

Title	Unmasking <i>Pachytriton labiatus</i> (Amphibia: Urodela: Salamandridae), with Description of a New Species of <i>Pachytriton</i> from Guangxi, China
Author(s)	Nishikawa, Kanto; Jiang, Jian-Ping; Matsui, Masafumi; Mo, Yun-Ming
Citation	Zoological Science (2011), 28(6): 453-461
Issue Date	2011-06
URL	http://hdl.handle.net/2433/216896
Right	© 2011 Zoological Society of Japan
Type	Journal Article
Textversion	publisher

Unmasking *Pachytriton labiatus* (Amphibia: Urodela: Salamandridae), with Description of a New Species of *Pachytriton* from Guangxi, China

Kanto Nishikawa^{1*}, Jian-Ping Jiang², Masafumi Matsui¹ and Yun-Ming Mo³

¹Graduate School of Human and Environmental Studies, Kyoto University,
Yoshida Nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan

²Chengdu Institute of Biology, Chinese Academy of Sciences,
Chengdu 610041, China

³Natural History Museum of Guangxi, Nanning 530012, China

Examination of the lectotype and paralectotypes of *Pachytriton labiatus* (Unterstein, 1930) from southern China revealed that the specimens do not represent a member of *Pachytriton*, but are identical with a newt of another genus, *Paramesotriton ermizhaoi* Wu et al., 2009 also described from southern China. We suggest that *Pac. labiatus* should be transferred to *Paramesotriton* as a senior synonym of *Par. ermizhaoi*. We compared the morphology of the northeastern and southwestern groups of newts previously called *Pac. "labiatus"*, with special reference to age and sexual variations. As a result, we confirmed that the two groups are differentiated sufficiently to be treated as different species. In this report, we revive the name *Pac. granulosus* Chang, 1933 to refer to the northeastern group of *Pac. "labiatus"*, and at the same time, describe a new species representing the southwestern group.

Key words: *Pachytriton labiatus*, *Paramesotriton*, synonym, new species, China

INTRODUCTION

Pachytriton labiatus (Unterstein, 1930) is one of the most popular species among Chinese salamandrids, because it has been traded as pets to many countries. Although morphological variation and breeding behavior in captivity of the species have been reported (e.g., Thiesmeier and Hornberg, 1997; Scholtz, 1998; Sparreboom and Thiesmeier, 1998), its biology in natural habitats is still poorly known.

Unterstein (1930) described this species on the basis of one male and three females from "Yaoshan, Kwangsi, China" (Mt. Dayao, Guangxi Zhuang Autonomous Region, China). All of the type specimens are stored in the Museum of Natural History, Humboldt University Berlin (ZMB). Unterstein (1930) provided measurement data on a subadult (*halbwüchsiges*) specimen, but did not indicate the register number of the specimen. Much later, Bauer et al. (1993) examined the syntypes and designated the lectotype and paralectotypes. However, no one has compared these type specimens with topotypic specimens to date.

Recently, Wu et al. (2010) reported that the newt then called *Pac. "labiatus"* is morphologically separated into two geographically isolated groups, "northeastern *P. labiatus*" (hereafter, NE group) and "southwestern *P. labiatus*" (SW

group), which are differentiated by a large genetic distance (calculated from mitochondrial and nuclear genes) comparable to interspecific levels in *Pachytriton* and other newt genera. Wu et al. (2010), thus concluded that the two groups are sufficiently differentiated to be treated as different species, and proposed to restrict *Pac. labiatus* sensu stricto to the SW group, as the type locality of the species is included in the area of distribution of the SW group.

For the NE group of *Pac. "labiatus"* sensu Wu et al. (2010), the name *Pac. granulosus* Chang, 1933 had been available (for taxonomic history of *Pac. granulosus*, see Nishikawa et al., 2009). However, Hou et al. (2009) revived the name as different genus *Pingia granulosa*. They considered that *Pin. granulosa* is a terrestrial newt, which is different from the sympatric and aquatic *Pac. "labiatus"*. However, Nishikawa et al. (2009) soon showed that *Pin. granulosa* is the immature terrestrial morph of *Pac. granulosus*, which becomes aquatic after maturation. Wu et al. (2010) seems to have followed Hou et al. (2009) and considered that the NE group of *Pac. "labiatus"* had no available name. They, thus, tentatively retained the name *Pac. labiatus* for the NE group, noting that the group was so differentiated in body coloration and genetic distance as to be separated into different taxa. However, as pointed out by Nishikawa et al. (2009), the NE group should be treated as *Pac. granulosus*.

In this paper, we examine the type specimens of *Pac. labiatus* and compare them with topotypic specimens of this species to solve the taxonomic confusion noted above.

* Corresponding author. Phone: +81-75-753-6848;
Fax : +81-75-753-2891;
E-mail: hynobius@zoo.zool.kyoto-u.ac.jp

Supplemental material for this article is available online.
doi:10.2108/zsj.28.453

MATERIALS AND METHODS

Survey on the type specimens of *Pac. labiatus*

We examined all of the four type specimens of *Pac. labiatus*: the lectotype (ZMB 34087) and three paralectotypes (ZMB 34088, 50280, and 50281). For comparison, we examined 35 topotypic *Pac. "labiatus"* (12 males, 17 females, and six juveniles) and 14 *Par. ermizhaoi* (three males, four females, and seven juveniles) in the collection of the Chengdu Institute of Biology (CIB). The specimens of *Par. ermizhaoi* had been catalogued as *Pac. labiatus* or *Par. chinensis* (Gray, 1859) in the CIB collection, but had characteristics that match well with the original description of *Par. ermizhaoi* (Wu et al., 2009): epibranchial nearly straight, maxilla oriented angular to the body axis, and skin slightly wrinkled or smooth without distinct dorsal ridge. By contrast, the topotypic *Pac. "labiatus"* has epibranchials wrapping the neck dorsolaterally, the maxilla connected with the pterygoid, and smooth skin without a dorsal ridge. *Paramesotriton chinensis* has similar skull characteristics with *Par. ermizhaoi*, but has more roughened skin than *Par. ermizhaoi*, with distinct dorsal and dorsolateral ridges. We analyzed complete sequences of mitochondrial cytochrome b gene (1141 bp) of two of the seven topotypic juveniles examined morphologically (CIB GX20070763: GenBank accession no. AB601425, CIB GX20070764: AB601426) and compared them with those of the holotype and a paratype of *Par. ermizhaoi* (CIB 88141: GQ303670, CIB 88140: GQ303671 [Wu et al., 2009]). These four sequences differed from each other by at most 0.17% in uncorrected *P*-distance. Thus the juveniles were confirmed as *Par. ermizhaoi*. Except for a *Par. ermizhaoi* (CIB 21146) collected from unknown locality in Guangxi, all the type specimens and the specimens we examined were collected from the type locality of *Pac. labiatus* and *Par. ermizhaoi*, Mt. Dayao.

