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Comparative Development of Anurans: Using Phylogeny to Understand Ontogeny[1]

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SYNOPSIS. **Hypotheses of relationships are critical to describing and understanding patterns of evolution within groups of organisms. But rarely has a comparative, historical approach been employed to study developmental change, particularly among anurans. A recent resurgence of interest in collecting basic ontogenetic information provides us with the opportunity to compare ontogenetic trajectories in a phylogenetic framework. Larval skeletons and osteological development were examined for 22 taxa and compared to two hypotheses of relationships—that of Cannatella, and one proposed herein based on 41 morphological characters from larvae and 62 from adults. Larval characters were mapped on the alternate cladograms using the ACCTRAN optimization criterion. Several larval features are highly conserved among some anurans, suggesting that there is some level of canalization of morphology early in ontogeny. In contrast, a number of morphologies vary among groups, supporting the fact that there have been major evolutionary modifications to anuran larval morphologies early in ontogeny and in the early evolutionary history of anurans.**

INTRODUCTION

Nearly all contemporary evolutionary biologists concede that an hypothesis of phylogeny, or historical relationships, is fundamental to describing and understanding patterns of evolution within any group of organisms. Thus, in a tangible and pragmatic sense, systematics is the framework for all comparative biology. However, until recently, most studies of developmental biology (and specifically, descriptive morphological studies) were conducted in an evolutionary vacuum. Embryonic and postembryonic development of various taxa were described as stand-alone projects in efforts to document and chart staged models of development so familiar to students of vertebrate embryology (*e.g.,* Taylor and Kollros, 1946; Patten, 1952).

Of vertebrates, anurans have been among the most common subjects of developmental studies, the results of which frequently are generalized to vertebrate systems despite the obvious morphological and developmental specializations of these amphibians. Moreover, the generalizations are based on the results of studies of a few model species chosen primarily for their hardiness as laboratory animals, such as *Xenopus laevis* (Nieuwkoop and Faber, 1956; Trueb and Hanken, 1992; Moon *et al.*, 1993); *Bombina orientalis* (Hanken and Hall, 1984); and *Rana pipiens* (Taylor and Kollros, 1946; Kemp and Hoyt, 1969). Given the limited taxonomic sampling, relatively little information can be extrapolated to describe overall patterns of postembryonic development. This is unfortunate because the skeletal reorganization experienced by anurans during metamorphosis is unparalleled among vertebrates.

A few studies of frog postembryonic development have been framed in the context of a phylogeny. Two studies (Maglia and Púgener, 1998; Trueb *et al.*, 2000) examined the timing of ossification of elements of an individual taxon relative to a hypothesis of relationships; one other (Davies, 1989) investigated changes in timing of bone formation among a small group of frogs within the context of a cladogram. A few other studies (Haas, 1997; Larson and

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de Sá, 1998; Pugener *et al.*, MS in preparation) used developmental characters to help elucidate the phylogenetic relationships of taxa.

Herein, we examine characters of the larval skeleton and patterns of anuran postembryonic development for a number of taxa within the framework of two hypotheses of relationships. By examining these taxa, and framing our results in a phylogenetic context, we gain a better understanding of the overall patterns of postembryonic development in anurans. We also determine the evolutionary history of individual morphologies, as well as identify suites of characters that may have evolved together as a result of phylogenetic, developmental, or functional constraints. Finally, we apply the results of our study to understand the evolutionary history of anurans in general.

MATERIALS AND METHODS

Morphological comparisons

Larval skeleton and osteological development were examined for 22 taxa: *Ascaphus truei* (Ascaphidae); *Bombina orientalis* (Bombinatoridae); *Alytes obstetricans, Discoglossus sardus* (Discoglossidae); *Hyla lanciformis* (Hylidae); *Leptodactylus fuscus* (Leptodactylidae); *Megophrys montana* (Megophryidae); *Pelobates cultripes, P. fuscus, Spea bombifrons, S. intermontana* (Pelobatidae); *Pelodytes punctatus* (Pelodytidae); *Xenopus borealis, X. laevis, X. muelleri, Silurana tropicalis, Pipa carvalhoi, P. parva, Hymenochirus boettgeri* (Pipidae); *Pyxicephalus adspersus* (Ranidae); *Rhinophrynus dorsalis* (Rhinophrynidae); and *Ambystoma talpoideum* (Urodela: Ambystomatidae). Most data for anurans were collected from ontogenetic series staged according to the developmental table of Gosner (1960) or Nieuwkoop and Faber (for pipoids; 1956); the salamander, *Ambystoma*, was staged according to Wilder (1925). Specimens were cleared and double-stained for bone and cartilage following the techniques of Taylor and Van Dyke (1985), Dingerkus and Uhler (1977), or Wassersug (1976). See Appendix 1 for a list of specimens examined.

