

Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): the effects of heterochronic changes during larval development and metamorphosis

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Heterochrony produces morphological change with effects in shape, size, and/or timing of developmental events of a trait related to an ancestral ontogeny. This paper analyzes heterochrony during the ontogeny of Ceratophryinae (*Ceratophrys*, *Chacophrys*, and *Lepidobatrachus*), a monophyletic group of South American frogs with larval development, and uses different approaches to explore their morphological evolution: (1) inferences of ancestral ontogenies and heterochronic variation from a cladistic analysis based on 102 morphological larval and adult characters recorded in ten anuran taxa; (2) comparisons of size, morphological variation, and timing (age) of developmental events based on a study of ontogenetic series of ceratophryines, *Telmatobius atacamensis*, and *Pseudis platensis*. We found *Chacophrys* as the basal taxon. *Ceratophrys* and *Lepidobatrachus* share most derived larval features resulting from heterochrony. Ceratophryines share high rates of larval development, but differ in rates of postmetamorphic growth. The ontogeny of *Lepidobatrachus* exhibits peramorphic traits produced by the early onset of metamorphic transformations that are integrated in an unusual larval morphology. This study represents an integrative examination of shape, size, and age variation, and discusses evolutionary patterns of metamorphosis. © 2008 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2008, 154, 752–780.

ADDITIONAL KEYWORDS: growth – *Lepidobatrachus* – peramorphosis – phylogenetic analysis – shape – size – tadpoles.

INTRODUCTION

The study of heterochrony has become one of the focal concepts forging the integration of many areas of evolutionary biology (Reilly, Wiley & Meinhardt, 1997). Different definitions have been used to explain heterochrony: e.g. ‘changes in the relative time of appearance and rate of development for characters already present in ancestors’ (Gould, 1977; Alberch & Alberch, 1981; Brusca, Brusca & Gilbert, 1997) and ‘pattern of evolutionary changes without implications regarding the underlying mechanism’ (Schlosser, 2001). Studies of heterochrony have been carried out from several perspectives, and the concept is of fundamental importance in comparative morphology

because it involves shape and size. Reilly *et al.* (1997) pointed out five basic assumptions used to study heterochrony: (1) it involves the development of traits and does not pertain to the whole organisms any more than homology pertains to whole organisms or lineages; (2) an accurate measurement or descriptor of the trait or shape under study must be available; (3) heterochronic patterns cannot be classified without information of the timing (age) of developmental events in the ancestral and descendant ontogenies; (4) the ontogenetic trajectory of a trait must be considered from its inception to its terminal shape; (5) a phylogenetic hypothesis is essential to determine the direction of the heterochronic change in the descendant species.

In anurans, morphological analyses of heterochrony have described derived patterns of paedomorphosis

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involved in miniaturization (Alberch & Gale, 1985; Clarke, 1996; Yeh, 2002a, b; Maglia, Púgener & Mueller, 2007). These studies analyzed the heterochronic variation of shape (especially skeletal traits) related to body size. However, integrative studies considering shape, size, and timing of developmental events are not available for this group.

Anurans are characterized by a biphasic life history, with distinct larval and adult phases joined by a major metamorphic event; tadpoles and post-metamorphic stages differ in most aspects, but share some key features, such as a truncated axial skeleton and the absence of discrete caudal vertebrae (Handrigan & Wassersug, 2007). Consequently, studies of morphological variation of shape and size, considering the timing of developmental events during the larval and postmetamorphic growth, become particularly appropriate for understanding the morphological effects of heterochronic changes in the larval body plan, and how they are integrated into the adult body plan during metamorphosis.

Here, we present a morphological study of heterochrony, following the five basic assumptions outlined by Reilly *et al.* (1997), based on ontogenetic series of the ceratophryine frogs. This clade is exceptional among anurans because it includes three genera that share peramorphic traits in skull shape, which do not seem to be constrained by different larval morphologies (Fabrezi, 2006).

The Ceratophryinae Tschudi, 1838 were considered a distinctive group of neotropical frogs in various systematics and taxonomic arrangements (see Table 1 for a historical review). Much has been discussed about the relationships of these genera, but there is a strong consensus for the monophyly of the clade (see Reig & Limeses, 1963; Lynch, 1971; Laurent, 1986; Maxson & Ruibal, 1988; Haas, 2003; Fabrezi, 2006; Frost *et al.*, 2006; Grant *et al.*, 2006, among others). The clade is composed of the following taxa: *Chacophrys* Reig & Limeses, 1963 (one species), *Lepidobatrachus* Budgett, 1899 (three species), and *Ceratophrys* Wied-Neuwied, 1824 (eight species).

Adults of *Chacophrys* and *Ceratophrys* are terrestrial, and adults of *Lepidobatrachus* are aquatic. *Ceratophrys* species are distributed in tropical areas of South America, and *Ceratophrys cranwelli* Barrio, 1980 and *Ceratophrys ornata* (Bell, 1843) are endemic to the Chaco region, and are sympatric with *Lepidobatrachus* species and *Chacophrys pierottii* (Vellard, 1948). As the Chaco region comprises semi-arid environments with wet summers, the anurans feed and breed during this season in temporary pools. Populations of different species of ceratophryines share places to breed. Adults of ceratophryines are megalophagous and are cannibals (Ruibal & Thomas, 1988; Hanken, 1993), and the Chaco species produce

an epidermal cocoon during the dry season that reduces water loss and protects them until the next rainy season (McClanahan, Ruibal & Shoemaker, 1994). Tadpoles in these genera are very different from each other: *Ceratophrys* and *Lepidobatrachus* have macrophagous and specialized carnivorous larvae (Ruibal & Thomas, 1988; Wassersug & Heyer, 1988), whereas the tadpole of *C. pierottii* (Faivovich & Carrizo, 1992; Quinzio, Fabrezi & Faivovich, 2006) may be characterized as a typical pond-type tadpole with generalized suspension feeding (Duellman & Trueb, 1986; Wassersug & Heyer, 1988). Consequently, the Ceratophryinae (being a monophyletic group) represents an excellent model to explore how larval features and anatomical metamorphic changes are integrated in the adult body plan.

Herein, we analyze larval and adult morphological characters, and use phylogenetic comparative methods to address a hypothesis of the ancestral ontogeny (assumption 5). Characters were recorded in four species of Ceratophryinae (*C. cranwelli*, *C. pierottii*, *Lepidobatrachus laevis* Budgett, 1899, and *Lepidobatrachus llanensis* Reig & Cei, 1963), and six nonceratophryine species [*Bombina variegata* (Linnaeus, 1758), *Discoglossus pictus* Otth, 1837, *Spea bombifrons* (Cope, 1863), *Odontophrynus lavillai* Cei, 1985, *Telmatobius atacamensis* Gallardo, 1962, and *Pseudis platensis* (Gallardo, 1961)], following the most recent phylogenies (Frost *et al.*, 2006; Grant *et al.*, 2006). This analysis allowed us to set those derived characters of ceratophryine larval and adult body plans, and to identify heterochronic variation (assumption 1).

We also present comparative data on the morphological variation during ontogeny (assumptions 2 and 4), extension of larval development, and postmetamorphic growth (assumption 3) in the Ceratophryinae, *T. atacamensis*, and *P. platensis*. *Telmatobius atacamensis* and *P. platensis* were selected for these comparisons for having tadpoles of comparable size, and for having aquatic adults like *Lepidobatrachus* spp. Furthermore, *T. atacamensis* belongs to the Telmatobiinae, the proposed sister group of the Ceratophryinae (Grant *et al.*, 2006), and *P. platensis* shares with *Lepidobatrachus* spp. the same places to breed and feed during the wet season. These comparisons show dissociation of metamorphic transformations defining unusual larval morphologies in *Lepidobatrachus* spp., and provide arguments for a more comprehensive interpretation of previous hypotheses of morphological evolution of metamorphosis, which included the concepts of gradual and abrupt metamorphosis (Wassersug & Hoff, 1982) and precocious metamorphosis (Hanken, 1993). It is hoped that the present study on heterochronic patterns involved in shaping the ceratophryine body plan will

Table 1. The historical synthesis of classification and relationships of ceratophryines since *Chacophrys* was described as a distinct genus (Reig & Limeses, 1963)

Author(s)	Type of data set	Suprageneric taxon	Comments	In-group relationships
Reig & Limeses (1963)	Morphological	Ceratophryidae	Probably related to the origin of Bufonidae	(<i>Lepidobatrachus</i> + (<i>Ceratophrys</i> + <i>Chacophrys</i>))
Lynch (1971)	Morphological	Ceratophryinae in Leptodactylidae	Ceratophryinae is a basal taxon of Leptodactylidae	(<i>Ceratophrys</i> + <i>Lepidobatrachus</i>)
Laurent (1986)	Morphological and cytological	Ceratophryinae in Leptodactylidae	Ceratophryinae (Odontophryini + Ceratophryini) is a basal taxon, probably related to the origin of Bufonidae	(<i>Lepidobatrachus</i> + (<i>Ceratophrys</i> + <i>Chacophrys</i>))
Maxson & Ruibal (1988)	Molecular	Ceratophryinae in Leptodactylidae	–	(<i>Ceratophrys</i> + (<i>Chacophrys</i> + <i>Lepidobatrachus</i>))
Haas (2003)	Morphological	Leptodactylidae	Leptodactylidae is paraphyletic. <i>Ceratophrys</i> and <i>Lepidobatrachus</i> could be related to hylids.	(<i>Ceratophrys</i> + <i>Lepidobatrachus</i>)
Fabrezi (2006)	Morphological	Ceratophryinae in Leptodactylidae	Leptodactylidae is paraphyletic.	(<i>Ceratophrys</i> + (<i>Chacophrys</i> + <i>Lepidobatrachus</i>))
Frost <i>et al.</i> (2006)	Molecular	Ceratophryini in Ceratophryidae	(Telmatobiinae + (Batrachylini + Ceratophryini))	(<i>Lepidobatrachus</i> + (<i>Ceratophrys</i> + <i>Chacophrys</i>))
Grant <i>et al.</i> (2006)	Molecular	Ceratophryinae in Ceratophryidae	(Batrachylinae + (Ceratophryinae + Telmatobiinae))	(<i>Lepidobatrachus</i> + (<i>Ceratophrys</i> + <i>Chacophrys</i>))

contribute to future works concerning the role of development in the evolution of anuran morphological diversity.

MATERIAL AND METHODS

Data in this study contribute to two different analyses: (1) cladistic analysis in order to address the relationships of the Ceratophryinae and to identify derived characters; and (2) comparisons of anatomical variation during the ontogeny between Ceratophryinae and two selected taxa (*P. platensis* and *T. atacamensis*), which could be considered as out-groups following the hypotheses proposed by Frost *et al.* (2006) and Grant *et al.* (2006). Both analyses included the study of morphological variation in ontogenetic series of *C. cranwelli*, *C. pierottii*, *L. laevis*, *L. llanensis*, *Odontophrynus americanus* (Duméril and Bibron, 1841), *O. lavillai*, *P. platensis*, and *T. atacamensis*.

Information for this study was obtained from six different sources: (1) microscopic examination of preserved specimens to describe external characters, and to identify neuromast lines; (2) manual dissection of specimens to study musculature; (3) examination of cleared and double-stained whole-mount specimens for cartilage and bone to describe skeletal characters; (4) examination of cleared and Sudan Black-stained whole-mount specimens for nerves, to observe peripheral cranial nerves; (5) serial sections of skin to describe tadpole and adult histological tegument structures; and (6) serial sections of metatarsal IV to infer age from lines of arrested growth (LAGs). For morphometric variables we used dial calipers accurate to 0.02 mm; measurements were given in millimetres. Terminology for neuromast topography was modified from Lannoo (1987). The technique for clearing and double-staining for bone and cartilage follows the protocol by Wassersug (1976). The technique for clearing and staining nerves with Sudan Black B follows the procedures described by Filipinski & Wilson (1984); Filipinski (1985); Nishikawa (1987). For histological sections, skin of the dorsum and Toe IV were separated from preserved specimens (Toe IV was decalcified), dehydrated, embedded in paraffin and sectioned at a thickness of 6 µm. Sections were stained with hematoxylin and eosin following the protocol described by Martoja & Martoja-Pierson (1970). The LAG analysis was performed according to the technique detailed in Hemelaar (1986). As the studied species live in environments characterized by the presence of a dry and cold season (May to September), each LAG could be interpreted as a year of life of the specimen.

Specimens were collected in the field and were fixed in neutral-buffered formalin (4%). Larval series were staged following the standard table of Gosner (1960).

Descriptions, illustrations and photographs were made with a stereo dissection microscope Nikon-SMZ1000 and light microscope Leica DM, equipped with a digital camera and camera lucida. Additional analyses of other anuran species were made in order to confirm some data of literature. Species, specimen numbers, and collection data are listed in Appendix 1.

The cladistic analysis was performed by scoring a total of 102 anatomical characters of tadpoles and adults for ten anuran species (see Appendices 2 and 3). *Bombina variegata* was chosen as an outgroup. Data for tadpoles of *Discoglossus*, *Spea*, *Bombina*, and some adult characters were obtained from literature (Wiens, 1989; Schlosser & Roth, 1995; Hall, Larsen & Fitzner, 1997; Púgener & Maglia, 1997; Hall & Larsen, 1998; Maglia & Púgener, 1998; Hall, Larsen & Fitzner, 2002; Haas, 2003; Manzano, Moro & Abdala, 2003). The whole data set for *C. cranwelli*, *C. pierottii*, *L. laevis*, *L. llanensis*, *O. lavillai*, *P. platensis*, and *T. atacamensis* were obtained in analyses of larval and adult specimens. *Telmatobius atacamensis* and *O. lavillai* were chosen to test previous hypotheses of Ceratophryinae relationships (Table 1). Some multistate characters were analyzed as non-additive because of the lack of any argument to justify an order in the codified states, but in others, the additivity was inferred from known ontogenetic sequences. Non-additive characters are indicated in the character list (Appendix 2). Phylogenetic analyses were performed using parsimony software (TNT, tree analysis using new technology, v1.0) by Goloboff, Farris & Nixon (2003). The data set was studied with the criterion of equally weighted character analysis (Goloboff, 1993). Heuristic searches with 1000 random-addition sequences replicates found minimal length topologies of 179 steps (consistency index = 0.715, retention index = 0.777). The branch-swapping algorithm used was tree bisection and reconnection (TBR), and replications of minimal-length trees led to one tree (Fig. 1A). Deactivating adult characters (61–101) or larval characters (0–60) in both analyses yielded trees that failed to recover the Ceratophryinae monophyly (Fig. 1B) or in-group relationships (Fig. 1C).

RESULTS

PHYLOGENETIC PATTERN

Cladistic analyses of the whole data set recovered the monophyly of Ceratophryinae. The single tree that resulted from the study of all of the larval and adult characters supports the Neobatrachia clade, with seven synapomorphies from larval body plans and six synapomorphies of adult characters. In this hypothesis *C. pierottii* represents the basal taxon of Ceratophryinae (Fig. 1A).

