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Cellular Structure of Terminal Baleen in Various Mysticete Species

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Introduction

Baleen plates of the Mysticeti are a unique integumentary specialization which has evolved over millions of years to allow these whales to sift water or mud-borne foodstuffs ranging in size from plankton to small fish or squid. These horny structures, referred to as whalebone by early whalers, grow from the edges of the hard palate of the upper jaw and ramify from long primary plates reaching in excess of 14 ft in bowhead whale (*Balaena mysticetus*) to secondary, tertiary and smaller structures terminating in bristles placed upon the inner or mouth side of the sieve. Charles Darwin in 'The Origin of Species' (1858) considered their possible evolution from duck-like lamellated beaks of the progenitors of modern baleen whales. Morphological descriptions of baleen have been reported since historical times, including accounts by Zorgdrager (1720), Hunter (1787), and Eschricht and Reinhardt (1866) which provided considerable detail about baleen of the Greenland right whale (bowhead whale). More recent descriptions of baleen have also focused on comparative size and numbers of baleen plates (Cousteau and Paccalet, 1988) in Mysticeti, on asymmetry of wear of baleen plates (Kasuya and Rice, 1970), or measurement of carbon and nitrogen isotopes in baleen in order to assess life history feeding patterns of humpback whales (*Megaptera novaeangliae*) as correlated with age (Ostrom *et al.*, 1991). Relatively little attention has been paid to microscopic analysis of baleen and information is entirely lacking in respect to its cellular and subcellular morphology. Accordingly, this report clarifies, in three mysticeti species, some of the ultrastructural morphology of baleen bristles which constitute the most terminal filtering structure.

Methods

Samples of baleen bristles were obtained either directly from freshly stranded whales on the Virginia shore or from the collection of preserved specimens at the Smithsonian Institution, Washington, DC. For this study specimens from the following animals were obtained; humpback whale, *Megaptera novaeangliae*, 4 animals; grey whale, *Eschrichtius robustus*, 2 animals; and sei whale, *Balaenoptera borealis*, 2 animals. The fresh samples were placed directly into 5% glutaraldehyde/3% formaldehyde in 0.1 M Na cacodylate buffer at pH 7.4, or into 10% buffered formaldehyde. The previously preserved specimens were fixed in 70% ethanol or were in some cases air dried. Samples were studied by light microscopy (1 μm specimens embedded in Poly/Bed 812; Polysciences, Inc. and stained with 1% toluidine blue in 1% sodium borate for 3 sec followed by 0.5% safranin in 0.5% sodium borate for 10 sec), by transmission electron microscopy (TEM), and by scanning electron microscopy (SEM). Resin-embedded thin sections doubly stained with lead citrate and uranyl acetate were studied with a JEOL 100 CX-II transmission electron microscope operating at 80 KV. Other baleen specimens were critical point dried, mounted and coated with gold in a SPI sputter coater and examined in a JEOL JSM 35C scanning electron microscope operating at 10 KV.

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Results

The terminal bristles of baleen of the humpback, grey and sei whales appeared in cross section by light microscopy to resemble closely the histology of hair of terrestrial mammalian species. The medullary core was either hollow or filled with a loose amorphous network of material resembling the membranous debris of disintegrating cells. The cortical layer consisted of closely packed squamous cells layered in excess of fifty cells deep.

Humpback whale

The baleen bristle shaft as viewed by SEM revealed slight overlapping of the outermost epithelial cells such as depicted at low magnification in Figure 1A. Occasionally, sloughed epithelial cells and, on air-dried specimens, fungal contamination could sometimes be observed on the surface. Cross sectional

