Short note

NASAL MUCOSA RESEMBLING AN OLFACTORY SYSTEM IN THE COMMON MINKE WHALE (*BALAENOPTERA ACUTOROSTRATA*)

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Abstract

Although it has long been assumed that modern cetaceans lack nervous system structures that mediate olfaction, recent studies suggest that mysticetes still maintain olfactory nerves. We collected samples of the mucous membrane covering the cribriform plate at the bottom of the dorsal nasal meatus from a mature female common minke whale. The samples were then thin-sectioned and stained with hematoxylin and eosin. Microscopic observations revealed that the mucosa was covered with a pseudostratified columnar epithelium with vessels, glands, and nerve plexuses in its lamina propria. These histological characteristics resembled those of the olfactory epithelium in terrestrial mammals, suggesting that mysticetes do indeed possess a sense of smell.

Key words Baleen whale, Olfaction, Histology, Nasal cavity, Olfactory epithelium.

In terrestrial mammals, the external nares and nasal passages function not only as a respiratory organ but also as an olfactory organ. Meanwhile, cetaceans use their blowhole as a respiratory tract, but their olfactory system seems to be highly degenerated. Extremely, odontocetes are reported to possess no nervous systems that mediate olfaction (Glezer, 2002; Oelschläger and Oelschläger, 2009).

In contrast, recent studies suggest that mysticetes can smell in air partly due to the presence of olfactory bulbs in their brain (Thewissen, George, Rosa and Kishida, 2011; Kishida, Thewissen, Usip, Suydam and George, 2015). The bony nasal passages of mysticetes branch into two sections: the ventral nasal meatus, which is broad and connects the blowhole and larynx, and the dorsal nasal meatus, a narrower passage the posterior part of which leads to the brain case (Godfrey, Geisler and Fitzgerald, 2013; Fig. 1). Because these structures resemble those of terrestrial mammals, previous studies support the presence of an olfactory system in mysticetes (Cozzi, Huggenberger and Oelschläger, 2016). Analyses of the olfactory marker protein gene further supports this, suggesting the existence of a functional olfactory system in mysticetes, even albeit much reduced compared with terrestrial mammals (Kishida and Thewissen, 2012; Springer and Gatesy, 2017).

Of the various functions and morphologies of the nasal epithelium, the portion known as the olfactory region affects an animal's olfactory ability (Kato and Yamauchi, 2003). The olfactory epithelium is a pseudostratified columnar epithelium composed of three types of cells: basal cells, sustentacular cells and olfactory receptor cells. Olfactory receptor cells have olfactory cilia projecting into the nasal cavity which react to odoriferous substances, stimulating the part of the brain known as olfactory bulbs (Wheater, Burkitt and Daniels, 1979) and subsequently the sense of smell will arise. The olfactory region is located near the cribriform plate of the ethmoid bone with great inter-species variations

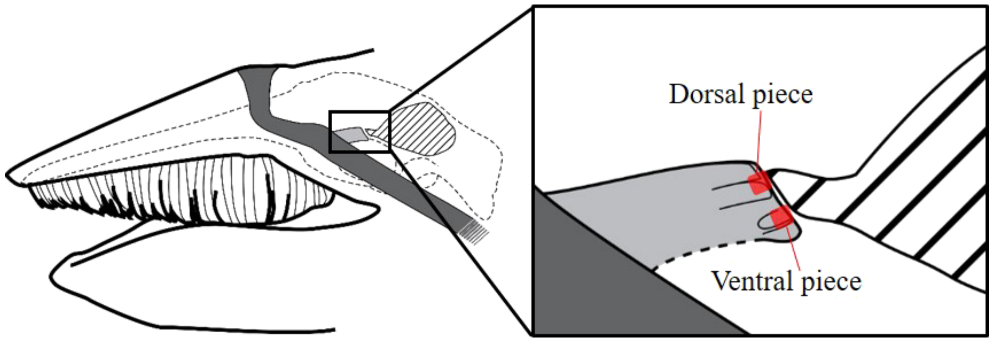

Fig. 1. The positions of mucosa sampling on the cribriform plate. Lateral view of the head showing structure of the ventral nasal meatus (dark gray), the dorsal nasal meatus (light gray), and brain case (stripes). There were a series of deep folds at the bottom of dorsal nasal meatus.

among mammals (Kato and Yamauchi, 2003). There are small serous glands below the olfactory epithelium known as Bowman's glands, the ducts of which penetrate into the epithelium and opening of the nasal cavity. Olfactory nerve fascicles are also found in the lamina propria.

