

The Lateral Line System in Anuran Tadpoles: Neuromast Morphology, Arrangement, and Innervation

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ABSTRACT

Anuran larvae have been classified into four morphological types which reflect intraordinal macroevolution. At present, complete characterizations of the lateral line system are only available for *Xenopus laevis* (Type I) and *Discoglossus pictus* (Type III). We analyzed the morphology, arrangement, and innervation of neuromasts related to the anterodorsal and anteroventral lateral line nerves in 10 anuran species representing Types I, II, and IV with the aim of interpreting the existing variation and discussing the evolution of the lateral line in anuran larvae. We found: (1) the presence of two orbital and three mandibular neuromast lines in all anuran larvae studied, (2) the ventral arrangement of mandibular neuromast lines appears to have evolved convergently in Larval Types I and II, and the lateroventral arrangement of mandibular lines of neuromasts appears to have evolved in Larval Types III and IV; (3) interspecific variation in the organization, size, and number of sensory cells per neuromast within the lines; and (4) the supralabial extension of the Angular line in *Lepidobatrachus* spp. and the tentacular location of the Oral neuromasts in *X. laevis* are concomitant with their particular morphologies. Based on the variation described we find that the lateral line system in anuran larvae seems to have been maintained without significant changes, with the exception of *Lepidobatrachus* spp. and *Xenopus*. These unique features added to other of *Lepidobatrachus* tadpoles are sufficient to propose a new Larval Type (V). Anat Rec, 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: anuran; larvae; neuromast lines; evolution; *Lepidobatrachus*

INTRODUCTION

In the beginning of the last century, most anuran classifications were based on adult morphology, especially of the skeleton. At the middle of the twentieth century new information on tadpole morphology provided by Orton (1953, 1957), Starrett (1973), and Sokol (1975, 1977) allowed the establishment of four morphological types for anuran larvae, which later were incorporated into the anuran macro-systematics (Sokol, 1975; Duellman and Trueb, 1986; Ford and Cannatella, 1993). These larval types were defined as I, II, III, and IV (Orton, 1953, 1957) and later renamed Xenoanura Savage, 1973 (filter tadpole), Scoptanura Starrett, 1973 (filter tadpole), Lemmanura and Acosmanura Savage, 1973 (beaked tadpoles) by Starrett (1973).

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Sokol (1975) resurrected the original denomination by Orton and proposed a suprafamilial hypothesis of relationships based only on the four larval types. He studied the condition of the trigeminal (nerve V) and facial (nerve VII) ganglia and described separate trigeminal (V) and geniculate (VII) ganglia in Type III tadpoles (Discoglossoid) as the basal condition among anuran larvae, and the fusion of trigeminal and facial ganglia in the prootic ganglion as the derived condition present in tadpoles of Types I (Pipoid), II (Microhyloid), and IV (Ranoid). He proposed the independent origin of filter tadpoles (Type I, pipoids and Type II, microhyloids) and suggested that these types could have evolved from either Type III or Type IV. Later, Ford and Cannatella (1993) proposed the clade Pipanura Ford and Cannatella, 1993 [Mesobatrachia (Pipoidea and Pelobatoidea) + Neobatrachia (with Microhyloidea)] based on the presence of the prootic ganglion, but this clade appeared paraphyletic when Haas (2003) analyzed anuran phylogeny based on larval morphology. The most recent anuran phylogeny, based on molecular data, shows taxa with Type III larvae as basal (Pyron and Wiens, 2011).

Although studies on anuran phylogeny have considered larval features in different hypotheses based on morphology and/or molecular data (Maglia et al., 2001; Haas, 2003; Faivovich, 2005; Frost et al., 2006), few studies have considered features of the larval lateral line system (Fabrezi and Quinzio, 2008).

The lateral line system in amphibians presents variation in their constituent components. Urodeles and caecilians possess both electroreceptor (ampullary) organs and mechanoreceptor organs (neuromasts)—although some caecilians have lost the mechanoreceptors. Anurans have lost electroreceptors and the lateral line system is constituted only by mechanoreceptors (Frittsch et al., 1984; Frittsch and Wake, 1986). The neuromasts are constituted by three cell types: sensory hair cells, supporting cells, and mantle cells embedded in the epidermis. Neuromasts are arranged in lines on the integumentary surface in a characteristic pattern on the head, trunk, and tail (Northcutt, 1989). The neuromast lines are innervated by fibers that are organized in four nerves: the anterodorsal and anteroventral lateral line nerves, related to the orbital and mandibular lines of neuromasts, and the middle and posterior lateral line nerves innervating the trunk and tail neuromast lines (Schlosser and Roth, 1995). These nerves represent a separate series of cranial nerves, based on their independent embryonic origin (Northcutt, 1993), and they have their own set of peripheral receptors and sensory ganglia (Northcutt, 1992, 1993). Even so, the lateral line nerves are physically associated with other cranial nerves to various degrees (Northcutt, 1993; Schlosser and Roth, 1995).

The anuran lateral line system is a distinctive feature of the aquatic life stages and is, therefore, generally restricted to larvae (Northcutt, 1989). The system is sensitive to water movements generated by prey and conspecifics (Dijkgraaf, 1962) and is completely lost during metamorphosis, except for the pipids, *Occidozyga laevis* Günther, 1858 and *Lepidobatrachus laevis* Budgett, 1899, which retain their lateral line system as adults (Frittsch et al., 1987; Wahnschaffe et al., 1987; Quinzio, 2011). Embryos of species with direct development lack a lateral line system (Northcutt, 1989; Schlosser, 2002a).

Although the lateral line system might be able to provide characters associated with the variation in larval morphology, the morphological variation of the different components of the system (e.g., morphology of the organs, organization of neuromasts in the lines, pattern of line distribution, innervation) has not been explored in all larval types. Paterson (1939), Holmgren and Pehrson (1949), Murray (1955), Shelton (1970), and Schlosser and Northcutt (2000) described the development, morphology, and innervation of the lateral line system in *Xenopus laevis* Daudin, 1802, and Schlosser and Roth (1995) described the distribution and innervation of lateral lines in *Discoglossus pictus* Otth, 1837. For Types II and IV larvae, Strong (1895), Lannoo (1987), Chou and Lin (1997), Hall et al. (2002), Quinzio et al. (2006), Fabrezi and Quinzio (2008), Fabrezi et al. (2009), and Fabrezi et al. (2012) described the interspecific variation in the arrangement of the lateral lines of about 70 species, showing variation in the number, extension, and relationship among the lines of neuromasts. From these studies, the orbital and trunk lines of neuromasts have been interpreted as the most conservative in anuran tadpoles in relation to their number and arrangement, while the mandibular lines of neuromasts appear to be the most variable. Importantly, features of the lateral line system were not included in descriptions of the larval types (Orton, 1953; Starrett, 1973; Sokol, 1977; Haas, 2003), even though mandibular neuromast lines are morphologically (and probably functionally) associated with the mouth (oral sensibility) and feeding, where the greatest differences among these larval types are found.

At present, the available information on the lateral line system of anuran tadpoles is dispersed and not equivalent, since some studies described variation which only refers to the arrangement of lines of neuromasts (Lannoo, 1987; Chou and Lin, 1997; Hall et al., 2002; Quinzio et al., 2006; Fabrezi and Quinzio, 2008; Fabrezi et al., 2009; Fabrezi et al., 2012) while other studies also consider the innervation of the neuromasts (Paterson, 1939; Schlosser and Roth, 1995; Schlosser and Roth, 1997; Schlosser and Northcutt, 2000). Studies presenting details of the nerves of the lateral lines (besides the arrangement of the organs), and an adequate terminology for the neuromast lines based on enough morphological information (arrangement of neuromasts and innervation) that ensures unambiguous identification of each line of neuromasts independently of the morphology of the species studied are desirable.

Here, we present the analysis of the neuromasts innervated by the anterodorsal and anteroventral lateral line nerves, including the morphology and arrangement of the neuromasts in a set of species whose larvae belong to Types II and IV. We also reanalyze these features in tadpoles of *X. laevis* (Type I) as the most comprehensive analyses of these features were provided in a study in which the anterior lateral line nerves were considered a single nerve (anterior lateralis nerve *sensu* Paterson 1939). In addition, we emphasize the relationship between the anterodorsal and anteroventral lateral line nerves (Ad and Av), and the trigeminal (V) and facial (VII) nerves. In *D. pictus* tadpoles, in which nerves V and VII present unfused roots and ganglia (Sokol, 1975), the innervation of the mandibular neuromasts involves the close association of the facial and the anteroventral

nerves, in which fibers of both nerves are joined in the *ramus mandibularis externus* of VII (Schlosser and Roth, 1995). The relationship of the anterodorsal and anteroventral lateral line nerves to the trigeminal and facial nerves was not considered by Sokol (1975) when he proposed the condition of trigeminal and facial ganglia as a character to distinguish basal from advanced anurans. So we also include the description of this feature for species of Larval Types I, II, and IV.

For this purpose, we selected larval specimens of *X. laevis*, representing Larval Type I (filtering), and *Dermatonotus muelleri* Boettger, 1885 and *Elachistocleis bicolor* Guérin-Ménéville, 1838, representing Larval Type II (filtering). *X. laevis* belongs to the Pipidae Gray, 1825, whereas *D. muelleri* and *E. bicolor* are included in the Gastrophryninae Fitzinger, 1843 clade of Microhylidae Günther, 1858 (Frost, 2013). Among larvae of Type IV, we chose different ecomorphotypes: tadpoles of *Ceratophrys pierottii* Vellard, 1948, *Pseudis paradoxa* Linnaeus, 1758, *Telmatobius atacamensis* Gallardo, 1962, and *Telmatobius oxycephalus* Vellard, 1946 represent the typical pond-type tadpole with generalized suspension feeding (Duellman and Trueb, 1986; Wassersug and Heyer, 1988); tadpoles of *Ceratophrys cranwelli* Barrio, 1980 and *Lepidobatrachus* spp. Budgett, 1899 present specialized carnivorous habits, *C. cranwelli* tadpoles are macrophagous while larvae of *Lepidobatrachus* spp. have megalophagous habits (Ruibal and Thomas, 1988; Wassersug and Heyer, 1988).

