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Chondrocranial and oral morphology of pipoid frogs

Charles Christopher Swart

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by

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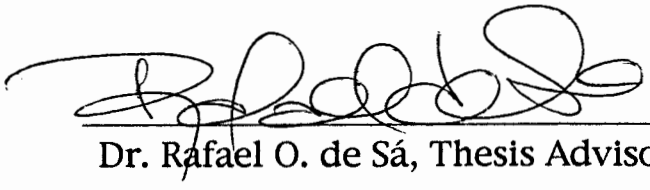

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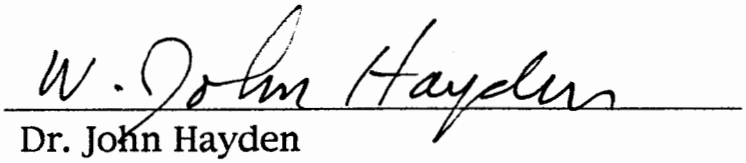
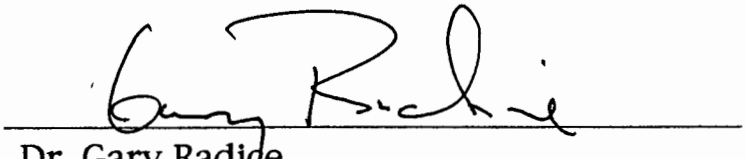


Dr. John Hayden



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Chondrocranial and Oral Morphology of Pipoid Frogs

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Abstract. The Pipoidea are a diverse group of frogs. Their diversity is demonstrated in their morphology, ecology, and behavior. One pipoid species, *Xenopus laevis*, has been used as a model system of developmental, physiological, and molecular studies of vertebrates. My work has focused on the developmental morphology of the chondrocranium and oral morphology of four pipoid taxa: *Hymenochirus boettgeri*, *Rhinophrynus dorsalis*, *Pipa carvalhoi*, and *Xenopus laevis*. Previous studies have suggested that the Anura may be diphyletic based on the unique characteristics of the chondrocanial morphology of pipoids. The chondrocranial and internal oral morphology of the pipoids indicates that as a group as well as individual species the Pipoidea are highly derived. However, the present study shows that the ethmoidal region of the Pipoidea is homologous to that of other Anura, refuting the proposed diphyletic origin of the Anura. An evaluation of chondrocranial characters in phylogenetic reconstruction is presented.

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By

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Table of Contents

List of Figures:

List of Tables:

Introduction

Frogs are prime candidates for studies of evolution because of their contrasting characteristics of constancy of form and, at the same time, vast diversity. The extant amphibians are the oldest lineage of vertebrates possessing four legs. They offer our best opportunity to study, not only the origin of tetrapoda but more importantly, to study diversity of form allowed by invasion of terrestrial environments by vertebrates (Duellman and Trueb, 1993). Because of their dual life history, amphibians represent extremes of freshwater and terrestrial habitat exploitation as larvae and adults, respectively. In addition, the drastic morphological transition from larvae to adult in amphibians provides an opportunity to study developmental processes and evolutionary changes related to shifts in timing of development, i.e., heterochronic mechanisms (Trueb and Alberch, 1985).

Of the three living orders of amphibians, the order Anura has been extensively studied. Anurans, i.e., frogs, are more speciose and conspicuous than the other two orders of amphibians. They are global in distribution, occupying nearly all habitat types (Duellman and Trueb, 1993). Many species are easily kept in captivity and breed readily, providing dozens, and sometimes hundreds of

offspring. Frogs and their tadpoles, while by no means simple organisms, are interesting and relatively easy to work with.

Historically, characteristics of adult morphology have been important in species descriptions (Noble, 1936; Funkhouser, 1957; Vial, 1973). These descriptions include all aspects of morphology (Duellman, 1973) including; external characteristics (Moore, 1944), skeletal structure (Trueb, 1973), musculature (Horton, 1982), and meristics (Moore, 1944), among others. During the second half of the twentieth century, characters derived from the study of mating calls (Ryan and Rand, 1995), cytogenetics (Bogart, 1974), behavior (Wells, 1977), molecular (Hillis and Davis, 1986), and developmental data (Trueb and Hanken, 1992) have also been used in species recognition. These new data sets have complemented and added new information about the evolutionary relationships suggested by adult morphology. Modern schools of systematics agree that, overall, the best hypotheses of evolutionary relationships are obtained from the integration of all available data. These attempts to resolve evolutionary relationships have become known as the "Total Evidence" approach (Kluge, 1989; Brooks and McClennan, 1991).

Anuran larvae are morphologically distinct from adults, but also they have different ecological requirements, food preferences, and behaviors. Consequently they can provide additional sets of data

to be used in phylogenetic inference. Descriptions of larval morphology have been widely used for tadpole identification (Altig, 1970; McDiarmid and Altig, 1989; de Sá et. al, 1997). The first attempt to use larval morphology in a large classification scheme was that of Orton (1956). From that time to present, tadpoles have captured the interest of a small but steadily growing number of herpetologists, developmental biologists, and systematists (Sokol, 1975; Haas, 1996).

Orton's arrangement has been questioned and revised on several occasions (Starrett, 1973; Sokol, 1975). Data on anuran larvae continue to accumulate from different sources. However, lack of baseline comparative data is the largest problem in studying anuran larvae. The larval stage remains unknown for several species. Currently, common sets of characters that have been reported include: external morphology (Altig and Johnston, 1986; 1989; Altig and Channing, 1993), chondrocranial morphology (de Sá, 1991; Haas, 1997), oral morphology (Wassersug, 1980; Wassersug and Heyer, 1988), and most recently, behavior (Wassersug and von Seckendorf Hoff, 1985; Waldman, 1989).

Recognition of the ecological importance of feeding specialization in larvae led Wassersug, (1977), to pioneer the study

of internal oral structures. These studies showed the usefulness of internal oral anatomy for diagnosing species (Wassersug, 1981).

Ecologically, adult members of the clade Pipoidea represent the extremes of adaptation for anurans. *Rhinophrynus* adults are highly adapted for terrestrial burrowing while all other pipoid adults are fully aquatic. However, all free-swimming pipoid larvae (with the exception of *Hymenochirus*) are filter feeders.

The focus of my work has been to understand the evolution and development of morphological characteristics in pipoid larvae. For this, I focused on describing the chondrocranial characteristics of three pipoid taxa: *Rhinophrynus dorsalis*, *Hymenochirus boettgeri*, and *Pipa carvalhoi*. The analysis of chondrocranial morphology led me to investigate the unique development of the ethmoidal region in pipoids, particularly to determine if the ethmoidal region of pipoids is homologous to that of other anuran larvae. In addition, I have described the internal oral anatomy of four pipoid taxa using scanning electron microscopy. Lastly, I evaluated the utility of chondrocranial and internal oral characteristics in phylogenetic reconstructions.

Materials and Methods

Histology

Histological cross-sections were used to verify tissue identification and to aid in illustrations. Large specimens were embedded in paraffin and serial cross-sectioned at 10μm. Paraffin sections were stained with Milligans trichrome for differentiation of bone, cartilage, and muscle (Presnell and Schreibman, 1997). Small specimens (<3mm) were imbedded in Glycol Methacrylate plastic polymer (JB4+, Polysciences, Inc.), serial sectioned at 2 μm and stained with Toluidine blue (Presnell and Schreibman, 1997).

Photomicrographs were taken under light microscopy with 35 mm film (Kodak Technical Pan developed in HC110). Film was processed in a standard dark room producing black and white prints demonstrating morphological differentiation of tissues. Color photographs were printed by a commercial laboratory.

Scanning Electron Microscopy

Preparation of specimens for scanning electron microscopy (SEM) was done as follows: specimens were dissected following Wassersug, 1976, ultrasonically cleaned for 15 minutes, fixed in 3-4%

glutaraldehyde for two hours at room temperature, followed by three 15 minute washes with 0.1 phosphate buffer. Specimens were post fixed for 1 hour in 1% osmium tetroxide, followed by an additional three washes of 15 minutes each in 0.1 M phosphate buffer. Dehydration was achieved with a graded ethanol series: 35, 50, 70, 80, 95, and three 100% changes. Specimens were critical point dried in CO_2, mounted on aluminum stubs and sputter coated with gold/palladium (30nm), using a Hummer VII sputtering system. Internal oral anatomy was observed in a Hitachi S-2300 scanning electron microscope at 15 kV and photographed using Polaroid 55 positive/negative film (de Sá and Lavilla, 1997).

Chondrocranial Description

Chondrocrania are described based on cleared and double-stained specimens complemented with observations of cross-sectioned specimens (see Histology). Clearing and differential staining for cartilage and bone was carried out according to the method of Dingerkus and Uhler, 1976. Drawings were obtained with the aid of a Wild M3C stereo-microscope fitted with a camera lucida attachment. Chondrocranial terminology follows that of de Beer (1937), de Jongh (1968), Sokol (1975), and Trueb and Hanken

(1993). Development of the ethmoidal region was described from serial cross-sections of both paraffin and plastic embedded specimens (see Histology).

Specimens

Rhinophrynus dorsalis larvae used in this study are deposited at the National Museum of Natural History, Smithsonian Institution. They were collected at two localities: Guanacaste, Costa Rica (USNM 515945—515958) and Aguacate Lagoon, Cayo District, Belize (USNM 515959—515974). Thirty larvae were cleared and double-stained and two specimens were embedded in paraffin and serial—sectioned at 10 µm. One specimen was dissected and prepared for SEM of internal oral morphology. Collection numbers, measurements, and stages, of material examined are given in Tables 1—3.

Xenopus laevis larvae were obtained from induced breeding of adults obtained from *Xenopus* I (Ann Arbor MI). Three *Xenopus* larvae were serial sectioned and one specimen was dissected for SEM of internal oral morphology.

Hymenochirus boettgeri adults and larvae were obtained from the pet trade (Blue Lobster Farms, Madeira CA). Additional specimens were obtained by natural breeding in the laboratory.

Sixty larval *Hymenochirus* purchased from the pet trade were cleared and double—stained. Thirteen *Hymenochirus* larvae were serial sectioned. Three *Hymenochirus* specimens were dissected and prepared for scanning electron microscopy of internal oral morphology.

Nieuwkoop and Faber's staging table for *Xenopus* was used to stage all larvae used in this study (Neiuwkoop and Faber, 1956). The similarity of external morphology of *Xenopus* and other pipoids during development makes this table preferable to Gosner's staging table (Gosner, 1960). *Hymenochirus boettgeri* follows the developmental sequence of *Xenopus laevis* up to approximately the onset of pigmentation (Neiuwkoop and Faber's stages 38—40). I decided to correlate the development of *Hymenochirus* with the staging table for *Xenopus* as closely as possible. This allows for comparison of my results with those published for other pipoid taxa. The stages of *Hymenochirus* between the completion of the retina (stage 38) and the emergence of the hind limb bud (stage 48) can be judged based on a few external changes including increase in pigmentation as well as degeneration of the cement gland and orientation of the mouth. Limb bud emergence is used as the main diagnostic feature for designation of stage 48. Thereafter,

development of the hind limb, forelimb, and resorption of the tail are used as diagnostic features for staging.

Thirteen larvae of *Pipa carvalhoi* were cleared and double-stained for description of chondrocranial morphology. One specimen was dissected and prepared for SEM for description of internal oral morphology. Specimens were collected at CEPLAC, Ilheus, Bahia, Brazil (H. Silva, M. Cox, and H. Zahyer, 4 Aug. 1989).

Any specimens obtained alive were anesthetized and killed using MS222, and fixed in 10% formalin. This procedure for euthanasia of specimens was reviewed and approved by the University of Richmond Animal Care and Use Committee (Protocol #96—3).

Phylogenetic applications

Phylogenetic analysis were performed under maximum parsimony using Paup version 3.1.1 (Swofford, 1993). Characters were run un-ordered and were polarized based on outgroup comparisons (see discussion). Statistical significance for the tree was tested using bootstrap analysis (100 replicates). Character coding is summarized in Table 4.

Background on pipoid Research

Phylogenetics of Pipoidea

The Pipoidea is composed of two families, the Pipidae, and the monotypic Rhinophrynidae (Ford and Cannatella, 1993). The phylogenetic relationships within the group have been proposed by several studies using morphology and DNA (Cannatella and de Sá, 1993; Hillis et. al, 1993; Maxson and Hedges, 1993, Fig. 1a). The extinct Palaeobatrachidae are considered most closely related to the extant pipoids (Sanchez and Rocek, 1996). The earliest fossil record of the Pipidae includes specimens from the lower Cretaceous of Israel, approximately 120 million years ago (MYA). This is one of the best fossil records for any extant group. Comparison of extant pipoid osteology with fossil pipoids indicates conservation of form in the genus *Xenopus* with later diversification into the more exotic forms of *Pipa* and the Hymenochirines (Estes and Reig, 1973).

Currently the family Pipidae includes 29 species grouped in four genera, *Pipa* (7), *Xenopus* (17), *Hymenochirus* (4), and *Pseudhymenochirus* (1). The diversity of life histories and the comparatively rich fossil record for pipoids indicates that the twenty-nine extant members are remnants of a much larger monophyletic group (Báez, 1981; see Fig. 1). Distribution of the

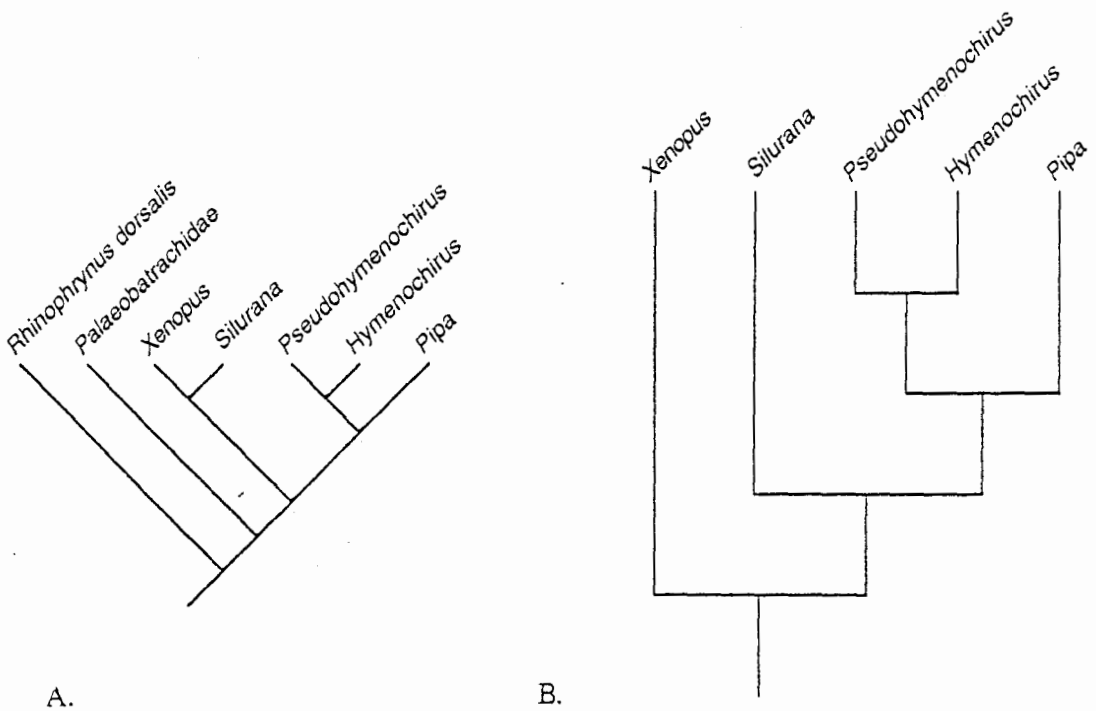


Fig. 1. A. Current accepted phylogeny for the Pipoidea based on adult morphology and Molecular data (from Cannatella and deSá 1993). B. Phylogeny proposed for living members of Pipidae based on adult morphology (from Cannatella and Trueb 1988a).

Pipidae is disjunct, ranging throughout tropical South America, east of the Andes and Panama, and in sub-Saharan Africa (Duellman and Trueb 1993). Most of the current understanding of pipoid biology (and to some degree anuran biology) is due to the large database on *Xenopus laevis* (Cannatella and de Sá, 1993; Tinsley and Kobel, 1996). Some pipoid synapomorphies include: modified ethmoidal region, strict aquatic habitat, filter feeding larvae, lack of a tongue, retention of the larval lateral line system in the adult, and clawed feet.

The phylogeny of the family Pipidae (Fig. 1) has been proposed using adult morphology and molecular data (Trueb and Cannatella, 1986; Cannatella and Trueb, 1988a; de Sá and Hillis, 1990). Relationships among pipoids are fairly well agreed upon.

Immunology, sperm protein and globin patterns, karyological data, and DNA content, agree on a monotypic *Xenopus*. Cannatella and Trueb's (1988) morphological study placed *Xenopus tropicalis* and *X. epitropicalis* in the genus *Silurana* (Fig. 1b). Subsequently, using molecular data de Sá and Hillis (1990) and Cannatella and de Sá (1993) determined that the separation of *Xenopus* and *Silurana* was unnecessary to render *Xenopus* monophyletic.

Cannatella and Trueb's (1988), phylogenetic analysis of the family Pipidae included one larval characteristic (dual branchial siphons). Due to the lack of a free swimming larvae in *Pipa pipa* and

the trend toward direct development in other species of *Pipa*, early larval characters are difficult to study in this genus.

Rhinophrynus dorsalis is the only extant member of the family Rhinophrynidae. *Rhinophrynus dorsalis* is currently endemic to the sub-humid lowland areas of Mexico and Central America (Duellman and Trueb, 1993). Included in the genus with *R. dorsalis* is one extinct taxa *R. canadiensis* (Holman, 1963, 1968, 1972) from the Eocene of Saskatchewan, Canada. In addition, the fossil record has yielded two other extinct genera of rhinophrynids, *Eorinophrynus* (middle Eocene of Wyoming, Hecht, 1959) and *Chelomophrynus* (middle Eocene of Wyoming, Henrici, 1991).

Rhinophrynus dorsalis is considered to be the sister group to Pipidae based on morphology, biochemical data and molecular data (Maxson and Daugherty, 1980; de Sá and Hillis, 1990; Hedges and Maxson, 1993; Hay et. al, 1995; see Fig. 1). Together with the Pipidae, *Rhinophrynus* and the Palaeobatrachidae form the clade Pipoidea (Fig 1, Ford and Cannatella 1993). The osteocranial morphology of *R. dorsalis* has been thoroughly described (Trueb, 1985; Trueb and Cannatella, 1988). The external morphology of the larvae of *Rhinophrynus* has been described in several papers (Taylor, 1942; Orton, 1943; Sokol, 1975).

Species Studied

Rhinophrynus dorsalis. — Taylor (1942) described pipid-like anuran larvae from a sample of tadpoles collected in Guerrero, Mexico. However, these larvae remained unidentified until, the tadpole of *Rhinophrynus dorsalis* was described by Orton (1943). Orton's description included a few chondrocranial characteristics, particularly focusing on the jaw apparatus. She noted the early formation of the lower jaw and suggested that this early development may be correlated with a macrophagous diet.

Subsequently, Sokol (1977, 1981) made references to the chondrocranial anatomy of *Rhinophrynus dorsalis* larvae while evaluating the use of larval characteristics in systematics. The sequence of cranial ossification for *Rhinophrynus* larvae was reported by Trueb, 1985. Adult osteology has been previously described (Trueb, 1973; Trueb and Cannatella, 1987).

