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Two New Species of *Pachytriton* from Anhui and Guangxi, China (Amphibia: Urodela: Salamandridae)

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Abstract: Two new species of the salamandrid genus *Pachytriton* are described from Anhui and Guangxi, China, based on molecular and morphological analyses. One of them from Huangshan, Anhui, has smaller body, longer limbs, and larger eye than the remaining congeners. The other from northeastern Guangxi possesses a larger body and head, and longer snout and tooth series than the remaining congeners. Also, we have found that two individuals of *Pachytriton* obtained from pet shops in Japan can represent an unnamed species, but do not describe them as new because we have no exact information on their original localities. The distribution patterns and ecological relationships among species of *Pachytriton* are discussed.

Key words: *Pachytriton*; molecular phylogeny; morphology; new species; China

INTRODUCTION

The salamandrid genus *Pachytriton* Boulenger, 1878 occurs in eastern and southern China (Fei et al., 2006). For a long time, only two species, *Pac. brevipes* and *Pac. labiatus*, had been known in the genus, but recently, *Pac. labiatus* was transferred to *Paramesotriton* (Nishikawa et al., 2011), while *Pac. granulosus* was revived (Nishikawa et al., 2011) and *Pac. archospotus* and *Pac. inexpectatus* were newly described (Shen et al. [2008] and Nishikawa et al. [2011], respectively). Thus, four species are currently known for *Pachytriton*. Discoveries of several new species in recent years imply that we still need more investigations on intra- and inter-specific variation by covering the whole distribution of the genus, with comprehensive analyses using morphological and molecular data.

Bearing this in mind, we performed a phylogenetic analysis using mitochondrial cytochrome b gene and inferred relationships among samples of *Pachytriton*. As a result, a total of seven lineages were recognized, which differed from each other by genetic distances comparable to those known between some newt species. Four of these corresponded to the four nominated species. Each of the remaining three lineages differed from them not only genetically but also by combinations of morphological characteristics. We therefore describe two of them, one from Anhui and the other from Guangxi, as new species in this article. For the remaining one, however, we refrain from description, because its representatives were

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obtained only from Japanese pet shops and their exact localities are unknown yet.

MATERIALS AND METHODS

Molecular phylogenetic analyses

We obtained sequence data of cytochrome b region (cyt b) of mitochondrial DNA (mtDNA) from muscle or liver tissue samples preserved in 99% ethanol. Methods for DNA extraction and, amplification and sequencing of the DNA fragment are same as those reported by Yoshikawa et al. (2008) but with a modification for primers. The PCR primers were HYD_Cytb_F1 (forward: Matsui et al., 2008) and Salamander_Cytb_RN2 (reverse: Matsui et al., 2008), and the cycle sequencing primers, H14778 (reverse: Matsui et al., 2007) and Pachy_Cytb_530F (forward: 5'-CTGTAGACA AGRCTACCYTGACACG-3'), were designed in the middle of the cyt b region, in addition to the above two primers. We then obtained sequences of complete cyt b gene. Prior to the phylogenetic analyses, we plotted the uncorrected sequence divergences (p-distance) against the number of transitions and transversions to test for saturation in substitutions within the ingroup samples of *Pachytriton*. We confirmed that cyt b does not show any signs of saturation (data not shown).

We reconstructed phylogenetic trees from the complete sequence of cyt b using our own samples of 83 specimens of *Pachytriton*, and single representatives of *Cynops orientalis* and *Paramesotriton chinensis*. For comparisons, published DNA sequence data for 37 specimens of *Pachytriton* (Wu et al., 2010) and a single specimen of *Par. hongkongensis* (Zhang et al., 2005) were obtained from GenBank and added to our analyses (Table 1, Fig. 1, Appendix 1). Outgroup taxa (*Cynops* and *Paramesotriton*) were selected based on the results of Chan et al. (2001) and Weisrock et al. (2006).

The optimum substitution models for each codon position (1st, 2nd, and 3rd positions) were selected by Kakusan4 (Tanabe, 2007). We then constructed phylogenetic trees by maximum likelihood (ML) and Bayesian inference (BI) methods using TREEFINDER ver. Oct. 2008 (Jobb, 2008) and MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001), respectively. The criteria used for model selection were AICc4 (ML) and BIC4 (BI).

For the ML analysis, the K80 (Kimura, 1980)+G (gamma shape parameter), HKY (Hasegawa et al., 1985), and HKY+G models were selected by Kakusan4 as the optimal models for the 1st, 2nd, and 3rd codon positions, respectively. For the Bayesian analyses, K80+G, HKY, the general time reversible model (GTR: Tavaré, 1986)+G were selected as the best substitution model for the 1st codon positions. Two independent runs of four Markov chains were conducted for 6 million generations. We sampled one tree every 100 generations and calculated a consensus topology for 30,001 trees after discarding the first 30,000 trees (burn-in=3,000,000).

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 sufficiently supported (Huelsenbeck and Hillis, 1993). For the Bayesian analysis, we considered posterior probabilities (bpp) 95% or greater as significant support (Leaché and Reeder, 2002).

Morphological comparisons

We compared morphology of adult male *Pachytriton* collected from 11 localities covering the whole distribution of the genus (Table 1, Fig. 1, Appendix 2), including type specimens of *Pac. archospotus*, *Pac. brevipes*, and *Pac. inexpectatus*, and specimens of *Pac. granulosus* from type locality of the lost holotype. We also studied specimens from two pet shops in Japan.

The following measurements were taken for comparisons: SVL (snout-vent length): from tip of snout to anterior tip of vent; HL (head length): from tip of snout to wrinkle of throat; HW (head width): measured at angle anterior to parotid grand; MXHW (maximum head width): measured at widest point; SL (snout

TABLE 1. Species studied (after Nishikawa et al., 2011), with localities and sizes of samples, and GenBank accession numbers of sequence data.

			Molecular analysis			Morphological analysis n		
Loc. no.	Species	Sampling locality	n	GenBank no.	Source	Male	Female	Juvenile
1	*Pachytriton granulosus*	Lin'an, Zhejiang	4	GQ303643-46	Wu et al. (2010)			
2	*Pac. granulosus*	Huangshan, Anhui	4	AB638629-32	This study	18	13	
3*	*Pac. granulosus*	Tiantai, Zhejiang	9	AB638633-41	This study	9		
4	*Pac. granulosus*	Ninghai, Zhejiang	3	GQ303647-49	Wu et al. (2010)			
5	*Pac. granulosus*	Jinhua, Zhejiang	2	GQ303661-62	Wu et al. (2010)			
6	*Pac. brevipes*	Jiangshan, Zhejiang	5	AB638642-46	This study	1		
7	*Pac. granulosus*	Wenzhou, Zhejiang	3	AB638647-49	This study			
8	*Pac. granulosus*	Longquan, Zhejiang	6	AB638650-55	This study			
9	*Pac. brevipes*	Zixi, Jiangxi	3	AB638656-58	This study			
10	*Pac. brevipes*	Wuyishan, Fujian	5	GQ303652-56	Wu et al. (2010)			
11*	*Pac. brevipes*	Nanfeng, Jiangxi	2	GQ303663-64	Wu et al. (2010)	1		
12	*Pac. brevipes*	Youxi, Fujiang	4	GQ303657-60	Wu et al. (2010)			
13*	*Pac. archospotus*	Guidong, Hunan	3	GQ303665-67	Wu et al. (2010)	3		
14	*Pac. inexpectatus*	Leishan, Guizhou	4	GQ303635-38	Wu et al. (2010)			
15	*Pac. inexpectatus*	Leishan, Guizhou	3	AB638659-61	This study	10		
16	*Pac. inexpectatus*	Ziyuan, Guangxi	29	AB638662-90	This study	4		1
17	*Pac. inexpectatus*	Xing'an, Guangxi	4	GQ303631-34	Wu et al. (2010)			
18	*Pac. inexpectatus*	Longsheng, Guangxi	11	AB638691-701	This study	16		
19	*Pac. inexpectatus*	Yizhang, Hunan				10		
20	*Pac. inexpectatus*	Hexian, Guangxi				10		
21	*Pac. inexpectatus*	Jinxiu, Guangxi	1	GQ303639	Wu et al. (2010)			
22*	*Pac. inexpectatus*	Jinxiu, Guangxi	7	AB638702-08	This study	14		
23	*Pac. granulosus*	Pet shop A, Japan	1	AB638709	This study	1		
24	*Pac. granulosus*	Pet shop B, Japan	1	AB638710	This study			
25	*Pac. granulosus*	Pet shop C, Japan	1	AB638711	This study	1		
26	*Pac. granulosus*	Pet market, Chengdu, Sichuan	3	GQ303640-42	Wu et al. (2010)			
27	*Pac. granulosus*	Pet market, Hangzhou, Zhejiang	2	GQ303650-51	Wu et al. (2010)			
28	*Paramesotriton chinensis*	Tiantai, Zhejiang	1	AB638712	This study			
29	*Para. hongkongensis*	Unknown locality, Hongkong	1	AY458597	Zhang et al. (2005)			
30	*Cynops orientalis*	Jiangshan, Zhejiang	1	AB638713	This study			

