

Revisiting the contribution of larval characters to an analysis of phylogenetic relationships of basal anurans

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The higher-level relationships of anurans have been explored by numerous studies, producing a variety of hypotheses. The relationships of the basal anurans ('archaeobatrachians') are, however, poorly known. In part, this may be because the adult morphology of basal anurans is derived and therefore may not provide suitable phylogenetic signal. Recently, several authors have shown the phylogenetic utility of information derived from anuran larvae. In this paper we conduct separate and combined analyses of anuran relationships based on adult and larval morphology. Our combined results suggest that anurans form two major clades – the pipoids and all other frogs. Evidence also suggests that, taken together, Neobatrachia and Pelobatoidea form a monophyletic group. We discuss support for various groupings as shown by the different data sets. We also comment on the consequences of our phylogenetic hypothesis for the interpretation of reduction of vertebral elements and evolution of Orton's tadpole types in anurans. © 2003 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2003, **139**, 129–155.

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INTRODUCTION

Anurans (frogs and toads) have attracted serious attention from systematic biologists who have attempted to resolve their relationships for the past 80 years. Although Anura is modestly sized (4837 species *vide* Frost, 2002), the relationships of the major groups of frogs and toads have proven intractable to resolution, despite molecular, morphological, and developmental tools having been applied separately and in tandem to the problem.

Anurans are highly derived and specialized amphibians; they have exploited the terrestrial environment with a success that has not been remotely paralleled by either of their sister groups, the salamanders and caecilians. At the risk of over-simplification, the explanation for their evolutionary success seems to be related primarily to the evolution of saltatorial locomotion in the group. The morphological modifications

that attend this specialization have contributed to our limited understanding of their phylogenetic relationships. The major changes in the body plan that facilitate jumping preclude facile morphological comparisons with fossil and extant sister groups (e.g. *Triadobatrachus* and salamanders). The success of this body plan has resulted in an astounding overall morphological similarity among anurans, as observed by Inger (1967), and the apparent homoplasy of the morphological variation that does occur has frustrated systematists for decades.

An alternate approach to resolution of anuran relationships involved phylogenetic arrangements based on features of external and oral morphology of larvae. Orton's (1953, 1957) early work was elaborated by Starrett (1973), although these typological approaches provided few characters that were phylogenetically informative or broadly congruent with results of analyses based on adult features. Sokol (1975, 1977, 1981) undertook the first comparative studies of larval chondrocrania. His results, although promising, had limited impact because data were available for so few taxa. In the last 20 years, we have learned a great deal about the morphology of many more taxa of anuran

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larvae, thanks to the efforts of many herpetologists (e.g. Haas, 1997, 2001; Larson & de Sá, 1998; Wassersug, 1980; Altig & McDiarmid, 1999). There is significant variation in larval structure that provides characters amenable to phylogenetic analysis. Our aim here is to apply some of these characters to an analysis of the relationships of basal anurans, in concert with features of the adult skeletal morphology, to investigate the relative powers of resolution of larval characters, adult characters, and a combination of larval and adult characters.

THE STATUS OF ANURAN CLASSIFICATION

Ford & Cannatella's (1993) analysis of relationships of the major clades of anurans reveals the minimal state of our understanding of the systematics of the group. More than 95% of the species reside in a largely unresolved terminal polytomy known as the Neobatrachia, or so-called 'advanced' anurans. The remaining 5% comprises the basal anuran taxa; this relatively small group is composed of 168 living species placed in 27 genera (Frost, 2002). Most authors concur that basal anurans form a paraphyletic group (e.g. Duellman & Trueb, 1986; Ford & Cannatella, 1993), but a few (e.g. Hedges & Maxson, 1993; Hay *et al.*, 1995) suggest that it is monophyletic based on molecular evidence.

The anuran phylogenies proposed throughout the last century derive mostly from Noble's (1922, 1931) arrangement, which was based on the adult structures of the vertebral column and pectoral girdle, the nature of thigh musculature, and the presence or absence of dentition. Noble (1931) divided anurans into five suborders. Three of these, Amphicoela (*Leiopelma* and *Ascaphus*), Opisthocoela (discoglossids and pipids), and Anomocoela (pelobatoids) included basal anurans. All other frogs, including rhinophrynids, were divided into Procoela and Displasiocoela. Noble's classification was widely accepted among researchers for nearly 30 years.

Based on larval morphology, Orton (1953, 1957) divided anurans into four groups. Anurans with Type-I larvae included pipids and rhinophrynids; Type-II larvae were present in microhylids. Type-III larvae included ascaphids (*Ascaphus* and *Leiopelma*) and discoglossids, and Type-IV included all other frogs. These types were arranged in order of increasing morphological complexity and Orton (1957) suggested that they might be in phylogenetic sequence. Although Orton did not use larval types as a basis for a formal reclassification of anurans, some authors adopted her concept of four major evolutionary lineages based on larval features (e.g. Savage, 1973; Starrett, 1973). Subsequent publications (e.g. Griffiths, 1963; Griffiths & Carvalho, 1965) objected to the larval types and attempted to show their lack of phylogenetic significance. In con-

trast, Hecht (1963) considered Orton's tadpole types as the only clear-cut key to the phylogeny of anurans. Based on the larval types and information from the fossil record, Hecht (1963) proposed that frogs with Type-I larvae (pipids and rhinophrynids) were most basal, and that pelobatoids were the sister to neobatrachians. Tihen (1965), however, argued that Orton's tadpole types were not in phylogenetic order and claimed that anurans with Type-III tadpoles were the most basal, with the other types having been derived from them. He suggested that pipids were paedomorphic, and that pelobatoids were transitional between anurans with Type-III and Type-IV tadpoles. Starrett (1973) reinvestigated the larval types of Orton, and concluded that there were no exceptions to the four categories. Following Hecht's (1963) suggested phylogenetic scheme, Starrett (1973) concluded that anurans with Type-I larvae were indeed the most basal; those with Type-II larvae were slightly more advanced, and those with Types III and IV larvae were similar and represented the most advanced anurans. Sokol (1975) also analysed Orton's larval types and proposed a phylogeny of anuran larvae wherein Type-III larvae were most basal, and Type-I larvae could have evolved from either Type-III or Type-IV tadpoles.

Reig (1958) proposed the name Archaeobatrachia, within which he placed discoglossids, rhinophrynids, and pelobatoids. The remaining basal anurans were included in Amphicoela (*Ascaphus*, *Leiopelma*, and †*Notobatrachus*) and Aglossa (pipids); all other anurans were placed within the newly named group Neobatrachia. Several other authors suggested different changes to the standard classification during the late 1950s and early 1960s. Of these, the most significant was Griffiths (1963), who based his classification on characters derived from adult skeletal and tongue morphology, thigh musculature, reproduction, and development. He considered ascaphids (*Ascaphus* and *Leiopelma*), pipids, and discoglossids to be basal, and suggested that pelobatoids were intermediate between basal and advanced frogs. Griffiths (1963) also proposed that rhinophrynids were closely related to pelobatoids.

Most of the aforementioned classification schemes were derived from a single character or a character complex. Later works (e.g. Inger, 1967; Kluge & Farris, 1969; Lynch, 1973) represent progressively more rigorous attempts to incorporate more information on additional anuran taxa with the goal of resolving the historical relationships of anurans. Duellman (1975) recognized that incongruities among earlier classifications could be correlated with analyses of larval data vs. those based on adult morphology. The disparities among some studies are so extraordinary that, as Duellman (1975) pointed out, it was as if frogs and tadpoles had evolved independently. To overcome this

obstacle, he combined some of the schemes proposed by earlier authors into a formal classification of anurans, dividing them into two major groups, Archaeobatrachia (*Ascaphus*, *Leiopelma*, discoglossids, pelobatoids, and pipoids) and Neobatrachia (all other anurans). Laurent (1979, 1986) subsequently recognized a new group, Mesobatrachia, to include pelobatoids and pipoids, leaving the discoglossids and leiopelmatids in Archaeobatrachia. Although Duellman & Trueb (1986) did not recognize groups above the family level, their phylogenetic analysis presented discoglossoids (*Ascaphus*, *Leiopelma*, and discoglossids) as monophyletic and the most basal clade of anurans, and pipoids as the sister to the clade (pelobatoids + neobatrachians). Reanalysis of Duellman & Trueb's (1986) data by Hillis (1991) rendered different results, including a paraphyletic Archaeobatrachia and a basal polytomy that included pipoids, neobatrachians, and the three pelobatoid families.

Ford & Cannatella's (1993) work on the evolutionary relationships of the major clades of frogs is an amalgamation of separate studies by Cannatella (1985) on archaeobatrachians and Ford (1989) on neobatrachians. Cannatella (1985) and Ford & Cannatella (1993) recognized Archaeobatrachia, discoglossoids (*Ascaphus*, *Leiopelma*, and discoglossids), and discoglossids as paraphyletic. Both Mesobatrachia (including pelobatoids and pipoids) and Neobatrachia, in contrast, were proposed to represent monophyletic clades. Based on their results, Ford & Cannatella (1993) proposed the informal use of the term 'archaeobatrachian' for those anurans that are not part of Neobatrachia. Reanalyses of Cannatella (1985) and Ford & Cannatella's (1993) data matrices by other workers (e.g. Hillis, 1991 and Lathrop, 1997, respectively) produced results differing from Ford & Cannatella's published arrangement and included Mesobatrachia as a paraphyletic taxon.

Most of our knowledge of the higher-level relationships of anurans is founded on morphological studies, but molecular data have been used to infer relationships within less inclusive clades (e.g. Miyamoto, 1983; Green, 1986; Hillis & Davis, 1986; de Sá & Hillis, 1990; Cannatella & de Sá, 1993; Pramuk, Hass & Hedges, 2001). Hillis *et al.* (1993) conducted a broad-scale phylogenetic analysis of anurans based on molecular data that supported the monophyly of mesobatrachians plus neobatrachians ('Pipanura' *sensu* Ford & Cannatella, 1993), which in turn indicated the paraphyly of archaeobatrachians. The monophyly of the clade (pelobatoids + pipoids) also was supported. In their phylogenetic analysis of lissamphibians based on molecular data, Hedges & Maxson (1993) found support for the monophyly of both Archaeobatrachia and Neobatrachia. Similar results were found by Hay *et al.* (1995).

The most recent analyses of the evolutionary relationships of basal frogs are those of Haas (1997, 2001) and Maglia, Pügener & Trueb (2001). Haas's (1997, 2001) phylogenetic analyses were based on characters of the larval hyobranchium and mandibular arch musculature, respectively; Haas found the basal frogs (*Ascaphus* and discoglossoids) to be paraphyletic, and ascaphids to be the most basal clade. His analyses also supported pipoids as the sister to the clade (pelobatoids + neobatrachians). Using characters of both larval and adult morphology, Maglia *et al.* (2001) found support for the monophyly of Pipoidea and the combined clade formed by the paraphyletic discoglossoids, pelobatoids, and Neobatrachia. Pelobatoidea and Neobatrachia were found to be monophyletic sister taxa.

METHODS

TAXA

Larval and adult morphology were examined in 21 anuran taxa, as follows: *Ascaphus truei* (Ascaphidae); *Bombina orientalis* (Bombinatoridae); *Alytes obstetricans*, *Discoglossus sardus* (Discoglossidae); *Megophrys montana* (Megophryidae); *Pelobates cultripes*, *P. fuscus*, *Spea bombifrons*, *S. intermontana* (Pelobatidae); *Pelodytes punctatus* (Pelodytidae); *Hymenochirus boettgeri*, *Pipa carvalhoi*, *P. parva*, *Silurana tropicalis*, *Xenopus laevis*, *X. muelleri* (Pipidae); and *Rhinophrynus dorsalis* (Rhinophrynidae). The neobatrachian taxa *Hyla lanciformis* (Hylidae), *Leptodactylus fuscus* (Leptodactylidae), and *Pyxicephalus adspersus* (Ranidae) also were included in the analyses. The salamander *Ambystoma talpoideum* (Urodela: Ambystomatidae) was used as an outgroup based on the sister-group relationship between Caudata [\dagger *Karaurus* + Urodela] and Salientia [\dagger *Triadobatrachus* + Anura] proposed by several authors (e.g. Milner, 1988, 1993; Trueb & Cloutier, 1991; Cannatella & Hillis, 1993; Hay *et al.*, 1995; Báez & Basso, 1996). Specimens examined are listed in Appendix 1. The choice of taxa was a compromise between selection of representative taxa and the availability of specimens or published data that allowed us to perform the character coding. The most inclusive anuran taxonomic names, different from the family level presented above, and used throughout the Results and Discussion are as follows: (1) Archaeobatrachians = informal name used in reference to those anurans that are not part of Neobatrachia; (2) Mesobatrachia = clade that comprises the most recent common ancestor of the living Pelobatoidea and Pipoidea and all of its descendants (*sensu* Ford & Cannatella, 1993); (3) Neobatrachia = clade that comprises the most recent common ancestor of living hylids and ranoids, and all of its descendants (*sensu* Ford & Cannatella, 1993); (4)

Pelobatoidea = clade that comprises the most recent common ancestor of living megophryids, pelobatids, and *Pelodytes*, and all of its descendants (*sensu* Ford & Cannatella, 1993); (5) Pipoidea = clade that comprises the common ancestor of the rhinophrynids, extinct palaeobatrachids, and pipids, and all of its descendants (*sensu* Báez & Trueb, 1997); (6) Pipinae = clade that comprises the most recent common ancestor of the living *Hymenochirus* and *Pipa* and all its descendants; and (7) Xenopodinae = clade that comprises the most recent common ancestor of the living *Silurana* and *Xenopus* and all its descendants.

