OSTEOLOGY OF CALLUELLA GUTTULATA (BLYTH 1855) AND ASSOCIATED COMMENTARY ON EVOLUTION IN THE FAMILY MICROHYLIDAE (ANURA)

BY

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ABSTRACT

Microhylid osteology is a morass of unusual structures and repetitive convergent evolution. The available phylogenetic information is limited, and the osteological information barely scratches the surface of the diversity present in the group, but even this much is enough to begin to identify certain patterns and areas of interest. Microhylinae, and to a lesser extent Gastrophryninae, in particular show high degrees of convergence both in pectoral and vomerine structure compared to Cophylinae and Asterophryinae. It remains to be seen whether other variable osteological structures, such as hyobranchial apparatuses or carpal arrangement, also show this level of convergence within these groups. Further taxonomic sampling is of utmost importance, both for osteology and phylogeny, as the few osteological descriptions available do not always correspond with the species used in existing phylogenies. Higher phylogenetic resolution could clarify many situations where the occurrence or number of occurrences of convergence is currently unclear.

Further sampling, too, will inevitably shed light on the functional significance of many microhylid traits. The hyobranchial apparatus, for example, is clearly a uniquely modified feature of the Microhylidae, but almost nothing is known about corresponding changes in the morphology of the attached muscles, and only a few examples of hyobranchial diversity have even been described. There are several possible avenues of study here where unique microhylid osteology might indicate a particularly unique functionality, all wide open to possible future research.

INTRODUCTION

Osteology and phylogenetics have long shared a close relationship. Historically, many phylogenies of anurans (Trueb & Cloutier, 1991; Ford and Cannatella, 1993), snakes (Lee & Scanlon, 2002), fish (Tyler, 1980), birds (Chu, 2005), and fossil vertebrates have been based largely on osteological characters. Osteological characters are widely considered less plastic and subject to environmental variation than external or soft-tissue morphology, while still divergent enough to be informative. In this era of

modern phylogenetics, in which skeletal morphology has come to take something of a back seat to molecular sequence data for developing phylogenies, osteology remains an important tool for understanding the evolutionary relationships between organisms. A molecular phylogeny may indicate that two genera are more closely related than ever before thought; this, however, is empty of information relating to the structural and morphological similarities between the frogs in question.

In a taxonomic group with a well-supported molecular phylogeny and a well-studied osteology, it is possible to combine the two and examine the history of the evolution of the osteological characters within the group. In the case of many anuran taxa, such as the family Microhylidae, there are phylogenetic hypotheses based on molecular data. Though the results of these studies are limited by taxon sampling and disagreement between different studies, they provide a framework in which the relationships among these anurans can be understood (Frost *et al.* 2006; Bocxlaer *et al.* 2006; van der Meijden *et al.* 2007). Parker (1934) was the first to document microhylid morphologies, covering a wide breadth of microhylid frogs in scant depth. Since that monograph, however, few complete osteological descriptions have been done, and nearly all of those (Carvalho 1954; de Sá and Trueb 1991; Lehr and Trueb 2007) focus on New World taxa within what is now recognized as the subfamily Gastrophryinae. Microhylidae, however, has a pantropical distribution, with approximately 430 currently recognized species, in 70 genera and nine subfamilies (Frost 2010). The external morphology of the group is confusing and highly similar, which has led to a morphology-based taxonomy overloaded with small and monotypic genera that molecular data are only just now beginning to resolve (Frost *et al.* 2006). Many microhylid frogs are small, fossorial, or leaf-litter dwellers. Both size and burrowing behavior have probably contributed to the evolution of extreme osteological variation within the group (Wells 2007; Yeh 2002). Thus, they are an ideal and fascinating group to begin to explore in more osteological depth.

Herein I describe the skeletal morphology of *Calluella guttulata* (Blyth 1855), a member of the subfamily Microhylinae. Adult *C. guttulata* have an snout-vent length of 40–50 mm. It is both the type species and one of the better-known exemplars of its genus (Parker 1934; Bourret 1942; Taylor 1954). *Calluella guttulata* occurs from the northern

end of Peninsular Malaysia north to southern Myanmar and west through Laos and Thailand into Vietnam (AmphibiaWeb 2009). A terrestrial and burrowing frog, *C. guttulata* is usually found in moist forest and lowland habitats, and it is common enough within its range to be listed as Least Concern (van Dijk 2009). Little is known about the osteology of the species; Parker, in his 1934 monograph, published a single illustration of the vomers and adjacent anterior cranial elements, in ventral view, but the skeleton has not been discussed since. Parker also described and illustrated the vomers of *Calluella brooksii* (*Colpoglossus brooksi*), and the sternal elements of the pectoral girdles of *C. brooksii* and *Calluella volzi*. Otherwise, the osteology of *Calluella* is entirely unknown.

Microhylidae is so little-studied that a full description of the osteology of *Calluella guttulata* alone constitutes a significant increase in knowledge of the group. Additionally, much of the pre-existing research has yet to be analyzed in a phylogenetic context. By combining this new description with a discussion of preexisting microhylid data in light of modern molecular phylogeny, this paper will shed light on the evolution of these morphological characters and the history of the group.

SYSTEMATICS OF THE MICROHYLIDAE

Relationships between microhylid subfamilies are still subject to debate. Microhylinae, Dyscophinae, and Asterophryinae clearly form a clade, but Frost *et al.* placed Scaphiophryne within this group as sister to Microhylinae, whereas Bocxlaer *et al.* and Van der Meijden *et al.* both produced trees that placed Dyscophinae as sister to Microhylinae, and Scaphiophryninae elsewhere in the group (Frost *et al.* 2006; Bocxlaer *et al.* 2006; Van der Meijden *et al.* 2007)(Fig. 1). Gastrophryninae and Cophylinae have been resolved both as sister groups and as descendents of successive branches off the microhylid lineage. The other five subfamilies, like Dyscophinae and Scaphiophryninae, have far fewer species; none contains more than 15 named species and only Hoplophryninae and Scaphiophryninae contain two genera. Like Scaphiophryninae, their phylogenetic placement has varied between trees, and poor taxonomic sampling in the smaller subfamilies means that any definitive conclusions would be premature.

With 69 species in 9 genera, Microhylinae is the second-most speciose subfamily of microhylid, being surpassed only by Asterophryinae (222 species) (Frost 2009). Its species range from Southeast Asia west to India. Microhylinae is well supported as a clade, but relationships within Microhylinae itself are only partially resolved (Fig. 2). There seems to be a consistently well-supported clade that includes *Microhyla*, *Calluella*, and *Glyphoglossus*, which is closely related to *Kaloula* or to a clade including *Kaloula*, *Ramanella*, *Uperodon*, and *Metaphrynella* (van der Meijden *et al.* 2006; Bocxlaer *et al.* 2006). Frost *et al.*, on the other hand, suggested that *Micryletta inornata* and at least one species of *Ramanella* are only distantly related to the rest of the Microhylinae.

MATERIALS AND METHODS

The description herein is based on the osteology of two adult male *Calluella guttulata* collected in Thailand and deposited in the collections of the Division of Herpetology of the Biodiversity Institute of the University of Kansas: DSM 1350 and DSM 1457. Frogs were cleared and double-stained for bone and cartilage according to Klymkowsky and Hanken (1991). All skeletons were examined and drawn using a stereomicroscope and a camera lucida.

Terminology is derived from Duellman and Trueb (1994) with some elaborations. Characters and terminology for the hyobranchial apparatus are taken from Trewavas (1933). Terminology for the manus and pes are taken from Fabrezi and Alberch (1996). Digits were numbered II–V based on homology (Alberch and Gale, 1985)

RESULTS

CRANIUM (FIG. 3)

Endocranium

Braincase: The posterior part of the braincase is enclosed by a pair of small, well-ossified exoccipitals. These are narrowly separated from the posterior edges of the frontoparietals and distinct from the prootics. The dorsal anterior margin of each bone is approximately a semicircle; thus the exoccipitals are widely separated anteriorly and only very narrowly separated at the dorsal edge of the foramen magnum, with cartilage forming the edge of the foramen magnum between them. Ventrally, their margins also curve medially, abutting the parasphenoid but not overlapped by it. The ventral edge of the foramen magnum is not completely encased in bone, but formed by heavily mineralized cartilage for approximately the medial third of its width, between the exoccipitals. The lateral and ventrolateral braincase anterior to the otic capsule is formed by the fully ossified portion of the prootics. The epiotic eminence is formed of mineralized cartilage, as is most of the remainder of the prootic.

Ossification of the paired sphenethmoids is limited to the lateral and ventrolateral regions of the anterior braincase. The bones do not meet dorsally; instead, ossification of the ethmoid cartilage extends across the roof of the braincase rostrad from the anterior edge of the frontoparietal fenestra to about the midlength of the nasal bones. The dorsal margin of each sphenethmoid lies ventrally adjacent to the lateral margins of the frontoparietal, which the sphenethmoid underlies in its posterior half. The orbitonasal foramina, margin complete in bone, opens beneath a small ridge in the far anterodorsal corner of the sphenethmoid. Ventrally, the medial edges of the sphenethmoids are hidden by the cultriform process of the parasphenoid such that it is impossible to tell if the sphenethmoids meet or not. The posterior margin of the sphenethmoid is located at about the midlength of the orbits, whereas the anterior margin curves laterally around the anterior end of the braincase just posterior to the dentigerous processes of the vomer. The anterior edge of the sphenethmoid continues nearly to the frontoparietals, separated from the nasal bone by a wide bar of the planum antorbitale. The planum antorbitale is lightly calcified in the area between the nasal bone and the anterior end of the pterygoid, but is mainly cartilaginous.

Otic Capsule: The otic capsules, apart from some few patches of organized bone, are almost entirely composed of mineralized cartilage. In keeping with the generally

widened aspect of the skull, the otic region is much wider (i.e., more elaborated laterally) than it is long (i.e., anterior-posterior dimension). The fully ossified exoccipitals form the posterior and ventralmost sides of the otic capsules. The roof of the otic capsule, exclusive of the epiotic eminence, is made of fully ossified prootic. The anterior and posterior epiotic eminence, the crista parotica, and the lateral, ventral and anterior walls of the otic capsule are cartilaginous with some mineralization. The bony rod of the pars media plectri extends anterolaterally at a wide angle—60° from the midline of the skull. The pars interna plectri is asymmetrical, expanding mostly dorsally to wrap around the anterior curve of the operculum. The operculum is oval and moderately domed, mostly cartilaginous with some ossification, particularly in the center. The pars externa plectri is entirely cartilaginous, elongate and ovoid in shape, and slightly flattened in the posterolateral/anteromedial aspect. It is about half as long and one quarter as wide as the tympanic annulus. The tympanic annulus itself is an incomplete oval with a deep notch in the posteromedial end, just below the pars media plectri, sides upturned to cup around the pars externa plectri.

Nasal Capsule: The paired olfactory capsules are large and for the most part without obvious mineralization. The oblique cartilage forms the anteromedial wall of the nasal capsule and extends dorsally as a flat bar diagonally across the capsule to form its posterolateral corner. Here the oblique cartilage descends to rest upon the lamina inferior, which forms a large, leaf-shaped plate underlying the lateral half of the nasal capsule. The crista subnasalis extends as a broad triangle from the lamina inferior, to buttress the wall at the anteromedial corner of the nasal capsule where oblique cartilage and tectum nasi meet. The tectum nasi extends posteriorly from here along the dorsomedial edge of the nasal capsule. The capsules are adjacent but separated by the septum nasi, which terminates anteriorly in a distinctly pointed medial prenasal process.

The two rods of inferior and superior prenasal cartilage extend together from the tip of the alary process of the premaxila, with no separation between them, to buttress the alary cartilage. The alary cartilage forms a relatively small cup, almost flat in a vertical plane, which curves posterolaterally from the prenasal cartilage around the front of the nasal capsules.

The small septomaxillae are complex in shape and situated posteroventral to the alary cartilages and just medial to the cristae subnasalis. The planum antorbitale lateral to the capsules is lightly mineralized. The solum nasi is highly calcified.

Exocranium

Dermal Investing Bones: The frontoparietals are narrowly separated medially, extending anteriorly from about the middle of the otic capsules three quarters of the way along the orbit. Each frontoparietal flares laterally over the anterior epiotic eminence, and covers most of the cartilaginous tectum synoticum anterior to the exoccipitals. The posterior margins of the bones are narrowly separated from the exoccipitals, whereas the anterior margins are almost perpendicular to the median axis of the skull, and distinctly separated from the posterior margins of the nasal bones.

The broad, paired nasals bones are narrowly separated medially. Dorsomedially the bones are irregularly rectangular and moderate in size. The posterior margin of each nasal is well separated from the frontoparietal, and the bone only covers the posterior half of the olfactory organ beneath. The anterolateral margin of the bone is concave, and extends to the planum terminale, whereas its concave posterolateral margin forms the anterior margin of the orbit in the region of the planum antorbitale. The lateral edge of the nasal bone is irregular, but basically horizontal, at about the level of the bottom of the braincase; the nasal is clearly separated from the pars facialis of the maxilla.

The T-shaped parasphenoid is distinguished by a long, relatively slender cultriform process and robust posterolateral alae. The margins of the cultriform process are not parallel. The process widens in the anterior part of the optic fenestra, and ventral to the sphenethmoid it is narrowed by shallowly concave margins. The terminus of the cultriform process is truncate and lies just posterior to the vomers. The alae are broad (in an anterior-posterior direction) and also quite wide, underlying the widest parts of the otic capsules posterior to the level of the pterygoids.