Hay et al (1995), placed *Rhinophrynus* as the sister group to Pipidae based on 12S and 16S rRNA sequence data. Previous morphological studies suggested the following synapomorphies for the Pipoidea: absence of mentomeckelian bones, lack of parasphenoid alae, presence of a single frontoparietal, greatly enlarged otic

capsules, and larvae with paired spiracles and lacking cornified beaks and denticles (Ford and Cannatella, 1993).

Hymenochirus boettgeri. — The genus *Hymenochirus* has the only strictly carnivorous larvae in the family Pipidae (Duellman and Trueb, 1993). These larvae lack filter plates and use the precociously formed lower jaw for feeding on copepods and other small prey while the adults specialize on aquatic insects and aquatic insect larvae (Sokol, 1962). The four species of *Hymenochirus* —*H. boettgeri*, *H. boulengeri*, *H. feae*, and *H. curtipes*—represent the smallest of the Pipid frogs (average SVL ≈ 30mm). The reduction in size may result in correlated altered chondrocranial structure (Hanken, 1984; Trueb and Alberch, 1985). The chondrocranial morphology of *Hymenochirus* larvae is highly divergent from that of all other pipids (Sokol, 1977).

The numerous autoapomorphies of the Hymenochirine chrondrocrania may be due to miniaturization (Hanken, 1984, 1996; Clarke, 1996), heterochrony (Trueb and Alberch, 1985) or adaptations (Reiss, 1997). All three processes would be expected to show effects in both larval and adult characters in anurans.

Larval characters shared between *Hymenochirus* and other pipoids are: a modified ethmoidal region, precocious formation of the

lower jaw, bilateral branchial siphons, and possession of a larval lateral line system (Orton, 1956; Sokol, 1975, 1977; Trueb and Hanken, 1992; Swart and de Sá, in press).

Pipa carvalhoi. — Molecular studies have grouped the clade made up of the genus *Pipa* and the Hymenochirines as the sister group to *Xenopus* (Cannatella and de Sá, 1993).

The chondrocrania of *Pipa carvalhoi* and *Pipa pipa* have previously been partially described. The description of *Pipa pipa* (Rocek and Vesely, 1989) focused on the development of the ethmoid plate and complete chondrocranial anatomy was not described. Sokol (1977) described the chondrocranium, musculature, nervous system, circulatory system, and hyobranchial apparatus of *Pipa carvalhoi* based on two specimens.

Chondrocranial Studies

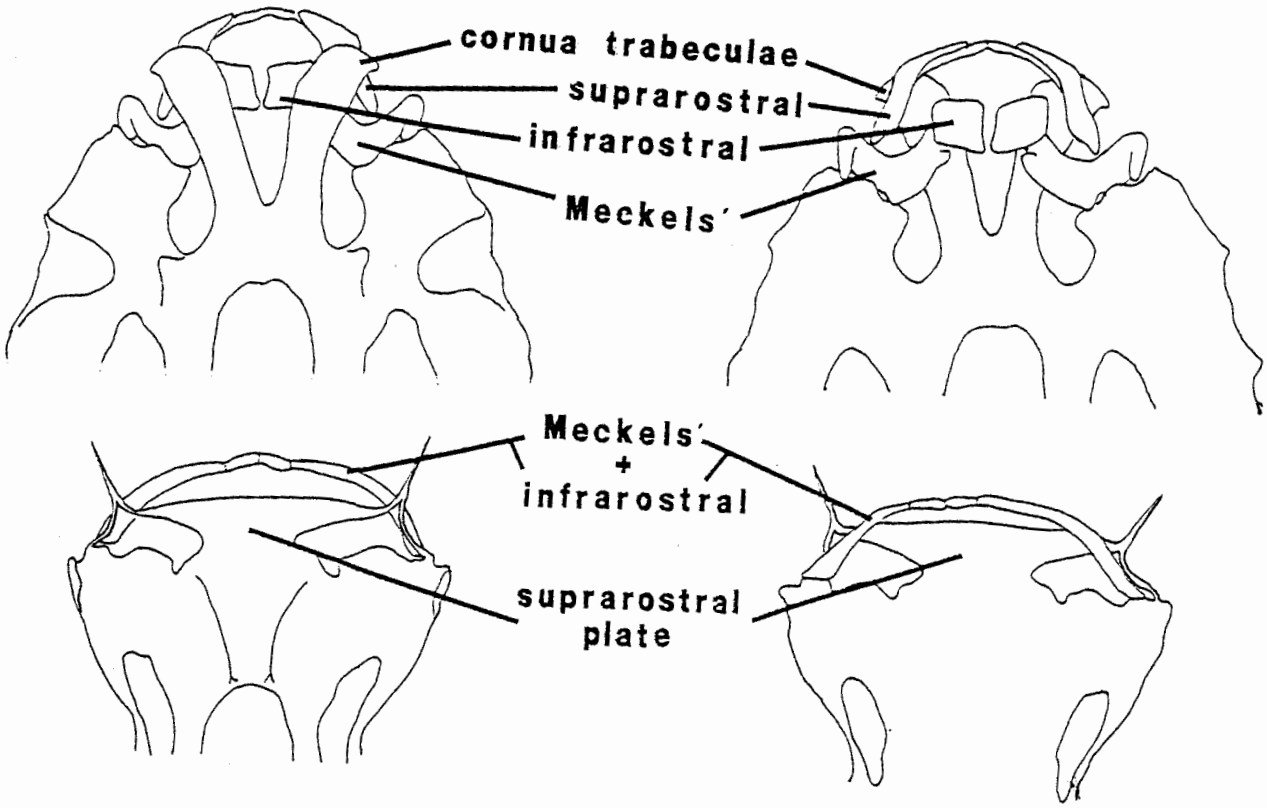
Descriptions of the chondrocrania of pipoids are limited to several descriptions of *Xenopus laevis* (Trueb and Hanken, 1993; de Beer, 1937; Kotthaus, 1933) and incomplete notes on *Rhinophrynus dorsalis*, *Xenopus tropicalis*, *Pipa carvalhoi,* and *Hymenochirus boettgeri* (Sokol 1975,1977). The overall appearance of the pipoid chondrocranium is broad and flat. Peculiarities shared by all

members include precocious formation of the lower jaw, fusion of the anterior elements of the chondrocranium into a single cartilaginous plate, and a high attachment of the suspensorium (Sokol, 1981).

The modification of the ethmoidal region in pipoids has resulted in different interpretations of homology of chondrocranial characters (Sokol, 1975, 1977; Rocek and Vesely, 1989; Trueb and Hanken, 1992). Some workers believe the modified ethmoidal region of pipoids indicates a drastically different evolutionary history for pipoids separate from anurans (Rocek and Vesely, 1989).

The ethmoidal region of non-pipoid larvae is composed of four pairs of independent cartilages that work together for feeding and respiration. These cartilaginous elements are the suprarostrals, infrarostrals, Meckels', and cornua trabeculae (Fig. 2). Early in development of non-pipoid anurans the cranial skeleton is composed of two pair of parallel, rod shaped cartilages. The parachordal cartilages are found posteriorly, while the trabecular cartilages are found anterior to and superior to the parachordals. The medial fusion of the trabecular cartilages and their fusion with the parachordal cartilages forms the intertrabecular plate. From this

Figure 2. Ethmoidal region of non-pipoid (A = dorsal, B = ventral) and pipoid (C = dorsal, D = ventral) Anura


cornua trabeculae
suprarostral
infrarostral
Meckels'
Meckels'
+
infrarostral
suprarostral
plate

plate, the pila ethmoidales grow dorsally forming the ethmoid plate, which serves as the anterior wall of the braincase. The cornua trabeculae grow forward from the anterior edge of the intertrabecular plate. The suprarostral cartilages articulate with the anterior tips of the cornua trabeculae (de Beer, 1937).

The pipoid ethmoidal region is composed of a flat plate having laterally expanded anterior tips. Sokol (1975, 1977) suggested that the suprarostral plate of pipoids may form as the fusion of the suprarostral alae, cornua trabeculae, prenasal process, and the ethmoid plate. However, he did not provide any data to support his hypothesis. Rocek and Vesely (1989) described the development of the ethmoidal region of a direct developing pipid, *Pipa pipa,* based on a limited number of specimens. They suggested that the ethmoidal region is formed by the fusion of the "planum internasale," "larval septum nasi," and the "ethmoid flanges." The lack of early developmental stages and the use of unconventional terminology, limits the usefulness of their descriptions. In their description of *Xenopus laevis,* Trueb and Hanken (1992) use Sokol's (1975, 1977) terminology and described the development of the chondrocranium from stage 45 through metamorphosis. Trueb and Hanken (1992) suggested that the suprarostral plate of pipoids is formed by either, the fusion and simplification of the cornua trabeculae of other Anura,

or that in pipoids, the cornua trabeculae are missing and a continuous cartilaginous plate develops from the intertrabecular plate in the ethmoidal region.

Internal oral morphology

Wassersug's (1977) paper defining standard terminology for description of internal oral structures initiated the gathering of useful descriptions of the internal oral anatomy of anuran larvae. Many groups of tadpoles have already been described (Wassersug, 1977, 1980); however, much work still needs to be done in this area for this set of characters to be useful for phylogenetic systematics among anurans.

An account of the internal oral morphology of *Rhinophrynus dorsalis*, with notes on the characteristics of *Xenopus laevis*, was reported using light microscopy (Wassersug, 1980, 1977). Wassersug (1980) noted that the variation of internal oral anatomy between *Rhinophrynus* and other pipoids is consistent with variation at the familial level. However, his observation is based only on two taxa: *Rhinophrynus* and *Xenopus*.

Results

The Chondrocranium of *Rhinophrynus dorsalis*

To facilitate comparisons with the available data for *Xenopus laevis*, the chondrocranium of *Rhinophrynus dorsalis* is illustrated at developmental stage 53 (Figs. 3 and 4). The parasphenoid and frontoparietals have begun ossification and are clearly visible at stage 53.

The most anterior region of the chondrocranium, the area roofing the buccal cavity, is highly modified when compared to other free swimming anuran larvae. In the ethmoidal region there is a single, broad, and flat element of approximately the same width as the braincase, the suprarostral plate. A short, blunt, and lateral trabecular process occurs on the lateral margin of the suprarostral plate. This is the anterior point of attachment for the ligamentum quadratoethmoidale, which runs posteriorly to the processus quadratoethmoidale on the commissura quadratocranialis anterior. A processus antorbitalis is present as a prominent ridge in the area of attachment of the commissura quadratocranialis anterior to the braincase. The lateral tips of the suprarostral plate are attached to the most antero-lateral edge of the pars articularis quadrati and to

Figure 3. Chondrocranium of *Rhinophrynus dorsalis* (USNM 515957, stage 53). A. Ventral view. B. Dorsal view. Stippling indicates bone and horizontal lines indicates notochord. CQA = commisura quadrato-cranialis anterior, i+MC = fused infrarostrals and Meckels' cartilages, FO = fenestra ovalis, LPPQ = ventrolateral process of the palatoquadrate, LOP = larval otic process, CP = larval crista parotica, OC = otic capsule, MPOC = muscular process of the otic capsule, PQE = processus quadratoethmoidale, PAO = processus antorbitalis, PM = processus muscularis, PQ = palatoquadrate, PA= processus ascendens, SC = symplectic cartilage, SP = suprarostral plate . Bar = 1mm.

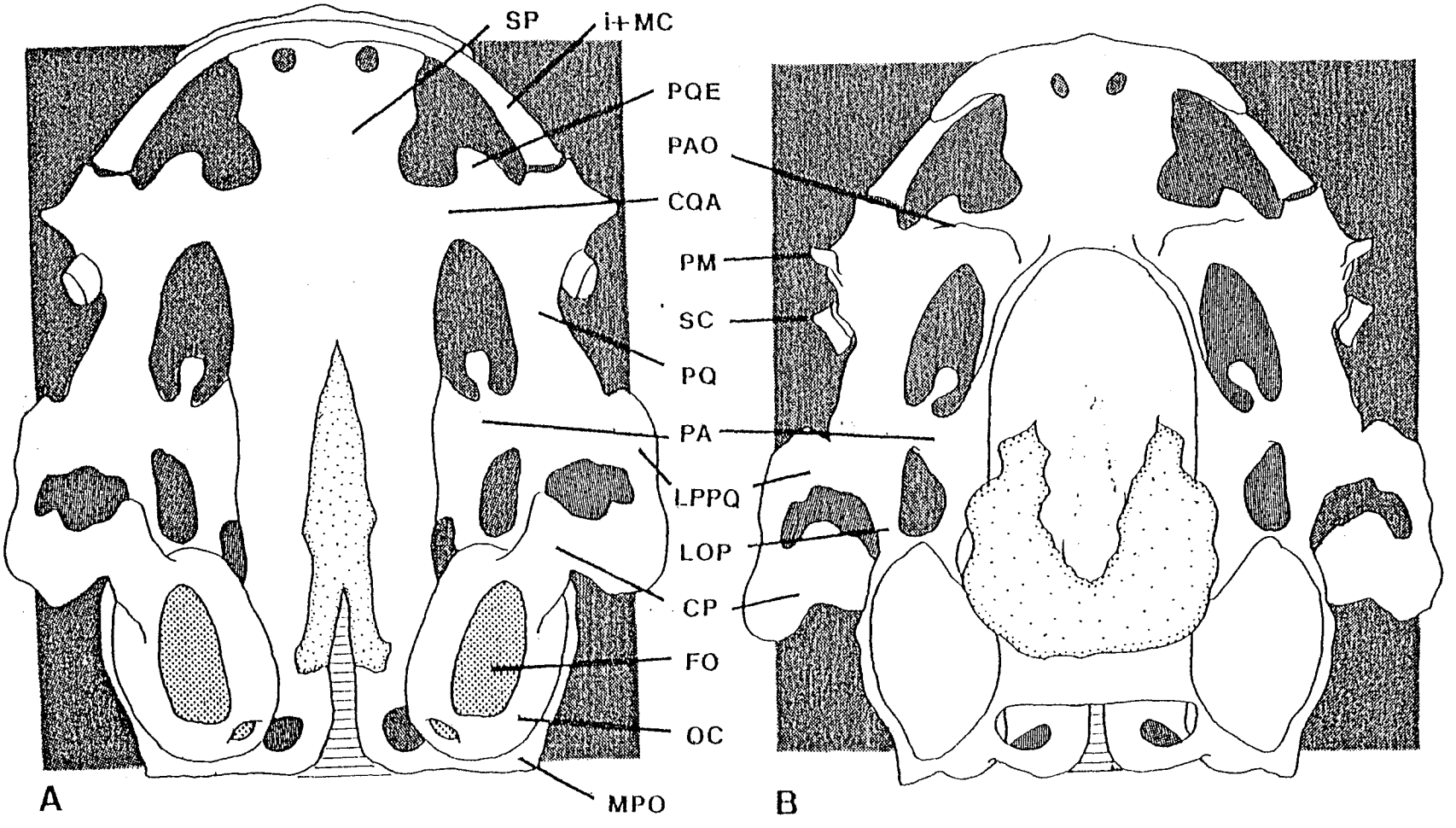

SP
i+MC
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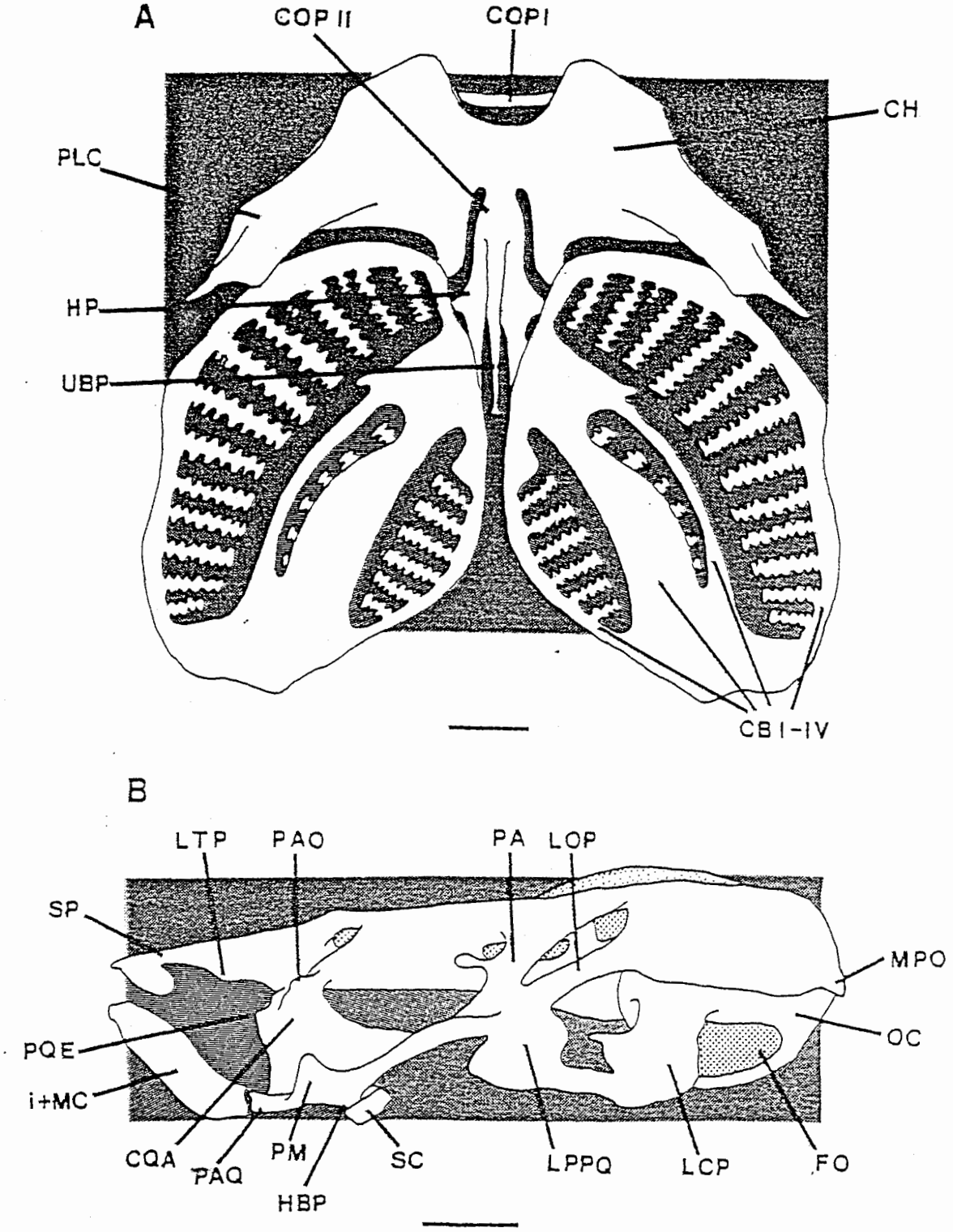
Fig. 4. Chondrocranium of *Rhinophrynus dorsalis* . . A. Hyobranchial apparatus (USNM 515958, stage 53). B. Lateral view (USNM 515957, stage 53). Stippling indicates bone. COP I = copula one, COP II = copula two, CH = ceratohyale, CBI—IV = ceratobranchial one—four, HP = hyobranchial plate, PLC = processus lateralis of the ceratohyal, UBP = urobranchial process. Other labels as in Fig. 3. Bar = 1mm.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i+MC
CQA
PAQ
PM
HBP
SC
LPPQ
LCP
FO

the commissura quadratocranialis anterior via a membranous sheet of connective tissue. This sheet of connective tissue covers the anterior area of attachment of the levator mandibulae muscle to Meckel's cartilage.

