the 3<sup>rd</sup> codon position of *ND2* and the flanking tRNAs, TrN+G. BI analyses were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Four incrementally heated Markov chains (using default heating values) were run for 5 000 000 generations each while sampling the chains at intervals of 100 generations. We discarded the first 25% of the trees as burn-in. Log-likelihood scores were visually inspected in Tracer 1.5 (Rambaut and Drummond, 2007) to assure attainment of stationarity. Two independent BI analyses were performed to assure consistency. ML was performed using RAxML 7.0.4 (Stamatakis *et al.*, 2006). MP analyses were conducted using PAUP\* 4.0b10 (Swofford, 2003) with TBR executed for 1000

random addition replicates. All characters were evaluated as equally weighted and unordered. Nonparametric bootstrapping with 1000 pseudoreplicates was employed to assess the reliability of each node in the ML and MP analyses, and the frequency of nodal resolution in the BI analyses was termed a Bayesian posterior probability.

### 3. Results

**3.1 MtDNA variation, differentiation, and genealogical relationships** Six newly sequences of the *ND2* and the adjacent tRNAs were determined and deposited in GenBank (Table 1). They contained 1143 bp of nucleotide positions. All the 40 aligned sequences of *ND2* and the adjacent tRNAs had 414 variable sites, of which 366 were potentially parsimony-informative. We did not observe indels. No premature stop codons occurred in *ND2* suggesting that nuclear pseudogenes were not sequenced. We also gathered 561 bp from the standard barcoding gene *COI* for six our samples (Table 1); comparative data for most species were not available in GenBank.

The BI, ML and MP analyses produced very similar topologies. They differed in relationships at poorly supported nodes only. Thus, only the BI tree was shown (Figure 2). All analyses recovered the *P. chinensis* and *P. caudopunctatus* groups with strong support. Within the former group, the BI tree depicted lineages A, B and C, but their relationships *inter se* were unresolved. Lineage A contained *P. yunwuensis*, *P. guangxiensis*,



**Figure 2** Bayesian inference (BI) tree based on mtDNA sequences of *ND2* and its flanking tRNAs (tRNA<sup>Tp</sup> and a partial tRNA<sup>Ala</sup>). *Cynops cyanurus*, *Pachytriton brevipes* and *Pac. granulosus* were used as outgroup taxa. Numbers on branches are maximum likelihood bootstrap values (BS), maximum parsimony (BS), and Bayesian posterior probabilities (BPP), respectively. “-” denotes low support (< 70% BS and < 95% BPP). Lineage designations used in text are given on nodes of the tree and vertical bars indicate assignment to the two species groups, sometimes considered to be subgenera. Numbers following the species’ name refer to localities shown in Figure 1 and Table 1.

*P. fuzhongensis*, and *P. deloustali*. Lineage B, which received high statistical support from BI only, consisted of *P. hongkongensis*, and the sister lineages *P. chinensis* and the new population from Qixiling Nature Reserve. Lineage C was comprised of *P. labitatus* only. The *P. caudopunctatus* group consisted of *P. caudopunctatus*, *P. wulingensis*, *P. zhijinensis*, *P. longliensis* and *P. maolanensis*.

The three matriline clusters together geographically (Figure 1). Within the *P. chinensis* group, Lineage A (localities 6, 7, 9 and 10) occupied the southwestern portion of the range, lineage B (localities 11–15) occurred in the east and lineage C (locality 8) occurred between these two. The *P. caudopunctatus* group occupied the northwestern-most portion of the range of *Paramesotriton*.

