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Morphology, composition, and deterioration of the embryonic rostral sheath of the smalltooth sawfish (*Pristis pectinata*)

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Abstract—Elongated rostra evolved in diverse animal groups as adaptations for feeding, defense, sensory perception, and reproduction. Sawfish rostra have tooth-like dermal denticles, referred to as *rostral teeth*, along their lateral margins. Embryos have a sheath, or covering, for the calcified rostral teeth during gestation, and it persists until after parturition. Little is known about the morphology and composition of the sheath. During 18 years of tagging juvenile smalltooth sawfish (*Pristis pectinata*), sheaths were documented for 36 neonates with stretch total lengths of 581–812 mm, and samples were collected from 6 specimens for laboratory evaluation. The multilayered, skin-like sheath, which cannot be easily removed manually, has a vascularized inner layer of connective tissue composed primarily of fibrous proteins (e.g., collagen, reticulin, and keratin) surrounded by an outer layer of columnar and spherical epithelial cells overlying a basement membrane. The columnar cells contain condensed chromatin and differentiate into the outermost spherical cells that contain carbohydrates. After birth, the sheath is shed evenly over 4 d, through sloughing and apoptosis, fully exposing the rostral teeth. The sheath is an ephemeral embryonic organ that protects the female and the embryos from injury during gestation and birth.

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Relationships between form and function have been the subject of many morphological and evolutionary studies (e.g., Dean et al., 2007; Kolmann et al., 2014). For example, elongated rostra have appeared in diverse animal groups as adaptations for feeding, defense (especially if rigid and armored), sensory perception, and reproduction (e.g., Breder, 1952; Thorson, 1976; Williams, 1984; Kells and Carpenter, 2011). Examples of chondrichthyan taxa that have elongated rostra include sharks (e.g., *Apristurus* spp.; *Deania* spp.; goblin shark, *Mitsukurina owstoni*; and *Pristiophorus* spp.), rays (e.g., *Pristis* spp.; *Aptychotrema* spp.; sixgill stingray, *Hexatrygon bickelli*; and Sclerorhynchoidea), skates (e.g., *Dipturus* spp.), and chimaeras (e.g., *Harriotta* spp. and *Rhinochimaera* spp.) (Last and Stevens, 2009; Castro, 2011; Welten et al., 2015).

Sawfish species (family Pristidae) and sawshark species (family Pristiophoridae) have among the most extreme examples of elongated rostra. Rostra of sawfish compose up to 25% of the total body length, and both sawfishes and sawsharks have tooth-like scales referred to as *rostral teeth* along the lateral margins of their rostra (Bigelow and Schroeder, 1953; Miller, 1974, 1995; Last and Stevens, 2009). These groups are thought to have evolved their rostra independently (Slaughter and Springer, 1968), indicating that broad or shared advantages of rostrum elongation span the deepwater habitats of sawsharks and the shallow-water habitats of sawfishes (Last and Stevens, 2009; Poulakis et al., 2011).

Sawfishes and sawsharks, as well as other chondrichthyans (e.g., stingrays and dogfishes), have evolved ephemeral embryonic coverings, often called sheaths, to encapsulate sharp body parts, such as spines and rostral teeth, during gestation (Whitley, 1940; Gudger, 1951; Babel, 1967). The gross morphology and microscopic anatomy of these sheaths has not been investigated, and for sawfishes, the coverings have been described as "transparent cartilaginous tissue" (Southwell, 1910), a "membrane" (Hussakof, 1912; Setna and Sarangdhar, 1948), and "gelatinous" (Abel and Grubbs, 2020). Although they apparently refer to the sheath as both "skin" and an unspecified "narrow band," Bigelow and Schroeder (1953) indicated that the rostral teeth of sawfish embryos are "entirely enclosed in the skin until birth, while a narrow band along each margin of the saw is naked, both below and above." Contrary to the description by Bigelow and Schroeder (1953) of rostral teeth as soft until birth, rostral teeth form and calcify early in gestation and the sheath forms around the entire tooth-bearing portion of the rostrum (Miller, 1995). The sheath prevents the rostral teeth of embryos from damaging 1) siblings and the uterus during gestation and 2) the female's cloaca during parturition (Setna and Sarangdhar, 1948). The purpose of this paper is to describe the macroscopic and microscopic morphology of the embryonic rostral sheath of the smalltooth sawfish (*Pristis pectinata*), including its organization, composition, and shedding process.

Materials and methods

Sample collection

Neonate smalltooth sawfish, defined as newborns having partial or complete rostral sheaths or being in the length range for size at birth, were caught in Florida between 2004 and 2022 primarily as part of ongoing, fishery-independent sampling in the Charlotte Harbor estuarine system and as opportunistic captures elsewhere in the southern half of the state (e.g., in the Indian River Lagoon). This research was conducted under endangered species research permit numbers 1475, 15802, 21043, and 25864 issued to the Florida Fish and Wildlife Conservation Commission.

Sampling was carried out during the day, primarily with gill nets that had panels of 102-mm (4-in) and 152-mm (6-in) stretch monofilament mesh. Some individuals were caught in seines, in cast nets, or by hand. Detailed gear descriptions and sampling protocols can be found in Scharer et al. (2017).

Prior to being released alive, neonates observed with partial or complete rostral sheaths were measured (in stretch total length [STL] in millimeters) and photographed. Small (2–4 mm long by 2–4 mm wide) samples of sheath tissue were collected when at least half the sheath remained. Samples were taken between the rostral teeth and placed in 95% ethanol or 10% neutral buffered formalin in the field.

Sample processing

Histology, scanning electron microscopy (SEM), micro-computed tomography, and elemental analysis were used to characterize the microscopic morphology, composition, and deterioration of the sheath and its association with the rostrum.

For histology, ethanol- and formalin-fixed samples were embedded in glycol methacrylate (JB-4 Embedding Kit[1], Polysciences Inc., Warrington, PA) at the Fish and Wildlife Research Institute in St. Petersburg, Florida. Most samples that included scales were decalcified through pretreatment with 90% formic acid for 5 d or 10% ethylenediaminetetraacetic acid for 3 weeks before embedding. Samples were sectioned at 4 µm by using a standard microtome and were stained with standard hematoxylin and eosin, with periodic acid Schiff's hematoxylin and metanil yellow (PASMY) to identify carbohydrates and mucus (Quintero-Hunter et al., 1991), with Masson's trichrome dye for collagen- and keratin-like tissue components (Sheehan and Hrapchak, 1987), and with a silver-based reticulin stain to highlight structural tissue and basement membranes (Quagio-Grassiotto[2]). Slides were retained.

