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A new species of the genus Paramesotriton (Caudata: Salamandridae) from Guangxi Zhuang Autonomous Region, southern China

YUNKE WU[1, 3], SEAN M. ROVITO[2], THEODORE J. PAPENFUSS[2], JAMES HANKEN[1]

[1] *Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, 02138, USA*

[2] *Museum of Vertebrate Zoology, University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, CA 94720-3160, USA*

[3] *Corresponding author. E-mail: yunkewu@fas.harvard.edu*

Abstract

A new species of Asian warty newt, *Paramesotriton ermizhaoi*, is described from Guangxi Zhuang Autonomous Region in southern China. This species is easily distinguished from other congeners by external morphology and osteology. Phylogenetic analyses based on mitochondrial data place *P. ermizhaoi* as a possible sister taxon to *P. hongkongensis* and *P. chinensis*. The number of trunk vertebrae varies from 12 to 13 in the new species. The relationship between *P. ermizhaoi* and a potentially new *Pachytriton* species (*Pachytriton* C), and the Guangxi population of *P. chinensis* is briefly discussed.

Key words: Caudata; Salamandridae; new species; *Paramesotriton ermizhaoi*; southern China

Introduction

New amphibian species are being discovered at an astonishingly high rate worldwide. Recent molecular technologies have accelerated this phenomenon by revealing cryptic species that were once undetected or misidentified by traditional taxonomic approaches (Hanken 1999).

The family Salamandridae ranges over almost the entire Holarctic region, with its diversity centering in temperate Europe and Asia (Duellman & Trueb 1994). In 2002, there were 58 formally described species in this family (Stuart & Papenfuss 2002). By the end of 2008, this number had jumped to 79, an increase of 36% in only six years (AmphibiaWeb). These salamanders comprise 20 genera, 5 of which occur in Asia, including *Cynops* (7 spp.), *Pachytriton* (3 spp.), *Paramesotriton* (9 spp.), *Echinotriton* (2 spp.), and *Tylototriton* (8 spp.) (AmphibiaWeb). The former three genera, recognized as modern Asian newts, form a monophyletic group that is well supported by various molecular data (Titus & Larson 1995; Chan *et al.* 2001; Weisrock *et al.* 2006; Steinfartz *et al.* 2007).

The genus *Paramesotriton*, commonly known as the Asian warty newts, currently comprises nine species, two of which were described recently (Li *et al.* 2008 a, b). The nine species are *P. caudopunctatus* Liu and Hu, *P. chinensis* Gray, *P. deloustali* Bourret, *P. fuzhongensis* Wen, *P. guangxiensis* Huang, Tang, and Tang, *P. hongkongensis* Myers and Leviton, *P. laoensis* Stuart and Papenfuss, *P. longliensis* Li, Tian, Gu, and Xiong, and *P. zhijinensis* Li, Tian, and Gu. Except for *P. chinensis*, which occurs mainly in southeastern China, all species are distributed in southern China, northern Vietnam and Laos. Monophyly of *Paramesotriton* is not supported by whole-mitochondrial genomic data, with *P. laoensis* consistently grouping with the genus *Pachytriton* (Zhang *et al.* 2008). Intrageneric relationships among species of *Paramesotriton* have been studied, yet they are not fully resolved (Chan *et al.* 2001; Lu *et al.* 2004; Weisrock *et al.* 2006; Zhao *et al.* 2008).

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Guangxi Zhuang Autonomous Region is located along the southern border of China, adjacent to northern Vietnam. Its subtropical moist climate, mountainous terrain, and well-preserved old growth forests make this region a suitable habitat for salamanders. Both *P. guangxiensis* and *P. fuzhongensis* were described from this province, and *P. chinensis* might be found here as well (Fei *et al.* 1999; Zhang & Wen 2000; Fei *et al.* 2006). A fourth *Paramesotriton* population also occurs in northeastern Guangxi (Fig. 1). These salamanders are morphologically different from all other known *Paramesotriton*. In July 2006, we acquired several specimens from this population in Guangxi Zhuang Autonomous Region and investigated its taxonomic identity and phylogenetic relationship to other *Paramesotriton*.