Interspecific genetic *P*-distances for *ND2* in the

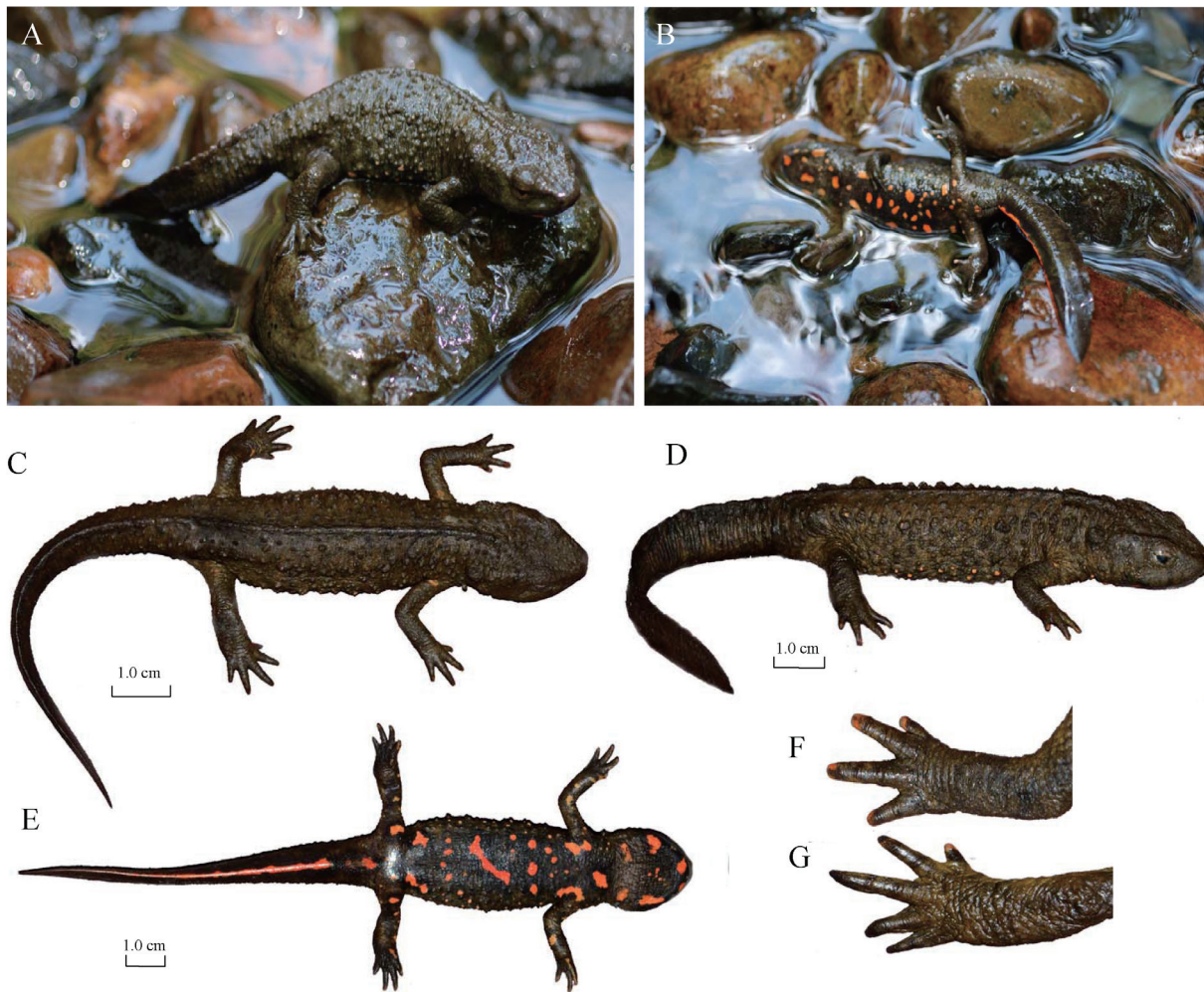
*P. chinensis* group ranged from 4.22% to 11.72% between species (Table 3). In the *P. caudopunctatus* group, *P*-distances ranged from 0.58% to 9.69%. The *P*-distances between the new population from Qixiling Nature Reserve and the other species ranged from 9.38% to 14.29%.

**3.2 Systematics** The genealogical relationships, extent of genetic difference and morphological distinctiveness necessitate the description of the population from Jiangxi as a new species. Thus, we describe:

***Paramesotriton qixilingensis* sp. nov. (Figures 3–7)**

**Holotype.** KIZ 022289, an adult female collected from Mt. Shenyuan, Qixiling Nature Reserve, Yongxin county, Ji’an, Jiangxi, China (26.75° N, 114.17° E; elevation 194 m) by Zhiyong YUAN on 10 August 2012.

