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The role of skeletal development in body size evolution of two North American frogs

Sarah Beth Havens

THE ROLE OF SKELETAL DEVELOPMENT IN BODY SIZE EVOLUTION OF TWO NORTH AMERICAN FROGS

by

SARAH BETH HAVENS

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Anne M. Maglia, Advisor
Jennifer Leopold
Melanie Mormile

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ABSTRACT

In order to better understand the evolution of miniaturization in *Acris blanchardi*, a North American Hylid with a unique life history and of ecological interest in the United States. The development and ossification sequences of 48 larvae, 5 juveniles and 5 adult *A. blanchardi* were examined. The adult was described by Maglia et al. (2007) to be a miniature and to display morphological novelties. In addition, 44 larvae, 2 juvenile and 5 adults of *Pseudacris crucifer* a closely related frog that has been suggested to be a miniature, were examined. The cranial and postcranial adult elements of this species, as well as development and ossification of the larvae were described. The onset of ossification is described for both species and, to better understand timing relative to other hylids, they are compared to a non-miniature, *Hyla lanciformis*. Ossification of the cranial elements in *A. blanchardi* begins at similar Gosner Stages as *Hyla lanciformis*, but most elements never fully ossify. *P. crucifer* begins ossification of cranial elements much later in development but continues ossification longer and ossifies more completely. In addition to poor ossification, *A. blanchardi* also adds novel endochondral ossification and random mineralization to the cranium to support under-ossified elements such as the nasals and otic capsule. Based on these results, the patterns of ossification leading to miniaturization in *A. blanchardi* are revealed, and no evidence of miniaturization in *P. crucifer* was discovered.

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1. LARVAL DEVELOPMENT OF *ACRIS BLANCHARDI*

1.1. ABSTRACT

Acris blanchardi is a small North American frog in the family Hylidae. It was found by Maglia et al. (2007) to be a miniature that displays morphological novelties in the adult. *A. blanchardi* has a unique life history because it has a relatively short life span of only 16-18 months. In order to understand the processes and reasons for miniaturization, herein the skeletal developmental patterns and ossification sequences of this species are described. The ossification sequence to that of *Hyla lanciformis,* a non-miniature hylid frog is compared to that of *A. blanchardi*. Ossification begins at similar Gosner stages in *A. blanchardi* and *H. lanciformis,* but, most elements do not fully ossify in *A. blanchardi* and development is arrested shortly after metamorphosis. In addition, ossification in *A.blanchardi* includes the addition of endochondral bone and mineralization in regions where ossification is reduced. This morphological novelty results from functional constraints. Finally, it is suggesed that the truncation of development in this species is a result of pressures to reach sexual maturity quickly.

1.2. INTRODUCTION

Acris blanchardi, commonly known as Blanchard's Cricket Frog, is a small North American species in the tree frog family Hylidae. Although *A. blanchardi* was considered to be a subspecies of *Acris crepitans* (e.g., Conant and Collins, 1991), a recent phylogenetic analysis of mitochondrial and nuclear genes by Gamble et al. (2008a) showed that *A. blanchardi* is a distinct species.

Contrary to most tree frogs, *Acris blanchardi* is semi-aquatic, and is most often found in and around ephemeral pools, small ponds, and wetlands (Johnson, 2000). Throughout most of its range, *A. blanchardi* breeds in the spring and fall, yet has a short life span of only about 18 months. Similar to other semi-aquatic North American hylids, it is a small frog, averaging 1.6--3.8 mm snout-vent length (Johnson, 2000).

Given its small size and adaptation to a semi-aquatic lifestyle from a tree frog ancestry, Maglia et al. (2007) examined the adult skeleton of *A. blanchardi* (identified as *A. crepitans*) to understand how it differed from that of other North American hylids. They found that adult *A. blanchardi* exhibit reduced cranial ossification and cranial morphological novelties, both of which are consistent with patterns of miniaturization described by Trueb and Alberch (1985).

The larval ontogeny of *A. blanchardi* was examined in an effort to identify the developmental patterns that have led to miniaturization in this species. Herein the skeletal morphology of the typical tadpole, the ontogeny of the larval skeleton, and the ossification sequence of the species are described with comments on the association between ecological adaptations, adult morphology, and skeletal development in this species.

1.3. MATERIALS AND METHODS

Forty-eight larvae, five juvenile and five adult wild-caught *Acris blanchardi* (originally identified as *A. crepitans blanchardi*) were examined. See the Appendix 1A for a list of specimens examined. Specimens were staged according to Gosner (1960), and cleared and double stained for bone and cartilage using methods adapted from Taylor and van Dyke (1985); see Appendix 1B for the complete protocol. Chondrocranial and osteological terminology follows that of Duellman and Trueb (1994), Fabrezi (1992, 1993), Fabrezi and Alberch (1996), Maglia et al. (2007b), and the Amphibian Anatomical Ontology (AAO; http://www.amphibanat.org; Maglia et al. 2007a; 2009). Numbers in parenthesis following anatomical terms refer to AAO reference numbers. All descriptions and illustrations were made using an Olympus SZX12 stereoscope equipped with a camera lucida and an Olympus QColor 5 digital camera.

1.4. RESULTS

1.4.1. Cranial Skeleton. The following description of the larval cranium is based on five tadpoles (Gosner Stage 37 and 38) that represent the typical tadpole morphology (i.e., stages in which there is complete chondrification of the larval cranium but prior to most ossification and metamorphic chondrocranial changes). Figure 1.1 shows dorsal, ventral and larval views of the larval cranium.

1.4.1.1. Structure of the Larval Cranium. The chondrocranium appears ovoid, with its width (at its widest, at the level of the palatoquadrate cartilages) approximately half its length (measured from the anterior tip of the upper jaw to the foramen magnum). The trabecular horns (AAO: 0010138) make-up approximately one-fourth the length of the tadpole cranium, and serve as the support for the suprarostral cartilages (AAO:0000998), the upper jaw of the tadpole. The horns form a V-shape in dorsal view, immediately diverging from one another at a 45° angle as they extend anteriorly from the ethmoid plate (AAO: 0010134). Each horn forms an arc in lateral view as it curves anterovententrally toward the jaw. At the base of each horn, near the fusion to the trabecular plate, there is a distinct triangular process (AAO:0010557) that serves as the attachment site for the quadratoethmoid ligament (AAO:0010558), which also attaches to the quadratoethmoid process (AAO:0010094) of the palatoquadrate (AAO:0000379). Each suprarostral is L-shaped (in anterior view) and comprises a lateral ala (AAO: 0010458) and a central corpus (AAO:0010458), which articulates dorsomedially with the anteromedial corner of the anterior margin of the trabecular horn via a ligamentous connection. The suprarostrals are connected to one another at the midline via a strong ligament.

Ventral to the suprarostral cartilages are the paired, rectangular infrarostral cartilages (AAO:00000237), which make up the medial portion of the tadpole's lower jaw. Medially, the infrarostrals articulate with one another via a strong ligamentous connection; laterally, each infrarostral forms a sinovial joint with Meckel's cartilage (AAO:0000289). Meckel’s cartilages are L-shaped in dorsal view and form the lateral portions of the tadpole lower jaw.

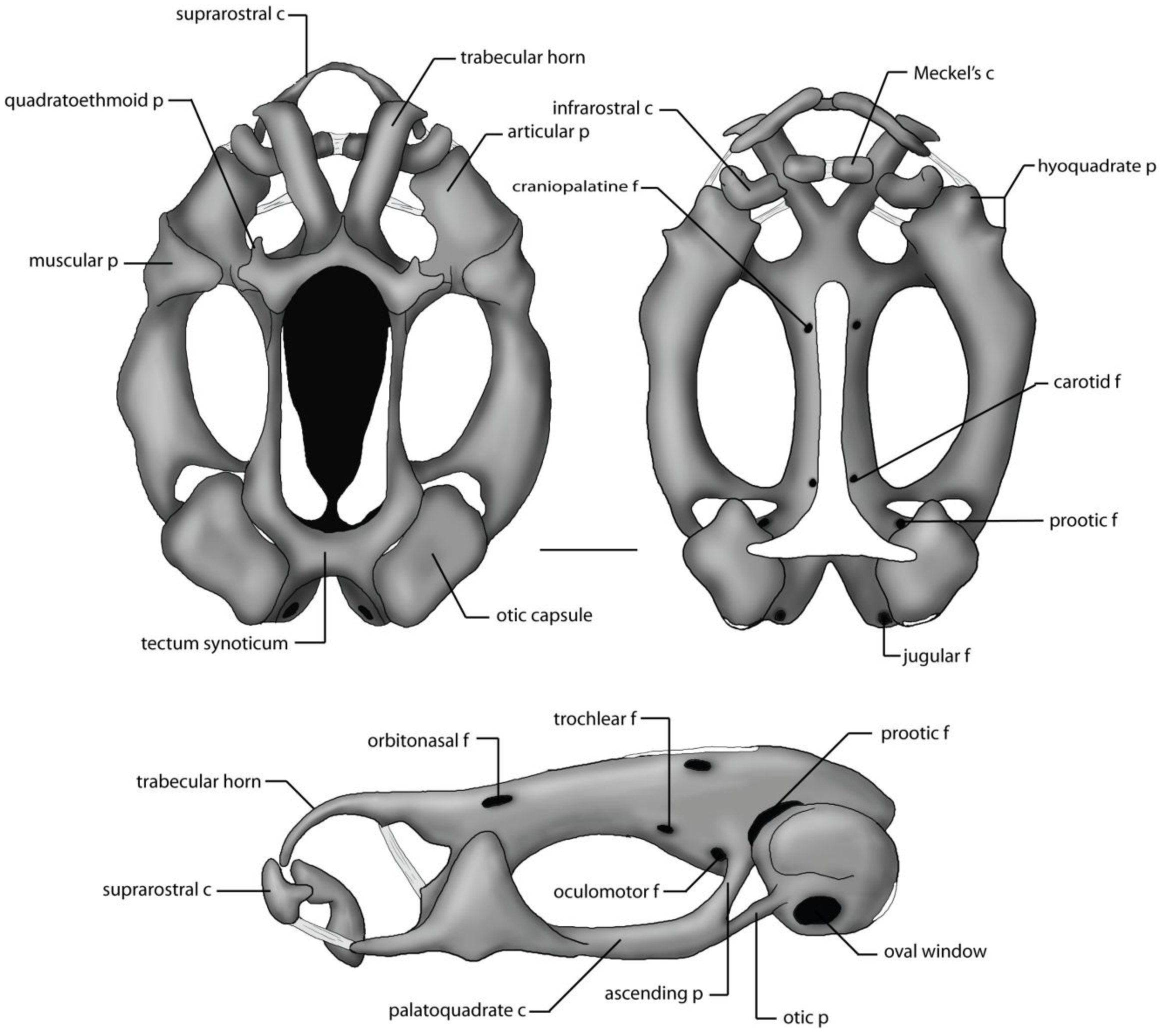

Fig. 1.1. Larval chondrocranium (Stage 38) of *Acris blanchardi* in: A) dorsal, B) ventral, and C) lateral view. Gray denotes cartilage, black denotes foramina. c = cartilage, f = foramen, p = process.

Laterally, each articulates with the anterior process (AAO:0010560) of the palatoquadrate via a cartilaginous bridge, the retroarticular process (AAO: 0010559).

The palatoquadrate is C-shaped in dorsal view and articulates with the lateral margin of the ethmoid plate anteriorly via the anterior quadratocranial commisure (AAO:0010061). Posteriorly, the palatoquadrate articulates with the pila antotic (AAO:0000431) via a thin ascending process (AAO:0000031) and with the crista parotica via the otic process (AAO:0010004). The suboccular arch (AAO: 0010820), the region between the anterior quadratocranial commisure and the ascending process, is wide and flat. The anterior process of the palatoquadrate is square in dorsal view, and just posterior to it lies a large, dorsally projecting, triangular muscular process (AAO: 0010005). Ventral to the muscular process is the hyoquadrate process (AAO:0010821), which project ventrally to articulate with the lateral process of the ceratohyal of the hyobranchial apparatus. Posterolaterally, the palatoquadrate bears a small articular process (AAO: 0010528), which serves as the connecting point for a strong otic ligament that attaches the palatoquadrate to the larval crista parotica (AAO:000016) of the otic capsule. The otic capsules are about a fourth the length of the larval chondrocranium, and are united with one another medially via a wide tectum synoticum (AAO:0010129). Ventrolaterally, each otic capsule is pierced by a large fenestra ovalis (AAO: 0000169). The posteroventral end of each otic capsule is pierced by the inferior perilymphatic foramen (AAO:0000195). Posterior to the otic capsules the jugular foramina (AAO:0000249) are visible in dorsal and ventral view; they open posteriorly at the level of the tectum synoticum. The notochord persists in the floor of the braincase between the otic capsules.

The cranial floor is formed by the basal plate (AAO:0010561), and is pierced anteriorly at the level of the anterior border of suboccular foramen (AAO:0010562) by a pair of craniopalatine foramina (AAO:0010563), and posteriorly, at the level of the ascending process, by a pair of carotid foramina (AAO:0010564).

The braincase is open dorsally via the frontoparietal fenestra (AAO: 0010525), and the taenia tecti medialis (AAO: 0010132) and taenia tecti transversalis (AAO: 0010130) are absent. The frontoparietal fenestra is bordered laterally by the taenia tecti marginalis (AAO: 0000599). The lateral wall of the braincase is pierced by four foramina. The most anterior of these is the optic foramen (AAO: 0000342), which lies between the pila preoptica (AAO: 0010565) and pila metoptica (AAO: 0000432), and accommodates the optic nerve (Cranial Nerve II [AAO: 0010467]) . Posterior to the pila metoptica, and just anterior to the ascending process of the palatoquadrate, is the oculomotor foramen [AAO: 0000332], which houses oculomotor nerve (Cranial Nerve III; [AAO: 0010468]). The trochlear foramen (AAO: 0000610), which houses the trochlear nerve (Cranial Nerve IV; [AAO: 0010469]), is present anterodorsal to the optic and oculomotor foramina. The prootic foramen (AAO: 0010566), the largest of the four foramina in the lateral briancase wall, accommodates the trigeminal (Cranial Nerve V; [AAO: 0010470]), abducens (Cranial Nerve VI; [AAO: 0010471]), and facial (Cranial Nerve VII; [AAO: 0010472]) nerves, opens just posterior to the ascending process of the palatoquadrate, and is separated from the oculomotor foramen by the pila antotica (AAO: 0000431).

1.4.1.2. Developmental Modifications of the Larval Cranium. The larval cranium undergoes substantial structural change during development (Fig. 1.2), including several modifications prior to metamorphosis, and there is substantial morphological variation among tadpoles of the same Gosner stage. Prior to Gosner Stage 33, the larval cranium is poorly chondrified and does not stain well. By Stage 33, the tectum synoticum (AAO: 0010129) is present as a faint bridge of cartilage spanning between the otic capsules. As development continues, this band of cartilage thickens, and by Stage 37 the tectum synoticum is fully chondrified and unites the otic capsules.

In early Gosner stages (pre-33), the basicranial fenestra (AAO: 0000037) is present in the floor of the braincase, but by Stage 33, the basicranial fenestra disappears, and the basal plate appears as a solid sheet of cartilage. By Stage 32, the trabecular plate (= ethmoid plate; [AAO: 0010137]) extends anteriorly from, and is confluent with, the basal plate, and it is pierced by the paired craniopalatine foramina anteriorly and internal carotid foramina posteriorly. By Stage 32 the trabecular horns are present as anterior extensions of the trabecular plate; they will increase in size, and by Stage 34 will articulate with the suprarostral cartilages. By Stage 41 the trabecular horns begin to erode, and by Stage 43 they are no longer present. By Stage 32 the suprarostral cartilages are fully formed, but small. By Stage 34, the suprarostrals are connected to one another via a strong ligament and articulate with the trabecular horns. As the adult jaws form, the suprarostrals begin to erode, the connection between them deteriorates, and they separate from one another around Stage 41. By Stage 44 the suprarostrals have eroded completely (and are no longer present). By Stage 32, the infrarostral cartilages are present as rectangular elements ventral to the suprarostral cartilages.

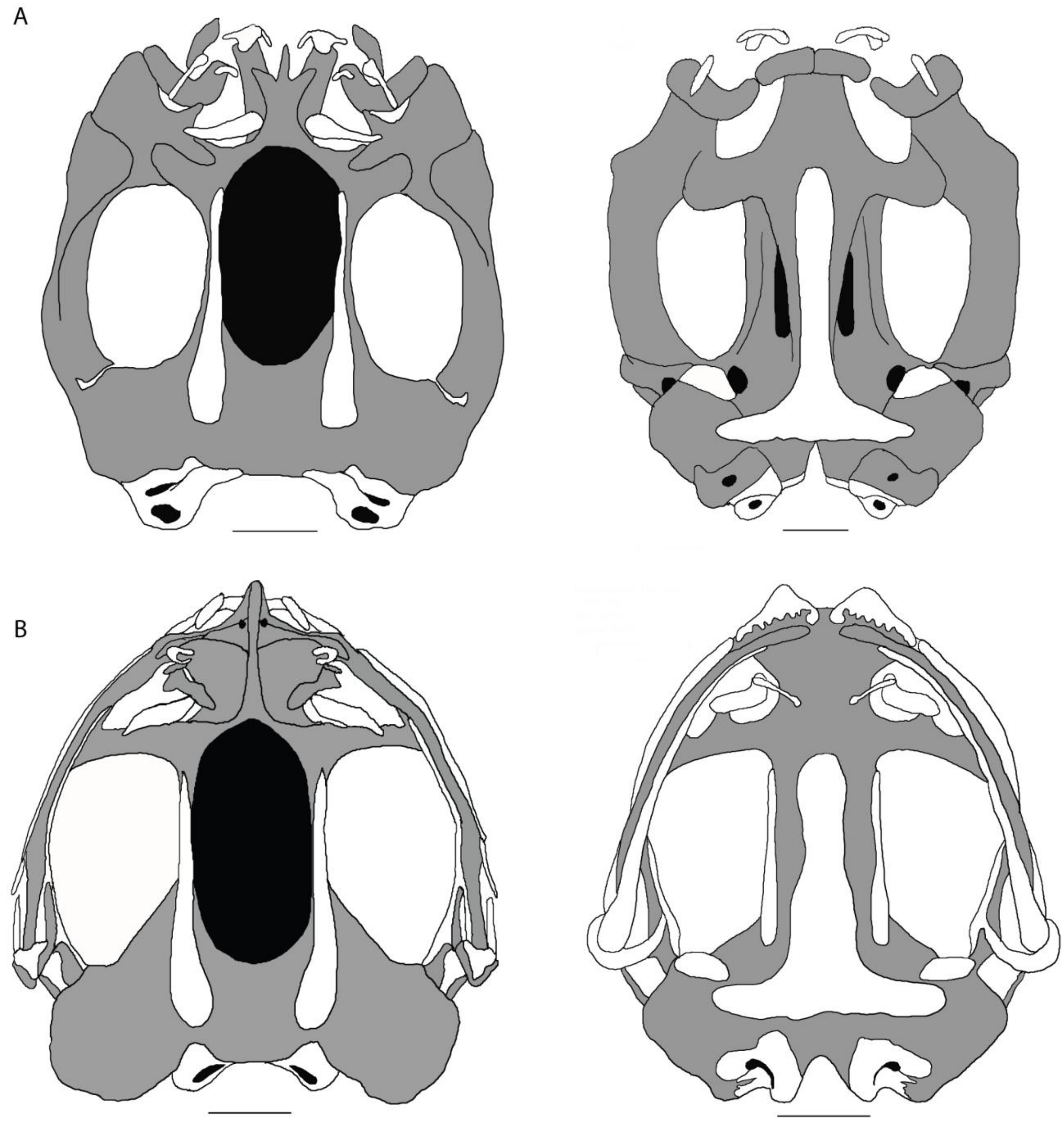

Fig. 1.2. Development of the cranium of *Acris blanchardi* in dorsal (left) and ventral (right) views. **A:** Stage 42. **B:** Stage 46. White denotes ossification; gray denotes cartilage; black denotes foramina.

By Stage 34 the infrarostrals are united at the midline via a weak ligamentous connection and articulate laterally with Meckel's cartilage. By Stage 35 the ligamentous connection strengthens and pulls the infrarostrals closer together at the midline. By Stage 41, the ligamentous connection between the infrarostrals disappears, and they begin to erode; by Stage 44, they are no longer present.

By Stage 32, the palatoquadrate resembles the typical larval morphology; subsequent to Stage 39, the palatoquadrate erodes and changes shape to reflect the adult morphology (described by Maglia et al., 2007). By Stage 45 the anterior portion of the palatoquadrate has eroded to form a narrow rod of cartilage lateral to the suboccular foramen; it is continuous anteromedially with the planum antorbitale (AAO: 0000434) and anterolaterally with the processus triangularis (AAO: 0010824), which terminates as the anterior maxillary process (AAO: 0000015). By Stage 44, the otic ligament chondrifies and the palatoquadrate becomes continuous with the crista parotica of the otic capsule. By Stage 46, the posterior remnants of the palatoquadrate forms the quadrate, a triangular cartilage lateral to the otic capsule that is invested anteriorly by the quadratojugal. By Stage 46, the ascending process of the palatoquadrate is absent and the palatoquadrate articulation with the lateral wall of the braincase is lost.

By Stage 32, the otic capsules are apparent as rectangular boxes of cartilages posterolateral to the braincase. The fenestra ovalis is present by Stage 34, and by Stage 35, a rudimentary operculum appears as a faint ossification in the fenestra ovalis. By Stage 36, ossification of the prootics begins along the anterior wall. By Stage 42, mineralization appears at the margin between the prootics and exoccipitals. The exooccipitals and prootics continue to grow and ossify through adulthood. By Stage 39 the occipital condyles are visible as ossified extensions of the posterior portion of the exoccipitals. The exocciptals will ossify to form the margins of the foramen magnum after metamorphosis. The crista parotica of the prootic begins ossification by Stage 46 as a lateral extension.

1.4.1.3. Development of the Nasal Capsule. In Stage 33 (the earliest available specimen) the septum nasi (AAO: 0000559) has already formed and is continuous with the chondrification of the taenia ethmoidalis. It appears as a faintly stained sheet of cartilage dorsal to the trabecular plate and between the olfactory foramina. By Stage 34, the lamina orbitonasalis (AAO: 0000262) have begun to chondrify. By Stage 38, the tectum nasi (AAO: 0000601) has chondrified to form a continuous piece of cartilage with the lamina orbitonasalis---these will form the adult postnasal wall. By Stage 39, the alary cartilages (AAO: 0000002) appear as minute triangles anterior to the lamina orbitonasalis, and the anterior prenasal cartilages (AAO: 0010827) appear near the anterior portions of the alary cartilages. By Stage 40, the minute beginnings of the superior prenasal cartilages (AAO: 0000586) are present near the anterior margins of the small alary cartilages. The paries nasi (AAO: 0000389) are apparent as triangular pieces of cartilage medial to the septomaxilla (which also appear by Stage 40, described below), and the anterior nasal wall has begun to chondrify. By Stage 41 the trabecular plate and anterior nasal wall fuse, and most of the trabecular plate forms the solum nasi. Also by this stage, the nasal bones begin to ossify. By Stage 42, the alary cartilages and tectum nasi begin to form the circular opening of the nasal passage. At Stage 43, the crista intermedia (AAO: 0000113), the laminae superior (AAO: 0000264) and inferior (AAO:

0000259), and oblique cartilages (AAO:0000329) appear. By Stages 43 and 44, the chondrocranium has eroded, the cranium resembles adult morphology, and most of the nasal structures have attained their adult morphology; the nasal structures continue to grow in size as the larval cranial structurs continue to erode. By Stage 45, mineralization of the tectum nasi is apparent, and by Stage 46, endochondral ossification of the tectum nasi and appears and partially fuses to the already ossified nasal bones.

1.4.2. Pre-Metamorphic Ossification of the Cranium. The postmetamorphic ossification sequence and adult skeletal morphology of *Acris (crepitans) blanchardi* were described by Maglia et al. (2007) and are not included here. The order of ossification sequence of cranial elements is shown in Table 1.1. Hanken and Hall (1988) observed that ossification can be detected in sectioned specimens several Gosner stages before it is visible in cleared-and-stained whole-mount preparations; thus, the results presented here should be considered estimates of the timing of ossification.

Parasphenoid.---The parasphenoid (AAO: 0000385) is the first cranial bone to ossify. By Stage 32, the cultriform process (AAO: 0000386) appears as a thin, unstained sliver underlying the newly formed floor of the braincase. The ala (AAO: 0000387) are present as thin, poorly stained fragments by Stage 33. By Stage 38 the parasphenoid resembles its adult shape but will continue to grow until maturity.

TABLE 1. Ossification Sequence of Acris Blanchardi

Stage	Cranial	Postcranial
32	Parasphenoid	
34		Vertebral column
36	Prootics	Femur
	Exoccipitals	
	Frontoparietals	
37		Scapula
		Humerus
		Radius
		Ulna
		Tibiofibula
		Tibiale-fibulare
		Metatarsals
		Foot Phalanges
38		Cleithrum
		Clavicles
		Coracoids
		Metacarpals
		Hand phalanges
		Ilium
40	Septomaxillae	Ischium
	Premaxillae	
41	Maxillae	
42	Nasals	
44	Vomers	
45	Dentaries	
	Angulosplenials	
46	Pterygoids	Carpal elements
	Quadratojugals	
	Quadrate	
	Squamosals	
	Mentomeckelian	
After Stage 46	Sphenethmoid	

Elements are listed at the first Gosner Stage at which they appear.