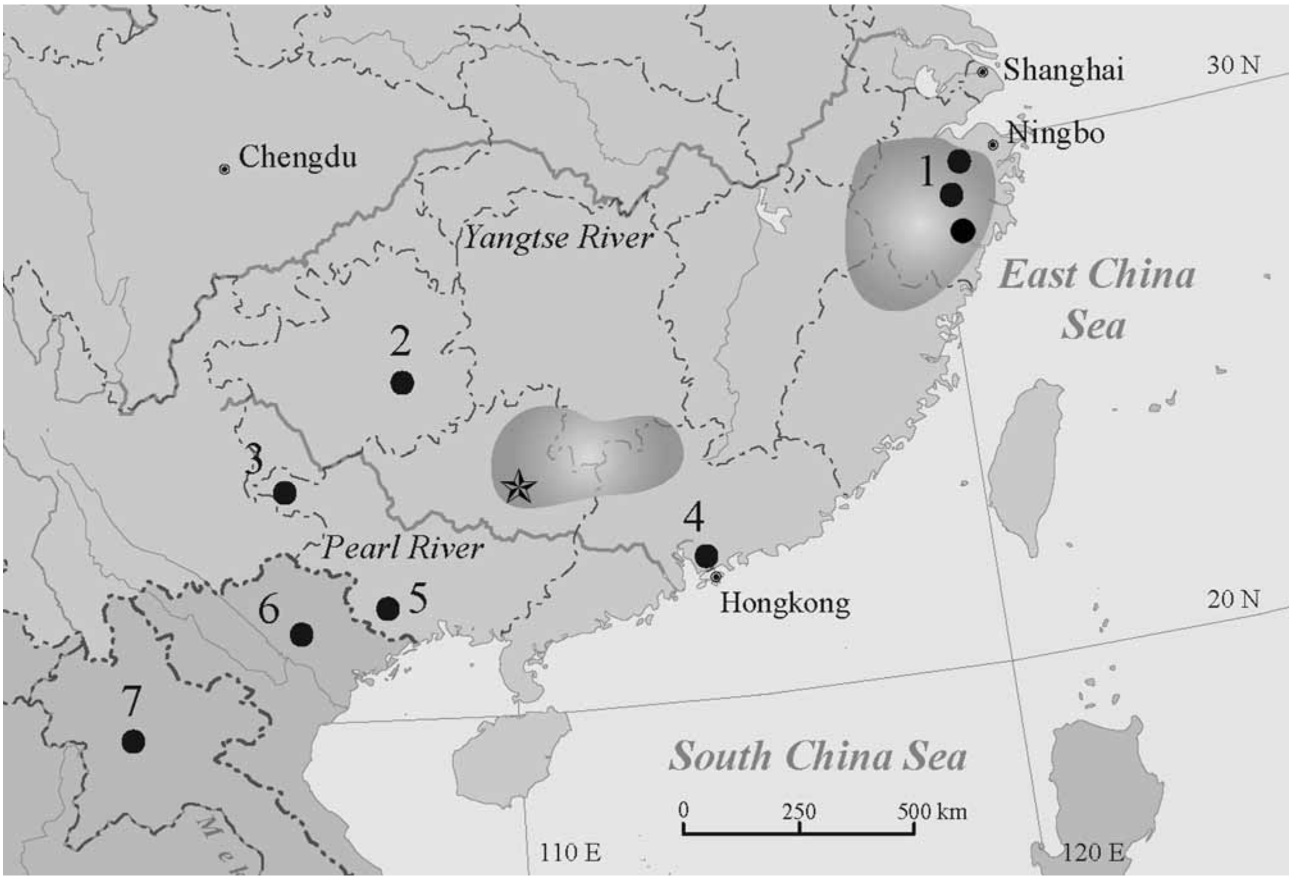

FIGURE 1. Sample localities used in this study. Except for *Paramesotriton fuzhongensis*, all species were collected from their respective type localities. 1: *P. chinensis*; 2: *P. caudopunctatus*; 3: *P. fuzhongensis*; 4: *P. hongkongensis*; 5: *P. guangxiensis*; 6: *P. deloustali*; 7: *P. laoensis*. Shaded areas represent the two disjunct distributions of *P. chinensis*. Asterisk denotes the type locality of *P. ermizhaoi*.

Materials and methods

Eleven specimens were examined. Five were collected from Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, in July 2006. Two of these (CIB 88140, 88141) were fixed in 10% neutral-buffered formalin before being permanently stored in 70% ethanol. Fresh liver tissues were preserved in 95% ethanol for genetic analyses. The other three animals (CIB 95998-96000) were brought back alive to the Chengdu Institute of Biology, the Chinese Academy of Sciences (CIB), and preserved in 95% ethanol. Six additional specimens (MVZ 230616-230621) were collected in March 1999 by E. Zhao from the same locality and catalogued under the name of *Paramesotriton chinensis*. These specimens were preserved in 95% ethanol. We examined comparative material from the Museum of Vertebrate Zoology, University of California at Berkeley (MVZ), the Chengdu Institute of Biology, the Chinese Academy of Sciences (CIB), and the Field Museum of Natural History (FMNH). Specimens are listed at the end of this paper.