* type localities of species of *Pachytriton*.

length): from tip of snout to anterior tip of upper eyelid; LJL (lower jaw length): from tip of lower jaw to jaw angle; ENL (eyelid-nostril length): minimum distance between eyelid and nostril; IND (internarial distance): minimum distance between the external nares; IOD (interorbital distance): minimum distance between upper eyelids; UEW (upper eyelid width): greatest width of upper eyelid; UEL (upper eyelid length): greatest length of upper eyelid; OL (orbit length): maximum length of orbit; AGD (axilla-groin distance): minimum distance between axilla and groin; TRL (trunk length): from wrinkle of throat to anterior tip of vent; TAL (tail length): from anterior tip of vent to tail tip; VL (vent length): from anterior to posterior tip of vent; BTAW (basal tail width): tail width measured at root of tail; MTAW (medial tail width): tail width measured at middle; BTAH (basal tail height): tail height measured at base of tail; MXTAH (maximum tail height): tail height measured at highest point; MTAH (medial tail height): tail height measured at middle; FLL (forelimb

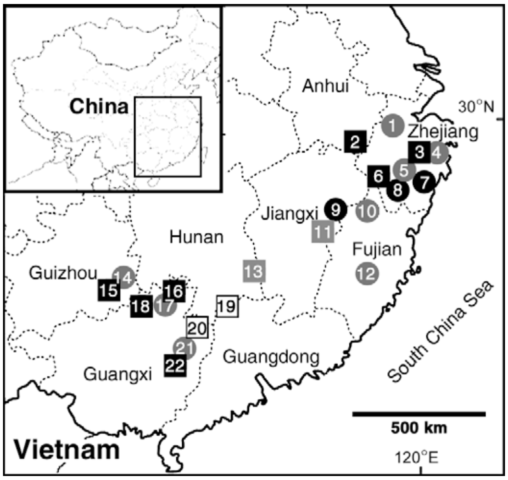

FIG. 1. A map of China showing sampling localities of *Pachytriton* species. Closed squares, meshed squares, open squares, closed circles, and meshed circles respectively show localities that both morphological and molecular data were obtained by this study, morphological data by this study but molecular data from GenBank, morphological data by this study without molecular data, molecular data by this study without morphological data, and molecular data from GenBank without morphological data. Locality numbers refer to those in Table 1.

length): distance from axilla to tip of longest finger; HLL (hindlimb length): distance from groin to tip of longest toe; UJTW (upper jaw teeth series width): greatest width of upper jaw teeth series; UJTL (upper jaw teeth series length): greatest length of upper jaw teeth series; VTW (vomerine teeth series width): greatest width of vomerine teeth series; VTL (vomerine teeth series length): greatest length of vomerine teeth series. All measurements were taken to the nearest 0.1 mm with dial calipers. We used a stereoscopic binocular microscope to measure characters when necessary. The sex and maturity status were checked for each specimen by minor dissection.

We compared SVL, 26 ratio values to SVL (R, %), and VTL/VTW ratio value among clades and subclades recognized in molecular analyses. Differences in SVL and ratio values were statistically tested between the two samples compared by Student's t-test and Mann-Whitney's U test, respectively. The significance level was 95% in all these statistical tests.

We conducted multivariate analysis to examine overall morphological variation among the clades and the subclades. Using a total of 27 $\log_e$-transformed metric values, we conducted Principal Component Analysis (PCA) by SAS (1990). We also examined skull morphology and counted the number of trunk vertebrae of some specimens (Appendix 3) by slight dissection or from soft X-ray photographs using Fuji Medical X-Ray Film.

RESULTS

Phylogenetic relationships and genetic divergence

We obtained 1141 bp sequences of complete cyt b region for 123 specimens including outgroups (Table 1). Of the 1141 nucleotide sites, 311 were variable and 278 were parsimony informative (sequence statistics available upon request from the senior author). From the 123 samples examined, a total of 83 haplotypes were found. The likelihood values of the ML tree were -5712.457. The mean likelihood score of the Bayesian analyses for all trees sampled at stationarity was -5955.323.

Phylogenetic analyses employing two different optimality criteria (BI and ML) yielded nearly identical topologies. We therefore present only the BI tree in Fig. 2. Monophyly of *Pachytriton* was fully supported in both trees (bpp=100% and bs=88%). Within *Pachytriton*, three major clades (Clades I–III) were recognized: Clade I from northeastern and central regions of the generic range and from pet shops; Clades II from the southwestern region, consisting of only *Pac. inexpectatus*; Clade III from the southwestern region and consisting of only six specimens. Although their relationships were unresolved, each of these major clades was highly supported (I: bpp=100% and bs=96%; II: 100 and 99%; III: 100 and 100%). High intra-population variation was found in samples from localities 16 and 18. Specimens of these samples were

