

The ontogeny of the olfactory system in ceratophryid frogs (Anura, Ceratophryidae)

Silvia I. Quinzio¹  | John O. Reiss²

¹Instituto de Bio y GeoCiencias del NOA (IBIGEO), Centro Científico Tecnológico CONICET- Salta. 9 de Julio 14. 4405. Rosario de Lerma, Salta, Argentina

²Department of Biological Sciences, Humboldt State University, Arcata, California

Correspondence

Silvia I. Quinzio, 9 de Julio 14, Rosario de Lerma, CP4406, Salta, República Argentina. Email: squinzio@conicet.gov.ar

Funding information

This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica PICT 2718 to S. Quinzio, PICT 510 and by a sabbatical leave granted to J. Reiss by Humboldt State University.

Abstract

The aquatic-to-terrestrial shift in the life cycle of most anurans suggests that the differences between the larval and adult morphology of the nose are required for sensory function in two media with different physical characteristics. However, a better controlled test of specialization to medium is to compare adult stages of terrestrial frogs with those that remain fully aquatic as adults. The Ceratophryidae is a monophyletic group of neotropical frogs whose diversification from a common terrestrial ancestor gave rise to both terrestrial (*Ceratophrys*, *Chacophrys*) and aquatic (*Lepidobatrachus*) adults. So, ceratophryids represent an excellent model to analyze the morphology and possible changes related to a secondary aquatic life. We describe the histomorphology of the nose during the ontogeny of the Ceratophryidae, paying particular attention to the condition in adult stages of the recessus olfactorius (a small area of olfactory epithelium that appears to be used for aquatic olfaction) and the eminentia olfactoria (a raised ridge on the floor of the principal cavity correlated with terrestrial olfaction). The species examined (*Ceratophrys cranwelli*, *Chacophrys pierottii*, *Lepidobatrachus laevis*, and *L. llanensis*) share a common larval olfactory organ composed by the principal cavity, the vomeronasal organ and the lateral appendix. At postmetamorphic stages, ceratophryids present a common morphology of the nose with the principal, middle, and inferior cavities with characteristics similar to other neobatrachians at the end of metamorphosis. However, in advanced adult stages, *Lepidobatrachus laevis* presents a recessus olfactorius with a heightened (peramorphic) development and a rudimentary (paedomorphic) eminentia olfactoria. Thus, the adult nose in *Lepidobatrachus laevis* arises from a common developmental 'terrestrial' pathway up to postmetamorphic stages, when its ontogeny leads to a distinctive morphology related to the evolutionarily derived, secondarily aquatic life of adults of this lineage.

KEYWORDS

anurans, *Lepidobatrachus*, nose, recessus olfactorius, secondary aquatic life

1 | INTRODUCTION

We examine the larval and adult morphology of the peripheral olfactory system in a clade of South American frogs, a clade that includes genera with terrestrial adults (*Ceratophrys* and *Chacophrys*) and with secondarily aquatic adults (*Lepidobatrachus*). The primary question we ask is whether this major difference in lifestyle is correlated with morphological changes in the peripheral olfactory system, and if so, which? We also seek to determine the overall pattern of olfactory ontogeny in the species of the clade, and how it differs among them.

Although direct development has evolved at least ten times independently among the anurans (Heinicke et al., 2009), the majority of

anuran species are characterized by a biphasic life cycle. In such life cycles, there are larval and adult phases, distinct in their morphology, physiology, and ecology, and united in ontogeny by the set of cellular and systemic changes that constitute metamorphosis (Handrigan & Wassersug, 2007). The morphological changes that take place during metamorphosis are generally related to three other changes: a change in habitat, a change in locomotion, and a change in the feeding mechanism (Reiss, 2002). In particular, for most anurans, metamorphosis involves the transformation from a swimming, microphagous, aquatic tadpole to a walking or hopping, carnivorous, terrestrial adult.

The change from water to air as a medium for sensation can require significant morphological changes for sensory systems, even

while there is often continuity of structural features between larval and adult body plans (e.g., Benzekri & Reiss, 2012; Hansen, Reiss, Gentry, & Burd, 1998; Hoskins, 1990; Witschi, 1949; see also Thewissen & Nummela, 2008). In this respect, anurans represent an outstanding model for studying the morphological and functional specializations of sensory systems in two different but continuous body plans, larval and adult, and the transformations that define the transition from one to another as part of normal development during metamorphosis.

Like other sensory systems, the olfactory system changes greatly during metamorphosis, and widely different larval and adult morphologies have been characterized in many anuran species (reviewed by Reiss & Eisthen, 2008). In the larval olfactory organ, the generalized morphology involves paired nasal organs, formed by two nasal sacs, which open to the exterior by the nares, and to the buccal cavity by the choanae. Each nasal sac consists of three cavities containing sensory epithelium (Figure 1a): a principal cavity, containing the main olfactory epithelium, a vomeronasal organ, containing vomeronasal epithelium, and a small lateral appendix, budding off the principal cavity. The principal cavity is divided into a dorsal region and a ventral region by the presence of a fold and groove along its lateral wall (crista ventralis and lateral groove). The olfactory epithelium is a pseudostratified columnar sensory epithelium. The external nares lead to the principal cavity through the entrance canal, which is lined with a nonsensory epithelium, as is the lateral wall of the principal cavity. The vomeronasal organ opens into the dorsomedial part of the anterior principal cavity; it consists of a sensory epithelium of variable thickness among species. This general morphology of the nose is found in a wide variety of anuran larvae (Jermakowicz et al., 2004; Jungblut, Pozzi, & Paz, 2011, 2012; Jungblut, Reiss, Paz, & Pozzi, 2017; Khalil, 1978; Rowedder, 1937; Tsui, 1946; Watanabe, 1936; Yvrou, 1966), although notable deviations are found in the ascaphid *Ascaphus truei* (Benzekri & Reiss, 2012; Pusey, 1943), the pipid *Xenopus laevis* (Föske, 1934; Paterson, 1939), and the microhylid *Kaloula borealis* (Tsui & Pan, 1946).

The anatomy of the adult anuran olfactory system has been studied extensively and a large number of morphological comparisons of this system are available (e.g., for some recent studies see Benzekri & Reiss, 2012; Jermakowicz et al., 2004; Junk, Wenzel, Vences, & Nowack, 2014; Královec, Zakova, & Muzakova, 2013). As in larvae, the general morphology of the adult olfactory system involves an organization into three functional cavities (Figure 1b): principal, middle, and inferior (Gaupp, 1904; Helling, 1938; Jurgens, 1971). The principal cavity contains the main olfactory epithelium, while the inferior cavity contains the vomeronasal epithelium. In most anurans, the middle cavity does not contain sensory epithelium. This organization is common to most anurans in which the metamorphic transformation of the nose is associated with the water-to-land transition. Pipid frogs, which are entirely aquatic as adults, constitute a remarkable exception to this generalized morphology; they have an accessory sensory cavity, proposed to be a morphological specialization for aquatic olfaction, related to their secondarily aquatic adult habits (Föske, 1934; Meyer, Fackler, Jadhao, D'Aniello, & Kicliter, 1997; Paterson, 1951; Reiss & Eisthen, 2008; Trahms, 1936).

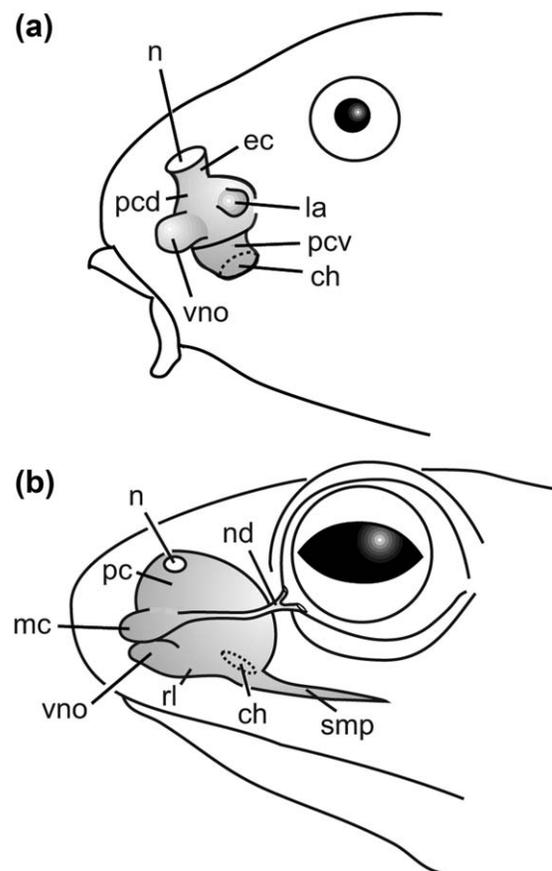


FIGURE 1 Schematic illustration of generalized morphology of nasal cavity of anurans. (a) Larval olfactory organ. (b) Adult olfactory organ. Abbreviations: ch, choana; ec, entrance canal; la, lateral appendix; mc, middle cavity; n, nares; nd, nasolacrimal duct; pc, principal cavity, pcd, dorsal part of principal cavity, pcv, ventral part of principal cavity, rl, lateral recess (of inferior cavity); smp, sulcus maxillopalatinus; vno, vomeronasal organ (= medial recess of inferior cavity)

Of course, there is a wide variety of degree of terrestriality among adult anurans, ranging from species that never enter water to those that are highly aquatic. From a broad survey of olfactory structure in anuran adults, Helling (1938) identified two features that seemed to be correlated with the degree of terrestriality of the species. One was the size of a small, distinct region of olfactory epithelium, devoid of Bowman's glands, in the anterolateral floor of the principal cavity, which he called the recessus olfactorius. He found the recessus olfactorius to be much larger in highly aquatic species, such as *Telmatobius hauthali*, and further suggested that this region is homologous to the accessory sensory cavity found only in pipids. Conversely, he found that the degree of development of the eminentia olfactoria, a raised ridge on the floor of the principal cavity, seemed to correlate with terrestriality—with highly aquatic species often (although not always) lacking an eminentia, and xeric-adapted and fossorial taxa having a highly developed eminentia. Others, both before and since (e.g., Jurgens, 1971; Ramaswami, 1934; de Villiers, 1933) have also suggested a relationship of the eminentia with terrestriality (but see also Ramaswami, 1939). Recent work on the semiaquatic, basal anuran *Ascaphus truei* (Benzekri & Reiss,

2012) supports both these correlations: in *Ascaphus* the entire anterior end of the principal cavity has been taken over by an expanded recessus olfactorius, and there is no eminentia developed.