The vomers are large and complex. It is unclear whether the postchoanal portion of the vomers have fused with the neopalatines or replaced them entirely. There is only one element in the adult frog that underlies the planum antorbitale and the olfactory organ anterior to the sphenethmoid. This element may represent a neopalatine that has fused to

the vomer; alternately, the neopalatine may be absent and the vomer hypertrophied to provide a functional replacement in the area of the planum antorbitale. The body of the vomer lies just medial to the choana, and nearly half the margin of the choana is encircled by the pre- and postchoanal processes, which are approximately equal in length. The short and robust anterior processes extend anteromedially toward the snout, but the solum nasi ventrally covers the tips. The posterior process extends flatly, as part of the floor of the nasal capsule, toward the tip of the parasphenoid.

The large dentigerous process is connected to the body of the vomer by a raised arm, which grows out of the body of the vomer at one end and projects from the medial end of the dentigerous process at the other. This arm extends in parallel to the posterior process of the vomer, but is ventrally separated from it, leaving a gap between the arm and the posterior process large enough for a narrow pin. The dentigerous processes either overlay or completely replace what would otherwise appear to be a pair of robust neopalatines. These processes are only narrowly separated medially, and extend laterally to form the entire ventral anterior margin of the orbit, nearly or all the way to the maxilla and the tip of the pterygoid. A raised tooth row bearing 14 teeth on each side, which may be the only true vomerine bone in this vomerine/neopalatine structure, cannot be visibly distinguished from the bone underneath it.

Suspensory Apparatus: The robust pterygoids are triradiate. The long, laterally arcuate anterior ramus invests the ventral, medial and dorsal surfaces of the cartilaginous pterygoid process. It extends anteriorly to meet the posterior corner of the pars palatina of the maxilla, and terminates at the level of the planum antorbitale. The robust medial and posterior rami are about equal in length. The medial ramus extends posteromedially, and curves dorsally in a half-cylinder around the anteroventral margin of the otic capsule. The posterior ramus is a thin, flat blade extending posterolaterally and descending to the level of joint articulation, where it invests the medial surface of the palatoquadrate. The ventral ramus of the Y-shaped squamosal is the largest; it descends ventrolaterally, and is angled about 10º to the posterior. The cylindrical ventral ramus swells near distal the end to wrap around the large ball of palatoquadrate dorsomedially, whereas the dorsally-projecting bar of palatoquadrate cartilage is medially invested by

the squamosal all the way to the junction of its rami. The flat zygomatic ramus extends anteromedially. The otic ramus extends posteromedially and forms a curved shell that invests the crista parotica.

Maxillary Arcade: Anteriorly, the quadratojugal is well articulated with the posterior ramus of the maxilla, such that at least a third of the quadratojugal invests the maxilla medially. Both bones are robust along this junction, and do not greatly at their articulation; thus the height of the maxillary arcade remains almost constant. Posterior to its articulation with the maxilla, the quadratojugal descends sharply, at an angle about 35° below horizontal, to terminate at a level markedly ventral to the horizontal axis of the maxilla. The quadratojugal invests the lateral surface of the palatoquadrate as it descends. A small dorsal flange of bone laterally overlaps the ventral ramus of the squamosal. The end of the bone is irregular, posteriorly convex and concave anteroventrally, distinct from the palatoquadrate beneath it.

The pars dentalis of the maxilla bears teeth from a low ridge extending past the vomer/neopalatines, an eighth to a quarter of the way along the orbit. The pars palatina is narrow throughout most of its length, and expands at the anterior end of the maxilla, near the articulation with the premaxilla. The pars palatina expands anterior to the vomer, and a wide flange of bone extends fom the maxilla nearly to the anterior process of the vomer and the lateral process of the pars palatina of the premaxilla, underlying and supporting the anterolateral corner of the nasal capsule. The pars facialis of the maxilla is broadly triangular, with its apex broadly separated from the anteroventral corner of the nasal bone. It is low and entirely unelaborated, without a hint of a preorbital process, and widely separated from the margins of the orbit.

The premaxillae bear robust, nearly rectangular alary processes slightly narrower at the tip than the base, inclined medially and curving posteriorly along the line of the snout. The pars dentalis is a low ridge, articulating laterally with the pars dentalis of the maxilla. The pars palatina is a large, flat shelf, posterior half divided into distinct lateral and medial processes. The lateral process curves posterolaterally, past the tip of the pars palatina of the maxilla toward the anteriormost extension of the vomer. The medial processes of the two premaxillae, slightly shorter than the lateral processes, abut each

other for most of their length; anteriorly the pars palatina of one premaxilla overlaps the other, but the posterior tips meet without overlapping.

MANDIBLE (FIG. 3, 4)

The mandible bears no teeth or other odontoid processes. The angulosplenial is robust and well-ossified, with a short, robust coronoid process raised along the posterior third of its length. The concave, semicartilaginous articular surface posterior to this is at an angle to cup the anteroventral surface of the palatoquadrate, such that, when articulated, the posterior corner of the coronoid process rests on a level with and anterior to the quadratojugal. Anteriorly, the angulosplenial articulates with the dentary along nearly half of its length. The anterior end of the dentary curves forwards and down, articulating with the posterior end of the mentomeckelian and briefly investing the lateral surface of Meckel's cartilage. A short, mineralized bar of Meckel's cartilage extends posteriorly from this mentomeckelian/dentary articulation, lying ventral to the rest of the mandible and extending nearly to meet the anteriormost end of the angulosplenial. The mentomeckelian bones come into contact along the midline, but are not fused.

HYOBRANCHIAL APPARATUS (FIG. 4)

The hyoid corpus is flat and broad, 1.5–2.0x wider, at its narrowest point between the anterolateral and posterolateral processes, than at its medial length. The hyoglossal sinus is wide and broadly V-shaped. Each hyale bends medially just anterior to its projection from the main corpus of the hyoid, reaching about two thirds of the way across the hyoglossal sinus from its greatest width, before sharply bending back on itself and curving laterally. The anterolateral processes are broadly expanded; each at its greatest width is about twice as broad as its total projection from the corpus of the hyoid plate, and 1.75–2.0x as broad as at the narrowest point of the stalk. The posterolateral processes are narrow and simple.

There are two entirely cartilaginous medial spurs that project from the ventral side of the hyoid plate. The most anterior of these is located directly at the middle of the

plate, is raised only slightly from the corpus, and extends posteriorly. The large posterior spur is oriented anteroventrally, and located at the posterior margin of the plate between the bony posteromedial processes. The anterior heads of these bony processes are broad and expanded medially. There is a narrow, raised ridge on each process along the anterior margin of the laryngeal sinus; they expand medially to bracket the medial cartilaginous projection between them. Posterior to this, the slender, rodlike processes are directed slightly dorsally, and bony for their full length, save a small cartilaginous tip. Each posteromedial process bears two bony flanges. A long, thin, flat flange extends along the lateral margin of about half the length of the bone; a thicker, rounded jut of bone projects from the medial side.

The large laryngeal cartilages are three times longer than the hyoid plate at its medial length, with a cricoid ring a third again as wide as the narrowest point of the hyoid plate and nearly as wide as the greatest flare of the anterolateral processes. The cricoid ring is complete and robust, with prominent cardiac processes and a short, wide esophageal process. Instead of a separate muscular and articular process on the dorsal side of the cricoid ring, a single long ridge extends from the esophygeal process to the midpoint of the ring. Slender, rodlike bronchial projections extend ventrally from the main ring, just anterior to the medial projections of the bony posteromedial processes that bracket the laryngeal cartilages. The arytenoid cartilages are elongate, semicircular, and nearly flat. The anterodorsal edges are straight and nearly touching along their full length, whereas the posterior opening is relatively narrow. Each arytenoid is pierced laterally by large, circular fenestra about halfway down its length.

POSTCRANIUM

Axial Column (Fig. 5)

Presacral Vertebrae: *Calluella guttulata* has eight nonimbricate presacral vertebrae. Presacrals I–VII are procoelous, whereas Presacral VIII seems to be amphicoelous. The neural arches are well separated from one another and about twice as wide as they are long. Presacrals IV–VIII bear only very low ridges, but I– III have distinct, cartilage-

tipped neural spines; the neural spines of Presacrals II and III project posteriorly but do not overlap the posteriorly adjacent vertebrae. The vertebral profile, in descending order of width, is: III > IV > II = S > V > VI = VII = VIII. The transverse processes of Presacrals II– IV are similar in being long and robust; the transverse processes of Presacrals II and IV flare very slightly at the tips. Presacrals V–VIII possess nearly identical, slender transverse processes that taper somewhat from their base. The transverse processes on Presacrals VI and VII are perpendicular to the axis of the spinal column; those of Presacrals II and VIII extend somewhat anteriorly, and those for Presacrals III–V are oriented posteriorly.

Sacrum: The sacral diapophyses are only slightly dilated, with the distal margins being about one and two-thirds the width of the base, and directed slightly backwards. The body of the sacrum bears a low dorsal ridge, more prominent than those on the neural crests of Presacrals V–VII. Each diapophysis also bears a large, U-shaped depression, its closed end lying nearer the body of the sacrum, about halfway between the midline of the sacrum and the lateral margin of the diapophysis. This depression occupies almost the entire width of the diapophysis. The sacrum has a bicondylar attachment with the urostyle,

Urostyle: This element is approximately 80–85% as long as the presacral potion of the spinal column. The slender urostyle is over all simple, with no vestigial transverse processes or any indication of postsacral vertebrae. It bears only a single low neural ridge, extending about a third the full length of the urostyle along its anterior portion, which lacks any additional knob or elaboration.

Pectoral Girdle (Fig. 6)

Zonal Elements: The clavicles in *Calluella guttulata* are somewhat reduced, very thin dermal bones that project medially from the glenoid cartilage in a parallel orientation to the coracoid bones. The clavicles taper to points that nearly meet at the midline. The bones do not invest procoracoidal cartilage and are attached to the coracoids medially by

only a minute projection of epicoracoidal cartilage. Each coracoid is symmetrically expanded to nearly three times its narrowest width at the sternal end, and asymmetrically expanded to the anterior to approximately twice its narrowest width at its glenoid end. The midline between the robust coracoids is narrow and entirely cartilaginous. The cartilaginous sternum, which bifurcates into two lobes approximately halfway down its length, is heavily mineralized from it anterior margin to slightly posterior to its the bifurcation.

Scapula: This endochondral bone is approximately cylindrical and as long as the coracoid. The glenoid end of the scapula is divided into distinct partes glenoidalis and acromialis, the latter of which does not articulate with the end of the coracoid or the clavicle, but is connected to them by a highly mineralized band of cartilage. Dorsolaterally, the scapula flattens and widens symmetrically to an edge approximately twice the width of its narrowest point.

Suprascapula: The bony cleithrum is scythe-shaped; it extends along the entire leading edge of the suprascapula, and continues along its anterior margin before curving to a point approximately one quarter of the way from the far end of the blade. Ossification of the suprascapular cartilage is centered at its posterior and ventral margin, just beyond the posterior edge of the cleithrum, but to a lesser degree invests the entire suprascapula.

Pelvic Girdle (Fig. 7)

Ilium: Viewed dorsally, the ilial shafts configure a relatively long and narrow U-shaped space that is one and two-thirds times as long as wide at the ilial tips; the ilia themselves are approximately twice as long as that widest gap between them. They are unfused, with a narrow gap between the ilial bases not even united by cartilage. There are no crests on the ilial shafts, but dorsal and just anterior to the acetabulum, each ilium has a distinct ridge with a small, dorsal prominence

Pubis and Ischium: The pubis is moderately calcified. It forms nearly a third of the circumference of the acetabulum, from the base of the ilium at the anteroventral, to nearly

the dorsolateral midpoint of the acetabulum at the posterior margin. The ischium is small, representing a little more than the posterodorsal quarter of the acetabulum, and completely ossified laterally. Medially the ischia do not meet, but are joined by a thick ridge of well-ossified cartilage. The acetabulum is oval, and higher than it is long, with a preacetabular angle of 90º.

Manus (Fig. 8)

Each manus has four digits, with a phalangeal formula of 2–2–3–3, and relative lengths, in decreasing order, IV > V > III > II. The terminal elements are cone-shaped, with a single expanded lobe at the tip. The prepollux has two segments; the distal one is mainly cartilage encased in a hollow cylinder of bone. Carpal elements include an ulnare, a larger radiale, and Element Y, as well as a large fused bone that seems to represent Carpals 3–5, and a smaller Carpal 2.

Pes (Fig.9)

The phylangeal formula for the pes is 2–2–3–4–3, and the toe length in decreasing order goes IV > III > V > II > I. The terminal elements, as in the manus, are tapered and expanded into a single lobe in the tip. The prehallux consists of two bones. Tarsal elements appear to include a large, flattened Element Y at the base of the prehallux, a much smaller Tarsal 1, and a thin but elongate bone representing the fusion of Tarsals 2 and 3.

DISCUSSION

The overwhelming dearth of information on microhylid osteology indicates how little is known about the conservation or variability of bony structures in this group; thus it is difficult to determine which osteological features of *Calluella guttulata* might be unique to the species or generally characteristic of the family. For this reason, it is important to establish a baseline for morphology within the Microhylidae to facilitate

future comparisons. There are a few full osteological descriptions of other microhylids available, all within the subfamily Gastrophryinae—one of *Hamptophryne boliviana* (de Sá and Trueb, 1991), and two species each in the genera *Nelsonophryne* and *Melanophryne* (Lehr and Trueb, 2002). Using these and *Calluella guttulata* to establish a small range of microhylid diversity, some striking commonalities emerge that distinguish these frogs osteologically from more "typical" ranoids such *Rana esculenta* as described in Gaupp's classic monograph (1986).

