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# Morphological Description of Dermic Denticles of Guitar Ray (*Pseudobatos horkelli*)

***Luana Félix de Melo[1]*; Bruna Grandisoli Andrade[2]; Jessica Ferreira de Souza[2]; Marcelly Giovanna Silva[1]; Helder Telles Stapait[3]; Edris Queiroz Lopes[4]; Rose Eli Grassi Rici[1]***

*[1]Department of Surgery, VCI, Postgraduate Program in Anatomy of Domestic and Wild Animals, Faculty of Veterinary Medicine and Animal Science FMVZ, University of São Paulo USP. São Paulo, SP, Brazil.*

*[2]Faculty of Biological Sciences, University City of São Paulo, UNICID, São Paulo, SP, Brazil.*

*[3]Postgraduate Program and Research in Environmental Management, University of the West of São Paulo, UNOESTE, Presidente Prudente, SP, Brazil.*

*[4]Institute of Marine Biology and Environment, IBIMM, Santa Cruz das Palmeiras, SP, Brazil.*

***Corresponding Author(s): Luana Félix de Melo**

Department of Surgery, VCI, Postgraduate Program in Anatomy of Domestic and Wild Animals-Faculty of Veterinary Medicine and Animal Science FMVZ, University of São Paulo USP. São Paulo, SP, Brazil; Av. Professor. Orlando Marques de Paiva, 87 - Butantã, São Paulo - SP, 05508-010, Brazil.

Email: luanafelix@usp.br

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**Abstract**

Dermal denticles or placoid scales can be classified as tooth-like structures that cover the body of the elasmobranchs. Which gives them a rough skin. However, if your skin were devoid of these structural dermal elements, that is, a smooth surface, this would cause turbulence, which would lead to a deficiency in your swimming, ability to protect yourself from biofouling and drag caused by swimming. The denticles are better preserved in fossil records than other structures. Cartilaginous skeletons degrade easily and quickly, the denticles resist this decomposition and, therefore, they become, in addition to being a valuable historical record, usable for possible phylogenetic identifications. For taxonomic purposes, its use is not very common due to the little existing study, so the objective was to analyze and describe the morphological characteristics of the dermal denticles of the viola streak through the scanning electron microscopy technique, and a greater predominance of crown denticles was observed smooth, thus providing greater physical protection than hydrodynamic protection.

**Keywords:** Bathoidea; Elasmobranchs; Placoid squama.

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## Introduction

The Chodrinchtyes, known as cartilaginous fish are formed by two subclasses: Holocephali represented by chimeras and Elasmobranchii represented by sharks and rays [1]. The guitar ray (*Pseudobatos horkelli*), belonging to the Pseudobatidae family, is an endemic species in Brazil and is present on the IUCN list of endangered animals due to the excess of its capture as by-catch fauna in trawling, occurring largely in the Southeast region and southern Brazil [2-4].

The guitar ray has a flat dorsal body and well-developed pectoral fins fused to the head forming a single contour, dorsal and caudal fin, five pairs of branchial slits present in the animal's belly [5]. The body has an anterior region in the form of a subtriangular disc, rigid snout with a long rostral cartilage with transparent sides, large eyes close to the spiracles and a row of dorsal spines of medium size, starting behind the eyes and ending between the dorsal fins. The long, broad tail is not demarcated from the rest of the body, with well-developed dorsal and caudal fins, a uniformly olive gray or brown back [6,7].

The external surface of the body is covered by placoid scales [1], structures similar to teeth, composed of a calcified base and a protrusion, the crown, covered by a layer of dentin and enamel [8], has its origin in the ectoderm [9], they accommodate bioluminescent and sensory organs, have a variety of functions such as: mechanical and biological protection, abrasion resistance and protection against parasites, friction reduction, aiding in the animal's hydrodynamics and greater impulse [10,11]. They are well developed, independent and vary in morphology, distribution, and function for each species [12,13].

Dermal denticles present, in their morphology, distinct and specific characteristics that are highly influenced by the animal's life habits, and in the way they are expressed in functions such as hydrodynamics and protection [14,15]. These dermal structures are better preserved in fossil records as they resist decomposition [5] and, therefore, become a valuable historical record, and usable for possible phylogenetic identifications [16] and have been used as a tool to produce guides and taxonomic studies [2,17]. work is to analyze and describe the morphological characteristics of the dermal denticles in different regions of the viola streak through the technique of scanning electron microscopy.

## Material and methods

### Animals

Three (3) juvenile specimens of guitar ray (*Pseudobatos horkelli*) were donated by the Institute of Marine Biology and Environment - IBIMM, with authorization from SISBio: 005/2018, and BioCEUA IBIMM: 0005/2019.

### Processing

Tissues were fixed in 10% formalin for 48 hours, washed in distilled water in an ultrasonic device, stored in 70% alcohol overnight, dehydrated in ethanol (80%, 90% and 100%), according to the protocol described by Melo et al. [18].