Linear measurements were taken from preserved specimens with dial calipers. We followed the procedure of Stuart and Papenfuss (2002) to take the following measurements: total length (TTL), snout-vent length (SVL), tail length (TAL), tail depth (TAD), head length (HL), head width (HW), interocular distance (IO), distance between front corner of eye and nostril (EN), internostril distance (IN), distance between axillae along left body side (AX), length of left forelimb (AL), and length of left hind limb (PL). Specimens were sexed by minor dissection. Skeletal characters were examined on digital radiographs at the Museum of Comparative Zoology, Harvard University (MCZ) and MVZ.

Phylogenetic analyses employed the mitochondrial sequences that cover a partial tRNA-Met, the complete NADH dehydrogenase 2 (ND2) and tRNA-Trp, and a partial tRNA-Ala. Our work was based mainly on the results of Weisrock *et al.* (2006), which included one of the six specimens (MVZ 230616) collected by E. Zhao. Sequences were retrieved from GenBank for seven of the nine *Paramesotriton* species (Table 1). New ND2 sequences were generated for the unidentified population. Weisrock *et al.* (2006) included only one specimen of *P. chinensis*, a species that is relatively wide-distributed in southeastern China (Fei *et al.* 1999; Fei *et al.* 2006). To avoid any unpredicted large genetic divergence within this species that may confound our molecular phylogeny, we supplemented the data with one *P. chinensis* collected from the type locality (inland from Ningbo in Zhejiang Province), and two other specimens from central Zhejiang. Additional ND2 sequences of two *P. deloustali* were also included. The genus *Pachytriton* was chosen as the phylogenetic outgroup based on its known sister relationship to *Paramesotriton* (Chan *et al.* 2001; Weisrock *et al.* 2006; Steinfartz *et al.* 2007).

TABLE 1. Specimens included in the molecular analysis.

Species	Museum No.	Locality	GenBank Accession No.
Ingroup			
Paramesotriton ermizhaoi	CIB 88141	Jinxiu, Guangxi, China	FJ744601
Paramesotriton ermizhaoi	CIB 88140	Jinxiu, Guangxi, China	FJ744602
Paramesotriton ermizhaoi	MVZ 230616	Jinxiu, Guangxi, China	DQ517801
Paramesotriton caudopunctatus	MVZ 236250	Leishan, Guizhou, China	DQ517799
Paramesotriton caudopunctatus	MVZ 236252	Leishan, Guizhou, China	EU880326
Paramesotriton chinensis	MVZ 230360	Yongjia, Zhejiang, China	DQ517800
Paramesotriton chinensis	CIB 95899	Ningbo, Zhejiang, China	FJ744603
Paramesotriton chinensis	CIB 95910	Jinhua, Zhejiang, China	FJ744605
Paramesotriton chinensis	CIB 95911	Jinhua, Zhejiang, China	FJ744604
Paramesotriton deloustali	MVZ 223627	Tam Dao, Vinh Phu, Vietnam	DQ517802
Paramesotriton deloustali	MVZ 223628	Tam Dao, Vinh Phu, Vietnam	FJ744599
Paramesotriton deloustali	MVZ 223629	Tam Dao, Vinh Phu, Vietnam	FJ744600
Paramesotriton fuzhongensis	MVZ 230363	Xiling, Guangxi, China	DQ517803
Paramesotriton guangxiensis	MVZ 220905	Ningming, Guangxi, China	DQ517804
Paramesotriton hongkongensis	MVZ 230367	Hongkong, China	DQ517805
Paramesotriton hongkongensis	MVZ 230365	Hongkong, China	DQ517807
Paramesotriton hongkongensis	MVZ 230369	Hongkong, China	DQ517806
Paramesotriton laoensis	FMNH 255452	Xiang Khouang, Laos	DQ517808
Outgroup			
Pachytriton brevipes	MVZ 231167	Dehua, Fujian, China	DQ517797
Pachytriton labiatus	CAS 194298	Jiaxing, Zhejiang, China	DQ517798

Genomic DNA was extracted from preserved liver tissue using QIAGEN DNeasy blood and tissue kits following the manufacturer's protocol. Amplifications of mitochondrial fragments were conducted under the condition of initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 5 min. Negative controls were added to detect contamination. Amplified products were purified with QIAquick PCR purification kits and sequenced on an ABI 3730 capillary sequencer. All regions were sequenced in both directions. PCR and sequencing primers are available from the authors upon request. Sequences were aligned manually in Se-Al 2.0 (Rambaut 1995). No indel or premature stop codon was detected in the protein-coding region, which supports the fact that we did not amplify any nuclear mitochondrial pseudogenes.