To better understand how the adult morphology of the anuran nose can be specialized to either a terrestrial or aquatic existence, we wanted to investigate the structure of the nose in a closely related group in which some species have highly terrestrial adults, others highly aquatic. Comparing the ontogeny of the olfactory system in a monophyletic clade with varying modes of adult life, allows us to identify whether the morphology of the nose diverges in accordance with the adult mode of life, or if it is phylogenetically conserved within the clade.

The South American frogs of the genera *Ceratophrys* Wied-Neuwied 1824, *Lepidobatrachus* Budgett 1899, and *Chacophrys* Reig and Limeses (1963), compose the monophyletic clade Ceratophryidae (Frost et al., 2006). The diversification of these frogs in semiarid regions of the South American Chaco has resulted in adults with distinctive lifestyles. In particular, the eight species of *Ceratophrys* and *Chacophrys pierottii* (monotypic) have terrestrial adults, while all three species of *Lepidobatrachus* have aquatic adults. Even though the monophyly of this clade has been well-supported by many morphological features (Fabrezi, 2006; Fabrezi & Quinzio, 2008; Frost et al., 2006; among others), these frogs exhibit marked morphological variation in their ontogenies. While the larvae of *Chacophrys* are generalized Orton (1953) type IV larvae with keratinized mouthparts that allow them to scrape food from the substrate (Faivovich & Carrizo, 1992; Quinzio, Fabrezi, & Faivovich, 2006), the larvae of *Ceratophrys* and *Lepidobatrachus* show a great reduction in oral structures (e.g., papillation of the roof and floor of the mouth, branchial filtration system) correlated with their carnivorous diet: macropagous in *Ceratophrys* and megalophagous in *Lepidobatrachus*. *Ceratophrys* also shows a strong development of mandibular and hyoid muscles, and *Lepidobatrachus* a mandibular configuration similar to that at the middle of metamorphosis in most frogs (Fabrezi, 2011; Fabrezi et al., 2016; Wassersug & Heyer, 1988). Many other unique larval morphological features distinguish the tadpole of *Lepidobatrachus* spp. (Bloom, Ledon-Rettig, Infante, Everly, & Hanken, 2013; Fabrezi, 2011; Fabrezi & Quinzio, 2008; Quinzio & Fabrezi, 2012; Quinzio et al., 2006) and this allowed the definition of a new larval type, Larval Type V, for this genus (Quinzio & Fabrezi, 2014).

The adult synapomorphies that support the Ceratophryidae include a number of derived characters that can be interpreted as morphological specializations to terrestrial and fossorial life. All three genera have exostotic (sculptured and hyperosified) skulls (Fabrezi, 2006; Wild, 1997) and keratinized metatarsal tubercles adapted to digging, and there are bony dorsal shields in species of both *Ceratophrys* and *Lepidobatrachus* (Fabrezi, 2006; Lynch, 1982; Quinzio & Fabrezi, 2012). In *Lepidobatrachus*, these features are integrated with others that appear related to an aquatic life: some examples include the complete webbing of the feet, a hyoglossal apparatus and associated musculature modified for feeding underwater (Fabrezi & Lobo, 2009) and the

postmetamorphic presence of the lateral line system (Fritsch, Drewes, & Ruibal, 1987; Quinzio & Fabrezi, 2014).

Hypotheses of intergeneric relationships in the group have varied greatly (Fabrezi, 2006; Fabrezi & Quinzio, 2008; Faivovich et al., 2014; Frost et al., 2006; Laurent, 1986; Maxson & Ruibal, 1988; Pyron & Wiens, 2011; Reig & Limeses, 1963). However, either of the most recent hypotheses—that *Chacophrys* is the sister group of *Lepidobatrachus*, with *Ceratophrys* the basal group (Castroviejo-Fisher et al., 2015; Fabrezi, 2006; Faivovich et al., 2014), or that *Chacophrys* is the basal group (Fabrezi & Quinzio, 2008)—is consistent with a secondarily aquatic lifestyle in adult *Lepidobatrachus*. This hypothesis is also in accord with the retained presence of presumed terrestrial features in the genus. Thus, this monophyletic group of frogs constitutes an excellent model system to analyze and interpret the morphology, physiology, and ecology of sensory systems in the evolution of complex life cycles.

In this article, we describe the morphology of the olfactory system in larval, juvenile, and adult ceratophryids to: (1) characterize morphological variation among ceratophryid ontogenies, (2) interpret this variation in relation to the adult life styles in the different genera and, (3) provide additional data that will be useful in interpreting the morphological and phylogenetic evolution of the group.

2 | MATERIALS AND METHODS

We studied the histology and morphology of the olfactory system in larval specimens (from Stages 32 to 38 of Gosner, 1960), midmetamorphic specimens, postmetamorphic specimens and adults of *Chacophrys pierottii*, *Ceratophrys cranwelli*, *Lepidobatrachus laevis*, and *L. llanensis*. Investigations involved three specimens of larval stages, one to two specimens for postmetamorphic stages, and one to two adult specimen of each species (except *Chacophrys*, in which only juvenils were analyzed) (Table 1). Specimens utilized belong to the Herpetological Collection of the Instituto de Bio y GeoCiencias, Salta, Argentina; after initial fixation in 10% neutral-buffered formalin, larvae were kept in 10% neutral-buffered formalin and adults in 70% alcohol.

Larval series were staged following the standard table of Gosner (1960), supplemented by Fabrezi and Quinzio (2008) for advanced larval stages of *Lepidobatrachus* spp. The nasal region was cut from the preserved specimens under a dissecting microscope; those of adults were decalcified in 5% formic acid. The pieces were dehydrated in a graduated series of ethanol and embedded in paraffin. Serial histological sections of 10 microns were cut using a rotary microtome and stained with hematoxylin and eosin using classical histological techniques. Descriptions and illustrations of the olfactory system were made with a Nikon-SMZ1000 stereo dissection microscope equipped with a Nikon Coolpix digital camera. Terminology for larval morphology largely follows Rowedder (1937) and Yvroud (1966), and for adult morphology Helling (1938) and Parsons (1967). Olfactory epithelium was distinguished from nonsensory (“respiratory”) epithelium by its characteristic columnar, pseudostratified appearance.

All aspects of the research complied with protocols approved by the Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno

TABLE 1 List of specimens analyzed in this study

Species	Stage/Size	Collection number ^a
<i>Ceratophrys cranwelli</i>	Larva Stage 34	IBIGEO A- 412
	Larva Stage 36/55 mm TL, 22 mm SVL	IBIGEO A- 1601
	Larva Stage 37/72 mm TL, 30 mm SVL	IBIGEO A- 1601
	Juvenile/30 mm SVL	IBIGEO A- 1022
	Juvenile/35 mm SVL	IBIGEO A- 1022
	Adult female/82 mm SVL	IBIGEO A- 188
<i>Chacophrys pierottii</i>	Larva Stage 35	IBIGEO A- 1027
	Larva Stage 38/68 mm TL, 33 mm SVL	IBIGEO A- 1029
	Larva Stage 38/87 mm TL, 42 mm SVL	IBIGEO A- 1029
	Juvenile/26 mm SVL	IBIGEO A- 1602
	Juvenile/28 mm SVL	IBIGEO A- 1603
<i>Lepidobatrachus laevis</i>	Larva Stage 34/51 mm TL, 22 mm SVL	IBIGEO A- 567
	Larva Stage 37/71 mm TL, 30 mm SVL	IBIGEO A- 567
	Larva Stage 38/76 mm TL, 32 mm SVL	IBIGEO A- 567
	Larva Stage 44/64 mm TL, 43 mm SVL	IBIGEO A- 567
	Juvenile/40 mm SVL	IBIGEO A- 979
	Juvenile/60 mm SVL	IBIGEO A- 1604
	Adult/103 mm SVL	IBIGEO A- 1806
	Adult/77 mm SVL	IBIGEO A- 1807
	Adult/85 mm SVL	IBIGEO A- 815
<i>Lepidobatrachus llanensis</i>	Larva Stage 33/47 mm TL, 20 mm SVL	IBIGEO A- 567
	Larva Stage 36/49 mm TL, 21 mm SVL	IBIGEO A- 567
	Larva Stage 38/52 mm TL, 24 mm SVL	IBIGEO A- 567
	Juvenile/52 mm SVL	IBIGEO A- 1605
	Juvenile/55 mm SVL	IBIGEO A- 1606
	Adult/69 mm SVL	IBIGEO A- 1805
	Adult/76 mm SVL	IBIGEO A- 081

^aIBIGEO = Instituto de Bio y GeoCiencias, CONICET, Salta, República Argentina.

de la Provincia de Salta, Argentina and adhered to the legal requirements of Argentine laws. Specimen collection permits were also issued by Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno de la Provincia de Salta, Argentina.