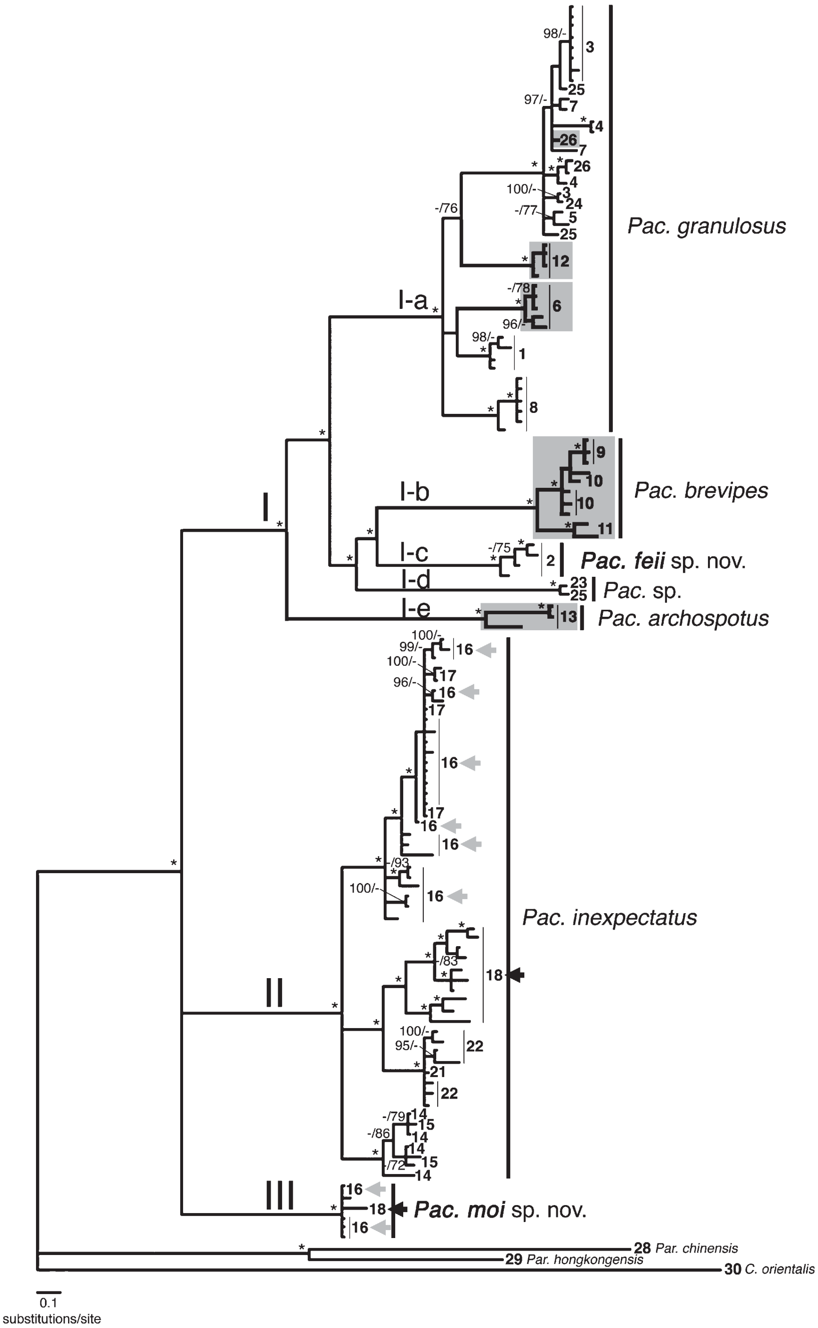

FIG. 2. Bayesian tree based on the complete cyt b for *Pachytriton* species and outgroups (see Table 1). Numbers above branches represent Bayesian posterior probability and bootstrap supports for ML inference (BI-bpp/ML-bs). Asterisks indicate nodes with BI-bpp >95% and ML-bs >70%. Meshed areas show specimens that have yellowish brown ground color on dorsum and black spots on the whole body (black-spotted type). Arrows show specimens from a same locality but separated into different clades.

TABLE 2. Measurements of types and other specimens of *Pachytriton* examined (means±SD of SVL (in mm) and medians of ratios of characters (R: %SVL) and VTL/VTW, with ranges in parentheses). Locality numbers (see Table 1) and sample sizes are also given. For character abbreviations, refer to text.

Subclade/Clade	I-c			III		I-d		I-e	I-b		I-a	II					
	Pachytriton. feii sp. nov.			*Pac. moi* sp. nov.		*Pac.* sp.		*Pac. archospotus*	*Pac. brevipes*		*Pac. granulosus*	*Pac. inexpectatus*					
Locality no.	2			18	16	23	25	13	11	6	3	22	15	16	18	19	20
Types	Holotype	Holotype and 30 Paratypes		Holotype	Paratype			2 Paratypes	Syntype		9 Topotypes	*					
Age/Sex and n	Male	18 Males	13 Females	Male	Juvenile	Male	Male	3 Males	Male	Male	9 Males	14 Males	10 Males	4 Males	15 Males	10 Males	10 Males
SVL	74.0	73.3±8.6	81.6±7.5	100.2	45.3	81.8	84.2	86.8±5.9	87.6	73.1	69.0±6.1	87.8±10.1	84.4±9.0	90.2±6.6	80.6±9.0	83.4±8.4	74.3±9.4
		(58.5–90.5)	(69.6–93.4)					(81.5–93.1)			(59.0–78.9)	(68.6–102.1)	(73.0–99.4)	(81.2–97.1)	(67.1–94.1)	(67.9–96.3)	(62.6–90.5)
RHL	27.0	27.1	25.2	35.9	32.0	25.4	25.2	25.5	25.6	24.1	26.6	28.7	26.4	26.7	29.1	24.9	28.5
		(24.5–29.1)	(22.1–28.0)					(23.5–29.4)			(24.5–29.0)	(24.6–31.6)	(24.7–28.3)	(25.6–27.9)	(26.4–32.5)	(22.6–25.3)	(25.3–31.5)
RHW	19.1	20.0	18.4	23.2	23.2	18.2	18.8	21.7	19.6	19.0	19.0	19.8	19.1	19.4	19.7	19.3	21.0
		(18.0–21.4)	(16.5–20.4)					(21.7–22.2)			(17.0–20.3)	(18.5–21.7)	(17.8–20.0)	(19.1–20.2)	(18.1–20.7)	(17.9–20.7)	(18.3–22.1)
RMXHW	20.4	21.2	19.7	25.3	24.5	19.3	20.5	24.3	21.9	20.4	20.0	22.3	20.8	21.7	22.1	21.1	22.5
		(19.9–22.6)	(18.2–21.3)					(23.8–24.6)			(17.9–21.5)	(20.7–25.2)	(19.7–21.2)	(20.7–23.3)	(20.8–23.6)	(19.1–23.1)	(20.1–23.8)
RSL	9.2	9.7	8.7	11.3	11.3	9.8	10.1	8.0	8.4	8.9	8.5	9.5	8.7	8.8	8.8	9.4	7.9
		(8.1–10.8)	(7.7–10.1)					(7.8–8.3)			(7.9–9.6)	(8.6–10.6)	(7.6–9.5)	(8.6–9.8)	(7.3–10.7)	(8.4–10.6)	(6.8–9.9)
RLJL	13.2	13.1	11.8	17.5	15.9	12.6	11.9	11.5	12.0	12.7	12.9	13.5	12.3	13.1	13.3	12.7	11.9
		(10.7–14.2)	(10.5–17.0)					(11.0–12.0)			(11.7–13.8)	(11.6–14.9)	(11.6–13.8)	(11.7–13.5)	(11.0–17.4)	(12.1–14.6)	(10.3–15.0)
RENL	7.0	7.2	6.3	8.6	9.1	7.3	7.2	5.7	6.6	6.6	6.6	7.0	6.6	6.7	7.1	7.3	6.5
		(6.3–8.2)	(5.7–7.4)					(5.4–5.9)			(6.2–7.4)	(6.4–7.9)	(5.8–7.5)	(6.1–7.0)	(6.3–7.7)	(6.6–7.8)	(5.5–6.9)
RIND	5.9	6.3	5.9	7.5	6.8	6.8	7.1	5.0	5.3	6.7	6.0	6.5	6.4	6.2	6.3	6.9	5.5
		(5.7–7.4)	(5.0–7.0)					(4.9–5.8)			(4.6–6.4)	(5.9–7.4)	(5.5–7.1)	(5.8–6.4)	(5.5–7.5)	(5.5–7.5)	(4.6–7.1)
RIOD	7.2	7.6	6.7	8.0	9.1	7.0	7.7	9.3	8.6	5.7	6.9	6.9	7.3	7.5	7.6	8.0	7.7
		(6.9–9.3)	(5.7–8.6)					(8.8–9.6)			(6.1–8.2)	(6.4–7.9)	(6.6–8.2)	(7.3–9.0)	(5.5–8.7)	(7.6–8.6)	(7.3–8.9)
RUEW	3.8	2.7	2.5	2.7	2.6	2.9	2.4	1.5	2.4	2.9	3.3	2.5	2.6	2.1	2.4	2.4	2.3
		(1.9–3.8)	(2.1–2.9)					(1.2–1.6)			(3.0–4.5)	(2.0–3.2)	(1.8–3.1)	(1.8–2.4)	(1.6–4.2)	(2.2–3.0)	(1.9–3.2)
RUEL	5.7	5.2	4.9	4.7	5.7	4.9	4.5	3.8	4.8	4.7	6.1	4.9	4.9	4.5	4.8	4.5	4.5
		(4.3–6.7)	(4.0–6.2)					(3.7–4.2)			(4.6–6.8)	(3.9–6.1)	(4.0–5.6)	(4.2–5.2)	(4.1–6.4)	(3.8–5.2)	(3.7–6.0)
ROL	2.8	3.6	2.9	3.7	4.6	3.0	3.7	2.6	3.5	3.3	3.0	2.9	3.0	3.0	3.0	2.5	2.9
		(2.8–4.1)	(2.2–3.6)					(2.1–2.8)			(2.7–3.7)	(2.4–3.5)	(2.6–3.6)	(2.9–3.2)	(2.5–3.5)	(2.0–2.8)	(2.4–3.5)
RAGD	49.7	50.1	51.3	52.1	46.8	48.5	51.7	50.1	51.6	52.1	51.7	49.5	51.4	49.3	50.0	50.3	46.9
		(45.7–52.3)	(49.4–57.8)					(49.2–50.9)			(48.6–52.9)	(43.7–53.7)	(48.0–54.4)	(47.8–50.0)	(44.8–54.3)	(45.5–53.2)	(42.8–57.7)
RTRL	73.0	72.9	74.8	64.1	68.0	74.5	74.8	74.5	74.4	75.9	73.4	71.3	73.6	73.3	70.9	75.1	71.5
		(70.9–75.5)	(72.0–77.9)					(70.6–76.5)			(71.0–75.5)	(68.4–75.4)	(71.7–75.3)	(72.1–74.4)	(67.5–73.6)	(74.7–77.4)	(68.5–74.7)