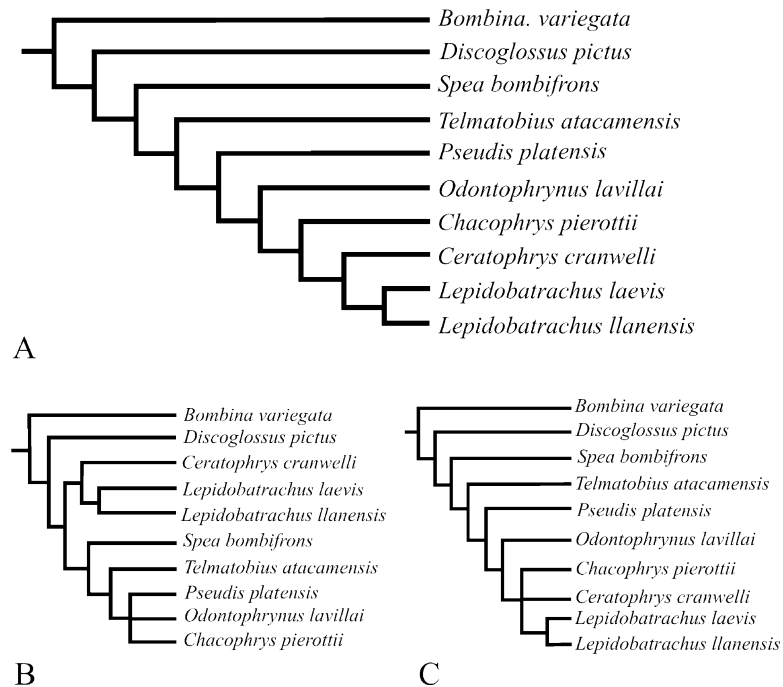


Figure 1. The hypothetical relationships among ten anuran taxa that resulted from the analyses of 102 morphological characters. A, the only tree that was obtained from the analysis of larval and adult characters. B, strict consensus of relationships obtained from the analysis of 61 larval characters. C, strict consensus of relationships obtained from the analysis of 41 adult characters.

Ceratophryinae is defined by 17 synapomorphies, from which four are larval features. Within Ceratophryinae, *Chacophrys* is the sister group of the clade formed by *Ceratophrys* and *Lepidobatrachus*.

The larval synapomorphies for Ceratophryinae are: oral disc papillation complete (20), anal tube at larval stage 37 cylindrical, short, and distinct (23) (Fig. 2), tectum cavum cranii almost completely chondrified (48) (Fig. 3), and trigeminal and facial nerves completely separated in tadpoles (59) (Fig. 4). Adult characters shared by the Ceratophryinae are: cranial exostosis (62), frontoparietal–squamosal arc (65), squamosal otic ramus expansive and overlapping the prootic (66), squamosal zygomatic ramus sutured to the maxilla (67), teeth monocuspid (68), teeth not pedicellate (69), mentomeckelian bone completely fused to the dentary (74), fangs in lower jaw (75), lower jaw articulation up to or beyond the craniovertebral joint (76), absence of anterolateral process of hyoid (78), ossification of the posteromedial processes invading the hyoid plate (79), cervical cotyles contiguous (81), and crest along the ilial shaft absent (91).

The clade composed by *C. cranwelli* + *Lepidobatrachus* spp. has 20 larval characters as synapomorphies, and a single character from adult morphology. These characters are: melanocytes in the dermis (1) (Fig. 5), epidermal mucous glands present

(2) (Fig. 5), spongiosum stratum of dermis differentiated (3) (Fig. 5), three or more epidermal layers (4) (Fig. 5), discontinuous supraorbital and infraorbital lines of neuromasts (6) (Fig. 6), supratemporal neuromasts (7) (Fig. 6), m. quadratoangularis absent (27), origin of m. suspensorioangularis posterior (29) (Fig. 7), mm. levatores arcuum branchialium I and II separated by a gap (30), processus pseudopterygoideus absent (34) (Fig. 8), cartilago labialis superior placed dorsally (38) (Fig. 8), pars alaris and pars corporis of cartilago labialis superior completely fused (40) (Fig. 8), partes corporum of cartilago labialis superior forming a single body (41) (Fig. 8), complete medial fusion of infrarostrals (45) (Fig. 8), infrarostral medial union bearing a pointed projection (46) (Fig. 8), retroarticularis processes of cartilago meckeli straight (47) (Fig. 8), commissurae proximalis absent (53) (Fig. 8), spiculae I–IV absent (54) (Fig. 8), processus anterolateralis of ceratohyalia absent (56) (Fig. 8), cartilaginous projections along ceratobranchialia absent (57) (Fig. 8), and origin of depressor mandibulae placed at squamosal and tympanic annulus in the adults (98).

Lepidobatrachus laevis and *L. llanensis* share 19 synapomorphies, 16 of which are larval characters. These derived features are: neuromasts of the infraorbital line descend towards the lateral margin of the

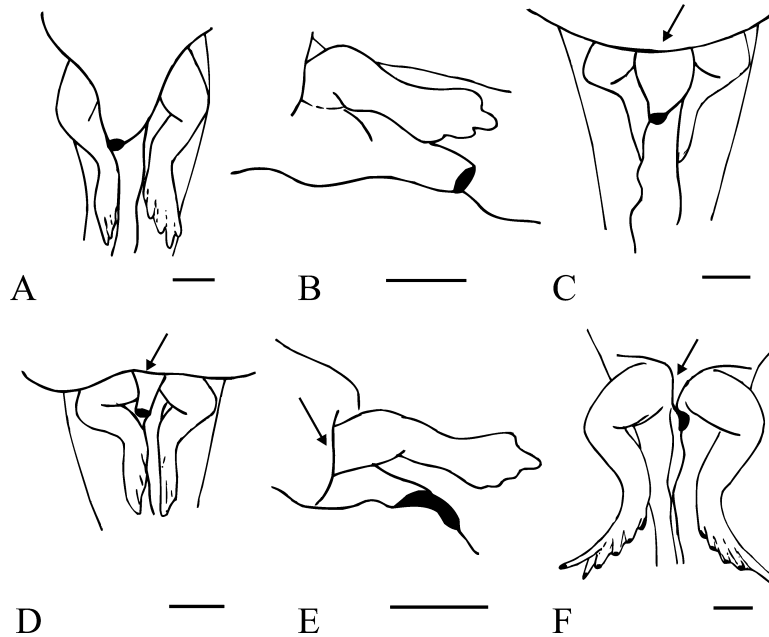


Figure 2. Anal tube morphology in anuran tadpoles. A, *Telmatobius atacamensis* (stage 36), ventral view of the funnel-shaped anal tube. B, *Odontophrynus lavillai* (stage 36), lateral view of the funnel-shaped anal tube. C, *Chacophrys pierottii* (stage 35), ventral view of the anal tube, which is short and cylindrical. D, *Ceratophrys cranwelli* (stage 36), the anal tube is cylindrical and smaller than the anal tube of *C. pierottii*. E, *Lepidobatrachus llanensis* (stage 34), lateral view of the anal tube. The anal tube is funnel shaped and short. F, *Lepidobatrachus llanensis* (stage 36), ventral view. The anal tube is short and its opening is wide. After this stage the anal tube disappears. Scale bars: 2 mm.

upper lip, and turn to become parallel with the lip up to the medial position (8) (Fig. 6), supralabial neuromasts (12) (Fig. 6), medial line of neuromasts diverges from the dorsal line (14) (Fig. 6), ventral line of neuromasts placed lateroventrally (16) (Fig. 6), keratodonts absent (17), keratinized jaw sheaths reduced (18), oral lower lip absent (19), spiracle paired (21), anal tube at larval stage 37 absent (23) (Fig. 2), m. mandibulolabialis absent (25), process quadratoethmoidalis absent (35) (Fig. 8), dorsal connection from processus muscularis to neurocranium by ligament only (36) (Fig. 8), posterior processus of pars alaris of cartilago labialis superior absent (42) (Fig. 8), lower jaw oriented transversally (44) (Fig. 8), hypobranchialia fused (51) (Fig. 8), process urobranchialis truncated (52) (Fig. 8), ceratohyalia discontinuous in adults (77), a single parahyoid dermal ossification (80), and subarticular tubercles in toes absent (99) (Fig. 9). *Chacophrys pierottii* has as synapomorphies an expansive chondrification of posterolateral projection of crista parotica (32) (Fig. 8), larval nasal appendix (58), and the femoral crest absent (92). *Ceratophrys cranwelli* presents the following derived characters: neuromasts of preorbital line cross the eyelid (5) (Fig. 6), processus anterolateralis of crista parotica forms larval processus oticus (31) (Fig. 6), short cornua trabeculae (39) (Fig. 6), and toes

bearing subarticular tubercles (99) (Fig. 9). *Lepidobatrachus llanensis* has as a synapomorphy well-keratinized claws (101). *Lepidobatrachus laevis* could not be defined by any synapomorphy.

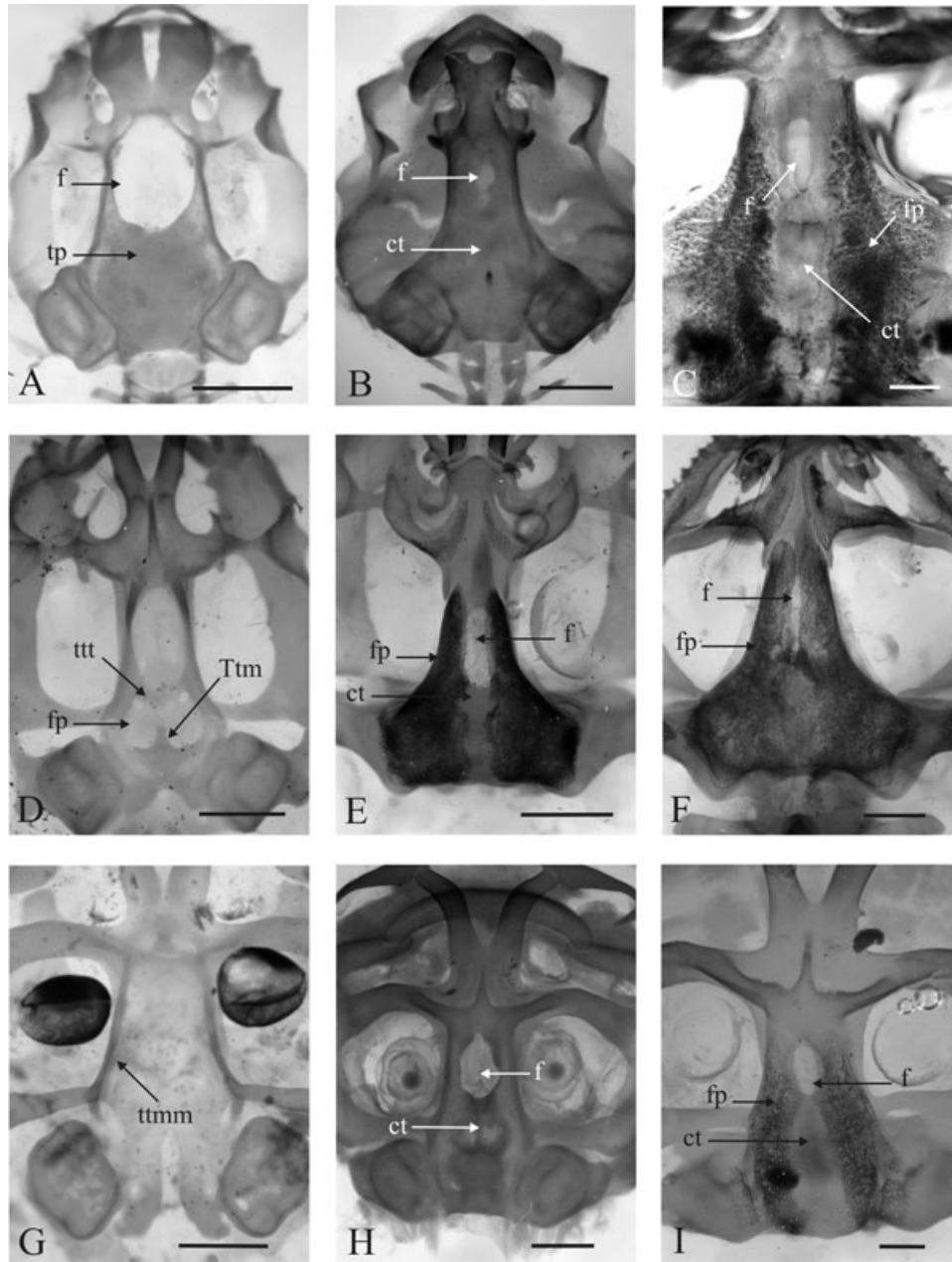
From this analysis, larval and adult characters support the monophyly of Ceratophryinae. Some larval derived features of *Lepidobatrachus* represent unique characters for anuran tadpoles.

ONTOGENETIC PATTERN

Tadpole size, adult size, and growth rates

Parameters to evaluate growth in anuran larvae are snout-vent length, tail length, total length, body mass, etc. The Ceratophryinae have medium-to-large tadpoles at advanced larval development. *Pseudis platensis* and *T. atacamensis*, having large tadpoles, were chosen in order to compare parameters of growth. Among the species compared, *C. cranwelli* tadpoles are the smallest and *P. platensis* are the largest (Fig. 10). *Pseudis platensis* exhibits an increase of total length (as a result of differential growth of the tail relative to the body length) compared with the other species.

Comparisons of snout-vent lengths before and during metamorphosis, and at adult stages (i.e. specimens with secondary sexual characters),



distinguished two groups: one formed by *C. pierottii*, *P. platensis*, and *T. atacamensis*, and the other composed of *C. cranwelli* and *Lepidobatrachus* spp. (Fig. 11). *Chacophrys pierottii*, *P. platensis*, and *T. atacamensis* exhibit limited postmetamorphic growth: they are characterized by an increase of 30% or less of snout–vent length compared with the metamorphs. The tadpoles of this group of species are thus larger than the adults. The second group displays a strong postmetamorphic growth, with an increase of more than 50% in the snout–vent length in adults. Developmental rates in these species vary. In *P. plat-*

ensis and *T. atacamensis*, the larval development takes place over a long period of time. Dixon, Mercolli & Yanosky (1995) reported 40 days to reach larval stage 39 from fertilization for *P. platensis*. Emerson (1988) presumed that *P. paradoxa* must have overwintering tadpoles. In contrast, our field data suggest that complete larval development could occur between November, when eggs and embryos were collected, and late April, when we found specimens at metamorphosis. For *T. atacamensis*, our field data indicated that larval development started at the end of winter (i.e. late August–early September), and that

metamorphs were already present at the beginning of autumn (late March–April). Larval development and metamorphosis in the Ceratophryinae species, however, are fast. *Lepidobatrachus* spp. and *C. pierottii* go from egg deposit to metamorphosis in 2 weeks, and *C. cranwelli* achieves this in 3 weeks (Quinzio *et al.*, 2006).

The postmetamorphic growth rate for these species can be assessed via skeletochronological analyses. Here, age is inferred from lines of arrested growth or LAGs. Our preliminary observations allowed us to approximate the relationship between age and size for adult specimens of ceratophryines, *P. platensis*, and *T. atacamensis* (Figs 11, 12). Postmetamorphic growth is fast in both species of *Lepidobatrachus*, and in particular in *L. laevis*, the females of which have twice the snout–vent length of *L. llanensis* males at the same age (7 LAGs). A large specimen of *C. cranwelli*, which reaches a similar size as *L. laevis*, is older than the latter (11 LAGs). This suggests a lower postmetamorphic growth than *L. laevis*. *Chacophrys pierottii* displays the slowest developmental rate of postmetamorphic growth among ceratophryines. *Pseudis platensis* and *T. atacamensis* present low developmental rates of postmetamorphic growth.

