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Article

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New species of *Pachytriton* (Caudata: Salamandridae) from the Nanling Mountain Range, southeastern China

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Abstract

New species of amphibians are being reported at an astonishingly fast rate. These include some that have been known to the commercial pet trade for years but have not been formally described due to uncertain origin. The distinctive phenotype of "*Pachytriton* B" among the Chinese stout newts (also known as paddle-tailed newts) is one such example. Through examination of museum specimens, we locate a population from Mt. Mang within the Nanling Mountain Range with morphology and coloration similar to *Pachytriton* B. Molecular phylogenetic analyses strongly suggest that this population and *Pachytriton* B belong to the same species, which differs from congeners morphologically and chromatically and is described here as a new species. This species is characterized by a large and stout body, uniformly light brown dorsum, and orange spots or blotches that extend ribbon-like along the dorsolateral sides of the body. A mitochondrial genealogy suggests that the new species is the sister taxon to the group (*P. brevipes* + *P. feii*). Morphologically, this species is significantly stouter than *P. feii* and has significantly longer limbs than *P. brevipes*.

Key words: Chinese stout newt; salamander; mitochondrial genealogy; principal-components analysis; *Pachytriton xanthospilos* sp. nov.

Introduction

Global amphibian species richness has increased by 28 percent since 2004 and 50 new species have already been described in 2012 (AmphibiaWeb, 2000). The astonishing rise in the number of named species confirms the unprecedented underestimation of amphibian diversity, especially in tropical regions (Meegaskumbura *et al.* 2002; Vieites *et al.* 2009; Funk *et al.* 2012). On the other hand, poorly surveyed temporal forests also harbor unexpected diversity, even in heavily populated areas such as southern China, from which 17 anuran species and 6 salamanders have been described or elevated to full species status since 2010 (e.g., Wu *et al.* 2009, 2010a, b; Nishikawa *et al.* 2011a, b; Li *et al.* 2012). Many new species have been discovered as a consequence of molecular phylogenetic analysis, which is now a routine tool in assessing amphibian diversity.

The Chinese stout newts (*Pachytriton*, also known as paddle-tailed newts) are a genus of highly specialized aquatic salamanders endemic to small montane streams in southeastern China. Together with *Cynops*, *Paramesotriton* and *Laotriton*, they constitute the sister clade to modern European newts, which include *Calotriton*, *Neurergus* and newts of the *Triturus* group (Steinfartz *et al.* 2007; Zhang *et al.* 2008). For over 130 years, there were only two named species of *Pachytriton*. Four additional species, however, have been described in the last four years. At this time, there are six species in the genus: *P. archospotus* Shen, Shen and Mo, *P. brevipes* Sauvage, *P. feii* Nishikawa, Jiang and Matsui, *P. granulosus* Chang, *P. inexpectatus* Nishikawa, Jiang, Matsui and Mo, and *P. moi* Nishikawa, Jiang and Matsui.

Unusual phenotypes of *Pachytriton* that do not match those of named species have long been observed in the European pet trade. Thiesmeier and Hornberg (1997) discuss two unnamed phenotypes, *Pachytriton* A and B. Until now, however, no molecular studies have assessed their phylogenetic relationship and taxonomic identities. During an examination of museum specimens in Chengdu Institute of Biology, the Chinese Academy of Sciences

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(CIB), we found population samples of *Pachytriton* that are consistent with the description of *Pachytriton* B, including a large body size, light-brown dorsum and orange dorsolateral spots. Subsequently, we acquired fresh tissue samples of *Pachytriton* B from the European pet trade and are now able to reveal its identity 28 years after it was first reported (Mudrack 1984).

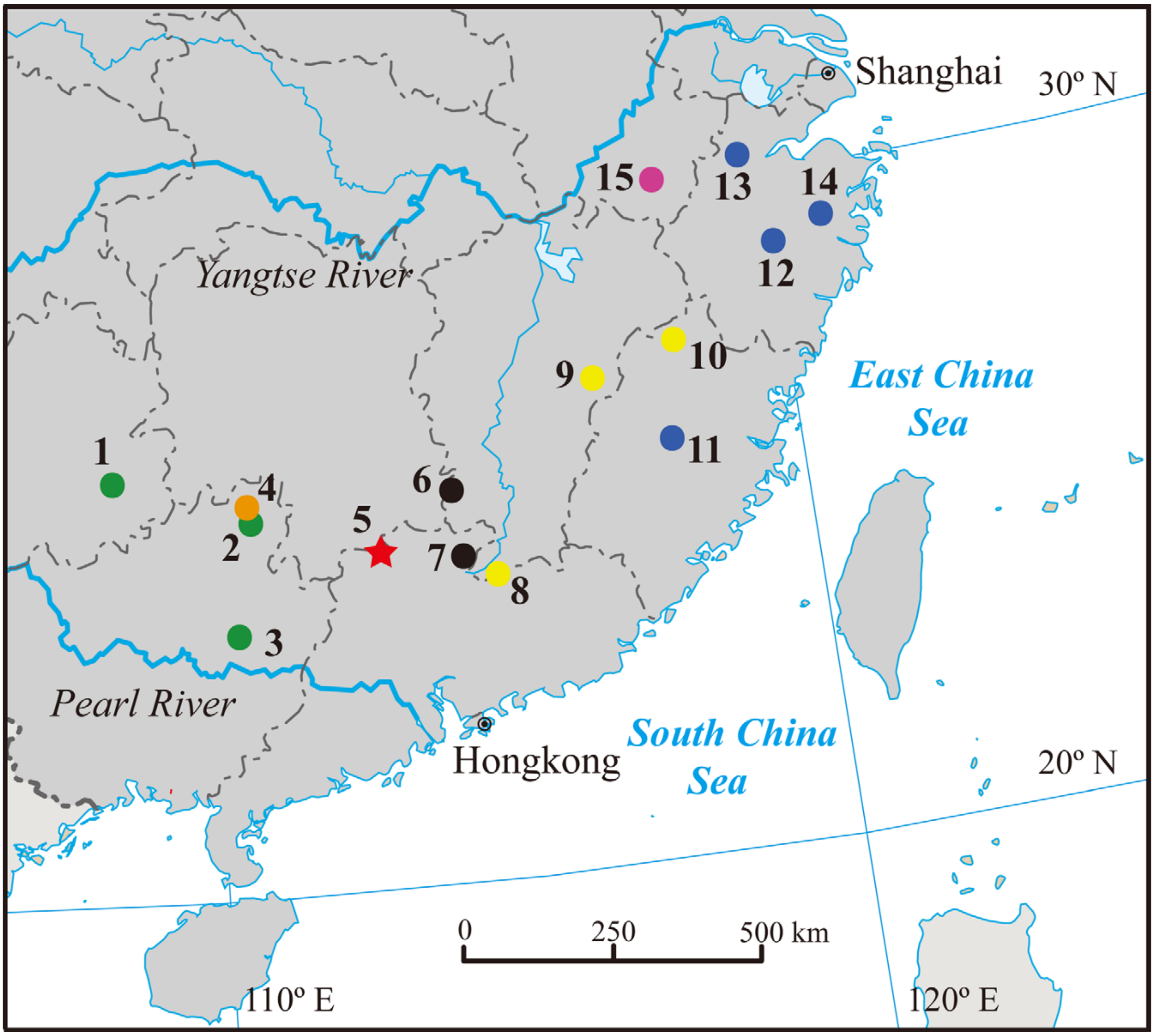

FIGURE 1. Sampling localities in southeastern China. 1–3 (green dots): *Pachytriton inexpectatus*; 4 (orange dot): *P. moi*; 5 (red star): *P. xanthospilos* sp. nov.; 6–7 (black dots): *P. archospotus*; 8–10 (yellow dots): *P. brevipes*; 11–14 (blue dots): *P. granulosus*; 15 (purple dot): *P. feii*.