Thus, since the presence of an olfactory epithelium indicates the existence of olfaction, it is a good starting point in discussing the feasibility of olfaction in mysticetes. In this study, we carried out histological observations (light microscopy) of nasal mucosa samples from the common minke whale to describe the olfactory anatomy in this species.

We analyzed a female common minke whale (*Balaenoptera acutorostrata*) with a body length of 7.68 m. This individual was captured in 2016 during the second phase of the Japanese Whale Research Program under the special permit in the Western North Pacific (JARPNII), and was appeared to be sexually matured based on analysis of its ovaries.

Samples were prepared immediately after the animal's death (approx. within 4.5 h). Since the location of the olfactory epithelium was thought to be inside the bony nasal passages, the head was separated into two halves along the mid-sagittal plane using a chainsaw. After carefully locating the right dorsal nasal meatus, two transverse sections were cut off to remove anterior part of the dorsal nasal meatus and posterior part of the ethmoid bone. Lateral bony parts were also removed, leaving the entire dorsal nasal meatus inside the specimen. Finally, the specimen was trimmed into a cube of approximately 10×5×20 cm using a hand saw, and the tissues were fixed in 10% formalin and preserved in 70% ethanol.

Three epithelium samples were collected from the mucosa on the cribriform plate. The location of the cribriform plate was assumed to be the posterior wall of the recess at the bottom of the dorsal nasal meatus (Fig. 1). Three square pieces of mucosa with 2–3 mm thick including the tissue beneath the epithelium were collected from the preserved specimen using a surgical knife. Two samples were collected from the dorsal side of the wall and one from the ventral side.

The epithelial samples were then processed according to standard histological techniques and stained with hematoxylin and eosin (H/E). The epithelium was observed under an optical microscope with magnification of 10× and 40×, paying particular attention to whether the epithelial cells consisted of a pseudostratified columnar epithelium typical of olfactory organs. The thickness of the epithelium and structure of the lamina propria were also determined.

Dorsal epithelial samples of mucosa, which constituted the posterior wall of the recess, showed features of a pseudostratified columnar epithelium 70–80 μm thick (Fig. 2). A similar epithelium was also observed on the ventral side; however, the thickness varied. On the dorsal portion of this ventral sample, the thickness was 80 μm or more, while the ventral portion was approximately 50 μm thick. Nerve plexuses and glandular cells were observed in the lamina propria in all three samples, and in one dorsal sample, some of these serum ducts appeared to open into the nasal cavity.

Thickness of the epithelium exceeds 100 μm in some areas, but less than 100 μm in other areas. In