Data for seven taxa were coded from the literature, as follow: *Leptodactylus fuscus,* Larson and de Sá (1998); *Megophrys montana,* Ramaswami (1943), Sokol (1975, 1981); *Pelobates fuscus,* Roček (1980); *Pelodytes punctatus,* Sokol (1981); *Pipa carvalhoi* and *P. parva,* Sokol (1977), and *Spea intermontana,* Hall and Larsen (1998). Additional data were collected from the following publications: de Beer (1937), de Sá (1988), Moore (1989), Wiens (1989), Trueb and Hanken, (1992), Púgener and Maglia (1997), Wang (1997), Maglia and Púgener (1998), de Sá and Swart (1999), Swart and de Sá (1999), and Trueb *et al.* (2000). Terminology is that of Gaupp (1896), Duellman and Trueb (1986), and de Sá and Trueb (1991).

A total of 41 characters (Appendices 2, 3) was coded from the larval skeletons and osteogenesis of the 22 taxa. Characters 33–36 were modified from Haas (1997). Characters of the larval skeleton were coded from specimens representing the ''typical'' larval skeleton of each taxon—*i.e.*, the morphology of the skeleton was relatively unchanged for at least one Gosner or Nieuwkoop and Faber stage prior, and subsequent, to the stage examined. Most of the morphological characters examined are illustrated in Figure 1.

Character evolution

Character evolution was examined using two similar, but slightly different methods. First, characters were optimized or ''mapped'' onto a cladogram of anuran relationships using MacClade 3.1.1 (Maddison and Maddison, 1992). Currently, there are several hypotheses of anuran relationships (*e.g.*, Duellman and Trueb, 1986; Ford and Cannatella, 1993; Hay *et al.*, 1995). Of these, the phylogeny presented by Ford and Cannatella (1993), based on morphological and life-history data, probably is the most widely accepted. Because this hypothesis is based on the separate studies of Cannatella (1985) on basal anurans and Ford (1989) on neobatrachians, we have chosen Cannatella's hypothesis (Fig. 2A) as a framework within which to consider our data.

The second method of examining char-

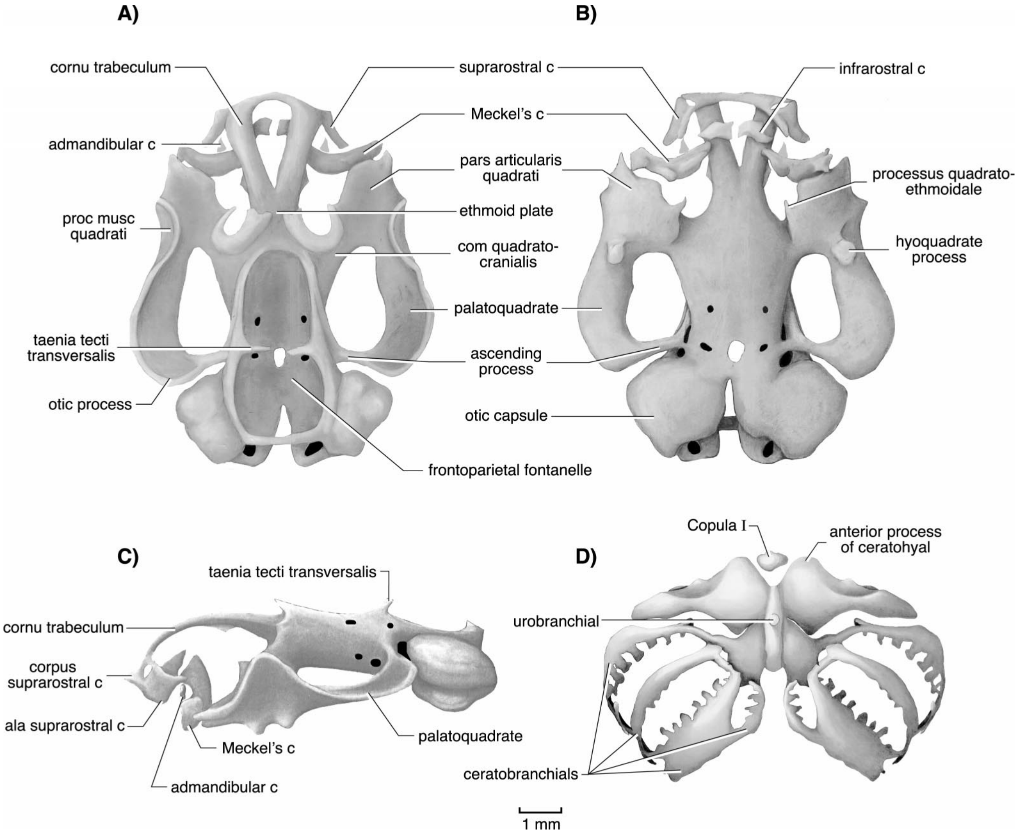

FIG. 1. *Bombina orientalis* (Gosner Stage 35; KU 223508). Larval chondrocranium is illustrated in **A.** dorsal, **B.** ventral, and **C.** lateral views. The hyobranchial apparatus is illustrated in **D.** ventral view. Gray denotes cartilage; black denotes foramina. Abbreviations: c = cartilage; com. = commissura; proc. musc. quadrati = processus muscularis quadrati.

