Comparative Osteology of the Genus *Pachytriton* (Caudata: Salamandridae) from Southeastern China

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Abstract Osteological evidence provides invaluable insights into patterns of amphibian biodiversity. In small montane streams of southeastern China, an endemic genus of salamanders (*Pachytriton*) displays remarkable aquatic specializations, many of which are reflected in skeletal morphology, but these specializations remain to be studied in an integrated perspective. Attempts to fully resolve the taxonomy within the genus also can benefit from knowledge of internal morphology. We present a detailed description of the adult skeleton of *P. brevipes*, *P. inexpectatus* and *P. archospotus* by analyzing both cleared-and-stained and radiographed specimens in a comparative framework. Compared to terrestrial and amphibious salamanders, the most distinctive osteological features of *Pachytriton* include a modified hyobranchial apparatus, a reduced frontosquamosal arch, and deep neural and haemal arches of the caudal vertebrae. The hyobranchial apparatus of *P. archospotus* is distinctly different from that of congeners and likely secondarily derived. Patterns of interspecific variation suggest that northeastern *P. inexpectatus* is more closely related to *P. brevipes* than it is to southwestern *P. inexpectatus*, thereby reinforcing results from earlier molecular phylogenetic analyses. We advocate assigning northeastern *P. inexpectatus* to *P. brevipes*.

Keywords montane amphibian, anatomy, aquatic specialization, interspecific variation, taxonomy

1. Introduction

With the growing application of DNA-sequence and other genetic data since the 1990s, osteological work has largely given way to molecular approaches in the study of amphibian biodiversity. Many influential works from the mid-20th century (e. g., Francis, 1934; Tihen, 1958; Hansen and Tanner, 1958; Wake, 1963; Özeti and Wake, 1969) are still cited today, but few such works are produced anew by contemporary biologists. However, as an independent source of data, osteological characters can provide invaluable insights into evolutionary relationships of living taxa that are as important as those derived from nucleotide substitutions. Homology and homoplasy are directly associated with biological functions.

The family Salamandridae, the second most diverse salamander group, exhibits remarkable differentiation in morphology and life history. One such extreme is exemplified by the Chinese stout newts (Pachytriton), which are highly specialized to inhabit small montane streams. Morphological adaptations include the loss of keratinized skin, a heavily ossified tongue skeleton that facilitates underwater feeding, and a paddle-like tail for efficient swimming (Özeti and Wake, 1969; Titus and Larson, 1995). Molecular phylogenies have defined relationships between Pachytriton and other salamandrid genera (Zhang et al., 2008), but the taxonomy of species within Pachytriton remains a source of contention. It is widely accepted that this genus contains at least three species, P. brevipes, P. labiatus and P. archospotus (Figure 1; Fei et al., 2006; Shen et al., 2008). Pachytriton archospotus previously was considered a population of P. brevipes due to the similar external morphology and coloration of the two forms (Shen et al., 2008). Indeed,

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Figure 1 Distribution of *Pachytriton brevipes* (blue solid line), *P. archospotus* (red dashed line) and *P. inexpectatus* (brown dashed line) in southeastern China. Note disjunct distribution of southwestern and northeastern *P. inexpectatus*. Modified from Wu *et al.* (2010).

P. archospotus has only recently been recognized as a distinct species based on osteological and molecular evidence (Shen *et al.*, 2008; Wu *et al.*, 2010). Another recent morphological study suggests that the lectotype of *P. labiatus* (Unterstein, 1930) is actually a member of the closely related genus *Paramesotriton* (Nishikawa *et al.*, 2011). Consequently, the specific epithet *labiatus* has been assigned to *Paramesotriton*, and the *Pachytriton* species originally known as *P. labiatus* given a new name, *P. inexpectatus* (Nishikawa *et al.*, 2011). We adopt this nomenclatural change herein.

Pachytriton inexpectatus has long been understood to comprise two disjunct populations (northeast vs. southwest) separated by several hundreds of kilometers (Zhao and Hu, 1984; Fei et al., 1999; Fei et al., 2006). However, molecular phylogenies based on mitochondrial and nuclear data indicate that northeastern P. inexpectatus is nested within P. brevipes, whereas the southwestern population represents the name-bearing species (Wu et al., 2010). To further tease apart phylogenetic relationships among these Pachytriton species, we analyzed osteological variation among them. Skeletal morphology of P. archospotus (misidentified as P. brevipes) and P. inexpectatus have been described in Chinese by Shen and Shen (1990) and Fan and Tian (1999), but neither of these works is sufficiently detailed to provide the basis for effective interspecific comparisons. In this paper

we present a comprehensive osteological study of four groups of *Pachytriton (P. brevipes, P. archospotus* and the two geographic populations of *P. inexpectatus*) to characterize how aquatic specializations have modified skeletal structures in this genus, to describe how characters vary among and within species, and to gain insight into interspecific phylogenetic relationships within *Pachytriton*.

2. Materials and Methods

Adult specimens were skinned, eviscerated, cleared and differentially stained for cartilage and bone (Klymkowsky and Hanken, 1991). Both sexes were included to evaluate sexual dimorphism. Because body length is linearly correlated with age in many salamandrid species until maximum length is reached (e. g., Caetano and Leclair, 1996; Lima et al., 2000; Üzüm, 2009), we chose similarsized specimens (140-150 mm total length) to minimize differences in ossification due to age. The sample of Pachytriton archospotus, a rare species in collections, was limited to two large females (about 190 mm total length). Skeletons were dissected and photographed by using a Leica MZ 12.5 stereomicroscope mounted with a JVC 3-CCD digital camera. For each specimen or view, a series of images were stacked in Auto-Montage (Synoptics Group) to produce a single high-resolution

image with maximum depth of field. Illustrations were prepared in Adobe Photoshop CS 3 and Illustrator 4 (San Jose, CA, USA). Radiographs of additional specimens were prepared with a Thermo Kevex X-ray System (Thermo Scientific) and used for interspecific comparisons. Skeletal nomenclature follows Francis (1934), except for the hyobranchial apparatus (Özeti and Wake, 1969) and the carpus and tarsus (Holmgren, 1933; Shubin and Wake, 2003). To test statistical significance among groups, two-sampled student's *t*-tests were performed in SPSS 13 (Chicago, IL, USA).

The following specimens were utilized: *P. brevipes*, 4 cleared-and-stained (Fujian), 23 x-rayed (18 from Fujian and 5 from Zhejiang); *P. archospotus*, 2 cleared-and-stained (Hunan, the type locality), 3 x-rayed (Hunan, the type locality); southwestern *P. inexpectatus*, 4 cleared-and-stained (Guangxi), 20 x-rayed (16 from Guangxi and 4 from Guizhou); northeastern *P. inexpectatus*, 4 cleared-and-stained (Zhejiang), 22 x-rayed (19 from Zhejiang and 3 from Anhui). Cleared-and-stained specimens of *Salamandra*, *Taricha* and *Cynops* were used for comparisons. Museum vouchers are listed in the appendix.

3. Results

3.1 Skull The skull of *Pachytriton* consists of two components: all cranial cartilages and bones that form within them (endochondral ossifications) and the dermal investing bones (dermal ossifications). The latter include the premaxilla, maxillae, nasals, frontals, prefrontals, parietals and squamosals on the dorsal side, and the parasphenoid, prevomers and pterygoids on the ventral

side (Figures 2-5).

Premaxilla: The single premaxilla comprises three regions: the tooth-bearing pars dentalis, which forms the anterior border of the upper jaw; the *pars palatina*, a posterior extension of the *pars dentalis* that forms the anterior part of the bony palate; and the dorsally ascending pars frontalis, which separates the paired nasals. The shapes and sizes of the first two regions are relatively conserved in the genus, but the shape and size of the pars frontalis are subject to substantial individual variation. The pars dentalis is a short, arched bar that extends laterally and forms the ventral border of the external naris. A perforation is present medially. The pars dentalis articulates with the anterior tip of the pars dentalis of each maxilla. An inconspicuous ridge marks the boundary between the pars dentalis and the pars palatina. The posterior edge of the pars palatina bears an acuminate projection that extends posteriorly between the paired prevomers. The pars frontalis ascends dorsally and forms the medial border of each external naris. It articulates with the nasal laterally and overlaps the frontal posteriorly. The frontal process expands in width and bifurcates posteriorly. The process is longest and most attenuate in P. brevipes and shortest and most robust in southwestern P. inexpectatus. Northeastern P. inexpectatus and P. archospotus are intermediate between the two extremes. A small internasal fontanelle is enclosed by the frontal process. The fontanelle is occasionally open posteriorly so that the frontal process appears deeply bifurcated.

