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RESEARCH ARTICLE

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Microstructural morphology of dermal and oral denticles of the sharpnose sevengill shark *Heptranchias perlo* (Elasmobranchii: Hexanchidae), a deep-water species

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Abstract

The dermal denticles are among the unique morphological adaptations of sharks, which have been acquired throughout their long evolutionary process of more than 400 million years. Species-specific morphological characteristics of these structures has been applied specially as tools for functional and taxonomic (family-level) studies. Nevertheless, few studies have explored the diversity of denticle structure in different around the body and oral cavity. In the present study, we described the morphological differences observed in skin and oral cavity of sharpnose sevengill shark *Heptranchias perlo*, using scanning electron microscopy. Our findings demonstrate substantial variation in morphological structure of the denticles of the body and oral cavity. Overall, the dermal denticles observed across body surface were overlapped, tricuspid, with the central cuspid being more pronounced, pointed, and triangular in shape compared with lateral ones. Unlike, the denticles on the tip of the nose had a smooth crown, with rounded edges, being compact, and overlapped. The oral denticles were found in the ventral and dorsal region of the oral cavity. They also were tricuspid, but with differences in arrangement and ridges. These results suggest a strict functional relationship with the morphological characteristics observed. Such morphological diversity body-region-dependent highlights the need for comparative studies that include oral denticles, since this structure has an important functional role in sharks and can be found in fossil and recent records.

KEYWORDS

elasmobranchs, functional morphology, oral cavity, placoid scales, taxonomic tool

1 | INTRODUCTION

Sharks are well known for their unique morphological adaptations, which have been acquired throughout their long evolutionary process of more than 400 million years (Grogan & Lund, 2004). Examples for such include a pair of copulatory organs (i.e., claspers), modified scales, termed dermal denticles, and a specialized sensory system (Hamlett, 1999). The dermal denticles, a structure with a neural crest origin of trunk, is composed of a calcified base and dentine protrusion covered by an enamel cap (Gillis, Alsema, & Criswell, 2017; Gravendeel, Van Neer, & Brinkhuizen, 2002). Denticle morphology has been well investigated in several studies, mainly considering their functional role on reducing hydrodynamic drag during locomotion, which has inspired several manufactured products (e.g., manufactured body suits and aeroengines, see references in Oeffner & Lauder, 2012). Additionally, denticle morphology has been used as an important tool for reconstruct shark communities and taxonomic identification specially related to fossil records (e.g., Cappetta, 1987, 2012; Dillon, Norris, & Dea, 2017; Kriwet & Benton, 2004; Kriwet, Kiessling, & Klug, 2008).

Some particularities of the denticles that make them interesting tools include: (a) their morphological aspects (i.e., shape, size, and arrangement) are highly variable intra and interspecifically; (b) they are correlated with shark ecology; (c) are very abundant across the body of sharks, including oral regions and nictitating membrane; and (d) are continually exchanged (e.g., Dillon et al., 2017; Poscai et al., 2017; Rangel et al., 2017). Although recent studies have addressed extensively about morphology, taxonomy, and function of denticles (e.g., Dillon et al., 2017), more studies are needed considering both the high shark diversity (~500 living species; Weigmann, 2016) and intra and interspecifically variability of denticles across the body and oral cavity (e.g., Ankhelyi, Wainwright, & Lauder, 2018; Dillon et al., 2017; Rangel, Salmon, Poscai, Kfoury Jr., & Rici, 2019).

Such a taxonomic tool seems to be especially interesting to explore areas where research is more difficult to perform such as in deep-water shark communities, despite its limited application to species and genus level identification (Dillon et al., 2017). Therefore, to contribute with information about the denticle morphology of deep-sea species, the present study described the morphological differences observed in the skin and oral denticles of sharpnose sevengill shark (*Heptranchias perlo*). The sharpnose sevengill shark is a little-known Hexanchiformes, with a wide geographic distribution, being found in tropical and temperate regions, in deep waters, continental regions, islands, and high slopes, at depths ranging from 27 to 720 m (Barnett, Braccini, Awruch, & Ebert, 2012; Ebert & Stehmann, 2013). Recently, the sharpnose sevengill shark was assessed as data deficient according to the World Conservation Union (IUCN) Red List (Soldo & Bariche, 2016). Thus, it is expected that the results of this study will contribute with descriptive information on the dermal and oral denticles of sharpnose sevengill shark, which can be used in future ecological and taxonomic studies.