A functional lower jaw is already formed at this stage. It is composed of the fused Meckel's and infrarostral cartilages. The three points of fusion among these elements are clearly visible. The Meckel's cartilages have their greatest diameter midway between the fusion with the infrarostrals and their articulation with the processus articularis. The mid-point of the fused infrarostrals forms a triangular ventral projection.

The palatoquadrate is firmly attached to the braincase anteriorly by a broad and sturdy commissura quadratocranialis anterior and, posteriorly, by an equally sturdy processus ascendens and by a well developed larval otic process. A fourth attachment of the palatoquadrate is accomplished via a thin, extensive, and convoluted larval crista parotica that fuses to the lateral process of the palatoquadrate. The ascending process is wide and from its anterior margin a rounded and cylindrical process projects into the sub-ocular space. On either side and below the ascending process, the optic and oculomotor foramina are found in the lateral wall of the braincase. The prootic foramen is large and anterior to the otic

capsules. The ascending process has a high attachment to the braincase. The muscular process is small and placed at a 45° angle with the sagittal plane of the body. Ventral and posterior to the muscular process, a rounded hyoquadrate process serves as the point of articulation between the palatoquadrate and the ceratohyal via the symplectic cartilage. The symplectic cartilage is small and biconcave. It remained unmodified in the more advanced stages available for this study (stage 62); consequently its developmental outcome could not be determined. At the level of the ascending process, the processus ventrolateralis is present and fused with the larval crista parotica.

The larval crista parotica is well developed, thin, convoluted, and variable in size. It attaches to the otic capsule above and anterior to the fenestra ovalis. It curves forward, slightly upward and attaches anteriorly to the ventrolateral process of the palatoquadrate.

The otic capsules are large, representing one fourth the chondrocranial length. The muscular process of the otic capsule forms a sharp posterolateral corner on the otic capsule. Posteriorly, the otic capsules have two foramina, the foramen jugulare and the foramen perilymphaticum inferior. The otic capsules have a large fenestra ovalis. The operculum is not yet formed.

The hyobranchial apparatus (Fig. 4) consists of a copula I, copula II, ceratohyalia, a chondrified pars reuniens, a long and thin urobranchial process, hypobranchial plates, and paired ceratobranchials I-IV. The ceratobranchials and the copula II are continuous with the hypobranchial plate. Ceratobranchial II has a small process close to its anterior end that projects toward ceratobranchial III, but does not contact ceratobranchial III. No spicules are present.

The taeniae tecti marginalis are not yet fused to the otic capsule early in stage 51 (6 of 9 specimens examined) leaving the foramen prooticum open dorsally. Three of the nine specimens have the lateral trabecular processes forming at this stage. The processus antorbitalis is present in two of the nine specimens at this stage.

The Chondrocranium of *Hymenochirus boettgeri*

The present description of *Hymenochirus boettgeri* is based on the analysis of the earliest cleared and double-stained larvae available, stage 49. This description is followed by notes on major developmental changes to the chondrocranial structure through metamorphosis. The sequence of ossification of the specimens examined is presented in Table 4.

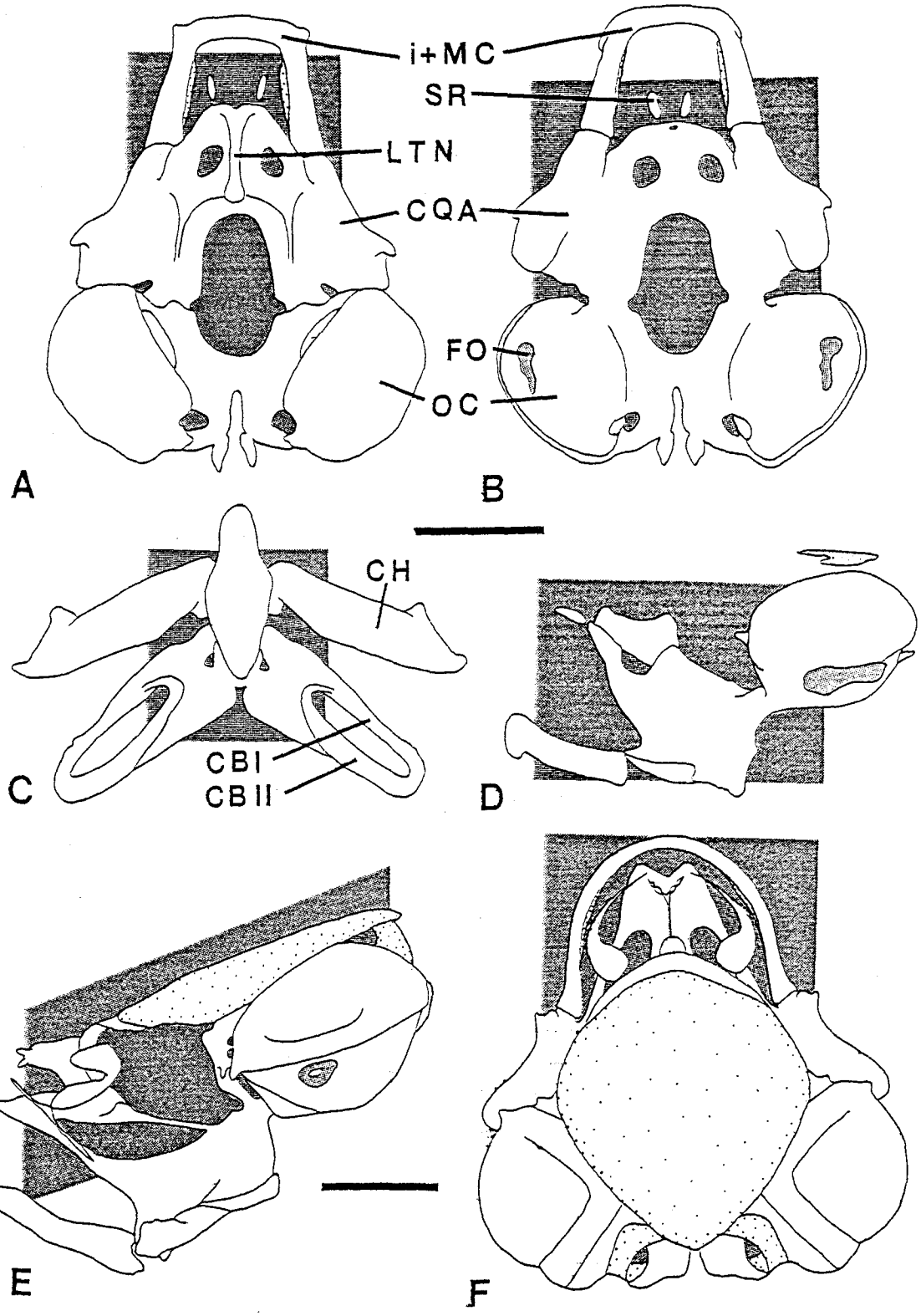
Stage 49 (Fig. 5). — Overall the chondrocranium is composed of a reduced number of robust, closely connected elements. The lower jaw consists of a single, continuous, and U-shaped cartilaginous element. Meckels' and infrarostral cartilages are not identifiable as separate cartilages. The parallel elements of this U-shaped structure form the lateral part of the lower jaw and overall, they are circular in cross-section. The anterior margin of the jaw consists of a dorso-ventrally flattened element.

The angulosplenials and frontoparietals have already begun ossification. The angulosplenials are present as splinters of bone developing over the latero-ventral lingual margin of the lower jaw. Overall they are triangular, being broad posteriorly and narrow anteriorly. The frontoparietals develop as a single ossification located medial and dorsal to the otic capsules.

The suprarostrals are present as two individual rod shaped cartilaginous elements projecting vertically from the anterior edge of the ethmoidal region. They are independent from each other and the ethmoidal region. They are embedded in non-cartilaginous connective tissue.

The ethmoidal region is divided medially by a prominent ridge, the larval septum nasi, which slopes sharply from the ethmoid plate

Figure 5. Chondrocranial morphology of *Hymenochirus boettgeri* at stages 49 and 63. A. — D. Dorsal view, ventral view, branchial basket, and lateral view of *H. boettgeri* at stage 49. SR = suprarostral alae. Other labeling as in Figs. 3 and 4. E — F. Lateral and dorsal views *of H. boettgeri* at stage 63. Bar = 1mm.


i+MC
SR
LTN
CQA
FO
OC
A
B
CH
CBI
CBII
C
D
E
F

to the anterior edge of the chondrocranium. Two large foramina, the foramina olfactoria, are located on the ethmoidal plate on either side of the larval septum nasi.

A small and lateral extension of the palatoquadrate is present in the area corresponding to the processus muscularis. This is very reduced in size compared to those of other pipoid taxa. The pars articularis quadrati protrudes prominently at the anterior tips of the palatoquadrate and articulates with the posterior edge of the Meckel's cartilage. The palatoquadrate is fused to the ventrolateral edge of the braincase by means of a broad and well-developed commissura quadratocranialis anterior. No ascending or otic processes are present, and the palatoquadrate does not fuse to the otic capsules. The sub-ocular fenestra is also absent.

The floor of the braincase has a large, oval, basicranial fenestra. The orbital cartilages are poorly developed. The occipital condyles are present as two prominent and posterior projections of the basal plate on either side of the notochord.

The otic capsules represent approximately 40% of the chondrocranial length and are fused to the chondrocranium along the entire length of their medio-ventral edge. The tectum synoticum is not yet formed. The long axis of the otic capsules, diverge from posterior to anterior forming an angle of approximately 40 degrees

with the chondrocranial axis. The ventral and lateral walls of the otic capsules are well formed and a large fenestra ovalis is present in the lateral walls.

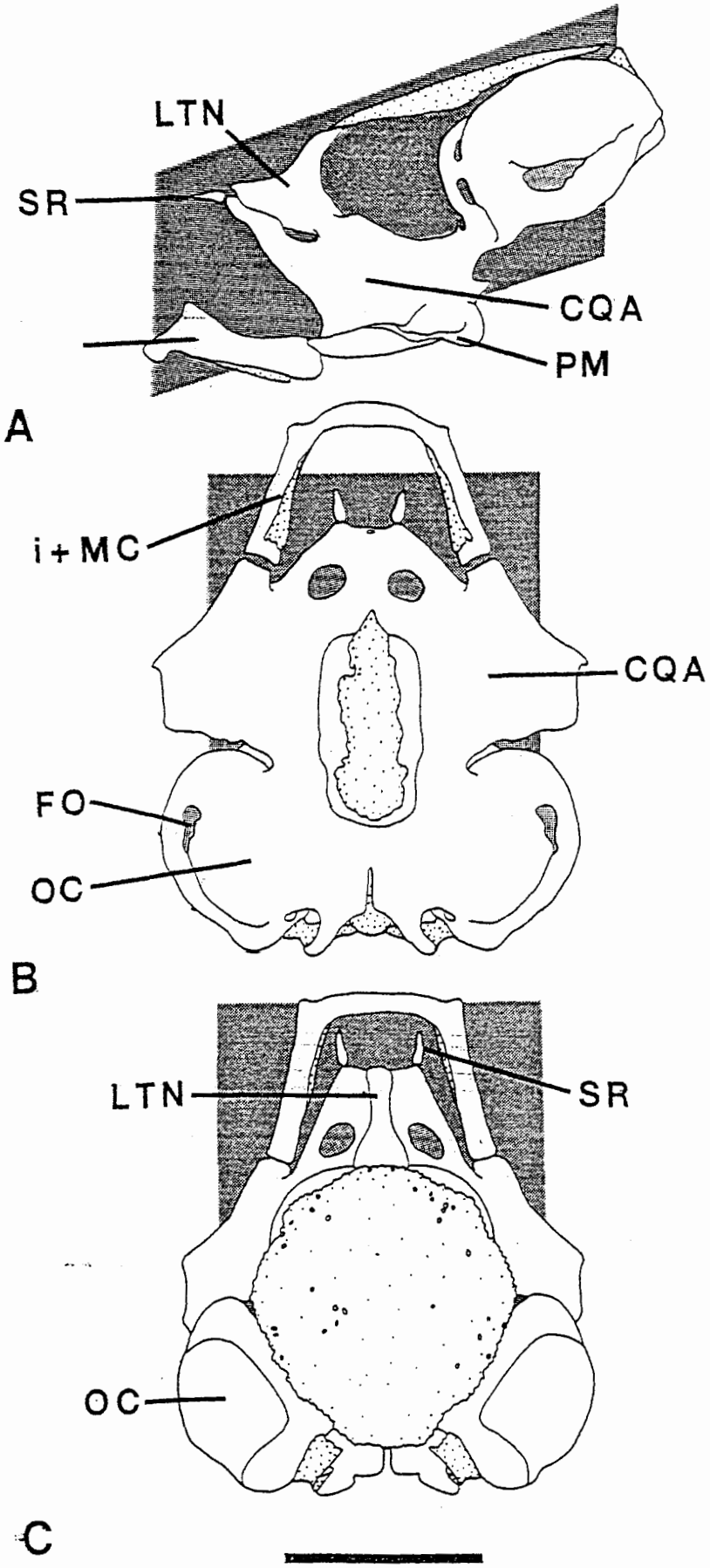
The hyobranchial apparatus (Fig. 5) consists of five independent elements. The lateral edges of the ceratohyalia form the articulation of the hyobranchial apparatus with the braincase. Medially, the ceratohyalia are connected to the single medial copula. There are only two pair of ceratobranchials; they are fused proximally and distally forming a oval shaped element that in turn, is attached to the posterior edge of the single medial copula.

Stage 53. — At this stage the orbital cartilages are slightly more developed and the larval septum nasi is more robust. The foramina carotica primaria have formed from lateral "notches" in the basal plate while the large basal foramen is still present in the floor of the braincase.

Stage 57. — The lower jaw is no longer in a vertical position but has rotated forward and the element is positioned horizontal to the body. At the same time, the suprarostrals have also rotated from vertical to a horizontal position.

Stage 58 (Fig. 6). — Moderate amounts of variation in both ossification and development are apparent. A small and ventrally projecting piece of cartilage appears at the antero-lateral edge of the

Figure 6. A. — C. Lateral, ventral and dorsal views of the chondrocranium of *Hymenochirus boettgeri* at stage 58. Bar = 1mm.


LTN
SR
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i+MC
CQA
FO
OC
B
LTN
SR
OC
C

otic capsules. This corresponds to the traditional attachment of the processus anterolateralis of the crista parotica or the larval processus oticus. In addition a small lateral process is present on the pila antotica corresponding to the remnant of the ascending process.

Stage 60. — The ethmoidal region undergoes drastic changes. The suprarostrals disappear. The adult septum nasi, tectum nasi, lamina orbitonasalis, and cupola anterior are present above the ethmoidal region. The tectum nasi appear as wings originating at the ethmoid plate and curving out and upward. The larval septum nasi previously present on the ethmoidal region has degenerated and anteriorly is being replaced by a newly formed adult septum nasi, which is fused to the transverse cupola anterior.

The ceratohyalia, ceratobranchalia, copula, and hypobranchial plates are greatly eroded. The mandible is modified from its boxy profile and is now smoothly arched. The connection of the palatoquadrate to the braincase is eroded leaving it free along most of its length. At this stage the lower jaw and palatoquadrate begin a counter clockwise rotation and posterior migration. The collumella is present as a cylindrical cartilage projecting from the fenestra ovalis.

Stage 62. — The ethmoidal region is greatly reduced. The nasal capsules are continuing to form. The ceratobranchalia and copula are

nearly gone while the ceratohyals remain and migrate posteriorly in association with the palatoquadrate.

Stage 63 (Fig. 5). — The nasal bones begin ossification dorsal to the well-formed cartilaginous elements of the nasal capsule. The sphenethmoid is present forming the antero-medial walls of the braincase. The palatoquadrate fuses with the antero-medial wall of the otic capsules.

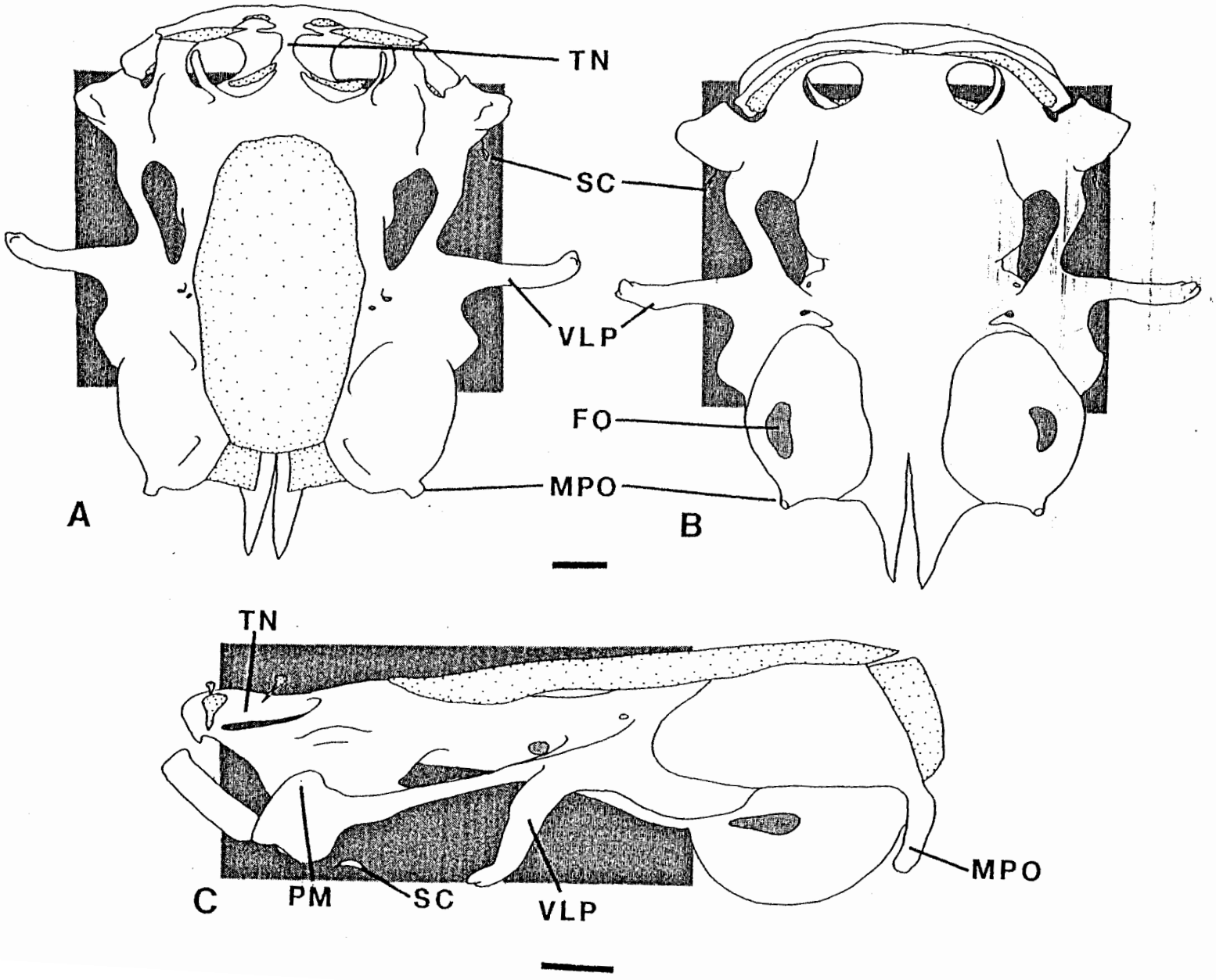
Stage 65. — The ceratohyals are fused to otic capsules posterior to the fusion of the palatoquadrate.