**Paratypes.** Three adult females: KIZ 022290–022292,



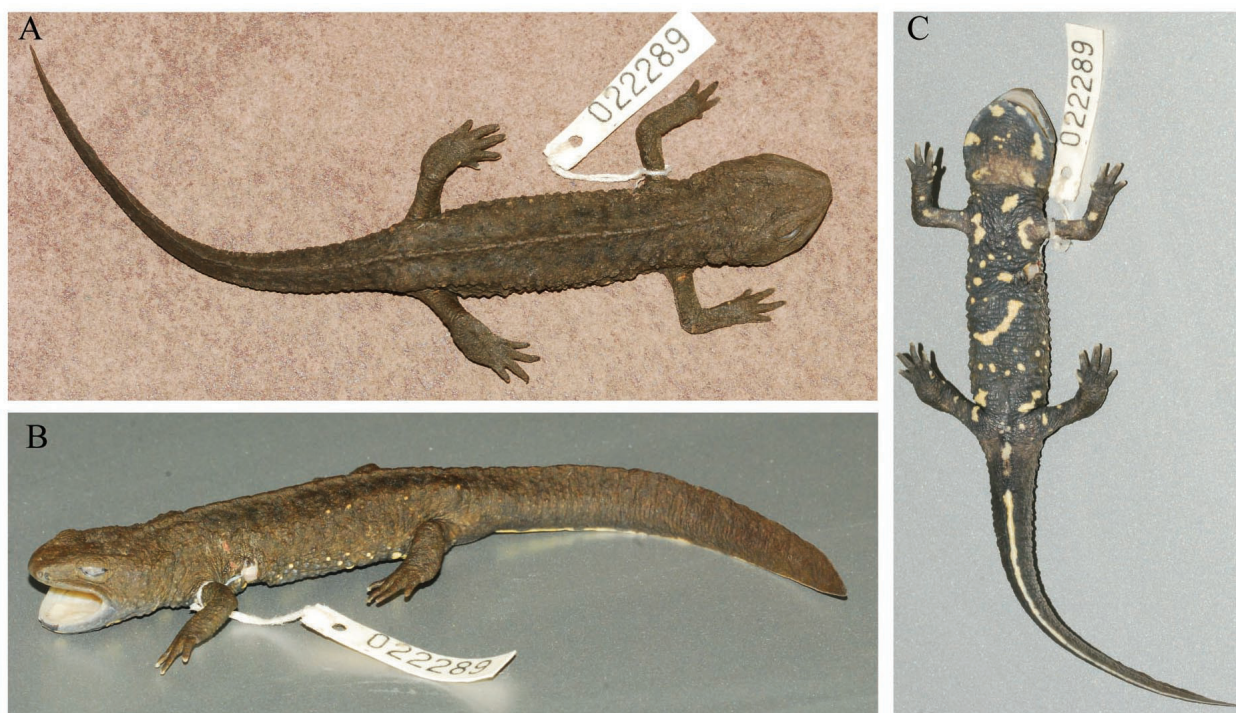
**Figure 3** (A) and (B): dorsal and ventral views of living male *P. qixilingensis* sp. nov. (KIZ 022290); (C), (D) and (E): dorsal, lateral and ventral views of female *P. qixilingensis* sp. nov. (KIZ 09790) after euthanization, respectively; (F) and (G): dorsal views of forelimb and hindlimb of the female *P. qixilingensis* sp. nov. (KIZ 022290) after euthanization, respectively.

and two adult males: KIZ 022293, KIZ 022294; same collection date and locality as the holotype.

