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Morphological Variation in *Pachytriton labiatus* and a Re-assessment of the Taxonomic Status of *P. granulosus* (Amphibia: Urodela: Salamandridae)

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Abstract: Variation in relation to age and sex in a population of *Pachytriton labiatus* from Mt. Huangshan, Anhui Province, China was examined. There appeared to be distinct morphological variation among age/sex groups. We suggest that some of this variation is associated with ecology and behavior. Examination of the newt species from the type locality and nearby localities of *Pachytriton granulosus* (= *Pingia granulosa*), a taxonomically problematic species, revealed that this taxon was almost indistinguishable from juvenile *P. labiatus*. We therefore conclude that *P. granulosus* is a junior synonym of *P. labiatus*.

Key words: Variation, Age, Sex, Juvenile Morphology, *Pachytriton*, *Pingia*.

INTRODUCTION

Pachytriton is a newt genus endemic to China, and, according to most recent classification (e.g., Frost, 2009), accommodates three species, *Pac. archospotus* Shen et al., 2008, *Pac. brevipes* (Sauvage, 1876), and *Pac. labiatus* (Unterstein, 1930). Adults of this genus inhabit montane streams where they breed and their larvae grow up. Their laterally compressed tail is considered adaptive in stream life, and because of this tail shape, they are sometimes called paddle tail newts. They have well-developed labial folds and a broad tongue,

which completely adheres to the mouth floor. These structures facilitate suction feeding in water. Although adults and subadults of *Pachytriton* are aquatic, some juveniles have been found on land, where they probably stay till subadulthood. From observations of animals in captivity, Thiesmeier and Hornberg (1997) reported that terrestrial juveniles showed morphological traits different from adults. In this study, we examined morphological variation of *Pac. labiatus* in relation to age and sex and tried to clarify the factors that have induced this variation.

We also examined the taxonomic status of *Pac. granulosus* Chang, 1933, which is now synonymized with other species by some authors (e.g., Frost, 2009; see Table 1). *Pachytriton granulosus* was described based on a juvenile

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TABLE 1. Historical change in the taxonomy of *Pachytriton granulosis*.

Species name	Author	Notes
<i>Pachytriton granulosis</i>	Chang (1933)	Described from Jietouzheng, Tiantai City, Zhejiang Province.
<i>Pingia granulosa</i>	Chang (1935)	New monotypic genus established.
<i>Pachytriton brevipes</i>	Pope and Boring (1940)	Synonymized.
<i>Cynops orientalis</i>	Freytag (1962)	Synonymized.
<i>Cynops orientalis</i>	Thorn (1968)	Synonymized.
<i>Pachytriton labiatus</i>	Cai (1985)	Synonymized after examination of specimens from the type locality.
<i>Pingia granulosis</i> [sic]	Hou et al. (2009)	Neotype described from Mt. Longwang, Zhejiang Province.
<i>Pingia granulosa</i>	Frost (2009)	Spelling of species epithet corrected.
<i>Hypselotriton (Pingia) granulosis</i>	Dubois and Raffaëlli (2009)	Relegated to subgeneric rank.

male (Total length [TOL]=87 mm) from a stream in Jietouzheng, Tiantai City, Zhejiang Province. Species of *Pachytriton* basically have a smooth skin (Fei et al., 2006), but *Pac. granulosis* has a granular skin. *Pachytriton granulosis* has a protrusible tongue (i.e., not completely adhering to the mouth floor) as illustrated in the original description, a character which is unique among *Pachytriton* species but is common in other newts like *Cynops* and *Paramesotriton*. Based on the unique skin character and skull morphology (cartilaginous connection between maxilla and pterygoid, and poorly developed frontal-squamosal arch) of this species, Chang (1935) established a new monotypic genus *Pingia*. However, Pope and Boring (1940) regarded *Pi. granulosa* as a juvenile of *Pac. brevipes*. By contrast, Freytag (1962) and Thorn (1968) synonymized both *Pac. granulosis* and *Pi. granulosa* with *Cynops orientalis* (David, 1873). Cai (1985) was the first author who actually studied specimens of *Pac. labiatus* from the type locality of *Pi. granulosa*. He found juveniles of *Pac. labiatus* with skin and skull characteristics that were identical to those of *Pi. granulosa* and, thus, synonymized *Pi. granulosa* with *Pac. labiatus*, while he did not examine *Pac. brevipes*, once considered as a senior synonym of *Pi. granulosa* by Pope and Boring (1940; see above). Recently, Hou et al. (2009) revived *Pingia*

granulosis [sic] based on specimens collected on Mt. Longwang, Zhejiang Province (240 km from the type locality) and designated a neotype. Dubois and Raffaëlli (2009), however, proposed to use the name *Hypselotriton (Pingia) granulosis* for this species.

So the taxonomic status of *Pac. granulosis* (or *Pi. granulosa*) has been controversial and confused. Unfortunately, the type specimen (H124, Biological Laboratory of Science Society of China [Chang, 1933]) seems to have been lost in WWII (Liang Fei, personal communication; Hou et al. [2009]). Thus, we collected newt specimens from the type locality of *Pac. granulosis* and studied them with reference to the original description of *Pac. granulosis* (Chang, 1933) and other newt species occurring in adjacent areas.

MATERIALS AND METHODS

We used *Pac. labiatus* from Huangshan (meaning "yellow mountain" in Chinese), Anhui Province, for examining ontogenetic and sexual variations in morphology. The peak of the mountain is located at 30°10'N, 118°11'E, and 1864 m asl (Fig. 1). The specimens examined were collected between 1972 and 2008 and stored at Chengdu Institute of Biology (CIB) and Graduate School of Human and Environmental Studies, Kyoto University

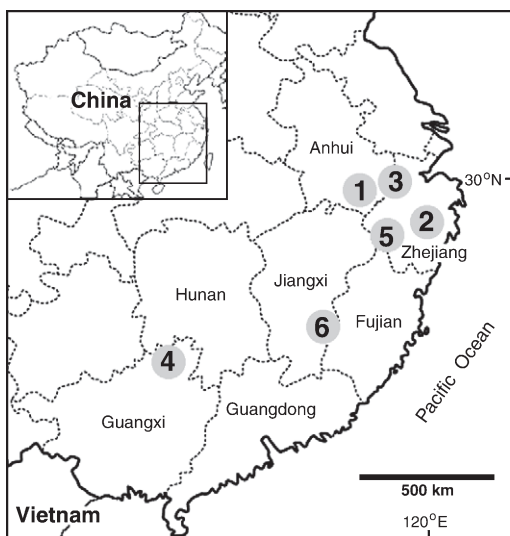


FIG. 1. A map of eastern China, showing sampling localities of newts examined in this study, and the type locality of *Pachytriton granulosus* (2) and the locality where the neotype of *Pingia granulosus* [sic] was collected (3). 1: Mt. Huangshan (*Pac. labiatus*); 2: Jietouzhzen, Tiantai (*Pac. labiatus* and *Paramesotriton chinensis*); 3: Mt. Longwang; 4: Longshen (*Pac. labiatus*); 5: Bao'an, Jiangshan (*Pac. brevipes*); 6: Ruijin (*Cynops orientalis*).

