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Microanatomy of Facial Vibrissae in the Florida Manatee: The Basis for Specialized Sensory Function and Oripulation

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Mammals · Sirenia · Manatee · Vibrissae · Hair · Bristles · Face

Abstract

Sirenians, including Florida manatees, possess an array of hairs and bristles on the face. These are distributed in a pattern involving nine distinct regions of the face, unlike that of any other mammalian order. Some of these bristles and hairs are known to be used in tactile exploration and in grasping behaviors. In the present study we characterized the microanatomical structure of the hair and bristle follicles from the nine regions of the face. All follicles had the attributes of vibrissae, including a dense connective tissue capsule, prominent blood sinus complex, and substantial innervation. Each of the nine regions of the face exhibited a distinct combination of these morphological attributes, congruent with the previous designation of these regions based on location and external morphological criteria. The present data suggest that perioral bristles in manatees might have a tactile sensory role much like that of vibrissae in other mammals, in addition to their documented role in grasping of plants during feeding. Such a combination of motor and sensory usages would be unique to sirenians. Finally, we speculate that the facial hairs and bristles may play a role in hydrodynamic reception.

Introduction

Sirenians possess a varied array of modified facial hairs that are used in tactile exploration, interactions with conspecifics, and feeding [Hartman, 1979; Marshall, 1997; Marshall et al., 1998a, 2000; Bachteler and Dehnhardt, 1999]. In manatees these include six types of perioral bristles and bristle-like hairs on the oral disk [Reep et al., 1998]. These are distinguished from other body hairs by their greater stiffness, which appears to result from smaller length/diameter ratios rather than from compositional differences. Hair having the external appearance of body hair is located on the supradisk portion of the face posterior to the orofacial ridge and on the chin [Reep et al., 1998].

As illustrated in figure 1, the six fields of perioral bristles on the face of the Florida manatee consist of four (U1–U4) on each side of the upper lips and oral cavity and two (L1–L2) on each side of the lower lip pad along the lip margin [Reep et al., 1998]. Each field has a characteristic location, number of bristles, and range of external bristle length and diameter. Branches of the infraorbital nerve innervate the bases of the largest bristles (U2 group) on the upper bristle pad, and the inferior alveolar nerve supplies the L1–L2 bristles of the lower bristle pad. These innervation patterns and the topographical locations of the six bristle fields suggest that the U1–U4 bristles are homologous to the mystacial vibrissae of other mammals, whereas the L1–L2 bristles correspond to the mental vibrissae [Reep et al., 1998]. Dorsal and ventral buccal branches of the facial nerve innervate

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Abbreviations

app	ring sinus appendages	epi	epidermis	ors	outer root sheath
bl	blood	gm	glassy membrane	rs	ring sinus
cap	follicle capsule	hs	hair shaft	trb	trabeculae of cavernous sinus
der	dermis	lcs	lower cavernous sinus	ucs	upper cavernous sinus
dp	dermal papilla	ms	mesenchymal sheath		

the superficial facial musculature, which is likely to be involved in bristle eversion and other movements that occur during feeding behavior [Marshall et al., 1998b; Reep et al., 1998].

Sirenians use the modified hairs of the face in a variety of behaviors. Uniquely among mammals, they use some of the perioral bristles (primarily those of the U2 and L1 fields) in a grasping fashion during feeding and oripulation of objects [Marshall et al., 1998a, 2000]. (We have coined the term 'oripulation' to refer to use of the mobile lips and associated perioral bristles in coordinated rhythmic grasping behaviors. This seems preferable to the term 'manipulation', which connotes use by the manus, or hand.) Tactile exploration without grasping typically involves the bristle-like hairs of the oral disk [Hartman, 1979; Marshall et al., 1998a; Bachteler and Dehnhardt, 1999]. Most mammals use their vibrissae in a purely sensory fashion, primarily to detect prey or to orient in low light environments. Vibrissae are capable of providing detailed textural information about surfaces and objects in the immediate environment [Dykes 1975; Brecht et al., 1997]. Psychophysical measures indicate that harbor seals may be able to use their vibrissae in sensory detection as effectively as monkeys use their hands in touch discrimination [Dehnhardt and Kaminski, 1995]. In rats the geometry of vibrissal lengths within a row and the angular arrangements of the vibrissae of each row relative to the body axis and the other rows are important variables influencing the behavioral use of the vibrissae [Brecht et al., 1997].

Among non-sirenians, movement of vibrissae occurs in several contexts. Rodents engage in whisking behavior in which the mystacial vibrissae are swept in a rhythmic fashion during tactile exploration of the external environment [Welker, 1964; Wineski, 1985; Carvell and Simons, 1990]. Pinnipeds move their long vibrissae during display behaviors and tactile exploration [Peterson and Bartholomew, 1967; Miller, 1975; Kastelein and Van Gaalen, 1988; Dehnhardt, 1994; Dehnhardt and Kaminski, 1995; Dehnhardt and Ducker, 1996]. When out of water with the snout pointed upward, walruses have been observed to move pieces of fish in the direction of the mouth by passing them from vibrissa to vibrissa in a wave-like manner [Marshall et al., 1998a], but they engage in no grasping. Freshwater river dolphins (Platanistidae) have reduced visual systems and relatively well developed bristle-like vibrissae along the upper and lower jaws. These non-mobile vibrissae are reportedly used to detect fish and crustacean prey along river bottoms [Norman and Fraser, 1948 as cited in Ling, 1977; Layne and Caldwell, 1964]. Collectively, these findings indicate that sensory detection in non-sirenians is often accompanied by movements of the vibrissae, but not by grasping.

One of the defining features of vibrissae in non-sirenian mammals is their organization as sinus hairs, in which a blood-filled sinus encircles the hair shaft and contains along its wall a substantial number of sensory nerve endings. Vibrissae in rodents, rabbits, and cats are innervated by a single deep vibrissal nerve containing 100–200 axons that ascend along the inner margin of the sinus wall and terminate in receptors located in the outer root sheath and mesenchymal sheath [Rice et al., 1986, 1997]. In monkeys the deep vibrissal nerve supplies each vibrissae with 80–100 axons entering through 2–3 nerve fascicles [Halata and Munger, 1980]. In addition to the deep vibrissal nerve, a superficial vibrissal nerve containing 20–30 unmyelinated axons innervates the rete ridge collar and outer conical body, components of the follicle located near the skin surface [Mosconi et al., 1993].

There has been no comparable study of hair follicle morphology and innervation in sirenians. Earlier studies reported morphological attributes of some of the facial bristles and hairs, but these were not based on systematic sampling methods and did not include nerve counts. Dosch [1915] reported that all body hairs on sirenians were sinus hairs, intermediate in form between pelage hairs and vibrissae. He expanded upon the earlier studies of Kükenthal and others [referenced in Dosch, 1915], and even used some of the same specimens used by Kükenthal. His conclusion has been supported in dugongs by the findings of Bryden et al. [1978], Kamiya and Yamasaki [1981], and Sokolov [1982], and in Antillean manatees by Sokolov [1986]. However, in

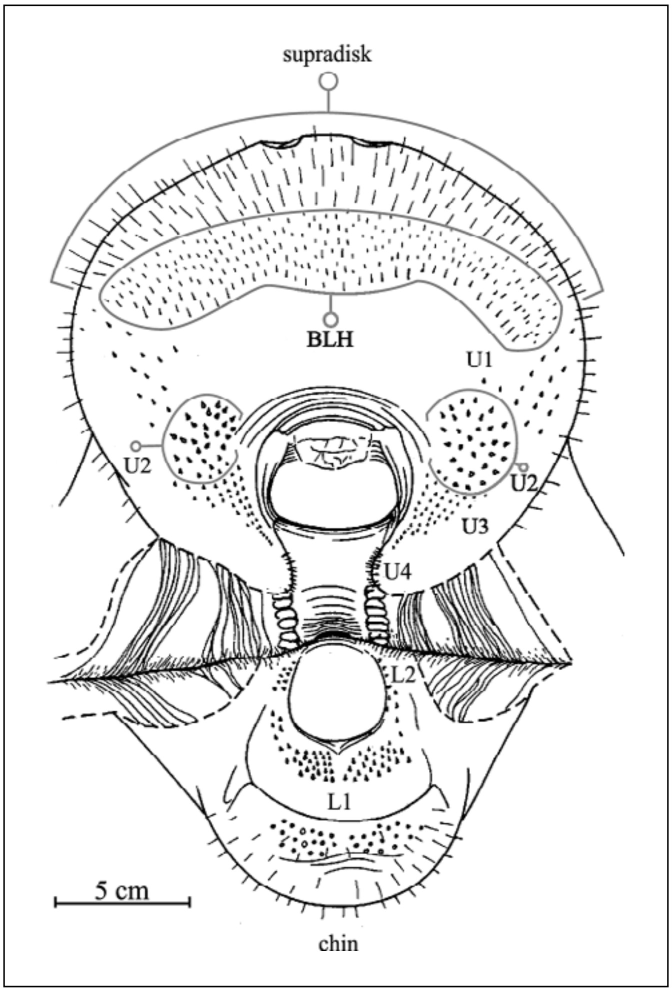


Fig. 1. Schematic diagram of the perioral region, showing the location of the upper (U1–4) and lower (L1–2) bristle fields, bristle-like hairs (BLH), supradisk, and chin. Dotted lines indicate cuts made in the cheek muscles to allow a fuller view of the oral cavity.