acter evolution is to trace the optimizations of characters used to create a phylogenetic hypothesis. Most systematic studies of morphology, such as Cannatella's (1985), include data derived primarily from adult specimens. However, the morphology of an organism is part of a continuous process of ontogeny that includes many different forms. Following the tenet of total evidence, because larval morphologies are considered part of the overall archetype of a taxon, they should be included in phylogenetic analyses. Therefore, we conducted an analysis of anuran relationships that included both characters coded on adult specimens (62 characters from Maglia, 2000) and the larval characters presented herein. A heuristic search was conducted using PAUP 4.0* (Swofford, 1999) with ACCTRAN optimization. All transformation series were weighed equally and unordered. Results of this analysis are presented in Figure 2B and were used as an alternate to Cannatella's (1985) phylogeny in considering larval character evolution.[3] Detailed results of the phylogenetic analysis will be discussed elsewhere (Púgener *et al.*, MS) and the resulting phylogeny presented here-

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[3] It has been argued that examining character evolution via optimizing on the tree they were used to build presents a circular argument. However, every character in a phylogenetic analysis represents an independent hypothesis of the evolutionary history of the group. Because phylogenetic analyses result in an hypothesis based on the most parsimonious congruence of many hypotheses of evolution (*i.e.,* characters), examination of single characters independently does not result in circularity.

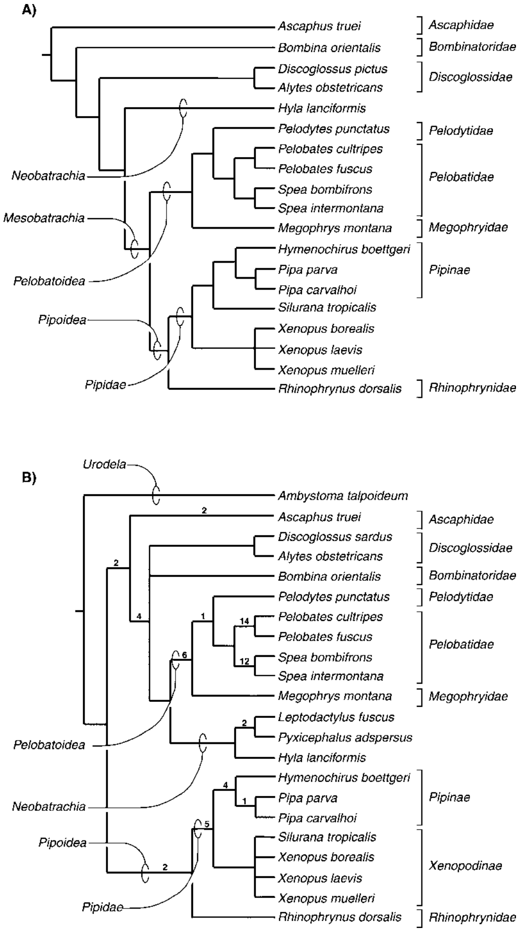

FIG. 2. **A.** Cannatella's (1985) phylogenetic hypothesis of relationships among anurans. Only the taxa for which developmental data were available are listed. **B.** Majority rule consensus (50%) of the 42 most parsimonious trees based on 62 characters from adult morphology (Maglia, 2000) and 41 characters from larval morphology (Appendices 2, 3). Tree length = 287; consistency index = 0.460; retention index = 0.728. Numbers indicate Bremer decay indices.

in should be considered only as a framework to test hypotheses of character evolution.

Alternate phylogenetic hypotheses

The phylogenetic hypotheses considered in this investigation differ substantially in the placement of a few major groups of anurans. Cannatella (1985) recognized Discoglossidae (originally including *Alytes*, *Barbourula*, *Bombina,* and *Discoglossus*) as paraphyletic (*i.e.*, not sharing a common ancestor). He recognized the name Bombinatoridae for the clade including *Bombina* (and *Barbourula*) and suggested that Discoglossidae is more closely related to the clade [Neobatrachia + [Pelobatoidea + Pipoidea]] than it is to Bombinatoridae. Cannatella (1985) also recognized Pipoidea as being more closely related to Pelobatoidea than either clade is to Neobatrachia. And within Pipidae, he found *Silurana* as the sister taxon to Pipinae (Fig. 2A).

We rooted our analysis (Fig. 2B) using the salamander *Ambystoma talpoideum* and found that, rather than *Ascaphus,* Pipoidea is the sister-taxon to all other anurans. Within Pipidae, we found *Silurana* and *Xenopus* (rather than Pipinae) to be sister taxa. Our results also suggest that Ascaphidae is the sister-taxon to the clade formed by [Discoglossidae + Bombinatoridae + [Neobatrachia + Pelobatoidea]]. Pelobatoidea and Neobatrachia (rather than Pipoidea) are sister-taxa. The combined clade [Neobatrachia + Pelobatoidea] forms an unresolved polytomy with Discoglossidae and Bombinatoridae.