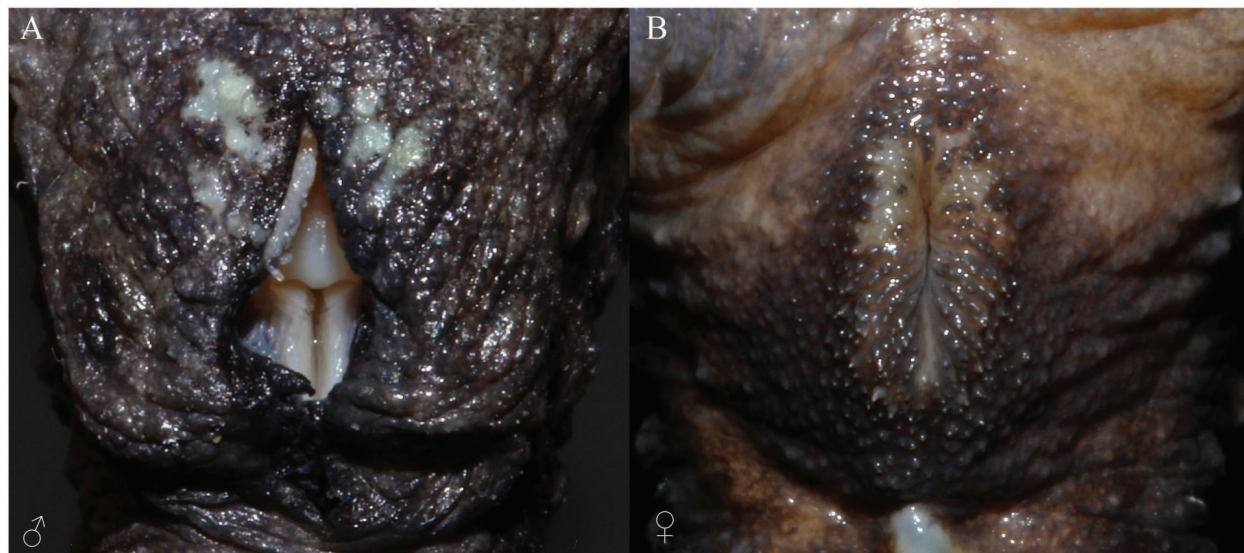
**Diagnosis.** *Paramesotriton qixilingensis* sp. nov. is assigned to its genus because of possessing due to the presence of a glandular ridge on the side of its head, warts covering dorsal and lateral surfaces, bright orange blotches on its ventral surface and a laterally compressed tail. Adults of the new species are diagnosed from congeners by a combination of the following characters: (1) skin rough with large clusters of conical dark brown warts covering the dorsum of head, lateral surface of body and dorsolateral ridges; (2) large body size (SVL 73.06 mm, TAL 66.80 mm); (3) small, irregular and orange-red spots present on ventral surface, chin, underside of axillae, and cloaca; (4) intermittent dorsal vertebral ridge that exhibits the same color as other parts of the dorsum;

(5) lateral ridge absent; (6) head large and broad with HL being slightly longer than HW; (7) distinct tail that gradually narrows from the base to the tip; (8) slightly developed velum and webbing on the fringe of fingers and toes; (9) cloaca relatively flat in females; (10) corner of mouth located just below after the posterior corner of orbit; and (11) long limbs that overlap when the forelimb and hind limb are adpressed.

**Description of the holotype** Specimen in good state of preservation. Measurements given in Table 2. Relatively large newt: TTL 139.86 mm; TAL shorter than SVL. Habitus very stout. Head large, much wider than neck; head length nearly equal with width (length/width = 1.01); snout truncate; nostrils close to snout tip, visible from above; corner of mouth located posterior to posterior margin of eye, but not extending beyond it; gular fold



**Figure 4** The holotype of *P. qixilingensis* sp. nov. (KIZ 022289) in preservative. A: dorsum; B: head; C: lateral view; and D: ventrum.