We reconstructed intrageneric relationships within *Paramesotriton* under maximum likelihood criterion (ML) in Garli v.0.951 (Zwickl 2006), with the best-fitted evolutionary model determined by Akaike Information Criterion (AIC) implemented in MODELTEST 3.7 (Posada & Crandall 1998). The search for the ML tree was terminated when the likelihood score had not been improved for 50,000 generations. Bootstrap values were calculated for 100 replicates. We also calculated bootstrap values under maximum parsimony criterion for 1000 replicates in PAUP 4.0b10. Lastly, we performed Bayesian inference (BI) for our molecular phylogeny in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Sequence data were partitioned into three portions: tRNA, the 1st and 2nd codon positions, and the 3rd codon position. We assigned independent GTR+I+G model to each partition. Trees were discarded before burn-in period, which was determined by TRACER v 1.4 (Rambaut & Drummond 2007).

Results and discussion

The aligned sequences yielded 1175-1176 base pairs from 20 specimens. The ML analysis chose TIM+G as the best-fit model and produced a topology completely consistent with that of the Bayesian tree based on data partitions (Fig. 2). Our phylogeny is also concordant with Chan *et al.* (2001), Lu *et al.* (2004) and Weisrock *et al.* (2006).

Paramesotriton laoensis and *P. caudopunctatus* were recovered as basal to all other *Paramesotriton* species, which form two major clades. The first clade comprises *P. hongkongensis*, *P. chinensis*, and samples from the new population from Guangxi. All three species are monophyletic with strong support. The *P. chinensis* group, which was collected in Zhejiang Province in Southeastern China, includes one specimen from the type locality (CIB 95899) and thus represents the name-bearing lineage. The new *Paramesotriton* population is the sister taxon to the group of *P. chinensis* and *P. hongkongensis*, albeit with low bootstrap support and posterior probability. The new population differs from the latter two species by 11% and 10% in the uncorrected pairwise distance, respectively. This is equivalent to interspecific divergence in other salamandrid genera (e.g., Babik *et al.* 2005; Steinfartz *et al.* 2007) and suggests long-term isolation.

The second major clade in *Paramesotriton* includes the closely related *P. deloustali*, *P. fuzhongensis*, and *P. guangxiensis*, with the former species being sister to the latter two. This result is similar to Weisrock *et al.* (2006) but inconsistent with Lu *et al.* (2004), which groups *P. deloustali* with *P. guangxiensis*. This discrepancy can be attributed to specimen misidentification. The *P. deloustali* specimen used by Lu *et al.* (2004) from Cao Bang, Vietnam, has since been re-catalogued as *Paramesotriton* sp. (Royal Ontario Museum). We included three *P. deloustali* from its type locality and derived the same topology as that of Weisrock *et al.* (2006).

Our analysis does not include the newly described *P. zhijinensis* and *P. longliensis* due to unavailability of tissue samples. However, the new population differs from these two species by conspicuous external morphological and osteological features (Li *et al.* 2008 a, b). It also has been suggested that *P. zhijinensis* is a separate lineage outside the two major clades discussed above based on mitochondrial data (Zhao *et al.* 2008). We conclude that the new population should be regarded as a distinct species in the genus *Paramesotriton*, which is described as follows.

