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Olfactory Metamorphosis in the Coastal Giant Salamander (*Dicamptodon tenebrosus*)

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ABSTRACT This study examined the gross morphology and ultrastructure of the olfactory organ of larvae, neotenic adults, and terrestrial adults of the Coastal Giant Salamander (*Dicamptodon tenebrosus*). The olfactory organ of all aquatic animals (larvae and neotenes) is similar in structure, forming a tube extending from the external naris to the choana. A nonsensory vestibule leads into the main olfactory cavity. The epithelium of the main olfactory cavity is thrown into a series of transverse valleys and ridges, with at least six dorsal and nine ventral valleys lined with olfactory epithelium, and separated by ridges of respiratory epithelium. The ridges enlarge with growth, forming large flaps extending into the lumen in neotenes. The vomeronasal organ is a diverticulum off the ventrolateral side of the main olfactory cavity. In terrestrial animals, by contrast, the vestibule has been lost. The main olfactory cavity has become much broader and dorsoventrally compressed. The prominent transverse ridges are lost, although small diagonal ridges of respiratory epithelium are found in the lateral region of the ventral olfactory epithelium. The posterior and posteromedial wall of the main olfactory cavity is composed of respiratory epithelium, in contrast to the olfactory epithelium found here in aquatic forms. The vomeronasal organ remains similar to that in large larvae, but is now connected to the mouth by a groove that extends back through the choana onto the palate. Bowman's glands are present in the main olfactory cavity at all stages, but are most abundant and best developed in terrestrial adults. They are lacking in the lateral olfactory epithelium of the main olfactory cavity. At the ultrastructural level, in aquatic animals receptor cells of the main olfactory cavity can have cilia, short microvilli, a mix of the two, or long microvilli. Supporting cells are of two types: secretory supporting cells with small, electron-dense secretory granules, and ciliated supporting cells. Receptor cells of the vomeronasal organ are exclusively microvillar, but supporting cells are secretory or ciliated, as in the main olfactory cavity. After metamorphosis two distinct types of sensory epithelium occur in the main olfactory cavity. The predominant epithelium, covering most of the roof and the medial part of the floor, is characterized by supporting cells with large, electron-lucent vesicles. The epithelium on the lateral floor of the main olfactory cavity, by contrast, resembles that of aquatic animals. Both types have both microvillar and ciliated receptor cells. No important changes are noted in cell types of the vomeronasal organ after metamorphosis. A literature survey suggests that some features of the metamorphic changes described here are characteristic of all salamanders, while others appear unique to *D. tenebrosus*. J. Morphol. 266:22–45, 2005.

KEY WORDS: olfaction; Urodela; Caudata; Amphibia; morphology; ultrastructure

Living amphibians consist of three clades: caecilians, salamanders, and frogs (Cannatella and Hillis, 1993). Most amphibians undergo a radical transformation from an aquatic larva to a terrestrial juvenile form. Metamorphosis allows amphibians to take advantage of food sources in two distinct habitats. To do so, however, sensory systems, such as the olfactory system, must adapt to maintain function in a new medium.

The gross morphological changes occurring during metamorphosis of the nose in frogs (Born, 1876; Hinsberg, 1901; Rowedder, 1937; Yvroud, 1966; Jermakowicz et al., 2004) and caecilians (Sarasin and Sarasin, 1890; Badenhorst, 1978) have been well known for a long time, but in spite of much relevant information available (see Discussion), apparently no previous study has focused on the structural changes occurring in the nose during salamander metamorphosis. Moreover, the ultrastructure of the olfactory epithelium in larval salamanders apparently has never been examined (Hansen et al., 1998).

The Coastal Giant Salamander, *Dicamptodon tenebrosus*, belongs to the family Dicamptodontidae, which is the basal sister group of the family Ambystomatidae (Larson and Dimmick, 1993; Wiens et al., 2005). It exhibits both aquatic (neotenic) and terrestrial adult life stages, allowing for a detailed comparison of structural and cellular differences between two different adult life stages, as well as a larval stage. Previous work on the nose of *Dicamptodon* spp. focused on the nasal capsule, rather than the olfactory organ itself (Jurgens, 1971), or provided only a brief overview (Saint Girons and Zylberberg, 1992a,b). The goals of this study were to compare larval, neotenic, and terrestrial *D. tenebrosus* regarding: 1) the gross morphology and distribu-

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tion of olfactory, vomeronasal, and respiratory epithelium; and 2) the specific cell types found in the epithelia.

MATERIALS AND METHODS

All procedures were carried out as approved by the Humboldt State University Institutional Animal Care and Use Committee. Specimens of *Dicamptodon tenebrosus* were collected in Jacoby Creek, Humboldt Co., Big French Creek, Cedar Flat Creek, Little French Creek, Mill Creek, and East Fork Willow Creek, Trinity Co., CA.

For purposes of description, animals were grouped into four general size classes. These were small larvae (n = 8, SVL [snout-vent length] 25–50 mm, $\bar{x}$ = 37 mm), advanced larvae (n = 7, SVL 51–78 mm, $\bar{x}$ = 67 mm), neotenes (n = 12, SVL 95–115 mm, $\bar{x}$ = 104 mm), and metamorphosed (n = 9, SVL 98–115 mm, $\bar{x}$ = 103 mm). All metamorphosed adults used were captured as larvae and underwent metamorphosis in captivity; they were sacrificed 1 week to a year postmetamorphosis.

Each salamander was anesthetized with 1:2,000 MS 222 (3-aminobenzoic acid ethyl ester, Sigma, St. Louis, MO) in water, adjusted to pH 7.0–7.4. Measurements were made of body mass, SVL, and total length (TL). Sex and sexual maturity were determined in larger salamanders, but this was not possible in very small larvae. Once the animals were thoroughly sedated and all measurements were taken the head was removed. Olfactory organs used for histological examination were left encased in bone and cartilage and placed in aqueous Bouin's solution for fixation and decalcification. After 2 weeks to a month of decalcification the tissue was embedded in Paraplast and serial-sectioned 8–10 μm thick, transversely, sagittally, or horizontally. Sections were mounted, dried, dewaxed, and stained with Delafield's hematoxylin and eosin (Humason, 1972). Photomicrographs were taken of representative sections using a Nikon Coolpix 4500 digital camera mounted to a Nikon Eclipse E400 compound microscope.

For TEM, tissue was fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Fixation occurred at room temperature for at least 2 h, followed by microwaving using a Pelco 3451 laboratory microwave system (Ted Pella, Redding, CA) for two 40-sec intervals separated by 5 min of cooling on ice. Postfixation was done in 1.5% osmium tetraoxide (in water) in two 40-sec microwave treatments. Dehydration and infiltration with ERL-Quetol low viscosity resin (Ted Pella) was also done in the microwave, followed by polymerization. Silver and gold sections (75–95 nm) were cut using a Pelco diamond knife mounted in a Leica Ultracut R microtome. Sections were poststained with 1% uranyl acetate and 0.4% lead citrate for 15 min each. Transmission electron microscopic examination of nasal organs was completed using a Philips EM 208S transmission electron microscope (TEM). Electron micrographs were taken at an accelerating voltage of 60 kV.

Multiple complete corrosion casts of the nasal cavities from each group were made by injecting Marcox resin (Ladd Research Industries, Burlington, VT) into the cavity through the internal naris after decapitation. A variety of techniques were tried to promote mucus removal and complete casting, including soaking in various concentrations and mixtures of sucrose, formalin, and Tween 20 as well as flushing with water. The best results were obtained by prompt and brief immersion of decapitated heads in an ice bath while the resin was mixed with its catalyst, followed by injection. After polymerization for 30 min at room temperature the head was placed in a 40°C water bath to completely cure the resin. Maceration of the tissue was accomplished in a 40°C, 20% NaOH bath for 1–2 h. Five to ten rinses with dH_2O were used to remove small remaining pieces of tissue and salt before a final rinse in ethanol. Clean casts were dried and mounted, sputter-coated with gold for 75 sec in a Denton Vacuum Desk II Cold Sputter Etch unit, and viewed with a Topcon ABT-32 scanning electron microscope.

RESULTS

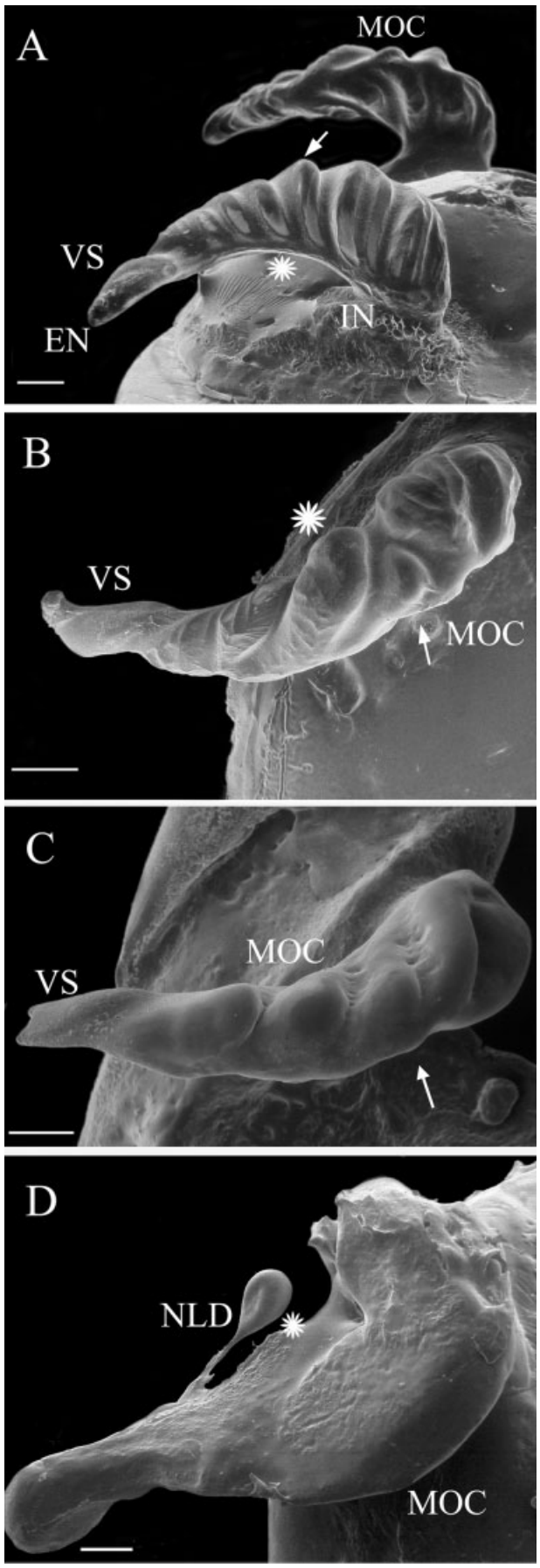
Gross Morphology and General Histology

Overall morphology of the nasal cavities. The nose in all *Dicamptodon tenebrosus* comprises two distinct chambers, the large main olfactory cavity and the smaller vomeronasal organ (Jacobson's organ). The vomeronasal organ is a diverticulum branching off the lateral side of the main olfactory cavity.

The nose is similar in all aquatic life stages, from small larvae through large neotenes. The nasal organ is a tube that arches posteriorly, medially, and ventrally from the external naris to the choana, or internal naris (Fig. 1A–C). A nonsensory vestibule connects the external naris to the main olfactory cavity. There are at least six dorsal and nine ventral ridges in the main olfactory cavity casts of small aquatic *Dicamptodon tenebrosus* (Fig. 1A,B), corresponding to valleys of olfactory epithelium. The basic pattern and number of valleys appears to be conserved in all aquatic life stages, although the valleys become deeper and further subdivide in the posterior main olfactory cavity (Fig. 1C). The valley pattern could be loosely described as helical. The vomeronasal organ extends along the lateral side of the olfactory organ (see below), but did not cast and so is not visible in Figure 1.

During metamorphosis the vestibule is lost as the external naris shifts posterolaterally. The dramatic ridges present in the main olfactory cavity of aquatic animals are lost and significant dorsoventral compression and widening occurs (Fig. 1D). The general shape of the main olfactory cavity in metamorphosed animals is an elongated flattened tube, with a narrow connection to the smaller lateral vomeronasal chamber, which is also flattened.

External nares and vestibule. In aquatic *Dicamptodon tenebrosus*, the external nares are directly anterior to the medial aspect of the eyes. They form upside-down teardrops with the tails pointing toward the mouth, resembling the nasolabial groove seen in terrestrial plethodontid salamanders (Dawley and Bass, 1988). The vestibule (Figs. 2A,B, 3B,C, 4A,B) runs from the external naris to the nasal tectum, which forms the cartilaginous roof over the olfactory organ proper (Figs. 2B, 3A–C). In small larvae, larger larvae, and neotenes, ~39%, 31%, and 21%, respectively, of the total length of the olfactory organ is dedicated to the vestibule. After leaving the external naris (Fig. 2A), the vestibule in small aquatic animals is oval in cross section until it meets the main olfactory cavity; then it becomes triangular. The triangular vestibule gradually merges into the thick olfactory epithelium of the main olfactory cavity until only the lateral point of the triangle remains as the beginning of the dorsolateral groove (see below). In neotenes, the vestibule is relatively shorter. It opens to the main olfactory cavity just posterior to the external nares and is here triangu-



lar in shape, similar to the caudal part of the vestibule in smaller aquatic animals (Fig. 2B). The nonsensory epithelium of the vestibule in aquatic *D. tenebrosus* is simple and squamous nearest the entrance of the nose, but promptly yields to stratified, more cuboidal cells (Fig. 2A). This type of epithelium is sparsely ciliated, and although stratified, does not appear to have any distinct layers.

The situation in metamorphosed animals is very different. The external nares are round, without any grooves, and are located more laterally, dorsally, and posteriorly than in aquatic animals. The external naris is fully supported by cartilage and communicates directly with the main olfactory cavity via the fenestra endonarina communis. The vestibule is thus lost as a distinct chamber. However, a small dorsal patch of nonsensory epithelium encircling the external nares strongly resembles the epithelium seen in the vestibules of aquatic *Dicamptodon tenebrosus* (Fig. 2C).

Main olfactory cavity. In aquatic *Dicamptodon tenebrosus*, as noted above, the main olfactory cavity exhibits at least six main valleys (separated by ridges) on the dorsal side (Figs. 1A–C, 3A–C), and nine valleys on the ventral side (Fig. 4B). In general, the valleys are lined by sensory epithelium and the ridges by respiratory epithelium. In larger aquatic animals lesser subsidiary undulations develop among the larger posterior ridges (Fig. 3D). The undulations are approximately perpendicular to the body axis near the choana, but twist outward as they progress anteriorly to meet the vestibule. Aside from the ridges, the lumen of the main olfactory cavity is approximately circular in cross section in larvae (Fig. 5A,B). As animals mature into neotenes the lumen flattens and widens (Fig. 5C). In small larvae all ventral ridges are reduced above the level of the vomeronasal organ except one that nearly halves the main olfactory cavity (Fig. 4A). In large larvae and neotenes this prominent ridge is present in conjunction with at least six other ridges on the medial side, which together form a channel or recess on the lateral side that appears to lead to the vomeronasal organ (Figs. 3D, 4B). The ridges in neotenes project deep into the lumen and often bend anteriorly or posteriorly, forming a flat plateau of respiratory epithelium over a pocket of sensory epithelium (Fig. 6B).