METHODOLOGICAL CONSIDERATIONS

Outgroup

The issue of outgroup inclusion is pertinent to the phylogenetic hypotheses considered herein. We included the salamander *Ambystoma talpoideum* as an outgroup in our phylogenetic analysis (based on the relationship Caudata [†*Karaurus* + Urodela] + Salientia [†*Triadobatrachus* + Anura] proposed by Milner, 1988, 1993; Hillis, 1991; Trueb and Cloutier, 1991; Cannatella and Hillis, 1993; Hay *et al.*, 1995; Báez and Basso, 1996). By including this outgroup taxon, all characters coded for anurans can be completely resolved (except for those related to the suprarostral cartilages—*de novo* structures in anuran larvae). Because Cannatella (1985) used *Ascaphus truei* as the outgroup taxon in his analysis (based on the proposition that this taxon is the sister-group to all other anurans; *e.g.*, Cannatella and Hillis, 1993; Haas, 1997), it is impossible to resolve (or polarize) some character transformations that include *Ascaphus.* Of the 41 larval characters mapped onto Cannatella's (1985) tree (Fig. 2A), Characters 12, 15, 27, and 33 are equivocal (*i.e.,* it is not possible to determine the plesiomorphic state). However, if *Ambystoma talpoideum* is included as an outgroup, all the characters become fully resolved.

Optimization criterion

Because hypotheses of character evolution are dependent upon patterns of character transformations mapped onto the phylogeny, the optimization criterion utilized will affect the resulting understanding of character evolution. Throughout our discussion of character evolution, we have chosen only to discuss ACCTRAN optimizations (favoring reversals).

DEVELOPMENTAL PATTERNS

By optimizing each of the characters on the two phylogenetic hypotheses, we identified patterns of conserved characters (or synapomorphies), as well as other characters that seem to lack pattern and, thus, be homoplastic.

Synapomorphy

Most of the larval morphologies examined are shared by a number of taxa. Many of these can be considered shared, derived features of natural groups. In other words, they seem to have evolved in the common ancestor of monophyletic taxa.

[Discoglossidae + Bombinatoridae + [Pelobatoidea + Neobatrachia]]. Five morphological features have a similar evolutionary history, as follow: divergence of the cornua trabeculae (Character 15); the otic process of the palatoquadrate (Character 26); hyobranchial spiculae (Character 35); the taenia tecti transversalis (Character 28); and the processus muscularis quadrati (Character 22). Each of these characters evolved either in the common ancestor to all frogs, with the exception of *Ascaphus truei* (when optimized on Cannatella's phylogeny) or in the common ancestor to [Discoglossidae + Bombinatoridae + [Pelobatoidea + Neobatrachia]] (when optimized on the phylogeny presented herein). The difference in optimization among the two trees relates to the placement of Pipoidea.

Discoglossidae and Bombinatoridae. One morphological feature, the presence of admandibulars (Character 12), masses of undifferentiated connective tissue lateral to the infrarostral cartilages, is found only in Discoglossidae and Bombinatoridae.

[Neobatrachia + Pelobatoidea + Pipoidea]. A single morphological character, the closed dorsal margin of one side of the suprarostral cartilage (Character 4), is shared by Neobatrachia, Pelobatoidea, and Pipoidea.

Neobatrachia and Pelobatoidea. The attachment of the cornua trabeculae laterally to the suprarostral alae (Character 14) evolved in the shared ancestor of [Neobatrachia + [Pelobatoidea + Pipoidea]] when optimized on the phylogeny of Cannatella (1985). This condition reversed to form a medial attachment to the corpus of the suprarostrals in Pipoidea. If mapped on the phylogeny presented herein, lateral attachment of the cornua trabeculae is a shared, derived character for [Pelobatoidea + Neobatrachia], having evolved in the common ancestor of these taxa.

Pelobatoidea and Pipoidea. Two morphological conditions are unique to pelobatoids and pipoids—*viz.,* fusion of middle portion of the suprarostral alae (Character 6), and medial fusion of the infrarostral cartilages (Character 10).

Neobatrachia. Two morphological conditions occur uniquely in neobatrachians. The ascending process of the palatoquadrate cartilage fuses to the braincase through what Sokol (1981) called a ''high'' attachment, dorsal to the oculomotor foramen (Character 25). The taenia tecti medialis (Character 29), a bar of cartilage on the dorsomedial surface of the chondrocranium that bisects the parietal portion of the frontoparietal fontanelle, evolved in the common ancestor to neobatrachians.

Pelobatoidea. Adrostral tissues (Character 9), rods of undifferentiated connective tissue lateral to the suprarostral cartilages, are present only in Pelobatoidea and seem to have evolved in the common ancestor to the group.

[Pelobatidae + Pelodytidae]. A single morphological feature, lack of medial fusion of the suprarostral corpus (Character 7)

evolved in the common ancestor of [Pelobatidae + Pelodytidae].

Pipoidea. Eight unique, shared-derived features occur in pipoids, as follow: position of the suprarostrals (Character 2); ethmoid plate between cornua trabeculae (Character 16); pars articularis quadrati/posterior margin of suprarostral cartilage (Character 19); hyoquadrate process (Character 23); urobranchial (Character 34); ceratobranchials (Character 36); primordia of epipubis (Character 39); and position of the eyes (Character 40).

