Chondrogenesis and Ossification of the Lissamphibian Pectoral Girdle

Rebecca M. Shearman*

ABSTRACT Knowledge of amphibian shoulder development is requisite for further understanding of gnathostome pectoral girdle evolution. Fish and amniotes share few pectoral girdle elements, but modern amphibians exhibit a unique combination of traits that bridge the morphological gap between these two groups. I analyzed patterns of chondrogenesis, ossification, and bone histology of the pectoral girdles of two anuran species (Xenopus laevis and Bombina orientalis) and two urodele species (Ambystoma mexicanum and Desmognathus aeneus) to provide new insight into the evolution of the tetrapod pectoral girdle. Comparisons reveal the following: 1) variation in the pattern of chondrogenesis among the anuran species analyzed correlates to variation in adult pectoral girdle morphology; 2) morphologically similar pectoral skeletons do not necessarily have similar patterns of bone histology; and 3) the urodele and anuran pectoral girdles included herein share a common morphology despite differences in patterns of chondrogenesis. J. Morphol. 269:479–495, 2008.

KEY WORDS: pectoral girdle; anuran; urodele; chondrogenesis; ossification; Xenopus; Bombina; Ambystoma; Desmognathus

Knowledge of modern amphibian skeletons is essential for the understanding of pectoral girdle evolution in gnathostomes. With few common pectoral girdle elements shared between modern fish and amniotes, extant amphibians uniquely exhibit a range of shoulder characteristics present in many vertebrate lineages. Fish typically have an extensive dermal pectoral skeleton composed of multiple elements and only a small endochondral skeleton that articulates with the fin. In contrast, amniotes possess an elaborate endochondral shoulder skeleton composed of several elements, with only a single dermal bone, the clavicle, remaining (Goodrich, 1958; Jarvik, 1980). Among extant amphibians, pectoral girdles vary in morphology, size of the dermal skeleton, and degree of endochondral ossification. In this article, I describe the ontogenetic variation of the shoulder in two species of frog and two species of salamander to provide new insight into the evolution of the tetrapod pectoral girdle.

Anura and Caudata (frogs and salamanders, respectively) are the two clades of Lissamphibia containing forelimb-bearing extant species. Within Anura, there is a wide range of shoulder girdle morphologies (Duellman and Trueb, 1986). The epicoracoid cartilages can be fused at the ventral midline, characteristic of most ranids, or overlapping across the ventral surface, common in more basal families such as Bombinatoridae (Fig. 1; Cope, 1864; Noble, 1922, 1926; Griffiths, 1963; Kluge and Farris, 1969; Emerson, 1983, 1984; Duellman and Trueb, 1986; Kaplan, 1995; Borkhvardt and Malashichev, 1997, 2000, 2003, 2004). Pectoral girdles with fused right and left epicoracoids often also exhibit a reduction in the sizes of the procoracoid cartilages, clavicles, and epicoracoid horns, and enlargement of the scapulae (Parker, 1868; Proctor, 1921; Trueb, 1973).

Salamanders do not exhibit the wide range of pectoral girdle morphologies observed in frogs (Duellman and Trueb, 1986). Urodele pectoral girdles lack all dermal elements, but as in anurans, the endochondral shoulder skeleton includes a scapula, coracoid, and procoracoid (Fig. 2). Despite a conserved shoulder morphology among salamanders, ossification varies from extensive bone formation in the scapula and coracoid to restriction of ossification to the scapula (Parker, 1868). Additionally, the left and right coracoids form large flat plates that overlap midventrally. This coracoid morphology, however, is not topographically analogous to the epicoracoid overlap in frog pectoral girdles (Francis, 1934).

Understanding lissamphibian evolution is complicated by several factors that make determining relationships within Anura and Caudata difficult. For instance, the overwhelming morphological diversity of each group confounds identification of primary homology (Duellman and Trueb, 1986;
Pough et al., 2001). Additionally, frogs, salamanders, and caecilians have undergone varying degrees of paedomorphosis (Duellman and Trueb, 1986; Pough et al., 2001) and the ancestral morphology for Lissamphibia is poorly understood (Zardoya and Meyer, 2001). Recent studies have lead to significant progress in understanding modern amphibian interrelationships (Bolt, 1991; Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Hedges and Maxson, 1993; Laurin and Reisz, 1997; Anderson, 2001; Bell and Wassersug, 2003; Schoch and Milner, 2004; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Carroll, 2007). Despite this, much remains unknown about the morphological evolution of Lissamphibia; however, embryology offers another avenue for studying amphibian evolution.