Based on examination of these taxa and data available in the literature we describe the variation of lines of neuromasts related to the anterodorsal and anteroventral nerves by comparing the four larval types, with the aim of interpreting this variation and discussing the morphological evolution within anuran larvae.

MATERIALS AND METHODS

Larval specimens of 10 anurans species belonging to five families and three larval types were analyzed: *X. laevis* (Pipidae, Type I); *D. muelleri* and *E. bicolor* (Microhylidae, Type II); *C. cranwelli*, *Ch. pierottii*, *L. laevis*, *L. llanensis* and *L. asper* (Ceratophryidae, Type IV); *P. paradoxa* (Hylidae, Type IV); *T. atacamensis* (Telmatobiidae, Type IV). We also studied one larval specimen of *Bombina variegata* Linnaeus, 1758 to describe the morphology of the neuromasts in tadpoles of Type III.

Larval specimens were staged by standard tables of Nieuwkoop and Faber, 1956 for tadpoles of *X. laevis*, and Gosner (1960) for tadpoles of Types II and IV. Five to ten specimens per species between Gosner Stages 35 and 37 of Types II and IV, and five specimens of *X. laevis* at Stage 57 of Nieuwkoop and Faber, were selected to conduct the observations and analyses. The stages studied correspond to the larval period at which the system is completely developed (Schlosser, 2002b).

Neuromasts were identified and described by direct observations of preserved specimens with a Nikon-SMZ1000 dissection stereomicroscope equipped with a Nikon Coolpix 8.1 MP digital camera. Lateral lines are defined by individual neuromasts or two or more neuromasts organized in stitches or grouped irregularly. In *Lepidobatrachus* spp., *D. muelleri*, *E. Bicolor*, and *X. laevis*, the observation of the lines is made difficult by the coloration of the larval integument; therefore, the specimens were lightly colored with methylene blue to enhance contrast between the integument and neuromasts.

Histological sections were obtained from separated pieces of the skin containing neuromasts of the **Oral**, **Angular**, and **Preopercular** lines of tadpoles of *B. variegata*, *C. cranwelli*, *Ch. pierottii*, *D. muelleri*, *L. laevis*, *L. llanensis*, *P. paradoxa*, *T. atacamensis*, and *X. laevis*. The skin pieces were dehydrated, embedded in paraffin, sectioned at 6 μ m, and stained with hematoxylin-eosin (Martoja and Martoja-Pierson, 1970). Descriptions, illustrations, and photographs were made with a Leica DM microscope, equipped with an 8.1 megapixel Nikon Coolpix digital camera. We examined the size of the neuromasts in transverse view and their shape and relation with the different integument layers, the size of the neuromasts is given in μ m as (height \pm the standard deviation \times width \pm the standard deviation). To estimate the mean size of the neuromasts we selected 5–12 organs at random from the histological preparation.

For scanning electron microscopy (SEM) we selected tadpoles of *C. cranwelli*, *Ch. pierottii*, *D. muelleri*, *L. laevis*, *L. llanensis*, *P. paradoxa*, *T. atacamensis*, and *X. laevis*. We used separated pieces of the skin containing neuromasts of the **Oral**, **Angular**, and **Preopercular** lines, which were processed following the technique of Bozola and Russell (1999). Observations were made in SEM at the LASEM (Laboratorio de Microscopía Electrónica de Barrido, NPCyT/UNSa/CONICET), Salta, Argentina. SEM images allowed us to document the following data on mechanoreceptor morphology: the size of each organ on the surface (major and minor axes), the average number of hair cells per neuromast, the arrangement of the organs in the lateral lines (single or in stitches) and; the number of neuromasts per stitch or group.

Innervation of the neuromasts was analyzed from whole mount preparations stained for peripheral nerves with Sudan Black B, following the procedures described by Filipinski and Wilson (1984) and Nishikawa (1987) (Fig. 1). We used the terminology by Schlosser and Roth (1995) to name lines of neuromasts, except for the **Jugal** line, which we instead named the **Oral** line (see Table 2 and Discussion). The lines of neuromasts are described in cephalo-caudal direction and are indicated in bold throughout the text to facilitate interpretation of results.

For cranial and lateral line nerves we applied the terminology provided by Schlosser and Roth (1995) for *D. pictus*, with some modifications taken from Northcutt (1992) for lateral line nerves of *Ambystoma mexicanum*. In the configuration of the anteroventral lateral line nerve, we noted the incorporation of the ramules innervating the **Angular**, **Oral**, and **Preopercular** lines into the *dorsal* and *ventral components* of the common *ramus mandibularis externus* of the facial and anteroventral nerves (VII–Av). We named the components of the nerves in a hierarchical order of branching, as follows: nerve, trunk, ramus, and ramule. The ramules of the anteroventral nerve were named according to the lines of neuromasts they innervate. The cranial and lateral line nerves are described in distal to proximal direction and the *rami* and *ramules* of cranial and lateral line nerves are written in italics throughout the text to facilitate the interpretation of results.

Specimens are deposited in the Herpetological Collection of the Museo de Ciencias Naturales (MCN),

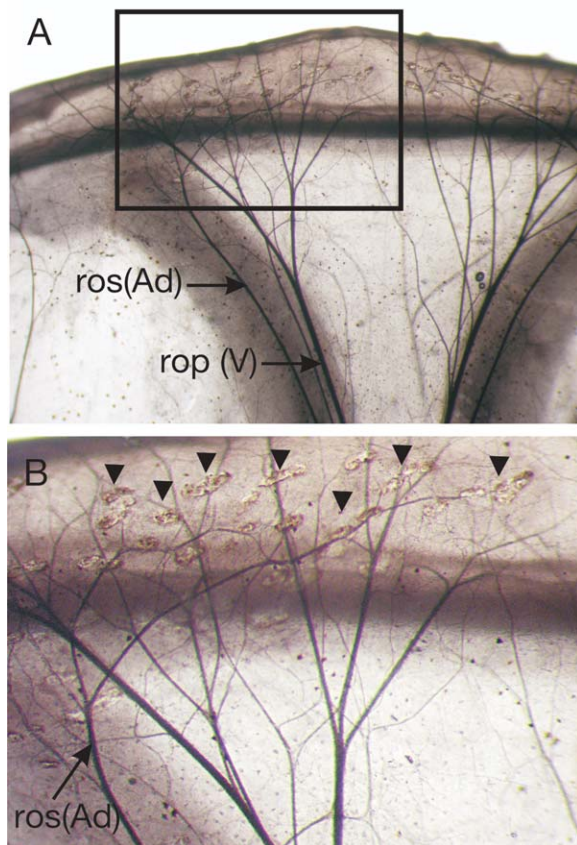


Fig. 1. *In toto* preparations with Sudan Black stain for peripheral nerves in tadpoles of *L. laevis* at Stage 37. (A) Detail of the anterior end of the upper jaw in which groups of supraorbital neuromasts are seen. (B) Magnification of the box showing in A in which are distinguished the branches of the superficial ophthalmic ramus of the anterodorsal nerve and the groups of supraorbital neuromasts (black arrowheads) that they innervate. Abbreviations used: ros (Ad) = ramus ophthalmicus superficialis of anterodorsal lateral line nerve; rp (V) = ramus ophthalmicus profundus of trigeminal nerve.

Universidad Nacional de Salta (Argentina) with the following collection data: *C. cranwelli* (MCN 425, MCN 427, MCN 1048); *Ch. pierottii* (MCN 1027, MCN 1028, MCN 1029); *L. laevis* (MCN 278, MCN 1109, MCN 1016, MCN 1118, MCN 1119); *L. llanensis* (MCN 1118, MCN 1119, MCN 1122, MCN 1178); *T. atacamensis* (MCN 1026, 1050); Hylidae: *P. paradoxa* (MCN 597, MCN 682, MCN 683, MCN 684, MCN 973, MCN 1015, MCN 1055, MCN 1056, MCN 1138, MCN 1197); Microhylidae: *D. muelleri* (MCN 961, MCN 1080, MCN 1102, MCN 1162, MCN 1180); *E. bicolor* (MCN 1090), *X. laevis* (MCN 490). Specimens of *L. asper* (LGE 1351) are deposited in the Colección Herpetológica del Laboratorio de Genética Evolutiva (LGE) de la Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Misiones (Argentina).

RESULTS

Morphology of Neuromasts

In superficial view the neuromasts have a central zone where the hair cells form a sensory cupule that is surrounded by the supporting cells, and mantle cells are

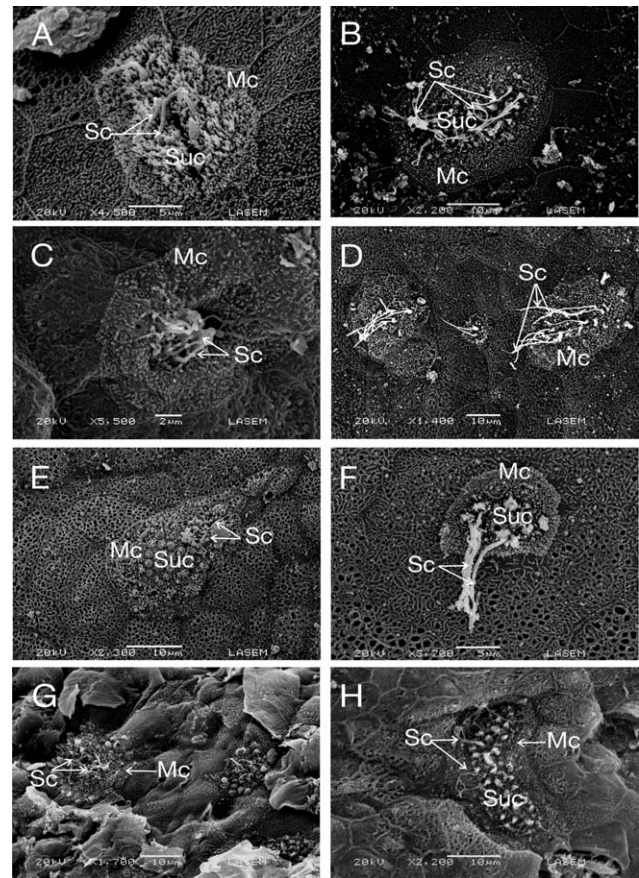


Fig. 2. SEM images of neuromast morphology in the mandibular lateral lines of anuran larvae at Stage 37. (A–E) Single oval-shaped neuromasts. (A) *Ch. pierottii*. General morphology of a single neuromast of the angular line showing the different cell types compound it. (B) *C. cranwelli*. Detail of a preopercular neuromast. (C) *P. paradoxa*. Detail of a neuromast of the oral line. (D) *T. atacamensis*. Two angular neuromasts. (E) *X. laevis* at Stage 57. General morphology of a neuromast of the oral line. (F) *D. muelleri*. Detail of an oral neuromast. (G) *L. laevis*. Two rounded neuromasts of the oral line. (H) *L. llanensis* one angular elongated neuromast. In all the images the cephalocaudal axis of the larvae is from left to right. Abbreviations used: Mc = mantle cells; Sc = sensory cells; Suc = supporting cells.