CHARACTER CODING

Forty-three characters of the larval morphology were included in this analysis. Most data were collected from ontogenetic series staged according to the developmental table of Gosner (1960) or Nieuwkoop & Faber (1956); the salamander, *Ambystoma talpoideum*, was staged according to Wilder (1925). Characters of the larval skeleton were coded from specimens representing the 'typical' larval skeleton for each taxon, i.e. the morphology of the skeleton was relatively unchanged for at least one Gosner, Nieuwkoop & Faber, or Wilder stage prior, and subsequent, to the stage examined. Specimens were cleared and double-stained for bone and cartilage following the techniques of Taylor & van Dyke (1985), Dingerkus & Uhler (1977), or Wassersug (1976). Larval data for seven taxa were coded from the literature, as follows: *Leptodactylus fuscus* (Larson & 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 & Larsen, 1998). Additional data were collected from the following publications: de Beer (1937), de Sá (1988), Moore (1989), Wiens (1989), Trueb & Hanken (1992), Púgener & Maglia (1997), Wang (1997), Maglia & Púgener (1998), de Sá & Swart (1999), and Swart & de Sá (1999). Terminology is that of Gaupp (1896), Duellman & Trueb (1986), and de Sá & Trueb (1991). Most characters are illustrated in Figures 1–9.

A total of 73 characters (Appendix 2) coded from adult skeletal morphology also was included in this analysis. Most characters have been identified in prior published studies (e.g. Cannatella & Trueb, 1988; Báez & Trueb, 1997; Báez & Púgener, 1998; Maglia, 1998), but all relevant material was reexamined; many characters were redefined and new ones were incorporated. Scoring of characters was based on dried skeletons and cleared-and-double-stained material. Twenty-seven of the 73 transformation series derived from adult specimens are multistate, and nine of the 43 transformation series derived from larval charac-

ters are multistate. In cases of inapplicability, the characters were coded as missing (?). The data matrix is presented in Appendix 3.

PHYLOGENETIC ANALYSES

Three different phylogenetic analyses were performed, as follows: (1) an analysis based only on characters derived from larval morphology; (2) an analysis based on characters from adult skeletal morphology; and (3) a combined analysis, i.e. combining both data sets. All trees were rooted using *Ambystoma talpoideum*. Maximum-parsimony analyses of the data were performed using PAUP* 4.0b8 (Swofford, 2000) with the branch-and-bound option; all transformation series were treated as unordered. The effects of equal and successive weighting schemes were explored. Under the latter, characters were weighted according to the rescaled consistency index and, when multiple most-parsimonious trees occurred, the maximum value of the index was used. In those cases in which more than one equally most-parsimonious tree resulted from a search, the strict consensus of these trees is presented, thereby depicting only those relationships that are shared among all shortest trees. The g_i statistic (Huelsenbeck, 1991; Hillis & Huelsenbeck, 1992) was used to evaluate the skewness of the tree-length frequency distributions generated from 10^5 trees randomly produced from the data. The stability of each node in the tree was assessed using bootstrap values (Felsenstein, 1985), with 500 pseudoreplicates, and Bremer decay indices (Bremer, 1988, 1994). The latter were calculated with TreeRot v. 2 (Sorenson, 1999). Bootstrap values $\geq 70\%$ are considered significant (Hillis & Bull, 1993). Analysis of character optimizations were performed using PAUP* 4.0b8 (Swofford, 2000) and MacClade 4.0 (Maddison & Maddison, 2000). Characters were optimized using ACCTRAN when various possible most-parsimonious transformations existed for a character.

RESULTS

DESCRIPTION OF LARVAL CHARACTERS

The 41 characters of the larval skeleton and two characters of the larval external morphology used in this analysis are described below. The data matrix is presented in Appendix 4.

Upper jaw cartilages

1. Suprarostrals. The suprarostal cartilages are de novo structures that support the upper beak of the tadpole (Figs 1–3). The cartilage may be single with a medial corpus flanked by lateral alae, or there may be

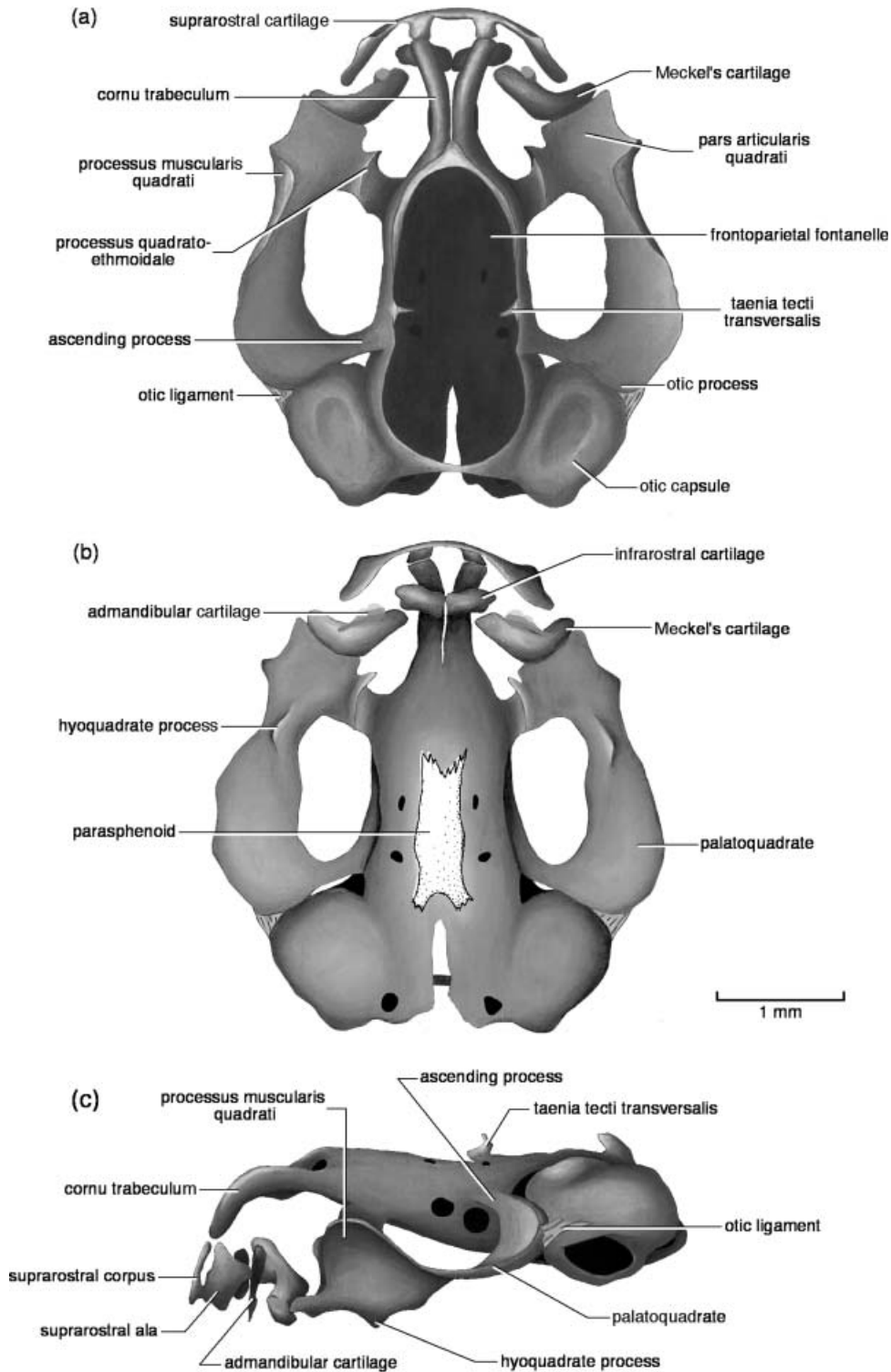


Figure 1. Larval chondrocranium of *Discoglossus sardus* (Gosner Stage 34; KU 222383) in (a) dorsal (b) ventral, and (c) lateral views. Cartilage is shown in grey, ossification in stippling, and foramina in black.

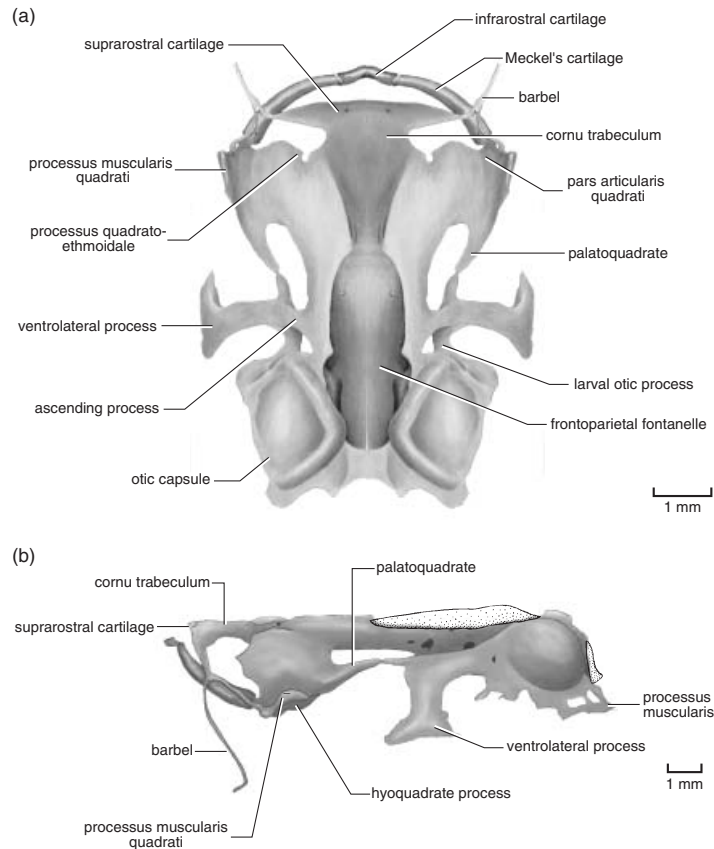


Figure 2. (a) Larval chondrocranium of *Xenopus laevis* (Nieuwkoop and Faber Stage 53; KU 217900) in dorsal view. (b) *X. borealis* (Nieuwkoop and Faber Stage 56; SMB 174) in lateral view. Cartilage is shown in grey, ossification in stippling, and foramina in black.

a pair of cartilages, each of which has a medial corpus and lateral ala. Suprarostrals are absent in salamanders (State 0), but present in most anuran larvae (State 1). De Sá & Swart (1999) reported the presence of reduced suprarostrale alae in *Hymenochirus* larvae; we located neither the alae nor a suprarostrale corpus in specimens that we examined.

2. Position of suprarostrals. In most anurans, the suprarostrale cartilages are dorsoventrally orientated, i.e. perpendicular to the longitudinal axis of the chondrocranium (State 0; Fig. 1). However, in pipoids they are orientated anteroposteriorly, i.e. parallel to the longitudinal axis of the chondrocranium (State 1; Fig. 2). This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

3. Suprarostrale alae. The suprarostrale alae are well-developed, rectangular structures (State 0; Fig. 3a–e) in all anurans except *Pipa* and the xenopodines (*Xenopus* and *Silurana*). In xenopodines, the alae are well-developed, but triangular (State 1; Fig. 3f), whereas in *Pipa*, they are greatly reduced (State 2). This charac-

ter does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

4. Dorsal margin of suprarostrale. In *Alytes*, *Ascaphus*, *Bombina*, *Discoglossus*, and *Rhinophrynus*, the dorsal margin of the suprarostrale is incomplete because the lateral alae are separated from the medial corpus (State 0). The dorsal margin is complete (State 1; Fig. 3; modified from Larson & de Sá, 1998: Character b) in *Hyla*, *Leptodactylus*, pelobatoids, pipoids (except *Hymenochirus*; Character 1), and *Pyxicephalus*. This character does not apply to *Ambystoma talpoideum* (Character 1).

5. Ventral margin of suprarostrale. In *Ascaphus*, *Hyla*, and *Leptodactylus*, the ventral margin of the suprarostrale is incomplete because the lateral alae are separated from the medial corpus (State 0). In all other taxa considered herein, the alae are connected ventrally to the corpus to form a complete ventral margin (State 1; Fig. 3; modified from Larson & de Sá, 1998: Character a). This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

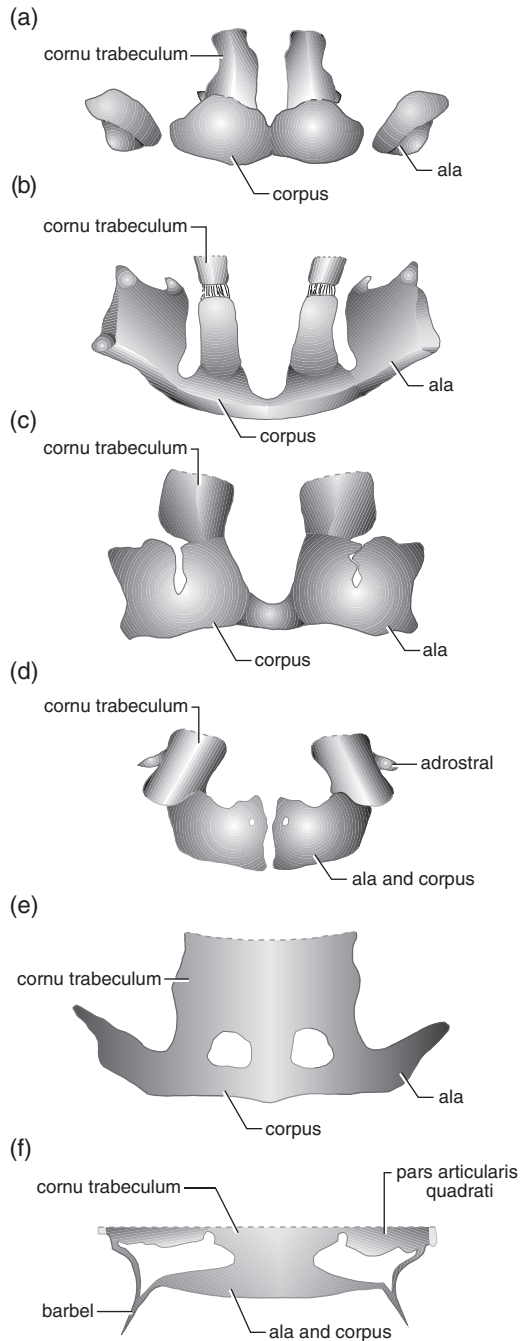


Figure 3. Anterior view of suprarostrals of (a) *Ascaphus truei* (Gosner Stage 36; TNHC 54025) (b) *Alytes obstetricans* (Gosner Stage 32/33; CAS 152175) (c) *Bombina orientalis* (Gosner Stage 35; KU 223509) (d) *Pelobates cultripes* (Gosner Stage 31; KU 110109) (e) *Rhinophrynus dorsalis* (Gosner Stage 32, KU 307147), and (f) *Xenopus laevis* (Nieuwkoop and Faber Stage 53; KU 217900). Grey denotes cartilage. Not to scale.