Prootics.---The prootics (AAO: 0000514) appear by Stage 36 as ossification of the anterior wall of the otic capsule. Ossification continues through Stage 46, progressively incorporating more of the anterior portions of the otic capsule. By Stage 46 the crista parotica (AAO: 0010152) has begun to ossify. The crista parotica continues to ossify into adulthood (Maglia et al., 2007).

Exoccipitals.---The exoccipitals (AAO: 0000147) form the posterior portions of the otic capsules and the margins of the foramen magnum (AAO: 0010826). By Stage 36, the exoccipitals begin to ossify around the jugular foramina (AAO: 0000249).

Exoccipital ossification continues anteriorly from the posterior margin of the otic capsule. The exoccipitals will not fully ossify until after metamorphosis, and the prootics and exoccipitals will unite via mineralization (rather than complete ossification) near adulthood.

Frontoparietals.---By Stage 36, the paired frontoparietals (AAO: 0000207) begin to ossify as long, narrow rods in the dorsolateral region of the neurocranium. As ossification continues, the bones lengthen and broaden posteriorly. The frontoparietals of the adult skull are thin and gracile, a condition achieved by Stage 42 (with only minor growth and ossification beyond this stage).

Septomaxillae.---By Stage 40, the septomaxillae (AAO: 0000556) begin to ossify and appear as small rectangular fragments dorsolateral to the triangular processes of the trabecular horns. Ossification continues, forming the adult shape by Stage 43. Beyond Stage 43, the septomaxilla continues to enlarge and migrates anteriorly as it develops.

Premaxillae.---The alary processes (AAO: 0000003) of the premaxillae (AAO:0000461) appear as tear-drop shaped ossifications dorsal to the trabecular horns by Stage 40. By Stage 43, the pars dentalis (AAO: 0000397) and pars palatina (AAO: 0000414) of each premaxilla are ossified.

Nasals.---The paired nasals (AAO: 0000312) appear by Stage 42 posterodorsal to the septomaxillae. They are tear-drop shaped by Stage 42, but become more rectangular as they grow larger and migrate anteriorly. Although the nasals remain gracile throughout life, postmetamorphic endochondral ossification of the nasal tectum will fuse to nasals in adulthood, thus making them seem more robust.

Maxillae.---By Stage 41, the pars facialis (AAO: 0000400) of the maxilla (AAO: 0000285) begins to ossify dorsomedial to the lateral margin of the palatoquadrate. The pars dentalis (AAO: 0000396) and tooth primordia appear by Stage 44. Fully developed maxillary teeth are evident by Stage 45.

Vomers.--- By Stage 44, the vomers (AAO: 0000633) appear as small fragments anterolateral to the rostral tip of the parasphenoid. By Stage 45, vomerine ossification continues anteriorly to form the anterior process (AAO: 0000649). The prechoanal process (AAO: 0000658), postchonanal process (AAO: 0000659), and vomerine teeth (AAO: 0000635) appear postmetamorphically.

Sphenethmoid.---The sphenethmoid (AAO: 0000572) does not ossify until after metamorphosis.

Pterygoid.---By Stage 46, ossification of the pterygoid (AAO: 0000521) is apparent on the medial margin of the palatoquadrate, anterior to the otic capsule and medial to the maxilla. The pterygoid completes ossification after metamorphosis.

Quadratojugal.---Quadratojugals (AAO: 0000532) appear by Stage 46 posterior to the maxillae and lateral to the posterior remnants of the palatoquadrate. The quadratojugals appear as long, narrow triangular bones that articulate anteriorly with the maxilla and posteriorly with the quadrate.

Quadrate.---The quadrate (AAO: 0000525) appears by Stage 46 as a small triangular bone medial to the squamosal and dorsolateral to the pterygoid.

Squamosal.---The squamosals (AAO: 0000574) appear by Stage 46 as small rectangular pieces of cartilage located lateral to the posterior remnants of the palatoquadrate (and ossifying quadrate). They will continue to grow ossify postmetamorphically.

Dentary.---The dentary (AAO: 0000124) begins ossification by Stage 45 and is apparent on the anterolateral surface of Meckel's cartilage. The dentary lacks any odontoids or ridges.

Angulosplenial.---The angulosplenials (AAO: 0000966) begin to ossify before Stage 45 along the posteromedial surface of Meckel's cartilage. By Stage 46, the coronoid process (AAO: 0000012) has formed on the posterior margin of each angulosplenial.

Mentomeckelian.---The mentomeckelian (AAO: 0000301) begins to ossify before Stage 46, and continues to ossify postmetamorphically to fuse posteriorly with the anterior portion of the dentary.

1.4.3. Hyobranchium

1.4.3.1. Structure of the Hyobranchial Skeleton. The following description of the larval hyobranchial apparatus is based on two Stage 37 specimens (Fig. 1.3).

Basibranchial I (AAO: 0010583) is absent; Basibranchial II (AAO: 0010585), two ceratohyals (AAO: 0000666), and four pairs of ceratobranchials (AAO: 0000953) are present. Each ceratohyal has two well-developed anterior processes and a prominent lateral and posterior process. The anterior processes project anteriorly such that the ceratohyal margin appears cup-shaped. The lateral processes are robust, with a rounded dorsal surface that articulates with the hyoquadrate process of the palatoquadrate.

The hypobranchial plates are broad sheets of cartilage that provide the articular surface for the ceratobranchials. Ceratobranchials I--IV are continuous with the hypobranchial plate, and each hypobranchial plate posesses three posterodorsally-projecting spicules that form along its posterior margin between the ceratobranchials. The ceratobranchials are long, thin bars of cartilage that possess poorly-developed ceratobranchial teeth; they articulate posteriorly with the commissura terminales (AAO: 0010828). A small branchial process is present on the anterior margin of Ceratobranchial III.

1.4.3.2. Developmental Modification of Hyobranchial Skeleton. Most of the developmental changes in the hyobrachial apparatus that result in the formation of the adult hyoid occur during or after metamorphosis. By Stage 41, the ceratobranchial teeth and hypobranchial spicules have begun to erode. By Stage 42, the spicules have disappeared completely, and the anterior processes of the ceratohyals have begun to erode. By Stage 43, the ceratohyals are significantly reduced and the ceratobranchials have begun to erode.

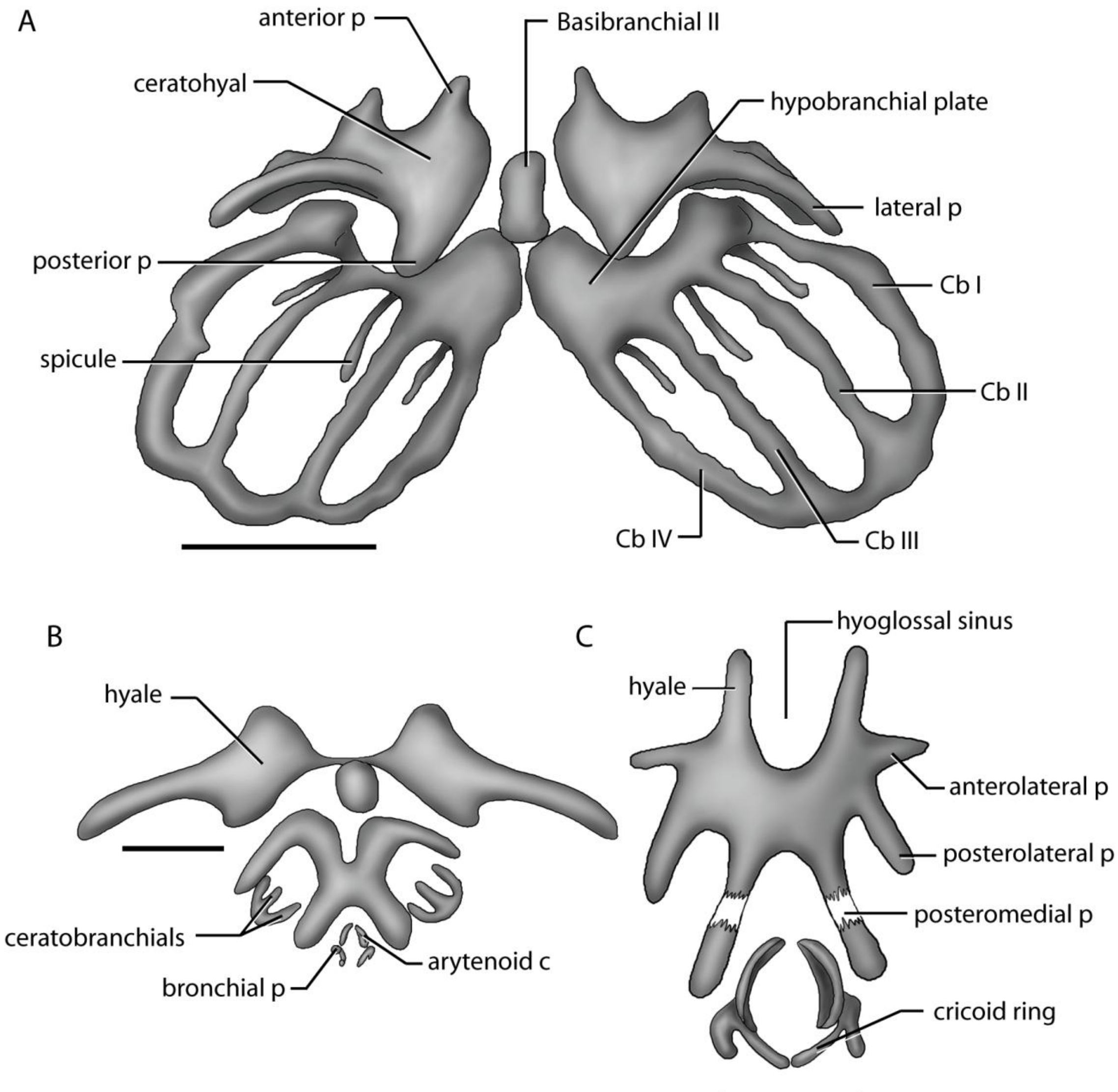

Fig. 1.3. Development of the hyobranchium of *Acris blanchardi* in ventral view. A) Stage 37. B) Stage 44. and C) Stage 46. White denotes ossification; gray denotes cartilage. c = cartilage; cb = ceratobranchial; p = process.

By Stage 44 the remnants of the ceratohyals have begun to fuse with the hypobranchial plate to form the rudiment of the adult hyoid plate. By Stage 44, early rudiments of the posteromedial processes of the hyoid plate appear along the posterior margins of the hypobranchial plate. By Stage 45, arytenoid cartilages (AAO: 0000674) appear as small triangular sheets of cartilage posterior to the hyoid plate, and a thin bar of cartilage, the bronchial process (AAO: 0000676), is apparent lateral to each arytenoid cartilage. By Stage 46, the anterior margins of the ceratohyals have begun to erode, but they are not completely separated from one another. Also by Stage 46, the cricoid cartilage (AAO: 0000675) is present, the posteromedial processes have begun to ossify proximally, and the hyale have elongated and narrowed. The remainder of the modifications to the hyobranchium take place postmetamorphically.

1.4.3.3. Ossification of the Hyobranchium. In *Acris blanchardi* the hyobranchial apparatus remains largely cartilaginous until after metamorphosis. Anterolateral (AAO: 0000671) and posterolateral (AAO:0000672) processes of the hyoid plate (AAO:0000664) are not present prior to metamorphosis, and the posteromedial processes (AAO:0000673) are the only portions to ossify.

1.4.4. Postcranial Skeleton Development. In contrast to the timing of cranial ossification, the postcranium is well formed in bone and before metamorphic climax (e.g., Gosner Stage 38) resembles the adult morphology. The pre-metamorphic postcranial ossification sequence is presented in Table 1.1.

1.4.4.1. Ossification of the Axial Skeleton. Note that the development of the axial skeletal of *A. blanchardi* was described by Pugener and Maglia (2009) and is not included here; however, the ossification sequence of the axial skeletal elements is described below (and included in Table 1.1). The axial skeleton of the adult consists of eight presacral vertebrae, the sacrum (AAO: 0000552)) and the urostyle (AAO: 0000732).

Presacral vertebrae.--- By early Stage 34, the centrum of the atlas (Vertebra I: AAO: 0000709) has begun to ossify as two center of ossification on the dorsolateral margins of the notochord. By late Stage 34, these ossifications fuse medially, and ossification of the centrum of Vertebra II is present as two medially-fused centers. Also by late Stage 34, the centra of Vertebrae III and IV are present as small (unfused) lateral centers of ossification, and the neural arches of the first four vertebrae have begun to ossify. Ossification of the presacral vertebrae continues in this manner in an anterior to posterior direction, and by Stage 37, fused ossification centers of the centra of all eight presacral vertebrae are present at the midline of the axis; ossification of the neural arches of all eight presacral vertebrae also is present. Ossification and growth of the presacral elements continues, and by Stage 46 the centra and neural arches are fully ossified and fused.

Sacrum and urostyle.---By Stage 36, the neural arches of the sacrum (AAO: 0000552) have begun to ossify. By Stage 37, the centra of the sacrum and Postsacral Vertebra 1 (AAO: 0000691) are present, each as two center of dorsolateral ossifications. Sacral ossification continues, and by Stage 40 the bony sacrum is small, but fully formed. By Stage 42, ossification of Postsacral Vertebra 2 is present as a posterior expansion of

Postsacral Vertebra 1. By Stage 43, Postsacral Vertebra 3 is present, and the three postsacral vertebrae have fused together to form the coccyx. By Stage 40, hypochord ossification is present. The hypochord continues to grow and expand, and by Stage 43 the hypochord fuses to the coccyx to form the urostyle.

1.4.4.2. The Anterior Appendicular Skeleton

Pectoral Girdle.---By Stage 35, pectoral girdle (AAO: 0000422) first appears in cartilage, although no elements are distinguishable. Chondrification continues rapidly, and by Stage 36, the scapula (AAO: 0000751) is evident as a spade shaped cartilage medial to the ovoid coracoid (AAO: 0000764) and procoracoid (AAO: 0000760) (Fig. 1.4). The scapula elongates, and by Stage 37, the suprascapula (AAO: 0000748) is present as a small sheet of cartilage articulating with the distal margin of the scapula. Also by Stage 37, early ossification of the scapula is present near the middle of the element. By Stage 38, the cleithrum (AAO: 0000750) has begun to ossify along the anterior margin of the suprascapula, the clavicle (AAO: 0000761) has begun to ossify and appears as a narrow strip of bone anterior to the procoracoid cartilage, and the coracoid has begun to ossify along its midline. By Stage 40, the pectoral girdle has increased in size, but there has been relatively little increases in ossification amount. By Stage 42 the pars acromialis (AAO: 0000752) of the scapula has begun to ossify. By Stage 42, the distal head of the scapula has nearly completed ossification. By Stage 43 the sternum appears as posterolateral cartilaginous processes extending from the epicoracoid cartilages. The pectoral girdle remains largely unchanged from Stage 44 until after metamorphosis, when it completes development (Maglia et al 2007).

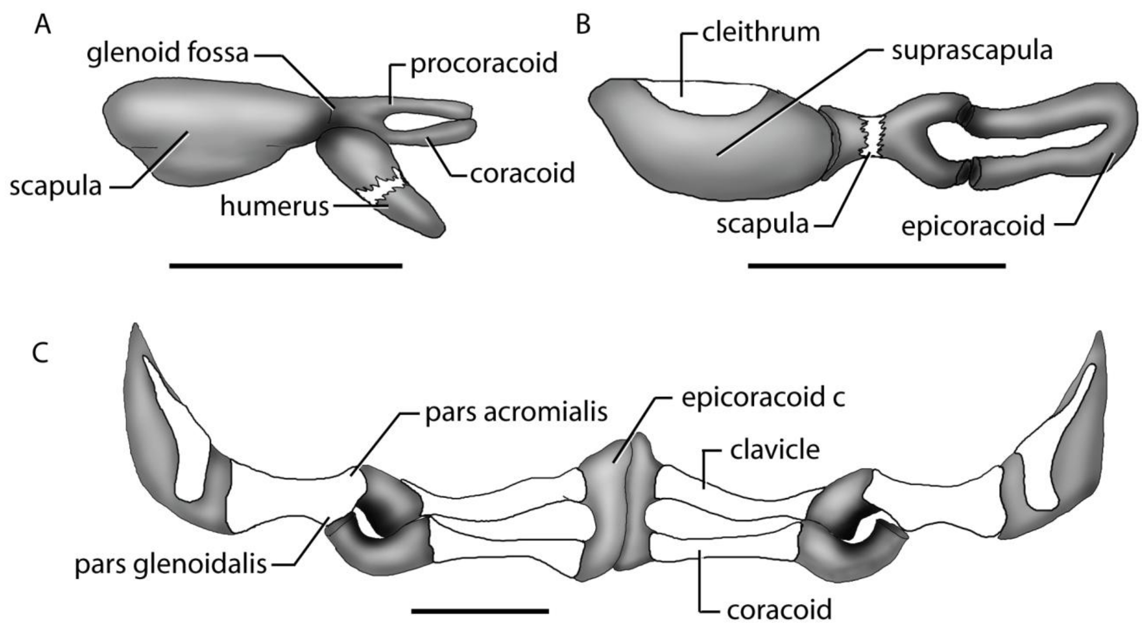

Fig. 1.4. Development of the pectoral girdle of *Acris blanchardi* shown in ventral view and the scapula and suprascapula averted ventrally into the plane. **A:** Right side, Stage 36. **B:** Right side, Stage 38. **C:** Stage 43. White denotes ossification; gray denotes cartilage. c = cartilage.

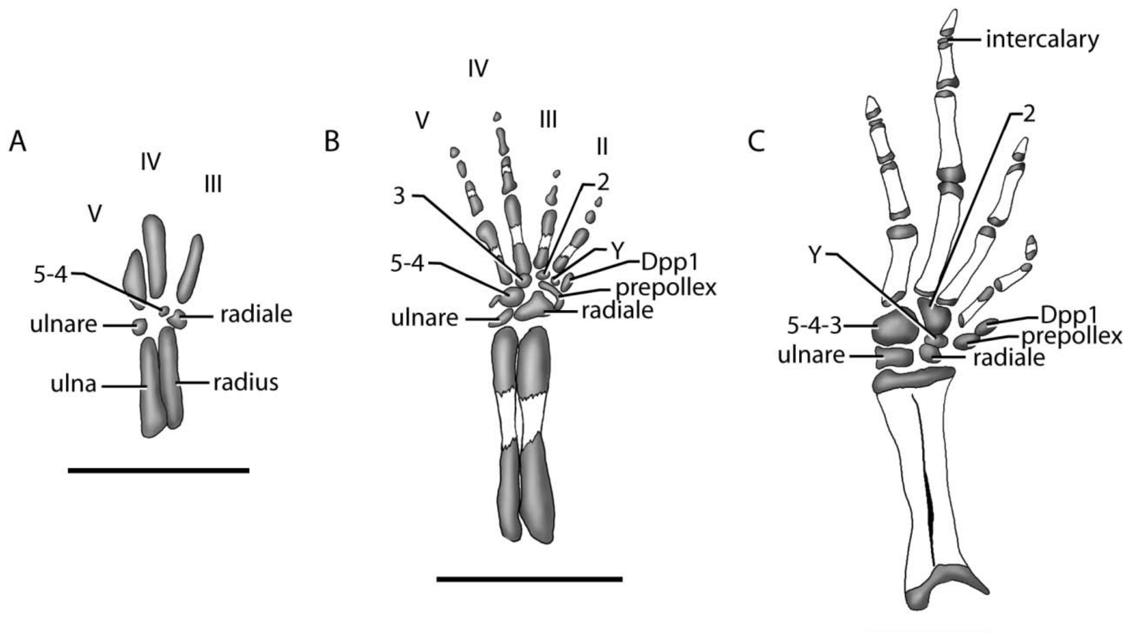

Fig. 1.5. Development of the right hand of *Acris blanchardi* shown in ventral view. **A:** Stage 36. **B:** Stage 39. **C:** Stage 46. White denotes ossification; gray denotes cartilage. Digits are numbered II–V (Fabrezi and Alberch, 1996). Prep, Prepollex; Y, Element Y.

Forelimb.---The adult forelimb consists of the humerus (AAO:0000679), the radioulna (AAO:0000901), and the manus (AAO:0010803). The manus, or hand, comprises eight ossified carpal elements, four metacarpals, and 10 phalanges, with a formula of 2-2-3-3 (Fig. 1.5). The cartilage precursors of the humerus, radius (AAO: 0000788), and ulna (AAO: 0000789) appear by Stage 35. Ossification of the humerus, radius, and ulna appears by Stage 37 around midshaft of each element. The radius and ulna remain separate until Stage 40 , at which time they begin to fuse in the midshaft region. By Stage 42, they are completely fused to one another, but remain distinguishable because of the presence of a medial groove, the sulcus intermedius (AAO: 0010829). The metacarpals and proximal phalanges of all of the digits begin ossifying by Stage 38. A small amount of ossification is present in the intermediate phalanges of all digits by Stage 39, and by Stage 40 the penultimate phalanges of Digits IV and V have begun to ossify. By Stage 41, the remaining phalanges have begun to ossify. The carpals are the last bones of the forelimb to ossify; Carpals 2-5, the radiale (AAO: 0000790), the ulnare (AAO: 0000845), Element Y (AAO:0000846), the prepollex (AAO: 0000852) and the distal prepollex (AAO:0010683) ossify postmetamorphically.

1.4.4.3. The Posterior Appendicular Skeleton

Pelvic girdle.---The adult pelvic girdle (AAO: 0000426) is composed of paired ilia (AAO:0000772) and ischia (AAO:0000860) and a fused pubis (AAO:0000861); these bones unite to form the oval acetabulum (AAO:0000770).

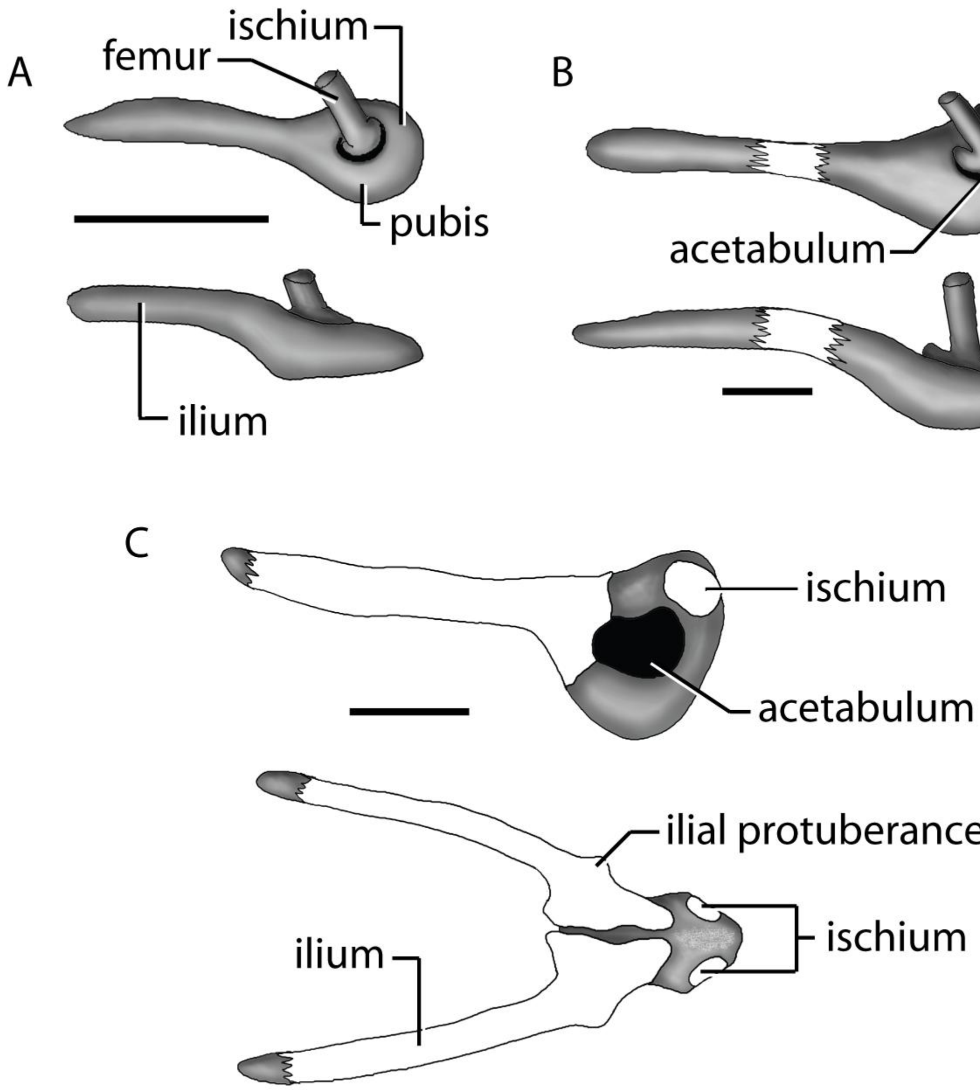

Fig. 1.6. Development of the pelvic girdle of *Acris blanchardi* in lateral (**left**) and ventral (**right**) views. **A:** Stage 34. **B:** Stage 38. **C:** Stage 44. White denotes ossification; gray denotes cartilage. acetab, acetabulum.

