Scanning Electron Microscopic Study on the Tongue and Lingual Papillae of the adult Spotted Seal, *Phoca largha*

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Summary: We observed the external surface and connective tissue cores (CTCs) of the lingual papillae (filiform, fungiform and vallate papillae) of adult Spotted seals (*Phoca largha*) using SEM and light microscopy. The tongue was V-shaped and its apex was rather rounded. On the dorsal surface from apex to the one-third posterior of the tongue, the lingual mucosa was densely covered by filiform papillae, with a scattered distribution of dome-like fungiform papillae, which have orthokeratinized epithelium. At the posterior part of the tongue, filiform papillae were totally diminished and their epithelium was parakeratinized. Approximately 6–7 vallate papillae were arranged in a V-shape on the posterior of the tongue. After removal of the epithelium, the CTCs of the filiform papillae that were distributed at apex consisted of a primary core and approximately 5–6 rod-shaped small accessory cores. The CTCs of filiform papillae that were distributed at anterior part of the tongue lacked primary protrusions and possessed approximately 10–15 rod shaped small accessory cores that were arranged in a horseshoe manner. The CTCs of fungiform papillae had cylindrical primary cores and were fringed with accessory protrusion. In the Vallate papillae, taste buds were only seen at the dorsal epithelium.

It is reported that there is various morphological variations of lingual papillae among mammal species. Sonntag (1920) made an observation regarding several mammalian lingual papillae macroscopically and there were many differences especially in the shape of tongues or the distribution of the different types of lingual papillae e.g. filiform, fungiform, vallate and foliate papillae depending on species. Furthermore, regarding three-dimensional morphological structure of the lingual papillae and their connective tissue cores (CTCs) after exfoliation of the epithelium, there are many differences originating in part from the various dietary habits of carnivorous, omnivorous and herbivorous land mammalian species (Kobayashi et al., 1987; Kobayashi and Iwasaki, 1988, 1989).

There have been some reports about morphological variations of lingual papillae of aquatic mammals (Semi-aquatic: Kubota et al., 1968; Kobayashi et al., 1994; Shimoda et al., 1996, Fully-aquatic: Yamasaki and Komatsu, 1980; Yoshimura 1983).

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**Table 1. Spotted seals tongue examined**

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Body length (cm)</th>
<th>Body weight (Kg)</th>
<th>Length of tongue (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 years</td>
<td>F</td>
<td>150</td>
<td>168</td>
<td>13</td>
</tr>
<tr>
<td>9 years</td>
<td>F</td>
<td>140</td>
<td>55.2</td>
<td>9.5</td>
</tr>
<tr>
<td>3 years</td>
<td>F</td>
<td>120</td>
<td>31.2</td>
<td>8</td>
</tr>
</tbody>
</table>

*¹: Average length between apex and most posterior position of lingual prominence.
*²: Average width at the broadest part of the tongue.
*³: Estimated value.
Yoshimura (2002) observed the three-dimensional morphological structure of the lingual papillae and their CTCs after exfoliation of the epithelium of the semi-aquatic mammal, California sea lion (*Zalophus californianus* californianus; Otariidae). It was reported that the morphological characteristics of the California sea lion’s tongue possess some structures similar to those of land mammals.

Regarding another pinnipedia species, *Phocidae*, Sonntag (1922) reported macroscopic observations; including those of the lingual papillae of the Harbour (or common) seal (*Phoca vitulina*). However, there had not been any study of the detailed three-

Fig. 1. Overview of the dorsal surface of the tongue of the Spotted Seal (*Phoca largha*) A: Anterior part. B: Antero-postero boundary. C: Root. Fu: Fungiform papillae Fi: Filiform papillae Vp: Vallate papillae. Scale bar = 1 cm.
dimensional morphological structure, especially for CTCs of lingual papillae of Phocidae. Phocidae are semi-aquatic mammal species and their dietary habit consist of fish and squid that is similar to other pinnipedia.

The aim of the present study was to observe both the three-dimensional morphological structures on the surface of the lingual papillae of the Spotted seal, and their CTCs after exfoliation of the epithelium on the dorsal surface of the tongue in detail.

**Materials and Methods**

**Tissue preparation**

The observation was performed on 3 adult Spotted seal’s (Phoca largha) tongues. The postmortem specimens of the tongues for autopsy were supplied from the Niigata City Aquarium. Table 1 represent the details. The tongues were fixed with 10% formalin. Shortly after that, blocks were then cut from various parts of the tongue.