(KUHE) (Appendix 1). The specimens were collected from several sites on the mountain ranging from 600 to 1235 m asl. We selected a total of 44 specimens (13 juveniles, 18 adult males, 13 adult females) for morphological survey, so as to cover body size variation in age/sex groups. We excluded individuals with regenerated or damaged characters. We identified enigmatic juveniles collected in Huangshan and Jietouzhzen, which had unique morphology, by comparing complete sequences (1141 bp) of the mitochondrial cytochrome b gene between these juveniles and adult newts occurring in and around these regions. Experimental protocol and primers were basically same as those reported in Yoshikawa et al. (2008).

Sex and maturity of specimens were determined by observation of gonad and cloaca (mature male possesses filaments in the cloacal slit; Fei et al., 2006). To examine morpho-

logical variation, we chose a total of 28 characters covering overall size and proportions of the newt (see Appendix 2). All measurements were taken to the nearest 0.1 mm with a dial caliper. We used a stereoscopic binocular microscope to measure characters when necessary.

Age and sex differences in snout-vent length (SVL) were examined with analysis of variance (ANOVA) with Tukey-Kramer test. We assessed age and sex differences in the remaining morphometric characters (except for TOL) by analysis of covariance (ANCOVA) with Tukey-Kramer test (Zar, 1984), using SVL as an independent variable. In this analysis, all metric values (x) were \log_{10} -transformed, and their allometric relationship to SVL was expressed by the standard formula (Huxley, 1932): $\log(x) = \alpha \log(\text{SVL}) + \log(b)$ (α : allometric constant; b : initial growth index). The values of α were statistically compared with the case of $\alpha=1$ in each character for each age/sex group ($P < 0.01$) and divided into three types: tachymetry ($\alpha > 1$), isometry ($\alpha = 1$), and bradymetry ($\alpha < 1$) (Matsui, 1984).

Age and sex differences in character ratios (R , % ratio of each character, except for TOL, to SVL) were examined by Kruskal-Wallis test with Dunn's multiple comparisons test (Zar, 1984). We studied the width/length ratio of the upper jaw tooth series (UJTW/UJTL) and vomerine tooth series (VTW/VTL) because shapes of these series are key characters for diagnosing populations of *Pac. labiatus* (Nishikawa et al., unpublished). Correlations of UJTW/UJTL and VTW/VTL with SVL in all sex/age groups combined were also examined by Spearman's rank correlation test.

For assessing the validity of *Pac. granulosus*, we examined three juvenile specimens of *Pac. labiatus* from Jietouzhzen, the type locality of *Pac. granulosus* ("Katoutsin" in the original description of Chang [1933]), Tiantai City, Zhejiang Province (29°09'N, 120°44'E, 349 m asl), six juveniles of *Pac. brevipes* from Bao'an, Jiangshan City, Zhejiang Province (28°22'N, 118°32'E, 819 m asl), and 10 adult males of *C. orientalis* from Zhejiang Province (locality

details unknown). *Pachytriton granulosus* is sometimes treated as conspecific and synonymized with these species (see above). Because granular skin in *Pac. granulosus* is also seen in the genus *Paramesotriton*, which has a sister group relationship with *Pachytriton* based on recent molecular phylogenetic studies (Chan et al., 2001; Weisrock et al., 2006), we also examined three juveniles of *Par. chinensis* (Gray, 1859) collected from 269 m asl of Jietouzhen (see Fig. 1). All these specimens had the body size similar to that of the holotype of *Pac. granulosus*. All these specimens are stored at CIB and KUHE (Appendix 1). We did not examine *Pac. archospotus* because the species occurs only in southeastern Hunan and adjacent areas (Shen et al., 2008) that are distant from our target localities. From Zhejiang Province, *Echinotriton chinghaiensis* (Chang, 1932) is also known, but we did not examine it, because *Echinotriton* has many characters (e.g., triangular head and protrusive ribs), which unquestionably distinguish the genus from the newt in question.

We compared our data with those given by Chang (1933) for *Pac. granulosus* and Hou et al. (2009) for the neotype of *Pi. granulosa*. For metric comparisons, we examined 11 characters shown by Chang (1933) and Hou et al. (2009) that were comparable with our data. Hou et al. (2009) seems to have measured "SVL" from tip of snout to posterior (anterior in our measurements, see Appendix 2) tip of vent, "TRL" from wrinkle of throat to posterior (anterior in our case) tip of vent, "TAL" from posterior (anterior in our case) tip of vent to tip of tail, and have not shown the value of vent length (VL), whereas Chang (1933) showed measurement data mostly comparable with ours and also provided values for VL. We thus used values of SVL+VL, TRL+VL, and TAL-VL instead of SVL, TRL, and TAL, respectively, in comparing our data with those given by Chang (1933) and Hou et al. (2009).

Presence or absence of a connection between maxilla and pterygoid, and a frontalsquamosal arch were examined for an adult

male *C. orientalis* from Zhejiang Province (detailed locality unknown), a juvenile and an adult male *Pac. labiatus* from Huangshan, a juvenile and an adult male *Pac. labiatus* from Jietouzhen, an adult male *Pac. brevipes* from Bao'an, and an adult male *Par. chinensis* from Jietouzhen.

All statistical analyses were performed with SAS (1990) and R 2.3.1 (R Development Core Team, 2006). The significance level was set at $P < 0.05$ unless mentioned otherwise.

RESULTS

All specimens of *Pac. labiatus* from Huangshan were found under the water surface except for one juvenile (CIB 200805046, TOL=89.8 mm), which was collected when it was moving on land about ten meters from a stream. It had granular dorsal skin, a paddle-like tail, and a typical labial fold, and, therefore, did not look like *Pachytriton*. Additionally, the juvenile had a protrusible tongue, which was also recognized in smaller juveniles found in water in Huangshan and Jietouzhen. We compared a complete sequence of the mitochondrial cytochrome b gene between the enigmatic juvenile and adults of *Pac. labiatus* from the same site in Huangshan for identification (sequence data are available from the senior author). These sequences were completely identical (Nishikawa et al., unpublished), thus we identified the juvenile as *Pac. labiatus*.