located more externally, delimiting each neuromast (Fig. 2). Neuromasts are oval in tadpoles of *Ch. pierottii*, *C. cranwelli*, *P. paradoxa*, *T. atacamensis*, and *X. laevis* (Fig. 2A–E) and rounded in *D. muelleri* and *L. laevis* (Fig. 2F,G). The shape of the neuromasts varies from rounded to oblong in *L. llanensis* tadpoles (Fig. 2H). Other variation, such as their size, number of sensory cells, and disposition of the neuromasts in the lines is detailed in Table 1.

In transverse sections, the neuromasts in *B. variegata*, *Ch. pierottii*, and *D. muelleri* are oval-shaped. The organs are completely engulfed in the epidermis, and it is difficult to distinguish the different cell types that compose them (Fig. 3A–C). In the remaining species, the neuromasts are pear-shaped and are housed in the epidermis, but the kinocilia of the sensory cells protrude slightly from the surface (Figs. 3D–F and 4). The sensory cells are surrounded by the supporting cells, which

TABLE 1. Morphological characterization by SEM examination of neuromasts innervated by the anteroventral lateral line nerve in different anuran species.

Taxon	Neuromast size (μm)	Number of hair cells/neuromast	Neuromast arrangement in the lines	Number of neuromasts per stitch
<i>Xenopus laevis</i>	$24 \pm 8 \times 18 \pm 4$ ($n = 10$)	6–15	Stitch	8–12
<i>Dermatonotus muelleri</i>	$15 \pm 3 \times 10 \pm 2$ ($n = 15$)	4–6	Group	4–6
<i>Discoglossus pictus</i>	–	2–5	Single	–
<i>Pseudis paradoxa</i>	$22 \pm 8 \times 15 \pm 6$ ($n = 11$)	3–6	Stitch	10–15
<i>Telmatobius atacamensis</i>	$28 \pm 5 \times 20 \pm 3$ ($n = 13$)	10–12	Stitch	2–6
<i>Chacophrys pierottii</i>	$22 \pm 8 \times 16 \pm 6$ ($n = 10$)	3–6	Single	–
<i>Ceratophrys cranwelli</i>	$29 \pm 7 \times 20 \pm 4$ ($n = 10$)	10–12	Single	–
<i>Lepidobatrachus laevis</i>	$29 \pm 5 \times 18 \pm 3$ ($n = 14$)	3–6	Group	2–6
<i>Lepidobatrachus llanensis</i>	$28 \pm 9 \times 16 \pm 3$ ($n = 12$)	3–6	Group	4–7

Size is given as major \times minor axes in surface view.

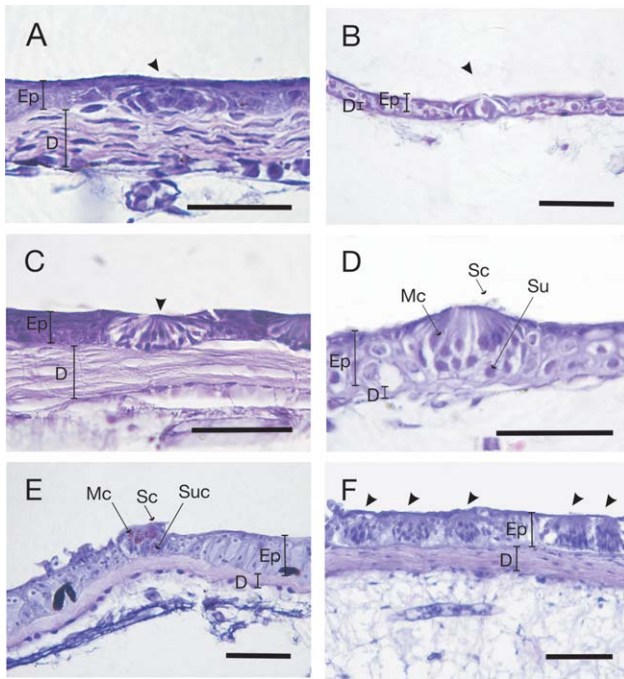


Fig. 3. Histological sections of throat skin containing neuromasts from the mandibular lines of anuran larvae at Stage 37. (A) *Ch. pierottii*. Single neuromasts of the oral line. (B) *B. variegata*, single neuromasts of the angular line. (C) *D. muelleri*, single neuromasts of angular line. (D) *X. laevis* at Stage 57. Single pear-shaped neuromasts of the angular line showing the different cell types compound it. (E–F) *P. paradoxa*. (E) One pear-shaped neuromast of the angular line. (F) Stitch of five neuromasts of the preopercular line. Abbreviations used: D = dermis; Ep = epidermis; Mc = mantle cells; Sc = sensory cells; Suc = supporting cells. Scale: 50 μm .

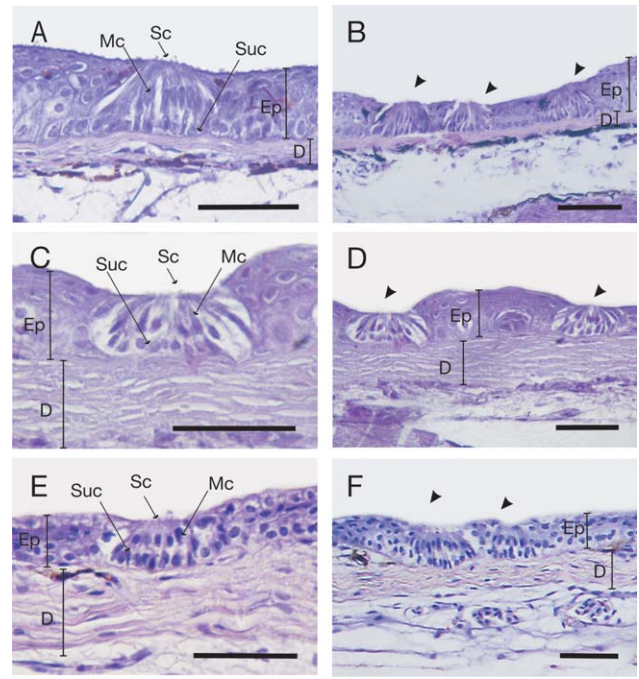


Fig. 4. Histological sections of throat skin containing neuromasts from the mandibular lines of anuran larvae at Stage 37 showing the different cell types compound it. (A–B) *T. atacamensis*. (A) Single neuromast of the oral line. (B) Stitch of three oral neuromasts. (C–D) *C. cranwelli*. (C) Single neuromast of the angular line. (D) Two paired neuromasts of the oral line. (E–F) *Lepidobatrachus* spp. (E) *L. llanensis*. Single neuromast of the oral line. (F) *L. laevis*. Two paired neuromasts from the angular line. Abbreviations used: D = dermis; Ep = epidermis; Mc = mantle cells; Sc = sensory cells; Suc = supporting cells. Scale: 50 μm .

are in contact with the basal membrane; supporting cells are large and columnar with basal nuclei. The neuromasts are delimited by mantle cells which are small and spindle-shaped (Figs. 3 and 4).

Lines of Neuromasts

We identified in the studied species two pairs of lines of neuromasts (**Supraorbital** and **Infraorbital**) innervated by the anterodorsal nerve, and three pairs of lines of neuromasts (**Oral**, **Angular**, and **Preopercular**)

supplied by the anteroventral nerve. The mandibular lines of neuromasts (**Oral**, **Angular**, and **Preopercular**) exhibit variation in their arrangement, length, and relationship with the **Supraorbital** and **Infraorbital** lines.