There are more than 5,424 known extant species of anurans and 557 extant species of urodeles (AmphibiaWeb, 2007). In this study, I provide a detailed description of the growth of the pectoral girdle in two anuran species, *Xenopus laevis* and *Bombina orientalis* and two urodele species, *Ambystoma mexicanum* and *Desmognathus aeneus*. *Xenopus laevis* and *B. orientalis* are both basal anurans (Duellman and Trueb, 1986; Pough et al., 2001; Frost et al., 2006). *Xenopus laevis* is a member of Pipidae, a clade of aquatic frogs that includes five extant genera, and *B. orientalis* is a smaller terrestrial frog that belongs to Bombinatoridae, a family containing only two extant genera (Fig. 3; Pough et al., 2002). The two salamander species included in this study are distantly related members of Salamandroidea. *Ambystoma mexicanum* is an aquatic salamander within Ambystomatidae, and *D. aeneus* is a terrestrial direct-developing plethodontid (Fig. 3; Pough et al., 2001; Frost et al., 2006). The four species studied herein are not representative of all of Batrachia (Anura + Caudata). However, this comparative study of broadly sampled extant amphibian species uses morphology, ontogeny, and bone histology to provide new insight into lissamphibian pectoral girdle development and evolution.

**MATERIALS AND METHODS**

**Animal Rearing and Collection**

Fertilized eggs of *Xenopus laevis* were purchased from Nasco Science (Fort Atkinson, WI) and reared through metamorphosis. Tadpoles were raised in filtered tap water at 22°C and housed at a rearing density of five tadpoles per liter of water. The animals were fed Nasco Frog Brittle daily and ~1/3 of the water was changed every 7 days. Tadpoles were collected once a day (n = 3) from the first appearance of hind limb buds through
metamorphosis and staged according to Nieuwkoop and Faber (1956). Adult frogs with a snout-vent length (SVL) of 28 mm were purchased directly from Nasco Science (Fort Atkinson, WI) and euthanized immediately. James Hanken (Harvard University) kindly provided fertilized eggs of *Bombina orientalis*. The eggs were reared under the same conditions as the *Xenopus laevis* eggs and tadpoles and staged according to the Gosner (1960) staging table. Tadpoles were reared until they began to metamorphose. Adult specimens with a SVL of 43 mm were purchased directly from The Aquarium Store (Chicago, IL).

Fertilized eggs of *Desmognathus aeneus* were collected by R. Adam Franssen from the Nantahala National Forest in Macon County, NC, and reared in the lab (Franssen et al., 2005). Specimens were staged according to Marks and Collazo (1998).

All animals were sacrificed using an overdose of MS-222 (0.2 g/100 ml) and then fixed in 4% Paraformaldehyde in PBS overnight. Samples were dehydrated through a graded methanol series and stored in 100% methanol at −20°C. Each species was staged using a different staging table (see earlier). For the sake of clarity, when referring to specific developmental stages the first letter of the genus being discussed is placed in front of the stage (i.e., Stage X10, B10, D10, and A10). Any given numeric stage is not comparable across species.

**Cartilage and Bone Staining**

A developmental series of each amphibian species was stained for cartilage and bone following modified versions of Dingerkus and Uhler (1977) and Hanken and Wassersug (1981) alcian blue and alizarin red protocols. Before staining, specimens were rehydrated, eviscerated, and bathed in distilled water overnight. The duration of each step varied owing to the size of the specimen. Larval specimens were immersed in the alcin blue solution for no more than 4 h and trypsin digestion varied from 45 min to 2 h. Adult specimens were bathed in trypsin solution overnight before staining with alizarin red. Following staining with alizarin red, specimens were bleached in 0.5% H2O2 in three parts KOH: 1 part glycerol. Cleared-and-stained specimens were stored in 100% glycerol.