3 | RESULTS

3.1 | Ontogeny of the olfactory system

3.1.1 | Larval morphology

Chacophrys pierottii

As in the general condition, the principal cavity of *Chacophrys* is divided into dorsal and ventral regions by a crista ventralis and lateral groove (Figure 2c). The olfactory epithelium begins anteriorly as a small pouch

in the roof of the mouth (Figure 2a), which extends caudally until the opening of the choana, where it is incorporated into the principal cavity and isolated from the oral cavity by the presence of the medial choanal valve (Figure 2b). In this region, the olfactory epithelium is divided into dorsal (dorsal olfactory epithelium) and ventral (ventral olfactory epithelium) sections in the corresponding parts of the principal cavity, separated by a nonsensory band of epithelium. The choana opens toward the buccal cavity in the anterior region of the ventral principal cavity, and is guarded by the lateral and medial choanal valves. The vomeronasal organ is a kidney-shaped structure located dorsolateral to the ethmoid plate and opening into the dorsal principal cavity, and its associated vomeronasal glands are present and well-developed (Figure 2a, b). In the middle region of the principal cavity, where it connects the external medium with the oral cavity through the nares and the

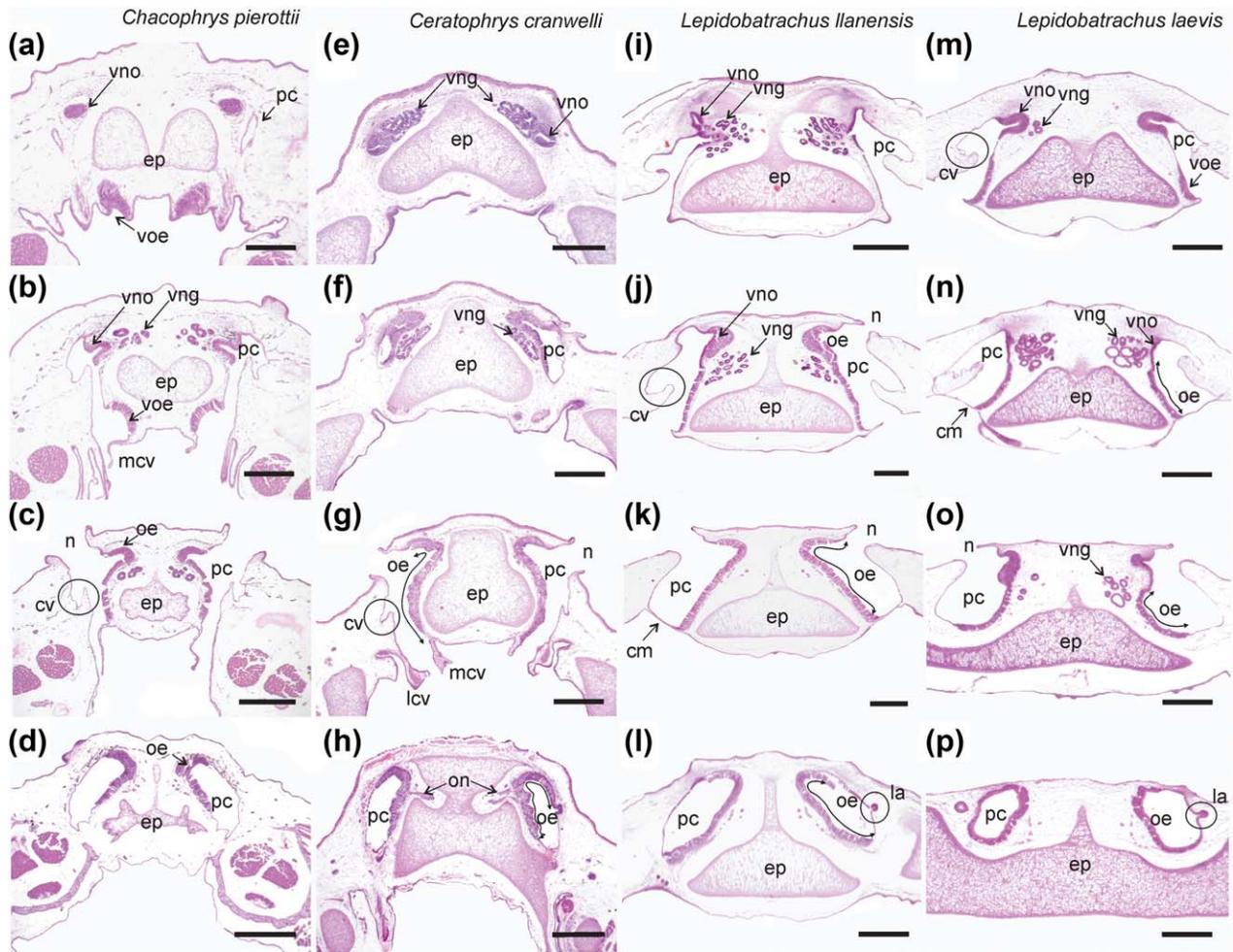


FIGURE 2 Histological transverse section of the nasal organ in larvae of ceratophryid frogs. (a–p) Stage 34 (Gosner) anterior to posterior sectioning. Sections at approximately corresponding levels across species, but adjusted to show special features. (a–d) *Chacophrys pierottii*. (e–h) *Ceratophrys cranwelli*. (i–l) *Lepidobatrachus llanensis*. (m–p) *L. laevis*. Abbreviations: cm, choanal membrane; cv, crista ventralis; ep, ethmoid plate; la, lateral appendix; lcv, lateral choanal valve; mcv, medial choanal valve; n, naris; oe, olfactory epithelium; on, olfactory nerve; pc, principal cavity; vng, vomeronasal glands; vno, vomeronasal organ; voe, ventral olfactory epithelium. Scale: 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

choanae, the olfactory epithelium becomes continuous along the dorsal and medial wall; the lateral wall, including the lateral groove, is covered by nonsensory epithelium (Figure 2b,c). Posteriorly, the principal cavity is reduced to its dorsal portion; this has an oval form in cross section (Figure 2d). A small lateral appendix is present (not shown).

Ceratophrys cranwelli

In *Ceratophrys*, as in *Chacophrys*, the crista ventralis and lateral groove divide the principal cavity into dorsal and ventral regions (Figure 2g). Unlike *Chacophrys*, the sensory epithelium of the principal cavity is continuous along the dorsomedial wall for its whole length; no distinct dorsal and ventral olfactory epithelia are formed (Figure 2g). However, the ventral part of the sensory epithelium forms a small pouch in the anterior wall of the choana, resembling that of the ventral olfactory epithelium in *Chacophrys* (Figure 2e,f). The vomeronasal organ and its associated glands are found dorsomedially above the ethmoid plate, in a position more anterior than in *Chacophrys* (Figure 2e,f). Medial and lateral choanal valves are present (Figure 2g). Posteriorly, behind the

choana, the principal cavity is reduced to its dorsal part and displays an oval shape; the lateral appendix projects from the lateral wall of the principal cavity, marking the ventral limit of the sensory epithelium, while the olfactory epithelium occupies the rest of the roof and medial wall (Figure 2h).

Lepidobatrachus laevis and *L. llanensis*

In both species of *Lepidobatrachus*, the external naris is rimmed by a thin flap along its medial side (Figure 2j,k,o). The principal cavity communicates directly with the oral cavity through the choana, which lacks both medial and lateral valves, although a thin choanal membrane spans the posterior part of the choana (Figure 2k,n). As in *Chacophrys* and *Ceratophrys*, the principal cavity is divided into dorsal and ventral sections by the crista ventralis and lateral groove. The crista ventralis has a free posterior end, which lies just above the choanal membrane (Figure 2n). Anteriorly, the olfactory epithelium forms a distinct section of ventral olfactory epithelium on the medial wall of the ventral principal cavity (Figure 2m). The vomeronasal organ and its associated gland

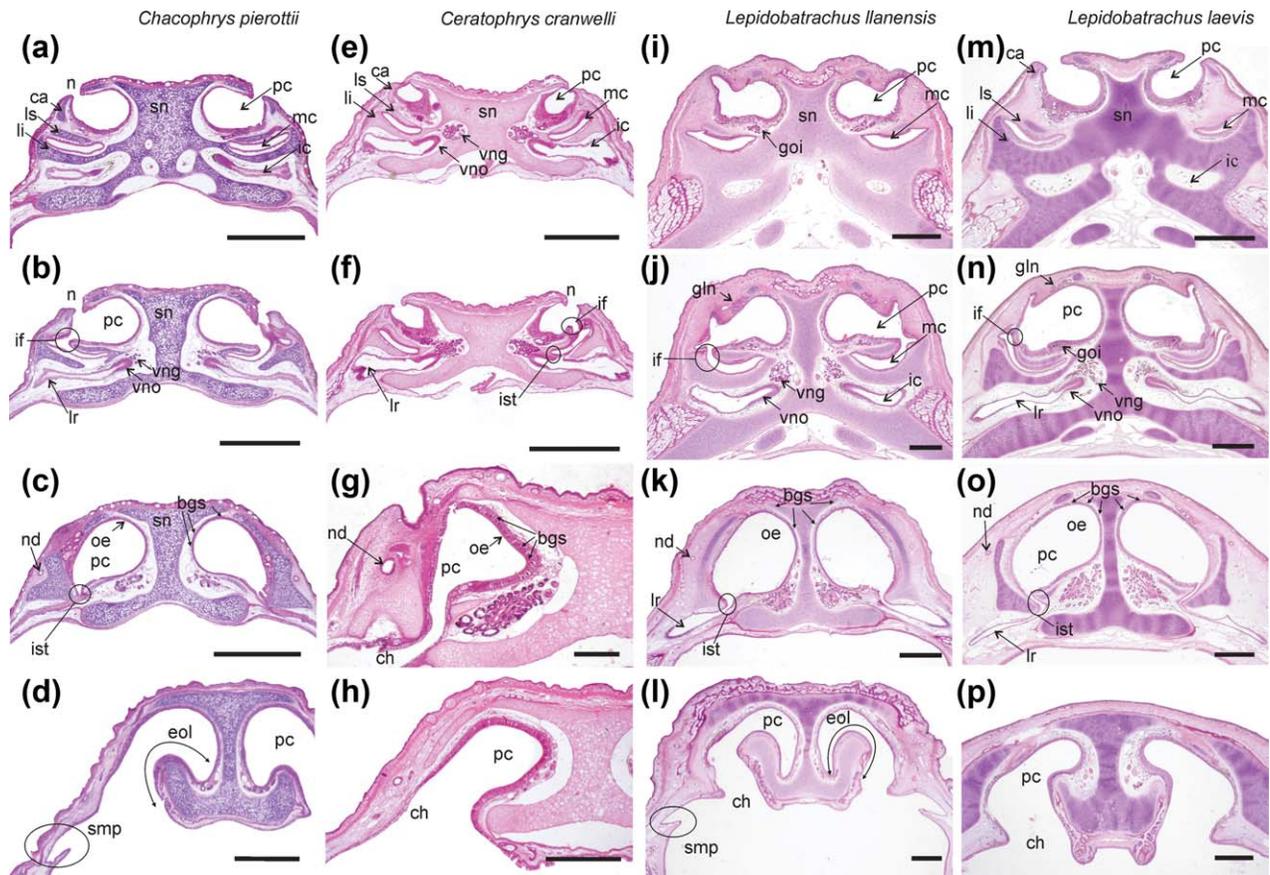


FIGURE 3 Histological transverse section of the nasal organ in postmetamorphic specimens of ceratophryid frogs. The generalized adult configuration of the system is acquired at metamorphosis in the four species analyzed; this organization involves the presence of the three functional cavities, principal, middle, and inferior. Sections at approximately corresponding levels across species, but adjusted to show special features. (a–d) *Chacophrys pierottii*. (e–h) *Ceratophrys cranwelli*. (i–l) *Lepidobatrachus llanensis*. (m–p) *L. laevis*. (d, h, l, p) Posterior portion of the principal cavity. The skeletal support of the eminentia olfactoria is well-developed in *C. pierottii* and *L. llanensis*, but not *C. cranwelli* and *L. laevis*. All the surface of the principal cavity is covered by respiratory and adult-like sensory epithelium. Abbreviations: bgs, Bowman’s glands; ca, cartilago alaris; ch, choana; eol, eminentia olfactoria; gln, glandula lateralis nasi; goi, glandula oralis interna; ic, inferior cavity; if, infundibulum; ist, isthmus; li, lamina inferior; lr, lateral recess; ls, lamina superior; mc, middle cavity; n, naris; nd, nasolacrimal duct; oe, olfactory epithelium; pc, principal cavity; smp, sulcus maxilopalatinus; sn, septum nasi; vng, vomeronasal glands; vno, vomeronasal organ. Scale: 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