At the University of Delaware, SEM was used to document surface features and degradation of the sheath. For visualizing sheath deterioration and rostral scales, a frozen rostrum from a dead neonate (reported to us by a member of the public) was thawed and scanned by using a SkyScan 1276 micro-computed tomography scanner (Bruker Corp., Billerica, MA) at a nominal resolution of 21.2 µm with a 0.25-mm thick aluminum filter and an applied X-ray tube voltage of 50 kV. Camera pixel binning was applied by combining each 2-by-2 grid of pixels into 1 pixel. The scan orbit was 180° with a rotation step of 0.9°. Reconstruction was performed with a modified Feldkamp algorithm by using the SkyScan NRecon software and NReconServer, vers. 1.7.4.2 (Bruker Corp.). Beam hardening correction was applied. With the same rostrum used for micro-computed tomography, energy-dispersive X-ray spectroscopy was performed by using an INCAx-act detector (Oxford Instruments, Abingdon, UK) attached to a Hitachi S-4700 field-emission scanning electron microscope (Hitachi Ltd., Tokyo, Japan) for elemental analysis to determine if neonates possessed a rostrum with uniformly calcified scales at birth.

Results

Live neonate smalltooth sawfish (sample size [*n*]=141) were caught primarily in southwest Florida between 2004 and 2022. Complete or partial embryonic rostral sheaths were observed on 36 individuals (range: 581–812 mm STL; mean: 733 mm STL), and multiple samples were collected from 6 individuals for laboratory analysis.

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA, or the Florida Fish and Wildlife Conservation Commission.

[2] Quagio-Grassiotto, I. 2014. Unpubl. data. Dep. Morphol., Inst. Biosci., Sao Paulo State Univ.-UNESP, Botucatu, São Paulo, 18618-689 Brazil.

Macroscopic morphology

The sheath, partial or complete, was firmly connected to the rostrum and could not be easily removed manually. The sheath covered the underlying calcified rostral teeth and rostral scales (Fig. 1, A and B; Fig. 2). The sheath also contains some scales near the rostrum (Fig. 3). Notably, during a parturition event, we observed the birth of a neonate with the tips (<1 mm) of its rostral teeth already exposed. The other neonate observed from the same event had a complete sheath (i.e., rostral teeth completely covered). Normally, the sheath receded evenly across the

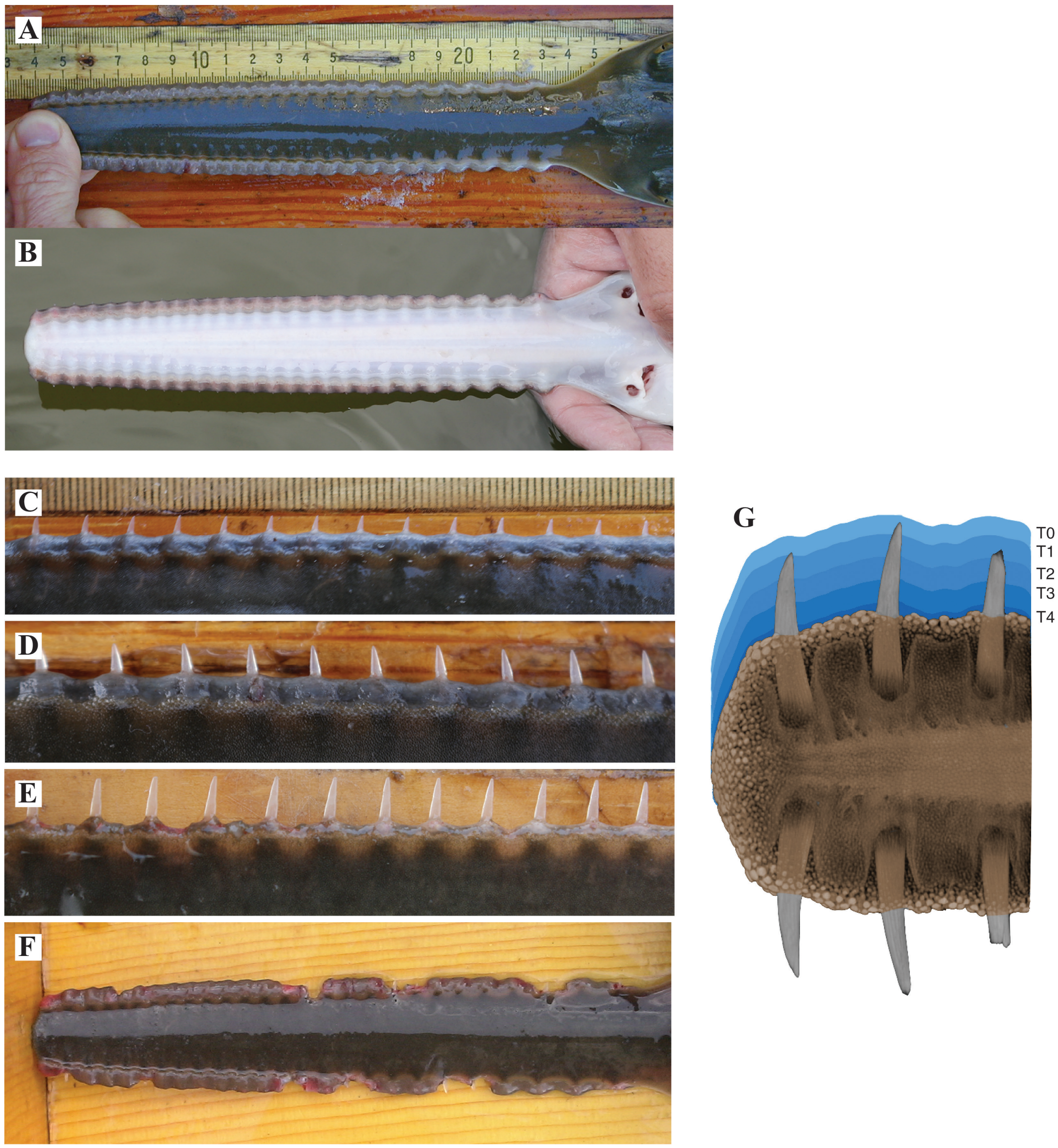

Figure 1

Photographs and an illustration showing the typical progression of deterioration of the embryonic rostral sheath of neonate smalltooth sawfish (*Pristis pectinata*) after parturition. The (**A**) dorsal and (**B**) ventral views of the complete rostral sheaths of specimens caught shortly after birth show that no teeth are exposed at this stage (labeled as T0 in panel G). The sheath usually recedes evenly across the entire rostrum, exposing more and more of the rostral teeth over time with (**C**) about 1 mm shed (stage T1) after 1 h of observation and (**D**) about 2 mm shed (stage T2) after 1 d. (**E**) After approximately 4 d, only a thin layer of remnant sheath is visible along the entire edge of the rostrum (stage T4). (**F**) Only 1 individual shed its sheath unevenly, with some teeth exposed before others. We hypothesize that this damage occurred because this individual began using the rostrum regularly before the sheath deteriorated much. The damage did not occur during capture because this fish was caught by hand. (**G**) This visualization shows the sequence of rostral sheath degradation.