**Light microscopy**

Light microscopy, specimens were dehydrated with a graded series of alcohol. Following dehydration, specimens were embedded with paraffin and sectioned 4 μm thick. Sections were stained with hematoxylin-eosin (HE). These slides were observed with a light-field lightmicroscope (BH-2, Olympus, Japan).

**Scanning electron microscopy**

For scanning electron microscopy, specimens were immersed with 3.5N-HCl for 2–3 weeks at room temperature (22–25°C). After immersion of the specimens, the epithelium was exfoliated from the underlying CTCs at the boundary of epithelium-connective tissue layer. Specimens were washed with tap water, and then treated with a 0.5% tannic acid solution. Post fixation was accom-

Fig. 2. Light micrograph of a sagittal section of filiform papillae distributed on the anterior part of tongue. Thick cornified layer is observable especially in the anterior epithelium of each filiform papillae. Scale bar = 500 μm.
plished by a 10 minute immersion in a 1% OsO₄ solution. Specimens were then washed and dehydrated with a graded series of ethanol.

After dehydration, specimens were dried with a t-butyl alcohol freeze-drying method (Inoue and Ohtake, 1988). Before observation, specimens were coated with Pt-Pd, and then observed with a scanning electron microscope (S-800, Hitachi-Hi-Technologies, Japan).

**Results**

1) Macroscopic overview

The appearance of the tongues of the Spotted seals (Fig. 1) were V-shaped. They were broadest at the posterior one-third, and narrower towards the apex. The apex of tongues had central grooves but were rather rounded, however, the Medianus linguae was not visible in the midline of the tongues. On the dorsal surface from apex to the one-third posterior of the tongues, the lingual mucosa was densely covered by filiform papillae. Dome-like fungiform papillae were scattered among filiform papillae. At the posterior part of the tongues, filiform papillae were absent and their epithelial surface was smooth but had numerous shallow folds and grooves. Approximately 6–7 vallate papillae were arranged in a V-shape on this area. The numerous shallow folds and grooves that were seen on the posterior part were also seen on the radix.
2) Microscopic observation

a) Filiform Papillae

i) Lightmicroscopic observation

The epithelium (Fig. 2) was of the orthokeratinized type and consisted of a thick cornified layer. A thick cornified layer was observable especially in the anterior epithelium of each filiform papilla. The anterior epithelium of the filiform papillae had numerous keratohyaline granules in the thick cornified layer. At the posterior part, the cornified layer was attenuated and lacked keratohyaline granules. No lingual glands were found in the lamina propria of the anterior part of the tongue (Fig. 1; A).

ii) SEM observation

The external appearance of the filiform papillae, which were distributed on the anterior part of the tongue, was clear and conical shaped (Fig. 4). Accessory processess were not seen around the main protrusion. The appearance of each connective tissue core (CTC) after removal of epithelium in this area was seen as a primary core associated with approximately 5–6 accessory processes (Fig. 5). Concavity was also observable in the front of each CTC. The CTCs of filiform papillae after exfoliation of epithelium distributed on the rather posterior parts, lacked primary protrusion. Each of these CTCs had approximately 10–15 accessory cores that were arranged in a horseshoe manner (Fig. 7).

Fig. 4. Scanning electron micrograph of dorsal surface of the anterior area of the tongue. Apex of each filiform papilla (Fi) has a somewhat sharp tip. Each filiform papilla lacked accessory protrusions. Dome-like fungiform papillae (Fu) are seen among filiform papillae. Scale bar = 200 μm.
The size of filiform papillae was varied. Filiform papillae that were situated in rather posterior part was bigger than filiform papillae that were distributed on the anterior part (Fig. 1; A). Length of the filiform papillae were 420–700 μm and their width was approximately 185–600 μm.

b) Fungiform Papillae
   i) Lightmicroscopic observation
   The top of the fungiform papillae (Fig. 3) were rod or cylinder in shape. A few taste buds were found in the dorsal surface of the epithelium. Vascular loops and nerves were seen in the lamina propria of fungiform papillae.

   ii) SEM observation
   The surface of fungiform papillae (Fig. 4) was smooth and dome-like, however their size varied (Diameter of fungiform papillae were approximately 257–450 μm). After removal of the epithelium, CTCs (Fig. 6) appeared a cylindrical primary core. At the lateral side of the cylindrical core, numerous rod-shaped accessory protrusions were arranged.

c) Vallate papillae
   i) Lightmicroscopic observation
   Six to seven dome-like vallate papillae could be seen, however circumferential furrows were quite
unclear. Several tastebuds were seen on the dorsal surface; however, no tastebuds were found on the lateral side of the papillae. Several mucous-rich lingual glands (mixed glands) were found in the lamina propria. The epithelium was para-keratinized and nuclei remained at the most superficial cells (Fig. 8).