**Histology**

Specimens were decalcified before sectioning using decalcifying solution from Richard-Allan Scientific (Kalamazoo, MI). The tissue was hydrated and rinsed in water for 1 h with multiple...
changes and then bathed in decalcifying solution for 45 min to 3 h, depending on the size of tissue. The pectoral girdles of adult frogs with a SVL of 64 mm were decalcified for 48 h with multiple changes before sectioning. Following decalcification, specimens were dehydrated through a graded ethanol series, cleared in xylenes, and imbedded in Richard-Allan Scientific Paraffin Type 9. Imbedded specimens were sectioned at 10 μm using a Heidelberg HM330 rotary microtome. Sections were mounted, deparaffinized, hydrated, and stained with Mayer’s hematoxylin and eosin stains. After staining with eosin, sections were rinsed in 95% ethanol and immersed in concentrated alcian blue (0.2 g/100 ml) for 15 s. All sections were then dehydrated, bathed in xylenes, and sealed with Permount under a cover slip.

Imaging and Figure Construction

Multiple digital images of a single pectoral girdle element were assembled to create high-resolution pictures of the cellular morphology of complete girdle elements. Images of histological sections were taken using a Leitz Laborlux S compound microscope and a Nikon DLX digital camera. In Adobe Photoshop (Ver. 7), the images were aligned using the individual nuclei of chondrocytes as morphological landmarks within the pectoral girdle. Composite images were created for Figures 4, 5, 7–9, and 11–14.

RESULTS

Adult shoulder morphology of *Xenopus laevis*, *Bombina orientalis*, *Desmognathus aeneus*, and *Ambystoma mexicanum*

There is a range of shoulder girdle morphologies within Anura (described above), and *Xenopus laevis* and *Bombina orientalis* represent one extreme in this continuum of girdle forms. In both species,
The length of the scapula is short relative to that of the suprascapula and coracoid. Specifically, the coracoid is twice the length of the scapula in *X. laevis* and 2.3 times the length of the scapula in *B. orientalis*. The ventral epicoracoids overlap at the midline in each species, although, the overlap is more extensive in *B. orientalis*. The glenoid fossa forms from the dorsolaterally extending coracoid and ventrolaterally projecting arm of the scapula. In *X. laevis*, the glenoid fossa and humerus project laterally, and in *B. orientalis* the glenoid opens ventrally. Both species have large dermal clavicles; however, the clavicle enlarges medially in *X. laevis* and laterally in *B. orientalis* (Fig. 1).

The salamanders *Desmognathus aeneus* and *Ambystoma mexicanum* have similar shoulder morphologies. In both species, each half of the girdle forms from a scapula, suprascapula, coracoid, and procoracoid. The glenoid fossa forms near the center of each half of the girdle, and there are no morphological boundaries between the scapula, coracoid, and procoracoid (Fig. 2). The most striking difference in shoulder form between *D. aeneus* and *A. mexicanum* is in their ossification patterns. The glenoid fossa is completely ossified in adult specimens of *D. aeneus*, and mineral deposition extends from the glenoid into the proximal regions of the scapula and coracoid. In the largest specimens of *A. mexicanum* analyzed (SVL 40 mm), ossification is limited to the scapula.

**Development of the Pectoral Girdle in *Xenopus laevis***

Chondrogenesis of the shoulder girdle of *Xenopus laevis* begins at Stage X52 with the condensation of mesenchymal cells proximal to and continuous with the developing forelimb bud (Fig. 4A–C). Both the scapula and coracoid are visible as two regions of densely packed cells, at Stage X54. However, the coracoid is already beginning to form a cartilage condensation while the presumptive scapula has not changed (Fig. 4D–F). By Stage X55, both elements are present as cartilaginous foci (Fig. 4G–I).

The suprascapula of *Xenopus laevis* develops from two chondrogenic foci within a continuous sheet of condensed mesenchyme. The ventral portion of the suprascapula derives from the dorsally growing scapula (Fig. 4G–I). A dorsal region of the suprascapula forms from a separate cartilage center that arises dorsal to the scapula and grows ventrally. In sections of the girdle at Stage X55, the dorsal condensation is only visible as densely...
packed cells distinct from the scapula (Fig. 4G–I). By Stage X58, the scapula and suprascapula form a single cartilage element (Fig. 5).

Endochondral ossification of the pectoral girdle of *Xenopus laevis* begins in the coracoid at approximately Stage X60. In whole-mount specimens, the diaphysis of the coracoid no longer stains with alcian blue because of the formation of a fibrous periosteum (Fig. 6A–C). At this stage, hypertrophic chondrocytes, within the coracoid, have begun to erode and the marrow cavity shows signs of expansion (Fig. 7A).