RTAL	101.6	97.4	96.5	90.5	97.4	101.1	105.1	95.7	101.1	91.2	101.6	90.6	96.0	97.0	93.6	99.8	99.8
		(87.7–105.4)	(78.0–103.7)					(93.5–95.8)			(94.7–104.8)	(85.4–98.7)	(85.1–102.9)	(92.6–100.4)	(84.4–99.6)	(94.6–102.6)	(92.8–108.0)
RVL	7.0	7.3	4.1	5.2	4.2	6.1	5.7	5.0	7.2	5.2	8.3	5.6	6.8	7.0	4.9	6.4	5.9
		(4.7–8.7)	(2.1–6.0)					(4.8–5.0)			(5.3–9.4)	(4.6–7.5)	(5.1–8.5)	(6.4–7.4)	(2.6–7.2)	(5.5–8.2)	(4.4–9.4)
RBTAW	14.3	12.4	12.1	13.2	13.7	12.2	12.0	15.8	14.3	15.0	14.7	14.7	13.4	13.5	13.8	13.2	15.1
		(10.6–14.3)	(9.2–12.9)					(15.7–17.2)			(14.0–15.7)	(12.3–16.5)	(11.9–14.2)	(12.0–14.5)	(11.5–15.5)	(12.7–16.4)	(12.0–16.7)
RMTAW	8.2	8.9	8.9	7.8	9.3	8.3	7.5	9.8	10.0	11.8	12.0	11.2	11.5	9.2	10.8	10.5	12.4
		(7.5–10.5)	(6.8–9.7)					(8.6–12.5)			(10.7–15.0)	(8.9–13.5)	(10.1–12.8)	(9.0–9.7)	(9.0–11.6)	(9.9–12.5)	(9.1–15.0)
RBTAH	10.9	11.5	11.5	12.8	12.4	10.8	10.0	15.9	12.9	13.5	12.9	12.2	11.7	11.8	11.8	12.2	11.5
		(10.3–14.2)	(8.8–13.2)					(14.0–16.0)			(11.4–13.5)	(10.3–14.2)	(10.7–12.8)	(11.0–13.2)	(10.3–13.6)	(10.6–13.6)	(9.7–15.0)
RMXTAH	15.0	15.6	15.3	14.4	14.3	15.0	15.4	17.5	17.1	23.5	16.2	14.9	14.1	13.5	14.0	16.2	13.6
		(13.0–16.6)	(10.4–17.0)					(16.1–18.0)			(13.3–19.1)	(12.4–15.7)	(12.0–16.8)	(12.2–15.9)	(11.8–16.2)	(14.5–21.1)	(12.2–18.0)
RMTAH	13.2	14.9	14.7	14.3	14.1	14.7	15.2	16.4	15.9	16.6	12.9	13.5	12.7	12.8	13.0	13.8	12.7
		(12.9–16.0)	(10.1–16.7)					(15.0–17.3)			(11.8–16.5)	(10.8–14.6)	(11.2–15.2)	(1.6–14.5)	(11.6–15.8)	(11.3–15.7)	(10.6–17.1)
RFLL	26.6	25.8	23.7	22.7	27.2	23.3	24.5	23.3	22.0	19.3	23.2	20.3	21.5	21.4	21.7	22.8	21.5
		(22.9–29.4)	(20.6–27.3)					(21.7–24.3)			(20.7–23.6)	(18.5–23.6)	(19.2–23.5)	(18.7–22.4)	(19.3–24.5)	(20.7–24.0)	(17.3–25.4)
RHLL	29.9	29.6	28.3	24.4	30.0	27.8	26.5	29.4	25.9	24.9	25.3	24.5	26.3	24.5	25.6	26.8	27.5
		(26.1–32.5)	(25.1–33.4)					(25.9–29.6)			(23.4–27.4)	(21.8–35.3)	(22.6–27.0)	(22.5–25.6)	(23.2–27.5)	(24.4–28.9)	(22.5–30.6)
RUJTW	9.7	9.5	8.9	13.3	12.4	10.6	11.2	9.4	8.7	9.3	9.3	9.8	8.6	9.0	8.8	8.7	9.6
		(7.8–11.4)	(7.7–9.5)					(8.2–9.8)			(7.9–10.1)	(8.2–10.9)	(7.6–9.3)	(8.6–9.8)	(6.6–10.9)	(8.4–10.7)	(8.6–11.2)
RUJTL	6.4	6.7	6.9	10.1	10.8	8.6	9.1	5.9	6.3	6.7	6.3	7.9	6.7	7.4	7.2	6.9	6.9
		(5.1–8.4)	(5.9–8.0)					(5.2–6.5)			(94.6–7.1)	(7.4–8.6)	(6.1–7.7)	(6.7–8.0)	(6.3–8.1)	(5.7–7.3)	(6.5–8.3)
RVTW	6.1	5.9	5.1	6.8	6.6	5.6	6.2	5.8	3.9	4.8	4.2	4.6	4.6	5.0	4.7	5.8	6.5
		(4.9–8.2)	(4.3–6.7)					(5.0–6.0)			(3.6–5.6)	(4.0–5.5)	(3.2–5.2)	(4.3–5.6)	(4.2–5.7)	(5.5–6.3)	(5.8–8.2)
RVTL	10.1	9.7	8.8	16.5	10.4	11.0	12.1	8.3	7.8	9.6	8.9	11.7	11.5	10.7	10.4	10.4	9.8
		(7.6–11.0)	(7.5–10.6)					(8.2–9.0)			(7.4–10.9)	(10.4–13.0)	(9.8–12.2)	(9.3–11.0)	(9.0–11.6)	(9.3–11.1)	(9.0–11.6)
VTL/VTW	1.7	1.6	1.7	2.4	1.6	2.0	2.0	1.5	2.0	2.0	2.1	2.4	2.5	2.0	2.3	1.8	1.5
		(1.3–2.0)	(1.4–2.0)					(1.4–1.7)			(1.7–2.4)	(2.1–2.9)	(2.2–3.0)	(1.9–2.5)	(1.7–2.5)	(1.5–2.0)	(1.3–1.7)

*including the holotype and two paratypes.

not grouped corresponding to locality, but separated into two lineages, Clades II and III.