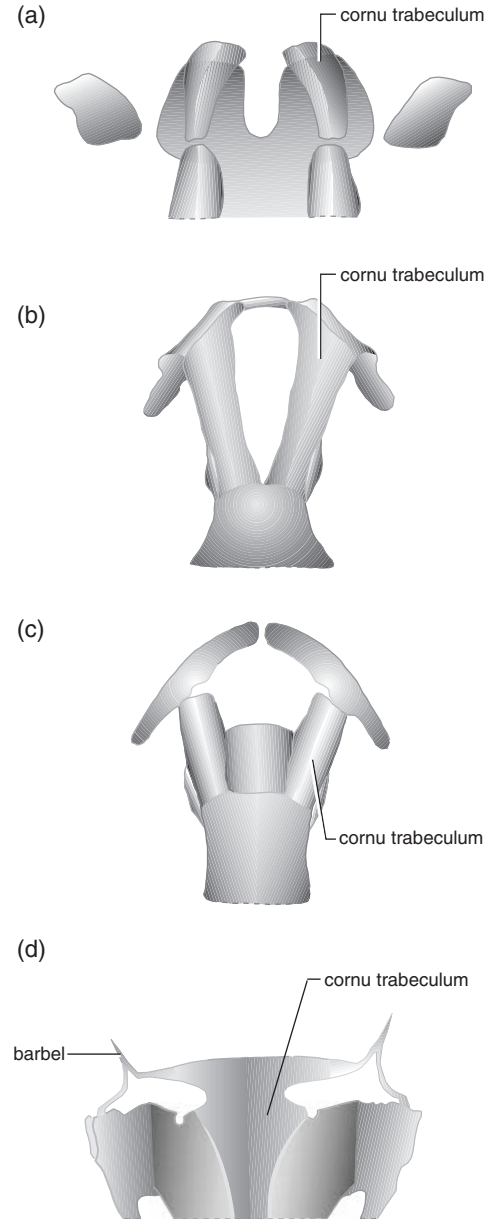


Figure 4. Dorsal view of cornua trabeculae and suprarostrals of (a) *Ascaphus truei* (Gosner Stage 36; TNHC 54025) (b) *Bombina orientalis* (Gosner Stage 35; KU 223509) (c) *Spea bombifrons* (Gosner Stage 36; KU 209876), and (d) *Xenopus laevis* (Nieuwkoop and Faber Stage 53; KU 217900). Grey denotes cartilage. Not to scale.

6. *Connection between suprarostral corpus and alae.* In *Alytes*, *Ascaphus*, *Bombina*, *Discoglossus*, *Hyla*, *Leptodactylus*, *Pyxicephalus*, and *Rhinophrynus*, the medial fusion of each suprarostral ala to the corpus is incomplete (State 0). In contrast, complete fusion between these elements produces a unipartite plate in pelobatoids and pipids (except *Hymenochirus*; Charac-

ter 3) (State 1; Fig. 3d, f). This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

7. *Corpora of suprarostrals*. The suprarostrum is represented by a pair of medially unfused corpora in *Pelodytes*, *Pelobates*, and *Spea* (State 0), whereas in other taxa, the corpus is single (State 1). This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

8. *Barbels*. Barbels (= tentacles of Nieuwkoop & Faber, 1956; Trueb & Hanken, 1992; Figs 2, 3f, 4d) are paired, filamentous structures located at each corner of the mouth. These structures are absent in all anuran larvae (State 0), except for *Silurana* and *Xenopus* (State 1). A rodlike cartilage formed by the fusion of a lateral prolongation of the suprarostrum ala and an anterior extension of the processus muscularis quadrati of the palatoquadrate supports each barbel. In addition, barbels are innervated and under muscular control (Sokol, 1977; Cannatella & Trueb, 1988). *Rhinophrynus* has a series of short, slender marginal structures around the mouth, also termed barbels by (Orton, 1943) and other authors. Lynch (1973) equated the paired barbels of xenopodines with the multiple barbels of *Rhinophrynus*. However, in the latter, these structures lack cartilaginous support, as well as muscles and nerves. Thibaudeau & Altig (1988) proposed that the structures present in *Rhinophrynus* are modified labial papillae; therefore, following Cannatella & Trueb's (1988) suggestion, we do not consider the structures around the mouth of *Rhinophrynus* to be homologues of the barbels of the xenopodines. We propose the term 'barbellae' (*sing.* barbella) for the oral structures of *Rhinophrynus* to avoid confusion with the barbels of xenopodines. Adults of *Xenopus* and *Barbourula* have a structure located at the anteroventral margin of the eye. This structure, usually called a 'tentacle', contains the terminal portion of the nasolacrimal duct; therefore, we assume that it is unrelated to the barbels observed in *Silurana* and *Xenopus* larvae. Because of the lack of anatomical specificity and the sundry structures that are termed 'tentacles' in anurans and other organisms, we recommend that the term not be applied to any structure in larval anurans. This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

9. *Adrostrals*. The paired adrostrals (= Suprarostrum 2; Hall & Larsen, 1998) are cartilaginous structures or unchondrified connective tissue masses; each adrostral is located next to the posterior margin of the suprarostrum alae (Fig. 3d). These structures are absent in *Ambystoma*, *Ascaphus*, *Bombina*, discoglossids, pipoids, and the three neobatrachian taxa included in this study (State 0), and present in pello-

batoids (State 1). Haas (1995) reported adrostrals in bufonids, dendrobatids, heleophrynids, hylids, leptodactylids, and at least some ranids; this observation suggests that the presence of this structure is a synapomorphy of a more inclusive group than Pelobatoidea. We could not determine whether adrostrals are present in *Spea intermontana* tadpoles.

Lower jaw cartilages

10. *Infrarostrals*. The infrarostral cartilages are de novo structures that support the lower beak of the tadpole (Figs 1, 2). They are absent in salamanders (State 0) and present in anuran larvae (State 1).

11. *Midline articulation of infrarostral cartilages*. Paired infrarostrals occur in *Ascaphus*, *Bombina*, discoglossids, *Hyla*, *Leptodactylus*, *Pelodytes*, *Pelobates*, *Pyxicephalus*, and *Spea* (State 0; Fig. 1b). The cartilages are fused medially to form a single structure in *Megophrys* and the pipoids (State 1; Fig. 2a). The lower jaw of *Hymenochirus* consists of a single, U-shaped cartilage. De Sá & Swart (1999) reported this mandibular cartilage to be formed by the fusion of Meckel's and infrarostral cartilages; therefore, we coded this taxon as having State 1. The character does not apply to *Ambystoma talpoideum* (Character 10).

12. *Connection of infrarostral and Meckel's cartilages*. Meckel's cartilage articulates medially with the infrarostral cartilage (Figs 1, 2) by means of either a ligament or connective tissue (State 0) in all taxa examined except *Hymenochirus* and *Rhinophrynus*, in which a cartilaginous bridge connects the two structures (State 1). This character does not apply to *Ambystoma talpoideum* (Character 10).

13. *Admandibular cartilages*. These small, paired elements (= paramandibular cartilages of de Beer, 1937) are located anterior to the anteroventral margin of Meckel's cartilages (Fig. 1). Admandibulars are absent in *Ambystoma* and all anuran larvae (State 0) except *Alytes*, *Bombina*, and *Discoglossus* (State 1).

Cornua trabeculum

14. *Attachment of cornua trabeculum and suprarostrum cartilages*. The rodlike, cartilaginous cornua trabeculum extend forward from the anterior wall of the braincase (Figs 1, 2, 4). Anteriorly, they are attached to the suprarostrum cartilages by cartilage or ligaments. A complete cartilaginous bridge (State 0) occurs in *Ascaphus*, *Bombina*, *Megophrys*, pipoids, and *Spea intermontana*. Discoglossids, *Hyla*, *Leptodactylus*, *Pyxicephalus*, and *Spea bombifrons* have a ligamentous attachment without a synovial joint (State 1), whereas *Pelodytes* and *Pelobates* have a ligamentous attachment with a synovial joint (State 2). This

character does not apply to *Ambystoma talpoideum* (Character 1).

15. *Position of attachment of cornu trabeculae to suprarostrum.* In *Ascaphus*, *Bombina*, discoglossids, and pipoids, each cornu trabeculae is attached to the corpus region of the suprarostrum cartilage (State 0; Fig. 3a–c), whereas in *Hyla*, *Leptodactylus*, pelobatoids, and *Pyxicephalus*, it is attached to the alar portion of the suprarostrum (State 1; Fig. 3d). This character does not apply to *Ambystoma talpoideum* and *Hymenochirus boettgeri* (Character 1).

16. *Ethmoid plate between cornua trabeculum.* The anterior process of the ethmoid plate (vertical anterior wall of the braincase) is a sheet of cartilage that extends anteriorly beyond the level of the planum antorbitale (Figs 1, 2, 4). In *Ambystoma* and most anuran taxa, the ethmoid plate lacks an anterior process (State 0). In *Spea* and *Megophrys*, this structure is present and distinct from the cornua trabeculum (State 1), whereas in pipoids it is continuous with the cornua (State 2).

17. *Divergence of cornua trabeculum.* In *Ambystoma*, *Ascaphus*, and discoglossids, the cornua are parallel (State 0; Fig. 4a). These structures diverge anterolaterally, describing a V-shape (State 1) in *Bombina*, *Hyla*, *Leptodactylus*, pelobatoids, and *Pyxicephalus* (Fig. 4b). It is not possible to assess the condition of the cornua trabeculum in pipoids owing to the presence of the anterior process of the ethmoid plate (Character 17).

18. *Dorsal configuration of cornua trabeculum.* The width of each cornu is uniform (State 0; Fig. 4a,c) in *Ambystoma*, *Ascaphus*, discoglossids, *Hyla*, *Megophrys*, *Pelodytes*, *Spea*, and *Pyxicephalus*, whereas in *Bombina*, *Leptodactylus*, and *Pelobates*, the cornu is expanded terminally (State 1; Fig. 4b). It is not possible to assess the condition of the cornua trabeculum in pipoids owing to the presence of the anterior process of the ethmoid plate (Character 17).

Palatoquadrate cartilage

19. *Palatoquadrate cartilage.* The paired palatoquadrates are C-shaped in dorsal aspect and located lateral to the lateral wall of the braincase. In *Ambystoma* and most anurans, each palatoquadrate is formed by a single element (State 0; Fig. 1), whereas in *Silurana* and *Xenopus*, it is composed of an anterior and a posterior element (State 1; Fig. 2).

20. *Pars articularis quadrati and posterior margin of suprarostrum cartilage.* The separation between the pars articularis quadrati of the palatoquadrate cartilage and the posterior margin of the alar portion of the suprarostrum cartilage depends on the relative length

of the cornu trabeculae. In *Ascaphus*, *Bombina*, discoglossids, *Hyla*, *Leptodactylus*, pelobatoids, and *Pyxicephalus*, the separation between these structures is more than 20% the total length of the chondrocranium (State 0; Fig. 1). In *Rhinophrynus* and *Xenopus*, the separation is about 10% the total length of the chondrocranium (State 1; Fig. 2). *Silurana* has an autapomorphic condition in which the pars articularis quadrati and the posterior margin of the suprarostrum ala are in contact (State 2). The state of this character cannot be determined in *Ambystoma* and *Hymenochirus* because of the absence of suprarostrum alae, and in *Pipa* because of the extreme reduction of these elements.

21. *Angle of suborbital cartilage relative to otic capsule.* The suborbital cartilage (= subocular bar of Trueb & Hanken, 1992) is the portion of the palatoquadrate cartilage parallel to the braincase between the commissura quadratocranialis anterior and the ascending process (Fig. 5). This cartilage forms a right or oblique angle relative to the otic capsule (State 0) in *Ambystoma*, *Ascaphus*, *Bombina*, discoglossids, *Hyla*, *Hymenochirus*, *Leptodactylus*, *Megophrys*, pelobatoids, *Pyxicephalus*, and *Rhinophrynus*. In *Pipa* and the xenopodines, the suborbital cartilage forms an obtuse angle relative to the otic capsule (State 1).