Ossification. — Table 5 summarizes the appearance of each bone in the specimens studied. The first appearance of many bones was variable and ranged typically across 3 or more stages. Ossification was initiated with the angulosplenial at stage 49 and typically ended with several different bones after metamorphosis. The majority of bones ossified during stage 65 and later.

The Chondrocranium of *Pipa carvalhoi*

Stage 55 (Fig. 7). — Overall the chondrocranium of *Pipa carvalhoi* is dorso-ventrally flattened, with the otic capsules representing approximately one-third the chondrocranial length. It possesses a

Figure 7. A — C. Dorsal, ventral, and lateral views of the chondrocranium of *Pipa carvalhoi* at stage 55. Bar = 1mm.


TN
SC
VLP
FO
MPO
A
B
TN
PM
SC
VLP
MPO
C

completely formed lower jaw and a typical pipid suprarostral plate lacking independent suprarostral cartilages and anteriorly projecting cornua trabeculae.

The lower jaw consists of fused Meckels' and infrarostral cartilages resembling closely the adult condition. The proximal part of the lower jaw is more robust than the distal portion. Separate Meckels' and infrarostral cartilages are not distinguishable. The angulosplenials are present along the latero-lingual margin of the lower jaw. They are triangular in shape being broader posteriorly and narrow anteriorly.

The anterior part of the suprarostral plate is thinner than the posterior part. The anterior margins of the suprarostral plate are laterally expanded. These lateral expansions lack a cartilaginous connection to the palatoquadrate; however, a cartilaginous commissura quadrato-ethmoidalis is present.

The most anterior part of the palatoquadrate consists of the pars articularis quadrati. The pars articularis quadrati is overall rectangular in shape and oriented at approximately a 45° angle relative to the main chondrocranial axis. Anteriorly, it articulates with the lower jaw. Immediately posterior to the pars articularis quadrati, and projecting dorsally from the palatoquadrate, is the processus muscularis palatoquadrati. At the level of the muscular

process a broad commissura quadratocranialis anterior is present. A well-developed processus antorbitalis is present at the junction of the commissura quadratocranialis palatoquadrati with the braincase. Ventral and lateral to the processus muscularis is a small cup shaped cartilage, the symplectic cartilage. The symplectic cartilage lies between the palatoquadrate and the ceratohyal. It is attached to both structures by ligamentous connections. Just posterior to the processus muscularis, the palatoquadrate consists of a slender subocular bar. Posteriorly, the subocular bar is continuous with the ascending and larval otic processes. The larval otic process and the ascending process are wide and almost indistinguishable in dorsal view. The ascending process has a high attachment to the braincase and the larval otic process is continuous posteriorly with the larval crista parotica of the otic capsule. A long and thin processus ventrolateralis palatoquadrati projects laterally, from the palatoquadrate, at the junction of the subocular bar with the ascending and larval otic processes.

The otic capsules are large. Their dorsal surface is flat, whereas ventrally they are sub-spherical. A large fenestra ovalis is present on the lateral wall of the otic capsules. A prominent larval crista parotica projects from the lateral sides of the otic capsules. A pair of foramina, the foramen auditivae and the foramen jugulare,

are present in the posterior wall of the otic capsules. The posterolateral edges of the otic capsules have a well-developed and cylindrical muscular process. The ventral edge of this process is fused to the posterior edge of the branchial basket.

The lateral walls of the braincase possess three pairs of foramina: the prootic, oculomotor, and optic foramina.

The hyobranchial apparatus is well developed. The ceratohyals are connected medially by the pars reuniens and posess short and broad processus anterior hyalis and processus antero-lateralis hyalis. There is a single attachment between each branchial basket and the hypobranchial plate. There are four ceratobranchials but no spicules are present. An intricate mesh of cartilage between the ceratobranchials forms the ventral surface of the branchial baskets. The urobranchial process and copula are absent. A notch is present at the distal end of ceratobranchials two and three; each notch possesses two small cartilaginous projections that connect the branchial basket to the cranium at the level of the larval crista parotica and the muscular process of the otic capsule.

A large and oval-shaped frontoparietal bone covers the braincase dorsally at this stage. Ventral to the braincase, the parasphenoid is present. The angulosplenials, maxillae, premaxillae, nasals, and exoccipitals have already begun ossification at stage 55.

Stage 59 (Figs. 8 and 9). — The chondrocranium at stage 59 is similar in overall appearance to that at stage 55. Differences are related to a higher degree of ossification and further chondrification of the chondrocranium. The nasal area is better developed having well-developed adult septum nasi, tectum nasi, alary, and oblique cartilages, anterior maxillary process, and lamina orbitonasalis. A cartilaginous collumela is present. The hyobranchial apparatus is present and very similiar to that described earlier, however; it is better chondrified.

Stage 62. — The ascending process, otic process, lateral process of the palatoquadrate, and the commissura quadratocranialis anterior have eroded away leaving the palatoquadrate free from the braincase. The palatoquadrate, along with the jaw articulation and ceratohyals, rotates ventrally and migrates posteriorly. Parts of the hyobranchial apparatus have also eroded leaving only the ceratohyals that are closely connected to the palatoquadrate by connective tissue. The ossification of the frontoparietal has expanded anteriorly and covers the entire frontoparietal fenestra. Alizarin stained teeth are present on the maxillae and premaxillae.

Figure 8. A. — B. Branchial basket and lateral view of the chondrocranium of *Pipa carvalhoi* at stage 59. Bar = 1mm.

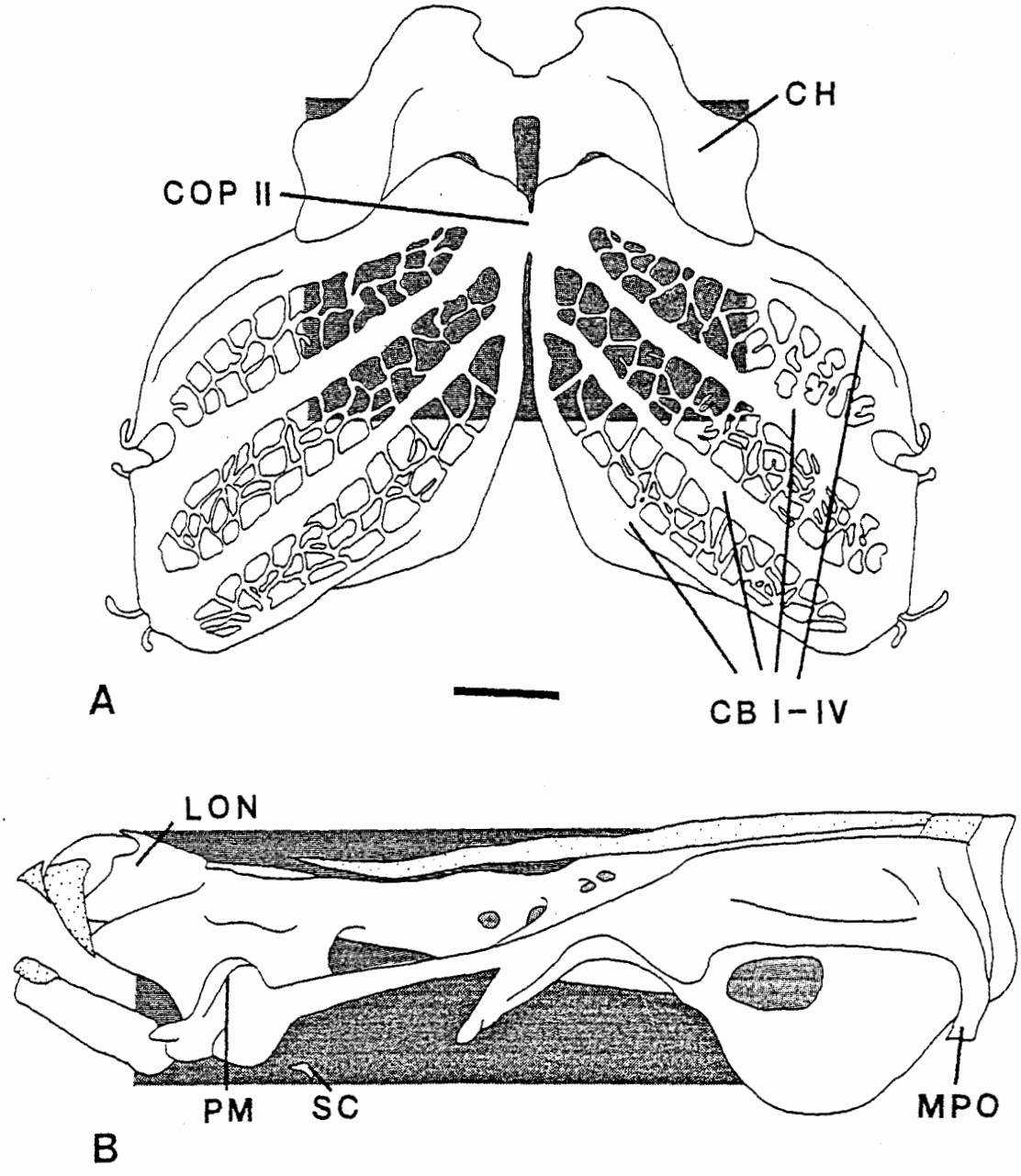
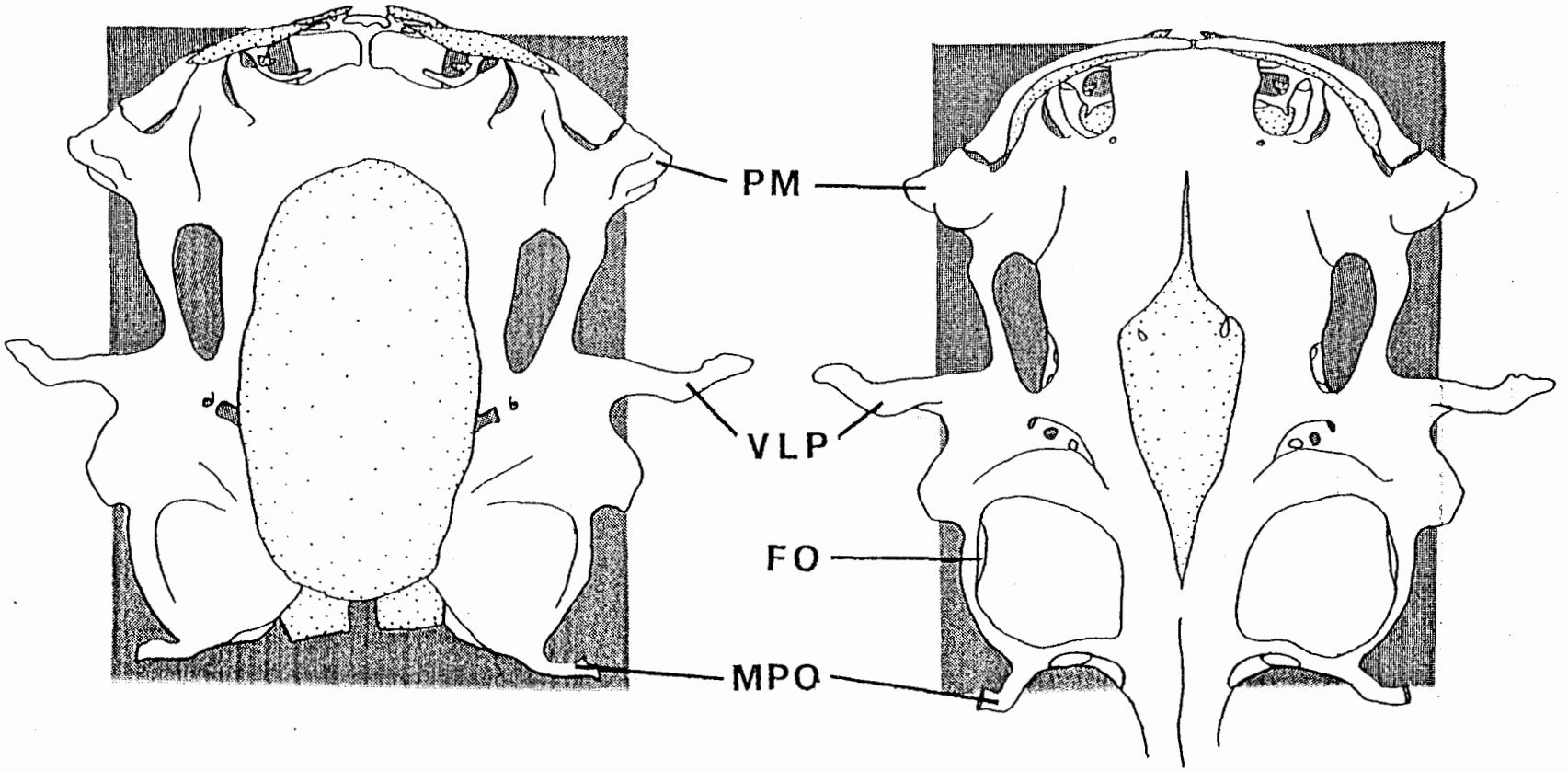

CH
COP II
A
CB I–IV
LON
PM
SC
MPO
B

Figure 9. Dorsal and ventral views of the chondrocranium of *Pipa carvalhoi* at stage 59. Bar = 1mm.


PM
VLP
FO
MPO

Stage 63. — The palatoquadrate and ceratohyals continue their posterior migration. The ceratohyals have greatly degenerated and remain attached to the ventral side of the palatoquadrate.

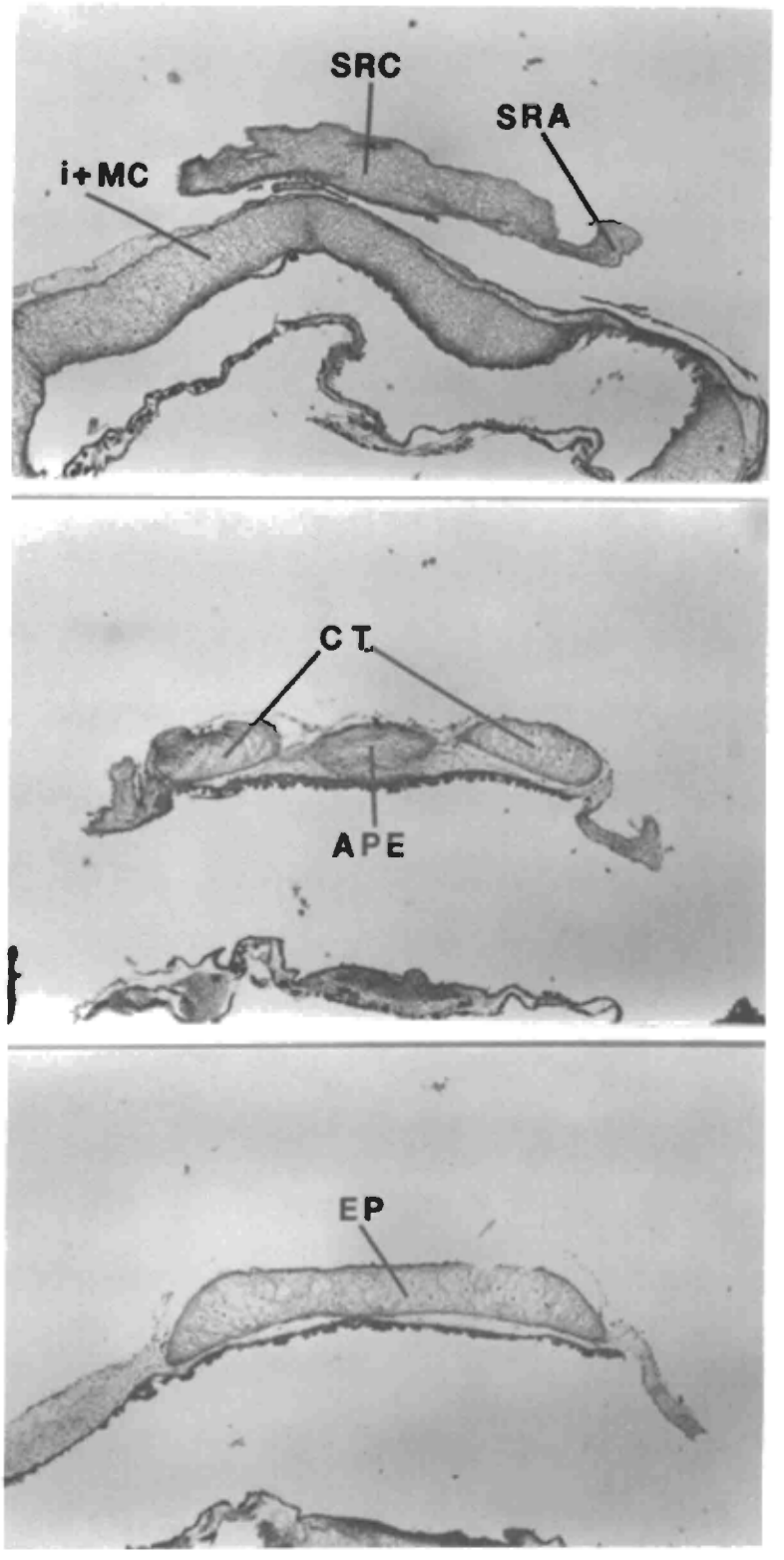
Development of the suprarostral plate

Rhinophrynus dorsalis

Paraffin embedded serial cross-sectioned specimens at stage 45 show the ethmoid plate (= anterior wall of the braincase) possessing two anteriorly projecting cartilaginous rods, the cornua trabeculae (CT, Fig. 10). The suprarostral cartilages are fused to the anterior tips of the CT. Whole-mounted cleared and stained early tadpoles, stages 48 to 51, continue to exhibit two CT that are fused anteriorly to the suprarostral cartilages. Between stages 48 and 51 a process, herein called the anterior process of the ethmoid plate (APE), grows anteriorly from the ethmoid plate. This process is sub-cylindrical in cross section and lies between the CT. In whole-mounted tadpoles at stage 51, this process is visible and also fused anteriorly with the suprarostral cartilages.

During later development, the space left between the APE and the CT "fills in" with cartilage, leaving two small anterior holes

Fig. 10. Cross-sections through the suprarostral plate of *Rhinophrynus dorsalis*, stage 51. A. Posterior section through the ethmoid plate region. B. Medial section of the suprarostral plate. C. Anterior section through the suprarostral area. EP = ethmoid plate, CT = cornua trabeculae, APE = anterior process of the ethmoid plate, SRA = suprarostral alae, SRC = suprarostral corpus, i + MC = fused infrarostral and Meckel's cartilages, arrows indicate thin cartilage connecting cornua trabeculae and the anterior process of the ethmoid plate. (Magnification = 45X)


SRC
SRA
i+MC
CT
APE
EP

(behind what would correspond to the posterior edge of the suprarostrals). This cartilaginous "filling" forms a continuous plate with the APE, the CT, and the suprarostrals, forming the suprarostral plate of later larval stages. In cross section, the suprarostral plate is thicker medially and laterally in the areas corresponding to the APE and the CT. Between these three thicker areas, the suprarostral plate is seen as a thin sheet of cartilage (Fig. 10).