Together the ilia form a U- shape in ventral view, and each comprises a simple shaft with a spade-shaped dorsal prominence (Fig. 1.6). Cartilaginous ilia are the first pelvic elements to appear by Stage 35. By Stage 38 the ilia have begun to ossify at their midshaft. The ischia and pubes first appear by Stage 39 as two rectangular cartilaginous rods posterior to the ilia; each rod comprises an ischium and a pubis fused to one another. By Stage 40 the pubes have synchondrotically fused to one another at the midline, and the ischia begin to ossify. By Stage 41, the ilia are fully ossified, the dorsal illial protuberances are well-developed, and the posterior margins of the ischia have begun to ossify. By Stage 43, the bony posterior margins of the ischia are more robust and the cartilaginous portions of the ischia and pubis have grown and expanded. By Stage 44, all of the pelvic girdle elements are well formed and resemble the adult morphology.

Hind limb.---The hind limb comprises the femur (AAO: 0000889), the tibiofibula (AAO: 0000902), the tibiale (AAO: 0000912), the fibulare (AAO: 0000913), and the pes (Fig. 1.7). The pes, or foot, is composed of a composite Tarsal 2-3 (AAO: 0000919), Tarsal 1 (AAO: 0000918), Element Y (AAO: 0000921), the prehallux (AAO: 0000922), several distal prehallicies (AAO: 0010685), five metatarsals, and 14 phalanges with the following formula: 2-2-3-4-3. The fibulare and tibiale are fused on the proximal and distal ends but are separated from one another medially along the shaft.

The femur and tibiofibula first appear as elongated cartilages by Stage 35. By Stage 36, the femur has begun to ossify midshaft; ossification of the tibiofibula, tibiale and the fibulare is present by Stage 37. By Stage 41 the proximal heads of the tibiale and fibulare are synchondrotically united, and the shafts of the tibiale and fibulare have further.

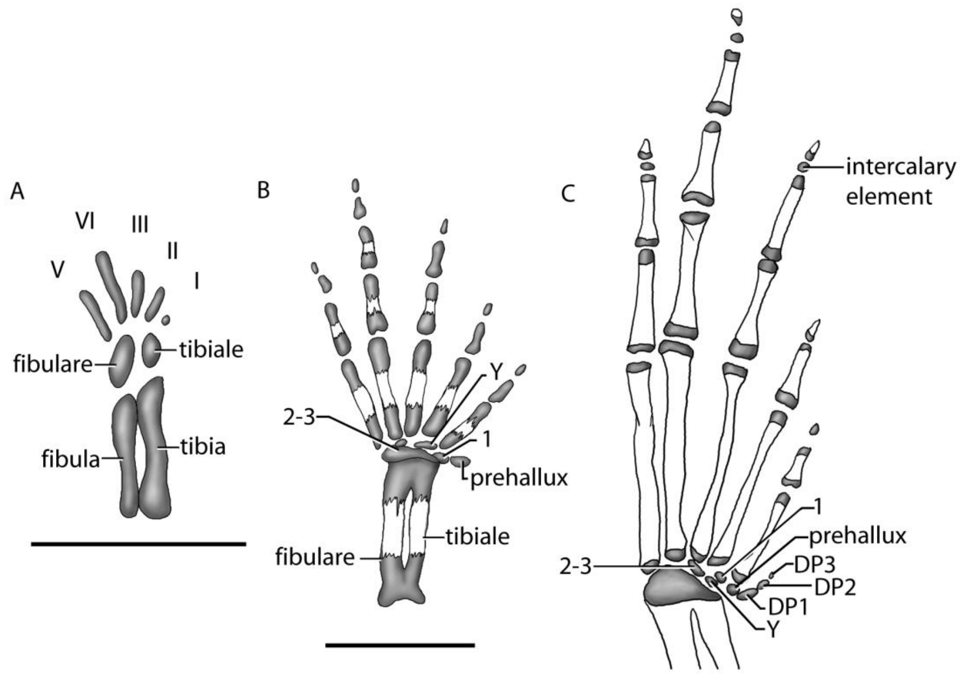

Fig. 1.7. Development of the right foot of *Acris blanchardi* shown in ventral view. **A:** Stage 34. **B:** Stage 38. **C**: Stage 46. White denotes ossification; gray denotes cartilage. Digits are numbered I–V (Fabrezi and Alberch, 1996). Y, Element Y.

The distal heads of the tibiale and fibulare are fully ossified by Stage 46, but do not synosthotically fuse until after metamorphosis.

The metatarsals and phalanges appear in cartilage by Stage 36, and by Stage 37, the fibula, tibia, Metatarsals II-V and the proximal and intermediate phalanges of Digits IV and V have begun to ossify. By Stage 38, Metatarsal I and the proximal phalanges of Digits II and III show ossification. Stage 39 all phalanges are present, and all but the ultimate phalanges have begun to ossify. By Stage 40, all elements of the digits have begun to ossify, and will continue to grow and ossify until they reach the adult form after metamorphosis.

1.5. DISCUSSION

Miniaturization is the process of a larger progenitor evolving into a smaller species (Hanken and Wake, 1993). There are many advantages to a species becoming smaller, including: the use of physically smaller niches (Swedmark 1964; Higgens and Thiel 1988; Clarke 1996; Yeh 2002); avoidance of predators (Clarke 1996; Blanckenhorn 200 and references therein; Yeh 2002); use of different food sources (Clarke 1996; Yeh 2002); the use of new habitats that have limited resources (Marshall and Corruccini 1978; Pregill 1986; Roth 1992; Yeh 2002); and speeding up development to reach reproductive maturity faster (Gould 1977; Griffith 1990; Blanckenhorn 2000 and references therein; Yeh 2002). Although increases in body size seem to require relatively long periods of time to evolve, miniaturization seems to happen much more quickly (Hanken and Wake 1993).

Throughout the evolution of amphibians, miniaturization has played a major role in shaping morphologies---e.g., the three extant amphibian orders (frogs, salamanders, and caecilians) are paedomorphic relative to the larger, more robust ancestors they from which they evolved (Bolt 1977, 1979; Milner 1988; Boy and Sues 2000). Within frogs, miniaturization has evolved many times (Yeh 2002).

Hanken and Wake (1993) described the characteristics of a miniature and the mechanisms by which miniatures developed. They showed that miniatures can evolve differently based on their life history, and that not all miniatures originate in the same way. They observed that paedomorphism (= retention of juvenile features in the adult via truncation of juvenile development) is responsible for most, but not all, cases of miniaturization (Hanken and Wake 1993).

Hanken and Wake (1993) also showed that most cases of miniaturization are accompanied by morphological novelty (relative to non-miniature progenitors). They categorized the effects of miniaturization as falling into three classes: 1) reduction and structural simplification, 2) morphological novelty, and 3) increased morphological variation. Trueb and Alberch (1985) showed that smaller frogs often lacked one or more skull bones, and the missing elements were typically those that normally appeared late in the developmental sequence. Yeh (2002) also found that the skull bones most frequently lost are those that typically ossify last. However, Yeh (2002) also showed that although certain cranial elements may ossify later in the developmental sequence in miniatures than in non-miniatures, they are never absent. In addition, bones such as the palatines, stapes, and quadratojugals are frequently reduced or loss in non-miniature species (Trueb 1973, 1993; Yeh 2002). Thus, Yeh (2002) concluded that despite the effects of paedomorphosis, there are functional constraints that limit the absence of some cranial elements, whereas other cranial elements can be easily lost.

In *Acris blanchardi* adults, Maglia et al. (2007) found that, although the postcranium was well-developed, the cranium was poorly ossified and several elements (such as the sphenethmoid) ossified post-metamorphically. Although there is no loss of cranial bones in *A. blanchardi*, there is a reduction in the size and extent of ossification in several elements (e.g., nasals, prootics and exoccipitals). In addition, Maglia et al. (2007) showed that the nasal bones of *A. blanchardi* are a composite of dermal and endochondral ossification, a state that they identified as a morphological novelty. Thus, unlike some other miniature anurans (e.g., *Scinax acuminata*, *Pseudacris ocularis*; Yeh 2002), *A. blanchardi* retains all cranial elements, yet many are gracile or poorly ossified. In an apparent need to compensate for the lack of ossification, *A. blanchardi* compensates by reinforcing functionally-important structures (e.g., nasals, prootics/exoccipitals, etc.) with disorganized mineralization. Overall, the development follows the patterns that were observed in the adult; there is some ossification of all the elements and they begin development much like *Hyla lanciformis* but they do not completely develop and expand. The postcranial skeleton retains many of the same developmental patterns as other hylids specifically *Hyla lanciformis*.

The timing of ossification in *Acris blanchardi* is very similar to that of *Hyla lanciformis*, a non miniature hylid species. In fact the postcranial ossification is nearly identical in timing when compared to *H. lanciformis,* as reported by de Sa (1988). It is evident that *A. blanchardi* begins ossification at the same relative times as that of non-miniature hylids, but the extent of ossification is reduced. *A. blanchardi* appears to exhibit a paedomorphic pattern of arresting ossification postmetamorphically. *Acris blanchardi* appears to require more support for certain elements that are lacking and adds mineralization of cartilaginous structures to compensate for poorly ossified bones. For example, this is seen in the nasals, which are small, thin, poorly ossified dermal elements that appear Stage 42, but never become robust through ossification. Instead, during the juvenile stage, the tectum nasi mineralizes and fuses to the poorly ossified dermal nasal elements.

Acris blanchardi has a short life span relative to closely related species such as *Pseudacris crucifer* and *Hyla lanciformis*. It lives approximately 16 months (Burkett,

1984) and breeds within the first year of its life. The growth of the juvenile individuals is very rapid in the fall with individuals reaching adult size prior to the winter (Maglia et al., 2007). Adults cease growing over the winter (Gray and Brown, 2005), and when they emerge in the spring, they have reached sexual maturity (Gray et al., 2005). Maglia et al. (2007) hypothesized that the short life span was responsible for miniaturization---i.e., the species is unable to ossify for one or two years of adulthood like many other hylids can. Another plausible factor contributing to miniaturization in this species is habitat use. In Missouri, *A. blanchardi* is found primarily in cattle ponds where resources are limited and the surrounding habitat is relatively sparse. Thus, the use of small microhabitats on the edges of these ponds, and limited food resources, may provide selective pressure for small size. *A blanchardi* also uses small ephemeral pools for breeding. Selective pressure for rapid development prior to ponds drying may result in a truncation of development.

Miniaturization is an important and interesting evolutionary phenomenon that is frequently observed in amphibians. With continued observation and studies, we will continue to increase our knowledge of morphology and development of miniature species, and the role miniaturization plays in the evolution of life history and morphological novelty.

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1.7. APPENDIX

A. SPECIMENS EXAMINED

KU No.	Stage	SVL(mm)	Preparation	KU No.	Stage	SVL(mm)	Preparation
307867	32	10.03	C&S	307889	40	15.38	C&S
307866	32	9.43	C&S	308891	40	16.77	C&S
307869	33	10.15	C&S	307897	40	13.92	C&S
307868	33	9.14	C&S	307893	41	18.15	C&S
307872	33	10.83	C&S	308892	41	14.89	C&S
307871	33	10.69	C&S	307875	41	16.03	C&S
307873	33	11.27	C&S	307900	41	14.04	C&S
307870	33	10.48	C&S	307903	42	13.26	C&S
307876	33	12.48	C&S	307902	42	13.73	C&S
307896	34	13.14	C&S	307994	42	12.75	C&S
307878	34	11.61	C&S	307995	42	13.17	C&S
307879	35	13.01	C&S	307901	43	12.39	C&S
307914	36	13.91	C&S	307905	44	11.69	C&S
307913	36	11.11	C&S	307904	44	11.49	C&S
307881	37	13.28	C&S	307910	45	12.79	C&S
307882	37	13.4	C&S	307908	45	13.02	C&S
307884	38	14.1	C&S	307907	45	14.03	C&S
307874	38	13.31	C&S	307899	46	12.27	C&S
307887	39	14.48	C&S	30936	Juvenile	13.11	C&S
307888	39	15.84	C&S	307942	Juvenile	17.71	C&S
307890	40	14.51	C&S	307931	Juvenile	19.24	C&S

B. CLEARING AND DOUBLE STAINING FOR BONE AND CARTILAGE

Protocol adapted from: Taylor, W.R. and Van Dyke, G. C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9(2): 107-119. Method assumes specimens have been stored in 10% formalin solution.

Dehydrating the specimens: Wash the specimens in water for 24 hours to rinse any remaining formalin. Place in 50% ETOH solution (50% deionized water) for 24 hours followed by 70% ETOH, and 95% ETOH solutions.

Staining for cartilage tissue: Place the specimens in 10 times alcian blue staining solution (80 parts 95% ETOH; 20 parts glacial acetic acid; 30 mg alcian blue powder per 100 milliliters solution). Leave specimen in solution until cartilaginous structures are stained deep blue.

Neutralization: Transfer specimens to 50 times their volume of saturated borate solution (excess sodium borate powder to several liters distilled water) for 24 hours.

Bleaching: Place neutralized specimens in approximately 30 times their volume of bleaching solution (10-20 parts hydrogen peroxide; 80-90 parts 1 % potassium hydroxide solution). Specimens should be left in until soft tissues are not blue, but without over bleaching cartilaginous structures.

Clearing/Digestion: Transfer specimens to 10-40 times their volume to stock enzyme solution (3 parts saturated borate solution; 7 parts distilled water; ¼ teaspoon trypsin). Specimens should be left to clear until soft tissues are cleared, but too long in solution will disarticulate bony elements.

Repeat: At this stage, some of the specimens will have lost their blue color. Repeating the steps starting with re-hydrating the specimens can be done.

Staining for bone tissue: Place digested specimens in 20 times their volume of alizarin stock solution (small quantity alizarin red powder in 1% potassium hydroxide solution) until bony elements have been stained red.

Rinsing: Place specimens in distilled water for no more than 24 hours.

Final clearing: If muscle is left on larger specimens they can be put through the clearing/digestion step again.

Storage: Transfer specimens to 40% glycerine solution (60% potassium hydroxide) for 3-4 days. Repeat with 70% glycerine solution (30% potassium hydroxide) for 3-4 days and 100% glycerine solution (adding 1-2 crystals of thymol to prevent fungal growth) for final storage.

PAPER

2. ADULT SKELETAL DESCRIPTION AND LARVAL SKELETAL DEVELOPMENT OF THE SPRING PEEPER, *PSEUDACRIS CRUCIFER* (ANURA: HYLIDAE)

2.1. ABSTRACT

Pseudacris crucifer is a common North American frog in the family Hylidae, which also includes the miniature frog species *Acris blanchardi*. These species share similar habitat, life history and size, and it has been suggested that *P. crucifer* is also a miniature. Herein, the adult skeleton and larval development of *P. crucifer* is described and compared to that of *A. blanchardi*, and a non-miniature hylid, *Hyla lanciformis*. Interesting patterns of development relating to the timing of onset and completion of ossification were found when *A. blanchardi*, *H. lanciformis*, and *P. crucifer* were compared. *P. crucifer* is unique because its ossification begins later than both *H. lanciformis* and *A. blanchardi*, but it ossifies more completely than *A. blanchardi*. Although *P. crucifer* is small, its pattern of ossification and adult osteology do not reflect those typically associated with miniaturization.

2.2. INTRODUCTION

Pseudacris crucifer is a small frog species commonly known as the Spring Peeper. In many parts of the country, they are the first species to breed, and can be heard calling as early as mid-February (Conant and Collins, 1991). Although they are members of the tree frog family Hylidae, they are semi-aquatic rather than arboreal, and can be found in grass and weeds in ephemeral pools during the breeding season (Conant and Collins, 1991). They are easily identified by their X-pattern on their back, and range from eastern Texas, north through eastern Oklahoma and Kansas, through Missouri, eastern Iowa, and eastern and northern Minnesota and continue east to eastern Canada to Northern Florida (e.g. Wright and Wright, 1949; Conant and Collins, 1991). The distribution of *P. crucifer* has remained the same as historical accounts, but populations have likely been diminished or lost due to human activity such as logging, mining, agriculture, road construction and urbanization (Butterfield et al, 2005). In a survey done in Michigan by Carpenter and Delzell (1951) they found *P. crucifer* to be the most abundant animals but 75% of the observed frogs were road killed.

The small size of *P. crucifer* and its similarity in life history to *Acris blanchardi*, a miniature semi-aquatic hylid, provides an ideal opportunity to examine the relationship between body size and life history in hylids. Although Gaudin (1969) conducted an extensive comparative study of the osteology of many North American hylids, it is difficult to extract species-specific information from his analysis. In addition, he studied dried skeletons representing adults, and much of the information required to study miniature species (e.g., larval morphology, onset of ossification, cartilaginous structures), is lacking. Thus, his data are not adequate to understand the evolution of small body size in North American hylids.

Herein, a detailed description of the adult skeletal and larval skeletal development of *P. crucifer* is provided and compared it to that of *A. blanchardi,* and a non-miniature hylid, *Hyla lanciformis*. Comments on miniaturization, developmental similarities, and ossification patterns in these species are included.

2.3. MATERIALS AND METHODS

Forty-four tadpoles of *P. crucifer* were examined. Eggs and day-old larvae were collected (Missouri Wildlife Collector's permit #13806 and 14043) during the spring of 2008 and 2009 and lab reared. Five adult wild-caught *Pseudacris crucifer* from the University of Kansas Natural History Museum and Biodiversity Research Center were used for adult descriptions. The lab reared specimens will be deposited in the University of Kansas Natural History Museum and Biodiversity Research Center at the conclusion of this study. See the appendix for a list of specimens examined. Tadpoles were euthanized (MS-222) and staged according to the table presented by Gosner (1960). Specimens were cleared and double-stained for bone and cartilages following protocols adapted from Taylor and van Dyke (1985). Chondrocranial and osteological terminology follows that of Duellman and Trueb (1994), Fabrezi (1992, 1993), Fabrezi and Alberch (1996), Maglia et al. (2007b), and the Amphibian Anatomical Ontology (AAO; Maglia et al. 2007a; 2009). The numbers following most anatomical terms in the results refer to Amphibian Anatomical Ontology (http://www.amphibanat.org) reference numbers. All descriptions and illustrations were made using an Olympus SZX12 stereoscope equipped with a camera lucida and an Olympus QColor 5 digital camera.

2.4. RESULTS

2.4.1. Cranium. The adult skull is nearly as wide at the exoccipitals as it is long, and has a rounded anterior (Fig. 2.1). In lateral view (Fig. 2.2), it is dome shaped with the highest point being dorsal to the optic foramen. The skull is fully ossified with parts of the nose, ear, and palatoquadrate cartilage remnants being the only cartilaginous elements. The frontoparietals do not meet, thus the frontoparietal fenestra (AAO: 0010525) is exposed through the frontoparietal fontanelle (AAO: 0000209).

2.4.1.1. Nasal Capsules. The nasal capsules (AAO: 0000313) comprise the olfactory region, which makes up the anterior 1/4 of the skull. The olfactory capsules are located anterior to the brain and supported anteriorly by the premaxillae (AAO: 0000461) and maxillae (AAO: 0000285). The nasals (AAO: 0000312) and vomers (AAO: 0000633) also support the nasal cartilages. The planum antorbitale (AAO: 0000434) forms the posterior wall of the nasal capsule, and is a robust, transverse wall of cartilage that separates the orbit from the nasal capsule. Ventral to the planum antorbitale, and dorsal to the solum nasi (AAO: 0000569), is the septum nasi (AAO: 0000559). It is a thin sheet of ossification that is a medial extension of the ethmoidal cartilage. In ventral view, the septum nasi is relatively broad at its posterior base. The posterior region of the septum nasi is more ossified than the anterior portion where it connects to the anterior rostral cartilages. Covering the opening of the nasal capsule is the oblique cartilage (AAO:0000329), which is a robust ovular piece of cartilage that spans from the tectum nasi (AAO: 0000601) posterolaterally to the the planum terminale (AAO: 0000440) ventrolaterally.

The solum nasi is a narrow ledge that is located between the septum nasi and the planum antorbitale. The solum nasi has an extension that traverses ventrolaterally to the planum terminale. The planum terminale continues anteriorly to the oblique cartilage.

2.4.1.2. Braincase and Otic Region. In *Pseudacris crucifer* the braincase is complete with a large dorsal frontoparietal fenestra (AAO: 0010525) flanked by the frontoparietals (AAO: 0000207). The braincase consists of the sphenethmoid (AAO: 0000572), paired prootics (AAO: 0000514) and paired exoccipitals (AAO: 0000147). The frontoparietals invest the braincase dorsally, while the parasphenoid (AAO: 0000385) invests it ventrally. The anterior braincase of *P. crucifer* is similar to the general anuran cranium structure (Duellman and Trueb, 1994).

Sphenethmoid. The sphenethmoid (AAO: 0000572) is located in the anterior region of the neurocranium and forms the anterior and anterolateral walls of the braincase. It is a U-shaped bone that also contributes to the medial and posterior walls of the nasal capsule. In *P. crucifer,* the sphenethmoid is ossified to the degree that it encircles the anterior portion of the brain throughout the orbital region.

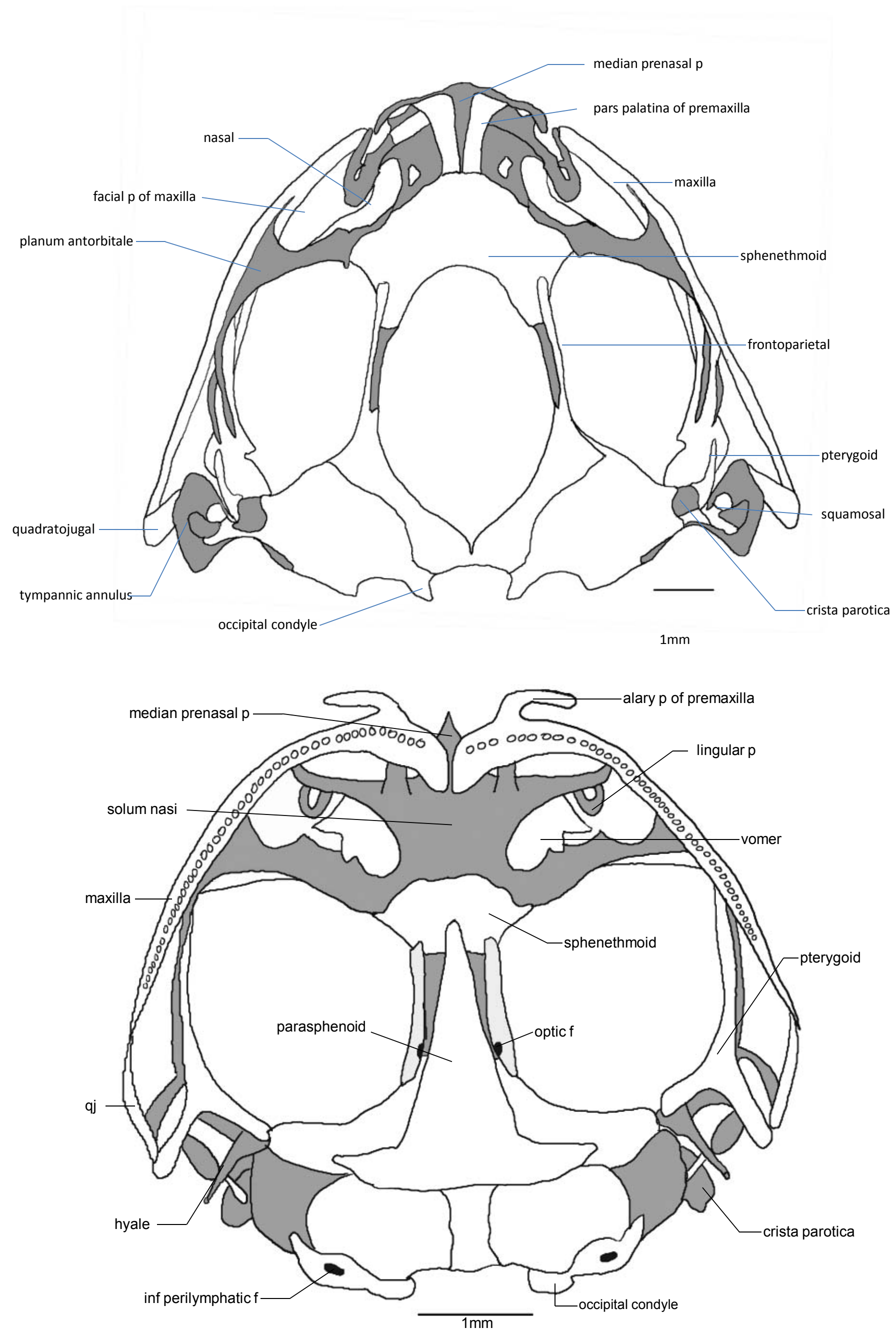


Fig. 2.1. Adult cranium of Pseudacris crucifer. **A**: Dorsal view and **B**: lateral view of adult cranium of *Pseudacris crucifer*. Gray denotes cartilage; white denotes bone. f, foramen; qj, quadratojugal; p, process.