22. *Quadrato-ethmoidalis ligament.* In most anuran larvae, this ligament attaches anteriorly to a small, lateral process of the cornu trabeculae and posteriorly to the processus quadrato-ethmoidalis of the commis-

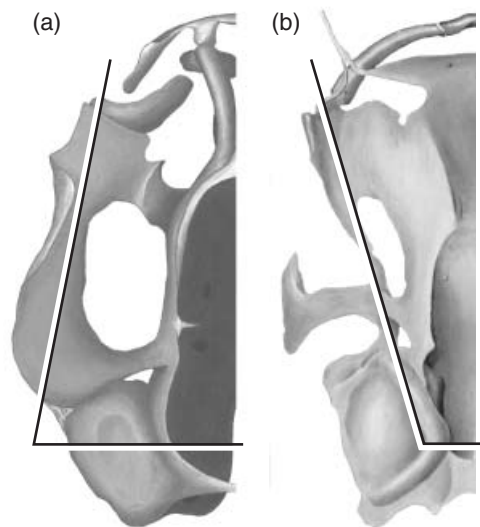


Figure 5. Dorsal view of the left side of the chondrocranium of (a) *Discoglossus sardus* (Gosner Stage 34; KU 222383) and (b) *Xenopus laevis* (Nieuwkoop and Faber Stage 53; KU 217900). Cartilage is shown in grey; solid lines illustrate the angle of the suborbital cartilage of the palatoquadrate relative to the otic capsule. Not to scale.

surra quadratocranialis of the palatoquadrate cartilage (Figs 1, 2; Sokol, 1977) (State 0). In *Hymenochirus* and *Pipa*, this element is chondrified (State 1). We could not determine the state of this character in *Ambystoma*.

23. *Processus muscularis quadrati*. This is a dorsal or dorsolateral expansion of the lateral margin of the anterior portion of the palatoquadrate cartilage (Figs 1, 2; modified from Larson & de Sá, 1998: character r). In *Ambystoma*, it is absent (State 0), while in *Ascaphus* and the pipoids, it is present but small (State 1). Sokol (1962, 1975) reported the absence of a muscular process of the palatoquadrate in *Hymenochirus*; however, we observed the presence of a reduced process in the specimens that we examined. Other anuran larvae have a large *processus muscularis quadrati* (State 2).

24. *Hyoquadrate process*. The hyoquadrate process projects ventrally from the suborbital portion of the palatoquadrate, at about the level of the pars articularis quadrati. This process bears a cotyle for the articulation with the ceratohyal cartilage of the hyobranchial skeleton (Figs 1, 2; modified from Larson & de Sá, 1998: character z). The hyoquadrate process is absent in *Ambystoma* (State 0). In most anuran larvae, it is present and large (State 1), whereas in pipoids it is present but reduced (State 2).

25. *Ventrolateral process of palatoquadrate*. In *Ambystoma* and most anurans, the posterior margin of the palatoquadrate is rounded (State 0). The palatoquadrate bears a ventrolateral process (= basal process of Kotthaus, 1933) at the level of the ascending process (i.e. the posterior connection of the palatoquadrate to the braincase) in *Pipa*, *Silurana*, and *Xenopus* (State 1; Fig. 2).

26. *Position of attachment of ascending process to braincase*. The ascending process of the palatoquadrate connects to the pila antotica, the cartilage that forms the lateral wall of the braincase between the oculomotor foramen anteriorly and the prootic foramen posteriorly (Figs 1, 2; Sokol, 1975; modified from Larson & de Sá, 1998: character n). The position of attachment varies in different taxa; in *Ambystoma* and most anuran larvae, it is above the oculomotor foramen (State 0), whereas in *Hyla*, *Leptodactylus*, and *Pyxicephalus*, it is at the level of, or below, the oculomotor foramen (State 1).

27. *Otic process of palatoquadrate*. The posterolateral corner of the palatoquadrate bears an otic process (Fig. 1). According to Sokol (1975, 1981), there are two types of otic process. A flat, platelike otic process is found in *Ambystoma*, *Ascaphus*, *Megophrys*, *Pelobates*, *Pelodytes*, and the pipoids (State 0). A vertically

curved, thick otic process occurs in *Bombina*, discoglossids, *Hyla*, *Leptodactylus*, *Pyxicephalus*, and *Spea* (State 1).

28. *Otic ligament and larval otic process*. The otic process of the palatoquadrate cartilage is attached to the anterior wall of the otic capsule by means of the otic ligament in discoglossids, *Hymenochirus*, *Leptodactylus*, *Pyxicephalus*, and *Spea* (State 0; Fig. 1). When chondrified, the otic ligament forms the 'larval otic process' (State 1; Fig. 2), as in *Ambystoma*, *Ascaphus*, *Hyla*, pipoids (except *Hymenochirus*), and pelobatoids (except *Spea*; Sokol, 1981; Maglia, 2000).

Tectum of braincase

29. *Taenia tecti transversalis*. This thin, cartilaginous bar extends across the frontoparietal fontanelle at about the level of the pila antotica, dividing the fontanelle into frontal (anterior) and parietal (posterior) portions (Fig. 1). Although absent in many anurans (State 0), the structure is present (State 1) in *Bombina*, discoglossids, *Hyla*, and *Pyxicephalus*.

30. *Taenia tecti medialis*. This thin, longitudinal cartilaginous bar extends across the midline of the parietal (posterior) portion of the frontoparietal fontanelle, dividing it into right and left portions. In most anuran larvae, this structure is absent (State 0), but it is present in *Hyla* and *Pyxicephalus* (State 1).

31. *Length of frontoparietal fontanelle relative to total length of chondrocranium*. The dorsal opening of the braincase is bordered by the taenia tecti marginalis laterally, the ethmoid region anteriorly, and the tectum synoticum posteriorly (Figs 1, 2). In *Ambystoma talpoideum* and most anuran larvae, the fontanelle is less than 70% the total length of the chondrocranium (State 0), whereas in Pipinae it is more than 70% the total length of the chondrocranium (State 1).

Otic capsules

32. *Size of otic capsules*. Most anuran larvae have otic capsules that are about 25% the total length of the chondrocranium (State 0). In Pipinae, however, the proportion is at least 40% (State 1; modified from Larson & de Sá, 1998: character i).

33. *Processus muscularis of otic capsule*. This flange of cartilage borders the lateral and posterolateral margins of the otic capsule. Medially, it is in contact with the crista parotica and posteriorly, it bears cartilaginous connections (the commissurae craniobranchiales) to the commissurae terminales of the hyobranchial apparatus (Fig. 2). The muscular process of the otic capsule is absent in all anuran larvae (State 0), except *Silurana* and *Xenopus* (State 1).

Parasphenoid

34. *Anterior end*. Typically, the parasphenoid is a T-shaped bone that invests the braincase ventrally (Fig. 1). A pair of posterolateral alae underlies the otic capsules, and the cultriform process extends anteriorly from the level of the prootic region (Duellman & Trueb, 1986). The parasphenoid is one of the first three bones to ossify (together with frontoparietals and exoccipitals), with ossification appearing near the level of the prootic foramen between Gosner Stages 30 and 36, depending upon the species and specimen. Ossification rapidly extends posteriorly to form the subotic alae (except in pipids, which lack subotic alae) and anteriorly to form the cultriform process. The incipient cultriform process is blunt anteriorly (State 0) in *Ambystoma*, *Alytes*, *Bombina*, *Discoglossus*, *Hyla*, *Leptodactylus*, and *Pyxicephalus*. In *Megophrys*, *Pelobates*, *Pelodytes*, *Pipa*, *Rhinophrynus*, *Silurana*, *Spea*, and *Xenopus*, it is acuminate (State 1), and in *Hymenochirus*, it is rounded (State 2).

Hyobranchial skeleton

35. *Copula I*. Copula I (= Basibranchial I of Duellman & Trueb, 1986; = copula anterior of Haas, 1995; = basi-hyal of Haas, 1997) is a single, median, cartilage that lies anterior to the pars reuniens (Fig. 6; from Haas, 1997: Character 2). According to Sokol (1975), Copula I is unique to anuran larvae. The element is absent in *Ambystoma*, *Ascaphus*, *Hyla*, *Leptodactylus*, *Pelodytes*, *Pipa*, *Pyxicephalus*, *Silurana*, *Spea*, and *Xenopus* (State 0) and present in discoglossids, *Pelobates*, and *Rhinophrynus* (State 1). The single median element of *Hymenochirus* consists of a slightly expanded pars reuniens fused posteriorly to Copula II; anteriorly, the pars reuniens has a robust process that might represent the fused Copula I.

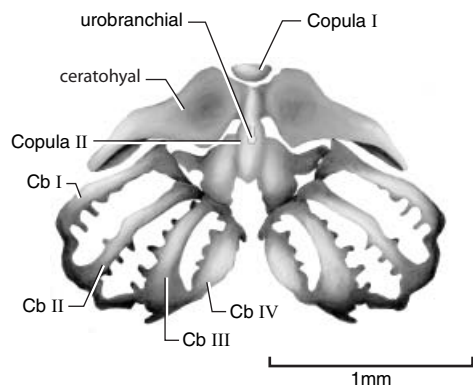


Figure 6. Larval hyobranchial skeleton of *Discoglossus sardus* (Gosner Stage 34; KU 222383). Grey denotes cartilage. Cb I–IV = Ceratobranchials I–IV.

36. *Urobranchial*. This ventral process (= urohyal of Worthington & Wake, 1971) is located at about the midline of Copula II (= Basibranchial II of Duellman & Trueb, 1986; = basibranchial of Haas, 1997 and Worthington & Wake, 1971; Fig. 6). In *Ambystoma* and most anuran larvae, the urobranchial is elongate and knoblike (State 0). In pipoids, it is ridgelike (State 1) and in *Ascaphus*, it is bifurcate (State 2).

37. *Hyobranchial spiculae*. These dorsal processes extend posteriorly from the commissurae proximales (modified from Haas, 1997: Characters 9 and 10). The spiculae are absent in *Ambystoma*, *Ascaphus*, and the pipoids (State 0), and present in the rest of the taxa (State 1).

38. *Ceratobranchials*. Four ceratobranchials usually form a basketlike framework that supports the larval gills. Each ceratobranchial is a barlike, cartilaginous structure that is associated anteriorly with the hyobranchial plate and the other ceratobranchials via the commissurae proximales. Posteriorly, the ceratobranchials are in contact with each other through the commissurae terminales (Fig. 6). In *Ambystoma* and most anuran larvae, the ceratobranchials are simple and not overlapping (State 0), whereas in pipoids, they are complex and overlapping (State 1). The exception within pipoids is *Hymenochirus*, in which the ceratobranchials are simple (i.e. State 0).

Axial skeleton

39. *Premetamorphic fusion of Presacral Vertebrae I and II*. In salamanders and most anurans, the first and second presacral vertebrae are separate, each arising from its own centre of chondrification (State 0; Fig. 7a). Presacral I bears cotyles for articulation with the occipital condyles and lacks transverse processes, whereas Presacral II bears a pair of short processes. A pair of spinal nerves leaves the spinal cord through the space between the two vertebrae. In *Hymenochirus*, *Pipa*, and *Silurana*, Presacral I has a pair of spinal nerve foramina and a pair of transverse processes, indicating fusion of Vertebrae I and II. In adult *Hymenochirus* and *Pipa*, the compound vertebra is about the same length as the posterior vertebrae; the spinal nerve foramina are minute and there is no evidence of a suture. Examination of young larvae reveals that the two vertebral centres of chondrification fuse before metamorphosis (State 1; Fig. 7b). In adult *Silurana tropicalis*, the length of the first vertebra is about twice that of any posterior vertebra; the spinal nerve foramina are larger than in *Hymenochirus* and *Pipa*, and a suture usually can be distinguished on the dorsum of the compound vertebra (State 2; Fig. 7c; Trueb, Pügener & Maglia, 2000).

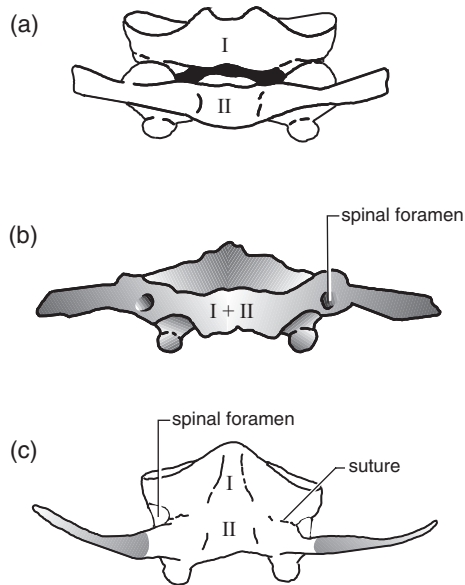


Figure 7. Ventral views of vertebrae I and II of (a) *Rhinophrynus dorsalis* (Gosner Stage 43, KU 307176) (b) *Pipa pipa* (12.5 mm, KU 204077), and (c) *Silurana tropicalis* (Nieuwkoop and Faber Stage 62, SMB 172). Not to scale.

40. Development of vertebral centra. In discoglossids, pipids, *Hyla*, *Pelobates*, and *Pelodytes*, vertebral centra ossify from the dorsal portion of the notochordal sheath (i.e. epichordal; State 0). In *Ambystoma*, *Ascapus*, *Leptodactylus*, *Megophrys*, *Pyxicephalus*, *Rhinophrynus*, and *Spea*, centra form from a sheath surrounding the entire notochord (i.e. perichordal; State 1).

41. Ribs. Of the taxa examined, free ribs are present in larvae and adults of *Alytes*, *Ambystoma*, *Ascapus*, *Bombina*, and *Discoglossus* (State 0). These ribs develop as independent centres of chondrification that later ossify. A pair of ribs is associated with each pre-sacral vertebra in *Ambystoma talpoideum*, whereas in the anuran taxa, there are only three pairs of ribs articulating with the transverse processes of Vertebrae II–IV. Free ribs also occur in the larvae of *Pipa*, *Silurana*, and *Xenopus*, but they fuse to the transverse processes around metamorphosis (State 1). In *Hyla*, *Leptodactylus*, *Megophrys*, *Pelobates*, *Pelodytes*, *Pyxicephalus*, *Rhinophrynus*, and *Spea*, free ribs are absent in larvae and adults (State 2). Cannatella (1985) coded *Hymenochirus* as having free ribs during the larval stages; however, we could not detect free ribs in the developmental series we examined.