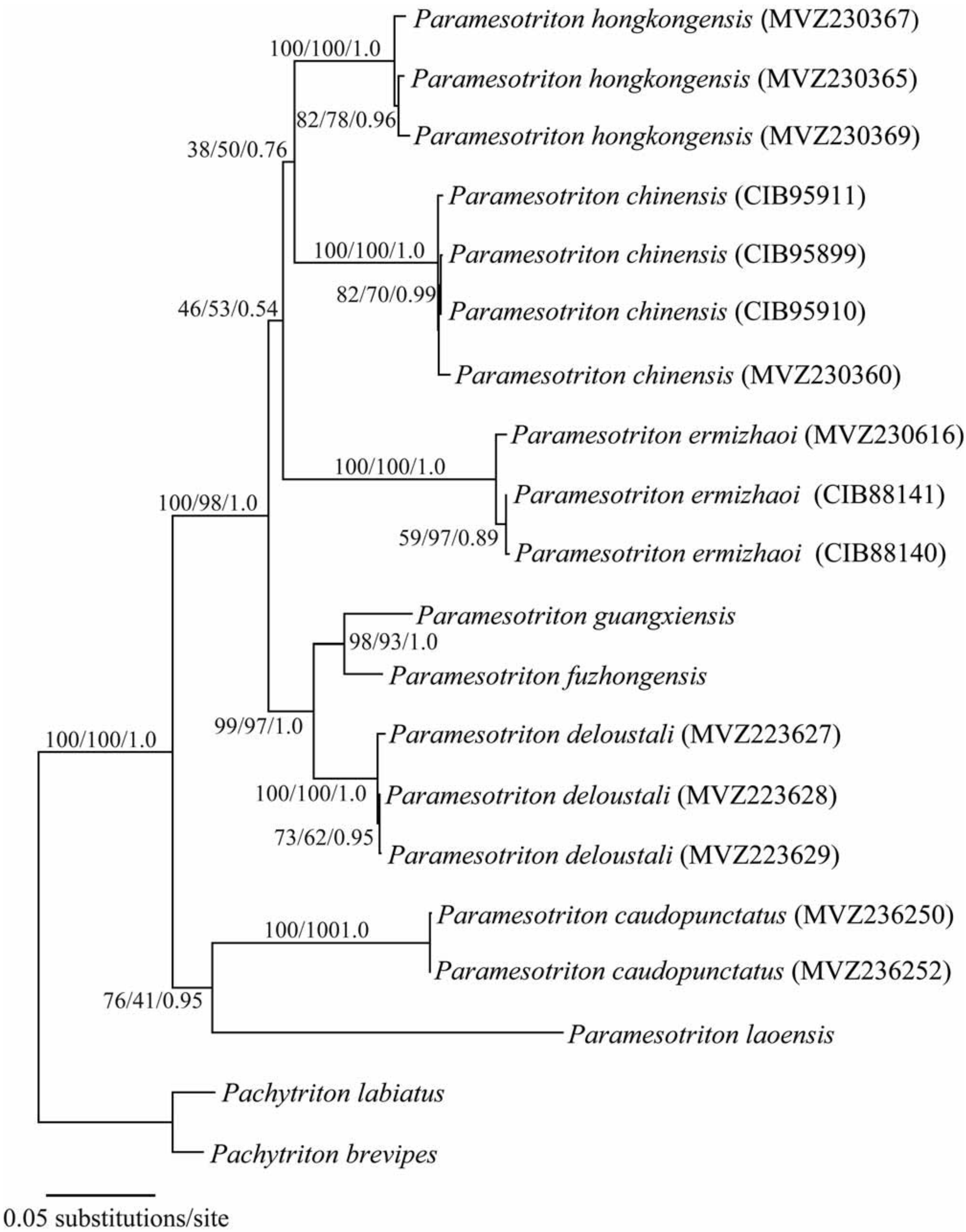

FIGURE 2. Maximum-likelihood tree based on ND2 sequences. Bayesian inference produced a consistent topology. Numbers on branches: boostrap support from ML analysis; bootstrap support from MP analysis; Bayesian posterior probability. Tree is rooted with *Pachytriton brevipes* and *P. labiatus*.

Paramesotriton ermizhaoi species nov.
(Fig. 3 ABC)

Holotype: CIB 88141, an adult male collected in Mt. Dayao (24°07´N, 110°13´E, 881m elevation), Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, P. R. China; collected by Jiatang Li on July 7, 2006.

Paratypes: Same locality as holotype: CIB 88140, CIB 95998-96000, collected with the holotype on the same date; MVZ 230616-230621, collected by E. Zhao in March 1999.