Simultaneous with the formation of the marrow cavity in the coracoid, hypertrophic cells at the center of both the scapula and coracoid are flanked by zones of stratified compact cells that neighbor resting mature chondrocytes (Fig. 7A,B). The scapula and coracoid meet at the gelenoid bridge, which is comprised of mature chondrocytes.

The scapula is the last shoulder element to ossify in *Xenopus laevis*. At Stage X60, a periosteum has not yet formed around the scapula. The endochondral coracoid and dermal clavicle and cleithrum have begun to ossify by Stage X62, but the scapula remains cartilaginous. All bony elements are ossified in postmetamorphic specimens with a SVL of 28 mm (Fig. 6D,E).

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The zonation of differentiated chondrocytes, at the transition between the scapula and suprascapula, resembles that of the epiphysial cartilage and growth zone of a young anuran long bone. The suprascapula is comprised of mature chondrocytes, the scapular/suprascapular joint is comprised of flattened cells, and the hypertrophic chondrocytes of the scapula abut the scapular/suprascapular joint (Fig. 7B). As in anuran long bones (Felisbino and Carvalho, 1999, 2001), the zones of differentiated chondrocytes within the scapula and suprascapula are not as distinct as those found in avian or mammalian long bones (Fell, 1925; Williams and Warwick, 1980).

The postmetamorphic scapula of *Xenopus laevis* (SVL 28 mm) has a thick periosteum and a lattice of trabeculae throughout the bone. The scapula contacts the cartilaginous procoracoid (Fig. 8A) and the coracoid to form the glenoid fossa. The marrow cavities of the scapula and coracoid extend into the glenoid bridge, but are separated by a zone of hyaline cartilage at the center of the bridge. The cellular organization of compact chondrocytes within the postmetamorphic scapular/
suprascapular joint resembles that of a premetamorphic shoulder girdle (Fig. 7A,B), albeit with more trabecular bone throughout the scapula and mineralization of the cartilaginous suprascapula (Fig. 8A). Just as the cellular organization of the scapular/suprascapular joint before metamorphosis resembles the histology of the epiphysis of a young anuran long bone, the scapular/suprascapular joint of the adult *X. laevis* girdle is similar to an adult epiphysis of an anuran long bone (Felisbino and Carvalho, 1999; Shearman, 2005).

**Development of the Pectoral Girdle in Bombina orientalis**

Early chondrogenesis in *Bombina orientalis* (Fig. 9) resembles that of *Xenopus laevis*. In *B. orientalis*, the scapula and coracoid first appear as two regions of densely packed cells within a homogeneous sheet of mesenchyme (Fig. 9A–C). By Stage B34, both the scapula and coracoid are present as cartilaginous foci; the coracoid already is larger than the scapula (Fig. 6F and 9D–F). Histological sections of the Stage-B34 girdle show that both elements are comprised of chondrocytes with large nuclei, and both stain with alcian blue indicating that these cells are already secreting extracellular matrix (ECM) rich in glycosaminoglycans.

The suprascapula of *Bombina orientalis* develops from both the scapular cartilage and a separate dorsal cartilaginous condensation. Histological sections at Stage B35 show the dorsal cartilaginous condensation distinct from the ventral scapular contribution to the suprascapula (Fig. 9G–I).
Periosteal formation and subsequent ossification occurs first in the coracoid and then the scapula. In whole-mount specimens stained with alcian blue and alizarin red, a colorless zone is present at the center of the coracoid at Stage B38. A similar colorless zone appears in the diaphysis of the scapula by Stage B41 (Fig. 6I,J). In serial sections, at Stage B41, the scapula is composed of hypertrophic cells, the periosteum around the coracoid is still thin, and the marrow cavity has not yet penetrated the coracoid cartilage (Fig. 7D). The dermal clavicle is present at Stage B41 in cross section, but the cleithrum has not yet formed. In adult specimens (SVL 43 mm), the endochondral scapula and coracoid are completely ossified and the cleithrum invests most of the suprascapula.

The scapulae and suprascapulae are strikingly different in postmetamorphic specimens of Bombina orientalis and Xenopus laevis despite the histological similarities of premetamorphic specimens. In B. orientalis trabecular bone of the scapula penetrates the glenoid bridge (Fig. 8C), and the scapula and suprascapula form two distinct elements. There is a boundary at the scapular/suprascapular joint (Fig. 8B) with cells of the scapula oriented at a 90° angle relative to the cells of the suprascapula.