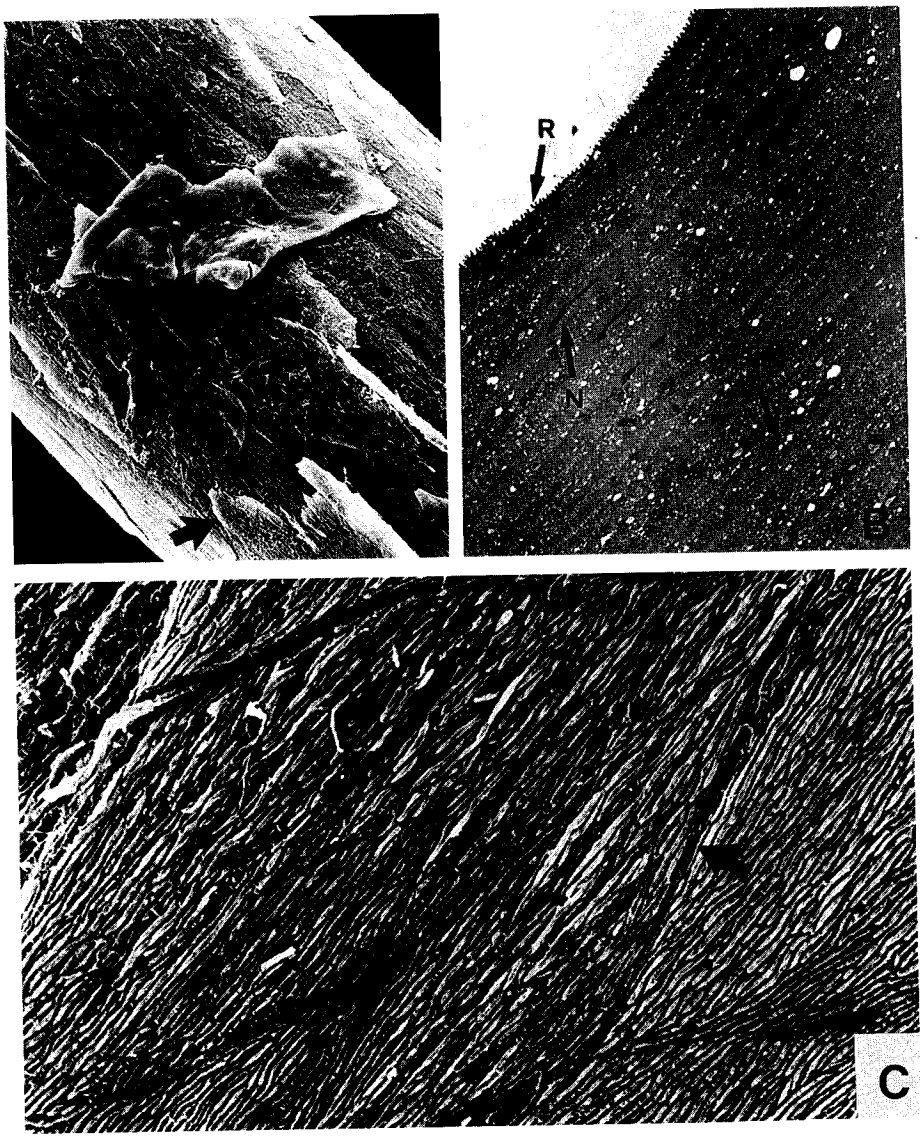

Figure 1A. Humpback whale. SEM of surface of baleen bristle. Cell boundaries (arrow) can be noted, as well as some detaching surface cells. × = 700.

Figure 1B. Humpback whale. TEM of cross section of baleen bristle. Full thickness of cortex is not shown here but multiple layers of squamous cells are clearly evident. Surface microridges (R) cut in cross section, remnants of cellular nuclei (N), and intercellular spaces (I) can be seen. × = 2100.

Figure 1C. Humpback whale. At high magnification by SEM the apical surface of parts of five cells are shown, with boundaries (arrow) between individual cells clearly demarcated. Note the parallel alignment of surface cellular microridges. × = 6000.

TEM viewing of the bristle revealed the compact stratification of the cortex seen by light microscopy (Fig. 1B). Occasional remnants of epithelial cell nuclei as well as enlarged intercellular spaces between the spinous processes were evident (Fig. 1B). The surfaces of the outermost epithelial cells showed in cross section by TEM a microvillous-like appearance; however, this fine structure was confirmed by SEM to be not microvilli but surface longitudinally aligned microridges (Fig. 1C). Also, at higher SEM