The following measurements were taken for comparison: TOL (total length): tip of snout to tip of tail; SVL (snout-vent length): tip of snout to anterior tip of vent; MXHW (maximum head width): measured at widest point; ENL (eye-nostril length): external nare to anterior tip of upper eyelid; IND (internarial distance): minimum distance between external nares; AGD (axilla-groin distance): minimum distance between axilla and groin; TAL (tail length): anterior tip of vent to tail tip; VL (vent length): anterior tip to posterior tip of vent; MXTAH (maximum tail height): tail height measured at highest point; FLL (forelimb length): distance from axilla to tip of longest finger; HLL (hindlimb length): distance from groin to tip of longest toe.

Wu et al. (2009) followed Stuart and Papenfuss (2002) in the measurements and took "TAL" from posterior (anterior in our case) tip of vent to tip of tail, without showing vent length (VL). We thus used values of TAL-VL instead of TAL, in comparing our data with those given by Wu et al. (2009). All measurements were taken to the nearest 0.1 mm with a dial caliper. We used a stereoscopic binocular microscope when necessary. The sex and maturity of the specimens were checked by slight dissections.

We conducted a linear discriminant analysis (DISCRIM; SAS, 1990) for topotypic specimens of *Pac. "labiatus"* (12 males, 17 females, and six juveniles) and *Par. ermizhaoi* (three males, three females, and six juveniles) from Mt. Dayao, Jinxiu using nine log-transformed morphometric characters. We then assessed the association of the type specimens of *Pac. labiatus* and *Par. ermizhaoi* (measurements from Wu et al. [2009]) with topotypic *Pac. "labiatus"* and *Par. ermizhaoi*, from which the linear discriminant functions were obtained.

We examined skull morphology and counted the number of trunk vertebrae of all the type specimens of *Pac. labiatus*, and each of three topotypic specimens of *Pac. "labiatus"* and *Par. ermizhaoi*. These characters were examined by minor dissection or by soft X-ray photographs using Fuji Medical X-Ray Film. For one specimen of *Pac. labiatus* (ZMB 50280) and one of *Par. ermizhaoi* (CIB 21194), whose heads were already skinned, we examined the skull

characters directly.

Morphological variation in *Pac. "labiatus"*

In order to assess taxonomic relationships of populations of *Pac. "labiatus"*, we compared a total of 10 characters noted above, and ratio values of eight characters to SVL. We compared differences in these characters between each topotypic specimen of 1) *Pac. "labiatus"* from a population in Mt. Dayao, Jinxiu (hereafter, JINXIU) that belongs to the SW group sensu Wu et al. (2010), represented by 12 males, 17 females, and six juveniles; and 2) *Pac. granulatus* from a population in Tiantai, Zhejiang (hereafter, TIANTAI), the NE group sensu Wu et al. (2010), nine males, 11 females, and three juveniles (Fig. 1).

Recently, Hou et al. (2009) designated the neotype of *Pingia granulosa* (= *Pac. granulatus* whose holotype seemed to be lost in WWII: see Nishikawa et al., 2009) collected from a locality ca. 200 km northeast of the type locality (Mt. Longwang, Zhejiang Province). The neotype seems to be a juvenile and, more problematically, a terrestrial morph, which is rarely encountered and shows morphology quite different from adults, which are much easier to find (Nishikawa et al., 2009). Thus, the neotype is not practically suitable for comparison with adult specimens, and Nishikawa et al. (2009) could not find any difference between the neotype and specimens from the type locality (although only juveniles were compared). Because of this situation, we used available adult samples from the type locality of *Pac. granulatus* (TIANTAI) as representing the NE group.

Differences in metric (mm) and meristic (ratio) values of adults were statistically tested between the two populations (JINXIU and TIANTAI) by Student's *t*-test and Mann-Whitney's U test, respectively. The significance level was assumed as 95% in all these statistical tests.

RESULTS

Notes on types of *Pac. labiatus*

The type specimens of *Pac. labiatus* were in good condition (Table 1, Fig. 2A–D). The measurements (in mm) of the lectotype were nearly identical to those shown in the

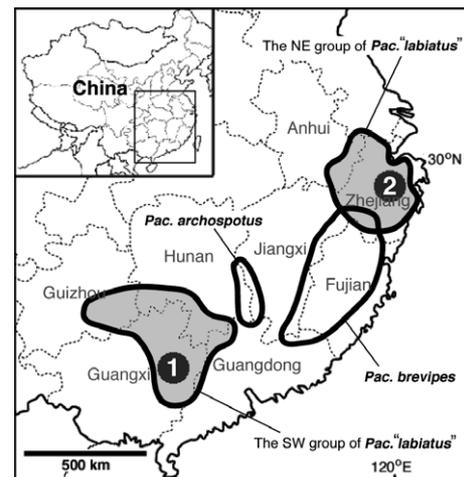


Fig. 1. A map of China showing populations examined (1 = JINXIU: a population from the type locality of *Pac. labiatus*, 2 = TIANTAI: a population from the type locality of *Pac. granulatus*) and distribution of *Pachytriton* species. Shaded areas showing range of *Pac. "labiatus"* currently recognized (data modified from Fei et al. [2006] and Wu et al. [2010]). For locality information of samples, refer to the text.