The small (TOL < 90 mm) juveniles collected in a stream in Jietouzhen were also identified as *Pac. labiatus* by the DNA analysis. These juveniles tended to have a granular skin like the unique terrestrial juvenile from Huangshan; tail was thin (sword-like in shape, not paddle-like as in adults), labial fold was poorly developed, and tongue was protrusible. These characters, however, changed to the conditions as seen in adults with growth. Especially, tongue morphology drastically changed from a fungi-like shape with free lateral margins (protrusible tongue: Fig. 2A, B) to a broad shape fully attached to the mouth floor (unpro-

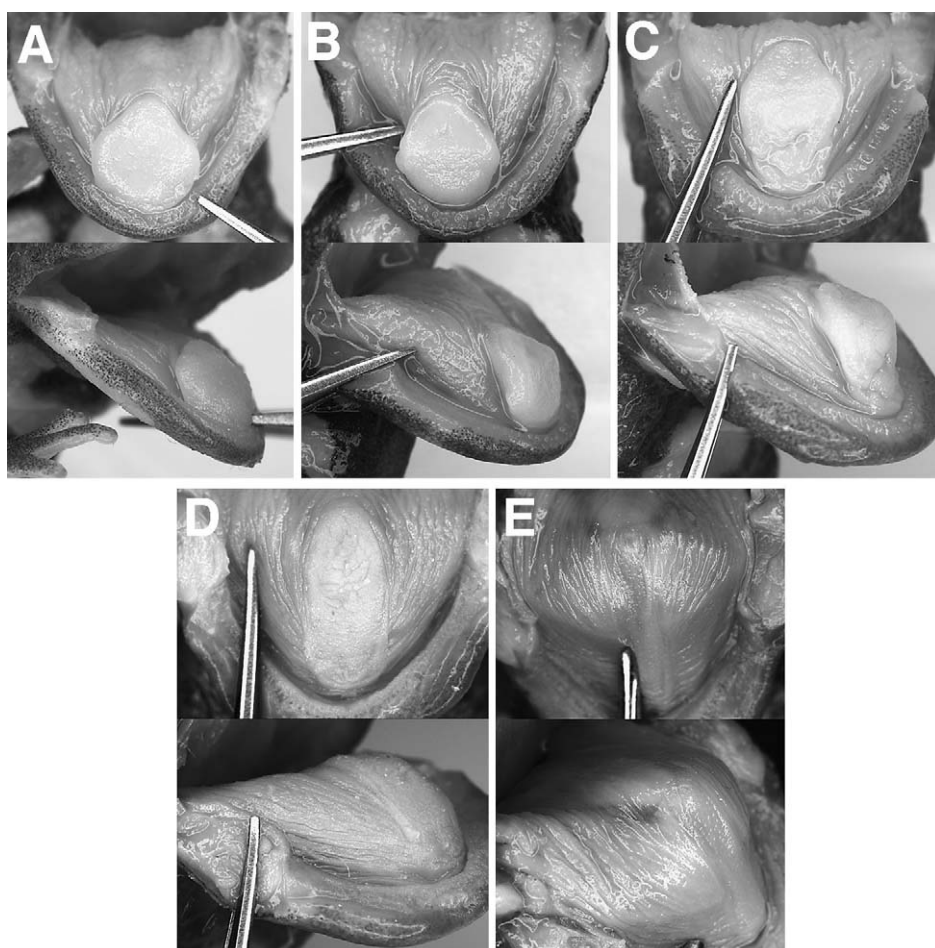


FIG. 2. Developmental change in tongue morphology in *Pachytriton labiatus* from Huangshan, showing dorsal (top) and anterolateral (bottom) views of mouth floor. A: juvenile, SVL=46.1 mm; B: juvenile, SVL=49.0 mm; C: juvenile, SVL=54.0 mm; D: adult male, SVL=58.5 mm; E: adult male, SVL=90.5 mm.

trusible tongue: Fig. 2D, E). Ventral color pattern of juveniles did not change basically until the subadult stage. In contrast, large adults, probably old ones, tended to have a more diffuse pattern than young and sometimes completely lacked red or orange ventral color. Mensural data for specimens are shown in Table 2.

Ontogenetic and sexual variations in morphology

Snout-vent length of *Pac. labiatus* significantly differed among juvenile, adult male, and adult female specimens from Huangshan

(ANOVA: $F_{2,41}=62.11$, $P<0.0001$). Adult females were largest, and adult males and juveniles followed in this order (Tukey-Kramer test: $P<0.05$). Males matured at 58.5 mm in SVL and females at 69.6 mm.

Analysis of covariance revealed significant regressions to SVL of the following characters: a total 11 characters (42.3% of all 26 characters examined: HL, HW, MXHW, ENL, IOD, AGD, TRL, TAL, FLL, HLL, and VTL) in all ages and sexes; eight characters (LJL, IND, BTAW, MTAW, BTAH, MXTAH, MTAH, and VTW) in juveniles and adult males; two characters (UJTW and UJTL) in juveniles and

TABLE 2. $\bar{x} \pm SD$ of SVL (in mm), medians of ratios of characters (R: %SVL), and ratios of tooth series (%), with ranges in parenthesis, and age/sex difference of *Pachytriton labiatus* from Huangshan. For character abbreviations, refer to Appendix 2 (J: juvenile; M: adult male; F: adult female).

	Age/Sex (n)			Difference
	J (13)	M (18)	F (13)	
SVL	50.4±5.3 (42.8–58.5)	73.3±8.6 (58.5–90.5)	81.6±7.5 (69.6–93.4)	F>M>J***
RHL	27.8 (25.6–30.0)	27.1 (24.5–29.1)	25.2 (22.1–28.0)	M, J>F***
RHW	20.7 (19.7–21.3)	20.0 (18.0–21.4)	18.4 (16.5–20.4)	M, J>F***
RMXHW	21.6 (21.0–22.4)	21.2 (19.9–22.6)	19.7 (18.2–21.3)	M, J>F***
RSL	10.2 (8.1–11.9)	9.7 (8.1–10.8)	8.7 (7.7–10.1)	M, J>F**
RLJL	14.8 (12.9–16.0)	13.1 (10.7–14.2)	11.8 (10.5–17.0)	J>M, F***
RENL	7.8 (7.2–8.5)	7.2 (6.3–8.2)	6.3 (5.7–7.4)	M, J>F***
RIND	6.6 (5.7–7.4)	6.3 (5.7–7.4)	5.9 (5.0–7.0)	J>F*
RIOD	8.0 (7.5–9.4)	7.7 (6.9–9.3)	6.7 (5.7–8.6)	J>F**
RUEW	3.5 (2.4–4.7)	2.7 (1.9–3.8)	2.5 (2.1–2.9)	J>M, F***
RUEL	6.6 (5.9–7.7)	5.2 (4.3–6.7)	4.9 (4.0–6.2)	J>M, F***
ROL	4.1 (3.6–5.2)	3.6 (2.8–4.1)	2.9 (2.3–3.6)	J>M>F***
RAGD	48.5 (45.5–51.9)	50.1 (45.7–52.3)	51.3 (49.4–57.8)	F>J**
RTRL	72.2 (69.9–74.4)	72.9 (70.9–75.5)	74.8 (72.0–77.9)	F>M, J***
RTAL	94.4 (90.5–111.6)	97.4 (87.7–105.4)	96.5 (78.0–103.8)	ns
RVL	5.7 (2.9–7.1)	7.3 (4.7–8.7)	4.1 (2.1–6.0)	M>F, J***
RBTAW	11.6 (10.2–14.0)	12.4 (10.6–14.3)	12.1 (9.2–12.9)	ns
RMTAW	8.4 (6.9–9.8)	8.9 (7.5–10.6)	8.9 (6.8–9.7)	ns
RBTAH	11.2 (10.0–13.1)	11.5 (10.3–14.2)	11.5 (8.8–13.2)	ns
RMXTAH	14.2 (11.7–16.1)	15.6 (13.0–16.6)	15.3 (10.5–17.0)	M>J*
RMTAH	13.7 (11.3–15.49)	14.9 (12.9–16.09)	14.7 (10.1–16.7)	ns
RFL	27.4 (23.8–30.2)	25.8 (22.9–29.4)	23.7 (20.6–27.3)	M, J>F***
RHLL	31.1 (28.9–34.2)	29.6 (26.1–32.5)	28.3 (25.1–33.4)	J>M, F***
RUIJTW	12.4 (10.9–14.4)	9.6 (7.8–11.5)	8.9 (7.7–9.5)	J>M, F***
RUIJTL	7.8 (7.4–9.4)	6.7 (5.1–8.4)	6.9 (5.9–8.0)	J>M, F***
RVTW	6.2 (5.2–7.5)	5.9 (4.9–8.2)	5.1 (4.3–6.7)	ns
RVTL	10.0 (9.5–12.2)	7.0 (5.3–8.9)	8.8 (7.5–10.6)	J>F***
UJTW/UJTL	153.5 (140.0–177.8)	143.8 (153.7–143.8)	126.7 (114.5–147.6)	J>M>F***
VTW/VTL	60.0 (48.5–74.4)	62.6 (50.6–79.2)	61.5 (49.4–74.1)	ns