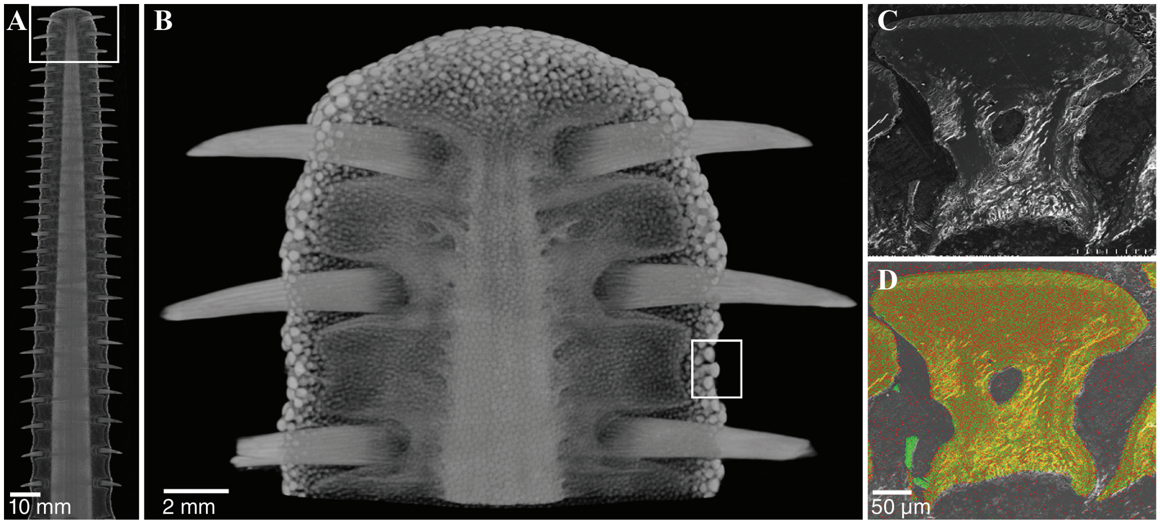

Figure 2

(**A**) A micro-computed tomography scan of a complete rostrum of a neonate smalltooth sawfish (*Pristis pectinata*) and (**B**) a close-up view of the tip of the rostrum from part of that scan (indicated by the box outlined in white in panel A) showing rostral scales, which are largest at the edges and tip of the rostrum. The 3D rendering applied to the scan makes the uniformly calcified rostral scales on the rostrum more conspicuous. Images from (**C**) scanning electron microscopy and (**D**) elemental analysis of cross sections of a rostral scale, from the region indicated by the box outlined in white in panel B, show deposition of calcium (green) and phosphorous (red) throughout the scale. The 50-µm scale bar in panel D also applies to panel C.

entire length of the rostrum (Fig. 1, C–E). However, on one occasion, an individual with a damaged sheath was observed (Fig. 1F).

One neonate was captured with a complete rostral sheath (i.e., sheath 5 mm wide, with no rostral teeth exposed; see the photographs of the full sheath in Figure 1, A and B, and the visualization of this stage [T0] in Figure 1G) and was observed on 4 successive occasions over 4 d, so that we could follow the progression of exposure of the rostral teeth. After 1 h of observation (at a water depth of 0.6 m and in surface and bottom temperatures of 30.0°C and 28.6°C and surface and bottom salinities of 16.7 and 17.5), 1 mm of the outermost sheath had been shed (stage T1 in Figure 1G). Then 2 mm of the sheath had shed after 1 d (stage T2 in Figure 1G), and 3 mm of it had shed (i.e., 60% of all individual rostral teeth were exposed) after 2 d (stage T3 in Figure 1G; in waters with temperatures of 22.3–30.0°C and salinities of 16.7–18.0 over those 2 d). After 2 more days (i.e., 4 d postpartum), only a thin layer of sheath remained at the base of the rostral teeth along the margin of the entire rostrum (stage T4 in Figure 1G).

Microscopic morphology and composition

Examination of cross sections revealed that the sheath is skin-like and composed of 2 main layers: a relatively thin outer layer of epithelial cells and a thicker inner layer composed of collagen-, reticulin-, and keratin-like connective tissues (Figs. 3–5). The 2 main layers are separated by a basement membrane spanning the entire cylindrical perimeter of the sheath.

Outer layer The outer layer of the sheath is composed of spherical and columnar epithelial cells. The spherical cells overlie the tips of the rostral teeth and penetrate between the scales on the rostrum (Figs. 4–6). Near the rostrum–sheath interface, groups of outer sheath cells surround the rostral scales, contributing to firm connectivity of the sheath to the rostrum (Fig. 6B).

Spherical cells are PASMY-positive (i.e., they contain carbohydrates) and are in direct contact with the environment. The diameter of the cells in the outermost half of the spherical cell layer (~8 cells thick) is approximately twice that of the interior spherical cells (Fig. 5B). Approximately half the cytoplasm of the outermost spherical cells is PASMY-positive and contains nuclei and glycoproteins, and the other half is PASMY-negative (i.e., they do not contain carbohydrates) (Fig. 5D). These cells also display cytoskeletal support with microfilaments (Fig. 5C). The outermost cells are hypothesized to maintain isolation of the tips of the rostral teeth during gestation by being constantly replaced and to begin gradually sloughing off after birth, evenly exposing more area of the rostral teeth.

Below the PASMY-positive spherical cells are columnar cells overlying a basement membrane. The columnar cells have darkly stained nuclei with condensed