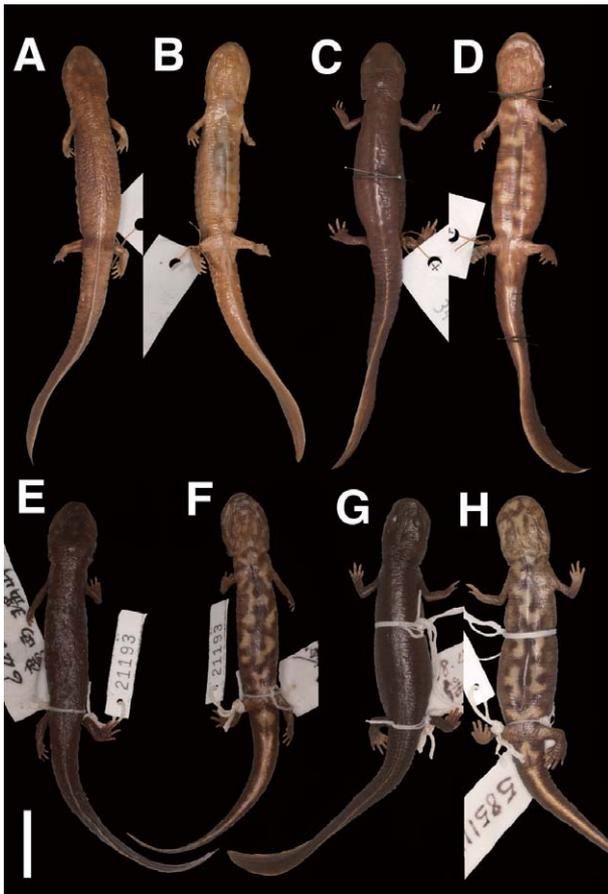


Fig. 2. Male lectotype (ZMB 34087: **A, B**) and female paralectotype (ZMB 34088: **C, D**) of *Pachytriton labiatus*, and male (CIB 21193: **E, F**) and female topotypes (CIB 21206: **G, H**) of *Paramesotriton ermizhaoi*; dorsal (**A, C, E, G**) and ventral views (**B, D, F, H**). Scale bar = 20 mm.

original description given by Unterstein (1930): TOL = 123, TAL = 58, TRL = 48, and HL = 17. In the original description, one male and three females were reported to be included in the syntypes. Bauer et al. (1993) admitted these contents and assigned one male to the lectotype (ZMB 34087). However our examination revealed that the former syntypes include two males (ZMB 34087 and 50281) and two females (ZMB 34088 and 50280), all of which are certainly mature except for one female (ZMB 50280, probably subadult).

Other than minor misidentifications in sex and maturity, the original description illustrates the four specimens well: "Body slender. Vomero-palatine teeth in two rows, beginning

slightly anterior to the line connecting anterior borders of choanae. Meeting together on top, then diverging in two weak arches; choana elongated, about three times as long as broad, obliquely extending posterolaterally. Tongue very wide, occupying almost whole breadth of mouth. Head oval, longer than broad. Head width comprises 4.5–5 times in length from snout tip to vent. Head length comprises 4–4.5 times in the same distance. Snout straightly cut anteriorly, projecting widely over mouth, in profile oblique cut posteriorly. Anterior part of head flat, swollen in the lump-like neck region, an anteriorly opened V-shaped protrusion. Canthus rostralis rounded but distinct. Lips laterally forming an arch and widely protruded over mouth slit. Mouth angle positioned at just below eye angle. Loreal region obliquely cut, distinctly concave. Nostrils small, positioned at angles of truncate snout. Space between nostrils equals to eye length. Gular fold distinct, forming an anteriorly convex arch. Parotoid flat, sharply cut at neck, thus giving an impression of being carved. Fore- and hindlimbs well developed, without webbing or palmar tubercles. Anteriorly stretched forelimb reaches mouth angle. A space about the length of a finger is left between closely set fore- and hindlimbs. Tail is laterally strongly compressed like a sword, posteriorly rounded, about as long as head and body. Dorsally and ventrally equipped with sharp skin edges. Anal lip strongly swollen, elongated, and equipped with long papillae in males, but small and elongated oval in females. Skin is smooth, or more or less transversely wrinkled, or folded. Color (in alcohol) dark gray brown on dorsal side, large spots united with yellow on ventral side. Throat clouded with yellow. Dorsal and ventral edges of tail yellowish, more or less sharply contrasting against ground color. On sides of tails in males, there are several irregularly scattered yellowish spots. (originally in German and translated with minor modification by authors)".

The following additional information will aid in defining *Pac. labiatus*: Maximum head width positioned at jaw angle (Fig. 2A–D). Upper jaw tooth series much wider than long (ca. 1.7 times). Fore- and hindlimbs thin. Dorsal tail ridge prominent light yellowish in color and sometimes reaching to posterior body. Tip of tail not so broad as in the remaining species of *Pachytriton* (*Pac. archospotus* and *Pac. brevipes*). On ventral side, many dark spots, often enclosing yellowish flecks. Epibranchial nearly straight, maxilla oriented angular to body axis and not connected with pterygoid (Fig. 3A, B), 12 trunk vertebrae (Fig. 4A).

Morphological comparisons between *Pac. labiatus* and *Par. ermizhaoi*

In the original description of *Pac. labiatus*, Unterstein (1930) noted the well-developed upper labium, as expressed in its specific name. But, the degree of labial fold development in the type specimens was much less than that found in topotypic specimens of *Pac. "labiatus"*, although it was similar to that of *Par. ermizhaoi* (lf in Fig. 3). Skins of the type specimens of *Pac. labiatus* were not as smooth as those of topotypic *Pac. "labiatus"*, but were slightly roughened or sometimes wrinkled, as in *Par. ermizhaoi*. The type specimens of *Pac. labiatus* and topotypes of *Par. ermizhaoi* had the

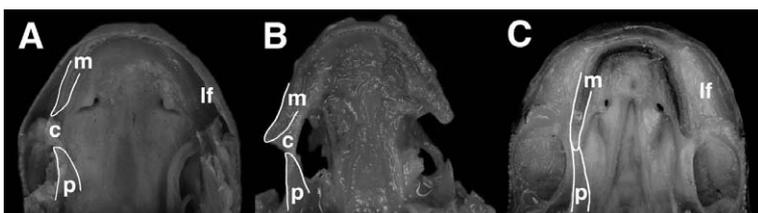


Fig. 3. Open mouths of paralectotypes (**A**) ZMB 34088, (**B**) ZMB 50280, of *Pachytriton labiatus* and a topotype of *Pac. "labiatus"* (**C**) CIB GX20090526. c: cartilage, lf: labial fold, m: maxilla, p: pterygoid.