TABLE 3. Age and sex differences in allometry in a population of *Pachytriton labiatus* from Huangshan (J: juvenile [n=13]; M: adult male [n=18]; F: adult female [n=13]). Abbreviations for growth type: tachymetry ($\alpha > 1$), I=isometry ($\alpha = 1$), B= bradymetry ($\alpha < 1$), —=no significant regression. For character abbreviations, refer to Appendix 2. (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: $P \geq 0.05$)

	Age/Sex	Slope				Y-intercept	
		a	Comparison with $\alpha=1$	Growth type	Age/sex difference	Log b	Age/sex difference
HL	M	0.798	$F_{1,16}=5.45^*$	I	ns	-0.193	M>F, J**
	F	0.572	$F_{1,11}=5.63^*$	I		0.214	
	J	0.818	$F_{1,11}=1.63$ ns	I		-0.243	
HW	M	0.768	$F_{1,16}=9.34^{**}$	B	ns	-0.269	M>F, J***
	F	0.697	$F_{1,11}=3.39$ ns	I		-0.160	
	J	0.938	$F_{1,11}=1.06$ ns	I		-0.579	
MXHW	M	0.829	$F_{1,16}=6.67^*$	I	ns	-0.355	M, J>F***
	F	0.778	$F_{1,11}=3.54$ ns	I		-0.283	
	J	1.008	$F_{1,11}=0.02$ ns	I		-0.678	
SL	M	0.674	$F_{1,16}=5.43^*$	I	ns (M, F)	-0.411	ns (M, F)
	F	0.438	$F_{1,11}=3.54$ ns	B		0.015	
	J	0.611	$F_{1,11}=0.02$ ns	—		-0.335	
LJL	M	0.777	$F_{1,16}=2.34$ ns	I	ns (M, J)	-0.479	ns (M, J)
	F	0.806	$F_{1,11}=0.24$ ns	—		-0.547	
	J	0.882	$F_{1,11}=0.50$ ns	I		-0.629	
ENL	M	0.647	$F_{1,16}=10.35^{**}$	B	ns	-0.484	M, J>F**
	F	0.689	$F_{1,11}=2.13$ ns	I		-0.597	
	J	0.948	$F_{1,11}=0.11$ ns	I		-1.021	
IND	M	0.901	$F_{1,16}=0.33$ ns	I	ns (M, J)	-1.013	ns (M, J)
	F	0.580	$F_{1,11}=2.40$ ns	—		-0.423	
	J	1.324	$F_{1,11}=2.65$ ns	I		-1.737	
IOD	M	0.530	$F_{1,16}=8.49^*$	I	ns	-0.233	M>F, J**
	F	0.170	$F_{1,11}=4.48$ ns	I		0.415	
	J	0.985	$F_{1,11}=0.01$ ns	I		-1.061	
UEW	M	0.199	$F_{1,16}=6.59^*$	—		-0.064	
	F	0.323	$F_{1,11}=5.41^*$	—		-0.304	
	J	-0.360	$F_{1,11}=21.02^{***}$	—		0.851	
UEL	M	0.497	$F_{1,16}=4.33$ ns	—		-0.349	
	F	0.714	$F_{1,11}=0.62$ ns	—		-0.766	
	J	0.604	$F_{1,11}=3.76$ ns	I		-0.508	
OL	M	0.626	$F_{1,16}=4.01$ ns	I		-0.756	
	F	-0.042	$F_{1,11}=15.16^{**}$	—		0.457	
	J	0.322	$F_{1,11}=6.47^*$	—		-0.210	
AGD	M	1.051	$F_{1,16}=0.31$ ns	I	ns	-0.400	F>M*
	F	1.037	$F_{1,11}=0.06$ ns	I		-0.353	
	J	1.139	$F_{1,11}=1.58$ ns	I		-0.550	
TRL	M	1.073	$F_{1,16}=5.22^*$	I	ns	-0.272	F, J>M*
	F	1.142	$F_{1,11}=5.81^*$	I		-0.396	
	J	1.071	$F_{1,11}=1.65$ ns	I		-0.263	
TAL	M	0.882	$F_{1,16}=1.42$ ns	I	ns	0.206	ns
	F	0.929	$F_{1,11}=0.09$ ns	I		0.113	
	J	1.291	$F_{1,11}=4.42$ ns	I		-0.511	
VL	M	0.591	$F_{1,16}=1.73$ ns	—		-0.385	
	F	0.413	$F_{1,11}=0.26$ ns	—		-0.288	
	J	0.456	$F_{1,11}=0.55$ ns	—		-0.355	