are present and open in the middle region of the principal cavity in a dorsomedial position, above the ethmoid plate (Figure 2i–k,m–o). The dorsal section of the olfactory epithelium first appears just behind the opening of the vomeronasal organ; it becomes continuous with the ventral section of the olfactory epithelium along the medial wall of the principal cavity more posteriorly. At the very back of the nasal sac the dorsal principal cavity closes and has a circular form in cross-section; here the lateral appendix is seen laterally, increasing the surface of the cavity covered by sensory epithelium (Figure 2l,p). By Stage 38, palatal (buccal) glands are apparent behind the choanae. No significant differences were noted between the two species examined.

3.1.2 | Postmetamorphic/juvenile morphology

In juvenile/postmetamorphic ceratophryids, as in anurans generally (Figure 1b), each olfactory organ consists of a principal cavity, a middle cavity, and an inferior cavity. In the anterior region of the olfactory organs, the principal cavity is located dorsally, within a cartilaginous

capsule formed by the tectum nasi dorsally, the cartilage alaris ventrolaterally, and the nasal septum medially; the middle cavity is seen ventral to the principal cavity between the lamina superior and lamina inferior of the crista intermedia, while the inferior cavity is the most ventral, and overlies the solum nasi (Figure 3a,e,i,m). The external nares open to the exterior from the anterior part of the principal cavity, and are located dorsolaterally (Figure 3a,b,f,m). Both the principal cavity and inferior cavity open to the buccal cavity at the choana (Figure 3d,h, l,p).

In all three genera, the principal cavity is more or less in the form of a cylinder running anteroposteriorly. The olfactory epithelium extends along the length of the medial wall of the principal cavity, and is also found posterodorsally; it is thick (generally 100–200 μm), pseudostratified, and shows many Bowman’s glands immersed among the epithelial cells (Figure 3a,c,e,g). Nonsensory (respiratory) epithelium is found principally in the lateral wall of the principal cavity (Figure 3). The glandula oralis interna is found in the anterior region of the

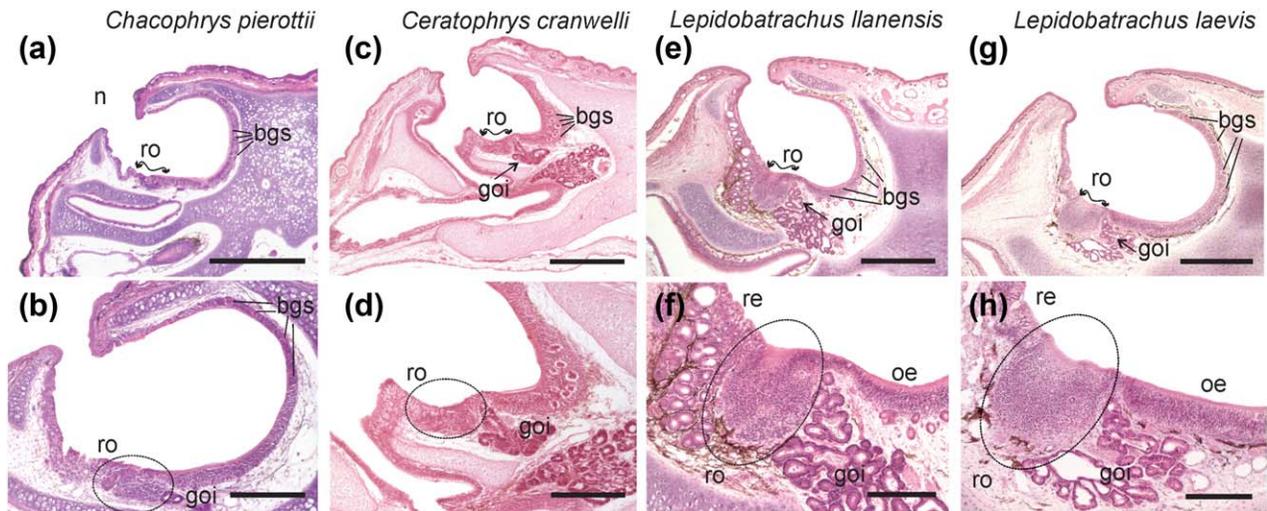


FIGURE 4 Enlarged view of the left principal cavity of the nasal organ in postmetamorphic specimens of ceratophryid frogs. (a, b) *Chacophrys pierottii*. (c, d) *Ceratophrys cranwelli*. (e, f) *Lepidobatrachus llanensis*. (g, h) *L. laevis*. (a, c, e, and f) Principal cavity medial to the external naris where the location of the recessus olfactorius is seen. (b, d, f, and h) Detail of the recessus olfactorius. The recessus olfactorius is a small patch of “larval type” sensory epithelium restricted to the lateral floor of the principal cavity in *Ceratophrys* and *Chacophrys* but in *Lepidobatrachus* species the epithelium of the recessus shows a marked thickening, acquiring a pocket configuration (e, f). Abbreviations: bgs, Bowman’s glands; goi, glandula oralis interna; n, naris; oe, olfactory epithelium; re, respiratory epithelium; ro, recessus olfactorius. Scale: a, c, e, and g 0.5 mm; b, d, f, and h 0.2 mm [Color figure can be viewed at wileyonlinelibrary.com]

principal cavity, lodged below the floor of the cavity (Figures 3 i,n and 4b,d,f,h). This gland opens into the principal cavity in a variable antero-posterior position among the ceratophryids, but in general at the same level as that in which the nares open to the external medium (Figure 4b,d,f,h). In the four species analyzed a “larval type” region of sensory epithelium (i.e., without Bowman’s glands) can be distinguished in the floor of the principal cavity, lateral to the duct of the glandula oralis interna; this patch of sensory epithelium is the recessus olfactorius (Figure 4). In postmetamorphic specimens of *Lepidobatrachus*, the development of the recessus olfactorius is remarkable with respect to *Chacophrys* and *Ceratophrys*, in these species the epithelium of the recessus is thickened and shaped like a pocket (Figure 4).

The middle cavity is entirely nonsensory; from an almost horizontal position in its medial part it curves dorsally in its lateral part to receive the nasolacrimal duct. The inferior cavity is found ventral to the principal cavity and the middle cavity in a horizontal position; in this cavity one distinguishes a lateral recess covered with nonsensory epithelium and a vomeronasal organ (medial recess) that abuts the nasal septum and opens laterally. The vomeronasal glands open into the vomeronasal organ (Figure 3b,e,j,n).

In the posterior region of the principal cavity, where it opens to the buccal cavity through the choanae, the surface area of the olfactory epithelium is increased by the presence of an eminentia olfactoria (Figure 3d,h,l,p), which in the postmetamorphic specimens examined, is well-developed in *Chacophrys pierottii* and *Lepidobatrachus llanensis* (Figure 3d,l) while in *Ceratophrys cranwelli* and *L. laevis* it is less-developed or even absent (Figure 3h,p). The glandula lateralis nasi is present in all species, behind the naris (Figure 3j,n). The sulcus maxillo-palatinus is absent in *Ceratophrys*, but present in juvenile *Chacophrys* and *Lepidobatrachus* (Figure 3d,l).

In juveniles, *Lepidobatrachus* species have the external nares located more anteriorly and dorsally than in *Chacophrys* and *Ceratophrys*, and the inferior cavity, including the vomeronasal organ, more posteriorly (Figure 3). The vestibule is absent in the species of *Lepidobatrachus* and the postchoanal region of the nasal sac is poorly developed.

3.1.3 | Adult morphology

Ceratophrys cranwelli adults differ from postmetamorphic stages mainly by the strong development of the eminentia olfactoria (Figure 5d), while in adult stages of *Lepidobatrachus* significant differences from immediately postmetamorphic stages involve the development of the recessus olfactorius (Figures 5e–g,i–k and 6). In both species of *Lepidobatrachus*, the recessus of adults is noticeably more extensive in a rostro-caudal direction, encompassing the floor of the main cavity from its distal to almost its posterior end, until the communication of the main cavity with the inferior cavity. This increased anteroposterior extent is apparent quantitatively: in *C. cranwelli* the recessus olfactorius occupies only 0.55% of SVL, while in *L. llanensis* and *L. laevis* it occupies 1.03% and 1.06%, respectively.

In *Lepidobatrachus llanensis* the recessus olfactorius of adults is a strip of epithelium on the ventromedial side of the principal cavity which lies principally on the lamina superior (Figure 6c,d). A large, well-developed glandula oralis interna lies medial to the recessus and its duct pierces the surface of the epithelium, splitting the recessus from the olfactory epithelium which lies more medially (Figure 6d).