Materials and methods

Twenty-six specimens collected from Mt. Mang, within the Nanling Mountain Range, were examined and measured (Fig. 1). Twenty of them were collected in 1975 and preserved in 70% ethanol; six were collected in 2009 and preserved in 95% ethanol. Tail muscle was harvested from the latter specimens for genetic analyses. Congeneric species were measured at CIB and at the Museum of Comparative Zoology, Harvard University (MCZ). Fifteen measurements were taken with digital calipers: TTL, total length; SVL, snout-vent length, measured from the tip of the snout to the posterior edge of the vent; HL, head length, measured from the tip of the snout to the posterior edge of the parotoid gland; HW, maximum head width; HD, head depth, measured at the posterior angle of the jaw; SL, snout length, measured from the tip of the snout to the posterior angle of the jaw; IC, intercanthal distance, distance between the anterior corner of each eye; IN, internostril distance, distance between nostrils; SF, snout-to-forelimb length, measured from the tip of the snout to the anterior edge of the forelimb insertion; SHW, shoulder width, distance between the bases of forelimbs; TAL, tail length, measured from the posterior edge of the vent to the tail tip; TAD, tail depth, measured at the posterior edge of the vent; TAW, tail width, measured at the posterior edge of the vent; AL, average length of both forelimbs; PL, average length of both hind limbs. Based on their phylogenetic affinity (see below), we performed a morphometric analysis among *Pachytriton brevipes*, *P. feii*, *P. granulosus* and specimens from Mt. Mang using principal-components analysis (PCA, TTL is excluded) in SPSS (ver. 16, Chicago, IL, USA). An average of 20 specimens was measured per species (see Appendix). The six specimens collected from Mt. Mang in 2009 were excluded from PCA due to their different preservation method. Homogeneity of covariance and error variance of principal-component (PC) scores were tested among species using Box's test and Levene's test, respectively. We applied a multivariate analysis of variance (MANOVA) and Bonferroni multiple comparisons to test if PC scores are statistically different among species.

Phylogenetic relationships within *Pachytriton* were inferred from a molecular genealogy reconstructed based on two mitochondrial DNA fragments, which cover the complete coding sequence of NADH dehydrogenase subunit 2 (ND2) and cytochrome *b* (cyt*b*) and flanking tRNAs. All six recognized species of *Pachytriton*, *Pachytriton* B and the population from Mt. Mang were included (Table 1). We acquired additional tissue samples of *Pachytriton* collected from Mt. Mang from the Kunming Institute of Zoology, the Chinese Academy of Sciences (KIZ). *Paramesotriton* and *Laotriton* were chosen as phylogenetic outgroups because of their sister relationship to *Pachytriton* (Zhang *et al.* 2008). Genomic DNA extraction, PCR and sequencing methods were performed as in Wu *et al.* (2009); PCR primers were available from Wu *et al.* (2010c). DNA sequences were aligned manually in Se-Al 2.0 (Rambaut 1995). Indels were found in tRNAs but not in the protein-coding region. According to Wu *et al.* (2010c), the ND2 and cyt*b* fragments in *Pachytriton* contain congruent phylogenetic signals and thus were concatenated. Uncorrected mean between-species distances were calculated in MEGA 5 (Tamura *et al.* 2011). One sequence of *P. brevipes* retrieved from Weisrock *et al.* (2006) lacks the cyt*b* fragment and thus was excluded from distance calculation. Mitochondrial genealogy was inferred from both maximum-likelihood analysis conducted in RAxML-HPC (Stamatakis 2006; Stamatakis *et al.* 2008) and Bayesian inference in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Both analyses were performed in computer clusters through the CIPRES Science Gateway (Miller *et al.* 2010). Prior to tree inference, two data-partitioning strategies were evaluated using Bayes Factors (BF) based on preliminary runs. The codon partitioning strategy applied one evolutionary model to the first and second codon positions, one model for the third codon position and one model for tRNAs; the gene partitioning strategy applied an independent model to each protein-coding region and tRNAs. The best-fit evolutionary model for each partition was selected by using the Akaike Information Criterion (AIC) implemented in MRMODELTEST 2.2 (Nylander 2004). A BF > 10 indicates strong evidence favoring the better partitioning strategy (Kass & Raftery 1995). In the maximum-likelihood analysis, bootstrap values for nodal support were calculated for 1000 replicates. In the Bayesian inference, two independent runs each with three heated chains and one cold chain were carried out for 10 million generations. Parameter convergence was assessed in Tracer ver. 1.5 (Rambaut & Drummond 2007). The first three million generations were discarded as burn-in.

Results and discussion

Principal-components analysis collapses the 14 measurements to two PCs with eigenvalues greater than one, which explain 79.7% of the total morphological variation (50.7% and 29.0%, respectively). Covariance and error variance of PC scores are homogeneous among species (Box's test, $p = 0.245$; Levene's test, $p_{\mathrm{PC1}} = 0.087$, $p_{\mathrm{PC2}} = 0.194$). The MANOVA using the two PC scores reveals significant separation in the morphospace among *Pachytriton brevipes*, *P. feii*, *P. granulosus* and the Mt. Mang population (Wilk's $\lambda = 0.165$, $F_{6,150} = 36.466$, $p < 0.001$; Fig. 2). Rotated component loadings suggest that PC1 captures salamander girth (e.g., measurements of width and depth), especially at the tail base, with higher values denoting stouter animals (Table 2). In contrast, limb length loads heavily on PC2, with higher values corresponding to longer front and hind limbs. Bonferroni multiple comparisons confirm that the Mt. Mang population is much stouter than both *P. feii* and *P. granulosus* ($p < 0.001$ and $p < 0.001$, respectively), and has significantly longer limbs than both *P. granulosus* and *P. brevipes* ($p < 0.001$ and $p = 0.001$, respectively).

TABLE 1. Sampling used in molecular phylogenetic analyses.

