A Quill Vibrating Mechanism for a Sounding Apparatus in the Streaked Tenrec (Hemicentetes semispinosus)

Authors: Endo, Hideki, Koyabu, Daisuke, Kimura, Junpei, Rakotondraparany, Felix, Matsui, Atsushi, et al.

Source: Zoological Science, 27(5) : 427-432

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.27.427

ZOOLOGICAL SCIENCE **27**: 427–432 (2010)

A Quill Vibrating Mechanism for a Sounding Apparatus in the Streaked Tenrec (*Hemicentetes semispinosus*)

Hideki Endo[1]*, Daisuke Koyabu[1], Junpei Kimura[2], Felix Rakotondraparany[3], Atsushi Matsui[4], Takahiro Yonezawa[5], Akio Shinohara[6] and Masami Hasegawa[5,7]

[1]*The University Museum, The University of Tokyo, Tokyo 113-0033, Japan*
[2]*Department of Veterinary Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*
[3]*Department of Animal Zoology, University of Anatananarivo, Anatananarivo 101, Madagascar*
[4]*Primate Research Institute, Kyoto University, Aichi 484-8506, Japan*
[5]*School of Life Sciences, Fudan University, Shanghai 200433, China*
[6]*Bio-resource Division, Department of Biotechnology, Frontier Science Research Center, University of Miyazaki 889-1692, Japan*
[7]*The Institute of Statistical Mathematics, Tokyo 190-8562, Japan*

The streaked tenrec (*Hemicentetes semispinosus*) is equipped with a quill vibrating mechanism on the dorsal side of the caudal trunk that has evolved as an extraordinary sounding apparatus for communication. An arrangement of 15 or 16 light-brown quills was observed. Thickened cutaneous muscles were confirmed beneath quills. We named this structure the "quill vibrator disc" (QVD). The QVD was 16.8 mm long and 8.55 mm wide in a typical adult. Longitudinal musculature symmetrical about the sagittal plane was developed in the QVD. Myocytes were found immunohistochemically to contain mainly fast myosin but not slow myosin. These findings indicate that the QVD is a specialized apparatus in the cutaneous muscle that contributes to the vibration of quills and to the production of sound for communication.

Key words: *Hemicentetes semispinosus*, myosin, quill, QVD, streaked tenrec

INTRODUCTION

The streaked tenrec (*Hemicentetes semispinosus*) has an extraordinary mechanism of sound communication that consists of hard, keratinous quills in the mid-dorsal region (Gould, 1965; Eisenberg and Gould, 1970; Novak, 1999). It has been suggested that vibration of these quills acts as a sounding apparatus in this species, allowing communication between the mother and young (Eisenberg and Gould, 1970; Stephenson, 2003). Certainly vibration of quills produces sound in living individuals; however, the functional-morphological mechanism of these vibrations remained unclear. The rarity of the streaked tenrec has prevented detailed study of its skin and subcutaneous structure, and no descriptive or functional-morphological data have been published regarding the sounding organ. In this study, we made macroscopic and microscopic morphological observations on this sound communication system. We also used immunohistochemical methods to identify the type of muscular myosin associated with the vibrating motor of the quills, since only fast myosin could produce the necessary quick, strong vibrations of the quill vibrator disc (QVD).

* Corresponding author. Phone: +81-3-5841-2848;
Fax : +81-3-5841-8451;
E-mail: hendo@um.u-tokyo.ac.jp
doi:10.2108/zsj.27.427

MATERIAL AND METHODS

Macroscopic examinations

Four fixed specimens of the streaked tenrec (*Hemicentetes semispinosus*) that have been stored in the Department of Animal Zoology of the University of Anatananarivo were used for macroscopic observations (Table 1). All specimens came from the Mandraka District of Madagascar. The animals had been fixed whole in 10% formalin. After washing the animals in water for 6 hours, we observed the quills with the naked eye. We then removed the skin of the dorsal area from the trunk and observed the reverse side of the skin.