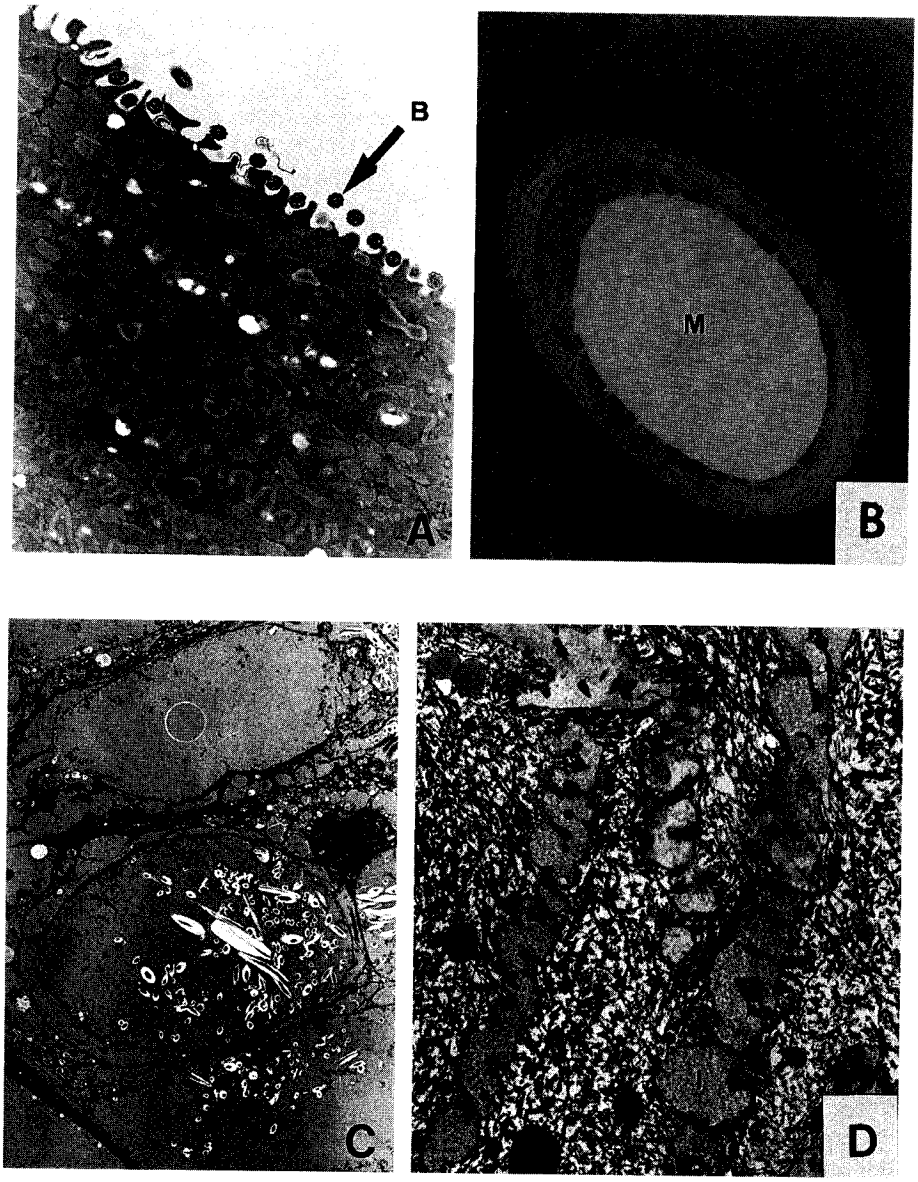

Figure 2A. Humpback whale. As revealed by this TEM cross section of the outer cortical cells of a baleen bristle, bacteria (B), reside in troughs of the surface microridges. Note the interdigitation between adjacent squamous cells, which give rise to the surface microridges when epithelial cells reach the outermost layer. × = 11 250.

Figure 2B. Humpback whale. The core of baleen bristle, i.e., medulla (M), was either hollow, or filled with amorphous remnants of cellular debris such as shown in this TEM illustration. × = 300.

Figure 2C. Humpback whale. This TEM shows the amorphous cellular debris which was observed in the medulla of an immature baleen bristle. × = 2030.

Figure 2D. Humpback whale. This TEM illustrates cross sections of four bristle cortical epithelial cells whose cytoplasm contains mainly keratohyalin fibers and occasional small lipid droplets (L). Note wide intercellular spaces (I). × = 4600.