most of these cases a lack of regional specification together with a paucity of figures make it impossible to determine the location from which follicle samples were taken. Sokolov [1986] did provide some quantitative morphological characterization of the 'thick vibrissae of the upper and lower lips' (probably corresponding to bristle fields U2 and L1) in the Antillean manatee *(T. manatus manatus)* but did not systematically explore other subfields of the face.

The nine regions of the manatee face are distinguishable based on location, distribution of bristles or hairs, external morphology of bristles or hairs, and the behavioral role played by the bristles and hairs during feeding and other behaviors. Therefore, in the present study we sought to define internal follicle microanatomy and innervation in these regions of the face. One goal was to determine whether all the facial hairs of the Florida manatee possess the structural characteristics of sinus hairs, as would be expected if they were homologous to the vibrissae of other mammals. A second goal was to obtain estimates of innervation density for the follicles in each of the nine regions of the face.

Materials and Methods

A total of 166 follicles were processed and 82 of these were analyzed quantitatively in this study (tables 1 and 2). They were collected from eleven fresh (w/in 24 h) postmortem manatee carcasses in conjunction with the statewide manatee carcass salvage program managed by the Marine Mammal Pathobiology Laboratory in St. Petersburg, Fla., USA (USFWS permit PRT-684532). All but one of the carcasses were in the subadult-adult range of body length, corresponding to 1.5+ years of age, as determined by the USGS Sirenia Project (unpubl. data). However, specimen SWFTM 9515 was much smaller, in the range of a small calf 1–6 months of age. As shown in table 1, follicles were taken from nine regions of the face which were defined in a previous study [Reep et al., 1998]. Bristle-like hairs were found in two locations; most were located on the oral disk (BLHod) but occasionally one was found among the bristles (BLHbr) in the U1 or U2 field.

A #11 scalpel blade was used to extract blocks of tissue (~5 × 5 mm at the surface and ~10–15 mm in length) that included single follicles and the immediately surrounding connective tissue. Specimens were stored in 4% phosphate buffered formalin (pH 7.4) then cut frozen at 40 µm on a Lipshaw sliding microtome. Serial longitudinal or cross sections were stored in dilute fixative in 24-well plastic tissue culture plates. Sections were mounted on gelatin coated glass slides, using a very dilute (0.5%) gelatin mixture to minimize background staining in the case of silver staining.

After drying overnight, slides with sections were hydrated and stained using hematoxylin and eosin, Masson's trichrome stain, or a modified Bodian stain. The longitudinal sections were used for measurements of follicle morphology and to identify deep vibrissal nerve bundles and their trajectories. The cross sections were used for counting the number of axons innervating a follicle.

Approximately 30 follicles were prepared using paraffin embedding and stained using Masson's trichrome or hematoxylin and eosin. Bodian staining of paraffin sections did not prove as reliable as that performed on frozen sections. The paraffin embedded sections were not used for quantitative measurements of follicle geometry, in order to avoid shrinkage artifacts.

In 57 selected longitudinal sections we measured maximum follicle length, maximum total sinus length (upper + ring + lower sinus), maximum ring sinus width, maximum follicle capsule thickness, and maximum hair shaft diameter at the level of the ring sinus. These measurements were made on an AIS/C imaging workstation (Imaging Research, Inc.) interfaced with a Zeiss Axiophot 2 microscope and Dage 72 video camera. Sections used for measurement were chosen based on their orientation as near as possible to the central longitudinal axis of the hair shaft. The left and right sides of each section were measured, and we retained only the maximum values. Data are presented as ranges due to low sample sizes and high variability.

Table 1. Number of follicle samples taken from eleven specimens

Animal	Sex	Weight (kg)	Length (cm)	U1	U2	U3	U4	L1	L2	BLHod	BLHbr	Chin	Supradisk
MNW 9425	M	458	301	2	3	5	2	2	2		3		1
SWFTM 9515	M	31	119						1	11		1	
SWFTM 9530	M	248	243		2								
SWFTM 9534	M	370	267		3		3	3			3		2
SWFTM 9607	M	387	278		2		1						
MEC 9903	M	300	257	3	3					3	5		
MSE 9210	M	NA	258	1									
MSW 9524	F	330	247	5	9	5	5	4	7			5	6
MSE 9807	F	321	250	5	9	6	7	6	6	5		2	5
MSTM 9908	F	134	220							3			
MEC 9952	F	123	171							4			

Table 2. Microanatomical attributes of perioral bristles and bristle-like hairs

	U1	U2	U3	U4	L1	L2	BLH (total)	BLH od	BLH br	Supra-disk	Chin
Follicle length (mm)	8.63–9.68	10.66–18.52	8.19–10.05	6.54–9.56	7.33–8.83	3.30–7.40	6.11–9.36	5.41–8.77	5.41–8.77	3.51–5.80	4.62–8.22
Max total sinus length (mm)	5.72–5.91	5.90–9.34	4.57–5.62	3.95–6.25	3.82–6.67	2.17–4.21	4.90–7.20	4.15–6.78	5.5–7.2	1.83–5.14	2.65–6.49
Max ring sinus width (μm)	509–698	546–1123	445–534	403–592	469–698	338–600	300–690	277–546	390–690	262–511	462–485
Max capsule thickness (μm)	601–655	588–866	385–503	394–591	396–761	198–529	225–560	225–548	504–560	144–423	263–586
Axons per follicle	61–84	210–254	89–116	88–100	184–202	82–108	49–74	49–60	50–74	34–42	40–48
Hair diameter (μm)	362–428	720–1800	486–662	435–494	315–798	328–546	131–362	92–218	131–362	62–118	108–362
Hair length/dia ratio	6	2–5	3	7	1–7	1	32	32	NA	80	var
# samples for geometry	2	14	4	6	4	5	14	10	4	5	3
# samples for nerve counts	2	3	2	2	2	3	5	3	2	3	2

Data for external hair length/diameter ratios are from Reep et al. [1998]. Top row gives abbreviations for the perioral fields described in the text.

For making axon counts, we used 33 cross sections in which axons appeared well stained by the modified Bodian procedure. We also required that these sections be located just apical relative to the region of nerve entry, in order to estimate the maximum number of axons per follicle. In such material one observes spaced bundles of axons, and individual axons are separately identifiable by their myelin sheath (fig. 4). However, due to the capricious nature of silver staining, it was rare that a section exhibited well defined staining throughout the circumference of the follicle. Rather, some axon bundles appeared dark and others appeared too light to permit accurate counting. In these cases we capitalized on the fact that just apical to their entry, the 2–6 large nerve bundles innervating each follicle disperse into smaller bundles of 2–5 axons each. These smaller bundles are uniformly spaced around the circumference of the follicle as they ascend toward their sites of termination along the sinus wall. Therefore, in many cases we estimated total axon number by performing direct axon counts on half of the total 360° and multiplying the result by two. In order to assess relationships between nerve counts and follicle geometry, we measured the perimeter of the innervated ring sinus wall and estimated the length of the ring sinus on the same follicles from which nerve counts were obtained. Perimeters were measured on the same sections from which axon counts were obtained, using the imaging system described above. Ring sinus length was estimated by counting the number of cross sections on which the ring sinus appeared as a distinct entity characterized by large width and a lack of trabeculae, then multiplying this number by section thickness. The beginning and end of the ring sinus were defined as those sections on which trabeculae were seen associated with less than 50% of the sinus area. This procedure necessitated the use of serial sections, which were available for 13 of the 24 follicles. The area of the ring sinus wall was estimated by multiplying the perimeter and length measurements.