TABLE 3. Continued

BTAW	M	0.904	$F_{1,16}=0.31ns$	I	ns (M, J)	-0.730	ns (M, J)
	F	0.684	$F_{1,11}=0.99ns$	—		-0.329	
	J	1.296	$F_{1,11}=1.21ns$	I		-1.433	
MTAW	M	0.808	$F_{1,16}=0.86ns$	I	ns (M, J)	-0.696	ns (M, J)
	F	0.466	$F_{1,11}=4.29ns$	—		-0.044	
	J	1.404	$F_{1,11}=1.95ns$	I		-1.768	
BTAH	M	0.899	$F_{1,16}=0.39ns$	I	J>M**	-0.747	ns (M, J)
	F	0.038	$F_{1,11}=12.77**$	—		0.885	
	J	1.314	$F_{1,11}=2.17ns$	I		-1.480	
MXTAH	M	0.889	$F_{1,16}=0.64ns$	I	J>M***	-0.606	ns (M, J)
	F	0.098	$F_{1,11}=6.47*$	—		0.897	
	J	1.757	$F_{1,11}=17.02**$	T		-2.140	
MTAH	M	0.965	$F_{1,16}=0.06ns$	I	J>M***	-0.770	ns (M, J)
	F	-0.064	$F_{1,11}=9.13*$	—		1.185	
	J	1.744	$F_{1,11}=13.25**$	T		-2.131	
FLL	M	0.641	$F_{1,16}=13.50**$	B	ns	0.080	M>F, J**
	F	0.600	$F_{1,11}=4.45ns$	I		0.135	
	J	0.593	$F_{1,11}=6.19*$	I		0.128	
HLL	M	0.620	$F_{1,16}=22.91***$	B	ns	0.172	ns
	F	0.627	$F_{1,11}=3.80ns$	I		0.165	
	J	0.785	$F_{1,11}=2.32ns$	I		-0.139	
UJTW	M	0.202	$F_{1,16}=27.76***$	—	F>J*	0.461	ns (F, J)
	F	0.893	$F_{1,11}=0.25ns$	I		-0.853	
	J	0.367	$F_{1,11}=21.33***$	B		0.175	
UJTL	M	0.401	$F_{1,16}=6.50*$	—	ns (F, J)	-0.056	ns (F, J)
	F	0.887	$F_{1,11}=0.15ns$	I		-0.950	
	J	0.816	$F_{1,11}=0.88ns$	I		-0.783	
VTW	M	1.009	$F_{1,16}=0.00ns$	I	ns (M, J)	-1.243	ns (M, J)
	F	0.596	$F_{1,11}=0.87ns$	—		-0.496	
	J	0.814	$F_{1,11}=0.42ns$	I		-0.897	
VTL	M	0.987	$F_{1,16}=0.00ns$	I	ns	-0.100	ns
	F	0.708	$F_{1,11}=1.06ns$	I		-0.500	
	J	1.044	$F_{1,11}=0.04ns$	I		-1.062	

adult females; one character (SL) in adult males and females.

The results of ANCOVA also showed significant differences in slopes (α) of the regression lines in four characters (BTAH, MXTAH, MTAH, and UJTW) between age groups (juveniles vs. adult males, or juveniles vs. adult females). Of these four, three were characters related to tail height (BTAH, MXTAH, and MTAH) and showed larger values in juveniles than in adults (Table 3).

Of the 11 characters, significantly regressed to SVL in all ages and sexes, eight did not differ in the slope (α), but differed in the y-intercept (log b) of the regression lines. Lines

for adult males showed larger y-intercept values than for adult females and juveniles in HL (Fig. 3), HW, IOD, and FLL, while adult males and juveniles showed larger y-intercept values than adult females in MXHW (Fig. 3) and ENL. In contrast, the regression lines for adult females and juveniles showed larger y-intercept values than for adult males only in AGD and TRL, and in TRL, respectively. The remaining three characters did not show significant differences in the y-intercept among age/sex groups.

Among a total of 57 regressions significant for all characters for each all age/sex groups (73.1% of all 78 regressions), 49 were judged

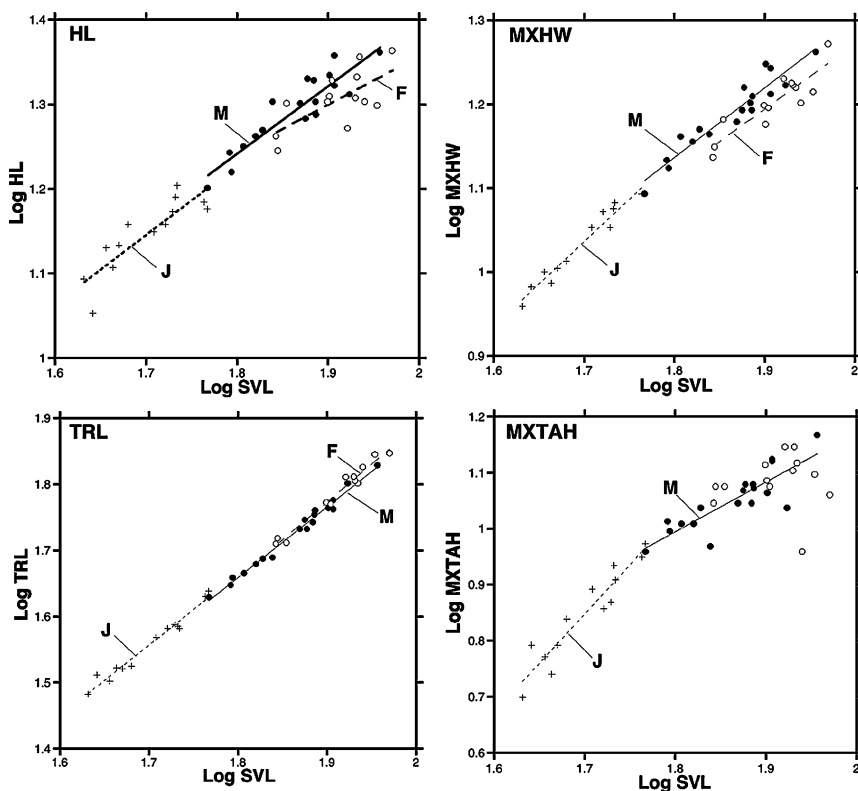


FIG. 3. Allometric relationships of HL, MXHW, TRL, and MXTAH against SVL in *Pachytriton labiatus*. Cross and dotted line=juvenile (J); closed circle and solid line=adult male (M); open circle and broken line=adult female (F). Only regression lines with significant correlations are shown.

as isometric, six bradymetric, and two tachymetric (Table 3). Tachymetric growth was observed only in two characters of tail height (MXTAH and MTAH: see Fig. 3).

At least one difference was found among age/sex groups in a total of 20 ratios to SVL (76.9% of all 26 ratios examined), but the remaining six (RTAL, RBTAW, RMTAW, RBTAH, RMTAH, and RVTW) did not differ among the groups. Juveniles showed larger values than adults in seven ratios, of which six were related to head (RLJL, RUEW, RUEL, ROL, RUJTW, and RUJTL). In the other six ratios, adult males and juveniles showed larger values than adult females, and five of these were also related to head (RHL, RHW, RMXHW, RSL, and RENL). In further three ratios related to head (RIND, RIOD, and RVTL), juveniles had larger values than adult

females. In RVL, adult males showed a larger value than adult females and juveniles.