The uncorrected p-distances among Clades I–III were remarkably large: I vs. II=7.3–9.9% (mean=8.4%); I vs. III=6.8–8.8% (mean=8.0%); II vs. III=6.5–7.7% (mean=7.1%).

Clade I was further divided into five subclades: Subclade I-a from Zhejiang and central Fujiang (bpp=100 and bs=100%) including the type locality of lost holotype of *Pac. granulosus* (Loc. 3) and localities of three samples of *Pac. brevipes* (Locs. 6, 12, 26), and from Chinese and Japanese pet shops; I-b from western Jiangxi and northern Fujiang (100 and 100%) including the type locality (Loc. 11) and localities of all *Pac. brevipes* exclusive of the three included in I-a; I-c from southern Anhui (100 and 99%); I-d from Japanese pet shops (100 and 100%); and I-e from southeastern Hunan (100 and 100%), the type locality of *Pac. archospotus* (Loc. 13). Divergence among the subclades was large [I-a vs. I-b=5.6–7.2% (mean=6.5%); I-a vs. I-c=4.8–6.6% (mean=5.7%); I-a vs. I-d=5.8–6.9% (mean=6.3%); I-a vs. I-e 6.8–8.7% (mean=7.9%); I-b vs. I-c=5.3–5.8% (mean=5.7%); I-b vs. I-d=6.4–6.9% (mean=6.7%); I-b vs. I-e=7.4–8.2% (mean=7.7%); I-c vs. I-d=6.1–6.2% (mean=6.1%); I-c vs. I-e=7.0–7.9% (mean=7.5%); I-d vs. I-e=7.8–8.1% (mean=7.9%)] and their relationships were well resolved. Subclade I-e was first separated from the remaining subclades, and I-a and I-d were separated in this order. All of these 10 averaged distances among sublades were greater than or equivalent to minimum values between species of other Eurasian salamandrids (≥5.0% in *Triturus*: Steinfartz et al., 2007) and Chinese hynobiids (≥5.3% in *Hynobius*: Nishikawa et al., 2010; ≥5.7% in *Batrachuperus*: Fu and Zeng, 2008).

Clade II was divided into three subclades, but divergence among them was small [2.0–3.8% (mean=2.9%)].

Clade III was composed of six specimens from two near localities with a very small divergence.

In subsequent morphological analyses, we compared difference among seven groups (Subclades I-a, I-b, I-c, I-d, and I-e, and Clades II and III) recognized in the molecular analyses, with special attention on morphological distinctness of Subclades I-c and I-d, and Clade III.

Morphological difference

A total of 98 specimens of adult males were used for comparison (Table 1). Values of SVL, ratios to SVL, and VTL/VTW ratio are shown in Table 2.

In SVL, the single specimen of Clade III was larger than the remaining specimens exclusive of the 14 male *Pac. inexpectatus* from Jinxiu (Loc. 22), but we could not test its statistic significance because of the sizes of available sample for the clade (1: see also Table 2). Samples of Clade II tended to show larger SVL values than the remaining samples. The smallest SVL was found in Subclade I-a (Loc. 3) and next was I-c (Loc. 2). Between the two, there was no significant difference (Student's t-test).

In measurement ratios, it is prominent that Clade III had larger values than the remaining samples in almost half of all characters examined (RHL, RHW, RMXHW, RSL, RLJL, RENL, RIND, ROL, RAGD, RUJTW, RUJTL,

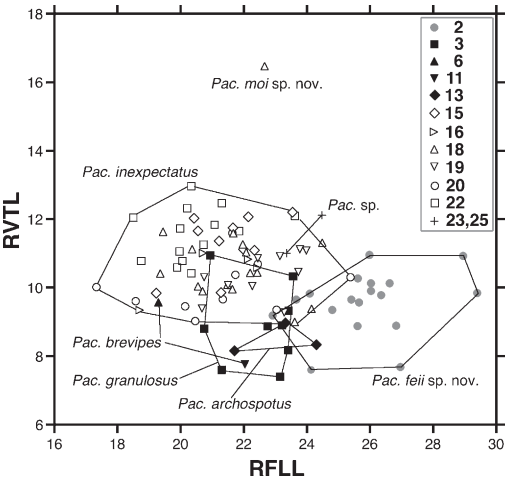

FIG. 3. Two dimensional plots of RVTL versus RFLL.

RVTW, and RVTL: Fig. 3) and that Subclade I-a exhibited many larger values in many characters (RUEW, RUEL, UTRL, RVL, RBTAH, RMXTAH, and RMTAH). Subclade I-b had smallest values in RUJTL, RVTW, and RVTL, whereas Subclade I-c had largest values in RFLL and RHLL, and relatively large values in RUEL and ROL. Although Subclade I-c is similar to I-a in body size and coloration, the former could be well distinguished from the latter by possession of significantly larger RHW, RMXHW, RSL, RENL, RIOD, ROL, RFLL, RHLL, and RVTW and significantly smaller RUEW, RBTAW, RMTAW, RBTAH, and VTL/VTW (Mann-Whitney's U-test). Subclade I-d had relatively large RSL and small RHW, RMXHW, RBTAW, RMTAW, and RBTAH. Subclade I-e exhibited small values in RLJL, RENL, RIND, RUEW, RUEL, and VTL/VTW. These results of comparisons in SVL and ratios indicate that each clade and subclade could be characterized by combinations of these characters.

The body coloration tended to be differentiated among localities. However, it did not always correspond to subclade or clade categorization and showed great variation within some samples from the same localities. In all samples exclusive of those from Locs. 6, 11, 13, and 18, the ground color on dorsal was uniformly dark brown, sometimes with orange spots along the dorsolateral regions, and venter was lighter than dorsum with strikingly reddish orange markings (unspotted type). In contrast, Locs. 6, 11, and 13 samples had bright yellowish brown ground color with numerous black spots on the whole body (black-spotted type). Although we did not examine body color of Locs. 12 and 26, their body coloration is same as that of the black-spotted type according to Wu et al. (2010). One large specimen (CIB GX20070009) of Loc. 18 most resembled the unspotted type but lacked reddish orange markings on venter. The remaining specimens of Loc. 18 were typical unspotted type with orange markings on venter. Samples from Locs. 23 and 25 of Subclade I-d had much brighter ground color with much more finely scattered orange markings on venter than the remaining samples.