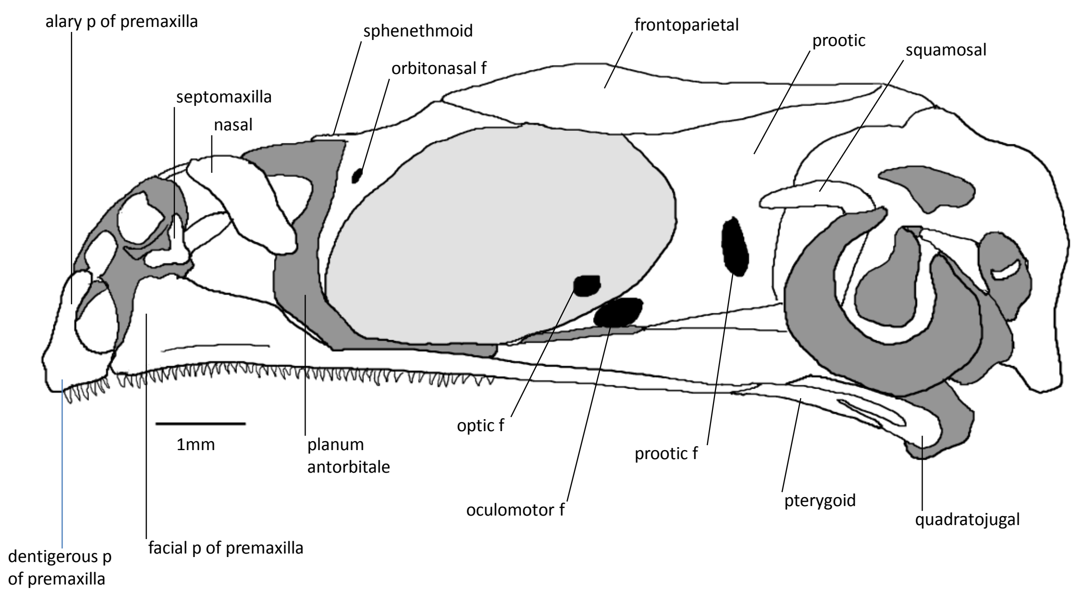


Fig. 2.2. Adult cranium of *Pseudacris crucifer* in lateral view. Gray denotes cartilage; white denotes bone; black denotes foramina. p, process; f, foramen.

When viewed dorsally, the sphenethmoid is overlapped by the frontoparietals along the posterolateral margins. In the ventral view, the cultriform process of the parasphenoid overlaps the posteroventral margin of the sphenethmoid. In all specimens examined the sphenethmoid is well ossified and completely separates the braincase from the nasal capsules.

The sphenethmoid accounts for approximately 1/5 of the entire braincase. It has a convex floor with posterior walls that are concave. The sphenethmoid is pierced by the orbitonasal formina (AAO: 0000344) laterally, and a pair of larger olfactory foramina are seen on the anterior wall.

Prootics. The prootics (AAO: 0000514) are paired endochondral bones that are located in the posterior region of the braincase and form all but the posteromedial wall of the otic capsules (AAO: 0010143). The prootics form approximately the middle third of the braincase wall and approximately two thirds of the otic capsule. The prootics are synostotically fused with the exoccipitals posteriorly. In *Pseudacris crucifer* the prootics and exoccipitals are fused together and when viewed dorsally there is no distinguishable line between the two.

When viewed dorsally, the posterior portion of the prootics is smooth with an epiotic eminence being visible on the surface of each otic capsule. Along the lateral edge of the prootics there is a cartilaginous crista parotica that extends over the plectral apparatus. The prootics are also pierced by two pairs of foramina: the oculomotor foramina (AAO: 0000332) and the prootic foramina (AAO: 0010566).

Exoccipitals. The exoccipitals (AAO:0000147) are square shaped, fully ossified elements that are located posterior and lateral to the prootics. They are paired endochondral bones that form the occipital condyles (AAO: 0000330) and flank the entire foramen magnum (AAO: 0010826) (except for the dorsal portion, which is formed by the posterior margin of the parasphenoid). The exoccipitals are fused to the prootics and comprise the posterior region of the otic capsule.

Plectral apparatus. The plectral apparatus (AAO: 0000443) is the sound conducting structure, which is composed of the pars interna plectri (AAO: 0000406), the pars media plectri (AAO: 0000408), pars externa plectri (AAO: 0000399), and the tympannic annulus (AAO: 0000615).

The pars interna plectri is a flat, round plate of calcified cartilage that lies in the posterior half of the fenestra ovalis and has a medial groove that is the site of attachment for the *m. opercularis*. The pars medial plectri is a bone that has a footplate that is anterior to the pars interna plectri and a rod of cartilage that projects anteriorly to synchondrotically fuse with the pars externa plectri. The pars externa plectri is a cartilaginous element that lies posterior to the tympannic annulus. The tympanic annulus is a half ring of cartilage that lies beneath the skin and supports the tympanic membrane.

2.4.1.3. Dermal Investing Bones. The dermal investing bones are the intramembranous elements that are formed without having a cartilaginous precursor. They include the parasphenoid, paired nasals, paired frontoparietals, paired vomers and paired palatines.

Parasphenoid. The parasphenoid (AAO: 0000385) is a median bone located ventral to the braincase, the sphenethmoid, prootics and exoccipitals. The parasphenoid is a fully ossified and consists of an anterior cultriform process (AAO: 0000386) and two posterior, laterally oriented subotic alae (AAO: 0000387); together, these form an inverted T-shape. The cultriform process extends posteriorly to the anterior region of the braincase to approximately to the anteromedial corner of the orbit. The anterior margin of the cultriform process invests the posteroventral margin of the sphenethomoid, but does not fuse to it. The median region of the parasphenoid is widened on each lateral margin and narrows prior to forming the alae. The alae extend posterolaterally from the cultriform process and form a point laterally. The alae invest the posteromedial margin of the prootics and the anteromedial margin of the exoccipitals.

Frontoparietals. The frontoparietals (AAO: 0000207) are paired dermal bones that are located on the dorsum of either side of the braincase. They are elongate bones that extend from the sphenethmoid to the prootics. The posterior part of the frontoparietals are wider and cover more of the frontoparietal fenestra than the anterior. Medially, the frontoparietals cover the entire tectum synoticum and invest the medial margins of the prootics. The lamina perpendicularis of each frontoparietal is fairly well developed and forms the lateral wall of the braincase. The frontoparietals are well developed in all specimens examined.

Nasals. The nasals (AAO: 0000312) are elongate bones that are located anterolateral to the sphenethmoid and anterior to the anterior margin of the planum antorbitale (AAO: 0000434). They act as the dermal roof of the nasal capsule. The nasals are well ossified with a widened anteromedial end and a narrow posterolateral end that comes to a point. The nasals do not touch medially and the septum nasi is visible between them.

Vomers. The vomers (AAO: 0000633) are paired, intramembranous bones that serve as a floor to the nasal capsule. Each is a triangular bone that is elongate anteroposteriorly and has small vomerine teeth. Each vomer is composed of an anterior (AAO: 0000649), a dentigerous (AAO: 0000657), a prechoanal (AAO: 0000658) and a postchoanal (AAO: 0000659) process. The anterior process of the vomer is cylindrical and extends anteriorly to the level of the lingular process of the planum terminale. The prechonal and postchoanal processes extend laterally from the body of the vomer; the prechonal process is long and narrow and forms a point. The postchoanal process is short and rounded and forms the medial margin of the choana. The dentigerous process bears 3-5 vomerine teeth (AAO: 000635), and it lies medial to the choana and anterior to the palatine.

Palatines. The palatines (AAO: 0000372) are present as slivers of visible in ventral view. They are posterior to the planum antorbitale and expand from the sphenethmoid to the maxilla, but do not articulate with either. The palatines do not have a transverse ridge and appear smooth.

2.4.1.4. Suspensorium. The suspensorium is the cranial complex that suspends and braces the jaws against the skull. It includes the palatoquadrate, quadrate, pterygoids and squamosals. The suspensory elements are mainly located in the posterior region of the skull lateral to the otic capsules.

Palatoquadrate. The adult palatoquadrate (AAO: 0000379) is what remains of the larval palatoquadrate cartilage. The larval palatoquadrate acted as a support for the eye, an articulation point for the hyobranchium and a connection point for several larval jaw muscles; in the adult, the palatoquadrate supports the suspecnsorium and upper jaw. In the adult the palatoquadrate remains cartilaginous in the anterior 3/4 of the maxillary arcade.

Pterygoids. The pterygoids (AAO: 0000521) are Y-shaped bones that lie on either side of the skull with an anterior, posterior and medial ramus. The anterior ramus lies medial to the maxilla and is the longest of the pterygoid rami. It has slightly wider posterior half than anterior half. The posterior ramus o the posterior wall of the palatoquadrate and is pointed toward the quadrate bone. The medial ramus is the shortest of the rami and is about 3/4 of the length of the posterior ramus. The medial ramus lies above the hyale cartilage and is curved to the middle of the skull.

Squamosals. The squamosals (AAO: 0000574) are T-shaped bones that lie at the posterolateral margins of the skull. They consist of three rami: the vental ramus (AAO: 0000628), the otic ramus (AAO: 0000367) and the zygomatic ramus (AAO: 0000639). The ventral ramus lies perpendicular to the maxilla and is the longest of the rami. The proximal half of the ventral ramus invests the the dorsolateral margin of the palatoquadrate, and the distal half invests the quadrate and articulates with the quadratojugal. The otic ramus is flat and rectangular and is shorter than the ventral ramus and comes to a square end. It points posteriorly from the intersection of the other two rami. The otic ramus invests the otic process and part of the lateral margin of the crista parotica. The zygomatic ramus projects anteromedially from the intersection point of the other two rami. It is about the same size as the otic ramus. In the specimens examined the squamosal was entirely ossified.

2.4.1.5. Upper Jaw. The upper jaw is composed of cartilaginous and bony elements. The cartilage elements include a pair of inferior prenasal cartilages, a pair of cristae subnasalis, and a pair of plana antorbitale. The bony elements consist of a pair of premaxillae, a pair of maxillae and a pair of quadratojugals.

Premaxillae. The premaxillae (AAO:0000461) are paired bones that form the anterior-most portion of the upper jaw. They are well developed and ossified with a narrow gap between one another medially. Laterally, they are overlapped slightly by the maxillae. The premaxillae each have three parts to them: the alary process (AAO: 0000003), the pars dentalis (AAO: 0000397) and the pars palatina (AAO: 0000414). The alary process is a wide, short bony extension that projects posterodorsal to the front of the premaxilla. The pars dentalis is the most anterolateral segment of the premaxilla, is well ossified, and contains 8-10 teeth. The pars palatina is a narrow lingual ledge that is located lateral on each side of the median prenasal cartilages when viewed dorsally.

Maxillae. The maxillae (AAO: 0000285) are intramembranous bones that make up the majority of the upper jaw. They are located on the lateral sides of the skull and contain the majority of the dentition with 28-33 recurved, pedicellate teeth. Each is composed of three sections: the pars dentalis (AAO: 0000396) which contains the teeth, the pars palatina (AAO: 0000413), which is a palatal ledge, and the pars facialis (AAO: 0000400), which is a facial flange.

The pars dentalis extends from the anterior end of the maxilla to the anterior end of the pterygoid fossa. The anterior end of the pars dentalis of the maxilla is narrower than the posterior end. The pars facialis is a facial flange that projects from the anterior region of the pars dentalis region of the maxilla. It acts as protection for the lateral aspect of the nasal capsule. It extends 1/3 of the length of the maxilla from the anterior margin to the level of the planum antorbitale. The posterior end of the maxilla ends in an acuminate point that extends to the level of the anterior wall of the otic capsule. The posterior end of the maxilla invests the anterior fourth of the medial margin of the quadratojugal. The pars palatina of the maxilla is a narrow portion of bone that runs from the anterior end of the maxilla the anterior end of the pterygoid fossa.

Quadratojugals. The quadratojugals (AAO: 0000532) are a small bones located in the posterior end of the upper jaw. They have been considered to be part of the suspensorium by several authors (Duellman and Trueb, 1994; Pugener and Maglia, 1997; Sheil, 1999), but were classified as part of the upper jaw by Maglia et al. (2007) in their paper on *Acris crepitans.* Each quadratojugal comes to a dagger-like point anteriorly and invests the lateral face of the posterior end of the maxilla. The posterior end of the quadratojugal is wide, completely ossified, and appears fused to the quadrate.

2.4.1.6. Lower Jaw. The lower jaw is formed by two mandibular arches on the anterior and lateral sides of the skull lying ventral to the maxillary arcade. The lower jaw consists of Meckel's cartilage, the mentomeckelian bones, dentaries, and angulosplenials. Similar to other anurans, the lower jaw is edentate.

Meckel's cartilage. Meckel's cartilage (AAO: 0000289) is a cylindrical, cartilaginous element that runs along the midline of the lower jaw. It extends between the dentary and the angulosplenial. On its anterior end it ossifies to form mentomeckelian bones. The articular head, which is a small sheet of cartilage on the very end of the mandibular arch, is the point of articulation of the lower jaw with the braincase. The articular head articulates with the quadrate posterior to the level of the crista parotica. Meckel's cartilage is visible in dorsal and ventral view.

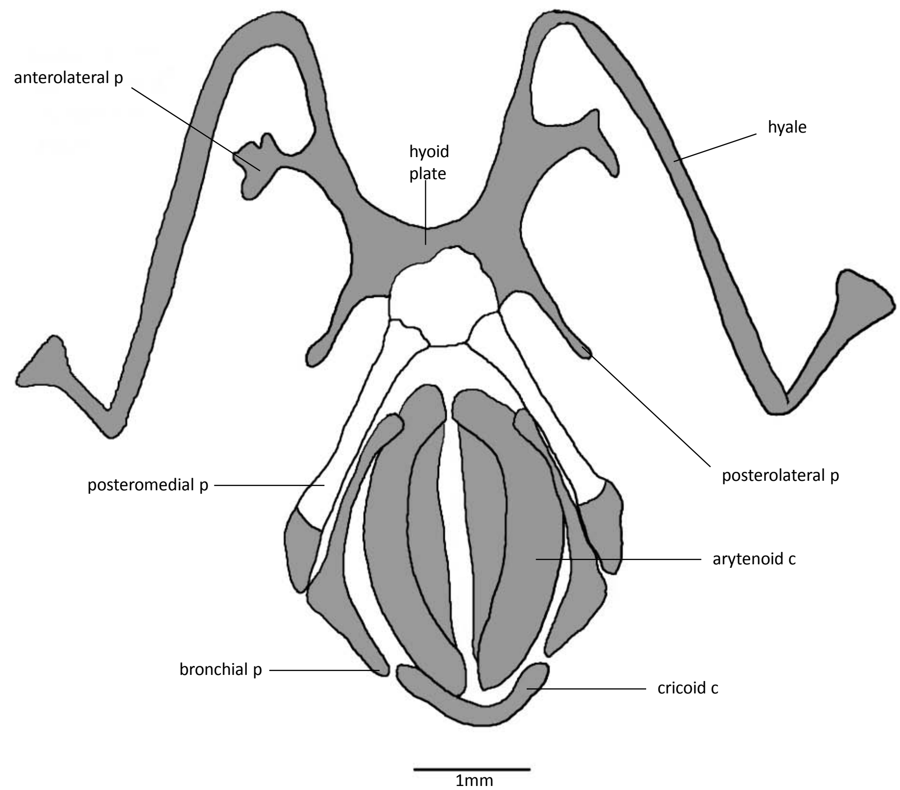
Mentomeckelian bones. The mentomeckelian bones (AAO: 0000301) are small endochondral bones that are located in the anteromedial region of the mandibular arch. They are small (~ 7-8% the length of the mandible) and cylindrical. Medial to the mentomeckelians, is the mandibular symphysis. In younger specimens the mentomeckelian bones are not fully ossified.

Dentaries. The dentaries (AAO: 0000124) are long, narrow bones of intramembranous origin. In *Pseudacris crucifer* they do not have odontoids or ridges, and they form the lateral margin of the mandible for the anterior three quarters. They invest the lateral margins of Meckel's cartilage and the mentomeckelian bones. In all specimens examined the dentaries were well ossified.

Angulosplenials. The angulosplenials (AAO: 0000966) are the largest bony component of the mandible. They are well ossified intramembranous bones that extend along the lingual margin of Meckel's cartilage. They extend approximately three-fourths of the length of Meckel's cartilage. The anterior half of the angulosplenial is recurved while the posterior half is oriented posterolaterally. The prearticular region of the angulosplenial also bears a well deveoloped laminar coronoid process for jaw muscle insertion. The coronoid process is flattened and wide and is about a fourth of the angulosplenial.

2.4.2. Hyolaryngeal Complex. The hyolaryngeal complex is composed of two units, the hyoid and the laryngeal apparatus (Fig. 2.3.). Both of these units are derived from the larval hyobranchial skeleton. The hyoid apparatus provides skeletal support for the mandibular, tongue and branchial muscles (Duellman and Trueb, 1994); thus, the hyoid apparatus functions in feeding and respiration. The laryngeal apparatus contains the vocal chords and is the sound producing structure.

Hyoid apparatus. The hyoid apparatus (AAO: 0000225) is located ventral to the mandibular arcade and in the mouth floor. The hyoid apparatus is composed of a central plate of cartilage, the hyoid plate (AAO: 0000664), which can be mineralized. The hyoid plate has four pairs of processes which include the hyale (= ceratohyal; AAO: 0000666), the anterolateral processes (AAO: 0000671), the posterolateral processes (AAO: 0000672) and the posteromedial processes (AAO: 0000673) (Fig. 2.3.). The hypoglossal sinus in females is very deep (~2 times the length of the medial length of the hyoid plate).



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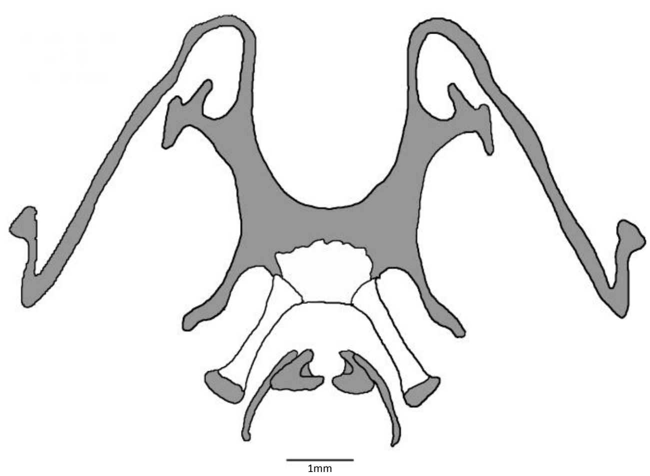

B

Fig. 2.3. Adult male and female hyoid apparatus of *Pseudacris crucifer* in ventral view. **A**: Male and **B**: female. Grey denotes cartilage; white denotes bone and mineralization. c, cartilage; p, process

In males the hypoglossal sinus is wider (~1.5 times the width of the females that were observed) and shallower. In other species such as *Acris crepitans* (Maglia et al., 2007) the shape of the sinus changes with age. In this study there was only one female available, so ontogenetic variation in sinus shape was not able to be assessed.

The hyales are cartilaginous elements that are doubly recurved and attach the hyoid apparatus to the skull. They begin at the anterolateral corners of the hyoid plate and extend anteriorly for approximately one seventh of their length; they then curve to continue posterodorsally for approximately five-sevenths of their length and recurve anterodorsally for two-sevenths of their length. They end in an expanded triangular cartilage that synchondrotically fuses with the palatoquadrate and basal process of the skull.

The anterolateral processes are located posterior to the point of attachment for the hyales and extend anterolaterally from the lateral margin of the hyoid plate. Each anterolateral process extends approximately one half of the length of the posteromedial processes and have irregular shaped expansions on the distal end. The posterolateral processes extend from the posterolateral margin of the hyoid plate, posterior to the anterolateral processes. They are long, slender cartilaginous elements and are approximately four-fifths the length of the posteromedial processes and one-half the width of the posteromedial processes.

The posteromedial processes extend from the posterior end of the hyoid plate and are the only ossified element of the hyoid apparatus. The proximal and distal heads of the posteromedial process are expanded. On the distal head the expanded section is cartilaginous. In males, the posteromedial processes are longer, being approximately twice the length of the hyoid plate, while females are shorter and approximately 1.5 times the length of the hyoid plate. In neither males nor females do the proximal heads meet.

Laryngeal apparatus. The laryngeal apparatus (AAO: 0000267) in *Pseudacris crucifer* is sexually dimorphic and consists of a pair of arytenoid cartilages (AAO: 0000674) that are held in place by the cricoid cartilage (AAO: 0000675). It is located posterior to the hyoid apparatus, and found between the lungs (AAO: 0010567) and buccal cavity (AAO: 0000960). The cricoid cartilage is ring shaped and acts as a base for the arytenoid cartilages. In the males, the laryngeal apparatus spans bewteen the posteromedial processes of the hyoid. In females the laryngeal apparatus is smaller. The arytenoid cartilages are lateral to the cricoid cartilage and ventromedially are thin, concave structures that support the vocal chords. In females, the arytenoid cartilage is more triangular shaped, while in males they are more oval shaped. The cricoid cartilage is a pair of ventrally concave half ovals. In males, the cricoid cartilages have a very narrow separation, while in females the space between the halves is greater.

2.4.3. Axial Skeleton. The axial skeleton (Fig. 2.4) is well developed, provides the anchor for the appendicular skeleton, and supports the head and internal organs. It extends from the base of the head to the tail and acts as one structure, but is made up of eight individual presacral a sacrum and several fused postsacral vertebrae. The presacral region is the largest region, and the eight vertebrae have nonimbricate neural arches. The sacral region is a single specialized vertebra in the middle of the axial skeleton. The postsacral region is the most posterior and includes the urostyle.

2.4.3.1. Presacral Region. The presacral region begins with the atlas (AAO: 0000709). The atlas lacks transverse processes but has special cervical cotyles that allow articulation with the occipital condyles of the skull. The cervical cotyles are located on the anterior ventrolateral margins of the atlas and appear as large projections. The cotyles have a wide separation and there is no centrum; thus the spinal cord is exposed between the atlas and the foramen magnum (AAO: 0010826). Each cotyle is a long pointed projection from the atlas and is completely ossified in all specimens examined. The atlas is completely ossified with no cartilage present between the neural arches.

The atlas bears postzygapophyses on the posterolateral corners that articulate with the prezygapophyses of the second vertebra. The postzygapophyses have a rounded shape, and are oriented ventrolatereally---this is typical of most anurans.

The seven postatlantal vertebrae all bear transverse processes along their lateral sides. The articular facets of the vertebral centrum are ossified in all specimens examined. Each of the transverse processes has a cartilaginous distal end. The second vertebra (or axis) has very little cartilage in all specimens examined. The transverse processes of the second vertebra are expanded anteroventrally and possess a projection from the lateral margin end that is anteriorly oriented. The transverse processes of Vertebra II do not extend laterally as far as the transverse processes of the remaining vertebrae. The third vertebra also has a small projection on the cartilaginous tip of the transverse process that is posteriorly oriented.

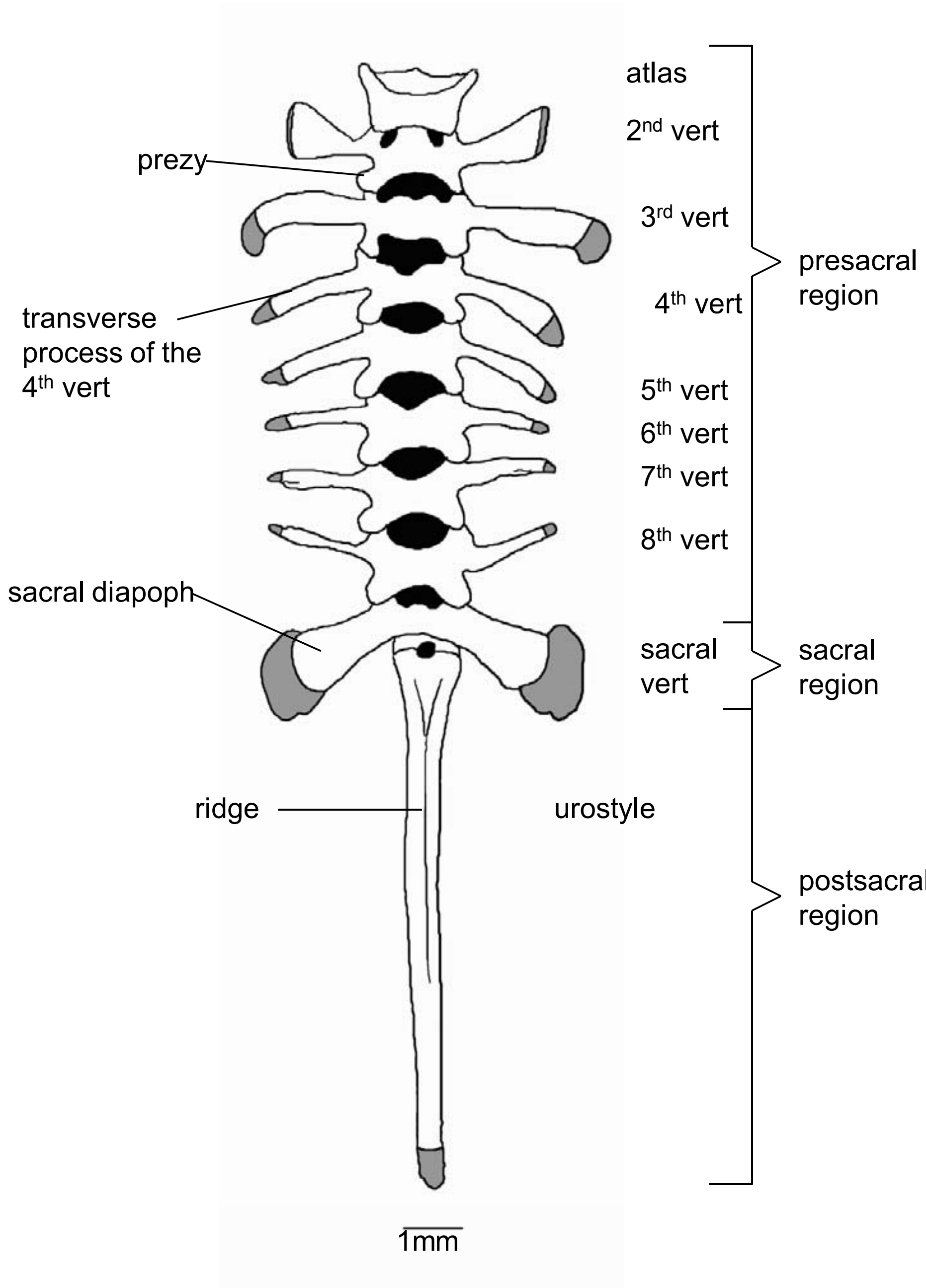

Fig. 2.4. Adult vertebral column of *Pseudacris crucifer*. Dorsal view. Gray denotes cartilage; black denotes exposed spinal cord; white denotes bone. diapoph, diapophyses; vert, vertebrae; prez, prezygapophyses

Vertebrae IV and V have narrow transverse processes that are oriented posteriolaterally. The transverse processes of the sixth vertebra are perpendicular to the axis of the column. The seventh and eighth vertebrae have transverse processes that are oriented anterolaterally. The relative lengths of the transverse processes in decreasing order of size are: III > IV >V =VI =VII = VIII > II.