In *X. laevis*, the **Oral**, **Angular**, and **Preopercular** neuromasts can be observed in ventral view (Fig. 5A). The **Preopercular line** has two segments of neuromasts (inferior and superior). With the exception of the neuromasts at the base of the tentacle, which are in disorganized groups, the **Angular** and **Preopercular** lines

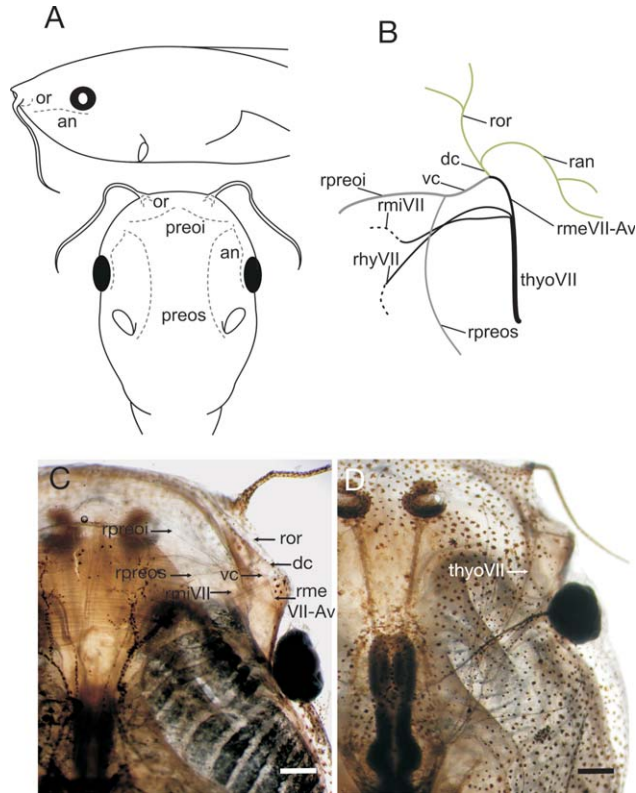


Fig. 5. Arrangement and innervation of mandibular lateral lines in tadpoles of *X. laevis* at Stage 57. **B**, **C**, and **D**, anterior to the top. **(A)** Schematic drawing of the mandibular lateral lines in lateral and ventral view. **(B)** Schematic drawing of the anteroventral nerve configuration, fibers of the facial nerve in black lines, fibers of the anteroventral nerve in green lines (dorsal components of the rmeVII-Av) and gray lines (ventral components of the rmeVII-Av). **(C-D)** *In toto* preparations with Sudan Black stain for peripheral nerves. **(C)** Dorsal view of the hyomandibular trunk of the facial nerve and its association with the anteroventral nerve. **(D)** Ventral view of the configuration of the external mandibular ramus of the facial and anteroventral lateral line nerves. Abbreviations used: an = angular line; dc = dorsal component of the external mandibular ramus of VII-Av; or = oral line; preoi = inferior preopercularis line; preos = superior preopercularis line; ran = angular ramule; rhyVII = hyoid ramus of the hyomandibular trunk of the nerve VII; rmeVII-Av = external mandibular ramus of the facial and anteroventral lateral line nerves; rmiVII = internal mandibular ramus of facial nerve; ror = oral ramule; rpreoi = ramule inferior preopercularis; rpreos = ramule superior preopercularis; thyoVII = hyomandibular trunk of facial nerve; vc = ventral component of the externus mandibular ramus. Scale: 1 mm.

are formed of neuromasts organized in stitches of 3 to 12 organs with their main axis parallel to the lateral line (Fig. 6A). The **Angular** lines extend from the oral commissure caudally up to level of the eye. These lines are innervated by the *angular ramules*, which present small branches at their distal ends; each *angular ramule* runs parallel to the neuromasts of the **Angular** line and descends to become incorporated into the dorsal component of the *mandibular externus ramus* of VII-Av. There is a group of neuromasts located at the base of the tentacle; these organs are innervated by two thin branches that join in the *oral ramule*; a similar innervation is found in the **Oral** lines of neuromasts of most anurans.

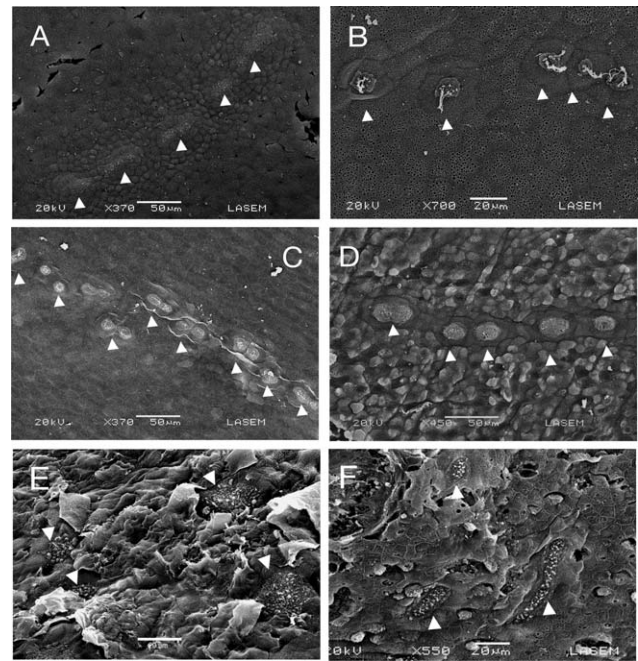


Fig. 6. SEM images of neuromast organization in the mandibular lateral lines of anuran larvae. **(A)** *X. laevis* at Stage 57. Longitudinal stitch of the neuromasts (white arrowheads) of the oral line. **(B-F)** Larval Stage 37. **(B)** *D. muelleri*. Group of irregularly arranged oral neuromasts. **(C)** *P. paradoxa*. Longitudinal stitch of the angular line. **(D)** *T. atacamensis*. Neuromasts of the angular line arranged in a longitudinal stitch. **(E-F)** *Lepidobatrachus* spp. *L. laevis*. Group of round and irregularly arranged neuromasts of the angular line housed in low depressions of the integument. **(F)** *L. llanensis*. Group of irregularly arranged angular neuromasts. In all the images the cephalocaudal axis of the larvae is from left to right.

The *oral ramule* together with the *angular ramule* join the *dorsal component* of the *mandibular externus ramus* of VII-Av (Fig. 5B-D). The inferior segment of the **Preopercular** line originates in continuity with the **Angular line** and extends up to the midline. The superior segment of the **Preopercular** line arises at the oral commissure and run caudally and ventrally to the **Angular line** to end at the base of the spiracle. The two segments of each **Preopercular** line are innervated by the ramules *preopercularis inferior* and *preopercularis superior* which run parallel to them. These *preopercular ramules* join in the *ventral component*; proximally, the *mandibular externus ramus* of VII-Av incorporates the *dorsal and ventral components* (Fig. 5). The *mandibular externus ramus* joins with the *mandibularis internus* and *hyoid rami* to form *hyomandibular trunk* of VII (Fig. 5B-D). The *hyomandibular trunk* runs posteriorly up to the anterior margin of the optic capsule, where it joins with the *palatine ramus* to complete the facial nerve. The ganglionic cells of the trigeminal, facial, anterodorsal, and anteroventral lateral line nerves form the prootic ganglion (Fig. 7A). The prootic ganglion is located at the prootic foramen, which is limited anteriorly by the orbital cartilage and posteriorly by the otic capsule. From the prootic ganglion, fibers of the trigeminal extend in to the brain to form its root, whereas the anterodorsal, anteroventral, and facial nerves emerge together in a single root (Fig. 7A).

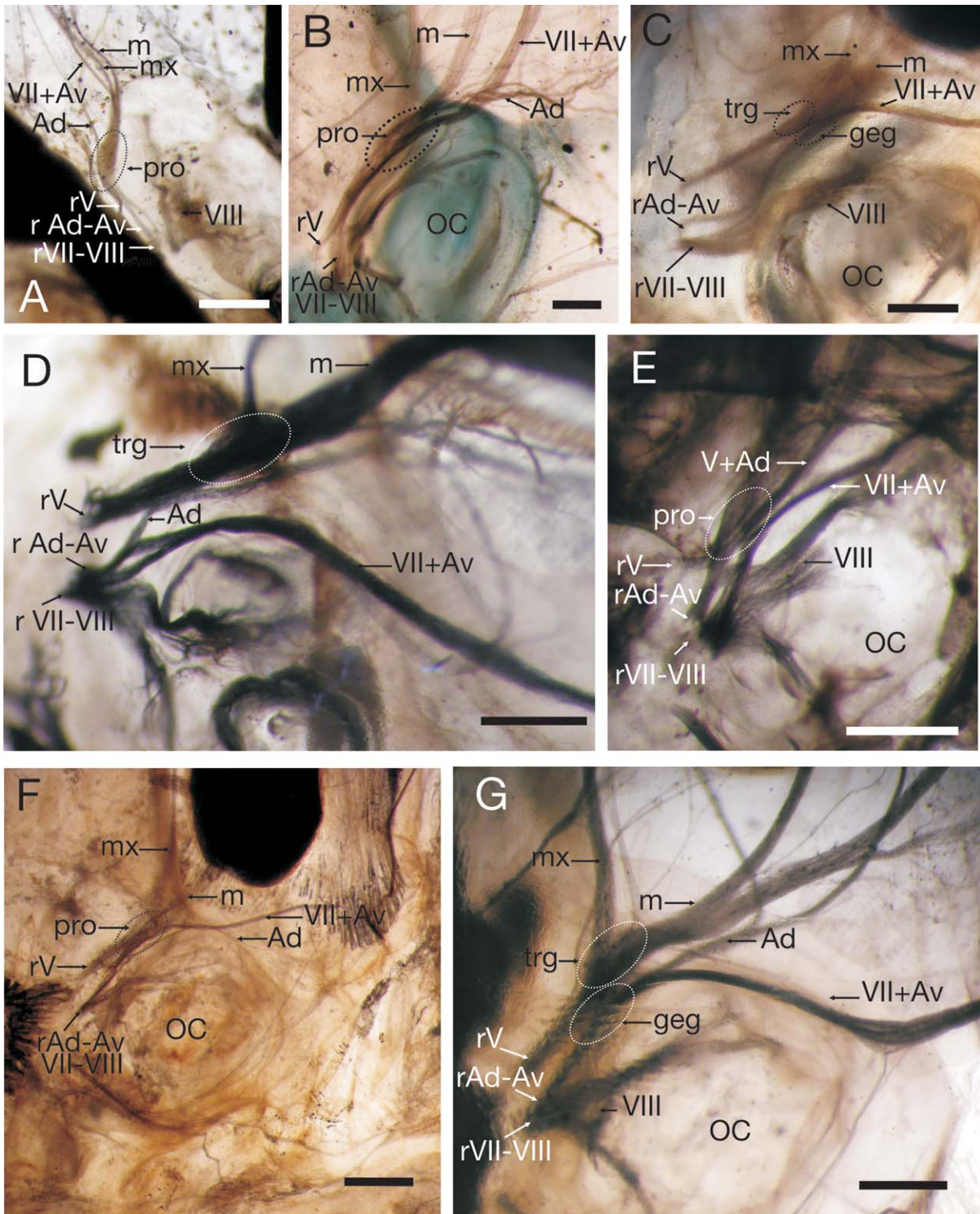


Fig. 7. *In toto* preparations for peripheral nerves of anuran tadpoles. (A) *X. laevis*. Ventral view of the roots of the anterior lateral line nerves and their relation with other cranial nerves at larval Stage 57. (B-G) Ventral view of the roots of the anterior lateral line nerves and their relation with other cranial nerves at larval Stage 37. (B) *D. muelleri*. (C) *T. atacamensis*. (D) *C. cranwelli*. (E) *Pseudis pradoxa*. (F) *Ch. pierottii*. (G) *L. laevis*. The dotted lines indicate the putative location of the sensory ganglia. A-G anterior to the top. Abbreviations used: Ad = anterodorsal lateral line nerve; geg = geniculate ganglion;

m = mandibular ramus of the trigeminal nerve; mx = maxilomandibular ramus of the trigeminal nerve; OC = otic capsule; pro = prootic ganglion; rAd-Av = common root of the anterior lateral line nerves; rAd-Av-VII-VIII = common root of the facial, vestibular and anterior lateral line nerves; rV = root of the trigeminal nerve; rVII-VIII = common root of the facial and vestibular nerves; trg = trigeminal ganglion; VIII = vestibular nerve; V + Ad = trigeminal nerve containing fibers of the anterodorsal lateral line nerve; VII + Av = facial nerve containing fibers of the anterodorsal lateral line nerve. Scale: 1 mm.