Maxilla: Similar to the premaxilla, three regions can be recognized in the paired maxillae, namely, the *pars dentalis*, the *pars palatina* and the *pars facialis*. The



Figure 2 The skull of *Pachytriton brevipes* (CIB 88208). Left: dorsal view; right: ventral view. The parasphenoid is shown with prevomers removed; the size of the medial spike varies among individuals. Blue shading indicates cartilage, and scale bar: 5 mm (the same below).



Figure 3 The skull of *Pachytriton archospotus* (CIB 95952) with eyes removed. Left: dorsal view; right: ventral view. Skeletal nomenclature follows Figure 2.



Southwestern P. inexpectatus

Figure 4 The skull of southwestern *Pachytriton inexpectatus* (CIB 88153) with eyes removed. Left: dorsal view; right: ventral view. Skeletal nomenclature follows Figure 2.



Northeastern P. inexpectatus

Figure 5 The skull of northeastern *Pachytriton inexpectatus* (CIB 88146) with eyes removed. Left: dorsal view; right: ventral view. Skeletal nomenclature follows Figure 2.

tooth-bearing pars dentalis, which joins the pars palatina medially and pars facialis dorsally, is the main component of the maxilla. It articulates with the pars dentalis of the premaxilla anteriorly and extends posteriorly (but not beyond the orbit) to form the arch of the upper jaw. In adult specimens, the posterior tip of the pars dentalis articulates with the anteriorly pointed pterygoid bone in a nearly straight line. In juveniles, tips of the two bones closely approach one another and are joined by a ligament. The maxillary-pterygoid joint is unique to Pachytriton among Asian salamandrids (Wake and Özeti, 1969; Titus and Larson, 1995). The ventral surface of the pars dentalis is populated by numerous pedicellate teeth but the posterior portion remains toothless. The toothless region measures about one third of the length of the maxilla in northeastern P. inexpectatus, one quarter in P. brevipes, and only one fifth in southwestern P. inexpectatus and P. archospotus. Northeastern P. inexpectatus also has the most slender pars dentalis compared to the other three groups. While the posterior tip of the pars dentalis expands laterally in all groups, southwestern P. inexpectatus has dorsal extensions from the buccal and lingual sides of the posterior half of the bone. These extensions produce a deep dorsal groove, which has not been observed in other groups. The pars palatina is the medial extension of the pars dentalis; it forms the anterolateral portions of the palate as well as the lateral border of each internal naris. The pars palatina attenuates and terminates near the toothless region of the pars dentalis. The pars facialis arises from the anterior tip of the pars dentalis and extends dorsally to reach the middle level of the orbit. This region is well developed into a large plate that measures half the length of the maxilla. It articulates with the nasal and overlaps the base of the prefrontal. The anterior margin of the pars facialis forms the lateral border of the external naris; the posterior margin forms the anteroventral border of the orbit. Perforations for nerves and blood vessels are abundant on the pars facialis.

Nasal: The nasals are paired bony plates that cover much of the dorsal surface of the cartilaginous nasal capsule. Each nasal is located above the anterior roof of the dorsal nasal fenestra and never extends beyond the posterior tip of the *pars frontalis* of the premaxilla. The nasal articulates with the *pars frontalis* medially, with the *pars facialis* of the maxilla laterally, and with the frontal and prefrontal bones posteriorly. The *pars frontalis* of the premaxilla always separates the two nasals. The shape of the nasal is subject to individual variation; it typically ranges from oval to rectangular, and sometimes

the bone is pointed posteriorly. The margin of the nasal is relatively smooth in northeastern *P. inexpectatus*, but serrated in southwestern *P. inexpectatus*, *P. brevipes* and *P. archospotus*. The nasal contacts the prefrontal posterolaterally in all but two specimens. One specimen of northeastern *P. inexpectatus* (CIB 88152) has its left nasal separated from the prefrontal by the *pars facialis* of the maxilla, whereas in another specimen (CIB 88137) from the same group the right nasal is fused with the prefrontal.

Prefrontal: The small amorphous prefrontal is located immediately anterior to the orbit. Its shape varies ontogenetically but is usually quadrangular, except for some northeastern *P. inexpectatus*, in which it is triangular. It forms the anterodorsal rim of the orbit. The prefrontal is partially covered by the *pars facialis* of the maxilla laterally, articulates with the nasal anteromedially, and overlies the anterior edge of the frontal medially.

Frontal: The paired frontal forms the anterior roof of the braincase. It is covered anteriorly by posterior edges of the *pars frontalis* of the premaxilla and of the nasal, and by the medial edge of the prefrontal. Posteriorly, the frontal overlies the anterior edge of the parietal. The commissure between left and right frontals is relatively straight in northeastern *P. inexpectatus* but strongly uneven in the other three groups. The lateral edge descends and meets the orbitosphenoid ventrally. A frontal process extends posterolaterally to form the dorsal rim of the orbit. When the frontal process connects with the squamosal arch. The complete arch is a synapomorphy of all true newts (Titus and Larson, 1995).

Due to the phylogenetic importance of this feature, the morphology of the frontosquamosal arch in Pachytriton has been evaluated extensively. A complete and robust arch (i.e., the frontal and the squamosal process articulate firmly) is reported in *P. archospotus* and southwestern *P.* inexpectatus (Shen and Shen, 1990; Fan and Tian, 1999; Shen et al., 2008). Cai (1985) described an attenuate frontosquamosal arch in adult northeastern P. inexpectatus but a more robust arch in juveniles. On the contrary, Chan et al. (2001) suggest that the arch of P. brevipes and northeastern P. inexpectatus is rarely complete and attenuate if present at all. Chang and Boring (1935) find substantial variation in P. brevipes and northeastern P. inexpectatus, from an incomplete arch (the frontal and squamosal process are widely separated) to a wellformed, robust arch. Our examination supports the claim of considerable variation both between and within groups. Most P. archospotus and southwestern P. inexpectatus

possess a complete, relatively firm frontosquamosal arch. Grooves and ridges may provide additional strength (Naylor, 1978). However, lack of contact between the two processes is occasionally observed and can occur on either side. In the four cleared-and-stained P. brevipes, two specimens display a complete but slender frontosquamosal arch, whereas the arch is incomplete in the other two specimens. No direct frontal-squamosal contact is observed in any northeastern P. inexpectatus examined, although most specimens have a spine-like frontal process that closely approaches the squamosal process.

Parietal: The posterior braincase is roofed by the large, paired parietal. The posterior half of each bone expands laterally to form a wing-like structure that is located most posteriorly in southwestern *P. inexpectatus*. The wing position is similar in northeastern P. inexpectatus, P. brevipes and P. archospotus. The posterior tip of the parietal reaches the caudal end of the skull. A sagittal crest is prominent in southwestern P. inexpectatus, less evident in P. brevipes and P. archospotus, and indistinct in northeastern P. inexpectatus. The parietal underlies the posterior edge of the frontal anteriorly, overlies the anteromedial portion of the occipital-otic complex, articulates with the posterior part of the orbitosphenoid ventrally and may loosely contact the squamosal laterally. In Pachytriton, it provides a partial origin for the jaw-closing muscle (levator mandibulae anterior), which passes through the large fenestra formed by the frontosquamosal arch and inserts on the coronoid process of the prearticular in the mandible.

Squamosal: The paired squamosal overlies the quadrate and lies lateral to the occipital-otic complex. This splintlike structure is T-shaped, with the largest process pointed ventrally. Its anterior process articulates with the frontal process, or approaches the frontal process if the frontosquamosal arch is incomplete. Its posterior process firmly articulates with the anterolateral portion of the occipital-otic complex and may contact the lateral side of the parietal wing. The ventral process, which overlies the quadrate, varies in shape and size among groups (Figure 6). In southwestern P. inexpectatus and P. archospotus, the ventral process expands in width and covers much of dorsal surface of the quadrate. In comparison, northeastern P. inexpectatus and P. brevipes have a much narrower and pointed ventral process, which leaves the quadrate largely exposed dorsally. In the latter two groups, the ventral process is located next to a vertical crest on the dorsal surface of the quadrate. The entire squamosal is thickened and rugose in southwestern P. inexpectatus and

P. archospotus, whereas it is relatively thin and smooth in northeastern P. inexpectatus and P. brevipes.



Figure 6 The squamosal and quadrate of southwestern P. inexpectatus and P. archospotus (left, CIB 88153) and northeastern P. inexpectatus and P. brevipes (right, CIB 88208) seen in left lateral view.