Results

Follicles from every region of the face exhibited the structural characteristics of vibrissae, including a well defined circumferential blood sinus, dense connective tissue capsule, and substantial innervation. Therefore, they may accurately be referred to as follicle-sinus complexes, or F-SCs [Rice et al., 1986]. Variations in F-SC geometry and innervation were seen among regions, but F-SCs within a given region tended to exhibit similar characteristics.

F-SC Microanatomy

Perioral bristles of the U1–4 and L1–2 fields are illustrated in figure 2. The basic F-SC morphology common to all bristle fields is illustrated by reference to the largest bristles, those of the U2 field (fig. 2B). A cylindrical capsule of dense connective tissue constitutes the exterior wall of the F-SC, and encloses the dermal papilla basally. The outer margin of the capsule abuts the dermal connective tissue in which it is embedded. The apical terminus of the capsule is located just beneath the epidermis. The interior wall merges with the epidermis apically, providing a circular opening through which the bristle shaft emerges. The interior wall of the F-SC is composed of three layers, from interior to exterior: outer root sheath (ors), glassy membrane (gm), and mesenchymal sheath (ms) (fig. 2C, 4E). A well defined circumferential blood sinus complex is located between the capsule and the interior wall and extends most of the length of the F-SC. The sinus complex consists of a lower cavernous portion spanned by connective tissue trabeculae and an intermediate ring sinus that is broader. A short upper cavernous sinus is sometimes present. The ring sinus lacks trabeculae but possesses multiple appendages that project from its inner margin. Unlike trabeculae, these appendages do not attach to the capsular side of the sinus (fig. 2C). The upper cavernous sinus is short and tapers apically so that the inner wall of the F-SC joins the capsule near the junction of the inner wall and epidermis. The lower cavernous sinus is more extensive than the upper, and surrounds the base of the dermal papilla. Several (~5) nerve bundles traveling through the dermis penetrate the capsule at a level between the F-SC base and the lower margin of the ring sinus (fig. 4A, B). Each of these bundles traverses the lower cavernous sinus along trabeculae and then divides into evenly spaced bundles of 3–5 axons that ascend along the mesenchymal sheath (fig. 3G, 4E) and terminate along the wall of the ring sinus.

Regional Variations in F-SC Geometry

The U2 bristle field contains the largest F-SCs by every measure (table 2). The U2 F-SCs are substantially longer than those of the other fields; they exhibit the widest ring sinuses, thickest capsules and thickest hair shafts. Consistent with these features, the exposed lengths of the U2 bristles are the longest of any of the bristles, 5–10 mm [Reep et al., 1998]. Of the remaining upper bristle fields, the U1 F-SCs tend to possess thinner bristles, fewer axons, wider ring sinuses, and thicker capsules, compared to the U3 and U4 F-SCs. In addition, the U1 F-SCs have an elongated lower cavernous sinus and shorter upper cavernous sinus, whereas in the U3 and U4 F-SCs the upper and lower cavernous sinuses appear to be nearer the same length (fig. 2D, E). The U3 and U4 F-SCs are similar to each other in many respects, but the U3 bristles are thicker. Although external bristle length is comparable in the U3 and U4 bristles, the greater thickness of the U3 bristles confers upon them a length/diameter ratio of 3 compared to a ratio of 7 for the U4 bristles [Reep et al., 1998]. In the lower perioral region, F-SCs of the L1 bristle field resemble those of the U3 field, but the L2 F-SCs are shorter, as are their bristles. Likewise, the sizes of the L2 sinuses and capsules are at the low end of the range of the L1 F-SCs.

Bristle-like hairs are found in two locations – on the oral disk (BLHod) and among the bristles (BLHbr). The latter are not found on every animal; when present they occur singly in the U1 or U2 field. The anatomical characteristics of bristle-like hair F-SCs are quite similar to those of the U3 and U4 F-SCs in most respects except for hair thickness. Even the thickest adult bristle-like hair is barely within the range of the thinnest bristle, and this is consistent with the distinct designation of the former based on external morphology. The BLHod also have fewer axons per F-SC than the U3 and U4 F-SCs (table 2).

Supradisk F-SCs are small and their hairs are thin compared to the F-SCs of the perioral and oral disk regions. They feature a relatively long lower cavernous sinus, resulting in a more superficial location of the ring sinus (fig. 2K). Supradisk F-SCs also have a reduced innervation compared to F-SCs from the other facial fields (table 2). Chin F-SCs are intermediate in size and resemble the U3 and U4 F-SCs in most respects except for having much thinner hairs. Their innervation is reduced and is similar to that of the supradisk F-SCs (table 2).