Microscopic and immunohistochemical examinations

The area of thick cutaneous musculature on the reverse side of the skin was named the "quill vibrator disc" (QVD). Since the QVD area corresponds to the region of the quills, morphological relationships between the quills and QVD were examined by light microscopy. The muscle tissues of the QVD were excised from the reverse side of the skin, post-fixed in Bouin's fixative for 2 hours, and washed in 70% ethanol. The tissues were dehydrated in ethanol and embedded in paraffin at 65°C. Tissue blocks were sectioned transversely and horizontally at 4 μm thickness. After removing the paraffin with xylene, sections were stained with haematoxylin and eosin for microscopic observations. For immunohis-

Table 1. Biological data for the four individuals examined in this study. QVD, quill vibrator disc; ND, no data were recorded.

Sex	Male	Male	ND	Female
Maturity	Juvenile	Juvenile	Subadult	Adult
Body weight (g)	27.5	21.0	ND	115.2
Head and body length (mm)	93.6	85.2	ND	158.0
Hindfood length (mm)	19.6	18.5	ND	25.9
Ear length (mm)	13.5	12.5	ND	13.3
Length of QVD	11.30	9.95	11.80	16.80
Width of QVD	4.05	4.00	5.30	8.55
Number of quills	15	16	16	16
Length of quills (range, mm)	8–9	8–9	8–9	8–9
Diameter of quills (range, mm)	0.4–0.5	0.4–0.5	0.4–0.5	0.8–0.9
Length of hard spines in dorsal part (range, mm)	10–14	9–14	10–14	12–15
Diameter of hard spines in dorsal part (range, mm)	0.4–0.5	0.4–0.5	0.4–0.5	0.5–0.7

tochemistry, sections were incubated in 3% H_2O_2 dissolved in methanol for 30 min to quench endogenous peroxidase activity and stained by using the avidin-biotin-peroxidase complex method (Hsu et al., 1981) with the monoclonal antibodies against fast (Sigma, Saint Louis, Missouri, USA, Product No. M4276, clone MY-32, 1:8000 dilution) and slow (Sigma, Saint Louis, Missouri, USA, Product No. M8421, clone NOQ7.5.4D, 1:400 dilution) skeletal myosin. After an overnight incubation at 4°C with the primary antibodies, sections were incubated with biotinylated anti-mouse immunoglobulin (Nichirei, Tokyo, Japan) for 30 min. Following this step, the avidin-biotin-peroxidase complex was applied to the sections for 5 min. Immunoreaction products were obtained by subsequently incubating the sections in a diaminobenzidine-H_2O_2 solution. Staining controls included omission of the primary anti-serum. Tissues from the cranial, middle, and caudal parts of the QVD were compared immunohistochemically to identify similarities or differences in the position of the musculature.

Fig. 1. Dorsal aspect of an adult streaked tenrec weighing 115.2 g. The quills are evident in the caudal part of the mid-dorsal area (arrow). The head is at the top.

RESULTS

In the streaked tenrec, specialized sounding quills are generally concentrated in a small area in the mid-dorsal region of the caudal part of the trunk (Fig. 1). This species possesses hard spines and normal long hairs that are morphologically different from the sounding quills (Fig. 2). Specimens used in this study had a total of 15 or 16 quills (Fig. 2). Macroscopic observations detected three rows of quills in the mid-dorsal region. Five quills were arranged laterally along each side and flanked five or six or

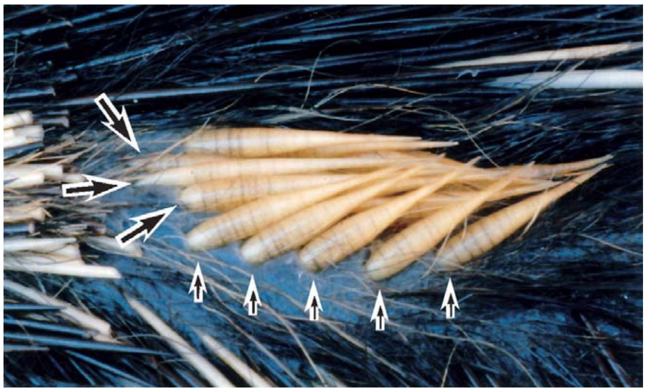

Fig. 2. Dorsal aspect of the quills, in the same individual as in Fig. 1. Sixteen light-brownish quills with circumferential dark step lines are concentrated in the caudal part of the mid-dorsal area. Five quills are arranged in the left lateral area (small arrows), and the group in the midline is evident (large arrows). Hair and spines have been partially cut away around the region to clearly show the quills. The cranial direction is to the left.