Orbitosphenoid: The vertically oriented orbitosphenoid is an endochondral ossification that forms the lateral side of the braincase. Its shape is nearly rectangular, with the anterior portion narrower than the posterior. Two foramina are entirely enclosed by bone. The larger opening, optic fenestra, is located one quarter of the distance from the caudal end of the orbitosphenoid. The smaller foramen oculomotorium is located posterodorsally to the optic fenestra. Pachytriton has a much smaller optic fenestra in comparison to those of some terrestrial and amphibious species of similar size (e.g., Salamandra and Paramesotriton), which correlates with its relatively small eyes. The orbitosphenoid contacts the nasal capsule anteriorly and the occipital-otic complex posteriorly, articulates with the ventral surface of the frontal and the parietal dorsally, and articulates with the parasphenoid ventrally.

Occipital-otic complex: The posterior region of the adult skull comprises a pair of large bones. Each forms via fusion of three distinct components: prootic, opisthotic and exoccipital (Trueb, 1993). The shape of the occipital-otic complex is often triangular or obliquely quadrangular in dorsal view. Left and right bones contact along the sagittal axis by a narrow commissure, the tectum synoticum. The dorsal surface of the occipital-otic complex, which encloses the membranous labyrinth of the auditory system, is depressed compared to its lateral margin. Three elevations outline the three semicircular canals, which are most prominent in northeastern P. inexpectatus. The dorsolateral margin contains a groove that accommodates the posterior process of the squamosal. The exoccipital portion of the occipital-otic complex encloses the foramen magnum. The occipital condyle arises as a cylindrical, bony extension lateroventral to the

foramen magnum; its flat head is capped with cartilage. The occipital condyle and the odontoid facet on the lateral wall of the foramen magnum articulate with the condylar facet and odontoid process of the atlas. The operculum is located along the posteroventral surface of the occipitalotic complex, covering the fenestra vestibule with a fibrous membrane. This partially mineralized plate is oval in shape, with both its external and internal surfaces slightly convex. A small bundle of muscle (musculus opercularis) connects the operculum to the suprascapula of the pectoral girdle. A distinct and separate columella is not observed; this structure is reported as fused to the occipital-otic complex in adult Pachytriton and the stylus portion is absent (Dunn, 1922). The occipital-otic complex articulates with the parietal anteriorly, with the squamosal dorsolaterally, and with the posterior margin of the parasphenoid ventrally. It also loosely contacts the posterior base of the pterygoid bone.

Suspensorium: The suspensory apparatus of the mandible is located at the lateral side of the occipital-otic complex and the ventral side of the squamosal. It consists of the pterygoid and quadrate bones; the latter in addition has three processes (ascending, otic and basal) that articulate with the lateral wall of the occipital-otic complex in a tripod-like manner. The ascending process is the most anterior and the most slender. The otic process is located on the dorsal side of the suspensorium and completely overlain by the squamosal. The basal process, which is the largest of all three, is situated posteroventrally to the otic process and connects the obliquely oriented quadrate. The ossified quadrate forms a direct lateral continuation of the cartilaginous basal process and is nearly perpendicular to the sagittal axis of the skull. An elevated crest runs vertically on the dorsal surface of the quadrate in northeastern P. inexpectatus and P. brevipes, whereas the dorsal surface is flat or slightly depressed in southwestern P. inexpectatus and P. archospotus. The quadrate is overlain dorsally by the squamosal, covers the pterygoid dorsolaterally, and articulates anteroventrally with the articular of the mandible. The pterygoid of Pachytriton has a dorsal groove that accommodates a cartilaginous rod, which originates from the joint between the basal and ascending process and extends anteriorly into the pars dentalis of the maxilla. The pterygoid has an elongate anterior ramus and a laterally expanded posterior region, which yields a spoon-like profile. The pterygoid firmly contacts the *pars dentalis* of the maxilla anteriorly in most specimens; together, the two bones form the ventral border of the orbit. Pachytriton archospotus has a distinct pterygoid with the anterior ramus curved medially

and large lateral and medial processes at the posterior region. In contrast, the ramus is relatively straight and the posterior region is more round in other groups. The pterygoid articulates with the occipital-otic complex dorsomedially, with the quadrate dorsolaterally and with the *pars dentalis* of the maxilla anteriorly.

Prevomer: The paired prevomer forms the dorsal and anterior roof of the oral cavity, as well as the posterior floor of the nasal capsule. Its anterior portion is a broad, toothless and dorsally convex plate that contacts the pars *palatina* of the maxilla laterally. Left and right prevomers enclose an internasal space medially. The middle-toposterior portion bears small teeth on its medial edge. Left and right dental ridges form an inverse-V. The posterior tip of the prevomer flares laterally and does not contact the parasphenoid. The distance between the two tips is about one half the length of either dental ridge. Laterally, the prevomer forms the medial rim of the internal naris. Each prevomer also articulates with the pars palatina of the premaxilla anteriorly, with the pars palatina of the maxilla anterolaterally, and with the parasphenoid dorsally.

Parasphenoid: This is the largest single bone in the skull; it forms the posterior roof of the oral cavity and the floor of the braincase. It also provides support for the posterior portion of the nasal capsule. Previous studies report its shape as sword-like with an acute anterior end (Shen and Shen, 1990; Fan and Tian, 1999). However, after removal of the articulated prevomers the parasphenoid appears nearly rectangular (Figure 2). Its anterior half is only slightly narrower than the posterior half. The dental ridge of the prevomer covers the lateral edge of parasphenoid, obscuring its shape. The parasphenoid is a broad and elongate plate. Its anterior tip is slightly bifurcated or trifurcated and underlies the cartilaginous planum internasale, which connects left and right nasal capsules ventrally. Its posterior end reaches the foramen *magnum*. A medial spike is present on the ventral surface; its size varies among individuals. The parasphenoid articulates with the orbitosphenoid dorsally, with the prevomers ventrally, and with the occipital-otic complex posterolaterally.

3.2 Mandible The mandible of *Pachytriton* is a semicircular structure comprised of two rami that join at a median symphysis. Each ramus consists of the Meckel's cartilage, the replacing mento-Meckelian and articular bones, and two dermal investing bones, the dentary and the prearticular (Figures 7, 8). Teeth are present on the anterior two thirds of each ramus. Individual variation is observed in the number of teeth and the shape of the



Figure 7 Mandible of *P. brevipes* (CIB 88208) in dorsal view. Meckel's cartilage and articular and prearticular bones are removed from the right side.

Pachytriton brevipes



Pachytriton archospotus



Figure 8 Mandible of *P. brevipes* (CIB 88208) and *P. archospotus* (CIB 95952) viewed from the lingual side.

prearticular. Otherwise, mandibular morphology is highly similar among groups.

Meckel's cartilage: Meckel's cartilage is the primary lower jaw skeleton in larvae and is retained in adult salamanders (Francis, 1934). In adults it extends from the posterior tip of the mandibular ramus and terminates a short distance from the median symphysis, where cartilage has ossified to form a mento-Meckelian bone that is fused with the dentary. The anterior portion is enclosed by the dentary but the posterior tip is exposed dorsally.

Articular: The posterior tip of Meckel's cartilage ossifies as the articular bone, which articulates with the quadrate of the skull to form the mandibular joint. The shape of the articular resembles an inverted flask with the dorsal surface expanded. The articular contacts the prearticular medially and ventrally and contacts the dentary laterally. **Dentary:** The dentary is the major bone of the mandibular ramus. Its anterior part forms a dorsomedial groove that accommodates numerous pleurodont teeth. The groove terminates anteriorly at a dentary process that is as high as or higher than the coronoid process of the prearticular posteromedially. The posterior part of the dentary resembles a vertically oriented thin blade. The length of the tooth row and the size of the dentary process are subject to individual variation. The dentary of *P. archospotus* is relatively straight, whereas that of the other three groups is more arched (Figure 10).

Prearticular: The wedge-like prearticular is inserted on the lingual side of the dentary, forming the lingual border of the posterior half of the ramus. It extends beneath the ventral surface of the articular and thus supports the jaw articulation. A coronoid process arises on its dorsal surface and inflects lingually. The apex of the coronoid process is often lower than the apex of the dentary process anterolaterally. The inflection results in an expanded broad shelf on the dorsal surface of the prearticular, where the adductor muscle levator mandibulae inserts. Southwestern P. inexpectatus has a broader shelf than the other three groups, perhaps indicating a large muscle and thus more powerful jaw closure. The prearticular of P. archospotus extends more posterior to the jaw joint than that of other Pachytriton groups (Figures 8, 10). Perforations on the lingual surface of the prearticular allow passage of nerves and blood vessels.