In *Lepidobatrachus laevis* (Figures 5 i–l and 6e,f), the recessus olfactorius extends more anteriorly in the principal cavity, and it is well-developed at the level of the external nares. As in *L. llanensis*, the recessus olfactorius extends principally on the lamina superior and reaches

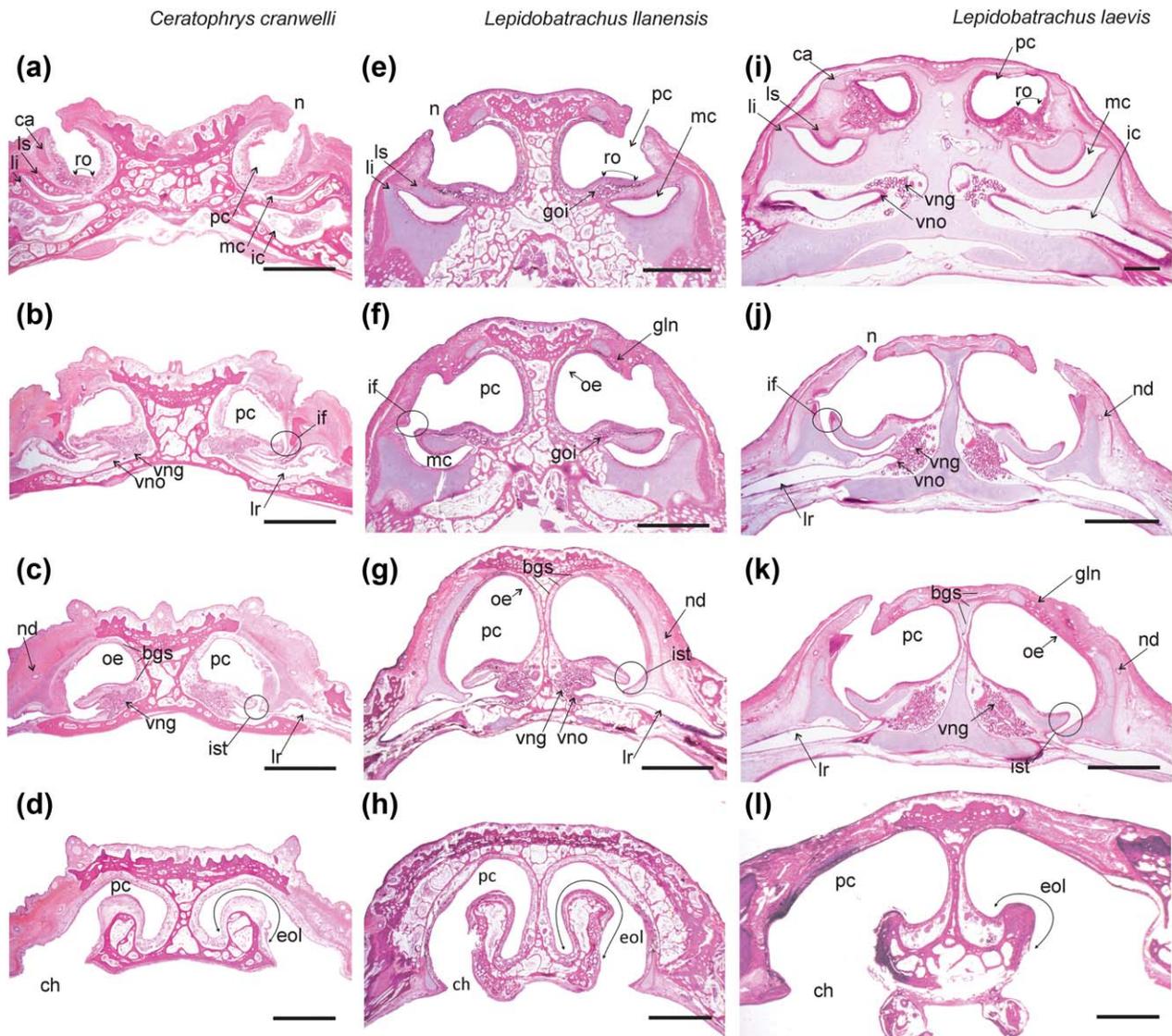


FIGURE 5 Selected cross sections (at anterior, mid, and posterior parts of the principal cavity) of the olfactory organ in advanced adult stages in ceratophryid frogs. (a–d) *Ceratophrys cranwelli*. (e–h) *Lepidobatrachus llanensis*. (i–l) *L. laevis*. Note the well-developed skeletal support of the eminentia olfactoria in *C. cranwelli* and *L. llanensis* (d, h) and the extension of the recessus olfactorius in species of *Lepidobatrachus* (e, i). Abbreviations: bgs, Bowman's glands; ca, cartilago alaris; ch, choana; eol, eminentia olfactoria; gln, glandula lateralis nasi; goi, glandula oralis interna; ic, inferior cavity; if, infundibulum; ist, isthmus; lr, lateral recess; ls, lamina superior; mc, middle cavity; n, naris; nd, nasolacrimal duct; oe, olfactory epithelium; pc, principal cavity; ro, recessus olfactorius; vng, vomeronasal glands; vno, vomeronasal organ. Scale: 1 mm [Color figure can be viewed at wileyonlinelibrary.com]

its maximum development by the midlevel of the principal cavity where it is invaginated forming a deep pit which increases the surface area of the recessus, this invagination also involves the cartilage of the lamina superior (Figure 6e,f). The glandula oralis interna is large, and lies on the medial side of the recessus (Figure 6e); its duct pierces the epithelium, as in *L. llanensis*.

More caudally, the adult nasal sac of *Lepidobatrachus* is less remarkable. The external naris enters at the dorsolateral side of the principal cavity; there is no real vestibule, but there is a groove running forward along the lateral wall from the naris to meet the nasolacrimal duct and middle cavity (Figure 5j,k), perhaps comparable to the recessus sacciformis + infundibulum of *Rana* (Gaupp, 1904). As described

for juveniles, the glandula lateralis nasi is present behind the naris, opening into the principal cavity (Figure 5f,k). In *L. llanensis*, a strongly developed eminentia olfactoria is present medial to the choana and extending posteriorly (Figure 5h), but in *L. laevis* the eminentia is very poorly developed (Figure 5l). In both species, the principal cavity ends blindly posteriorly as a small postchoanal recess.

4 | DISCUSSION

The morphology of the larval nose in all three genera of Ceratophryidae conforms to the generalized pattern for anuran larvae described above. For example, the vomeronasal organ and its associated glands

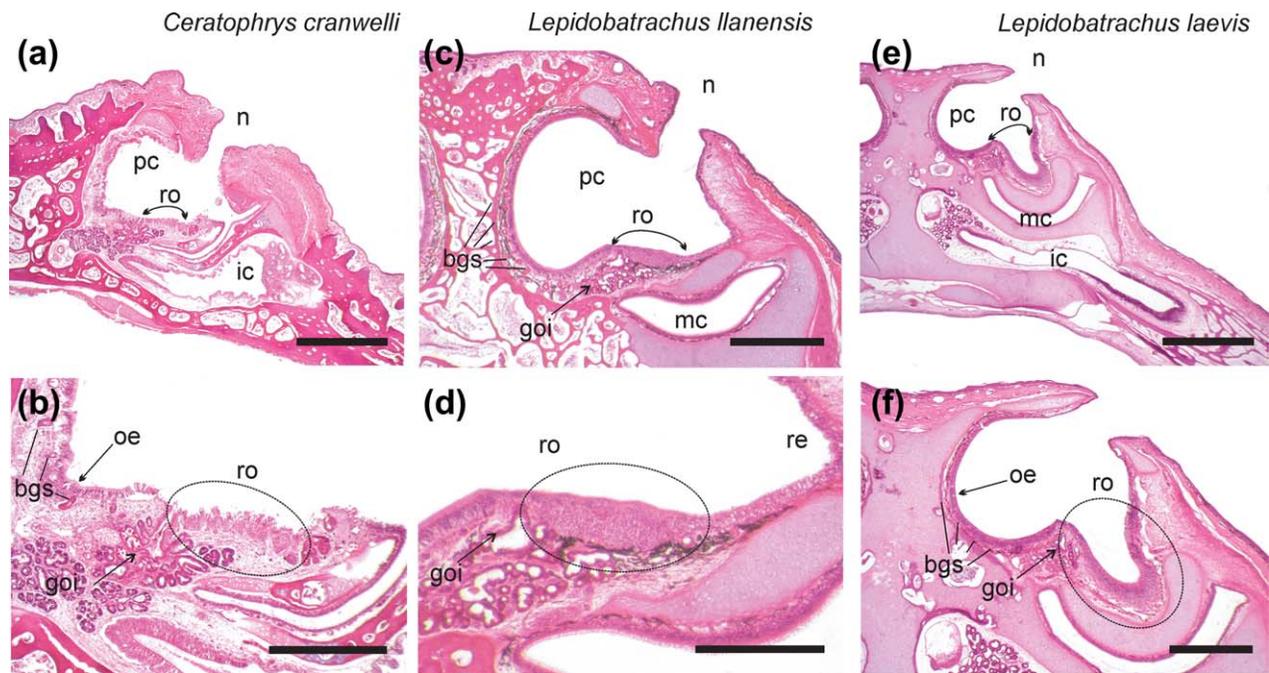


FIGURE 6 Selected cross sections of the olfactory organ in advanced adult stages of *Ceratophrys cranwelli*, *Lepidobatrachus llanensis*, and *L. laevis*. (a, b) *Ceratophrys cranwelli*. (c, d) *Lepidobatrachus llanensis*. (e, f) *L. laevis*. (a, c, and e) Principal cavity medial to the external naris, where the recessus olfactorius is seen. (b, d, and f) Detail of the recessus olfactorius. Abbreviations: bgs, Bowman's glands; goi, glandula oralis interna; ic, inferior cavity; mc, middle cavity; n, naris; oe, olfactory epithelium; pc, principal cavity; re, respiratory epithelium; ro, recessus olfactorius. Scale: a, c, and e 1 mm; b, d, and f, 0.5 mm [Color figure can be viewed at wileyonlinelibrary.com]

of the species examined differentiate in early larval stages; they are present and completely developed by Gosner Stage 34, as in most other anuran larvae (Cooper, 1943; Jungblut et al., 2012; Khalil, 1978; Nieuwkoop & Faber, 1994; Taniguchi, Toshima, Saito, & Taniguchi, 1996; Tsui, 1946; Wang, Zhao, Tai, & Zhang, 2008).

However, there are a few unusual features. In *Chacophrys*, a part of the ventral olfactory epithelium is exposed to the oral cavity at the anterior border of the choana (the "buccal exposed olfactory epithelium" of Jungblut et al., 2017). This is a feature that appears sporadically in a variety of anuran larvae (Benzekri & Reiss, 2012; Jermakowicz et al., 2004; Jungblut et al., 2011, 2012, 2017; Khalil, 1978; Rowedder, 1937; Tsui, 1946; Wassersug, 1980; Yvroud, 1966), and has been suggested to function as a chemosensory detector for sampling of oral cues during the larval period, complementing the role of taste buds (Jungblut et al., 2017; Wassersug, 1980). It is of interest that only the microphagous ceratophryid shows this feature. However, a small part of the olfactory epithelium does appear along the medial border of the choana in the other genera.