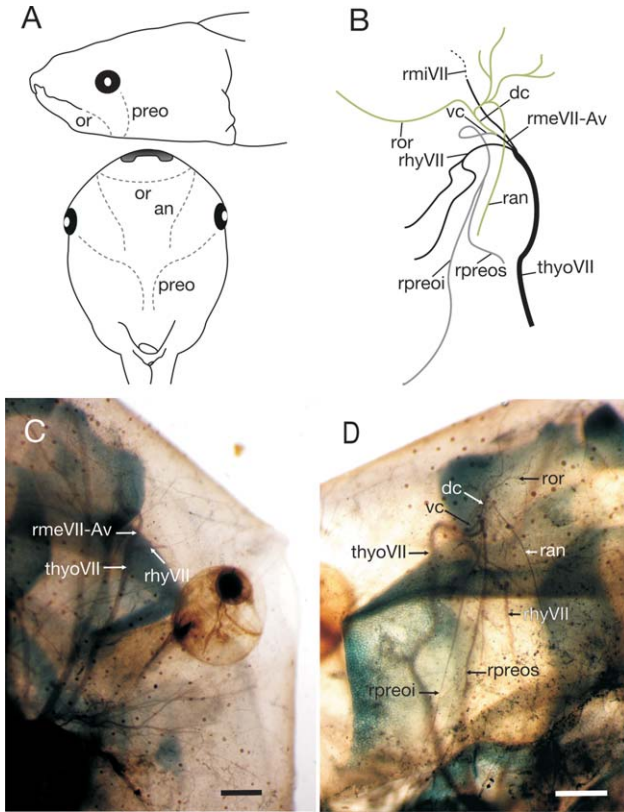


Fig. 8. Arrangement and innervation of mandibular lateral lines in tadpoles of *D. muelleri* at Stage 37. **(A)**, **(B)**, **(C)**, and **(D)**, anterior to the top. **(A)** Schematic drawing of the mandibular lateral lines in lateral and ventral view. **(B)** Schematic drawing of the Av configuration, fibers of the facial nerve in black lines, fibers of the anteroventral nerve in green lines (dorsal components of the rmeVII-Av) and gray lines (ventral components of the rmeVII-Av). **(C-D)** *In toto* preparations with Sudan Black stain for peripheral nerves. **(C)** Dorsal view of the hyomandibular trunk of the facial nerve and its association with the anteroventral nerve. **(D)** Ventral view of the configuration of the external mandibular ramus of the facial and anteroventral lateral line nerves. Abbreviations used: an = angular line; dc = dorsal component of the external mandibular ramus of VII-Av; or = oral line; preo = preopercularis line; ran = angular ramule; rhyVII = hyoid ramus of the hyomandibular trunk of the nerve VII; rmeVII-Av = external mandibular ramus of the facial and anteroventral lateral line nerves; rmiVII = internal mandibular ramus of facial nerve; ror = oral ramule; rpreoi = ramule inferior preopercularis; rpreos = ramule superior preopercularis; thyo-VII = hyomandibular trunk of facial nerve; vc = ventral component of the externus mandibular ramus. Scale: 1 mm.

D. muelleri and *E. bicolor* present rounded neuro-masts organized in groups of 3 to 6 and disposed irregularly (Fig. 6B). The **Angular**, **Oral**, and **Preopercular** lines are placed ventrally on the gular surface. Each **Oral** line extends from the oral commissure, runs ventrally and parallel to the lower lip and joins with its opposite **Oral** line (Fig. 8A). Each **Angular** line descends caudally from the oral commissure, runs ventrally parallel to the other and ends behind the level of the eyes (Fig. 8A). Each **Preopercular** line extends from behind the level of the eye up to the midline, where it turns posteriorly and extends in caudal direction, ending near the spiracle (Fig. 8A). In *D. muelleri*, the **Angular** lines of neuromasts are innervated by the

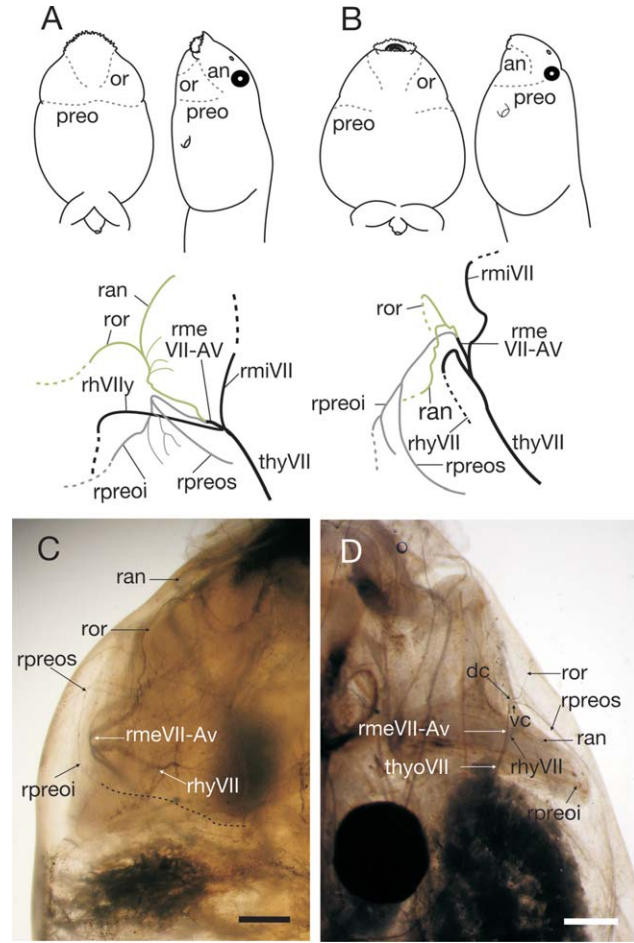


Fig. 9. Arrangement and innervation of mandibular lateral lines in selected Type IV tadpoles at Stage 37. Anterior to the top. **(A-B)** Schematic drawing of the mandibular lateral lines and anteroventral nerve configuration, fibers of the facial nerve in black lines, fibers of the anteroventral nerve in green lines (dorsal components of the rmeVII-Av) and gray lines (ventral components of the rmeVII-Av). **(A)** *C. cranwelli*. **(B)** *Ch. pierottii*. **(C-D)** *In toto* preparations with Sudan Black stain for peripheral nerves of the hyomandibular trunk of the facial nerve and the configuration of the external mandibular ramus of the facial and anteroventral lateral line nerves. **(E)** *T. atacamensis* dorsal view. **(C)** *C. cranwelli*, ventral view. **(D)** *Ch. pierottii*, dorsal view. Abbreviations used: an = angular line; dc = dorsal component of the external mandibular ramus of VII-Av; or = oral line; preo = preopercularis line; racc = ramule accessory; ran = ramule angular; rpreoi = ramule preopercularis inferior; rpreos = ramule preopercularis superior; rhyVII = hyoid ramus of the hyomandibular trunk of the facial nerve; rmeVII-Av = external mandibular ramus of the facial and anteroventral lateral line nerves; rmiVII = internal mandibular ramus of the facial and anteroventral lateral line nerves; ror = oral ramule; thyo-VII = hyomandibular trunk of the facial nerve; vc = ventral component of the externus mandibular ramus of the facial and anteroventral lateral line nerves. Scale: 1 mm.

angular ramules, which run parallel to the organs. The *angular ramules* are incorporated into the *dorsal component of the mandibularis externus ramus* of the VII-Av. The neuromasts of the **Oral** lines are innervated by the *oral ramules*, which extends ventrally and parallel to the oral disc. The *angular ramules* join the *dorsal component of the mandibularis externus ramus* of the