3.3 Hyobranchial apparatus The hyobranchial apparatus constitutes the tongue skeleton. The apparatus consists of paired ceratohyals, a central basibranchial that bears a pair of radials, paired first and second ceratobranchials, and paired epibranchials (Figure 9). The hyobranchial skeleton of *Pachytriton* is highly ossified, whereas those of other salamandrid genera are largely cartilaginous. Extensive ossification provides extra strength and rigidity to the apparatus, which facilitates suction feeding in water (Özeti and Wake, 1969).

Ceratohyal: The paired ceratohyal is the largest element in the hyobranchial apparatus. Its anterior third is cartilaginous and laterally expanded into a spatula shape. The anterior tip inflects medially and closely approaches its counterpart from the opposite side. The posterior two-thirds are ossified and gradually attenuate into a rod. Individuals vary in the shape of the border between cartilaginous and ossified parts. The border is highly curved (S- or C-shaped) in larger and thus older salamanders, but relatively straight in smaller animals. The posterior portion of the ceratohyal flares dorsolaterally; its tip is expanded and capped with



Figure 9 Hyobranchial apparatus of *P. archospotus* (left, CIB 95952) and other *Pachytriton* (represented by *P. brevipes*, right, CIB 88208) in ventral view. The interradial cartilage, which is found on the dorsal side of the first basibranchial, is not visible.



Figure 10 Relative position of the hyobranchial apparatus and skull in dorsal view. Posterior portions of the first and second ceratobranchials (yellow) are visible posterior to the skull in *P. archospotus* (right, CIB 95953) but not in other species (such as northeastern *P. inexpectatus*, left, CIB 88146).

cartilage. In *P. archospotus* the ceratohyal is located more anteriorly than in other groups; the posterior tip barely reaches the joint between the first ceratobranchial and the epibranchial. In the other three groups the posterior tip extends caudally far beyond this joint.

Basibranchial I: The rod-like first basibranchial is the only basibranchial present in *Pachytriton*. In all specimens examined, the anterior tip remains cartilaginous, from which extends a pair of cartilaginous radials that point posterolaterally. An interradial cartilage bridges the two radials at their proximal ends dorsal to the first basibranchial. The posterior half of the first basibranchial is mostly cartilaginous and slightly flattened dorsoventrally, but its central portion is mineralized to variable extent. It articulates with the proximal ends of the first ceratobranchials. The posterior tip is diamond-shaped and articulates with the proximal ends of the second ceratobranchials. Our observations differ from those of Özeti and Wake (1969), who report that the radials of *Pachytriton* point laterally and the entire basibranchial is ossified.

Ceratobranchial I: The paired first ceratobranchial in *Pachytriton* is a stout, well-ossified and strongly bowed bone. Both proximal and distal ends are capped with cartilage. The proximal end articulates with the ventral surface of the basibranchial; the distal end articulates with the proximal tip of the epibranchial. The first ceratobranchial of *P. archospotus* is elongate; the distal tip may reach the atlas of the vertebral column and is visible posterior to the skull in radiographs (Figure 10). In other *Pachytriton*, the first ceratobranchial is smaller and the distal tip does not exceed caudally beyond the occipital

condyle.

Ceratobranchial II: The paired, rod-like second ceratobranchial is much more slender and slightly shorter than the first ceratobranchial and bows in an opposite direction from it. Both proximal and distal ends are capped with cartilage. The second ceratobranchial articulates with the posterior tip of the basibranchial proximally, and with the proximal tip of the epibranchial posterolaterally. The second ceratobranchial is much larger in *P. archospotus* than in the other three groups. Consequently, the distal tip of each second ceratobranchial of *P. archospotus* is also visible posterior to the skull in dorsal view (Figure 10).

Epibranchial: The robust epibranchial is a solid, paired bone with both ends capped by cartilage. The proximal portion is expanded laterally to accommodate the distal ends of ceratobranchials I and II. The shape of the distal portion, which is sheathed by thick muscle, is subject to interspecific variation. In all groups except P. archospotus, the distal portion elongates, bows and flares dorsolaterally to wrap around the neck; it reaches the height of the upper limit of the quadrate. In contrast, P. archospotus has a straight epibranchial. This difference offers the most convincing morphological evidence to support P. archospotus as a separate species from P. brevipes (Shen and Shen, 2008). Its rod-like distal portion does not wrap around the neck but instead is elevated and tapers caudally. Pachytriton archospotus also differs from other groups by the absence of a flange on the medial side of the epibranchial. Özeti and Wake (1969) hypothesize that the function of the epibranchial flange is to strengthen the hyobranchial apparatus during aquatic feeding. Lack of this feature in P. archospotus may suggest modified feeding mechanics in this species.

3.4 Dentition Teeth are borne on four bones of the skull: premaxilla, maxilla, dentary and prevomer. Tooth shapes, sizes and numbers are subject to ontogenetic and among-group variation. The teeth are pedicellate and similar to those of other amphibians; each consists of a pedicel and crown connected by fibrous tissue. Premaxillary, maxillary and dentary teeth are pleurodont and prevomerine teeth are acrodont. The following tooth counts are based on cleared-and-stained specimens, except for the premaxillary tooth count, which includes additional radiographed specimens.

Premaxillary teeth are elongated and saber-like. A chisel-like lingual cusp and an inconspicuous labial cusp constitute the crown. Eleven southwestern *P. inexpectatus* have 12–16 premaxillary teeth, mean 14.1, which are fewer than reported by Fan and Tian (1999): 14–18. Twelve northeastern *P. inexpectatus* have 17–21 teeth,

mean 18.8; and fourteen *P. brevipes* have 13–18, mean 16.4. Five *P. archospotus* have 12–15 teeth, mean 13.8, which is more than reported by Shen and Shen (1990): around 11. Two-sampled *t*-tests indicate that southwestern *P. inexpectatus* and *P. archospotus* have the fewest premaxillary teeth (P < 0.05), but these two groups are not significantly different from each other (P = 0.067). Northeastern *P. inexpectatus* possess significantly more premaxillary teeth than any other group (P < 0.01).

The recurved maxillary teeth are similar to the premaxillary teeth. Teeth decrease in size posteriorly and are more conical with a single cusp. There are total 33–55, mean 41.8, maxillary teeth in the four southwestern *P. inexpectatus*; 35–40, mean 37.0, in the four northeastern *P. inexpectatus*; 42–46, mean 43.5, in the four *P. brevipes*; and 29 and 32 teeth, mean 30.5, in the two *P. archospotus*. Tooth counts for southwestern *P. inexpectatus* and *P. archospotus* are consistent with those from Fan and Tian (1999) and Shen and Shen (1990). We did not perform statistical tests of these differences due to limited sample sizes.

Dentary teeth are longer than teeth on the upper jaw. The lingual cusp is recurved and the labial cusp is inconspicuous. Teeth decrease in size posteriorly and are more conical with a pointed cusp. There are total 50–62, mean 53.5, dentary teeth in the four southwestern *P. inexpectatus*; 53–66, mean 58.0, in the four northeastern *P. inexpectatus*; 55–70, mean 63.0, in the four *P. brevipes*; and 44 and 53 teeth, mean 48.5, in the two *P. archospotus*.

Prevomerine teeth, which are smaller than oral teeth, have a sharp, recurved and unicuspid tip. The teeth decrease in size posteriorly but to a lesser extent than do oral teeth. Their number varies greatly among individuals. There are total 84–91, mean 86.7, prevomerine teeth in the four southwestern *P. inexpectatus*; 74–88, mean 79.0, in the four northeastern *P. inexpectatus*; 74–82, mean 78.7, in the four *P. brevipes*; and 71 and 89 teeth, mean 80.0, in the two *P. archospotus*.

3.5 Vertebral column The vertebral column of *Pachytriton* consists of a single cervical vertebra (the atlas), typically twelve trunk vertebrae, one sacrum and several caudal vertebrae. The number of caudal vertebrae varies greatly among individuals and groups. All vertebrae are opisthocoelous. Ribs are typically borne on the trunk and sacral vertebrae.