CRANIAL MORPHOLOGY AND FEEDING APPARATUS

Microhylid skulls, with their anteriorly displaced jaw joints, are unmistakable. They are all much broader than long, in contrast to the skulls of Rana, which are only about 90–95% as wide at its broadest point as it is long. This overall change in proportion is directly related to the point of articulation of the jaw in these microhylids, which is shifted anteriorly by a significant margin. All the microhylids compared here have distinctively small mouths, with palatoquadrate and jaw joint anterior to the midline of the auditory bulla. The jaw joint sits farthest posterior in *Hamptophryne boliviana,* and in the other taxa almost as far forward as the posterior margin of the orbit. In conjunction with this shift, several structural elements including the palatoquadrate, the maxillary arcade, and the bones of the suspensorium, are rotated and reshaped compared to typical frogs. The pterygoid in particular differs from that in *Rana esculenta* and other typical ranoids. It seems that in microhylids, the medial ramus of pterygoid meets the anterior and not the lateral edge of the otic capsule, and the ventral ramus sits far more laterally than is observed in other frogs, to accommodate the anterolateral position of the jaw joint.

The microhylid feeding apparatus is a subject ripe for research. Microhylids project their tongues via hydrostatic elongation, unlike most other frogs; hydrostatic elongation has only been observed outside of microhylids in *Rhinophrynus dorsalis* and a few ranoids (Nishikawa 2000; Trueb and Gans 1983). In this method of feeding, the tongue is thought to work as a muscular tube that expands when lymph is pumped into a central sinus, with a network of collagen fibers around the circumference of the tongue ensuring that it only grows longer and not wider (Nishikawa *et al.* 1999). Hydrostatic projection

tends to be both much slower and much more accurate a method for projecting the tongue in anurans than inertial elongation. Interestingly, those non-microhylids which feed by hydrostatic elongation are almost all specialized burrowers feeding on termites and ants. (Nishikawa 2000).

The forward position of the microhylid jaw relative to the jaw in other frogs necessarily changes the mechanics of opening and closing the mouth, although all of the major sites for muscle attachment on the mandible remain, both on the skull and the mandible. The most obvious functional constraint imposed here is gape, which must necessarily be restricted as the overall length of the jaw is reduced. In terms of muscular function, the mandible is a simple lever, usually raised and lowered by muscles exerting mostly vertical force. With the mandibular joint shifted forward relative to the cranium and the sites at which the jaw muscles usually attach, the angle of this force changes, pulling up and back instead of simply up. Basic mechanics would suggest that this actually reduces the speed and force with which the mouth can open and close, although any extensive comparative examination of the kinematics of microhylid feeding has yet to be performed. It has been seen in *Hemisus marmoratus* that hydrostatic elongators do not move their jaws as far or as quickly during feeding as frogs that project their tongues via either inertial elongation or mechanical pulling (Nishikawa 2000). This suggests that hydrostatic elongators, including microhylids, can afford the theoretical loss of mechanical advantage accompanying a shorter, slower jaw with a more anterior jaw joint, due to the different degree of movement necessary for successful tongue protraction and feeding. It is unclear how much slower and less forceful a microhylid jaw actually is, in comparison to the jaw of a typical inertial elongater with a much more posterior jaw joint such as *Rana esculenta*. It is also unknown whether this shorter jaw confers some other mechanical advantage upon microhylids, which might compensate for any loss of speed and force.

Beyond overall shape, other characteristics of microhylid skulls, such as degree of ossification, are far more variable. The prootics of *Hamptophryne boliviana*, like those of *Calluella guttulata*, are largely mineralized cartilage; the prootics of *Nelsonophryne* and *Melanophryne* are fused with the exoccipitals, entirely bony in *Nelsonophryne*, distally giving way to mineralized cartilage in *Melanophryne*. On the whole, none of the

microhylid skulls have highly ossified braincases, with the frogs of *Nelsonophryne* being the boniest, but the dermal bones are all rather large to compensate. The frontoparietals of all species are well developed and roof the whole braincase, with narrow median separation, but the posterior end widens much more drastically in *C. guttulata* posterior to the orbit. The parasphenoid is highly variable even between species within *Nelsonophryne* and *Melanophryne*. The alae are smallest where they underlie the fully ossified auditory capsules in *Nelsonophryne*, and spread the widest in *Calluella guttulata*, not because the auditory capsules are significantly more cartilaginous than in *H. boliviana*, but because the capsules themselves extend the farthest laterally. Likewise, all of the nasal capsules are quite well covered by the nasal bones, although the nasal capsules are large and not roofed completely.

As a dermal bone, the nasals are diverse among anurans, varying from vast plates fused to each other and the frontoparietal, to small strips of bone that barely cover the nasal capsule or articulate with the maxillary arcade. Each of the microhylids compared here has relatively large, plate-like nasal bones that do not entirely cover the entirety of a pair of large nasal capsules. More interesting is the nasal capsule itself. Jurgens, in his 1971 study, examined the nasal capsules of microhylids *Hypopachus cuneus*, *Elachistocleis ovalis*, *Gastrophryne carolinensis* (then *Microhyla*), and *Rhombophryne testudo*, and exemplars of the genus *Phrynomantis* (then *Phrynomerus*), and brevicipitids from the genera *Breviceps*, *Probreviceps*, and *Spelaeophryne*, as well as other ranoids from *Anhydrophryne*, *Arthroleptis*, and *Rana*. *Rana esculenta* demonstrates the usual anuran state of having nasal capsules that sit just anterior to the forebrain, whereas the nasal capsules of many of these microhylids actually project backwards beneath the forebrain, their posterior ends either ventral or ventrolateral to the braincase. This is most prominent in *Hypopachus cuneus* and *Elachistocleis ovalis*, whereas members of the genus *Phrynomantis* seem to have retained or regained the plesiomorphic condition, having capsules that do not underlie the brain. The brevicipitids and some few of the other ranoids also have nasal capsules that extend along the brain case, but these tend to be ventrolateral or lateral to the brain. This suggests a mechanism designed to fit large nasal capsules such as those possessed by *C. guttulata* in the space of a shorter, broader snout created by widening the angle of the maxillary arcade.

The hyobranchial apparatus serves as the attachment site for muscles responsible for tongue protraction and retraction, and also the muscles that control the laryngeal cartilage. Microhylid hyoids, like the one in *Calluella guttulata*, are unusual among anurans; the laryngeal structure of *C. guttulata*, on the other hand, is unique even within the Microhylidae. Based on Trewavas' (1933) examination of hyobranchia across Anura, the midventral bump of hyoid cartilage and the spike seen between the origin of the posteromedial processes are uniquely microhylid characteristics, and ubiquitous within the group. The long flanges on the posteromedial processes are also seen in some form, as flanges or bulges, in nearly all examined microhylids; in *Rana esculenta* or other typical ranoids such as *Breviceps* or *Hemisus*, the posteromedial processes tend to be cylindrical and rod-like, sometimes flaring at the tips but generally without significant bulging in the middle of the bone (Trewavas 1933). There is no evidence that other microhylids possess the enormous laryngeal cartilages found in *C. guttulata*.

One might assume that the extensive modifications to the microhylid hyoid are in some way correlated with tongue protraction and feeding mode, because hydrostatic elongation is so unusual among anurans.

Yet many of the modified parts of the hyoid attach specifically to muscles that are not known to have any function in feeding at all (Trewavas 1933). The middle of the hyoid plate, which in addition to the thickening of cartilage seen in *Calluella guttulata* is also mineralized in some microhylid taxa, serves as the origin for the m. constrictor laryngis anterior, which closes the arytenoid cartilage of the larynx. The mm. sternohyoideus and petrohyoideus, which retract and depress the hyoid, attach to either side of the corpus, usually lateral to the central area that is raised and thickened in *C. guttulata* (Trewavas 1933). Contrary to expectation, there seems to be no muscle attaching to the spike between the heads of the posteromedial processes. The anterior edge of the posteromedial processes, and the long flange it often bears in microhylids, acts as an attachment site for the m. geniohyoideus, which serves to protract the hyoid, along with another slip of the m. sternohyoideus. The constrictor laryngis externis attaches to the medial edge of the posteromedial processes, where some microhylids have raised flanges; in *C. guttulata* these are especially large, which likely is correlated with the huge size of the larynx.

Thus, the two hyper-developed portions of the hyoid serve specifically as attachment sites for muscles that constrict the larynx, and at least one area which serves as an attachment site for muscles that move the hyoid plate. It is still uncertain exactly what role movement of the hyoid plate serves in feeding in these anurans; Emerson theorized that movement of the hyoid was a necessary element of tongue protraction and retraction in inertial elongators, but was later disproven, whereas no study of hyoid movement in hydrostatic elongators has been undertaken (Emerson 1977; Nishikawa 2000). The expanded flanges for the attachment of the mm. geniohyoideus and sternohyoideus in microhylids suggest that such an investigation might well be worthwhile. The expanded sites of attachment for the m. constrictor laryngis, on the other hand, suggest a unique functionality for the larynx even in the case of those microhylids in which it is not as greatly enlarged as in *Calluella guttulata*. It is possible that the muscle attachments, and the size of the laryngeal cartilages in *C. guttulata*, are all ultimately related to vocalization; however, the hyperdevelopment is not sexually dimorphic. Future investigation, not the least of which will involve examining variation in these unique characteristics across Microhylidae as a whole, is clearly warranted.

POSTCRANIAL MORPHOLOGY

The postcranial skeleton of *Calluella guttulata* resembles the other microhylid taxa under consideration. The vertebral column is largely similar in all four genera. With the exception of *Nelsonophryne aterrima*, all have seven procoelous vertebrae with Presacral VIII amphicoelous; all have slightly to moderately expanded sacral diapophyses, and the vertebral profile is similar or the same for all of them. Among these four genera, one notaeable difference is that the spinal columns of the gastrophrynine species exhibit some imbrication (only Presacrals V and VI show true imbrication in *Nelsonophryne aequatorialis*), whereas the vertebrae of *C. guttulata* are entirely non-imbricate. Gaupp described the vertebral column of *Rana esculenta* with non-imbricate vertebrae and relatively prominent neural arches. The sacrum is small and distally unexpanded. *Rana esculenta*, and indeed most other ranids and ranoids, are generally diplasiocoelous, although this has been shown to vary within the same genus and even between individuals of the same species (Holman 1963). The relative width and length

of vertebrae in *Calluella guttulata*, and the vertebral profile, are typical, but the sacrum and urostyle, and their relationship with the pelvic girdle, require additional study.

The vertebral column and pelvic girdle tend to vary together as a functional unit. The pelvic girdles for these microhylids are generally similar. *Calluella guttulata* has a pre-acetabular angle of nearly 90º and a nearly straight ilial shaft, whereas most of the other microhylids examined here have somewhat more acute preacetabular angles and both members of *Melanophryne* and *Nelsonophryne* show a distinct downward curve to the ilium. *Rana esculenta* demonstrates the same slightly acute preacetabular angle and curved ilial shaft, but none of the microhylids share its prominent ilial crest, which indicates a different set of muscle attachments.

Much of the examination that has been done of variation in anuran vertebral columns and pelvic girdles involves the ilio-sacral articulation. Emerson (1979) identified three different modes of attachment. *Calluella guttulata* belongs to Type IIA, based on the distinct indentations of ligament scars on its sacrum. It is known that microhylids are variable in attachment type, (either Type I or Type IIA), whereas *Rana esculenta*—indeed, all ranoids outside of the Microhylidae—have a Type IIB articulation. Type I and Type IIA articulations are both loosely correlated with walking and burrowing behaviors, although with a high degree of functional variation. They do not lend themselves to long-distance jumping, typical of Type IIB articulations. What is not known is how many or which microhylids fall into which type, or how many times in the evolution of the group the state has changed. For example, there was insufficient information available to categorize the gastrophryines in this study, as designations are mainly based on ligament attachments.

The phalanges of *Calluella guttulata* are simple and nearly identical to the phalanges of other microhylids considered here. Like *Nelsonophryne* and *Melanophryne*, the phalangeal formula is typical, although *Hamptophryne boliviana* is missing one segment in Digit IV of the hand for a phalangeal formula of 2-2–3–2. More interestingly, the terminal elements of *Melanophryne*, *Nelsonophryne*, and *H. boliviana* are distinctly bilobed, whereas the distally expanded discs on the terminal elements of the manus and pes of *C. guttulata* have only one lobe. Parker (1929) described the group as having phalanges with T- or Y-shaped dilations only.

Andersen (1978) considered all microhylids to have Carpal Type 4, corresponding to a wrist with only three elements: a separate radiale and ulnare, and the fusion of Element Y and distal carpals 2-5. However, the only microhylid Andersen examined was *Gastrophryne olivacea*, and he did not illustrate the manus of the specimens examined. Results of this study indicate that *Calluella* matches Andersen's Carpal Type 2, which is characterized by the presences of five carpal elements: a radiale, an ulnare, element Y, distal carpal element 2, and the fusion of distal carpals 3-5. This pattern is also seen in *Hamptophryne boliviana* and both species of *Melanophryne*. It is clear that some microhylids do have Carpal Type 4, but it is not a universal trait. Furthermore, both members of the genus *Nelsonphryne* have been seen to contain six carpal elements instead of five, with a distal carpal element 3 that is not fused to distal carpals 4 and 5. This corresponds precisely to Andersen's Carpal Type 1, which he considered a synapomorphy for the Myobatrachidae.