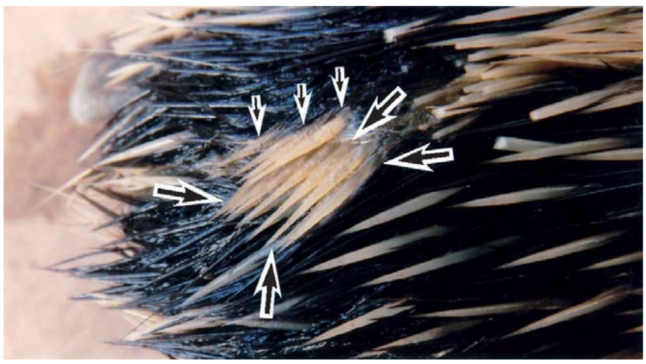

Fig. 3. Dorsal aspect of a juvenile streaked tenrec weighing 21.0 g. Five quills are arranged on the left side (small arrows), and the other groups in the midline and on the right side are evident (large arrows). Hair and spines have been partially cut away around the region to clearly show the quills. The cranial direction is to the right.

medial quills.

Quills consisted of a hard, keratin-like spiny structure. They were entirely light brown, except for the surface in the basal half of the quills, which had ten darker, circumferential step lines (Fig. 2). The quill points were sharp, fine, and devoid of darker lines. The basal parts of quills in the lateral area were thicker than those in the medial region in the adult streaked tenrec we examined.

Table 1 gives the number, length, and diameter of quills, along with data on normal hard spines. Quills in juveniles were similar in length to those in adults, but were much smaller in diameter. The arrangement of quills in juveniles was similar to that in adults, comprising three rows in the midline and the adjacent areas bilaterally (Fig. 3).

The quill vibrator disc (QVD) (Fig. 4) had a cranio-caudally elongated oval shape at the macroscopic level (Fig. 5). The area of the structure corresponded exactly to the area in which the sounding quills occurred. The muscular direction was longitudinal or corresponded to the margin of the QVD (Fig. 5). The size of QVD is indicated in Table 1. In the adult individual, the QVD was 16.8 mm in cranio-caudal length and 8.55 mm in transverse width. The size of the QVD increased with increasing body size (Table 1).

We observed transverse (Figs. 6, 7) and horizontal (Figs. 8, 9) sections of the musculature. Muscle bundles consisted of thin, longitudinally curved skeletal muscle. Some muscle bundles and the accompanying connective

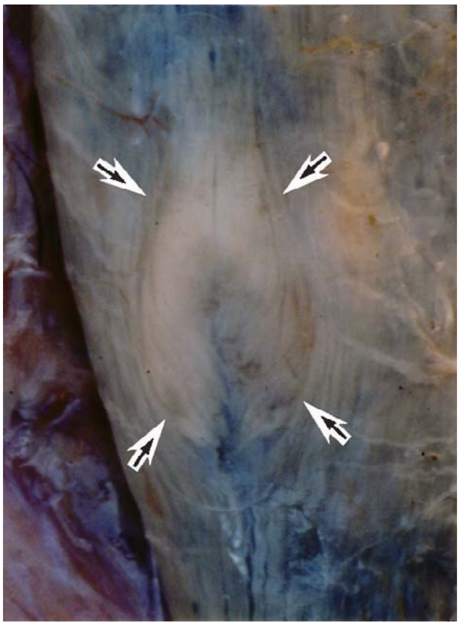

Fig. 4. Reverse side of the skin in the adult specimen. The QVD (arrows) appears elliptical and shows thick cutaneous muscles. The cranial direction is toward the top.

Fig. 5. Elongated oval shape of the thick longitudinal musculature of the cutaneous muscles evident at the macroscopic level in the QVD of the adult specimen. The cranial direction is to the right.