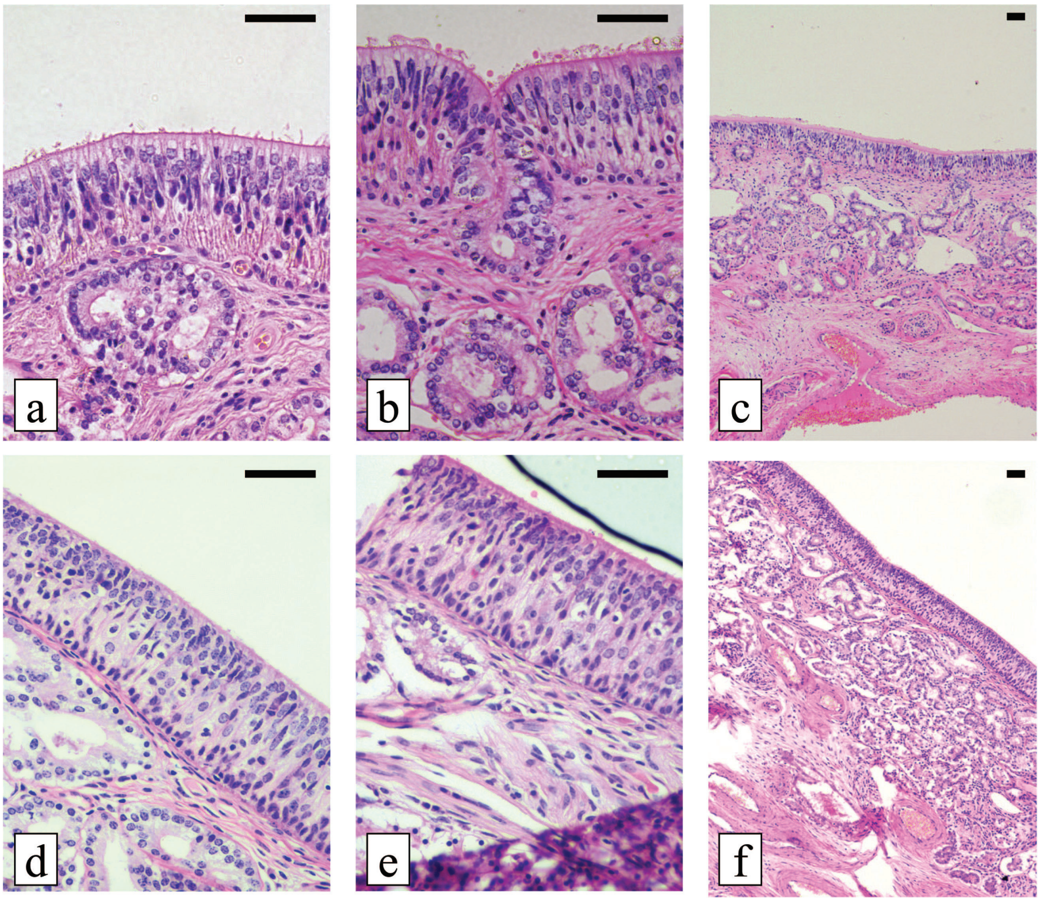

Fig. 2. Sections showing the epithelium samples from the cribriform plate. Sections were 6 μm thick and stained with H/E. Scale bars = 50 μm. The lumen (nasal cavity) is located at the top of the pictures. Close-up of epithelial cells (a, b; 40×) and epithelial tissue showing the lamina propria (c; 10×) from the dorsal side of the cribriform plate. Similar images from the ventral side (d, e, f).

terrestrial mammals, the olfactory epithelium is typically composed of a pseudostratified columnar epithelium approximately 100–120 μm thick (Kato and Yamauchi, 2003). The epithelial samples of nasal mucosa observed here show the morphological characteristics similar to those in terrestrial mammals.

The lamina propria of the thick pseudostratified columnar epithelium contained numerous glands, and these glands opened into the lumen in the dorsal sample. Although more detailed examination is required to conclude, we assume that these are Bowman's glands. Numerous blood vessels were also observed, suggesting a role in helping warm inhaled air. Near these vessels, peripheral nerve fascicles were also observed. Overall, the distribution of these tissues in the lamina propria resembled the olfactory region of the nasal mucosa in other mammals (Kato and Yamauchi, 2003; Harkema, Carey and Wagner, 2006; Chamanza and Wright, 2015).

Areas of pseudostratified columnar epithelium were located near the olfactory bulbs. Meanwhile, in megascopic observations of parasagittal sections of the animal's head, the bony canal representing the olfactory tract, or anterior elongation of the brain case, faced the olfactory recess. This elongated area is occupied by the olfactory bulb, and the bone dividing the brain case and bony nasal passage is the cribriform plate of the ethmoidal bone (Thewissen *et al*., 2011; Godfrey *et al*., 2013; Ichishima, 2016). Epithelium samples of nasal mucosa from the cribriform plate therefore resembled the olfactory epithelium in terrestrial mammals, supporting the feasibility of olfaction in mysticetes.

In general, mysticetes have left and right nasal passages completely isolated from each other (Berta, Ekdale and Cranford, 2014), suggesting that left and right nasal passages could serve as independent olfactory organs. That is, since the left and right auditory organs are able to locate a sound source, these two olfactory organs may be capable of locating the origin of an odoriferous substance via different stimuli of chemical substances between the left and right nasal cavities (Kikuta, Sato, Kashiwadani, Tsunoda, Yamasoba and Mori, 2010). We therefore hypothesize that mysticetes have two nasal openings with symmetrical nasal passages, which they use in olfaction (Kishida, 2016).

The microscopic structure of the epithelium samples observed in this study strongly supports the possibility of an olfactory epithelium. However, it should be noted that similar morphologies do not always suggest an identical function. Further analyses of the role of this epithelium are required to confirm the existence of olfaction in the common minke whales.

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