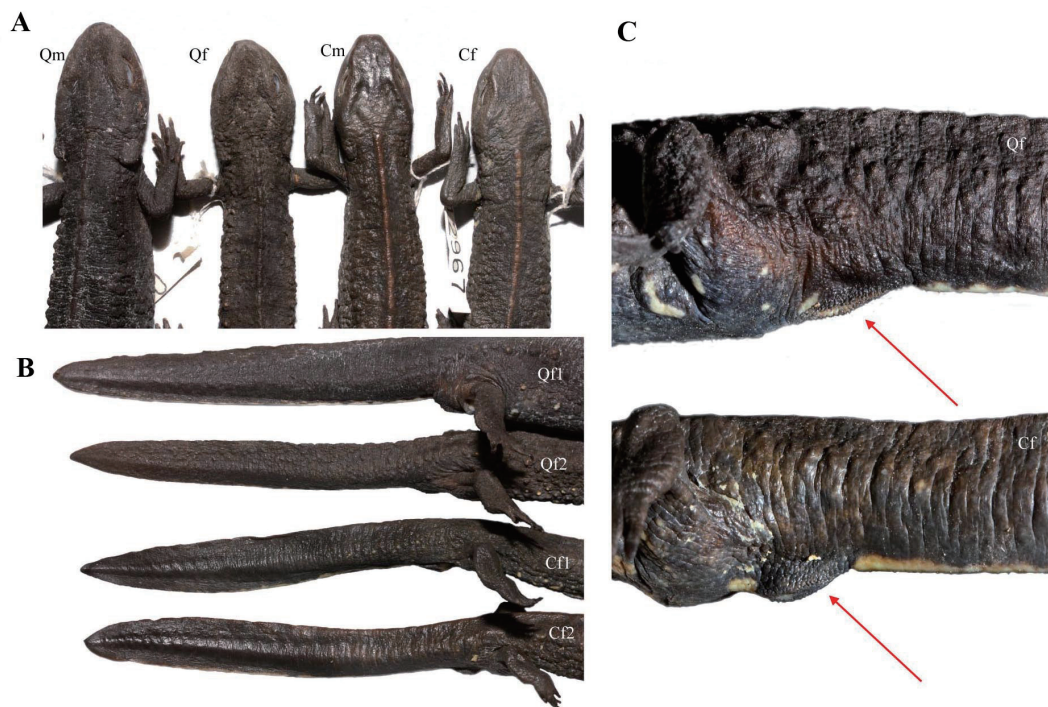


**Figure 5** The cloacae of male *P. qixilingensis* sp. nov. (A, KIZ 022293, paratype) and female *P. qixilingensis* sp. nov. (B KIZ 022289, holotype) in preservative.

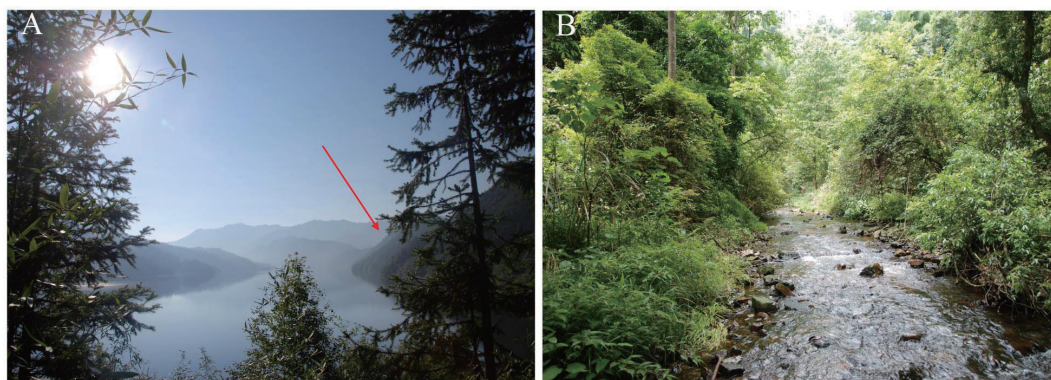
present. Head slopes steeply downward anteriorly in lateral view; labial fold well developed on lateral side of upper jaw. Vomerine tooth patch  $\Lambda$ -shaped; tongue elliptical, adheres to mouth floor but with free lateral margins. Parotoid gland prominent. Vertebral ridge conspicuous. Forelimb long, when extended rostrally reaches posterior margin of eye; palm and tarsus of

forelimb and hindlimb overlap when adpressed against flank; four fingers and five toes with lightly developed velum and webbing; relative lengths of fingers:  $1 < 4 < 2 < 3$ ; relative lengths of toes:  $1 < 5 < 2 < 4 < 3$ . Tail long, wide, depth of tail gradually narrows from tail-base to tail-tip; tail laterally compressed; dorsal caudal fin evident on posterior half of tail; ventral caudal fin inconspicuous;