Table 1. Measurements of type specimens, and means \pm SD of 10 characters (in mm) and medians of ratios of characters (R: %SVL) of the female of *Par. ermizhaoi* were skinned, thus some head characters of the specimens are lacking. Asterisks showing significantly larger values 0.01; ***: $P < 0.001$; ns ≥ 0.05). For character abbreviations, refer to text. #measurements from Wu et al. (2009).

	<i>Pac. labiatus</i>				<i>Par. ermizhaoi</i>			
	ZMB 34087	ZMB 50281	ZMB 34088	ZMB 50280	CIB 88141#	CIB		
	Lectotype Male	Male	Female	Female	Holotype Male	3 Males	4 Females	7 Juveniles
TOL	122.6	112.4	114.3	113.1	126.0	141.6 \pm 10.7 (131.8–153.0)	144.3 \pm 21.9 (117.2–169.4)	86.8 \pm 15.4 (72.9–118.5)
SVL	61.7	58.6	57.4	–	63.5	72.3 \pm 4.0 (68.5–76.5)	73.3 \pm 11.9 (59.4–87.0)	43.7 \pm 7.7 (38.4–60.0)
MXHW	12.5	12.6	11.6	–	12.1	14.8 \pm 1.4 (13.8–16.4)	13.8 \pm 1.9 (11.4–16.0)	9.9 \pm 1.6 (8.2–12.59)
ENL	4.3	4.2	3.8	–	4.9	5.1 \pm 0.5 (4.6–5.6)	4.8 \pm 0.7 [n = 3] (4.1–5.5)	3.7 \pm 0.7 [n = 6] (3.1–5.1)
IND	3.0	2.9	2.9	2.4	3.7	4.2 \pm 0.6 (3.8–4.9)	3.7 \pm 1.0 (2.4–4.7)	2.7 \pm 0.5 (2.1–3.6)
AGD	32.5	30.5	28.0	30.1	31.3	40.5 \pm 3.5 (36.5–42.6)	38.5 \pm 8.9 (31.4–50.4)	20.5 \pm 3.9 (17.2–25.8)
TAL-VL	57.6	48.1	54.0	51.5	57.7	64.6 \pm 5.6 (59.3–70.5)	68.1 \pm 9.5 (55.6–78.6)	41.2 \pm 7.7 (32.8–56.2)
MXTAH	10.1	10.1	8.9	9.0	7.5	11.2 \pm 0.6 (10.6–11.8)	10.3 \pm 1.1 (8.8–11.2)	6.3 \pm 1.1 (5.0–8.1)
FLL	14.4	15.1	13.8	13.4	15.6	15.8 \pm 0.4 (15.4–16.1)	17.0 \pm 2.5 (14.1–19.8)	11.5 \pm 1.2 (9.9–13.2)
HLL	16.6	16.5	16.0	14.3	16.0	18.0 \pm 1.2 (16.7–19.1)	18.4 \pm 2.5 (15.4–21.4)	12.6 \pm 2.0 (10.5–16.3)
R (% of SVL)								
RMXHW	20.3	21.5	20.2	–	19.1	20.7 (19.2–21.4)	19.2 (16.6–20.5)	13.8 (12.9–17.7)
RENL	7.0	7.2	6.6	–	7.7	6.8 (6.7–7.8)	6.9 [n = 3] (5.5–7.1)	8.3 [n = 6] (8.0–8.6)
RIND	4.9	4.9	5.1	–	5.8	5.5 (5.4–6.4)	5.3 (3.5–6.0)	6.1 (5.4–6.7)
RAGD	52.7	52.0	48.8	–	49.3	55.7 (50.8–61.9)	52.2 (46.6–57.9)	47.5 (44.3–49.0)
R (TAL-VL)	93.4	82.1	94.1	–	90.9	88.9 (86.6–92.2)	92.0 (90.0–98.4)	95.2 (84.5–97.9)
RMXTAH	16.4	17.2	15.5	–	11.8	15.4 (15.4–15.5)	14.5 (12.9–14.8)	13.8 (12.9–17.7)
RFL	23.3	25.8	24.0	–	24.6	21.4 (21.0–23.4)	23.3 (22.8–23.7)	27.4 (22.0–28.5)
RHLL	26.9	28.2	27.9	–	25.2	25.0 (23.2–26.6)	25.3 (24.0–26.0)	29.4 (27.2–31.2)

MXHW at jaw angle, protrusive tongue with free lateral margin, and no or very weakly developed webbing on fingers and toes, but most of the topotypes of *Pac. "labiatus"* had MXHW at level of parotoid gland, tongue fully attached to mouth floor, and well-developed webbing on fingers and toes.

Coloration of the type specimens of *Pac. labiatus* was obscure, but the pattern was recognizable and was almost the same as that of *Par. ermizhaoi*. They had irregularly connected blotches and many dark spots. By contrast, they differed from topotypes of *Pac. "labiatus"*, by possessing more isolated blotches without dark spots on ventral side (Figs. 2, 5).

In measurement and ratio values, the type specimens of *Pac. labiatus* and the holotype and topotypes of *Par.*

ermizhaoi were also similar, but together they were different from the topotypic *Pac. "labiatus"* (Table 1). The type specimens of *Pac. labiatus* and the holotype and topotypes of *Par. ermizhaoi* had smaller values in almost all the characters measured and in relative size related to head (RMXHW) and snout (RENL, RIND) than topotypic *Pac. "labiatus"*. In contrast, the type specimens of *Pac. labiatus* and the holotype and topotypes of *Par. ermizhaoi* had larger relative values in trunk length (RAGD), tail length [R (TAL-VL)], tail height (RMXTAH), and limb lengths (RFL and RHLL) than the topotypic *Pac. "labiatus"*.