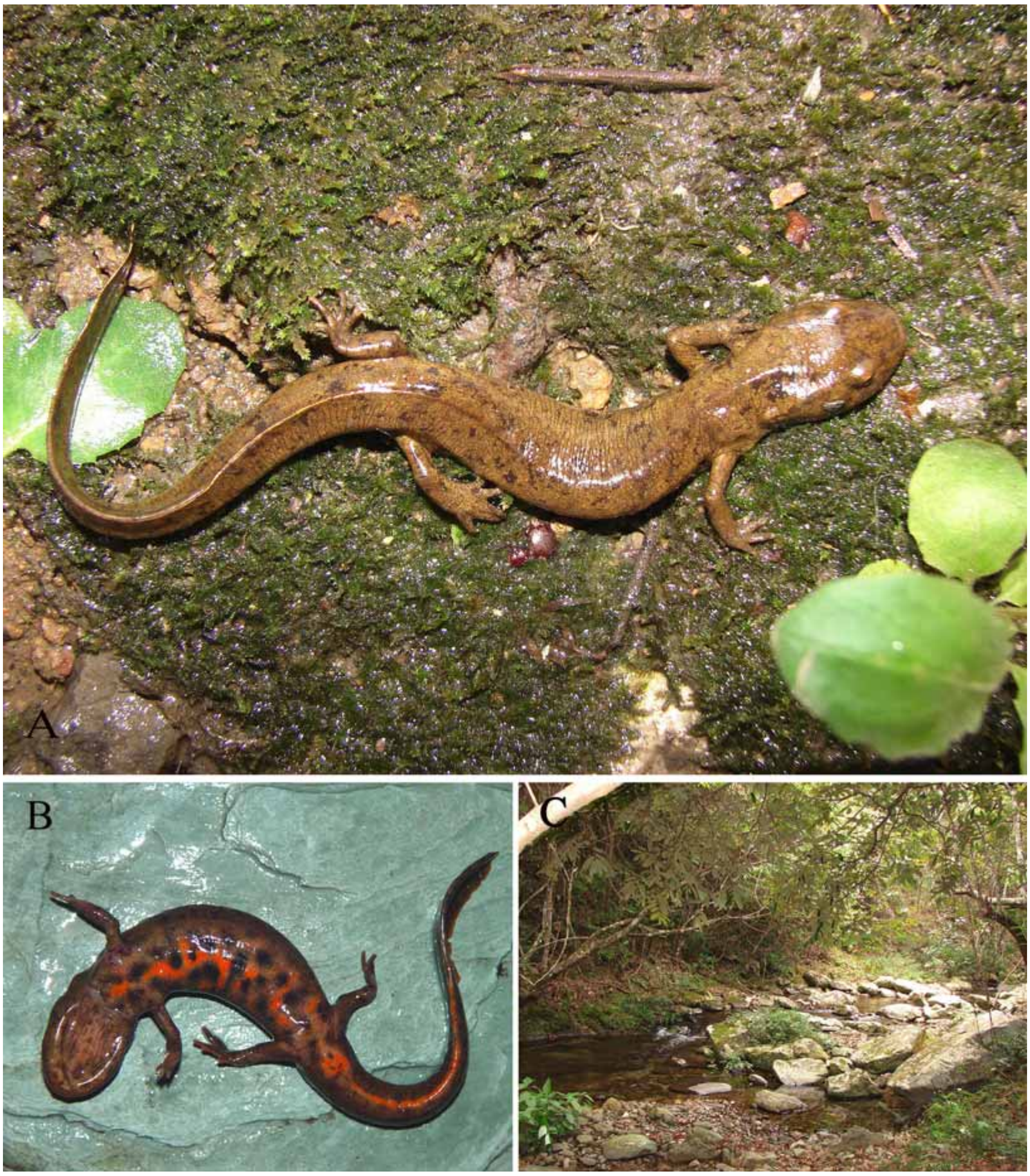

FIGURE 3. A: Dorsal view of living *P. ermizhaoi* (CIB 95998, paratype: ♀); B: Ventral view of living *P. ermizhaoi* (CIB 88141, holotype: ♂). C: habitat of *P. ermizhaoi* in Mt. Dayao, Jinxiu Yao Autonomous County, Guangxi Zhuang Autonomous Region, China.

Diagnosis: *Paramesotriton ermizhaoi* is assigned to the genus *Paramesotriton* because of its slender and nearly straight epibranchial bones, maxillary bones oriented angular to the body axis, and laterally compressed tail. This species can be diagnosed from other congeners by the following combination of characters: skin relatively smooth; granular warts absent on head and body; vertebral ridge not prominent; head depressed and nearly flat in profile; habitus slender and depressed; limbs short—when adpressed against flank, fingers and toes hardly meet; dorsum olive brown with irregular black flecks; ventral orange-red blotches irregular in shape.

Description of the holotype: A slender and flat newt, preserved with mouth open. Head oval in shape, depressed and nearly flat in profile. Snout truncate and slightly projecting beyond lower mandible. Nostrils close to snout tip. Labial fold evident throughout posterior half of upper jaw. Skull relatively narrow, with maxillary bones oriented angular to body axis. Maxillary tips lie anterior and lateral to pterygoid bones; no contact between maxillary and pterygoid. Fronto-squamosal arch complete but not very robust. Epibranchial bones slender, nearly straight, only slightly flared dorsally. Vomerine tooth patch Λ-shaped, tooth rows converging anteriorly and exceeding the anterior limit of choanae. Tongue adhering to mouth floor with free lateral margins. Parotoid region evident, but not enlarged. Gill filaments absent. Gular fold present. Skin relatively smooth on head and body, with small transverse wrinkles. Vertebral ridge thin, slightly elevated. Lateral dorsal ridge absent. Thirteen trunk vertebrae. Four fingers, five toes, without webbing. Tail laterally compressed; posterior dorsal caudal fin evident, ventral caudal fin indistinct; tail extremity round in profile. Cloaca swollen, with a few papillae near posterior limit.