Andersen (1978) suggested that Carpal Type 2, the state found in most other ranoids as well as Bufonidae, Hylidae, and Leptodactylidae, directly led to Carpal Types 3 and 4, and either evolved from Type 1 or gave rise to it. He presumed the common ancestor of microhylids and ranids to have either Carpal Type 1 or Type 2. Based on the microhylids observed here, these two possibilities indicate an independent evolution of either a Type 2 or Type 1 wrist, respectively, as well as the unique evolution of Carpal Type 4. Either way, it is clear that number and structure of wrist elements is distinctly variable in the Microhylidae, contrary to Anderson's concept of a conserved carpal structure within the family.

In contrast, the tarsal arrangements of Microhylidae show almost no variation in number or type of elements. Results of this study correspond to those of Fabrezi (1993) who found the structure of the tarsals to be consistent within *Dermatonotus, Elachistocleis, Gastrophryne,* and *Phrynomantis*. The number of prehallical elements is variable; *Calluella guttulata* and most other examined microhylids have two, unlike *Melanophryne* (three) and *Hamptophryne boliviana* (two). The conservation of the rest of the tarsal structure is hardly limited to Microhylidae, as Fabrezi found a similar structure in *Breviceps* and almost all other ranoid frogs. It seems that regardless of lifestyle, the structure of the ankle is highly conserved, with most adaptation left to the

wrist as discussed above.

RE-ANALYSIS OF CHARACTER STATES FROM PARKER (1934)

Vomers and neopalatines

Parker (1934) studied vomers and neopalatines across the Microhylidae extensively. Vomers and neopalatines vary across Anura, but most families show somewhat more conservation of form than Microhylidae. Parker reported a tremendous amount of variation, ranging from huge, complex vomers like those found in *C. guttulata,* to minute vomers limited just to the anteromedial margin of the choana such as those in *Nelsonophryne* and *Melanophryne*. Likewise the neopalatines can be present, absent, or obscured by by postchoanal vomerine processes. Rather than grouping frogs based on the structure of their vomers, Parker considered these various different forms to be an evolutionary series, with more reduced forms derived from complex, plate-like ancestral vomers containing both a pre- and postchoanal portion united in one bone. Thus he included in the same subfamily genera as diverse as *Kaloula*, *Glyphoglossus,* and *Uperodon*, with their large vomerine structures, and *Gastrophryne*, *Elachistocleis,* and *Microhyla,* which usually lack the posterior portion of their vomers. If Parker's large, plate-like vomer truly was ancestral to the rest of Microhylidae, then it seems to have evolved uniquely within the family. The typical ranid vomer consists of a robust, variably complex, frequently dentigerous prechoanal portion, with a complete neopalatine and no postchoanal vomerine process (Ramaswami 1939). Other ranoids are similar. Almost all African ranoids, for example, possess neopalatines, and though in some taxa the vomers are limited to the anterior margin of the choana whereas others extend posteriorly to meet the neopalatine, they are never divided (Clarke 1981).

By combining modern molecular phylogenies with Parker's observations, we can begin to examine the character state changes within Microhylidae. Postchoanal vomerine structures are nearly unknown within the Gastrophryninae, except for a small vestige described in *Hamptophryne boliviana* (de Sá and Trueb 1991)(Fig. 10). This may in theory result from the reduction of a smaller ancestral vomer similar to those found in other ranoids, but Parker (1934) suggested that it may represent the prechoanal portion of

a much larger, ancestral vomer that divided some time before Gastrophryninae arose and lost the postchoanal portion relatively soon thereafter. Cophylinae is distinguished by a divided vomer with a toothy postchoanal portion that often overlies the ever-present neopalatine (Fig. 11). This toothy postchoanal portion has been reduced once in *Stumpffia*, which has lost all vomerine teeth, and is lost entirely in *Anodonthyla*, resulting in a state very like the gastrophrynine palatal region (Parker 1934). The relationship between Gastrophryninae and Cophylinae remains unclear (Frost *et al.* 2006, Boxclaer *et al.* 2006, van der Meijden *et al*. 2007), but if, as Frost *et al.* suggested, the subfamilies are more closely related to each other than either is to the Asterophryinae/Dyscophinae/Microhylinae clade, then the general state of vomers in cophyline frogs combined with the remnants of a postchoanal vomer in *Hamptophryne*, supports the hypothesis that the ancestral state is a large, divided vomer.

The neopalatine is of additional interest. Parker (1934) correlated the loss of the neopalatine with the loss of a postchoanal process, and indeed both have been lost in most of the gastrophryninae, but separately, with the postchoanal process being lost first. *Nelsonophryne* and *Melanophryne* both retain robust neopalatine bones, indicating that the loss of the postchoanal process does not necessarily equate to the loss of the neopalatine (Fig. 10). It is unclear how many times the neopalatine has been lost within Gastrophryninae. According to Frost *et al*. (2006), *Nelsonphryne* is closely related to *Ctenophryne*, in which the state of the neopalatines is unknown. That clade is sister to a clade containing the other gastrophrynines in the analysis, all of which lack neopalatines; this may represent a single loss of the neopalatine in that clade alone. The relationship of *Meanophryne* to the rest of the Gastrophryninae, or the state of the neopalatines in *Ctenophryne*, may suggest further occurrences of loss within the group.

Members of the Asterophryinae/Dyscophinae/Microhylinae clade bear vomers that are typically—though not universally—robust (Fig. 12). This is particularly evident within asterophrynes. Asterophrynine vomers are huge, frequently extending all the way to the premaxilla, and cover the entire neopalatine region to such an extent that it is impossible to determine whether a neopalatine remains without a developmental series or histological staining. *Dyscophus* has large, unified vomers that overgrow the neopalatine and typify Parker's (1934) concept of an ancestral state.

Nowhere within Microhylidae is the amount of variation in vomerine structure more obvious than in the Subfamily Microhylinae. If we presume an ancestral state in Microhylinae of having robust, unified vomers similar to Dyscophinae and Asterophryinae, then the vomers have divided and become reduced at least twice within the *Kaloula/Ramanella/Uperodon* clade (Fig. 13). In both *Ramanella* and *Metaphrynella*, the posterior portion of a divided vomer is either reduced to slivers or entirely lost, yet *Uperodon*, considered sister to *Ramanella* (Bocxlaer *et al* 2006), contains both species with undivided vomers with postchoanal processes that extend across the neopalatine region, and divided vomers with robust postchoanal portions (Parker 1934). *Kaloula pulchra* has been recovered as sister to all other taxa within this clade (Bocxlaer *et al* 2006, van der Meijden *et al* 2007), and it has a robust, undivided vomer growing across the neopalatine region, similar to that of *Dyscophus* and of certain species of *Uperodon*. If *Uperodon* and *Ramanella* are truly monophyletic, then the postchoanal process may have been split off and been reduced or lost independently within *Metaphrynella*, *Ramanella*, and some species of *Uperodon*. Alternatively, and contrary to the assumptions made by Parker, a divided, reduced vomer may have actually redeveloped in some species of *Uperodon*.

The neopalatine, too, seems to have a particularly odd evolutionary history within this group. In *Kaloula pulchra* and *Uperodon*, the neopalatine is either absent or so reduced as to be completely hidden beneath the robust postchoanal processes. The neopalatine is absent in *Metaphrynella*, but remains, reduced to slivers of bone, in *Ramanella*. If no neopalatine is present in *Kaloula*, then the reduced neopalatine may have been lost repeatedly during the evolutionary history of this clade, a question potentially answerable via developmental series or histological section of *Kaloula pulchra* or *Uperodon*.

Additionally, although the taxa used to construct these molecular phylogenies belong to the same genera as the species illustrated by Parker (1934), they are not in all cases the same species. For example, Parker illustrated *Ramanella triangularis* as an exemplar of the genus, and explicitly indicated that it was similar to *Ramanella variegata* and *Ramanella obscura*, the taxa used in the molecular phylogenies here (Frost *et al* 2006, Bocxlaer *et al* 2006, van der Meijden *et al* 2007); however Frost *et al*. (2006)

found *Ramanella* to be polyphyletic. According to Parker (1934), *Uperodon* shows variation in vomerine structure within the genus, but only *Uperodon systoma* has been included in a molecular study (Bocxlaer *et al* 2006). It is possible that *Uperodon* should be found paraphyletic with respect to *Ramanella*, for instance, which would indicate only one incidence of vomerine division and loss. Only more thorough taxonomic sampling, both in building molecular phylogenies and in examining the vomerine structures of frogs that Parker did not address, can properly resolve these questions.

The patterns of vomerine and neopalatine evolution seen in the *Microhyla*/*Calluella/Glyphoglossus* clade of microhylines are even more complex (Fig. 14). Like *Calluella guttulata*, *Glyphoglossus molossus* possesses large, undivided vomers that lack true teeth but bear large knobs and ridges (Parker 1934). It is impossible to tell whether the postchoanal portion of the vomer in *C. guttulata* has completely replaced the neopalatines along the entire margin of the orbit, or simply fused so completely with them as to leave no seam, but in *G. molossus* at least it seems very clear that the neopalatine has disappeared completely. *Calluella guttulata* and *Glyphoglossus molossus* almost certainly comprise a clade closely related to the genus *Microhyla* (Frost *et al.* 2006, Bocxlaer *et al.* 2006, van der Meijden *et al.* 2007), yet the postchoanal process was lost in every exemplar of *Microhyla* that Parker examined (1934). *C. guttulata* and *G. molossus* bear vomers much larger than even those in *Kaloula*, easily the biggest and most complex of any microhylines, whereas vomers seen in *Microhyla* are some of the most reduced. *Micryletta inornata* may or may not be closely related to this clade, as it has been recovered both as sister to the rest of this group (van der Meijden *et al.* 2007), and as an entirely unrelated microhylid (Frost *et al.* 2006), but any light it might shed here is obscured by the bizarre morphology of its palatine region. Parker (1934) stated that *M. inornata* has lost both the postchoanal portion of the vomer and the neopalatine, and illustrated the area with the ventral sphenethmoid extending along the neopalatine region and anteriorly beneath the nasal capsules. If the neopalatine is truly lost in *M. inornata*, it is evidence for another incidence of convergence within the subfamily, because *Microhyla* contains frogs with everything from very robust neopalatines to none at all. Between the *Calluella*/*Glyphoglossus* clade, the genus *Microhyla*, and *Micryletta inornata*, this is at least two or three independent

losses of the neopalatine, combined with at least one and possibly more losses in the *Kaloula/Ramanella/Uperodon* clade. Without a well-supported and adequately sampled phylogeny of species within Microhylinae, it is difficult to fully understand the evolutionary history of the neopalatines in this group.

Pectoral girdle

All ranoids, including all members of the Microhylidae, have firmisternal pectoral girdles, but the presence and robustness of clavicles and procoracoid and epicoracoid cartilage varies significantly within the group. These elements have been considered informatively variable for microhylid taxonomy since Parker (1934). Degrees of ossification, fusion, and reduction are relatively variable among anurans, but particularly so in Microhylidae; Parker determined that clavicles and procoracoids have each been reduced several times in obviously different ways. *Rana esculenta* possesses a full compliment of the bones found in firmisternal pectoral girdles, including thick styles of bone projecting anteriorly and posteriorly as part of the omosternum and sternum, which are absent from almost all microhylids. The overall trend is of reduction, but no further pattern within Microhylidae is immediately obvious. Parker firmly believed that presence and reduction of ventral pectoral girdle elements was systematically relevant within Microhylidae itself, but as several of Parker's groups have been overturned in the past century, this information bears further examination.

The pectoral girdle of *Calluella guttulata* (Fig. 6) shows only limited amounts of reduction from this basic plan. Though it lacks an omosternum entirely, as well as most procoracoid cartilage, it clearly retains clavicles that, if not as robust as some, still extend nearly the entire length of the coracoid. The development of the zonal portion of the pectoral girdle in *C. guttulata* is surprising, given the reduced nature of these elements among other microhylines. The clavicles of *Calluella volzi* have been illustrated as two-thirds as long those of *C. guttulata*, whereas *Calluella brooksii* bears no more than vestigial knobs of bone and cartilage at the glenoid end of its coracoids, in those individuals that retain any remnants at all (Parker 1934)(Fig. 15). Most other microhylines are characterized by a total lack of clavicle or any procoracoid cartilage, a characteristic so ubiquitous that it seems a strong synapomorphy for the group.

Only *Kaloula pulchra* has been shown to retain any hint of procoracoid, and that remains as small vestiges connected to the omosternum, whereas those members of *Calluella* appear to have lost clavicle and procoracoid from the medial end first and retain it at the distal end longest. Given this, and *Calluella*'s phylogenetic position nested between genera that clearly all lack clavicles, it would seem that microhylines have actually reduced the ventral elements of their pectoral girdle repeatedly, probably from both ends.

Reduction and loss of clavicles has occurred at least once within each of the larger subfamilies of Microhylidae, and at least once within the smaller subfamilies. Among the smaller subfamilies, Dyscophinae, Kalophryninae, Melanobatrachinae, and Otophryninae are characterized by robust clavicles and complete procoracoid cartilage, with no known reduction among them (Parker 1934). Another unique loss has occurred within the Hoplophryninae, as *Parhoplophryne usambaricus* possesses well-developed clavicles and procoracoid cartilage, and between the two members of Hoplophryne only *Hoplophryne uluguruensis* retains the vestiges of procoracoid cartilage, along the interomedial corner of the scapula. Nothing is known about the Scaphiophryninae or Phrynomerinae (Parker 1934).