In PCA, the first three principal components accounted for 74.6% of the total variation. A two dimensional plot of PC2 versus PC1 (Fig. 4, top) indicated that all the nominated species exclusive of *Pac. brevipes* tended to be separated. The greatest variation was found in *Pac. inexpectatus* (Locs. 15–16, 18–20, and 22). Especially, the specimen from Loc. 18 (CIB GX20070009) was greatly separated from the remaining specimens of *Pac. inexpectatus*. The three lineages of Subclades I-c (Loc. 2

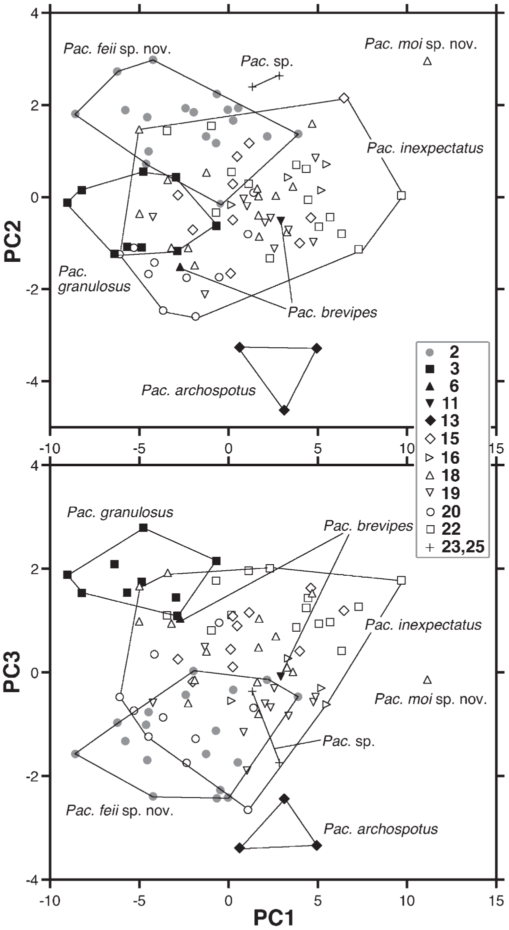

FIG. 4. Two dimensional plots of the first principal component (PC1) versus the second (PC2) (top), and PC1 versus the third (PC3) (bottom).

sample) and I-d (Locs. 23 and 25 samples), and Clade III (the single specimen from Loc. 18) recognized in the molecular analyses were also separated from the remaining specimens in the plots. The two dimensional plots of *Pac. brevipes* were largely overlapped with those of *Pac. inexpectatus*. Of the samples identified to these two species, Loc. 6 sample was much closer to plots of *Pac. granulosus* (Loc. 3) than Loc. 11 (a syntype of *Pac. brevipes*). In a plot of PC3 versus PC1 (Fig. 4, bottom), similar tendency to that of PC2 versus PC1 was recognized.

In the molecular and morphological analyses, similar results were obtained. A total of seven groups were recognized, of which Subclade I-a corresponds to *Pac. granulosus*, I-b to *Pac. brevipes*, I-e to *Pac. archospotus*, and Clade II to *Pac. inexpectatus*, because the topotypes of each species was included in these four clades or subclades. The Loc. 6 sample, whose coloration is similar to *Pac. brevipes*, could be treated as a slight variant of *Pac. granulosus*, because both genetic distance and morphological difference between this sample and topotypic *Pac. granulosus* is actually very small. Although we could not examine morphology by ourselves, Locs. 12 and 26 samples also should be treated as *Pac. granulosus* because of small genetic distance.

For the remaining Subclades I-c and I-d, and Clade III, there are no former available names. We therefore describe two of them (Subclade I-c and Clade III) as new species, but keep the remaining one (Subclade I-d: samples from pet shops) as *Pac.* sp. because we could not detect its locality of collection:

SYSTEMATICS

Pachytriton feii sp. nov.

Fig. 5

Diagnosis

A small-sized newt of *Pachytriton*, male 73.3 mm and female 81.6 mm SVL; skin smooth; limbs long and thin; tips of fore- and hindlimbs adpressed on body slightly separated (one to 1.5 costal fold); tip of tail broad; uniform dark brown on dorsum, usually without bright orange dots along dorsolateral regions in adult; no black spots over the body. Most similar to *Pac. granulosus*, but with significantly wider head, longer snout and limbs (tips of fore- and hindlimbs adpressed on body more widely [1.5 to 2.5 costal folds] separated in *Pac. granulosus*), larger eyes, thinner tail, and wider vomerine tooth series.

FIG. 5. Male holotype (CIB 200805012) of *Pachytriton feii*; dorsal (A) and ventral views (B). Scale shows 10 mm.

Etymology

The specific epithet is dedicated to Dr. Liang Fei of Chengdu Institute of Biology, Chinese Academy of Sciences, who has made great contribution to our understandings of the batrachofauna of China.

Holotype

CIB 200805012, an adult male from Tangkou (Mt. Huang), Huangshan, Anhui Province, People's Republic of China (30°06' N, 118°10' E, 670 m asl), collected on 28 April 2008 by Jian-Ping Jiang, Liang Fei, and Chang-Yuan Ye.

Paratypes

A total of 30 specimens from Mt. Huang, Huangshan, Anhui. CIB 20070666 collected on unknown date of June 2007 by X.-B. Wu; CIB 200805014–15 collected on 29 April and 1 May 2008 by Jian-Ping Jiang, Liang Fei, and Chang-Yuan Ye; CIB 21064–65, 70, 73–74, 77–79, 82, 85–86, 92–96, 99–102, 104, 106, and 108–110 collected in 1972 and 1974 by unknown collectors; KUHE 36808–9 collected on 22 July 1992 by Masafumi Matsui.

Description of holotype

Body slender and flat. Skin smooth. Head oval in shape, depressed and nearly flat in profile. Snout truncate, slightly beyond lower jaw. Nostrils close to snout tip. Labial fold evident, especially in posterior half of upper jaw. Skull narrow. Maxillary connecting with pterygoid, forming nearly straight line. Front-squamosal arch complete, but not robust. Epibranchial relatively short, curving as wrapping neck dorsolaterally. Vomerine tooth series long, inverted V-shaped, tooth rows converging anteriorly, slightly exceeding anterior limit of choanae. Tongue fully attached to mouth floor. Parotoid region evident. Gular fold present, but weak. No vertebral or dorsolateral ridges. Number of costal grooves between axilla and groin 11. Adpressed limbs separated by 1.5 costal folds. Number of trunk vertebrae 12. Fingers and toes without webbing. Tail laterally compressed, dorsal fin evident posteriorly.

Measurements of holotype (measurements in mm)

SVL 74.0, HL 20.0, HW 14.1, MXHW 15.1, SL 6.8, LJL 9.8, ENL 5.2, IND 4.4, IOD 5.3, UEW 2.8, UEL 4.2, OL 2.1, AGD 36.8, TRL 54.0, TAL 75.2, VL 5.2, BTAW 10.6, MTAW 6.1, BTAH 8.1, MXTAH 11.1, MTAH 9.8, FLL 19.7, HLL 22.1, UJTW 7.2, UJTL 4.7, VTW 4.5, VTL 7.5.

Color in life

Dorsum uniformly dark brown. Venter lighter than dorsum, with reddish orange markings. Markings diffusing in adults but bright and fine in juveniles. Underside of tail and cloaca light orange.

Color in preservative

Dorsal coloration tending to fade, becoming light brown to cream. Ventral bright markings fading to cream.

Eggs and larvae

Eggs and larvae have never been found.