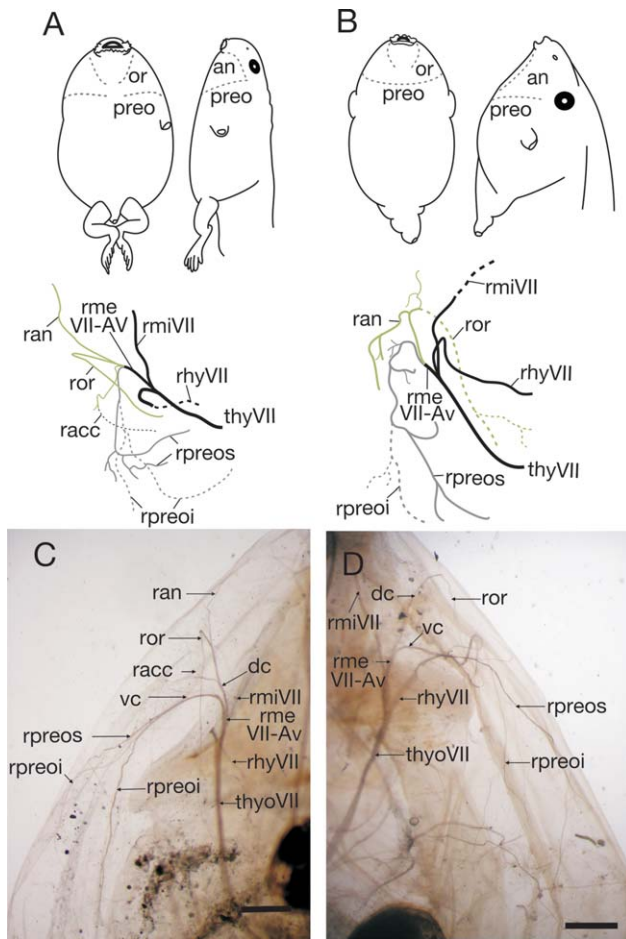


Fig. 10. Arrangement and innervation of mandibular lateral lines in selected Type IV tadpoles at Stage 37. Anterior to the top. (A–B) Schematic drawing of the mandibular lateral lines and anteroventral nerve configuration, fibers of the facial nerve in black lines, fibers of the anteroventral nerve in green lines (dorsal components of the rme-VII) and gray lines (ventral components of the rme-VII–Av). (A) *T. atacamensis*. (B) *P. paradoxa*. (C–D) *In toto* preparations with Sudan Black stain for peripheral nerves of the hyomandibular trunk of the facial nerve and the configuration of the external mandibular ramus of the facial and anteroventral lateral line nerves. (B) *T. atacamensis* dorsal view. (C) *P. paradoxa*, dorsal view. Abbreviations used: an = angular line; dc = dorsal component of the external mandibular ramus of VII–Av; or = oral line; preo = preopercularis line; racc = ramule accessory; ran = ramule angular; rpreoi = ramule preopercularis inferior; rpreos = ramule preopercularis superior; rhyVII = hyoid ramus of the hyomandibular trunk of the facial nerve; rmeVII–Av = external mandibular ramus of the facial and anteroventral lateral line nerves; rmi-VII = internal mandibular ramus of the facial and anteroventral lateral line nerves.; ror = oral ramule; thyoVII = hyomandibular trunk of the facial nerve; vc = ventral component of the externus mandibular ramus of the facial and anteroventral lateral line nerves. Scale: 1 mm.

VII–Av posterior to the *oral* ramules (Fig. 8B–D). The **Preopercular** lines are supplied by two thin ramules called *preopercularis superior* and *preopercularis inferior*; these ramules are incorporated into the *ventral component* of the *mandibularis externus ramus* of the VII–Av (Fig. 8B–D). The ganglion cells of the trigeminal, facial, anterodorsal, and anteroventral lateral line nerves form the prootic ganglion (Figs. 7B, 11A).

In *C. cranwelli*, *Ch. pierottii*, *T. atacamensis*, *T. oxycephalus*, and *P. paradoxa* (Figs. 9A–B and 10A–B), the **Angular**, **Oral**, and **Preopercular** lines are observed in lateral and ventral views with little variation in their arrangement. In *C. cranwelli* and *Ch. pierottii*, the lateral lines are formed by single neuromasts but sometimes they seem to be arranged in pairs (Fig. 4D), whereas in *P. paradoxa* and *Telmatobius* spp. the neuromasts are organized in stitches of 10–15 and 3–6 organs, respectively, with their main axis parallel to the lateral line (Fig. 6C,D). The **Oral** lines descend from the oral commissures, and end separately in the gular region (Fig. 9A,B). The anterior end of each **Angular** line is contiguous with the anterior end of each **Preopercular** line (in lateral view); the **Angular** lines extend anteriorly to reach the oral disc, where they end dorsally to the **Oral** lines (Fig. 9A,B). Each **Preopercular** line descends from the inferior margin of the eye to the midline; they join the opposite line in *C. cranwelli* and *P. paradoxa* but end separately in *Ch. pierottii* and *Telmatobius* spp. (Figs. 9A–B and 10A–B).

In *Telmatobius* spp., the **Angular** and **Oral** lines are innervated by the *angular* and *oral* ramules, respectively, which are incorporated into the *dorsal component* of the *mandibularis externus ramus* of VII–Av (Fig. 10A,C). The **Preopercular** lines are innervated by the *preopercularis superior* and *preopercularis inferior* ramules. A thin ramule that extends caudally and is related to the **Preopercular** lines (named the *accessory ramule*) also joins the *dorsal component* of the *mandibularis externus ramus* of the VII–Av (Fig. 9A,C). Fibers of the anterodorsal lateral line nerve are seen on top of the trigeminal ganglion while the ganglionic cells of the anteroventral nerve are fused with the geniculate ganglion of the facial nerve. The trigeminal fibers form a single root whereas the fibers of the anterodorsal nerve run dorsally and posteriorly and join up near the root of the trigeminal with fibers of the anteroventral and facial nerves (Fig. 7C).

In *P. paradoxa*, the **Angular** lines are absent (Fig. 10B); concomitantly, each *dorsal component* of the anteroventral lateral line nerve is formed by a single *oral ramule*, which innervates the neuromasts of the **Oral** lines (Fig. 10D). *P. paradoxa* and *C. cranwelli* share a bilateral arrangement of the **Preopercular** lines, which join ventrally (Fig. 10B). The innervation of these lines involves two ramules, *preopercularis superior* and *preopercularis inferior*, the *preopercularis superior* ramule bifurcates and gives rise to dorsal and ventral branches (Fig. 10B,D). In *P. paradoxa*, the sensory cells of the anterior lateral line nerves are incorporated in the prootic ganglion with the trigeminal and facial nerves (Fig. 7E). In *C. cranwelli*, the anterodorsal lateral line nerve is dorsal to the anteroventral and facial nerves. In these species, the anterodorsal and anteroventral nerves form a common root that enters the brain posterior to the root of the trigeminal (Fig. 7C, D). In *Ch. pierottii*, the **Preopercular** lines are supplied by the two *preopercularis* ramules mentioned above (Fig. 9B, D). From the prootic ganglion, the roots of the nerves emerge; fibers of the trigeminal form a discrete root, whereas the anterodorsal, anteroventral and facial nerves are joined together in a single root (Fig. 7F).

In *Lepidobatrachus* spp. (Fig. 11), the lateral lines are formed by neuromasts arranged in groups of 3–7 organs

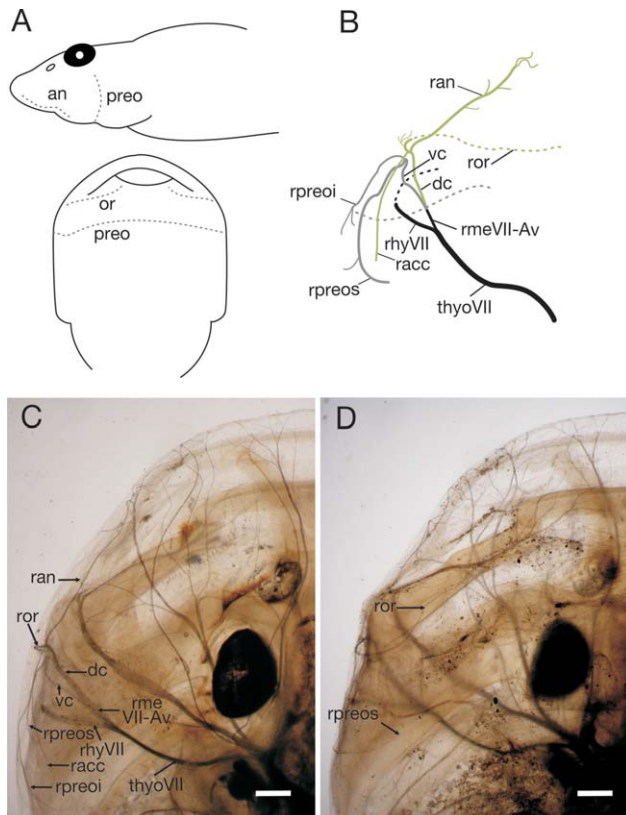


Fig. 11. Arrangement and innervation of mandibular lateral lines in *L. laevis* tadpoles at Stage 37. **B**, **C**, and **D**, anterior to the top. **(A)** Schematic drawing of the mandibular lateral lines in lateral and ventral view. **(B)** Schematic drawing of the anteroventral nerve configuration, fibers of the facial nerve in black lines, fibers of the anteroventral nerve in green lines (dorsal components of the rmeVII-Av) and gray lines (ventral components of the rmeVII-Av). **(C–D)** *In toto* preparations with Sudan Black stain for peripheral nerves. **(C)** Dorsal view of the hyomandibular trunk of the facial nerve and its association with the anteroventral nerve. **(D)** Ventral view of the configuration of the external mandibular ramus of the facial and anteroventral lateral line nerve. Abbreviations used: an = angular line; dc = dorsal component of the external mandibular ramus of the facial and anteroventral lateral line nerves; or = oral line; preo = preopercularis line; racc = ramule accessory; ran = ramule anterior; rpreoi = ramule preopercularis inferior; rpreos = ramule preopercularis superior; rhyVII = hyoid ramus of the hyomandibular trunk of the facial nerve; rmeVII-Av = external mandibular ramus of the facial and anteroventral lateral line nerves; ror = oral ramule; thyoVII = hyomandibular trunk of the facial nerve; vc = ventral component of the external mandibular ramus of the facial and anteroventral lateral line nerves. Scale: 1 mm.