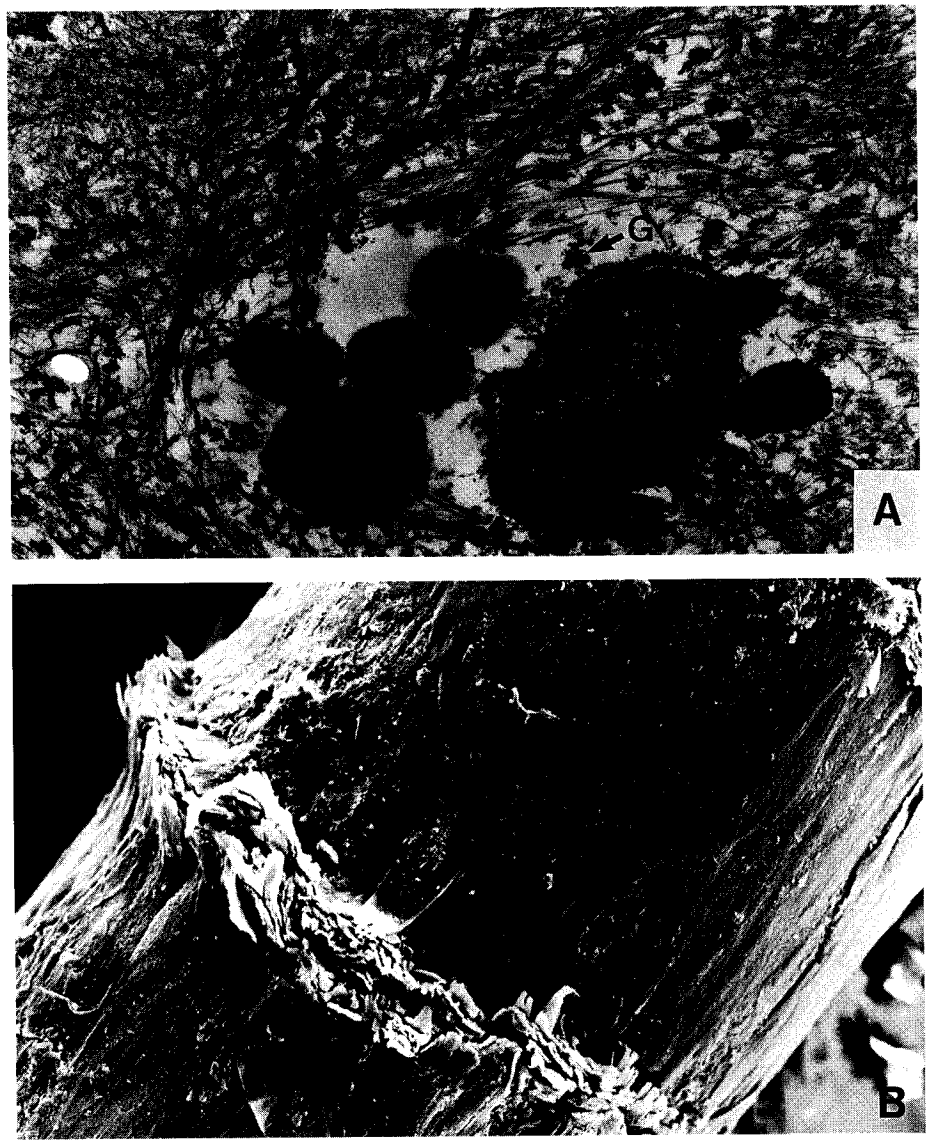

Figure 3A. Humpback whale. A higher TEM magnification of cytoplasmic content of a cortical cell illustrates several lipid granules (L) in the perinuclear region, a nucleus (N), small glycogen-like granules (G), and numerous fibrils of keratohyalin. × = 13 000.

