

Comparative Morphology of the Tongue of *Miniopterus schreibersi fuliginosus* and *Pipistrellus savii*

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긴가락박쥐 (*Miniopterus schreibersi fuliginosus*)와 검은집박쥐 (*Pipistrellus savii*) 혀의 비교형태

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ABSTRACT

A SEM study on morphology of lingual papillae of Korean long-fingered bat (*Miniopterus schreibersi fuliginosus*) and Savi's Pipistrelle (*Pipistrellus savii*) was conducted. Three kinds of lingual papillae were observed: filiform, fungiform, circumvallate papillae. Filiform papillae were divided into two types; the type 1 had a group of needle-like projections, and was distributed throughout the front half of the tongue; the type 2 had a smooth and thick body, and was found in rear half of the tongue. 35 to 45 fungiform papillae were found on the dorsal surface of the tongue in both species. They were observed along the lateral margins and were also found on front and rear end part of the tongue. There were two to three noticeably large fungiform papillae arranged in a straight line on the region between lingual prominence and circumvallate papillae. There were two circumvallate papillae close to the rear end of the tongue. They were large and round, each having two layers of pads. The overall morphology of lingual papillae of *M. schreibersi fuliginosus* and *P. savii* was found to be similar with other Chiroptera. However, few but noticeable differences were found among the filiform papillae and fungiform papillae. Type 2 filiform papillae differed in that bifid and trifid configuration were found in *M. schreibersi fuliginosus* unlike in *P. savii*. In addition, numbers of large fungiform papillae located in the center of posterior region of the tongue were different with *M. schreibersi* have three while *P. savii* having only two.

Keywords : Tongue, Lingual papilla, *Miniopterus schreibersi fuliginosus*, *Pipistrellus savii*

INTRODUCTION

Tongue is a taste sensor located in oral cavity that plays an important role in digestion among many vertebrates (Fawcett,

1986; Kanazawa & Yoshie, 1996). Tongue primarily conducts several tasks as a tactile and taste organ, but is also involved in vocalization and transportation of food. On the surface of tongue, there are various kinds of lingual papillae including filiform, fungiform, circumvallate and foliate papillae, each having

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different morphological structure and shape. Distribution of these lingual papillae has been considered to be related to species' eating habits and vocalization (Fawcett, 1986).

Accordingly, many vertebrate species have been studied specifically for their lingual papillae and their distribution. Those include primates (Kobayashi et al., 2004), rodents (Fernandez et al., 1978; Shimizu et al., 1979, 1980; Iida et al., 1985; Kullaa-Mikkonen et al., 1987; Meisel et al., 1987; Kobayashi, 1990; Iwasaki et al., 1997, 1999; Toprak, 2006) and cattles (*Bos taurus*) (Steflik et al., 1983).

Bats, or species under the order *Chiroptera*, consist more than 20% of the entire mammal species, and as a result, there have been vigorous studies on the lingual papillae of bats: *Rhinolophus ferrumequinum* (Son et al., 2000), *Pteropus vampyrus* (Emura et al., 2002), *Leptonycteris nivalis*, *L. sanborni* (Greenbaum & Phillips, 1974), *Miniopterus schreibersi fuliginosus* (Kobayashi & Shimamura, 1982), *Pipistrellus abramus* (Iwasaki et al., 1986) and *P. pipistrellus* (Pastor et al., 1993).

Miniopterus schreibersi fuliginosus is commonly known as long-fingered bat inhabiting in southern part of Korea. It grows up to 46.5~56.6 mm with a wingspan of 40.75~50 mm. It has characteristically long wings and square-shaped ears. Many caves serve as common habitat for this species. It does not migrate much throughout its longevity and shows a strong tendency for group behaviors. It produces single offspring in July and August, and provides care in a group-wise manner with other individuals. It hibernates in a cave from November to February in the following year.

Pipistrellus savii, or commonly known as Savi's Pipistrelle, is a small-size bat that grows up to 36~48 mm with the wingspan of 32~36 mm. It is primarily brown, but has a beige hair on the belly. This common bat inhabits under the roof of the house or between cracks on the wall, as indicated from its common name. It produces up to four offspring in July and hibernates from November to March. This species can be found throughout the East Asia.

This research compares the morphological structure of lingual papillae of *M. schreibersi fuliginosus* and *P. savii* through scanning electron microscopy, and focuses on the differences in their distribution and morphological shapes.