**Figure 6** Comparison of the main differences between *P. qixilingensis* sp. nov. and *P. chinensis*. A: dorsum; B: tail; C: cloacae. The red arrows indicate the cloacae of *P. qixilingensis* sp. nov. Qm = male *P. qixilingensis* sp. nov.; Qf = female *P. qixilingensis* sp. nov.; Cm = male *P. chinensis*; Cf = female *P. chinensis*; Qf1 = female *P. qixilingensis* sp. nov. (KIZ 022289); Qf2 = female *P. qixilingensis* sp. nov. (KIZ 022290); Cf1 = female *P. chinensis* (KIZ 012963); Cf2 = female *P. chinensis* (KIZ 012964).



**Figure 7** A: habitat at the type locality of *Paramesotriton qixilingensis* sp. nov., Qixiling Nature Reserve, Yongxin county, Jiangxi, China; arrow points to the stream in Mt. Shenyuan where the new species was collected. B: The stream where the holotype of *P. qixilingensis* sp. nov. was collected.

tail-tip cusped. Cloaca swollen, with transverse wrinkles; few papillae on cloacal wall.

Overall coloration in life black brown. Dorsal and lateral surfaces of head, body, and tail darker brown. Single pale-orange dot on dorsal side of base of forelimbs. Ventral surfaces of head, body and tail slightly darker black than dorsal surfaces. Irregular orange-red spots on venter, chin, underside of axillae, underside of the four limbs, and cloaca. Ventral, fresh orange-red stripe from

cloaca to end of tail-tip. In preservative, dorsal and lateral surfaces of head, body, and tail chocolate brown; ventral surfaces darker than in life; orange-red coloration fades to milky white.

**Variation.** Table 2 summarizes the linear measurements. Morphologically, the paratypes resemble the holotype except that the cloaca is wider and more swollen in males than in females, and the tail is proportionally shorter but wider in males. Adult dorsal coloration ranges from olive

brown to darker brown; ventral color pattern varies in having black background with a few orange-red spots. Number, shape and position of ventral orange-red spots varies among individuals. Two males present a bluish-white caudal stripe on posterior half of tail, perhaps present only in the breeding season.

**Etymology.** The specific epithet is named for the type locality, Qixiling, Yongxin county, Jiangxi.

**Habitat and distribution.** The species was found in a stream in a valley of a broadleaf forest on Mt. Shenyuan. Shrubs and vines occurred on both sides of the stream, which was near a reservoir (Figure 7). The slow-current stream was mostly flat and shallow with a slight gradient only, 3–5 m wide, with a substrate of silt, gravel, rocks, and submerged rock faces. The water was cold. All the newts were found at the bottom and near the edge of the stream in daytime. Fishes, shrimps, and small aquatic invertebrates were abundant. Many similar streams occurred in the reserve and they may have also contained this newt. Currently, the new species is known only from the type locality (Figures 1, 7). Further investigations are required to determine the extent of its distribution.

**Comparison.** *Paramesotriton qixilingensis* sp. nov. differs from *P. labitatus*, *P. hongkongensis* and *P. maolanensis* by having very rough skin. It differs from *P. caudopunctatus* and *P. wulingensis* by having only one dorsal ridge that is the same color as other parts of the dorsal surface; it also differs in the absence of colored spots on tail of males. It differs from *P. zhijinensis* by absence of vestigial gills and gill filaments. It differs from *P. longliensis* by having a relatively flat cloaca and a slightly developed velum and webbing on the fringe of fingers and toes. It differs from *P. deloustali*, *P. fuzhongensis*, *P. guangxiensis*, and *P. yunwuensis* by having many small irregular orange-red spots on its chin, venter, underside of axillae, and cloaca. *Paramesotriton qixilingensis* sp. nov. resembles but is nevertheless distinct from *P. chinensis* by having the following characters: intermittent vertebral ridge color is the same as other parts of the dorsum; distinct tail that gradually narrows from the base to the tip; tail fins relatively underdevelopment; the dorsal and lateral sides of the trunk have many clustered conical warts; and the cloaca of females is not raised but relatively flat (Figure 6).