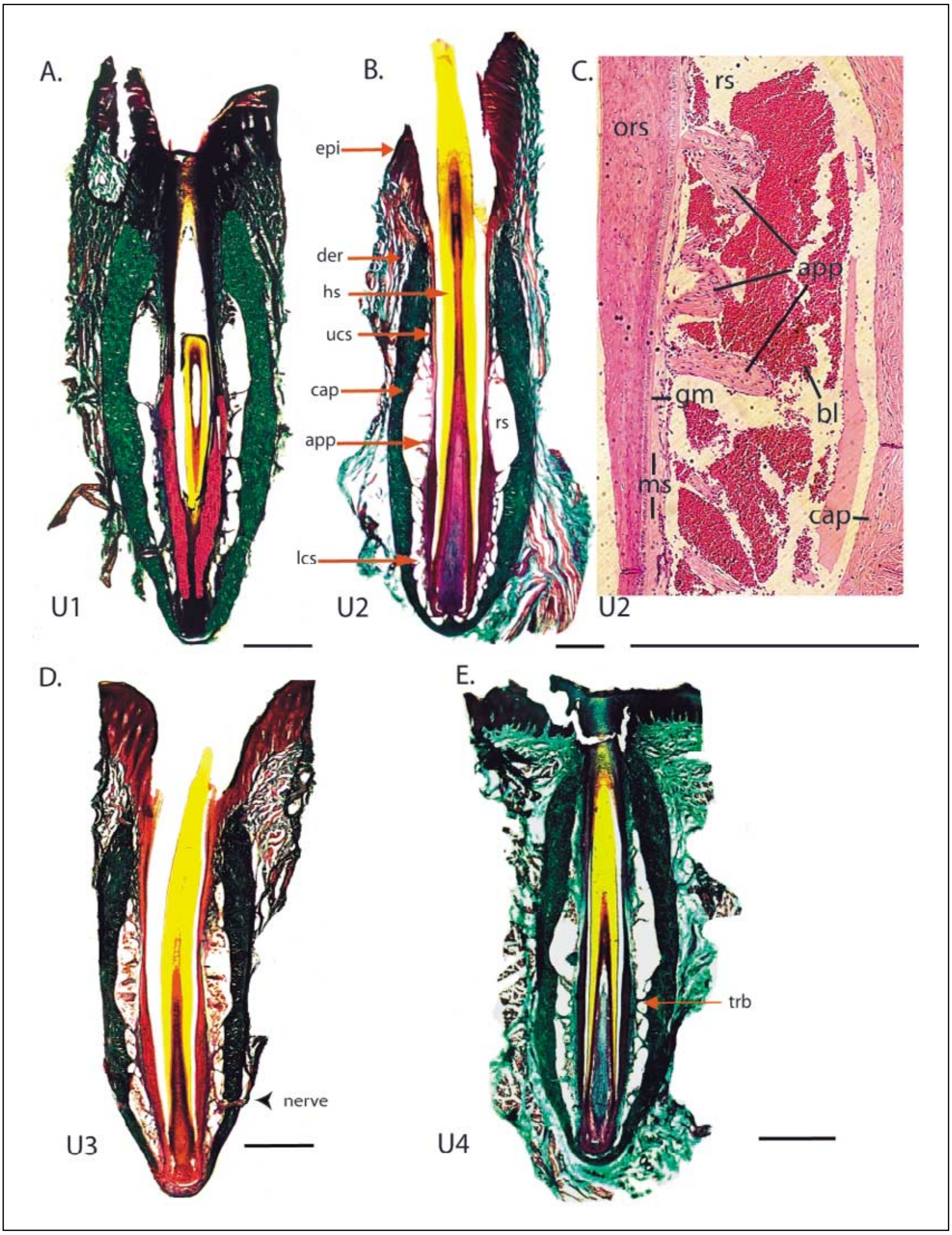
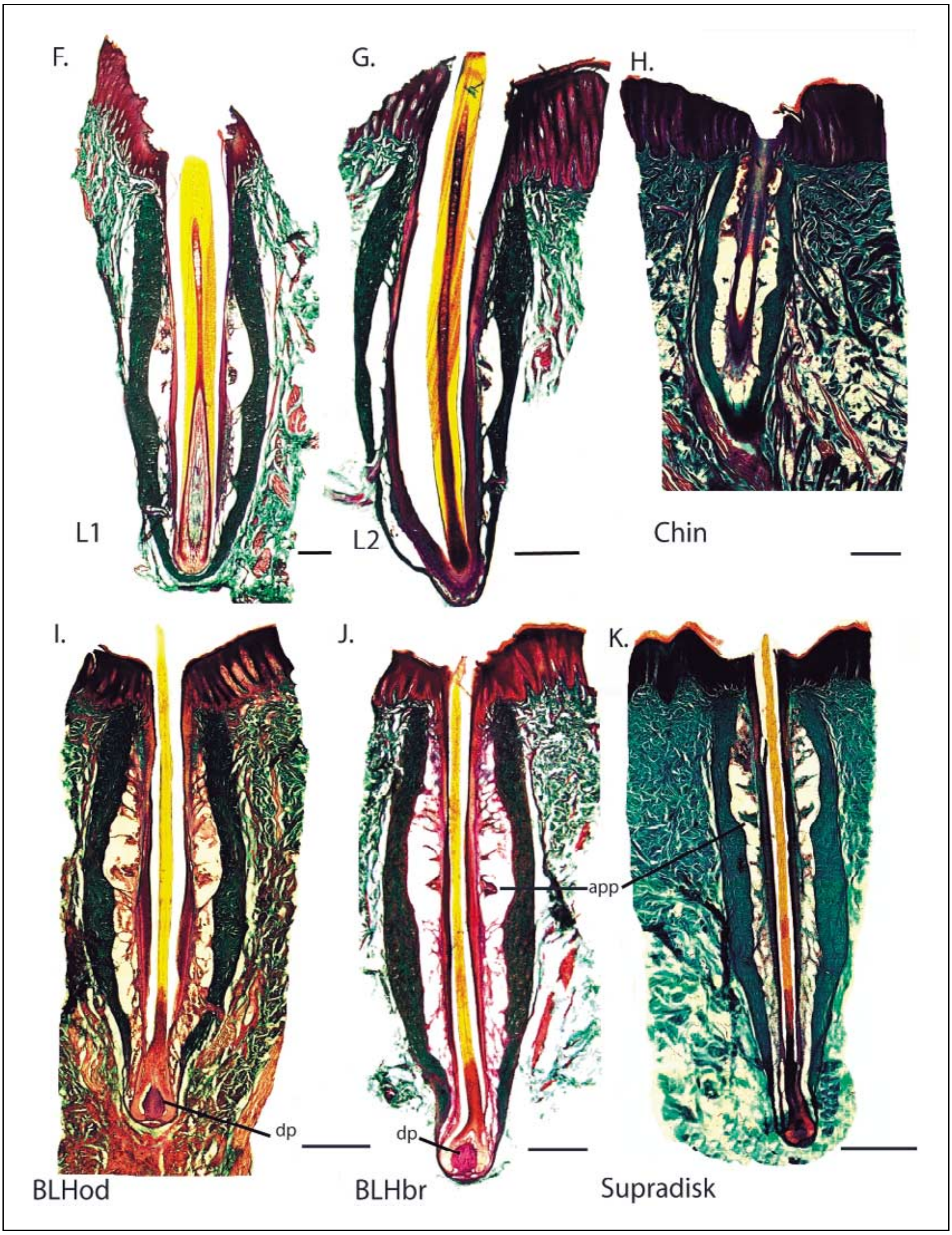


Fig. 2. Morphology of follicles from nine regions of the face, as seen in longitudinal frozen sections along the central axis of the follicle. Masson's Trichrome stain. Scale bars in all figures = 1 mm. **A** U1 bristle follicle, specimen 105. **B** U2 follicle, specimen 93. The indicated structural features are present in the other follicles as well. **C** Ring sinus region on the right side of a longitudinally sectioned U2 follicle, specimen OH72-5. **D** U3 follicle, specimen 106. Arrowhead denotes nerve bundle penetrating the capsule. **E** U4 follicle, specimen 107. **F** L1 follicle, specimen 133. **G** L2 follicle, specimen 108. **H** Chin follicle, specimen 260. **I** Oral disk bristle-like hair follicle, specimen 91. **J** Bristle-like hair follicle from the U1 bristle field, specimen 36. **K** Supradisk follicle, specimen 259.