Non-skeletal characters

42. Eyes. In *Ambystoma* and most anuran tadpoles, the eyes are dorsal to the palatoquadrate cartilage

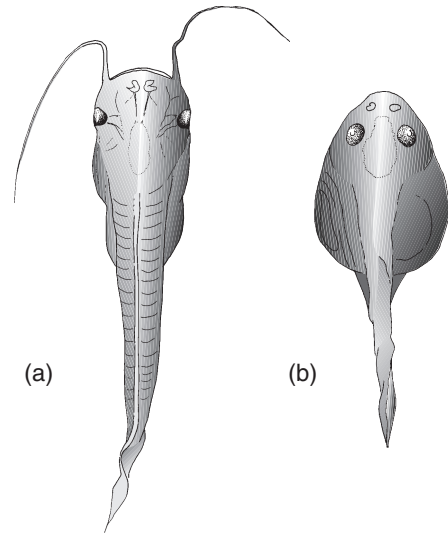


Figure 8. Dorsal view of (a) *Xenopus laevis* and (b) *Discoglossus sardus* tadpoles. Not to scale.

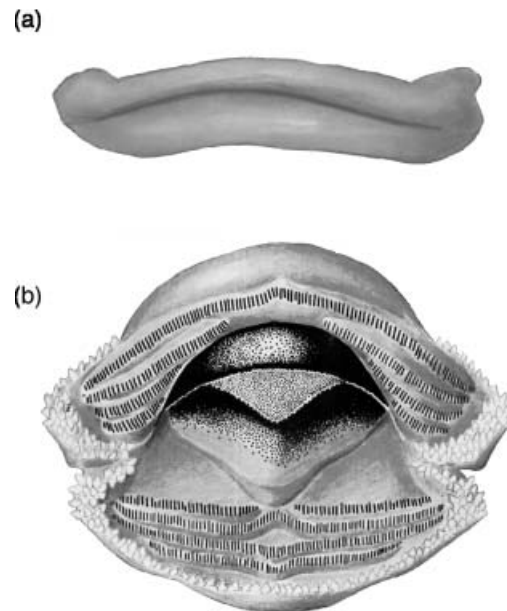


Figure 9. Anterior view of mouths of (a) *Pipa myersi* and (b) *Rana palmipes*. Not to scale.

(State 0), but in pipoids, they are lateral to the palatoquadrate (State 1; Fig. 8).

43. Oral keratinous structures. Following the terminology proposed by Altig & McDiarmid (1999), we use 'oral apparatus' to refer to the oral disc and its associated keratinized and soft structures. Oral keratinous structures (i.e. jaw sheath and labial

teeth) are absent in pipoid tadpoles (State 0; Fig. 9a). All other anuran taxa included in this analysis bear keratinous structures associated to their oral apparatus (State 1; Fig. 9b). *Ambystoma* larvae have jaw sheaths, but these seem to differ structurally from those of anuran larvae (Altig & McDiarmid, 1999). Thus, we do not consider the jaw sheath of *Ambystoma* homologous to those of anuran tadpoles (State 2).

PHYLOGENETIC HYPOTHESES

The phylogenetic analysis based on larval characters produced 12 most parsimonious trees of 84 steps (TL); the retention index (RI) is 0.83 and the consistency index (CI) is 0.64, and the rescaled consistency index (RC) is 0.53. After successive weighting of the characters according to the RC, the analysis produced eight most parsimonious trees of 46 steps (RI = 0.93; CI = 0.84; RC = 0.78). The analysis based on adult characters resulted in three most parsimonious trees of 214 steps (RI = 0.74; CI = 0.50; RC = 0.37). After successive weighting of the characters, the analysis produced one most parsimonious tree of 78 steps (RI = 0.86; CI = 0.68; RC = 0.58). Finally, simultaneous analysis of all data resulted in one most parsimonious tree of 304 steps (RI = 0.76; CI = 0.53; RC = 0.40). After successive weighting of the characters, the number of steps was reduced to 121 (RI = 0.88; CI = 0.72; RC = 0.63). The latter (Fig. 10) shows total resolution and reasonable Bremer and bootstrap support at most nodes; two major clades occur at the base of the tree – Pipoidea and a clade containing the remaining taxa. This basal split also was recovered by the analysis of adult characters (Fig. 11b). However, in the tree resulting from analysis of larval characters (Fig. 11a), the basal dichotomy differs markedly in the position of *Ascaphus*, which is the sister taxon of pipoids, instead of discoglossids, pelobatoids, and neobatrachians. The frequency distributions of tree lengths in all analyses were highly left skewed, with g_1 scores of -0.68 (larval characters), -0.77 (adult characters), and -0.79 (combined data sets). These values are well beyond the $P = 0.05$ and 0.01 significance levels (Hillis & Huelsenbeck, 1992), suggesting considerable 'hierarchical' signal in the data sets.

Each analysis established the monophyly of the following lineages: (1) the clade formed by *Bombina*, discoglossids, neobatrachians, and pelobatoids; (2) pelobatoids; and (3) pipoids. Each of these clades is supported by strong bootstrap values and Bremer indices in the combined tree. Other portions of the combined tree, however, are present in only one of the topologies obtained based on the data partitions. For example, a monophyletic [*Alytes* + *Bombina* + *Disco-*

glossus] was found by the analysis based on larval characters, but was not supported by the adult dataset. In contrast, the position of *Ascaphus* as the sister taxon to the clade formed by *Bombina*, discoglossids, pelobatoids, and neobatrachians, and the sister relationship between *Megophrys* and *Pelobates* were recovered only by the phylogeny based on adult characters.

The phylogenetic positions of *Ascaphus truei*, *Bombina orientalis*, *Hyla lanciformis*, *Megophrys montana*, and *Pelodytes punctatus*, are problematic when comparing the tree topologies obtained based on the different data partitions and the combined data. *Ascaphus* appears as the sister taxon to the clade formed by all anurans, except pipoids, in the phylogenies based on combined data as well as adult characters (Figs 10, 11b). In both cases, there is robust support for the position held by *Ascaphus*. In contrast, in the phylogeny based on larval characters, *Ascaphus* appears as the sister taxon to pipoids (Fig. 11a). The latter arrangement, however, has weak support. *Bombina* is closely related to *Alytes* and *Discoglossus* in a polytomy or sister-group arrangement in the phylogenies based on combined data and larval characters (Figs 10, 11a). However, in the phylogeny based on adult characters, *Bombina* is the sister taxon to the clade formed by *Alytes*, *Discoglossus*, and [Neobatrachia + Pelobatoidea]. All of these arrangements are supported by relatively high bootstrap values, but that generated by larval characters has the best Bremer support. The monophyly of Neobatrachia was recovered by the analyses based on data partitions (Fig. 11), whereas the analysis based on combined data shows *Hyla* as the sister taxon to pelobatoids. None of these arrangements, however, is strongly supported. The ambiguity probably reflects the limited sample of neobatrachians, along with the equivocal relationships among the pelobatoids. *Megophrys* is the sister taxon of *Pelobates* in the tree topologies based on combined data and adult characters, and has high Bremer and bootstrap support in both cases (Figs 10, 11b). But in the phylogeny based on larval characters, *Megophrys* appears in a polytomy with *Spea bombifrons* and *S. intermontana* (Fig. 11a). *Pelodytes* has a slightly different position in the three phylogenetic hypotheses; it is the sister taxon of [*Megophrys* + *Pelobates*] in the topology based on combined characters (Fig. 10), the sister taxon of *Pelobates* in the tree based on larval characters (Fig. 11a), and the sister taxon of [[*Megophrys* + *Pelobates*] + *Spea*] in the topology based on adult characters (Fig. 11b). Bremer indices are the same for all arrangements, but bootstrap values give better support to the grouping generated by larval characters.

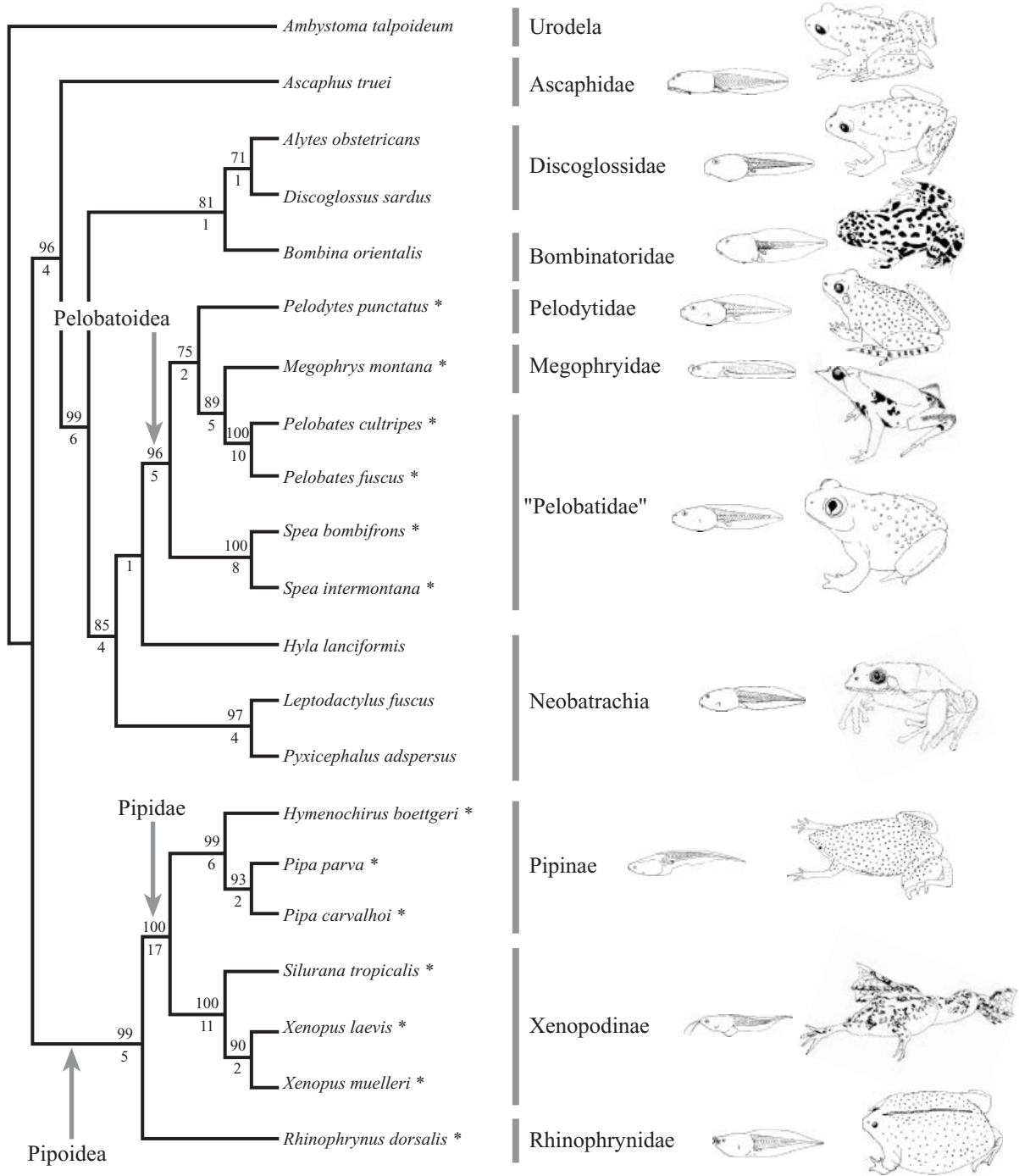


Figure 10. Most parsimonious tree based on 43 characters from larval morphology and 73 from adult skeletal morphology (TL = 121; RI = 0.88; CI = 0.72; RC = 0.63 after successive weighting of the characters). Bootstrap values are shown above each node and Bremer decay indices are presented beneath. Asterisks indicate taxa included in Mesobatrachia *sensu* Ford & Cannatella (1993).