In the analyses of the shape of tooth series, UJTW/UJTL significantly differed among age/sex groups (juveniles>adult males>adult females: Kruskal-Wallis test, $H_c=23.02$, $P<0.0001$), but VTW/VTL did not differ among the groups. When all age/sex groups were combined, VTW/VTL did not significantly correlate with SVL, but UJTW/UJTL negatively correlated with SVL (Spearman's rank correlation, $r=-0.696$, $S=24068.27$, $P<0.0001$).

Comparison of Pachytriton granulatus with other species

Measurement data and illustration from the original description of *Pac. granulatus* (Chang, 1933) are reproduced in Table 4 and Fig. 4, respectively. Diagnostic external characteris-

TABLE 4. $\bar{x} \pm SD$ of TOL and SVL+VL (in mm), and medians of ratios of a metric character (R: % [SVL+VL]), with ranges in parenthesis, of newts from Zhejiang Province (only *Pi. granulosa* and *C. orientalis* are adult). For character abbreviations, refer to Appendix 2.

Species Age/Sex (n) Locality	<i>Pac. granulosa</i> * J (1) Jietouzhen	<i>Pac. labiatus</i> J (3) Jietouzhen	<i>Pi. granulosa</i> ** M (1) Mt. Longwang	<i>Pi. granulosa</i> *** F (1) Mt. Longwang	<i>Pac. brevipes</i> J (6) Bao'an	<i>Par. chinensis</i> J (3) Jietouzhen	<i>C. orientalis</i> M (10) unknown
TOL	87.0	98.6±7.7 (92.0–107.0)	95.9	94.4	84.2 (44.9–109.9)	92.5 (59.8–116.0)	66.0±4.5 (59.1–76.1)
SVL+VL	48.0	53.0±3.9 (49.2–56.9)	51.1	51.2	48.1 (43.9–56.9)	46.5 (30.8–59.5)	40.7±2.5 (35.9–45.3)
RHL	25.0	26.0 (24.7–26.8)	26.1	27.0	27.9 (27.2–29.2)	27.4 (26.6–28.9)	25.4 (23.6–29.5)
RMXHW	21.3	19.3 (19.0–19.5)	17.6	17.8	20.8 (20.6–21.0)	21.3 (20.7–23.7)	21.0 (20.4–24.8)
RENL	6.5	7.0 (6.7–7.2)	4.5	5.2	7.5 (6.7–8.1)	7.6 (7.1–7.7)	NA
RIND	5.2	5.7 (5.3–6.0)	4.5	5.2	5.9 (4.7–6.7)	5.5 (4.9–5.8)	5.3 (4.6–6.4)
RIOD	7.5	6.7 (6.0–7.2)	7.1	6.6	6.5 (5.5–7.5)	6.7 (5.4–8.4)	NA
R(TRL+VL)	75.0	74.0 (73.2–75.3)	73.9	73.0	72.1 (70.8–72.8)	72.6 (71.1–73.4)	74.6 (70.5–76.4)
R(TAL-VL)	83.3	85.8 (82.5–88.0)	87.7	84.5	89.2 (84.9–94.1)	95.0 (94.2–106.5)	63.2 (49.1–68.1)
RFL	27.7	25.2 (24.2–26.0)	27.8	25.5	24.9 (22.7–27.2)	32.1 (30.8–34.1)	29.0 (26.6–32.7)
RHLL	28.8	27.5 (26.2–28.5)	28.0	25.9	28.0 (27.6–29.2)	35.0 (32.8–36.2)	32.6 (26.9–37.0)

* Holotype, H124, measurements from Chang (1933).

** Neotype, HM08z0001, measurements from Hou et al. (2009).

*** HM08z0002, measurements from Hou et al. (2009).

tics of the species given by Chang (1933) were: no dorsal ridge, numerous minute granules on skin, and orange-red spots along each side of body (Fig. 4).

Specimens of *C. orientalis* examined were all mature, but their mean TOL, 66.0 mm, was smaller than TOL of juvenile samples of *Pachytriton* and *Paramesotriton* examined, and of the neotype of *Pi. granulosa*. *Cynops orientalis* had a smooth skin, well-developed parotoid gland, and short tail (Table 4 and Fig. 5D), and the combination of these conditions was not found in other species. *Paramesotriton chinensis* had a granular skin like *Pac. granulosa*, but had a distinct dorsal ridge. The species had isolated reddish orange spots

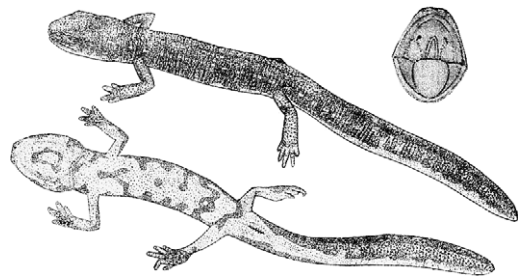


FIG. 4. The holotype of *Pachytriton granulosa* shown in the original description (Chang, 1933). Dorsolateral (top) and ventral (bottom) aspects, and mouth cavity (top right).

on ventral body, and a distinct yellowish small spot on the dorsal base of each limb (Fig. 5C), which is characteristically long (Table 4), while such characters were not found in *Pac. granulatus*. In the remaining samples, *Pac. brevipes* (also *Pac. archospotus* from literature source) was most differentiated in coloration, with typical black spots on whole body surface in adults but only on dorsum in juveniles (Fig. 5B). Such spots were not recognized in the remaining samples and not mentioned in the description of *Pac. granulatus* (Chang, 1933).

In the newts examined, juveniles of *Pac.*

labiatus most resembled *Pac. granulatus* as described (Chang, 1933) in the character ratios and coloration. Especially, their ventral color pattern was nearly identical: large reddish orange marking and isolated dark brown spots, and lines like vermiculation (Figs. 4 and 5A). In the type locality of *Pac. granulatus*, Jietouzheng, we unfortunately could not find any newt that completely agreed with the original description of the taxon. The smallest juvenile of *Pac. labiatus* collected, however, had a few granules on dorsum, protrusible tongue, poorly developed labial fold, and reddish