Anatomical variation and metamorphosis

For anuran development, Etkin (1932) distinguished between prometamorphosis (up to stage 41), which is the period of continued growth, especially of the limbs, and the metamorphic climax, which is the period of radical changes that culminate in the loss of most of the larval characters (stages 42–46).

Table 2 summarizes the morphological changes that were proposed to define and characterize prometamorphosis and metamorphic climax for most anurans, following Gosner (1960), and compares *C. pierottii*, *C. cranwelli*, *Lepidobatrachus* spp., and *P. platensis*.

As is evident, *Lepidobatrachus* spp. and *P. platensis* exhibit clear deviations from the pattern of most anurans.

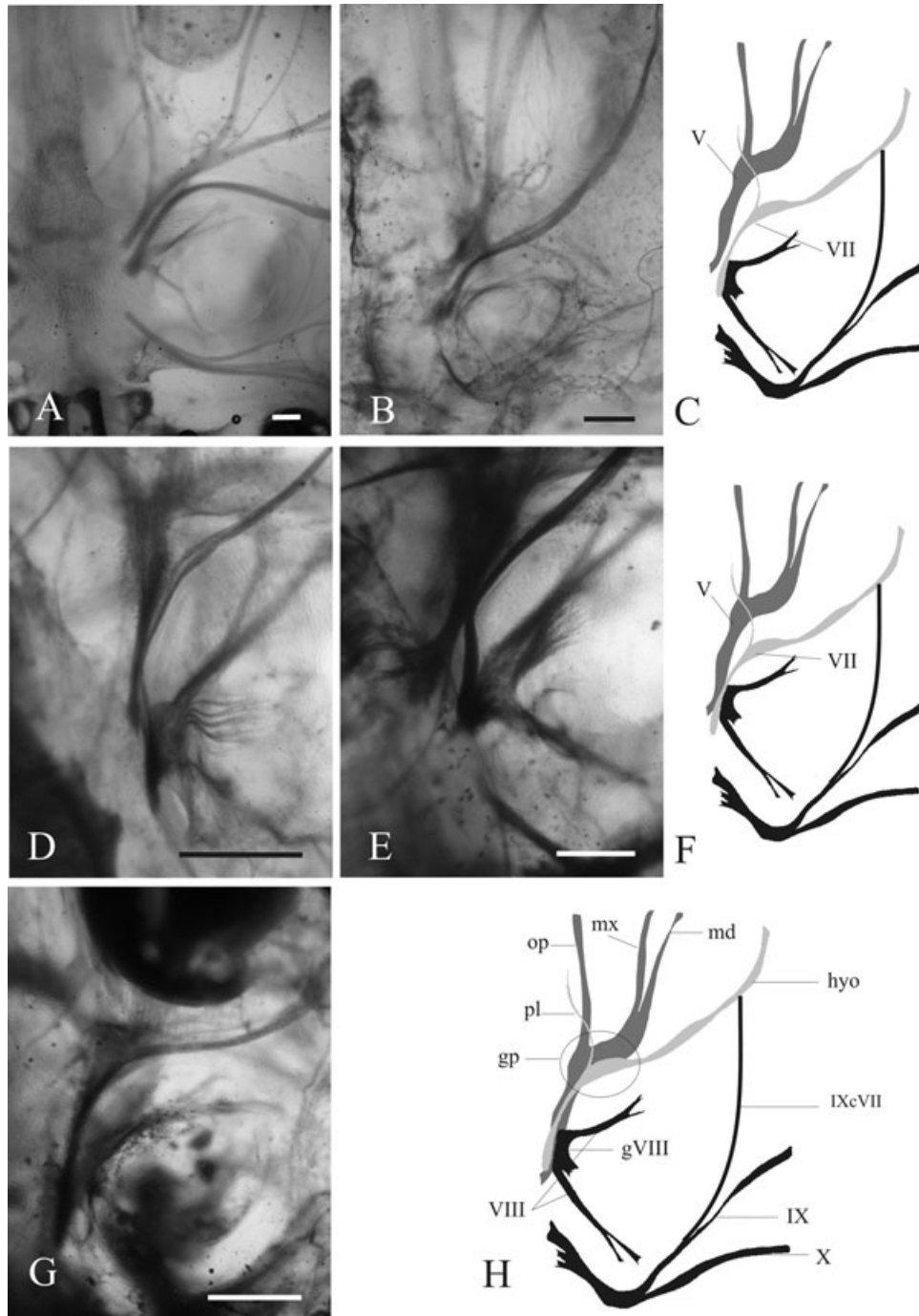
Metamorphic changes related to mouth elongation seem to be shared by *C. pierottii*, *C. cranwelli*, and *Lepidobatrachus* spp. For most anurans, the end of metamorphosis is denoted by the position of the angle of mouth, in reference to the margin of the eye, and the complete resorption of the tail (Gosner, 1960). In Ceratophryinae, the angle of the mouth is caudal to the eye well before the tail is reduced to a stub (Fig. 13).

In *Lepidobatrachus* spp. and *C. cranwelli* the metamorphic climax is characterized by fewer events (specifically, tail resorption, and mandible and hyobranchial changes). Skin and gut transformations that occur at this period in most species have already occurred during prometamorphosis. In *L. laevis* there is no degeneration of the lateral line system, and it is present throughout adult life.

Other changes that occur early during prometamorphosis in *Lepidobatrachus* spp. include: early loss of the anal tube, reduction of oral structures, and the absence of development of subarticular tubercles. *Lepidobatrachus llanensis* tadpoles develop keratinized claws at prometamorphic larval stages.

To varying degrees, the ceratophryines exhibit larval development in which some features that indicate metamorphic changes are predisplaced (early onset). In contrast, larval development shows postdisplacement (later offset) in the occurrence of some transformations in *P. platensis*. Loss of the anal tube, for example, which is characteristic of the end of prometamorphosis, occurs after the forelimbs emerge and the larval mouth structures disappear. Tail musculature persists well after the angle of the mouth has reached the caudal margin of the eye (Fig. 13).

Figure 3. Variation of cartilaginous roofing of the cavum cranii in tadpoles. A, *Trachycephalus venulosus* (Laurenti, 1768) (stage 36), continuous tectum parietale in front and confluent with tectum synoticum. B, *Ceratophrys cranwelli* (stage 35), tectum of cavum cranii almost completely chondrified. C, *Lepidobatrachus llanensis* (stage 45), tectum of cavum cranii almost completely chondrified. Frontoparietal ossification is not fused medially. D, *Chacophrys pierottii* (stage 35), differentiation of taenia tecti transversalis and medialis that will fuse to form fenestrae parietales. E, *Chacophrys pierottii* (stage 41), tectum of cavum cranii almost completely chondrified. Frontoparietal ossifications are in progress. F, *Chacophrys pierottii* (end of metamorphosis), tectum of cavum cranii almost completely chondrified. Frontoparietal ossifications are not fused medially. G, *Lepidobatrachus laevis* (stage 28). There are incipient taenia tecti marginalis; other cartilaginous elements of tectum cranii are absent. H, *Lepidobatrachus laevis* (stage 35), tectum of cavum cranii almost completely chondrified. I, *Lepidobatrachus laevis* (stage 39), tectum of cavum cranii almost completely chondrified, and frontoparietal ossifications are developing. Ontogenetic variation in the roof of the skull is observed. *Ceratophrys cranwelli* and *Lepidobatrachus* spp. exhibit the complete roofing of cavum cranii at early larval stages (from stage 32), whereas chondrification of the roof of the cavum cranii in *C. pierottii* recapitulates other states of characters, until reaching the extensive chondrification of the cavum cranii at larval stage 39. Scale bars: 1 mm in (G); 2 mm in (A)–(F), (H), and (I). Abbreviations: ct, chondrified tectum; f, frontoparietal fontanelle; fp, frontoparietal bone; tp, tectum parietale; ttm, taenia tecti medialis; tmm, taenia tecti marginalis; ttt, taenia tecti transversalis.



DISCUSSION

MORPHOLOGICAL VARIATION AND RELATIONSHIPS

Molecular data (Frost *et al.*, 2006; Grant *et al.*, 2006) have added support to the previous hypotheses of monophyly for the genera *Ceratophrys*, *Chacophrys*, and *Lepidobatrachus* (Reig & Limeses, 1963; Lynch, 1971; Laurent, 1986; Maxson & Ruibal, 1988; Hanken, 1993; Fabrezi, 2006). These hypotheses,

however, did not resolve the affinity of this clade to other taxa. In addition, the relationships within the clade are controversial (Table 1).

In this study, we found a set of characters that resulted in synapomorphies defining the Ceratophryinae clade, and supporting *Ceratophrys* and *Lepidobatrachus* as the sister group of *Chacophrys* (Fig. 1A). The morphological diagnosis of the group was well supported by adult characters. Some

Figure 4. Variation of trigeminal and facial nerves relationships during larval development at larval stage 37. A, *Lepidobatrachus laevis*; B, *Ceratophrys cranwelli*. The trigeminal and facial nerves are completely separated, as schematized in (C). D, *Odontophrynus lavillai*; E, *Pseudis platensis*. There is an incipient fusion of the trigeminal and facial ganglia, but the roots of these nerves remain separated, as shown in (F). G, *Telmatobius atacamensis*, the roots and ganglia of the trigeminal and facial nerves are completely fused, as schematized in (H). The presence of the commissura prefacialis (a cartilaginous bridge dividing the passage of nerves V and VII), and the unfused condition of the roots and ganglia of nerves V and VII, were considered as two related features that were only present in type-III larvae, and the absence of the commissura and the fusion of the roots and ganglia of nerves V and VII was regarded as the derived condition of neobatrachia (Sokol, 1975; Duellman & Trueb, 1986; Ford & Cannatella, 1993). However, different degrees in the condition of these nerves related to the absence of the commissura prefacialis were described for tadpoles of *L. llanensis*, *C. cranwelli*, *Ceratophrys ornata*, and *Pelodytes caucasicus* Boulenger, 1896 (Fabrezi & Chalabe, 1997; Haas, 2003). Abbreviations: gp, ganglion prooticum (fused ganglia of nerves V and VII); gVIII, ganglion vestibulocochlearis; hyo, ramus hyomandibularis of VII; md, ramus mandibularis of V; mx, ramus maxillaris of V; op, ramus ophthalmicus profundus of V; pl, ramus palatinus of VII; V, trigeminal nerve; VII, facial nerve; VIII, vestibulocochlearis nerve; IX, glossopharyngeal nerve; IXcVII, ramus communicans IX cum VII; X, vagus nerve. Scale bars: 0.5 mm.

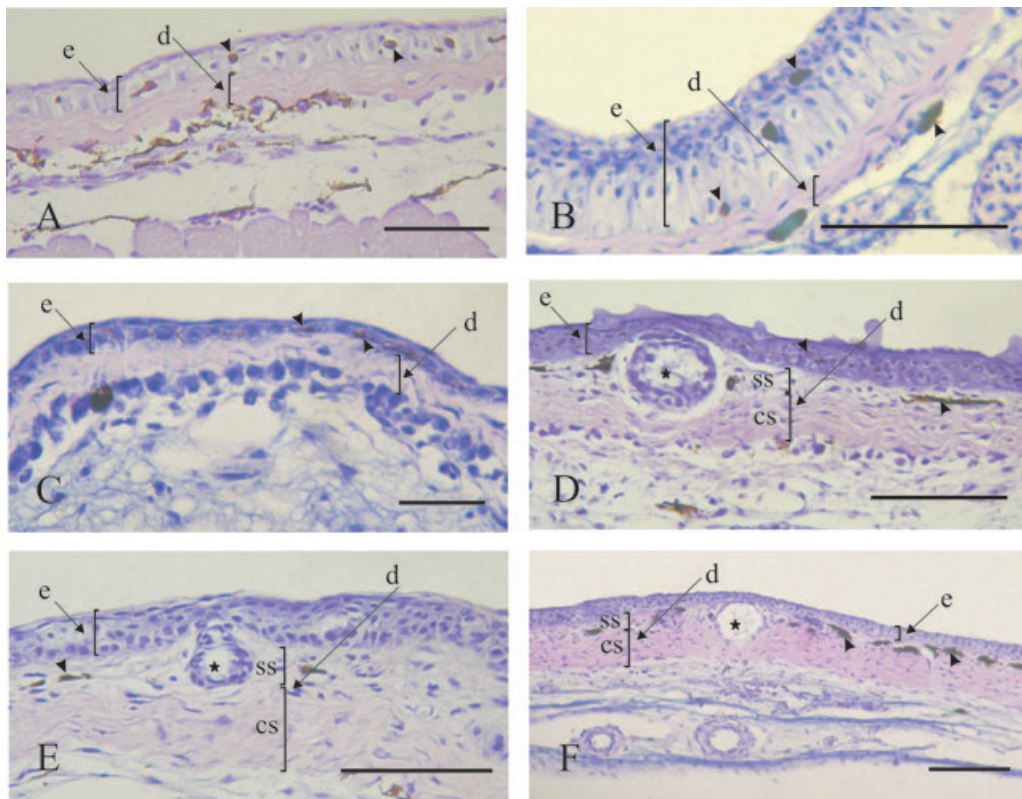


Figure 5. Cross sections of the skin of the dorsum in selected tadpoles at larval stage 37. A, *Odontophrynus lavillai*. The epidermis is double layered with cells organized in an internal layer of columnar cells and an external layer of flattened cells. Melanocytes are in the epidermis. The dermis is a single layer, with a compact stratum composed of collagenous fibres. B, *Telmatobius atacamensis*. The thick epidermis is composed of an inner layer of tall and large cells, and at least three external layers of flattened cells. Melanocytes are in the epidermis. The dermis is thin and has only a compact stratum. C, *Chacophrys pierottii*. The epidermis is double layered. Melanocytes are in the epidermis. The dermis is single and thin. D, *Ceratophrys cranwelli*. Five layers of cells form the epidermis. Melanocytes are in the epidermis and dermis. Two layers form the dermis: the outer stratum spongiosum that has mucous glands, and the underlying stratum compactum. E, *Lepidobatrachus llanensis*. The epidermis is composed of three or four layers of flat cells. The dermis has the stratum spongiosum with glands and the stratum compactum. There are scarce melanocytes in the dermis. F, *Lepidobatrachus laevis*. Three layers of cells form the epidermis. The dermis consists of stratum spongiosum and compactum. The stratum spongiosum has small glands and melanocytes. In *C. cranwelli*, *L. llanensis*, and *L. laevis* the dermis thickness is greater than twice the epidermis thickness. Abbreviations: cs, compactum stratum of the dermis layer; d, dermis; e, epidermis; ss, spongiosum stratum of the dermis layer. Symbols: arrowheads point to melanocytes; stars indicate mucous glands. Scale bars: 0.1 mm.