Variation

Juveniles often have orange spots dorsolaterally and much more finely scattered orange or reddish orange markings on venter than adults. Ventral orange markings tend to be obscure against ventral ground color in larger (probably, older) adults. Terrestrial juveniles have a protrusible tongue, which will change to adhere to the mouth floor before their maturation in the stream.

Range

Southern Anhui Province, People's Republic of China. The species is known only from Mt. Huang.

Natural history

This species is common in streams and their branches on Mt. Huang over ca. 600 m asl. The adults are nocturnal and prey under the water, but can be seen also in the daytime under the cloudy or rainy weather. Its breeding season seems to start by late spring and continue to early summer. A terrestrial juvenile was found near a stream at the end of April, 2008, which suggests the species to enter the stream before sexual maturation. Several mites of *Hygrobates* (Acari, Hydrachnidia, Hygrobatidae) were found on limbs and inside of the mouth in adults.

***Pachytriton moi* sp. nov.**

Figs. 6, 7

Diagnosis

A large, stout-bodied newt of the genus *Pachytriton*, male 100.2 mm SVL; skin smooth;

head large, snout long; limbs thin; tips of fore- and hindlimbs adpressed on body slightly separated; tip of tail broad; in adults, uniform dark brown dorsally, without bright orange dots dorsolaterally; no black spots over body; no reddish orange markings on venter; most similar to *Pac. inexpectatus*, but with larger body, longer snout, and wider head; in juveniles, snout longer and ventral markings more finely scattered than *Pac. inexpectatus*.

Etymology

The specific epithet is dedicated to Mr. Yun-Ming Mo of Guangxi Natural History Museum who actively elucidated faunal diversity in Guangxi and helped our field surveys.

Holotype

CIB GX20070009, an adult male from Chujiang Station, Huaping National Nature Reserve, Longsheng Gezu Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China (25°36' N, 109°54' E, 922 m asl), collected on 13 March 2007 by Kanto Nishikawa, Jian-Ping Jiang, and Yun-Ming Mo.

Paratype

CIB GX200905007, a juvenile specimen from Jiuniutang, Mt. Mao'er, Ziyuan County, Guangxi Zhuang Autonomous Region (25°54' N, 110°27' E, 1525 m asl), collected on 13 May 2009 by Kanto Nishikawa, Jian-Ping Jiang, and Yun-Ming Mo.

Referred specimens

CIB GX20070086, 2007012005–6, and 8, a total of four juveniles from the locality of paratype.

Description of holotype

Body robust and flat. Skin smooth. Head squarish oval in shape, depressed and nearly flat in profile. Snout long, truncate, slightly beyond lower jaw. Nostrils close to snout tip. Labial fold evident, especially in posterior half of upper jaw. Skull wide. Maxillary connecting with pterygoid, forming nearly straight line. Front-squamosal arch complete and very robust. Epibranchial very long, curving as wrapping neck dorsolaterally. Vomerine tooth series long, inverted V-shaped, tooth rows converging anteriorly, greatly exceeding the anterior limit of choanae. Tongue fully attached to mouth floor. Parotoid region evident. Gular fold present, but weak. No vertebral or dorsolateral ridges. Number of costal grooves between axilla and groin 10. Adpressed limbs separated by one costal fold. Number of trunk vertebrae 12. Fingers and toes without webbing. Tail laterally compressed, posterior dorsal fin evident.

FIG. 6. Male holotype (CIB GX20070009) of *Pachytriton moi*; dorsal (A) and ventral views (B). Scale shows 10 mm.

FIG. 7. Male holotype (CIB GX20070009) of *Pachytriton moi* in life.

Measurements of holotype (measurements in mm)

SVL 100.2, HL 36.0, HW 23.2, MXHW 25.4, SL 11.3, LJL 17.5, ENL 8.6, IND 7.5, IOD 8.0, UEW 2.7, UEL 4.7, OL 3.7, AGD 52.2, TRL 64.2, TAL 90.7, VL 5.2, BTAW 13.2, MTAW 7.8, BTAH 12.8, MXTAH 14.4, MTAH 14.3, FLL 22.7, HLL 24.4, UJTW 13.3, UJTL 10.1, VTW 6.8, VTL 16.5. The numbers of upper jaw teeth 83, lower jaw teeth 91, vomerine teeth 192.

Color in life

Dorsum uniformly dark brown. Venter light brown with several small orange spots in adults. Underside of tail partly light orange.

Color in preservative

Dorsal coloration tending to fade, becoming light brown. Ventral bright markings fading to cream.

Eggs and larvae

Eggs and larvae have not been found.

Variation

Juveniles of paratypes are generally similar in morphology and vary only slightly. Some of juveniles have orange spots along dorsolateral regions and finely scattered orange or reddish orange markings on venter.

Range

Northeastern Guangxi Zhuang Autonomous Region, People's Republic of China. The species is known only from Mt. Mao'er, Ziyuan County and Huaping National Nature Reserve, Longsheng Gezu Autonomous County.

Natural history

The adult was collected at ca. 2200 h in a montane stream. The adult must be thus nocturnal and prey under the water. Its breeding ecology and life history are poorly known. Because the holotype collected in mid March had swollen testis, its breeding seems to start by late spring and presumably continue to early summer. Several mites of *Hygrobates* were found around cloaca and on hindlimbs of the holotype. Of the total five juveniles collected, three were dug out from under gravels in stream head in the daytime, another was found swimming in a small pool near a stream in the daytime, and the other was dipped in a plunge pool at night. In both of the two known localities, this species is much rarer than the sympatric *Pac. inexpectatus*.

DISCUSSION

Although species of *Pachytriton* have been very popular as pets in the world, their taxonomy, distribution, and ecology in nature have been poorly examined. Recent, vigorous taxonomic studies resulted in the descriptions of two new species (Shen et al., 2008; Nishikawa et al., 2011), in which another species was revived (Nishikawa et al., 2011) and discordance between long used diagnostic characters in body coloration and the actual extents of species was demonstrated (Wu et al., 2010). In the present study we described two additional species and suggested the presence of one undescribed species. We still need more intensive taxonomic studies on *Pachytriton*.

One of the two new species described here, *Pac. feii*, is found only from Mt. Huang (Loc. 2). This species is similar to *Pac. granulosus* from Tiantai, type locality of holotype (Loc. 3), in body size and coloration, but differentiated from it in other morphological characters. Because the holotype of *Pac. granulosus* has been lost (see Nishikawa et al., 2009), Hou et al. (2009) designated a neotype of *Pac. granulosus* from Mt. Longwan, Zhejiang. *Pachytriton granulosus* from the locality of the neotype might possibly be specific with that from Mt. Huang. However, a GenBank sample from Lin'an, (Loc. 1), which is very close to Mt. Longwan, and the sample was not differentiated from Tiantai at the species level. We thus consider that the newt from Mt. Huang is unnamed.

According to the distribution map in Fei et al. (2006), *Pac. brevipes* is distributed in northeastern Guangxi including the type locality of *Pac. moi*, one of the two new species described here. Recently Shen et al. (2008) separated western populations of *Pac. brevipes* as *Pac. archospotus*, and we thus could treat populations of *Pac. brevipes* in Guangxi as *Pac. archospotus*. Anyhow, *Pac. archospotus*, *Pac. brevipes*, and some populations of *Pac. granulosus* are reported to be black-spotted, and the authors have never found such *Pachytriton* in Guangxi in spite of intensive field surveys. It is also the case that *Pac. moi* might have been confused with the black-spotted species, because one of the species, *Par. brevipes*, has no orange markings on venter like *Pac. moi*. In order to clarify distribution of *Pachytriton* species, we must continue field survey in areas that have not yet been explored.