MATERIALS AND METHODS

The sample *M. schreibersi fuliginosus* and *P. savii* used in this study were captured in exhausted mines located near Tong-

yeong and Haman in South Korea respectively. Two adult individuals were captured and were used for research. After the sample has been anesthetized with ethyl ether, its tongue was excised and was fixed with 3% glutaraldehyde for three hours. Then, it was rinsed in buffer solution (Millonig's buffer, pH 7.4), and was fixed again with 1.33% OsO₄ for two hours. The fixed tissue was dissociated in 8N-HCl at 60°C to eliminate mucus and other non-tissue matters from the sample tongue. After cleansing, the sample tissue was dehydrated through graded series of alcohol (60, 70, 80, 90, 95, 99, 100% concentration). Then, the alcohol inside the tissue was substituted with hexamethyldisilazane (HMDS). Finally, the tissue was coated with Pt for 90 seconds in ion coater (E-1030, Hitachi), and was observed using scanning electron microscope (FESEM, S-4200, Hitachi)

RESULTS

The tongue of *M. schreibersi fuliginosus* was approximately 7 mm long. The tip of the tongue was obtuse with oval sides.

Table 1. Comparison of the cell types, shapes, sizes and total numbers of the filiform, fungiform and circumvallate papillae of *M. schreibersi fuliginosus* and *P. savii*

		<i>M. schreibersi fuliginosus</i>	<i>P. savii</i>
Filiform papillae			
Type 1 :	Front	171 μm	79 μm
	Rear	115 μm	71 μm
Type 2 :	Front	95 μm	82 μm
	Rear	120 μm	144 μm
Fungiform Papillae			
Middle :	Width	90 μm	80 μm
	Length	120 μm	80 μm
Rear-1 :	Width	84 μm	90 μm
	Length	96 μm	110 μm
Rear-2 :	Width	135 μm	137 μm
	Length	155 μm	173 μm
Amount		40~45	35~40
Circumvallate Papillae			
Left :	Width	330 μm	280 μm
	Length	410 μm	390 μm
Right :	Width	360 μm	300 μm
	Length	390 μm	400 μm

Type 1 filiform papillae indicate ones in the anterior half of the tongue; Type 2 signify the ones in the posterior half of the tongue; middle fungiform papillae indicate fungiform papillae found in the middle region in the tongue; rear-1 fungiform papillae signify ones found in the rear region of the tongue; the rear-2 fungiform papillae indicate three large fungiform papillae located in the rear part of the longitudinal axis.

The front section of the tongue was 2.4 mm wide, and the mid-rear section of the tongue was 3.1 mm wide. Overall shape of the sample tongue resembled a thick rod with a pointed tip. Three types of lingual papillae were observed: filiform papillae, fungiform papillae, and circumvallate papillae (Table 1).

The filiform papillae were observed throughout the whole tongue. Their shapes varied according to their location within the tongue. The ones in the very front (Type 1) were long and sharp, and they were bent toward the rear (Fig. 2A). Their slim figure resembled a group of thorns (Fig. 2A and 2B). The shape of the filiform papillae remained same throughout the front half of the tongue, but their directions changed. The ones close to the middle part of the tongue were directed toward the tip of the tongue, an opposite direction of the way filiform papillae found in the very front were headed (Fig. 2B). The directions of filiform papillae located between were indiscriminate, some directed toward the tip and some toward the rear. The ones found in the rear part of the tongue (Type 2) were noticeably thicker and larger than the ones in the front. They no longer had the needle-like projections, but instead had one large triangular body. Almost all filiform papillae found in the rear part of the tongue were directed toward the rear end. Noticeable characteristic of type 2 filiform papillae was that some of them, especially ones close to the middle, had bifid and trifid ends.

The fungiform papillae were also observed in all parts of the tongue, but their numbers were far smaller (40~45) than those of filiform papillae. They had an unchanging circular shape, with radius ranging from 40 to 90 micrometers. They were evenly distributed along the lateral margins, possibly in symmetric pattern. The ones in the posterior region were clearly distributed in pattern along the symmetrically curved line on each side. A characteristic feature of fungiform papillae was found in the rear section of the tongue, right on the main axis. There were three discernable large fungiform papillae right above the two circumvallate papillae. Their sizes were the largest of all other fungiform papillae.

There were two circumvallate papillae in the rear part of the tongue, one on the left and the other on the right. They were doughnut-shaped, and had the center region seemingly independent from the other part. The outer ring of circumvallate papilla was somewhat integrated with the surrounding part of the tongue.

P. savii showed slightly different, but mostly similar results with *M. schreibersi fuliginosus*. The tongue of the *P. savii* was 6.6 mm long, and 2 mm wide in the middle while 2.7 mm wide in the rear. It had pointed tip and rear end.