Fig. 1. Corrosion casts of the nasal organs. SEM. Anterior to the left. **A:** Advanced larva. Left lateral view of the olfactory organs. Note the transverse ridges, corresponding to valleys in the olfactory epithelium. **B:** Same larva. Dorsal view of right olfactory organ. **C:** Neotene. Dorsal view of right olfactory organ. Note retention of larval form. In **A–C**, the arrows point to a distinct reference point used to locate the approximate position of the vomeronasal organ (asterisk in **A,B**), which did not cast. **D:** Terrestrial adult. Dorsal view of the right olfactory organ. Note the broadening of the main olfactory cavity (MOC) and the striking absence of ridges; the vomeronasal organ (asterisk) and the nasolacrimal duct (NLD) can also be seen. EN, external naris; IN, internal naris; VS, vestibule. Scale bars = 600 μm.

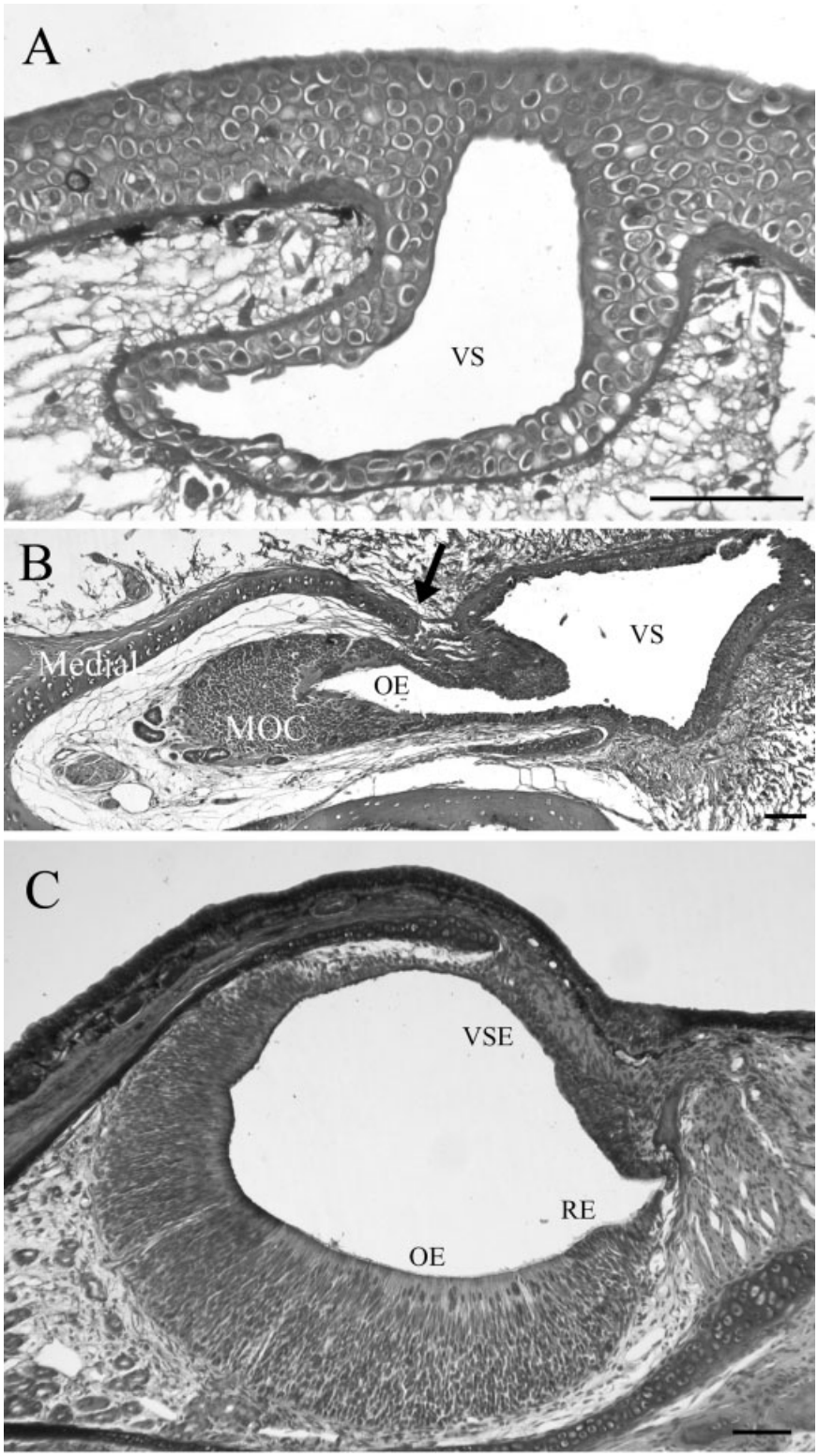


Fig. 2. Transverse sections, immediately posterior to the external naris, of a small larva (**A**), a neotene (**B**) and a terrestrial *Dicamptodon tenebrosus* (**C**). Lateral to right. Note the relatively anterior position of the main olfactory cavity (MOC) in **B** and the lack of a vestibule in **C**. The arrow in **B** points to the anterior cupula, which separates the vestibule (VS) from the main olfactory cavity. OE, olfactory epithelium; VSE, vestibular type epithelium; RE, respiratory epithelium. Scale bars = 300 μm.

In aquatic animals most of the main olfactory cavity is dedicated to olfactory epithelium, except on ridge tops, near the choana, and along the lateral side near the opening to the vomeronasal organ (Figs. 3A–D, 4A,B, 5A–C). The olfactory epithelium is thickest on the medial side of the main olfactory cavity (Fig. 5A–C). The ridge tops are composed of respiratory epithelium. A capillary network is present in the connective tissue below the basal lamina and is particularly noticeable near the ridge

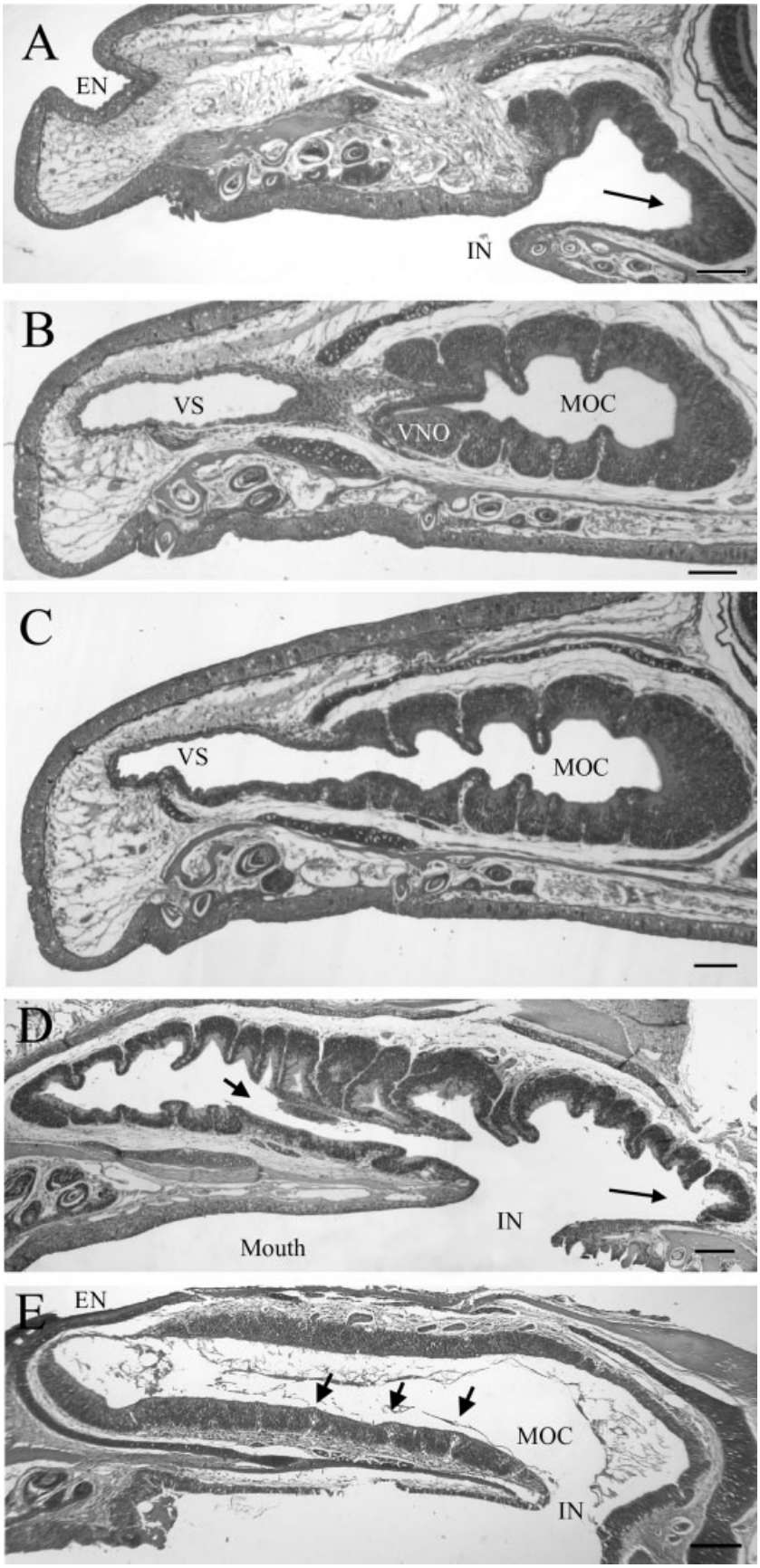


Fig. 3. Sagittal sections through the nose of a small larva (**A–C**; from lateral to medial), a neotene (**D**) and a terrestrial *Dicamptodon tenebrosus* (**E**). Anterior to the left. **A:** Note the presence of olfactory epithelium (arrow) dorsal and posterior to the internal naris, characteristic of aquatic *D. tenebrosus*. **B:** The vestibule (VS), main olfactory cavity (MOC), and vomeronasal organ (VNO) are all visible. The roof of the vomeronasal organ is poorly developed. **C:** The vestibule connects to the main olfactory cavity. **D:** In neotenes, the folding of the olfactory epithelium is greatly increased. Note the uniting of ridges to form a channel (arrow on left) just medial to the vomeronasal organ. **E:** In metamorphosed animals, the lumen of the main olfactory cavity is smooth and sensory epithelium is lacking behind the internal naris. Short arrows point to mini-ridges of respiratory epithelium, possibly representing the location of former ridge-tips. EN, external naris; IN, internal naris. Scale bars = 200 μm in **A–C**, 300 μm in **D,E**.

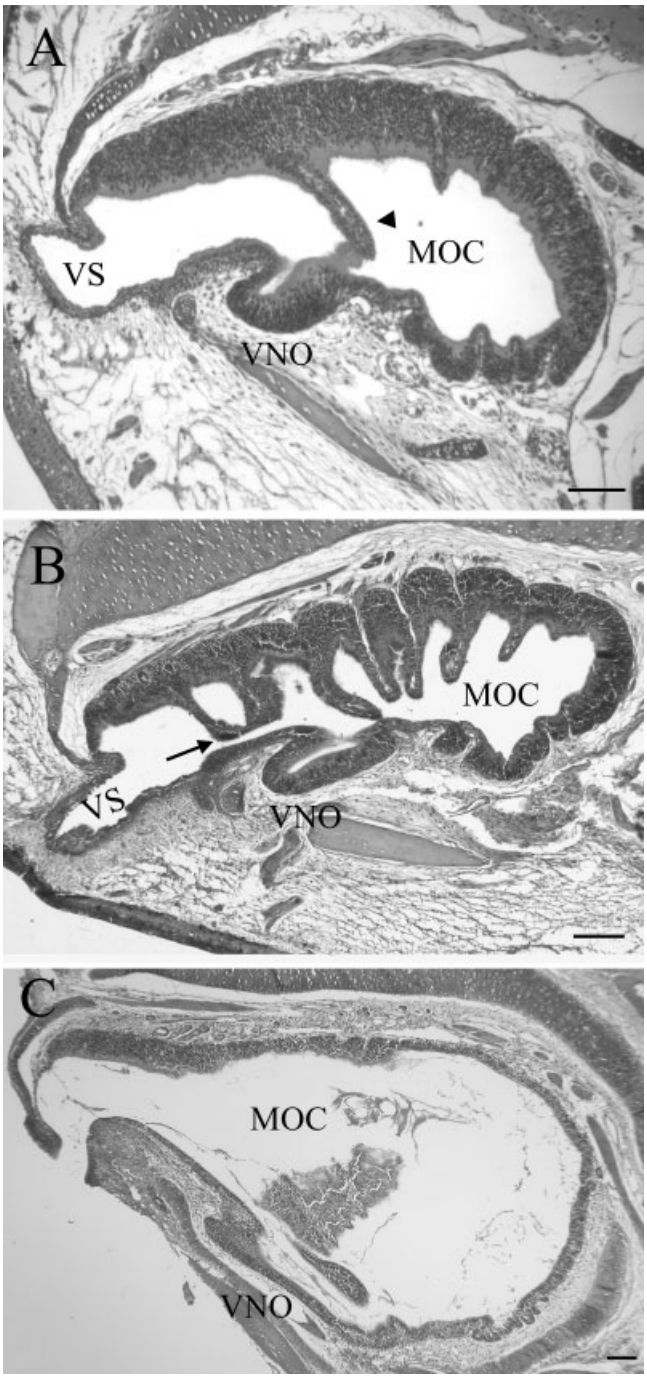


Fig. 4. Horizontal sections through the nasal organ of a small larva (**A**), an advanced larva (**B**), and a terrestrial *Dicamptodon tenebrosus* (**C**). Anterior to left, medial to top. **A:** The vestibule (VS), main olfactory cavity (MOC), and vomeronasal organ (VNO) are all visible. A laterally projecting ridge (arrowhead) divides the ventral main olfactory cavity into anterior and posterior parts immediately behind the opening of the vomeronasal organ. **B:** The laterally projecting ridges are more developed, but the main ridge is still present. The arrow points in the presumed direction of water flow into the channel leading to the vomeronasal organ. **C:** After metamorphosis the ridges are lost, as is the vestibule, and the main olfactory cavity broadens considerably. The floor of the main olfactory cavity has been grazed medial to the vomeronasal organ; the division into bands of sensory and nonsensory epithelium can be seen. Scale bars = 200 μm in **A**, 300 μm in **B,C**.