DISCUSSION

The most profound difference between the phylogenetic arrangement proposed herein and other recent hypotheses based on living anurans is in the place-

ment of the pipoids. The group was considered a sister taxon to pelobatoids + neobatrachians by Inger (1967), Kluge & Farris (1969), Lynch (1973), and Duellman & Trueb (1986), whereas more recent studies proposed pipoids + pelobatoids to constitute

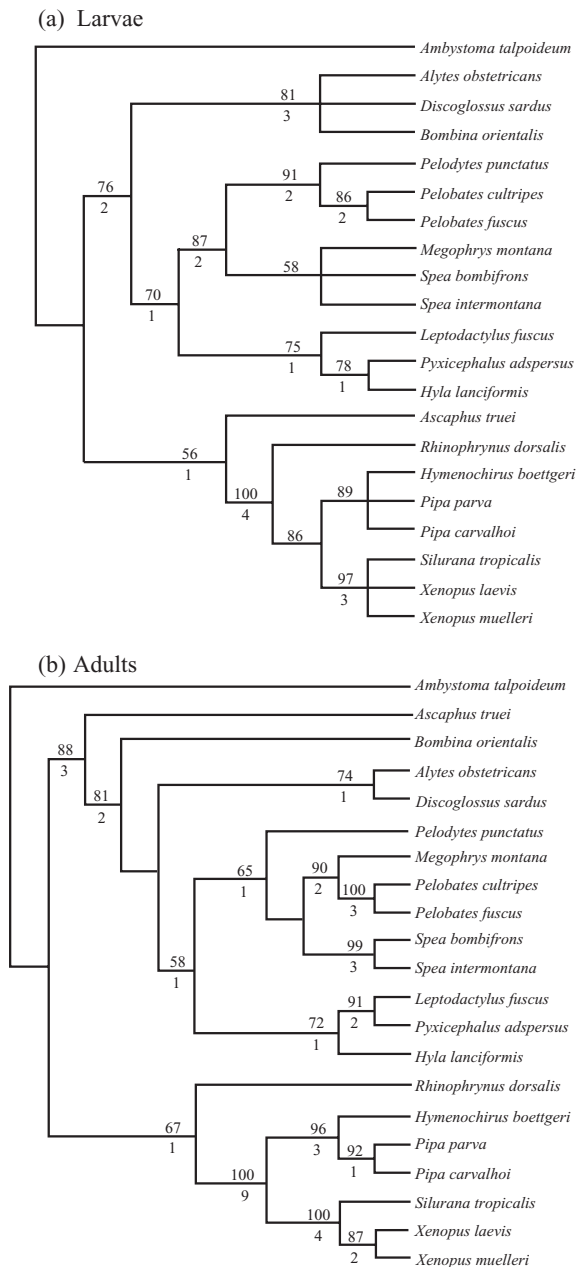


Figure 11. (a) Strict consensus of eight most parsimonious trees based on 43 larval characters (TL = 46; RI = 0.93; CI = 0.84; RC = 0.78, after successive weighting of the characters). (b) Most parsimonious tree based on adult skeletal characters (TL = 78; RI = 0.86; CI = 0.68; RC = 0.58, after successive weighting of the characters). Bootstrap values are shown above each node and Bremer decay indices are presented beneath.

the sister clade to neobatrachians (Cannatella, 1985; Ford & Cannatella, 1993; Hillis *et al.*, 1993; Maglia, 2000). In contrast, our results indicate that pipoids are the sister clade of *Ascaphus* + all other

anurans. In addition, our results, like those of Haas (1997), fail to recover a well-supported, monophyletic Neobatrachia. This suggests that future analyses need to address the monophyly of Neobatrachia and relationships between advanced and pelobatoid anurans.

TAXONOMIC CONSIDERATIONS

Given the phylogenetic arrangement proposed herein, we recommend some modifications to common anuran nomenclature. The monophyly of Mesobatrachia, as proposed by Ford & Cannatella (1993), has been largely unchallenged. However, Hillis (1991) demonstrated Pelobatoidea to be paraphyletic, with Neobatrachia nested within the group. Our results indicate a basal position for pipoids and a sister relationship between Pelobatoidea and Neobatrachia; therefore, we suggest that the term 'Mesobatrachia' be used with caution. We found no evidence of the monophyly of Archaeobatrachia; this is in agreement with Ford & Cannatella's (1993) results, but is contrary to the findings of Hedges & Maxson (1993) and Hay *et al.* (1995) based on molecular analyses. Therefore, we suggest the names 'Archaeobatrachia' and 'archaeobatrachians' should not be used. Rather, we recommend referring to indisputably monophyletic groups by name (e.g. pipoids, discoglossoids), or simply referring to anurans outside of the clade Neobatrachia + Pelobatoidea as 'basal anurans.' At the moment, we choose not to suggest a name for the group *Ascaphus* + all other anurans minus Pipoidea, because until the relationships of anurans are resolved completely, recognition of a new name potentially adds more confusion than clarity. Likewise, we note the paraphyly of Pelobatidae with respect to Megophryidae, but prefer not to make a formal taxonomic change based on these analyses of a limited number of taxa in an historically problematic group.

INTERPRETATION OF CHARACTERS

The arrangement proposed herein has interesting consequences for the interpretations of evolutionary patterns of morphological characters and complexes that, classically, have been used to interpret relationships of the major groups of anurans. Kluge & Farris (1969) provided the best historical summary and explanation of these characters. Two of these features are especially worthy of consideration, given our emphasis on larvae: the development of the vertebral column and the revision of the evolutionary patterns of tadpole structures based on the larval types as defined by Orton (1953).

Presacral vertebrae

It is generally agreed that there is a trend toward decreasing the number of presacral vertebrae from 14 in †*Triadobatrachus massinoti*, the sister taxon of Anura, to nine or ten in †*Vieraella herbstii*, and nine in †*Notobatrachus degiustoi* and *Ascaphus truei*, arguably the most primitive of the anurans (Báez & Basso, 1996). As pointed out by Lynch (1973), all other families have eight. However, the first and second presacrals may be fused or the last presacral may fuse to the sacrum, resulting in a functional reduction to seven or fewer independent vertebrae. In bufonids and pipids there may be as few as five presacrals, owing to the fusion of anterior presacrals and incorporation of posterior presacrals into the sacrum. In the case of most frogs and toads having fewer than eight presacrals, synostosis is evidenced by the presence of multiple pairs of spinal nerve foramina and the increased size of fused elements. In Pipinae (*Hymenochirus*, *Pseudhymenochirus*, and *Pipa*), reduction occurs by synchondrosis of the anterior vertebrae early in larval development, with the result that the cervical vertebra is short (Fig. 12: Character 39). Thus, shortening of the vertebral column apparently has occurred independently in several anuran lineages; however, the developmental mechanism in some pipid frogs seems to be unique. This, along with the elaboration of the pre- and postzygapophyses and complex neural arches to form interlocking mechanisms between adjacent vertebrae in xenopodines (*Xenopus* and *Silurana*) suggests that vertebral modifications in this clade may have evolved along an evolutionary trajectory quite independent of those of the other anuran lineages.

Orton's tadpole types revisited

The basal dichotomy between pipoids and all other anurans in our tree does not support the contention of several authors (e.g. Orton, 1957; Hecht, 1963; Savage, 1973; Starrett, 1973), who visualized evolution proceeding from simple to more complex morphologies; neither does it corroborate the assertions of other authors (e.g. Ford & Cannatella, 1993; Lynch, 1973; Sokol, 1975) that larval Type III most closely resembles the tadpole of the hypothetical ancestral anuran. The following discussion is based primarily on the concepts elaborated by the contrasting studies of Sokol (1975) and Starrett (1973). Cannatella's (1999) statement that the continued acceptance of Orton's tadpole types diverts attention from the issue of evolution of larval structures is probably true. However, our understanding of this part of the life cycle of anurans is still poor and the available data, at least with regard to larval Types I, III, and IV, do not seem to contradict Orton's (1957) definitions when broadly taken. To facilitate discussion, we use Type I to refer to

pipoid larvae, Type III in reference to *Ascaphus* and discoglossoid tadpoles, and Type IV for the larvae of the rest of the anurans included in our study.

Starrett (1973) assumed that structural simplicity is plesiomorphic and argued that the lack of keratinous oral structures (Fig. 12: Character 43) and the presence of a simpler skeletal system (e.g. fused suprarostrals [Character 6]; elongate Meckel's cartilage; small, fused infrarostrals [Character 11]) of pipoid larvae are plesiomorphic for anurans (Cannatella, 1999); therefore, she proposed that larval Types II, III, and IV evolved from larval Type I. However, Sokol (1975) argued that there is no compelling reason to think that the lack of keratinous mouth structures is a primitive feature of anuran larvae, even though the absence of keratinized oral parts in salamanders and caecilians would suggest the plesiomorphy of this character.

In addition, Sokol (1975) rejected the notion of structural simplicity as being plesiomorphic in anuran larvae; he argued that because fusion of the cartilaginous elements prevents mobility of the jaws (e.g. in some Type-III tadpoles), it is apomorphic. Following Sokol's (1975) line of reasoning, the straight, fused suprarostal (Fig. 12: Character 6) and the small, fused infrarostral cartilages (Fig. 12: Character 11) would derive from taxa having larvae with beak-bearing cartilages.

However, Sokol (1975) also acknowledged that, based on anatomical comparison, it is difficult to decide whether suprarostal and infrarostral cartilages originated as a support for the beak or as a 'stiffener' for the upper and lower lips. Because suprarostal and infrarostral cartilages are de novo structures of anuran larvae, the results of our analysis do not provide insights as to their probable morphology and function in the common ancestral tadpole. Based on outgroup comparison, however, we can estimate that the first anuran larva probably lacked keratinized mouth structures. Furthermore, if we follow Sokol's (1975) assumption that unfused rostral cartilages promote mobility of the mouthparts, then it seems likely that the first tadpole had at least partially fused cartilages that stiffened the lips; these cartilages underwent little or no change in the lineage that gave rise to pipoids, whereas they evolved to bear alae and corpora in the other lineage.

The larval feeding mode and its relation to respiration was one of the key aspects of Starrett's (1973) interpretation of the evolutionary history of anurans based on Orton's (1953) tadpole types. Starrett considered filter feeding to be the primitive mode of procuring food, because the larvae pump water into the mouth, through the pharynx, and over the gills, thereby entrapping food particles with their specialized buccopharyngeal filter apparatus. Thus, both feeding and

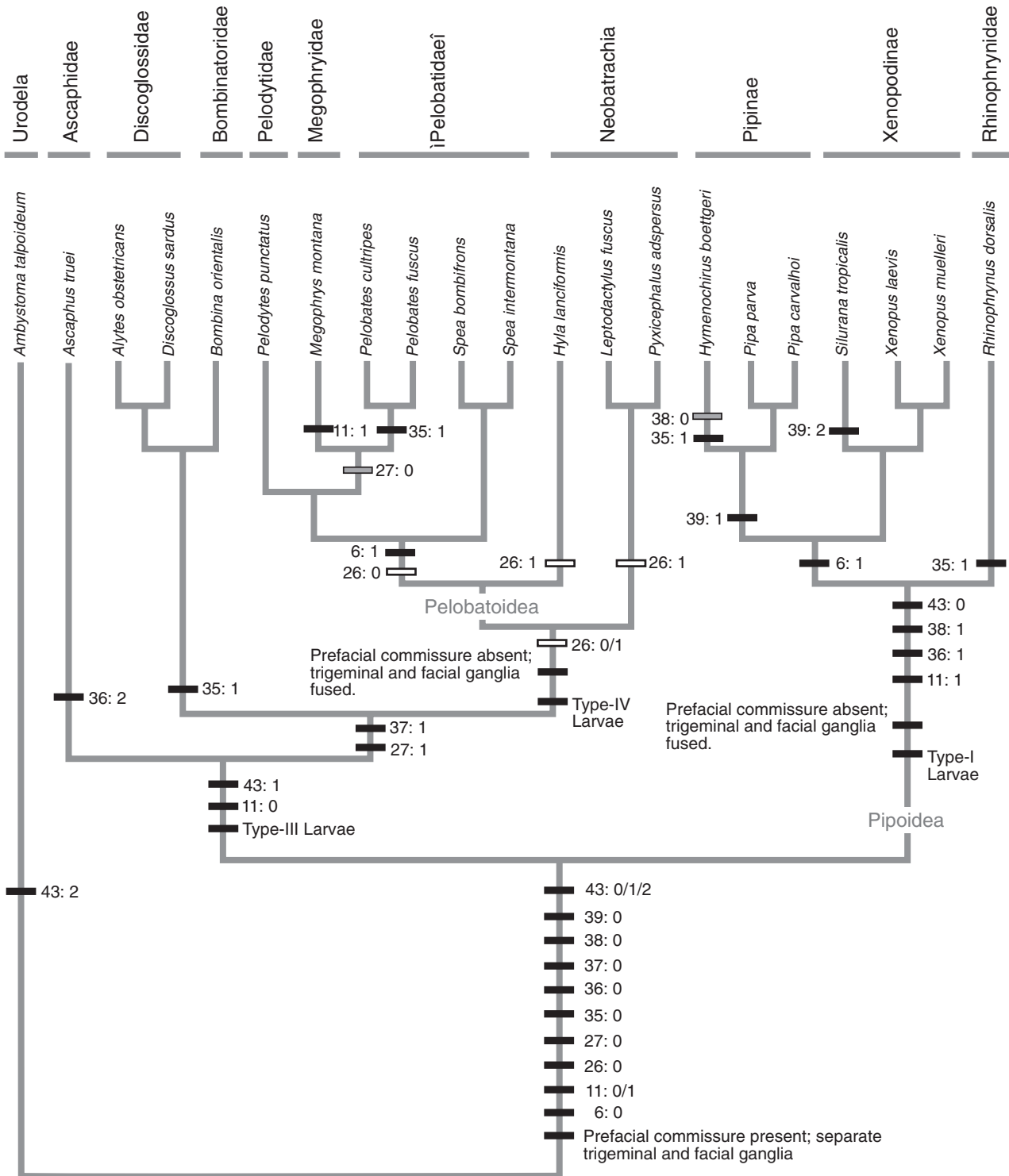


Figure 12. Most parsimonious tree (Fig. 10) with characters addressed in Discussion mapped on tree. Characters in italics are homoplastic. Those with grey bars are reversals, and the states of characters associated with white bars are equivocal.

respiration would involve the same jaw actions. Because some pipoid larvae filter feed, Starrett (1973) suggested that the basal anuran larva might have resembled Recent pipoid larvae. She also claimed that the evolution of keratinized mouthparts contributed to the functional separation of feeding and respiration.

However, most tadpoles are filter feeders (Wassersug, 1972); larvae lacking keratinized mouthparts filter particles suspended in the water column and those with keratinized oral structures scrape food from the substrate and filter the suspension (Viertel & Richter, 1999). Moreover, it seems that, at least in *Xenopus*, the feeding capability does not differ significantly from the typical beaked larvae (Kenny, 1969; Wassersug, 1972). In addition, and as correctly pointed out by Cannatella (1999), feeding in pipoid larvae is more diverse than Starrett (1973) recognized. The larvae of *Rhinophrynus* – the most basal living taxon within Pipoidea (Cannatella & de Sá, 1993; Báez & Trueb, 1997; Báez & Púgener, 1998) – are known to feed not only microphagously, but also macrophagously on other tadpoles (Stuart, 1961; Satel & Wassersug, 1981; Swart & de Sá, 1999).