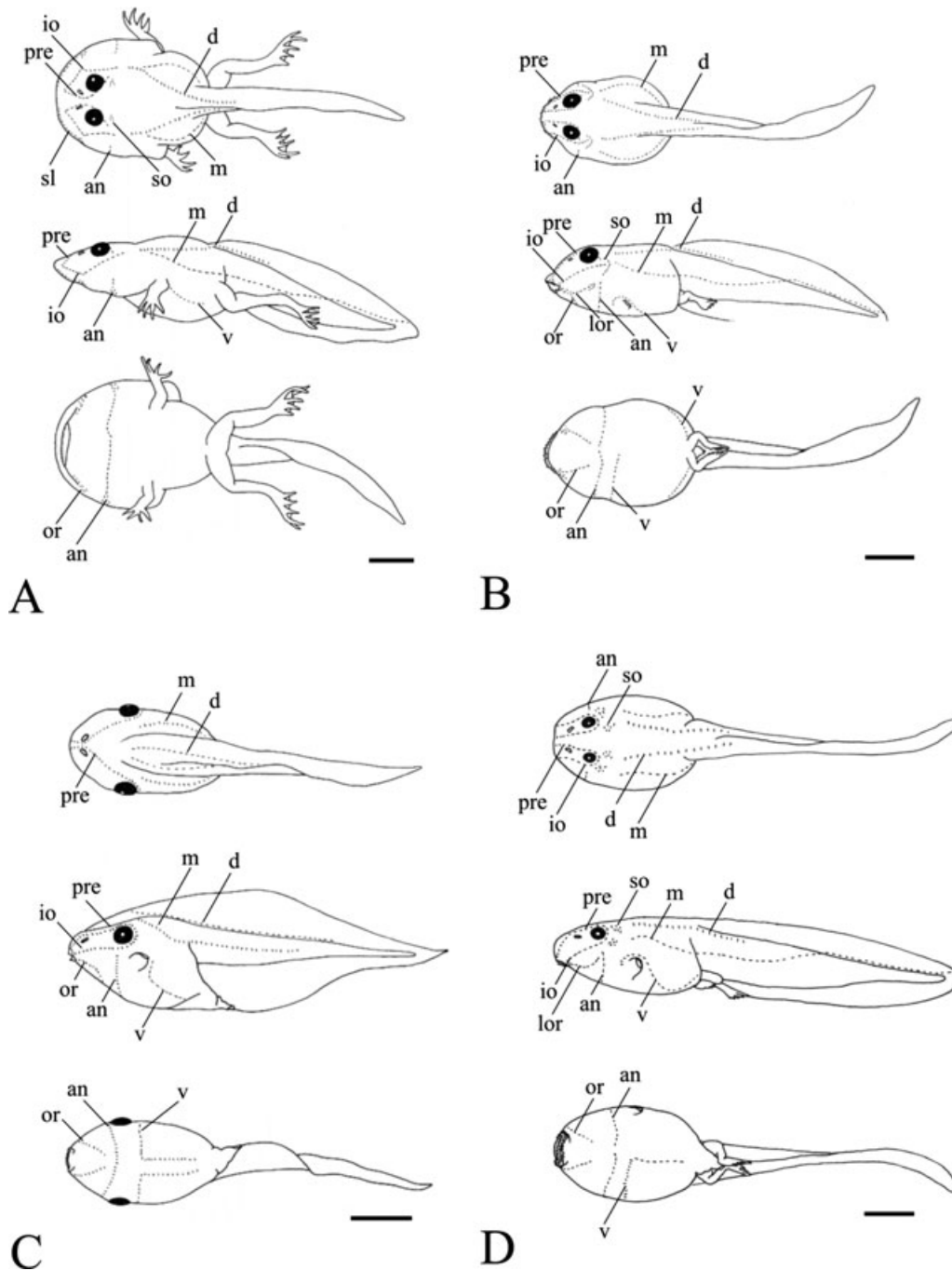


Figure 6. Pattern of the lateral line system of tadpoles at larval stage 37 in dorsal, lateral, and ventral views. A, *Lepidobatrachus laevis*. B, *Ceratophrys cranwelli*. C, *Pseudis platensis*. D, *Telmatobius atacamensis*. Discontinuous lines represent neuromasts. Abbreviations for the anterior lateral line system: an, angular line; io, infraorbital line; lor, oral longitudinal line; or, oral line; pre, preorbital line; sl, supralabial line, and st, supratemporal line. Abbreviations for the posterior lateral line system: d, dorsal line; m, medial line; v, ventral line. Scale bar: 10 mm.

larval characters shared by *Lepidobatrachus* and *Ceratophrys* were proposed to define Ceratophryini (Haas, 2003; Frost *et al.*, 2006). The absence of some of them in tadpoles of *Chacophrys* would therefore

imply that they were reversed if *Lepidobatrachus* was the basal taxon (Reig & Limeses, 1963; Laurent, 1986; Frost *et al.*, 2006; Grant *et al.*, 2006).

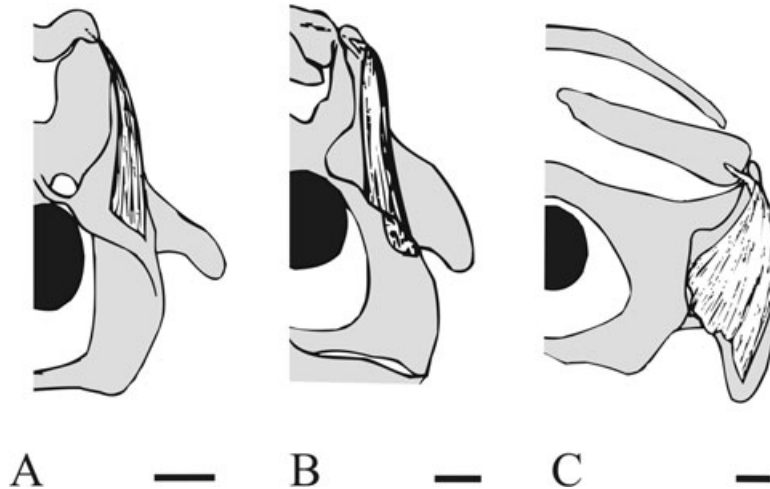


Figure 7. Variation in the origin of the m. suspensorioangularis in tadpoles (stage 37). A, in *Chacophrys pierottii*, the m. suspensorioangularis has a preorbital origin, its fibres originate at the posterior base of the process muscularis. B, in *Ceratophrys cranwelli*, the m. suspensorioangularis is long, and has fibres originating posterior to the base of the processus muscularis at the ventral surface of the palatoquadrati, and are covered by the m. hyoangularis. The origin of this muscle in this species is at the level of the eye. C, in *Lepidobatrachus llanensis*, some fibres of the m. suspensorioangularis originate from the lateral expansion of the palatoquadrati, behind the posterior base of the processus muscularis, and other fibres that are joined by connective tissue link the origin of this muscle with the ceratohyal. The origin of this muscle is postorbital. Haas (2003) recognized a posterior origin of the m. suspensorioangularis in basal anurans –differing from the preorbital origin observed in neobatrachians. Scale bars: 1 mm.

We distinguished three types of derived characters: (1) characters that are only present in the larval body plan; (2) characters that have continuity between the larval and adult body plans; and (3) characters that belong to the adult body plan.

1. Characters that are only present in the larval body plan

From our phylogenetic analysis, two derived characters of the larval body plan defined the Ceratophryinae: complete oral disc papillation, and anal tube short and almost cylindrical (Fig. 2). The presence of continuous papillation has been reported in other anurans (Haas, 2003) as a synapomorphy for the clade formed by Batrachylini (Frost *et al.*, 2006), or for the three ceratophryine genera (Quinzio *et al.*, 2006). The anal tube morphology of ceratophryines (Quinzio *et al.*, 2006) differs from that of most anuran larvae, and could represent an advanced morphology before its early disappearance at larval stage 39, as Hall *et al.* (1997) described in *Spea intermontana* (Cope, 1883) at larval stage 38.

Many derived characters shared by *C. cranwelli* and *Lepidobatrachus* spp. are characters of the larval chondrocrania (Fig. 8) and muscles (i.e. absence of m. quadratoangularis, and mm. levatores arcuum branchialium I and II separated by a gap). Some of these traits were proposed by Haas (2003) to define

the clade composed of *Ceratophrys* + *Lepidobatrachus*, and are distinctive traits noted in anatomical descriptions of tadpoles of these taxa (Lavilla & Fabrezi, 1992; Wild, 1997; Vera Candioti, 2005). Other characters of the tadpoles of *C. cranwelli* and *Lepidobatrachus* spp., such as neuromast topography (Fig. 6), are potentially significant, but are poorly understood. *Ceratophrys* and *Lepidobatrachus* share discontinuous supraorbital and infraorbital lines, and the presence of supratemporal neuromasts. Schlosser & Roth (1995) mentioned a similar group of supratemporal neuromasts in tadpoles of *Discoglossus pictus*, which could represent either a posterior extension of the infraorbital line supplied by the buccal ramus of the ADLLN, or a rudimentary otic lateral line supplied by an otic lateral line nerve that is completely fused with the ADLLN. This last interpretation would mean a vestigial feature of an ancient condition.

Although a lateral line system that disappears at metamorphosis is typical of anuran tadpoles, metamorphosis does not invariably lead to the loss of these organs (Fritzsche, 1990). The retention of neuromasts, afferents, and second-order neurons in some aquatic anurans – *L. laevis*, *Occydozyga lima* (Gravenhorst, 1829), and the Pipidae (Fritzsche, Drewes & Ruibal, 1987) – may be interpreted as being even further derived from the typical anuran transformation, and is most likely to reflect a

Table 2. Comparisons of relative timing of developmental events during the whole larval period, following the standard table for staging anurans (Gosner, 1960)

Morphological features	<i>Chacophrys pierottii</i>	<i>Ceratophrys cranwelli</i>	<i>Lepidobatrachus</i> spp.	<i>Pseudis platensis</i>
(1) Toe differentiation. Stages 31–37.	Stages 31–37	Stages 31–37	Stages 31–37	Stages 31–37
(2) Inner metatarsal tubercle differentiation. Stages 38–40.*	Stage 36	Stage 36	Stage 36	Stage 38–40
(3) Subarticular tubercles differentiation. Stages 38–40.	Stage 42	Stage 42		
(4) Disappearance of anal tube. Stage 41.	Stage 39	Stage 39	Stage 37	Stage 43
(5) Forelimb emergence. Stage 42.	Stage 42	Stage 42		Stage 42
(6) Larval teeth and horny beaks disappearance. Stage 42.	Stage 42	Stage 42	Stage 42	Stage 42
(7) Angle of mouth in front of nostril. Stage 42.	Stage 42	Stage 42		Stage 42
(8) Tail shortening. Stage 42.	Stage 42	Stage 42	Stage 42	Stage 43
(9) Angle of mouth between nostril and eye. Stage 43.	Stage 43	Stage 43	Stage 36	Stage 43
(10) Caudal fins regression. Stage 43.	Stage 43	Stage 43	Stage 43	Stage 45
(11) Angle of mouth at level of eye. Stage 44.	Stage 44	Stage 43	Stage 42	?
(12) Tail greatly reduced. Stage 44.	Stage 44	Stage 44	Stage 44	?
(13) Angle of mouth at posterior margin of eye. Stage 45.	Stage 45	Stage 44	Stage 43	Stage 45
(14) Tail reduced to stub. Stage 45.	Stage 45	Stage 45	Stage 45	?
(15) Tail completely resorbed. Stage 46.	Stage 46	Stage 46	Stage 46	?
(16) Angle of mouth beyond the posterior margin of eye.	Stage 46	Stage 46	Stage 44	
(17) Shortening of the gut and stomach formation. Stage 42–46.†	Stage 42–46	Earlier than Stage 42	Earlier than Stage 42	Stage 42–?
(18) Increase of skin complexity. Stage 42–46.	Stage 42	Stage 37	Stage 37	?
(19) Lateral line system degeneration. From Stage 42.‡	From Stage 42	From Stage 42	?	?

Dark-grey areas indicate predisplaced features and light-grey areas indicate postdisplaced features.

*At larval stages 36–37, the hypertrophic prehallical distal element and prominent inner metatarsal tubercle were described in *Odontophrynus americanus*, *Odontophrynus lavillai*, *Ceratophrys cranwelli*, and *Lepidobatrachus laevis* (Fabrezi, 2001a), and in *Spea intermontana* (Hall *et al.*, 1997). Keratinization of the spade takes place at larval stage 39 in *Lepidobatrachus llanensis* and in *Chacophrys pierottii*, and after metamorphosis in *C. cranwelli* and *L. laevis*.

†In *Ceratophrys cranwelli* and *Lepidobatrachus* spp. an adult-like stomach and a short intestine are features present in tadpoles before stage 42 (Ulloa Kreisel, 2001, 2002, 2003). Carrol *et al.* (1991) described pepsinogen secretions in *Lepidobatrachus laevis* tadpoles.

‡Wahnschaffe, Bartsch & Fritsch (1987) described changes in degeneration in the lateral-line nerves, lateral-line organs and lateral-line nuclei during anuran metamorphosis, where degeneration of peripheral nerves and ganglion cells occurs last of all, including the postmetamorphic stages. Fritsch *et al.* (1987) reported the complete absence of the lateral line system in *Pseudis paradoxa*, although our specimen of *P. platensis* at larval stage 43 retains neuromasts lines on the tail.

