# Nasal cavity of green sea turtles contains three independent sensory epithelia

Daisuke Kondoh[1]*, Chiyo Kitayama[2], Yohei Yamaguchi[1], Masashi Yanagawa[3], Yusuke K. Kawai[4], Chihiro Suzuki[1], Raito Itakura[1], Atsuru Fujimoto[5], Tadatoshi Satow[5], Satomi Kondo[2], Takayuki Sato[2]

[1]Laboratory of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

[2]Everlasting Nature of Asia (ELNA), Ogasawara Marine Center, Ogasawara, Tokyo 100-2101, Japan

[3]Department of Applied Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080–8555, Japan

[4]Laboratory of Toxicology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080–8555, Japan

[5]Division of Environmental and Agricultural Engineering, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

Correspondence to be sent to: Daisuke Kondoh, Laboratory of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan. email: kondoh-d@obihiro.ac.jp

**Keywords:** computed tomography; histology; odorant receptor; olfactory system; reptile; vomeronasal organ.

**Abstract**

The morphological and histological features of the nasal cavity are diverse among animal species, and the nasal cavities of terrestrial and semi-aquatic turtles possess two regions lined with each different types of sensory epithelium. Sea turtles can inhale both of volatile and water-soluble odorants with high sensitivity, but details of the architectural features and the distribution of the sensory epithelia within the sea turtle nasal cavity remain uncertain. The present study analyzed the nasal cavity of green sea turtles using morphological, computed tomographic and histological methods. We found that the middle region of the sea turtle nasal cavity is divided into anterodorsal, anteroventral and posterodorsal diverticula and a posteroventral excavation by connective tissue containing cartilages. The posterodorsal diverticulum was lined with a thin sensory epithelium, and the anterodorsal and anteroventral diverticula were occupied by a single thick sensory epithelium. In addition, a relatively small area on the posteroventral excavation was covered by independent sensory epithelium that differed from other two types of epithelia, and a single thin bundle derived from the posteroventral excavation comprised the most medial nerve that joins the anterior end of the olfactory nerve tract. These findings suggested that the posteroventral excavation identified herein transfers stimuli through an independent circuit and plays different roles when odorants arise from other nasal regions. (214 words)

## Introduction

Nasal morphological features are diverse among animal species, and the nasal cavity in vertebrates contains sensory epithelia that function as an olfactory organ. Sensory epithelia associated with olfactory system in most tetrapods are generally divided into the olfactory and vomeronasal epithelia that project to the main and accessory olfactory bulbs, respectively (Taniguchi and Taniguchi 2014). Both types of epithelia are histologically pseudostratified and generally comprises the supporting, receptor and basal cells. Nuclei of the supporting cells are generally oval and form a zone at the top region of the epithelium. Nuclei of the receptor cells form the zone of round nuclei in the middle of the epithelium. Basal cells are progenitors of receptor and supporting cells and are located at the bottom of the epithelia (Gunasegaran 2010).

In terms of phylogenetical theory, most fishes possess only a single type of sensory epithelium associated with olfaction, whereas amphibians have a vomeronasal epithelium that covers the ventral region of the nasal cavity, and squamates and most mammals have a vomeronasal organ that is completely separate from the nasal cavity (Romer and Parsons 1977; Kondoh et al. 2010; Taniguchi and Taniguchi 2014). In contrast, crocodiles and birds have lost this organ and have only olfactory epithelium in the nasal cavity (Romer and Parsons 1977; Kondoh et al. 2011). Turtles do not have a separate vomeronasal organ, but they have two regions in the nasal cavity lined with different types of sensory epithelia (Graziadei and Tucker 1969; Nakamuta N et al. 2016; Nakamuta S et al. 2016). However, whether any of these types of epithelia corresponds to vomeronasal epithelium remains debatable.

Turtles split from the bird-crocodilian lineage approximately 250 million years ago (Wang et al. 2013). The oldest fossil of a candidate sea turtle, *Odontochelys*, is from the

Triassic period about 220 million years ago (Li et al. 2008), but sea turtles existed during the Lower Cretaceous period about 108 million years ago (Hirayama 1998). Sea turtles belong to the suborder Testudines, the order Cryptodira, superfamily Chelonioidea, and are presently classified into seven species (two families and six genera). Green sea turtles (*Chelonia mydas*) reach sexual maturity after 20 - 50 years (Balazs 1982; Frazer and Ehrhart 1985) and lay eggs on beaches. These eggs hatch after two months, and the hatchlings move to the horizon according to "low horizon elevation" (Limpus and Kamrowski 2013), although the ecology of sea turtles during this period remains largely unknown. Small juveniles are polyphagous and spend time offshore, whereas developed turtles (straight carapace length: > 0.3 m) populate coastal areas and become mainly herbivorous. Sea turtles make developmental and seasonal migrations. Green sea turtles return to the beach where they were born (homing behavior) to breed (Bowen and Karl 2007), but how and when they imprint the home beach remains unknown.

Sea turtles intake both volatile and water-soluble odorants with high sensitivity (Manton et al. 1972; Endres et al. 2009; Endres and Lohmann 2013), although their lifestyle is uniquely marine. The structure of the sea turtle nasal cavity is more complex than those of terrestrial or semi-aquatic turtles, and reports indicate that it has three diverticula (Parsons 1968; Saito et al. 2000). However, details of the architectural features and the distribution of the sensory epithelia within the sea turtle nasal cavity remain uncertain because few individuals have been analyzed and non-destructive verification is scant. Here, we analyzed the nasal cavity of juvenile green sea turtles in detail using morphological and histological methods as well as computer tomographic (CT) imaging.

## Materials and methods

### Animals

We analyzed seven juvenile turtles (age, < 6 months) of unknown sex that were hatched, bred and died naturally at Ogasawara Marine Center (Japan), and a juvenile turtle that became stranded on the coast of Chichi-Jima in the Ogasawara Islands, Japan. The heads of the former seven turtles were rapidly fixed and preserved in natural formalin. The head of the stranded turtle was stored frozen for CT imaging. This study proceeded in accordance with the guidelines for the Regulations on the Management and Operation of Animal Experiments, and the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine approved the experimental protocol (Notification number 28-44).

### Morphological procedures

The nasal regions of four turtles were assessed in the left-lateral view after removing the encircling maxillary and prefrontal bones. The lateral half of the left nasal regions was then removed to evaluate the internal structures of the nasal cavity. The remaining medial half of the left nasal regions of three specimens were removed, and then the distribution of nerves derived from the right nasal cavity to the olfactory bulb was determined. The remaining specimen was cleared and double stained with Alcian blue (pH 2.5) and alizarin red to visualize cartilages and bones, respectively.