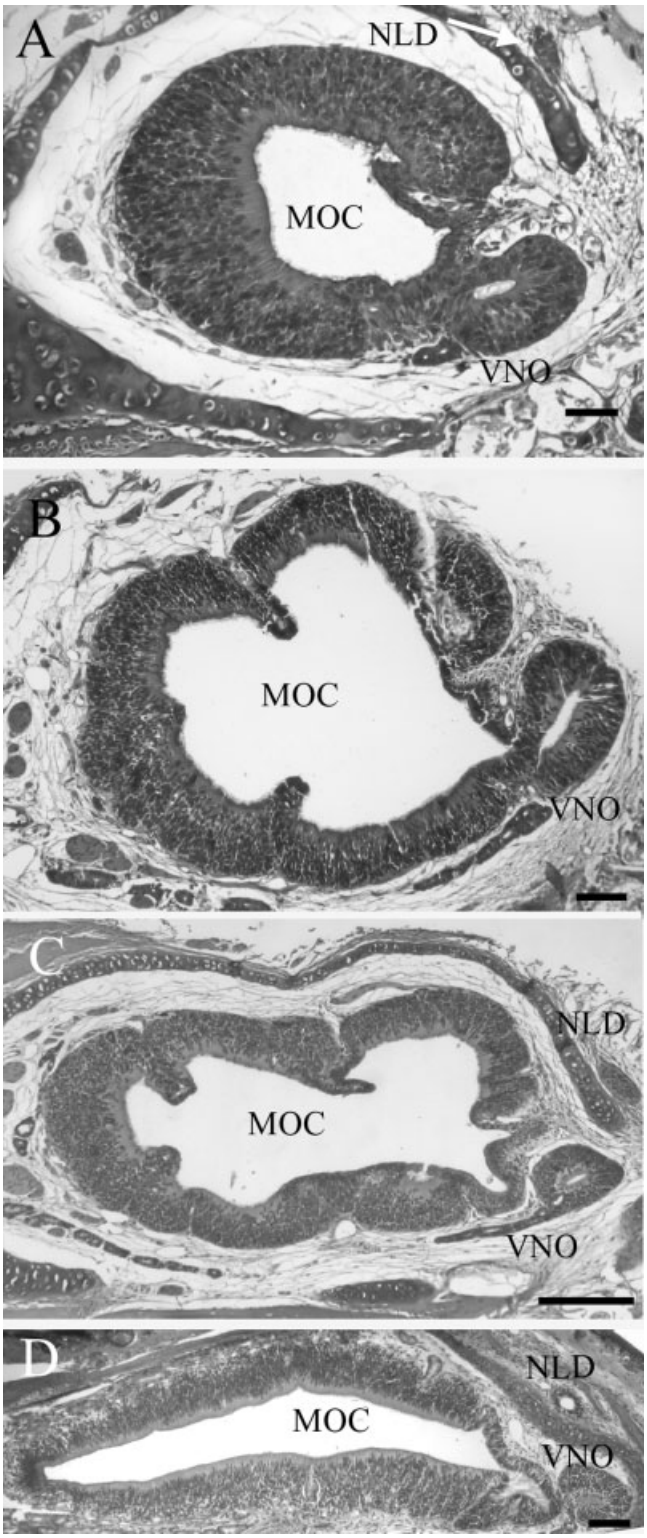
tops, between the thick block-like sections of olfactory epithelium. Two grooves lined by respiratory epithelium extend along the lateral side of the main olfactory cavity anterior to the opening into the vomeronasal organ, together corresponding to the lateral nasal groove of Seydel (1895). The dorsolateral groove continues from the external naris along the lateral side of the vestibule into the main olfactory cavity (Fig. 5A–C). The ventrolateral groove begins in the lateral wall of the main olfactory cavity and extends back toward the opening of the vomeronasal organ (Fig. 5B,C), with which it is continuous. In larvae the dorsolateral channel dwindles posteriorly as it reaches the point where the ventrolateral channel begins (Fig. 5A,B), but in neotenes the two overlap, possibly forming a continuous channel from the outside world to the vomeronasal organ (Fig. 5C).

Metamorphosed *Dicamptodon tenebrosus*, in contrast to aquatic animals, possess a wide dorsoventrally flattened main olfactory cavity without major internal ridges (Figs. 1D, 3E, 4C, 5D). The main olfactory cavity bulges slightly anteriorly with respect to the external nares (Fig. 4C). This pre-narial region accounts for 3% of the total length of the olfactory organ. The majority of the main olfactory cavity is formed by a ventral and a dorsal sheet of olfactory epithelium, connected anteromedially.

The olfactory epithelium of metamorphosed adults can be divided into two classes according to the presence of Bowman's glands and the types of supporting cells present. The first class, termed herein the "predominant olfactory epithelium," occurs on over half of the ventral floor and nearly the entire dorsal roof of the main olfactory cavity. Bowman's glands are present (Figs. 5D, 7C) and the supporting cells are large, with a loosely packed vesicular cytoplasm, distinguishable at the level of light microscopy (Fig. 7C). The second class, termed herein the "lateral olfactory epithelium," occurs mostly on the ventrolateral floor of the main olfactory cavity, but also in a thin strip along the dorsolateral roof (Figs. 2C, 5D). This class never has Bowman's glands and contains granular and large ciliated supporting cells. These two epithelial classes will be described in greater detail below.

Olfactory epithelium is absent dorsolateral and posterior to the choana in metamorphosed animals (Fig. 3E), unlike in aquatic animals. This area is composed of densely ciliated respiratory cells and goblet cells. Although some elongated narrow cells resembling receptor neurons can be seen in this region, they do not have typical olfactory knobs. Sensory epithelium is also lacking along the lateral side in or near the opening to the vomeronasal organ and in the anterior (dorsolateral and ventrolateral) and posterior grooves that lead in or out of the vomeronasal organ. Several small mini-ridges or crests of nonsensory epithelium occur within the main olfactory cavity, running from anteromedial to

posterolateral, approximately parallel to the lateral side of the head (Figs. 3E, 4C, 6C, 11A). These mini-ridges may be remnants of the transverse ridges found in aquatic specimens (see Discussion). They are present dorsally, but are more abundant ventrally. The mini-ridges of respiratory epithelium become completely flat medial to the choana. As in larger aquatic animals, terrestrial animals have connected dorsolateral and ventrolateral grooves of respiratory epithelium that lead from the external naris to the vomeronasal organ (Fig. 5D). In addition, a newly developed groove leads posteriorly from the vomeronasal epithelium to the mouth through the choana; it will be discussed in the vomeronasal organ section.



Bowman's glands. Multicellular Bowman's glands are found in the main olfactory cavity of all *Dicamptodon tenebrosus*, both aquatic and terrestrial. In aquatic animals, the base of the gland often appears to be embedded between basal cells (Fig. 7A). The glands appear compressed in small larvae, but are fuller and present in greater numbers in neotenes (Fig. 7B). Bowman's glands are usually present in aquatic specimens directly dorsal to the opening of the vomeronasal organ into the main olfactory cavity. They appear to be confined exclusively to olfactory epithelium, although this is not always easy to distinguish from nonsensory epithelium.

In metamorphosed animals, Bowman's glands are much more abundant. Some are embedded in the plane of the epithelium, but others protrude down into the lamina propria (Fig. 7C). The greatest concentration of Bowman's glands can be found in broad bands along the floor and roof of the main olfactory cavity. They never occur in the lateral olfactory epithelium, but they do occur in the respiratory epithelium at the posterior end of the main olfactory cavity.

Nasolacrimal duct. The nasolacrimal duct is incompletely formed and dwindles before connecting with the main olfactory cavity in small larvae (Fig. 5A). In more mature animals, it connects to the lateral aspect of the main olfactory cavity in the dorsolateral groove of respiratory epithelium, anterior to the opening of the vomeronasal organ. However, the anterior portion of the nasolacrimal duct in larger larvae and neotenes does not appear to be patent (Fig. 5C). By contrast, the nasolacrimal duct in metamorphosed specimens is fully formed and possesses a large round clear lumen (Fig. 5D). It

Fig. 5. Transverse sections of the nasal organ of a small larva (**A**), an advanced larva (**B**), a neotene (**C**), and a terrestrial *Dicamptodon tenebrosus* (**D**). Lateral to the right. **A:** The lumen of the main olfactory cavity (MOC) compresses during the transition through a ridge. Note the dorsolateral groove in the main olfactory cavity above the vomeronasal organ (VNO). The nasolacrimal duct (NLD) can be seen at upper right. **B:** As larvae develop the ridges project deeper into the round lumen. **C:** In neotenes, the lumen of the main olfactory cavity is dorsoventrally compressed. Note the presence of both dorsolateral and ventrolateral grooves. **D:** After metamorphosis the nasal organ is greatly broadened and dorsoventrally compressed. The nasolacrimal duct is now patent. Scale bars = 100 μm in **A,B**, 300 μm in **C**, 200 μm in **D**.

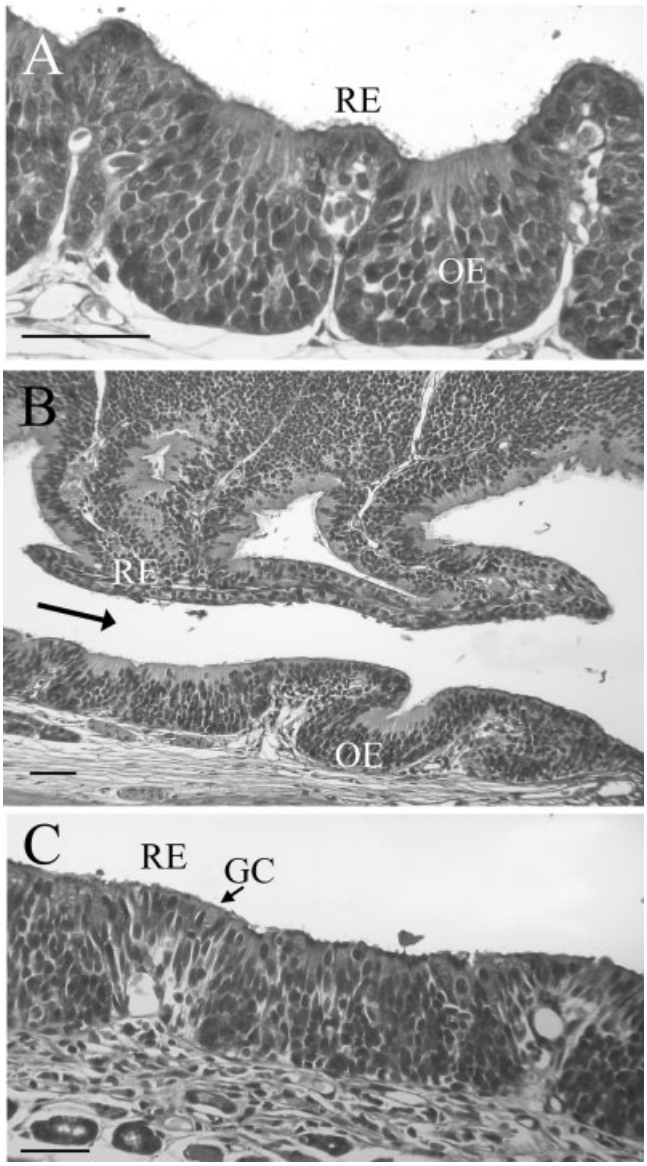


Fig. 6. Sagittal sections showing details of the lateral half of the nose of a small larva (**A**), a neotene (**B**), and a terrestrial *Dicamptodon tenebrosus* (**C**). **A:** In small larvae, shallow ridges of respiratory epithelium (RE) separate blocks of olfactory epithelium (OE) in the main olfactory cavity. **B:** In neotenes, ridges of respiratory epithelium combine to form a channel (arrow) leading back to the vomeronasal organ. **C:** In metamorphosed adults, strips of respiratory epithelium containing goblet cells (GC) separate regions of olfactory epithelium. Scale bars = 100 μm.

connects to the main olfactory cavity in a similar fashion as in older aquatic specimens.

Vomeronasal organ. In all life stages the dorsoventrally flattened lumen of the vomeronasal organ branches off the lateral side of the middle of the main olfactory organ (Figs. 4, 5). In aquatic stages, the anterior portion of the vomeronasal organ is ventral to the caudal end of the dorsolateral groove originating in the vestibule (Fig. 5A–C). Ciliated respiratory epithelium surrounds the opening of the vomeronasal organ into the main olfactory cavity. A groove lined by respiratory epithelium continues back from the caudal end of the vomeronasal organ to the choana.

The vomeronasal organ mass in smaller larvae is triangular in appearance in horizontal sections and oval in sagittal or transverse sections (Figs. 4A, 5A). The vomeronasal organ is stretched in an anterior–posterior direction in larger aquatic animals, giving the organ a cylindrical appearance from a dorsal perspective (Fig. 4B). The dorsoventral height of the lumen of the vomeronasal organ increases slightly with maturity. In small larvae, sensory epithelium is restricted to the ventral and lateral walls of the vomeronasal organ. The dorsal part of the vomeronasal organ is composed of thinner ciliated nonsensory epithelium (Figs. 3B, 5A). In large larvae and neotenes, typical vomeronasal epithelium replaces the nonsensory epithelium on the dorsal roof of the vomeronasal organ as well (Fig. 5B,C). The vomeronasal epithelium extends 10% of the length of the main olfactory cavity in small larvae and 30% in large larvae and neotenes.

In metamorphosed animals, the vomeronasal organ is similar to that of large larvae and neotenes, but even better developed. The vomeronasal epithelium extends 35% of the length of the main olfactory cavity. The wall of the vomeronasal organ is composed of thick, mature sensory epithelium (Figs. 5D, 8A). Unlike in aquatic stages, the vomeronasal organ does not narrow as it progresses posteriorly. Instead, the vomeronasal epithelium is replaced dorsally, then ventrally, and finally laterally by a respiratory epithelium (Fig. 8B,C), which lines a posterior groove that is equal to the length of the sensory portion of the vomeronasal organ. This epithelium is composed of densely decorated columnar cells and goblet cells (see above). The posterior groove leads out of the vomeronasal organ, passes through the choana, and continues in the mouth (Fig. 8D) as the lateral palatal groove.

Ultrastructure of the Nasal Epithelia

Examination of the ultrastructure of olfactory and vomeronasal epithelia in each life stage focused on comparing supranuclear regions; here the distinction between cell types is most evident. Our detailed review begins with the main olfactory epithelium.