Xenopus laevis

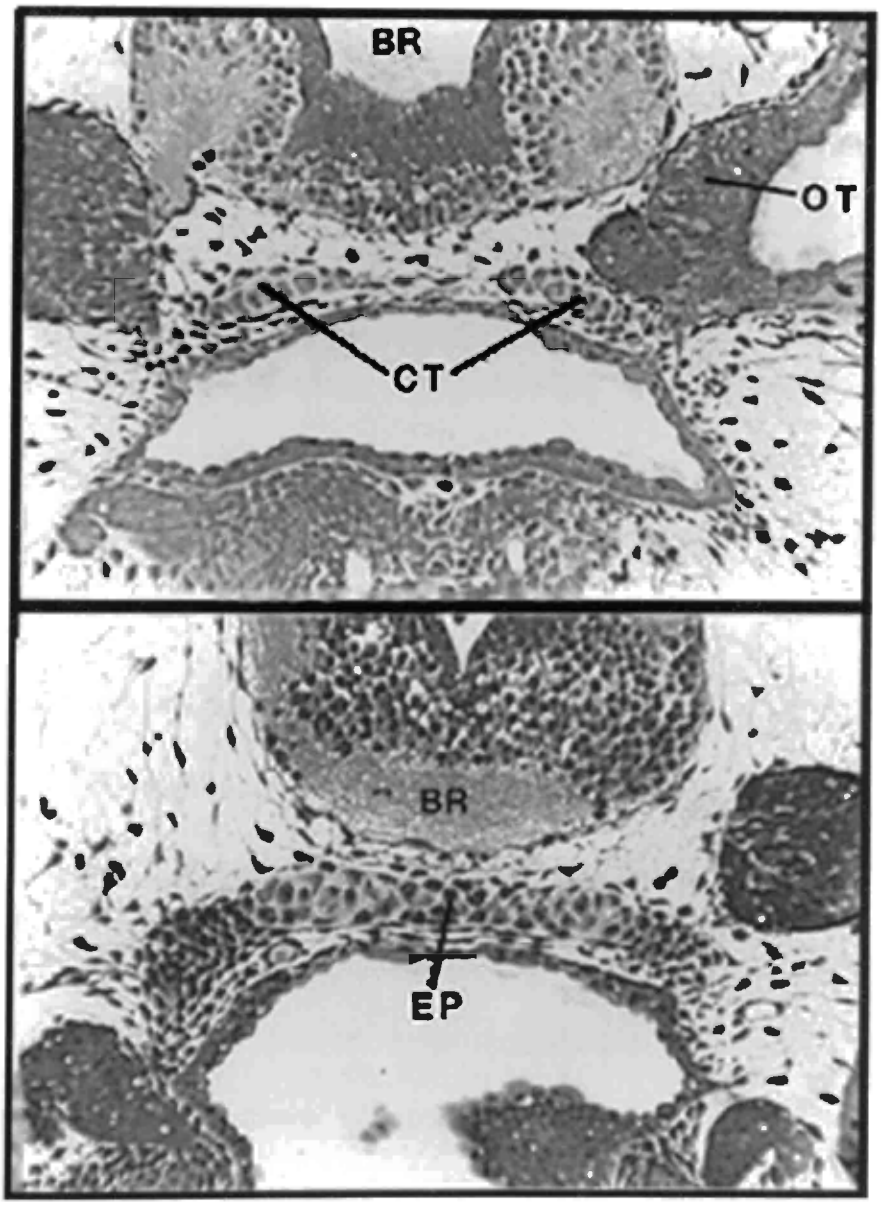
At stage 41 serial cross-sections show an ethmoid plate with two anteriorly projecting CT. The suprarostral cartilages are not yet formed.

Early stage 43 specimens have two separate rod-shaped CT free at their anterior ends and no suprarostral cartilages have developed (Fig. 11).

In later stage 43 specimens, a shorter medial rod-shaped element is visible projecting from the ethmoid plate medial to the CT. No suprarostral elements are visible at this stage but they form between stages 43 and 45. Cartilaginous "fill-in" of the area between the CT, the anterior process of the ethmoid plate, and the

Figure 11. Cross-sections through the ethmoidal region of *Xenopus laevis* at stage 43. A. Section demonstrating the anteriorly projecting cornua trabeculae of *X. laevis*. B. Section demonstrating the flat ethmoid plate *of X. laevis*. OT = olfactory tissue, BR = brain tissue, CT = cornua trabeculae, EP = ethmoid plate.

(Mag.= 450x)


BR
OT
CT
BR
EP

suprarostrals during later development produces the suprarostral plate.

Hymenochirus boettgeri

In early stage 38, a single median cartilaginous rod-shaped anterior process of the ethmoid plate represents the anterior region of the chondrocranium of *Hymenochirus boettgeri*. The ethmoid plate is poorly developed, flat, and posteriorly fused to the parachordal cartilages (Fig. 12). Neither CT nor suprarostral cartilages are visible. Late stage 38 specimens show two short cartilaginous rod-shaped elements fused to the ventrolateral edges of the APE (Fig. 13). Suprarostral cartilages are not present.

During stages 41 and 42, the two cylindrical cartilages ventral to the APE project an average of 12 µm past the anterior tip of the APE. The suprarostral cartilages are present at stage 41 and all later stages until their resorption. The suprarostral cartilages of *Hymenochirus* are paired, antero-posteriorly oriented, rod-shaped elements. Cross-sections of stage 41 specimens clearly show CT, suprarostrals and the APE (Fig. 14).

At stage 43 and 45 the CT are better developed (Fig. 13) and range in length from 6 to 16µm. In older specimens, from stages 45 to 48, cartilage fills in the space between the anterior of the CT

Figure 12. Cross-sections through the ethmoidal region of *Hymenochirus boettgeri* at stage early 38. A. Section demonstrating the anterior process of the ethmoid plate. B. Section posterior to A demonstrating the ethmoid plate. APE = medial process of the ethmoid plate, EP = ethmoid plate, BR = brain tissue, E = eye, OC = oral cavity. (Mag. = 450x)

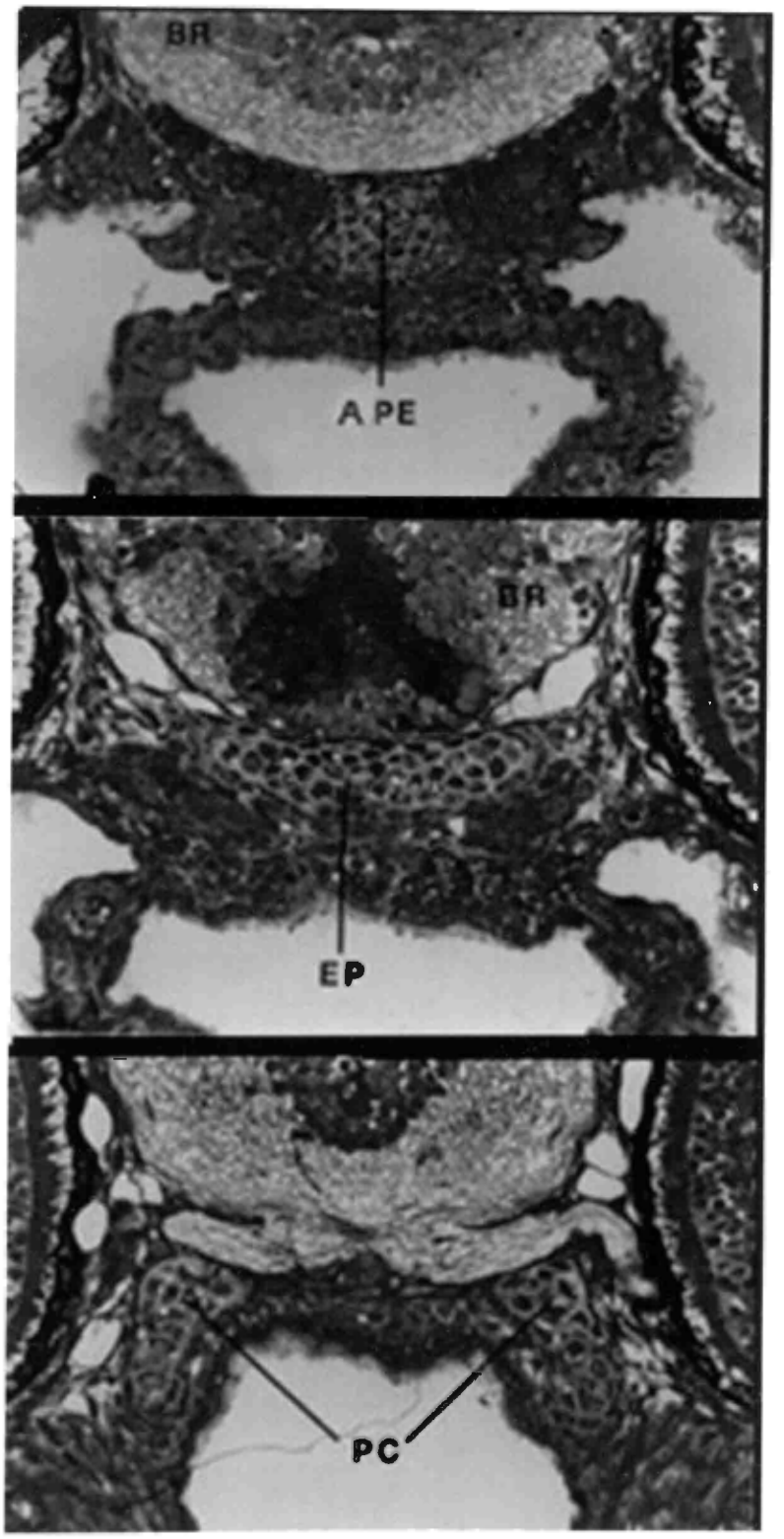

BR
APE
BR
EP
PC

Figure 13. Cross-sections through the ethmoidal region of *Hymenochirus boettgeri* at later stage 38. A. Cross-section demonstrating the fusion of the cornua trabeculae to the ventro-lateral edge of the anterior process of the ethmoid plate. B. Cross-section posterior to A through the ethmoid plate. C. Cross – section posterior to B showing the separate trabecular cartilages. BR = brain, E = eye, EP = ethmoid plate, CT = cornua trabeculae, TC = trabecular cartilages, APE = anterior process of the ethmoid plate (Magnification = A and B = 450, C = 115).

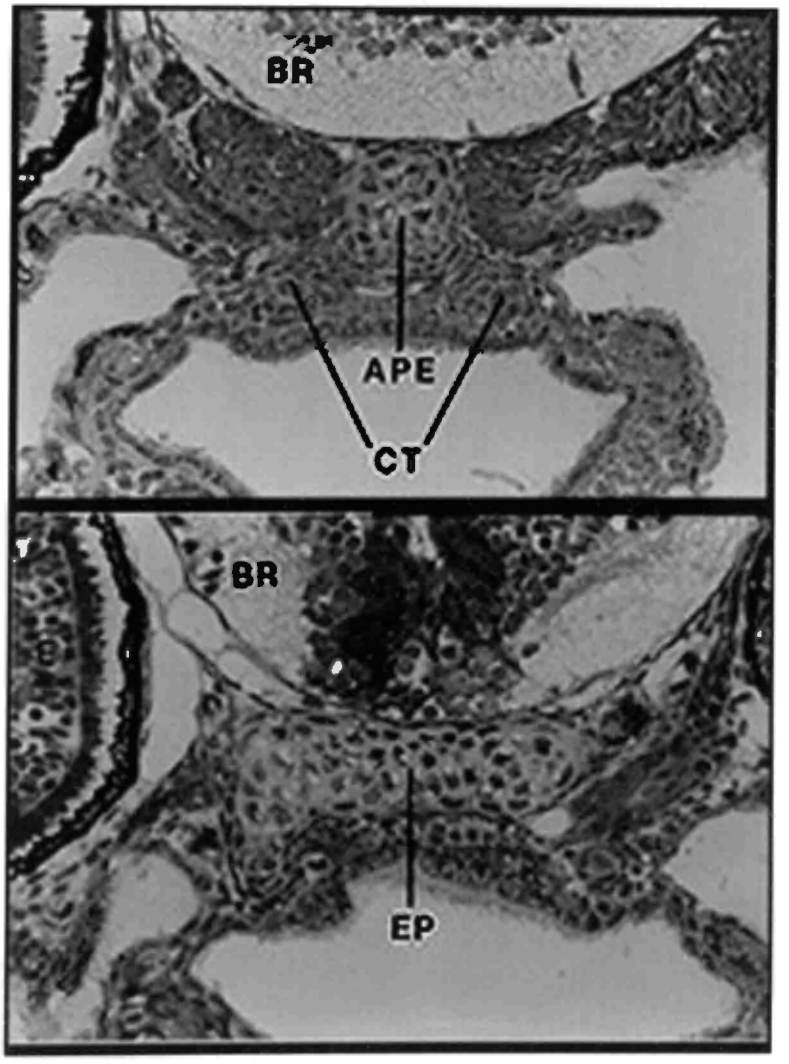
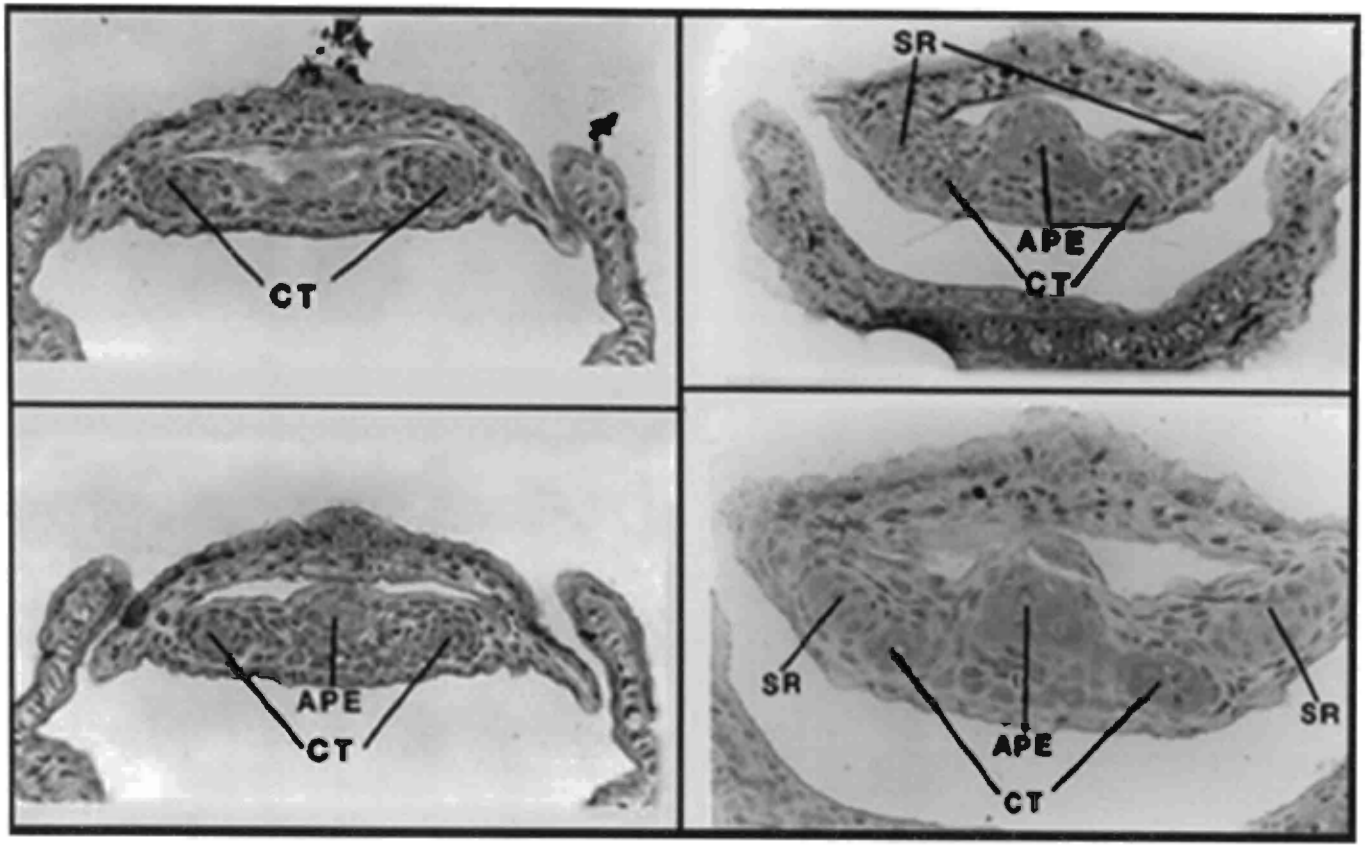

BR
APE
CT
BR
EP

Fig. 14. Cross-sections through the ethmoidal region of *Hymenochirus boettgeri* at stage 41. A. Section showing free cornua trabeculae anteriorly. B. Section showing the anterior process of the ethmoid plate and the cornua trabeculae. C. Cross-section through the ethmoid plate demonstrating the fusion of the anterior process of the ethmoid plate with the cornua trabeculae and the independent suprarostral cartilages. D. Section posterior to C indicating same. APE = anterior process of the ethmoid plate, BR = brain tissue, E = eye, CT = cornua trabeculae. (Magnification = A – B, 450x).


CT
SR
APE
CT
APE
CT
SR
APE
CT
SR

and leaves small holes on either side of the medial element. From stage 48 on, the CT and the APE form a continuous cartilaginous plate. Furthermore, the APE grows dorsally to form the larval septum nasi.

Internal oral morphology of Pipoid frogs

Rhinophrynus dorsalis

Buccal Roof (Fig. 15). — The upper lip is smooth, does not curve inward and is free of keratinized structures. The nares are positioned diagonally just posterior to the upper lip. They are laterally placed and lack narial valves. The buccal roof is slightly concave. A ridge is found running along the midline of the buccal roof. A small transverse canal divides this ridge at approximately one-third of its length. Pustulations are found on the entire surface of the roof but are more concentrated along the median ridge. Three to four prominent papillae are found on the posterior part of the buccal roof. They are attached to each other along their bases by a thin membrane.

Buccal Floor (Fig. 16). — The lower lip is sturdy with a strong posterior curvature and a few prominent papillae present on the

Figure 15. Scanning electron microscopy photograph of the buccal roof of *Rhinophrynus dorsalis* at stage 52(15Kv, 40x, bar = 1mm).