### CT image analysis

The frozen head of a turtle was assessed by CT using an Aquilion TSX-201A scanner (Toshiba Medical Systems Corporation, Otawara, Japan) under the following

116 conditions: 120 kV, 250 mA and 0.5 mm slice thickness. Imaging data stored in DICOM
117 format were reconstructed into three-dimensional images using an AZE VirtualPlace
118 Fujin workstation (AZE Ltd., Tokyo, Japan). The internal architecture of the nasal cavity
119 was visualized using the software mode for outline detection of the lungs.
120
121 **Simulation of water inflow into the nasal cavity**
122 The DICOM files were processed using Fiji/ImageJ software (http://fiji.sc/Fiji).
123 The internal structure of the nasal region was extracted from sequenced 8-bit inverted
124 images on a threshold range of 200 to 255, reconstructed into three-dimensional images
125 with a 3D Viewer plugin (display as surface; threshold, 100; resampling factor, 2) and
126 imported as STL files, which were then trimmed using MeshLab software
127 (http://meshlab.sourceforge.net), smoothed (factor, 0.50; repeat, 1) and solidified
128 (thickness, 0.10) using Blender software (www.blender.org). These data were output at
129 three times the original size using 3DPrint Software (3D Systems Inc., Rock Hill, SC,
130 USA) and a ProJet 4500 printer (3D Systems). A transparent silicon mold (Zoukei-Mura
131 Inc., Kyoto, Japan) was made of the right side of the nasal cavity. One hole at each of the
132 anterior and posterior ends was drilled through the mold, which was gently submerged in
133 water at various angles.
134
135 **Histological procedures**
136 The nasal regions of three turtles were post-fixed in Bouin fixative overnight and
137 decalcified using Plank-Rychlo solution for four hours at room temperature. Samples
138 were embedded in paraffin using standard procedures and sliced into 5-μm thick sections
139 at 20-μm intervals. Some sections were deparaffinized, rehydrated and stained with

hematoxylin-eosin and others were processed for immunohistochemistry.

**Immunohistochemical procedures**

Receptor cells were immunohistochemically detected using the antibody A-21271 (Molecular Probes, Eugene, OR, USA) against HuC and HuD, which are RNA-binding proteins that serve as markers of developing neurons in most vertebrate species, including turtles (Nakamuta et al. 2018). Deparaffinized, rehydrated sections in Tris-EDTA buffer (0.01 M, pH 9.0) were heated in a microwave oven for antigen retrieval. Thereafter, the sections were incubated with 0.3% $H_2O_2$ in methanol for 30 min to eliminate endogenous peroxidase, rinsed in phosphate-buffered saline (0.01 M, pH 7.4) three times and incubated with 3% normal goat serum for one hour at room temperature to block nonspecific binding. The sections were incubated with 1.0 μg/mL of A-21271 antibody overnight at 4 °C, rinsed three times, and then incubated with 7.5 μg/mL of BA-9200 biotinylated secondary antibody (Vector Laboratories Inc., Burlingame, CA, USA) for one hour at room temperature. After three rinses, the sections were reacted with PK-6100 avidin-biotin complex reagent (Vector) for 30 min, rinsed again, and then colored with Tris-HCl buffer containing 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.006% $H_2O_2$ for 10 min at room temperature. The sections were mounted in MGK-S (Matsunami Glass Industries, Osaka, Japan) and assessed using a Microphot-FX microscope (Nikon, Tokyo, Japan) equipped with a Digital Sight DS-5Mc camera (Nikon).

## Results

### Morphological features of the nasal cavity

The nasal meatus of green sea turtles comprised a pair of straight tubes that respectively opened as nostrils and choanae at the anterior and posterior ends, forming a respiratory tract (Figure 1). The middle region of each tube contained distinct anterodorsal, anteroventral and posterodorsal diverticula and a posteroventral excavation separated by connective tissue containing cartilages (Figure 1C, D). The anterodorsal and anteroventral diverticula appeared compressed compared with the longitudinal axis on CT images, whereas the posterodorsal diverticulum assumed the form of water drops (Figure 2).

### Water inflow into the nasal cavity

We simply simulated water inflow by placing the nasal cavity mold from downwards (-90°) to upwards (90°) in water (Figure 3). Water filled the respiratory tract, anteroventral diverticulum and anteroventral excavation, but hardly entered the posterodorsal diverticulum positioned from -90° to about 40°. Water did not enter the anterodorsal diverticulum when positioned horizontally (0°), but filled most of this area in both the upward (about 40°) and downward (-90°) positions.

### Projection patterns of nerve bundles derived from the nasal cavity

The left and right olfactory nerve tracts projected into each one of a pair of well-developed olfactory bulbs that were located anterior to the cerebrum (Figure 4A). A thick nerve bundle derived from the posterodorsal diverticulum formed the dorsolateral part of the olfactory nerve tract (Figure 4B). The anterodorsal and anteroventral diverticula projected several thin nerve bundles that formed the ventromedial part of the olfactory

185 nerve tract (Figure 4C, D). A single thin bundle derived from the posteroventral
186 excavation comprised the most medial nerve that ran across the nerves derived from the
187 anterodorsal and anteroventral diverticula, to join the anterior end of the olfactory nerve
188 tract (Figure 4C, D). Figure 4E summarizes these projections.
189
190 **Distribution pattern and histological features of three types of sensory epithelia**
191 Most of the respiratory tract from the nostrils to the choanae of the green sea turtles
192 was covered by non-sensory epithelium (Figure 5, black line), and three types of
193 sensory epithelia were separated by non-sensory epithelium in the middle of the nasal
194 cavity (Figure 5, blue, red and green lines).
195 The anterodorsal and anteroventral diverticula, as well as the medial side of the
196 respiratory tract between them, were lined with a thick epithelium (Figure 5, blue line).
197 Zones of oval and round nuclei were respectively located at the upper third and lower
198 two-thirds of the epithelium (Figure 6A). Nuclei of supporting cells arranged at the top
199 region were large and bright. Cells in the zone of round nuclei composed those resembling
200 supporting cell-like cells with large bright nuclei, and bipolar receptor cells with small
201 dark nuclei (Figure 6B). Some cells in the zone of round nuclei were positive for
202 HuC/HuD, but others were negative (Figure 6C). Basal cells were located at the bottom
203 of the epithelium, and the lamina propria did not contain gland structures (Figure 6A).
204 Sensory epithelium that occupied a relatively small area on the posteroventral
205 excavation (Figure 5, red line) also had similar histological features to the epithelium
206 covering the anterodorsal and anteroventral diverticula (Figure 7).
207 Relatively thin epithelium that exclusively lined the posterodorsal diverticulum
208 (Figure 5, green line) also contained the zones of oval and round nuclei (Figure 8A). The

209 zone of round nuclei almost completely consisted of HuC/HuD-positive bipolar receptor
210 cells (Figure 8B, C), unlike other two types of epithelia. The basal cells were located at
211 the bottom, and some well-developed olfactory glands were located in the lamina propria
212 (Figure 8A).
213