Species	Locality	Voucher No.	Locality information	GenBank Acc. No.		Reference
				ND2	cyt*b*	
Ingroup						
Pachytriton xanthospilos sp. nov.	5	CIB 97900	Mt. Mang, Hunan, China	JX237740	JX237760	this study
		CIB 97901	Mt. Mang, Hunan, China	JX237741	JX237761	this study
		CIB 97902	Mt. Mang, Hunan, China	JX237742	JX237762	this study
		CIB 97903	Mt. Mang, Hunan, China	JX237743	JX237763	this study
		CIB 97904	Mt. Mang, Hunan, China	JX237744	JX237764	this study
		CIB 97905	Mt. Mang, Hunan, China	JX237745	JX237765	this study
		KIZ 06750	Mt. Mang, Hunan, China	JX237732	JX237752	this study
		KIZ 06751	Mt. Mang, Hunan, China	JX237733	JX237753	this study
		KIZ 06752	Mt. Mang, Hunan, China	JX237734	JX237754	this study
Pac. B		N/A	Pet trade	JX237730	JX237750	this study
Pac. brevipes	9	CIB 95926	Mt. Junfeng, Jiangxi, China	GQ303626	GQ303663	Wu *et al.* (2010c)
		CIB 95930	Mt. Junfeng, Jiangxi, China	GQ303627	GQ303664	Wu *et al.* (2010c)
	10	CIB 88221	Mt. Wuyi, Fujian, China	GQ303615	GQ303652	Wu *et al.* (2010c)
		CIB 88194	Mt. Wuyi, Fujian, China	GQ303616	GQ303653	Wu *et al.* (2010c)
		CIB 88188	Mt. Wuyi, Fujian, China	GQ303617	GQ303654	Wu *et al.* (2010c)
		CIB 88197	Mt. Wuyi, Fujian, China	GQ303618	GQ303655	Wu *et al.* (2010c)
		CIB 88192	Mt. Wuyi, Fujian, China	GQ303619	GQ303656	Wu *et al.* (2010c)
	8	N/A	Mt. Jiulian, Jiangxi, China	DQ517796	N/A	Weisrock *et al.* (2006)
Pac. feii	15	KIZ 04228	Mt. Huang, Anhui, China	JX237735	JX237755	this study
		KIZ 04229	Mt. Huang, Anhui, China	JX237736	JX237756	this study
		KIZ 04230	Mt. Huang, Anhui, China	JX237737	JX237757	this study
		KIZ 04231	Mt. Huang, Anhui, China	JX237738	JX237758	this study
		KIZ 04232	Mt. Huang, Anhui, China	JX237739	JX237759	this study
Pac. granulosus	11	CIB 88207	Youxi, Fujian, China	GQ303620	GQ303657	Wu *et al.* (2010c)
		CIB 88189	Youxi, Fujian, China	GQ303621	GQ303658	Wu *et al.* (2010c)
		CIB 88190	Youxi, Fujian, China	GQ303623	GQ303660	Wu *et al.* (2010c)
		CIB 88185	Youxi, Fujian, China	GQ303622	GQ303659	Wu *et al.* (2010c)
	13	CIB 88145	Mt. Tianmu, Zhejiang, China	GQ303606	GQ303643	Wu *et al.* (2010c)
		CIB 88137	Mt. Tianmu, Zhejiang, China	GQ303607	GQ303644	Wu *et al.* (2010c)
		CIB 88152	Mt. Tianmu, Zhejiang, China	GQ303608	GQ303645	Wu *et al.* (2010c)
		CIB 88139	Mt. Tianmu, Zhejiang, China	GQ303609	GQ303646	Wu *et al.* (2010c)
	14	CIB 88143	Mt. Tiantai, Zhejiang, China	GQ303610	GQ303647	Wu *et al.* (2010c)
		CIB 88161	Mt. Tiantai, Zhejiang, China	GQ303611	GQ303648	Wu *et al.* (2010c)
		CIB 88169	Mt. Tiantai, Zhejiang, China	GQ303612	GQ303649	Wu *et al.* (2010c)
	12	CIB 95997	Mt. Dapan, Zhejiang, China	GQ303624	GQ303661	Wu *et al.* (2010c)
		CIB 95996	Mt. Dapan, Zhejiang, China	GQ303625	GQ303662	Wu *et al.* (2010c)

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TABLE 1. (Continued)

Species	Locality	Voucher No.	Locality information	GenBank Acc. No.		Reference
				ND2	cyt*b*	
Ingroup						
Pac. archospotus	6	CIB 95953	Mt. Qiyun, Hunan, China	GQ303628	GQ303665	Wu *et al.* (2010c)
		CIB 95950	Mt. Qiyun, Hunan, China	GQ303629	GQ303666	Wu *et al.* (2010c)
		CIB 95949	Mt. Qiyun, Hunan, China	GQ303630	GQ303667	Wu *et al.* (2010c)
	7	CIB 97863	Shixing, Guangdong, China	JX237731	JX237751	this study
Pac. moi	4	KIZ 07767	Mt. Mao'er, Guangxi, China	JX237746	JX237766	this study
		KIZ 07768	Mt. Mao'er, Guangxi, China	JX237747	JX237767	this study
Pac. inexpectatus	3	CIB 88165	Mt. Dayao, Guangxi, China	GQ303602	GQ303639	Wu *et al.* (2010c)
	2	CIB 88182	Mt. Mao'er, Guangxi, China	GQ303594	GQ303631	Wu *et al.* (2010c)
		CIB 88173	Mt. Mao'er, Guangxi, China	GQ303595	GQ303632	Wu *et al.* (2010c)
		CIB 88158	Mt. Mao'er, Guangxi, China	GQ303596	GQ303633	Wu *et al.* (2010c)
		CIB 88156	Mt. Mao'er, Guangxi, China	GQ303597	GQ303634	Wu *et al.* (2010c)
	1	CIB 88162	Mt. Leigong, Guizhou, China	GQ303598	GQ303635	Wu *et al.* (2010c)
		CIB 88148	Mt. Leigong, Guizhou, China	GQ303600	GQ303637	Wu *et al.* (2010c)
		CIB 88170	Mt. Leigong, Guizhou, China	GQ303601	GQ303638	Wu *et al.* (2010c)
		CIB 88147	Mt. Leigong, Guizhou, China	GQ303599	GQ303636	Wu *et al.* (2010c)
Outgroup						
Laotriton laoensis		FMNH 255452	Phoukhout, Xiang Khouang, Laos	EU880328	EU880328	Zhang *et al.* (2008)
Paramesotriton caudopunctatus		MVZ 236252	Mt. Leigong, Guizhou, China	EU880326	EU880326	Zhang *et al.* (2008)
Paramesotriton chinensis		CIB 95910	Mt. Dapan, Zhejiang, China	FJ744605	JX237749	this study
Paramesotriton hongkongensis		MVZ 230368	Hongkong, China	GU980582	JX237748	this study
Paramesotriton deloustali		MVZ 223628	Tam Dao, Vinh Phu, Vietnam	EU880327	EU880327	Zhang *et al.* (2008)

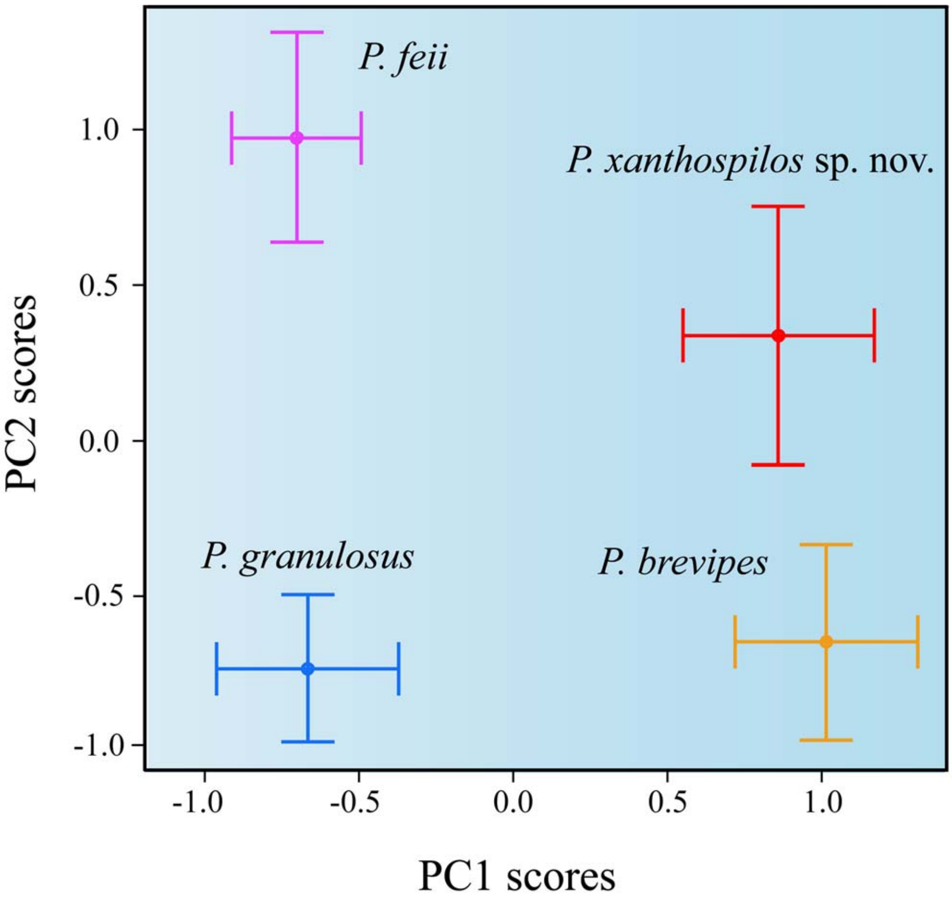

FIGURE 2. Results of morphometric analysis. Principal-component (PC) scores are plotted as means with 95% confidence intervals. Higher values in PC 1 represent stouter animals; higher values in PC 2 represent animals with longer limbs.