Aquatic stages: main olfactory epithelium. The cellular composition of the main olfactory epithelium in all aquatic *Dicamptodon tenebrosus* appears similar. As in all vertebrates, the epithelium is pseudostratified, with receptor cell nuclei deeper in the epithelium than nuclei of supporting cells. Four types of receptor cell dendrites can be distinguished (Figs. 9, 10). The most common type has both cilia and short (0.4–0.5 μm) microvilli (Fig. 9B). Other receptor cells are generally similar, but appear to have only cilia (Figs. 9, 10). These cilia are

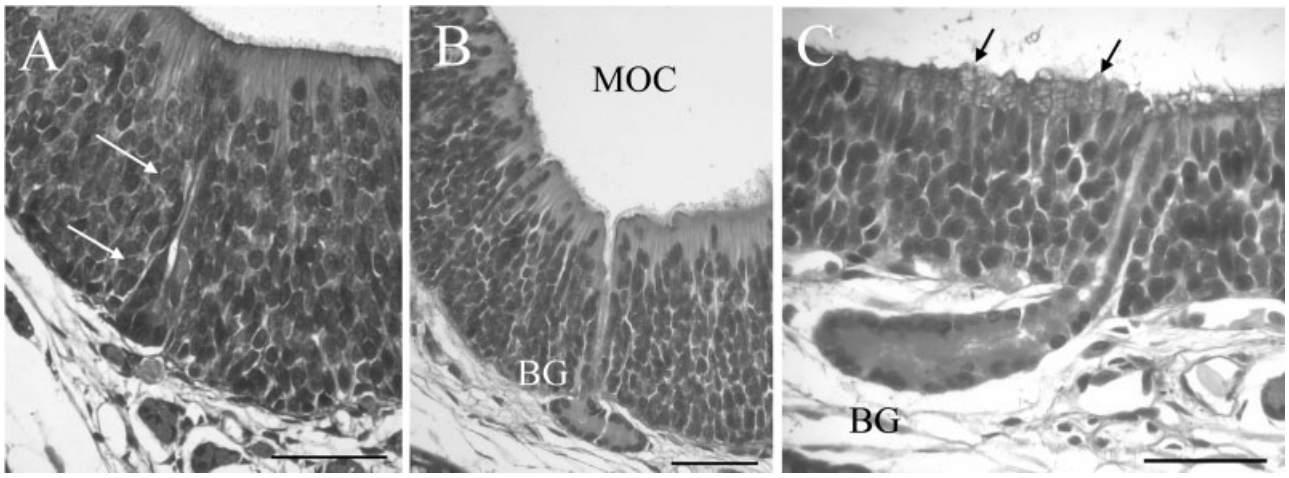


Fig. 7. Transverse sections of Bowman's glands in the main olfactory cavity of a small larva (**A**), a neotene (**B**), and a terrestrial *Dicamptodon tenebrosus* (**C**). **A:** In larvae, Bowman's glands (white arrows) appear immature; this one is from the dorsomedial side of the main olfactory cavity (MOC). **B:** In neotenes, Bowman's glands (BG) are better developed; this one is from just above the opening of the vomeronasal organ. **C:** In metamorphosed adults, Bowman's glands tend to drop into the lamina propria. Note also the presence of loosely packed (vesicular) supporting cells in the sensory epithelium (arrows). Scale bars = 50 μm in **A,C**, 100 μm in **B**.

~9–15 μm long, have diameters of 0.25 μm, and are attached to a basal body. A third type of receptor cell has only short microvilli (Fig. 9B); these receptors tend to have low projecting dendrites, similar to those of the ciliated receptor cells. Finally, the fourth type of receptor cell has very long microvilli (9–15 μm), which form dense clusters on the knobby ends of the dendrites (Fig. 10B). Long microvilli never occur on ciliated receptor cells. Long microvillar receptor cells are greatly outnumbered by receptor cells with cilia and those with only short microvilli. All microvilli, both long and short, have a diameter of 0.1 μm.

The cytoplasm in the apical end of microvillar and ciliated receptor cells is similar, although some receptor cells appear more electron dense than others, without an apparent correlation to type of projection. All receptor cells contain many mitochondria, longitudinally oriented filaments, and usually several basal bodies near the surface (Figs. 9B, 10B). The nuclei of receptor cells in the main olfactory cavity and vomeronasal organ are more ovoid than the circular nuclei of supporting cells around them. Receptor cells throughout the main olfactory cavity and throughout all aquatic life stages are similar in appearance, although the cilia and microvilli are slightly longer in neotenes than in smaller larvae.

Supporting cells are nonsensory cells that are intermixed with sensory cells in the olfactory epithelium. As with the receptor cells, the supporting cells in all aquatic animals are virtually indistinguishable among life stages. There are always at least two main types of supporting cells. The most common supporting cell type has a long thin irregular apical process with a concentration of bicolored granules at the apical edge (Figs. 9, 10). These granules have a diameter of ~0.3 μm. Often the free edge of the cell projects up to 4 μm above surrounding dendrites. No cilia or microvilli are present on these projections. The number of dark granules in supporting cells appears to be slightly greater in the sensory epithelium located posterior to the vomeronasal organ on the lateral side. Few supporting cells show signs of secretion, which may indicate a low rate of secretory activity. The second type of supporting cell is very large, ciliated, and is much less common than supporting cells with granules (Fig. 10A). The large ciliated supporting cells have superficial, electron-dense, oval nuclei. The free edge of these cells is the widest part of the cell and is covered with cilia that are at least 10 μm long, intermixed with short 0.5 μm microvilli. Both of these cell types are present in all aquatic *Dicamptodon tenebrosus* wherever olfactory epithelium is found.

Metamorphosed animals: main olfactory epithelium. As noted above, the olfactory epithelium of the main olfactory cavity in metamorphosed animals can be divided into two types, a predominant and a lateral olfactory epithelium. Nevertheless, the receptor cells are relatively uniform throughout the epithelium. As in aquatic animals, both microvillar and ciliated receptor cells are found, and ciliated receptor cells seem to be more prevalent than microvillar receptor cells (Fig. 11). Many of the ciliated receptor cells once again have short (0.5 μm) microvilli. The cilia and microvilli on the receptor cells are approximately the same length and width as in aquatic specimens. Cilia are always attached to a basal body. Some slight differences between receptor cells of predominant and lateral epithelium are apparent. The cytoplasm in receptor cells from the predominant epithelium tends to be more electron dense than in those in the lateral region. Although cilia and microvilli are similar in each, in the pre-

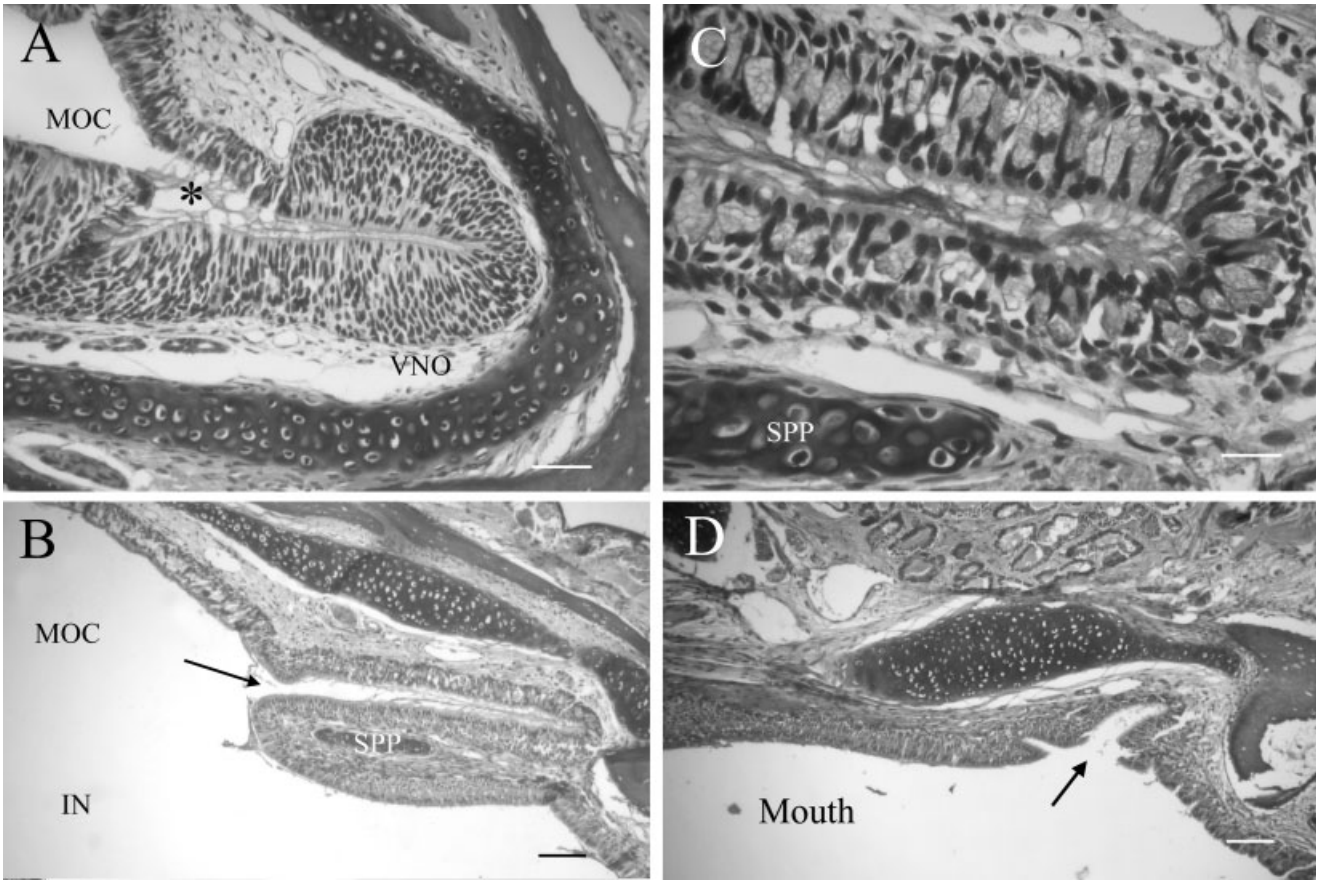


Fig. 8. Successive transverse sections through the posterior part of the nasal organ of a terrestrial *Dicamptodon tenebrosus*, to show lateral nasal groove connecting vomeronasal organ to palate. Lateral to the right. **A:** The vomeronasal organ (VNO) is separated from the main olfactory cavity (MOC) by a deep groove. Note mucus (*) in vomeronasal organ. **B:** The main olfactory cavity is here open to the mouth via the internal naris (IN). The arrow points to the lateral nasal groove leading back out of the vomeronasal organ, above Seydel's palatal process (SPP). **C:** The lateral nasal groove is completely overrun with ciliated respiratory epithelium containing abundant goblet cells. **D:** Behind the internal naris the lateral nasal groove is continued by the lateral palatal groove (arrow). Scale bars = 100 μm in **A**, 200 μm in **B,D**, 50 μm in **C**.

dominant olfactory epithelium the cilia and microvilli are usually matted and can only be followed about 6 μm before they bend to become parallel to the apical edge. In the lateral olfactory epithelium the cilia and microvilli are relatively untangled and extend up to 15 μm.

Unlike the receptor cells, supporting cells differ between the predominant and lateral olfactory epithelium. Supporting cells of the predominant olfactory epithelium are uniformly secretory, and contain what appears to be a loosely packed secretory product (Fig. 11B,C). The large (15 μm) towering edges are full of electron-lucent vesicles which are 1.2 μm in diameter; these cells lack any cilia or microvilli. Loosely packed supporting cells are not present in aquatic specimens. By contrast, supporting cells in the lateral olfactory epithelium closely resemble those found throughout the main olfactory cavity in aquatic stages. The most common type of supporting cell in the lateral olfactory epithelium has long slender granulated projections without cilia and may have a few microvilli (Fig. 11D,E). The granules in the supporting cells are moderately electron dense and have a very small (0.13 μm) electron-dense core. The less prevalent large ciliated supporting cells have both cilia and small microvilli-like projections (Fig. 11E). The boundary between the two types of epithelium is not sharp and some mixing of supporting cell types occurs here (Fig. 11B).

Respiratory epithelium of the main olfactory cavity. In aquatic *Dicamptodon tenebrosus*, as noted above, respiratory epithelium is found on ridges, on plateaus formed by the confluence of ridges, in and near the choana, and surrounding the opening into the vomeronasal organ. This epithelium is generally very uniform in apical appearance (Fig. 12A), although it varies in thickness from simple squamous, thinly covering the floor of the main olfactory cavity around the choana, to pseudostratified cuboidal or columnar on ridges, and then to a thicker stratified columnar on plateaus. The nuclei of respiratory epithelial cells vary in electron den-

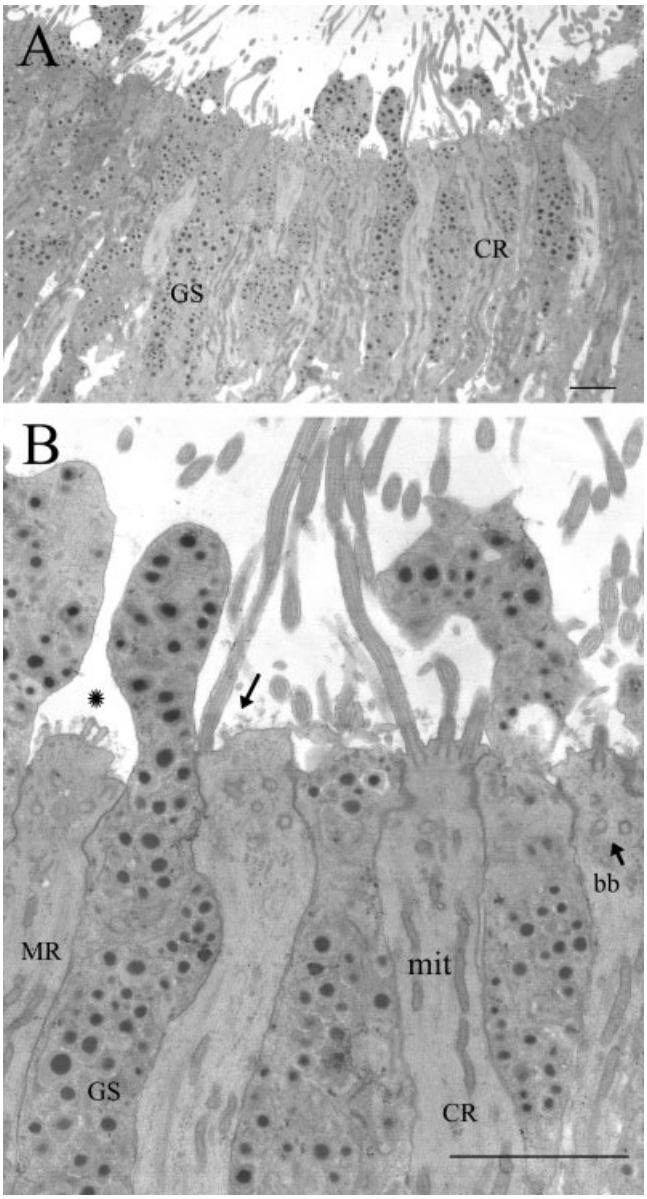


Fig. 9. The apical part of the main olfactory epithelium of a small larval *Dicamptodon tenebrosus*. TEM. **A:** Olfactory receptor cell dendrites are interspersed with granule-filled secretory supporting cells. **B:** The secretory supporting cells lack microvilli or cilia. The dendrites have short microvilli (asterisk) or short microvilli and cilia (arrow) or cilia only. Many mitochondria and a few basal bodies are present near the free edge of the dendrite. bb, basal bodies; CR, ciliated receptor cell; GS, granular secretory supporting cell; mit, mitochondria; MR, short microvillar receptor cell. Scale bars = 3 μm.

sity. Many of these cells have both cilia and short microvilli-like projections, although some have neither. The respiratory cells with electron-dense nuclei strongly resemble large ciliated supporting cells, but are distinguished by a more granulated nucleoplasm.