## Discussion

The structure of the nasal cavity that functions in olfaction and respiration is diverse among animal species. Comparative morphological investigations of the nasal cavity helps to understand how odorants are sensed and how animals phylogenetically adapt to the environment in terms of olfaction and respiration. We identified four distinct structures that arise from the respiratory tract in the middle of the nasal cavity of green sea turtles, and these four structures were also significant in the nose of the adult sea turtle (manuscript in preparation). Among them, three have been described as diverticula (Parsons 1968), but the present morphological and CT analyses uncovered a fourth distinct structure, namely, a posteroventral excavation. The nasal cavity of turtles in general is separated into olfactory (regio olfactoria) and intermediate (regio intermedialis) regions (Parsons 1959; Bartol and Musick 2003; Schwenk 2008) that were each lined with different types of epithelium. These have also been described as upper and lower chambers respectively (Taniguchi and Taniguchi 2014; Nakamuta N et al. 2016; Nakamuta S et al. 2016). The olfactory region refers to the sector of the nasal cavity that contains mostly air to receive volatile odorants (Bartol and Musick 2003), and the findings of our water inflow simulation supported the notion that the posterodorsal diverticulum corresponds to the olfactory region of sea turtles (Parsons 1959; Bartol and Musick 2003). The anterodorsal and anteroventral diverticula and the posteroventral excavation seem to be components of the intermediate region.

The olfactory region of green sea turtles was covered by a relatively thin type of sensory epithelium containing olfactory glands in the lamina propria. The histological features of this epithelium corresponded to those of the sensory epithelium in the olfactory region of other turtles (Graziadei and Tucker 1969; Nakamuta N et al. 2016; Nakamuta S

et al. 2016) including loggerhead sea turtles (Saito et al. 2000), and it is similar to the olfactory epithelium of mammals. Therefore, our findings suggested that the olfactory region of sea turtles plays a role in the reception of volatile odorants.

The intermediate region where water enters, contained two thick types of sensory epithelia without olfactory glands, indicating that these epithelia receive water-soluble odorants. Among structures in this region, the posteroventral excavation was lined by a type of sensory epithelium that differed from that covering the anterodorsal and anteroventral diverticula, and a nerve bundle derived from this excavation extended solely to the olfactory nerve tract. These findings suggested that the excavation and the other intermediate region receive different odorants and transfer the information through a different circuit.

The zone of round nuclei in sensory epithelia in the intermediate region contained HuC/HuD-negative cells. Because HuC/HuD proteins are mainly expressed in developing neurons, it is possible that receptor cells that differentiated a long time ago did not react with anti-HuC/HuD antibody. In addition, supporting-like cells were histologically found in the zone of round nuclei, suggesting that these cells reflected to a high number of HuC/HuD-negative cells. Because this zone in the intermediate region of terrestrial and freshwater turtles contains few non-receptor cells (Graziadei and Tucker 1969; Nakamuta N et al. 2016; Nakamuta S et al. 2016), the supporting-like cells seem unique in sensory epithelia in the intermediate region of sea turtles.

Olfactory stimuli via air and water flow are recognized by odorant receptors expressed in receptor cells, and odorant receptors in vertebrates are mainly classified as olfactory (OR), vomeronasal types 1 (V1R) and 2 (V2R), and trace amine-associated (TAAR) receptors. Wang et al. (2013) revealed the draft genome of green sea turtle and

showed that it contains intact 159 Class I OR that generally seem hydrophilic, and intact 95 Class II OR that seem hydrophobic. Genbank refseq database shows that the genome of green sea turtles also encodes twelve intact TAAR (Supplementary Table 1). On the other hand, only two intact V1R and a single V2R are encoded in green sea turtle genome (Supplementary Table 1), indicating that both V1R and V2R families have degenerated in sea turtles. Therefore, the sensory epithelia in the intermediate region in the nasal cavity of sea turtles might receive water-soluble odorants mainly via Class I OR and/or TAAR families, although further evaluation *in situ* and embryological studies are required to understand the features of the sensory epithelia in the nasal cavity of sea turtles.

The present study revealed structural details of the nasal cavity and three independent sensory epithelia that project nerve bundles to the olfactory bulbs of juvenile green sea turtles. These findings provide a basic understanding of olfactory sensing in juveniles, and further studies will focus on adult sea turtles.

## Conflict of interests

The authors have no conflicts of interest to declare.

## Funding

This study was supported by the Okinawa Churashima Foundation [No. 205 to C.K.].

## Acknowledgments

We thank the staff of the Everlasting Nature of Asia (ELNA) for cooperation with this study, and Mr. Hidenori Otsubo and the staff of the Information Processing Center, Obihiro University of Agriculture and Veterinary Science for advice regarding three-dimensional printing.

289 **References**


290 Bartol SM, Musick JA. 2003. Sensory biology of sea turtles. In: Lutz PL, Musick JA,
291 Wyneken J (eds) The Biology of Sea Turtles, vol II. CRC Press, Boca Raton. pp. 79–
292 102.

293 Bowen BW, Karl SA. 2007. Population genetics and phylogeography of sea turtles. *Mol*
294 *Ecol*. 16:4886–4907.

295 Endres CS, Putman NF, Lohmann KJ. 2009. Perception of airborne odors by loggerhead
296 sea turtles. *J Exp Biol*. 212:3823–3827.

297 Endres CS, Lohmann KJ. 2013. Detection of coastal mud odors by loggerhead sea turtles:
298 a possible mechanism for sensing nearby land. *Mar Biol*. 160:2951–2956.

299 Frazer NB, Ehrhart LM. 1985. Preliminary growth models for green, *Chelonia mydas*,
300 and loggerhead, *Caretta caretta*, turtles in the wild. *Copeia*. 1985:73–79.

301 Graziadei PPC, Tucker D.1970. Vomeronasal receptors in turtles. *Z Zellforsch*. 105:498–
302 514.

303 Gunasegaran JP. 2010. Textbook of Histology and a Practical Guide, 2nd ed. New Delhi,
304 Elsevier.

305 Hirayama R. 1998. Oldest known sea turtle. *Nature*. 392:705–708.

306 Kondoh D, Nashimoto M, Kanayama S, Nakamuta N, Taniguchi K. 2011. Ultrastructural
307 and histochemical properties of the olfactory system in the Japanese jungle crow,
308 *Corvus macrorhynchos*. *J Vet Med Sci*. 73:1007–1014.

309 Kondoh D, Yamamoto Y, Nakamuta N, Taniguchi K, Taniguchi K. 2010. Lectin
310 histochemical studies on the olfactory epithelium and vomeronasal organ in the
311 Japanese striped snake, *Elaphe quadrivirgata*. *J Morphol*. 271:1197–1203.