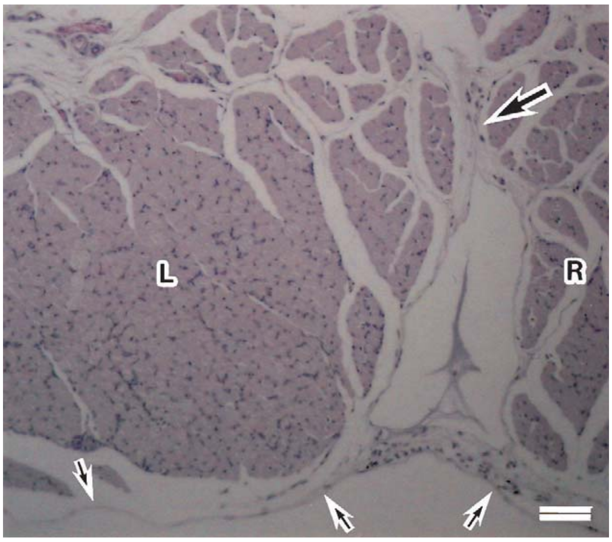

Fig. 6. Transverse section of the QVD, stained with hematoxylin and eosin, showing the left (L) and right (R) sides of the ellipse of the QVD separated by thin connective tissue (large arrow). The structure is symmetrical in the sagittal plane on each side of this connective tissue. The basement membrane (small arrows) is evident in the ventral area of the OVD. Dorsal is toward the top and the left lateral direction is to the left. Scale bar: 100 μm.

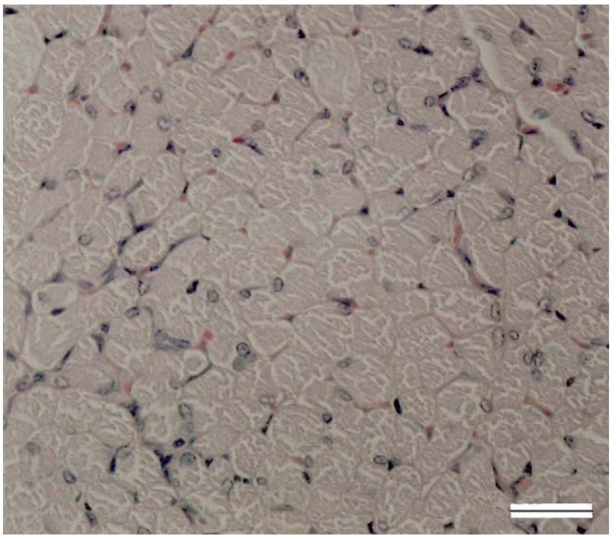

Fig. 7. Transverse section of the QVD, stained with hematoxylin and eosin. The musculature consists of many myocytes, each containing an oval nucleus peripherally. Scale bar: 30 μm.

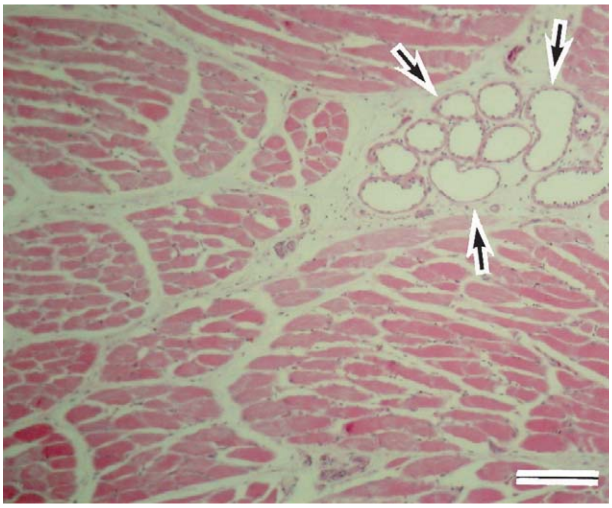

Fig. 8. Frontal section of the QVD, stained with hematoxylin and eosin. The muscles are divided into muscle bundles by thin connective tissue. Since the musculature is slightly dorsoventrally curved, an oblique section of each muscle is evident in the exactly frontal section. Apocrine sweat glands (arrows) are evident among the muscles. Scale bar: 100 μm.