Another unusual, if not unique, feature, is that in larvae of both *Chacophrys pierottii* and *Lepidobatrachus* spp. (but not *Ceratophrys cranwelli*), the olfactory epithelium splits anteriorly into dorsal and ventral sections of epithelia, similar to the condition described in larvae of bufonids (Jermakowicz et al., 2004; Jungblut et al., 2011; Khalil, 1978). While this arrangement of the olfactory epithelium has been suggested to be a synapomorphy for Bufonidae (Jungblut et al., 2011), its clear occurrence in larvae of *Chacophrys* and *Lepidobatrachus* justifies a broader taxonomic review of this character.

As expected, the larval nose of *Lepidobatrachus* diverges most from the generalized type. The most unusual feature is the absence of choanal valves; the olfactory cavity is instead only partially separated from the buccal cavity by a thin choanal membrane. Possibly correlated with this is the presence of a free posterior end to the crista ventralis, not hitherto reported in any anuran larvae. The absence of choanal valves was noted in a previous study on the larval oral cavity (Wassersug & Heyer, 1988), and is only shared with microhylid tadpoles, in which the absence of choanal valves occurs in association with the absence of external nares (Wassersug & Heyer, 1988). The choanal valves prevent the passage of water into the nasal cavity during buccal compression (Gradwell, 1969), and thus enhance the uptake of oxygen by branchial respiration. The presence of paired spiracles and the simplification of the branchial baskets in *Lepidobatrachus* tadpoles could facilitate the circulation of water, and the thin choanal membranes might provide a partial physical barrier to the entrance of water into the nasal cavity during buccal compression. Functional studies analogous to those of Gradwell (1969) could test this hypothesis. Regardless of its precise functional significance, the presence of a thin choanal membrane in *Lepidobatrachus* can be considered a derived, simplified morphology, highlighting the uniqueness of *Lepidobatrachus* tadpoles.

The morphology of the olfactory system in adult stages has been studied in great detail in ranids (Døving, Trotier, Rosin, & Holley, 1993; Gaupp, 1904; McCotter, 1917; Tsui, 1946), in basal anurans (Benzekri & Reiss, 2012; Stephenson, 1951; Wagner, 1934), and in pipids (Dittrich, Kuttler, Hassenklöver, & Manzini, 2016; Hansen et al., 1998; Paterson, 1939; Reiss & Burd, 1997, among others), as well as in

surveys across anurans (Helling, 1938; Jurgens, 1971). These studies have shown that the general morphology and organization of this system are shared by the majority of anurans, with the exception of the pipids and ascaphids, in which certain morphological peculiarities have been described as related to the aquatic habits of adults (Paterson, 1951; Reiss & Eisthen, 2008), and in the recently described deviant anatomy of the mantellid *Mantidactylus betsileanus* (Junk et al., 2014; Nowack & Vences, 2016), where the functional significance appears to relate to perception of femoral gland pheromone secretions (Nowack et al., 2017). The analysis of the adult morphology of the nose in ceratophryid frogs also reveals a somewhat deviant morphology in adult stages of the aquatic *Lepidobatrachus* spp. with respect to the terrestrial genera (Figures 3–6).

The presence of the recessus olfactorius is a common characteristic in the adult nose of the majority of anurans. The recessus olfactorius generally constitutes a small, depressed region of the olfactory epithelium, which lacks Bowman's glands and which is usually located in the anterolateral floor of the principal cavity (Helling, 1938; Jungblut et al., 2017; Nowack, Jordan, & Wittmer, 2013). A strip of respiratory epithelium separates the recessus olfactorius from the main olfactory epithelium, and the duct of the glandula oralis interna (internal rostral nasal gland) opens in this strip. This is the condition in postmetamorphic specimens of all ceratophryids. However, in adults of *Lepidobatrachus laevis* the recessus is further developed due to its pocketed structure that increases its surface area. This morphology is unique among that described for other anuran species (see Benzekri & Reiss, 2012; Helling, 1938; Jermakowicz et al., 2004; Junk et al., 2014; Královec et al., 2013).

Helling (1938) suggested a relation between the degree of development of the recessus olfactorius and the habitats of the adult frogs, grounded in the observations that (1) in aquatic frogs such as *Telmatobius hauthali*, the recessus is highly developed and occupies around 30% of the floor of the principal cavity, and (2) since the epithelium of the recessus olfactorius lacks Bowman's glands, unlike the adult main olfactory epithelium, but like that of larvae, it likely indicates a sensory epithelium capable of detecting chemical stimulants in the water in adult animals (Helling, 1938). Secretions of the Bowman's glands play a fundamental role in aerial olfaction, contributing to the mucus coat that covers the sensory epithelium. This mucus not only prevents desiccation of the olfactory epithelium in terrestrial environments, but also contains proteins that combine with odor molecules and transport them to membrane receptors of the sensory neurons (Millery et al., 2005). Further support for the hypothesis that the recessus olfactorius of the adult nose is specialized for aquatic olfaction, or at least for a different function than the main olfactory epithelium, comes from evidence that the receptor and supporting cell types present differ from the main olfactory epithelium, and have distinct lectin-binding patterns (Nowack et al., 2013) and N-CAM expression patterns (Jungblut et al., 2011).

The recessus olfactorius is also notably expanded in two other lineages with secondarily aquatic adults. In *Ascaphus truei*, the anterolateral region of the principal cavity is lined by a "larval type" sensory epithelium, and appears to be homologous with the recessus olfactorius of other forms (Benzekri & Reiss, 2012). In the pipids, an accessory sensory cavity is present (Bancroft, 1895; Paterson, 1939, 1951;

Trahms, 1936). This is the so-called "middle cavity" of *Xenopus laevis*, which contains a sensory epithelium—lacking Bowman's glands—in contrast to the middle cavity of the remainder of anurans (Föske, 1934; Hansen et al., 1998; Helling, 1938; Paterson, 1951), and is thought to be homologous to the recessus olfactorius of other forms (Helling, 1938; Reiss & Eisthen, 2008). Thus, the enlargement of the recessus olfactorius in adult stages of *Lepidobatrachus* (particularly *L. laevis*) while not as extensive as that in *Ascaphus* or the pipids, appears to be a specialization to the aquatic lifestyle of these frogs.

The presence and degree of development of an olfactory eminence in the floor of the principal cavity of adult anurans is a character whose functional significance is somewhat controversial. The studies that have contributed most to the understanding of the presence of this structure are likewise those carried out by Helling (1938). He noted that the eminentia olfactoria is better developed in anurans with a terrestrial or fossorial mode of life, such as *Bufo viridis*, *Leptodactylus mystacinus*, and *Breviceps mossambicus*, while completely absent in the pipids and in aquatic neobatrachians such as *Telmatobius hauthali*. A correlation of the presence of the eminentia with terrestriality and/or fossoriality was independently suggested by de Villiers (1933), who also noted its extreme development in *Breviceps*, and was supported by Ramaswami (1934). However, Ramaswami (1935a, 1939) later questioned the relation, noting that aquatic forms like *Euphlyctis (Rana) hexadactylus* and *E. (R.) cyanophlyctis* have a fairly well-developed olfactory eminence, even though it is absent in other aquatic species like *Clinotarsus (R.) curtipes*.

Among the ceratophryids, the development of the olfactory eminence takes place during metamorphosis in *Chacophrys pierottii* and *Lepidobatrachus llanensis* and in postmetamorphic stages in *Ceratophrys cranwelli*. The floor of the nasal cavities elevates and forms a skeletal support for the eminentia, which is very notable in postmetamorphic stages of *C. pierottii* and adult stages in *C. cranwelli* and *L. llanensis*. In comparison, the eminentia olfactoria in *L. laevis* can be considered absent or at least very rudimentary.

The elevation of the floor of the nasal cavities, and the consequent formation of the eminentia olfactoria, contributes to an increased surface area of the olfactory epithelium in the principal cavity, increasing the sensory area available for olfaction in an aerial environment. In ceratophryids (and other anurans as well), it does appear that its presence is related to the degree of terrestriality of the species, in spite of the doubts raised by Ramaswami (1939). It is clear that in species of *Ascaphus*, *Bombina*, and *Telmatobius hauthali* (Benzekri & Reiss, 2012; Helling, 1938; de Villiers, 1934). Also, the eminentia olfactoria is very poorly developed, similar to the condition in *Lepidobatrachus laevis*. Conversely, as noted, the eminentia is generally highly developed in bufonids, ranids, brevicipitids, scaphiopodids (Ramaswami, 1935b) and in the terrestrial species and in *L. llanensis* within Ceratophryidae. Perhaps the most accurate generalization is that this structure can be present both in terrestrial and in aquatic forms, but that when it is absent it is always absent in aquatic forms. Thus, one can reasonably consider its absence in neobatrachians such as *L. laevis* a derived condition related to secondarily aquatic habits.