Respiratory epithelium of the main olfactory cavity in metamorphosed animals is distinguished from that of aquatic animals by the presence of goblet cells (Fig. 12C,D). Nuclei of goblet cells have light nucleoplasm and prominent nucleoli. The large vesicles within the goblet cells have a diameter of roughly 1.25 μm, and are of variable electron density. Each vesicle often has smaller dense granules on the inside. Many of the goblet cells have released the majority of their vesicles, possibly an artifact due to manipulation. Goblet cells are concentrated on the lateral side of the main olfactory cavity, in the grooves anterior and posterior to the vomeronasal organ, and are occasionally found in sensory epithelium.

In metamorphosed animals, the strip of respiratory epithelium that extends from the external naris, around the opening of the vomeronasal organ, and continues to the internal naris undergoes a gradual transition from squamous to cuboidal and finally to columnar epithelium, with goblet cells near the vomeronasal organ. The cells generally resemble those of larval respiratory epithelium. However, there are also distinctive cuboidal to columnar cells with a fan-shaped apical edge 7 μm above an electron-dense nucleus (Fig. 12B). These cells are densely decorated with many long cilia intermixed with short microvilli. The cilia and microvilli are ~7.3 μm and 0.7 μm long, respectively. The cilia originate at a basal body and have a lateral basal foot. These cells are concentrated on the lateral roof above and in the anterior groove leading to the vomeronasal organ. They are also found on the medial wall of the main olfactory cavity at the same level as the opening into the vomeronasal organ, and surrounding the choana, in regions that appear to be olfactory epithelium. This type of respiratory cell dominates the nonsensory epithelium in the main olfactory cavity in terrestrial animals. It is unclear whether the cuboidal and columnar cells are truly different cell types or merely a different conformation of the same cell type. The densely decorated respiratory cells, like the goblet cells, are not found in respiratory epithelium of aquatic animals.

Vomeronasal organ. The cellular composition of the vomeronasal organ in aquatic and metamorphosed *Dicamptodon tenebrosus* will be discussed together, since no apparent change occurs at metamorphosis. Dendrites of receptor cells in the vomeronasal organ of *D. tenebrosus* are exclusively microvillar (Fig. 13). The receptor cells contain basal bodies and mitochondria, similar to receptor cells of the main olfactory cavity. The lengths of microvilli are at least 1.3 μm, 8.5 μm, 10 μm, and 7.0 μm in small larvae, large larvae, neotenes, and metamorphosed animals, respectively. The widths of all microvilli are ~0.1 μm, just as in the main olfactory organ.

There are two classes of supporting cells in the vomeronasal organ: granular supporting and ciliated supporting cells. These resemble those in the main olfactory cavity of aquatic stages and in the

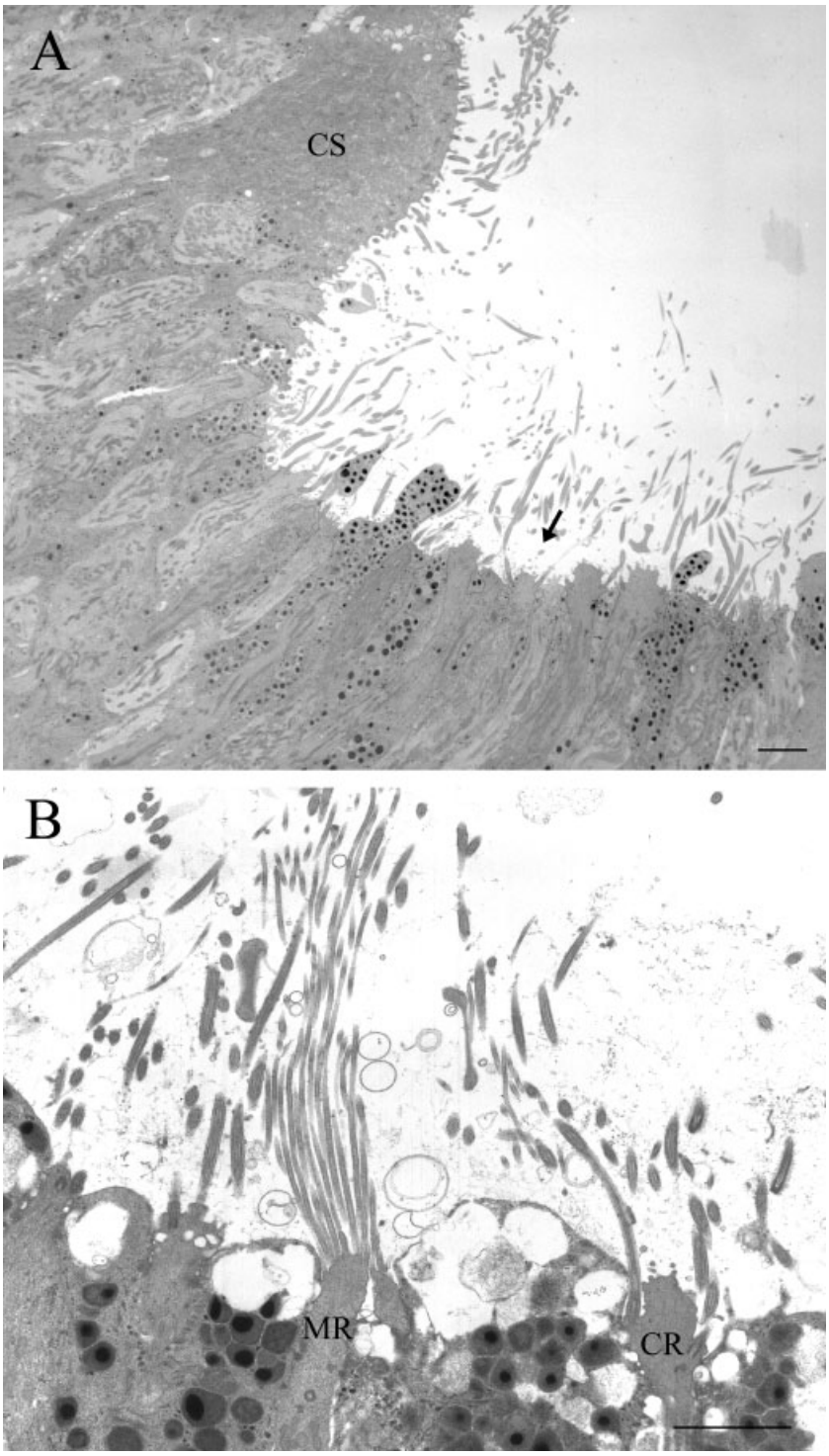


Fig. 10. The apical part of the main olfactory epithelium of a neotene (**A**) and a small larval *Dicamptodon tenebrosus* (**B**). TEM. **A:** The olfactory epithelium of neotenes closely resembled that of larvae. Note the large ciliated supporting cell (CS), along with granule-filled supporting cells and olfactory receptor cells interwoven with granular supporting cells similar to those in Figure 9. **B:** Microvillar receptor cells with long microvilli (MR) were rare in the larval main olfactory cavity, compared with short microvillar and ciliated receptor (CR) cells. The vesicular and ruptured ends of granule-containing support cells are likely an artifact. Scale bars = 3 μm.

lateral olfactory epithelium of terrestrial stages. There are relatively fewer granular supporting cells in comparison to the main olfactory cavity. The granules in all life stages are ~0.5 μm in diameter. Some have a dark ring around a light center, while others are uniformly dark. Some granule-containing supporting cells have small 0.8 μm microvilli. In contrast to the main olfactory cavity, ciliated supporting cells are abundant in the vomeronasal organ (Fig. 13A,D). The cilia have lengths of at least 13 μm in the middle of the vomeronasal organ and are mounted to basal bodies. Intermixed with the very long cilia are also cut fragments of microvilli, suggesting that receptor cells might have longer microvilli than estimated above.

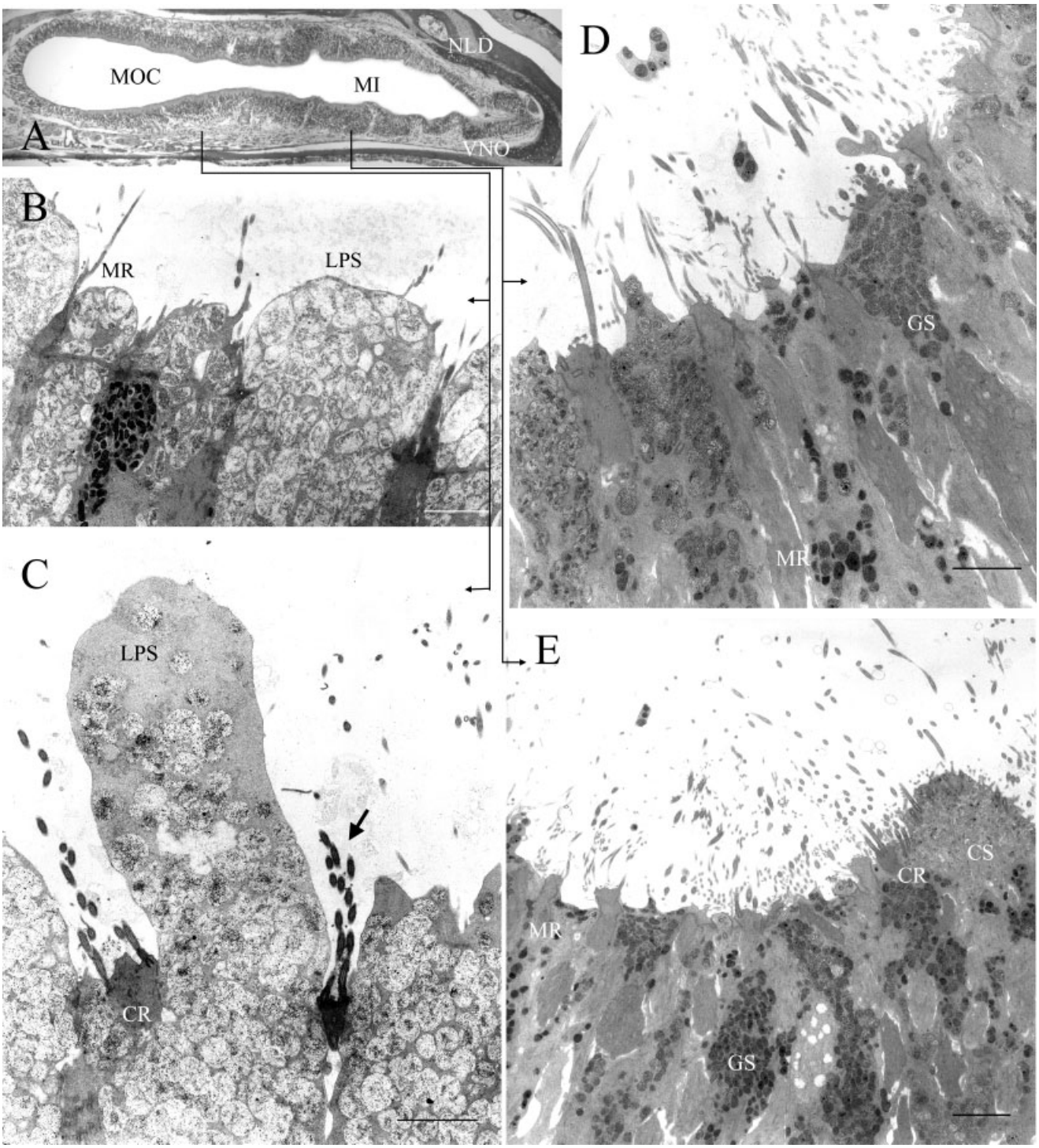


Fig. 11. The nasal organ of a terrestrial *Dicamptodon tenebrosus*. TEM. **A:** Division of ventral olfactory epithelium of the main olfactory cavity (MOC). Note that the predominant olfactory epithelium of the medial half is continuous, whereas the lateral olfactory epithelium is divided by longitudinal mini-ridges (MI) of respiratory epithelium. The low elevation in the floor of the main olfactory cavity approximately separates these regions. The arrows point to the approximate location of the TEMs. **B:** In the predominant epithelium, the supranuclear secretory contents of the supporting cells (LPS) appear loosely packed. Note also microvillar receptor cells (MR). **C:** The apical edge of loosely packed supporting cells is without cilia or microvilli and can be greatly extended above receptor cells, which here are ciliated (CR; arrow). **D:** In the lateral olfactory epithelium microvillar and ciliated receptor cells are present between granular supporting cells (GS). The general appearance is similar to the olfactory epithelium of aquatic animals. **E:** As in aquatic animals, the lateral olfactory epithelium also contains large ciliated supporting cell (CS). Scale bars = 200 μm in **A**, 3 μm in **B–E**.