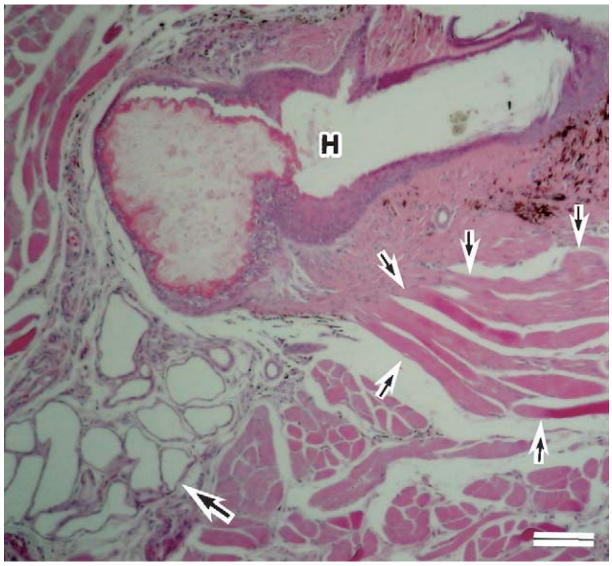

Fig. 10. Apocrine sweat glands (large arrow) and a hair follicle (H) are evident among the muscle bundles. Arrector pilorum muscles (small arrows) are attached to the hair follicle. Stained with hematoxylin and eosin. Scale bar: 100 μm.

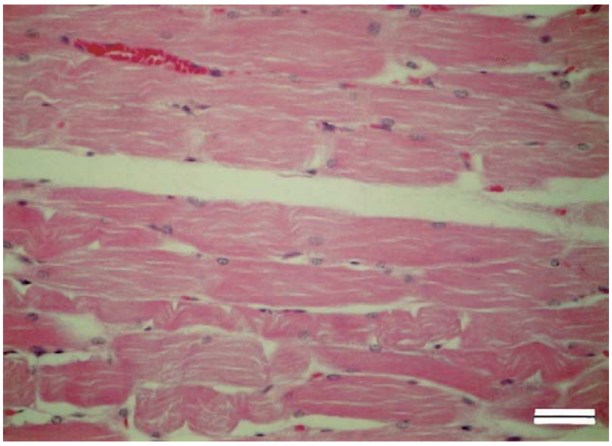

Fig. 9. Frontal section of the QVD, stained with hematoxylin and eosin. Myocytes comprise the longitudinal musculature. An oval nucleus is evident peripherally in each myocyte. Scale bar: 30 μm.

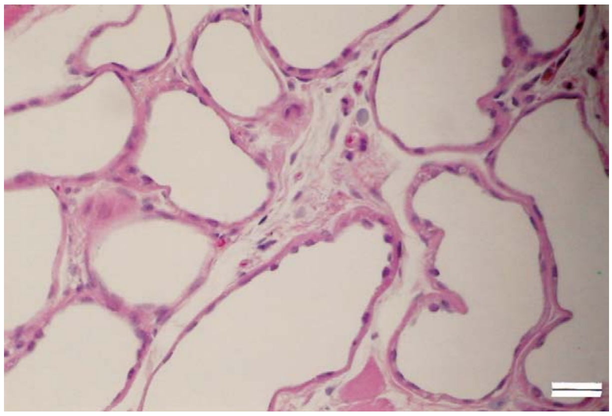

Fig. 11. Apocrine sweat glands among the musculature. Stained with hematoxylin and eosin. Scale bar: 30 μm.

tissues composed the elliptical tissue structure of the QVD. The elongated oval structure was symmetrical in the sagittal plane, and thin connective tissues divided the muscular structure into two lateral parts (Fig. 6). Striated myocytes containing oval-shaped nuclei formed the musculature of the QVD (Figs 7, 9). The basement membrane was located in the ventral area of the QVD (Fig. 6).

In the dorsal layer of the QVD, we observed sweat glands and hair follicles among the muscle bundles (Fig. 10). Apocrine sweat glands were concentrated in particular areas (Fig. 11). Arrector pilorum muscles were attached to hair follicles (Fig. 10).