2 | MATERIALS AND METHODS

Skin and oral cavity samples were obtained from three adult female' sharpnose sevengill sharks (95.0, 103.0, and 114.5 cm total length). The individuals were obtained during the monitoring of longline fishing targeting tuna in Santos and Guarujá, São Paulo, from August 1997 to February 1999. The specimens were preserved in ice in the boats and subsequently fixed in a 10% formaldehyde solution until the analysis. The individuals were donated from Instituto de Pesca to the Surgery Department of Faculdade de Medicina Veterinária da Universidade de São Paulo (FMVZ-USP). Sample use was approved from the Ethics Committee on the use of Animals (CEUA) n° 4245050214, FMVZ-USP.

Samples were obtained from different body regions: (a) anterior oral cavity, (b) tip of the nose, (c) anterior dorsal, (d) medial dorsal, (e) anterior ventral, and (f) medial ventral. Scanning electron microscopy (LEO 435VP) was used to examine the three-dimensional microstructure of dermal and oral denticles. In order to scan, tissue samples dehydrated in series of increasing ethanol densities (70, 80, 90 and 100%, with a half-hour intervals between each one). After dehydration, the samples were dried in a Balzers CPD 020 critical-point device, mounted onto metal stubs with carbon adhesive, and sputtered with gold in an Emitech K550 sputter apparatus for scanning.

3 | RESULTS

3.1 | Dermal denticles

The three-dimensional aspects of the dermal denticles reveled variation among body regions (Figure 1). Overall, the denticles observed across the body's surface were overlapped, with the apex pointing in a posterior direction (Figure 1). No differences were found among the specimens analyzed. The denticles on the tip of the nose had a smooth crown, with rounded edges, being compact and overlapped (Figure 1b). Denticles of the dorsal anterior body regions have crowns slightly less elongated shapes and more rounded cusps (Figure 1c). Three to four less pronounced ridges were identified on the crown in these denticles.

Denticles of the dorsal (central, Figure 1d) and ventral (anterior, Figure 1e and central, Figure 1f,g) were similar. Denticles were tricuspid, with the central cuspid being more pronounced, pointed, and triangular in shape. Three ridges were identified on the crown, extending to the base until the end of the cusp, with the median ridge being the most prominent (Figure 1d-g). On the dorsal anterior surfaces of the body were observed pores of the ampullae of Lorenzini (Figure 1e).

3.2 | Oral denticles

The oral denticles were found in the ventral and dorsal region of the oral cavity (Figure 2). The results illustrate the similarities in denticle shape across the oral cavity. In the ventral region, there is the basihyal attached to the hyoid arch of the mandible (Figure 2a). Scanning electron microscopy of the ventral region revealed a sparce distribution of the denticles throughout the oral epithelium (Figure 2b). The denticles were distributed with little overlapping each other, with irregular spaces (Figure 2b,c). They were tricuspid, with the central cuspid being more pronounced, pointed, and triangular in shape compared with lateral ones. Four to five fine ridges were identified on the crown of oral denticles of ventral region (Figure 2f).

In dorsal region of oral cavity, the denticles were heavily distributed in anterior regions (Figure 2h). The overlapping observed in denticles may be a result of sample preservation (Figure 2i). In other regions, the denticles were found with little overlapping (Figure 2i). They were also tricuspid, with the central cuspid being more pronounced, pointed, and triangular in shape. Three ridges were identified on the crown, with the median ridge being the most prominent (Figure 2h,i).

4 | DISCUSSION

Our findings demonstrate substantial variation in morphological structure of the denticles of the body and oral cavity of sharpnose