Cervical vertebra: The cervical vertebra is hourglassshaped in dorsal view with its anterior end wider than the posterior (Figure 11). A well-developed odontoid process arises from the ventral side and extends into the *foramen magnum* of the skull. Lateral to the odontoid process are two cervical condylar facets that accommodate the condylar processes of the occiput. A lateral process is present posterior to the facet near the middle of the atlas. The centrum portion is limited to the posterior end of the vertebra, which is conical-shaped and opens posteriorly to accommodate the intervertebral cartilage of the first trunk vertebra. Dorsally, the atlas is covered by a broad neural arch, which bears an indistinct neural spine on its rugose dorsal surface. The neural arch is constricted at the middle and expands posteriorly. The disc-like postzygapophysis arises at the lateral side of the posterior margin and articulates with the prezygapophysis of the first trunk vertebra. All articulating surfaces are sheathed with cartilage. There are three major perforations laterally (Francis, 1934). The anterior foramen is located just posterior to the condylar facet, which allows passage of the first spinal nerve. Posterodorsally, the second foramen allows passage of blood vessels. The third opening, on the lateroventral side of the centrum, conducts the second spinal nerve. The shape and size of the atlas are subject to ontogenetic and individual variation, but there is no obvious group-specific variation.

Trunk vertebrae: Typically *Pachytriton* have 12 trunk vertebrae. The one exception we observed is a northeastern *P. inexpectatus*, which possesses one additional trunk vertebra. Chan *et al.* (2001) examine 15 specimens of *Pachytriton* and find one individual with thirteen trunk vertebrae as well. Each vertebra consists of two portions, a ventral centrum and a dorsal neural arch. The centrum is slightly shorter than the neural arch. One pair of ribs is borne on the transverse processes of the centrum. The shape and size of the vertebral column, as well as rib length, are subject to ontogenetic variation, but there is no group-specific variation. The following description is based on the eighth vertebra, which is taken as a representative of the entire trunk series (Figure 12).

The trunk vertebrae are strongly opisthocoelous. The centrum bears an anterior intervertebral cartilage that is solidified in adults as a condyle, which fits into the hollow cavity of the previous vertebra. The transverse section of the centrum is rounded anteriorly and oval posteriorly, with its breadth wider than height. The ventral surface is almost flat and the central region is depressed to various extents with several fossae. The dorsal part of the vertebra is formed by the neural arch, which covers the neural canal below. The anterior opening of the neural canal is triangular and the posterior opening is rounded. A thin, plate-like neural spine arises on the dorsal surface of the neural arch. The anterior margin of the neural arch is notched and covered by the posterior margin of the previous vertebra. The wing-like prezygapophysis arises anterolaterally and bears a disc-like articulating facet dorsally. The posterior margin of the neural arch expands laterally to form the postzygapophysis, which bears a ventral articulating facet. The prezygapophysis extends slightly anterior to the intervertebral condyle, and the postzygapophysis extends well posterior to the centrum. The posteromedial portion of the neural arch is elevated and thickened. It provides the origin for the inter-spinal muscle that inserts on the dorsal surface of the neural arch of the vertebra next behind (Francis, 1934).

Near the midpoint of the vertebra two transverse processes extend posterolaterally to articulate with the rib. The diapophysis arises from the lateral side of the neural arch dorsally, and the parapophysis arises from the lateral side of the centrum ventrally. In all clearedand-stained specimens, the parapophysis is slightly anteroventral to the diapophysis except for the first three trunk vertebrae, in which the former process is directly beneath the latter. The two transverse processes are of similar length and connected by a thin bony plate. Knoblike cartilages are borne on their distal tips, which support the two corresponding proximal heads of the rib. Ribs are longest in the middle region of the trunk and shorten into acuminate spines with fused proximal heads near the sacrum. The distal tip of the rib bears a small cartilage that is absent from the last two or three trunk vertebrae. In the terrestrial genus Salamandra, the distal cartilage on ribs of the first and second trunk vertebrae expands into a spatula-like structure, to which attach muscles from the scapula that suspend the trunk and therefore support the upper body (Francis, 1934). In contrast, distal cartilages remain knob-like throughout the whole trunk series in aquatic Pachytriton. The first three-to-four ribs are bent downwards at about their middle or distal one-third, where a dorsal spine arises. Posteriorly along the vertebral column, the distal tip of the rib lacks a process but instead has a dorsal groove or fossa, which provides attachment for costal muscles. Perforations on the vertebra allow passage of spinal nerves and blood vessels.

Sacral vertebra: The sacral vertebra resembles trunk vertebrae except for its modified ribs. Its centrum and neural arch exhibit no major difference from those of the most caudal trunk vertebra, but the diapophysis and parapophysis are much stouter and longer. Unlike trunk vertebrae, the distal tips of the two transverse processes are not attenuate. The rib is modified into a very robust spine, with the two proximal heads not divergent from each other. The rib is oriented posterolaterally, but its distal one-third is curved forward and articulates anteriorly



Figure 11 Atlas of *P. brevipes* (CIB 88208) in dorsal (left) and lateral (right) views. A: foramen for the first spinal nerve; B: foramen for blood vessels; C: foramen for the second spinal nerve.



Figure 12 The eighth trunk vertebra and associated ribs of *P. brevipes* (CIB 88208) in lateral (left) and ventral (right) views.

with the cartilaginous tip of the ilium of the pelvic girdle. A well-developed dorsal flange borne on the curved region increases muscle attachment surface area. A knoblike cartilage is present at the distal tip of the rib. Some specimens develop a bony process near the proximal end. Caudal vertebrae: The caudal vertebrae of Pachytriton are largely compressed laterally as an adaptation for swimming in permanent montane streams (Figure 13). The first caudal vertebra resembles the anterior sacrum but bears no ribs. In rare cases, an acuminate appendage is attached to the first caudal vertebra. The transverse process arises near the midpoint of the vertebra and is not differentiated into diapophysis and parapophysis. The process diminishes posteriorly to a mere osseous thickening on the last one-third of the caudal series and is ultimately lost in the last three to five vertebrae. The caudal zygapophyses reduce from disc-like articulations of the trunk and sacral vertebrae to pointed contacts that are absent completely after the middle of the caudal series. The neural arch is similar to those of trunk and sacral elements. An elevated neural spine is borne on the dorsal surface of the neural arch; jointly, the two structures form a Y-shaped chevron in dorsal view. The haemal arch arises on the ventral surface of the second caudal vertebra, where it is restricted to the posterior ventral margin. Beginning with the third vertebra, the haemal arch completely covers the ventral surface of the centrum and allows the passage of caudal blood vessels. A prominent haemal spine is developed along the mid-ventral line, resembling the neural spine dorsally. The haemal arch and spine also



Figure 13 The ninth to twelfth caudal vertebrae of *P. archospotus* (CIB 95952) in right lateral view. Individual transverse processes are not differentiated into a diapophysis and parapophysis. The articulation between adjacent prezygapophyses and postzygapophyses is absent after the eleventh caudal vertebrae.

form a Y-shaped chevron in ventral view. Caudal vertebral size decreases posteriorly. The last 5-to-7 vertebrae each consist of only a tiny centrum with no arch or spine.

Caudal vertebral number is subject to individual and group-specific variation. Variation is least in southwestern P. inexpectatus, which has 28 to 31 caudal vertebrae, mean 29.4 (n = 19). In contrast, the number is most variable in *P. brevipes*, where it ranges from 27 to 34, mean 30.5 (n = 17). Northeastern *P. inexpectatus* has 29 to 34 caudal vertebrae, mean 31.4 (n = 15). Our sample of P. archospotus comprises only five specimens, which have 27 to 31 caudal vertebrae, mean 28.4. These data are consistent with those of Fan and Tian (1999) for southwestern P. inexpectatus but not with those of Shen and Shen (1990) for P. archospotus, who reported only 21 and 23 caudal vertebrae in two specimens. Two-sampled t-tests indicate that southwestern P. inexpectatus and P. archospotus have significantly fewer caudal vertebrae than northeastern P. inexpectatus and P. brevipes (P <0.005 for all tests). However, the former two groups do not differ significantly from each other (P = 0.289), nor do the latter two groups (P = 0.113).

3.6 Pectoral girdle As is typical for salamanders, each half of the pectoral girdle consists of the scapula, procoracoid and coracoid (Figure 14). All three components converge to the glenoid cavity, which articulates with the proximal head of the humerus. Most of the pectoral girdle remains cartilaginous in adults except for the ossified region that surrounds the glenoid cavity. The extent of ossification and how left and right coracoids overlap are subject to individual variation. No group-specific difference is apparent.