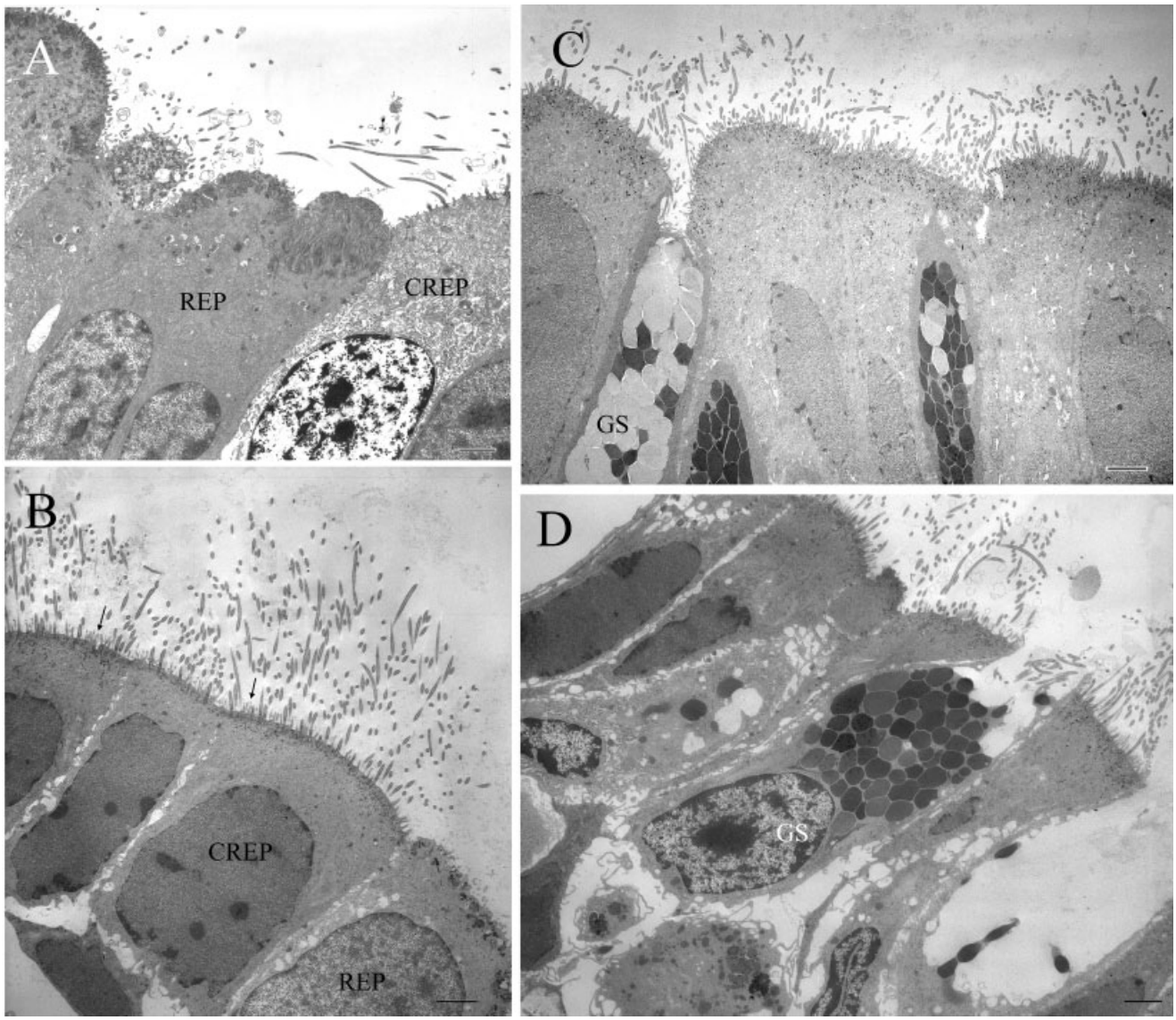


Fig. 12. Respiratory epithelium in the main olfactory cavity of a small larva (**A**) and terrestrial *Dicamptodon tenebrosus* (**B–D**). TEM. **A:** In aquatic animals, ciliated respiratory cells (CREP) have cytoplasm and nucleoplasm that is relatively electron lucent. Other more electron-dense respiratory cells (REP) lack cilia and may lack microvilli. **B:** In metamorphosed adults, some regions of respiratory epithelium contain cuboidal cells that can be ether ciliated or nonciliated. The ciliated cells have more abundant cilia than those found in aquatic animals; some also appear to have microvilli (arrows). **C:** Other regions contain abundant goblet cells (GS) and large columnar ciliated cells. This is from an area anterior to the vomeronasal organ. **D:** In this region some of the goblet cells have lost their vesicles. Scale bars = 3 μm.

DISCUSSION

General Morphology of the Nasal Cavities

Perhaps the most surprising result to emerge from this study is the great difference in gross morphology between the olfactory organ of all aquatic animals (both larvae and neotenes) and that of metamorphosed animals. Most recent literature suggests that the differences between larval and metamorphosed salamanders are minimal, with the main difference being the folding of the larval epithelium into ridges and grooves (e.g., Dawley, 1998).

However, a careful reading of the literature suggests that at least some of the other metamorphic changes seen in *Dicamptodon tenebrosus* are not unusual for salamanders. Seydel (1895) compared the morphology of the adult nasal cavities in several neotenic adult salamanders (*Proteus anguinus*, *Siren lacertina*, *Ambystoma mexicanum* [as "*Siredon pisciformis*"]) with that of metamorphosed adults salamanders (*Triturus vulgaris*, *T. alpestris*, *Salamandra salamandra* [as "*S. maculata*"]), and included a mid-metamorphic *S. salamandra*, as well as some observations on larvae of other species. He found

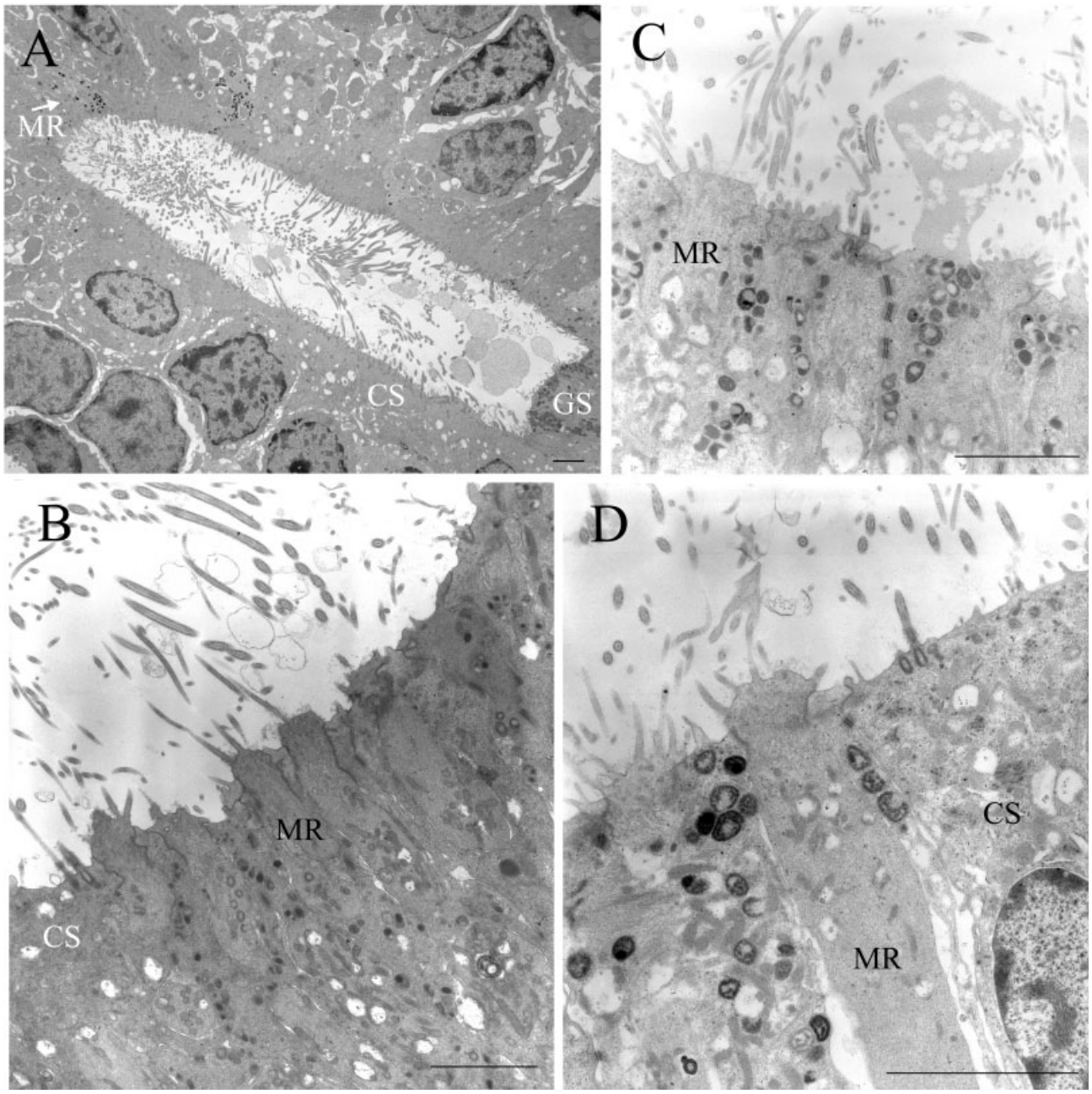


Fig. 13. The vomeronasal organ (VNO) of a small larva (**A**), a neotene (**B**), and a terrestrial *Dicamptodon tenebrosus* (**C,D**). TEM. **A:** Cilia from the wide apical edge of ciliated supporting cells (CS) fill the lumen of the vomeronasal organ. Microvillar receptor cells (MR) and granular supporting cells (GS) are relatively rare. **B:** In neotenes, microvillar receptor cells are more abundant. Projections from deep granular and ciliated supporting cells can also be seen. **C:** Note the abundance of granules on either side of microvillar dendrites. **D:** Ciliated supporting cells of terrestrial animals resemble those of aquatic animals. Scale bars = 3 μm.

that the neotenes generally had a vestibule ("Atrium") and a tubular nasal sac with ridges, unlike the broad, smooth nasal sac of metamorphosed animals. He noted the presence of a continuation of the lateral nasal groove ("seitlichen Nasenrinne") from the vomeronasal organ posteriorly, through the choana and above a palatal process ("Gaumenfortsatz"), to form the lateral palatal groove of metamorphosed animals; this was lacking in neotenes and larvae. Schuch (1934) observed similar changes in the metamorphosis of the European alpine newt, *T. alpestris*. He also noted a medial choanal valve in larvae that was absent after metamorphosis. The structure and function of this valve was investigated by Bruner (1914b) in a number of larval and neotenic salamanders; interestingly, such a valve does not occur in *D. tenebrosus* larvae or neotenes. In the

plethodontid salamander *Eurycea bislineata*, Wilder (1925) noted a number of metamorphic changes in the nose, including the formation of the nasolacrimal duct, the shift of the external naris dorsally, the formation of the nasolabial groove, and an anterior growth of the vomeronasal organ past the opening of the nasolacrimal duct into the lateral wall of the principal cavity. This anterior extension of the vomeronasal organ is apparently unique to plethodontids; it is related to a functional connection formed between the vomeronasal organ and the nasolabial grooves found only in this group (Dawley and Bass, 1988, 1989).

As noted above, the arrangement of the sensory epithelium of aquatic salamanders in grooves separated by ridges is well known. In larvae and neotenes, sensory epithelium is confined to low valleys, while respiratory epithelium dominates the ridges between them (Blaue, 1884; Seydel, 1895; Anton, 1908, 1911; Matthes, 1934; Farbman and Gesteland, 1974; Graziadei and Monti Graziadei, 1976; Eisthen et al., 1994). However, the ridges in most other neotenes are relatively poorly developed; only *Necturus maculosus* (Anton, 1911, as "*Menobranchus lateralis*") seems to approach the condition seen in *Dicamptodon tenebrosus*. Interestingly, Matthes (1927) found that metamorphosed newts, *Triturus vulgaris*, reversibly develop larger ridges in their water- than their land-phase.

Eisthen et al. (1994) reported that *Ambystoma mexicanum* larvae have much deeper grooves than the neotenic adults and that both have the same pattern of ridges. It is worth noting that *A. mexicanum* is naturally unable to undergo metamorphosis due to a deficiency in release of thyroxin (Kalthoff, 2001). This deficiency may not fully inhibit changes that occur in the nasal organ associated with metamorphosis, possibly explaining why the nasal organs of adult aquatic *A. mexicanum* have only low ridges, and thus are more similar to metamorphosed than to aquatic *Dicamptodon tenebrosus*.

The pattern of ridges and grooves appears to be variable among species. In *Dicamptodon tenebrosus*, the ridges are approximately transverse; in larval *Triturus cristatus*, they are oblique (Blaue, 1884); and in larval *T. alpestris*, they appear to be longitudinal (Schuch, 1934). In the neotenic proteids *Proteus anguinus* and *Necturus maculosus*, there is a central ventral longitudinal fold with lateral transverse branches (Blaue, 1884; Anton, 1911; Matthes, 1934; Farbman and Gesteland, 1974). However, Schmalhausen (1958, cf. 1968) figured a series of transverse folds in larvae of *Ambystoma tigrinum* and the hynobiids *Ranodon sibiricus* and *Salamandrella ("Hynobius") keyserlingii*.

Terrestrial *Dicamptodon tenebrosus* have an olfactory organ similar to that of other metamorphosed salamanders in shape and distribution of epithelium. As in *D. tenebrosus*, the main olfactory cavity in most other salamanders is a long dorsoventrally flattened tube composed of olfactory epithelium on the floor and roof, with a narrow connection to the smaller lateral vomeronasal cavity, which is also flattened (Bawden, 1894; Seydel, 1895; Matthes, 1934; Schuch, 1934; Breipohl et al., 1982; Dawley and Bass, 1988). In the main olfactory cavity of metamorphosed Tiger Salamanders, *Ambystoma tigrinum*, there are long parallel ridges of respiratory epithelium ventrolaterally, starting at the external nares and ending at the choana (Breipohl et al., 1982). Similar ridges are found in the Spotted Salamander, *A. maculatum* (Bawden, 1894), and both land- and water-phase newts, *Triturus vulgaris* (Blaue, 1884; Matthes, 1927, 1934; Saint Girons and Zylberberg, 1992b), but in the Fire Salamander, *Salamandra salamandra*, even these ridges are lost with postmetamorphic growth (Blaue, 1884). These low ridges in adults appear to be remnants of the larval ridges. Similarly, the dramatic transverse folds present in aquatic *D. tenebrosus* appear to be almost completely lost dorsally in metamorphosed animals; however, the very small ridges of respiratory epithelium found on the ventrolateral floor of the main olfactory cavity, which run from anteromedial to posterolateral, are possibly a remnant of the ridges found in the premetamorphic life stage.

In *Dicamptodon tenebrosus*, these mini-ridges of respiratory epithelium on the lateroventral floor of the main olfactory cavity segregate the distinctive lateral olfactory epithelium from the predominant postmetamorphic type and partition it into strips. This lateral olfactory epithelium resembles the epithelium seen in the aquatic life stage (see below). The region of the lateral olfactory epithelium appears to correspond to the region in the Tiger Salamander, *Ambystoma tigrinum*, which Breipohl et al. (1982) observed divided into strips by intervening respiratory epithelium, and which Getchell et al. (1986) called the "adult immature olfactory mucosa."

In terrestrial *Dicamptodon tenebrosus*, unlike aquatic animals, only respiratory epithelium is present around and behind the choana and along the posteromedial and posterior walls of the main olfactory cavity, in contrast to the ample sensory epithelium in this region in aquatic *D. tenebrosus*. This appears to be a common phenomenon: a similar reduction or lack of olfactory epithelium posteriorly has been noted in terrestrial Fire Salamanders, *Salamandra salamandra* (Seydel, 1895, as "*S. maculata*"), newts, *Triturus vulgaris* (Seydel, 1895; Matthes, 1934), Tiger Salamanders, *Ambystoma tigrinum* (Breipohl et al., 1982), and Eastern Redbacked Salamanders, *Plethodon cinereus* (Dawley and Bass, 1988).