312 Li C, Wu XC, Rieppel O, Wang LT, Zhao LJ. 2008. An ancestral turtle from the Late

313 Triassic of southwestern China. *Nature*. 456:497–501.
314 Limpus C, Kamrowski RL. 2013. Ocean-finding in marine turtles: the importance of low
315 horizon elevation as an orientation cue. *Behaviour*. 150:863–893.
316 Manton M, Karr A, Ehrenfeld DW. 1972. Chemoreception in the migratory sea turtle,
317 *Cheronia mydas*. *Biol Bull*. 143:184–195.
318 Nakamuta N, Nakamuta S, Kato H, Yamamoto Y. 2016. Morphological study on the
319 olfactory systems of the snapping turtle, *Chelydra serpentina*. *Tissue Cell*. 48:145–
320 151.
321 Nakamuta S, Yokosuka M, Taniguchi K, Yamamoto Y, Nakamuta N. 2016.
322 Immunohistochemical analysis for G protein in the olfactory organs of soft-shelled
323 turtle, *Pelodiscus sinensis*. *J Vet Med Sci*. 78:245–250.
324 Nakamuta S, Kusuda S, Yokosuka M, Taniguchi K, Yamamoto Y, Nakamuta N. 2018.
325 Immunohistochemical analysis of the development of olfactory organs in two species
326 of turtles *Pelodiscus sinensis* and *Mauremys reevesii*. *Acta Histochem*. 120: 806–813.
327 Parsons TS. 1959. Nasal anatomy and the phylogeny of reptiles. *Evolution*. 13:175–187.
328 Parsons TS. 1968. Evolution of the nasal structure in the lower tetrapods. *Am Zoologist*.
329 7:397–413.
330 Romer AS, Parsons TS. 1977. The Vertebrate Body, 5th ed. Philadelphia, Saunders.
331 Saito K, Shoji T, Uchida I, Ueda H. 2000. Structure of the olfactory and vomeronasal
332 epithelia in the loggerhead turtle *Caretta caretta*. *Fish Sci*. 66:409–411.
333 Schwenk K. 2008. Comparative anatomy and physiology of chemical senses in nonavian
334 aquatic reptiles. In: Thewissen JGM, Nummels S. (eds) Sensory Evolution on the
335 Threshold. Adaptations in Secondarily Aquatic Vertebrates. Berkeley, Univ. of
336 California Press. pp. 65–81.

337 Shi P, Zhang J. 2007. Comparative genomic analysis identifies an evolutionary shift of
338 vomeronasal receptor gene repertoires in the vertebrate transition from water to land.
339 *Genome Res*. 17:166–174.
340 Taniguchi K, Taniguchi K. 2014. Phylogenic studies on the olfactory system in
341 vertebrates. *J Vet Med Sci*. 76:781–788.
342 Wang Z, Pascual-Anaya J, Zadissa A, Li W, Niimura Y, Huang Z, Li C, White S, Xiong
343 Z, Fang D, *et al*. 2013. The draft genomes of soft-shell turtle and green sea turtle yield
344 insights into the development and evolution of the turtle-specific body plan. *Nat Genet*.
345 45:701–706.
346

## Figure Legends

**Figure 1.** Morphological features of nasal cavity of green sea turtles.

(A) Left lateral view of the head. Boxed area indicates the nasal area and corresponds to panels (B-D). (B) Outer nasal cartilages after removing bones encircling nasal area. (C) Internal structure of nasal cavity after removing left half. (D) Alcian blue (pH 2.5) and alizarin red stained image corresponding to panel (C). Connective tissue containing cartilages (arrowheads) positive for Alcian blue distinctly separates anterodorsal (1) and anteroventral (2) diverticula, posteroventral excavation (3) and posterodorsal diverticulum (4). *Nostrils.

**Figure 2.** Internal structures of nasal cavity of green sea turtles.

(A) Three-dimensional image of head reconstructed from computed tomographic (CT) images. Air in nasal cavity is extracted in yellow to visualize internal structure of nasal cavity. Boxed area corresponds to panels (B-E). (B) CT image of nasal cavity shows four distinct structures. (C-E) Left lateral (C), frontal (D) and dorsal (E) views of three-dimension reconstructed images of the nasal cavity. Anterodorsal (1) and anteroventral (2) diverticula, posteroventral excavation (3) and posterodorsal diverticulum (4). *Nostrils.

**Figure 3.** Simulation of water inflow into nasal cavity.

Model of internal structure of nasal cavity (upper). Silicon molding submerged in water at horizontally (0°), upwards (about 40°) and downwards (90°) (middle), and schemes of water inflow at each location (lower). Upper side is water surface. Minimal amount of water enters posterodorsal diverticulum (arrows). Water fills anterodorsal diverticulum

371 (arrowheads) in upward and downward positions. Blue and white areas in nasal cavity
372 indicate water and air, respectively. *Nostrils.
373
374 **Figure 4.** Projection of nerve bundles derived from nasal cavity.
375 (A) Dorsal view of nasal cavity and anterior brain region after removing skull. Left side
376 is anterior. Left and right olfactory nerve tracts (arrow) originate from nasal cavity (NC)
377 and reach olfactory bulbs (OB) located at front of cortex (Cor). *Orbit. (B) Dorsal view
378 of right olfactory nerve tract. Left olfactory nerve tract is removed. Dorsolateral part (dl)
379 is derived from posterodorsal diverticulum (4), and ventromedial part (vm) is derived
380 from other regions of nasal cavity. (C and D) Left lateral view (upper) and trace (lower)
381 of nerve distribution in right nasal cavity before (C) and after (D) removing nasal septum.
382 Several thin nerve bundles derived from anterodorsal (1) and anteroventral (2) diverticula
383 form ventromedial part (vm) of olfactory nerve tract (blue lines). Single thin bundle of
384 nerves (arrows) is derived from posteroventral excavation (3) and runs in most medial
385 region (red lines). Dorsolateral part (dl) of olfactory nerve tract is derived from
386 posterodorsal diverticulum (green lines). (E) Scheme of left lateral view of nerve
387 distribution in right nasal cavity according to panels (C and D).
388
389 **Figure 5.** Distribution of epithelia in nasal cavity.
390 Lateral view of nasal cavity region (top) and frontal section of nasal cavity based on
391 histological findings (bottom). Straight lines with alphabetical letters in top panel indicate
392 positions corresponding to images with same letters. Blue, red and green lines indicate
393 separate types of sensory epithelia. Black line indicates non-sensory epithelium.
394 Anterodorsal (1) and anteroventral (2) diverticula, posteroventral excavation (3) and

posterodorsal diverticulum (4).

**Figure 6.** Histological features of sensory epithelium covering anterodorsal and anteroventral diverticula.

(A) Whole epithelium stained with hematoxylin-eosin. Arrowheads indicate basal cells. ZON, zone of oval nuclei; ZRN, zone of round nuclei. (B) High magnification of ZRN. Arrows and arrowheads indicate nuclei of supporting-like cells and bipolar receptor cells, respectively. (C) Anti-HuC/HuD immunoreaction in ZRN. Arrows and arrowheads indicate negative and positive cells, respectively. Bar = 30 μm.