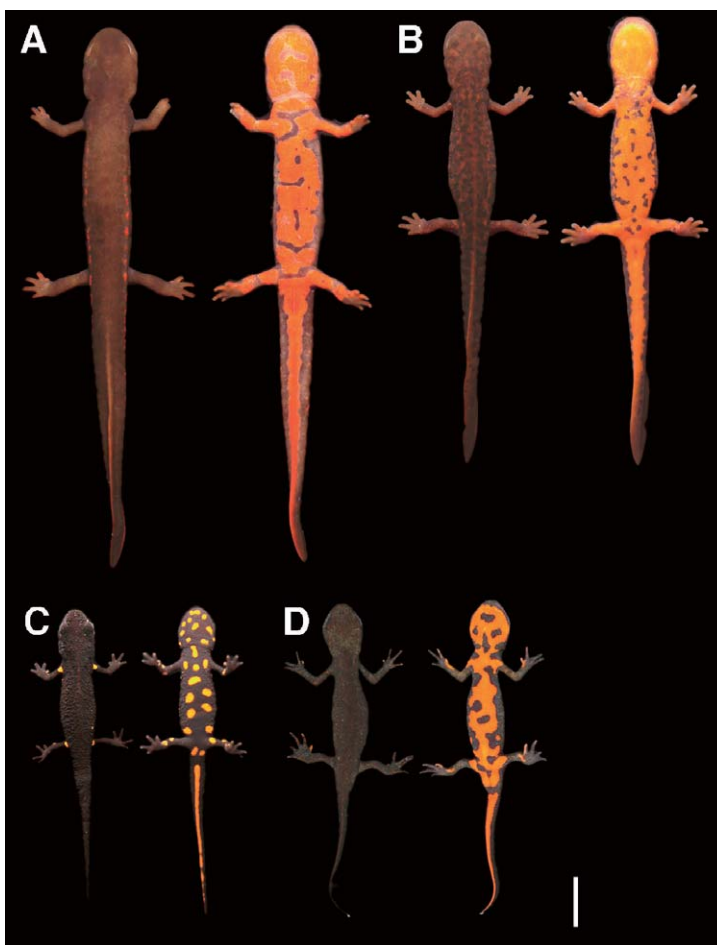


FIG. 5. Dorsal (left) and ventral (right) views in life of subadult *Pachytriton labiatus* from Jietouzheng (A), juvenile *Pac. brevipes* from Bao'an (B), juvenile *Paramesotriton chinensis* from Jietouzheng (C), and adult male *Cynops orientalis* from Ruijin, Jiangxi Province (D). Scale shows 10 mm. Photo of *C. orientalis* in life from Zhejiang Province was unavailable but the color pattern was almost identical with D.

orange dots on dorsolateral ridges, all of which were found in the illustration of original description of *Pac. granulosa* (Fig. 4).

In most ratio values derived from the original descriptions, the neotype, as well as a female, of *Pi. granulosa* (Hou et al., 2009) did not differ much from the holotype of *Pac. granulosa* (Chang, 1933). However, they did not overlap in ratios of MXHW and ENL (Table 4), and were also dissimilar in ventral color pattern, as far as illustrations showed. Likewise, *Pi. granulosa* neotype differed in MXHW and ENL ratios or in the ventral color pattern from juvenile *Pac. labiatus* from Jietouzheng (type locality of *Pac. granulosa*) examined by us, although adult *Pac. labiatus* from the same locality were similar to *Pi. granulosa* neotype (see Plate I in Hou et al., 2009).

In the present samples, neither the connection of the maxilla with the pterygoid, nor the bony arch in the frontal-squamosal was well formed in juveniles, but both tended to be well formed and calcified in adults. *Pachytriton brevipes* and *Pac. labiatus* had the maxilla and pterygoid well-connected and forming a nearly straight line, but lacked (adult *Pac. brevipes*) or possessed at most a thin frontal-squamosal arch (*Pac. labiatus* and juvenile *Pac. brevipes*). On the other hand, *Cynops* and *Paramesotriton* lacked the bony connection, but possessed a well-developed frontal-squamosal arch.

DISCUSSION

Morphological variation in Pachytriton labiatus

Although available samples were small in number, especially for allometric analysis, characters that differed sexually in allometric patterns largely corresponded to those that exhibited sexual dimorphism in ratios. This result is meaningful, since few authors have ever tried to study allometry prior to the use of ratios in elucidating intraspecific variations in newts, mainly because the lack of good series of samples of various ages and body sizes. Our results indicated the validity of the comparison

of ratios in taxonomic studies on at least newt species, and would be also applicable to other animal groups. This result is significant, because it is now becoming more and more difficult to collect a large number of specimens from a wild population.

Our results also suggest that in examining interpopulation variation we must compare age/sex groups separately for some characters that are differentiated in the groups. For example, values of UJTW/UJTL are differentiated among age/sex groups, and adult males have larger RVL than adult females and juveniles. Possession of a longer vent slit by adult males than adult females has been reported in species of *Pachytriton* (Fei et al., 2006; Shen et al., 2008), but the tendency has not previously been examined statistically.

In *Pac. labiatus*, juveniles possessed relatively large eyes (RUEW, RUEW, and ROL) and jaws (RLJL, RUJTW, and RUJTL) compared with adults, indicating that these head organs are essential for the earlier stages of life. Most characters examined were isometric or bradydymetric in growth in all age/sex groups. Tachymetry was found only in the characters related to tail height (BTAH, MXTAH, and MTAH) of juveniles (Table 3, Fig. 3). As noted above, juveniles of *Pac. labiatus* probably spend their life on land after metamorphosis until the subadult stage. A similar phenomenon is well-known in the American salamandrid, *Notophthalmus* spp. Juveniles of this genus are terrestrial for several years after metamorphosis and are called "efts", which show a rougher skin and less compressed tail than aquatic adults (e.g., Noble, 1929; Petranksa, 1998). The terrestrial juveniles of *Pachytriton* spend a secretive life and have been collected on a few occasions (e.g., Longshen, Guangxi Zhuang Autonomous Region: Nishikawa unpublished; Anhui Province: Hou et al. [2009]), making further study difficult.

The tail of one unique terrestrial juvenile from Huangshan was not extremely high, i.e., not paddle-like as seen in adults. As clarified by our allometric analysis, the speed of growth of the tail is higher than that of SVL

(tachymetry) in juveniles, and this morphological change accompanies habitat change from land to a stream. Not only the tail, but also the other characters unique to terrestrial juveniles show drastic changes for the aquatic life. The skin changes to soft and smooth as in the larva so as to enable better skin respiration. The labial fold develops and tongue adheres to the mouth floor for effective suction feeding (Fig. 2). All these changes are adaptive for a secondary aquatic life and make the morphology of adult *Pachytriton* similar to that of their larvae.

Adult specimens exhibit a general morphological trend found in many urodeles, such as males with organs relatively larger than in females, except for trunk characters (see above). Generally in urodeles, males have a higher tail than females, but this trend was not observed in the species studied here. Like in other salamandrids, males of *Pachytriton* use the broad tail for courting females (Sparreboom and Thiesmeier, 1998). Because brooding females are known to care for their eggs to some extent (Thiesmeier and Hornberg, 1997), females may use the relatively high tail for fanning fresh water against their eggs, but such a behavior pattern has not been reported thus far. It is possible that similarly well-developed tails in both sexes are just an adaptation to a stream habitat.

Pachytriton is inferred to be the sister group of *Paramesotriton* (e.g., Weisrock et al., 2006), whose members are aquatic but with a morphology less adapted to an aquatic habitat than *Pachytriton*. *Pachytriton* has unique characteristics for effective suction feeding such as epibranchials that wrap the neck dorsolaterally (Chan et al., 2001) and a tongue that is completely attached to the mouth floor. Such morphology must have been derivatively acquired in *Pachytriton*. Another Asian aquatic newt, *Hypselotriton wolterstorffi*, possesses larval characteristics like external gills, compressed tail, slender limbs, and smooth skin, but because the genus seems to be fully aquatic throughout its life, these characteristics must be considered an extension of the larval stage.