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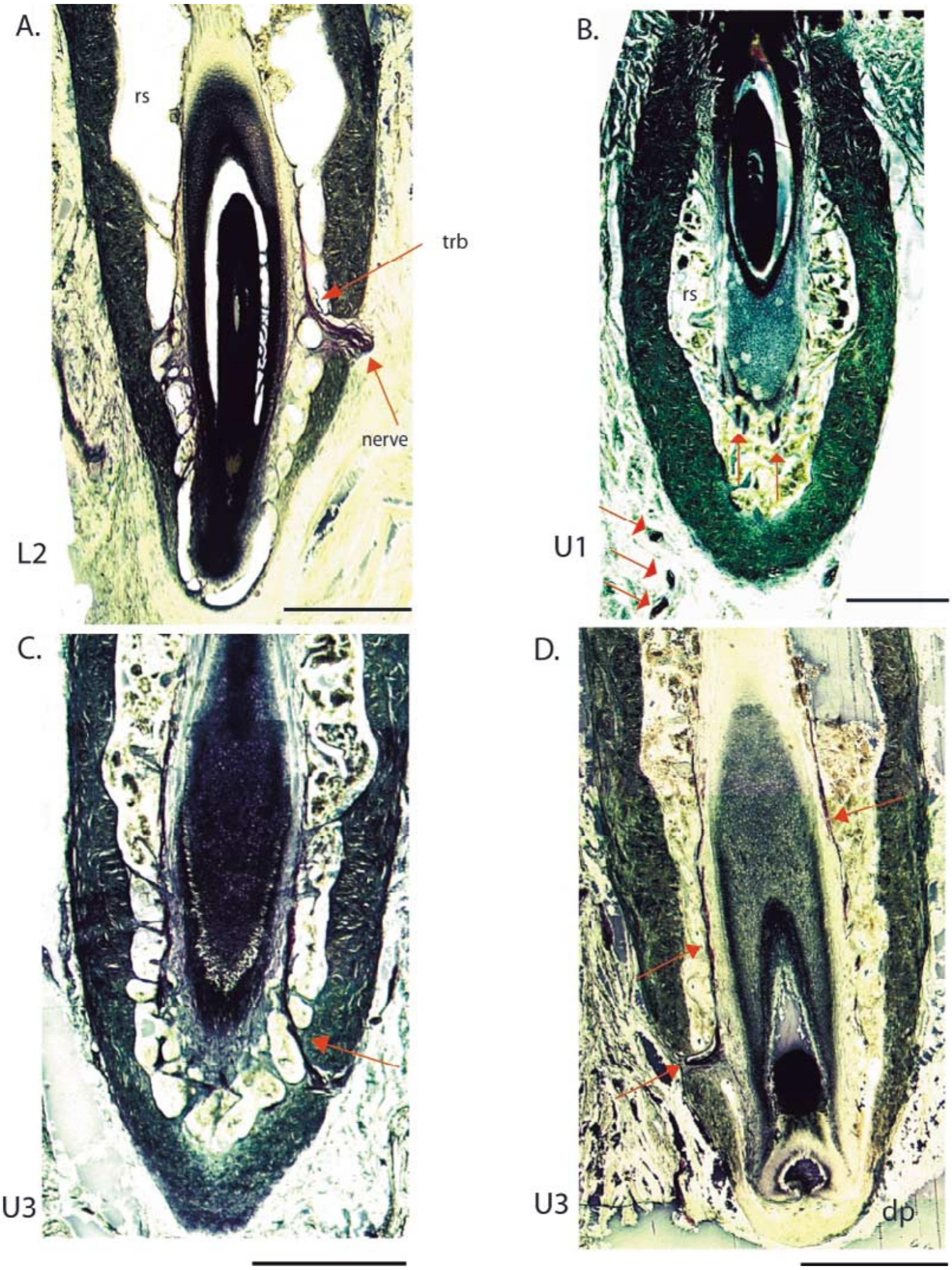
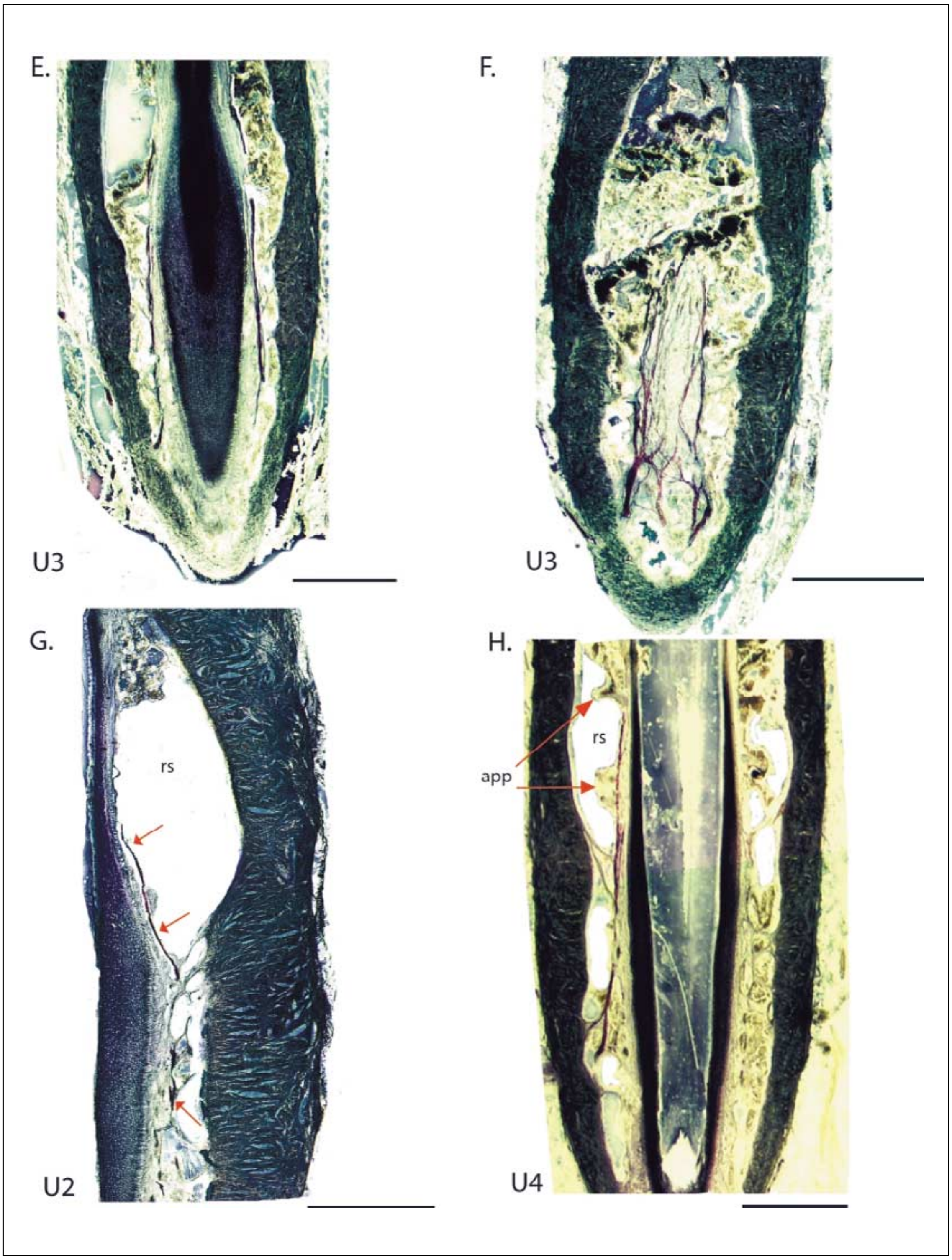


Fig. 3. Nerves innervating follicles, seen in longitudinal sections. Some are slightly displaced from the central axis of the follicle, others are oblique. Modified Bodian stains. **A** Arrows on right indicate a nerve penetrating the capsule of a L2 follicle, crossing the lower cavernous sinus along a trabecula, then ascending toward the wall of the ring sinus. Another nerve with a similar trajectory is seen on the left. Specimen 103. **B** Oblique section of a U1 follicle. Arrows indicate several nerve bundles in the follicle and external connective tissue. Specimen 94. **C** Nerves ascending along the wall of the lower cavernous sinus of a U3 follicle. Arrow at lower right denotes penetration of nerve through the follicle cap-



sule. Specimen 96. **D** Nerves ascending toward the ring sinus wall in a U3 follicle. Arrow at lower left indicates penetration of capsule by nerve. Specimen 97. **E** Nerves ascending toward the ring sinus wall in a U3 follicle. Specimen 97. **F** Para-axial section of the same follicle as in **E**, showing several nerve bundles. **G** Arrows indicate a nerve bundle traveling in the mesenchymal sheath along the ring sinus wall in a U2 follicle. Specimen 104. **H** Nerve ascending and traveling along the mesenchymal sheath of the ring sinus in a U4 follicle. Specimen 98.