The alignment of two mitochondrial fragments includes 2357 base pairs from 56 specimens. Within *Pachytriton*, 629 sites are variable and 571 of them are parsimony-informative. Uncorrected mean genetic distance between the Mt. Mang population and each congener ranges from 6.1% (*P. feii*) to 10.2% (*P. inexpectatus*) (Table 3). These distances are comparable to interspecific divergence values among currently recognized species of *Pachytriton*. Uncorrected genetic distances are only around 1.4% between *Pachytriton* B (commercially identified phenotype) and specimens collected from Mt. Mang. These distances are much lower than intraspecific divergence values within *P. archospotus* (2.5%), *P. inexpectatus* (3.8%) and *P. granulosus* (3.9%).

For phylogenetic reconstruction, the harmonic mean of the likelihood of the codon partitioning strategy is −11737.655; that of the gene partitioning strategy is −12176.374. Together, these values yield a BF = 2[−11737.655 − (−12176.374)] = 877.438, which strongly favors the former partitioning strategy. Therefore, and according to the AIC in MRMODELTEST 2.2, we assigned an HKY+G model to tRNAs, a single HKY+G+I model to the first and second codon positions, and a GTR+G+I model to the third codon position in the Bayesian inference. Because RAxML-HPC in the CIPRES Science Gateway only allows GTR models, three independent GTR+G+I models are assigned to the above three partitions for the maximum-likelihood analysis. Tree topologies recovered by both analyses are nearly identical; here we depict only the maximum-likelihood tree (Fig. 3).

Monophyly of *Pachytriton* is supported by all analyses. Phylogenetic relationships within the genus are highly congruent with those of Wu *et al.* (2010c) and Nishikawa *et al.* (2011b). Two major clades are recognized based on our mitochondrial sequence data. The first clade includes *P. inexpectatus* and the recently described *P. moi*. The latter species is regarded as the third major clade in *Pachytriton* by Nishikawa *et al.* (2011b), but our data strongly support a sister relationship between *P. inexpectatus* and *P. moi*. Interestingly, the geographic range of *P. moi* lies within that of *P. inexpectatus* and the two species occur in sympatry at some localities (Nishikawa *et al.* 2011b). Nuclear gene and ecological data are necessary to further investigate interactions between these two species.

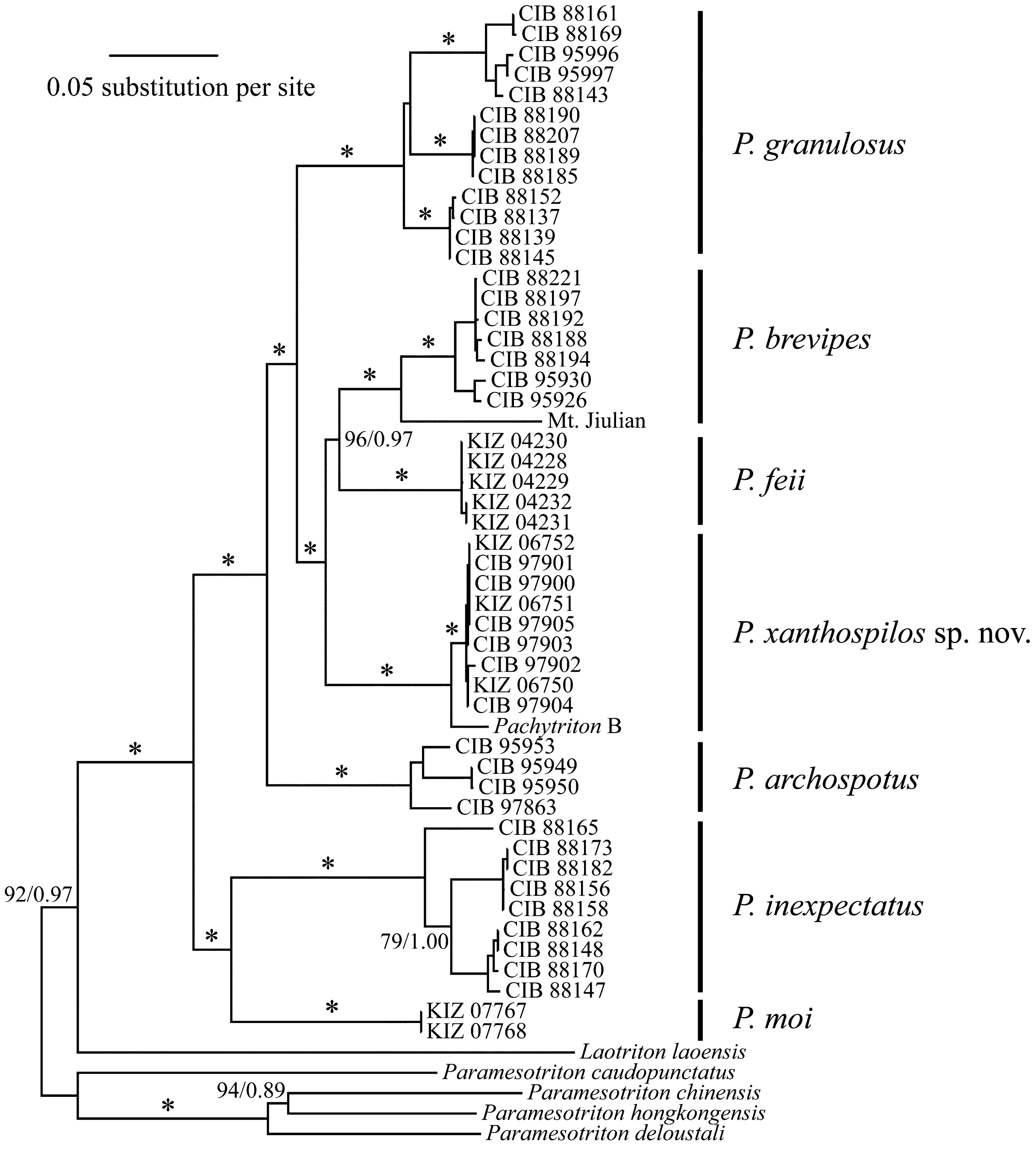

FIGURE 3. Maximum-likelihood (ML) tree inferred from mitochondrial DNA sequence data. Numbers on branches are ML bootstrap values and Bayesian posterior probabilities, respectively. Asterisks indicate > 95% support in ML analysis and > 0.99 support in Bayesian inference. The genera *Laotriton* and *Paramesotriton* are included as outgroups.