**Figure 7.** Histological features of sensory epithelium covering posteroventral excavation.

(A) Whole epithelium stained with hematoxylin-eosin. Arrowheads indicate basal cells. ZON, zone of oval nuclei; ZRN, zone of round nuclei. (B) High magnification of ZRN. Arrows and arrowheads indicate nuclei of supporting-like cells and bipolar receptor cells, respectively. (C) Anti-HuC/HuD immunoreaction in ZRN. Arrows and arrowheads indicate negative and positive cells, respectively. Bar = 30 μm.

**Figure 8.** Histological features of sensory epithelium covering posterodorsal diverticulum.

(A) Whole epithelium with olfactory gland (asterisk) in lamina propria, stained with hematoxylin-eosin. Arrowheads indicate basal cells. ZON, zone of oval nuclei; ZRN, zone of round nuclei. (B) High magnification of ZRN. Arrows indicate nuclei of bipolar receptor cells. (C) Anti-HuC/HuD immunoreaction in ZRN. Arrowheads indicate positive cells. Bar = 30 μm.

**Supplementary Table 1.**
TAAR, V1R and V2R genes in green sea turtle based on the genomic information.

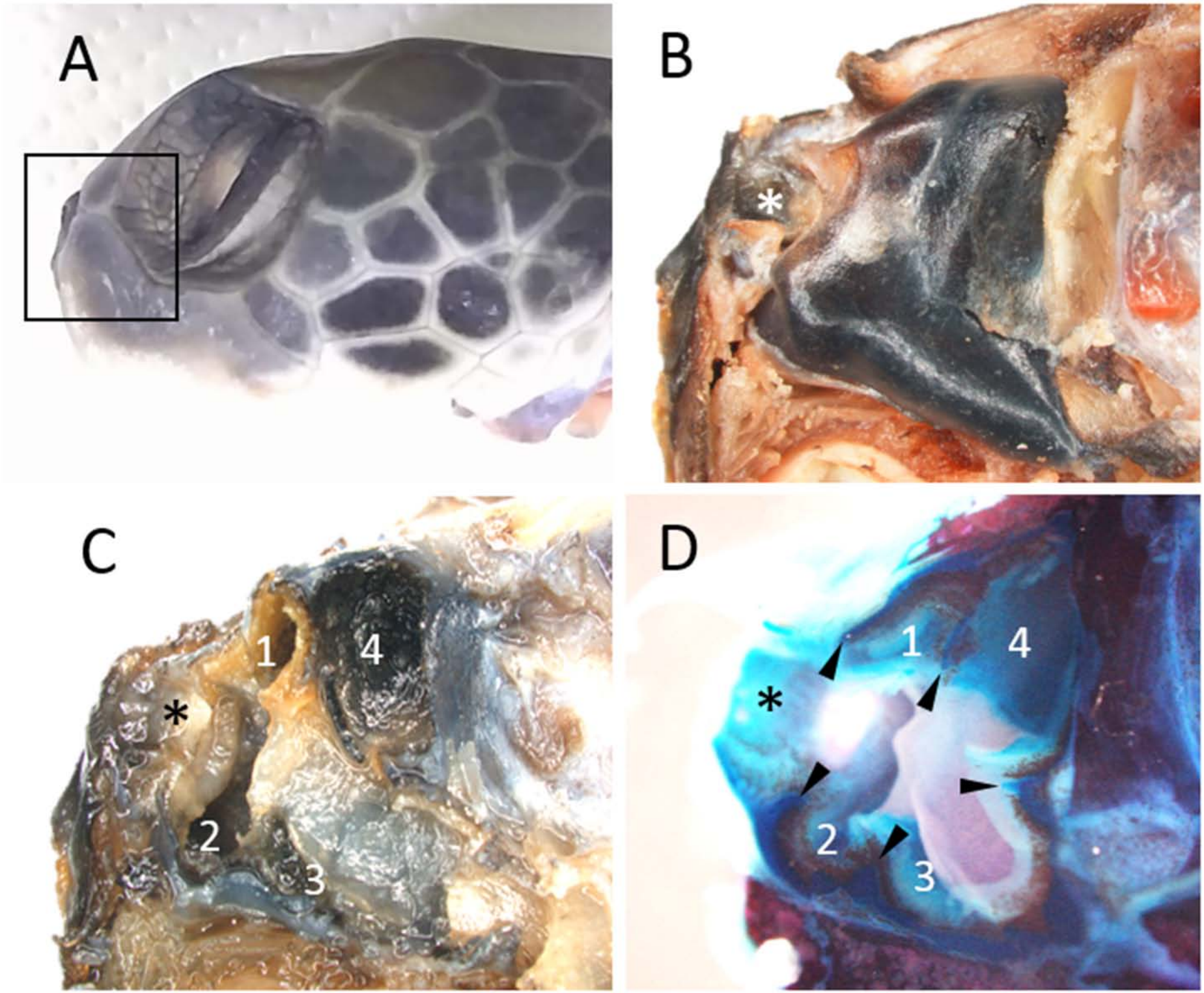
| Receptor family | Genbank accession |
|---|---|
| TAAR* | XM_007062262.2 |
| | XM_007062261.1 |
| | XM_007062260.2 |
| | XM_007062259.2 |
| | XM_007062257.1 |
| | XM_007062256.1 |
| | XM_007062255.1 |
| | XM_007062254.1 |
| | XM_007062253.2 |
| | XM_007062247.2 |
| | XM_007062246.2 |
| | XM_027824652.1 |
| | XR_443470.2 |
| V1R† | XM_007059608.1 |
| | XM_007067150.1 |
| V2R† | (EMP28964.1; protein database)‡ |