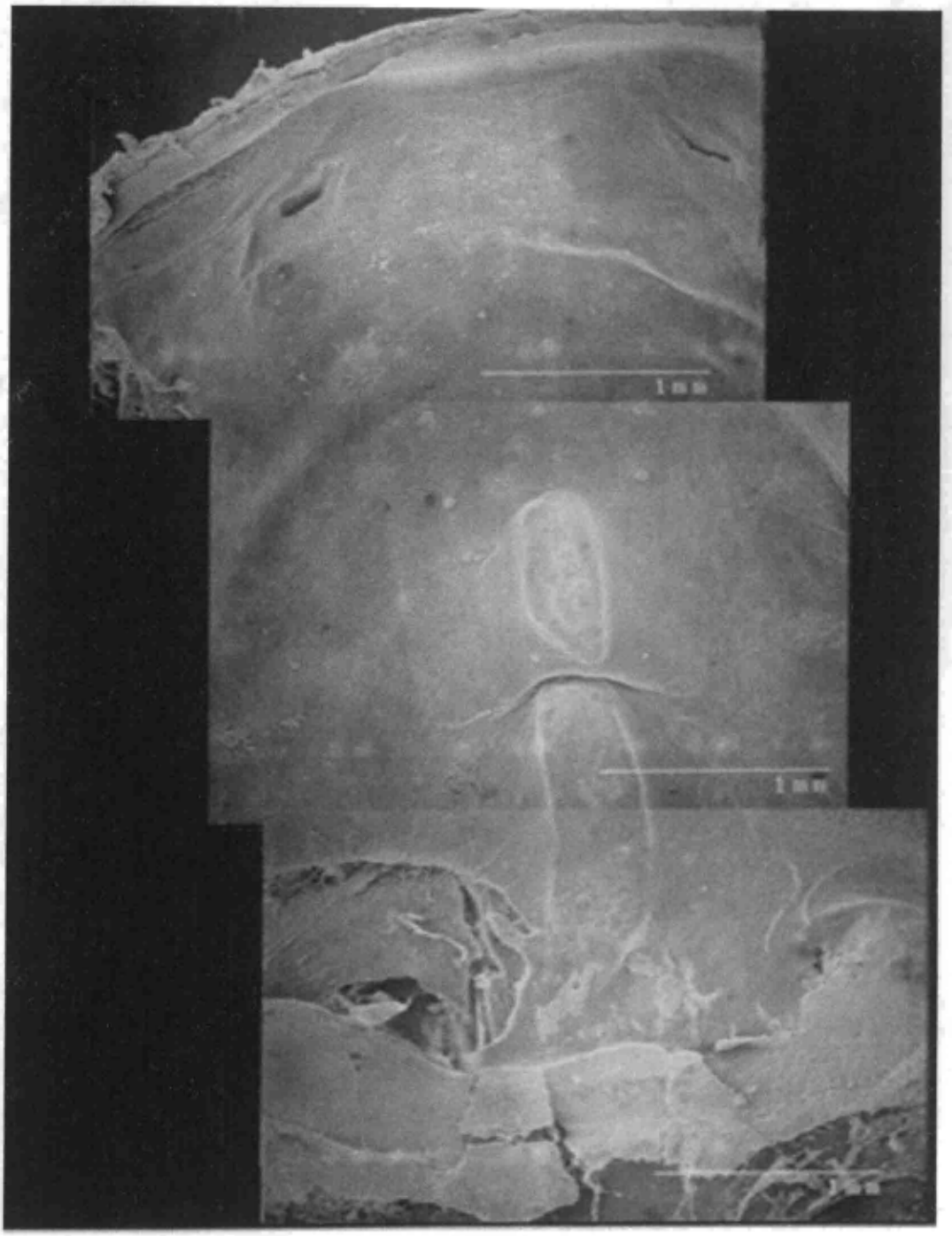

1mm
1mm

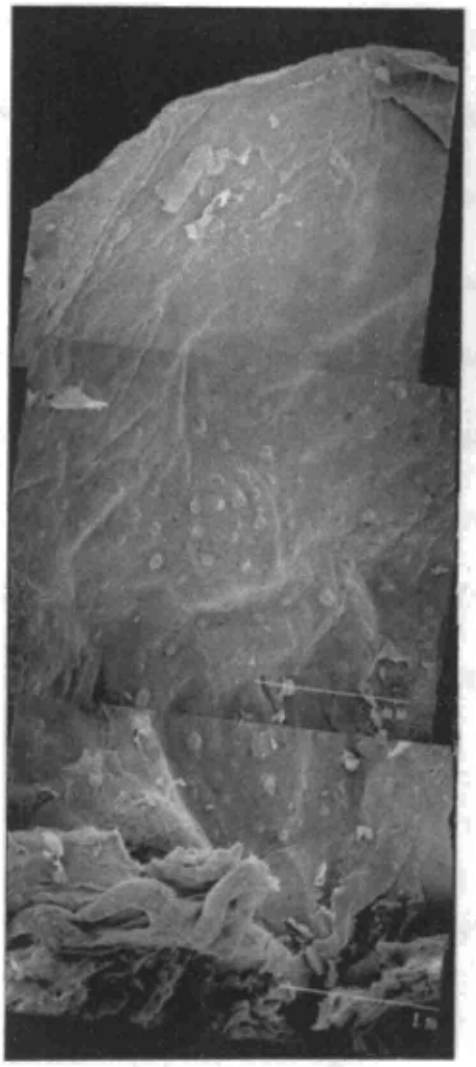
Figure 16. Scanning electron microscopy photograph of the buccal floor of *Rhinophrynus dorsalis* at stage 52 (15Kv, 40x, bar = 1mm).

external surface. A narrow, but fairly well defined, antero-posterior groove is found at the midline of the floor. The groove begins at about one-third the length of the buccal. Few pustulations are scattered throughout the surface of the buccal floor. In addition to the small pustulations, a few randomly placed, broad based, low, and wrinkled pustulations were observed. Arranged in a crescent pattern located at the posterior edge of the buccal floor, just anterior to the glottis, there is a row of slender papillae. The glottis is found at the posterior edge of the floor and has the typical midline slit shape with well defined.

Pipa carvalhoi

Buccal Roof (Fig. 17). — The dorsal lip is thin and lacks an incurving fold. The nares are close to the anterior edge of the roof. They lack narial valves but the anterior and posterior edges have small rugosities. The area between the nares has a few randomly placed pustulations. The most striking feature of the buccal roof is a prominent median ridge that divides the roof into right and left halves. It begins at approximately the midpoint of the roof and ends just anterior to the posterior margin. This median ridge is divided into anterior and posterior sections by a transverse fold. The

Figure 17. Scanning electron microscopy photograph of the internal oral anatomy of the buccal roof of *Pipa carvalhoi* at stage 59 (15Kv, 40x, bar = 1mm).



posterior edge of the buccal roof is defined by a broad and glandular, buccal pressure cushion.

Buccal Floor (Fig. 18). — The lower lip is firm and well defined but lacks keratinized structures. The anterior part of the buccal floor has a broad V-shaped, posteriorly pointing, concavity approximately 1/4 the width of the floor of the mouth. The area anterior to the V-shaped concavity is smooth and free of projections. Immediately behind this V-shaped depression, are found three short antero-posterior ridges. The edges of these ridges have large and blunt nodules. Lateral to these ridges, the ventral floor has numerous randomly arranged pustulations. Posteriorly, the filter plates and branchial baskets occupy approximately 75% of the buccal floor.

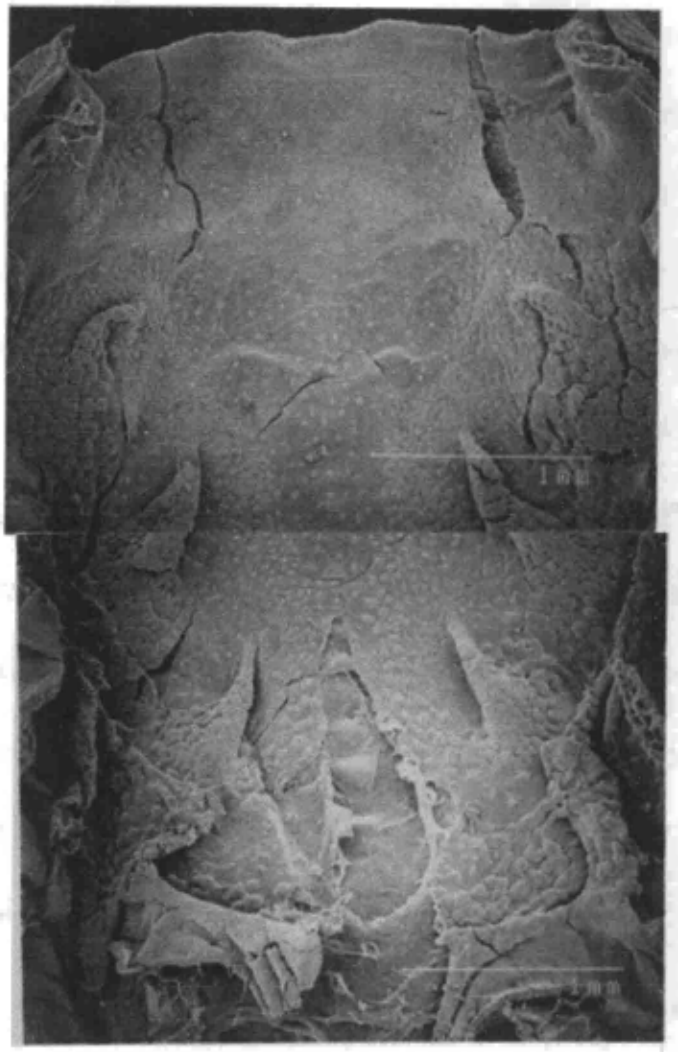
Xenopus laevis

Buccal Roof (Fig. 19). — The dorsal lip is thin with a very smooth edge. The internal nares are small and widely separated. They are oriented transverse to the body and lack narial valves, but their edges have short blunt pustulations. Overall the dorsal roof is funnel shaped with a slight medial concavity and three pair of prominent and glandular folds of skin along the lateral edges. These folds are oriented diagonal to the midline of the dorsal roof. There are

Fig. 18. Scanning electron microscopy photograph of the internal oral anatomy of the buccal floor of *Pipa carvalhoi* at stage 59 (15Kv, 40x, bar = 1mm).


1 mm

Figure 19. Scanning electron microscopy photograph of the internal oral anatomy of the buccal roof of *Xenopus laevis* at stage 53 (15Kv, 40x, bar = 1mm).



numerous pustulations in the median concavity and they are more abundant in the lateral portions of the buccal roof. The posterior limit of the buccal cavity is defined by a smooth, ventrally projecting flap of skin, the dorsal velum. It was damaged during specimen preparation so further description is not possible in this specimen.

Buccal Floor (Fig. 20). — The ventral lip of *Xenopus* is thick with a strong posterior curvature. It lacks keratinized structures. Anteriorly, the buccal floor is free of projections but has a small antero-posteriorly oriented median ridge. This ridge is fairly thin anteriorly and only slightly broader posteriorly. There is a very thick collection of pustulations in posterior part of the ventral floor, medial to the branchial sacs. The branchial basket occupies approximately 75% of the buccal floor.

Hymenchirus boettgeri

Buccal Roof (Fig. 21). — Overall, the buccal roof is pear-shaped and about three times long as wide. The dorsal lip is much larger than the lower lip. Just inside the dorsal lip there is a rectangular patch of keratinized, teeth-like structures. No internal nares were found. Just posterior to the tooth patch is a medially placed longitudinal row of four broad and elongate knobs. A group of pustulations are found on the lateral walls of the buccal roof on either side of the ridge.

Figure 20. Scanning electron microscopy photograph of the internal oral anatomy of the buccal floor of *Xenopus laevis* at stage 59 (15Kv, 40x, bar = 1mm).

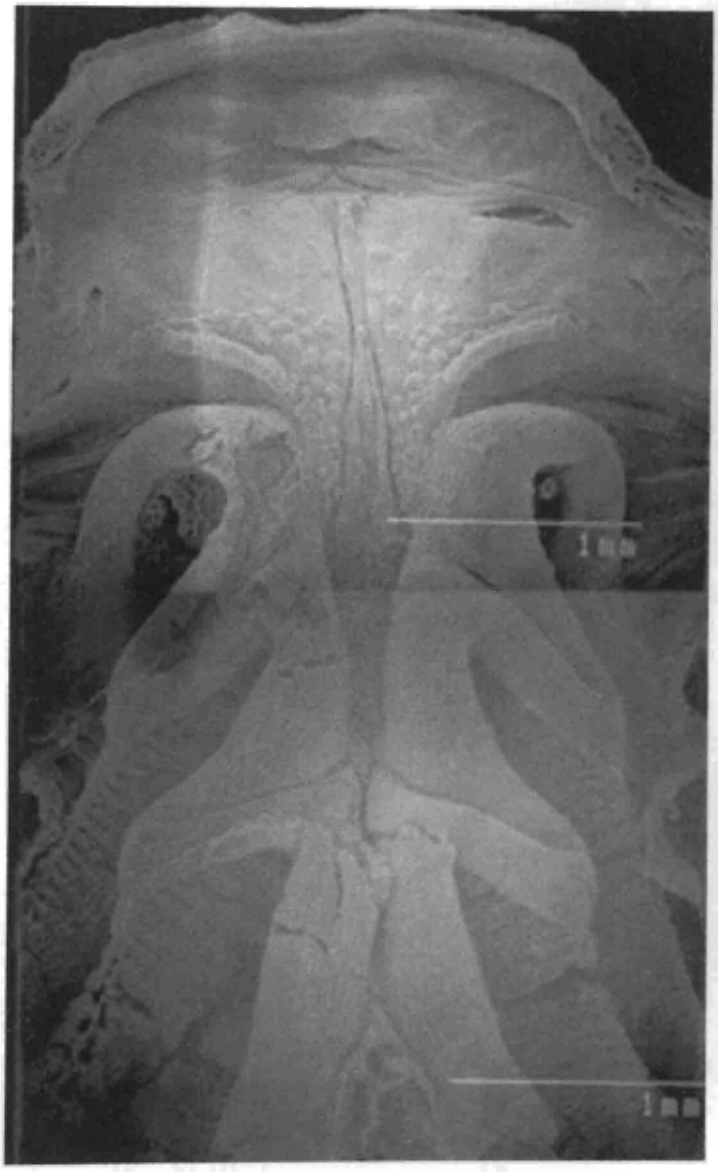
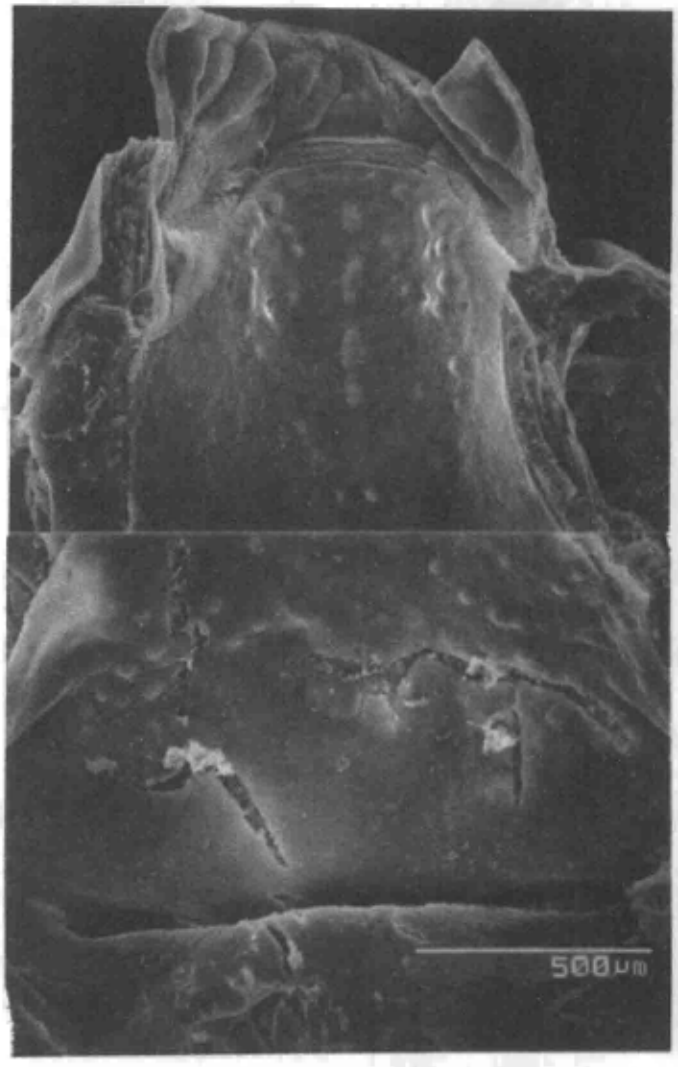

1 mm
1 mm

Figure 21. Scanning electron microscopy photograph of the internal oral anatomy of the buccal roof of *Hymenchirus boettgeri* at stage 52 (15Kv, 40x, bar = 1mm).


500µm

Two more groups of small pustulations are located in the postero-lateral margin of the buccal roof. Other than these few papillae, the dorsal roof is smooth with only a few randomly placed small pustulations. A deep channel spans the entire roof at its posterior limit.

Buccal Floor (Fig. 22). — Anteriorly, lining the entrance to the mouth is a rectangular grouping of teeth-like structures. They are placed just inside the thin but firm lower lip (Fig. 23). The buccal floor of *H. boettgeri* is dominated by a large posteriorly attached tongue-like structure. Cross-sections indicate this tongue is composed of the long median copula of the hyobranchial apparatus. It is free from the ventral floor anteriorly. Three longitudinally arranged rows of pustulations are found on the dorsal part of this tongue-like structure. Branchial sacs are present but lack filter plates. The branchial basket fills about 40% of the buccal floor.

Figure 22. Scanning electron microscopy photograph of the internal oral anatomy of the buccal floor of *Hymenochirus boettgeri* at stage 52 (15Kv, 40x, bar = 1mm).