Taking a broader overview, the ontogeny of the olfactory system in species of *Chacophrys* and *Ceratophrys* is not significantly different from that seen in the majority of anurans (Benzekri & Reiss, 2012;

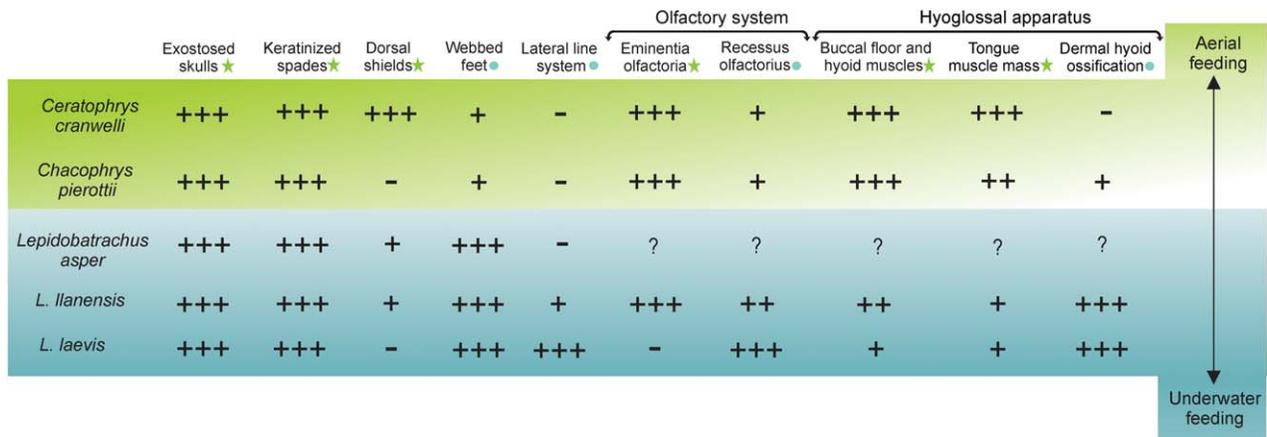


FIGURE 7 Graphical representation of general evolutionary trends in the morphology of adult ceratophryids. *Ceratophrys* and *Chacophrys* share a set of derived morphological traits that have historically been associated with terrestrial habits (green stars). In *Lepidobatrachus*, these are integrated with other features associated with aquatic habits (blue circles). These aquatic features are developed to different degrees among the species of *Lepidobatrachus*. They define a distinctive adult body plan in *L. laevis* unique among Neobatrachia, which suggests a strong trend in morphological change within the genus, related to a terrestrial to secondarily aquatic (green to blue area) shift. Based on data from present work, and literature (Fabrezi, 2006; Fabrezi & Lobo, 2009; Fabrezi & Quinzio, 2008; Fabrezi et al., 2014; Quinzio & Fabrezi, 2012, 2014). Symbols: -, absent; +, weakly developed; ++, moderately developed; +++, strongly developed [Color figure can be viewed at wileyonlinelibrary.com]

Dittrich et al., 2016; Jermakowicz et al., 2004; Junk et al., 2014; Královic et al., 2013). However, *Lepidobatrachus* spp. shows unusual features in larvae (as the absence of choanal valves) which then converge to a morphology shared with other ceratophryids (and neobatrachians in general) in immediately postmetamorphic stages, and then diverge again, leading to a distinctive morphology of the olfactory system in adult stages of these aquatic frogs. This morphology of the nose does not involve the development of a new structure but a heightened (peramorphic) development of the recessus olfactorius, and a reduced (paedomorphic) development of the eminentia olfactoria.

The enhancement of the recessus olfactorius and the rudimentary state of the eminentia olfactoria in adult stages of *Lepidobatrachus laevis*, represent common and convergent features in the nose of adult frogs with aquatic habits (Benzekri & Reiss, 2012; Helling, 1938). Nevertheless, these characteristics of the recessus and the absence of the eminentia in *L. laevis* are uniquely integrated with other derived morphological traits defining a distinctive body plan, both within the group and with respect to other Neobatrachia. The early ontogenetic acquisition of many of these features, reducing the morphological differences between larval and adult body plans, seems to be correlated with the absence of the aquatic to terrestrial shift in these frogs (Fabrezi, 2006; Fabrezi & Lobo, 2009; Fabrezi & Quinzio, 2008; Fabrezi et al., 2016; Quinzio & Fabrezi, 2012, 2014).

Our results on the adult morphology of the olfactory system support previous work (Fabrezi, 2006; Fabrezi & Lobo, 2009; Fabrezi & Quinzio, 2008; Fabrezi et al., 2016; Quinzio & Fabrezi, 2012, 2014) showing that *Chacophrys* and *Ceratophrys* show an ancestral typical terrestrial morphology, while in *Lepidobatrachus* there is a trend within the genus of morphological change related to a derived aquatic life, with *L. asper* the least aquatic and *L. laevis* the most aquatic (Figure 7). Thus, we added data, from the olfactory system, to the derived morphology of adult *L. laevis* and its evolutionary trend to a secondarily aquatic life.

ACKNOWLEDGMENTS

Specimen collection permits were issued by the Secretaría de Medio Ambiente y Desarrollo Sustentable, Gobierno de la Provincia de Salta, Argentina. Authors thank M. Fabrezi for her comments in the early draft of the manuscript. This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica PICT 2718 to S. Quinzio, PICT 510 and by a sabbatical leave granted to J. Reiss by Humboldt State University.

AUTHOR CONTRIBUTIONS

SIQ and JOR conceived the study and participated in the acquisition, interpretation, and discussion of data. SIQ designed, performed and wrote the manuscript, and prepared the majority of figures, with input from JOR. Both authors read and approved the manuscript.

ORCID

Silvia I. Quinzio  <http://orcid.org/0000-0002-3930-914X>

REFERENCES

- Bancroft, I. R. (1895). The nasal organ of *Pipa americana*. *Bulletin of the Essex Institute*, 27, 101–107.
- Benzekri, N. A., & Reiss, J. O. (2012). Olfactory metamorphosis in the coastal tailed frog *Ascaphus truei* (Amphibia, Anura, Leiopelmatidae). *Journal of Morphology*, 273, 68–87.
- Bloom, S., Ledon-Rettig, C., Infante, C., Everly, A., & Hanken, J. (2013). Developmental origins of a novel gut morphology in frogs. *Evolution and Development*, 15, 213–223.
- Castroviejo-Fisher, S., Padial, J. M., De La Riva, I., Pombal, J. P., Jr., Da Silva, H. R., Rojas-Runjaic, F. J. M., ... Frost, D. R. (2015). Phylogenetic systematics of egg-brooding frogs (Anura: Hemiphractidae) and the evolution of direct development. *Zootaxa*, 4004, 1–75.

- Cooper, R. S. (1943). An experimental study of the development of the larval olfactory organ of *Rana pipiens* Schreber. *Journal of Experimental Zoology*, 93, 415–451.
- Dittrich, K., Kuttler, J., Hassenklöver, T., & Manzini, I. (2016). Metamorphic remodeling of the olfactory organ of the African clawed frog, *Xenopus laevis*. *Journal of Comparative Neurology*, 524, 986–998.
- Døving, K. B., Trotier, D., Rosin, J. F., & Holley, A. (1993). Functional architecture of the vomeronasal organ of the frog (genus *Rana*). *Acta Zoologica*, 74, 173–180.
- Fabrezi, M. (2006). Morphological evolution of Ceratophryinae (Anura, Neobatrachia). *Journal of Zoological Systematics and Evolutionary Research*, 44, 153–166.
- Fabrezi, M. (2011). Heterochrony in Growth and Development in Anurans from the Chaco of South America. *Evolutionary Biology*, 38, 390–411.
- Fabrezi, M., & Lobo, F. J. (2009). Hyoid skeleton, related muscles, and morphological novelties in the frog *Lepidobatrachus* (Anura, Ceratophryidae). *Anatomical Record*, 292, 1700–1712.
- Fabrezi, M., & Quinzio, S. I. (2008). Morphological evolution in Ceratophryinae frogs (Anura, Neobatrachia): The effects of heterochronic changes during larval development and metamorphosis. *Zoological Journal of the Linnean Society*, 154, 752–780.
- Fabrezi, M., Quinzio, S. I., Goldberg, J., Cruz, J. C., Chuliver Pereyra, M., & Wassersug, R. J. (2016). Developmental changes and novelties in ceratophryid frogs. *EvoDevo*, 7, 5.
- Faivovich, J., & Carrizo, G. R. (1992). Descripción de la larva de *Chacophrys pierottii* (Vellard, 1948) (Leptodactylidae, Ceratophryinae). *Alytes*, 10, 81–89.
- Faivovich, J., Nicoli, L., Blotto, B. L., Pereyra, M. O., Baldo, D., Barriovenue, J. S., ... Haddad, C. F. B. (2014). Big, bad, and beautiful: Phylogenetic relationships of the horned frogs (Anura: Ceratophryidae). *South American Journal of Herpetology*, 9, 1–21.
- Föske, H. (1934). Das Geruchsorgan von *Xenopus laevis*. *Zeitschrift Für Anatomie Und Entwicklungsgeschichte*, 103, 519–550.
- Fritsch, B., Drewes, R., & Ruibal, R. (1987). The retention of the lateral-line nucleus in adult anurans. *Copeia*, 1987, 127–155.
- Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., ... Wheeler, W. C. (2006). The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1–370.
- Gaupp, E. (1904). *A. Ecker's und R. Wiedersheim's Anatomie des Frosches. Dritte Abteilung: Lehre von den Eingeweiden, dem Integument und den Sinnesorganen*. Braunschweig, Germany: Friedrich Vieweg und Sohn.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 83–190.
- Gradwell, N. (1969). The function of the internal nares of the bullfrog tadpole. *Herpetologica*, 25, 120–121.
- Handrigan, G. R., & Wassersug, R. J. (2007). The anuran Bauplan: A review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. *Biological Review*, 82, 1–25.
- Hansen, A., Reiss, J. O., Gentry, C. L., & Burd, G. D. (1998). Ultrastructure of the olfactory organ in the clawed frog, *Xenopus laevis*, during larval development and metamorphosis. *Journal of Comparative Neurology*, 398, 273–288.
- Heinicke, M. P., Duellman, W. E., Trueb, L., Menas, D. B., Macculloch, R. D., & Hedges, S. B. (2009). A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. *Zootaxa*, 2211, 1–35.
- Helling, H. (1938). Das Geruchsorgan der Anuren, vergleichend-morphologisch betrachtet. *Zeitschrift Für Anatomie Und Entwicklungsgeschichte*, 108, 587–643.
- Hoskins, S. G. (1990). Metamorphosis of the amphibian eye. *Journal of Neurobiology*, 21, 970–989.
- Jermakowicz, W. J., Dorsey, D. A., Brown, A. L., Wojciechowski, K., Gismombe, C. L., Graves, B. M., ... Eyck, G. R. (2004). Development of the nasal chemosensory organs in two terrestrial anurans: The directly developing frog, *Eleutherodactylus coqui* (Anura: Leptodactylidae), and the metamorphosing toad, *Bufo americanus* (Anura: Bufonidae). *Journal of Morphology*, 261, 225–248.
- Jungblut, L. D., Pozzi, A. G., & Paz, D. A. (2011). Larval development and metamorphosis of the olfactory and vomeronasal organs in the toad *Rhinella* (*Bufo*) *arenarum* (Hensel, 1867). *Acta Zoologica*, 92, 305–315.
- Jungblut, L. D., Pozzi, A. G., & Paz, D. A. (2012). A putative functional vomeronasal system in anuran tadpoles. *Journal of Anatomy*, 221, 364–372.
- Jungblut, L. D., Reiss, J. O., Paz, D. A., & Pozzi, A. G. (2017). Quantitative comparative analysis of the nasal chemosensory organs of anurans during larval development and metamorphosis highlights the relative importance of chemosensory subsystems in the group. *Journal of Morphology*, 278, 1208–1219. <https://doi.org/10.1002/jmor.20705>
- Junk, A., Wenzel, S., Vences, M., & Nowack, C. (2014). Deviant anatomy of the olfactory system of the Malagasy frog *Mantidactylus betsileanus* (Anura: Mantellidae). *Zoologischer Anzeiger*, 253, 338–344.
- Jurgens, J. D. (1971). The morphology of the nasal region of Amphibia and its bearing on the phylogeny of the group. *Annale Universiteit Van Stellenbosch*, 46A, 1–146.
- Khalil, S. H. (1978). Development of the olfactory organ of the Egyptian Toad, *Bufo regularis* Reuss. *Folia Morphologica*, 26, 69–87.
- Královec, K., Zakova, P., & Muzakova, V. (2013). Development of the olfactory and vomeronasal organs in *Discoglossus pictus* (Discoglossidae, Anura). *Journal of Morphology*, 274, 24–34.
- Laurent, R. F. (1986). Sous-classe des lissamphibiens (Lissamphibia). Systématique. In M. Delsol (Ed.), *Grassé Traité de zoologie: Anatomie, systématique, biologie, tome XIV, Batraciens, fasc. 1B* (pp. 594–798). Paris: Masson.
- Lynch, J. D. (1982). Relationships of the frogs of the genus *Ceratophrys* (Leptodactylidae) and their bearing on hypotheses of Pleistocene forest refugia in South America and punctuated equilibria. *Systematic Zoology*, 31, 166–179.
- Maxson, L., & Ruibal, R. R. (1988). Relationships of frogs in the leptodactylid subfamily Ceratophryinae. *Journal of Herpetology*, 22, 228–231.
- McCotter, R. E. (1917). The vomeronasal apparatus in *Chrysemys punctata* and *Rana catesbeiana*. *Anatomical Record*, 13, 51–67.
- Meyer, D. L., Fackler, I. R., Jadhao, A. G., D'aniello, B., & Kicliter, E. (1997). Differential labelling of primary olfactory system subcomponents by SBA (lectin) and NADPH-d histochemistry in the frog *Pipa*. *Brain Research*, 762, 275–280.
- Millery, J., Briand, L., Bézirard, V., Blon, F., Fenech, C., Richard-Parpaillon, L., ... Gascuel, J. (2005). Specific expression of olfactory binding protein in the aerial olfactory cavity of adult and developing *Xenopus*. *European Journal of Neuroscience*, 22, 1389–1399.
- Nieuwkoop, P. D., & Faber, J. (1994). *Normal table of Xenopus laevis (Daudin)* (252. p.). New York: Garland Publishing.
- Nowack, C., Jordan, S., & Wittmer, C. (2013). The recessus olfactorius: A cryptic olfactory organ of anuran amphibians. In M. East & M. Dehnhard (Eds.), *Chemical signals in vertebrates* (pp. 37–48). New York: Springer.
- Nowack, C., Peram, P. S., Wenzel, S., Rakotoarison, A., Glaw, F., Poth, D., ... Vences, M. (2017). Volatile compound secretion coincides with modifications of the olfactory organ in mantellid frogs. *Journal of Zoology*, 303, 72–81 <https://doi.org/10.1111/jzo.12467>