#### 4. Discussion

Our phylogeny is a matrilineal genealogy only, and that trees based on matrilineal versus species (nuclear DNA, morphology) may differ (Toews and Brelsford,

2012). Genetic interactions, especially introgressive hybridization, may drive the difference (Wu, 2001). In the case of *Paramesotriton*, we believe the matrilineal tree reflects the phylogeny of the genus. In cases of cytonuclear discordance, species often live side-by-side, thus providing an opportunity for hybridization to occur. In our case, the species groups cluster together yet geographically apart (Figures 1, 2). This pattern precludes inter-group hybridization. If cytonuclear discordance were to occur, then it would most likely exist within species groups only. Although sample sizes are limited, all species consist of a single, highly differentiated matriline. This pattern indicates long-term isolation. No evidence indicates incomplete lineage sorting (Figure 2). Thus, all evidence suggests that our matrilineal genealogy reflects the phylogeny of the species. Certainly, additional analyses involving other genes, and both genomes, are required to test further this initial hypothesis of relationships.

Our genealogical reconstructions confirm the results of previous studies, which recovered two species groups that are sometimes recognized as the subgenera *Paramesotriton* and *Allomesotriton*. The former *P. chinensis* species group now contains eight species including *P. qixilingensis* sp. nov. Within this group, and upon sampling five species, [Gu et al. \(2012b\)](#) placed *P. chinensis* as the sister to a clade of *P. fuzhongensis*; *P. guangxiensis*; *P. hongkongensis* and *P. deloustali*. They applied the same model of nucleotide substitution to all genes and codon positions without testing for the best model. Our partitioned re-analyses of their data obtains the same result as ours. Our more complete taxonomic sampling resolves three distinct lineages (A, B, and C) within the *P. chinensis* group, although their relationships to one another are not resolved. Differing from the results of [Gu et al. \(2012b\)](#), *P. chinensis*, *P. qixilingensis* sp. nov. and *P. hongkongensis* unite as Lineage B (Figure 2) and with strong support in the BI tree, but not ML and MP. These results are consistent with those of [Wu et al. \(2010a\)](#). Because different genes and different codons may have different evolutionary rates ([Degnan and Rosenberg, 2009](#)), analyses may obtain conflicting results when applying the same untested model for different genes.

The high levels of genetic differentiation (*P*-distances > 9.38%) imply that species in the *P. chinensis* group, as well as within *Paramesotriton*, diverged long ago. The *P*-distance between the new species and *P. chinensis* is 9.97%. Thus, all analysis support the recognition of *P. qixilingensis* sp. nov.

The diagnosing of species within the *P. chinensis* group often presents a challenge because all taxa are very similar superficially in having a dark brown dorsum. Further, substantial variation occurs in the ventral spotting, not only between species but also within populations. Thus, it is not surprising that *P. qixilingensis* sp. nov. was mistakenly identified initially as *P. chinensis* in the report of a new record in Jiangxi (Song *et al.*, 2006). Consequently, *P. chinensis* remains unknown from Qixiling Nature Reserve; our samples came from the same stream collected by Song *et al.* (2006).

Our resolution of relationships within the *P. caudopunctatus* species group conforms to those of Gu *et al.* (2012a, 2012b). Compared with deep genetic divergences among species in *P. chinensis* group (all *P*-distances > 4.22%; Table 3), several species within the *P. caudopunctatus* group show little difference (e. g., *P*-distance = 0.58% between *P. longliensis* and *P. maolanensis*). However, most adult *P. zhijinensis* have vestigial gills characteristic of neotenic newts. *Paramesotriton maolanensis* has smooth skin and degenerate eyes. Neither feature occurs in *P. longliensis*. The irony of little genetic difference yet great morphological distinctiveness requires additional investigation. Future analyses integrating greater sampling and larger numbers of loci, including nuclear gene sequences, may point to either rapid speciation via environmental adaptation (e.g., to karst), or recent gene flow.