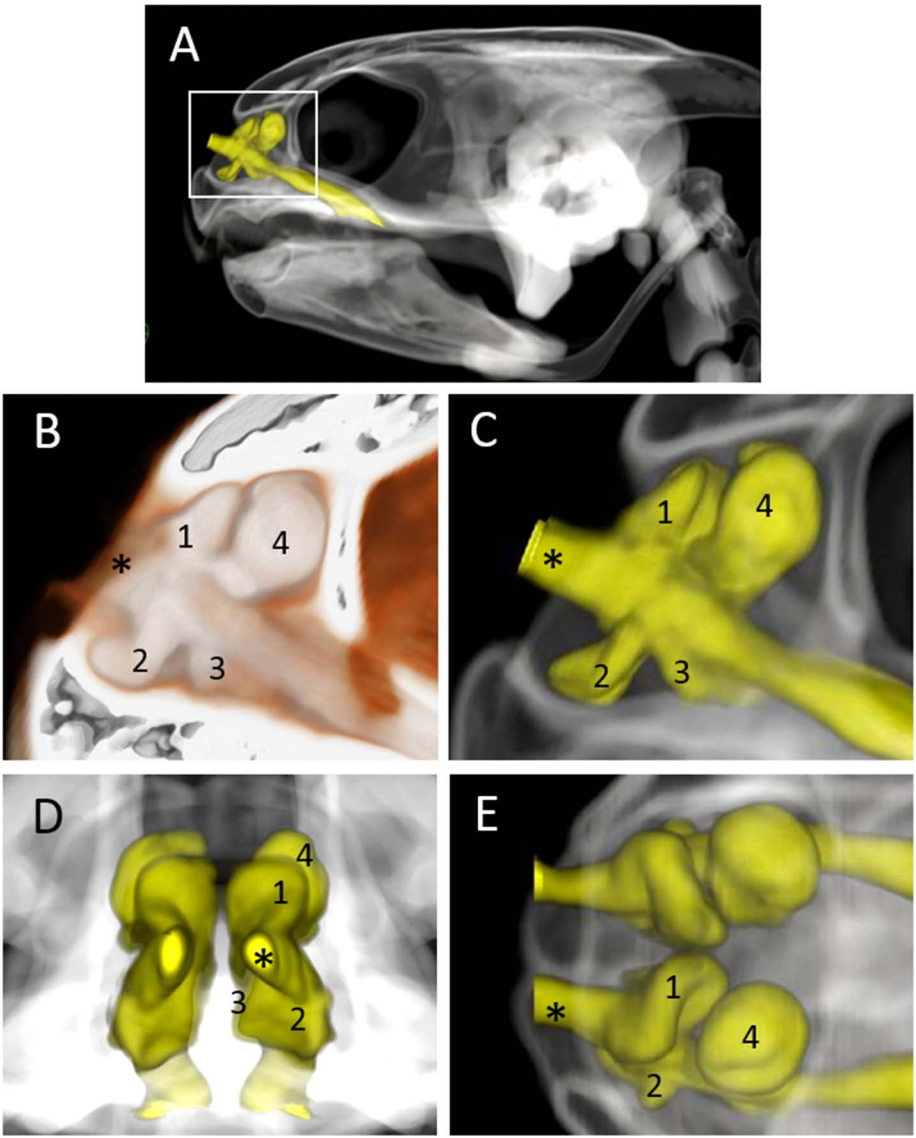
* The locus of TAAR on chromosome are conserved among reptiles, and TAAR genes are located near vanin 1 gene to form a gene cluster. We counted the number of TAAR genes on contig: NW_006637070.1.

† TBLASTN search (Camacho et al. 2009) were performed on refseq RNA sequences to retrieve green sea turtle V1R and V2R genes. Query sequences were total 244 and 215 protein sequences of V1R and V2R, respectively, in four vertebrates; zebrafish (*Danio rerio*), western clawed frog (*Xenopus tropicalis*), mouse (*Mus musculus*) and Chinese soft-shell turtle (*Pelodiscus sinensis*).

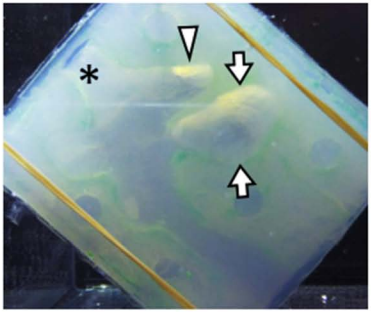
‡ Blast results did not show any V2R in green sea turtle, but protein database suggests one V2R and genome information indicates one pseudo gene (Gene ID: 102943443) in green sea turtles.

*Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST plus: architecture and applications. BMC Bioinformatics. 10:1.*

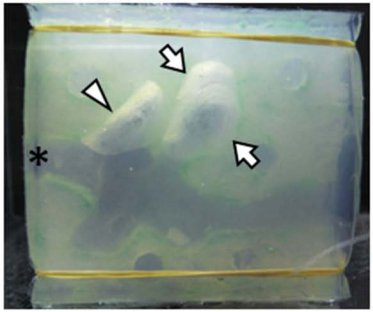

A
B
*
C
1
4
*
2
3
D
1
4
*
2
3


A
B
1
4
*
2
3
C
1
4
*
2
3
D
4
1
*
3
2
E
1
*
4
2

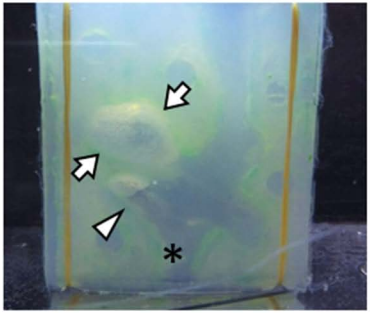
Upward (about 40˚)

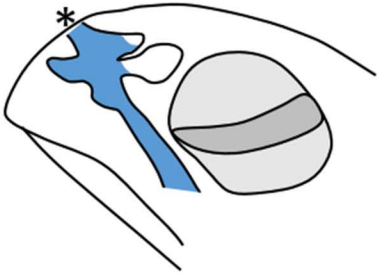


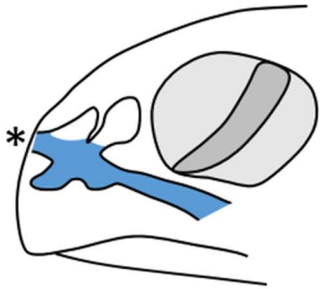
Horizontal (0˚)