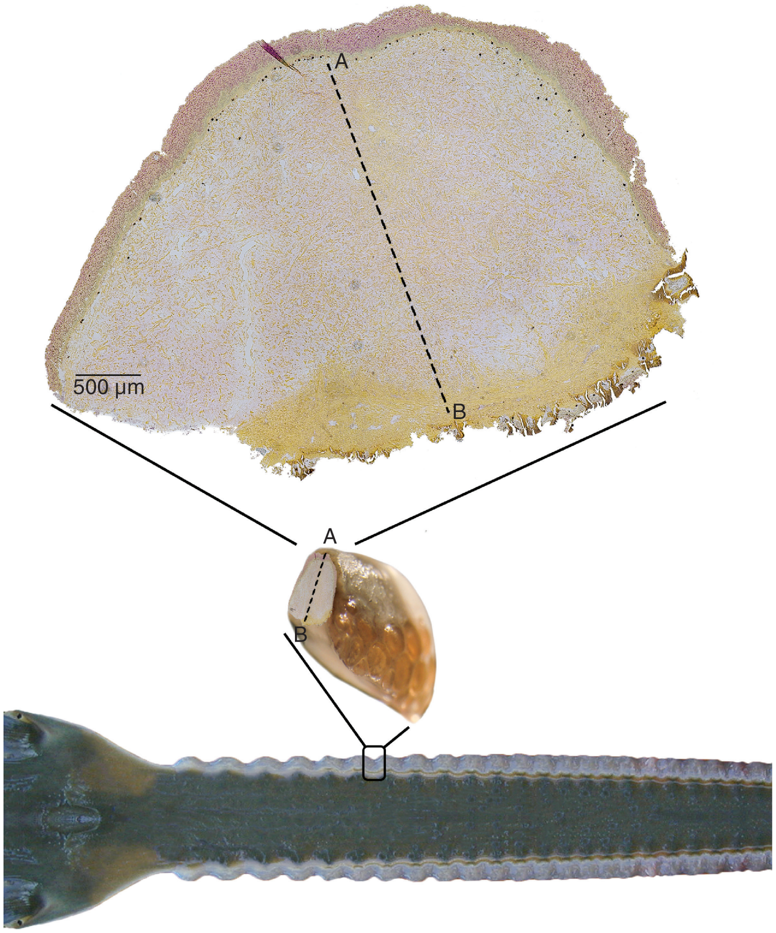

Figure 3

Cross section (top image) of a sample of an embryonic rostral sheath (middle image) taken between rostral teeth (the box outlined in black in the bottom image) of a neonate smalltooth sawfish (*Pristis pectinata*). Circular scales are visible on the outside of the gross sheath sample (middle image) and in the bottom margin of the histology (dark brown areas in the top image). The cross section shows the general orientation of the sheath and its components and was stained with periodic acid Schiff's hematoxylin and metanil yellow. Samples were taken from multiple locations along the rostrum for analysis.

chromatin and are PASMY-negative (Fig. 5, E and F). The columnar cells are hypothesized to be derived from stem cells adjacent to the basement membrane that maintain the cellular composition of the entire outer layer of epithelial cells (Fig. 5E).

Inner layer Below the basement membrane lies an inner layer of connective tissues containing fibrocytes, melanocytes, and blood vessels (Figs. 6–8). The connective tissues increase in density nearest the rostrum (Figs. 4C and 6A), where bases of the outermost rostral scales, some of which are likely shed with the sheath (see Figure 3), are embedded in interwoven collagen- and keratin-like connective tissues (Fig. 6, E and F). Fibrocytes were observed throughout the inner layer, often near collagen-like connective tissue (Fig. 7). Melanocytes were found in both layers of the sheath but were concentrated at the margin of the inner layer near the basement membrane (Fig. 8).

Degradation and surface features The sheath is hypothesized to be shed through a combination of sloughing, during which the outer layer contacts the environment, and apoptosis. Morphological evidence of apoptosis (e.g., pyknosis or karyorrhexis) was observed in nuclei of the deteriorating sheath cells (Fig. 9). Broader signs of degradation, such as gaps in the basement membrane and the presence of eosinophils, were also noted.

The SEM revealed an uneven texture of the spherical epithelial cells on the sheath surface as they were losing connectivity and integrity (Fig. 10, A and B). In addition, surface pores (Fig. 10, C and D), rostral scales (Fig. 10E), and degradation of the junction between scales and abutting cells of the sheath were observed (Fig. 10F).

Discussion

Elongated rostra have evolved in different animal groups, including fishes (Breder, 1952; Bigelow and Schroeder, 1953; Wueringer et al., 2012). We hypothesize that the embryonic rostral sheath of sawfishes evolved to protect females while gravid and during parturition. However, because there is no parental care, the sheath deteriorates within 4 d, as the neonates begin using their toothed rostrum to feed. Therefore, few neonates have been caught with any remnants of sheath during almost 2 decades of fishery-independent surveys.

The smalltooth sawfish feeds primarily on other fishes (Poulakis et al., 2017; Hancock et al., 2019), capturing prey by quickly swinging the rostrum side to side (Wueringer et al., 2012). Quick deterioration of the sheath and exposure of the rostral teeth benefits neonates by allowing them to begin feeding soon after birth. Maternally derived resources have been shown to aid neonatal hammerhead sharks (*Sphyrna* spp.) by increasing survivorship during the first weeks after birth (Lyons et al., 2020). Whether sawfish rely on similar provisions is unknown. However, sheath-retaining neonates were

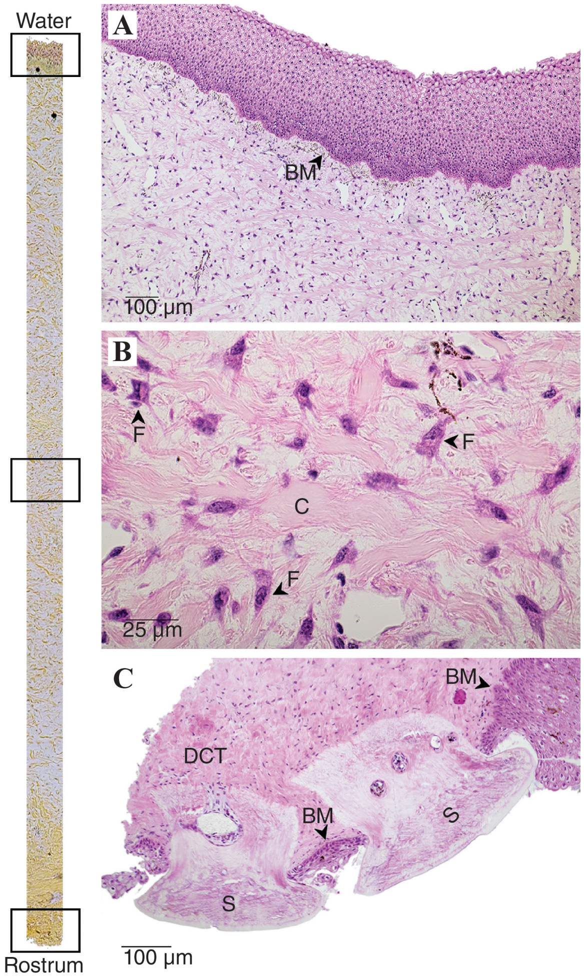

Figure 4

Cross sections of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*) showing the morphology of the (**A**) outermost, (**B**) central, and (**C**) innermost areas. The boxes outlined in black and adjacent to the micrographs are meant to aid orientation and correspond to the general area of the sheath from which each micrograph came. The morphological features shown include the basement membrane (BM), fibrocytes (F), collagen-like fibers (C), dense connective tissue (DCT), and scales (S). These cross sections were stained with standard hematoxylin and eosin.

captured in productive estuarine nursery areas with ample food supplies; therefore, a few days are unlikely to be detrimental to early survivorship, especially if some residual maternal resources, such as an internal yolk sac, are available.

Rostral teeth and scales presumably form and calcify early in gestation (Miller, 1995). For this reason, the sheath likely evolved with a robust, firm connection to the rostrum to maximize its durability during a 1-year gestation period (Brame et al., 2019). On the basis of these data, we describe the sheath and its connection to the rostrum as a continuum between the sheath and the rostrum rather than as each being a discrete entity. This approach allowed us to describe the morphology and composition of the sheath and to show a theoretical sequence of sheath deterioration and exposure of the rostral components, despite the limitations of studying a species that is listed as endangered under the U.S. Endangered Species Act (Federal Register, 2003) and as critically endangered on the IUCN Red List of Threatened Species (Carlson et al., 2022) (e.g., low sample size and inability to sample through the sheath deep into the rostra of live specimens). Still, questions remain. For example, we were not able to fully elucidate all aspects of the connectivity of the sheath to the rostrum because there is skin on the rostrum and the sheath also appears to be skin, indicating a "double skin" morphology. In addition, the sheath contains some scales (see Figure 3) that are likely lost as the sheath fully deteriorates because of the intimate association between the sheath and rostral scales.