As a result of DISCRIM, both topotypes of *Pac. "labiatus"* and *Par. ermizhaoi* were separated into two different groups each with a high rate (91.4% in *Pac. "labiatus"* and 100% in *Par. ermizhaoi*). Based on the functions obtained, it was

specimens examined. Ranges in parenthesis. Heads of a paralectotype (ZMB 50280) and a between the SW and NE groups of *Pac. "labiatus"* in corresponding sex (*: $P < 0.05$; **: $P <$

the SW group of <i>Pac. "labiatus"</i> (JINXIU) <i>Pac. inexpectatus</i> sp. nov.			the NE group of <i>Pac. "labiatus"</i> (TIANTAI) <i>Pac. granulatus</i>		
CIB			CIB		
12 Males	17 Females	6 Juveniles	9 Males	11 Females	3 Juveniles
163.5 ± 20.4**	176.0 ± 17.7***	108.9 ± 14.6	138.6 ± 11.6	140.4 ± 16.0	98.6 ± 7.7
(128.2–196.9)	(144.1–206.6)	(90.2–127.1)	(120.8–159.1)	(116.1–165.8)	(92.0–107.0)
85.9 ± 9.6***	92.3 ± 9.4***	56.6 ± 8.0	69.0 ± 6.1	70.9 ± 7.7	49.9 ± 3.8
(68.6–99.1)	(75.9–108.6)	(45.6–65.8)	(59.0–78.9)	(59.9–81.6)	(46.1–53.6)
19.4 ± 2.5***	20.3 ± 2.3***	12.4 ± 1.9	13.8 ± 1.4	13.8 ± 1.6	10.2 ± 0.6
(15.4–23.5)	(16.1–24.6)	(9.3–14.2)	(12.3–16.9)	(11.8–17.6)	(9.6–10.8)
6.0 ± 0.7***	6.2 ± 0.6***	4.2 ± 0.5	4.6 ± 0.3	4.6 ± 0.3	3.7 ± 0.2
(5.0–6.8)	(5.0–7.2)	(3.5–5.0)	(3.9–5.1)	(4.2–5.1)	(3.5–3.8)
5.6 ± 0.7***	5.8 ± 0.7***	3.5 ± 0.8	4.0 ± 0.3	4.6 ± 0.4	3.0 ± 0.2
(4.6–6.6)	(4.3–6.8)	(2.4–4.4)	(3.4–4.4)	(3.4–4.8)	(2.8–3.2)
42.3 ± 5.7**	46.8 ± 6.1***	27.3 ± 3.8	35.3 ± 3.3	37.6 ± 5.0	26.4 ± 1.5
(31.1–52.0)	(35.9–54.4)	(23.1–31.8)	(31.2–41.2)	(30.8–45.8)	(24.8–27.7)
72.8 ± 10.6*	79.6 ± 8.7***	49.8 ± 6.4	64.2 ± 5.1	65.9 ± 8.4	45.5 ± 4.0
(56.1–92.7)	(64.8–94.4)	(42.2–58.1)	(57.7–73.4)	(52.8–80.7)	(42.8–50.1)
12.4 ± 1.9ns	13.9 ± 2.5**	7.6 ± 1.9	11.2 ± 1.7ns	11.2 ± 2.2	7.1 ± 0.5
(9.7–15.0)	(10.0–20.6)	(4.6–9.3)	(9.2–14.6)	(9.2–16.6)	(6.8–7.6)
17.6 ± 1.8**	18.0 ± 1.7**	13.4 ± 1.0	15.5 ± 0.9	16.0 ± 1.4	13.4 ± 1.3
(15.2–20.1)	(14.7–20.6)	(11.7–14.4)	(13.9–16.8)	(14.4–18.4)	(12.5–14.8)
21.6 ± 1.9***	22.5 ± 2.3***	15.8 ± 1.2	17.3 ± 1.3	17.7 ± 1.2	14.5 ± 0.5
(19.1–24.2)	(18.3–26.6)	(13.9–16.8)	(15.3–18.7)	(15.8–19.2)	(14.0–14.9)
22.3***	21.5**	22.2	20.0	19.2	20.6
(20.7–25.2)	(20.6–24.6)	(20.4–22.8)	(17.9–21.5)	(17.6–25.1)	(20.1–20.8)
7.0ns	6.6ns	7.5	6.6ns	6.8ns	7.6
(6.4–7.9)	(5.8–7.3)	(6.7–7.7)	(6.2–7.4)	(5.5–7.3)	(7.1–7.6)
6.5**	6.2*	6.1	6.0	5.5	6.1
(5.9–7.4)	(5.7–7.1)	(5.3–6.7)	(4.6–6.4)	(4.8–6.8)	(5.6–6.4)
49.5ns	50.1	48.1	51.7ns	52.7**	53.7
(43.7–53.7)	(46.0–59.6)	(44.8–51.0)	(48.6–52.9)	(50.5–56.1)	(51.7–53.8)
83.1	86.1	88.6	93.0***	92.8**	92.8
(80.1–93.5)	(76.7–95.4)	(82.5–92.5)	(87.4–99.5)	(87.6–98.9)	(87.6–93.5)
14.9	14.7ns	14.1	16.2*	15.4ns	14.8
(12.4–15.5)	(11.7–19.8)	(10.1–14.9)	(13.3–19.1)	(12.9–20.3)	(112.7–15.2)
20.3	19.5	22.6	23.1**	22.5***	27.1
(18.5–23.6)	(16.8–22.0)	(21.3–29.4)	(20.7–23.6)	(20.9–24.5)	(25.7–27.6)
24.8ns	24.1ns	26.7	25.3ns	25.4ns	29.5
(23.2–35.3)	(22.2–27.3)	(25.5–35.3)	(23.4–27.4)	(21.8–27.8)	(27.8–30.4)

shown that all of the type specimens of *Pac. labiatus* and the holotype of *Par. ermizhaoi* were associated with the topotypes of *Par. ermizhaoi*.

In the type specimens of *Pac. labiatus* (Figs. 3A, B, 4A) and topotypes of *Par. ermizhaoi*, the posterior tip of maxilla was anterior or lateral to the anterior tip of pterygoid (both bones were connected by a cartilage) and the tip of epibranchial was nearly straight. These conditions were not found in the topotypic *Pac. "labiatus"* (Figs. 3C, 4B, C), in which maxilla and pterygoid were well united, nearly forming a straight line, and the tip of epibranchial was strongly curved. The difference in these characters was stable irrespective of sex and age (Fig. 4).

Soft X-ray photographs showed no asymmetric connection between sacrum and ilium, and all the specimens exam-

ined had 12 trunk vertebrae (Fig. 4).