2.4.3.2. Sacral Region. The sacrum (AAO: 0000552) or sacral vertebra is a specialized vertebra that is located anterior to the urostyle and posterior to the presacral vertebrae. The sacrum bears two diapophyses that are well developed with cartilage on the distal ends. The distal ends of the diapophyses articulate with the ilium of the pelvic girdle. The diapophyses are wider at the distal end than the proximal ends and originate from the lateral margins of the neural arch pedicels. They are also cylindrical in shape when viewed laterally. The diapophyses are also positioned more dorsally than the transverse processes of the presacral vertebrae. For relative size they are approximately as long as the transverse processes of the third presacral vertebra.

That centrum of the sacrum is entirely ossified and it is of similar size to Presacral Vertebra II. The anterior end of the centrum is flat while the posterior end of the sacrum has two condyles that articulate with the cotyles of the urostyle. There is a small space between the condyles and cotyles. The condyles are ossified and convex when viewed dorsally. The neural arch lamina is flat and unornamented. The centrum is also unornamented and there are no neural spines observed. The sacrum has no prezygapophyses present but the eighth presacral vertebra has postzygapophyses present that articulate with the sacrum.

2.4.3.3. Postsacral Region. The urostyle (AAO: 0000732) is the only element that makes up the postsacral region. The urostyle is a unique structure this is found only in anurans. It is a long element that is the fusion of the dorsal coccyx and the ventral hypochord (Griffiths, 1963; Branham and List, 1979; Maglia and Pugener, 1998). The urostyle is located posterior to the sacral region and is approximately 45% of the entire length of the axial column. It is located between the ilia when viewed dorsally and it extends posteriorly to the pelvic girdle symphysis. When viewed in cross sections the urostyle is rounded, and when viewed dorsally it is narrow. The anterior end of the urostyle has two cotyles that articulate with the sacrum. The cotyles are ossified in all specimens examined. The anterior end is two times as wide as the cartilaginous posterior end. It has a longitudinal ridge that is seen dorsally and formed by the caudal neural arches. The ridge is highest and widest at the anterior end of the urostyle. When viewed laterally the urostyle is pierced by a pair of very small spinal nerve foramina. The foramina are located near then anterior end of the urostyle at the base of the longitudinal ridge that is observed dorsally. The urostyle does not have transverse processes or pre- or postzygapophyses.

2.4.4. Appendicular Skeleton. The appendicular skeleton consists of the pectoral girdle (AAO: 0000422), pelvic girdle (AAO: 0000426), forelimbs (AAO: 0000205) and hind limbs (AAO: 0000219). The pectoral and pelvic girdle are attached to the vertebral column with the limbs attaching to their respective girdle. The fore- and hind limbs all have digits with intercalary elements or small, bony elements between the terminal phalanx and proximal phalanx.

2.4.4.1. Pectoral Girdle. *Pseudacris crucifer* has a typical arciferal arrangement with the epicoracoids uniting in the interclavicle area and free but overlapping posterior to the clavicles (Fig. 2.5.). The pectoral girdle consists of two regions: the suprascapula-scapula region that is dorsal to the glenoid fossa and the zonal area that is ventral to it. The zonal area can be further separated into the prezonal, zonal and postzonal areas. The prezonal area contains the omosternum, the postzonal area contains the sternum, and the zonal area contains the epicoracoid cartilage, the coracoids and the clavicles. The glenoid fossa is the depression for the forelimb to articulate with the pectoral girdle, and is formed by the scapula, coracoid and clavicle.

Suprascapulae. The suprascapulae (AAO: 0000748) are blade shaped elements of the pectoral girdle that form in the shoulder region. The suprascapulae are approximately one-quarter times the length of the scapula. The suprascapular ossification in all specimens examines was of similar degree. The suprascapulae have three cartilaginous margins with ossification starting along the anterior margin to form the cleithrum. The distal margin of the suprascapulae is cartilaginous and bears a hook like anterior projecting process.

Scapulae. The scapulae (AAO: 0000751) are paired endochondral bones located between the clavicles and the suprascapulae. Dorsoventrally, each scapula is about 1/4 the glenoid fossa (AAO: 0000749) length. It is well ossified with the center being narrower than either end. The ends are concave with the clavicular end having a prominent pars acromialis (AAO: 0000752) that forms the anterior portion of the ventral margin of the scapula. The pars glenoidalis (AAO: 0000753) forms the posterior portion of the ventral margin of the scapula and it is somewhat concave. The dorsal margin of the scapula is wide and flat and is a broad point of articulation with the suprascapula.

Omosternum. The omosternum (AAO: 0000759) is an entirely cartilaginous element located anterior to the clavicles and epicoracoids. The posterior region is a long, slender triangular element that extends to form a fan-shaped anterior region. The posterior margin is rounded and articulates with the clavicles. The anterior region has uneven margins and lies ventral to the hyoid region.

Procoracoids. The procoracoids (AAO: 0000760) make up the anterolateral portion of the zonal region of the pectoral girdle. They are cartilaginous and form the anterior margins of the pectoral fenestrae. They are continuous with the epicoracoid cartilage and extend laterally in a triangular shape posterior to the clavicle toward the glenoid fossa.

Clavicles. The clavicles (AAO: 0000761) are slender dermal bones that invest the anterior margin of the procoracoids. The anterior margin of each clavicle is concave, while the posterior margin is convex. The clavicle is slightly longer than the scapula. The lateral end of the clavicle is irregularly shaped and overlaps the procoracoid cartilage entirely. The medial end comes to a rounded point and is slightly flattened in ventral view.

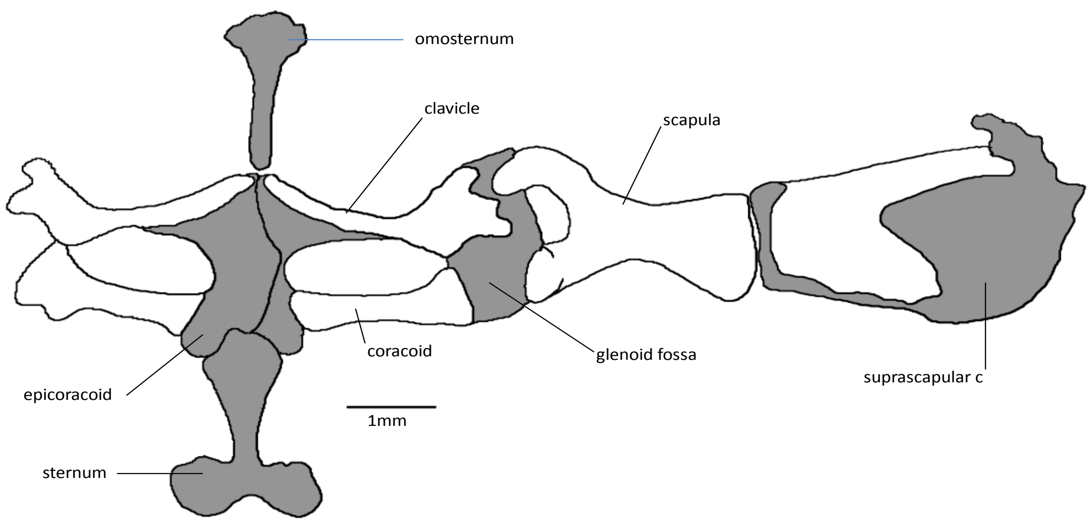

Fig. 2.5. Adult pectoral girdle of *Pseudacris crucifer*. Ventral view. Dorsal and lateral elements have been reflected ventrally into zonal plane, and right scapula and suprascapula have been removed. Gray denotes cartilage; white denotes bone. c, cartilage.

Epicoracoids. The epicoracoids (AAO: 0000762) are paired cartilaginous elements that compose the central region of the zonal area. They connect the clavicle and coracoid of each half of the pectoral girdle. The anterior area of the epicoracoids is synchondrotically fused with posterior portion, and the right epicoracoid appears to overlap the left epicoracoid when viewed ventrally. The length of the epicoracoid cartilage is roughly equal to the length of the scapula. In *Pseudacris crucifer* there is no epicoracoid bridge, but there is a well-defined cartilaginous articulation between the epicoracoids and the omosternum. The epicoracoids articulate with the sternum along their posterior margins.

Coracoids. The coracoids (AAO: 0000764) are paired endochondral bones located posterior to the clavicles and lateral to the epicoracoid cartilage. They are concave along the posterior and anterior margins, and have a widened sternal and glenoid head. They are slender bones that are approximately 3/4 the length of the scapula. The sternal head articulates with the epicoracoid cartilage and the glenoid head forms the medial part of the glenoid fossa.

Sternum. The sternum (AAO: 0000765) is a cartilaginous element that is posterior to the epicoracoids. It has a wide, rounded anterior region that narrows before widening posteriorly to form two rounded points of irregular shape. The sternum t is approximately equal in length to that of the epicoracoid cartilages. The sternum articulates with the posterior margin of the epicoracoids via a cartilaginous bridge that, when viewed ventrally, appears to overlap the epicoracoids. The distal region of the sternum has two equal rounded posterolateral extensions.

2.4.4.2. Forelimbs. The forelimbs are the anterior paired appendages and comprise the humeri, radioulnae, carpals, metacarpals and phalanges (Fig. 2.6.).

Humeri. The humeri (AAO: 0000679) are long, endochondral bones that form the upper arm. They are well ossified and narrow at mid shaft. The humeri are stout, with the length being approximately 8-9 times the width. The entire humerus is about one-third the length of the entire arm. The crista ventralis (AAO: 0000736), which is located on the proximal half of the humerus, is well developed. There is a small crista medialis (AAO: 0000837), but a crista lateralis is absent. The glenoid head (AAO: 0000835) of the humerus is large with some cartilaginous portions but mostly ossified in the specimens examined. A ventral projection extends from the glenoid head that forms a loop of cartilage, and the crista ventralis is ossified.

The distal end of the humerus has a widened round humeral head. In the specimens examined, the humeral head is completely ossified and has two epicondyles that are mostly ossified with a cartilaginous ring on each. Both epicondyles are similar in size with the lateral epicondyle being a little larger; thus, the distal end of the humerus is asymmetrical when viewed in dorsal and lateral views. The distal end of the humerus also possesses a ventral fossa that is small and rounded and located between the epicondyles.

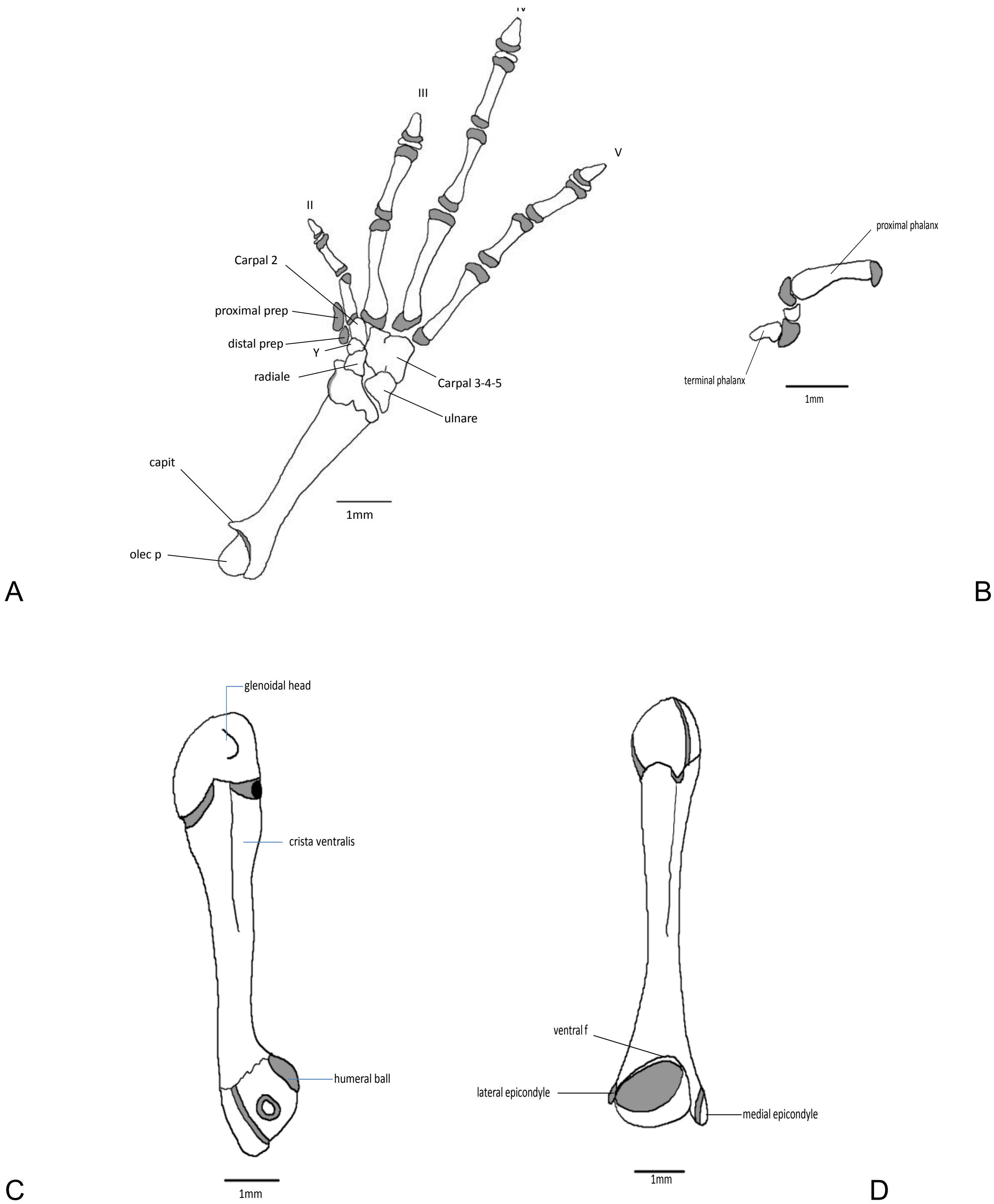

Fig. 2.6. Right forelimb elements of *Pseudacris crucifer*. **A**: Dorsal view of the manus and radioulna. **B**: Lateral view of Digit III. **C**: Lateral view of the humerus. **D**: Ventral view of the humerus. Gray denotes cartilage; white denotes bone. capit, capitulum; olec, olecranon; p, process, Y, Element Y.

Radio-ulnae. The radio-ulnae (AAO: 0000901) are compound endochondral bones that form the forearm. They are well ossified, their length is about 5-6 times their width, and they are about 3/4 of the length of the humerus. They make up about 1/3 of the entire length of the arm. The sulcus is deeper and more evident in the distal end of the radio-ulna, but is present in the proximal end. The proximal head consists of a olecranon (AAO: 0000843) and a capitulum (AAO: 0000840). The distal head is covered by a flat, almost rectangular calcified structure that articulates with the carpal elements. The distal portion was ossified in all specimens examined but had some cartilage covering the bone to aid in articulation.

Manus. The hands of *Pseudacris crucifer* are similarly arranged to Morphology C of Fabrezi (1992). The hand contains five carpals, two prepollical elements, four metacarpals, and ten phalanges. The length of the manus is approximately 1/3 of the entire length of the arm.

The radiale (AAO: 0000790) and the ulnare (AAO: 0000845) make the basal row of carpals and they are completely ossified. They are articulate with the ulnare and radiale portions of the radio-ulna. The ulnare is slightly larger and has a more triangular shape than the radiale. The radiale articulates with Element Y (AAO: 0000846) anteromedially. Carpal 3-4-5 is distal to ulnare and radiale and it is a large square ossified element that articulates with the metatarsals of Digits 2-3-4. Medial to Carpal 3-4-5 is Carpal 2, which is a small oval shaped element that is fully ossified. The medial margin of Carpal 2 (AAO: 0000848) articulates with n oval shaped cartilaginous proximal prepollex (AAO: 0000852). A rectangular shaped distal prepollex (AAO:

0000852) articulates with the prepollex. There is some mineralization of the proximal prepollex in all the specimens examined.

Posterior to the carpals are the metacarpals which are long cylindrical bones. Metacarpal II and III articulate with Carpal 2. Metacarpal IV articulates with 3-4-5. The metacarpals all have a fibrous cartilage cap on each end. The metacarpal lengths relative to each other in decreasing order of size are IV (AAO: 00000010615) > III (AAO: 0000854) > V (AAO: 0000855) > II (AAO: 0000853). The digits as whole have relative lengths of IV > V > III > II.

2.4.4.3. Pelvic Girdle. *Pseudacris crucifer* has a V-shaped pelvic girdle (AAO: 0000426) when viewed dorsally (Fig. 2.7). It is located posterior to sacral region and articulates with the sacrum. The articulation type is IIB (Emerson, 1979). Three paired elements the ilia, ischia and pubes make up the pelvic girdle. These three elements come together to form the oval acetabulum, which is the cavity for articulation with the hind limb. Their convergence however is not at the acetabular depression, so there is a large triangular space that is left open in the center off the articular region. The cartilaginous acetabular ring surrounds this space.

Ilia. The ilia (AAO: 0000772) are paired endochondral bones that make up the anterior portion of the pelvic girdle. They are long narrow bones that, when viewed in cross section, are rounded. Each ilium consists of a long shaft that articulates with the diapophysis of the sacrum and has an expanded posterior portion that forms the anterior portion of the acetabulum. Each shaft has a cartilaginous anterior terminus and an ilial ridge that runs from the ilial protuberance to just posterior to the cartilaginous anterior terminus.

The ilia come together in the posterior region of the pelvic girdle to form the ilial corpus. This portion of the ilium has an expansion of bone that forms the anterior border of the acetabulum (AAO: 0000770). The dorsal portion of the ilial corpus, which is known as the supra-acetabular expansion is well ossified and elongates about 1 1/2 times the width of the ilial shaft. The supra-acetabular expansion also articulates with the anterior supra-acetabular expansion of the ischium. The ilial corpus also has a small triangular dorsolaterally projecting protuberance that is located anterior to the supra-acetabular expansion. The ilial corpus also has a ventral preacetabular expansion that forms the dorsal margin of the acetabulum and articulates with the anterior margin of the pubis. In all specimens examined the ilia are well ossified.

Ischia. The ischia (AAO: 0000860) are endochondral bones located in the posterior region of the pelvic girdle. In the adult, the ischia are synostotically fused together medially. The ischia form the posterior part of the acetabulum, and possess a pronounced dorsal ridge of bone called the interischiadic crest. The anterior margin of the ischium is the point of articulation with the supra-acetabular expansion of the ilia, and the posteroventral margin is synchondrotically fused to the pubis. In all specimens examined the ischia are entirely ossified with a clear line between the pubis and ischium.

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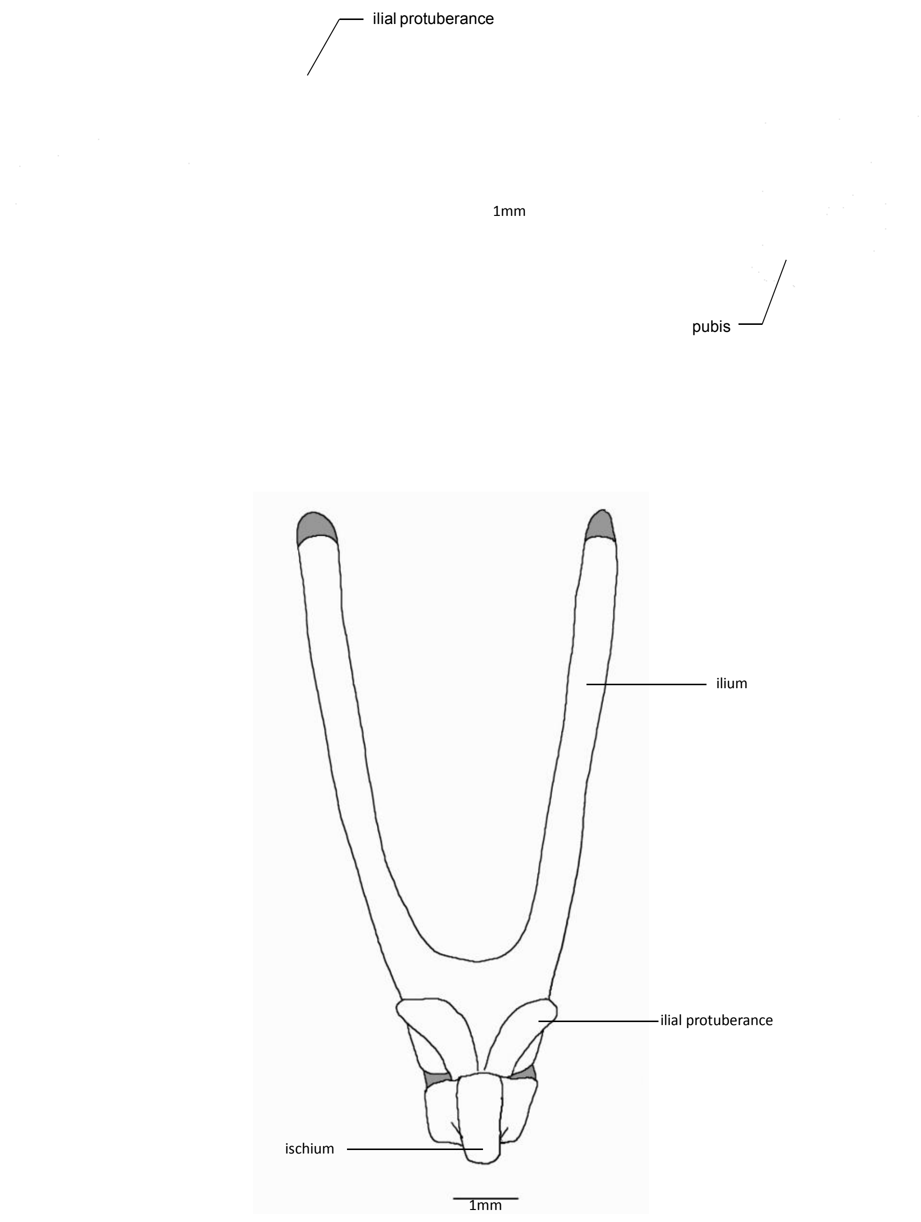

Fig. 2.7. Adult pelvic girdle of *Pseudacris crucifer.* In **A**: lateral view and **B**: ventral view. Gray denotes cartilage; white denotes bone; black denotes open area between bones; white speckling denotes mineralization.

Pubes. The pubes (AAO: 0000861) are cartilaginous elements that have a large amount of mineralization that are located in the posteroventral portion of the pelvic girdle. They are fused together synchondrotically, articulate with the ilia and ischia ventrally, and form the posterior part of the acetabulum. In all specimens examined the pubes were cartilaginous with heavy amounts of mineralization

2.4.4.4. Hind limbs. The hind limbs are paired posterior appendages that articulate with the pelvic girdle. There are five basic segments of the hind limbs and they are listed in proximal to distal sequence: femora (AAO: 0000889), tibiofibulae (AAO: 0000902), tarsals, metatarsals and phalanges (Fig. 2.8). Each foot of *Pseudacris crucifer* has five digits, like all anurans.

Femora. The femora are the most proximal bones of the hind limb and articulate with the pelvic girdle. They are long, endochondral bones that are well ossified. The femora have a slight curvature that is anterior to the proximal head. The femora are approximately 12 times longer than wide, and are about 1/3 of the total length of the leg. They are the same length as the tibiofibula. The proximal head, which articulates with the acetabulum of the pelvic girdle is slightly smaller than the distal head which articulates with the tibiofibula. The heads of the femora are both ossified but have sheets of cartilage that cover parts and allow for smoother articulation. There are two small lateral and medial condyles on the proximal head. The femora in all specimens examined were completely ossified.

Tibiofibulae. The tibiofibulae (AAO: 0000902) connect the femora to the pes. They are compound, endochondral bones that are completely ossified. The tibiofibula are about 1/3 of the total length of the leg and is approximately 12 times its width. Each tibiofibula has a sulcus that runs between the tibia and fibula which indicates that it is a compound bone. The sulcus is even visible on each head of the tibiofibula. The distal head articulates with the tarsal elements of the pes and the proximal head articulates with the distal head of the femur. Both heads are similar in size and are completely ossified with some fibrous cartilage on the surface of each one. There is a foramen visible at the midpoint that is for the tibial artery and vein.