Notably, during a parturition event in the field, we observed the birth of 2 neonates, an individual with a complete sheath and another with the tips of its rostral teeth already exposed, indicating that the sheath sometimes begins to recede in utero. This observation supports our contention that these 2 neonates were not born prematurely.

On one occasion, a rostrum with a damaged sheath was observed (see Figure 1F). We hypothesize that this damage occurred because this individual began using the rostrum regularly before the sheath deteriorated much. The damage did not occur during capture because this fish was caught by hand.

We observed deterioration and sloughing of the rostral sheath of one neonate smalltooth sawfish. This individual was first caught (and tagged) in the Peace River, Florida, where neonates tend to remain (Scharer et al., 2017); therefore, we returned to the area to acoustically relocate the individual. Observations made during a series of recaptures corroborate our hypothesis that the sheath normally recedes evenly across the entire length of the rostrum and allowed us to refine the shedding time. Previous observations of an individual recaptured after 10 d have been used to conservatively estimate a shedding time of within 2 weeks (Poulakis et al., 2011), but our latest research has shortened that estimate. On the basis of the observation time series and observations from other captured neonates, we hypothesize that the last layer of sheath to deteriorate corresponds to the dense connective tissue layer at the junction between the rostrum and sheath (see Figures 4C and 6A).

The protected status of the smalltooth sawfish makes obtaining specimens and resolving interpretation uncertainties inherently difficult because there are no regular sources of specimens, such as recreational or commercial fisheries. In this study, one of the most difficult things to describe was the association between the

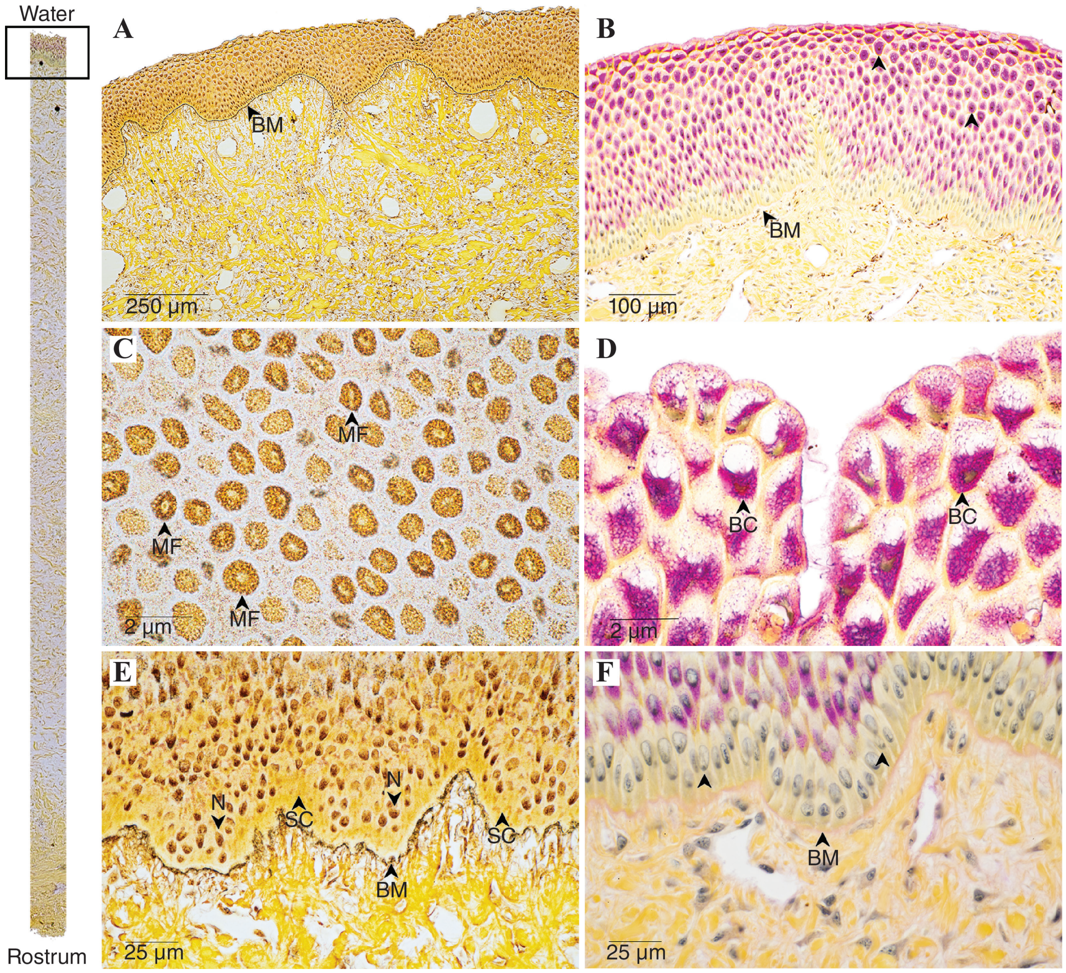

Figure 5

Cross sections of the outer layer of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*). The cross sections were stained with a silver-based reticulin stain (left panels) to highlight structural tissue and basement membranes and periodic acid Schiff's hematoxylin and metanil yellow (PASMY, right panels) to identify carbohydrates. (**A**) This micrograph shows the outermost portion of the sheath above a basement membrane (BM) and the cells within it. (**B**) This micrograph shows the spherical epithelial cells above the BM that are in direct contact with the environment; the magenta color indicates that cells are PASMY-positive (i.e., they contain carbohydrates). The innermost epithelial cells in the yellow area above the BM are columnar and PASMY-negative. About half the spherical cell layer (~8 cells thick), closest to the water, are about twice the diameter of the more interior spherical cells (compare the diameters of cells at unlabeled arrowheads). (**C**) This micrograph shows spherical cells that have cytoskeletal support with microfilaments (MF). (**D**) In this close-up view of the outermost spherical cells shown in panel B, about half the area of cytoplasm of these cells is PASMY-positive, contains the nuclei, and contains glycoproteins, and the other half is PASMY-negative (BC). (**E**) The columnar epithelial cells in this micrograph contain densely and darkly stained nuclei (N). The columnar cells are hypothesized to be derived from stem cells (SC) adjacent to the basement membrane and appear to create and maintain the entire outer layer of the sheath. (**F**) This micrograph provides a close-up view of the innermost epithelial cells (indicated with unlabeled arrowheads) and their columnar shape in the outer layer of the sheath (yellow area) shown in panel B. The box outlined in black and adjacent to the micrographs is meant to aid orientation and corresponds to the general area of the sheath from which each micrograph came.