Invalidity of Pachytriton granulosis or Pingia granulosa

In and around Zhejiang Province, five salamandrids have been recorded (*C. orientalis*, *E. chinhaiensis*, *Pac. brevipes*, *Pac. labiatus*, and *Par. chinensis*; Fei et al., 2006). Of these, *E. chinhaiensis* can be distinguished easily from *Pac. granulosis* as already noted. In the type locality of *Pac. granulosis* and adjacent areas in Zhejiang, juvenile *Pac. labiatus* most resembled the description of *Pac. granulosis* in coloration, morphometric ratios, and skull characters among the newts with similar body size, including the neotype of *Pi. granulosa*. Newt specimens with numerous granules on the skin were collected, unfortunately not from the type locality of *Pac. granulosis*, but from Huangshan. This *Pac. granulosis*-like unique juvenile from Huangshan looked similar to the holotype of *Pac. granulosis* but was completely identical with adult *Pac. labiatus* from the same locality in mtDNA sequences. Although we could not examine DNA data of the neotype, we now have no other choice but to conclude that *Pac. granulosis* is the juvenile of *Pac. labiatus* based on morphological evidence. Our present results also deny the validity of the subgenus *Pingia* proposed by Dubois and Raffaelli (2009).

There has been much confusion in the taxonomy of *Pac. granulosis* or *Pi. granulosa* until now. *Pachytriton labiatus* had been treated as conspecific or at most as a subspecies of *Pac. brevipes* until Zhao and Hu (1984) validated its specific status. After Zhao and Hu (1984), Cai (1985) studied *Pac. labiatus* from Jietouzheng and synonymized *Pi. granulosa* with *Pac. labiatus*. Later Hou et al. (2009) considered that Cai (1985) had failed to collect adult *Pingia* and revived *Pingia* by assigning a neotype based on a specimen not from the type locality of *Pac. granulosis*. However, the specimen that is Hou et al.'s (2009) neotype possesses a morphology similar to that of the terrestrial juvenile of *Pachytriton* (see also the photo of terrestrial juvenile in Thiesmeier and Hornberg [1997] and the comment on *Pac. granulosis* by Thiesmeier [1997]). Its dorso-

lateral and ventral color pattern is very similar to that of adult *Pac. labiatus* from the locality where the neotype was collected, but is different from that of *Pac. labiatus* collected in the original type locality of *Pac. granulosis* (see Plate I in Hou et al. [2009] and our Figs. 4 and 5).

Hou et al. (2009) mentioned that *Pachytriton* was fully aquatic throughout its whole life but that *Pingia* was terrestrial. Nevertheless, the unique individual known as the latter (holotype, juvenile male) was collected from a river by a fishing net (Chang, 1933). Juveniles of *Pac. labiatus* we collected from a stream in Jietouzhen possessed some characteristics of *Pac. granulosis*. We surmise that the holotype of *Pac. granulosis* is a juvenile *Pac. granulosis* just coming back to a stream from the land. Although we can not completely exclude the possibility that the newt assigned to the neotype by Hou et al. (2009) is a new terrestrial species, the holotype of *Pac. granulosis* is doubtlessly a juvenile *Pac. labiatus*. Detailed examinations on the degree of maturity and skull characters, as well as genetic identification, if possible, of the neotype of *Pi. granulosa* are needed to clarify its exact taxonomic status.

Chang (1935) established *Pingia* based not only on granular skin but also skull characteristics. However, these characters vary with age and/or geographically in *Pachytriton* (Chang and Boring, 1935). Based on such observations, Pope and Boring (1940) synonymized *Pi. granulosa* with *Pac. brevipes*, while Cai (1985) synonymized it with *Pac. labiatus*. Shen et al. (2008) agreed that the degree of development in frontal-squamosal arch was variable between populations, but they nevertheless suggested that the character was useful for species-level identification in *Pachytriton*. Our survey indicated that the straight line formed at the connection between maxilla and pterygoid is one of the diagnostic characteristics of *Pachytriton* whereas the degree of development of frontal-squamosal arch varies with age at least in *Pac. labiatus*. Further study on the skull characters of *Pachytriton*

species is needed for reliable taxonomic revision of this genus.

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APPENDIX 1

Specimens examined

See text for institutional acronyms.

Pachytriton labiatus from Mt. Huangshan, Anhui Province (n=44), CIB 20070666–68, CIB 200805012, 14–15, 46, CIB 21064–66, 70–75, 77–79, 82–86, 88–89, 92–102, 104, 106, 108–110, KUHE 36808–09.

Pachytriton labiatus from Jietouzhen, Tiantai City, Zhejiang Province (n=3), CIB

ZJ200806013, ZJ200806101–02.

Pachytriton labiatus from Longshen, Guangxi Zhuang Autonomous Region (n=1), CIB 21202.

Pachytriton brevipes from Bao'an, Jiangan City, Zhejiang Province (n=6), CIB ZJ200806127, 29–33.

Paramesotriton chinensis from Jietouzhen, Tiantai City, Zhejiang Province (n=3), CIB ZJ200806112, 17, 18.

Cynops orientalis from unknown locality, Zhejiang Province (n=10), CIB 20353, 58–61, 69–70, 74, 76–77.

Cynops orientalis from Ruijin City, Jiangxi Province (n=1), KUHE 42323.

APPENDIX 2

Character definitions

TOL (Total length): tip of snout to tip of tail; SVL (snout-vent length): tip of snout to anterior tip of vent; HL (head length): tip of snout to wrinkle of throat; HW (head width): measured at jaw angle; MXHW (maximum head width): measured at widest point; SL (snout length): tip of snout to anterior tip of upper eyelid; LJJ (lower jaw length): tip of lower jaw to the angle of jaw; ENL (eye-nostril length): external nare to anterior tip of upper eyelid; IND (internarial distance): minimum distance between the external nares; IOD

(interorbital distance): minimum distance between the upper eyelids; UEW (upper eyelid width): greatest width of the upper eyelid; UEL (upper eyelid length): greatest length of upper eyelid; OL (orbital length): maximum diameter of orbit; AGD (axilla-groin distance): minimum distance between axilla and groin; TRL (trunk length): wrinkle of throat to anterior tip of vent; TAL (tail length): anterior tip of vent to tail tip; VL (vent length): anterior tip 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 length): distance from axilla to tip of the longest finger; HLL (hindlimb length): distance from groin to tip of the longest toe; UJTW (upper jaw teeth width): the greatest width of upper jaw teeth series; UJTL (upper jaw teeth series length): the greatest length of upper jaw teeth series; VTW (vomerine teeth series width): the greatest width of vomerine teeth series; VTL (vomerine teeth series length): the greatest length of vomerine teeth series.

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