- Nowack, C., & Vences, M. (2016). Ontogenetic development of the derived olfactory system of the mantellid frog *Mantidactylus betsileanus*. *Anatomical Record*. <https://doi.org/10.1002/ar.23351>
- Orton, G. L. (1953). The systematics of vertebrate larvae. *Systematic Zoology*, 2, 63–75.
- Parsons, T. S. (1967). Evolution of the nasal structure in the lower tetrapods. *American Zoologist*, 7, 397–413.
- Paterson, N. F. (1939). The olfactory organ and tentacles of *Xenopus laevis*. *South African Journal of Science*, 36, 390–404.
- Paterson, N. F. (1951). The nasal cavities of the toad *Hemipipa carvalhoi* Mir. Rib. and other Pipidae. *Proceedings of the Zoological Society of London*, 121, 381–415.
- Pusey, H. K. (1943). On the head of the Leiopelmid frog *Ascaphus truei*. *Quarterly Journal of Microscopical Science*, 84, 105–185.
- Pyron, R. A., & Wiens, J. J. (2011). A large-scale phylogeny of Amphibia with over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
- Quinzio, S. I., & Fabrezi, M. (2012). Ontogenetic and structural variation of mineralizations and ossifications in the integument within ceratophryid frogs (Anura, Ceratophryidae). *Anatomical Record*, 295, 2089–2103.
- Quinzio, S. I., & Fabrezi, M. (2014). The lateral line system in anuran tadpoles: Neuromast morphology, arrangement, and innervation. *Anatomical Record*, 297, 1508–1522.
- Quinzio, S. I., Fabrezi, M., & Faivovich, J. (2006). Redescription of the tadpole of *Chacophrys pierottii* Vellard, 1948 (Anura, Ceratophryidae). *South American Journal of Herpetology*, 1, 202–209.
- Ramaswami, L. S. (1934). Contributions to our knowledge of some ranid genera of frogs—Part I. *Proceedings of the Indian Academy of Sciences, Section B*, 1, 80–95.
- Ramaswami, L. S. (1935a). Contributions to our knowledge of the cranial morphology of some ranid genera of frogs—Part II. *Proceedings of the Indian Academy of Sciences, Section B*, 2, 1–20.
- Ramaswami, L. S. (1935b). The cranial morphology of some examples of Pelobatidae (Anura). *Anatomischer Anzeiger*, 81, 65–96.
- Ramaswami, L. S. (1939). Some aspects of the anatomy of Anura (Amphibia)—A review. *Proceedings of the Indian Academy of Sciences, Section B*, 10, 41–80.
- Reig, O. A., & Limeses, C. E. (1963). Un nuevo género de anuros ceratofrinidos del distrito chaqueño. *Physis*, 24, 113–128.
- Reiss, J. (2002). The phylogeny of amphibian metamorphosis. *Zoology*, 105, 85–96.
- Reiss, J. O., & Burd, G. D. (1997). Cellular and molecular interactions in the development of the *Xenopus* olfactory system. *Seminars in Cell and Developmental Biology*, 8, 171–179.
- Reiss, J. O., & Eisthen, H. L. (2008). Chemical senses: Comparative anatomy and physiology in amphibians. In J. Thewissen & S. Nummela (Eds.), *Sensory evolution on the threshold: Adaptations in secondarily aquatic vertebrates* (pp. 43–63). Berkeley: University of California Press.
- Rowedder, W. (1937). Die Entwicklung des Geruchsorgans bei *Alytes obstetricans* und *Bufo vulgaris*. *Zeitschrift Für Anatomie Und Entwicklungsgeschichte*, 107, 91–123.
- Stephenson, E. M. (1951). The anatomy of the head of the New Zealand frog, *Leiopelma*. *Transactions of the Linnean Society of London*, 27, 255–305.
- Taniguchi, K., Toshima, Y., Saito, T. R., & Taniguchi, K. (1996). Development of the olfactory and vomeronasal organ in the Japanese Reddish Frog, *Rana japonica*. *Journal of Veterinary Medical Science*, 58, 7–15.
- Thewissen, J. G., & Nummela, S. (2008). Sensory evolution on the threshold: Adaptations. In J. G. Thewissen & S. Nummela (Eds.), *Secondarily aquatic vertebrates* (358 p.). Berkeley, California: University of California Press.
- Trahms, O. K. (1936). Das Geruchsorgan von *Pipa americana*. *Zeitschrift Für Anatomie Und Entwicklungsgeschichte*, 105, 678–693.
- Tsui, C. L. (1946). Development of olfactory organ in *Rana nigromaculata*. *The Quarterly Journal of Microscopical Science*, 87, 61–90.
- Tsui, C. L., & Pan, T. H. (1946). The development of the olfactory organ of *Kaloula borealis* (Barbour) as compared with that of *Rana nigromaculata* Hallowell. *The Quarterly Journal of Microscopical Science*, 87, 299–316.
- de Villiers, C. G. S. (1933). *Breviceps* and *Probreviceps*: Comparison of the cranial osteology of two closely related Anuran genera. *Anatomischer Anzeiger*, 75, 257–276.
- de Villiers, C. G. S. (1934). Studies of the cranial anatomy of *Ascaphus truei* Stejneger, the American “Leiopelmid”. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 77, 1–38.
- Wagner, D. S. (1934). On the cranial characters of *Liopelma hochstetteri*. *Anatomischer Anzeiger*, 79, 65–77.
- Wang, H., Zhao, H., Tai, F., & Zhang, Y. (2008). Postembryonic development of the olfactory and vomeronasal organs in the frog *Rana chensinensis*. *Zoological Science*, 25, 503–508.
- Wassersug, R. J. (1980). Internal oral features of larvae from eight anuran families: Functional, systematic, evolutionary and ecological considerations. *University of Kansas Publications, Museum of Natural History*, 68, 1–146.
- Wassersug, R. J., & Heyer, W. R. (1988). A survey of internal oral features of leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology*, 457, 1–99.
- Watanabe, M. (1936). Über die Entwicklung des Geruchsorgans von *Rhacophorus schlegelii*. *Zeitschrift Für Anatomie Und Entwicklungsgeschichte*, 105, 519–561.
- Wild, E. R. (1997). Description of the adult skeleton and developmental osteology of the hyperossified horned frog, *Ceratophrys cornuta* (Anura: Leptodactylidae). *Journal of Morphology*, 232, 169–206.
- Witschi, E. (1949). The larval ear of the frog and its transformation during metamorphosis. *Zeitschrift Für Naturforschung B*, 4, 230–242.
- Yvrou, M. (1966). Développement de l'organe olfactif et des glandes annexes chez *Alytes obstetricans* Laurenti au cours de la vie larvaire et de la métamorphose. *Archives D'Anatomie Microscopique Et De Morphologie Experimentale*, 55, 387–410.

How to cite this article: Quinzio SI, Reiss JO. The ontogeny of the olfactory system in ceratophryid frogs (Anura, Ceratophryidae). *Journal of Morphology*. 2017;00:000–000. <https://doi.org/10.1002/jmor.20751>