Pipidae. The obtuse angle of the suborbital cartilage (Character 20) and the ventrolateral process of the palatoquadrate cartilage (Character 24) are unique, derived characters shared by pipids. Five additional pipid features seem to have evolved in the ancestor to Pipoidea when optimized on Cannatella's (1985) phylogenetic hypothesis: suprarostral alae (Character 3), barbels (Character 8), palatoquadrate (Character 18), and processus muscularis of the otic capsule (Character 32).

Xenopodinae. Four morphological features have similar evolutionary histories in the clade [*Xenopus* + *Silurana*]. These characters include the following: alae of the suprarostrals triangular (Character 3), presence of barbels (Character 8), formation of the palatoquadrate cartilage (Character 18), and processus muscularis of the otic capsule (Character 32).

Pipinae. Five unique, shared-derived characters are found in the clade [*Pipa* + *Hymenochirus*], as follow: suprarostrals reduced (Character 3); quadrato-ethmoidalis ligament (Character 21); length of frontoparietal fontanelle versus total length of chondrocranium (Character 30); size of otic capsule (Character 31); and premetamorphic fusion of first two presacral vertebrae (Character 37).

Homoplasy

Several of the larval morphological conditions considered have an evolutionary history that includes two or more independent derivations, regardless of which phylogenetic hypothesis is considered.

Two derivations. An incomplete or open ventral margin of one side of the suprarostral cartilage (Character 5) occurs in *Ascaphus, Hyla,* and *Leptodactylus.* Mapped onto the phylogenies, this condition either (1) evolved in the ancestor of *Ascaphus* and closed in the common ancestor to all frogs excluding *Ascaphus,* or (2) evolved a second time as an open state in the common ancestor to neobatrachians and then closed in the ranid *Pyxicephalus.*

Similarly, a cartilaginous fusion of Meckel's cartilage and the infrarostral cartilages (Character 11) evolved at least two times—once in the ancestor to *Rhinophrynus* and once in the ancestor to *Hymenochirus.*

Multiple derivations. Five larval morphological features seem to have evolved at least three times in the taxa considered. These are, as follow: terminally expanded cornua trabeculae (Character 17); the presence of Copula I (Character 33); the presence of an otic ligament (Character 27); the type of articulation between the cornua trabeculae and the suprarostral cartilages (Character 13); and the mode of vertebral development (Character 38).

Regardless of which hypothesis of relationships is considered, the cornua trabeculae evolved to be terminally expanded three times—in *Bombina,* in the common ancestor of *Pelobates,* and in *Leptodactylus.*

The presence of Copula I of the hyobranchial apparatus evolved at least four times. Optimized on Cannatella's (1985) hypothesis, it first appeared in the common ancestor of all frogs, excluding *Ascaphus.* Subsequently, Copula I was lost in the common ancestor to [Neobatrachia + [Pelobatoidea + Pipoidea]], but appeared again in the common ancestor to *Pelobates,* in *Rhinophrynus,* and in *Hymenochirus.* Optimization on our phylogenetic hypothesis is similar, with the copula evolving in parallel in the common ancestor to [Discoglossidae + Bombinatoridae], the common ancestor to *Pelobates,* in *Rhinophrynus,* and in *Hymenochirus.*

The otic ligament extends from the posterolateral margin of the palatoquadrate to the otic capsule. Typically, this ligament is chondrified and is referred to as the larval otic process. Relative to Cannatella's hypothesis (1985), a ligamentous connection evolved in the common ancestor to all anurans excluding *Ascaphus,* subsequently reversed to a chondrified connection in the ancestor to [Neobatrachia + [Pelobatoidea + Pipoidea]] and evolved as a ligamentous connection again in the common ancestor to *Spea* and in *Hymenochirus.* Mapped onto the phylogeny presented herein, a ligamentous connection evolved in parallel three times—in the common ancestor to [Discoglossidae + Bombinatoridae], in the common ancestor to *Spea,* and in *Hymenochirus.*

The type of articulation between the cornua trabeculae and the suprarostral cartilages has evolved several different morphologies including a cartilaginous, ligamentous, or synovial attachment. Optimizing on the tree of Cannatella (1985), basal anurans possess a cartilaginous articulation and a ligamentous attachment evolved in the common ancestor to [Discoglossidae + [Neobatrachia + [Pelobatoidea + Pipoidea]]]. Subsequently, the articulation reversed to a cartilaginous one in the common ancestor to [Pelobatoidea + Pipoidea]. The articulation evolved as a synovial joint in the common ancestor [Pelobatidae + Pelodytidae], but evolved as both cartilaginous and ligamentous in *Spea.* Mapping the morphologies onto our hypothesis, it seems that a ligamentous articulation evolved in the common ancestor to Discoglossidae and Neobatrachia, and at least one species of *Spea.* A synovial articulation seems to have evolved in the common ancestor to [Pelobatidae + Pelodytidae], but to have reversed to a cartilaginous articulation in *Spea.*