The second clade in *Pachytriton* consists of *P. archospotus*, *P. brevipes*, *P. feii*, *P. granulosus*, *Pachytriton* B and salamanders collected from Mt. Mang, with *P. archospotus* as the sister taxon to the other species. The long-known phenotype of *Pachytriton* B from the commercial pet trade is closely related to the Mt. Mang population. Morphology and coloration of the two groups also match. Therefore, it is highly likely that *Pachytriton* B originated from Mt. Mang or nearby mountains. In the mitochondrial genealogy, the Mt. Mang population forms the sister taxon to *P. brevipes* and *P. feii* with strong support. Until recently, *P. brevipes*, *P. feii*, *P. granulosus* and the Mt. Mang population were considered a single species bearing the name *P. brevipes* (Wu *et al.* 2010c), which was the first species described in the genus. Nishikawa *et al.* (2009, 2011a, b) recognize *P. feii* and *P. granulosus* as separate species based on mitochondrial DNA and morphological data. They further report deep divergences among all named species and a few specimens obtained from pet shops in Japan, which have a similar phylogenetic position as the Mt. Mang population in the mitochondrial gene tree presented here. It is likely that those specimens are *Pachytriton* B, which was first reported from European pet stores (Mudrack 1984; Thiesmeier & Hornberg 1997). However, DNA sequences from those Japanese specimens are not available from GenBank at this time. Given the intrageneric relationships recovered in our phylogenetic analysis, the Mt. Mang population should be recognized as a new species of *Pachytriton*, which we describe below.

TABLE 2. Varimax-rotated loadings of principal components summarized from morphological measurements.

	Component	
	1	2
SVL	.797	.518
HL	.649	.612
HW	.831	.220
HD	.813	.383
SF	.707	.574
SL	.419	.708
IC	.798	.470
IN	.632	.538
SHW	.824	.405
TAL	.727	.419
TAD	.918	.143
TAW	.928	.119
AL	.118	.866
PL	.222	.871

***Pachytriton xanthospilos* species nov.**

(Figs. 4, 5)

Holotype: CIB 97902, an adult female from Mangshan National Forest Park, Mt. Mang, 24.93ºN 112.97ºE, elevation 1375 m, near the border between Hunan and Guangdong provinces, P. R. China, collected by Yuhong Guo on July 8, 2009.

Paratypes: CIB 97900–01, 97903–05, same data as the holotype; CIB 21034–35, 21037–38, 21040–41, 21043–45, 21048–55, 21057, 21060–61, from Mt. Mang, Yizhang, Hunan province, collected June 25–27, 1975.

Diagnosis: *Pachytriton xanthospilos* sp. nov. is assigned to the genus *Pachytriton* based on its phylogenetic position derived from molecular data and by the following morphological characters: adult total length exceeds 150 mm; skin very smooth; limbs short and do not meet when forelimb and hind limb are adpressed against flank; digits short, flat and with limited webbing; posterior half of tail extremely laterally compressed; dorsal caudal fin conspicuous. The species can be diagnosed from congeners by the following combination of characters: body size large and very robust; dorsal color uniformly brown to light brown in life; large bright-orange spots or blotches extend ribbon-like dorsolaterally in most specimens; orange blotches sometimes present on the head and dorsum.

Description of the holotype: This is a moderately sized newt; SVL equals 82.6 mm. Head is flat and oval to rectangular. Head width measured at the posterior angle of the jaw almost equals width measured at the parotoid gland. Head much longer than wide. Snout truncate, projects slightly beyond mandible. Nostril at snout tip. Eye very small and does not bulge. Labial fold prominent on upper jaw. Tongue pad elliptical, poorly differentiated from the mouth floor. Posterior tip of maxillary bone contacts pterygoid bone; the two bones are arrayed in an approximately straight line. Vomerine tooth patch ʌ-shaped. Tooth rows converge anteriorly at the anterior limit of the internal choanae and extend posteriorly into the oral cavity. Parotoid gland prominent. Gular fold present but inconspicuous. Skin very smooth. Vertebral groove present along the dorsal midline. A few longitudinal wrinkles on throat, and numerous transverse wrinkles on flanks and venter. Limbs very short; digits remain well separated when forelimb and hind limb are adpressed against flank. Four fingers and five toes, with limited webbing at base of digits. Relative length of fingers, 1 < 4 < 2 < 3; relative length of toes, 1 < 5 < 2 < 4 < 3. Tail highly laterally compressed. Prominent dorsal caudal fin extends from base of tail to tail tip; ventral caudal fin conspicuous. Tail tip rounded. Cloaca small and not swollen.

TABLE 3. Uncorrected mean interspecific genetic distances based on mitochondrial DNA sequence data.

	P. xanthospilos sp. nov.	*P. brevipes*	*P. feii*	*P. granulosus*	*P. moi*	*P. archospotus*	*P. inexpectatus*
P. xanthospilos sp. nov.	–						
P. brevipes	6.1%	–					
P. feii	6.1%	5.7%	–				
P. granulosus	7.0%	7.1%	6.6%	–			
P. archospotus	7.7%	7.8%	7.1%	8.1%	–		
P. moi	8.9%	9.1%	9.1%	9.0%	8.6%	–	
P. inexpectatus	10.2%	10.6%	9.9%	10.4%	10.3%	9.0%	–

Color of the holotype: In preservative, dorsal color uniformly brown, but slightly lighter on head. Upper edge of dorsal caudal fin pale-gold. Pale-gold spots present along dorsolateral flanks, most conspicuous near pectoral and pelvic regions. Venter brown with large, irregular, pale-gold blotches; coloration very light on chin. Cloaca, underside of limbs and tail also pale-gold.

Variation: Linear measurements are summarized in Table 4. Males have larger and slightly swollen cloacae with a few papillae on the cloacal wall. Unlike the poorly differentiated, small adult tongue, the tongue of juveniles is larger, more elevated and with free lateral margins (Fig. 6). This contrast is consistent with the ontogenetic change observed in *Pachytriton feii* (Nishikawa *et al.* 2009). The pale-gold spots in preserved specimens are bright orange in live salamanders (Fig. 7). The dorsum is also lighter in life. Dorsolateral spots are large and conspicuous in most specimens but can be small or even absent. Orange blotches may be present on the head and dorsum. Ventral coloration varies from a brown ground color with orange blotches to nearly entire orange. Ventral orange blotches have defined margins in smaller animals but are more diffuse in the largest specimens (TTL > 180 mm).

Etymology: The specific epithet *xanthospilos* is derived from the characteristic orange (*xantho*-) spots (-*spilos*) along the dorsolateral side of body. It is used as a noun in apposition to the generic name.

Habitat and distribution: This species occurs in montane streams at elevations above 800 m (Fig. 7). Streams are covered by canopies of lush broad-leaf forest, under which flourish dense bushes and bamboos. Tall grasses grow along the adjacent stream banks. Streams are about 2–3 m wide and 0.5–1 m in depth; water is cold and clear. Large boulders are scattered in streams or on banks. Stream substrate includes fallen leaves, gravels and sand. Salamanders are usually found in pools (> 2.5 m^2 surface area and deeper than 0.5 m) along the stream, where the water current is slow (Xu *et al.* 2002). They are most active at night, but can be seen resting at the bottom of pools during the day. Large numbers of tadpoles of *Leptobrachium liui* co-inhabit the same stream. At night, *Trimeresurus stejnegeri* are found in bushes and on bamboos along stream banks. The known geographic distribution of *P. xanthospilos* sp. nov. includes Mt. Mang from the Mangshan National Forest Park. The species very likely occurs in nearby mountains from the Guangdong Nanling National Forest Park. Those mountains are located in the middle-to-eastern section of the Nanling Mountain Range.