Pedes. The foot of *Pseudacris crucifer* contains five tarsals, four prepollical elements, five metatarsals and fourteen phalanges. The arrangement of the foot is similar to the arrangement that Fabrezi (1993) described for Hylidae. The pes is approximately 1/3 of the length of the entire leg although 40% of that is the tibiale and fibulare. The most proximal element of the pes is the preaxial fibulare (AAO: 0000913) and the postaxial tibiale (AAO: 0000912), which are elongate bones. The fibulare and tibiale are the basal row of tarsals. They are separate along the length of each but are fused medially at the distal and proximal heads. The proximal and distal heads are similar in size and each is a cup shaped ossified structure. There is some cartilage that surrounds the heads for articulation. The distal head of the tibiale and fibulare articulates with the prehallux and distal tarsals anteromedially, and with the metatarsals of Digits II and III anterolaterally.

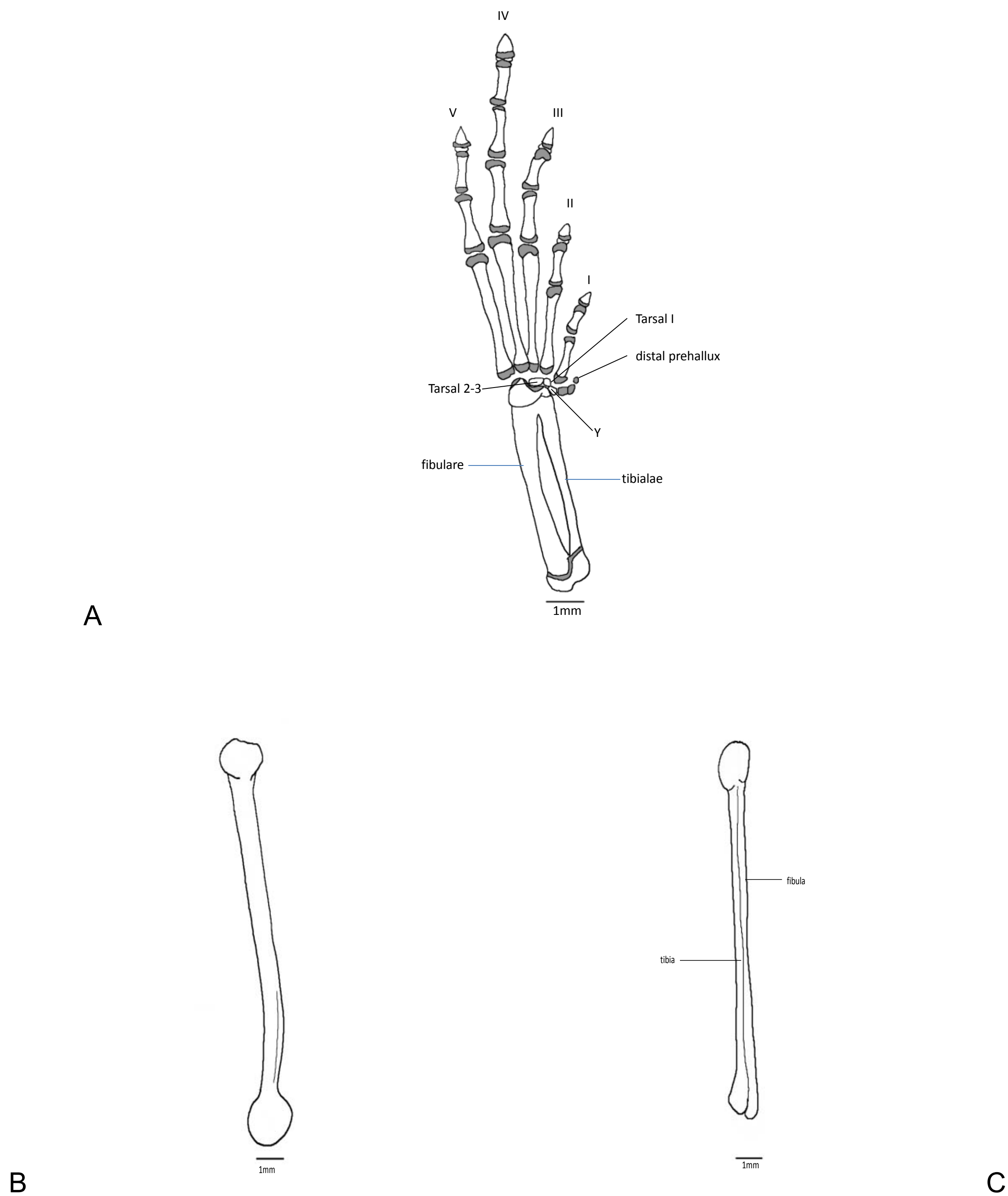

Fig. 2.8. Adult hind limb of *Pseudacris crucifer*. **A**: Dorsal view of the pes. **B**: Ventromedial view of the femus. **C**: Ventral view of the tibiofibula. Gray denotes cartilage; white denotes bone.

The largest tarsal is 2-3 (AAO: 0000919) and is a regtangular shape that lies proximal to the metatarsals of Digits II and III. Medial to Tarsal 2-3 is Tarsal 1. Tarsal 1 (AAO: 0000918) is a small round ossified element that articulates with the metatarsals of Digits II and III. Posteroventral to Tarsal 1 lies the ossified Element Y (AAO: 0000921), which is also a small round element. Medial to the Tarsal 1 is a rectangular, cartilaginous distal prehallux 1 (AAO: 0000922). A cartilaginous oval shaped Prehallux 2 (AAO: 0000922) articulates with the distal end of Prehallux 1 and the proximal end of Prehallux 3 (AAO: 0000922). Prehallux 3 is a small round cartilaginous element that forms the distal end of the prehallux.

Anterior to the tarsals are the metatarsals, which are elongate, cylindrical bones. They have a distal and proximal heads that are similar in size and covered by fibrous cartilage; in some metatarsals the heads are mineralized. Metatarsal 4 is the longest, and it is approximately four-fifths the length of the tibiale. The relative lengths of the metatarsals, in decreasing order of size, are: IV (AAO: 0000928) > III (AAO: 0000927) > V (AAO: 0000929) >II (AAO: 0000926) > I (AAO: 0000925).

The digits have a phalangeal formula in medial to lateral sequence of 2-2-3-4-3 and have relative lengths of IV (AAO: 0010656) > V (AAO: 0010658) = III (AAO: 0010646) > II (AAO: 0010645) > I (AAO: 0010644). The terminal phalanges (AAO: 0010676) are claw shaped when viewed laterally because they possess an intercalary element. The intercalary elements are small rectangular shaped ossified elements that articulate with the anteroventral ends of the penultimate phalanges (AAO: 0010677). The proximal and central phalanges are similar in shape and structure to the metatarsals and are cylindrical slender bones. Each head of the proximal and central phalanges has fibrous cartilage covering it.

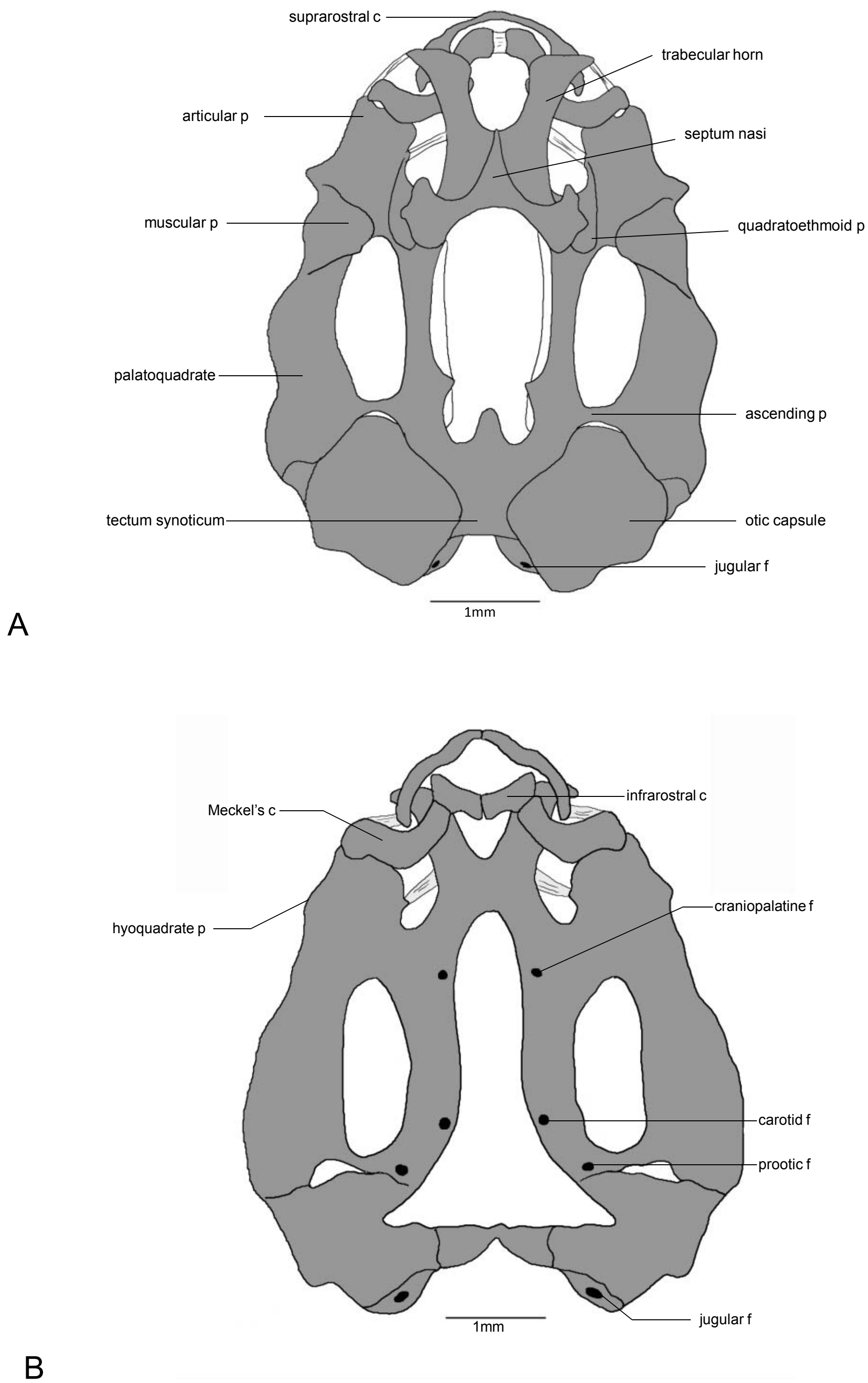

Fig. 2. 9. Larval chondrocranium (Stage 37) of *Pseudacris crucifer*. **A**: Dorsal view. **B**: Ventral view. Gray denotes cartilage; white denotes bone; black denotes foramina. c, cartilage; f, foramina; p, process.

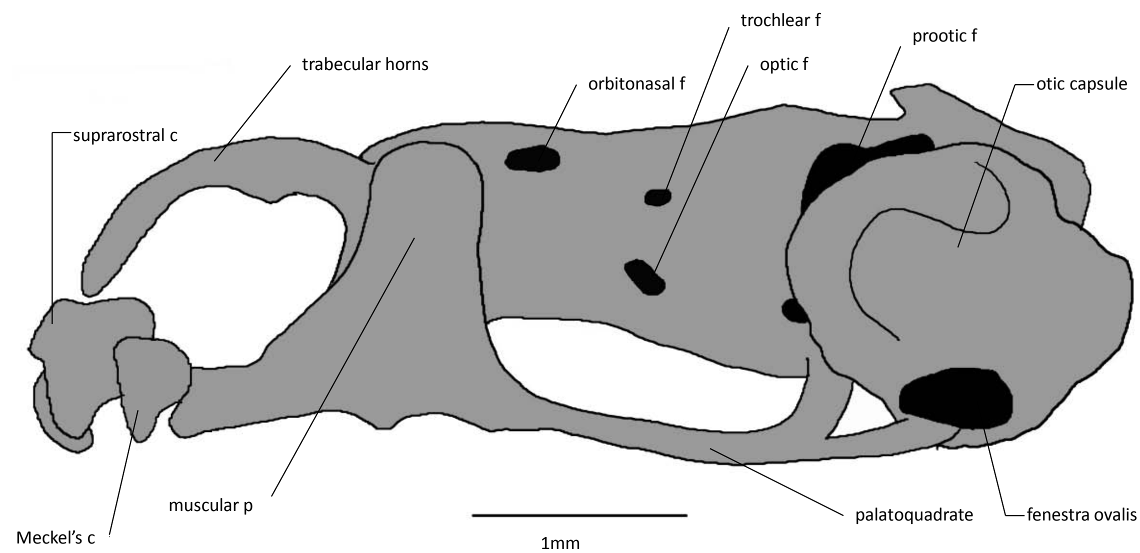

Fig. 2.10. Larval chondrocranium (Stage 37) of *Pseudacris crucifer* in lateral view. Gray denotes cartilage; black denotes foramina. c, cartilage; p, process; f, foramina.

2.4.5. Larval Skeleton

2.4.5.1. Structure of the Larval Cranium. The following description is based on five tadpoles (Gosner Stage 37 and 38) that represent the typical tadpole morphology (i.e., the stages that represent complete chondrification of the larval chondrocranium, yet are prior to most ossification or metamorphic chondrocranial changes) (Fig. 2.9) (Fig. 2.10)

The chondrocranium appears arrow shaped, with its width (at its widest portion, at the level of the palatoquadrate cartilages) approximately two-thirds its length (measured from the anterior tip of the upper jaw to the foramen magnum). The trabecular horns (AAO: 0010138) make-up approximately one-fifth the length of the tadpole cranium, and serve as the support for the suprarsotral cartilages, the upper jaw of the tadpole. They are relatively short and thick compared to other species. The horns form a V-shape in dorsal view, immediately diverging from one another at a 45° angle as they extend anteriorly from the ethmoid plate. In the middle of the trabecular horn the horn extends anteriorly to form a more rectangular shape. Each horn forms an arc in lateral view as it curves anterovententrally toward the jaw. At the base of each horn, near the fusion to the trabecular plate, there is a distinct triangular process that serves as the attachment site for the quadratoethmoid ligament (AAO: 0010558). Each suprarostral cartilage (AAO: 0000998) is L-shaped (in anterior view) and contains a lateral ala and a central corpus, which articulates dorsomedially with the anteromedial corner of the anterior margin of the trabecular horn via a sinovial joint. The suprarostrals are connected to one another at the midline via a strong ligament.

Ventral to the suprarostral cartilages are the paired, rectangular infrarostral cartilages (AAO: 0000237), which make up the medial portion of the tadpole's lower jaw. Medially, the infrarostrals articulate with one another via a strong ligamentous connection; laterally, each forms a sinovial joint with Meckel's cartilage. Meckel's cartilages are L-shaped in dorsal view, and form the lateral portions of the tadpole lower jaw. Laterally, each articulate with the quadratoethmoid process (AAO: 0010094) (anterior portion) of the palatoquadrate via a cartilaginous bridge, the retroarticular process (AAO: 0010559). Laterally, the palatoquadrate has a triangular shaped muscular process (AAO: 0010005). The muscular process is large and extends one half of the distance from the edge of the palatoquadrate to the commissura quadratocranialis anterior.

Anteriorly the palatoquadrate is attached to the neurocranium by the commissura quadratocranialis anterior and is attached posteriorly by the processus ascendens (AAO: 0000486). The processus ascendens is thin. The palatoquadrate continues posteriorly to connect with the otic capsule. The palatoquadrate connection to the otic capsule is a very fine ligamentous connection. The otic capsule is somewhat spherical and somewhat small compared to other species. It is only about ¼ of the entire larval chondrocranium (AAO: 0010560).

The cranial floor is a large sheet of cartilage that is formed by the basal plate (AAO: 0010561). The floor is pierced anteriorly by a pair of craniopalatine foramina (AAO: 0010563), and posteriorly by a pair of carotid foramina (AAO:0010564). The craniopalatine foramina are small and round and located at the same level as the suboccular foramen. The lateral walls of the braincase consist of the orbital cartilages.

In dorsal view, the braincase has a large frontoparietal fenestra that is formed by the ethmoid plate (AAO: 0010134) anteriorly and tectum synoticum (AAO: 0010129) posteriorly. It is bordered laterally by the taenia tecti marginalis (AAO: 0000599). *Pseudacris crucifer* also has taenia tecti tranversalis (AAO: 0010130) that are located in the posterior end of the braincase and are relatively small. There is also a taenia tecti medialis (AAO: 0010132) that is located in the most posterior region of the braincase. It extends to the anterior from the middle of the tectum synoticum.

The lateral walls of the braincase are pierced by four foramina. The optic foramen (AAO: 0000342) is the most anterior and lies between the pila preoptica (AAO: 0010565) and pila metoptica (AAO: 0000432), and it is where the optic nerve (Cranial Nerve II [AAO: 0010467]) exits the braincase. Posterior to the optic nerve and anterior to the ascending process of the palatoquadrate, the oculomotor foramen [AAO: 0000332] pierces the lateral wall. The oculomotor foramen accomodates the oculomotor nerve (Cranial Nerve III; [AAO: 0010468]). The trochlear nerve (Cranial Nerve IV; [AAO: 0010469]) is accomodated by the trochlear foramen (AAO:0000610), is present in the anterodorsal region of the lateral wall. The prootic foramen (AAO: 0010566) is the largest of the four foramina because it accommodates the trigeminal (Cranial Nerve V; [AAO: 0010470]), abducens (Cranial Nerve VI; [AAO: 0010471]), and facial (Cranial Nerve VII; [AAO: 0010472]) nerves. The prootic foramen is located posterior to the ascending process of the palatoquadrate near the cartilaginous prootics. The pila antotica is a strip of cartilage that separates the prootic foramen from the oculomotor foramen.

2.4.5.2. Developmental Modifications of the Cranium. During development the larval cranium will change dramatically in structure and will undergo several modifications prior to completion of metamorphosis. There is significant morphological variation among tadpoles of the same Gosner stage; thus, this description is based on earliest seen ossification and modifications. Prior to Gosner Stage 31, the larval cranium is very small and does not stain well because there is a lack of chondrification. By Stage 32, the tectum synoticum (AAO: 0010129) is visible as a faint bridge of cartilage that connects the otic capsules. The tectum synoticum continues to chondrify over time and by Stage 38 it is a wide piece of cartilage that completely unites the otic capsules. In early Gosner stages (pre-31), the basicranial fenestra (AAO: 0000037) is seen in the floor of the braincase, the basicranial fenestra will disappear by Stage 31 and the basal plate will chondrify and appear as a sheet of cartilage that covers the braincase floor. By Stage 31, which is the earliest stage examined, the trabecular plate (AAO: 0010137) extends anteriorly from the basal plate with which it is confluent. The trabecular plate is pierced by two sets of foramina: the craniopalatine foramina and the carotid foramina.

By Stage 31 the trabecular horns are chondrified and established as elongated extensionsof the trabecular plate that extend anteriorly.

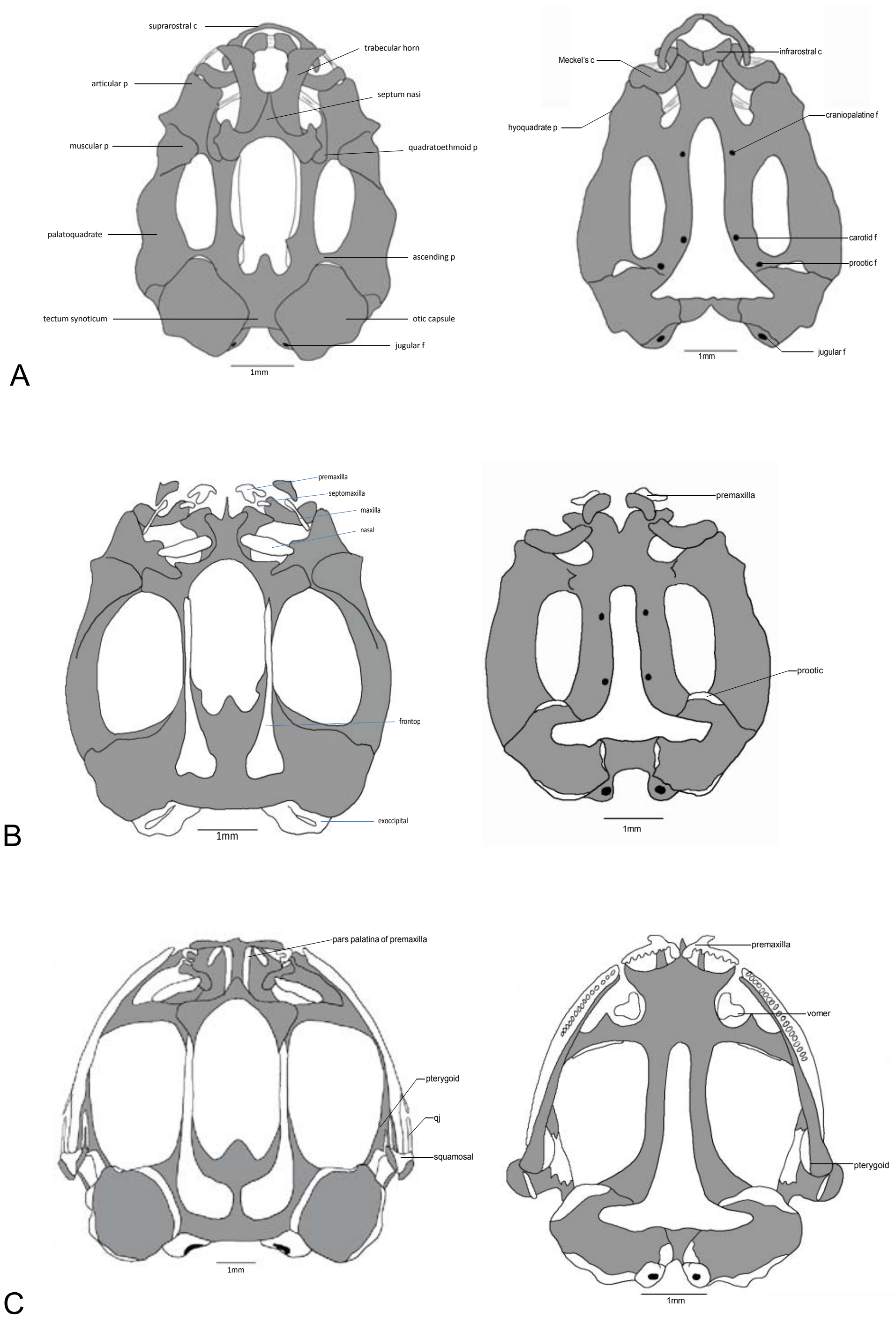

Fig. 2.11. Development of the larval cranium of *Pseudacris crucifer*. In dorsal (left) and ventral (right) views. **A**: Stage 38. **B**: Stage 43. **C**: Stage 46. White denotes bone; gray denotes cartilage; black denotes foramina. qj, quadratojugal.

They continue to increase in size, and by Stage 33 they articulate with the suprarostral cartilages. By Stage 34 the trabecular horns also have formed a ligamentous connection to the quadratoethmoid process (AAO: 0010094) of the palatoquadrate. By Stage 40 the trabecular horns have reached their largest size and begin to erode. By Stage 44 the trabecular horns have completely eroded and are no longer present (Figure 2.11). At Stage 31 the suprarostral cartilages are chondrified and visible. They remain separate until Stage 33 when they synchondrotically fuse to one another and articulate with the trabecular horns. Around Stage 42 the suprarostrals begin to erode as new elements of the adult jaw form. At this stage they also separate from one another and move apart. By Stage 45 the suprarostrals have completely eroded and are not longer present. Posteroventral to the suprarostrostrals are the infrarostrals, which are chondrified and are visible by Stage 31. They are rectangular elements that are part of the larval mouth structure. By Stage 35 there is a ligamentous connection between the infrarostrals, and they articulate with Meckel's cartilage along their lateral margin. The ligamentous connection strengthens, and the infrarostrals pull closer together along their medial margins where they remain until Stage 42. By Stage 42 the ligamentous connection disappears and the infrarostrals begin to deteriorate. By Stage 44, the infrarostrals have disappeared completely.

The palatoquadrate is chondrified and resembles the typical larval structure by Stage 31, and by Stage 40 the palatoquadrate has begun to erode and change to resemble the adult morphology (described previously). By Stage 44, the otic ligament chondrifies and the palatoquadrate is united to the crista parotica of the otic capsule. The anterior portion of the palatoquadrate erodes to leave only a narrow cylinder of cartilage that lies lateral to the suboccular foramen, and continues anterolaterally with the processus triangularis, which forms the anterior maxillary process by Stage 45. In addition, the palatoquadrate has a narrow band of cartilage continues to the planum antorbitale (AAO: 0000434). The quadrate ossifies by Stage 46. By Stage 45 the ascending process of the palatoquadrate has completely eroded and the palatoquadrate no longer articulates with the lateral wall of the braincase.

By Stage 31 the otic capsules have formed as ovoid cartilages that are posterolateral to the braincase. The fenestra ovalis (AAO: 0000169) appears by Stage 33 as a circular opening when the otic capsule is viewed laterally. In the fenestra ovalis, a faint ossification, which is the beginning of the operculum, appears around Stage 35. By Stage 38 the prootics have begun to ossify along the anterior wall near the braincase. By Stage 41, the margin between the prootics and occipitals shows signs of mineralization. The prootics and exoccipitals ossify throughout and grow in size through adulthood. The occipital condyles are extensions of bone that articulate with atlas of the axial skeleton and they have begun ossification by Stage 45. They extend posteriorly from the exoccipitals. Later during the juvenile stage the exoccipitals will form the foramen magnum (AAO: 0010826) and the crista parotica (AAO: 0000116) will ossify. It is during the juvenile stage that the exoccipitals will form the foramen magnum.