Within Pipidae, *Silurana* and *Xenopus* have an extreme microphagous condition. Free-swimming *Pipa* larvae are adapted to feed on medium-sized planktonic organisms; the direct-developing *Pipa* species do not feed as larvae. *Hymenochirus* is a carnivore, specialized for feeding on large planktonic organisms (Sokol, 1977; Wassersug, 1980; Cannatella, 1999). Thus, the variation of feeding modes observed among pipoid tadpoles parallels that present among the larvae of the rest of the anurans. *Hymenochirus*, *Pipa* (Sokol, 1977), *Rhinophrynus* (Sokol, 1975), and *Xenopus* (Gradwell, 1971; Sokol, 1977; Wassersug, 1996) larvae lack persistent (= internal) gills and are obligate air breathers; the latter is made possible by the early ontogenetic differentiation of lungs (Viertel & Richter, 1999). As a consequence, movement of water through the mouth does not have a respiratory function (Cannatella, 1999).

Sokol (1975) considered persistent gills to be de novo structures of anuran larvae; these gills develop as ventral expansions of the transient (= external) gills, which disappear during the earliest feeding stages (Schmalhausen, 1968; Viertel & Richter, 1999). Sokol (1975) assumed that the presence of gills on all four branchial arches is plesiomorphic among anuran larvae. However, he noted that the absence of persistent gills also could be interpreted as a genuinely plesiomorphic character, but rejected this hypothesis because pipoid larvae lack well-developed transient gills. Sokol (1975) reasoned that the poor development of transient gills probably triggered the suppression of the development of persistent gills. Thus, the absence of persistent gills in pipoids would repre-

sent a secondary loss and should be considered a derived condition.

Based on the results of our analysis, we can infer that the first tadpole was a filter feeder because this feeding mode is present in most tadpoles and is a feature of the basal taxa of both major clades (i.e. pipoids and the remaining anurans). In addition, this ancestral larva probably had transient gills, but lacked persistent gills; the latter probably evolved later on the lineage that includes all nonpipoid anurans.

The hyobranchial skeleton of anuran larvae consists of a pair of anterior ceratohyals that articulate with the palatoquadrates and are joined to the branchial baskets by cartilaginous basihyobranchial elements. Copula I, a structure unique to anuran larvae, occurs in the tadpoles of most anuran taxa, but is absent in all pipoids except *Rhinophrynus* and *Hymenochirus* (Fig. 12: Character 35). Sokol (1975) assumed the presence of Copula I most likely to be plesiomorphic. Hypobranchial elements of salamanders are usually connected by ligaments; Sokol (1975) therefore inferred that the presence of the ligamentous attachments between the ceratobranchials and hypobranchial plates in the branchial baskets of some Type-III larvae is plesiomorphic, whereas the fusion of these elements in pipid larvae is derived. However, *Rhinophrynus* larvae, overall, are the most generalized Type-I larvae and their filter apparatus resembles in certain aspects those of beaked larvae (Sokol, 1977; Wassersug, 1980).

Sokol's (1975) hypothesis that the larval hyobranchial apparatus of Type-III larvae retains the most numerous plesiomorphic characters among anurans is supported by Haas's (1997) phylogenetic analysis based on characters derived from the larval hyobranchial apparatus. Our phylogenetic arrangement also supports the hypothesis that the hyobranchial apparatus of the ancestral larval anuran probably resembled those of *Ascaphus*, discoglossoids, and *Rhinophrynus*, but offers a different explanation for the evolutionary history of the hyobranchial apparatus than that presented by Haas (1997). The hyobranchial apparatus was significantly modified twice. One modification occurred in the pipoid lineage (Fig. 12: Characters 35, 36, and 38), and resulted in larvae that inhabit the middle of the water column in lotic situations and, in some cases, are limited to filter feeding on fine suspended particulate matter. Another modification of the hyobranchial apparatus occurred in the lineage that includes the rest of the anurans, and generated a wide diversity of larvae adapted to macrophagous feeding in a variety of aquatic habitats (Fig. 12: Characters 36, 37).

In salamanders, the trigeminal and facial ganglia are separated, and a median wall of the otic capsule (the prefacial commissure) divides the trigeminal fora-

men for the trigeminal nerve rami from the palatine foramen for the palatine ramus of the facial nerve (Pusey, 1938; Sokol, 1981; Púgener & Maglia, 1997). When the trigeminal and facial ganglia fuse, the pre-facial commissure is lost and a single prootic foramen is present (Pusey, 1938; Sokol, 1975).

In anurans, a cartilaginous or bony pre-facial commissure is present in *Ascaphus* (de Villiers, 1934; Pusey, 1938), *Barbourula* (pers. obs.), *Bombina* (Cannatella, 1999; pers. obs.), *Discoglossus* (Púgener & Maglia, 1997), and *Leiopelma* (Wagner, 1934; Pusey, 1938). *Alytes* also has a pre-facial commissure, but apparently in this taxon, the trigeminal and facial ganglia are partially fused into a prootic ganglion (Pusey, 1938). Sokol (1975) suggested that all anuran tadpoles, except for the aforementioned, lack a pre-facial commissure and have a single prootic ganglion and only the prootic foramen. Furthermore, Cannatella (1999) identified the absence of the pre-facial commissure as a synapomorphy of Pipanura ([Pelobatoidea + Pipoidea] + Neobatrachia, *sensu* Ford & Cannatella, 1993). However, a pre-facial commissure unquestionably is present in the living taxa *Xenopus clivii* and *X. muelleri* (pers. obs.), and the fossil *Shelania laurenti* (Báez & Púgener, 1998) and *X. romeri* (Estes, 1975a,b).

Our phylogenetic arrangement (Fig. 12) suggests that the ancestral tadpole had a pre-facial commissure separating trigeminal and palatine foramina and separate trigeminal and facial ganglia, and that the disappearance of the pre-facial commissure and fusion of the ganglia occurred independently in the two basal anuran lineages. This hypothesis seems to be supported by Wassersug's (1980) investigations on the internal oral features of anuran larvae. He suggested that the fusion of the fifth and seventh ganglia is an indirect result of an anterior displacement of the palatoquadrate bar and an expansion of the ceratohyal in nondiscoglossoid larvae. Based on the differences in the form of the palatoquadrate in pipoid and nonpipoid, nondiscoglossoid tadpoles, he concluded that it could be possible that the modifications of these cartilaginous elements, as well as the changes in the ceratohyal and consequent fusion of trigeminal and facial ganglia evolved twice, once in the pipoid clade and a second time in the clade that includes all remaining anurans.

The attachments of the posterior end of the palatoquadrate to the otic capsule and the neurocranium constitute the palatoquadrate suspensorium. Sokol (1975) determined that two basic types of suspensorium are found among anurans: a high suspensorium, found in *Ascaphus* and discoglossoids, and a low suspensorium found in most other nondiscoglossoid, nonpipoid anurans. Pipoids, according to this author, have a derived high suspensorium. We considered that the

suspensorium should not be analysed as a whole but as three different characters: (1) position of the attachment of the ascending process of the palatoquadrate to the braincase; (2) condition of the otic process of the palatoquadrate; and (3) condition of the attachment of the otic process of the palatoquadrate to the wall of the otic capsule (i.e. presence of an otic ligament or a larval otic process). The results of our analysis suggest that the ancestral anuran larva presumably possessed a high attachment of the ascending process of the palatoquadrate cartilage (i.e. above the oculomotor foramen) and a flat otic process of the palatoquadrate that probably was connected to the anterior wall of the otic capsule through a 'larval otic process' (Fig. 12: Characters 26, 27). Our results also indicate that in the hypothetical ancestral tadpole, the eyes were probably located dorsally on the head. In addition, the palatoquadrate cartilage had a small processus muscularis quadrati. The frontoparietal fontanelle lacked cartilaginous bars (i.e. taenia tecti transversalis and medialis), and free ribs were present.

In summary, our phylogenetic arrangement suggests that both discoglossoid and pipoid tadpoles retain numerous plesiomorphic features. We think that the common ancestor to all anurans had a larva that was, in many respects, intermediate between the typical Type-I and Type-III larvae, and that all anurans with Type-IV larvae (i.e. pelobatoids and most neobatrachians) arose from an ancestor with a Type-III tadpole. We did not include taxa with a Type-II larva in our study. It is noteworthy that the hypothesis of an ancestral tadpole with intermediate characteristics between those of Type-I and Type-III larvae is supported by the studies of Viertel (1991) and Viertel & Richter (1999) on the buccopharyngeal system of anuran larvae. Furthermore, some of the findings reported by Haas (2001) on the mandibular arch musculature of anuran tadpoles seem to provide additional support for our hypothesis.

The a priori notion that pipoid larvae and adults are so 'aberrant' that they could not be regarded as basal has guided most of the research on anuran evolution for the last 40 years. This preconception has probably been driven, at least in part, by the fact that most living anurans have tadpoles with oral keratinous structures (Types III and IV). Most of these larvae develop into terrestrial adults, whereas beakless larvae (Type I), usually metamorphose into aquatic adults; such anurans are a minority and therefore unusual. In addition, the most recent phylogenetic analyses based on adult characters indicate that the discoglossoids, and hence Type-III tadpoles, are plesiomorphic and pipoids with their Type-I larvae are derived. The results presented herein demonstrate, for the first time in the context of a phylogenetic analysis, that there was an early divergence of anurans reflected in

both the morphology of the adults and in the larvae; one clade evolved into aquatic adults with a unique vertebral column especially adapted for such environment and larvae feeding on suspended food particles, and the second clade gave rise to terrestrial, jumping frogs and larvae that are able to scrape or bite food from the substrate. Thus, our study resolves the long-lasting incongruence between the phylogenies derived from adult characters and those based on larval characters, and provides an answer to Sokol's (1975 : 21) question: 'Why, in view of the wide range of food items available to beaked larvae, should beakless forms evolve?'

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APPENDIX 1

Specimens examined. Abbreviations: A = adult; G = Gosner Stage (Gosner, 1960); N & F = Nieuwkoop and Faber Stage (Nieuwkoop & Faber, 1956); W = Wilder Stage (Wilder, 1925). Codes for institutional collections: CAS = California Academy of Sciences; DJM = D. J. Meinhardt specimen (uncatalogued at KU); 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; and UMMZ = University of Michigan Museum of Zoology.

LARVAL SPECIMENS

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), 222384 A–B (G35), 222384 C–E (G36), KU 222385 A–B (G37/38), KU 222384 C (G38), KU 222384 D–E (G38/39), KU 222386 (G41), 222387 A–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 220958, 220959, 220972 (G31), 220944–220946 (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 laevis*: KU 217919 (N & 57), 217924 (N & F58), 217925 (N &

F58), 217927 (N & F59), 217934 (N & F60). *Xenopus muelleri*: KU 206507 (N & F56).

ADULT SPECIMENS

Alytes obstetricans: KU 148616; MNCN 15098, 15090. *Ambystoma talpoideum*: KU 204729, 204733, 204736, 204737, 204738. *Ambystoma tigrinum*: KU 20416. *Ascaphus truei*: KU 153186, 153187, 153208, 153209; TNHC 53301. *Barbourula busuangensis*: KU 79003. *Bombina bombina*: KU 129238. *Bombina orientalis*: KU 129702, 129703. *Bombina variegata*: KU 94388. *Discoglossus galganoi*: MNCN 15143–44. *Discoglossus jaeneae*: MNCN 11844. *Discoglossus pictus*: MNCN 19710. *Discoglossus sardus*: KU 129239. *Hyla lanciformis*: KU 99299. *Leptodactylus fuscus*: KU 92957, 167678. *Megophrys aceras*: MCZ 23436, 23437. *Megophrys montana*: KU 79010, 147205. *Megophrys monticola*: MCZ 22635. *Pelobates cultripes*: MNCN 18047, 18039, 20241. *Pelobates fuscus*: KU 129240; MCZ 1012–3. *Pelodytes punctatus*: KU 153435; MCZ 1616. *Pipa carvalhoi*: KU 92370, 128761; MCZ 97278. *Pipa parva*: USNM 115775. *Pipa snethlagae*: MCZ 85572. *Pyxicephalus adspersus*: KU 170526, 220957. *Rhinophrynus dorsalis*: KU 62137, 69084, 84883. *Silurana tropicalis*: KU 195667. *Spea bombifrons*: KU 22142, 73383. *Spea intermontana*: KU 204563. *Xenopus borealis*: KU 206861. *Xenopus clivii*: KU 195642–643. *Xenopus laevis*: KU 195934, 195935, 203844. *Xenopus muelleri*: KU 97203, 129699–700; MCZ 51689, 85213.

APPENDIX 2

Characters and character states derived from adult morphology.

CRANIAL FEATURES

1. Skull rounded (State 0) or wedge-shaped (State 1) in lateral profile.
2. Septum nasi not ossified (State 0) or ossified for half its length or more (State 1).
3. Margin of optic foramen cartilaginous or incompletely ossified (State 0) or completely bound in bone (State 1).
4. Anterior margin of prootic foramen not completely ossified (State 0) or completely formed by bone (State 1).
5. Eustachian canal absent (State 0) or present (State 1).
6. Inferior perilymphatic foramen present (State 0) or absent (State 1).
7. Superior perilymphatic foramen present (State 0) or absent (State 1).
8. Prootic and palatine foramina separate, prefacial commissurae present (State 0) or foram-

ina fused, prefacial commissurae absent (State 1).

9. Teeth present, pedicellate (State 0); present, non-pedicellate (State 1); or absent (State 2).
10. Ornamentation absent (State 0) or present (State 1).