Conservation status: *Pachytriton xanthospilos* sp. nov. has long been collected for the commercial pet trade. Technically, the type locality and nearby mountains are protected from logging and poaching at the national level. Nevertheless, there is frequent illegal collection of salamanders and recent habitat destruction from tourism. In the near future, this species could qualify for a threatened category of the IUCN Red List ver. 3.1 (http://www.iucnredlist.org). Therefore, we regard *P. xanthospilos* sp. nov. as Near Threatened.

TABLE 4. Linear measurements (in mm) of *Pachytriton xanthospilos* sp. nov. See text for abbreviations.

Measurements	Holotype	Females from 2009 (N = 2)		Males from 2009 (N = 3)		Juvenile	Females from 1975 (N = 17)		Males from 1975 (N = 3)	
unit: mm	(CIB 97902)	Range	Mean ± SE	Range	Mean ± SE	from 2009	Range	Mean ± SE	Range	Mean ± SE
SVL	82.6	82.6–82.6	82.6 ± 0.0	77.5–90.1	84.7 ± 6.5	57.3	75.5–101.3	91.7 ± 7.1	76.2–94.5	87.0 ± 9.6
TTL	157.0	157.0–158.0	157.5 ± 0.7	140.5–173.5	156.8 ± 16.5	103.0	144.9–196.2	175.9 ± 14.5	145.5–178	163.7 ± 16.6
HL	21.6	21.4–21.6	21.5 ± 0.1	20.4–24.0	22.4 ± 1.8	17.6	18.2–23.9	21.2 ± 1.6	18.4–22.5	20.4 ± 2.1
HW	15.4	15.4–15.7	15.6 ± 0.2	13.2–17.8	15.2 ± 2.3	12.2	14.0–18.7	16.8 ± 1.4	15.7–18.9	17.0 ± 1.7
HD	7.2	7.2–7.3	7.3 ± 0.1	6.8–9.4	8.1 ± 1.3	5.8	8.1–11.0	9.8 ± 1.0	7.9–9.9	9.0 ± 1.0
SL	7.6	7.6–7.9	7.8 ± 0.2	7.5–9.3	8.4 ± 0.9	6.9	6.9–8.9	8.1 ± 0.6	7.5–8.6	8.2 ± 0.6
IC	8.8	8.6–8.8	8.7 ± 0.1	7.2–9.6	8.4 ± 1.2	6.8	7.4–9.8	8.8 ± 0.7	8.2–9.4	8.7 ± 0.6
IN	5.3	5.3–5.9	5.6 ± 0.4	4.9–6.2	5.6 ± 0.7	3.6	4.7–6.3	5.5 ± 0.5	4.4–6.0	5.2 ± 0.8
SF	25.1	25.1–26.0	25.6 ± 0.6	22.4–28.0	24.6 ± 3.0	19.3	22.6–30.3	26.9 ± 2.0	24.5–28.3	26.7 ± 2.0
SHW	14.3	14.0–14.3	14.2 ± 0.2	11.5–16.4	13.9 ± 2.5	10.7	13.3–18.4	15.9 ± 1.5	15.1–16.4	15.6 ± 0.7
TAL	74.4	74.4–75.4	74.9 ± 0.7	63.0–83.4	72.1 ± 10.4	45.7	69.7–94.9	84.2 ± 7.8	69.3–83.5	76.7 ± 7.1
TAD	8.2	8.2–8.4	8.3 ± 0.1	6.4–10.4	8.7 ± 2.1	6.5	7.9–11.2	9.8 ± 0.9	8.4–10.3	9.3 ± 1.0
TAW	9.9	8.9–9.9	9.4 ± 0.7	7.0–11.6	9.5 ± 2.3	6.5	9.5–13.3	11.4 ± 1.0	10.0–12.4	11.2 ± 1.2
AL	18.1	18.1–19.3	18.7 ± 0.8	17.0–18.7	18.1 ± 0.9	13.7	16.3–21.0	19.3 ± 1.3	17.6–20.3	19.0 ± 1.4
PL	19.5	19.5–20.6	20.1 ± 0.8	17.8–19.9	19.0 ± 1.1	14.7	17–22.4	20.3 ± 1.5	18.1–21.7	19.8 ± 1.8

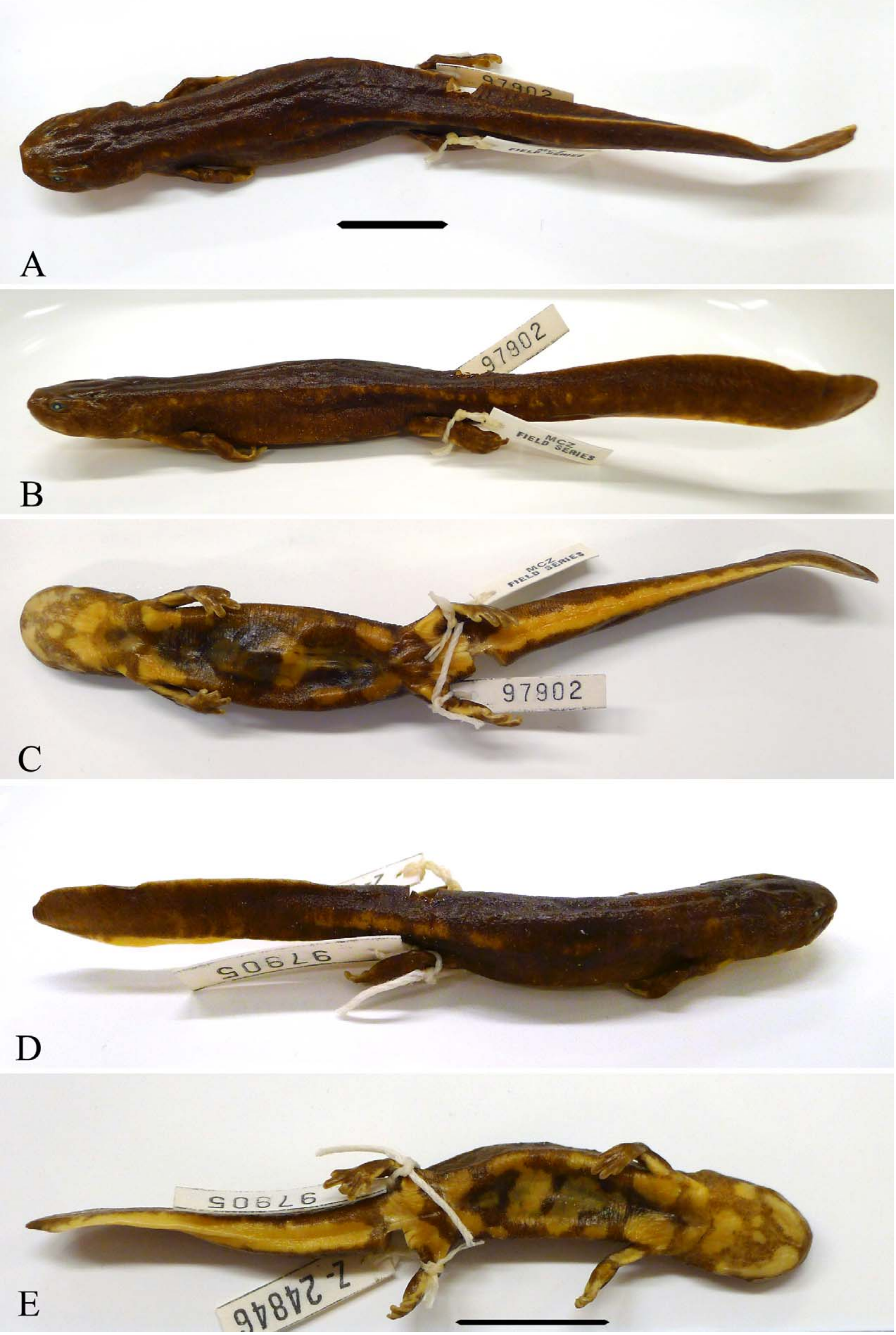

FIGURE 4. *Pachytriton xanthospilos* sp. nov. collected in 2009. A–C: holotype (CIB 97902); D–E: a juvenile paratype (CIB 97905). Scale bar = 2 cm.