ii) SEM observation

Six to seven vallate papillae were arranged at the boundary part between anterior part and radix of the tongue (Fig. 1; B). Circumferential furrows were obscure on most of the vallate papillae. On the CTC (Diameter: 555–975 μm) that were situated beneath of these vallate papillae, primary CTCs appeared to be hemi-spherical and covered with fine-distributed secondary cores. Among these secondary cores, some concavities were seen.

e) Radix
i) Lightmicroscopic observation

The epithelium was para-keratinized and nuclei were remained at the most superficial cells (Fig. 11). Mucous-rich large mixed salivary glands were distributed in the lamina propria beneath this area.
ii) SEM observation

The surface of the epithelium was smooth, but had numerous shallow ridges and grooves (Fig. 12). After their epithelium was exfoliated, CTC was also displayed shallow ridges and grooves densely covered with small cores. Several orifices of salivary glands were also seen among these small cores (Fig. 13).

Discussion

Lingual papillae and their connective tissue cores

The tongue of the Spotted seal had an each filiform, fungiform and vallate papillae that were quite similar with those found on the California sea lion (Yoshimura et al., 2002) and Steller’s sea lion (Kobayashi, 1982). In the epithelial surface, all of these species possessed clear and conical shaped filiform papillae, which had lacked accessory protrusions. Furthermore, CTCs after exfoliation of their epithelium had many similarities; primary core associated with approximately 5–6 accessory processes, concavity in the front of each filiform CTCs, cylindrical primary cores of fungiform CTCs, rod-like accessory protrusions distributed at the lateral side of the fungiform CTCs, obscure circumferential furrow of the vallate papillae and hemi-spherical CTCs covered with fine-distributed secondary cores on the vallate CTCs. Our present observations
however found clear morphological differences especially on the radix. In the radix part of the tongue of spotted seal, the surface of the epithelium was rather smooth, and their CTCs were flat and possessed only shallow ridges and grooves densely covered with small cores. On the other hand, numerous large conical papillae were densely distributed on the epithelial surface on the radix part of California sea lion (Yoshimura et al., 2002) and Steller’s sea lion (Kobayashi, 1982). Kubota (1968) reported about the lingual mucosa of the Northern fur seal that lacked a description regarding the papillae on the radix, even though large conical shaped papillae were also shown on his macroscopic drawing. In Sonntag’s report (1922) about the surface and macroscopic observation of the Harbour (or common) seal (Phoca vitulina), fold-like structures were seen on his drawing. A smooth surfaced radix of spotted seal was also quite similar to the lingual mucosa that was found on the bottlenosed dolphin which is a fully aquatic mammal (Yoshimura and Kobayashi, 1997). The flatted root epitheliums that were seen in these species may represent adaptation of aquatic life.

The spotted seal is a carnivorous mammalian species and there are some reports regarding the CTCs of lingual papillae found on the tongue of terrestrial carnivorous species. Inatomi et al. (1999) reported on the CTC of the lingual papillae of the carnivorous, Japanese black bear. The CTCs of filiform papillae of Japanese black bear possessed concavities and were surrounded by 10–15 rod-like protrusions. The rod-like protrusions of Japanese black bear that were situated at the posterior part were somewhat long and large. The CTCs of fungiform papillae were columnar or cone-like. At the side of the CTCs, fringe-like protrusions were situated across each CTC. Kobayashi et al., (1988a, 1989, 1992) reported on the CTCs of lingual papillae on the cat tongue. The CTCs of filiform papillae on the cat tongue also possessed central concavities and several to 10 or more rod-like small protrusions that surrounded central concavities. Main protrusion of filiform CTCs were found on the posterior part of each central concavity. Regarding the lingual papillae of the dog tongue (Kobayashi et al., 1987, 1988b,c, 1992), the CTCs of the filiform papillae also exhibited central concavities and main protrusions that were situated at posterior part of each CTC. These structural characteristics of each fungiform and filiform CTCs exhibited similarity with present observations. In our study of the Spotted seal, CTCs of filiform papillae possessed central concavities and primary cores associated with approximately 5–6 accessory processes. These morphological characteristic found on filiform

Fig. 8. Light micrograph of a sagittal section of a vallate papilla. A taste bud is seen on the top epithelium of the papilla (arrowhead). Scale bar = 200 μm.
CTCs seems to be commonly found amongst carnivores. Present observations of the filiform CTCs of Spotted seals support that Spotted seal possess morphological similarity with terrestrial carnivores mammals.