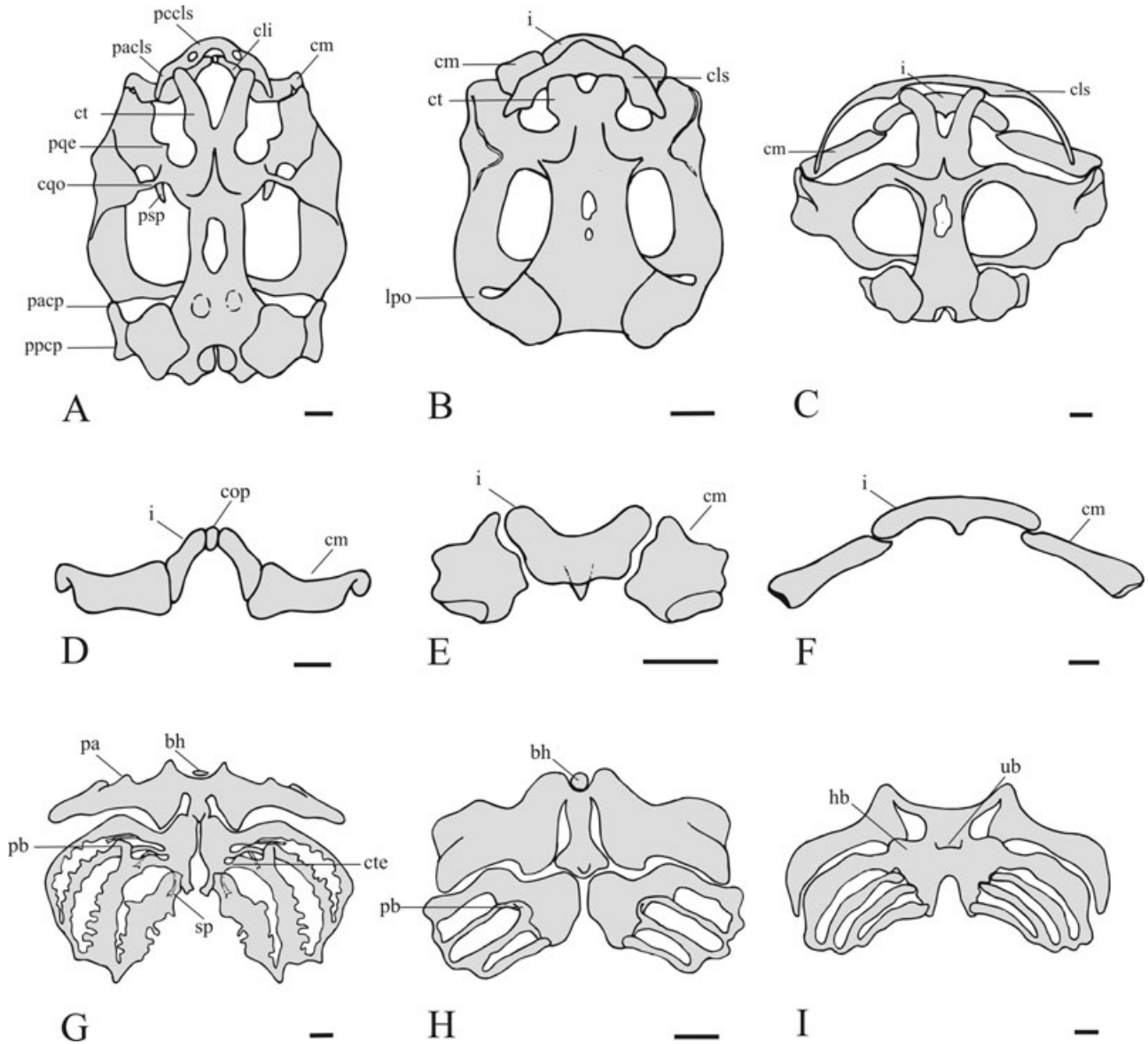


Figure 8. A–C, dorsal view of the chondrocranium. D–F, ventral view of the lower jaw. G–I, hyobranchial skeleton at larval stage 37. A, D, and G, *Chacophrys pierottii*. B, E, and H, *Ceratophrys cranwelli*. C, F, and I, *Lepidobatrachus laevis*. Abbreviations: bh, basihyal; cli, cartilage labialis inferior; cls, cartilago labialis superior; cm, cartilago meckeli; cop, copula mandibularis; cqo, commissura quadratoorbitalis; ct, cornua trabeculae; cte, commissura terminalis; hb, hypobranchialia; i, infraostrals; lpo, larval process oticus; pa, processus anterolateralis of ceratohyal; pacls, pars alaris cartilago labialis superior; pacp, processus anterolateralis crista parotica; pb, processus branchialis; pccls, pars corporea cartilago labialis superior; pqe, processus quadratoethmoidalis; ppcp, processus posterolateralis crista parotica; psp, processus pseudopterygoideus; sp, spicula; ub, processus urobranchialis. Scale bar: 1 mm.

specific adaptation, rather than a simple conservation of the ancestral condition (Frittsch, 1990). Despite Ceï's (1981) report of the absence of the lateral line system in adults of *L. llanensis* and *Lepidobatrachus asper* Budgett, 1899, there are no data in these species indicating the complete loss of the lateral line system in the postmetamorphic indi-

viduals. Adults of *Lepidobatrachus* spp. could share the derived condition of the retention of components of the lateral line system.

In *C. pierottii*, the presence of a nasal appendix would represent an autapomorphy, because this structure has not yet been described in anuran tadpoles (Faivovich & Carrizo, 1992; Quinzio *et al.*, 2006).

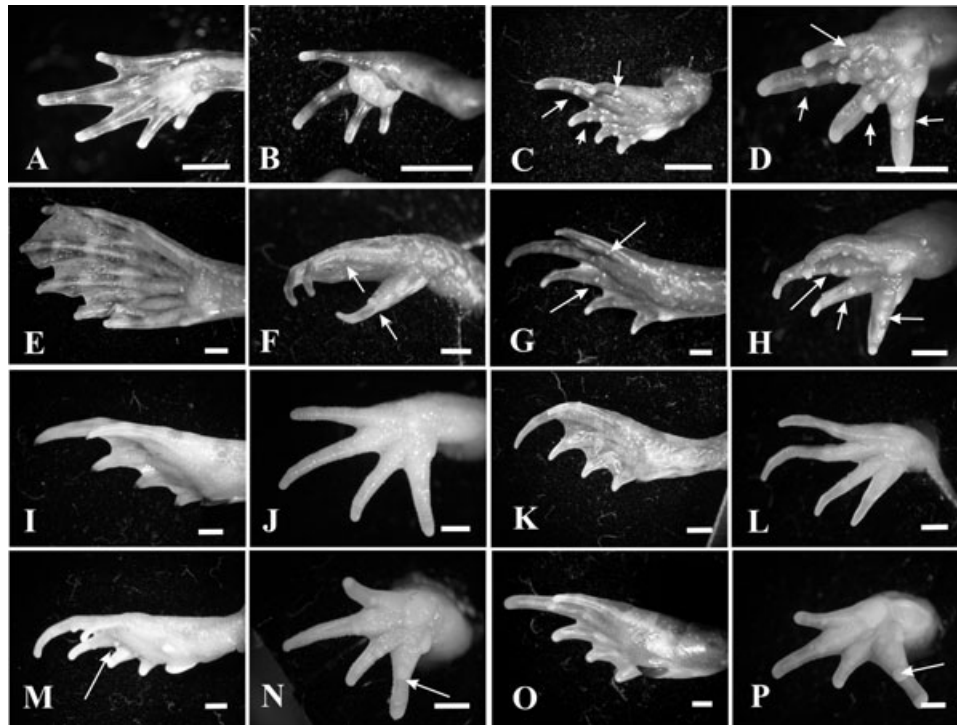


Figure 9. Palmar and plantar views of the autopodia in anurans at the end of metamorphosis, where subarticular tubercles are already well defined. A, *Bombina variegata* (foot): subarticular tubercles are absent and metatarsal tubercles are poorly developed. B, *Bombina variegata* (hand): subarticular tubercles are absent. C, *Odontophrynus americanus* (foot): subarticular tubercles are evident, and the inner metatarsal is not yet keratinized. D, *Odontophrynus americanus* (hand): subarticular tubercles are strongly developed. E, *Pseudis platensis* (foot): tubercles are absent and the inner metatarsal tubercle is defined. F, *Pseudis platensis* (hand): small subarticular tubercles are evident. G, *Telmatobius atacamensis* (foot): small subarticular tubercles and the inner metatarsal tubercle are present. H, *Telmatobius atacamensis* (hand): subarticular tubercles are better defined than in the foot. I, *Lepidobatrachus llanensis* (foot): subarticular tubercles are absent. The well-developed inner metatarsal tubercle and toe tips are keratinized. J, *Lepidobatrachus llanensis* (hand): subarticular tubercles are absent. K, *Lepidobatrachus laevis* (foot): subarticular tubercles are absent, and the inner metatarsal tubercle presents incipient keratinization. L, *Lepidobatrachus laevis* (hand): subarticular tubercles are absent. M, *Ceratophrys cranwellii* (foot): small subarticular tubercles are defined, and the inner metatarsal tubercles are poorly keratinized. N, *Ceratophrys cranwellii* (hand): small subarticular tubercles are present. O, *Chacophrys pierottii* (foot): subarticular tubercles are not prominent. Inner metatarsal tubercle has keratinization. P, *Chacophrys pierottii* (hand): subarticular tubercles are well developed. The pattern of distribution of subarticular tubercles is considered as diagnostic in most species, and development of these structures takes place at larval stages 38–40 (Gosner, 1960). Subarticular tubercles in manus and pes are also absent or poorly developed in *Xenopus*, *Hymenochirus*, *Pipa*, *Ascaphus*, *Leiopelma*, *Alytes*, *Discoglossus*, *Madecassophryne*, *Stumpffia*, *Rhinoderma*, *Brachycephalus*, *Truebella*, and *Ansonia* (Guibé, 1978; Graybeal & Cannatella, 1995; Matsui, Nabhitabhata & Panha, 1998; Pombal & Gasparini, 2006, among others). Scale bar: 0.2 mm.

2. Characters that have continuity between the larval and adult body plans

The almost complete chondrification of the cavum cranii (Fig. 3) was proposed as a synapomorphy for Ceratophryini (Haas, 2003): this hypermorphic condition is present in the three genera. In the Ceratophryinae, larvae have nerves V and VII completely separated (Fig. 4). Given that the adults of neobatrachian species have fused trigeminal and facial roots and ganglia, the late fusion of trigeminal and facial

ganglia and roots could be a synapomorphic condition for the Ceratophryinae.

The characters of skin (epidermal glands present, spongiosum stratum of dermis present, and three or more epidermal layers) shared by tadpoles of *Ceratophrys* and *Lepidobatrachus* spp. (Fig. 5) reveal more complexity than the typical tadpole skin (Duellman & Trueb, 1986). Skin transformations take place during metamorphosis, are highly influenced by thyroid hormones (Yoshizato, 1992), and have a

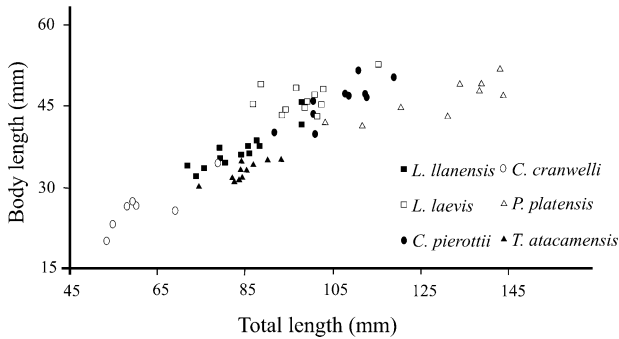


Figure 10. Total length (TL) versus snout–vent length (SVL) plotted for larval specimens of six species between stages 39 and 41. *Ceratophrys cranwelli* tadpoles ($N = 8$, $TL = 61.8 \pm 8.2$ mm, $SVL = 26.3 \pm 3.8$ mm) are the smallest. *Pseudis platensis* larvae ($N = 9$, $TL = 129.5 \pm 14.4$ mm, $SVL = 46.3 \pm 3.6$ mm) are the largest. *Lepidobatrachus laevis* ($N = 12$, $TL = 98.3 \pm 7.2$ mm, $SVL = 46.6 \pm 2.8$ mm) and *Chacophrys pierottii* ($N = 10$, $TL = 106.5 \pm 8.0$ mm, $SVL = 46.3 \pm 3.9$ mm) share with *P. platensis* similar values of SVL. *Lepidobatrachus llanensis* ($N = 15$, $TL = 83.7 \pm 7.5$ mm, $SVL = 36.7 \pm 3.4$ mm) and *Telmatobius atacamensis* ($N = 11$, $TL = 84.6 \pm 4.8$ mm, $SVL = 32.9 \pm 3.4$ mm) are similar in total length, but *L. llanensis* tadpoles have greater body sizes.

sequential expansion from the lateral sides of the body to the dorsal and ventral regions (Tamakoshi, Oofusa & Yoshizato, 1998). In *Ceratophrys* and *Lepidobatrachus*, the derived traits of tadpole dorsal skin reflect a coordinated predisplacement of those metamorphic transformations in different epidermal and dermal structures that are already advanced at larval stage 37.

The origin of depressor mandibulae from the squamosal and tympanic annulus in *Ceratophrys* and *Lepidobatrachus* (Manzano *et al.*, 2003) is an adult character that appears as a synapomorphy for these genera. This muscle originates as a set of larval muscles that are: *m. suspensoriohyoideus*, which is present in the Ceratophryinae (Fig. 14); *m. orbitohyoideus*; *m. hyoangularis*; *m. suspensorioangularis*; and *m. quadratoangularis*. The absence of *m. quadratoangularis* in *C. cranwelli* and *Lepidobatrachus* spp. results in a synapomorphic condition, in agreement with the previous hypothesis of Haas (2003). We also observed a difference from other anurans in the origin of *m. suspensorioangularis* in *C. cranwelli* and *Lepidobatrachus* spp. (Fig. 7).

Lepidobatrachus laevis and *L. llanensis* share the absence of subarticular tubercles on the autopodia (Fig. 9). The available information suggests some hypotheses that could be tested in a more extensive study of intraordinal variation: (1) the absence represents a primitive condition of basal anurans; (2)

poorly developed or absent subarticular tubercles may reflect paedomorphic traits related to digital reduction in some microhylids and bufonids; (3) reduction or absence of subarticular tubercles may occur in pes, manus, or in both; (4) the complete lack of subarticular tubercles on the manus and pes of *Lepidobatrachus* spp. is an unusual condition among neobatrachians.

3. Characters belonging to the adult body plan

Adult synapomorphies for Ceratophryinae are well known. The three genera share exostotic skulls that involve directly or indirectly the development of a frontoparietal–squamosal arch, the expansive development of the otic ramus of the squamosal, and the sutured zygomatic ramus of the squamosal with the maxillary. In *Chacophrys*, the frontoparietal arch and supraorbital plate are present at juvenile stages, whereas in *Ceratophrys* and *Lepidobatrachus* the supraorbital plate is developed at the end of metamorphosis, although the fusion of the squamosal and frontoparietal occurs later. The exostosed bones of the Ceratophryinae present two types of similarities: (1) similarity in the skull configuration, because the squamosal rami are well developed, the zygomatic is fused to the maxillary, and the otic ramus forms the frontoparietal–squamosal arch and grows into the supraorbital plate overlapping the otic capsules, and (2) similarity in the squamosal development, because the squamosal rami differentiate at the beginning of the metamorphosis, from three distinct ossification centers in *Ceratophrys* (Wild, 1997) and *Lepidobatrachus*, or two centers in *Chacophrys* (Wild, 1999).

Other adult synapomorphies of Ceratophryinae involve derived traits in the jaws. Monocuspid and nonpedicellate teeth have been previously used to diagnose the Ceratophryinae (Reig & Limeses, 1963; Lynch, 1971; Cei, 1981; Laurent, 1986; Fabrezi, 2006). Like *Pyxicephalus adspersus* Tschudi, 1838 (Smirnov & Vasil'eva, 1995; Haas, 1999), the nonpedicellate teeth of the Ceratophryinae were also interpreted as accelerated development of calcification, invading the zone dividing the pedicel from the crown (Fabrezi, 2001b). The mentomeckelian bone completely fused to the dentary and fangs on the lower jaw are related features involved in early metamorphic events of lower jaw development, which may be integrated with the absence of the pars palatina of the premaxillae (Fabrezi & Emerson, 2003). With respect to the posterior articulation of the lower jaw, Fabrezi (2006) interpreted the enlargement of the jaws in the Ceratophryinae as a consequence of peramorphosis – extension of trait offset shape (Reilly *et al.*, 1997) – in the skull configuration.