With the description of *P. qixilingensis* sp. nov., *Paramesotriton* contains 13 species, which is almost a twofold increase in species diversity in 5 years only. In part, this increase may reflect the recent upsurge in field survey work in unexplored or incompletely surveyed areas across South China. However, detailed analyses of morphological diversity, microhabitat preferences and gene sequence data (e.g., Li *et al.*, 2008a, 2008b; Wu *et al.*, 2009, 2010a; Zhao *et al.*, 2008) also contribute to the increase. This high rate of discovery also occurs in *Pachytriton* and *Cynops* in South China (Nishikawa *et al.*, 2011a, 2011b, 2012; Wu *et al.*, 2010b, 2012; Yuan *et al.*, 2013). The diversity of amphibians within the region likely remains underestimated, especially in Jiangxi and Fujian given the paucity of field surveys (Zhao and Hu, 1984; Fei *et al.*, 2006).

As for many of the modern Asian newts, we know very little about the natural history, biology and distribution of *P. qixilingensis* sp. nov. This situation challenges the effective conservation of species (Wilson, 2000;

Xie *et al.*, 2007). Although the forest inside of Qixiling Nature Reserve is well preserved, local people often secretly glean the stream for food and other resources. Such activity may negatively impact the survival of *P. qixilingensis* sp. nov. Most species of *Paramesotriton* apparently have extremely small distributions. We suspect that *P. qixilingensis* sp. nov. qualifies for listing as being threatened or endangered by IUCN and CITES, as well as for protection in China.

## 5. Key to the species of *Paramesotriton*

The following key synthesizes previous work (Fei *et al.* 2006; Wu *et al.*, 2009, 2010a; Gu *et al.*, 2012a, 2012b).

- 1 Skin relatively smooth.....2  
Very rough skin.....4
- 2 Few granular warts on the head and body; eyes not reduced.....3  
Granular warts absent on the head and body; reduced eyes.....*P. maolanensis*
- 3 Body slender and flat; tail long; vertebral ridge inconspicuous.....*P. labiatus*  
Body not slender and flat; tail short; vertebral ridge conspicuous.....*P. hongkongensis*
- 4 Body small; dorsolateral ridge with yellow or orange tint.....5  
Body robust; dorsal ridge is the same color as other parts of the dorsal surface.....8
- 5 Scent glands absent on snout; vestigial gills and gill filaments absent.....6  
Scent glands on snout; 3 gill filaments behind head.....  
.....*P. zhijinensis*
- 6 No fleshy protuberance present in branchial region; three colored spots on tail of males.....7  
Fleshy protuberance present in branchial region; three colored spots on tail of males.....*P. longliensis*
- 7 Dorsum olive brown; frontal branch of pterygoid arrived at the posterior edge of maxillary.....  
.....*P. wulingensis*  
Dorsum pale yellow; frontal branch of pterygoid not contacting posterior edge of maxillary.....  
.....*P. caudopunctatus*
- 8 Small, irregular orange-red spots on its chin, venter, underside of axillae, and cloaca.....9  
Large irregular orange-red spots on its chin, venter, underside of axillae, and cloaca.....10
- 9 Tail gradually tapers from base to tip without expanding posteriorly; tail fins underdevelopment; dorsal and lateral sides of the trunk with many clustered, conical warts; cloaca of females relatively

- flat.....*P. qixilingensis*  
Tail expands posteriorly to form tail fin; warts small;  
cloaca of females raised.....*P. chinensis*  
10 Vertebral ridge flat or low; few granular warts.....  
.....*P. yunwuensis*  
High vertebral ridge; densely granulated warts.....11  
11 Digit tips only overlap when forelimbs and hind limbs  
adpressed.....12  
Palm and tarsus overlap when forelimbs and hind limbs  
adpressed.....*P. fuzhongensis*  
12 Extended forelimb reaches midpoint of eye.....  
.....*P. deloustali*  
Extended forelimb reaches posterior edge of eye only...  
.....*P. guangxiensis*

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