Immunohistochemical reactions against fast myosin were observed largely in striated myocytes in the QVD musculature (Figs. 12, 13), whereas no reaction was observed against slow myosin (Fig. 14). Reactions within myocytes

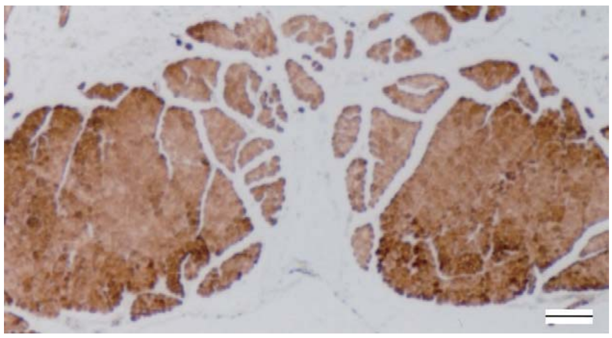

Fig. 12. Transverse section of the QVD, at a sectional angle similar to that in Fig. 6. Fast myosin is distributed largely in the myocytes, indicated by immunostaining, which is not evident in any structures except the musculature. Dorsal is toward the top, and the left lateral direction is to the left. Scale bar: 100 μm.

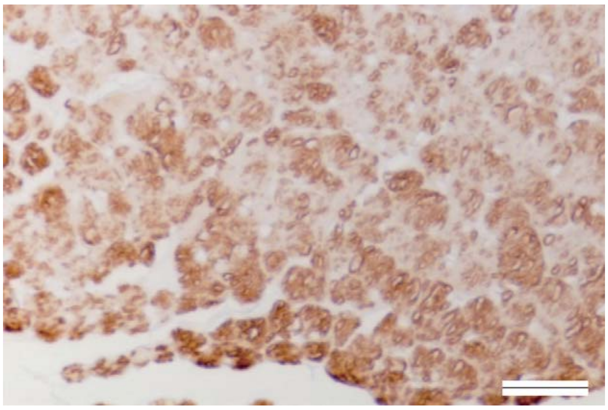

Fig. 13. Diffuse immunostaining within myocytes of the QVD. No reaction is evident in the nuclei. Scale bar: 30 μm.

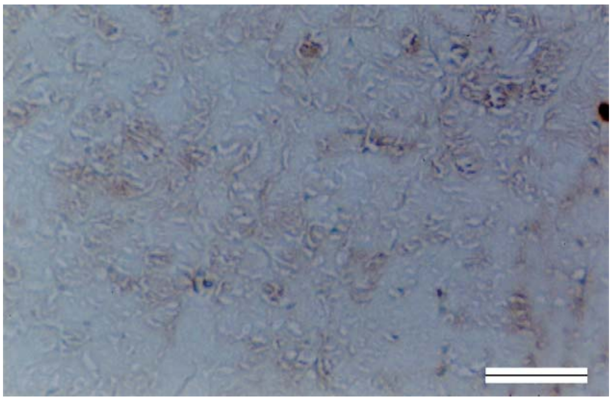

Fig. 14. No immunostaining for slow myosin is evident in the musculature. Scale bar: 30 μm.

were diffuse (Fig. 13), and no structures except the musculature reacted (Fig. 12). There was no difference in immunoreactivity among individuals or in sampling positions in the QVD.

DISCUSSION

Renrecs are noteworthy for their phylogenetic position showing the parallel evolution of the Lipotyphla (Asher, 1999; Asher et al., 2003), and the functional-morphological convergence (for example, in limbs as the locomotor apparatus) between these two phylogenetic groups has been examined in detail and discussed (Endo et al., 2006, 2007). The quill vibration system of the streaked tenrec represents one of the most extraordinary sounding mechanisms in mammals. Sound communication and echolocation in mammals are generally realized by vocalization related to the air pathway. Species of *Sorex*, *Neomys*, and *Diplomesodon* in the Lipotyphla communicate intraspecifically by emitting vocal signals within their tunnels (Movchan and Shibkov AA, 1982). In species of Lipotyphla, sound communication may contribute to defense, attracting mates, and actively searching for moving prey. Why vocalization has not developed in tenrecs has remains unclear, but the quill vibration mechanism of the streaked tenrec may correspond functionally to vocal communication in the Lipotyphla. We suggest that sounding by quill vibrations may be reasonable for spiny animals such as the streaked tenrec, since hard, keratinous quills and the vibrating motor could have easily evolved from spines and cutaneous muscles, respectively.