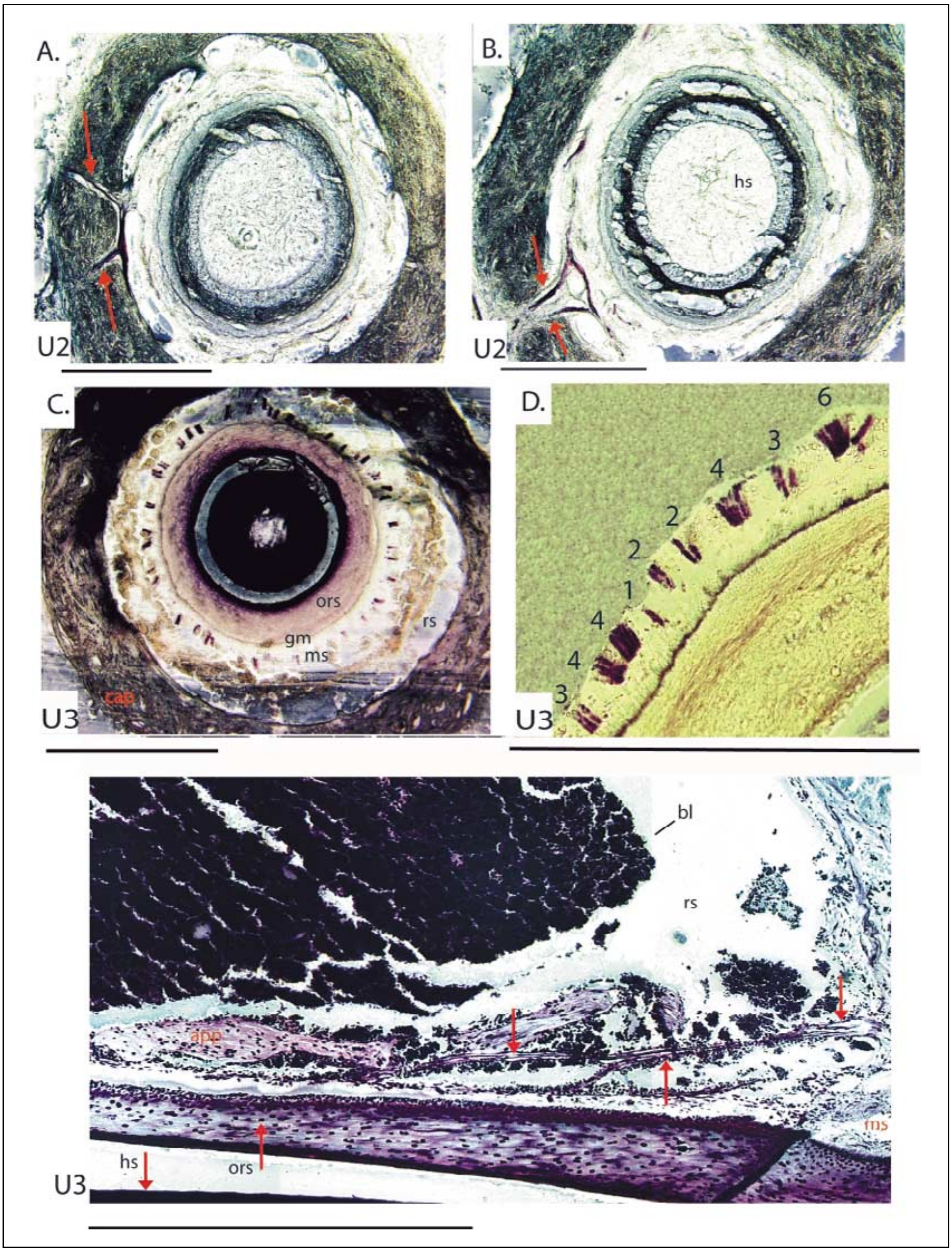


Fig. 4. A, **B** Two cross sections of a U2 follicle at the level of the lower cavernous sinus. Nerve bundles (arrows) can be seen piercing the follicle capsule and then spreading radially. Modified Bodian stain. Specimen 128. **C** Cross section of a U3 follicle at the level of the ring sinus. Visible are numerous evenly spaced groups of 2–5 axons in the mesenchymal sheath. Modified Bodian stain. Specimen 110. **D** High magnification view of axon bundles in a L1 follicle. Numbers indicate counts of axons in each bundle. Specimen 240, modified Bodian stain. **E** High magnification view of the ring sinus wall of a U2 follicle. Arrows designate stained axons entering the region of the ring sinus from the right. The basal aspect of the follicle is toward the right. Paraffin section, Bodian stain. Specimen OH72-50.

F-SC Innervation

In all F-SCs in which nerves were observed, nerve bundles enter the capsule, traverse the lower cavernous trabeculae, and divided into smaller bundles that ascend the mesenchymal sheath (fig. 3). The number of axons per F-SC ranges from 34–254, as shown in table 2. The U2 F-SCs received greater than 200 axons each, and the L1 F-SCs had counts in the range of 200. The U3, U4, and L2 F-SCs had about half as many axons, about 100 each. Among the perioral bristles, the U1 F-SCs had the fewest axons. Bristle-like hairs received 49–74 axons each, a range that overlaps with the range of the U1 bristles. F-SCs of the supradisk and chin had far fewer axons, ranging from 34–48 each.

In longitudinal sections the number of stained axons appears to decrease as one approaches the apical aspect of the ring sinus. This was verified in case 196, in which nerves were counted on three cross sections in the region of the ring sinus of a U1 F-SC, and in case 239, where similar counts were made in an L1 F-SC. In both cases axon number decreased from the most basal to the most apical section (84, 76 and 63 axons in case 196; 184, 176 and 158 axons in case 239), suggesting that, as in other taxa, many axons terminate in the region of the ring sinus wall.

Innervation Density

Is innervation density simply a function of area innervated? F-SCs vary in size, and most axons terminate on the mesenchymal sheath and outer root sheath lining the sinus. Therefore, circumference of the outer root sheath at the level of the sinus may be a critical variable determining innervation density. In order to test this, we measured the perimeter of the outer root sheath at the level of the ring sinus on 13 F-SC cross sections for which axon counts had been obtained. As illustrated in figure 5, there is a significant positive correlation between nerve count and ring sinus perimeter ($r^2 = 0.62$; $n = 13$, $p < 0.01$) and a similar correlation between nerve count and estimated sinus area ($r^2 = 0.53$; $n = 13$, $p < 0.01$).

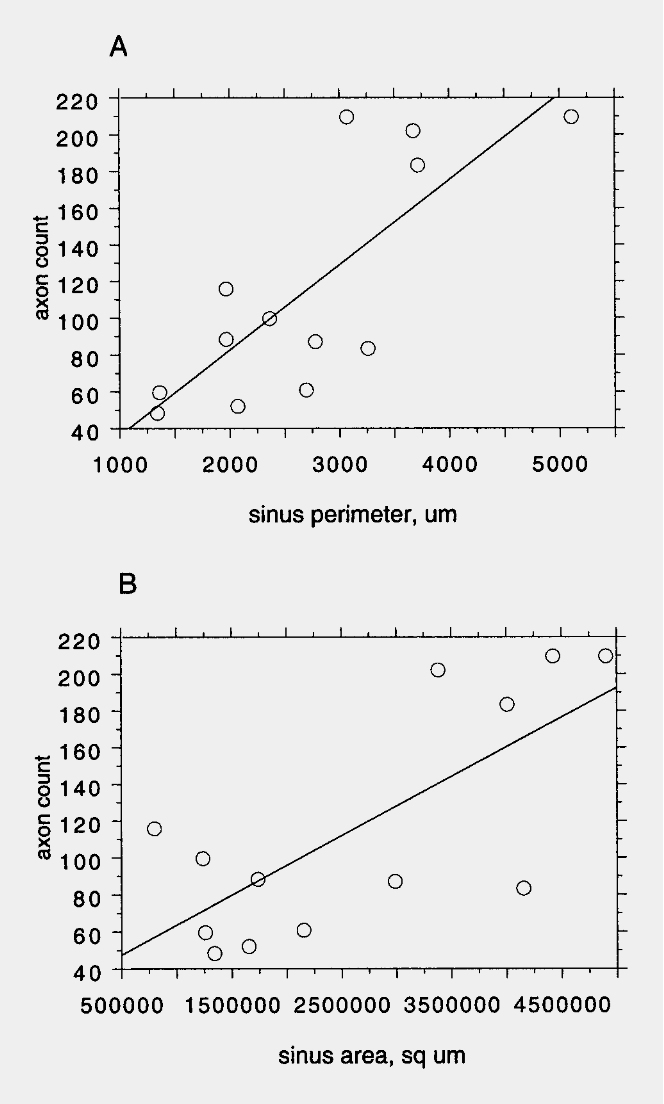


Fig. 5. Axon number changes with follicle geometry. **A** Relationship between axon counts and sinus perimeter. **B** Relationship between axon counts and sinus area. n = 13 in each case.

Discussion

A major finding of the present study is that in Florida manatees all the hairs and bristles of the facial region are vibrissae, characterized by a prominent blood sinus complex, dense connective tissue capsule, and substantial innervation. Each of the nine regions of the face exhibits a distinct combination of morphological attributes, congruent with the designation of these regions based on location and external morphological criteria [Reep et al., 1998].

The bristle-like hairs of the U1 and U2 bristle fields are rather enigmatic. It is unclear whether they are best considered bristle-like hairs or arrested U1 or U2 bristles. They are rare and isolated in occurrence, which would tend to support the latter conjecture.

Comparative F-SC Morphology

The blood sinus configuration we have described for manatees, consisting of a ring sinus with appendages and upper and lower cavernous sinuses, may be compared to the variations seen among other mammalian vibrissae. The

Table 3. Total axon counts by field

Vibrissal field	Mean number of follicles per animal	Mean number of axons per follicle	Total number of axons per field
U1	26	72	1,872
U2	46	225	10,350
U3	60	102	6,120
U4	20	94	1,880
L1	56	193	10,808
L2	16	108	1,728
Oral disk	602	54	32,508
Supradisk	710	37	26,270
Chin	411	44	18,084
Grand total			109,620

Data on mean number of follicles per animal are from Reep et al. [1998], data on mean number of axons per follicle are from the present study.

mystacial vibrissae of rodents exhibit a circumferential ring sinus with a ringwulst (ring body), as well as a ventrally adjacent trabeculated cavernous sinus [Wineski, 1985; Rice et al., 1986, 1997; Dehnhardt et al., 1999]. Ringed seals [Hyvarinen and Katajisto, 1984], California sea lions [Stephens et al., 1973] and southern elephant seals [Ling, 1966] possess elongated vibrissae F-SCs having trabeculated cavernous sinuses superficial and deep to the ring sinus, which has a ringwulst. The ringwulst in southern elephant seals is relatively small compared to the ringwulst in rats [Ling, 1966]. Ling [1977], in a review of vibrissal structure in marine mammals, noted a variety of sinus configurations among the cetaceans. The vibrissae of rhesus monkeys [Van Horn, 1970], tammar wallabies [Marotte et al., 1992] and brush-tailed possums [Hollis and Lyne, 1974] lack a ring sinus and ringwulst and have only cavernous tissue. Hippopotamus facial vibrissae appear to have a similar configuration as these three species, with umbrella-like appendages containing Merkel cells [Bachteler et al., 1998].