The way in which the vertebral centra ossify varies among taxa. In *Ascaphus,* Bombinatoridae, Discoglossidae, Pelobatidae, Pelodytidae, and *Hyla* (neobatrachian), ossification originates from only the dorsal portion of the notochordal sheath (=epichordal). In all other taxa, ossification includes the entire sheath surrounding the notochord (=perichordal; Duellman and Trueb, 1986; Maglia, 2000). Relative to Cannatella's (1985) phylogeny, the epichordal development of the vertebral centra evolved in the common ancestor to all anurans excluding *Ascaphus.* The perichordal condition evolved in the common ancestor to [Pelobatoidea + Pipoidea], reversed to epichordal in the ancestor of [Pelobatidae + Pelodytidae], and then perichordy evolved again in *Spea.* Epichordy developed another independent origin in the common ancestor to Pipidae. Mapping on our tree, perichordy is the plesiomorphic (*i.e.,* ''primitive'') condition in anurans and epichordy seems to have evolved four different times—*viz.,* in the ancestor of [Discoglossidae + Bombinatoridae], in the ancestor of [Pelobatidae + Pelodytidae], in the ancestor of Pipidae, and in *Hyla.*

CONCLUSIONS

By examining larval morphologies and postembryonic development in the framework of phylogenetic hypotheses of anuran relationships, we identified some patterns of character evolution. Specifically, our results suggest that most of the morphologies examined were shared by various taxonomic groups, and some taxa are characterized by as many as nine shared, derived features. Also, within some groups, larval morphologies are highly conserved—*viz.,* there are few or no differences in the morphologies among the various species. This seems to support von Baer's (1828) suggestion that there is some level of canalization in early ontogeny. In other words, morphologies of closely related taxa are very similar at early stages, with phenotypic differentiation appearing later in ontogeny (through additions and deletions).

In the case of pipoids, most of the apomorphic morphologies (*e.g.,* presence of a continuous ethmoid plate, lack of mouthparts, complex ceratobranchials) are associated with an extreme degree of filter feeding. Therefore, canalization in these taxa may be the result of functional constraints associated with larval feeding. However, the morphologies (*e.g.,* divergence of the cornua trabeculae, otic process of the palatoquadrate, hyobranchial spiculae) shared by other, more ecologically and anatomically diverse taxa do not seem to be the result of functional constraint, but rather developmental or phylogenetic constraint. But because most of these morphologies characterize more inclusive taxonomic groups (*i.e.,* families, genera), it is not likely that they are recent occurrences.

Our results also showed that, to some degree, larval morphologies do vary among taxa. Some morphologies are unique to specific groups (*e.g.,* ‘‘high’’ attachment of ascending process of the palatoquadrate, presence of taenia tecti medialis in neobatrachians; presence of adrostrals in pelobatoids), suggesting that there has been a number of major evolutionary modifications to larval morphologies in early ontogeny.

Also, several morphologies seem to have evolved in parallel (*e.g.,* ligamentous connection of otic process, terminal expansion of cornua trabeculae). This information is useful because it allows us to determine if multiple evolutionary events have given rise to similar morphologies, which may in fact, not be so similar. These data may also help us to recognize potential functional or ecological constraints (in the case of convergence), as well as to help us recognize the complexity of developmental processes. Moreover, recognition of parallelisms demonstrates that patterns of development themselves are evolving, and may provide useful insights into the homologies of adult morphologies.

Finally, we can apply the information gained from this exercise to the evolutionary history of the taxa examined. Specifically, the nature of the characters unique to pipoids gives us insight into their evolutionary history. All pipoids (except *Rhinophrynus*) remain aquatic as adults and have a rather uncommon appearance, compared to other anurans. These features may explain, at least in part, the historical controversy about higher-level relationships among basal anurans.

In the context of Cannatella’s (1985) phylogenetic hypothesis, the most basal anuran lineages are Ascaphidae, Bombinatoridae, and Discoglossidae—all groups that include terrestrial adults and larvae with mouthparts. Accordingly, the common ancestor to all anurans would have had a larva resembling that of *Bombina, Discoglossus,* or any other related taxon. Given this hypothesis of relationships, pipids are assumed to have reverted to an ontogeny reminiscent of salamanders by abandoning larval specializations associated with feeding (Cannatella, 1999).

Our hypothesis offers an alternate interpretation. Early in the evolution of anurans, two major lineages appeared—pipoids on one hand and the rest of the anurans on the other. Thus, based on outgroup comparison, the ancestor to all anurans could have had a larva that resembled the tadpole of *Rhinophrynus* or *Xenopus,* at least with regard to some of its feeding specializations. Interestingly, the basal condition of pipoid frogs proposed herein is congruent with the scheme proposed by Orton (1953, 1957) and Starrett (1973), despite the fact that their conclusions were not made in the context of a phylogenetic analysis.

By using a historical approach, we were able to determine overall patterns of conservation in larval morphology and hypothesize about evolution in early ontogeny. In a reciprocal manner, because of the fairly conserved nature of larval morphologies, developmental information should be highly useful in reconstructing hypotheses of relationships and understanding evolutionary histories, particularly among more inclusive taxonomic groups.

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APPENDIX 1

SPECIMENS EXAMINED

Institutions are as follow: CAS = California Academy of Sciences; DJM = Daniel J. Meinhart personal collection; KU = The University of Kansas Natural History Museum; MNCN = Museo Nacional de Ciencias Naturales, Madrid; SMB = S. M. Brown specimen (uncatalogued at KU); TNHC = Texas Natural History Collection; UMMZ = University of Michigan. Abbreviations: G = Gosner Stage (Gosner, 1960); N&F = Nieuwkoop and Faber Stage (Nieuwkoop and Faber, 1956); W = Wilder Stage (Wilder, 1925).