The rounded or triangular glenoid cavity is bordered by the scapula laterally, by the procoracoid anteriorly and by the coracoid medially. The scapula arises dorsally from the glenoid region and expands laterally, attaining the greatest width at the connection with the suprascapula, which is the cartilaginous extension of the scapula. The spatulate suprascapula is inflected at its proximal onethird and attaches to the lateral side of the trunk through muscles and connective tissues. On the ventral side, the cartilaginous procoracoid extends rostrally and expands laterally; its distal end may reach as far as the occipital condyles. The procoracoid is separated from the coracoid by a deep, U-shaped notch, the incisura coracoidea. A large opening, foramen supracoracoideum, is completely enclosed in bone and conducts nerves and blood vessels (Francis, 1934). The largest component of the pectoral girdle is the coracoid, which includes a bony part that extends medially from the glenoid region and a large sector-shaped distal cartilage that overlaps medially with its bilateral counterpart. In some specimens the right coracoid overlies the left, whereas others have the opposite condition. Both arrangements occur at about equal frequency. The extent of overlap also differs among specimens, varying from half the width of the cartilage to its entire width. The sternum is a thickened rhomboid cartilage at the ventral midline. It has two grooves that accommodate posteromedial edges of the cartilaginous coracoid. One groove is located on the dorsal surface and the other one on the ventral surface, which gives the sternum a notched anterior tip.

3.7 Forelimb The forelimb of *Pachytriton* is similar to that of other tetrapods, which consists of the humerus (Figure 15), the radius and ulna (Figure 16), and the carpus, metacarpals and phalanges (Figure 16). Epiphyses of long bones and all articulating surfaces are cartilaginous.

Humerus: The humerus is a single straight element. Its proximal head expands anteroposteriorly and is capped with hemispherical cartilage that fits into the glenoid cavity of the pectoral girdle. The cartilage extends distally to a prominent crest, the crista ventralis humeri, on the anteroventral surface of the shaft. On the opposite side, the crista dorsalis humeri arises as a smaller and more pointed crest. Both crests serve as insertions for locomotor muscles. The distal head of the humerus rotates 90° as it expands dorsoventrally and is equipped with two distinct cartilaginous condyles. The preaxial radial condyle is much larger than the postaxial ulnar condyle. Group-specific variation is observed in the size of the dorsal and ventral crests. Southwestern P. inexpectatus and P. archospotus develop a massive crista ventralis humeri that extends to the distal end of the humerus. In contrast, in northeastern P. inexpectatus and P. brevipes the ventral crest is confined to the proximal one-third of the shaft. The dorsal crest of southwestern P. inexpectatus and *P. archospotus* is also much more prominent than that of northeastern P. inexpectatus and P. brevipes.

Radius: The radius is a long, straight bone with expanded ends. The cartilaginous epiphysis on the proximal end is bowl-shaped, which creates a concavity that receives the radial condyle of the humerus. The epiphysis on the distal end is asymmetrical with the preaxial portion elongated, which creates an oblique articulation with the radiale and the amalgamated intermedium+ulnare.

Ulna: The ulna is slightly bowed, with a shaft longer than the radius. A horseshoe-shaped cartilage at the proximal end forms the olecranon process, which articulates with both radial and ulnar condyles of the humerus. The distal



Figure 14 The right pectoral girdle (left) and sternum (right) of *P. brevipes* (CIB 88208) in ventral view.



Figure 15 The right humerus of northeastern *P. inexpectatus* and *P. brevipes* (upper, CIB 88208) and southwestern *P. inexpectatus* and *P. archospotus* (lower, CIB 88160) in ventral view.

end bears only a small knob of cartilage that articulates with the amalgamated intermedium+ulnare.

Manus: Proximodistally, the manus is composed of the carpus, metacarpals and phalanges. The carpus comprises seven elements: the proximal radiale and the amalgamated intermedium+ulnare; a single central; the preaxial elementy (Holmgren, 1933; Shubin and Wake, 2003); and three basal carpals. The element y is designated as the prepollex by Francis (1934) and Duellman and Trueb (1994), and as the centrale 1 by Schaeffer (1941) and Wake (1963). The carpus is cartilaginous in juveniles but ossifies gradually in older animals. All articulating surfaces remain cartilaginous. Except for obvious deformities, all four *Pachytriton* groups share the same *manus* morphology.

The largest element in the carpus is the amalgamated intermedium+ulnare. Shubin and Wake (2003) make a distinction between amalgamation and fusion, reserving the former term for cartilages that fail to separate during embryonic development and the latter term for initially independent cartilaginous condensations that subsequently merge. In salamanders, the intermedium and ulnare are connected in early ontogenetic stages (Blanco and Alberch, 1992; Wake and Shubin, 1998). Therefore, the union of these two elements should be regarded as amalgamation. In Pachytriton, the intermedium+ulnare is always the first element to begin ossification. The intermedium+ulnare is oval in shape and bordered by cartilage, which is thicker at the postaxial margin. It articulates with the ulna and radius proximally, with the centrale and the distal carpal 4 distally, and slightly with the radiale preaxially. The quadrangular radiale is a small element, only one-third the size of the intermedium+ulnare. It is the penultimate carpal element to ossify, always slightly earlier than the neighboring element y. The radiale articulates with the radius proximally, with the intermedium+ulnare and the centrale postaxially, and with the element y preaxially. In many adult Pachvtriton, the radiale and the element v are the only completely cartilaginous elements. The triangular element y, which is about half the size of the radiale, loosely contacts the centrale and articulates extensively with the basale commune postaxially. There is no direct contact between the element y and the metacarpal 1, thus the former does not support the first digit. The small, irregular-shaped centrale is located in the center of the carpus. In rare cases, one or two extra centralia are present (Chang and Boring, 1935). Distally, the basale commune is the largest basal carpal; it represents the amalgamated distal carpals 1 and 2. This unit is quadrangular to pentagonal in shape and supports metacarpals 1 and 2 distally. It articulates with the element y preaxially,



Figure 16 Right forelimb of northeastern *P. inexpectatus* (CIB 88152) in dorsal view. I–IV: digits I–IV. Scale bar: 2 mm.

with the centrale proximally, and with distal carpal 3 postaxially. The triangular or round distal carpal 3 is the smallest basal carpal. It supports metacarpal 3 and articulates extensively with the *basale commune* and distal carpal 4. It articulates loosely with the centrale. The round distal carpal 4 supports metacarpal 4. It articulates with the centrale and distal carpal 3 preaxially and with the intermedium+ulnare proximally. A variant carpal morphology is found in one northeastern *P. inexpectatus*, which has amalgamated distal carpals 3 and 4 in the right hand.

Four metacarpals are present in *Pachytriton*. The first one is the shortest; metacarpals 3, 4 and 5 are of about equal length. All metacarpals are dumbbell-shaped and capped with cartilage at both ends. Proximally, both metacarpals 1 and 2 are supported by the *basale commune* and metacarpals 3 and 4 are supported by distal carpals 3 and 4, respectively. Phalanges are also dumbbell-shaped except for the terminal ones, which are triangular with an expanded distal tip. The phalangeal formula of *Pachytriton* is 1-2-3-2; digits, in order of decreasing length, are: 3 > 2 > 4 > 1. One *P. brevipes* (CIB 88186) exhibits extra phalanges in the left hand (phalangeal formula 2–3–3–2) and the entire left carpus is cartilaginous.

3.8 Pelvic girdle The pelvic girdle consists of three components: ilium, pubo-ischium plate and vpsiloid cartilage (Figure 17). The rod-like ilium points dorsally from the acetabulum with its proximal end expanded anteroposteriorly. The distal one-fourth remains cartilaginous and tapers to a pointed tip, which attaches to the curved tip of the sacral rib posteriorly by fibrous connective tissues. The pubis and ischium are fused to form the rectangular pubo-ischium plate. The anterior portion of the plate is the cartilaginous pubis, which is thickened at its anterolateral margin and forms an indistinct prepubic process. A large perforation at the center of each pubis, the obturator foramen, allows passage of the obturator nerve (Francis, 1934). The posterior portion, the ischium, is ossified except at the anterolateral margin and the midline symphysis. Each ischium bears a posteriorly pointed spine with a cartilaginous tip. The acetabulum is formed by the proximal end of the ilium dorsally, by the lateral margin of the ischium posteriorly, and by the lateral margin of the pubis anteriorly. The acetabulum receives the large cartilaginous head of the femur. The ypsiloid cartilage is located along the midline, immediately anterior to the pubo-ischium plate, but there is no direct articulation between the two structures. Instead, the cartilage connects to the plate via a short connective tissue. The two tips of the ypsiloid cartilage are usually longer than its stem. All four groups of *Pachytriton* share the same pelvic girdle morphology. The extent of ossification at the pubo-ischium plate varies individually.

3.9 Hind limb The hind limb skeleton of *Pachytriton* comprises the femur (Figure 18), the tibia and fibula (Figure 19), and the tarsus, metatarsals and phalanges (Figure 19). Epiphyses of long bones and all articulating surfaces are cartilaginous.