(Fig. 6E–F). The **Angular**, **Oral**, and **Preopercular** lines can be observed in dorsal, lateral, and ventral views. *Lepidobatrachus* spp. are the only species exhibiting the **Angular** lines with a supralabial arrangement, extending from the oral commissure dorsally on the upper lip. The **Oral** lines run from the oral commissure ventrally and parallel to the lower lip. Each **Preopercular** line descends from the eye to join the other **Preopercular** line and forms a continuous transverse line (Fig. 11A). The neuromasts of each **Angular** line are innervated by a single ramule, which has many thin branches along its length (Fig. 11C,D), whereas the **Oral** lines are innervated by the *oral ramules*, which

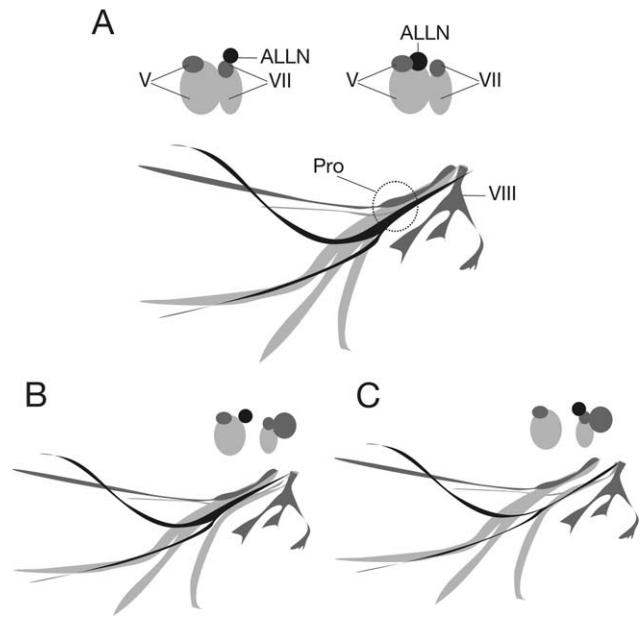


Fig. 12. Schematic representation of the relationships of V, VII, VIII, and anterior lateral line nerves. Sensory fibers are indicated in dark gray, the motor fibers in light gray and the lateral line fibers in black. **(A)** Transverse section of the prootic ganglion to show the relationships of the anterior lateral line nerves and trigeminal, and facial. The prootic ganglion (Pro) joins the nerves V, VII, and anterior lateral line nerves; the ganglionic components of the anterior lateral line nerves and sensory branches of V (ophthalmicus profundus V_1) and VII (palatine ramus) are dorsally situated in the ganglion. The roots of these nerves are aligned in cephalo-caudal orientation; a single root of V emerges from the lateral and anterior portion of the rhombencephalon; the anterodorsal lateral line nerve root is posterior and dorsal to the root of the V. The anterodorsal lateral line nerve root is placed between the roots of V and VII, near V in *X. laevis* and *D. muelleri*, or near the root of VII in *Ch. pierottii* and *P. paradoxa*. In addition, the facial nerve shares its root and part of its path with the anteroventral lateral line nerve. **(B, C)** In tadpoles of *T. atacamensis*, *Lepidobatrachus* spp., and *C. cranwelli* nerves V and VII are well-separated as the prootic ganglion is still not developed. The anterodorsal lateral line nerve root is near nerve V (**B**, in *T. atacamensis*) or contiguous with nerve VII in the remaining species (**C**). Abbreviations used: ALLN = anterior lateral line nerves; Pro = prootic ganglion; V = trigeminal nerve; VII = facial nerve; VIII = vestibular nerve.

run parallel to the neuromasts. Each *angular ramule* joins anteriorly to the *oral ramule* and, together, they form the *dorsal component* of the *mandibularis externus ramus* of the VII-Av. As in *Telmatobius* spp., an *accessory ramule* joins with the *angular* and *oral ramules* in the *dorsal component*; this *accessory ramule* seems to innervate a group of neuromasts close to the **Preopercular** lines. Most of the neuromasts of the **Preopercular** lines are innervated by the *preopercularis superior ramule*, which runs parallel to the *hyoid ramus* of the facial nerve (Fig. 11B–D), and by the *preopercularis inferior ramule*, which runs parallel to the *accessory ramule*. The *preopercularis superior* and *inferior ramules* converge in the *ventral component* of the *mandibularis externus ramus* of VII-Av (Fig. 10). The *external mandibular ramus* joins the *internal mandibular* and *hyoid rami* into the *hyomandibular trunk* of the facial nerve (Fig. 11C). The *hyomandibular trunk* joins the *palatine ramus* of the facial nerve and forms a small ganglion

TABLE 2. Comparisons of the lateral line system among the anuran tadpoles considering the four types defined by Orton (1953), Starrett (1973), and Sokol (1977).

Taxon and Tadpole Type	Views of lateral lines	Lateral lines	Lateral line nerve	V, Ad, Av and VII ganglia	V, Ad, Av, VII and VIII roots
Pipidae <i>Xenopus laevis</i> Type I	Dorsal	Supraorbital Infraorbital (Postorbital + Infraorbital)	<i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Ventral	Angular (Preorbital) Oral (Tentacular) Preopercular inferior (Mandibular) Preopercular superior (Hyomandibular)	<i>Angular</i> / Posterior ramule /rmed <i>Oral</i> / Anterior ramule /rmed <i>Preopercular superior</i> / Posterior ramule		
	Dorsal	Supraorbital Infraorbital Oral	<i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Angular ramule</i> /rmed	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Ventral	Angular Preopercular Supraorbital Infraorbital	<i>Preopercular ramule</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Angular ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
Discoglossidae <i>Discoglossus pictus</i> Type III	Dorsal	Angular (Jugal) Preopercular (Oral + Angular) Preopercular (Preopercular)	<i>Preopercularis inferior and preopercularis superior ramules</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Supraorbital Infraorbital Oral Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
Hylidae <i>Pseudis paradoxa</i> Type IV	Dorsal	Supraorbital Infraorbital Oral	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V (Ad+Av-VII+VIII)
Telmatobiidae <i>Telmatobius atacamensis</i> and <i>T. oxycephalus</i> Type IV	Dorsal	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior, preopercular inferior ramules</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
Ceratophryidae <i>Chacophrys pierottii</i> Type IV	Dorsal	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Fused in the prootic ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
Lepidobatrachidae <i>L. laevis</i> and <i>L. llanensis</i> Type IV	Dorsal	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
<i>Lepidobatrachus asper</i> , <i>L. laevis</i> and <i>L. llanensis</i> Type IV	Dorsal	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)
	Lateroventral	Supraorbital Infraorbital Oral Angular Preopercular	<i>Preopercular superior and preopercular inferior</i> /rmed <i>Ophthalmicus superficialis ramule</i> /Ad <i>Buccalis ramule</i> /Ad <i>Oral ramule</i> /rmed	Trigeminal and Geniculate ganglion	V and (Ad+Av-VII+VIII)

Abbreviations: Ad, anterodorsal lateral line nerve; Av, anteroventral lateral line nerve; rmed, dorsal component of mandibularis externus ramus of the facial and anteroventral lateral line nerves; rmev, ventral component of the mandibularis externus ramus of the facial and anteroventral lateral line nerves; V, trigeminal nerve; VII, facial nerve. The names of the mandibular lines used by Shelton (1970) in *Xenopus laevis* and Schlosser and Roth (1995) in *Discoglossus pictus* are in bold.

(geniculate ganglion). The anterodorsal lateral line nerve is located very close to the facial nerve, dorsal to it (Figs. 7G and 12C).

DISCUSSION

The variation recorded in the lateral line system among the four anuran tadpole types allow us to discuss some interpretations of patterns of variation: (1) the presence of two pairs of orbital lateral lines and three pairs of mandibular lateral lines is shared by all the anuran larvae in which the system has been studied; (2) the ventral arrangement of mandibular neuromast lines appears to have evolved convergently in Larval Types I and II (filtering tadpoles), and the lateroventral arrangement of mandibular lines of neuromasts appears to have evolved in Larval Types III and IV (beaked tadpoles); (3) interspecific variation of lateral lines is found in the organization, size, and number of the neuromasts within the lines; and (4) the supralabial extension of the **Angular** line in *Lepidobatrachus* spp. and the tentacular location of the **Oral** neuromasts in *X. laevis* are concomitant with the unique morphologies of their larvae and imply spatial changes involving additional organs.

Number and Arrangement of Neuromast Lines Innervated by the Anterodorsal and Anteroventral Lateral Line Nerves

We identified in tadpoles of Types I, II, and IV the three pairs of neuromast lines (**Angular**, **Oral**, and **Preopercular**) described previously in *D. pictus* (Schlosser and Roth, 1995). The **Angular**, **Oral**, and **Preopercular** lines exhibit morphological variation in some traits among the four larval types, which allowed us to establish some generalizations for each Type. By contrast, the **Infraorbital** and **Supraorbital** lines, related to the anterodorsal nerve, show a similar pattern without variation among larval types (Table 2).

In agreement with previous studies (Shelton, 1970; Lannoo, 1987; Schlosser and Roth, 1995; Chou and Lin, 1997, 1997; Hall et al., 2002; Quinzio et al., 2006; Fabrezi and Quinzio, 2008; Fabrezi et al., 2009; Fabrezi et al., 2012) the arrangement of the **Infraorbital** and **Supraorbital** lines does not display variation. The anterodorsal lateral line nerve presents a superficial bifurcation behind the eyes giving rise to two rami: the *ophthalmicus superficialis* innervating the neuromasts of the **Supraorbital** line, and the *buccal ramus* for the neuromasts of the **Infraorbital** line. Variation of the anterodorsal nerve is observed in the relationships of its root with trigeminal, anteroventral and facial roots (Table 2; Figs. 7 and 12).