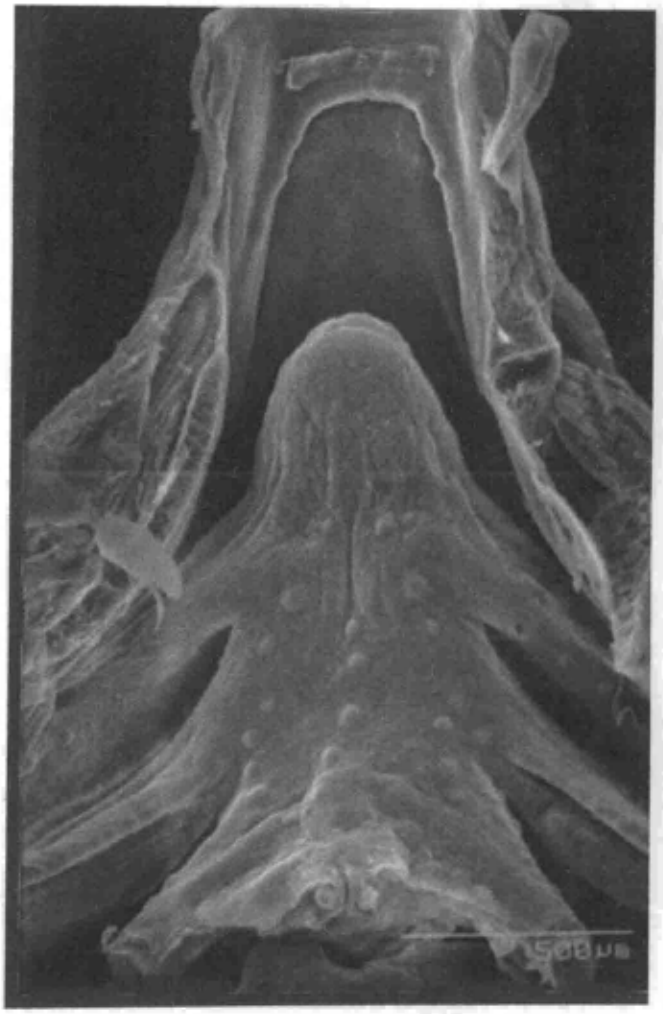
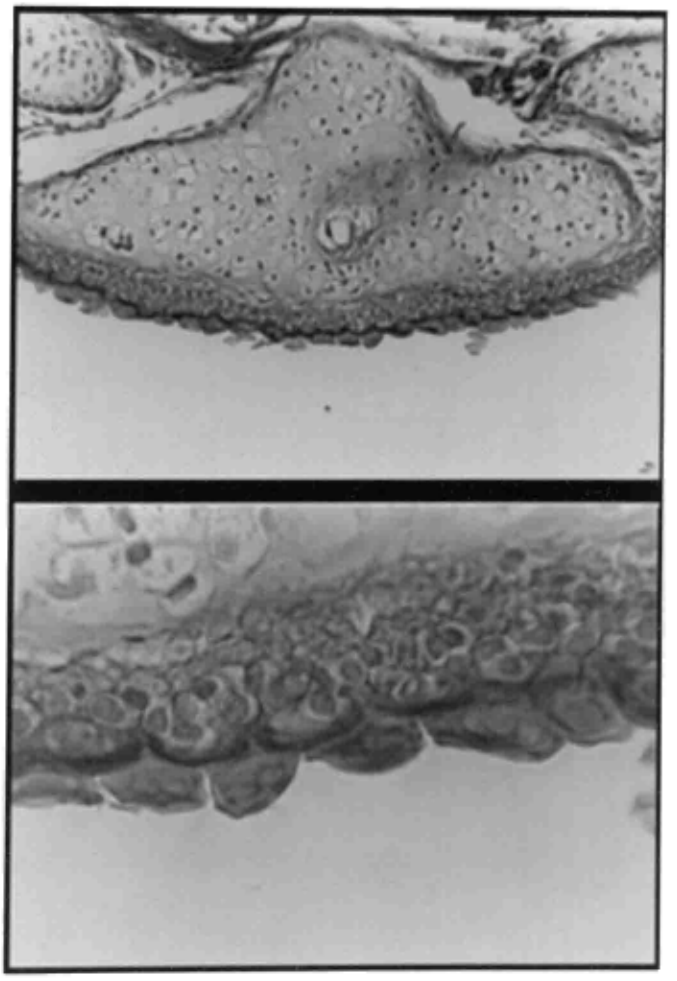

1500µm

Fig. 23. Cross-sections through the teeth patches of *Hymenchirus boettgeri* at stage 58. (Magnification — A = 25X, B = 120X)



Larval Characters for Phylogenetic Analysis

Based on the previous anatomical descriptions and the available literature for: *Ascaphus truei* (Reiss, 1997), *Leiopelma archeyi* (Stephenson, 1951), *Bombina orientalis* (Maglia and Púgener, in press), *Spea bombifrons* (Weins, 1989), *Discoglossus sardus* (Púgener and Maglia, 1997), and *Hyla chrysocelis* (pers. observ), I identified the following sets of characters:

1. musc. A dorsally projecting processus muscularis palatoquadrati at the anterior edge of the palatoquadrate. This character is absent in *Ascaphus*, *Lieopelma*, and *Hymenochirus* and present in all other taxa. 0= present, 1= absent
2. subo. Sub-ocular bar of the palatoquadrate. A subocular bar is absent in *Hymenochirus* and present in all other taxa. 0= present, 1= absent.
3. Subb. Wide subocular bar of the palatoquadrate. The sub-subocular bar of the palatoquadrate of *Xenopus*, *Pipa*, *Lieopelma*, and *Ascaphus* is thin (ratio of width to length < .4). *Hymenochirus* lacks a subocular bar (coded as N). All other taxa have a wide subocular bar of the palatoquadrate (ratio of width to length >.5). 0= robust, 1= thin.

4. pmca. Procesus muscularis capsulae auditivae. This character is present in *Rhinophrynus* and *P. carvalhoi* and absent in all other taxa. 0= present, 1= absent.

5. lapp. Lateral process of the palatoquadrate. The lapp is present in *Xenopus*, *Pipa*, and *Rhinophrynus*, and is absent in all other taxa. 0= absent, 1= present.

6. supr. Condition of the suprarostral cartilages. 0 = identifiable as separate elements (all non-pipoids), 1 = unfused to the cornua trabeculae (*Hymenochirus*), 2 = fused to suprarostral plate (*Pipa*, *Xenopus*, *Rhinophrynus*).

7. proa. Processus ascendens. The processus ascendens is absent in *Hymenochirus* and present in all other taxa. 0 = present, 1 = absent.

8. pras. Processus ascendens. The ascending process in *Pipa* is fused to the anterior edge of the processus oticus along its entire length. The processus ascendens is free from the otic capsule in all other tax. 0= free from otic process, 1= fused to otic process.

9. symp. The symplectic cartilage. 0= absent (non-pipoids, *Xenopus*, and *Hymenochirus*), 1 = present and biconcave (*Rhinophrynus*), 2 = present and uniconcave (*Pipa*).

10. tent. Cartilaginous tentacles. These are present in *Xenopus* and absent in all others. 0= absent, 1= present.

11. cera. Ceratohyalia. The ceratohyalia of *Rhinophrynus* and *Pipa* is broader than the branchial basket. The other taxa have ceratohyalia narrower than the branchial basket. 0= wide, 1= narrow.

12. otpr. Otic process. The otic process is absent in *Hymenochirus*, *Hyla*, *Spea*, *Discoglossus*, and *Bombina*. 0 = present, 1 = absent

13. cerb. Ceratobranchia. *Hymenochirus* has two ceratobranchials while all other taxa have four. 0= 4 ceratobranchalia, 1= 2 ceratobranchalia.

14. pphy. Processus posterior hyalis. The processus posterior hyalis is absent in *Hymenochirus*, and *Lieopelma* and present in all other taxa. 1= absent, 0= present.

15. papi. Papillae surrounding the glottis. *Rhinophrynus* and *Pipa* possess elongate papillae surrounding the entrance to the glottis. These papillae are absent in all other taxa. 0= present, 1= absent.

16. brrg. Buccal roof ridge. A ridge is present in the buccal roof of *Rhinophrynus* and *Pipa* and absent in all other taxa. 0 = absent, 1 = present.

17. cb3e. Ceratobranchial three. Ceratobranchial III is greatly expanded (twice the width of CB 1, 2, and 4) in *Rhinophrynus*,

Ascaphus, *Discoglossus*, and *Bombina*. 0 = greatly expanded ceratobranchial three, 1 = thin ceratobranchial three.

18. ttmg. Taenia tecti marginalis. Present in *Bombina*, *Hyla*, *Discoglossus*, and *Lieopelma*. 0 = present, 1 = absent.

19. cqca. Commissura quadratocranialis anterior. The cqca of *Hymenochirus*, *Rhinophrynus*, *Xenopus*, *Pipa*, is broad. All other taxa have a narrow cqca. 0 = narrow, 1 = broad.

20. fimc. Early fusion of infrarostral and Meckel's cartilage. All pipoids have a fusion of the components of the lower jaw at an early stage. All other taxa lack fimc early in development. 0 = fused, 1 = unfused.

21. lcpo. Larval crista parotica of the otic capsule. *Rhinophrynus*, *Xenopus*, *Pipa*, *Hymenochirus*, and *Spea* have a well-developed lcpo. All other taxa lack a lcpo. 0 = absent, 1 = present.

22. aspq. Attachment of the palatoquadrate suspensorium. All pipoids have a high attachment of the palatoquadrate suspensorium. All other taxa lack a high aspq. 0 = not high attachment, 1 = high attachment.

Discussion

Chondrocranial Morphology

Chondrocranial synapomorphies uniting pipoids include: 1) plate-like ethmoidal region, 2) broad commissura quadratocranialis anterior, 3) small processus muscularis palatoquadrati, 4) precocious formation of the lower jaw, and 5) prominent larval crista parotica. In addition, all pipoids described to date, except the highly divergent *Hymenochirus,* share the following chondrocranial synapomorphies: 1) lateral process of the palatoquadrate, 2) suprarostral alae fused to the suprarostral plate, 3) presence of a muscular process of the otic capsule, 4) slender sub-ocular bar of the palatoquadrate, and 5) a high attachment of the palatoquadrate suspensorium.

Hymenochirus larvae are unique. Sokol (1977) noted the unique chondrocranial morphology of *Hymenochirus* larvae and suggested that it was due to adaptation to carnivory on small aquatic insects. Given its small size as an adult (<30mm average SVL) and its placement on the currently proposed phylogeny (Fig. 1) another possible explanation for its uniqueness relates to a miniaturization process in this lineage. Several studies of miniaturization among amphibians (Hanken, 1982, 1983; Hanken and Wake, 1993; Trueb and Alberch, 1985) have suggested three characteristics of

miniaturized taxa. These three characteristics are expressed in the larval chondrocranium of *Hymenochirus boettgeri*. The first trait is reduction and structural simplification of miniaturized taxa. *Hymenochirus* is not only the smallest genus in the family, but also has a reduced number of chondrocranial components (e.g., lack of otic and ascending processes, absence of the subocular bar of the palatoquadrate, loss of two ceratobranchials, etc.). Secondly, miniaturized amphibians are characterized by morphological novelty. This is represented in *Hymenochirus* by free suprarostral alae and the larval septum nasi. The suprarostral cartilages can not be identified as separate cartilages in pipoids (Trueb and Hanken, 1992; Swart and de Sá, in press; Rocek and Vesely, 1989). However, in *Hymenochirus* two small and free cartilages are present at the anterior end of the chondrocranium where suprarostrals would be placed in non-pipoid larvae. These two small cartilages have been previously considered to correspond to the suprarostral alae by Sokol (1977). The larval septum nasi is unique by its absence in other taxa. Finally, miniaturized amphibians are characterized by increased variability. This trait is seen most clearly in the sequence of cranial ossification in *Hymenochirus*. The variation in the appearance of cranial bones in the specimens examined had ranged from three to eleven stages. This variation in ossification is similar

to that reported for the miniaturized urodelan genus *Thorius* (Hanken, 1984).

An alternative explanation for the unique chondrocranial morphology of *Hymenochirus* would be to argue that heterochrony has played a role in the evolution of *Hymenochirus*. Trueb and Hanken (1992) described the development of the palatoquadrate of *Xenopus laevis* from two separate chondrifications, an anterior and a posterior chondrification. Since *Xenopus* is considered basal in the family Pipidae (Fig. 1), the lack of the posterior chondrification of the palatoquadrate in *Hymenochirus* may be explained as a heterochronic event from the ancestral state exhibited in early *Xenopus* larvae.

Two chondrocranial characters shared by *Rhinophrynus* and *Pipa* are worth noting. These characters are the presence of a symplectic cartilage and the processus muscularis capsulae auditivae (PMCA). Sokol (1977) first named and described the PMCA in two pipid species. According to this author, the PMCA is chondrified in *Pipa parva* and it remains unchondrified in *Pipa carvalhoi*. Furthermore, Sokol (1977) stated that the PMCA is absent in *Xenopus tropicalis* and it has not been reported for any other pipoid taxa. Additionally, the symplectic cartilage has only been reported in *Pipa carvalhoi* (this work) and *Rhinophrynus* (Swart and de Sá, in press).

The most parsimonious placement on the current phylogenetic tree would require convergent gain of these two characters in *Rhinophrynus* and *Pipa*.

Development of the Suprarostral Plate

As described before, the chondrocranium of pipoids is characterized by having a suprarostral plate in the ethmoidal region. Rocek and Vesely (1989) argued that the suprarostral plate of pipoids and the cornua trabeculae of non-pipoids are not homologous, i. e., they can not be derived from one another. Subsequently, Trueb and Hanken (1992) while describing the suprarostral plate of *Xenopus* suggested that in pipoids either: 1) the cornua trabeculae fail to develop and an anterior extension of the intertrabecular plate (= planum internasale of Trueb and Hanken) develops in their place or 2) that the suprarostral plate corresponds to the "fusion and simplification" of the cornua trabeculae of non-pipoids.

Actually four other possibilities can be considered: 1) fusion of the cornua trabeculae along their midline, 2) posterior expansion of the suprarostrals with fusion to the ethmoid plate, 3) anterior expansion of the ethmoid plate with fusion to the suprarostrals, or

4) posterior expansion of the suprarostrals and anterior expansion of the ethmoid plate.

The serial cross-sections of early *Xenopus* and *Rhinophrynus* specimens showed a similar pattern of development for the ethmoidal region. Initially, a pair of cartilaginous rods, herein identified as the cornua trabeculae, grow anteriorly from the ethmoid plate. The anterior tips of the cornua trabeculae fuse to the suprarostral cartilages. In addition, a third cartilaginous rod, herein called the anterior process of the ethmoid plate (APE), also grows anteriorly from the ethmoid plate, between the cornua trabeculae, and fuses to the suprarostrals. Later, most of the space between the cornua trabeculae and the APE is occluded by cartilage. This filling creates a continuous cartilaginous plate that includes the cornua trabeculae, the APE, and the suprarostrals forming the suprarostral plate of later larvae. The most anterior part of the intertrabecular space, behind the suprarostrals, does not fill with cartilage and is present in the suprarostral plates of both species as conspicuous.

Serial sections through the rostral region of early specimens of *Hymenochirus boettgeri* show a different pattern of development. First, the anterior process of the ethmoid plate grows anteriorly. Subsequently, the cornua trabeculae grow anteriorly

from the ethmoid plate, but ventral to the APE. During development, the cornua trabeculae grow past the anterior tip of the APE. Finally, cartilage "fills-in" between the anterior tips of the cornua trabeculae forming, a cartilaginous plate. This plate becomes continuous with the APE, except two holes that are left between the cornua trabeculae and the APE.

Development of the ethmoidal region of pipoid frogs has been controversial (Rocek and Vesely, 1989; Trueb and Hanken, 1992). If the differences in chondrocranial structure of pipoid and non-pipoid larvae could not be explained by deviation of an ancestral developmental pattern, then we would have to consider a diphyletic origin of Anura. Evidence supporting the monophyly of Anura is abundant (see review by Trueb and Cloutier, 1991).

Concerning the controversy over the ethmoidal region of pipoids, the first question that needs to be answered is: Do the cornua trabeculae of non-pipoid Anura appear in pipoids? The present study showed that in early stages of *Xenopus*, *Hymenochirus*, and *Rhinophrynus*, rod-like anterior projections of the ethmoid plate (= intertrabecular plate) are present. Although they are small and transitory these structures are considered to be homologous (i.e., in position and origin) with those of non-pipoid larvae.

The second question to be addressed is: Can the suprarostral plate of pipoid larvae be derived from the rostral structures present in the ethmoidal region of non-pipoid larvae? The answer is yes. The serial cross-sections showed that during development, the intertrabecular space fills in with cartilage forming a continuous plate between the cornua trabeculae, the suprarostrals, and the anterior process of the ethmoid plate.

Answering the previous two questions showed that: First, the ethmoidal region of pipoids and non-pipoids are homologous. This data refutes Rocek and Vesely's (1989) argument for a diphyletic origin of Anura. Second, the cornua trabeculae do form in pipoids and the suprarostral plate develops not from a single fusion and simplification of the cornua trabeculae, as suggested by Trueb and Hanken (1992), but from a complex fusion of the cornua trabeculae, anterior process of the ethmoid plate, and suprarostrals, complemented by the appearance of new cartilage between these elements.

The anterior process of the ethmoid plate is homologous to Sokol's description of a prenasal process in *Rhinophrynus* (Sokol, 1975). A prenasal process has also been reported for *Pelodytes punctatus* (Sokol, 1977) and *Pipa pipa* (Rocek and Vesely, 1989). Herein this structure is called the anterior process of the ethmoid

plate because it forms very early in development as an anterior process of the ethmoid plate, when the cartilages of the nasal capsules are not yet formed. In pipoids, this process seems to be enlarged and it will provide support to the septum nasi. Interestingly, a prenasal process has been reported for Urodela and its absence in Anura has been considered a major difference between Anura, Urodela, and Gymnophiona (de Beer, 1937).

Once the homology of the ethmoidal region of pipoids and non-pipoids has been explained, a third question needs to be addressed: How can the different developmental patterns within Pipoidea be explained? Herein, I propose that the cornua trabeculae and the anterior process of the ethmoid plate (prenasal process of Sokol) are homologous in *Rhinophrynus*, *Xenopus*, and *Hymenochirus*. The only difference is that the cornua trabeculae of *H. boettgeri* are not flanking but they are ventral to the APE. This developmental rearrangement is likely the result of the process of miniaturization in *Hymenochirus*, which together with the predacious habit of these larvae, resulted in a shift of the cornua trabeculae to a ventral position relative to the APE.

Internal oral morphology of Pipoid frogs

The larvae of pipoids is characterized by an overall simplification of oral structures, e.g., papillae, ridges, pustulations, etc. Wassersug (1977) suggested that this reduced complexity might be correlated with the filtering mechanism involving dual branchial siphons in these taxa.

Rhinophrynus is the only anuran with a posteriorly attached tongue and no other pipid adult (or larvae) has a tongue, including adult *Hymenochirus*. The tongue-like structure in larval *Hymenochirus* is undoubtedly not homologous to that of adult anurans. Cross-sectioned material indicates that it is composed of the median copula of the hyobranchial apparatus and lacks musculature. This structure was noted by Sokol, (1977) but its function or adaptive purpose has not been pursued. The "teeth patches" are also unique to *Hymenochirus* among pipids. They were briefly noted by Wassersug, (1980), but their importance as phylogenetic characters or their possible homologies have not been investigated. Cross-sections and SEM indicate they are composed of individually keratinized cells that are each shaped like a spade (Fig. 19). Further work needs to be done to determine whether they are truly keratinized but they are enucleate and are birefringent under

polarized light. They may be correlated with the carnivorous habit of *Hymenochirus* and function in crushing the prey.

One notable oral character shared in the group is the ridge present in the dorsal roof of *Rhinophrynus dorsalis* and *Pipa carvalhoi*. Although Wassersug (1981) fails to note this character in his description of *Rhinophrynus*, it was distinctly present in this study. Alternatively this ridge may be an artifact of preparation.

The four genera examined have numerous autapomorphies associated with its internal oral morphology. In the phylogenetic analysis I have coded three characters that seem to be useful among the four taxa that I have studied. The characters that I consider to be phylogenetically informative are: papilla surrounding the glottis (present in *Xenopus* and *Pipa*), dorsal pressure cushions (present in *Rhinophrynus* and *Xenopus*), and a thick in-curved upper lip (present in *Rhinophrynus* and *Hymenochirus*).