Morphological difference between two populations of *Pac. "labiatus"*

In both sexes, the JINXIU (the SW group) showed significantly larger values than the TIANTAI (the NE group) in all measurements, with the exception of male MXTAH (Table 1). Labial fold and webs tended to be much better developed in JINXIU than in TIANTAI.

In character ratios to SVL, JINXIU had significantly larger RMXHW and RIND than TIANTAI in both sexes. In contrast, TIANTAI had larger R (TAL-VL) and RFLL than JINXIU in both sexes, RAGD in females, and RMXTAH in males (Table 1). We could not determine the statistical difference between juveniles of the two populations, due to the small number of specimens available, but tendencies similar to adults were recognized. In summary, JINXIU had relatively large head, and TIANTAI tended to have relatively long trunk, tail, and limb, and high tail.

In addition to differences in body size and proportion, body coloration tended to differ between the two populations. Generally, the dorsal ground color was darker in TIANTAI than JINXIU, and the former population tended to possess black spots on dorsum and prominently on lateral side of tail, which made some individuals look like *Pac. brevipes*. TIANTAI often had reddish orange dots on dorsolateral ridges. Adults of JINXIU rarely had such dots, but juveniles sometimes had orange dots, which usually disappear through growth. Male adults of TIANTAI had silvery markings laterally on tail tip, but such markings were not seen in JINXIU.

From the present examination of the type specimens, it is evident that

Pac. labiatus is quite different from the newt now known by that name, but is identical with *Par. ermizhaoi* Wu et al. (2009). Thus, *Par. ermizhaoi* is relegated to a junior synonym of *Pac. labiatus*. Because morphological data (and also molecular one by Wu et al. [2009]) showed that the species belonged not to *Pachytriton* (type species: *Pac. brevipes*) but to *Paramesotriton*, *Pachytriton labiatus* must be called "*Paramesotriton labiatus*".

The present survey indicated that the NE group should be referred to as *Pac. granulatus*. Meanwhile, the SW group has no name available in the nomenclature, as the present study shows that the type specimens of *Pac. labiatus* are different from the newts in this group. We, therefore, describe the SW group as a new species.

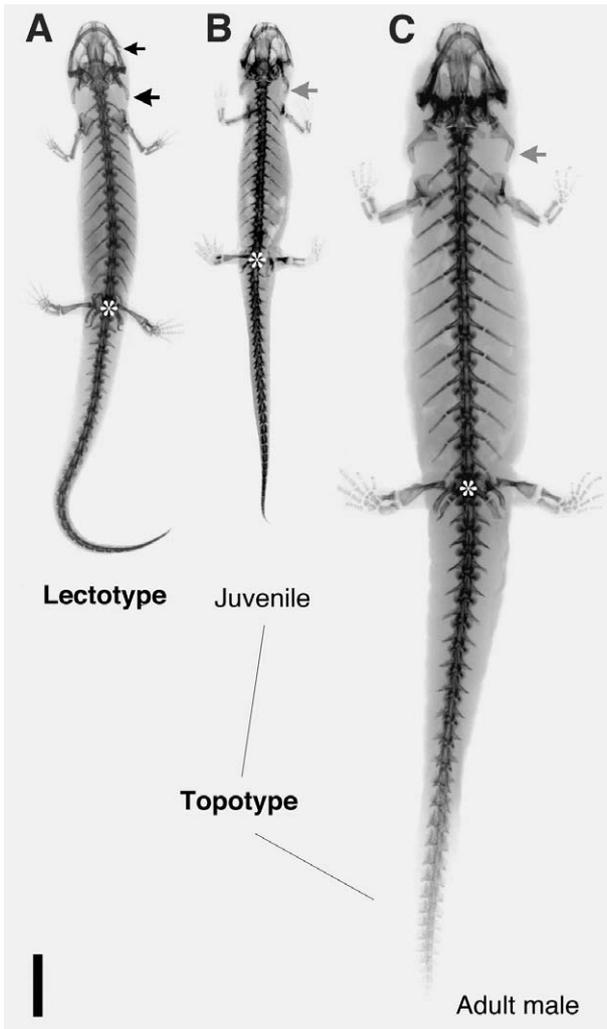


Fig. 4. X-ray photographs of the lectotype (A: ZMB 34087) and topotypes of a juvenile (B: CIB GX20081029) and an adult male (C: CIB GX20081006) of *Pachytriton labiatus*. Large arrows showing the tip of epibranchials (black: nearly straight; grey: curving as if wrapping the neck). A small arrow showing the tip of maxilla oriented angular to the body axis. Asterisks showing the sacrum vertebrae. Scale bar = 10 mm.

SYSTEMATICS

Pachytriton inexpectatus sp. nov.
(Figs. 5, 6)

Diagnosis

A large-sized newt of the genus *Pachytriton*, males 68.6–99.1 mm (n = 12), females 75.9–108.6 mm (n = 17) SVL; body stout; skin smooth; tips of fore- and hindlimbs adpressed on body widely separated; tip of tail broad; uniformly dark or pale brown on dorsum, usually without bright orange dots dorsolaterally; no black spots over body; similar to *Pac. granulatus*, but has larger and more robust body and wider head.

Etymology

The specific epithet, a Latin adjective, refers to our “unexpected” finding of this new species, which has been a



Fig. 5. Male holotype (CIB GX20081006) of *Pachytriton inexpectatus* (formerly, the SW group of *Pac. “labiatus”*); dorsal (A) and ventral views (B). Scale bar = 20 mm.



Fig. 6. Female paratype (CIB GX20081008) of *Pachytriton inexpectatus* (formerly, the SW group of *Pac. “labiatus”*) in life.

common pet animal but has never been considered as unnamed.

Holotype

CIB GX20081006, an adult male from Mt. Dayao, Jinxiu

Yao Autonomous County, Guangxi Zhuang Autonomous Region, People's Republic of China (24°5' N, 110°13' E, 1140 m a.s.l.), collected on 10 October 2008 by Kanto Nishikawa.

Paratypes

A total of 10 specimens, all from the type locality. CIB GX20070734 collected in 2007 by Fu-Cai Gong; CIB GX20071101 and 20071102 collected on 25 November 2007 by Kanto Nishikawa, Jian-Ping Jiang, and Yun-Ming Mo; CIB GX20081002-04 collected on 9 October 2008 by Kanto Nishikawa and Masafumi Matsui; CIB GX20081007 and 20081008 collected on 10 October 2008 by Kanto Nishikawa and Jian-Ping Jiang; CIB GX20081028 and 20081029 collected on 10 October 2008 by Jian-Ping Jiang.