The filiform papillae of the *P. savii* were observed in the every part of the tongue, but their shapes differed according to their position on the tongue. The ones in the anterior part of the tongue (Type 1; Fig. 6A) were relatively long and slim, and had brush-like textures, but the ones in the posterior region (Type 2; Fig. 6B) were thick and triangular with smooth textures. There was a sudden change of direction of filiform papillae in the middle section where a group of large filiform papillae was visible. The section anterior to the middle region had filiform papillae heading toward the rear end of the tongue, while the section posterior to the middle region showed filiform papillae heading toward the opposite direction. The noticeable group of filiform papillae located in the middle region showed conspicuously large and thick body, easily observable in the low magnification. They were directed toward the tip of the tongue, directly opposite from the filiform papillae right in front of them.

There were about 35 to 40 fungiform papillae on the dorsal surface of *P. savii*' tongue. They were round-shaped with sizes ranging from 84 micrometers wide and 96 micrometers long to 150 micrometers wide to 160 micrometers long. They were distributed along the margins on each side, but were rarely found in the rear part of the tongue where thick filiform papillae were densely populated. There were two distinguishably large fungiform papillae in the posterior section on the main axis of the tongue.

Two circumvallate papillae were found in the posterior part of the tongue. They were flat and were also doughnut shaped, and had irregular troughs in the center alongside with large circular trough around the inner part. Each circumvallate papilla was about 350 micrometers wide and 400 micrometers long.

DISCUSSION

In terms of distribution of filiform papillae, *M. schreibersi fuliginosus* and *P. savii* showed similar results with other bats including long-nosed bat (Greenbaum & Phillips, 1974), Korean greater horseshoe bat (Son et al., 2000) and lesser dog-faced fruit bat (Emura et al., 2001). The filiform papillae of those two species were distributed throughout the entire dorsal surface of the tongue. Despite their large numbers, basically two different types of filiform papillae were found. Type 1 filiform papillae were mostly found in the anterior half of the tongue, and were hair-shaped with 10 to 20 needle-like projections. As Iwasaki et al. (1987) suggested, these filiform papillae in the

anterior part of the tongue seemed to have participated in touch and attachment to the food by making the surface of the tongue rough and fractious. Type 2 filiform papillae were triangular with one whole, bifid or trifid body. They differed from the first type in that they had smooth surfaces and thick bodies. They, on the other hand, may enhance the transportation of food through smooth and relatively large surface. In *P. savii*'s tongue, several large type 2 filiform papillae were found in lingual prominence, or the middle region where the type of the filiform papillae present changes. It might be possible to consider them as another type, but they seemed more like oversized type 2 filiform papillae with slightly sharper tips. They may contribute in holding the food until it is swallowed (Pastor et al., 1993; Son et al., 2000). There was only a small variation between type 1 filiform papilla of *M. schreibersi fuliginosus* and that of *P. savii*. The former papillae were larger in radius, but had fewer but sharper projections, and were less worn out. The latter papillae were longer in terms of the two longest projections that resembled pincers of an earwig. They were relatively flat due to a significant attrition, but it was possible to notice that they once had many long, slender projections. Type 2 filiform papillae found in both species showed similar resemblance of each other. Both species had similar sized type 2 filiform papillae, although *P. savii* generally had slightly bigger ones. Only significant difference was that the type 2 filiform papillae of *M. schreibersi fuliginosus* had bifid or trifid configuration while those of *P. savii* did not. In comparison with *Myotis macrodactylus*, the aforementioned two species had similar filiform papillae in the anterior regions of their tongues. Hwang and Lee noted that the filiform papillae of the *M. macrodactylus*' tongue were crown or thorn shaped and scale or conical shaped, which show a very similar resemblance with the type 1 and type 2 filiform papillae of *M. schreibersi fuliginosus* and *P. savii* (Hwang & Lee, 2007), except that only the type 2 filiform papillae of *M. schreibersi* had unique bifid and trifid ends.

The fungiform papilla has been reported to contain many taste buds and function as a taste sensor. Its numbers are known to be proportional to both the range of food a species consume (Hwang & Lee, 2007) and the size of the species' tongue (Chung & Kwun, 1977). Hence, humans (180~220) and other primates that are omnivores and have relatively large tongue, have more fungiform papillae than rabbits (90~120) or moles (80~120) (Chung & Kwun, 1977). In this study, *M. schreibersi fuliginosus* and *P. savii* had a smaller number (35~45) of fungiform papillae than aforementioned mammals. This might

be the result of those species' insectivorous food habit, but it is more probable that this small number of fungiform papillae is due to relatively small tongue sizes as Chung and Kwun have conjectured. The distribution of fungiform papillae is usually divided into four types. The first type indicates an even distribution throughout the whole area of dorsal surface as found in dogs; the second type refers to a focused distribution in the boundaries including side edges, tip and rear end section as in cats, moles and humans; the third type designates a concentrated distribution only in the tip and side edge areas as in rabbits; the fourth type indicates a center and tip distribution seen in mice (Chung & Kwun, 1977). *M. schreibersi fuliginosus* and *P. savii* basically showed edge-tip distribution of fungiform papillae that is similar with the second type.