From the present morphological findings, we conclude that the QVD is a specialized apparatus in the cutaneous muscle of the trunk. Arrector pilorum muscles are not functional-morphologically related to the control of the QVD, or to the movement of non-specialized hairs (Fig. 10). Since enlarged arrangements and fast movement of the musculature are needed to vibrate quills, we suggest that general arrector pilorum muscles cannot be adapted for this functional role. Although vibrations have not been analyzed by filming living animals, we suggest that the bases of the quills, with circumference step lines, may rub against each other to produce sound. The longitudinally and marginally running cutaneous musculature may effectively act as a rubbing motor for the three groups of quills arranged in the midline and bilaterally in the dorsal region. In the future, however, we need to explain functional-morphologically how sound is produced by the circumference step lines of the quills.

In this study, we examined the growth of quills by comparing adults with juveniles (Table 1). The number and arrangement of quills did not alter during growth. The length of quills did not increase during growth like that of the normal hard spines, whereas the quill diameter increased from 0.4–0.5 mm in juveniles to 0.8–0.9 mm in adults. Although differences in the vibrating function between juveniles and adults remain unclear, the restricted variation in quill number and early development of quill length suggest that sounds produced by quill vibrations may contribute to sound communication and echolocation even in juveniles. This is consistent with data demonstrating that sounding is important in helping the streaked tenrec mother and young locate one another (Eisenberg and Gould, 1970; Novak, 1999).

The QVD is also equipped with apocrine sweat glands. This suggests that the skin surface near the QVD may secrete exocrine chemical signals related to the sense of smell. Our results confirm the presence of systems contributing to non-visual communication via the QVD in this species.

The structure of the cutaneous musculature cannot be readily analyzed. Our study was related to work done on the sugar glider (*Petaurus brevicepis*) (Endo et al., 1998), which indicated that observations made skillfully from the reverse side of the skin provide macroscopic data on the cutaneous muscles. We used similar techniques in our observations of the QVD in the streaked tenrec.

ACKNOWLEDGMENTS

This study was supported financially by the Grants-in-Aid for Scientific Research (Nos. 21370033, 21658103, and 20405016) from the Ministry of Education, Science and Culture, Japan, and by the JSPS core-to-core program HOPE.

REFERENCES

Asher RJ (1999) A morphological basis for assessing the phylogeny of the “Tenrecoidea” (Mammalia, Lipotyphla). Cladistics 15: 231–252

Asher RJ, Novacek MJ, Geisler JH (2003) Relationships of endemic African mammals and their fossil relatives based on morphological and molecular evidence. J Mammal Evol 10: 131–194

Eisenberg JF, Gould E (1970) The tenrecs: a study in mammalian behavior and evolution. Smithson Contr Zool 27: 1–138

Endo H, Yokokawa K, Kurohmaru M, Hayashi Y (1998) Functional anatomy of gliding membrane muscles in the sugar glider (*Petaurus brevicepis*). Ann Anat 180: 93–96

Endo H, Yonezawa T, Rakotondraparany F, Sasaki M, Hasegawa M (2006) The adaptational strategies of the hindlimb muscles in the Tenrecidae species including the aquatic web-footed tenrec (*Limnogale mergulus*). Ann Anat 188: 383–390

Endo H, Oishi M, Yonezawa T, Rakotondraparany F, Hasegawa M (2007) The semifossorial function of the forelimb in the common rice tenrec (*Oryzorictes hova*) and the streaked tenrec (*Hemicentetes semispinosus*). Anat Histol Embryol 36: 413–418

Gould E (1965) Evidence for echolocation in the Tenrecidae of Madagascar. Proc Am Philos Soc 109: 352–360

Hsu SM, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 19: 577–580

Movchan VN, Shibkov AA (1982) Structural patterns of acoustic signals in shrews (Soricidae). Zool Zh 61: 1695–1705

Nowak RM (1999) Family Tenrecidae. In “Walker’s Mammals of the World” Ed by RM Nowak, Johns Hopkins University Press, Baltimore, pp 186–199

Stephenson PJ (2003) *Hemicentetes*, streaked tenrec, Sora, Tsora. In “Natural History of Madagascar” Ed by SM Goodman, JP Brensted, University of Chicago Press, Chicago, pp 1278–1281

(Received July 9, 2009 / Accepted December 4, 2009)