Figure 3B. Sei whale. This low power SEM reveals the similarity of outer surface of a baleen bristle to that shown in Fig. 1A of the humpback whale, but the distinctive 'joint' or ridge which separates longitudinal sections of the bristle. × = 325.

magnification the boundaries between adjacent surface cells were clearly demarcated (Fig. 1C). In some instances unidentified bacteria were found to reside in close association with the cell surface in the troughs of the microridges' ridges (Fig. 2A), as shown by TEM. The medulla of the humpback baleen bristle was hollow in mature bristles (Fig. 2B), or contained, as shown in Figure 2C, cellular debris as this zone subsequently becomes hollow in the mature bristle. The cytoplasm of squamous cells comprising the cortex was filled with deposits of fibrous keratin (keratohyalin) but other organelles

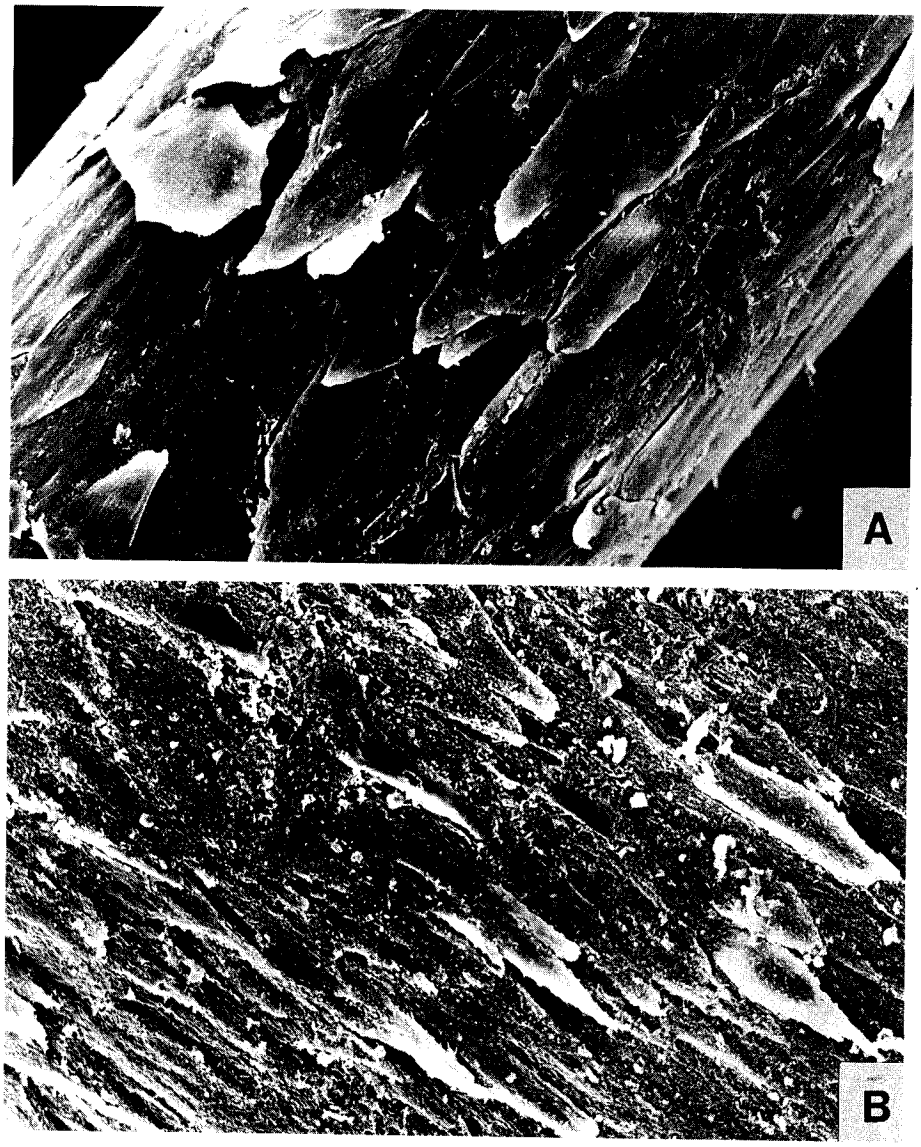

Figure 4A. Sei whale. This SEM illustration demonstrates the flaking of the outermost epithelial cells on the baleen bristle cortex. × = 370.