2.4.5.3. Development of the Nasal Capsule. By Stage 31 (the earliest available specimen) the septum nasi (AAO: 0000559) has chondrified and is connected to the taenia ethmoidalis. It first appears anterior to the braincase and dorsal to the trabecular plate. It is only evident as a faint sheet of cartilage. By Stage 32 the lamina orbitonasalis

(AAO: 0000262) is apparent. By Stage 36, the lamina orbitionasal has joined with the tectum nasi, which is newly chondrified, to form a sheet of cartilage that will subsequently form the adult postnasal wall. By Stage 39, the alary cartilages (AAO: 0000002) have chondrified anterior to the lamina orbitonasalis. The anterior prenasal cartilages (AAO: 0010827) also chondrify near the anterior portion of the alary cartilage by this stage. The superior prenasal cartilages chondrify on the anterior margin of the already present alary cartilages by Stage 40. The paries nasi (AAO: 0000389) appear as a rhomboidal shaped pieces of cartilage by Stage 40. They are located medial to the septomaxilla, which also first appears by Stage 40. By Stage 41, the nasals begin ossification lateral to the trabecular plate. By Stage 42, the trabecular plate and anterior nasal wall will fuse and the majority of the trabecular plate will become the solum nasi. By Stage 43, the alary cartilages and tectum nasi begin to appear as the rounded opening of the nasal passage. By Stage 43, the crista intermedia (AAO: 0000113), the laminae superior (AAO: 0000264), laminae inferior (AAO: 0000259), and the oblique cartilages (AAO: 0000329) appear in the nasal capsule. During Stages 44 and 45, the chondrocranium has almost achieved the adult morphology, and the bony and cartilaginous elements of the nasal capsule have nearly attained their adult form. They will continue to grow and the remaining larval structures will erode entirely. By Stage 46, the tectum nasi (AAO: 0000601) will ossify.

2.4.5.4. Pre-Metamorphic Ossification of the Cranium. The order of ossification sequence of cranial and postcranial elements is presented in Table 2.1. Hanken and Hall (1988) reported that bony elements may show signs of ossification when sectioned several Gosner stages before ossification is apparent in cleared-and-stained whole-mount preparations; thus, the results shown here should be considered relative estimates.

Parasphenoid.---The first cranial bone to ossify is the parasphenoid (AAO:0000385), which begins as a thin, sliver of bone underneath the braincase by Stage 33. The cultriform process (AAO: 0000386) is the first part that forms, and the ala (AAO: 0000387) form later in Stage 34 as thin, poorly stained fragments on the posterolateral portion. The parasphenoid resembles its adult form by Stage 39, but continues to enlarge through metamorphosis.

Prootics.---The prootics (AAO:0000514) begin ossification by Stage 38 along the anterior wall of the otic capsules. Ossification continues through the juvenile stage and progressively incorporates more of the anterior portions of the otic capsule, eventually fusing with the exoccipitals. The crista parotica begins ossification from the lateral margins of the prootics by Stage 46 and will continue to ossify into adulthood (as described above).

Exoccipitals.---The posterior portion of the otic capsules is formed by the exocciptals, which fuse with the prootics and form the margins of the foramen magnum. The exoccipitals (AAO: 0000147) begin ossification around the jugular foramina just prior to the prootics by Stage 37 (AAO: 0000249). From the exoccipitals, the occipital condyles extend posteriorly to articulate with the atlas of the axial skeleton. The exoccipitals continue ossification through metamorphosis and into adulthood. By Stage 46 the exoccipitals and prootics have fused together through ossification.

TABLE 2.1. Ossification sequence of *Pseudacris crucifer*

Stage	Cranial	Postcranial
33	Parasphenoid	
34		Vertebral column
35		Femur
36		Suprascapula
		Humerus
		Radius
		Ulna
		Illium
37	Exoccipitals	Scapula
	Frontoparietals	Cleithrum
		Tibiofibula
		Tibiale
		Fibulare
38	Prootics	Clavicle
		Coracoid
		Metatarsals
		Foot Phalanges
40		Metacarpals
		Hand Phalanges
41		Ischium
42	Septomaxillae	
	Premaxillae	
	Maxillae	
43	Nasals	
45	Vomers	
	Angulosplenials	
46	Pterygoids	Carpal elements
	Quadratojugals	
	Quadrate	
	Squamosals	
	Mentomeckelian	
After Stage 46	Sphenethmoid	

Elements are listed at the first Gosner Stage at which they appear.

Frontoparietals.---The frontoparietals (AAO: 0000207) are among the earlier elements to ossify in the cranium; ossification is evident by Stage 37. They appear as long rods of cartilage that form along the dorsolateral region of the neurocranium. The frontoparietals continue to grow through metamorphosis, and by Stage 39 they have formed medial extensions that will eventually articulate over the posteromedial region of the braincase. The frontoparietals reach their adult form by Stage 45.

Septomaxillae.---By Stage 42, the septomaxillae (AAO: 0000556) appear as small bones that lie dorsolateral to the triangular process of the trabecular horns. They continue to ossify through metamorphosis. They will elongate and form the spiral shape seen in adult specimens by Stage 45. Beyond Stage 45 the septomaxillae will migrate anteriorly and enlarge until they reaches their adult position and size.

Premaxillae.---Each adult premaxilla consist of an alary process (AAO: 0000003), a pars dentalis (AAO: 0000397), and a pars palatina (AAO: 0000414). The alary process is the first to ossify and appears as an oval ossification dorsal to the trabecular horns by Stage 42. By Stage 44, the pars dentalis and pars palatina have begun to ossify.

Nasals.---The paired nasals (AAO:0000312) appear by Stage 43 anterior to the planum antorbitale and lateral to the septum nasi. They are tear shaped bones with the wider end being the medial portion. They continue to ossify and grow in size throughout metamorphosis and the juvenile stage.

Maxillae.---The pars facialis (AAO:0000400) of the maxilla (AAO:0000285) begins to ossify medially along the lateral margin of the palatoquadrate by Stage 42. It continues to ossify and elongate through metamorphosis to form the adult morphology. The pars dentalis (AAO:0000396) appears by Stage 45 along with tooth primordia. Maxillary teeth are seen by Stage 46.

Vomers.---The vomers (AAO:0000633) begin ossification by Stage 45 and first appear as small fragments located medial to the maxilla and anterolateral to the rostral tip of the parasphenoid. By Stage 46, the vomers ossify anteriorly and enlarge to form the anterior process (AAO: 0000649). The postchoanal process (AAO: 0000659), prechoanal process (AAO: 0000658) and vomerine teeth (AAO: 0000635) will form during the juvenile period.

Sphenethmoid.---Ossification of the sphenethmoid (AAO: 0000572) occurs during the juvenile period.

Pterygoid.---The pterygoid (AAO: 0000521) has begun to ossify in the medial region of the cartilaginous precursor by Stage 46. It is a sliver of bone that lies anterior to the otic capsule and posterior to the squamosal. The pterygoid completes growth and ossification during the juvenile period.

Quadratojugal.---Quadratojugals (AAO: 0000532) begin ossification by Stage 46 posterior to the maxillae and lateral to the squamosal. They appear as small triangular bones that increases in size during the juvenile period. They articulate anteriorly with the maxilla and posteriorly with the quadrate.

Squamosal.---The squamosals (AAO:0000574) begin ossification by Stage 46 and are small, rectangular piece of bone that are lateral to the posterior end of what remains of the palatoquadrate. They continues to ossify both anteriorly and posteriorly, and will be fully developed by the end of the juvenile period.

Dentary.---The dentary (AAO:0000124) appears by Stage 46 and lacks odontoids or ridges.

Angulosplenial.---By Stage 45, the angulosplenial (AAO:0000966) starts ossification. The ossification appears as a sliver of bone along the posterior region of the Meckel's cartilage and will continue to ossify anteriorly and posteriorly. By late Stage 46 the coronoid process (AAO:0000012) has formed and articulates with the palatoquadrate.

Mentomeckelian.---By Stage 46 the mentomeckelian (AAO:0000301) begins to ossify. This bone continues to ossify and develop during the juvenile period and will fuse with the anterior margins of the dentaries.

2.4.5.5. Hyobranchium

2.4.5.5.1. Structure of the Hyobranchial Skeleton. The hyobranchial apparatus is described here based on two Stage 37 specimens (Fig. 2.12). Basibranchial I (AAO: 0010583) is absent. They hyobranchial apparatus consists of a Basibranchial II (AAO: 0010585), two ceratohyals (AAO: 0000666), four pairs of ceratobranchials (AAO: 0000953) and three pairs of spicules (AAO: 0010830).

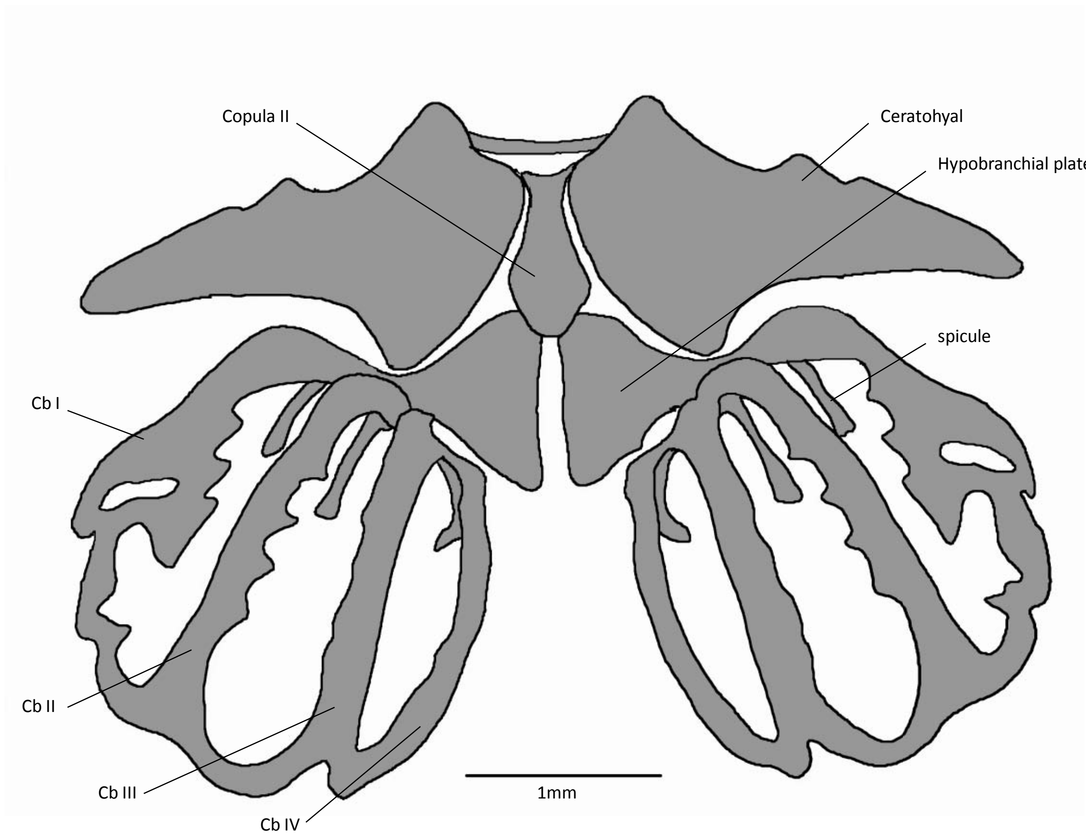

Fig. 2.12. Larval hyobranchium of *Pseudacris crucifer.* Shown in ventral view at Stage 37. Cb I–IV, ceratobranchials I–IV.

The ceratohyals each have two well-developed anterior processes and a large lateral process. The ceratohyal margin between the two anterior processes appears as an inverted triangle. The lateral processes are large and possess rounded points that provide a surface for articulation with the hyoquadrate process of the palatoquadrate. The ceratobranchials connect to the hypobranchial plates, which are flat, triangular shaped sheets of cartilage. The hypobranchial plate is continuous with Ceratobranchials I--IV. Ceratobranchial I has an ovoid foramen that is located in proximal region. There are three posterodorsally projecting spicules that form along the posterior margin of the hypobranchial plate between the ceratobranchials. Each ceratobranchial is a long thin rod of cartilage that extends posteriorly from the hypobranchial. All of the ceratobranchial possess ceratobranchial teeth but they are most developed on Ceratobranchial I. The ceratobranchials articulate posteriorly with the commissura terminales (AAO: 0010828). Basibranchial II is rounded cartilaginous element that is located medial to the ceratohyals.

2.4.5.5.2. Developmental Modification of Hyobranchial. The majority of the developmental modifications of the hypobranchial apparatus to form the adult hyoid occur postmetamorphically; however, there are some changes that occur during the larval stage (Fig 2.13). The ceratobranchial teeth of Ceratobranchials II-IV and the hypobranchial spicules have begun to erode by Stage 42. By Stage 43 the spicules have eroded completely. By Stage 43 the anterior processes have begun to erode and the ceratohyals have decreased in size.

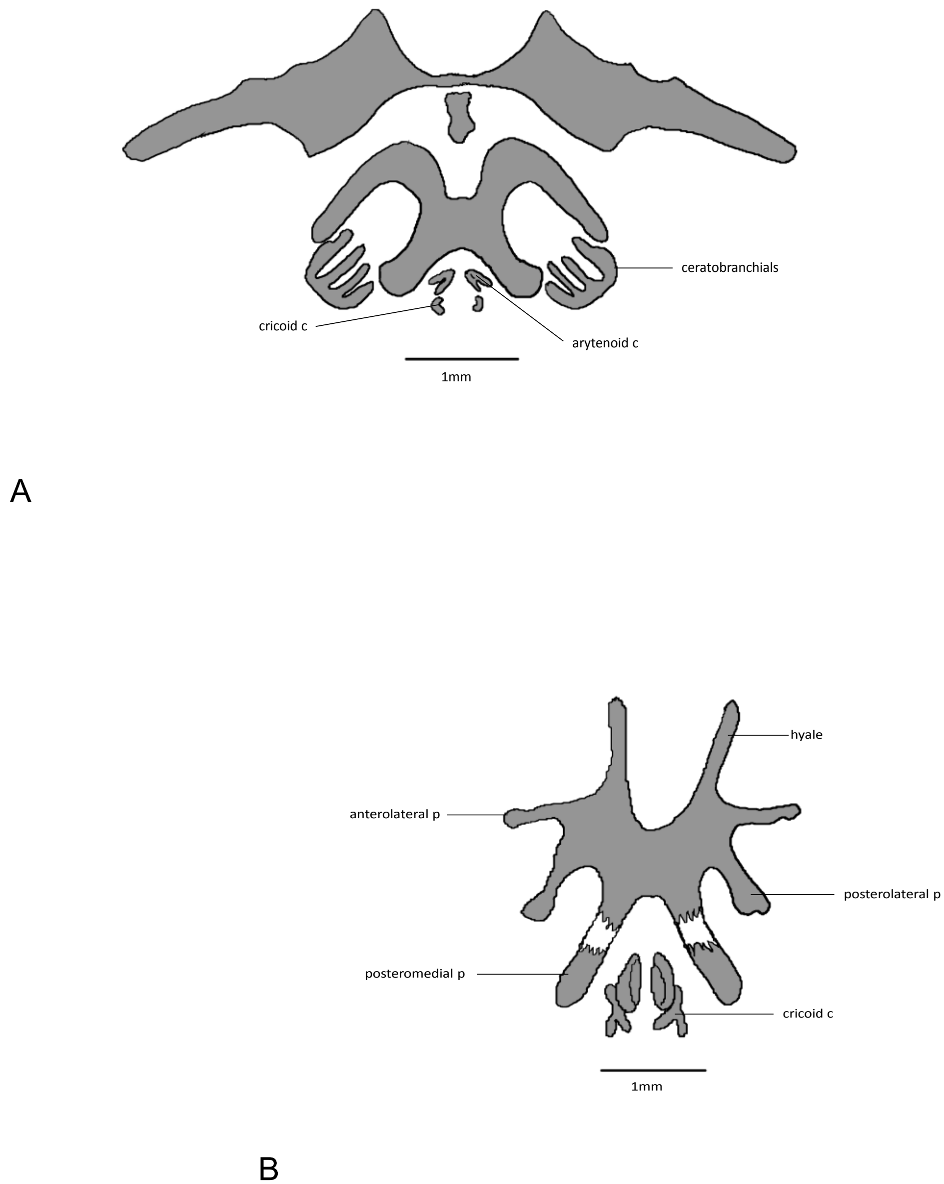

Fig. 2.13. Development of the hyobranchium of *Pseudacris crucifer*. Shown in ventral view. **A:** Stage 43. **B:** Stage 46. White denotes ossification; gray denotes cartilage. c, cartilage; f, foramen; p, process.

In addition, the ceratobranchials have detached from the hypobranchial plate and have dramatically eroded. By Stage 44 the rudimentary adult hyoid plate has begun to form from the remaining cartilage of the ceratohyals and hypobranchial plate that have fused together. By Stage 44 the posteromedial process of the hyoid plate has also formed along the posterior margin of the remaining hypobranchial plate. The arytenoid cartilage (AAO: 0000674) develops as triangular cartilaginous elements posterior to the developing hyoid plate. Lateral to the arytenoid cartilage the bronchial process (AAO: 0000676) appears as a thin bar of cartilage by Stage 45. By Stage 46 the ceratohyals have eroded completely and the adult hyoid plate has formed the adult shape but not size. By Stage 46 Basibranchial II has completely eroded and the cricoid cartilage (AAO: 0000675) has developed. The posteromedial process begins ossification by Stage 46 in the middle of the cartilaginous bars, and the hyal has lengthened and thinned. The hyobranchial skeleton will continue its development during the juvenile period.

2.4.5.5.3. Ossification of the Hyobranchium. The hyoid of *Pseudacris crucifer,* like that of many related anurans, remains mostly cartilaginous until after metamorphosis. Anterolateral (AAO: 0000671) and posterolateral (AAO: 0000672) processes of the hyoid plate (AAO: 0000664) appear during the juvenile stage, and in the adult the posteromedial processes (AAO: 0000673) of the hyoid plate are the only portions to ossify. However there is mineralization evident on the medial region of the hyoid plate.

2.4.5.6. Postcranial Skeleton Development. The postcranium of *Pseudacris crucifer* is well developed and complete.

2.4.5.6.1. Development and Ossification of the Axial Skeletal. The axial skeleton of the adult consists of eight presacral vertebrae, the sacrum (AAO: 0000552) and the urostyle (AAO:0000732).

Presacral vertebrae. Each of the presacral vertebra ossify from two epichordal centers (around the notochord) and from lateral centers that will form the neural arches. By Stage 31, the beginnings of the eight presacral vertebrae are apparent as cartilaginous rectangles dorsal to the notochord. The presacral vertebrae continue to chondrify and develop in an anterior to posterior direction. The anterior vertebrae will remain more advanced in development and ossification until adulthood.

The atlas (Vertebra I: AAO: 0000709) is the most anterior vertebra and it begins ossification along with Vertebrae II-IV by Stage 34. Ossification begins around the centrum of vertebra continues laterally on each sides. By Stage 35 the atlas has fused to Vertebra II (Fig. 2.14). Ossification spreads within each vertebra to each lateral side and continues posteriorly from vertebra to vertebra. The neural arches do not fuse to each other to ossify by this stage. By Stage 37, the neural arches and vertebral column have continued to ossify but do not fuse beyond the atlas to Vertebra II. By Stage 40 the neural arches have ossified to fuse with the vertebral column. In Stage 43 the notochord is still visible within the vertebral column and it will remain visible until adulthood. By Stage 46 the vertebral column has fully ossified and the neural arches have fused to the centrum in all eight presacral vertebrae.

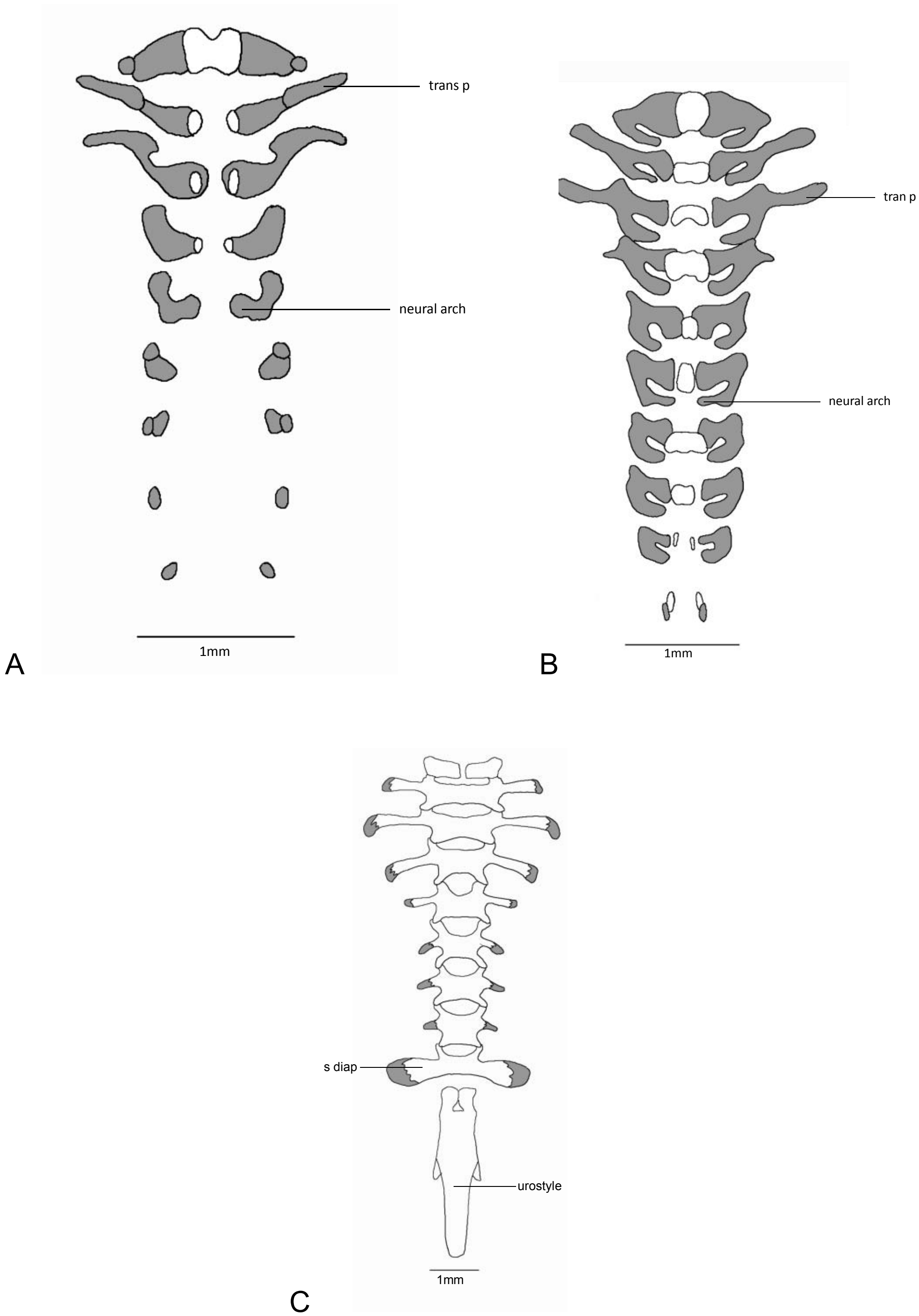

Fig. 2.14. Development of the axial skeleton of *Pseudacris crucifer*. In dorsal view. **A:** Stage 35. **B:** Stage 38. **C:** Stage 44. White denotes ossification; gray denotes cartilage. s diap, sacral diapophysis; trans p, transverse process.

Sacrum and urostyle. The sacrum is posterior to the presacral vertebrae and anterior to the urostyle. It is composed of fused Vertebrae IX and X and articulates with the ilium of the pelvic girdle. By Stage 35 the neural arch of Vertebra IX has chondrified and by Stage 36 Vertebra X will also appear. They independently begin ossification by Stage 39. By Stage 44 they have completely fused and ossified to form the adult sacrum. The hypochord is visible in Stage 38 and it will form the posterior portion of the adult urostyle. By Stage 39 ossification is evident on and around the middle of the hypochord. The ossification continues anteriorly and posteriorly and by Stage 44 the hypochord is completely ossified. By Stage 46 the urostyle has ossified and formed to resemble the adult element.

2.4.5.6.2. Development and Ossification of the Anterior Appendicular Skeleton. By Stage 34 the cartilaginous beginnings of the pectoral girdle (AAO: 0000422) appear as an undifferentiated mass of cartilage. The scapula (AAO: 0000751), the coracoids (AAO: 0000764) and the procoracoids (AAO: 0000760) have formed by Stage 35 (Fig. 2.15). The scapula is spade shaped and is the most medial element of the pectoral girdle. The procoracoids and coracoids appear as ovoid cartilages with the procoracoids lying anterior and the coracoids posterior. By Stage 36 the suprascapula (AAO: 0000748) has chondrified as a sheet of cartilage that articulates with the scapula. A small amount of ossification is visible along the suprascapula's ventral margin by Stage 37, and the suprascapula has an anteromedial extension on its distal end. By Stage 37 the cleithrum (AAO: 0000750) begins ossification and appears as a crescent shaped ossification along the anterior margin of the suprascapula.