Femur: The femur is the longest bone in the hind limb. The shaft constricts in the middle. Its proximal end bears a large cartilaginous condyle that fits into the acetabulum of the pelvic girdle. Depressions are present on both preaxial and postaxial surfaces of the condyle. Ligaments that insert on depressions attach the condyle to the pubis and ilium. The trochanter crest arises on the ventral surface of the proximal one-third of the shaft; it provides the insertion for the ventral caudal muscle (musculus caudalifemoralis), which flexes the tail or retracts the femur (Francis, 1934). The tip of the crest remains cartilaginous. Cartilaginous tibial and fibular condyles arise on the distal end of the shaft. Groups vary in the size of the trochanter crest, which is spine-like and rapidly merges into the shaft surface in northeastern P. inexpectatus and P. brevipes. In southwestern P. inexpectatus, a second crest arises as an extension of the trochanter crest and extends to the distal end of the femur. This second crest is also present, but less distinct, in P. archospotus. Therefore, the femur is slender in northeastern P. inexpectatus and P. brevipes but very stout in the other two groups.

Tibia: The tibia is a straight bone that is dilated at both ends. In transverse section, the proximal end is a flat oval and the distal end is circular. The shaft constricts near the midpoint. A prominent, proximally pointed tibial spine arises on the dorsal surface; its length varies among specimens. In some the spine extends beyond the proximal end of the shaft, but in others the spine is much shorter. The tibia articulates proximally with the femur and distally with the tibiale and intermedium.

Fibula: The fibula is the same length as the tibia, but much wider. The slightly bowed fibula is dorsoventrally compressed with a concave dorsal surface, which yields a relative thin shaft. The fibula articulates proximally with the femur and distally with the fibulare and intermedium.

Pes: Much resembling the *manus*, the *pes* consists of the tarsus, metatarsals and phalanges. The tarsus further comprises eight elements: tibiale, intermedium and fibulare in the proximal row; one centrally located centrale; one preaxial element y (Holmgren, 1933; Shubin and Wake, 2003); and three basal tarsals. In adults the



Figure 17 Pelvic girdle of *P. brevipes* (CIB 88208) in dorsal view. Scale bar: 5 mm (the same below).



Figure 18 Right femur of northeastern *P. inexpectatus* and *P. brevipes* (upper, CIB 88208) and southwestern *P. inexpectatus* (lower, CIB 88160) in anterodorsal view. The femur of *P. archospotus*, while similar to that of southwestern *P. inexpectatus*, has a less prominent secondary crest.

tibiale and element y are frequently cartilaginous but other elements are ossified in the center. All *Pachytriton* groups share the same *pes* morphology, except for obvious deformities observed in individual specimens.

The triangular-or-round fibulare is the largest element in the tarsus. Unlike the ulnare of the *manus* that has amalgamated with the intermedium, the fibulare is separate from but articulates extensively with the intermedium. It also articulates extensively with the amalgamated distal tarsals 4 and 5. It only loosely contacts the centrale. The oval intermedium is slightly smaller than the fibulare and is wedged between the distal ends of the fibula and tibia. The intermedium articulates preaxially with the tibiale and distally with the centrale. The irregularly shaped tibiale is only half the size of the intermedium. Similar to the radiale in the *manus*, the tibiale is the penultimate element to be ossified in the tarsus, slightly earlier than the element y [prehallux of Francis (1934) and Duellman and Trueb (1994)]. The triangular element y articulates postaxially with the centrale and distally with the *basale commune* of the tarsus. The centrale is located in the center of the tarsus and is about half the size of the fibulare. This sector-shaped element articulates with all other tarsal elements. In the distal row, the first two basal tarsals are amalgamated into the oval basale commune, which is larger than distal tarsal 3 but smaller than amalgamated distal tarsal 4+5. The basale commune articulates proximally with the centrale and element y and postaxially with distal tarsal 3, and supports metatarsals 1 and 2 distally. The rounded distal tarsal 3 is only half the size of the basale commune and supports metatarsal 3. The distal tarsal 4+5 of Pachytriton is a single amalgamated element that is slightly larger than the centrale. It supports both metatarsals 4 and 5. One northeastern P. inexpectatus (CIB 88137, Figure 20) is unique in having separate distal tarsals 4 and 5 (in this case, tarsal 5 is much smaller than tarsal 4), which resembles the ancestral state in Paleozoic temnospondyls, Salamandra and Taricha (Francis, 1934; Shubin et al., 1995). Another atavistic condition is observed in one P. brevipes (MVZ 204299, Figure 20), which has a small bone that is surrounded by distal tarsal 4+5, centrale, fibulare and intermedium. This extra bone represents the m element of Schmalhausen (1917). Chang and Boring (1935) describe a *P. brevipes* that has the intermedium, centrale and element y all fused to the tibiale.



Figure 19 Left hind limb of southwestern *P. inexpectatus* (CIB 88160) in dorsal view. I–V: digits I–V.

Metatarsals resemble metacarpals of the *manus*. All five dumbbell-shaped bones are capped with cartilage at each end. The first and fifth metatarsals are much shorter than metatarsals 2–4, which are of similar length. Distally, each metatarsal supports 1–3 phalanges, which are successively shorter towards the toe tip. Each phalanx is also dumbbell-shaped except for the terminal one, which is triangular and has an expanded distal tip. The phalangeal formula of *Pachytriton* is 1–2–3–3–2; digits, in order of decreasing length, are: 3 > 4 > 2 > 5 > 1. Rare cases of toe loss and polydactyly are described by Chang and Boring (1935).

4. Discussion

4.1 Aquatic specialization of *Pachytriton* As adults, Pachytriton are permanently aquatic, inhabiting small montane streams (Pope, 1931; Özeti and Wake, 1969; Titus and Larson, 1995). However, molecular phylogenies suggest that this genus descended from an amphibious ancestor and evolved special adaptations for its obligate aquatic lifestyle (Zhang et al., 2008). Among the most remarkable specializations is its heavily ossified hyobranchial apparatus. Basibranchial and second ceratobranchial elements typically remain cartilaginous in terrestrial salamanders (Özeti and Wake, 1969), but they are solid bones in Pachytriton. Cartilage retained in the ceratohyal also is more reduced than in any other salamandrid species (Özeti and Wake, 1969). Posterior components, which include the first ceratobranchial, the osseous portion of the ceratohyal and the epibranchial, are all laterally expanded, a configuration that provides extra rigidity to resist mechanical distortion during "gape and suck" feeding (Özeti and Wake, 1969). During feeding, one group of muscles anchors the anterior part of the apparatus while a second group of muscles pulls the posterior part forward; this causes rapid depression of the throat and generates a negative pressure for suction. Extreme ossification and robustness of the tongue skeleton in *Pachytriton* enables rapid expansion of the oral cavity in these salamanders, which are much more efficient at prey capture than are amphibious salamanders (Miller and Larsen, 1989). All Pachytriton except P. archospotus have a strongly bowed epibranchial, which flares dorsolaterally and wraps around the neck with the distal tip extending further posteriorly. In contrast, the epibranchial is relatively short and straight in terrestrial or amphibious genera (Özeti and Wake, 1969). This modification allows the epibranchial to be pulled further downward and forward, thereby increasing both the volume of the oral cavity and the negative pressure generated. The posterior extension of the epibranchial also extends the length of the sheathing muscle (subarcualis rectus I) yielding a stronger pulling force. Finally, all Pachytriton except P. archospotus possess a unique epibranchial flange on the medial side, which provides additional rigidity. These specializations of the hyobranchial apparatus are crucial for prey capture in montane streams.

Two synapomorphies are shared by the "true newts" (Salamandridae: Pleurodinae): keratinized skin and a frontosquamosal arch (Titus and Larson, 1995). However, even though *Pachytriton* belongs to this clade, it has lost the first feature and exhibits reduction of the second one. Both specializations of *Pachytriton* may be due to its permanently aquatic habitat. Constant immersion in water relieves *Pachytriton* from the risk of evaporative water loss, which in terrestrial and amphibious newts is resisted by bound phospholipids and free fats that are linked to keratin side chains in the epidermis (Spearman, 1968). The skin of *Pachytriton* is very smooth and has abundant capillary vessels, which facilitate underwater gas exchange (Xu, 2007). In contrast, the granular skin of terrestrial and amphibious newts absorbs water more



Figure 20 Left: right hind limb of CIB 88137, representing the typical condition of *Pachytriton*; middle: left hind limb of CIB 88137, the red arrow points to a separate distal tarsal 5; and right: left hind limb of MVZ 204299, the red arrow points to an extra small bone.

efficiently from the substrate through capillarity (Toledo and Jared, 1993). In true newts, the frontosquamosal arch may act as a defensive mechanism that adds strength to the skull and protects the retracted eyeball during encounters with predators (Naylor, 1978). This arch is attenuate or incomplete in *P. brevipes* and northeastern *P. inexpectatus* and relatively firm in *P. archospotus* and southwestern *P. inexpectatus*. In comparison, the closely related, amphibious *Paramesotriton* has a much stouter frontosquamosal arch (Chang and Boring, 1935; Chan *et al.*, 2001). Arch reduction in *Pachytriton* may be associated with the absence of predators in shallow montane streams.