Figure 4B. Grey whale. As shown in this SEM the baleen bristle surface in this species in rough and epithelial cells is elongated. × = 550.

were sparse (Fig. 2D), and lipid droplets were present. Spinous processes bridging the enlarged intercellular spaces were seen, as well as an electron lucent granular deposit within the spaces (Fig. 2D). Figure 3A illustrates at higher TEM magnification the fibrils of cellular keratin, lipid droplets, a nucleus, and intracellular small granules resembling glycogen within the cytoplasm. Occasionally, intracellular melanosomes were also evident.

Sei whale

The baleen bristles of the sei whale were distinctive in that they appeared truncated, with regularly spaced circumference ridges, resembling the shafts of bamboo. One such ridge is shown in Figure 3B, but it can be seen that otherwise the epithelial surface is similar to that of humpback baleen. The desquamation of individual aged surface cells is also clearly evident by SEM on sei whale baleen bristles (Fig. 4A). In cross

Figure 5. Grey whale. The irregularity of epithelial cell boundaries (B) and arrangement of surface microridges (R) were also distinctive for the grey whale baleen bristle. × = 930.

section the cellular morphology of sei whale bristles was similar to that of the humpback whale.

Grey whale

The surface of baleen bristles of the grey whale was distinctive from that of humpback and sei whales as shown by SEM, with the epithelial cells showing a more elongated configuration (Fig. 4B). Higher SEM magnification also revealed other distinctions. The peripheral contour of the squamous epithelial cells was more irregular than in humpback and sei whales, and the surface microridges were arranged in a less linear pattern (Fig. 5). Cross sections of the bristles were nondistinctive.

Discussion

Cetacean baleen has not previously been described at the ultrastructural level, so the present data serve as an initial perspective of some of its cellular and subcellular morphology. Of the three Mysticeti species examined, there was general similarity in baleen bristle microanatomy although some minor variations were observed on the surface structure of the grey whale bristles. It is remarkable how closely the baleen bristles resemble the structure of hair of terrestrial mammals in view of the facts that: (a) cetaceans have generally lost hair as an integumentary adaptation during their evolution in an aquatic environment, and (b) the unique structure, baleen, is derived from integument within the oral cavity.

As reported by earlier workers the baleen of mysticetes varies considerably at the macroscopic level, both in length of plates and in average numbers of pairs of primary plates. The plates range from 160 pairs (grey whale) to 360 pairs in the fin whale (*Balaenoptera physalus*), and approximate maximal width ranges from 4 inches in the pygmy right whale (*Caperea marginata*) to 15.5 inches in the sei whale (Cousteau and Paccalet, 1988). Great surface area is added to the primary plates, however, and counting the smaller plate pairs up to the smallest fifth level, it has been estimated that up to 6390 pairs of plates can be found in the fin whale (Cousteau and Paccalet, 1988) and accordingly, the numbers of bristles are enormous.

At the cellular and subcellular level the squamous epithelial cells comprising the baleen cortical layer have features in common with cetacean integumentary cells of other sites as well as integumentary cells of unrelated aquatic vertebrates across phylogenetic lines. Differences also exist. Specifically, a unique feature of cetacean skin cells, in contrast to that of other mammals, is the high prevalence of lipid droplets in stratum corneum cells of skin of most sites. This was also noted in baleen bristle cortical cells, although its metabolic significance in bristle remains dubious. The presence of intracellular keratohyalin fibrils and melanosomes within the epithelial cells of baleen bristles, and wide intercellular spaces very closely resemble these common features of terrestrial, mammalian integument. Also at the cellular level, the surface microridges seen on the cetacean bristle surface closely match surface ridges reported by SEM analyses of skin of fish, such as on gill secondary lamellae (Hughes and Datta Munshi, 1978) and on body surface skin of amphibia such as we reported for the Japanese newt (Pfeiffer *et al.*, 1989). Although these surface microridges do increase the surface area markedly, and the transport of respiratory gases, electrolytes and waste products is a vital function of the integument of some aquatic vertebrates, the surface microridges may only reflect the remnants of intercellular adhesion structures such as desmosomes and spinous processes. Certainly, in the case of baleen bristles important transport functions would not be anticipated.

The above findings are only a preliminary look at the ultrastructure of baleen which has been limited to bristles, and further study of more proximal regions of this integumentary specialization will be useful.

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