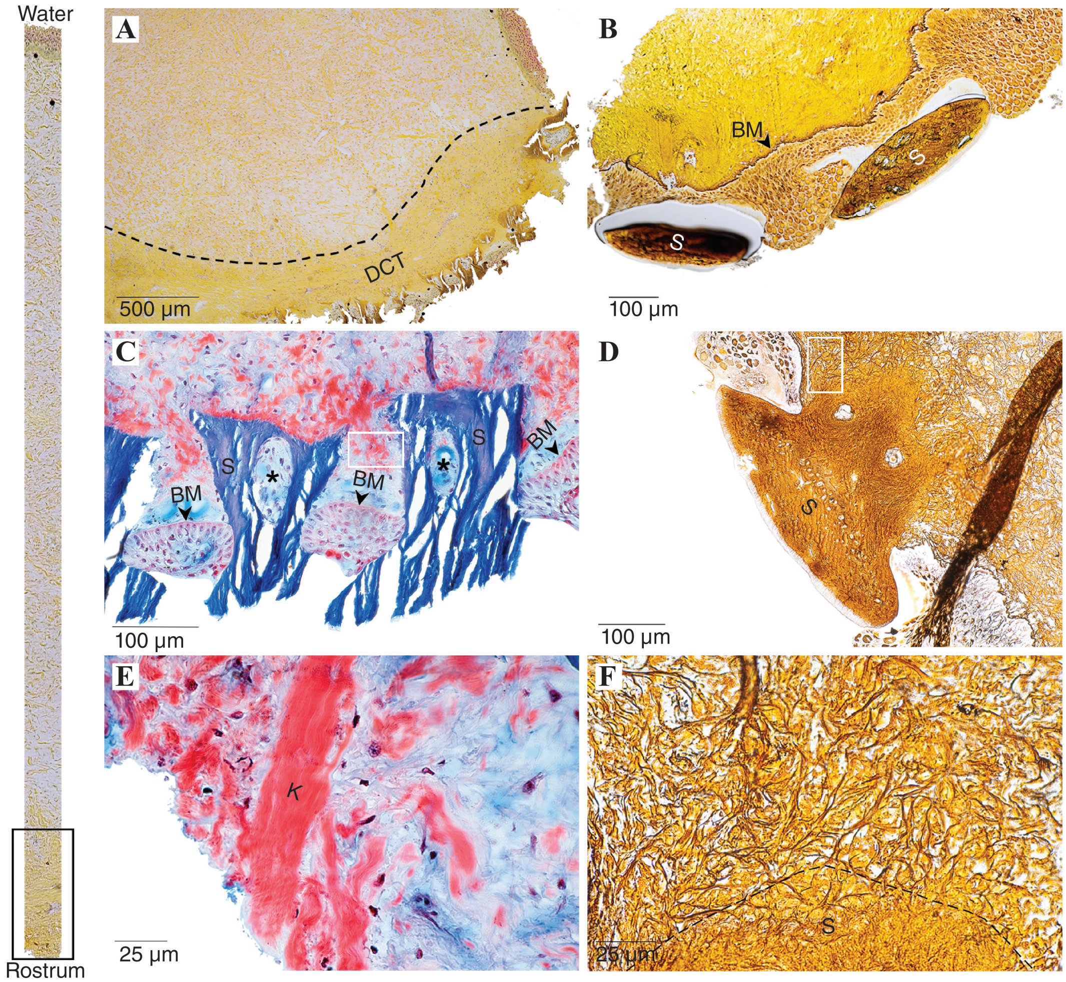

Figure 6

Cross sections of the innermost layer of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*). (**A**) This micrograph shows the layer of dense connective tissue (DCT, below the dashed line) near the scales (which are dark brown along the margin) of the rostrum. (**B**) In this micrograph, the basement membrane (BM), which produces the outer layer of the sheath, is continuous and exists behind the rostral scales (S). Note how groups of spherical cells in the outer layer curve around the largest scale, helping to secure the sheath to the rostrum. (**C**) The BM appears between the scales in this micrograph, which also shows the locations of pulp cavities of scales (*). The scales were not decalcified in this early sample. The box outlined in white indicates the approximate area shown in panel E. (**D**) The scale in this micrograph is embedded in interwoven collagen- and keratin-like connective tissues. The box outlined in white indicates the approximate area shown in panel F. The dark area on the right side of the micrograph is folded tissue. (**E**) This micrograph provides a close-up view of the presence of keratin (K) in the area outlined in panel C. (**F**) In this micrograph, the partial scale (indicated with the dashed line) at the bottom of the panel blends in with the interwoven tissues of the DCT layer. The cross section in panel A was stained with periodic acid Schiff's hematoxylin and metanil yellow, the cross sections in panels C and E were stained with Masson's trichrome dye, and the cross sections in panels B, D, and F were stained with a silver-based reticulin stain. The box outlined in black and adjacent to the micrographs is meant to aid orientation and corresponds to the general area of the sheath from which each micrograph came. Collectively, aspects of the outer and inner layers of the sheath establish a firm connection between the sheath and the rostrum. The sheath is not easily separated from the rostrum and cannot be removed manually.

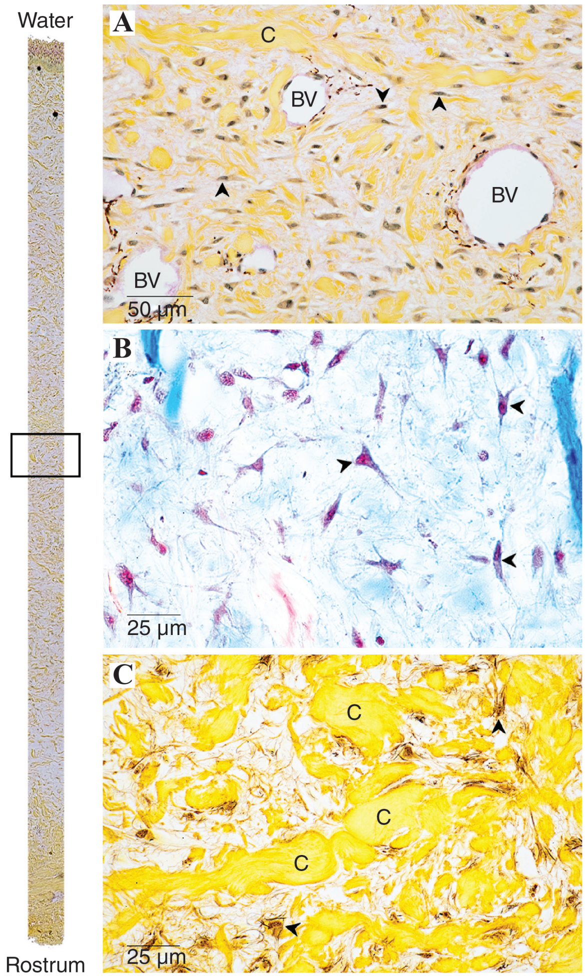

Figure 7

Cross sections of the central, inner structural tissue layer of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*) stained with (**A**) periodic acid Schiff's hematoxylin and metanil yellow, (**B**) Masson's trichrome dye, and (**C**) a silver-based reticulin stain. The micrographs show collagen-like fibers (C) and fibrocytes (indicated with unlabeled arrowheads) interspersed with blood vessels (BV). The box outlined in black and adjacent to the micrographs is meant to aid orientation and corresponds to the general area of the sheath from which each micrograph came. The fibrocytes can also be seen in a cross section stained with standard hematoxylin and eosin in Figure 4B.

sheath and the rostrum—where does each begin and end? In the future, if dead gravid females are obtained, and if the embryos can be adequately preserved, examining cross sections of the sheath that extend into the rostrum may help answer this question. Similarly, if the endogenous origin of the sheath can be determined, perhaps the hypothesis of a double skin morphology could be resolved also.