In comparing the metamorphic changes in the nose of salamanders with those of other amphibians, one finds that there are both similarities and differences. In frogs, the larval olfactory organ is typically a complicated system of interconnected sacs and

outpocketings that extends nearly vertically from the external naris to the choana (Born, 1876; Hinsberg, 1901; Watanabe, 1936; Rowedder, 1937; Tsui, 1946; Tsui and Pan, 1946; Yvroud, 1966; Khalil, 1978; Jermakowicz et al., 2004). One of the outpocketings is the vomeronasal organ, which lies on the anterior (morphologically ventral) aspect of the organ, another is the larval "lateral appendix." Both respiratory and olfactory epithelium are present in various regions, but there is no indication of the ridge and valley arrangement seen in salamander larvae. After metamorphosis, the olfactory organ resembles that of metamorphosed salamanders (reviewed by Jurgens, 1971), although the simple lateral nasal groove of salamanders is represented by more complicated, interconnected middle and inferior nasal cavities, with the vomeronasal organ located along the medial side ("medial recess") of the inferior cavity. A raised olfactory eminence is often found on the floor of the principal cavity—it is best developed in terrestrial forms. Four metamorphic changes that especially resemble those seen in salamanders are the development of the nasolacrimal duct, the loss of the larval vestibule, or "entrance canal," the loss of the larval choanal valve (lacking in *Dicamptodon tenebrosus*), and the posterior continuation of the vomeronasal organ by the lateral recess of the inferior cavity and the lateral palatal groove. Metamorphic changes in the nose of the basal anuran *Ascaphus truei* (Baard, 1987) and of *Xenopus laevis* (Föske, 1934; Paterson, 1939) differs from that of more typical representatives, but not in any way especially resembling that of salamanders.

In caecilians, metamorphic changes have been best described in *Ichthyophis glutinosus* (Sarasin and Sarasin, 1890; Badenhorst, 1978; Schmidt and Wake, 1990). The larval nasal sac resembles that of salamanders in general form, with a large vestibule ("external nasal tube") and a simple, dorsoventrally flattened principal cavity. Unlike salamanders, however, there is no division of sensory epithelium by ridges of nonsensory epithelium. The anterior part of the vomeronasal organ is located laterally, as in salamanders, but it shifts to lie ventromedially at the level of the choana. A choanal valve is apparently absent in *I. glutinosus*, but a larval choanal valve has been described in *Uraeotyphlus oxyurus* (Wilkinson, 1992). A small choanal slime sac ("Choanenschleimbeutel") lies at the anterior border of the choana. During metamorphosis the olfactory organ becomes less similar to that of salamanders. A large longitudinal ridge arises in the floor of the principal cavity, separating it into lateral and medial portions (this somewhat resembles the olfactory eminence of frogs). The anterior end of the vomeronasal organ bends laterally and connects to the nasolacrimal (tentacular) duct. The choanal slime sac greatly enlarges. Unlike frogs and salamanders, no lateral nasal and palatal grooves form. Only two metamorphic changes specifically resemble those in salamanders (and frogs): the loss of the vestibule and the formation of the nasolacrimal duct.

Finally, it is worth noting that, although no other amphibians have a larval olfactory epithelium with ridges of respiratory epithelium separating troughs of sensory epithelium like that of larval salamanders, similar structures have been described in early larval stages of lungfishes (Bartsch, 1993). In later stages, this arrangement persists in the "receptor grooves" on the surface of the primary gill lamellae (Derivot, 1984). The arrangement shared by salamander larvae and lungfishes contrasts with the situation in most fishes, in which the primary olfactory lamellae are largely covered by olfactory epithelium, rather than nonsensory epithelium (Kleerekoper, 1969). Such an arrangement of epithelium is thus likely a primitive feature of salamander larvae, lost in other amphibians. Young lungfishes also have a nonsensory vestibule, resembling that of larval amphibians (although the different position of the incurrent naris makes their homology somewhat questionable).

If we accept the current consensus phylogeny with a monophyletic Lissamphibia, and frogs and salamanders as sister groups (e.g., Mauro et al., 2005), the common ancestor likely had an olfactory metamorphosis resembling that of salamanders. In this interpretation, frogs and caecilians retained the ancestral metamorphic loss of the vestibule and formation of the nasolacrimal duct, but independently lost the ancestral ridge-and-valley arrangement of olfactory epithelium. A larval choanal valve was present in the ancestor, and independently lost in some caecilians, salamanders (including *Dicamptodon tenebrosus*), and frogs. Adults of the batrachian lineage (frogs + salamanders) evolved a lateral palatal groove leading through the choana to the lateral nasal groove (lateral recess) and the vomeronasal organ (alternatively, caecilians may have lost this groove). Frog tadpoles greatly specialized their nose in association with the unique tadpole morphology. Likewise, frog adults greatly specialized the lateral nasal groove as the inferior nasal cavity. Finally, caecilian adults evolved a new association between the nasolacrimal ducts and vomeronasal organ in association with the evolution of the tentacle (cf. Billo and Wake, 1987). This evolutionary interpretation is consistent with our current knowledge, although not highly constrained. Further information on the diversity of olfactory structure within living amphibians would help to confirm or reject it.

Ultrastructure of Amphibian Olfactory Epithelium

Our knowledge of the changes that occur at the ultrastructural level during metamorphosis in all three groups of living amphibians is also incomplete (see Tables 1, 2). This is the only study yet to exam-

TABLE 1. Olfactory receptor and supporting cell types in selected amphibians

	Frogs[a] (*Xenopus, Rana*)		**Caecilian**[b] (*Typhlonectes*)
	Larvae	Adults	Aquatic adult
Principal cavity			
Receptor cells	microvillar, ciliated	ciliated	microvillar*, ciliated
Supporting cells	secretory, ciliated	secretory	secretory
Middle cavity (*Xenopus* only)			
Receptor cells	—	microvillar, ciliated	
Supporting cells	—	secretory, ciliated	
Vomeronasal organ			
Receptor cells	microvillar	microvillar	microvillar
Supporting cells	ciliated	ciliated	secretory

	Salamanders and newts		
	Ambystoma mexicanum[c] Neotenic adult	*Ambystoma tigrinum*[d] Terrestrial adult	*Cynops pyrrhogaster*[e] Aquatic adult
Main olfactory cavity			
Receptor cells	microvillar, ciliated	microvillar, ciliated	microvillar, ciliated
Supporting cells	secretory (granular)	secretory (loosely packed)	secretory (loosely packed)
Vomeronasal organ			
Receptor cells	microvillar	microvillar	microvillar
Supporting cells	secretory, ciliated	secretory, ciliated	secretory

[a]Hansen et al., 1998; Taniguchi et al., 1996
[b]Saint-Girons and Zyberberg, 1992b.
[c]Eisthen et al., 1994.
[d]Zielinski et al., 1988; Eisthen and Schroeder, 1992.
[e]Jones et al., 1994.
*Found only in the anteroventral region of the nasal cavity.

ine multiple life stages of one species of salamander. The only frogs to have been examined before and after metamorphosis are *Xenopus laevis* (Hansen et al., 1998) and *Rana japonica* (Taniguchi et al., 1996). Only one species of caecilian (*Typhlonectes compressicauda*), a secondarily aquatic animal, has been studied at the ultrastructural level (Saint Girons and Zylberberg, 1992b).

Receptor cells of the main olfactory cavity. All amphibian olfactory receptor cells are bipolar neurons. Each neuron contains a single dendrite and a single axon at opposite ends of the cell body. Receptor cells can be divided into two groups: 1) olfactory receptor cells, located only in the main olfactory cavity; and 2) vomeronasal receptor cells, located only in the vomeronasal organ.

In all salamanders examined to date, including both aquatic and terrestrial forms, both microvillar and ciliated receptor cells are found in the main olfactory cavity (Farbman and Gesteland, 1974; Eisthen, 1992; Eisthen and Schroeder, 1992; Saint Girons and Zylberberg, 1992b; Eisthen et al., 1994; Jones et al., 1994; Dumas and Chris, 1998; Eisthen, 2000). In *Ambystoma mexicanum*, neotenic adults there are many olfactory receptor cells which terminate in microvillar dendrites and a few of which terminate in motile cilia with 9 + 2 microtubules (Eisthen et al., 1994). By contrast, in aquatic *Dicamptodon tenebrosus* at least as many, if not more, olfactory receptor cells terminate in cilia (with or without microvilli) as microvilli alone in the main olfactory cavity.

TABLE 2. Cell types found in the olfactory epithelia of Dicamptodon tenebrosus

	Larvae and neotenes	**Terrestrial adults**	
Main olfactory cavity		*Predominant epithelium*	*Lateral olfactory epithelium*
Receptor cells	microvillar, ciliated	microvillar, ciliated	microvillar, ciliated
Supporting cells	secretory (granular), ciliated	secretory (loosely packed)	secretory (granular), ciliated
Respiratory cells	ciliated, nonciliated		
Vomeronasal organ		ciliated, nonciliated, goblet, "densely decorated"	
Receptor cells	microvillar	microvillar	
Supporting cells	secretory (granular), ciliated	secretory (granular), ciliated	

Although in *Dicamptodon tenebrosus* the main olfactory organ contains receptor cells that terminate in either microvilli or cilia, it is unclear if this is a fundamental distinction between cell types. Based on overall morphology of the dendrite and comparison with other taxa, it appears rather that the cells terminating in long microvilli may be one type, while those with cilia, short microvilli, or a combination of both may be variant forms of another. Farbman and Gesteland (1974) reported finding receptor cells with long microvilli only, cilia only, or both cilia and short microvilli (but none with short microvilli only) in the olfactory cavity of neotenic salamanders (*Necturus maculosus*). Larvae of the frog *Xenopus laevis* have just two types of receptor cells: ciliated and long microvillar. The ciliated receptor cells have small protrusions between the cilia on receptor cells (Hansen et al., 1998). These protrusions somewhat resemble the short microvilli seen in *Dicamptodon tenebrosus*, but are less defined. Similar small protrusions are also seen in the micrographs of ciliated receptor cells of neotenic *Ambystoma mexicanum* (Eisthen et al., 1994).

More generally, it is unclear if having cilia is a permanent or transitory phase in the life cycle of olfactory receptor cells. Cells destined to develop cilia contain many basal bodies (centrioles) to anchor the bases of the cilia and to initiate growth of the cilia. Microvillar olfactory and vomeronasal receptor cells in many vertebrates contain multiple basal bodies, suggesting that ciliated receptor cells may have given rise to microvillar cells or originate from them in ontogeny or phylogeny (Eisthen et al., 1994; Eisthen, 1997). However, ciliated, short microvillar, ciliated with short microvilli, and long microvillar receptor cells may all nevertheless be functionally distinct.

Ciliated and microvillar cells of neotenic *Ambystoma mexicanum* are found in clusters with little overlap (Eisthen et al., 1994), unlike *Dicamptodon tenebrosus,* which has an even distribution of all receptor cell types. The width of the microvilli (0.1 μm) and cilia (0.25 μm) are the same for *D. tenebrosus* and *A. mexicanum*. Cilia (10 μm) are slightly longer than microvilli (7 μm) on receptor cells, similar to *D. tenebrosus*, and both cilia and (long) microvilli are within the same general range as *D. tenebrosus* (9–15 μm).

In *Dicamptodon tenebrosus*, the receptor cells of the main olfactory cavity have no apparent differences after metamorphosis (Table 2). Not only are both ciliated and long and short microvillar receptors still present, but the length of the cilia and microvilli appears similar in both cases. Although no other comparable study at the TEM level has been conducted in salamanders, Schuch (1934), in his careful light microscopical study of olfactory metamorphosis in the alpine newt *Triturus alpestris*, noted an increase in length of the cilia at metamorphosis, along with an overall thinning of the olfactory epithelium. He noted a reverse shortening of the cilia in metamorphosed animals that had returned to the water. A similar change in ciliary length with change in medium in metamorphosed *T. vulgaris* and *T. cristatus* was reported by Matthes (1927), who found that the shortening of cilia occurred within 1 h of placing terrestrial animals into water, apparently due to osmotic effects, while the lengthening of cilia upon bringing aquatic animals on land took 4–5 days. He correlated this change in length of cilia with the ability of the animals to smell in the two media; terrestrial animals could immediately locate food by smell in water, but aquatic animals could not do so on land until their cilia had lengthened.

A similar situation in seen in frogs, where the metamorphic changes have previously been best studied (Table 1). Larval frogs have long microvillar and ciliated receptor cells in the principal cavity (equivalent to the salamander main olfactory cavity), but lose the microvillar receptor cells as the olfactory epithelium shifts to chemoreception in air during metamorphosis (Table 1; Taniguchi et al., 1996; Hansen et al., 1998). The ciliated receptor cells in the adult have much longer cilia than those of premetamorphic frogs (Hansen et al., 1998).

Interestingly, in the aquatic adult of the caecilian *Typhlonectes compressicauda*, two types of olfactory epithelium are present, somewhat resembling the situation in *Dicamptodon tenebrosus*. One type, found anteroventrally, has both microvillar and ciliated receptors and lacks Bowman's glands. The other, found posterodorsally, has only ciliated receptors and has Bowman's glands (Saint Girons and Zylberberg, 1992b).

Supporting cells of the main olfactory cavity. In *Dicamptodon tenebrosus*, there are at least three different types of supporting cells (see Table 2). Two of these are found in aquatic *D. tenebrosus*. The most common is a long, slender secretory supporting cell containing electron-dense granules; each granule typically contains a cluster of smaller, more electron-dense matter inside. The second type of supporting cell is large and ciliated; the cilia are apparently motile. The secretory supporting cells seen in larvae resemble those seen in neotenic axolotls *Ambystoma mexicanum*, as well as *Amphiuma tridactylum*, *Necturus maculosus*, and *Proteus anguinus* (Farbman and Gesteland, 1974; Eisthen et al., 1994; Dumas and Chris, 1998; Eisthen, 2000). However, the large ciliated supporting cells do not seem to have been observed previously in the main olfactory cavity of salamanders, although similar cells are present in the principal cavity of larval frogs in general and the middle cavity of adult *Xenopus* (Taniguchi et al., 1996; Hansen et al., 1998; Oikawa et al., 1998), as well as in respiratory epithelium of aquatic salamanders (see below). The ciliated supporting cells of frogs are much more slender and have fewer cilia than those seen in *D. tene-*

brosus. Farbman and Gesteland (1974) reported a third type of supporting cell in *N. maculosus*, containing abundant smooth endoplasmic reticulum (ER) in the supranuclear cytoplasm. These resemble the nonciliated cells of the respiratory epithelium in aquatic *D. tenebrosus*.

In metamorphosed *Dicamptodon tenebrosus*, both larval types of supporting cells are still found in the lateral olfactory epithelium, although the electron-dense granules inside the granular supporting cells in terrestrial specimens are smaller and generally less electron dense than those of aquatic specimens. However, a third type of supporting cell in terrestrial adults is found exclusively throughout the predominant type of olfactory epithelium. This is a secretory supporting cell which is full of large electron-lucent vesicles. We have called these "loosely packed" supporting cells. The loosely packed supporting cells in the predominant epithelium of metamorphosed *D. tenebrosus* are quite similar in appearance to the supporting cells in the terrestrial Tiger Salamander *Ambystoma tigrinum* (Zielinski et al., 1988) and in the aquatic adult Japanese Newt *Cynops pyrrhogaster* (Jones et al., 1994).