Shelton (1970) described in *X. laevis* four pairs of lateral lines of neuromasts **Hyomandibular**, **Mandibular**, **Preorbital**, and **Tentacular** lines. Based on the reanalysis of *Xenopus* tadpoles we conclude that the **Preorbital** and **Tentacular** lines innervated by the dorsal component of *mandibularis externus ramus* of the VII–Av are equivalent to the **Angular** and the **Oral** lines, respectively, as is observed in tadpoles of Types II and IV (Table 2). The **Oral** line in *Xenopus* has a unique arrangement among anuran tadpoles at the base of the tentacle. The **Mandibular** and **Hyomandibular** lines of *Xenopus* tadpoles (Shelton, 1970) have the same con-

figuration of the two segments of the **Preopercular** line described for *Discoglossus* by Schlosser and Roth (1995), and they are innervated by the ramules *preopercularis inferior* and *preopercularis superior* of the *ventral component* that incorporates to the *mandibularis externus ramus* of VII–Av (Fig. 5). From this comparative analysis, it appears that the **Mandibular** and **Hyomandibular** lines described by Shelton (1970) represent the two segments (**inferior** and **superior**) of the **Preopercular** line (Table 2).

In *D. pictus*, the **Oral** and **Angular** lines are described as two different lines by Schlosser and Roth (1995) because they are supplied by two thin ramules (an *anterior* and short ramule and an *oral ramule*), which branch from the *mandibularis externus ramus* of VII–Av in an anterior position. However, these lines are continuously arranged and could be interpreted as a single line and equivalent to the **Angular** line described in this study. The arrangement and innervation of the **Preopercular** line of *D. pictus* with its superior and inferior parts have the same configuration as is observed in the **Preopercular** lines described for tadpoles of Types I, II and IV.

From this study, it is possible to provide the following generalization: anuran larvae have two pairs of orbital lines of neuromasts (**Infraorbital** and **Supraorbital**) innervated by the anterodorsal lateral line nerve and three pairs of mandibular lateral lines (**Angular**, **Preopercular**, and **Oral**) innervated by the anteroventral nerve.

Larval Types I and II represent filtering tadpoles and Types III and IV are beaked tadpoles; most of their morphological differences are found in those structures related to their feeding mechanism. Sokol (1975) proposed and the current anuran phylogeny (Pyron and Wiens, 2011) supports the independent origin of those taxa with tadpole Type I (Pipoidae) and II (Microhylidae). The arrangement of the lines of neuromasts and their nerves in ventral or lateroventral position seems to be features that could have evolved concomitant with the larval feeding mechanism.

Interspecific Variation Within Lines of Neuromasts

Among the analyzed taxa, independently of the tadpole types, we found some variation in the relative size of the neuromasts, their organization in the lines, and the number of sensory cells per organ.

The size of the neuromasts appears to be related to integumentary features as larger organs are present in those species with advanced development of the skin (i.e., adult-like with three or more epidermal layers, a well-differentiated dermis and a thick stratum compactum) as in *Lepidobatrachus* spp. (Fig. 4E,F). Conversely, small neuromasts were observed in those species in which the skin presents the typical larval condition (*B. variegata* and *Ch. pierottii*) (Fig. 3A–C).

The organization of the neuromasts in the lateral lines has been analyzed from different perspectives. Schlosser (2002b) observed the presence of longitudinal stitches in the lungfish *Neoceratodus* and *Leiopelmatidae*, and proposed that organization in stitches may represent the plesiomorphic condition for anurans. Lannoo (1988) considered transverse stitches as the generalized

TABLE 3. Major phenotypic differences between tadpoles of *Lepidobatrachus* spp. and the remaining anuran tadpoles.^a

<i>Lepidobatrachus</i> ' tadpoles	Anuran tadpoles (Types I, II, III, and IV)
Feeding from living zooplankton. Megalophagous.	Feeding from planktonic primary production (Wassersug, 1975). Usually microphagous
Flat head. Maximum width at level of the lower jaw articulation in dorsal view	Tall head. Uniform width in dorsal view
Continuous branchial chambers with bilateral openings formed by cutaneous lateral flaps (Ruibal and Thomas, 1988)	Separated branchial chambers with: bilateralventrolateral openings (Type I), a single ventroposterior opening (Type II), or a single midventral opening (Type III). Continuous branchial chambers with a single sinistral opening (Type IV) (Starret, 1973)
Forelimbs develop exposed to the external environment. The gut is divided by a constriction separating stomach from duodenum and the terminal segment of the ileum (rectum) presents a dilated caecum (Fabrezi, 2011; Bloom et al., 2013)	Forelimbs develop covered by skin The intestine does not present regional differentiation and is spiraled, long, has a uniform diameter and occupies the abdominal cavity.
Supralabial and lower jaw cartilages transversally elonged	Supralabial and lower jaw cartilages short
Suspensorioangularismuscle with postorbital origin (Fabrezi and Quinzio, 2008)	Suspensorioangularis muscle with antorbital origin (Haas, 2003; Fabrezi and Quinzio, 2008)
Trigeminal rami (V ₁ , V ₂ , and V ₃) anterior to the levator mandibulae set of muscles (Fabrezi, 2011)	Trigeminal rami (V ₂ , and V ₃) posterior the mm. levator-mandibulaeexternus and l. m. internus (Haas, 2001)
Hyoid and branchial elements fused in a single piece (Fabrezi and Lobo, 2009)	Hyoid and branchial elements articulated or united by ligaments (Haas, 2003)
Angular line of neuromasts long and extended on the upper labium	Angular line of neuromasts ends at the angle of the mouth
Large neuromasts, up to 45 × 65 µm in transverse view	Small to medium sized neuromasts, up to 25 × 60 µm in transverse view

Roelants et al. (2011) demonstrated that homoplasy limited the origin of novel morphologies and promoted clade overlap in morphospace during anuran larval evolution; exceptions are represented by tadpoles of Pipoidea (Larval Type I), Microhyloidea (Larval Type II), and *Lepidobatrachus* spp. Uniqueness of *Lepidobatrachus* tadpoles resulted from evolutionary changes in several specific developmental pathways that occurred simultaneously or sequentially from a generalized larval type (Bloom et al., 2013). Interestingly, within the *Lepidobatrachus* lineage derived phenotypic traits of the larvae seem to reduce morphological differences between larval and adult body plans, representing a good model to study the evolution of ontogenies.

condition for anurans and single neuromasts as the derived condition (Lannoo, 1987, 1988). Furthermore, the organization of the neuromasts has also been related to some ecological traits such as habitat and diet (Lannoo, 1987).

Here, we described variation in the morphological characteristics of larval neuromasts such as their size in superficial view or the number of sensory cells per organ, and three different conditions of neuromast organization in the lateral lines: single, aligned in stitches, or grouped irregularly (Table 1, Fig. 6). Both the characteristics of neuromast morphology and their organization in the lateral lines are features that vary among tadpole types without a clear relationship to each type.

Roots and Nerve Ganglia

As proposed by Sokol (1975), the presence of a single prootic ganglion could represent the generalized condition of tadpoles of Types I, II, and IV, although the prootic ganglion is not present in all neobatrachian larvae (Table 2; Fabrezi and Chalabe, 1997; Fabrezi and Quinzio, 2008).

We found different conditions when the trigeminal and facial nerves are completely separated or are fused in a common ganglion (Fig. 12). When the prootic ganglion is present, the neurons of the anterodorsal and anteroventral lateral line nerves are attached to the ganglion, and it is difficult to distinguish by dissection the identity of each component. When nerves V and VII are separated

(*Discoglossus* and *Lepidobatrachus* spp., *C. cranwelli*, *Telmatobius* spp.), the relationships of the anterior lateral lines nerves with the V and VII varies (Fig. 12).

In agreement with previous descriptions (Schlosser and Roth, 1995, 1997), we found a close association between facial and anteroventral lateral line nerves, in which the *hyomandibular trunk* and the *ramus mandibularis externus* of the facial contains the fibers of the anteroventral nerve. The ganglionic cells of the anteroventral nerve are indistinguishable from the facial ganglionic cells and their *ramules* are only evident just near their innervating targets. Regarding the configuration of the *ramules* of the anteroventral nerve, we found similarities with the descriptions of Northcutt (1992) for *Ambystoma*, in which the different *ramules* join into two clearly separate components (Table 2; Figs. 5, 8–12).

Tadpole Types and Lateral Line System

Most of the variation observed in the lateral lines and nerves allows us to distinguish Larval Type I from Types II, III, and IV by the arrangement of the *oral ramule* of the anteroventral nerve (at the base of the tentacle, as in *Xenopus*) and *Lepidobatrachus* tadpoles from the other types (I, II, II, IV) by the dorsal arrangement of the *angular ramule* of the anteroventral nerve. The Larval Type IV (except *Lepidobatrachus* spp.) exhibits scarce variation related to the anteroventral nerve, which seems to be limited to the presence or absence of

the *accessory ramules* for the **Preopercular** lines of neuromasts.

Here, based on the examination of neuromast morphology, arrangement, and the innervation of neuromast lines in different species of anuran larvae and on the variation described, we found that the lateral line system in anuran larvae seems to have been maintained without significant changes and/or distinctive patterns with the exception of *Lepidobatrachus* spp. and *Xenopus*.

Tadpoles of *Lepidobatrachus* spp. have a distinctive pattern with an **Angular** line of supralabial arrangement, which is conserved in the adult stages of *L. laevis*. Comparison between adult stages of *L. laevis* (Quinzio, 2011) and *X. laevis* (Shelton, 1970) demonstrated similarities in the location of mandibular lines of neuromasts, although their innervation is different. This convergence could be explained by aquatic feeding, which would explain the similar increase of the number of neuromasts on the upper jaw of *Lepidobatrachus* and *Xenopus*.

The **Angular**, **Oral**, and **Preopercular** lateral lines have distinctive features that remain unchanged in the adult of *L. laevis*, suggesting that the larval lateral line system has evolved along with other adult-like morphologies (such as those oral structures related to megalophagy) by reduction of the morphological-ecological differences between tadpoles and adults (Fabrezi, 2011). Many larval morphological features in *Lepidobatrachus* spp. (Quinzio et al., 2006; Fabrezi and Quinzio, 2008; Fabrezi, 2011; Quinzio, 2011; Quinzio and Fabrezi, 2012; Bloom et al., 2013) support the uniqueness of their tadpoles. The findings of this study emphasize this uniqueness and are strong enough to support the proposal of a new type of anuran larva, Type V, represented by the tadpole of *Lepidobatrachus* (Table 3).

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