Conclusions

The intact, embryonic rostral sheath of sawfishes is not easily removed manually, a finding that is inconsistent with terminology used in previous descriptions. For example, to describe a structure as "gelatinous" or a simple "membrane" (see Southwell, 1910; Hussakof, 1912; Setna and Sarangdhar, 1948; Abel and Grubbs, 2020) implies fragility and weak attachments to the underlying tissue. Given its morphology, the embryonic sheath of the smalltooth sawfish is a skin-like, ephemeral embryonic organ with specialized structure rather than a discrete layer of surrounding tissue or secretions. Whether analogous chondrichthyan embryonic sheaths of tail spines of rays (e.g., *Hypanus* spp.; *Rhinoptera* spp.; round stingray, *Urobatis halleri*; Babel, 1967; senior author, unpubl. data), dorsal-fin spines of dogfishes (e.g., spiny dogfish, *Squalus acanthias*; roughskin dogfish, *Cirrhigaleus asper*; velvet belly lanternshark, *Etmopterus spinax*; Whitley, 1940; Gudger, 1951; Castro[3]), or oral teeth of neonates in this class (e.g., bull shark, *Carcharhinus leucas*; A. Wooley, personal observ.) have similar morphologies is unknown. Their study and comparison to the embryonic rostral sheath of the smalltooth sawfish would inform our understanding of their form and function.

Resumen

Los rostros alargados evolucionaron en diversos grupos de animales como adaptaciones para la alimentación, la defensa, la percepción sensorial y la reproducción. Los rostros de los peces sierra tienen a lo largo de sus márgenes laterales, dentículos dérmicos, denominados dientes rostrales. Los embriones tienen una cubierta o envoltura para los dientes rostrales calcificados durante la gestación, que persiste hasta después del parto. Se sabe poco sobre la morfología y composición de la cubierta. Durante 18 años de marcado de juveniles de pez sierra peine (*Pristis pectinata*), se documentaron las cubiertas de 36 neonatos con longitudes totales estiradas de 581-812 mm, y se recogieron muestras de 6 especímenes para su evaluación en laboratorio. La cubierta multicapa, similar a la piel, la cual no puede extraerse manualmente con facilidad, tiene una capa interna vascularizada de tejido conectivo compuesta principalmente de proteínas fibrosas (ej. colágeno, reticulina y queratina) rodeada por una capa externa de células epiteliales columnares y esféricas que recubren una membrana basal. Las células columnares contienen cromatina condensada y se diferencian en las células esféricas más externas que contienen carbohidratos. Tras el nacimiento, la cubierta se desprende uniformemente a lo largo de 4 días, mediante descamación y apoptosis, exponiendo completamente los dientes rostrales. La cubierta es un

[3] Castro, J. I. 2023. Personal commun. Southeast Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 263 13th Ave. South, St. Petersburg, FL 33701.

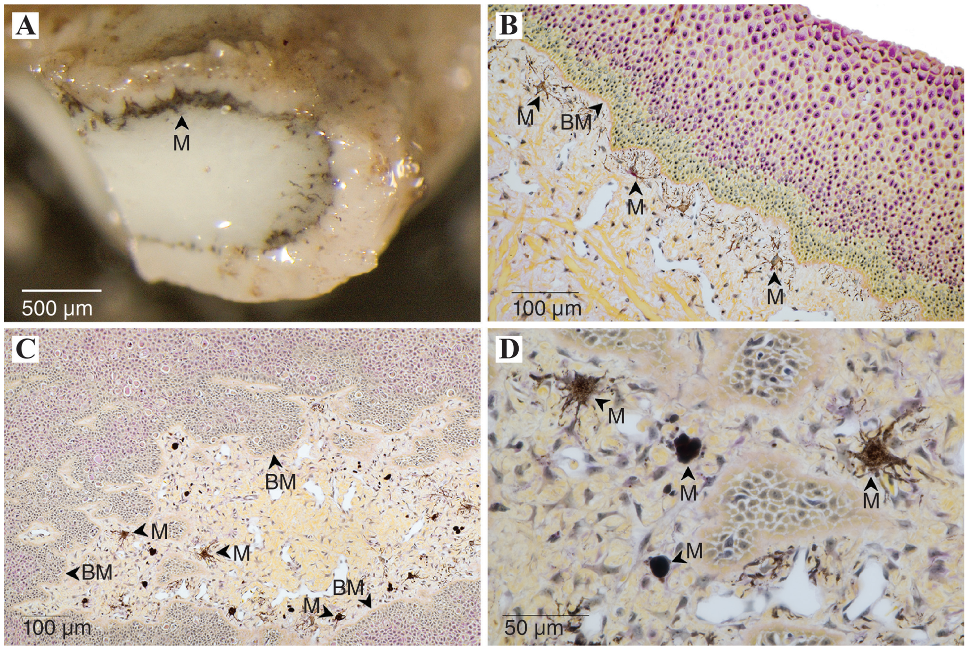

Figure 8

(**A**) Photograph and (**B**–**D**) histological sections of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*). The cross sections in panels B and D and the tangential section in panel C show the prevalence of melanocytes (M) in the inner connective tissue layer, just below the basement membrane (BM). The histological sections were stained with periodic acid Schiff's hematoxylin and metanil yellow.

órgano embrionario efímero que protege a la hembra y los embriones de lesiones durante la gestación y el nacimiento.