Several asterophryne frogs also retain the complete pectoral girdle, which along with the state of the Dyscophinae supports the hypothesis that robust clavicles and complete procoracoid cartilage are ancestral within Microhylinae and all reduction and loss took place within the subfamily itself. Asterophryines have been shown to have lost clavicles and procoracoids multiple times, probably at least three times (Köhler and Günther 2008). Unlike Microhylinae, Asterophryinae also contains several intermediate species with elements reduced but still distinctly present (Fig. 16). Interestingly, within microhylines procoracoid cartilage seems to have been lost along with or, in the case of *Calluella guttulata*, before the clavicles; asterophrynes, however, have clearly reduced and lost their clavicles before their procoracoid cartilage; several species retain a full bar of procoracoid cartilage from glenoid to omosternum despite having vestigial clavicles or none at all (Parker 1934). A similar pattern is seen within Cophylinae, where all known species retain at least vestiges of procoracoid cartilage, despite many genera having reduced or no clavicles (Fig. 17). In this subfamily, the group of species with reduced or

missing clavicles may or may not be monophyletic. Members of *Cophyla*, *Rhombophryne*, and *Plethodonthyla* all lack clavicles, but have not all been included in phylogenetic analysis, and thus it is unclear whether the lack of a clavicle is evidence for monophyly or homoplasy. *Plethodonthyla* also contains species with full clavicles, but the genus has been shown to be polyphyletic (Fig. 1)(Parker, 1934, van der Meijden *et al.* 2007).

Gastrophrynine pectoral girdles seem to fit into three categories—those with complete clavicles and procoracoid cartilage, as is found in *Dermatonotus*, *Hypopachus*, and *Stereocyclops*; those entirely lacking cartilage and clavicles, as in *Ctenophryne*, *Dasypops*, *Gastrophryne*, and *Nelsonophryne*; and those with clavicles reduced at the distal end, extending only from the midline of the sternum along half the coracoid or less, and attached to the middle of that bone with procoracoid cartilage (Fig. 18). The last condition is found in *Chiasmocleis, Elachistocleis, Relictivomer, Hamptophryne,* and *Melanophryne*, with varying degrees of loss of procoracoid along the length of whatever clavicle is left (Parker 1934; de Sa and Trueb 1994; Trueb and Lehr 2006). Despite the fact that the intermediate state is so morphologically consistent across several species in the group, which might suggest an evolutionary series ending in a single loss event, the phylogenetic relationships within Gastrophryninae indicate a much more complex history. The interrelationships of the subfamily are still not fully known, but the group of frogs lacking clavicles, or even the group of frogs with reduced and absent clavicles, is clearly not monophyletic. *Hypopachus*, with complete clavicles and procoracoids, is thought to be sister to *Gastrophryne*, which has none at all, and either *Chiasmocleis* or the clade of *Nelsonophryne* + *Ctenophryne* are thought to be sister to the rest of the subfamily, although *Chiasmocleis* has the intermediate state and *Nelsonophryne* and *Ctenophryne* have both lost clavicles and procoracoid cartilage entirely (Parker 1934; Lehr and Trueb 2006; Frost *et al.* 2006; van der Meijden *et al.* 2007). Two things about this are most interesting. First, as with the Microhylinae, the pectoral girdle has clearly been reduced several times within this subfamily. Second, unlike the Microhylinae, all of the reduction seems to have happened in very much the same way. The intermediate-form pectoral girdle of *Elachistocleis* looks very similar to the one found in *Chiasmocleis*, even though *Elachistocleis* is more closely related to a clade

containing *Dermatonotus* and *Hypopachus* and must have become reduced independently.

Ultimately, this adds up to several independent occasions of convergence, of clavicle and procoracoid loss, reacquisition, or both. This is even more noteworthy seeing as how no other frog is known to lack clavicles at all (Duellman and Trueb 1994). It would seem likely that a single case of clavicular reduction might result in several subsequent losses, but most cases reveal frogs with fully robust pectoral girdles nested between and even within the clades showing losses. This pattern of loss, not obviously correlated with body size or ecomorphology, is indicative not so much of a newfound evolutionary pressure to lose clavicles and procoracoid as the relaxation of some strong evolutionary pressure to keep them. Between them, the clavicles and the procoracoid cartilage generally serve as attachment points for three superficial muscles, the mm. deltoideus, coracoradialis, and pectoralis, which work to move the upper arm and elbow. How these muscles attach in frogs that lack clavicles and procoracoids entirely is relatively unknown. The m. deltoideus may shift to a wholely scapular origin, but though the m. coracoradialis has been seen to shift its origin from clavicle to procoracoid cartilage when the former is missing, little is known about its attachment site when the procoracoid, too is gone (Duellman and Trueb 1994).

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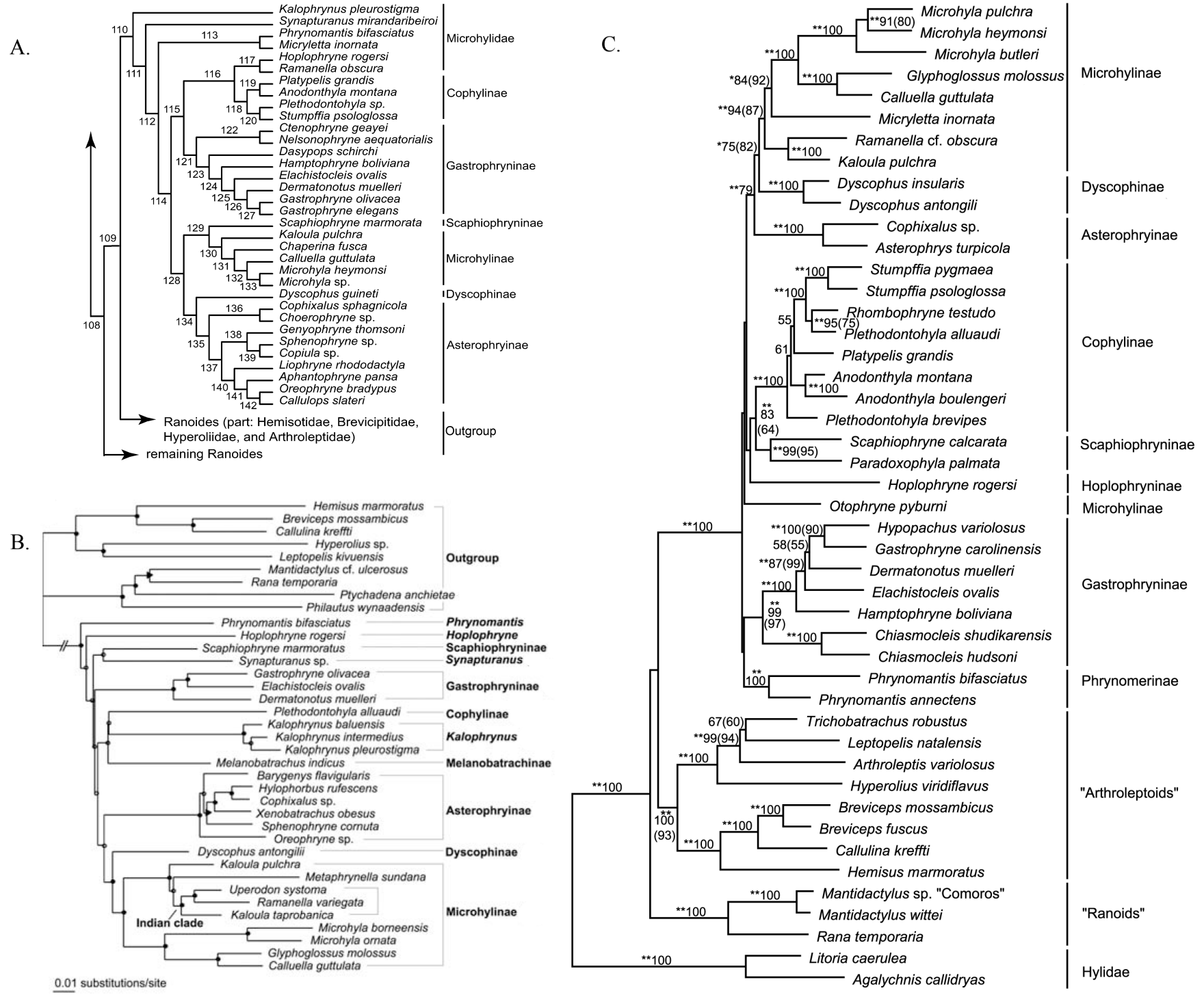

Figure 1: Three current molecular phylogenies of the family Microhylidae. A) from Frost et al. 2006; B) from Bocxlaer et al. 2006; C) from van der Meijden et al. 2007

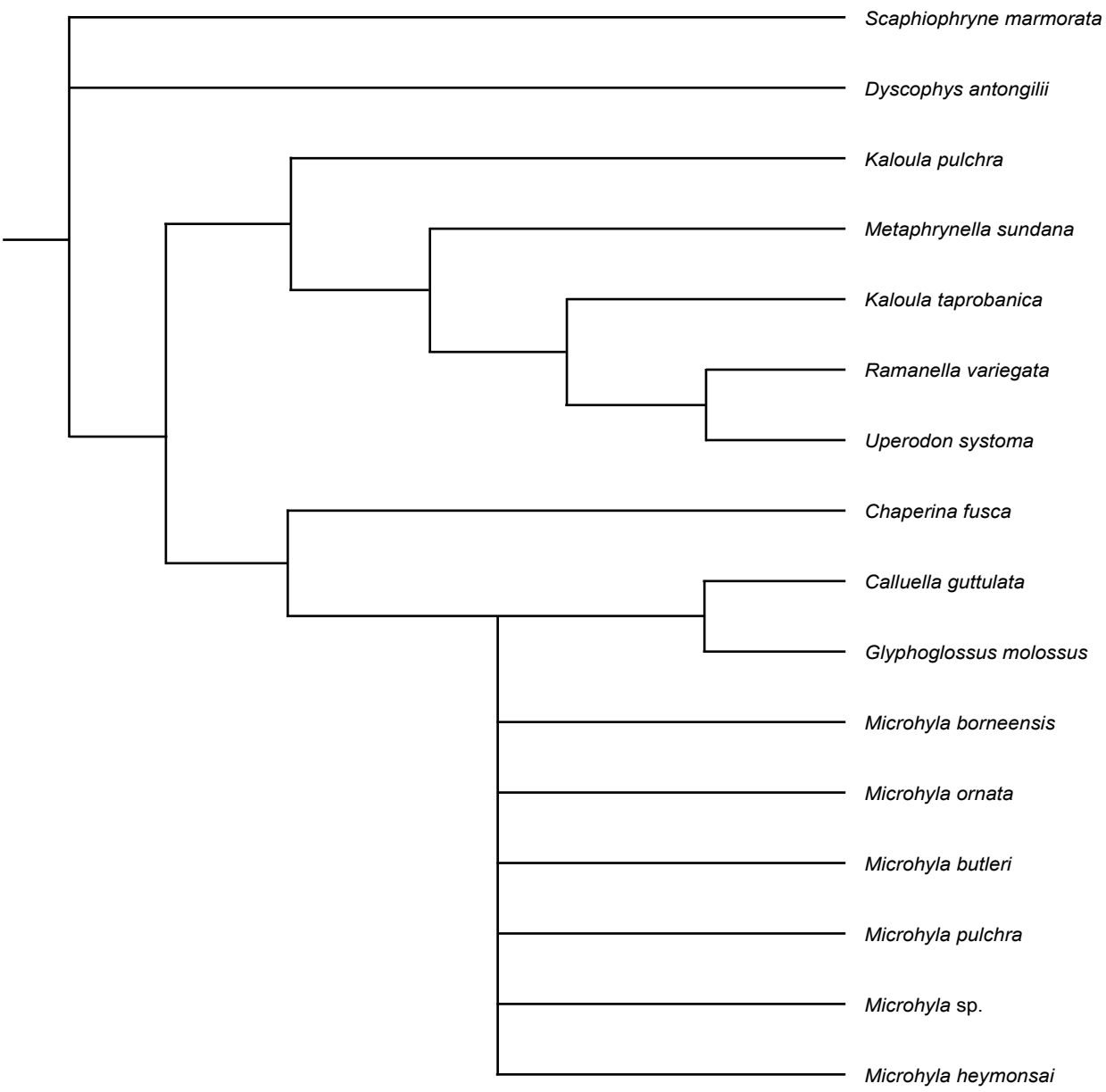

Figure 2: Consensus tree of three phylogenies of the subfamily Microhylinae, removing *Micryletta inornata* and *Ramanella* cf. *obscura* of van der Meijden *et al.*, which Frost *et al.* do not consider part of Microhylinae. (Frost *et al.* 2006, Bocxlaer *et al*. 2006, van der Meijden *et al.* 2007)

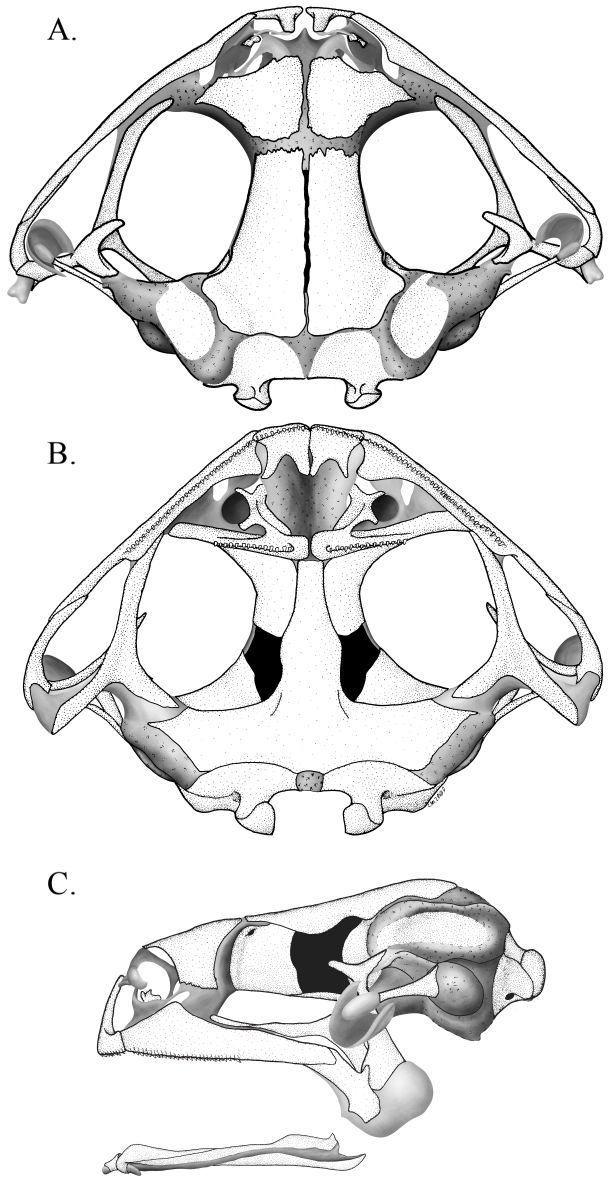

Figure 3: Cranium of *Calluella guttulata* (DSM 1457) in (A) dorsal view; (B) ventral view; (C) lateral view, with mandible