Color of holotype in life: Dorsum olive brown with irregular black flecks. Vertebral ridge with orange tint. Venter paler brown. Irregular orange-red blotches on venter and anterior portion of cloaca with indistinct black margin; some blotches connected into short irregular stripes. Orange-red on underside of tail.

In preservative, dorsum and venter black. Vertebral ridge inconspicuous. Ventral bright orange fading to yellow to milky white; black margins invisible.

Variation: Morphology of paratypes resembles that of holotype except that females have smaller and non-swollen cloaca without papillae. Linear measurements are summarized in Table 2. Color of ventral blotches ranges from orange to red, spreading to chin and underside of axillae, varying in shape and arrangement. Black marks border those blotches or intersperse on venter.

TABLE 2. Linear measurements of *Paramesotriton ermizhaoi*. See text for abbreviations. Holotype is included in the measurements of males. Unit: mm.

	Holotype	Females (N = 6)		Males (N = 5)	
Measurements	(CIB 88141)	Range	Mean ± SE	Range	Mean ± SE
SVL	63.5	46.6–66.8	56.3 ± 2.7	46.4–63.5	56.1 ± 3.1
TTL	126.0	94.0–137.5	112.8 ± 5.9	92.2–127.6	111.5 ± 6.8
TAL	57.7	44.7–65.5	53.4 ± 3.0	43.3–60.2	51.1 ± 3.3
TAD	7.5	5.5–8.1	7.0 ± 0.4	5.5–8.5	7.3 ± 0.5
HL	17.7	14.4–17.4	15.9 ± 0.5	14.8–17.8	16.1 ± 0.7
HW	12.1	8.8–12.6	10.7 ± 0.5	9.1–12.3	10.9 ± 0.6
IO	7.1	5.3–6.9	6.0 ± 0.2	5.4–7.1	6.4 ± 0.3
EN	4.9	3.2–4.2	3.8 ± 0.2	3.1–4.9	4.0 ± 0.3
IN	3.7	2.7–3.6	3.1 ± 0.1	2.4–3.7	3.1 ± 0.2
AX	31.3	20.2–36.7	27.7 ± 2.2	21.4–31.3	27.6 ± 1.7
AL	15.6	12.6–15.4	13.8 ± 0.4	12.5–17.4	14.6 ± 0.9
PL	16.0	13.1–15.6	14.2 ± 0.4	13.5–17.4	15.2 ± 0.7

Etymology: The new species is named after Ermi Zhao, a prominent Chinese herpetologist and educator, for his great contribution to the development of herpetological study and the training of a new generation of scientists in China.

Habitat: Stream in broadleaf forest with herbaceous plants and vines. Stream is 3-4 meters wide and shallow, flowing slowly in a valley (Fig. 3C). Substrates include gravels, scattered small rocks, and semisubmerged larger rocks. Along the stream are pools with a very slow current. Newts are found at the stream bottom, usually under rocks or between crevices during daytime. Fishes and small aquatic invertebrates coexist with *Paramesotriton ermizhaoi*, and *Pachytriton labiatus* is found in the same stream drainage at a higher elevation.