Alytes obstetricans: CAS 152175 (G27–41); *Ambystoma talpoideum*: KU 204668 (WIII), 204699 (WIII), 204701 (WIII), 204712 (WVI), 204714 (WVI), 204715 (WVI), 204693 (WIV); *Ascaphus truei*: TNHC 54023 (G34), 54026 (G37), 54031 (G44); *Bombina orientalis*: KU 223508–09 (G35), 223510–15 (G36), 223516–24 (G37), 223526–32 (G38), 223533–40 (G39), 223541–46 (G40); *Discoglossus pictus*: UMMZ 143266 (G28–46); *Discoglossus sardus*: KU 222383 (G34), 222384A–B (G35), 222384C–E (G36), KU 222385A–B (G37/38), KU 222384C (G38), KU 222384D–E (G38/39), KU 222386 (G41), 222387A–B (G42); *Hyla lanciformis*: KU 205966 (G38), 205967 (G39), 205968 (G40), 205970 (G41), 205972 (G42), 205982 (G33), 205983 (G36), 205984 (G37), 205981 (G31); *Hymenochirus curtipes*: DJM 024 (N&F58), 025 (N&F55), 027 (N&F54), 029 (N&F58); *Pelobates cultripes*: MNCN 288646 (G30), 288647 (G31), 288648 (G32), 288649 (G33), 288650 (G34), 288651 (G35), 288652 (G36), 288653 (G37), 288654 (G38), 288655 (G39), 288656 (G40), 288659 (G41), 288660 (G42); *Spea bombifrons*: KU 209862 (G30), 209863 (G31), 209865 (G32), 209867 (G33), 209870 (G34), 209872 (G35), 209873 (G35), 209879 (G36), 209880 (G37), 209884 (G38), 209887 (G39), 209890 (G40), 209908 (G42); *Silurana tropicalis*: SMB 141 (N&F53), 151 (N&F57), 152 (N&F60), 168 (N&F56), 169 (N&F58), 174 (N&F56); *Pyxicephalus adspersus*: KU 220963–66 (G31), 220967–220969 (G34); *Rhinophrynus dorsalis*: KU 307147 (G32), 307145 (G38), 307154 (G33) 307155 (G34), 307156 (G35), 307157 (G36), 307158 (G37), 307169 (G38), 307160 (G39), 307161 (G40), 307167 (G42), 307168 (G41), 307175 (G40); *Xenopus borealis*: SMB 173 (N&F 55), 175 (N&F 56); *X. laevis*: KU 217919 (N&57), 217924 (N&F58), 217925 (N&F58), 217927 (N&F59), 217934 (N&F60); *X. muelleri*: KU 206507 (N&F56).

APPENDIX 2
Characters and character states derived from larval morphology.

	Character states		
Character name	0	1	2
1. Suprarostrals	Absent	Present	—
2. Position of suprarostrals	Perpendicular to longitudinal axis of chondrocranium	Parallel to longitudinal axis of chondrocranium	—
3. Suprarostral alae	Well-developed, rectangular	Well-developed, triangular	Reduced
4. Condition of dorsal margin of one side of suprarostral	Open or incomplete	Closed	—
5. Condition of ventral margin of one side of suprarostral	Open or incomplete	Closed	—
6. Condition of midline portion of corpus/ala connection	Incomplete fusion	Complete fusion	—
7. Condition of corpora of suprarostrals	Not fused at midline	Fused at midline	—
8. Barbels	Absent	Present	—
9. Adrostrals	Absent	Present	—
10. Articulation of infrarostrals at midline	Not fused	Fused	—
11. Connection of infrarostrals and Meckel's cartilages	Not cartilaginous	Cartilaginous	—
12. Admandibulars	Absent	Present	—
13. Attachment of cornua trabeculae to suprarostrals	Cartilage	Ligaments, synovial joint absent	Ligaments, synovial joint present
14. Position of attachment of cornua trabeculae to suprarostral	Attach to corpus	Attach to ala	—
15. Divergence of cornua trabeculae	Extend parallel to one another	Diverge laterally	—
16. Ethmoid plate between cornua trabeculae	Absent	Present, distinct from cornua	Present, continuous with cornua
17. Shape of cornua in dorsal view	Width consistent throughout	Terminally expanded	—
18. Palatoquadrate	Formed by a single element	Formed by anterior and posterior portions	—
19. Pars articularis quadrati/posterior margin of suprarostral cartilage	Separation more than 20%	Separation about 10%	In contact
20. Angle of suborbital cartilage relative to otic capsule	Right or oblique angle	Obtuse angle	—
21. Quadrato-ethmoidalis ligament	Ligamentous	Chondrified	—
22. Processus muscularis quadrati	Small	Large	—
23. Hyoquadrate process	Large	Reduced	—
24. Ventrolateral process of palatoquadrate	Absent	Present	—
25. Position of attachment of ascending process to braincase	Above the occulomotor foramen	Below or at the level of the occulomotor foramen	—
26. Otic process of palatoquadrate	Flattened	Rounded	—
27. Otic ligament/larval otic process	Ligamentous	Cartilaginous	—

APPENDIX 2
Continued.