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pula. The scapula and suprascapula are also separated by connective tissue, unlike the scapular/suprascapular joint in *X. laevis*. In *B. orientalis*, the suprascapula consists of hyaline cartilage, with chondrocytes evenly dispersed throughout the ECM. The scapula has a large marrow cavity threaded with trabeculae, hypertrophic cells abut the marrow cavity, and compact cells lie between the hypertrophied chondrocytes and the suprascapula (Fig. 8B).

**Development of the Pectoral Girdle in the salamander, Ambystoma mexicanum**

The pectoral girdle of *Ambystoma mexicanum* is formed from a scapula, suprascapula, coracoid, and procoracoid (Fig. 10I). Each half of the shoulder skeleton develops from a single mesenchymal condensation. Before the uptake of alcian blue in the humeral cartilage condensation, the shoulder girdle is visible as a single cartilaginous
center (Fig. 10A–C). The cartilage condensation grows along the dorsal–ventral axis and eventually forms the scapula, suprascapula, and proximal portion of the coracoid. By Stage A41, the coracoid and procoracoid branch off of the original cartilage condensation (Fig. 10C). The coracoid develops into a cartilaginous plate and forms the coracoid foramen by growing around the preexisting brachial nerve and blood vessels (Fig. 10E–G).

The shoulder girdle in Ambystoma mexicanum forms from a single chondrogenic condensation adjacent to the limb-bud mesenchyme (Fig. 11). Between Stages A38 and A40, the girdle’s proximo-distal width is rarely more than two cells thick and both the dorsal and ventral extensions are only a single cell wide (Fig. 11). As A. mexicanum grows from a SVL of 10 mm (Stage A45; Fig. 12) to a SVL of 20 mm, the large cells of the girdle remain uniformly distributed throughout the scapula, suprascapula, coracoid, procoracoid, and glenoid bridge (Fig. 13). The shoulder girdle in a juvenile A. mexicanum specimen (SVL of 20 mm) is cartilaginous and the peristeum has not yet developed. At this stage, the relative proportion of chondrocytes and extra cellular matrix is uniform throughout the girdle, and the cells have large lacunae and darkly staining nuclei (Fig. 13). Additionally, chondrocytes remain undifferentiated and are uniformly distributed throughout the glenoid as well as the zone between the scapula and suprascapula.

Periosteal formation and ossification is restricted to the scapula in the shoulder girdle of Ambystoma mexicanum. The peristeum first appears as a col-

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**Fig. 11.** Histological sections through anterior, median, and posterior portions of the left shoulder girdle of Ambystoma mexicanum in anterior view. A–C: Stage A38. D–F: Stage A40. Scale bar = 0.25 mm.
orless zone in the center of the scapula in whole-mount specimens with a SVL of 38 mm (Fig. 10H). When *Ambystoma mexicanum* reaches a SVL of ~40 mm, the scapula is calcified and stains with alizarin red; however, the coracoid remains entirely cartilaginous (Fig. 10I). The morphological boundary between the scapula and suprascapula is not discernable until ossification of the scapula is complete (Fig. 10I).

Sections of a shoulder girdle from a 40-mm SVL specimen show that neither marrow nor trabecular endochondral bone has penetrated the scapula (Fig. 14). Chondrocytes have begun to differentiate throughout the girdle, although the zones of hypertrophic cells, stratified chondrocytes, and hyaline cartilage are not as distinct as those in a frog shoulder or long bone (Fig. 14). Chondrocytes within the scapula and adjacent to the periosteum are hypertrophic, but this zone is not flanked by a region of compact cells, typical of several frog scapulae and many vertebrate long bones (Fell, 1925; Haines, 1938, 1942; Dickson, 1982; Baleeva, 2001; Felisbino and Carvalho, 2001; Shearman, 2005). The remaining girdle elements, including the suprascapula, procoracoid, coracoid, and glenoid...
bridge are still comprised of uniformly distributed chondrocytes. Indeed, when comparing the shoulder girdle of a 40-mm SVL specimen to a younger Stage A45 specimen, the most notable difference is that the chondrocytes within the older girdle are more compact with smaller lacunae (Fig. 14).