Other features of the adult body plan, such as the absence of anterolateral processus of the hyoid, which

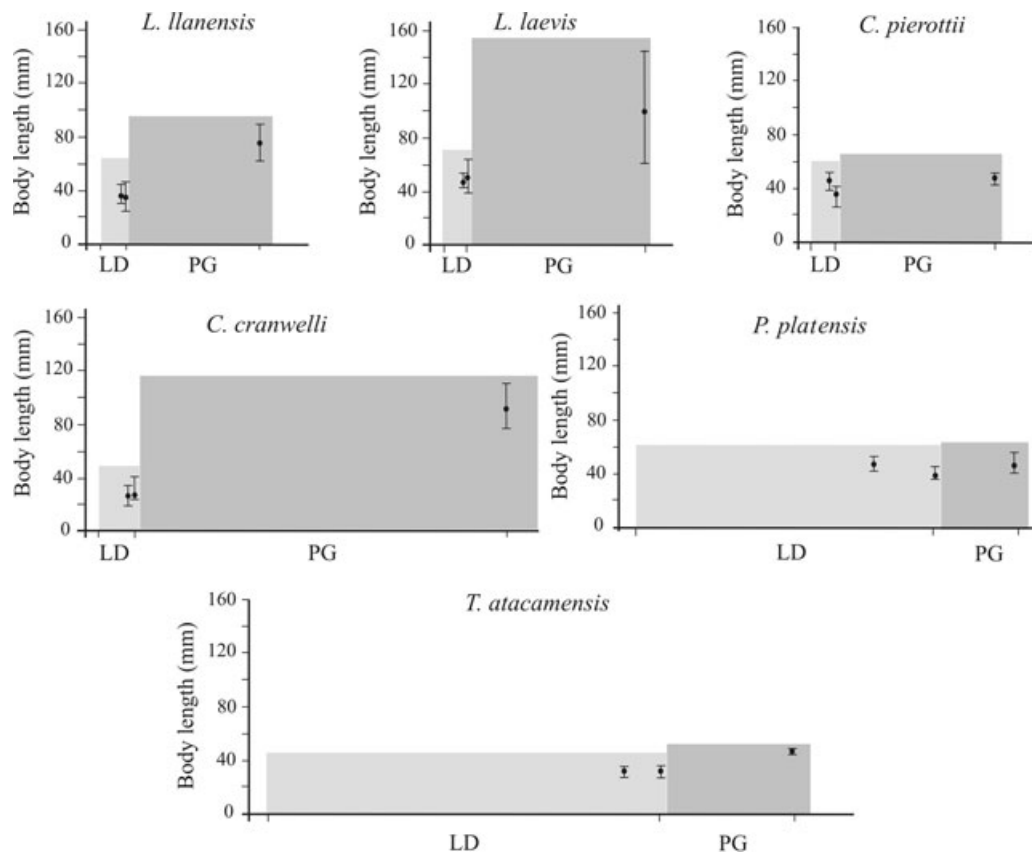


Figure 11. Mean, maximum, and minimum values of snout-vent lengths (SVLs) in advanced tadpoles (stages 38–41), during metamorphosis (stages 42–46), and in adults. Values of SVL are given in mm. Light-grey areas approximately represent the extension of larval development (LD) in months, and dark-grey areas refer to postmetamorphic growth (PG) in lines of arrested growth (LAGs). *Lepidobatrachus llanensis*: SVL in tadpoles ($N = 15$, $SVL = 36.7 \pm 3.4$ mm); SVL of metamorphic specimens ($N = 26$, $SVL = 36.3 \pm 5.8$ mm); SVL of adults ($N = 16$, $SVL = 76.5 \pm 7.5$ mm). Larval development takes place over a period of 2 weeks. Postmetamorphic growth estimated in LAGs = 6 years. *Lepidobatrachus laevis*: SVL in tadpoles ($N = 12$, $SVL = 46.6 \pm 2.8$ mm); SVL of metamorphic specimens ($N = 26$, $SVL = 49.9 \pm 7.8$ mm); SVL of adults ($N = 13$, $SVL = 99.2 \pm 22.1$ mm). Larval development takes over a period of 2 weeks. Postmetamorphic growth estimated in LAGs = 7 years. *Chacophrys pierottii*: SVL in tadpoles ($N = 10$, $SVL = 46.3 \pm 3.9$ mm); SVL of metamorphic specimens ($N = 28$, $SVL = 36.3 \pm 2.6$ mm); SVL of adults ($N = 15$, $SVL = 47.8 \pm 2.7$ mm). Larval development takes place over a period of 2 weeks. Postmetamorphic growth estimated in LAGs = 6 years. *Ceratophrys cranwelli*: SVL in tadpoles ($N = 8$, $SVL = 26.3 \pm 3.8$ mm); SVL of metamorphic specimens ($N = 18$, $SVL = 27.5 \pm 4.4$ mm); SVL of adults ($N = 10$, $SVL = 91.9 \pm 12.7$ mm). Larval development takes place over a period of 3 weeks. Postmetamorphic growth estimated in LAGs = 14 years. *Pseudis platensis*: SVL in tadpoles ($N = 9$, $SVL = 46.3 \pm 3.6$ mm); SVL of metamorphic specimens ($N = 8$, $SVL = 37.4 \pm 2.7$ mm); SVL of adults ($N = 7$, $SVL = 45.3 \pm 5.0$ mm). Larval development takes place over a period of 6 months. Postmetamorphic growth estimated in LAGs = 3 years. *Telmatobius atacamensis*: SVL in tadpoles ($N = 11$, $SVL = 32.9 \pm 3.4$ mm); SVL of metamorphic specimens ($N = 30$, $SVL = 31.0 \pm 1.8$ mm); SVL of adults ($N = 2$, $SVL = 45.2 \pm 1.0$ mm). Larval development takes place over a period of 8 months. Postmetamorphic growth estimated in LAGs = 5 years.

is shared by the three genera, appears in several anuran taxa (Trewavas, 1933). The extensive ossification of the posteromedial processus invading the hyoid plate and mineralization, both progressing during the postmetamorphic growth of *Ceratophryinae*, also suggest peramorphosis in features of the hyoid skeleton (Fabrezi, 2006).

Two features of the hyoid skeleton – discontinuous ceratohyalia and parahyoid dermal ossification – in *L. laevis* and *L. llanensis* could represent reversions to the condition of basal anurans (Fabrezi, 2006). *Lepidobatrachus llanensis* also exhibits a derived feature convergent with Pipidae, represented by the presence of toes with well-keratinized claws. Interest-

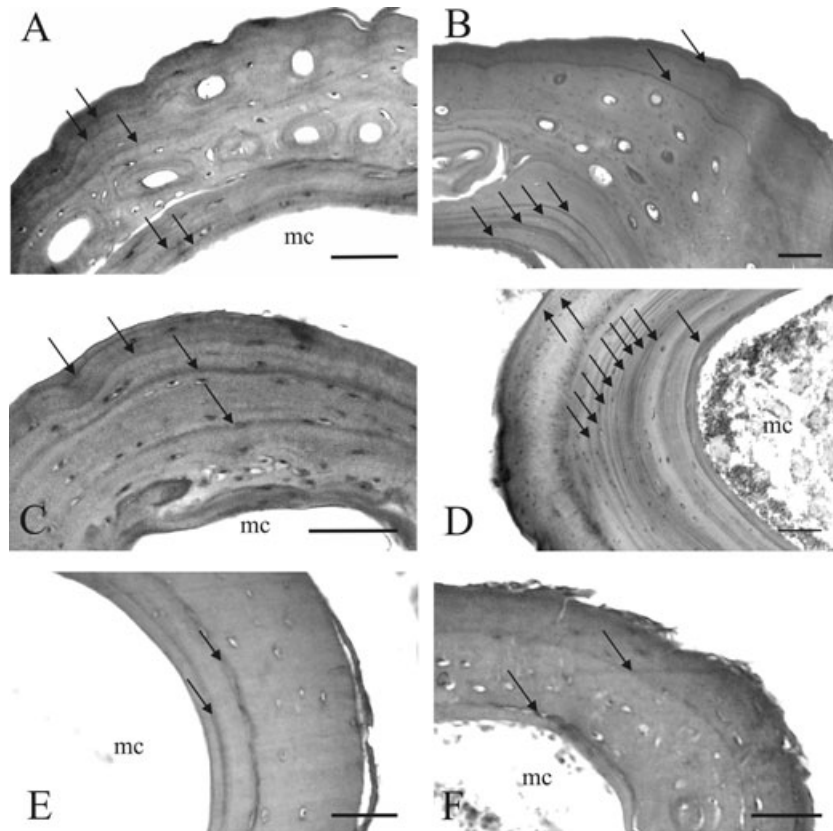


Figure 12. Hematoxylin–eosin cross sections (6- μ m thick) at the diaphyseal level of metatarsal IV in postmetamorphic specimens. Black arrowheads indicate lines of arrested growth (LAGs). A, *Lepidobatrachus llanensis*, five LAGs male [data for two adult males; 5 ± 1 (SVL 74.5 mm) and 6 ± 1 (SVL 74.1 mm)]. B, *Lepidobatrachus laevis*, six LAGs female [data for two adult females; 6 ± 1 (SVL 144 mm) and 7 ± 1 (SVL 111 mm)]. C, *Chacophrys pierottii*, four LAGs male [data for two adult males; 6 ± 1 (SVL 49.9 mm) and 4 ± 1 (SVL 45.2 mm)]. D, *Ceratophrys cranwelli*, 11 LAGs male [data for three adult males; 14 ± 1 (SVL 84.6 mm), 11 ± 1 (SVL 81 mm), and 13 ± 1 (SVL 74.3 mm)]. E, *Pseudis platensis*, two LAGs female [data for two adults (female and male); 2 ± 1 (SVL 46 mm) and 3 ± 1 (SVL 44.6 mm)]. F, *Telmatobius atacamensis*, juvenile specimen, two LAGs [data for an adult male; 5 ± 1 (SVL 45.4 mm) and for the juvenile 2 ± 1 (SVL 45 mm)]. Abbreviations: mc; marrow cavity. Scale bars: 0.05 mm.

ingly, the claws are already differentiated at those larval stages in which the digits become separated (stage 36–37). This is quite different from *Xenopus laevis* (Daudin 1802), where claw development occurs only after metamorphosis (Nieuwkoop & Faber, 1967).

Overall, our morphological data support the monophyly of Ceratophryinae, and recognize *Chacophrys* as the basal taxon. *Ceratophrys* and *Lepidobatrachus* share most of the derived features of larval body plans. Furthermore, the larval body plan of *Lepidobatrachus* spp. displays characters that are unique among anurans. Those characters that have continuity between larval and adult body plans are developed later in *Chacophrys* than in the other genera. Many derived features of the adults, especially those related to skull configuration, involve peramorphic development of dermal ossification.

INTEGRATED DEVELOPMENTAL PATHWAYS

The morphological evolution of the anuran life cycle involves either direct development or larval development with metamorphosis. One aspect about the evolution of the anuran metamorphosis was analyzed by Wassersug & Hoff (1982), who explored the reorientation of the suspensorium at metamorphosis. These authors proposed that evolution in anurans has increased the difference between larvae and adults, which shows an inverse relationship between the size of the angle of the jaw suspension in the larva and the size of the angle of the jaw suspension in the adult. Frogs recognized as basals tend to have the least difference in this feature between larvae and adults (gradual metamorphosis), whereas advanced frogs have the greatest difference (abrupt metamorphosis).

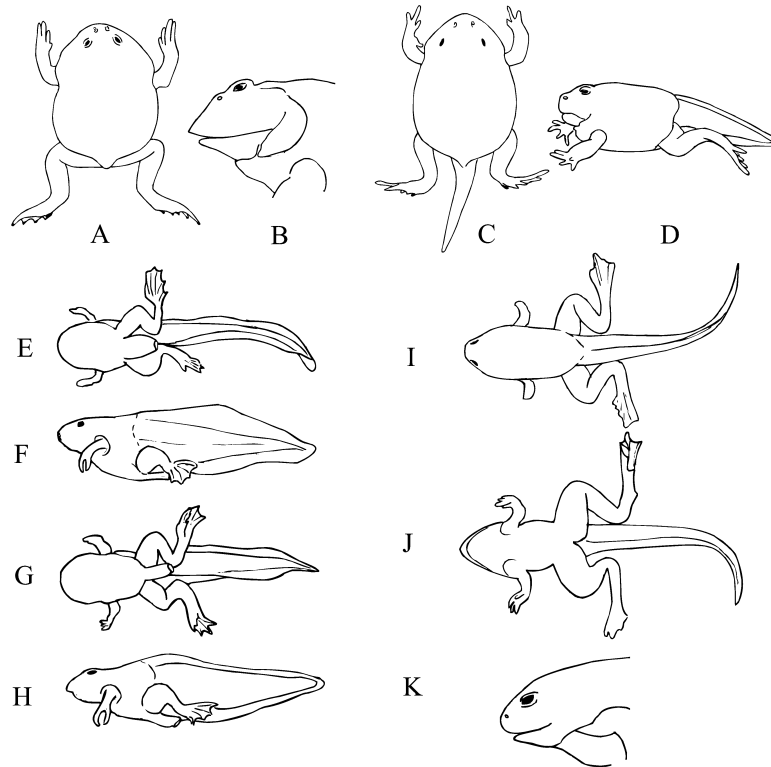


Figure 13. Variation in structural changes in some external features. A, dorsal view of *Lepidobatrachus llanensis* at the end of metamorphosis. A small tail stub is present. B, lateral view of the same specimen in (A) showing the angle of the mouth far beyond the posterior margin of the eye. C, dorsal view of *Chacophrys pierottii* during metamorphosis. The tail has started to reduce. D, lateral view of the same specimen in (C). The disappearance of the caudal fin is advanced, and the angle of the mouth reaches the posterior margin of the eye. E and F, ventral and lateral views of a *Pseudis platensis* tadpole at the beginning of metamorphosis. The oral disc and keratinized buccal structures are still present, and the forelimbs have emerged, but the anal tube remains well developed. G and H, ventral and lateral views of a *P. platensis* tadpole during metamorphosis. Larval mouthparts have disappeared; tail regression has started with the reduction of the fins, whereas the disappearance of the anal tube is delayed. I, dorsal view of *P. platensis* at an advanced metamorphic stage. The tail is conserved and has reduced caudal fins. J, ventral view of the same specimen showing the absence of the anal tube, and features of the mouth that are similar to those present in most anurans at the end of metamorphosis. K, detail in lateral view of the position of the angle of the mouth posterior to the eye.

The higher values for the angle difference reported by Wassersug & Hoff (1982) correspond to *Anotheca spinosa* (Steindachner, 1864) (102), *Occidozyga laevis* (Günther, 1858) (100), *Leptodactylus wagneri* (Peters, 1862) (111), and *Heleophryne purcelli* Sclater, 1898 (97). By comparison, the value for *C. pierottii* is 96, *C. cranwelli* 113, *L. llanensis* 130, and *L. laevis* 139, and also reveal abrupt metamorphosis.

The features of Ceratophryinae suspensoria thus, on average, reflect an increase in the level of metamorphic change with respect to other anurans. This is a consequence of the peramorphic patterns in jaw development that produce enlarged jaws and a posterior position of the articulation of the mandible, which provides these frogs with exceptionally large and wide mouths (Fabrezi, 2006). Skull metamorphosis involves the transformation of cranial cartilage

and skull ossification, which are influenced by the thyroid hormone (T3), although morphological and temporal integration between these tissues (cartilage and bone) is in part the result of each tissue responding independently to the hormonal influence, and it is not the consequence of tight developmental coupling between them (Hanken & Summers, 1988; Hanken, Summers & Hall, 1989). As the Ceratophryinae have different cartilaginous skull templates, the shared features that characterize their abrupt metamorphosis are concomitant with skull ossification, rather than with cartilage transformation.