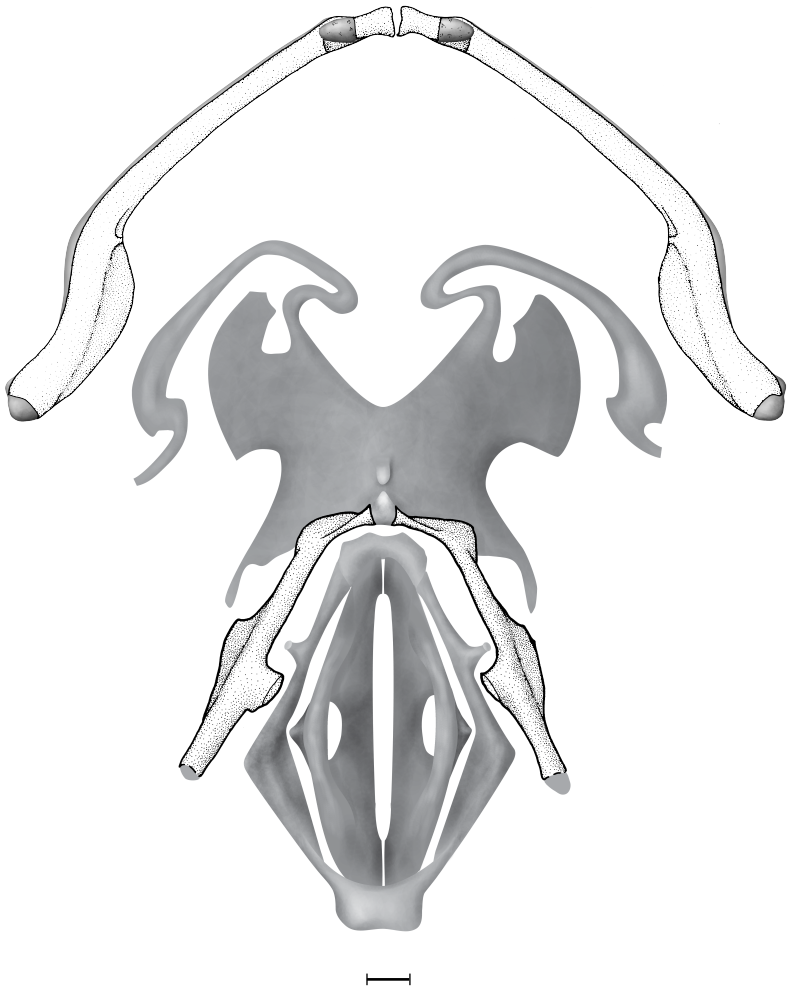

Figure 4: Hyobranchial apparatus and mandible of *Calluella guttulata* (DSM 1350)

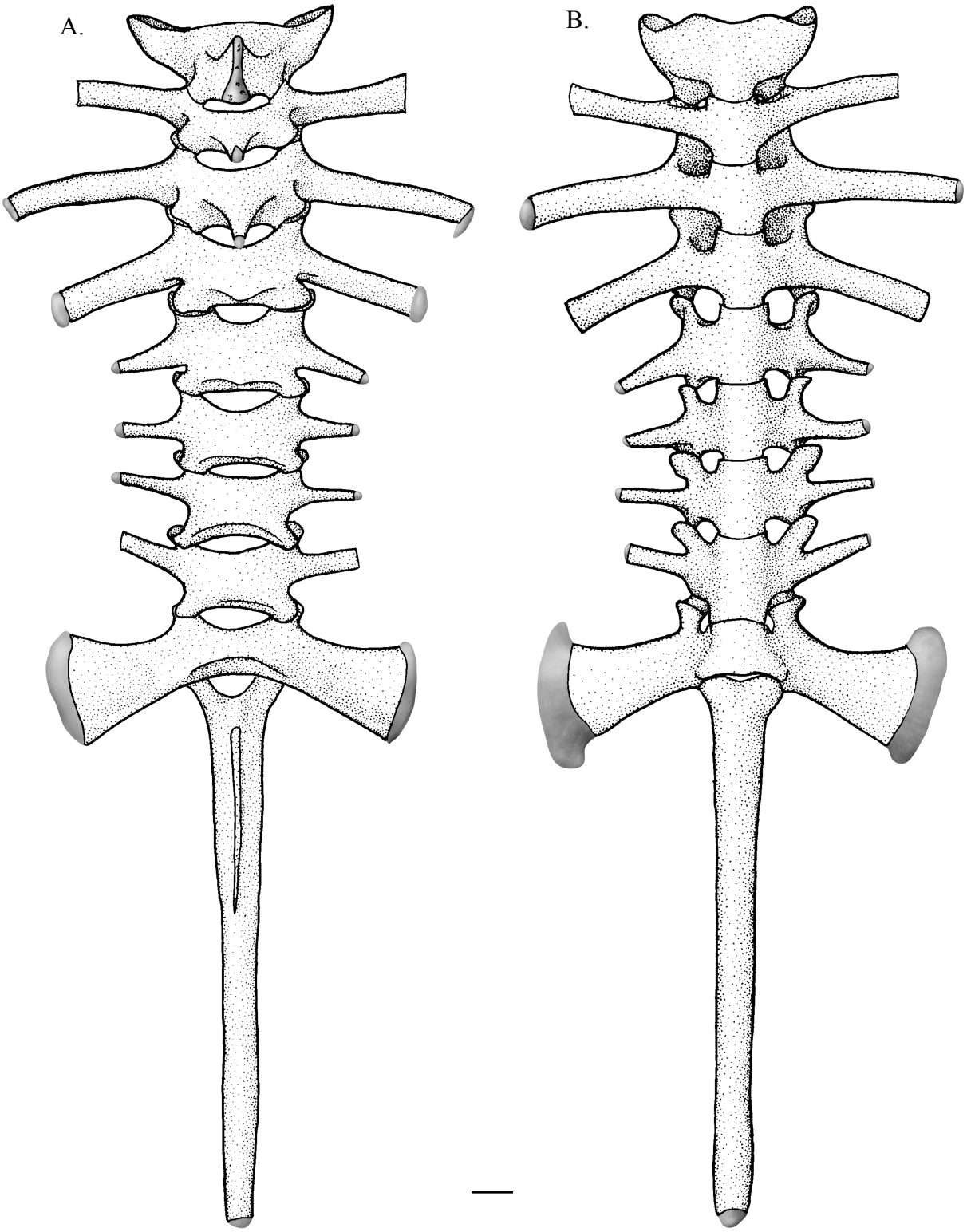

Figure 5: Vertebral column of *Calluella guttulata* (DSM 1457), in (A) dorsal view, (B) ventral view.

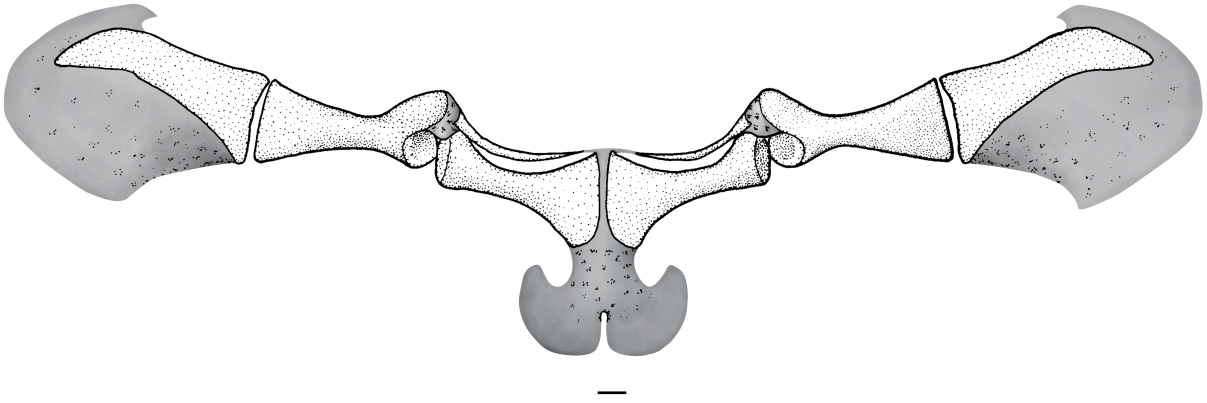

Figure 6: Pectoral girdle of *Calluella guttulata* (DSM 1457), ventral view

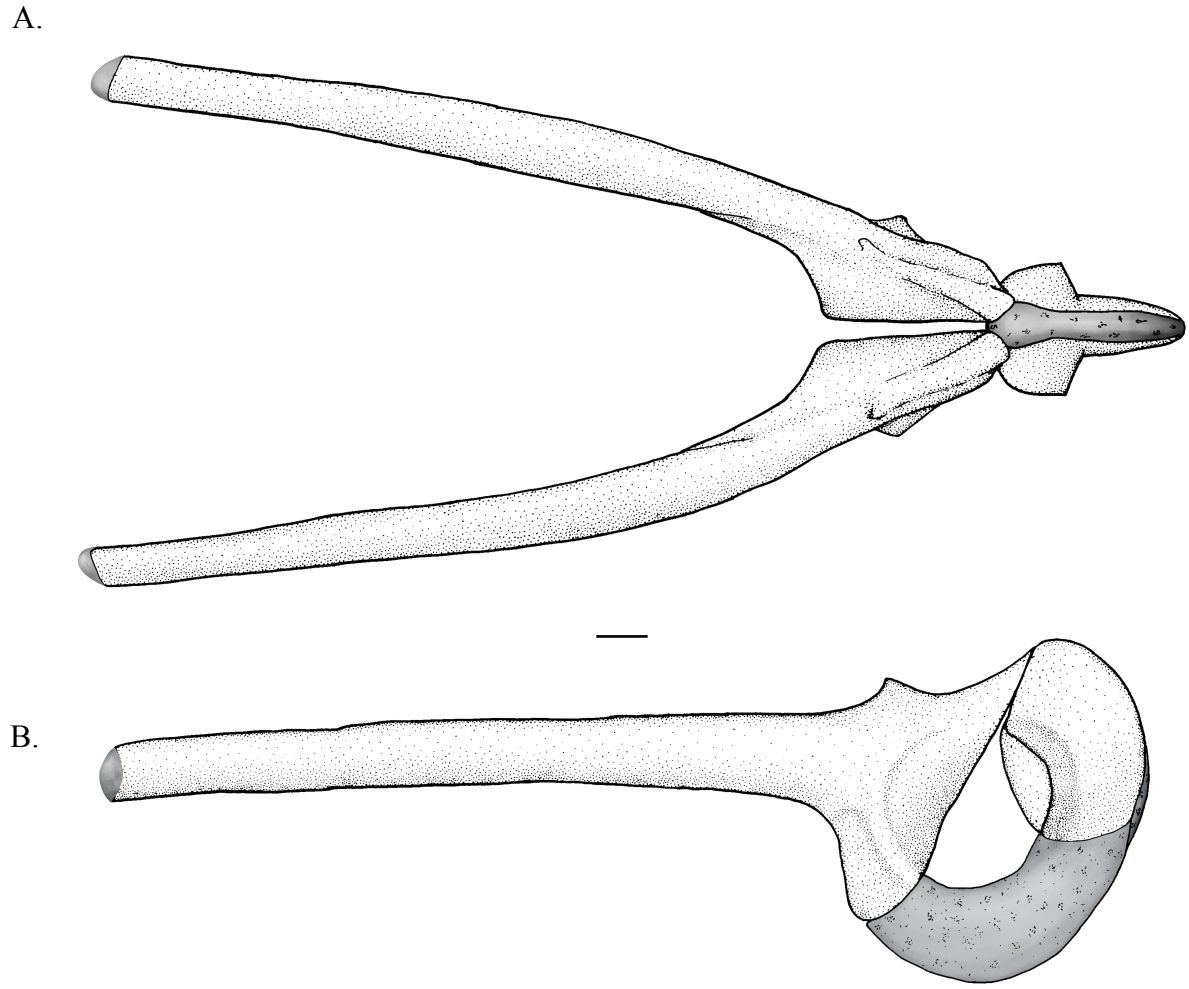

Figure 7: Pelvic girdle of *Calluella guttulata* (DSM 1457), in (A) dorsal view; (B) lateral view