Development of the Shoulder Girdle in Desmognathus aeneus

The pattern of pectoral girdle chondrogenesis in Desmognathus aeneus, a direct-developing terrestrial salamander, resembles that of Ambystoma mexicanum, a paedomorphic aquatic salamander. The pectoral girdle of D. aeneus develops as a single cartilage condensation and by Stage D27, the scapula, coracoid, and procoracoid are present (Fig. 10J). By Stage D30, the shoulder girdle is formed in cartilage and the coracoid foramen is visible (Fig. 10L). In an adult girdle (from a specimen with a SVL of 24 mm), the scapula and coracoid are ossified and the suprascapula, procoracoid, and epicoracoid remain as flat cartilage plates (Fig. 10N).

DISCUSSION

Variation in Pectoral Girdle Chondrogenesis of Anurans

Differences in adult pectoral girdle morphology of the two anuran species examined here can be
correlated to variation in the relative timing of chondrogenesis of the coracoid, procoracoid, and scapula. Because the scapula and suprascapula form a single cartilage element before ossification, it has been hypothesized that the boundary between the two elements is determined late in development. By studying the length ratio of the ossified scapula to the cartilaginous suprascapula in adult frogs, Schmalhausen (1917) hypothesized that the scapula evolved from being very short, as in Bombina orientalis, to slender and elongate, as in Rana pipiens, by the progressive ossification of the suprascapula. More recently, Baleeva (2001) and Shearman (2005) suggested that the boundary between the scapula and suprascapula does not fall between the ventral condensation of the scapula and the dorsal condensation of the suprascapula as originally suggested by Braus (1909). Instead, these two cartilage foci fuse to form a single cartilage element. Baleeva (2001) also suggests that the boundary between the scapula and suprascapula is determined earlier than the ossification of the scapula and is instead determined by the position of the attachment site of the m. interscapularis.

The size of the scapula is not only determined by the boundary between the scapula and suprascapula, but by the relative timing of the appearance of the scapula and coracoid cartilage. The scapula, coracoid, and procoracoid form distinct cartilage condensations within the forelimb bud mesenchyme. The scapula is the first shoulder girdle element to chondrify in Rana pipiens (Shearman, 2005), but is second to the coracoid in Xenopus laevis and Bombina orientalis (Fig. 15). Importantly, in each species, the first element to form as a cartilage anlagen in the tadpole becomes the largest bone in the adult. In an adult R. pipiens (SVL 64 mm), the scapula is 1.4 times the length of the coracoid (Shearman, 2005) but the coracoid is between two and 2.5 times the length of the scapula in adult specimens of X. laevis and B. orientalis. This suggests that the size of an individual girdle element is partially determined by the length of time it has to develop as a cartilage condensation.

**Cellular Organization and Morphology of the Lissamphibian Pectoral Girdle**

Skeletogenesis of the endochondral elements of lissamphibian pectoral girdles resembles that of long bones. For example, in the anuran and urodele scapulae and coracoids included in this study, chondrocytes are organized into regions of hypertrophic, proliferating, and resting cells typical of long bone epiphyses. Those differences in the bone histology observed between anuran and urodele shoulder girdle elements, including variation in the columnar organization of proliferating cells and the presence of trabecular bone, are the same differences described for frog and salamander long bones (Eggeling, 1911; Froböse, 1927; Lubosch, 1927, 1938; Haines, 1942; Castanet, 1975; deRicqlès, 1979; Francillon, 1981; Dickson, 1982).

In contrast to the histological similarities of developing anuran and urodele shoulder girdles, the cellular organization of postmetamorphic scapulae in the observed frogs differs regardless of similarities in adult morphology. Xenopus laevis has short scapulae like those of Bombina orientalis, yet bone histology is more similar between X. laevis and the long-scapula form Rana pipiens. There is a gradual transition of hypertrophic, proliferating, and resting chondrocytes in pre- and postmetamorphic scapulae of X. laevis and R. pipiens, a pattern typical of a long bone epiphysis. In both species, the marrow cavities of the scapula and coracoid penetrate the glenoid bridge as the girdle continues to ossify. Each element, however, remains separated by a zone of hyaline cartilage. The histology of the glenoid bridge resembles that of two long bones contacting each other at the epiphyses. In B. orientalis, however, the scapular/suprascapular joint changes dramatically after metamorphosis, and there is a distinct boundary of connective tissue between scapula and suprascapula (Fig. 16). This connective tissue is similar to an articular ligament or synchondrosis (Braus, 1909), a joint that develops because of a zone of flexibility between two stronger (i.e., ossified) elements (Williams and Warwick, 1980).