The ring sinus appendages we observed in manatee F-SCs are quite unlike the ringwulst seen in other species. Whereas the latter are club shaped in longitudinal section and constitute a single bulbous ring around the inner wall of the sinus, the appendages in manatees are multiple in number and appear flat rather than bulbous on their ends. The function of the ring sinus appendages is unclear. We did not observe axons entering the appendages, and the ringwulst seen in most other species is also not highly innervated [Rice et al., 1997]. Stephens et al. [1973] suggested that the ringwulst may function to transmit vibratory energy from the hair shaft to the ring sinus, and this conjecture may be applied to the appendages in manatees as well.

Innervation

The nine regions of the Florida manatee face are innervated by a total of approximately 110,000 axons originating from the deep vibrissal nerves (table 3). (These axon counts exclude innervation by the superficial vibrissal nerves, which were not examined in the present study.) The perioral bristles and oral disk bristle-like hairs, all of which are known to be involved in tactile exploration, account for about 65,000 of these axons. This constitutes a significant sensory array devoted to relaying tactile information to the central nervous system. By comparison, the sensory innervation of the tactile star in the star-nosed mole is innervated by approximately 100,000 axons [Catania and Kaas, 1997].

In the present study, the U2 F-SCs received the largest number of axons, in the range of 250 per F-SC. By comparison, facial vibrissae in rodents, rabbits, cats, and monkeys are innervated by 80–200 axons originating from the deep vibrissal nerves [Halata and Munger, 1980; Rice et al., 1986, 1997]. An exception is the Australian water rat in which each vibrissa receives over 500 myelinated axons [Dehnhardt et al., 1999]. Nerve estimates in cetacean F-SCs ranged from 40–290 axons per F-SC [Ling, 1977]. Therefore, from this perspective the U2 F-SCs may be considered very well innervated. However, Hyvarinen and Katajisto [1984] estimated that a single vibrissa in the ringed seal receives 1,000–1,500 myelinated axons. This expanded innervation is hypothesized to be the basis for orientation of the ringed seal using tactile and acoustic cues in a dark water environment [Hyvarinen, 1989].

All seven major hair types (vibrissae, carpal, hemisinus, eyelashes, tylotrich, guard, and vellus) are associated with innervated mechanoreceptors [Zelena, 1994]. However, sinus hairs are the most extensively innervated and the most profusely supplied with mechanoreceptors, including Merkel cells, lanceolate endings, and free ('blebbed') nerve endings. Receptor types and innervation patterns have been studied in a variety of mammals including rodents [Munger and Rice, 1986; Rice et al., 1986, 1997; Mosconi et al., 1993], rabbits and cats [Rice et al., 1986], primates [Halata and Munger, 1980], California sea lions [Stephens et al., 1973], ringed seals [Hyvarinen, 1995], hippopotamuses [Bachteler et al., 1998], and tammar wallabies [Marotte et al., 1992]. The densest accumulation of receptors is along the mesenchymal sheath and outer root sheath that line the ring sinus [Stephens et al., 1973; Hyvarinen, 1995; Rice et al., 1997]. Similarly, we found that axon counts decreased as one ascended the mesenchymal sheath in the region of the ring sinus, suggesting that manatee receptors are likely to be similarly distributed. Because some axons innervate single receptors and other axons branch to supply several, there is no simple relationship between axon number and receptor number. Thus the axon counts made in the present study represent only a first order approximation of innervation pattern, in the absence of data on receptor types and numbers in manatee F-SCs. Our finding of a correlation between number of axons and area innervated suggests that to some extent high axon counts, as in the U2 bristles, may simply be related to the large size of these F-SCs.

Functional Considerations

It is not known exactly how sinus hairs work. The integrity of the sinus capsule is necessary for normal function; discharge patterns of afferents innervating the vibrissa cease upon opening the sinus capsule [Gottschaldt et al., 1973]. Blood pressure may set the mechanical tension (and thus response sensitivity) of the tissue in which lanceolate endings and Merkel cells are located [Woolsey et al., 1981; Rice et al., 1986]. Thus the neural response properties of individual F-SCs would depend on at least the following factors at the microanatomical level: (1) hair length, diameter, and stiffness, both external to the skin surface and within the F-SC; (2) F-SC geometry, especially F-SC length and diameter, ring sinus wall thickness, and ring sinus size and shape; (3) innervation density; (4) quantity, types, and distribution of receptor endings. A more complete appreciation of the functional capacities of manatee vibrissae now awaits the characterization of receptor types and their distribution patterns.

One implication of the present findings is that perioral bristles in manatees may have a tactile sensory role much like that of vibrissae in other mammals, in addition to their documented role in grasping of plants during feeding. This combination of motor and sensory usages would be unique to sirenians. In support of this conjecture, the U2 and L1 bristles, which are the major fields involved in grasping plants during feeding [Marshall et al., 1998a], also have the most densely innervated F-SCs. It seems likely that a major function of the sensory innervation of the U2 and L1 F-SCs is to provide tactile feedback concerning contact with grasped objects. In contrast, tactile exploration is done predominately by the bristle-like hairs of the oral disk, with the perioral bristles playing a secondary role [Bachteler and Dehnhardt, 1999]. The oral disk contains approximately 600 bristle-like hairs [Reep et al., 1998], each innervated by approximately 50 axons (present study), for a total of approximately 30,000 axons. This represents an impressive sensory array, congruent with the role of the oral disk in tactile scanning and exploration [Hartman, 1979; Marshall et al., 1998a; Bachteler and Dehnhardt, 1999].

The elaboration of vibrissae in manatees may suggest the existence of hydrodynamic receptive capacities not available to terrestrial animals. Pressure waves, including compression waves (sound), travel 4.5 faster in water than air (depending on temperature, depth, and salinity), and undergo less rapid energy dispersal than in air [Urick, 1983; Kalmijn, 1989]. Therefore they are potentially useful stimuli in the denser aquatic environment in which visibility is often reduced. Dehnhardt and colleagues have demonstrated that harbor seals can use their facial vibrissae to detect water movements of small amplitude [Dehnhardt et al., 1998, 2001]. Poulter and co-workers discussed a similar possibility with regard to acoustic reception in California sea lions [Stephens et al., 1973]. Thus vibrissae in manatees may function in hydrodynamic distance reception in addition to their known roles in tactile exploration and oripulation.

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References

Bachteler, D., and G. Dehnhardt (1999) Active touch performance in the Antillean manatee: evidence for a functional differentiation of facial tactile hairs. Zoology, *102:* 61–69.

Bachteler, D., G.J. Klauer, and G. Dehnhardt (1998) The morphology of the vibrissae of *Hippopotamus amphibious.* Euro-American Mammal Congress Abst., 669.

Brecht, M., B. Preilowski, and M.M. Merzenich (1997) Functional architecture of the mystacial vibrissae. Behav. Brain Res., *84:* 81–97.

Bryden, M.M., H. Marsh, and B.W. MacDonald (1978) The skin and hair of the dugong, *Dugong dugon.* J. Anat., *126:* 637–638.

Carvell, G.E., and D.J. Simons (1990) Biometric analyses of vibrissal tactile discrimination in the rat. J. Neurosci., *10:* 2638–2648.

Catania, K.C., and J.H. Kaas (1997) Somatosensory fovea in the star-nosed mole: behavioral use of the star in relation to innervation patterns and cortical representation. J. Comp. Neurol., *387:* 215–233.