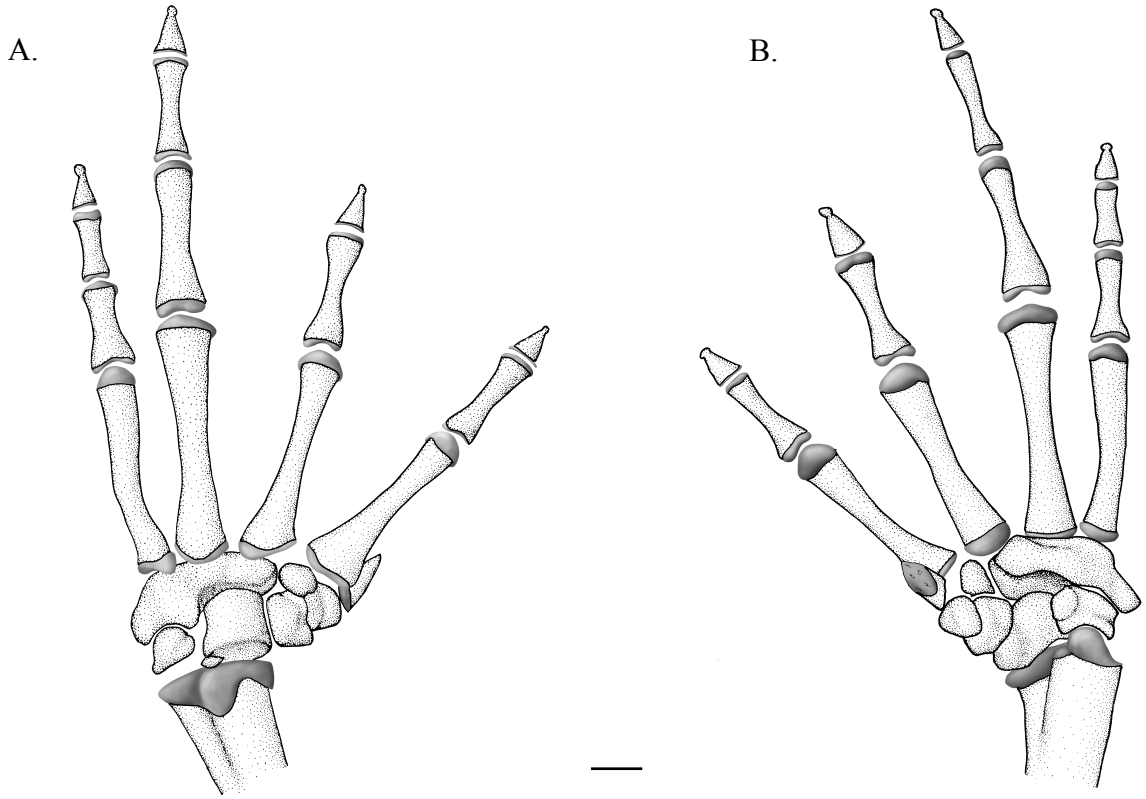

Figure 8: Manus of Calluella guttulata (DSM 1457), in (A) dorsal view; (V) lateral view

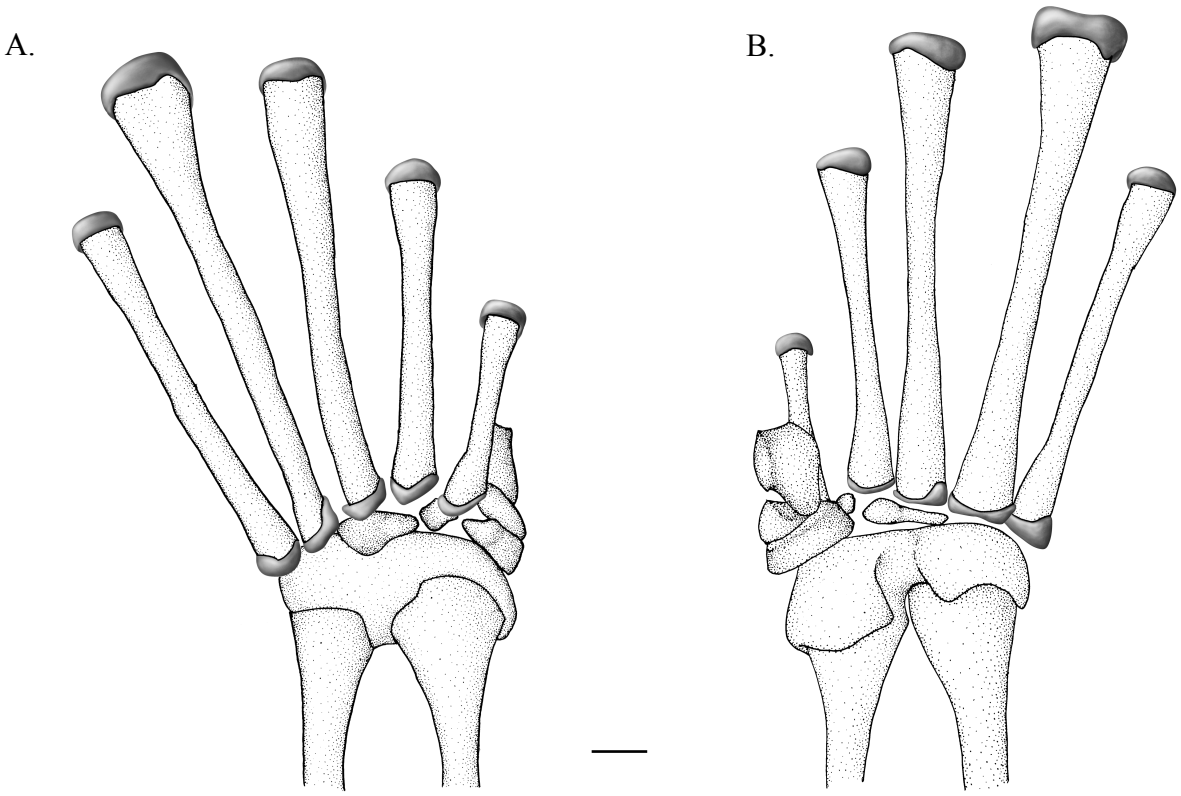

Figure 9: Tarsal elements of Calluella guttulata (DSM 1350), in (A) dorsal view; (V) lateral view

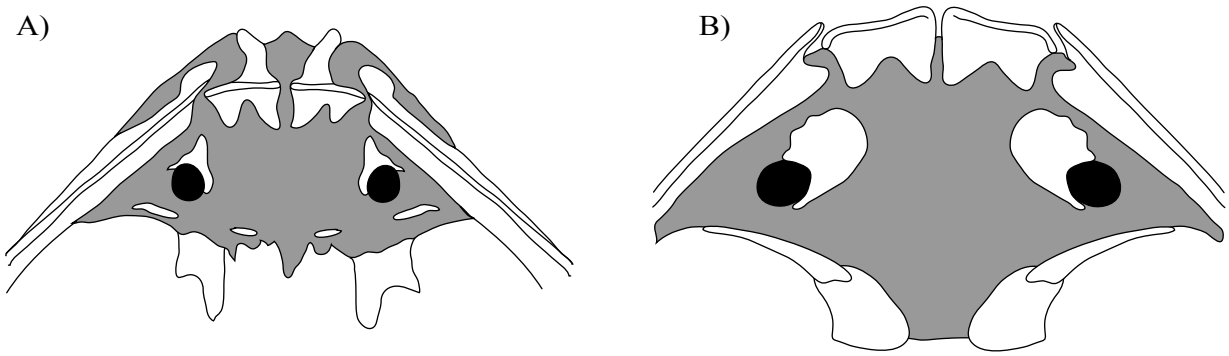

Figure 10: Palatine region of gastrophrynines: (A) *Hamptophryne boliviana* (modified from de Sa and Trueb 1991), (B) *Nelsonphryne aterrima* (modified from Lehr and Trueb 2006)

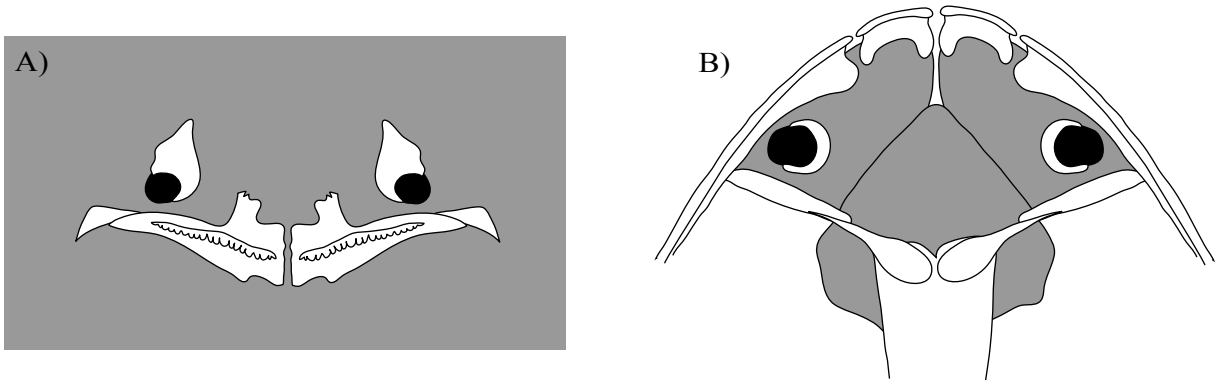

Figure 11: Palatine region of cophylines: (A) *Plethodonthyla notosticta*, (B) *Stumpffia psologlossa*. Drawings modified from Parker (1934)

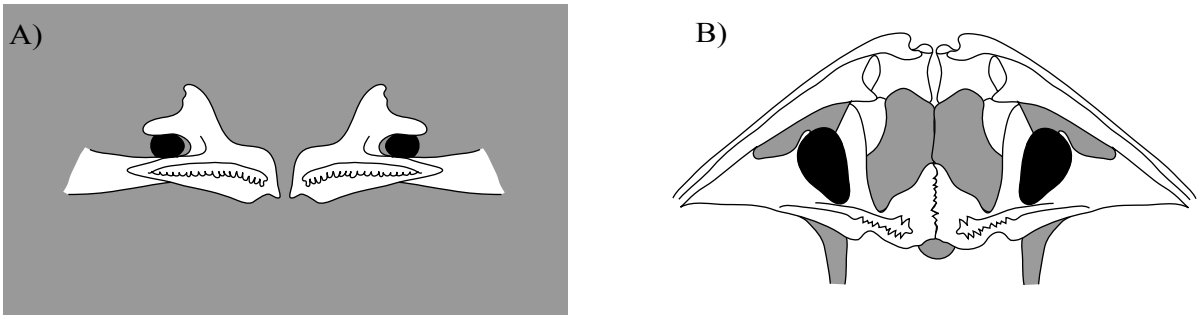

Figure 12: Palatine region of (A) *Dyscophus antongili*, (B) *Genyophryne thomsoni*. Drawings modified from Parker (1934)

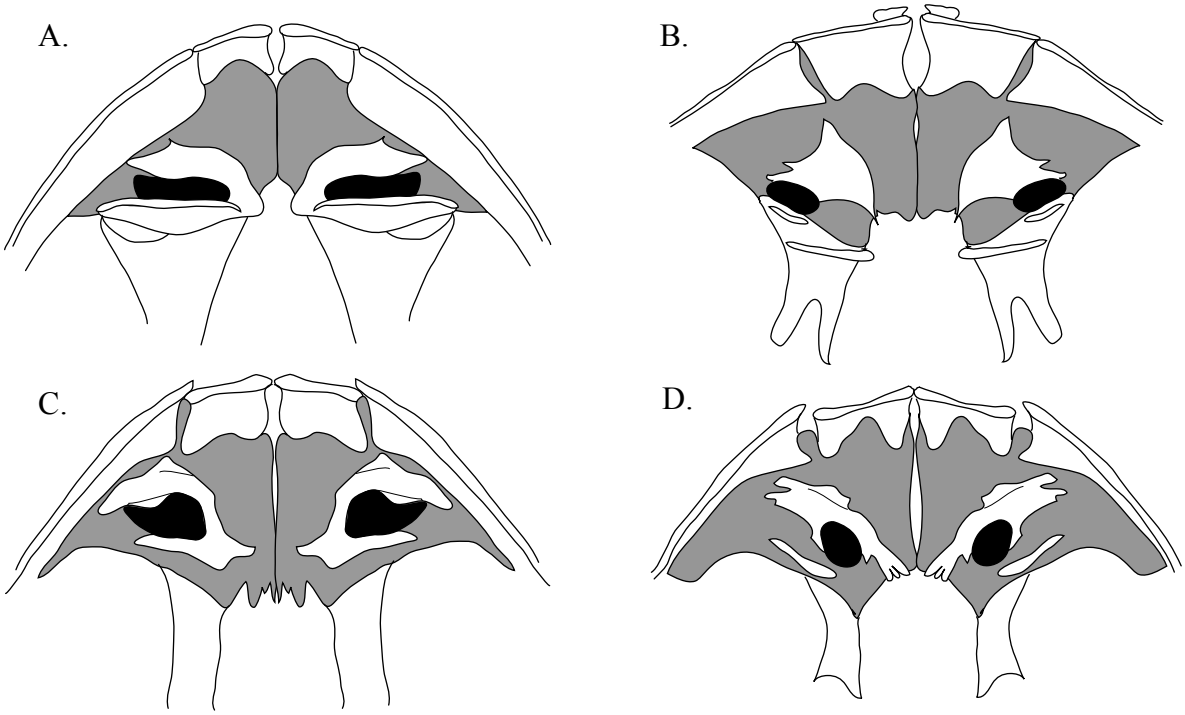

Figure 13: Palatine region of microhylines: (A) *Kaloula pulchra*, (B) *Ramanella triangularis*, (C) *Uperodon globulosus*, (D) *Uperodon systoma*. All drawings modified from Parker (1934)