Phylogenetic Applications

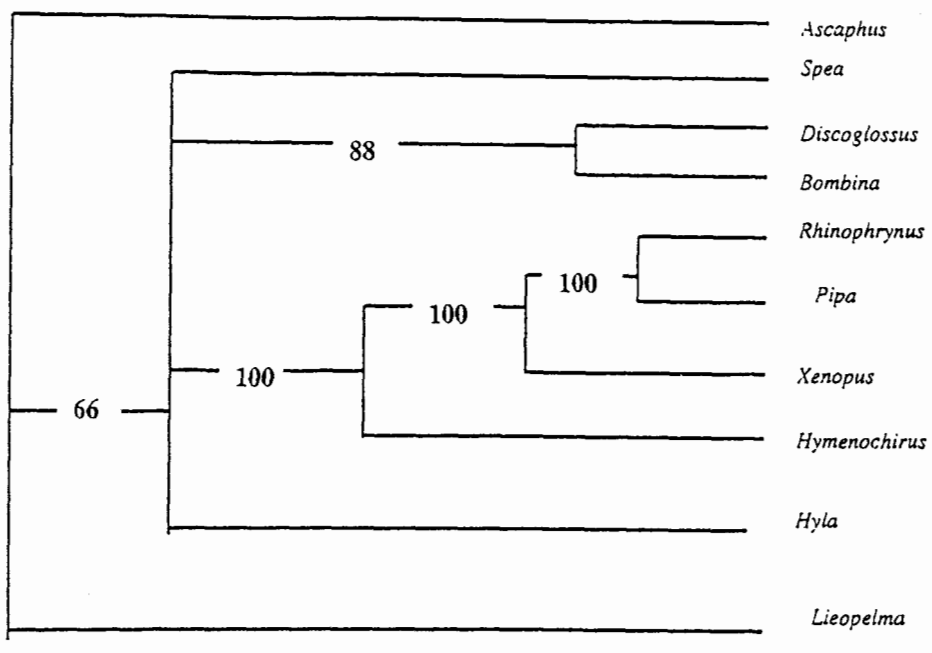
Among pipoids, comparable chondrocranial data are now available for *Rhinophrynus dorsalis, Xenopus laevis, Pipa carvalhoi,* and *Hymenochirus boettgeri.*

The most recent review of the phylogenetic relationships among the Anura is that of Ford and Cannatella, 1993. According to this phylogeny there are six monophyletic lineages of anurans: *Ascaphus truei, Leiopelma*, Bombinatoridae, Discoglossidae, Mesobatrachia (including Pelobatoidea and Pipoidea) and Neobatrachia. *Ascaphus truei* is considered the most basal extant anuran and is the sister to all other anurans. Chondrocranial data are now available for representatives of each of the six groups and internal oral anatomy descriptions are available for representatives of five of the six groups, except *Lieopelma* (Reiss, 1997; Stephenson, 1951; Maglia and Púgener, in press; Weins, 1989; Púgener and Maglia, 1997; Wassersug, 1981). Based on this phylogeny *Ascaphus truei* and *Lieopelma* are used here as outgroups for phylogenetic analyses.

The first phylogenetic analysis, based on twenty-two characters identified in this work, produced eight equally parsimonious trees (C.I. = 0.68, tree length = 25). The 50% majority-rule consensus tree is shown in Figure 24. In this analysis all eight trees failed to yield a monophyletic Pipidae. Monophyly of the Pipidae is well supported (Ford and Cannatella, 1993).

In order to understand the possible evolution of chondrocranial morphology, a second analysis was performed. In this analysis,

A.



B.

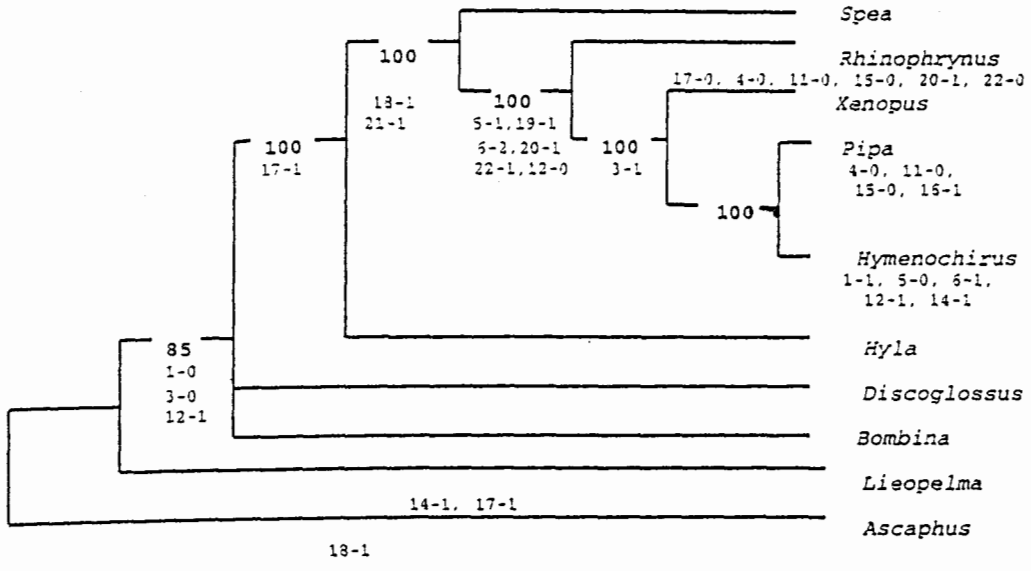


Fig. 24. A. 50% Majority-rule consensus of 8 trees based on the unconstrained data matrix. B. 50% Majority-rule consensus of 2 trees produced by the constrained topology of Ford and Cannatella (1993). Numbers in bold are bootstrap values. Numbers below each branch indicate character states at that branch.

topological constraints that agree with Ford and Cannatella's (1993) phylogeny were enforced during the search. This resulted in two equally parsimonious trees (Fig. 25, C.I. = 0.57, tree length = 30 steps). The overall difference between the two trees is the relationship of *Discoglossus* and Bombinatoridae.

Below I discuss the possible evolutionary pathways of chondrocranial characters under the topological relationships of anurans presented in Ford and Cannatella's phylogeny.

The absence of the muscular process (character 1) represents the plesiomorphic state for anurans. The presence of the muscular process is the derived condition. The absence of the muscular process in *Hymenochirus* represents a secondary loss in this lineage.

The presence of the larval otic process (character 12) is plesiomorphic for anurans. This character is present in the outgroups but is lost in the common lineage of all other anurans. The otic process is regained in Pipoidea (*Rhinophrynus* and Pipidae) and is uniquely lost in *Hymenochirus*.

Having a thin subocular bar of the palatoquadrate (3) is the plesiomorphic state present in *Ascaphus* and *Lieopelma*, and is convergent with the thin subocular bar in *Xenopus* and *Pipa*. A subocular bar is absent in *Hymenochirus*.

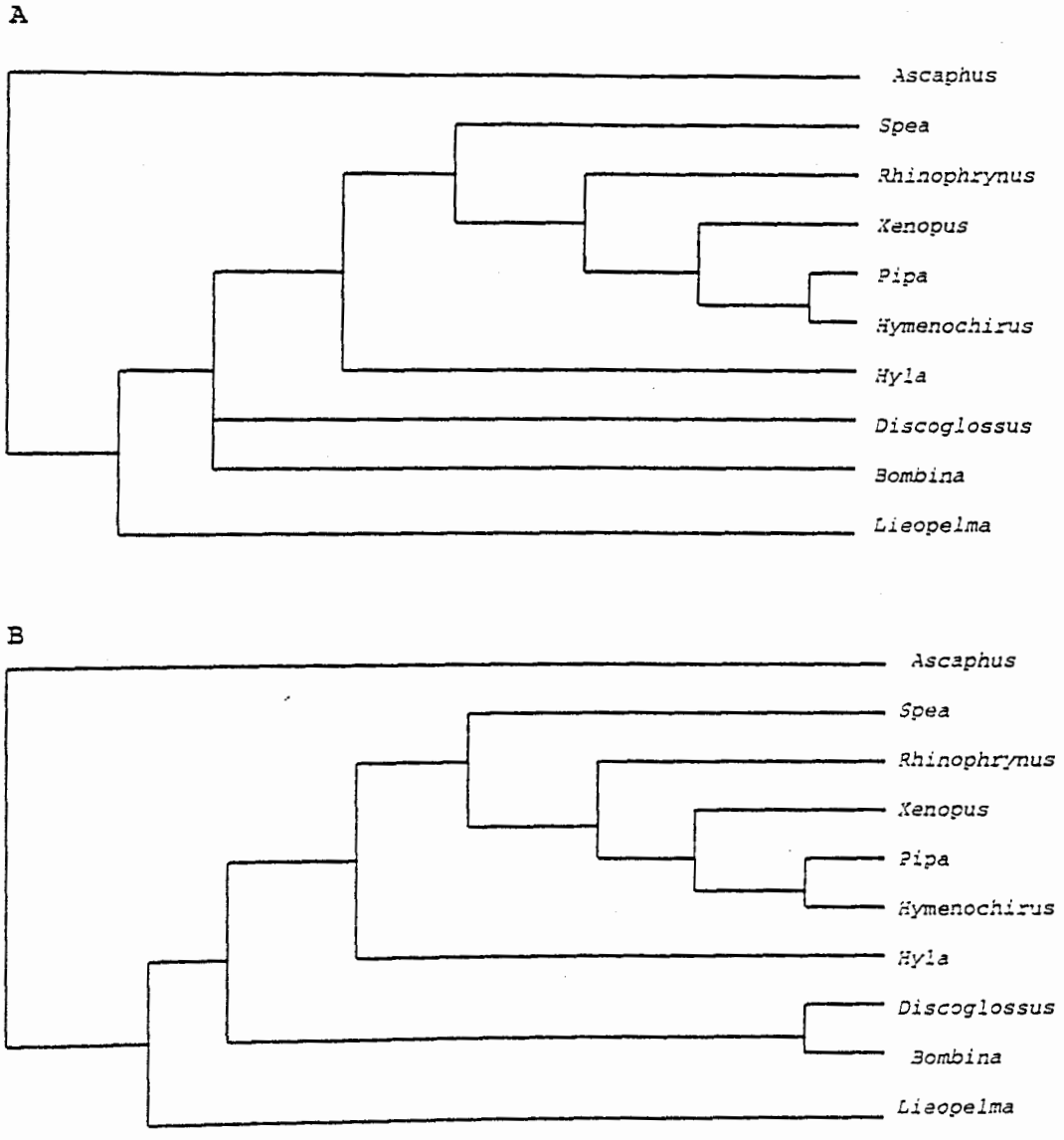


Figure 25. Two most parsimonious trees produced using the constrained topology of Ford and Cannatella (1993). C.I. = 0.57, Tree length = 30 steps.

Having independent suprarostral cartilages (character 6, i. e. not fused to the cornua trabeculae) is plesiomorphic for anurans. Integration of the suprarostrals into a suprarostral plate is a synapomorphy for pipoids. Presence of a suprarostral plate and separate suprarostral alae is uniquely derived for *Hymenchirus*.

Fusion of the posterior edge of the ascending process with the anterior edge of the larval otic process (character 8) is an autapomorphy for *Pipa*.

A greatly expanded ceratobranchial III (character 17) is plesiomorphic for anurans and is independently lost in *Lieopelma* and the common lineage leading to the Neobatrachia and Mesobatrachia. This character is a reversion to the ancestral state in *Rhinophrynus*.

The presence of a well-developed taenia tecti marginalis (18) could be either the plesiomorphic state for anurans independently lost in Ascaphus and Mesobatrachia, or derived after the divergence of *Ascaphus* and secondarily lost in the Mesobatrachia.

The following characters: having a wide commissura quadratocranialis anterior (19), early fusion of the lower jaw elements (20), high attachment of the ascending process (22), and presence of a lateral process of the palatoquadrate (5), are

synapomorphies for Pipoidea. The lateral process of the palatoquadrate is secondarily lost in *Hymenochirus*.

A larval crista parotica (character 21) is present and represents a synapomorphy of the Mesobatrachia.

Five characters: having a processus muscularis capsulae auditivae (4), presence of a symplectic cartilage (9), having ceratohyalia broader than the branchial basket (11), papillae surrounding the glottis (15), and possessing a buccal roof ridge (16) are primitively absent in anurans and convergent in *Rhinophrynus* and *Pipa*.

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Table. 1. List of stages and measurements of specimens of *Hymenochirus boettgeri*, *Rhinophrynus dorsalis* and *Xenopus laevis* used in this work. BL = body length, TL = total length, SEM = specimen dissected and prepared for scanning electron microscopy, E-G = Plastic embedded specimen, P-Xsect = paraffin sectioned specimen, C&S = cleared and stained.

Hymenochirus boettgeri

Number	Stage	BL	TL	Status
H045	35	1.03	2.11	E-G
H046	E38	1.06	2.89	E-G
H047	L38	0.96	2.56	E-G
H048	41	1.04	3.23	E-G
H001	49	3.21	9.43	C&S
H076	49	4.15	10.49	P-Xsect
H002	50	3.54	7.80	C&s
H027	50	3.44	10.03	C&S
H003	51	3.42	10.31	C&S
H004	51	3.93	10.90	C&S
H019	51	3.48	9.93	C&S
H028	51	3.54	10.82	C&S
H029	51	3.36	10.16	C&S
H020	52	4.03	10.44	C&S
H005	52	4.53	10.60	C&S
H006	52	4.09	11.55	C&S
H007	53	4.80	12.38	C&S
H018	53	4.48	11.69	P-Xsect
H026	53	3.73	11.65	C&S
H030	53	4.67	10.99	C&S
H008	54	4.27	10.81	C&S
H021	54	4.27	10.66	C&S
H031	54	3.62	11.00	C&S
H075	54	4.78	14.27	P-Xsect
H009	55	4.32	13.69	C&S
H032	55	5.15	15.10	C&S
H010	56	6.21	15.63	C&S
H033	56	5.54	14.05	C&S
H011	57	5.53	16.37	C&S

Number	Stage	BL	TL	Status
H022	57	4.85	14.25	C&S
H012	58	5.81	15.89	C&S
H023	58	5.29	14.24	C&S
H034	58	5.49	15.35	C&S
H035	58	6.02	17.17	C&S
H036	58	5.29	14.67	C&S
H077	58	6.75	17.62	P-Xsect
H013	59	7.76	19.90	C&S
H037	59	7.15	17.80	C&S
H038	59	7.34	18.19	C&S
H039	59	6.92	18.04	C&S
H014	60	8.33	18.87	C&S
H040	60	8.22	20.78	C&S
H015	61	8.59	20.39	C&S
H016	62	8.95	19.49	C&S
H017	63	9.51	14.61	C&S
H041	63	8.70	13.20	C&S
H042	63	8.89	16.14	C&S
H043	63	8.77	13.55	C&S
H024	64	8.41	12.55	C&S
H070	64	8.87	11.65	C&S
H025	65	10.11	10.58	C&S
H071	65	---	9.62	C&S
H073	65	12.32	12.40	C&S
H072	66	---	9.85	C&S
H074	66	--	9.80	C&S
H069	66+	---	11.88	C&S
H044	Adult	---	19.83	C&S

Xenopus laevis

	stage	BL	TL	Status
X1	41	2.75	6.14	E-G
X2	E43	2.12	6.03	E-G
X3	L43	2.08	6.40	E-G

Hymenochirus boettgeri - SEM
H049 St. 50 BL = 3.50, TL = 9.85
H050 St. 52 BL = 3.57, TL = 9.86
H051 St. 58 BL = 4.44, TL = 12.02

Rhinophrynus dorsalis -SEM
USNM 515953 St. 52 BL = 13.58 TL = 33.64

Table 2. Specimens of *Rhinophrynus dorsalis*. Measurements are in mm.

Specimen	Stage	Body Length	Total Length	
USNM 515955		53	14.28	36.03
USNM 515952		52	14.79	34.60
USNM 515956		53	15.05	35.40
USNM 515953		52	13.58	33.64
USNM 515957		late 53	15.35	37.72
USNM 515958		53	15.16	36.54
USNM 515954		53	14.73	36.54
USNM 515945		Late 54	19.63	37.04
USNM 515946		54	22.20	49.54
USNM 515947		54	18.57	44.43
USNM 515948		Late 54	19.44	46.30
USNM 515949		Late 54	19.61	48.53
USNM 515950		55	19.43	49.20
USNM 515951		Late 54	19.05	46.14
USNM 515959		54	16.55	36.39
USNM 515960		51	12.37	26.17
USNM 515961		45	5.58	13.67
USNM 515962		51	9.46	20.07
USNM 515963		51	10.17	17.98
USNM 515964		51	11.36	19.74
USNM 515965		52	11.09	20.33
USNM 515966		50	10.14	21.21
USNM 515967		50	10.21	18.19
USNM 515968		48	6.47	14.50
USNM 515969		51	10.42	19.07
USNM 515970		51	10.13	21.43
USNM 515971		51	10.05	20.21
USNM 515972		45	5.23	11.04
USNM 515973		49	6.40	14.15
USNM 515974		45	4.95	10.02

Table 3. Stages and measurements of specimens of *Pipa carvalhoi* used in this study. C&S = cleared and stained, SEM = dissected and prepared for scanning electron microscopy.

Specimen	Stage	Total length	Body length	Status
1	57	60.27	20.88	C&S
2	56	55.40	20.23	C&S
3	55	54.29	19.22	C&S
4	55	54.08	19.55	C&S
5	56	58.35	20.60	C&S
6	56	62.48	20.36	C&S
7	59	69.58	24.73	C&S
8	61	55.69	22.50	C&S
9	62	56.93	21.86	C&S
10	60	62.58	23.36	C&S
11	63	39.37	22.16	C&S
12	58	66.67	23.58	C&S
13	56	39.40	13.44	C&S
14	59	64.61	23.93	SEM

Table 5. Data matrix for phylogenetic analysis.

	Characters																					
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ascaphus	1	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Spea	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0	1	0
Discoglossus	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Bombina	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Lieopelma	1	0	1	1	0	0	0	0	0	0	1	0	0	1	N	N	1	0	0	0	0	0
Rhinophrynus	0	0	0	0	1	2	0	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1
Xenopus	0	0	1	1	1	2	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1	1
Pipa	0	0	1	0	1	2	0	1	2	0	0	0	0	0	0	1	1	1	1	1	1	1
Hymenochirus	1	1	N	1	0	1	1	0	0	0	1	1	1	1	1	0	N	1	1	1	1	1
Hyla	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0

Table 4. Ossification sequence of *Hymenochirus boettgeri* (dashes indicate all specimens have the bone, 66+ indicates one month post metamorphosis).

Appearance of Bone	Developmental stage (Neiuwkoop and Faber 1956)																			
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	66+	
Frontoparietal	1/1	1/2	3/5	1/3	2/3	2/3	1/2	---	---	---	---	---	---	---	---	---	---	---	---	---
Angulosplenial	1/1	1/2	2/5	1/3	1/3	1/3	1/2	1/2	2/3	4/5	2/3	---	---	---	---	1/4	---	---	---	---
Exoccipital			2/5	1/3	1/3	1/3	1/2	2/2	3/3	5/5	3/3	---	---	---	---	---	---	---	---	---
Parasphenoid								1/2	2/3	1/5	1/3	2/2	---	---	---	---	---	---	---	---
Maxilla													1/3	1/5	1/4	0/2	2/3	2/2	---	
Nasal															1/4	0/2	1/3	2/2	---	
Sphenethmoid															2/4	1/2	3/3	2/2	---	
Squamosal																	2/3	2/2	---	
Prootic																	1/3	2/2	---	
Dentary																	1/3	2/2	---	
Pterygoid																	1/3	2/2	---	
Tympanic annulus																	1/3	1/2	1/1	
Septomaxilla																		2/2	---	
Premaxilla																		2/2	1/1	
Collumela																		1/2	1/1	
Pars externa plectri																			1/1	
Vomer																				

VITA

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