The locomotor system of Pachytriton is specialized for an aquatic habitat as well. In terrestrial salamanders such as the genus Salamandra, ribs of the first two trunk vertebrae bear large spatulate distal cartilages, to which insert muscles that originate from the scapula of the pectoral girdle (Francis, 1934). Consequently, the anterior body is suspended above the ground by the pectoral girdle. In contrast, distal cartilages of the first two ribs in Pachytriton are knob-like. This reduction suggests that there may be less muscle attached from the scapula, and thereby less support for the axial skeleton by the pectoral girdle. Furthermore, the limbs of Pachytriton are short and gracile in comparison to its stout body; terrestrial locomotion is correspondingly cumbersome (Wu, unpublished). However, Pachytriton is an efficient swimmer. Its powerful, paddle-like tail is supported by caudal vertebrae with prominent neural and haemal spines. These maintain their size posteriorly until the terminal one-fifth of the tail, whereas other vertebral components (e. g., transverse processes, zygapophyses) gradually reduce in size towards the tip. The length of caudal spines may even increase slightly near the middle of the tail. In comparison, caudal spines in the ponddwelling Cynops orientalis taper gradually. The laterally compressed tail skeleton of *Pachytriton* is wrapped by thick muscles, which enable the salamander to chase fastswimming prey and avoid being washed downstream.

4.2 Hyobranchial apparatus of *P. archospotus* For most of the time since the genus was first described in 1876, *Pachytriton* comprised just two named species, which clearly differ in external coloration: the spotted *P. brevipes* (Sauvage, 1876) and the unspotted, uniformly dark brown *P. inexpectatus* (Unterstein, 1930). A recent study, however, revealed unique phenotypic features in a geographic segment of spotted *Pachytriton*, which on that basis was described as a new species, *P. archospotus* (Shen *et al.*, 2008). Subsequent molecular phylogenetic

analyses confirm the validity of *P. archospotus* as a distinct species (Wu *et al.*, 2010).

The most reliable characteristic that easily distinguishes P. archospotus from congeners is the morphology of the hyobranchial apparatus. In P. archospotus, the ceratohyal is placed more anteriorly than it is in other Pachytriton groups. First and second ceratobranchials also are greatly enlarged such that their distal ends, including their articulations with the epibranchial, are visible posterior to the skull in dorsal view. Unlike the strongly bowed and flared epibranchial that is characteristic of other Pachytriton, the epibranchial of P. archospotus is straight and rod-like, resembling that of many species of Paramesotriton and Cynops (Özeti and Wake, 1969). Furthermore, P. archospotus lacks the distinct epibranchial flange that is unique to Pachytriton among all other salamandrids. One hypothesis that can explain this distribution of characters is that P. archospotus is the most basal species in the genus and its hyobranchial apparatus retains the ancestral condition. However, neither nuclear nor mitochondrial sequence data support this hypothesis; P. archospotus is likely the sister species to the group P. brevipes+northeastern P. inexpectatus (Wu et al., 2010; Wu et al., unpublished). Instead, the first divergence within Pachytriton occurs between southwestern P. inexpectatus and the common ancestor of P. archospotus, P. brevipes and northeastern P. inexpectatus (Wu et al., 2010). The most parsimonious explanation of hyobranchial variation among species of Pachytriton is that the typical Pachytriton hypotranchial apparatus evolved once in the common ancestor of the genus and is retained in all but P. archospotus, which evolved specializations that resemblethose in *Cynops* and Paramesotriton. This resemblance, however, is superficial. The apparatus is much stouter and more laterally expanded in P. archospotus, for example, and the extraordinary enlargement of ceratobranchials I and II is not seen in any other Asian salamandrid. The derived hyobranchial anatomy in *P. archospotus* may indicate the evolution of a modified feeding habit in this species.

4.3 Relationship between northeastern *P. inexpectatus* **and** *P. brevipes* As currently understood, *Pachytriton inexpectatus* has a disjunct geographic distribution: two widely separated populations are located on either side of the range *P. brevipes* (Zhao and Hu, 1984; Fei *et al.*, 1999; Fei *et al.*, 2006). Salamanders from both populations share the same external coloration, which at one time was the primary character used to diagnose species in the genus (Fei *et al.*, 2006). However, based on recent molecular phylogenetic analyses, the shared

color pattern is the product of homoplastic evolution and the northeastern population is instead nested within P. brevipes (Wu et al., 2010). Several osteological characters discussed above exhibit among-group variation, and in each case northeastern P. inexpectatus is more similar to P. brevipes than it is to southwestern P. inexpectatus. For example, southwestern P. inexpectatus develops dorsal extension on the buccal and lingual sides of the pars dentalis of the maxilla, but this distinct feature is absent in both northeastern *P. inexpectatus* and *P. brevipes*. Conversely, in both northeastern P. inexpectatus and P. brevipes the squamosal has a narrow ventral process, which is located next to a vertical ridge on the dorsal surface of the quadrate. In southwestern P. inexpectatus, the ventral process instead is broad and covers the flat dorsal surface of the quadrate. The frontosquamosal arch is attenuate or incomplete in northeastern P. inexpectatus and P. brevipes but relatively firm in southwestern P. inexpectatus. The humerus of northeastern P. inexpectatus and P. brevipes is a slender, rod-like bone, but in southwestern *P. inexpectatus* the humerus is laterally dilated due to its massive dorsal and ventral humeral crests. Likewise, the trochanter crest on the femur of the former two groups is much smaller and shorter than that of southwestern P. inexpectatus, which develops a second crest that extends to the distal tip of the femur. The mean number of caudal vertebrae shows no statistical difference between northeastern P. inexpectatus and P. brevipes, whereas southwestern P. inexpectatus has significantly fewer caudal vertebrae. In sum, osteological comparisons corroborate the phylogenetic affinity between northeastern P. inexpectatus and P. brevipes. Given that northeastern P. inexpectatus is nested within P. brevipes in molecular phylogenies (Wu et al., 2010), we assign northeastern P. *inexpectatus* to *P. brevipes*, which eliminates paraphyly of the former species and restore monophyly of the latter species. The southwestern population should be regarded as the nominal *P. inexpectatus*.

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Appendix

Cleared-and-stained specimens

Pachytriton brevipes CIB 88186 \bigcirc , 88191 \circlearrowleft , 88207 \bigcirc , 88208 \textdegree Pachytriton archospotus CIB 95951 \bigcirc , 95952 \bigcirc

Southwestern Pachytriton inexpectatus CIB 88153 \Diamond , 88157 \bigcirc , 88160 \bigcirc , 88173 \Diamond

Northeastern *Pachytriton inexpectatus* CIB 88137 \bigcirc , 88146 \bigcirc , 88152 \bigcirc , 88155 \bigcirc

Salamandra salamandra MCZ 8048, 99122 Taricha granulosa MCZ 1207, 125980

Cynops orientalis From pet trade, not yet catalogued.

Radiographed specimens

Pachytriton brevipes CIB 88187, 88188, 88192–88197, 88199– 88201, 88203–88205, 88221, 95965, 95972, 95973, 95975, 95978; MCZ 204107, 204108; MVZ (Museum of Vertebrate Zoology) 204299

Pachytriton archospotus CIB 95949, 95950, 95953

Southwestern *Pachytriton inexpectatus* CIB 88134, 88147, 88148, 88156, 88158, 88161–88166, 88170, 88178, 88180, 88182, 95944–96948

Northeastern *Pachytriton inexpectatus* CIB 88135, 88136, 88138, 88139, 88143–88145, 88149–88151, 88154, 88159, 88169, 88179, 88181, 95912–95914; CAS 194298, 194299, 194300

Paramesotriton hongkongensis MCZ 109447, 116047, 116048

Paramesotriton longliensis CIB 97884, 97885

Cynops wolterstorffi MCZ 1786, 8149, 8156, 9623

Cynops orphicus MVZ 22471, 22472, 22503