Keratinization

In the present study, the lingual epithelium on the tongue of the Spotted seal exhibited both parakeratosis and orthokeratosis. Fundamental and common features of the lingual epithelium of mammals are stratification and keratinization (Iwasaki, 2002; Review). It is not unusual for masticatory mucosa to show a variation of keratinization as parakeratinization. In parakeratinized epithelium, shrunken (or pyknotic) nuclei are retained in many or all of the squames. Keratohyaline granules may be present in the underlying granular layer, though usually fewer than in orthokeratinized areas, so that this layer is difficult to recognize in histologic preparations (Squier CA and Hill MW, 1994; Textbook). Many terrestrial mammals exhibit orthokeratinized epithelia on the lingual mucosa, therefore, the nuclei of epithelial cells in the most superficial part of the epithelium in these mammals is absent. (Dog: Kobayashi et al., 1987, 1988b,c; Cat: Kobayashi et al., 1988a; Kobayashi and Iwasaki, 1989; Mongoose: Iwasaki et al., 1984; Japanese black bear and Mountain goat: Inatomi et al., 1999; American Beaver: Shindo et al., 2006). On the other hand,

![Fig. 9. SEM micrograph of external surface of vallate papilla. Circular furrow was quite unclear. Scale bar = 200 μm.](image-url)
many aquatic mammals have parakeratinized lingual epithelia and as the result, nuclei of epithelial cells remain even at the most superficial layers (Bottle-nosed, Striped and Pacific white-sided dolphins: Yoshimura and Kobayashi, 1997; Manatee and Dugong: Yamasaki and Komatsu 1980; California sea lion: Yoshimura et al., 2002; Northern fur seal: Kubota et al., 1968; Steller’s sea lion: Kobayashi, 1982). On the dorsal surface of tongue of the Spotted seals, the epithelium situated on the anterior part of the tongue exhibited orthokeratinized type that consisted of a thick cornified layer. However, the epithelium, which was distributed at the vallate papillae and root, appeared to be parakeratinized and nuclei remained in the most superficial cells. Authors (Yoshimura et al., 2002) that observed other pinnipedia, such as the California sea lion’s tongue found that entire part of the lingual epithelia involving lingual papillae e.g. filiform, fungiform, vallate and large conical papillae exhibited a parakeratinized type of epithelium and there are no differences in each region. Both California sea lions (Zalophus californianus californianus) and Spotted seals (Phoca largha) are quite similar aquatic mammalian species. Both of them are carnivores and the diet of both species is quite similar; mostly fish, cephalopods like squid and octopus (Jefferson et al., 1993). Moreover, both

Fig. 10. SEM micrographs of CTC of the vallate papillae after epithelial removal. Rod shaped small cores were densely distributed especially on the bottom of primary core(s). Scale bar = 200 μm.
species are pinnipedia and have semi-aquatic lives. In contrast, lingual mucosae that have thick (Kobayashi et al., 2005) and/or keratinized epithelium, are exhibited among terrestrial animals reflecting their diet and living environment. The Bactrian Camel that has a thorny, stiff and rough inorganic plant diet may be associated with the development of thick and keratinized lenticular papillae situated on the lingual torus (Eerdunchaolu et al., 2001). In regards to the oral mucosa of Homosapiens, Caffesse et al., (1982) reported that mechanical stimulation of the sulcular epithelium plays a role in promoting its keratinization. Shimoda et al., (1996) examined the lingual mucosa of the aquatic mammal, the Sea Otter (Lutrinae: Enhydra lutris) and found a thin, but orthokeratinized cornified layer. The diet of sea otters consists of abalones, sea urchins, rock crabs and shellfish (Jefferson et al., 1993). According to description of Jefferson et al., (1993), adult Spotted seals sometimes feed larger crustaceans. Franks et al., (1985) reported that differences of mastication between anthropoid primates and herbivores can be attributed to differences in anatomy of oral apparatus e.g. soft or hard palate. Orthokeratinized lingual epithelium on the tongue of the Spotted seal may imply difference in food intake, especially in mastication.

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References

Fig. 12. SEM micrographs of the external surface of a lingual radix. Numerous ridges and grooves consisting of rather smooth epithelium. Scale bar = 500 µm.


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Fig. 13. SEM micrographs of CTCs of the root after removal its epithelium. Numerous small cores were densely distributed on the ridges and grooves of CTCs. Scale bar = 500 µm.