The number of circumvallate papillae in bats is known to depend on the types of food species consume. Usually, fruit-eating bats have three circumvallate papillae while insectivorous bats have two (Pastor et al., 1993; Son et al., 2000; Emura et al., 2001, 2002; Gregorin, 2003; Hwang & Lee, 2007). *M. schreibersi fuliginosus* and *P. savii* are insectivores, and consequently showed two circumvallate papillae on their tongue. There were large circular troughs around the inner part of the circumvallate papillae, which seem to enhance the accessibility of food to the taste buds present at the papillae's sides.

This research has studied morphological aspects of tongue of *M. schreibersi fuliginosus* and *P. savii*. It discovered main characteristics of lingual papillae and their distribution, and compared both with each other and with other species under the order Chiroptera. The morphology of lingual papillae was mostly similar between two species, and it contained several characteristics found in many other Chiropteran species. However, few but noticeable differences were found among the filiform papillae and fungiform papillae. Type 2 filiform papillae differed in that bifid and trifid configuration were found in *M. schreibersi fuliginosus* unlike in *P. savii*. In addition, numbers of large fungiform papillae located in the center of posterior region of the tongue were different with *M. schreibersi* have three while *P. savii* having only two.

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< 국문 초록 >

주사전자현미경을 이용하여 한국산 긴가락박쥐 (*Miniopterus schreibersi fuliginosus*)와 검은집박쥐 (*Pipistrellus savii*)의 혀유두의 형태를 관찰하였다. 본 연구에서 두 종 모두 세 가지의 혀유두, 즉, 실유두, 버섯유두 그리고 성곽유두가 관찰되었다. 실유두는 다시 두 종류로 세분화되었으며, 침상의 돌기물을 갖는 유형 1은 혀의 전반부의 1/2 지점까지 분포하며, 유형 2는 밋밋하고 두꺼운 돌기물을 갖고 있으며 혀의 후반부의 1/2에 분포한다. 두 종 모두 35~45 개의 버섯유두가 혀의 표면에서 관찰된다. 이들 버섯유두는 혀의 측면과 혀의 전반과 후반부의 끝 쪽에서 나타난다. 두 종의 버섯유두는 각각 두 개 혹은 세 개의 매우 큰 유두로 혀의 후반부 중앙에 일직선으로 배열되어 있다. 두 개의 성곽유두는 혀의 후반부 끝 쪽 가까이에 분포하고 있다. 이들 성곽유두는 크고 둥글며 각각은 두 층의 pad를 갖고 있다. 긴가락박쥐와 검은집박쥐의 혀유두의 전체적인 형태는 다른 익수류와 유사하였다. 그럼에도 불구하고, 두 종 사이에 드물지만 주목할 만한 차이점이 실유두와 버섯유두에서 나타났다. 검은집박쥐와는 달리 긴가락박쥐의 유형 2의 실유두는 두 개 혹은 세 개의 돌기를 가지고 있었다. 혀의 후반부 중앙에 위치하고 있는 큰 버섯유두의 경우, 긴가락박쥐에서는 3개, 검은집박쥐에서는 단지 2개만 관찰되었다.

FIGURE LEGENDS

Fig. 1. SEM micrographs of the dorsal surface of the tongue of the *M. schreibersi fuliginosus*.

Fig. 2. SEM micrographs of the filiform papillae of *M. schreibersi fuliginosus*: 2A and 2B show type 1 filiform papillae in front and middle region respectively; 2C and 2D show type 2 filiform papillae in the mid-rear and rear region correspondingly.

Fig. 3. SEM micrographs of the fungiform papillae of *M. schreibersi fuliginosus*: 3A shows a fungiform papilla in the anterior region of the tongue, 3B shows one in the middle region of the tongue.

Fig. 4. SEM micrograph of the posterior half of the tongue shows one of the three large fungiform papillae located in the rear part of the longitudinal axis (4A) and the left circumvallate papilla (4B).

Fig. 5. SEM micrographs of the dorsal surface of the tongue of the *P. savii*.

Fig. 6. SEM micrographs of the filiform papillae of *P. savii*: 6A shows type 1 filiform papillae in front region and 6B show type 2 filiform papillae in the mid-rear region.

Fig. 7. SEM micrographs of the fungiform papillae of *P. savii*: 7A shows a fungiform papilla in the anterior region of the tongue, 7B shows one in the middle region of the tongue, 7C shows another one in the middle region of the tongue and 7D shows one of the two large fungiform papillae located in the rear part of the longitudinal axis.

Fig. 8. SEM micrograph of the posterior half of the tongue (8A) and the left circumvallate papilla (8B).