Skull metamorphosis implies enlargement of lower jaw cartilage and displacement of the lower jaw–palatoquadrati articulation posteriorly. Tadpoles of *Lepidobatrachus* spp. have chondrocrania with infrarostral and enlarged cartilage meckeli similar

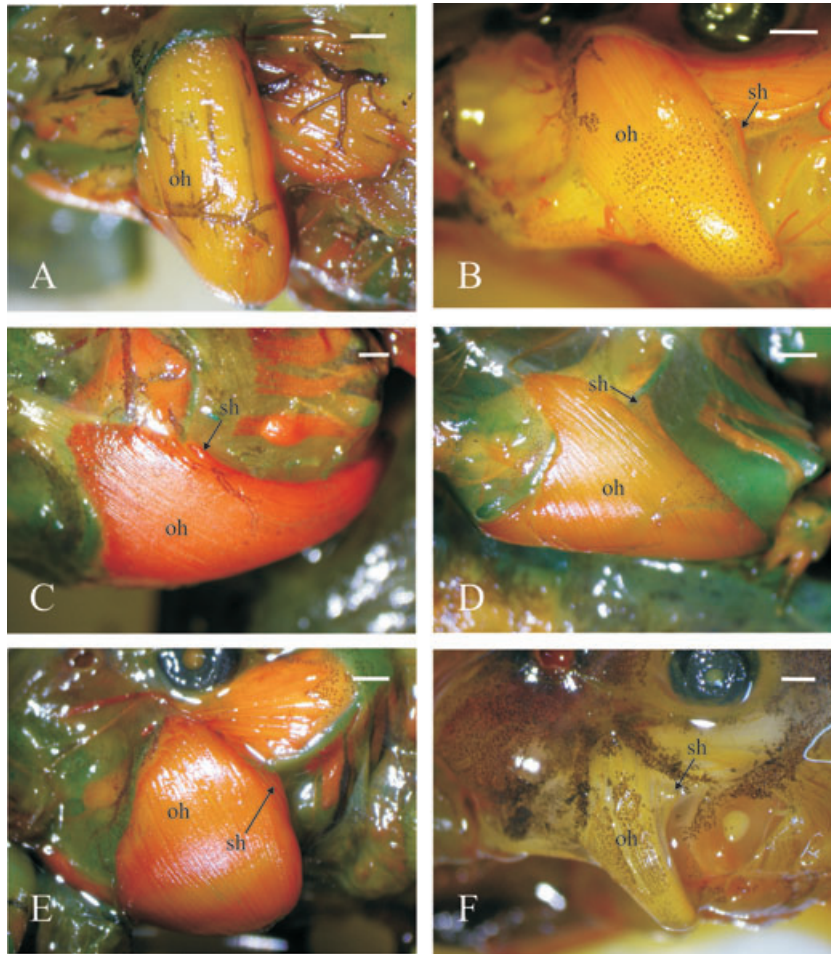


Figure 14. Presence and absence of *m. suspensoriohyoideus* among selected anuran tadpoles, following the criteria proposed by Haas (2003) at larval stage 37. A, *Pseudis platensis*. The muscle is absent. B–F, the muscle is present. B, *Telmatobius atacamensis*. C, *Lepidobatrachus laevis*. D, *Lepidobatrachus llanensis*. E, *Ceratophrys cranwelli*. F, *Chacophrys pierottii*. The absence of *m. suspensoriohyoideus* was reported for *Ceratophrys ornata*, *L. laevis* (Ruibal & Thomas, 1988; Haas, 2003), and *C. cranwelli* (Vera Candiotti, 2005), but Palavecino (1999) described it in *C. ornata* and *C. cranwelli*. Haas (2003) proposed that the absence of *m. suspensoriohyoideus* is a synapomorphy for the *Ceratophrys* and *Lepidobatrachus* clade, and Frost *et al.* (2006) extended the synapomorphy for Ceratophryini. Our data demonstrate the presence of the *m. suspensoriohyoideus* in tadpoles of the three genera of Ceratophryinae. Abbreviations: oh, *m. orbi-tohyoideus*; sh, *m. suspensoriohyoideus*. Scale bars: 1 mm.

to those of most anuran tadpoles in the middle of metamorphic climax. Hanken (1993) described these unique characteristics of the larval chondrocranium of *Lepidobatrachus* as a consequence of precocious differentiation of a cartilaginous configuration typical of the metamorphic skull. He identified this process as a derived metamorphosis pattern, and called it 'precocious metamorphosis'. Thus, the adult skull of the Ceratophryinae is the result of a derived abrupt metamorphosis, which seems to be more intensive in *Lepidobatrachus*, and may occur as a consequence of precocious changes in chondrocranial configuration.

The Ceratophryinae metamorphosis involves other morphological changes that are interpreted as having deviated from the stereotypical sequence of metamorphic events (Fig. 15, Table 2). Tadpoles of *Ceratophrys* and *Lepidobatrachus* show the disappearance of the anal tube before digit differentiation is complete. Similarly, they have a short gut, an adult-like stomach with mucous secretory cells (Ulloa Kreisel, 2001, 2002, 2003), gastric secretion (Carroll, Seneviratne & Ruibal, 1991), and more complexity in the organization of skin (Fig. 5) earlier than the beginning of metamorphosis. In addition, *Lepidobatrachus* exhibits two unique features among anuran tadpoles

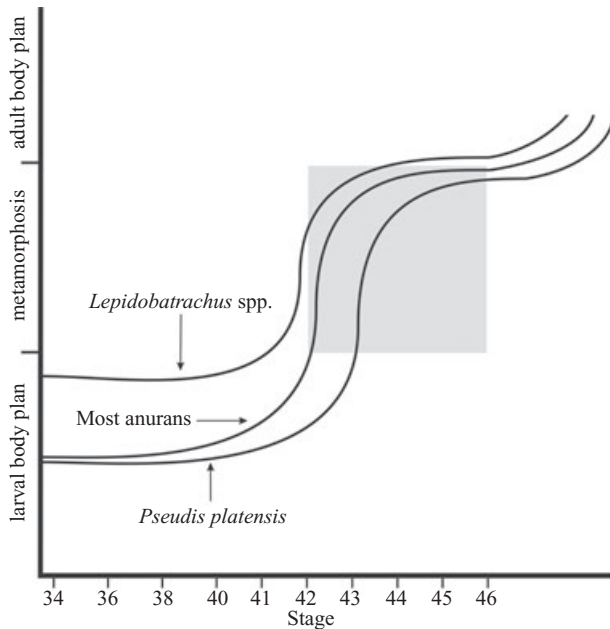


Figure 15. Comparison of the changes between the larval and adult body plans during larval development. The horizontal axis represents developmental stages (Gosner, 1960). The curves plotted depict structural modifications, and the grey area represents metamorphic events that take place during the metamorphic climax (stages 42–46) for most anurans. The early occurrence of metamorphic events (predisplacement) is observed in the ceratophryine frogs, especially in *Lepidobatrachus* spp., that have precocious metamorphosis. Delayed metamorphic events take place in the development of *Pseudis platensis*, a species in which some morphological changes that imply the end of metamorphosis for most anurans have yet to finish.

– external forelimbs and enlarged lower jaw cartilage, with simplified mouthparts – that are characteristic of the metamorphic climax. The predisplacement of metamorphic morphologies in *Lepidobatrachus* larval development reduces the level of change at the metamorphic climax. This metamorphosis could thus be called ‘precocious metamorphosis’, as Hanken (1993) proposed for the chondrocranial configuration, with some features of the ‘precocious metamorphosis’ insinuated in the larval development of the other ceratophryines. *Pseudis platensis* has, in contrast, ‘delayed metamorphosis’. In this species the events related with the loss of the anal tube and resorption of the tail are postdisplaced, taking place after the larval mouth structures have disappeared and the skull transformations are complete.

For most anurans, the gap between larval and adult body plans is marked by the metamorphic transformations. This gap becomes less constrained in *Pseudis platensis*, with delayed metamorphosis,

and *Lepidobatrachus* spp., with precocious metamorphosis (Fig. 15). Delayed metamorphosis produces (with a slow developmental rate), an early distinctive and paedomorphic adult body plan (Fig. 13). Precocious metamorphosis (with a fast developmental rate), builds a mature and peramorphic larval body plan. Both are derived developmental programs that show different developmental rates for the whole larval development, independent of the postmetamorphic growth rates. Larval development, including metamorphosis, is rapid and produces large tadpoles and metamorphs in *Lepidobatrachus* and *Chacophrys*. These genera show different patterns of postmetamorphic growth, being fast in *Lepidobatrachus* and low in *Chacophrys*. *Ceratophrys* larval development takes place with a lower rate, and reaches medium-sized tadpoles and metamorphs, and an extended postmetamorphic growth produces large adults. Despite the interspecific differences in the rates of larval and postmetamorphic growth in the Ceratophryinae, they shared a common history from which their characteristic adult body plan has been maintained. However, the changes in the timing of developmental events of metamorphosis (heterochrony) that are more notorious in *Lepidobatrachus* spp. seem to have had consequences in shaping a quite unique tadpole body plan.

Wassersug & Heyer (1988) proposed the morphological specializations of tadpoles of *Ceratophrys* and *Lepidobatrachus* had been independently derived. Our data suggest that the Ceratophryinae conserved a similar adult body plan, even for different life styles, which is the result of three different larval developments. The larval development of *Chacophrys* seems to represent the plesiomorphic condition from which the distinctive larvae of *Lepidobatrachus* and *Ceratophrys* could be derived, sharing some features of precocious metamorphosis.

To conclude, the Ceratophryinae share: (1) derived skull features involving the peramorphic development of the suspensorium that are the result of an abrupt metamorphosis; (2) fast larval development and medium-to-large metamorphs; (3) morphological features that are typical of the metamorphic climax, appearing at larval prometamorphic stages and reducing the events of the metamorphosis; (4) metamorphosis is highly precocious in *Lepidobatrachus* and results from a quite unique larval body plan.

Larval development and metamorphosis within Ceratophryinae show derived patterns relative to other anurans produced by heterochronic events, defining morphological features from which peramorphic development predominates and integrates acceleration, predisplacement, and hypermorphism (Reilly *et al.*, 1997). However, development of ceratophryines

exhibits some traits that could be interpreted as paedomorphic, such as the delayed fusion of trigeminal and facial roots and ganglia, delayed differentiation or absence of differentiation of subarticular tubercles in the autopodia, and persistence of the lateral line system in *L. laevis*.

The derived features of Ceratophryinae seem to be in agreement with phylogenetic hypotheses proposing *Chacophrys* as the basal taxon of the group from which larval development and metamorphosis have evolved in unusual directions.

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APPENDIX 1

ABBREVIATIONS OF MUSEUMS

FML: Instituto de Herpetología, Fundación Miguel Lillo, Tucumán, Argentina.
 MCN: Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

SPECIMENS EXAMINED

Ceratophryidae: Ceratophryinae

Chacophrys pierottii: MCN 1027 (35 larval specimens, stages 26–31); MCN 1028 (40 larval specimens, stages 31–36), skin cross sections, muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 1029 (56 larval specimens, stages 35–46), skeleton whole mounts; MCN 987 (nine males), two specimens for skeletochronology; MCN 1045 (one female); FML 9011 (two males), skeleton whole mounts; FML 9043 (four males).

Ceratophrys cranwelli: MCN 021 (23 larval specimens, stages 28–34), skeleton and peripheral nerves whole mounts; MCN 045 (32 larval specimens, stages 32–36); MCN 669 (45 larval specimens, stages 33–46), muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 188 (one male), skeleton whole mount; MCN 413 (12 larval specimens, stages 41–46); MCN 425 (72 larval specimens, stages 34–46), skin cross sections, muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 819 (three males), two specimens for skeletochronology and skeleton whole mount; MCN 933 (one male), skeletochronology; MCN 943 (one male); MCN 1034 (27 larval specimens, stages 37–43).

Lepidobatrachus laevis: MCN 647 (25 larval specimens, stages 33–40); MCN 663 (15 larval specimens, stages 36–41), muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 666 (seven larval specimens, stages 38–43 and one froglet); MCN 668 (one froglet), skeleton whole mount; MCN 695 (two juvenile specimens), skeleton whole mounts; MCN 696 (one juvenile specimen); MCN 814 (six larval specimens, stages 36–38), skin cross sections; MCN 815 (one adult and three juveniles), adult skin cross sections; MCN 817 (three juveniles); MCN 931 (three adults); MCN 934 (one juvenile); MCN 935 (one juvenile); MCN 936 (one juvenile); MCN 989 (one female), skeletochronology; MCN 1174 (female), skeletochronology.

Lepidobatrachus llanensis: MCN 970 (32 larval specimens, stages 33–46); MCN 081 (one female), skeleton whole mount; MCN 664 (12 larval specimens, stages 26–30); MCN 665 (70 larval specimens, stages 36–41), muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 932 (one male); MCN 986 (one female and eight males), two males for skeletochronology; MCN 1118 (45 larval specimens, stages 36–46), skin cross sections; MCN 1122 (seven larval specimens, stages 42–46), skeleton whole mounts.

Ceratophryidae: Telmatobiinae

Telmatobius atacamensis: MCN 937 (15 larval specimens, stages 33–41, and two juvenile specimens), one juvenile for skeletochronology; MCN 1026 (30 larval specimens, stages 34–46, and one adult), adult for skeletochronology, tadpoles for muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 1050 (15 larval specimens, stages 35–44), skin cross sections.

Cyclorhamphidae: Alsodinae

Odontophrynus lavillai: MCN 230 (one adult and 38 larval specimens, stages 33–41), skin cross sections, muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 244 (three adult and 45 larval specimens, stages 37–42, 44–46).

Odontophrynus americanus: MCN 267 (one male); MCN 315 (four males).

Cyclorhamphidae: Cyclorhamphinae

Rhinoderma darwinii: MCN 020 (six adult specimens).

Hylidae: Hylinae

Pseudis platensis: MCN 812 (one female), skeleton whole mount; MCN 968 (43 larval specimens, stages 27–34); MCN 969 (two males); MCN 988 (43 larval specimens, stages 32–39), muscle dissections, and skeleton and peripheral nerves whole mounts; MCN 1015 (five larval specimens, stages 34–37, 46); MCN 1047 (53 larval specimens, stages 34–43); MCN 1055 (53 larval specimens, stages 32–43); MCN 1120 (two males and one female), two specimens for skeletochronology; MCN 1141 (one female); MCN 1171 (34 larval specimens, stages 32–42).

Trachycephalus venulosus: MCN 403 (13 larval specimens, stages 34–42), skeleton whole mounts.

Alytidae

Discoglossus pictus: FML 3945 (one adult), skeleton whole mount.

Bombinatoridae

Bombina variegata: MCN 810 (one adult), skeleton whole mount; MCN 1002 (seven larval specimens, stages 34, 37, 39, 41, 42, 44, and 46).

Scaphiopodidae

Spea bombifrons: MCN 809 (one female), skeleton whole mount.

APPENDIX 2

CHARACTER AND CHARACTER STATES EXAMINED IN TEN ANURAN TAXA

Characters 0–60 are larval characters.

Characters 61–101 are adult characters.

Characters 10, 13, 23, 36, 40, 41, 42, 70, 85, 86, and 98 are non-additive.