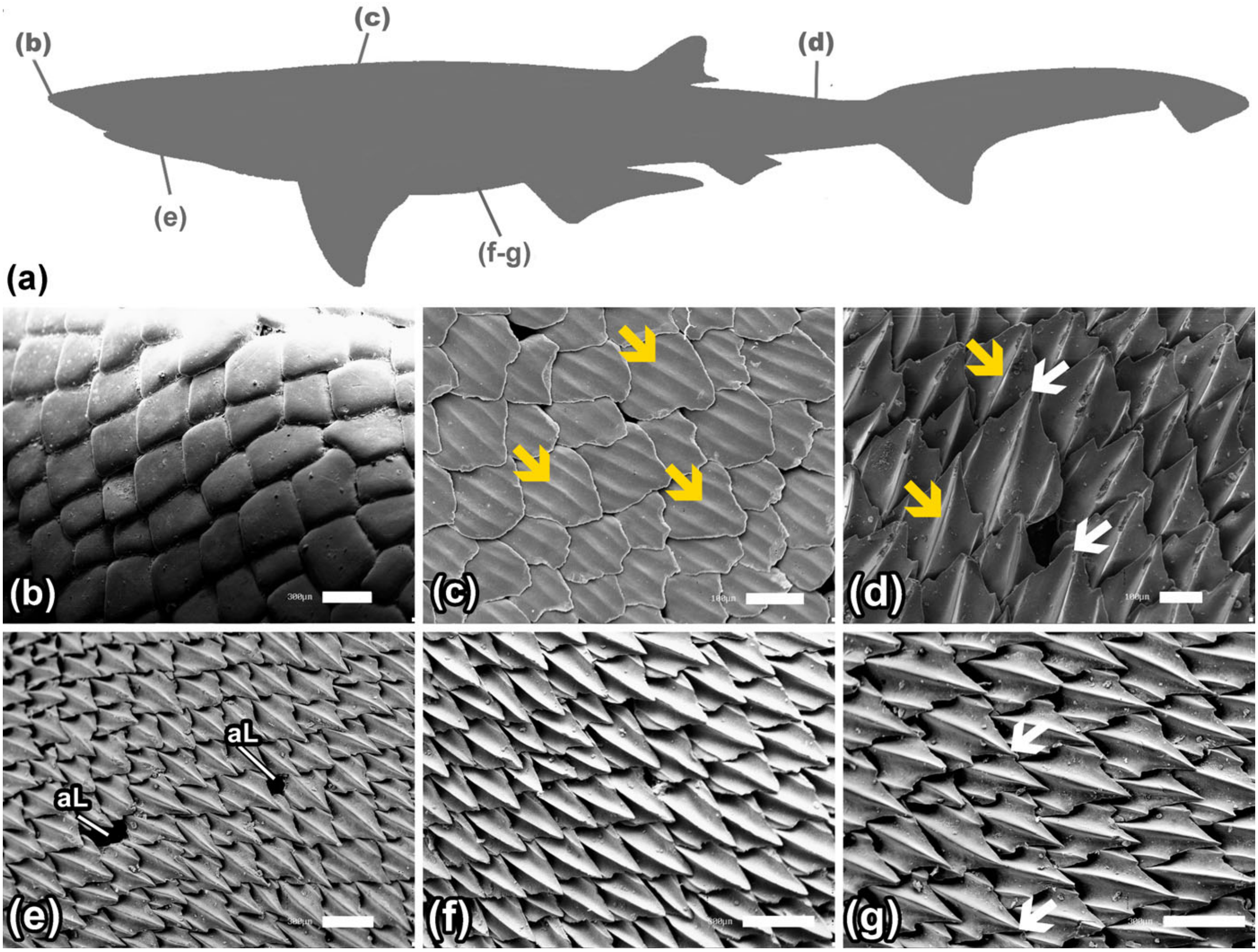

FIGURE 1 (a) Body regions of sharpnose sevengill shark *Heptranchias perlo*. In scanning electron microscopy, dermal denticles of the (b) tip of the nose, (c) Doral anterior, (d) dorsal central, (e) ventral anterior, showing the ampullae of Lorenzini (aL), and (f and g) ventral central region. Dermal denticles of some regions of dorsal and all of ventral region were found with cuspid (write arrow) and with ridges on the crown (yellow arrow). Scale bars: (b, e, f, g) 300 μm; (c, d) 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

sevengill shark, *H. perlo*. Such morphological diversity being body-region-dependent highlights the need for comparative studies that include different regions (Ankhelyi et al., 2018). While the denticles on the ventral and dorsal (central and posterior) regions of the body were more similar to each other, the oral denticles and those found on the nose and dorsal anterior regions had more a distinct shape and arrangement. These results suggest a strict functional relationship with the morphological characteristics observed.