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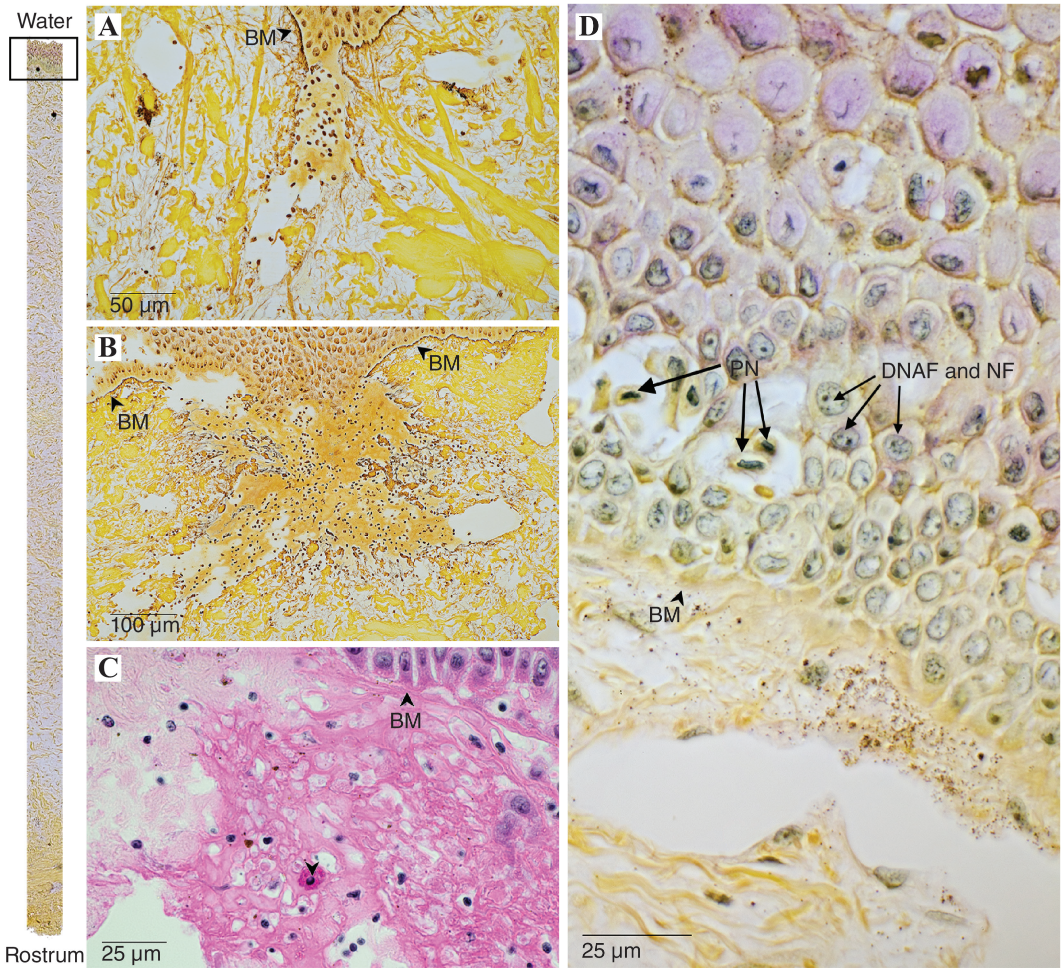

Figure 9

Cross sections of degraded portions of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*). (**A** and **B**) The integrity of the outer and inner layers of the sheath is disturbed in these micrographs, a result of the failed coherence of the basement membrane (BM) caused by the apoptosis that occurs during sheath degradation. (**C**) This micrograph shows an eosinophil (indicated with the unlabeled arrowhead). (**D**) In this micrograph, signs of apoptosis, including pyknotic nuclei (PN), DNA fragmentation (DNAF), and nuclear fading (NF), are apparent. The box outlined in black and adjacent to the micrographs is meant to aid orientation and corresponds to the general area of the sheath from which each micrograph came. The cross sections in panels A and B were stained with a silver-based reticulin stain, the cross section in panel C was stained with standard hematoxylin and eosin, and the cross section in panel D was stained with periodic acid Schiff's hematoxylin and metanil yellow.

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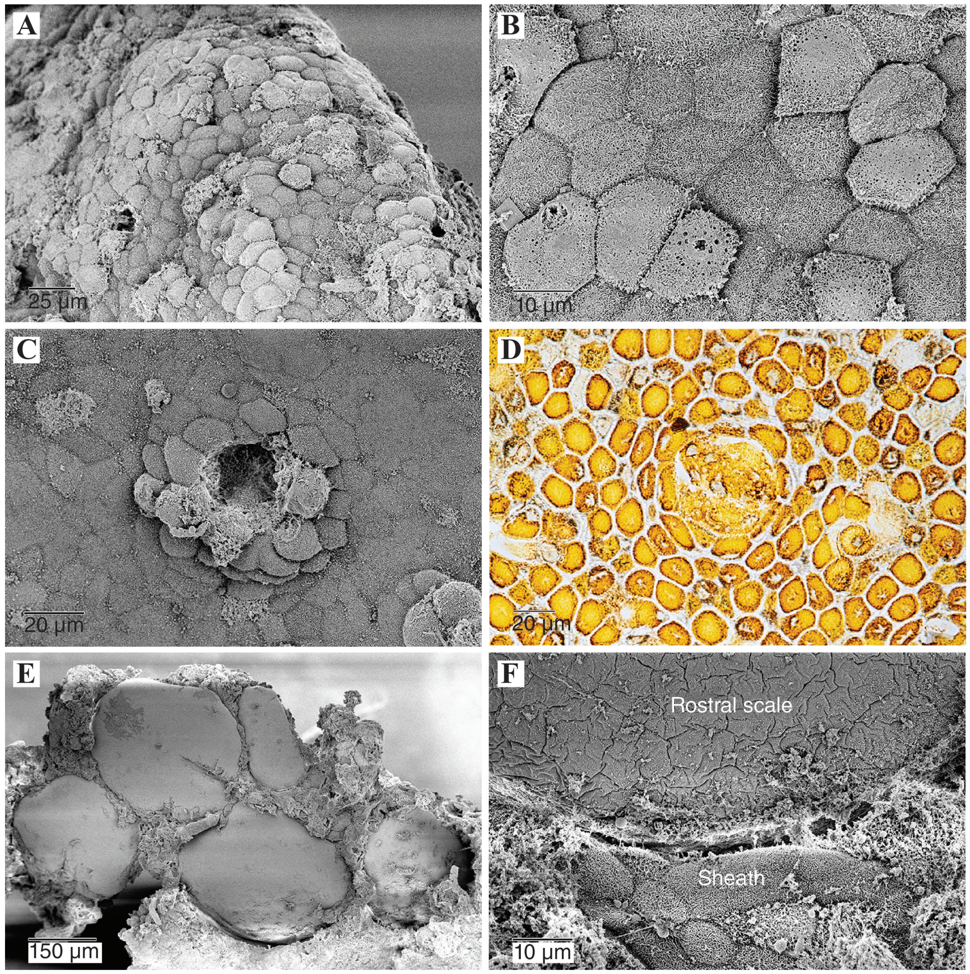

Figure 10

Scanning electron micrographs and a histological image of a cross section of the embryonic rostral sheaths of neonate smalltooth sawfish (*Pristis pectinata*). In (**A**) the first micrograph and (**B**) the close-up view, the outermost spherical epithelial cells have an uneven texture, a result of the cells starting to lose their integrity shortly after the birth of the fish. Evidence of degradation is also visible in (**C**) the scanning electron micrograph and (**D**) the histological image (of a cross section stained with a silver-based reticulin stain) that show surface pores and in (**E**) the micrograph that shows rostral scales. (**F**) This micrograph shows the junction between the sheath and a rostral scale as the sheath was degrading.

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