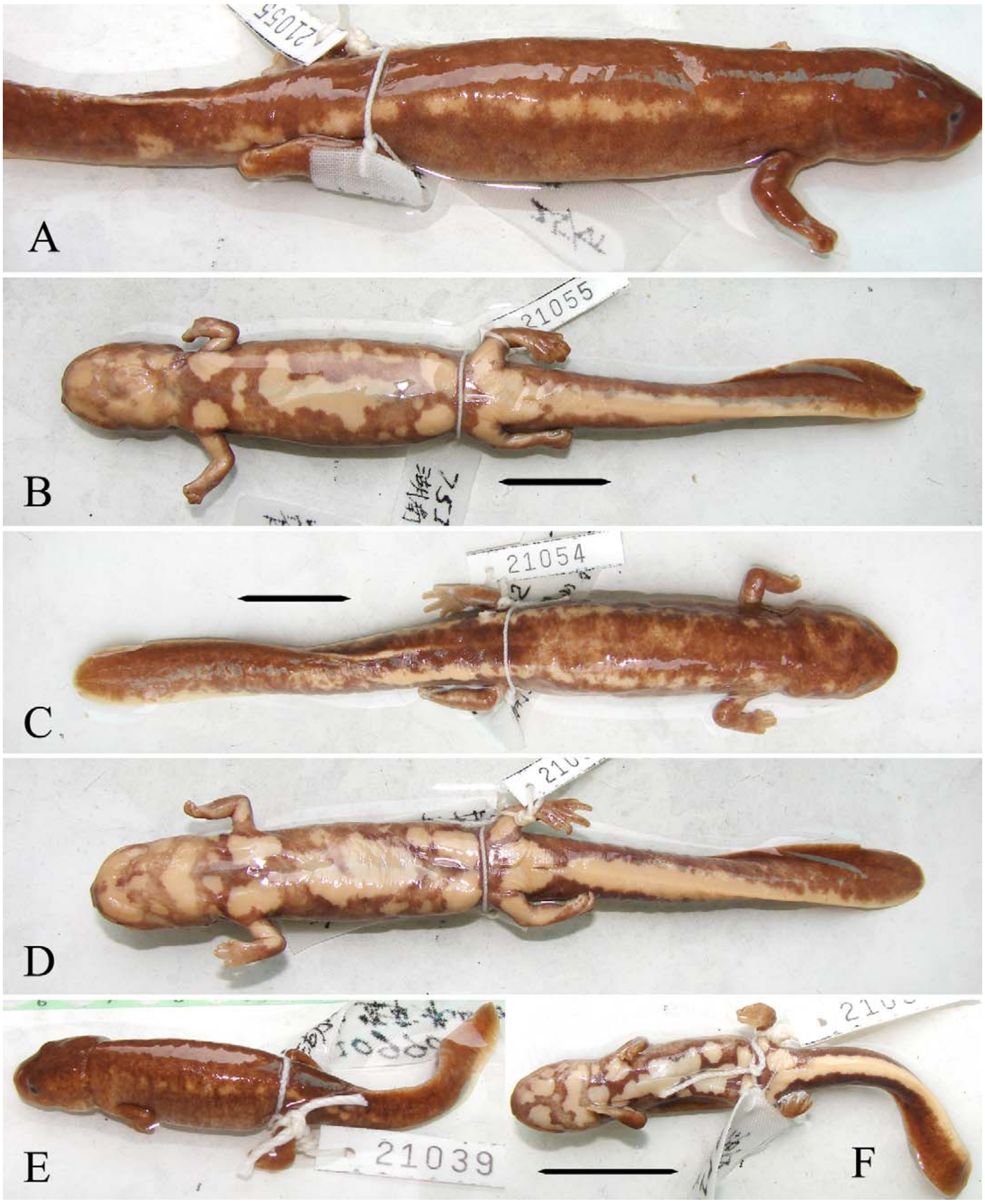

FIGURE 5. *Pachytriton xanthospilos* sp. nov. collected in 1975. A–B: paratype (CIB 21055), showing the dorsolateral blotches; C–D: paratype (CIB 21054); E–F: a juvenile paratype (CIB 21039). Scale bar = 2 cm.

Taxonomy of *Pachytriton*: The genus *Pachytriton* was long understood to contain just two species, *P. labiatus* and *P. brevipes*, with the former species comprising two disjunct populations (northeast vs. southwest) separated by several hundred kilometers (Zhao & Hu 1984; Fei *et al.* 1999; Fei *et al.* 2006). Nishikawa *et al.* (2011a) argue that the lectotype of *P. labiatus*, which is from the southwest population, is actually a species of the genus *Paramesotriton*; they thus designate a new name, *Pachytriton inexpectatus*, for the former *P. labiatus*. We adopt this nomenclatural change but do so with caution, because external and x-ray images of the lectotype in Nishikawa *et al.* (2011a, cf. fig. 2A and 4A) appear to represent different specimens.

Recent phylogenetic analyses reveal that the two populations of *P. inexpectatus* are remarkably distinct and suggest that the northeast population represents a separate species that is more closely related to *P. brevipes* (Wu *et al.* 2010c; Nishikawa *et al.* 2011a). Within the geographic range of the northeast population of *P. inexpectatus*, Chang (1933, 1935) described the salamandrid genus *Pingia* with its monotypic species *Pingia granulosa*. Based on morphological comparison, Nishikawa *et al.* (2009) synonymize *Pingia granulosa* with the northeast population of *Pachytriton inexpectatus*, which now they treat as *Pachytriton granulosus* (Nishikawa *et al.* 2011a). The holotype of *Pingia granulosa* was lost during World War II but additional specimens recently identified as *Pingia granulosa* are available for study (Hou *et al.* 2009). No analysis has yet assessed the relationship between *Pingia granulosa* and the northeast population of *Pachytriton inexpectatus* in a molecular phylogenetic context. Here we temporarily follow Nishikawa *et al.* (2011a) in the recognition of *Pachytriton granulosus*.

Shen *et al.* (2008) describe the fourth species of *Pachytriton* named *Pachytriton archospotus*, which is distinct from congeners in osteological, morphological and genetic characters (Shen *et al.* 2008; Wu *et al.* 2010c; Wu *et al.* 2012). Recently, two more species, *Pachytriton feii* and *Pachytriton moi*, are described based on mitochondrial gene tree and morphological comparisons (Nishikawa *et al.* 2011b). The new species described here, *Pachytriton xanthospilos* sp. nov., represents the seventh species in this genus. It is closely related to *Pachytriton feii*, *Pachytriton brevipes* and *Pachytriton granulosus*.