The dermal denticle morphology has been linked to the ecological traits of the shark, consequently with the specific functions on the shark's body (Dillon et al., 2017; Raschi & Musick, 1986; Raschi & Tabit, 1992). Therefore, several functional morphotypes are observed across the body, such as protection or drag reduction (e.g., Raschi & Tabit, 1992). Among the functional morphotypes observed in sharks (i.e., drag reduction, abrasion strength, ridged abrasion strength, defense, luminescence, and generalized functions), sharpnose sevengill sharks have been classified as having generalized functions (Dillon et al., 2017). However, by observing other regions, it was possible to identify specific functional morphotypes, such as abrasion strength found on the tip of the nose, probably providing protection during feeding. This morphotype is commonly observed in other sharks in these regions (e.g., *Mustelus canis*, Ankhelyi et al., 2018). Similarly, the denticles found in the dorsal anterior region were similar to the described ridged abrasion strength denticles (Dillon et al., 2017).

The morphology of denticles of the other regions analyzed (i.e., tricuspid with ridges in the crown) were similar to those described to Bigelow and Schroeder (1948), and the functional morphotype seems to be more related to hydrodynamic drag reduction (Dillon et al., 2017). The ridges present on the crown surface are known to improve hydrodynamic efficiency, reducing turbulence as water flows around the body (e.g., Dillon et al., 2017; Raschi & Musick, 1986). Despite this small shark being a demersal species, it has a wide distribution (i.e., 27–1,000 m, Ebert & Stehmann, 2013), indicating that it is a strong swimmer and that this morphotype is consistent with its vertical movement behavior.

Although denticles are strongly related to the shark ecology, some studies have shown that phylogeny may have an important role in the