No previous study of metamorphosed salamanders appears to have noted a special lateral epithelium containing larval-type electron-dense granule-containing and ciliated supporting cells, as found in terrestrial *Dicamptodon tenebrosus*. However, using light microscopy Getchell et al. (1984, p. 556) noted that supporting cells of larval Tiger Salamanders *Ambystoma tigrinum* had small basophilic secretory granules, whereas adult supporting cells had "vesicular material" that "resembled mucigen droplets seen in goblet cells." In juvenile animals immediately after metamorphosis both types of cells were present in different regions; their precise distribution was not described. In a later article from the same laboratory (Getchell et al., 1986), the ventrolateral olfactory epithelium in *A. tigrinum* was described as the "immature adult olfactory epithelium," but they noted that the supporting cells here had large secretory vesicles, as in the predominant or "mature olfactory epithelium," but unlike the larval supporting cells, which contained small secretory granules. No differences in the olfactory epithelium of this region in *A. tigrinum* have been reported at SEM (Breipohl et al., 1982) or TEM (Eisthen and Schroeder, 1992) levels. Moreover, physiological studies (e.g., Mackay-Sim et al., 1982) and mapping of odorant receptor expression patterns (Kauer, 2002; Marchand et al., 2004) also show no important distinction between this region and other areas of the main olfactory cavity.

It is thus unclear whether conditions with respect to supporting cells in *Dicamptodon tenebrosus* are typical of salamanders in general. It appears likely that the change from small electron-dense granules to larger electron-lucent vesicles is a common feature of supporting cell metamorphosis, because it has been noted previously in Tiger Salamanders and corresponds to differences generally observed between neotenic and metamorphosed forms. On the other hand, the presence of ciliated supporting cells in larvae and the retention of a larval-type lateral olfactory epithelium of adults appears to be unique to *D. tenebrosus* among described salamanders. It is worth noting that this epithelium was observed in all metamorphosed *D. tenebrosus*, from 1 week to a year postmetamorphosis, and so its presence is unlikely to be merely a transient stage in development.

A change in supporting cell types at metamorphosis resembling that of *Dicamptodon tenebrosus* also occurs in frogs. The principal cavity of the frogs *Xenopus laevis* and *Rana japonica* at metamorphosis transitions from chemoreception in water to air; in the process it loses the ciliated supporting cells and maintains a population of secretory supporting cells (Taniguchi et al., 1996; Hansen et al., 1998; Oikawa et al., 1998). Moreover, the secretory supporting cells appear to change morphology. However, the secretory supporting cells in the principal cavity of premetamorphic *X. laevis* contain vesicles that are electron lucent, like those of terrestrial *D. tenebrosus* (although fewer in number). After metamorphosis in *X. laevis*, the vesicles become more electron dense and now often contain a more electron-dense core (Hansen et al., 1998). They are thus more similar to those present throughout the main olfactory cavity of aquatic phase and on the ventrolateral side of the main olfactory cavity of metamorphosed *D. tenebrosus*. In adult *X. laevis*, however, the vesicles are only of moderate density (cf. Saint Girons and Zylberberg, 1992b; Oikawa et al., 1998), and resemble those of other frogs (Graziadei, 1973).

In the caecilian *Typhlonectes compressicauda*, only secretory supporting cells are present in both regions of the main olfactory epithelium (Saint Girons and Zylberberg, 1992b).

Respiratory epithelium. In the main olfactory cavity of all aquatic salamanders, ridges of respiratory epithelium separate regions of olfactory epithelium. This respiratory epithelium generally appears similar to that observed in aquatic *Dicamptodon tenebrosus*, containing both ciliated and nonciliated cells, but lacking goblet cells (Farbman and Gesteland, 1974; Graziadei and Monti Graziadei, 1976; Saint Girons and Zylberberg, 1992b; Eisthen et al., 1994). However, Getchell et al. (1984, 1986) reported both goblet and "mucoid" cells present in larval Tiger Salamanders, although noting that the goblet cells in larvae had "scant secretory material." Goblet cells have also been reported in larval newts, *Triturus alpestris* (Schuch, 1934). Ciliated and microvillar respiratory epithelium lacking goblet cells is also present on ridges of the main olfactory cavity in secondarily aquatic adult newts (Matthes, 1927; Schuch, 1934; Jones et al., 1994). Schuch (1934) argued, on the basis of his careful observations, that

these strips of respiratory epithelium derived from supporting cells by transdifferentiation, but there has been no experimental test of his view.

After metamorphosis, the respiratory epithelium of *Dicamptodon tenebrosus* contains both goblet cells and cells densely decorated with cilia, as well as cells resembling the ciliated and nonciliated cells present in aquatic animals. Goblet cells and densely ciliated respiratory cells have also been reported in the adult respiratory epithelium of a number of other terrestrial as well as secondarily aquatic salamanders (Saint Girons and Zylberberg, 1992b; Jones et al., 1994). A marked increase in the secretory activity of the goblet cells in terrestrial vs. aquatic newts was noted by Matthes (1927). He also noted the loss of cilia on the strips of respiratory epithelium separating regions of olfactory epithelium in the main olfactory cavity.

In frogs, respiratory epithelium containing both goblet and ciliated cells is common in adults (e.g., Gaupp et al., 1904; Saint Girons and Zylberberg, 1992b). We have been unable to determine from the literature whether goblet cells are also present in larvae. In caecilians the adult nasal cavities have extensive regions of respiratory epithelium containing both goblet cells and ciliated cells (Sarasin and Sarasin, 1890; Schmidt and Wake, 1990). Goblet cells are apparently absent in larvae (Sarasin and Sarasin, 1890).

Bowman's glands. Bowman's glands, in conjunction with secretory supporting cells, are responsible for secreting the layer of mucus present on the olfactory epithelium in terrestrial vertebrates. Bowman's glands are present in the olfactory epithelium of all *Dicamptodon tenebrosus*, but are less abundant in aquatic than terrestrial animals. In small larval *D. tenebrosus*, Bowman's glands are embedded in the epithelium and appear underdeveloped, but have visible ducts aiming toward the surface, suggesting that the gland may be functional. As *D. tenebrosus* mature, the Bowman's glands enlarge and often protrude down into the lamina propria. They also become more common, although the source of the new glands is not clear. However, Bowman's glands are absent from the larval type lateral olfactory epithelium.

Bowman's glands are also present in larval *Ambystoma tigrinum* and neotenic *A. mexicanum* (Getchell et al., 1984, 1986; Eisthen et al., 1994) and neotenic *Amphiuma* (Eisthen, 2000), but other neotenes in the families Proteidae and Sirenidae completely lack these glands (Anton, 1911; Farbman and Gesteland, 1974; Saint Girons and Zylberberg, 1992b; Eisthen, 2000). In *A. tigrinum*, as in *Dicamptodon tenebrosus*, Bowman's glands are much better developed in adults than larvae (Getchell et al., 1986). The development of the glands has been well described in *Triturus alpestris* (Schuch, 1934), which resembles *D. tenebrosus* closely in this respect. Bowman's glands are always present in the main olfactory cavity of metamorphosed salamanders.

In most frogs, Bowman's glands appear in the principal cavity only during metamorphosis (Rowedder, 1937; Hansen et al., 1998). The premetamorphic principal cavity (as well as the water-filled middle cavity of adult *Xenopus laevis*) completely lacks Bowman's glands. Likewise, in the caecilian *Ichthyophis glutinosus*, Bowman's glands appear only during metamorphosis (Sarasin and Sarasin, 1890; Badenhorst, 1978). Thus, in frogs and caecilians, the appearance of Bowman's glands in the principal cavity is a metamorphic event; in salamanders the same change is drawn out over a greater period of premetamorphic larval development. Interestingly, the secondarily aquatic caecilian *Typhlonectes compressicauda*, like *Dicamptodon tenebrosus*, lacks Bowman's glands in the distinctive anteroventral olfactory epithelium, but has them in the posterodorsal epithelium (Saint Girons and Zylberberg, 1992b).

Vomeronasal epithelium. The vomeronasal organ is present in all life stages of *Dicamptodon tenebrosus* and is located on the lateral wall of the main olfactory cavity. This position is similar to that in most other salamanders, although the position and morphology of the vomeronasal organ among salamander families varies (Seydel, 1895; Anton, 1911; Jurgens, 1971; Eisthen, 2000). Larval *D. tenebrosus* have a relatively small vomeronasal organ, which largely lacks receptor cells on its roof. This supports the argument that the vomeronasal organ is behaviorally more significant for adult salamanders. The vomeronasal epithelium is indistinguishable among all life stages of *D. tenebrosus*. No apparent change occurs at metamorphosis.

The vomeronasal epithelium of *Dicamptodon tenebrosus*, like that of most other salamanders and other tetrapods (Eisthen, 1992, 2002; Eisthen et al., 1994), contains exclusively microvillar receptor cells. The lengths and widths of the microvilli of *Ambystoma tigrinum* and *A. mexicanum* are also comparable to those of *D. tenebrosus*. On the other hand, the newts *Triturus alpestris*, *T. cristatus*, and *Pleurodeles waltl* have both microvillar and ciliated receptors in the vomeronasal organ (Saint Girons and Zylberberg, 1992b).

In *Dicamptodon tenebrosus*, two types of supporting cells are present in the vomeronasal epithelium (see Table 2). The first type is similar to the granule containing supporting cells found in the main olfactory cavity of aquatic *D. tenebrosus*, except there are fewer granules and they are more electron lucent. The second type of supporting cell is ciliated and is similar to ciliated supporting cells of the main olfactory cavity. Both of these supporting cell types have been seen in neotenic axolotls, *Ambystoma mexicanum* (Eisthen et al., 1994), and *Amphiuma tridactylum* (Eisthen, 2000), but the ciliated cells are lacking in *Siren lacertina* (Eisthen, 2000). Among

metamorphosed salamanders, ciliated supporting cells are apparently present in the vomeronasal organ of Tiger Salamanders, *A. tigrinum* (Eisthen and Schroeder, 1992), and all known salamandrids (Schuch, 1934; Saint Girons and Zylberberg, 1992b), but are lacking in plethodontids (Dawley and Bass, 1988; Saint Girons and Zylberberg, 1992b).

In all frogs examined to date, the vomeronasal organ of both tadpoles and adults contains only microvillar receptors and ciliated supporting cells (Franceschini et al., 1991; Eisthen, 1992; Taniguchi et al., 1996; Hansen et al., 1998). On the other hand, in the caecilian *Typhlonectes compressicauda*, the only species for which ultrastructural data are available, the vomeronasal organ contains microvillar receptors, but secretory supporting cells (Saint Girons and Zylberberg, 1992b).

Implications for Function

It has been known for a long time that amphibians are able to smell both in water and in air (reviewed by Dawley, 1998; Jorgensen, 2000). The significant changes observed in the gross morphology and ultrastructure of the olfactory organ at metamorphosis in *Dicamptodon tenebrosus* suggest a functional relation with the change in environment, from aquatic to terrestrial. At the gross morphological level, the prominent ridges in the main olfactory cavity of aquatic salamanders, including *D. tenebrosus*, suggest an important function: perhaps they increase odorant perception by increasing turbulence and directing the flow of water. Interestingly, in newts, which return to the water for extended periods after metamorphosis from the larval phase, the ridges of respiratory epithelium again increase in height (Matthes, 1927; Schuch, 1934), supporting the idea that the ridges have an important function. The great development of these ridges in *D. tenebrosus*, particularly in older larvae and neotenes, argues for a more important role here than in other salamanders. Perhaps there is a relationship to its fast-flowing stream habitat; most other aquatic salamanders are found in ponds or slow streams. The membranous vestibule of aquatic animals may serve only as a connection from the external naris to the main olfactory cavity, but it is possible that it plays some role in regulating the flow of water through the cavity. The absence in *D. tenebrosus* of the choanal valves typically seen in other salamanders (Bruner, 1914b) certainly suggests that some other mechanism for preventing reverse flow of water through the nose may exist; a small striated muscle that runs anteriorly along the medial border of the choana may play a role here.

The expanded, smooth nasal sac seen in terrestrial *Dicamptodon tenebrosus* is typical for terrestrial salamanders, as is the development of the lateral palatal groove as a posterior extension of the lateral nasal groove. As has been suggested many times (e.g., Seydel, 1895; Bruner, 1914a), the lateral palatal groove may function to conduct odorous substances from the mouth cavity into the vomeronasal organ of adults.

At the ultrastructural level, the most dramatic changes that take place during metamorphosis of the olfactory epithelium are in the olfactory supporting cells. As noted above, the transition from supporting cells with electron-dense vesicles to the electron-lucent loosely packed appears to be typical of all metamorphosing salamanders. The increase in number and maturity of Bowman's glands and the appearance of goblet cells in respiratory epithelium suggests, rather unsurprisingly, that increased mucus secretion occurs in the terrestrial forms. This is likely necessary not only to protect the epithelium from desiccation, but also to secrete odorant-binding proteins to aid in transporting volatile molecules across the layer of mucus. Interestingly, although no obvious morphological changes occur in the receptor cells at metamorphosis, physiological studies of Tiger Salamanders (Arzt et al., 1986) have shown that the larval epithelium responds more effectively to amino acids in solution, the adult epithelium to volatile compounds in air. This difference may be mediated by changes in the chemistry of the mucus (e.g., a change in odorant-binding protein expression). Alternatively, olfactory receptor cells may change expression of odorant receptors without showing any morphological changes.

The presence of a larval-type lateral olfactory epithelium in terrestrial adults was an unexpected result of this study. The obvious interpretation is that this epithelium enables *Dicamptodon tenebrosus* to continue smelling in water after metamorphosis. In the secondarily aquatic frog *Xenopus laevis*, two olfactory chambers are present, one of which is responsible for smelling in air, the other for smelling in water (Altner, 1962; Freitag et al., 1995; Hansen et al., 1998). However, in *D. tenebrosus*, unlike *X. laevis*, the larval-type epithelium is not anatomically segregated from the predominant olfactory epithelium, and it is unclear how selective olfaction would work. A similar question arises with respect to the secondarily aquatic caecilian *Typhlonectes compressicauda*, which likewise has a region of larval-type epithelium (as distinguished by the lack of Bowman's glands and the presence of both ciliated and microvillar receptors) (Saint Girons and Zylberberg, 1992b). Moreover, metamorphosed *D. tenebrosus* are generally not aquatic, although they do return to water to breed (Nussbaum et al., 1983). It will be interesting to see if any other salamanders likewise display a larval-type epithelium in metamorphosed forms. The observations of Matthes (1927) and Schuch (1934) in *Triturus vulgaris* and *T. alpestris* show that there is a "secondary metamorphosis" of the olfactory organ, similar to that seen in other organ systems, but their study was done only at the LM level. The morphological and physiologi-

cal basis for the ability of the salamander olfactory system to function in both aquatic and terrestrial environments clearly deserves further study.

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