Dehnhardt, G. (1994) Tactile size discrimination by a California sea lion *(Zalophus californianus)* using its mystacial vibrissae. J. Comp. Physiol. A, *175:* 791–800.

Dehnhardt, G., and G. Ducker (1996) Tactual discrimination of size and shape by a California sea lion *(Zalophus californianus).* Animal Learning Behav., *24:* 366–374.

Dehnhardt, G., and A. Kaminski (1995) Sensitivity of the mystacial vibrissae of harbour seals *(Phoca vitulina)* for size differences of actively touched objects. J. Exp. Biol., *198:* 2317–2323.

Dehnhardt, G., H. Hyvarinen, A. Palviainen, and G. Klauer (1999) Structure and innervation of the vibrissal follicle-sinus complex in the Australian water rat, *Hydromys chrysogaster.* J. Comp. Neurol., *411:* 550–562.

Dehnhardt, G., B. Mauck, and H. Bleckmann (1998) Seal whiskers detect water movements. Nature, *394:* 235–236.

Dehnhardt, G., B. Mauck, W. Hanke, and H. Bleckmann (2001) Hydrodynamic trail-following in harbor seals *(Phoca vitulina).* Science, *293:* 102–104.

Dosch, F. (1915) (translated by D.A. Sinclair) Structure and development of the integument of Sirenia. Tech. trans. No. 1626 National Research Council of Canada, Ottawa 1973. (Bau und Entwicklung des Integuments der Sirenen. Janaische Zeitschr., *53:* 805–854, 1914–1915.)

Dykes, R.W. (1975) Afferent fibers from mystacial vibrissae of cats and seals. J. Neurophysiol., *38:* 650–662.

Gottschaldt, K.-M., A. Iggo, and D.W. Young (1973) Functional characteristics of mechanoreceptors in sinus hair follicles of the cat. J. Physiol., *235:* 287–315.

Halata, Z., and B.L. Munger (1980) Sensory nerve endings in rhesus monkey sinus hairs. J. Comp. Neurol., *192:* 645–663.

Hartman, D.S. (1979) Ecology and behavior of the manatee *(Trichechus manatus)* in Florida. Am. Soc. Mammal. Spec. Pub., *5:* 1–153.

Hollis, D.E., and A.G. Lyne (1974) Innervation of vibrissa follicles in the marsupial *Trichosurus vulpecula.* Aust. J. Zool., *22:* 263–276.

Hyvarinen, H. (1989) Diving in darkness: whiskers as sense organs of the ringed seal *(Phoca hispida saimensis).* J. Zool. Lond., *218:* 663–678.

Hyvarinen, H. (1995) Structure and function of the vibrissae of the ringed seal (*Phoca hispida* L.). *In* Sensory Systems of Aquatic Mammals (ed. by R.A. Kastelein, J.A. Thomas, and P.E. Nachtigall), De Spil Publishers, Woerden, The Netherlands, pp. 429–445.

Hyvarinen, H., and H. Katajisto (1984) Functional architecture of the vibrissae of the ringed seal (*Phoca hispida* Schr.). Acta Zool. Fennica, *171:* 27–30.

Kalmijn, A.J. (1989) Functional evolution of lateral line and inner ear sensory systems. *In* The Mechanosensory Lateral Line, Neurobiology and Evolution (ed. by S. Coombs, P. Gorner, and H. Munz), Springer-Verlag, New York, pp. 188–215.

Kamiya, T., and F. Yamasaki (1981) A morphological note on the sinus hair of the dugong. *In* The Dugong (ed. by H. Marsh), Dept. of Zoology, James Cook University of North Queensland, Australia, pp. 111–113.

Kastelein, R.A., and M.A. Van Gaalen (1988) The tactile sensitivity of the mystacial vibrissae of a Pacific Walrus *(Odobenus rosmarus divergens).* Part 1. Aqu. Mamm., *14:* 123–133.

Layne, J.N., and D.K. Caldwell (1964) Behavior of the Amazon dolphin, *Inia geoffrensis* (Blainville), in captivity. Zoologica, *49:* 81–108.

Ling, J.K. (1966) The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.). I. The facial vibrissae. Aust. J. Zool., *14:* 855–866.

Ling, J.K. (1977) Vibrissae of marine mammals. *In* Functional Anatomy of Marine Mammals, Vol. 3 (ed. by J.B. Harrison), Academic Press, London, pp. 387–415.

Marotte, L.R., F.L. Rice, and P.M.E. Waite (1992) The morphology and innervation of facial vibrissae in the tammar wallaby, *Macropus eugenii.* J. Anat., *180:* 401–417.

Marshall, C.D. (1997) Characterization of the dugong oral disk, perioral bristles and bristle-like hairs. Am. Zool., *37:* 56A.

Marshall, C.D., L.A. Clark, and R.L. Reep (1998b) The muscular hydrostat of the Florida manatee *(Trichechus manatus latirostris)* and its role in the use of perioral bristles. Marine Mamm. Sci., *14:* 290–303.

Marshall, C.D., G.D. Huth, V.M. Edmonds, D.L. Halin, and R.L. Reep (1998a) Prehensile use of perioral bristles during feeding and associated behaviors of the Florida manatee *(Trichechus manatus latirostris).* Marine Mamm. Sci., *14:* 274–289.

Marshall C.D., P.S. Kubilis, G.D. Huth, V.M. Edmonds, D.L. Halin, and R.L. Reep (2000) Prehensile use of perioral bristles by Florida manatees during feeding upon several species of aquatic vegetation. J. Mammal., *81:* 649–658.

Miller, E.H. (1975) A comparative study of facial expressions of two species of pinnipeds. Behaviour, *53:* 268–284.

Mosconi, T.M., F.L. Rice, and M.J. Song (1993) Sensory innervation in the inner conical body of the vibrissal follicle-sinus complex of the rat. J. Comp. Neurol., *328:* 232–251.

Munger, B.L., and F.L. Rice (1986) Sequential maturation of cutaneous receptors in the rat mystacial pad. J. Comp. Neurol., *252:* 404–411.

Norman, J.R., and F.C. Fraser (1948) Giant Fishes, Whales and Dolphins. Putnam, London.

Peterson, R.S., and G.A. Bartholomew (1967) The natural history and behavior of the California sea lion. Am. Soc. Mammal. Spec. Pub., *1:* 1–79.

Reep, R.L., C.D. Marshall, M.L. Stoll, and D.M. Whitaker (1998) Distribution and innervation of facial bristles and hairs in the Florida manatee *(Trichechus manatus latirostris).* Marine Mamm. Sci., *14:* 257–273.

Rice, F.L., B.T. Fundin, J. Arviddson, H. Aldskogius, and O. Johansson (1997) Comprehensive immunofluorescence and lectin binding analysis of vibrissal follicle sinus complex innervation in the mystacial pad of the rat. J. Comp. Neurol., *385:* 149–184.

Rice, F.L., A. Mance, and B.L. Munger (1986) A comparative light microscopic analysis of the innervation of the mystacial pad. I. Vibrissal follicles. J. Comp. Neurol., *252:* 154–174.

Sokolov, V.E. (1982) Mammal Skin. University of California Press, Berkeley, Calif.

Sokolov, V.E. (1986) Manatee – Morphological Description. Nauka Press, Moscow. (In Russian.)

Stephens, R.J., I.J. Beebe, and T.C. Poulter (1973) Innervation of the vibrissae of the California sea lion, *Zalophus californianus.* Anat. Rec., *176:* 421–442.

Urick, R.J. (1983) Principles of Underwater Sound, 3rd Edition. McGraw Hill Book Co., New York.

Van Horn, R.N. (1970) Vibrissae structure in the rhesus monkey. Folia Primatologica, *13:* 241–285.

Welker, W.I. (1964) Analysis of sniffing of the albino rat. Behavior, *22:* 223–244.

Wineski, L.E. (1985) Facial morphology and vibrissal movements in the golden hamster. J. Morph., *183:* 199–217.

Zelena, J. (1994) Nerves and Mechanoreceptors. Chapman and Hall, London.

Woolsey, T.A., D. Durham, R.M. Harris, D.J. Simons, and K.L. Valentino (1981) Somatosensory development. Dev. Percept., *1:* 259–292.