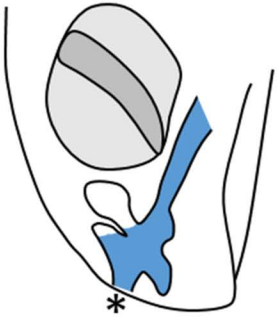


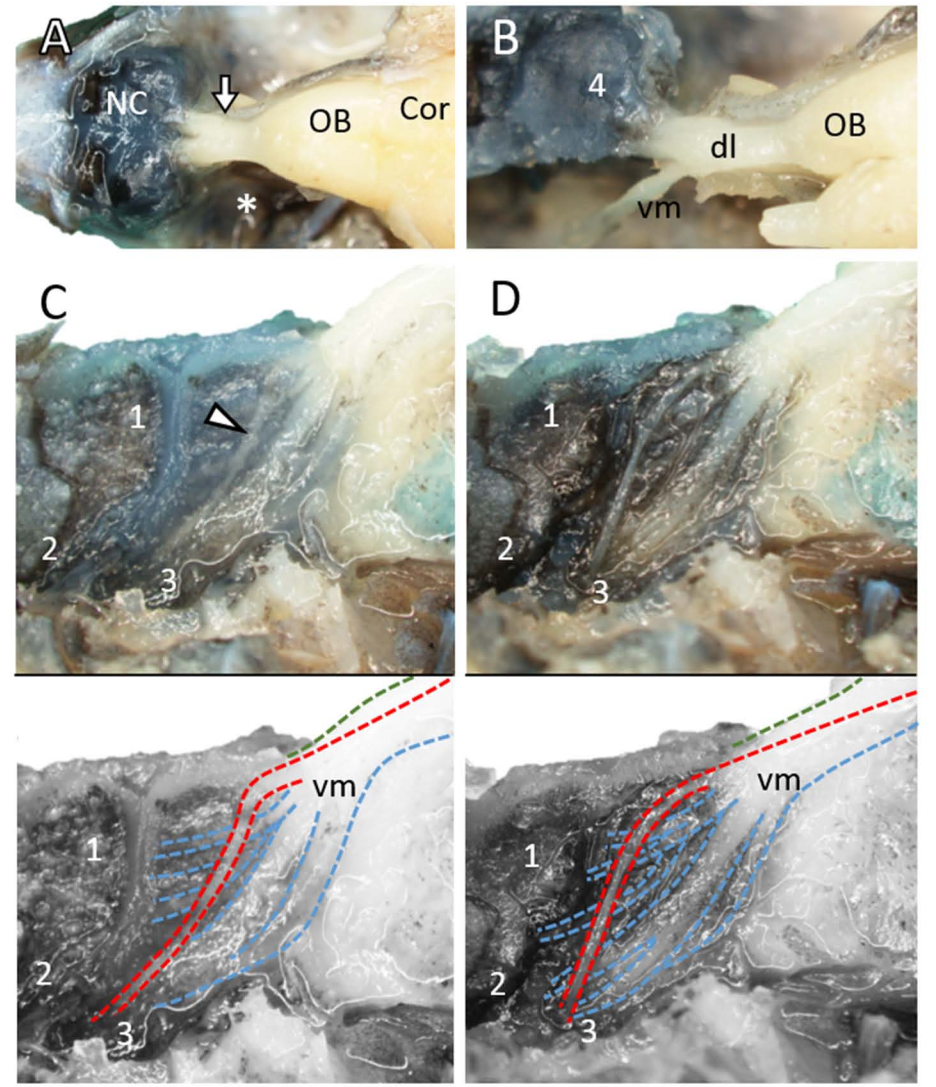
Downward (-90˚)