0. *Melanocytes shape*: (0) melanocytes with elongated projections forming a reticulate pattern; (1) melanocytes irregular, without projections.
1. *Position of melanocytes (Fig. 5)*: (0) in the epidermis; (1) in the dermis.
2. *Epidermal glands (Fig. 5)*: (0) absent; (1) present.
3. *Spongiosum stratum of dermis (Fig. 5)*: (0) absent; (1) present.
4. *Number of epidermal layers (Fig. 5)*: (0) two; (1) three or more.
5. *Preorbital line of neuromasts (Fig. 6)*: (0) crossing the eyelid; (1) lateral to the eyelid.
6. *Preorbital and infraorbital lines of neuromasts (Fig. 6)*: (0) continuous; (1) discontinuous.
7. *Supratemporal neuromasts (Fig. 6)*: (0) absent; (1) present.
8. *Infraorbital line of neuromasts (Fig. 6)*: (0) descendent towards marginal invagination of oral disc; (1) descendent towards lateral margin of upper lip, and turning parallel with the lip up to medial position.
9. *Angular line of neuromasts (Fig. 6)*: (0) continuous with infraorbital line, immediately below the eye; (1) discontinuous, separated by a gap from infraorbital line.
10. *Oral line of neuromasts (Fig. 6), non-additive*: (0) forming a row and a group; (1) forming a row; (2) forming a group.
11. *Longitudinal oral line of neuromasts (Fig. 6)*: (0) absent; (1) present.
12. *Supralabial neuromasts (Fig. 6)*: (0) absent; (1) present.
13. *Dorsal line of neuromasts (Fig. 6), non-additive*: (0) runs parallel with the trunk and dorsal fin base; (1) runs parallel with the trunk and epaxial caudal musculature.
14. *Medial line of neuromasts (Fig. 6)*: (0) continuous with the dorsal line; (1) discontinuous with the dorsal line.
15. *Ventral lines of neuromasts (Fig. 6)*: (0) symmetrical; (1) asymmetrical, the left one interrupted by the spiracle.
16. *Position of ventral line of neuromasts (Fig. 6)*: (0) lateroventral; (1) ventral.
17. *Keratodonts*: (0) present; (1) absent.
18. *Keratinized jaws sheaths*: (0) present, well developed; (1) reduced.
19. *Oral lower lip*: (0) present; (1) absent.
20. *Oral disc papillation*: (0) complete; (1) discontinuous with a dorsal diastema.
21. *Spiracle*: (0) single; (1) double.
22. *Spiracle position*: (0) medial; (1) lateral.

23. *Anal tube at larval stage 37 (Fig. 2), non-additive*: (0) funnel shaped; (1) cylindrical; (2) absent.
24. *Origin of m. geniohyoideus*: (0) ceratobranchial III; (1) planum hypobranchiale.
25. *m. mandibulolabialis*: (0) present; (1) absent.
26. *m. suspensoriohyoideus (Fig. 14)*: (0) present; (1) absent.
27. *m. quadratoangularis*: (0) present; (1) absent.
28. *m. hyoangularis*: (0) present; (1) absent.
29. *Origin of m. suspensorioangularis (Fig. 7)*: (0) anterior; (1) posterior.
30. *mm. levatores arcuum branchialium I and II*: (0) separated by a gap; (1) fused.
31. *Processus anterolateralis of crista parotica (Fig. 8)*: (0) absent; (1) present; (2) forming the larval processus oticus.
32. *Posterolateral projection of crista parotica (Fig. 8)*: (0) absent; (1) present; (2) present, as expansive chondrification.
33. *Commissura prefacialis*: (0) present; (1) absent.
34. *Processus pseudopterygoideus (Fig. 8)*: (0) absent; (1) present.
35. *Processus quadratoethmoidalis (Fig. 8)*: (0) absent; (1) present.
36. *Dorsal connection from processus muscularis to neurocranium (Fig. 8), non-additive*: (0) ligament only; (1) ligament and pointed processus antorbitalis; (2) commissura quadratoorbitalis.
37. *Union cartilago labialis superior and cornua trabeculae*: (0) ligamentous or synchondrosis; (1) articulation.
38. *Position of cartilage labialis superior (Fig. 8)*: (0) frontal; (1) dorsal.
39. *Cornua trabeculae (Fig. 8)*: (0) long, more than 20% of skull length; (1) short, less than 15% of skull length.
40. *Pars alaris and pars corporis (Fig. 8), non-additive*: (0) separated by a deep distal notch; (1) partially fused; (2) completely fused.
41. *Pars corporis (Fig. 8), non-additive*: (0) medially separated; (1) with distal confluence; (2) forming a single body.
42. *Posterior processes of pars alaris (Fig. 8), non-additive*: (0) two processes, dorsal and ventral; (1) a single dorsal processus; (2) absent.
43. *Adrostral*: (0) absent; (1) present.
44. *Orientation of infrarostral cartilages (Fig. 8)*: (0) U-shaped; (1) transverse.
45. *Infrarostrals medial union (Fig. 8)*: (0) connective; (1) complete medial fusion of infrarostrals.
46. *Pointed medial projection of infrarostrals (Fig. 8)*: (0) absent; (1) present.
47. *Shape of cartilago meckeli (Fig. 8)*: (0) L-shaped; (1) a straight bar.
48. *Cartilaginous roofing of the cavum cranii at larval stage 40 (Fig. 3)*: (0) absent; (1) taenia transversalis present only; (2) taenia transversalis and medialis; (3) tectum parietale; (4) tectum cavum cranii almost completely cartilaginous.
49. *Union ceratobranchial I and hypobranchial*: (0) connective; (1) fusion.
50. *Free basihyal (Fig. 8)*: (0) present; (1) absent.
51. *Medial union of hypobranchialia (Fig. 8)*: (0) connective; (1) fusion.
52. *Processus urobranchialis (Fig. 8)*: (0) pointed; (1) wide and truncated.
53. *Commissura proximalis (Fig. 8)*: (0) absent; (1) present.
54. *Spiculae I–IV (Fig. 8)*: (0) present; (1) absent.
55. *Processus branchialis (Fig. 8)*: (0) open; (1) closed.
56. *Anterolateralis process of ceratohyal (Fig. 8)*: (0) present; (1) absent.
57. *Cartilaginous projections along ceratobranchyalia (Fig. 8)*: (0) present; (1) absent.
58. *Nasal appendix*: (0) absent; (1) present.
59. *Trigeminal and facial nerves (Fig. 4)*: (0) completely separated in larvae and adults; (1) completely separated in tadpoles at larval stage 37; (2) partially fused in tadpoles at larval stage 37; (3) completely fused in tadpoles at larval stage 37.
60. *Lateral line system*: (0) regresses during metamorphosis; (1) persists in adults.
61. *Nasals*: (0) separated; (1) in medial contact.
62. *Craneal exostosis*: (0) absent; (1) present.
63. *Sphenethmoid dorsal exposition*: (0) visible; (1) hidden by nasals and frontoparietals.
64. *Frontoparietals*: (0) separated; (1) in medial contact.
65. *Frontoparietal–squamosal arch*: (0) absent; (1) present.
66. *Ramus otic of squamosal*: (0) short, poorly developed; (1) reaching the prootic; (2) expansive and overlapping the prootic.
67. *Squamosal zygomatic ramus*: (0) short; (1) moderated; (2) long and sutured to the maxilla.
68. *Tooth shape*: (0) bicuspid; (1) monocuspid.
69. *Teeth condition*: (0) pedicellate; (1) non pedicellate.
70. *Pars palatina of premaxillae, non-additive*: (0) present, with processus palatine; (1) reduced to the processus palatinus only; (2) completely absent.
71. *Processes alares of premaxillae*: (0) parallel in frontal view; (1) divergent in frontal view.
72. *Pars facialis of maxillae*: (0) low; (1) high, expansive.

73. *Neopalatine*: (0) absent; (1) present.
74. *Mentomeckelian bone*: (0) distinct from the dentary; (1) completely fused to the dentary.
75. *Fang in lower jaw*: (0) absent; (1) present.
76. *Position of lower jaw articulation*: (0) anterior to the craniovertebral joint; (1) up to or beyond the craniovertebral joint.
77. *Ceratomyalia*: (0) continuous; (1) discontinuous.
78. *Anterolateral process of the hyoid*: (0) present; (1) absent.
79. *Ossification of the posteromedial processes*: (0) reaching the hyoid plate; (1) invading the hyoid plate.
80. *Parahyoid dermal ossification, non-additive*: (0) absent; (1) present single; (2) present branched.
81. *Cervical cotyles*: (0) contiguous; (1) widely separated.
82. *Presacral vertebrae*: (0) opisthocoelous; (1) procoelous.
83. *Neural arches*: (0) as spines projected posteriorly; (1) as plates.
84. *Ribs*: (0) absent; (1) present.
85. *Transverse processes of presacral vertebrae II–IV, non-additive*: (0) shorter than the posterior ones; (1) longer than the posterior ones; (2) strongly longer than the posterior ones.
86. *Sacral diapophyses, non-additive*: (0) widely expanded; (1) slightly dilated; (2) almost cylindrical.
87. *Postsacral transverse processes*: (0) present; (1) absent.
88. *Dorsal shields*: (0) absent; (1) present.
89. *Omosternum*: (0) a cartilaginous plate; (1) absent.
90. *Length of the scapulae, non-additive*: (0) short; (1) as long as ventral elements; (2) longer than ventral elements.
91. *Crest along the ilial shaft*: (0) present; (1) absent.
92. *Femoral crest*: (0) present; (1) absent.
93. *Distal tarsalia 2 and 3*: (0) free; (1) fused.
94. *Prehallux*: (0) two or more distal elements; (1) a well-differentiated distal element; (2) a hypertrophic distal element.
95. *Carpal torsion*: (0) absent; (1) present.
96. *Distal carpalia 5, 4, and 3*: (0) free; (1) fused.
97. *Intercalary elements*: (0) absent; (1) present.
98. *Origin of m. depressor mandibulae, non-additive*: (0) dorsal fascia; (1) dorsal fascia and squamosal; (2) squamosal and tympanic annulus.
99. *Subarticular tubercles in toes (Fig. 9)*: (0) present; (1) reduced; (2) absent.
100. *Subarticular tubercles in fingers (Fig. 9)*: (0) present; (1) reduced; (2) absent.
101. *Keratinized claws in toes*: (0) absent; (1) present.

APPENDIX 3
DATA MATRIX OF CHARACTER STATES SCORED FOR TEN ANURAN SPECIES

Character																								
Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Bombina variegata</i>	0	0	0	0	0	?	?	?	0	?	?	?	0	0	?	?	?	0	0	0	0	0	0	0
<i>Discoglossus pictus</i>	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Spea bombrifrons</i>	?	0	0	?	0	0	1	0	1	1	1	1	0	2	1	?	0	0	0	0	0	0	1	0
<i>Odontophrynus lavillai</i>	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0
<i>Pseudis platensis</i>	1	0	0	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	1	0
<i>Telmatobius</i>	1	0	0	0	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0
<i>atacamensis</i>																								
<i>Chacophrys pierottii</i>	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	1	1
<i>Lepidobatrachus laevis</i>	1	1	1	1	1	1	1	1	1	2	0	1	0	0	0	0	0	1	1	1	0	1	1	2
<i>Lepidobatrachus</i>	1	1	1	1	1	1	1	1	1	1	2	0	1	0	0	0	0	1	1	1	0	1	1	2
<i>llanensis</i>																								
<i>Ceratophrys cranwelli</i>	1	1	1	1	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0	1	1

Character																								
Species	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45		
<i>B. variegata</i>	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	2	0	0	0	0	0	
<i>D. pictus</i>	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	
<i>S. bombrifrons</i>	1	0	0	0	1	0	1	1	0	1	0	1	0	0	0	1	2	1	1	0	0	0	0	
<i>O. lavillai</i>	1	0	0	0	1	0	1	1	1	1	1	1	2	1	0	0	0	1	1	0	0	0	0	
<i>P. platensis</i>	1	0	1	0	1	0	1	2	2	1	1	1	0	1	0	0	1	1	1	1	1	0	0	
<i>T. atacamensis</i>	1	0	0	0	1	0	1	1	1	1	1	1	0	1	0	0	0	0	1	1	1	0	0	
<i>C. pierottii</i>	1	0	0	0	1	0	1	1	2	1	1	1	2	1	0	0	1	1	1	1	0	0	0	
<i>L. laevis</i>	1	1	0	1	1	1	0	1	1	1	0	0	0	1	1	0	2	2	2	0	0	1	1	
<i>L. llanensis</i>	1	1	0	1	1	1	0	1	0	1	0	0	0	1	1	0	2	2	2	0	0	1	1	
<i>C. cranwelli</i>	1	0	0	1	1	1	0	2	0	1	0	1	1	1	1	1	2	2	1	0	0	0	1	

Character		46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
Species		46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
<i>B. variegata</i>		0	0	1	0	1	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>D. pictus</i>		0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. bombifrons</i>		0	0	0	1	1	0	0	1	0	0	1	0	0	?	0	1	0	0	1	0	0	0
<i>O. lavillai</i>		0	0	3	1	1	0	0	1	0	0	0	0	0	2	0	1	0	1	1	0	1	1
<i>P. platensis</i>		0	0	3	1	1	0	0	1	0	1	0	0	0	2	0	1	0	0	1	0	1	1
<i>T. atacamensis</i>		0	0	2	1	1	0	0	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0
<i>C. pierottii</i>		0	0	4	1	0	0	0	1	0	1	0	0	1	1	0	1	1	1	1	1	1	2
<i>L. laevis</i>		1	1	4	1	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	2	2
<i>L. llanensis</i>		1	1	4	1	0	1	1	0	1	0	1	1	0	1	?	1	1	1	1	1	2	2
<i>C. cranwelli</i>		1	1	4	1	1	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	2	2
Character		68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
Species		68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
<i>B. variegata</i>		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
<i>D. pictus</i>		0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
<i>S. bombifrons</i>		0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	2	0	0	0	0
<i>O. lavillai</i>		0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	2	1	1	0	1
<i>P. platensis</i>		0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	2	1	0	0
<i>T. atacamensis</i>		0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0
<i>C. pierottii</i>		1	1	2	0	1	1	1	1	1	0	1	1	?	0	1	1	0	2	1	1	0	1
<i>L. laevis</i>		1	1	2	0	1	1	1	1	1	1	1	1	1	0	1	1	0	2	1	1	0	1
<i>L. llanensis</i>		1	1	2	0	1	1	1	1	1	1	1	1	1	0	1	1	0	2	1	1	1	1
<i>C. cranwelli</i>		1	1	2	0	1	1	1	1	1	0	1	1	0	0	1	1	0	2	1	1	2	1
Character		90	91	92	93	94	95	96	97	98	99	100	101										
Species		90	91	92	93	94	95	96	97	98	99	100	101										
<i>B. variegata</i>		0	0	1	0	0	0	0	0	0	2	2	0										
<i>D. pictus</i>		0	0	1	0	0	0	0	0	0	2	2	0										
<i>S. bombifrons</i>		1	1	0	1	2	0	0	0	0	2	2	0										
<i>O. lavillai</i>		2	0	1	1	2	1	1	0	1	0	0	0										
<i>P. platensis</i>		0	0	0	1	1	1	1	1	1	2	0	0										
<i>T. atacamensis</i>		1	0	1	1	0	1	1	0	1	1	0	0										
<i>C. pierottii</i>		2	1	0	1	2	1	1	0	1	1	1	0										
<i>L. laevis</i>		2	1	1	1	2	1	1	0	2	2	2	0										
<i>L. llanensis</i>		2	1	1	1	2	1	1	0	2	2	2	1										
<i>C. cranwelli</i>		2	1	1	1	2	1	1	0	2	0	0	0										