Frontal and parietal (frontoparietal)

11. Not in contact medially or in contact posteriorly only (State 0); in contact for most of medial margin (State 1); fused, two centres of chondrification (State 2); or fused, three centre of chondrification (State 3).
12. Frontal (frontoparietal) and nasal not in contact (State 0) or in contact (State 1).
13. Supraorbital flange absent (State 0) or present (State 1).
14. Lateral foramen of lamina perpendicularis absent (State 0) or present (State 1).
15. Pathway for occipital vessels open (State 0) or roofed by bone (State 1).

Nasal

16. Nasals paired, not in contact medially (State 0); paired, in contact medially (State 1); or fused medially (State 2).
17. Posterior divergence one third or less the length of the bones (State 0) or at least half the length (State 1). *Note:* The length does not include the rostral process.
18. Maxillary process of nasal absent (State 0) or present (State 1).

Septomaxilla

19. Small, U-shaped (State 0) or large, arcuate (State 1).

Premaxilla

20. Alary process notably taller than wide (State 0); about as tall as wide, not expanded distally (State 1); about as tall as wide, expanded distally (State 2); or lower than wide, scarcely evident (State 3).
21. Lingual process absent (State 0) or present, medially overlapping maxilla (State 1).
22. Palatine process well developed, daggerlike (State 0) or greatly reduced or absent (State 1).

Maxilla

23. Pars facialis not extending to alary process of premaxilla (State 0); extending to slightly overlap pars facialis of premaxilla (State 1); or extending to, or overlapping, entire lateral margin of alary process or premaxilla (State 2).
24. Preorbital process absent (State 0) or present (State 1).

25. Postorbital process absent (State 0) or present (State 1).

26. Maxilla not extending posteriorly beyond about half the length of the orbit (State 0) or extending for most of the length of the orbit (State 1).
27. Pars facialis poorly developed (State 0) or relatively well developed (State 1).
28. Pterygoid process absent (State 0) or present (State 1). From Maglia (1998).
29. Anterior process of pars palatina absent (State 0) or present (State 1).
30. Palatine process of pars facialis absent (State 0) or present (State 1).

Quadratojugal

31. Absent (State 0) or present (State 1).

Palatine (Neopalatine)

32. Absent (State 0) or present (State 1).

Vomer

33. Vomeres present, paired (State 0); present, fused (State 1); or absent (State 2).
34. Postchoanal process of vomer absent (State 0); present, short (State 1); or present, long (State 2).
35. Dentigerous process of vomer present, at about the level of posterior margin of planum antorbitale (State 0); present, anterior to posterior margin of planum antorbitale (State 1); or absent (State 2).

Parasphenoid

36. Not fused to braincase (State 0) or fused to braincase (State 1).
37. Anterior terminus not reaching maxillary arcade (State 0) or reaching maxillary arcade (State 1).
38. Subotic alae present (State 0) or absent (State 1).
39. Posteromedial process ending near ventral margin of foramen magnum (State 0) or ending well anterior to ventral margin of foramen magnum (State 1).

Pterygoid

40. Anterior ramus present, short, not reaching maxilla (State 0); present, abuts maxilla (State 1); present, medially overlapping maxilla (State 2); or absent (State 3).
41. Ventral flange of anterior ramus absent (State 0); or present (State 1).
42. Auxiliary dorsal process of anterior ramus absent (State 0) or present (State 1).
43. Medial ramus present, not expanded (State 0); present, slightly expanded, investing anterolateral region of otic capsule (State 1); present, expanded to form an otic plate (State 2); or absent (State 3).

Squamosal

44. Rectangular (State 0); T-shaped (State 1); or conch-shaped (State 2).
 45. Zygomatic ramus of squamosal absent (State 0); present, short (State 1); or present, long (State 2).
 46. Otic ramus of squamosal barely overlapping lateral margin of crista parotica (State 0) or forming otic plate (State 1).

Pletral apparatus

47. Stapes absent (State 0); present, shorter than or extending entire length of ear cavity (State 1); or present, much longer than ear cavity (State 2).

Lower jaw

48. Coronoid process of angulosplenial subtriangular (State 0); rectangular, bladeliike (State 1); or greatly reduced (State 2).

Hyolaryngeal apparatus

49. Parahyoid bone absent (State 0); present, single (State 1); present, paired (State 2).
 50. Hyale of hyoid continuous, unbroken (State 0); divided, otic portion present (State 1); divided, otic portion absent (State 2); or entirely absent (State 3).
 51. Hyoglossal sinus present, not bounded by laminar expansions of hyalia (State 0); present, partially bounded by laminar expansions of hyalia (State 1); or absent, hyalia fused forming foramen for hyoglossal nerve (State 2).
 52. Anterolateral process of hyoid absent (State 0); present, not fused to hyale (State 1); or present, fused to hyale, forming foramen for hypoglossal nerve (State 2).

POSTCRANIAL CHARACTERS

Vertebral column

53. Zygapophyseal articular surfaces flat (State 0); bearing sulci and ridges (State 1); or postzygapophyses curved to cover margin of prezygapophyses (State 2).
 54. Spinous process of vertebrae single, if any (State 0) or paired parasagittal spinous processes (State 1).
 55. Transverse processes of Vertebra III longest (State 0); about as long as transverse process of Vertebra IV (State 1); or transverse process of Vertebra IV longest with terminal cartilaginous plate (State 2).
Note: ribs have been included when considering the entire length of the transverse process for those species in which these elements are present.
 56. Uncinate processes on transverse processes of Vertebra III absent (State 0) or present (State 1).
 57. Length of sacral diapophyses less than or equal to width (State 0); length greater than width, ante-

rior and posterior lateral angles rounded (State 1); or length greater than width, anterior and posterior lateral angles acuminate (State 2).

58. Sacrum and urostyle articulating, no fused (State 0); fused, 'webbing' absent (State 1); or fused, 'webbing' present (State 2).
 59. Urostyle shorter than combined length of presacral vertebrae (State 0); about as long or longer than combined length of presacral vertebrae (State 1); or absent (State 2).

Pectoral girdle

60. Pectoral girdle arciferal (State 0); firmisternal (State 1); or pseudofirmisternal (State 2). *Note:* in this analysis *Ambystoma* is assumed to have an arciferal type of pectoral girdle.
 61. Diameter of glenoid fossa about one third total length of scapula (State 0) or more than one third total length of scapula (State 1).
 62. Clavicle and scapula not fused (State 0) or fused (State 1).
 63. Clavicle approximately equal in length to coracoid (State 0) or clavicle longer than coracoid (State 1).
 64. Medial end of clavicle slender or acuminate (State 0) or expanded and wider than lateral end (State 1).
 65. Prezonal element absent (State 0); present, cartilaginous (State 1); or present, ossified (State 2).
 66. Sternum absent (State 0); present, elongate rod with or without rounded posterior plate (State 1); present, plate-like (State 2); or present, sickle-shaped (State 3).

Forelimb

67. Torsion of Digit II of the forelimb absent (State 0) or present (State 1).

Pelvic girdle

68. Supra-acetabular ilium laterally compressed in dorsal view, expanded and bladeliike in lateral view (State 0) or wide in dorsal aspect and lacking dorsal expansion in lateral aspect (State 1).
 69. Preacetabular (ventral) region of ilium narrow (State 0) or wide (State 1).
 70. Dorsal crest on ischium greatly reduced or absent (State 0) or present, well developed (State 1).
 71. Pubis well developed, cartilaginous, with or without mineral deposits (State 0) or poorly developed and usually ossified (State 1).
 72. Epipubis present, rodlike (State 0); present, forming an expanded plate (State 1); or absent (State 2).

Hind limb

73. Proximal phalanx of digit I longer than wide (State 0) or about as long as wide (State 1).

APPENDIX 3

Table of adult characters

Characters 1–40	0	0	1	1	1	2	2	2	3	3	3	4	
	1	5	0	1	5	0	1	5	0	1	5	0	
<i>Ambystoma talpoideum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alytes obstetricans</i>	0	0	0	0	0	0	1	1	1	0	0	0	0
<i>Ascaphus truei</i>	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Bombina orientalis</i>	0	0	0	0	0	0	1	0	0	0	1	0	0
<i>Discoglossus sardus</i>	0	0	0	0	0	0	1	0	0	0	1	0	0
<i>Hyla lanciformis</i>	0	0	0	0	0	0	1	1	0	1	0	0	0
<i>Hymenochirus boettgeri</i>	0	1	1	1	1	0	1	0	0	1	0	0	0
<i>Leptodactylus fuscus</i>	0	1	1	0	0	0	1	0	0	1	0	0	0
<i>Megophrys montana</i>	0	0	0	0	0	0	1	0	1	1	1	0	0
<i>Pelobates cultripes</i>	0	0	0	0	0	0	1	1	1	1	1	0	0
<i>Pelobates fuscus</i>	0	1	0	0	0	0	1	1	1	1	1	0	0
<i>Pelodytes punctatus</i>	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>Pipa parva</i>	1	1	1	1	0	1	0	0	0	0	0	0	0
<i>Pipa carvalhoi</i>	1	1	1	1	0	1	0	0	0	0	0	0	0
<i>Pyxicephalus adspersus</i>	0	1	0	1	0	0	0	1	0	1	1	1	0
<i>Rhinophrynus dorsalis</i>	0	0	0	0	0	0	1	0	0	0	0	1	0
<i>Silurana tropicalis</i>	0	0	1	1	0	1	0	0	0	0	0	0	0
<i>Spea intermontana</i>	0	1	?	0	0	0	0	1	0	0	1	0	0
<i>Xenopus laevis</i>	0	0	1	1	0	1	0	0	0	0	0	0	0
<i>Xenopus muelleri</i>	0	0	1	1	0	1	0	0	0	0	0	0	0

Characters 41–73	4	4	5	5	5	6	6	6	7	7	7	
	1	5	0	1	5	0	1	5	0	1	3	
<i>Ambystoma talpoideum</i>	1	0	0	0	0	1	1	0	0	0	0	0
<i>Alytes obstetricans</i>	1	0	0	1	1	0	0	0	0	0	0	0
<i>Ascaphus truei</i>	0	0	0	1	0	0	0	1	0	0	0	0
<i>Bombina orientalis</i>	0	0	0	1	0	0	0	1	0	0	0	0
<i>Discoglossus sardus</i>	1	0	0	1	1	0	0	1	0	0	0	0
<i>Hyla lanciformis</i>	0	0	0	1	0	0	0	1	0	0	0	0
<i>Hymenochirus boettgeri</i>	?	?	2	2	1	0	2	1	0	2	1	1
<i>Leptodactylus fuscus</i>	0	0	0	1	0	0	0	1	0	2	1	0
<i>Megophrys montana</i>	0	0	1	1	1	0	0	2	1	0	0	0
<i>Pelobates cultripes</i>	1	1	1	2	1	0	0	1	0	1	1	0
<i>Pelobates fuscus</i>	1	1	1	2	1	0	0	1	0	1	1	0
<i>Pelodytes punctatus</i>	0	0	0	1	0	0	0	2	0	1	0	0
<i>Pipa parva</i>	1	0	2	2	0	0	2	1	0	2	0	1
<i>Pipa carvalhoi</i>	1	0	2	2	0	0	2	1	0	2	0	1
<i>Pyxicephalus adspersus</i>	0	1	1	2	2	1	0	0	1	0	0	0
<i>Rhinophrynus dorsalis</i>	0	0	3	1	0	0	0	1	0	0	0	0
<i>Silurana tropicalis</i>	1	0	2	2	0	2	1	0	1	0	2	0
<i>Spea bombifrons</i>	0	0	1	1	0	0	1	0	1	0	1	0
<i>Spea intermontana</i>	0	0	0	1	0	0	0	1	0	1	1	0
<i>Xenopus laevis</i>	1	0	2	2	0	2	1	0	1	0	2	0
<i>Xenopus muelleri</i>	1	0	2	2	0	2	1	0	1	0	2	0

APPENDIX 4

Table of larval characters

Characters 1–43	0	0	1	1	1	2	2	2	3	3	3	4	4	4		
Taxa	1	5	0	1	5	0	1	5	0	1	5	0	1	3		
<i>Ambystoma talpoideum</i>	0	?	?	?	?	?	?	?	?	?	?	?	?	0	0	2
<i>Alytes obstetricans</i>	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
<i>Ascaphus truei</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Bombina orientalis</i>	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1
<i>Discoglossus sardus</i>	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
<i>Hyla lanciformis</i>	1	0	0	1	0	0	0	1	0	1	1	1	1	1	1	1
<i>Hymenochirus boettgeri</i>	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
<i>Leptodactylus fuscus</i>	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1
<i>Megophrys montana</i>	1	0	0	1	1	1	0	0	0	1	0	0	0	1	0	1
<i>Pelobates cultripes</i>	1	0	0	1	1	0	0	0	2	1	0	1	1	0	0	0
<i>Pelobates fuscus</i>	1	0	0	1	1	0	0	0	2	1	0	1	1	0	0	0
<i>Pelodytes punctatus</i>	1	0	0	1	1	0	0	0	2	1	0	1	0	0	0	0
<i>Pipa parva</i>	1	1	2	1	1	1	0	0	0	1	1	0	0	1	0	0
<i>Pipa carvalhoi</i>	1	1	2	1	1	1	0	0	1	1	2	1	0	0	1	0
<i>Pyxicephalus adspersus</i>	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0
<i>Rhinophrynus dorsalis</i>	1	1	0	0	1	0	0	0	2	?	?	?	?	?	?	?
<i>Silurana tropicalis</i>	1	1	1	1	1	1	0	0	0	2	?	?	?	?	?	?
<i>Spea bombifrons</i>	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0
<i>Spea intermontana</i>	1	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0
<i>Xenopus laevis</i>	1	1	1	1	1	1	0	0	0	2	?	?	?	?	?	?
<i>Xenopus muelleri</i>	1	1	1	1	1	1	0	0	0	2	?	?	?	?	?	?