Although partial sequences of cyt b (307 bp) of *Pac. moi* have been available from GenBank (Accession numbers: AY079489, and AY233146–7; locality: Ziyuan, Guangxi, same as the paratype of the species) and some of them were used as *Pac. labiatus* in Lu et al. (2004), no one has compared these sequences with the remaining species of *Pachytriton*. Because *Pac. moi* is distributed with *Pac. inexpectatus* sympatrically but is much less abundant, the species might have been considered as a variant or much aged individual of *Pac. inexpectatus*.

By the present study, distribution of each species of *Pachytriton* is changed as shown in Fig. 8. Wu et al. (2010) first showed that body coloration, unspotted or black-spotted, did not always delimit species in the genus. In our Subclade I-a (*Pac. granulosus*), we also found both of the colorations, but the genetic and morphological variation in the subclade was not large enough to be separated into different species. From these lines of information, sympatry of *Pac. granulosus* [formerly, *Pac. labiatus*: see Nishikawa et al., 2011] and *Pac. brevipes* in Zhejiang reported previously (e.g., Zhao and Hu, 1984) is doubtful.

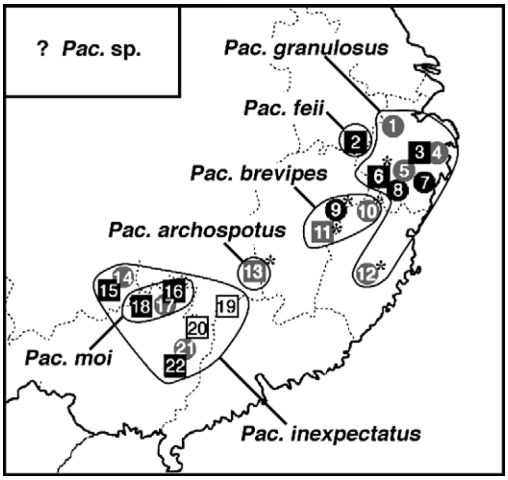

FIG. 8. Distribution of *Pachytriton* spp. suggested by this study. Asterisks show localities of newts having yellowish brown ground color with black spots on the whole body (black-spotted type: see text).

Black-spotted populations of *Pac. granulosus* could have been induced by gene introgression from black-spotted species like *Pac. archospotus* or *Pac, brevipes* through interbreeding. However, Wu et al. (2010) examined nuclear gene variation and found no evidence indicating such an introgression. According to our result, we must hypothesize at least three times of introgressions in order to explain the presence of three black-spotted lineages in Subclade I-a, but such a situation seems unlikely. As shown in Fig. 8, the black-spotted populations tend to locate closely in the middle area of distribution of the genus. If the possibility of introgressions could be rejected, we may assume a selection of black-spotted coloration in the area. However, we presently do not have any data supporting this hypothesis.

It has been known that in *Pachytriton* both sexes show aggressive territorial conflict in captivity. We found many bite scars on the head, body, and tail of specimens collected in the wild. The pattern of distribution, allopatry or sympatry, might be explained by such behavioral or ecological interactions between species. We found sympatric distribution of *Pac. moi* and *P. inexpectatus* in northern Guangxi. To the present, this is the only positive proof of sympatry in *Pachytriton* species.

In the type locality of *Pac. moi*, we found the holotype about four to five meters from the riverbank, but *Pac. inexpectatus* were found assembling in pools or very slow currents near the bank. The two species might be differentiated in their microhabitat and, probably, in the breeding season. The morphological analyses showed that *Pac. moi* was most differentiated among the species examined, including sympatric *Pac. inexpectatus*. *Pachytriton moi* has the largest body, and longest snout and vomerine tooth series, which might make it possible to establish sympatric distribution with *Pac. inexpectatus* by food and/or habitat differentiation associated with its differentiated morphology. Further ecological studies of the two sympatric species, as well as the remaining species, are needed for our better understandings on the pattern of their distributions.

It was shown that *Pac. moi* represents the third major clade in *Pachytriton*. Of the three clades (Clades I–III), two occur sympatrically in southern China. *Pachytriton* is known to be phylogenetically close to *Paramesotriton*, which also contains many species that are endemic in the region (*Par. caudopunctaus*, *Par. fuzhongensis*, *Par. guanxiensis*, *Par. labiatus*, *Par. longliensis*, and *Par. yunwuensis*). The southern China might have played an important role in the diversification of these two genera through unknown reasons.

The present study found that even an unnamed and thus unrecognized species has been exploited for international pet-trading. In the result of molecular analyses, samples closely located geographically tended to form a clade. The unnamed species from pet shop was grouped with samples from the northern half area of the genus' range. Thus the species is likely to occur in the area. In order to protect the species from commercial-based collection, we must first detect its locality with certainty and describe it as soon as possible.

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APPENDIX 1

Specimens newly sequenced by us.

For locality numbers, see Table 1.

Locality number (Loc.) 2: CIB 200805012–14 and 200805046; Loc. 3: CIB ZJ200806006, 9–10, 13, 15, 17, 101–102, and 105; Loc. 6: CIB ZJ200806123, 125, 130, and 132–133; Loc. 7: CIB 2007012015–17; Loc. 8: CIB JJPZJ047–52; Loc. 9: CIB JX200806238–40; Loc. 15: CIB Wg20080718004–6; Loc. 16: CIB GX20070086, GX2007012004–09, GX200905001– 02, 04–10, 12–16, 18–20, 22–23, and 30–32; Loc. 18: CIB GX20070003–10, 36, and 69–70; Loc. 22: CIB 200805122, JX20071101–2, GX200807001–02, and 50–51; Loc. 23: KUHE 39763; Loc. 24: KUHE 39764; Loc. 25: KUHE 39832; Loc. 28: CIB ZJ200806081; Loc. 30: CIB ZJ200806473.

APPENDIX 2

Specimens examined for morphometric and ratio comparisons.

For locality numbers, see Table 1.

Pachytriton archospotus: HNUL 870526C, 870526504 (paratypes) from Loc. 13 and HNUL 790678 from Lingxian, Hunan.

Pac. brevipes: MNHN 5072 (Syntype) from Loc. 11, CIB ZJ200806125 from Loc. 6.

Pac. granulosus: CIB ZJ200806006, 7, 10, 12, 16–17, 22, and 103–104 from Loc. 3, CIB 20070666, 200805012, 14–15, 21064–65, 70, 73–74, 77–79, 82, 85–86, 92–96, 99–102, 104, 106, 108–110, KUHE 36808–9 from Loc. 2, KUHE 39763 and 39832 obtained in pet shops (Locs. 23 and 25).

Pac. inexpectatus: CIB 20071101, 20081002, 6–7, GX200905096, 109, CIB 21137, 39–40, 43–45, 47, and 1 no number from Loc. 22; CIB 20877–78, 80, 82, 91, 94, 98, 900-1, and 21012 from Loc. 15; CIB GX200905001, 15, 18, and 30 from Loc. 16; CIB GX20070003, 6, 9, 69–70, CIB 21149–50, 53, 57, 61, 63–67, and 69 from Loc. 18; CIB 21016–17, 19–21, 23–24, 28, 31, and 33 from Loc. 19; CIB 20928, 35, 37–38, 47–49, and 56–58 from Loc. 20.

APPENDIX 3

Specimens examined for skull characteristics and number of trunk vertebrates.

KUHE 36808 and 36809 (paratypes of *Pac. feii*), CIB GX20070009 (holotype of *Pac. moi*), KUHE 39763 and 39832 (*Pac.* sp.).

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