Referred specimens

CIB 21137, 21139, 21140, 21143–45, 21147, 21175–84, 21186–89, and 21195 collected from the type locality.

Description of holotype

An adult male with the following measurements (mm; in preservative): SVL 99.1, head length 28.7, head width at jaw angle 20.1, MXHW 23.1, tip of snout to anterior tip of upper eyelid 8.6, tip of lower jaw to angle of jaw 11.7, ENL 6.4, IND 6.3, interorbital 7.8, greatest width of upper eyelid 2.5, greatest length of upper eyelid 4.0, maximum diameter of orbit 2.9, AGD 49.6, fold of throat to anterior tip of vent 70.4, TAL 97.8, VL 5.1, tail width measured at base of tail 15.6, tail width measured at middle 11.7, tail height measured at base of tail 13.2, MXTAH 15.0, tail height measured at middle 13.4, FLL 19.7, HLL 23.2, greatest width of upper jaw tooth series 9.4, greatest length of upper jaw tooth series 7.8, greatest width of vomerine tooth series 4.1, greatest length of vomerine tooth series 10.5. Numbers of upper jaw teeth 68, lower jaw teeth 77, and vomerine teeth 127. Tongue fully attached to mouth floor. Costal grooves between axilla and groin 11. Adpressed limbs separated by 3.5 costal folds. Fingers and toes slightly webbed.

Color in life

Dorsum uniformly dark brown. Venter lighter than dorsum. Reddish orange markings on venter, some in contact, forming two longitudinal lines. Small reddish orange markings scattered on throat and underside of limbs. Underside of tail and cloaca reddish orange.

Color in preservative

Dorsal coloration tended to fade and become light brown. Ventral bright markings faded to pale cream.

Eggs and larvae

Eggs or larvae are rarely found in nature. Only Fei et al. (2006) has reported developed embryos from Leishan, Guizhou Province in June. The number of eggs found in ovaries of six females from Guangxi Zhuang Autonomous Region ranged from 32 to 89 (mean = 51.8). The mean diameter of ova found in ovaries of the above six females ranged from 3.4 to 4.7 (gross mean = 3.9) mm. Both the animal and the vegetal poles of ripe eggs were beige, and unripe ones were cream in color.

Variation

Individuals of type series are generally similar in morphology and vary only slightly within each age and sex. Females are larger than males in body size. In proportion, males have relatively large heads and long limbs. Ventral reddish orange markings tend to be much brighter in juveniles and diffused in larger adults. Some individuals, especially juveniles, have an orange spot on the dorsal base of each limb. The degree of development of webbing on fingers and toes are variable. Each testis of larger males tends to be segmented up to four lobes.

Range

Western Guizhou Province, southwestern Henan Province, northwestern Guangdong Province, and northern and western Guangxi Zhuang Autonomous Region, People's Republic of China (also see Fig. 1).

Natural history

The adults are nocturnal and usually found in montane streams. Some adults are found in very steep and shallow branch streams about 90 m from the main stream. Adults prey under the water. Their stomach contents include aquatic insect larvae such as Diptera, Ephemeroptera, Plecoptera, and Trichoptera, as well as Annelida (earthworm) and Decapoda (fresh water crab). They also predate terrestrial animals including larval Hymenoptera (sawfly), probably fallen down from vegetation near the stream. Breeding ecology and life history in nature are poorly known. Because females with ripe eggs were collected in May and June, breeding seems to occur in early summer. Some adults of both sexes seem to be bitten by competitive individuals. Small juveniles are rarely found in the stream, and are probably terrestrial.

DISCUSSION

The present study shows that *Par. labiatus* is a senior synonym of *Par. ermizhaoi*. All characteristics of *Par. labiatus* were identical between the present study and Wu et al. (2009), except for the number of trunk vertebrae. Wu et al. (2009) reported that 55% of 11 specimens had 13 trunk vertebrae (remaining 45% had 12). Although we could only examine a smaller number of specimens (N = 7), we did not find any variation in the number (all 12). Wu et al. (2009) showed an X-ray photograph of one specimen, which they interpreted to have 13 trunk vertebrae. However, vertebrae articulating with ilium seem to be the 13th on the left side and the 14th on the right side in the photograph. In this case, the number of trunk vertebrae of the specimen should be counted as 12. In order to clarify the reason for the high variation in the counts in Wu et al. (2009), we need to carefully examine more specimens.

In the present study, we could not examine detailed geographic morphological variations in *Pac. inexpectatus* and *Pac. granulosus*, but have found some variations, at least, in body coloration. For example, populations of *Pac. inexpectatus* from Guizhou and Henan Provinces often have dorsolateral dots, even in adults (rare in the type locality in Guangxi). Meanwhile, a population of *Pac. granulosus* from Huangshan, Anhui Province lacks the dots in most of adults (frequent in the type locality in Zhejiang). Because dorsolat-

eral dots are also found in *Pac. brevipes* (Wu et al., 2010), the presence or absence of dots (also black spots, see above) cannot always diagnose the congeners.

It has been considered that species of *Pachytriton* have smooth skin and lack lateral and dorsal ridges in contrast to *Paramesotriton*, whose skin is roughened and ridges are distinct (Fei et al., 2006). Wu et al. (2009) first revealed that these morphological differences do not always differentiate the two genera, because *Par. labiatus* (as *Par. ermizhaoi* in their paper) has a relatively smooth skin without distinct ridges. The external morphology of the species was much similar to *Pachytriton* than to *Paramesotriton* at a glance, but its skull character indicates closer relationship to the latter than to the former genus. The genus *Pachytriton* could be characterized by the possession of a nearly straight bony connection between maxilla and pterygoid, while in *Paramesotriton*, these bones usually do not contact and, when they do, they do not form a straight line (see also Chang, 1933; Nishikawa et al., 2009).