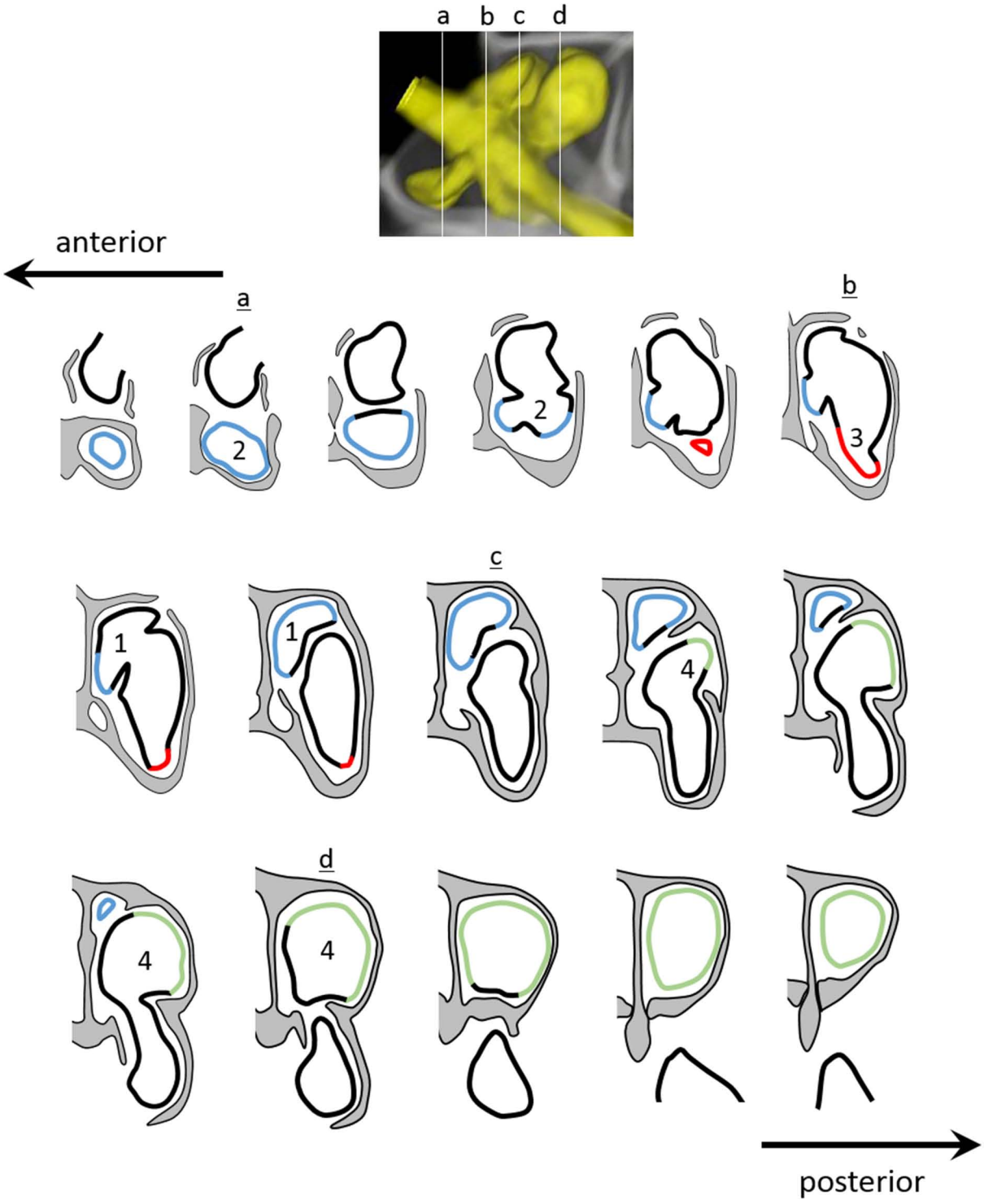


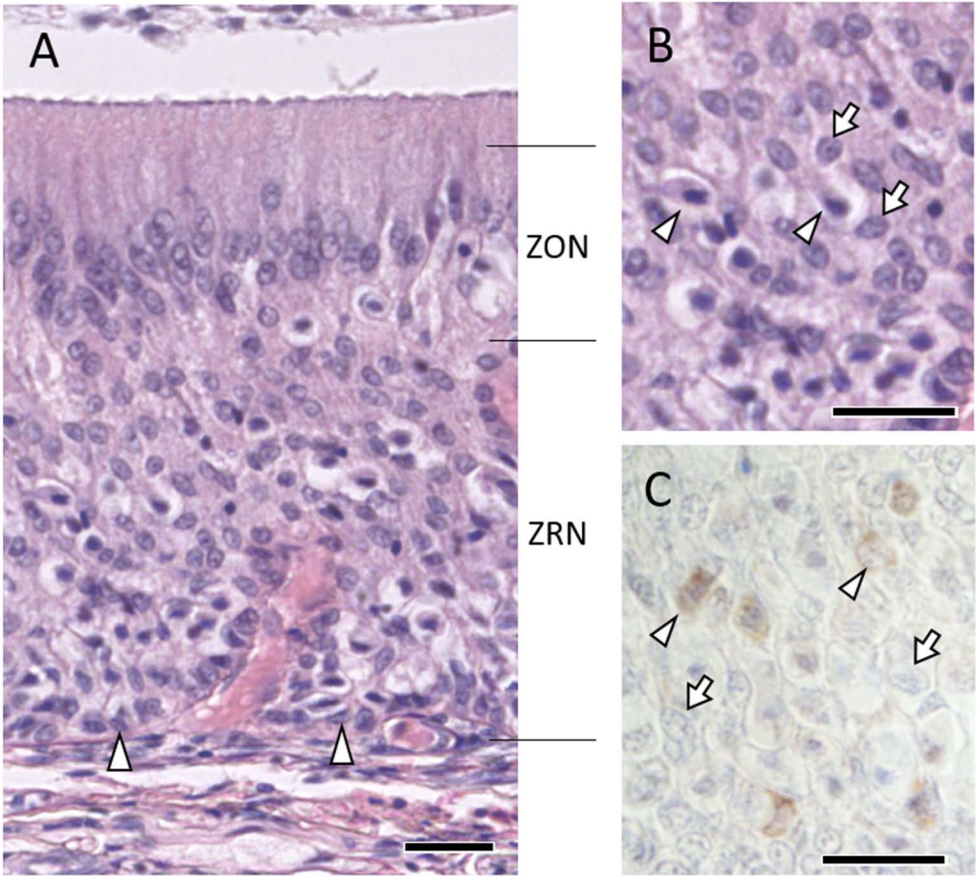


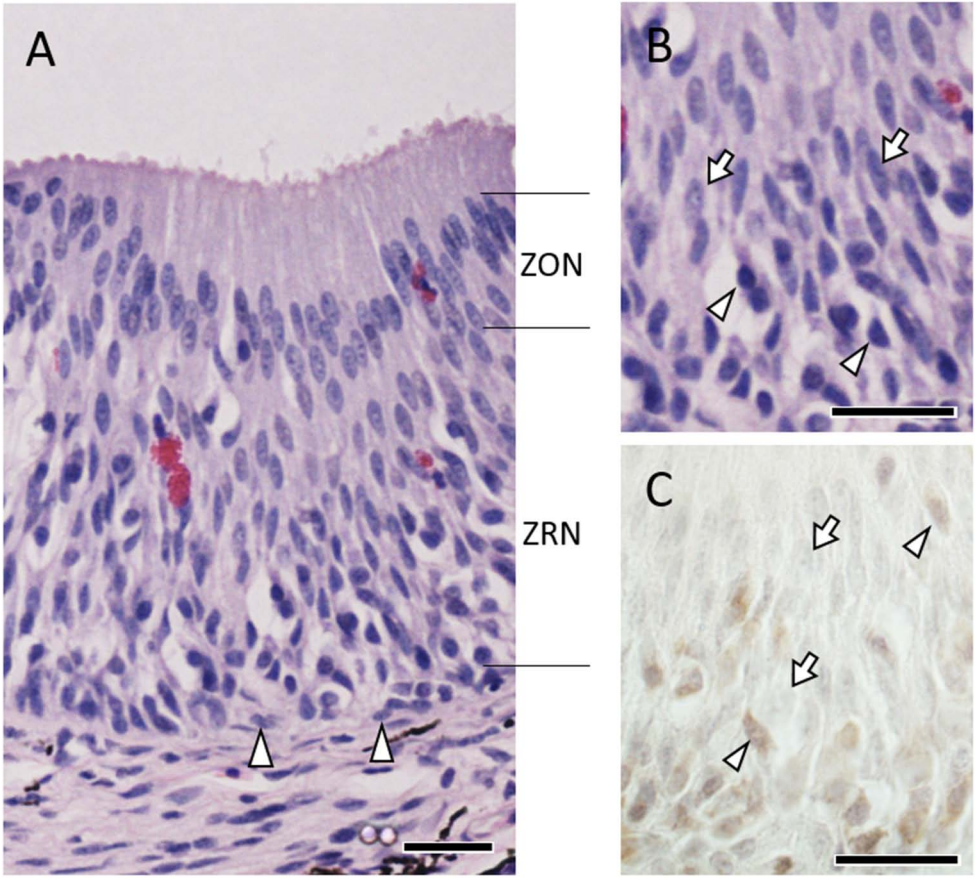


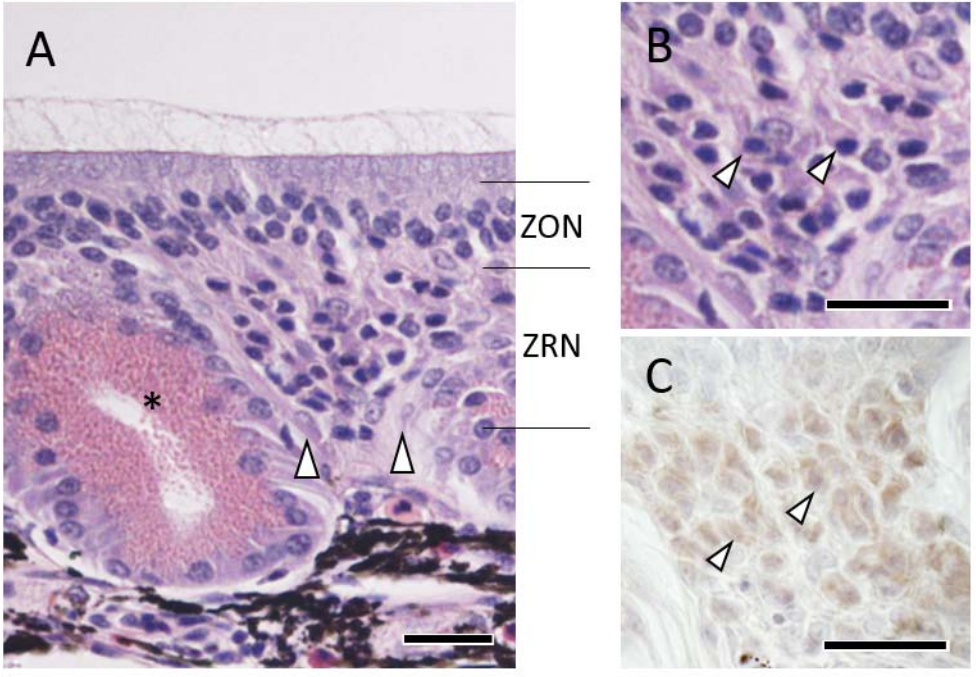



A
NC
OB
Cor
*
B
4
dl
OB
vm
C
1
2
3
D
1
2
3
vm
1
2
3
vm
1
2
3
E
dl
1
4
vm
2
3


a b c d
anterior
a
b
c
d
1
2
3
4
posterior


A
ZON
ZRN
B
C


A
ZON
ZRN
B
C


A
ZON
ZRN
*
B
C