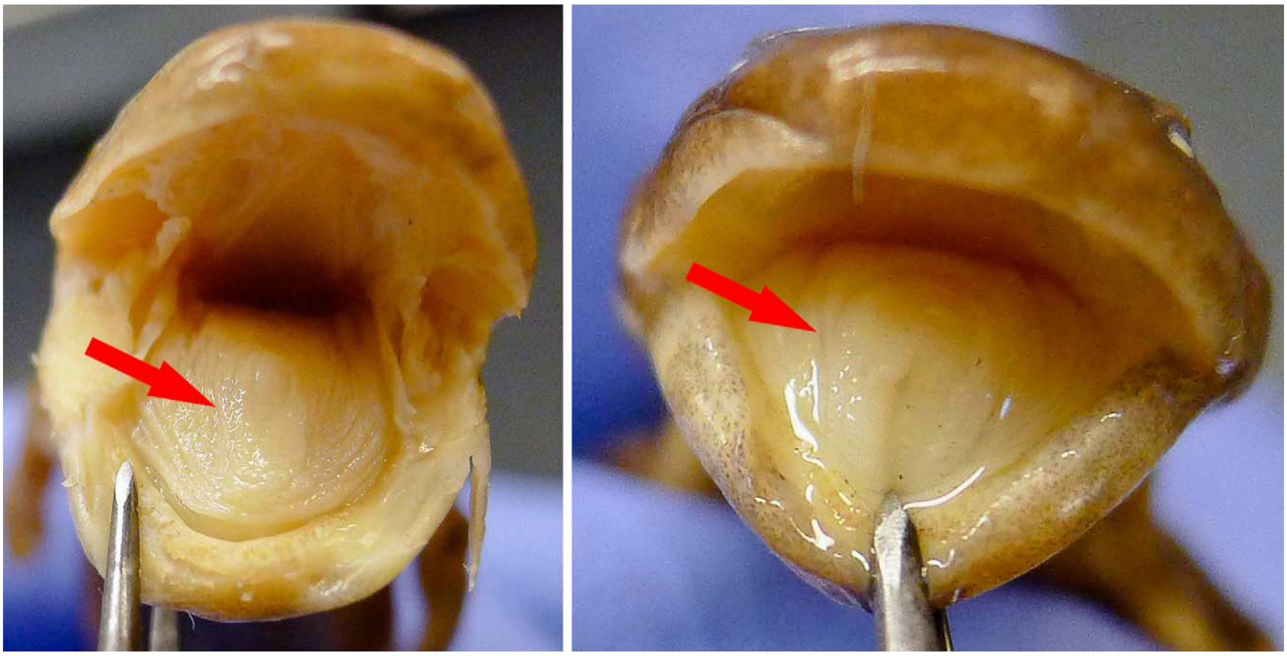

FIGURE 6. Tongue morphology in adult (left, CIB 97900) and juvenile (right, CIB 97905) *Pachytriton xanthospilos* sp. nov. Red arrow points to the tongue.

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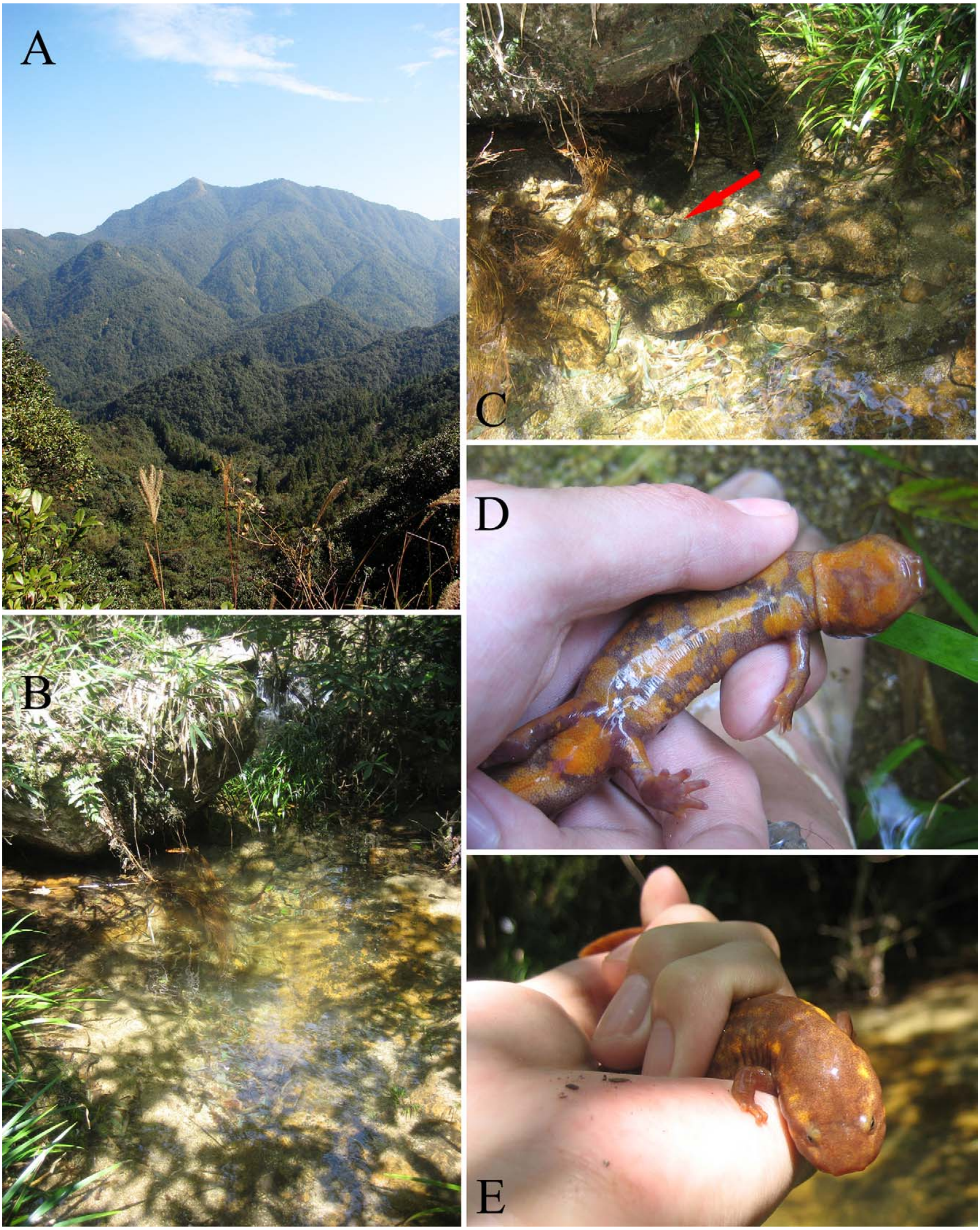

FIGURE 7. A: Mt. Mang, near the border between Hunan and Guangdong provinces. B: At the type locality, the stream is covered by canopy. C: Red arrow points to a newt; water depth is about 0.5 m. D–E: ventral and dorsal views of the newt in C, which was released back to the stream. Photography by X. Zhang.

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Appendix

Specimens measured for morphometric analysis: *Pachytriton brevipes* (CIB 20688, 20691, 20693, 20695, 20698–99, 20701–02, 20708, 20710, 20712–13, 88186, 88208, all from Mt. Wuyi, Fujian province), *P. feii* (CIB 21064–65, 21069–70, 21073–74, 21077–78, 21082, 21085, 21093, 21095–96, 21099–21102, 21104, 21107–08, 21110, all from Mt. Huang, Anhui province), *P. granulosus* (CIB 20719, 20726–28, 20794, 20796, 20815, 20817, 20868, 88135–38, 88143–46, 88149–50, 88152, 88155, 88179, 88181, 88191, 88207, all from Mt. Daiyun, Fujian province and Mt. Tianmu, Zhejiang province).