**Anuran and Urodele Pectoral Girdle Morphology**

There has been a long debate surrounding the identity and homology of the individual elements within the salamander shoulder girdle (Gegenbaur, 1865; Parker, 1868; Goette, 1877; Howell, 1935; Hoffman, 1936). Unlike anuran pectoral girdles, urodele shoulder skeletons are predominantly cartilaginous with bone ossifying around the glenoid fossa. Joints do not develop between the coracoid and procoracoid. Indeed, the procoracoid has been described variably as part of the coracoid, scapula, and as an independent element (Gegenbaur, 1865; Detwiler, 1918; Engler, 1929; Chen, 1935).

The adult pectoral girdles of the salamanders included in this study have a scapula, suprascapula, coracoid, and procoracoid. These shoulder elements are also present in most anurans, but there are several differences between urodele and anuran pectoral skeletons. Notably, only the anurans in this study possess epicoracoids extending from the procoracoid to the coracoid. Additionally, the scapula and coracoid in Xenopus laevis and Bombina orientalis are distinct ossified elements. The shoulders of Desmognathus aeneus are ossified around the glenoid fossa and only the scapula is
ossified in *Ambystoma mexicanum*. Although the ossification extends in the coracoid and scapula in *D. aeneus*, there are no morphological boundaries between individual elements.

The overall architecture of the anuran and urodele shoulder remains similar despite variations in ossification. Each half of the shoulder forms a triradiate structure with a dorsally extending scapula and suprascapula, a coracoid projecting posterovertrally, and a procoracoid extending anterovertrally. This similarity appears during chondrogenesis and is most striking just before ossification. All of the amphibians studied herein share a stage of development in which each half of the shoulder forms a triradiate shape that is cartilaginous and lacks any morphological boundaries between the scapula, suprascapula, coracoid, and procoracoid (Figs. 6C,H and 10G,M).

This triradiate architecture appears during chondrogenesis and persists into adulthood, albeit with specific modifications in each lissamphibian lineage. Importantly, several species of anurans and salamanders arrive at this similar morphology differently. Previous descriptions of salamander pectoral girdle development differ. Some reports conclude that the shoulder skeleton forms from a single cartilage condensation and others claim the girdle forms from multiple chondrogenic centers.
aeneus reveal a complex combination of shared specimens of connective tissue and chondrocytes within the served. However, differences in the organization of members of Lissamphibia exemplify the degree to shoulder elements of four distantly related mem-
differentiated chondrocytes in the developing pectoral girdle. Similarities in the organization of and variable traits related to the growth of the pectoral limb recorded in the literature for taxa with missing data in an analysis of the Lepospondyli (Vertebrata, Tetrapoda). Syst Biol 50:160–170.

The pectoral girdle of the Jurassic Eocaecilia micropodia (Jenkins and Walsh, 1993), the only known caecilian relative with limbs, is reminiscent of the ossified scapula and coracoid of many salamander shoulder skeletons. As in salamanders, the shoulder of E. micropodia is ossified around the glenoid fossa with a broad coracoid and a scapula that is narrow at the shaft and wide along the superior margin. Although there is no evidence of a procoracoid in E. micropodia, the morphological similarities between E. micropodia and the adult anuran and salamander shoulder girdles studied here hints at the possibility that the triradiate form may be the primitive condition for Lissamphibia.

Evolution of the vertebrate skeleton includes not only changes in morphology, but also changes in the pattern and process of bone formation. The comparisons Xenopus laevis, Bombina orientalis, Ambystoma mexicanum, and Desmognathus aeneus reveal a complex combination of shared and variable traits related to the growth of the pectoral girdle. Similarities in the organization of differentiated chondrocytes in the developing shoulder elements of four distantly related members of Lissamphibia exemplify the degree to which the pattern of bone formation can be conserved. However, differences in the organization of connective tissue and chondrocytes within the scapular/suprascapular joint of postmetamorphic specimens of X. laevis and B. orientalis illustrate that, despite similarities in morphology, cellular patterns similar during ontogeny can become variable in adults. Data from this study provide new insight into the variation in ontogeny and bone histology of the lissamphibian shoulder skeleton, and may also serve as a guide for species sampling in future studies on the skeletal evolution of modern amphibians.

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