It is known from molecular phylogenetic analyses that *Pachytriton* and *Paramesotriton* form a monophyletic group (Weisrock et al., 2006; Steinfartz et al., 2007). In spite of their close phylogenetic relationship, their habitat preferences are contrasting. Species of *Paramesotriton* occur in variable habitats such as streams, ponds, and forest floor, while *Pachytriton* occurs only in montane streams, except for in terrestrial juvenile stages. In adaptation to stream habitat, *Pachytriton* must have achieved several derived characters, such as a smooth body for reducing water resistance and improving cutaneous respiration, strong and broad tail for increasing propulsive force, and specialized skull, hyoid elements, and tongue for suction feeding. *Paramesotriton labiatus* has an exceptionally smooth skin without prominent ridges that are widely seen in the genus, and this trait might have been one of first adaptations for life in the montane stream. This species is found in streams at lower altitudes than *Pac. inexpectatus* on Mt. Dayao (our personal observation; Wu et al., 2009). The ancestor of *Pachytriton* may have been a newt such as *Par. labiatus*, and later become a pioneer using the habitat of higher altitude (i.e., steeper) streams by acquiring adaptive characteristics noted above.

Interestingly, not only the smooth skin, but also other unique characteristics of *Pachytriton* are shared with at least one species of *Paramesotriton*. In adult *Pachytriton*, the tongue is fully attached on the mouth floor, and this condition is also found in *Par. laoensis* (see Stuart and Papenfuss, 2002). The tongue is protrusive in juveniles but changes to unite with the mouth floor through growth for suction feeding in *Pachytriton* (Nishikawa et al., 2009). Ontogenetic change of the tongue from protrusive to be attached is likely to have evolved in parallel in *Pachytriton* and *Par. laoensis*.

In the eastern area of China, habitats of montane streams are occupied by *Pachytriton* and some *Paramesotriton*. However, in the central and western areas, such habitats are monopolized by aquatic hynobiid salamanders, such as *Batrachuperus*, *Liua*, and *Pachyhynobius*. There has been no report of sympatric occurrence of these aquatic salamanders and hynobiids in China, but several species belonging to the same family can be found in a single stream. This phenomenon could be caused by their phylogeographic history, climate preference, and/or an extinction of one group

through competition with the other. Before testing this hypothesis, we first delineate species boundary more accurately and revise the taxonomy of these aquatic newts and salamanders.

In the present study, the newts formerly misidentified as *Pac. labiatus* are separated into two species (*Pac. inexpectatus* and *Pac. granulatus*). Habitats of these two species have been severely damaged by the recent extensive exploitation of the natural environment in China. Further, these species are actively collected and traded as pets, both domestically and internationally. These situations may lead to threats of extinction to the two species in the near future. Legal protection of their habitat (including the terrestrial one for juveniles), and regulation of excessive commercial collection will be important measures for their conservation.

ACKNOWLEDGMENTS

We would like to thank Liang Fei for help in obtaining literature, Natsuhiko Yoshikawa for laboratory assistance, Mark-Oliver Rödel (ZMB) and Yue-Zhao Wang (CIB), for allowing us to examine specimens under their care, and Max Sparreboom and an anonymous reviewer for improving the earlier version of the manuscript. This work was partly supported by grants of the Ministry of Education, Science and Culture, Japan (No. 20770066) and the Kyoto University Foundation in 2008 to KN and by the National Natural Science Foundation of China (NSFC-31071906, 30730029) to JPJ.

REFERENCES

- Bauer AM, Good DA, Günther R (1993) An annotated type catalogue of the caecilians and salamanders (Amphibia: Gymnophiona and Caudata) in the Zoological Museum, Berlin. Mitt Zool Mus Berlin 69: 285–306
- Chang MLY (1933) On the salamanders of Chekiang. Contri Biol Lab Sci Soc China, Nanjing 9: 305–328
- Fei L, Hu SQ, Ye CY, Huan YZ (2006) Fauna Sinica Amphibia. Beijing Science Press, Beijing
- Hou M, Zhou ZC, Li PP, Lü SQ (2009) Rediscovery of *Pingia granulatus* (Chang, 1933), and description of the neotype specimens. Sichuan J Zool 28: 15–18
- Nishikawa K, Jiang JP, Matsui M, Chen CS (2009) Morphological variation in *Pachytriton labiatus* and a re-assessment of the taxonomic status of *P. granulatus* (Amphibia: Urodela: Salamandridae). Cur Herpetol 28: 49–64
- SAS (1990) SAS/STAT User's Guide. SAS Institute Incorporation, Cary
- Scholtz KP (1998) Über eine rauhhäutige *Pachytriton*-Art. Salamandra 34: 375–380
- Sparreboom M, Thiesmeier B (1998) Courtship behaviour of *Pachytriton labiatus* (Caudata: Salamandridae). Amphibia-Reptilia 20: 339–344
- Steinfartz S, Vicario S, Arntzen JW, Caccone A (2007) A Bayesian approach on molecules and behavior: reconsidering phylogenetic and evolutionary patterns of the Salamandridae with emphasis on *Triturus* newts. J Exper Zool 308B: 139–162
- Stuart BL, Papenfuss TJ (2002) A new salamander genus *Paramesotriton* (Caudata: Salamandridae) from Laos. J Herpetol 36: 145–148
- Thiesmeier B, Hornberg C (1997) Paarung, Fortpflanzung und Larvalentwicklung von *Pachytriton* sp. (*Pachytriton* A) nebst Bemerkungen zur Taxonomie der Gattung. Salamandra 33: 97–110
- Unterstein W (1930) Beiträge zur Lurch- und Kriechtierfauna Kwangsi's: II. Schwanzlurche. Sitzungsber Ges Naturforsch Freunde Berlin 1930: 313–315

Weisrock DW, Papenfuss TJ, Macey JR, Litvinchuk SN, Polymeni R, Ugurtas IH, et al. (2006) A molecular assessment of phylogenetic relationships and lineage accumulation rates within family Salamandridae (Amphibia, Caudata). *Mol Phylogen Evol* 41: 368–383

Wu Y, Rovito SM, Papenfuss TJ, Hanken J (2009) A new species of the genus *Paramesotriton* (Caudata: Salamandridae) from

Guangxi Zhuang Autonomous Region, southern China. *Zootaxa* 2060: 59–68

Wu Y, Wang Y, Jiang K, Hanken J (2010) Homoplastic evolution of external colouration in Asian stout newts (*Pachytriton*) inferred from molecular phylogeny. *Zool Scr* 39: 9–22

(Received July 13, 2010 / Accepted November 16, 2010)