Character name	Character states		
	0	1	2
28. Taenia tecti transversalis	Absent	Present	—
29. Taenia tecti medialis	Absent	Present	—
30. Length of frontoparietal fontanelle *vs.* total length chondrocranium	Less than 70%	More than 70%	—
31. Size of otic capsules	About 25% the total length of the chondrocranium	At least 40% the total length of the chondrocranium	—
32. Processus muscularis of the otic capsule	Absent	Present	—
33. Copula I	Absent	Present	—
34. Urobranchial	Single, elongate or knoblike	Bifurcated	Single, ridgelike
35. Hyobranchial spiculae	Absent	Present	—
36. Ceratobranchials	Not overlapping, simple	Overlapping, complex	—
37. Pre-metamorphic fusion of Vertebrae I and II	Absent	Present, early in development	Present, around metamorphosis
38. Development of vertebral centra	Epichordal	Perichordal	—
39. Primordia of epipubis	Absent	One	Two
40. Position of the eyes	Dorsal to palatoquadrate	Lateral to palatoquadrate	—
41. Mouthpart	Absent	Present	—

APPENDIX 3.
Matrix of larval characters.

Taxon	Characters																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ambystoma talpoideum	0	?	?	?	?	?	?	?	0	?	?	0	?	?	0	0	0	0	?	0
Alytes obstetricans	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0
Ascaphus truei	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Bombina orientalis	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0
Discoglossus sardus	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0
Hyla lanciformis	1	0	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0
Hymenochirus boettgeri	?	?	?	?	?	?	?	?	0	1	1	0	0	?	?	2	?	0	?	0
Leptodactylus fuscus	1	0	0	1	0	0	1	0	0	0	0	0	1	1	1	0	1	0	0	0
Megophrys montana	1	0	0	1	1	1	1	0	1	1	0	0	0	1	1	1	0	0	0	0
Pelobates cultripes	1	0	0	1	1	1	0	0	1	0	0	0	2	1	1	0	1	0	0	0
Pelobates fuscus	1	0	0	1	1	1	0	0	1	0	0	0	2	1	1	0	1	0	0	0
Pelodytes punctatus	1	0	0	1	1	1	0	0	1	0	0	0	2	1	1	0	0	0	0	0
Pipa parva	1	1	2	1	1	1	1	0	0	1	0	0	0	0	?	2	?	0	?	1
Pipa carvalhoi	1	1	2	1	1	1	1	0	0	1	0	0	0	0	?	2	?	0	?	1
Pyxicephalus adspersus	1	0	0	1	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0
Rhinophrynus dorsalis	1	1	0	0	1	0	1	0	0	1	1	0	0	0	?	2	?	0	1	0
Silurana tropicalis	1	1	1	1	1	1	1	1	0	1	0	0	0	0	?	2	?	1	2	1
Spea bombifrons	1	0	0	1	1	1	0	0	1	0	0	0	1	1	1	1	0	0	0	0
Spea intermontana	1	0	0	1	1	1	0	0	?	0	0	0	0	1	1	1	0	0	0	0
Xenopus borealis	1	1	1	1	1	1	1	1	0	1	0	0	0	0	?	2	?	1	1	1
Xenopus laevis	1	1	1	1	1	1	1	1	0	1	0	0	0	0	?	2	?	1	1	1
Xenopus muelleri	1	1	1	1	1	1	1	1	0	1	0	0	0	0	?	2	?	1	1	1

Appendix 3.
Continued.

	Characters																				
Taxon	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Ambystoma talpoideum	?	?	?	?	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	2
Alytes obstetricans	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1
Ascaphus truei	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	1
Bombina orientalis	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1
Discoglossus sardus	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1
Hyla lanciformis	0	1	0	0	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1
Hymenochirus boettgeri	1	0	1	0	0	0	0	0	0	1	1	0	1	2	0	0	1	0	2	1	0
Leptodactylus fuscus	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
Megophrys montana	0	1	0	0	0	0	1	0	0	0	0	0	?	0	1	0	0	1	0	0	1
Pelobates cultripes	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1
Pelobates fuscus	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1
Pelodytes punctatus	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Pipa parva	1	0	1	1	0	0	1	0	0	1	1	0	0	2	0	1	1	0	2	1	0
Pipa carvalhoi	1	0	1	1	0	0	1	0	0	1	1	0	0	2	0	1	1	0	2	1	0
Pyxicephalus adspersus	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1
Rhinophrynus dorsalis	0	0	1	0	0	0	1	0	0	0	0	0	1	2	0	1	0	1	0	1	0
Silurana tropicalis	0	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	2	0	2	1	0
Spea bombifrons	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
Spea intermontana	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
Xenopus borealis	0	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	0	0	2	1	0
Xenopus laevis	0	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	0	0	2	1	0
Xenopus muelleri	0	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	0	0	2	1	0