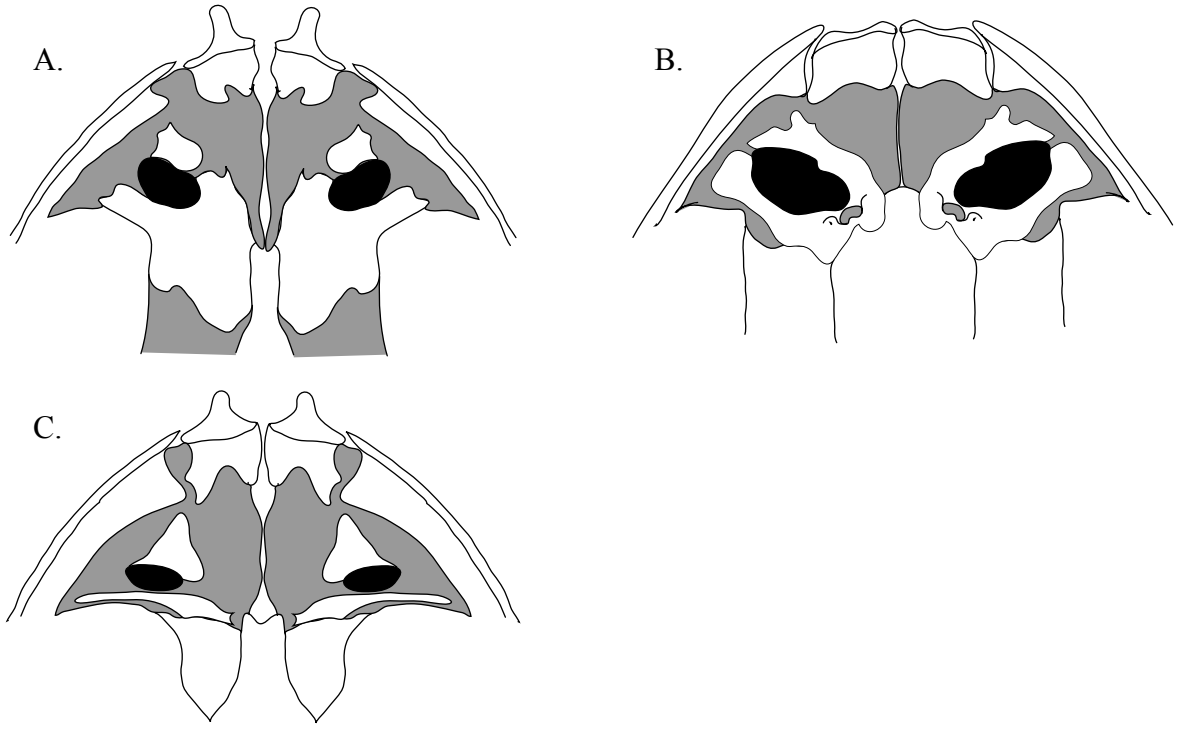

Figure 14: Palatine region of microhylines: (A) *Micryletta inornata*, (B) *Glyphoglossus molossus*, (C) *Microhyla berdmorei*. All drawings modified from Parker (1934)

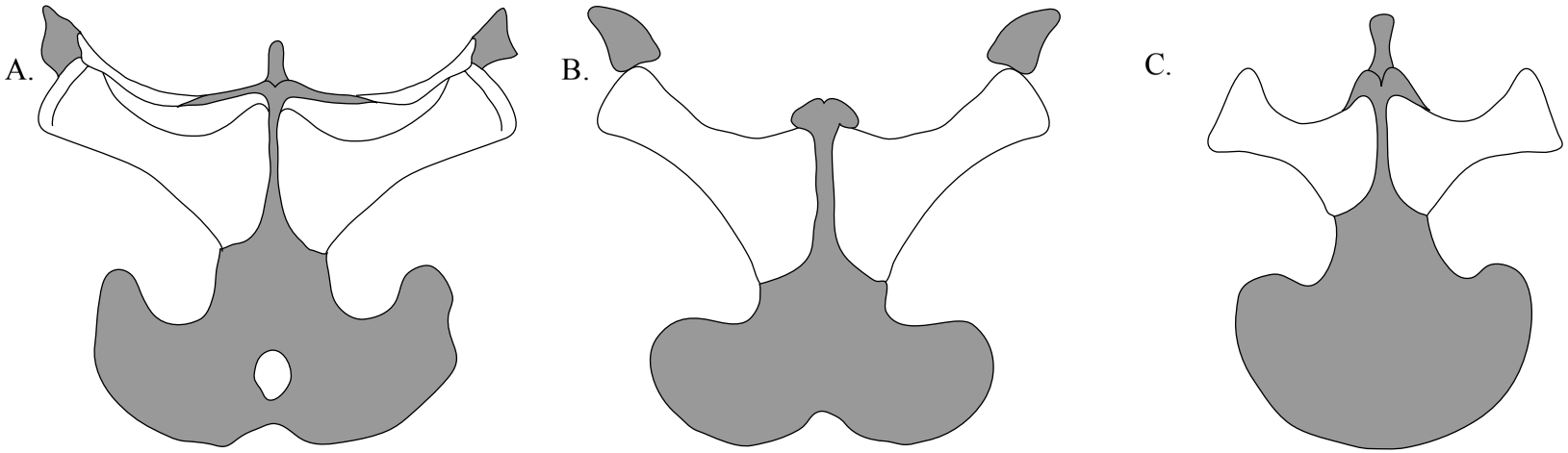

Figure 15: Ventral pectoral elements of microhylines: (A) *Calluella volz*i, (B) *Calluella brooksii*, (C) *Kaloula pulchra*. All drawings modified from Parker (1934)

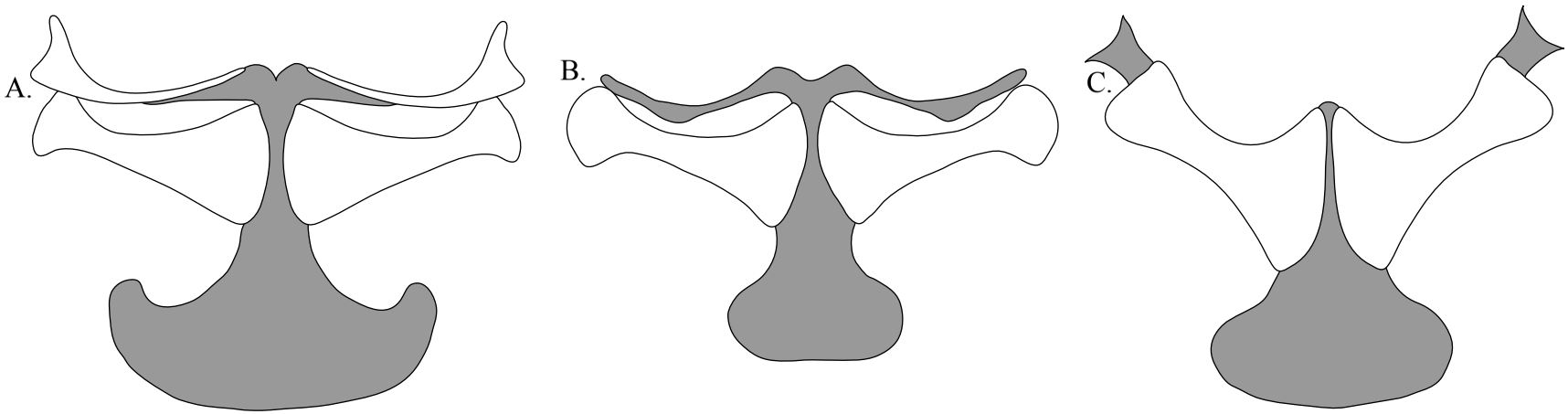

Figure 16: Ventral pectoral elements of asterophrynines: (A) *Sphenophryne cornuta*, (B) *Genyophryne thomsoni*, (C) *Asterophrys doriae*. All drawings modified from Parker (1934)

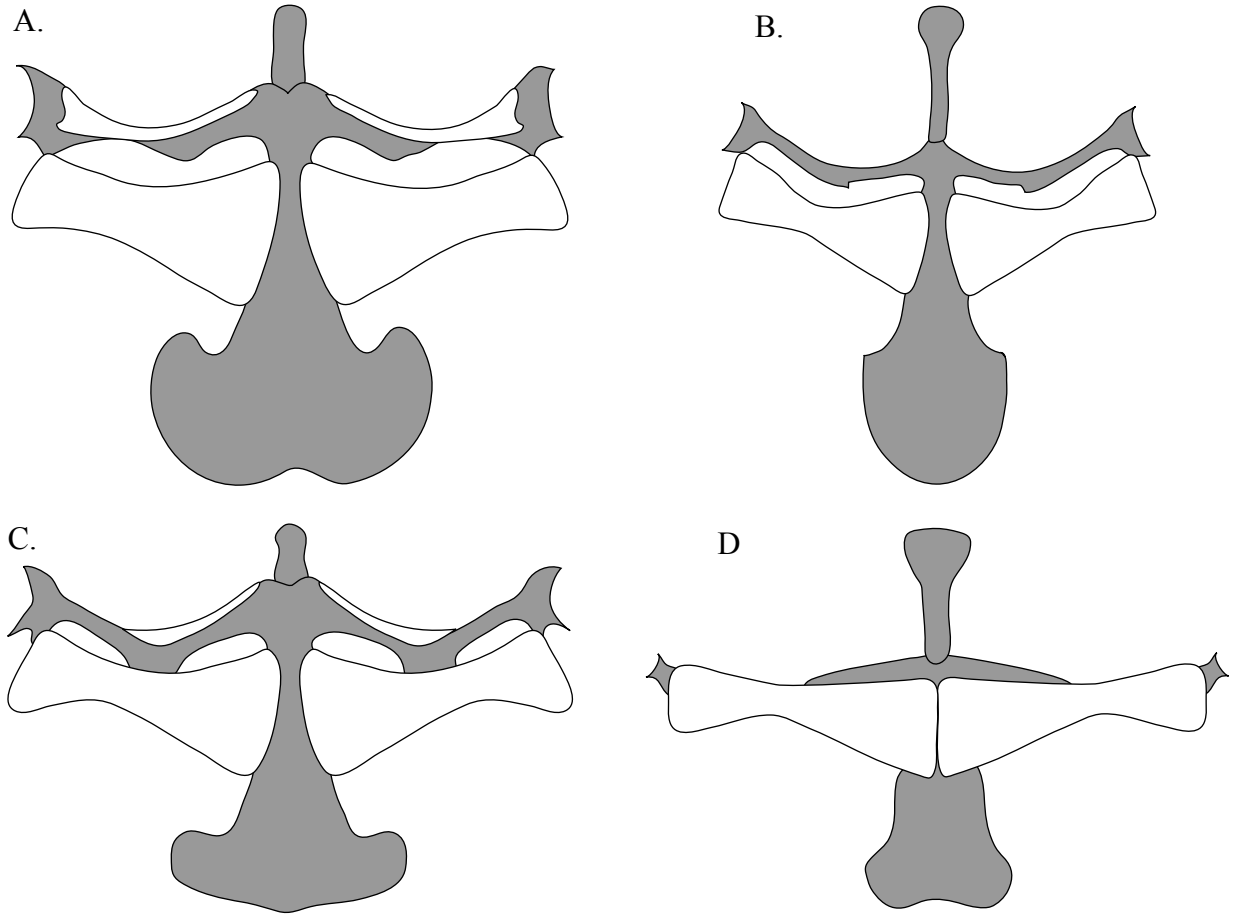

Figure 17: Ventral pectoral elements of cophylines: (A) *Plethodonthyla inguinalis*, (B) *Plethodonthyla notosticta*, (C) *Platypelis grandis,* (D) *Cophyla phylodactyla*. All drawings modified from Parker (1934)

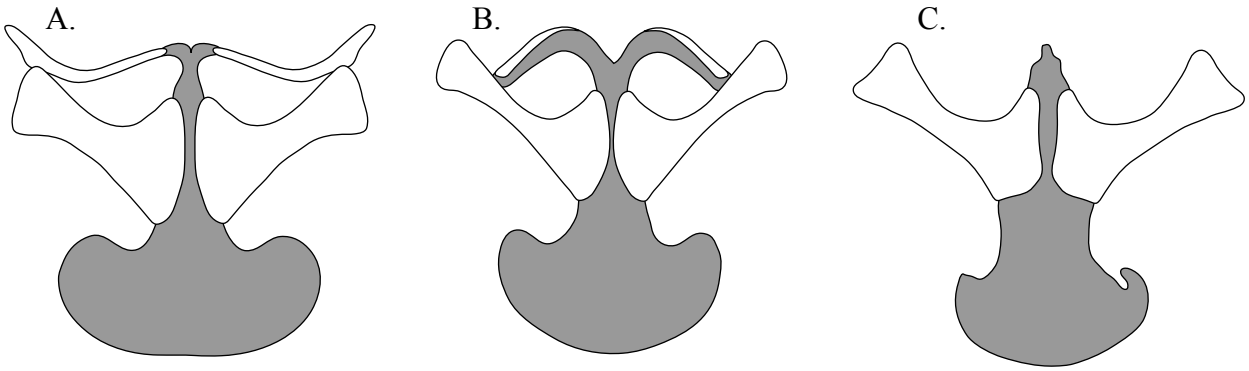

Figure 18: Ventral pectoral elements of gastrophrynines: (A) *Hypopachus variolosus* (modified from Parker 1934), (B) *Chiasmocleis albopunctata* (modified from Parker 1934), (C) *Nelsonphryne aequatorialis* (modified from Lehr and Trueb 2006)