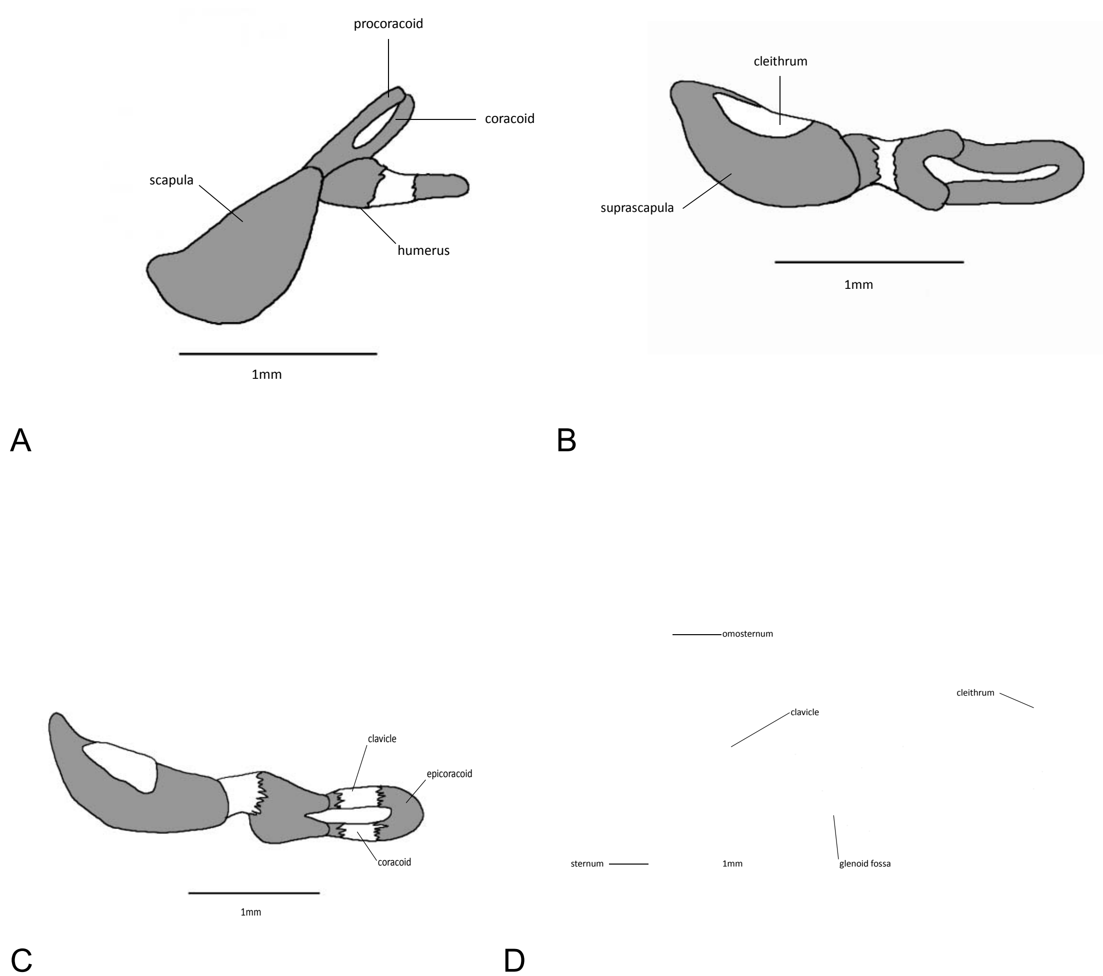

Fig. 2.15. Development of the pectoral girdle of *Pseudacris crucifer.* Shown in ventral view and the scapula and suprascapula averted ventrally into the plane. **A:** Right side, Stage 36. **B:** Right side, Stage 39. **C:** Stage 41. **D**: Left side, Stage 46. White denotes ossification; gray denotes cartilage.

By Stage 37 the scapula begins ossification proximally and will continue ossify distally until the entire scapula is ossified and resembles the adult scapula. The clavicle (AAO: 0000761) has also begun ossification by Stage 38 and appears as a sheet of mineralization dorsal to the procoracoid cartilage. The coracoids also have begun ossification by Stage 38. The coracoid begins ossification proximally and will continue to ossify laterally and medially. By Stage 41 the pectoral girdle has increased in size but ossification has increased very little proportionally. The pars acromialis (AAO: 0000752) and pars glenoidalis (AAO: 000753) appear by Stage 43 and articulate with the coracoid and clavicle. They will be completely ossified by Stage 45. By Stage 42 the sternum appears and begins as a lateral process of the epicoracoid cartilages.

The omosternum (AAO: 0000759) also appears by Stage 43 as a sheet of rectangular cartilage anterior to the epicoracoid. From Stage 44 the pectoral girdle increases in size but will remain in the same condition through adulthood.

Forelimb.--- The humerus (AAO: 0000679) and radioulna (AAO: 0000901) chondrify by Stage 32 and appear as three pieces of cartilage, with radius and ulna completely separated. By Stage 34 the phalanges and metacarpals have chondrified and appear as rectangular pieces of cartilage on the distal end of the forelimb. By Stage 36 the mid shaft of the humerus, the radius and the ulna have begun ossification (Fig. 2.16). The radius and ulna ossify independent of one another at their distal margins. By Stage 44 the radius and ulna will fuse along their medial margins but they can be distinguished from one another through adulthood by a lateral groove, the sulcus intermedius (AAO: 0010829).

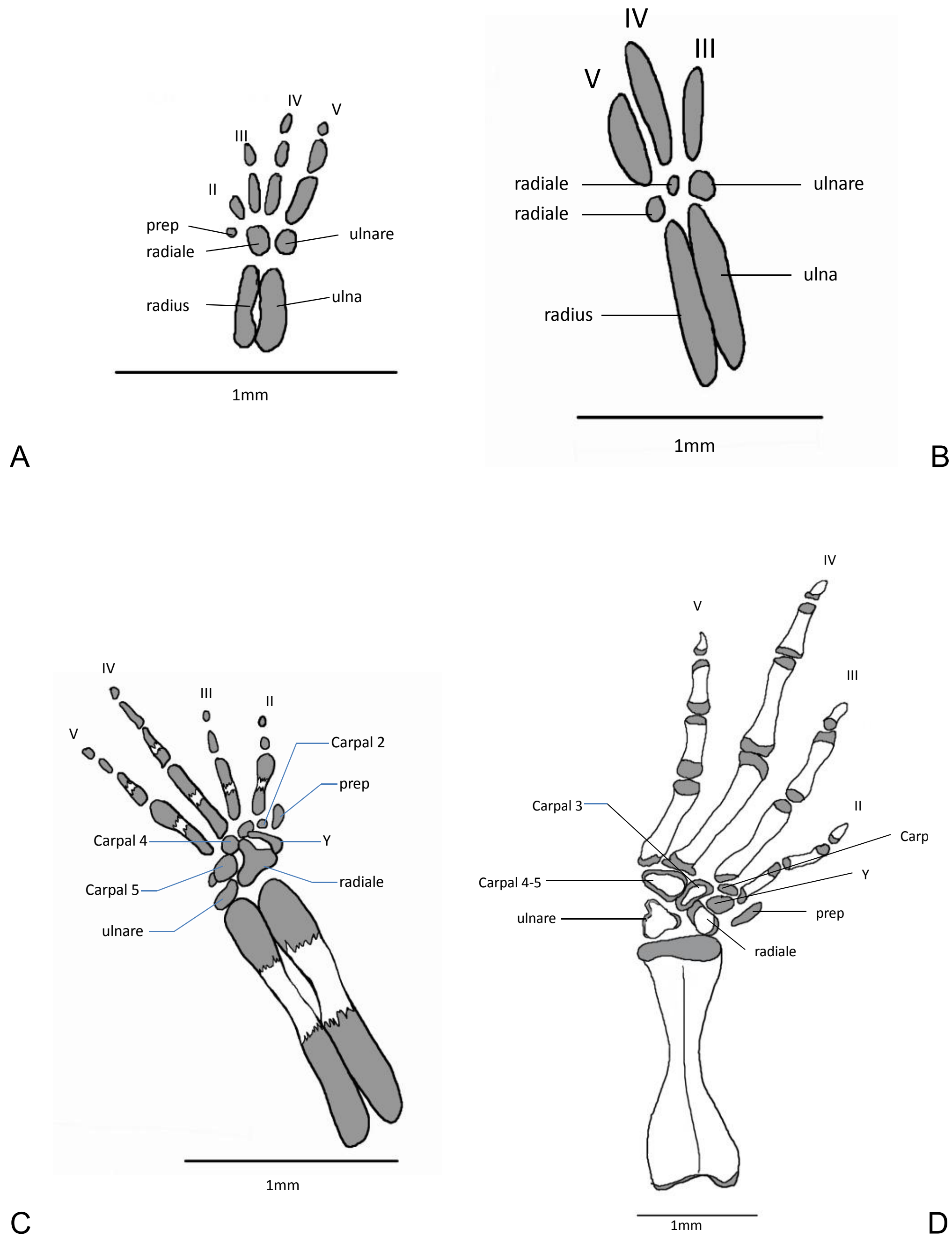

Fig. 2.16. Development of the hand of *Pseudacris crucifer*. Shown in ventral view. **A:** Stage 36. **B:** Stage 39. **C:** Stage 42. **D:** Stage 46. White denotes ossification; gray denotes cartilage. Digits are numbered II–V (Fabrezi and Alberch, 1996). Prep, Prepollex; Y, Element Y.

Metacarpals II-V and Phalanges IV-1, V-1 begin ossification in the middle of the cylinders of cartilage. By Stage 40 Phalanges II-1-2, III-1-2, IV-1-3, and V-1-3 have all begun proximal ossification, and by Stage 41 the metacarpals and phalanges are mostly bony. The carpals are the last bones of the manus to ossify. Carpals 2-5, the radiale (AAO: 0000790), ulnare (AAO: 0000845), and Element Y (AAO: 0000846) will ossify during the juvenile period. The prepollex (AAO: 0000852) and the distal prepollex (AAO: 000853) will remain cartilaginous through adulthood.

2.4.5.6.3. Development and Ossification of the Posterior Appendicular Skeleton. The bones of the pelvic girdle (AAO: 000426) are all endochondrally derived. The first cartilages of the pelvic girdle are apparent by Stage 34 with the beginnings of the ilia (AAO: 0000772) (Fig. 2.17). The ilia first appear as shafts of cartilage with a spade shaped dorsal extensions; the two come together medially at the dorsal end to form a U-shaped structure. The tibiofibula (AAO: 0000902) articulates with the ilia on the medial margins of the dorsal extensions. The ilia begin ossification by Stage 36 in the middle of each cartilaginous shaft, and will continue to ossify anteriorly and posteriorly until the posterior ossification joins the acetabulum.

The ischia (AAO: 0000860) and pubes (AAO: 0000861) appear posterior to the ilia by Stage 38. They first appear as a single structure made of cartilage but continue to develop. The ischia will ossify but the pubes will remain as mineralized cartilaginous through adulthood.

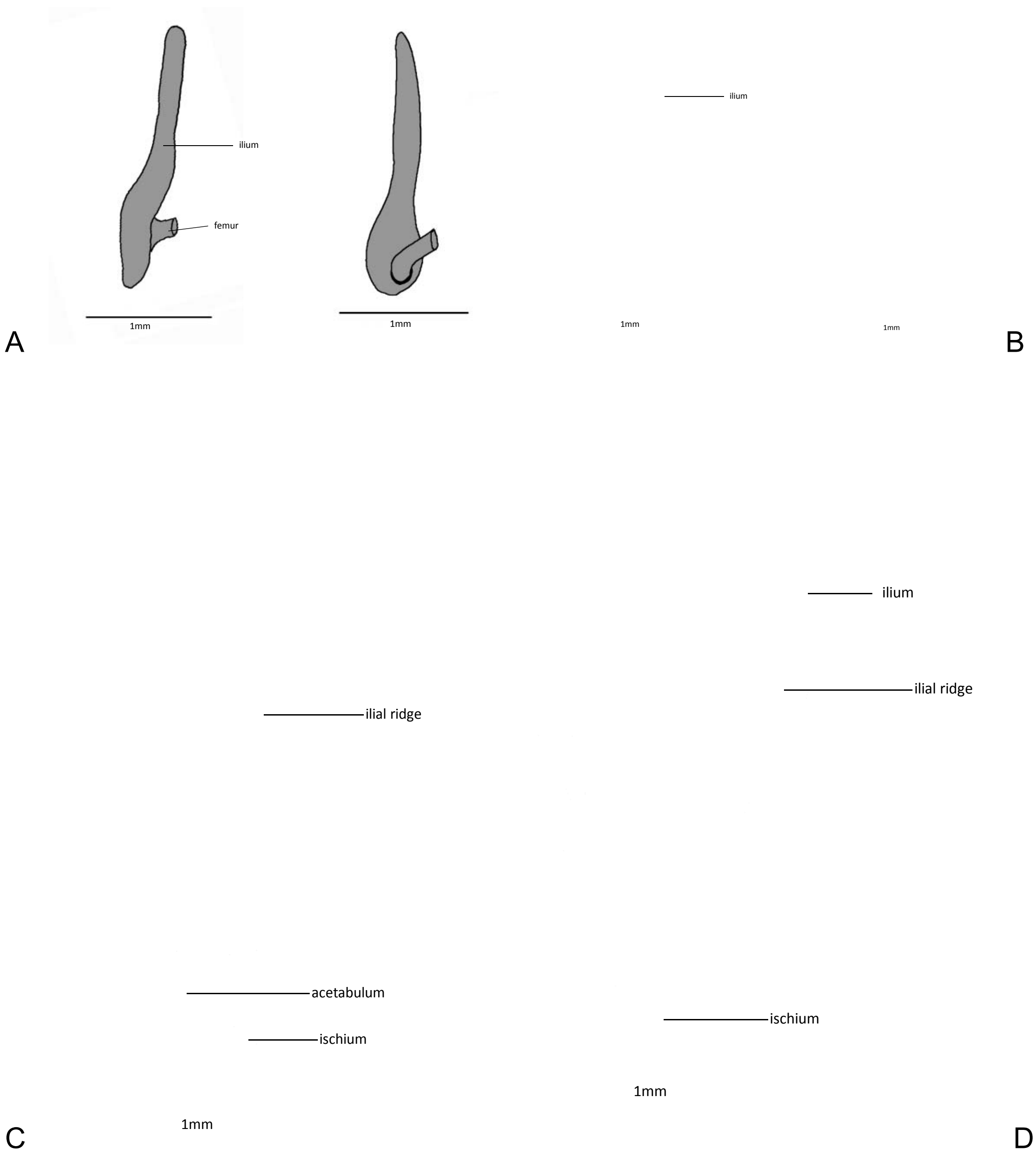

Fig. 2.17. Development of the pelvic girdle of *Pseudacris crucifer* in lateral (**left**) and ventral (**right**) views. **A:** Stage 34. **B:** Stage 38. **C:** Lateral view, Stage 44. **D**: Ventral view, Stage 46. White denotes ossification; gray denotes cartilage.

The ischia unites synstotically by Stage 40 and the interischiadic crest is visible. By Stage 41 there is a ridge along the ilia, ilial protuberance (AAO: 0000869), and the ischia have begun ossification along their posterior margins. By Stage 42 the ilia have nearly completed ossification but have not begun articulation with the sacrum. Through the rest of the larval stage the ischia will continue to ossify and the entire pelvic girdle will grow to resemble the adult morphology by Stage 46.

Hind limb.---The femur (AAO: 0000889) and tibiofibula (AAO: 0000902) form as rectangles of cartilage at approximately Stage 32. The femur is the first element of the hind limb to ossify by Stage 35 (Fig. 2.18).

Ossification of the femur begins proximally and continues in the distal and proximal directions. Ossification of the tibiofibula, the tibiale (AAO: 0000912) and the fibulare (AAO: 0000913) begins in a similar manner by Stage 37. The femur continues to ossify and the cartilaginous ends expand and widen. The femur also becomes more recurved. The tibiale and fibulare begin as separate elements and ossify independent of one another. By Stage 40 the medial heads of the tibiale and fibulare appear to have fused synchondrotically but the tibiale and fibulare continue to ossify along their respective shafts. The lateral heads of the tibiale and fibulare are completely ossified before they fuse to one another leaving the medial margins separate.

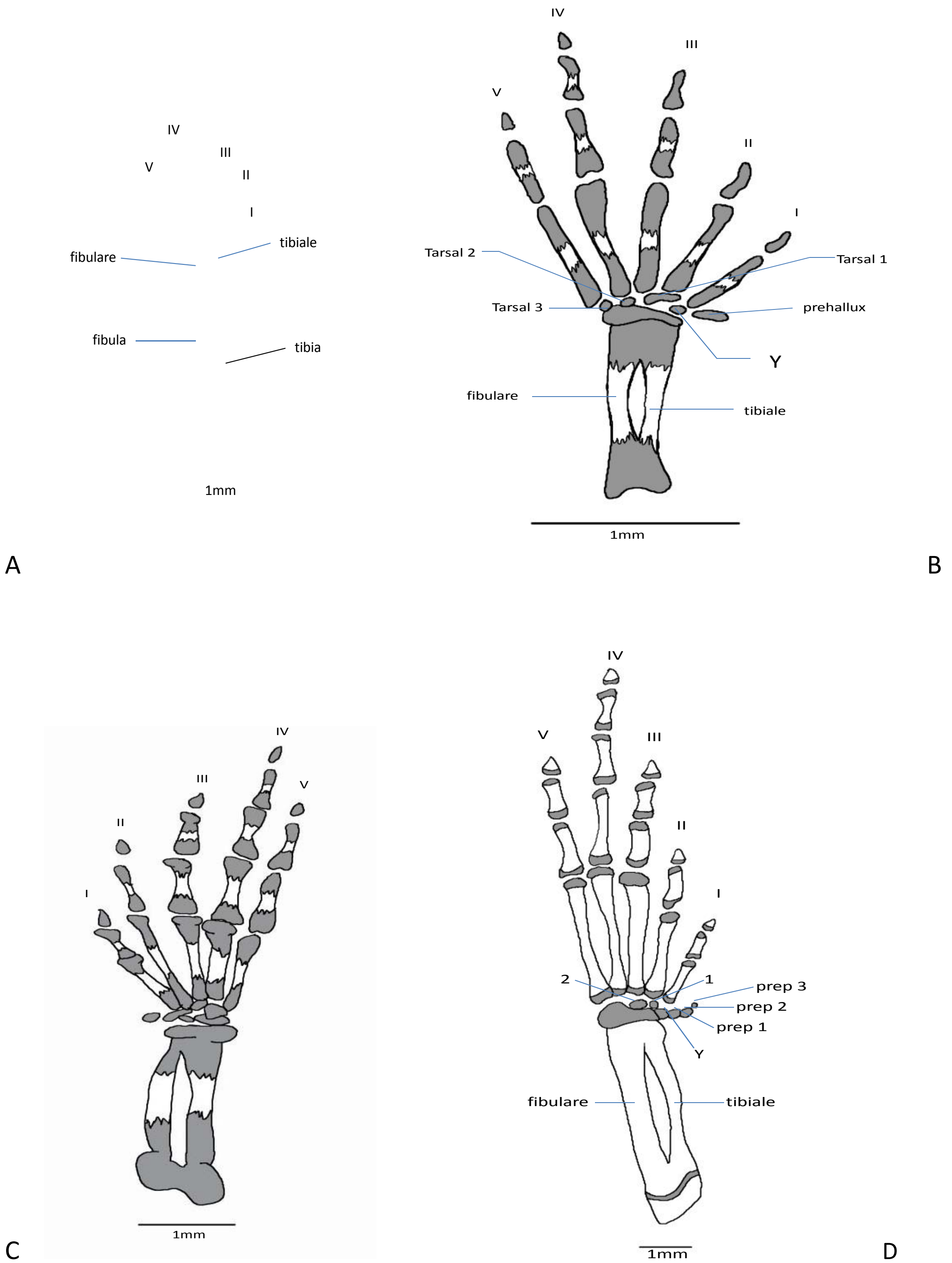

Fig. 2.18. Development of the foot of *Pseudacris crucifer*. Shown in ventral view. **A:** Stage 34. **B:** Stage 38. **C**: Stage 41. **D**: Stage 43. White denotes ossification; gray denotes cartilage. Digits are numbered I–V (Fabrezi and Alberch, 1996). Y, Element Y.

The metatarsals and phalanges appear by Stage 34 as rectangular pieces of cartilage on the distal end of the tibiofibula. By Stage 37 there is proximal ossification of Metatarsals II (AAO: 0000926), III (AAO: 0000927), IV (AAO: 0000928) and V (AAO: 0000929), Phalanges IV-1-2 and V-1-2. Metatarsal I (AAO: 0000925) and Phalanges III-1 and II-1 begin proximal ossification by Stage 38 (Fig. 2.18). By Stage 38 the prehallux is also evident as a small oval of cartilage. By Stage 39 all five metatarsals are developed and ossified at mid shaft with Tarsals 2 (AAO: 0000919), 3 (AAO: 0000920), and 4 (AAO: 0000932) articulating with Metarsals II-IV. By Stage 41 the phalanges are well ossified and bear proximal and distal caps of articular cartilage.

2.5. DISCUSSION

Pseudacris crucifer is a small hylid that is fairly common in the Eastern United States. Given its small size, it could be classified it as a miniature based on Yeh's (2002) miniature anuran parameters. Despite its small size, *P. crucifer* ossifies its elements extensively and does not display the same mineralization in cartilage that is seen in *Acris blanchardi* adults. When compared to *A. blanchardi*, which is considered a miniature species, a general observation is that *P. crucifer* begins ossification of most of the cranial elements much later than *A. blanchardi*. In addition, the elements appear to ossify in similar sequences but *Pseudacris crucifer* has more growth during its juvenile stage and ossifies the cranial elements to a greater extent than *A. blanchardi*.

Trueb and Alberch (1985) examined miniaturization in anurans and the loss of cranial elements. They observed that in some miniature species there was hyperossification, and in other miniature species, there was a loss of late ossifying elements in the cranium and digits. My personal observation, and previous studies, have shown that miniature species have great variability from individual to individual (Trueb and Alberch, 1985; Hanken and Wake, 1993). The number of adult and juvenile specimens in this study was limited due to specimen availability, but the specimens that were observed all displayed average ossification with all elements of the cranium and post cranium being well developed and functional. There were no signs of hyperossification in any individuals studied either.

Because of patterns seen in other miniatures, the elements of most interest for examination were the nasals, prootics, exoccipitals and dentaries. In *A. crepitans* the nasals are considered to be morphologically novel because the nasal bone does not ossify extensively and as compensation for needed support, the tectum nasi mineralizes and fuses to the dermal nasal bone. *P. crucifer* does not require the additional mineralization because it has a well ossified nasal bone (and skull in general). The other elements examined did not show patterns of novelty.

The timing of ossification and development was also of interest. The postcranial ossification *P. crucifer* is very similar in timing that of *A. crepitans* and *H. lanciformis*. In addition, when compared to *Pseudacris ocularis,* a closely related miniature species, the timing of ossification of cranial elements was also similar (Meinhardt, 2002). The only difference is that *P. ocularis* lacks a mentomeckelian, which is among the latest element to ossify. This is consistent with the predictions of Trueb and Alberch (1985) that miniatures will lose late developing elements.

Miniaturization evolves for numerous reasons. How miniaturization evolves is not always clear, but developmental timing and extent of development gives some insight into anuran miniaturization. Meinhardt (2002) suggested that miniaturization was determined early in embryogenesis, within the egg. He found that *P. ocularis* had a smaller egg than the other *Pseudacris* genera and a shorter metamorphosis. Although this is interesting, it does not explain why or how morphological novelty is seen in miniature species.

Although it has small body size, there is not enough evidence to indicate that *Pseudacris crucifer* is a miniature. It possesses a well-developed skull, no missing elements and no morphological novelty. The timing of ossification is similar to other

Pseudacris species, but the genus as a whole ossifies later than in other related Hylids. Timing is an important factor in paedomorphism, which is the most widely accepted theory for miniaturization, however, there is no evidence that delayed onset of ossification in *P. crucifer* has led to miniaturization.

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2.7. APPENDIX

A. Cleared and Stained *Pseudacris crucifer* specimens

Number	Stage	Number	Stage
SH000101	31	SH000015	40
SH000105	32	SH000012	41
SH000024	32	SH000132	41
SH000034	32	SH000138	41
SH000112	32	SH000106	42
SH000008	33	SH000136	42
SH000022	34	SH000109	43
SH000113	35	SH000133	43
SH000010	35	SH000102	44
SH000021	36	SH000107	44
SH000130	36	SH000141	44
SH000020	36	SH000100	45
SH000114	37	SH000103	45
SH000009	37	SH000110	45
SH000115	37	SH000125	45
SH000006	37	SH000143	45
SH000111	37	SH000011	46
SH000116	38	SH000142	46
SH000003	38	SH000120	Juvenile
SH000117	38	KU207429	Adult
SH000126	38	KU207431	Adult
SH000121	39	KU84668	Adult
		KU207434	Adult
		KU84669	Adult

VITA

Sarah Beth Havens was born in Phoenix, AZ on October 7, 1981. She graduated from Francis Howell Central High School in St. Charles, MO in 2000. In the fall of 2000, she started her post-secondary education at the University of Missouri-Columbia. She became active in the School of Natural Resources. After graduating from the University with a Bachelors of Science in Resource Forest Management in 2004, she began her career in forestry. In the fall of 2007 she began her graduate work at the Missouri University of Science and Technology under the mentorship of Dr. Anne Maglia. In 2010 she completed a Masters of Science in Applied and Environmental Biology. After graduation, she plans to find employment in ecology or bioinformatics and eventually go on for a PhD.