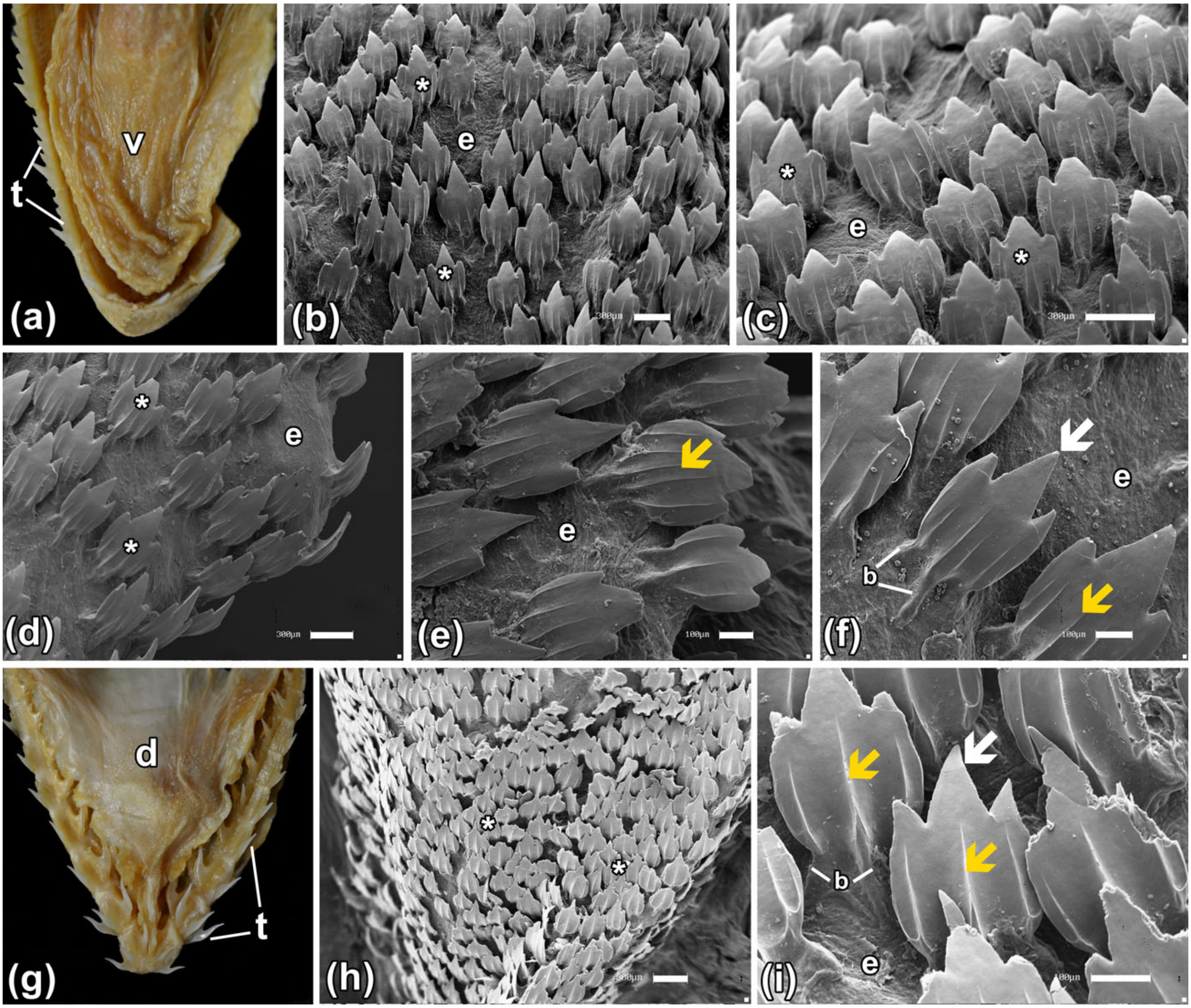

FIGURE 2 Oral cavity of sharpnose sevengill shark *Heptranchias perlo*. (a) Jaw with teeth (t) and ventral region of oral cavity (v). In scanning electron microscopy (b-f) oral denticles (*) distributed with little overlapping by epithelium (e) of ventral regions. (g) Jaw with teeth (t) and dorsal region of oral cavity (d). (h and i) in scanning electron microscopy oral denticles (*) distributed with more overlapping by epithelium of dorsal regions. All oral denticles were tricuspid, with the central cuspid being more pronounced (write arrow) with four to five fine ridges on the crown (yellow arrow) of ventral region and three ridges on the crown of dorsal region that extending from the base (b). Scale bars: (b, c, d, h) 300 μm; (e, f, i) 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

morphological diversity found (e.g., Mello, de Carvalho, & Brito, 2013). This is evident in the microstructures found on the crown, which are more specialized in sharks of more derived phylogenetic position (i.e., sphyrnids; Mello et al., 2013; Rangel et al., 2019). Therefore, the absence of these microstructures linked to hydrodynamics (e.g., ornaments on the crown) may be related to phylogeny; likely because the sharpnose sevengill shark belongs to an ancestral family of sharks (i.e., Hexanchidae), with evolutionary divergence estimated 82 million years (Tanaka et al., 2013).

While dermal denticles are relatively well known, only recent studies have investigated the morphology and distribution of oral denticles (e.g., Atkinson & Collin, 2012; Rangel et al., 2019). The oral denticles of sharpnose sevengill sharks were completely distinct from dermal denticles around the body. Similarly, the oral denticles seems to have a functional morphotype for drag reduction. This morphotype has been described in other shark species, such as *Prionace glauca* (Rangel et al., 2017), *Isurus oxyrinchus*, *Alopias superciliosus*, *Sphyrna* spp. (Rangel et al., 2019), *Rhizoprionodon lalandii* (Ciena et al., 2016), and some batoid species (e.g., *Zapteryx brevirostris*, Rangel et al., 2016; *Aptychotrema rostrata*, Atkinson & Collin, 2012). The reduction of hydrodynamic drag may be mainly beneficial for pelagic sharks, including sharpnose sevengill, which have RAM ventilation (i.e., the water flows through the mouth during swimming; Atkinson & Collin, 2012; Rangel et al., 2019). Additionally, other functions of oral denticles have been suggested, including protection of the oral epithelium from parasites and injury caused by food items, and to facilitate food retention (Atkinson & Collin, 2012; Rangel et al., 2016).

In summary, our results showed that, as seen in other species (e.g., Ankhelyi et al., 2018), the denticles vary considerably throughout the body, including the oral cavity. Considering the great potential of the use of denticles as a tool for taxonomic and ecological studies (e.g., Dillon et al., 2017; Mello et al., 2013), understanding how such variation is species-specific is essential, especially because the intraspecific variation in dermal and oral denticles were body-region-dependent. In addition, it is strongly recommended to include such morphological descriptions of oral denticles, since this structure have an important functional role in sharks and can be found in fossil and recent records, as well dermal denticles from other body regions (e.g., lateral regions).

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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