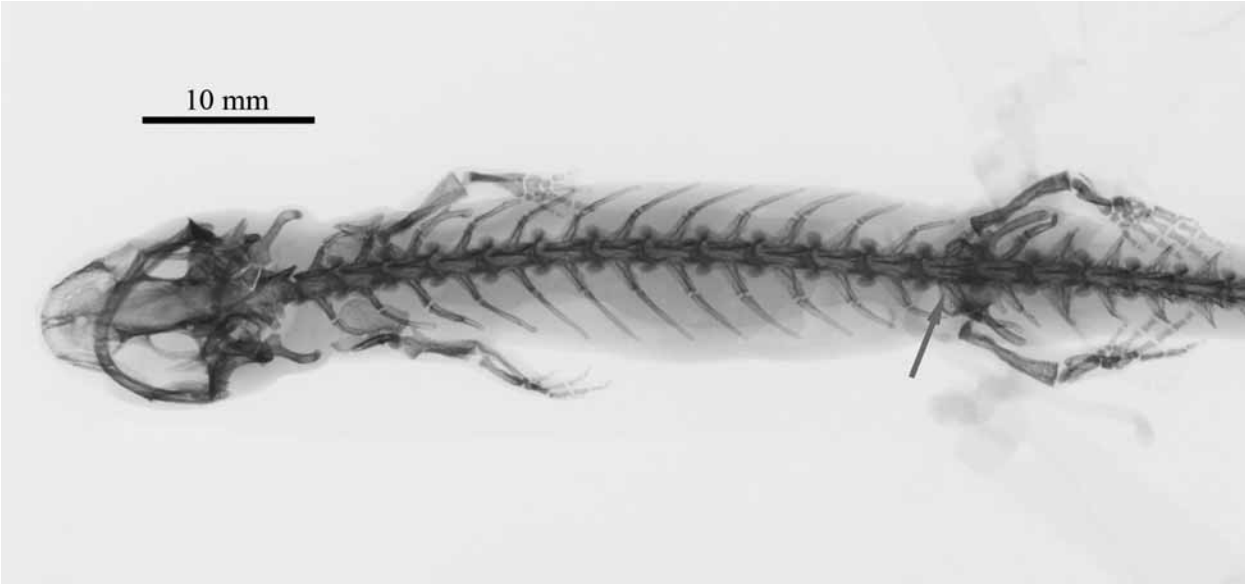

FIGURE 4. Radiograph of *P. ermizhaoi* (CIB 88141, holotype: ♂). Arrow points to the 13th trunk vertebrae.

Discussion: *Paramesotriton ermizhaoi* is unusual insofar as a large portion of individuals possess 13 trunk vertebrae; other congeneric species normally have 12 trunk vertebrae (Chan *et al.* 2001). Although variation has been observed in other *Paramesotriton*, it is rare. However, six of eleven *P. ermizhaoi* (including the holotype) have 13 trunk vertebrae (Fig. 4); the other five specimens have 12. The number of trunk vertebrae does not appear to be correlated with gender. A similar situation occurs in the European salamandrid *Ommatotriton ophryticus*, in which the modal number of trunk vertebrae varies from 12 to 13 in different geographic populations (Litvinchuk *et al.* 2005). Orska and Imiolek (1962) reported the correspondence of vertebral number to developmental temperature in salamanders. Future research is necessary to evaluate the correlation between environmental temperature and embryonic development in *P. ermizhaoi.*

Paramesotriton ermizhaoi may have been long misidentified as another Asian salamandrid species. Scholz (1998) reported a potentially new species of *Pachytriton* from the pet trade, named *Pachytriton* C. In fact, most descriptions of morphology, coloration, and behavior of *Pachytriton* C match with *P. ermizhaoi*. Due to lack of molecular data and known locality, Scholz (1998) did not make a definitive taxonomic statement on *Pachytriton* C. We suspect that the two names refer to the same species.

The only known locality of *P. ermizhaoi*, Mt. Dayao, is located within the putative Guangxi population of *P. chinensis* (Fei *et al.* 1999; Zhang & Wen 2000; Fei *et al.* 2006), which was first described in rivers at inland from Ningbo, Zhejiang Province (Gray 1859). However, *P. ermizhaoi* has been identified mistakenly as *P. chinensis* despite their different morphologies. For instance, the paratypes of *P. ermizhaoi* from MVZ were originally catalogued as *P. chinensis*. We also note that the *P. chinensis* used by Lu *et al.* (2004) from Mt. Dayao actually are *P. ermizhaoi*, based on mitochondrial sequences. To our knowledge, no true *P. chinensis* has been collected from Mt. Dayao. It is possible that claims of a disjunct Guangxi population of *P. chinensis* are incorrect due to misidentification. However, Zhang and Wen (2000) provide an account on Guangxi *P. chinensis* that matches the diagnostic characters of this species. More fieldwork is needed to determine if *P. chinensis* occurs in Guangxi Zhuang Autonomous Region.

Comparative material examined: *P. caudopunctatus* (MVZ 236250-236254 from Guizhou, China), *P. chinensis* (MVZ 230360, CIB 95899, CIB 95907-95911 from Zhejiang, China), *P. fuzhongensis* (MVZ

230622-230625 from Guangxi Zhuang Autonomous Region, China), *P. hongkongensis* (MVZ 230365-230370 from Hong Kong, China), *P. deloustali* (MVZ 222122-222123, MVZ 223627-223629 from Tam Dao, Vietnam), *P. laoensis* (FMNH 255450, FMNH 257850, FMNH 257852, FMNH 257853 from Xiang Khouang, Laos).

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