### Scanning Electron Microscopy - SEM

The SEM analysis was performed to assess the Three-Dimensional (3D) structure of the dermal denticles. After dehydration, the samples were dried in a critical point apparatus with CO2LEICA EM CPD 300, glued with carbon glue on aluminum metallic bases (stub) and metallized ("sputting") with gold in the EMITECH K550 metallizing apparatus, and were analyzed and photodocumented in Scanning Electron Microscope (SEM) LEO 435VP, at the Advanced Diagnostic Imaging Center - CADI-FMVZ-USP. Melo et al. [19,20,18]describe the same procedure in samples from sea turtles and in elasmobranch organs.

## Results & discussion

The dermal denticles showed different morphologies according to the region and the function they play, and although some regions have similar characteristics, it is possible to identify 04 groups of different morphologies, grouped from the modifications in the crown, such as expansion, cusps and keels (Figure 01A). The morphology of the denticles was presented as: denticles with a surface without keels and without the presence of cusp (Figure 01B), denticles with a smooth surface, without keels and with the presence of cusp (Figure 01C), denticles that present on their dorsal surface keels to the medial region and with the presence of a cusp (Figure 01D) and denticles that have keels along their entire length and with the presence of a cusp (Figure 01E) on their dorsal surface.

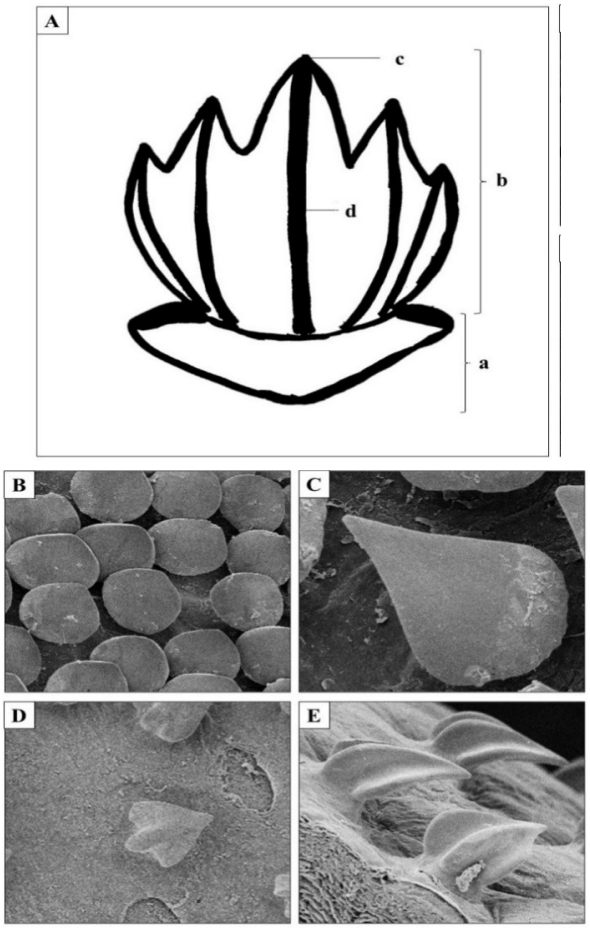


**Figure 1:** Schematic of dermal denticle morphology. In A: description of the topography of the denticle showing the base **(a)**, crown **(b)**, cusp **(c)** and keel **(d)**. In B: denticle without keel and without cusp, C: denticle without keel and with cusp, D: denticle with keel and without cusp and E: denticle with keel and with cusp.

Source: Adapted from Reif (1985) and Thies (1995) [23,28].

The same pattern adopted for the morphological criteria of the denticles was used by Johns and group [21], in studies with ichthyoliths and by Oliveira et al. [22] in studies with sharks, these standardizations being adapted from the works of Reif [23].

Pectoral fin microscopy (Figure 02I) can observe denticles with a more dispersed distribution, without covering the entire surface, enabling the visualization of the epidermis. When observed in its distal margin, the denticles present in the pectoral fin present modifications in the crown, when compared to the dorsal and ventral surfaces. The crown has a leaf shape, without the presence of keels on the ventral surface of the fin (Figure 02 IA) and with the presence of keels on its dorsal surface, the main and most developed keel being medially positioned, with a single crown facing the caudal region. Of the animal, elevated at an angle of 45° in relation to the epidermis (Figure 02 IABC). In the pelvic fins, both on the dorsal and ventral sides (Figure 02 II), first dorsal fin (Figure 02 III) and second dorsal fin (Figure 02 IV), the denticles presented a wide expansion of the crown, leaving its rounded shape, without the presence of keels and presenting cusp, with uniform distribution and overlapping of the denticles, covering the entire surface of the epidermis (Figure 02 IIABC; IIIABC; IVABC). In the caudal fin (Figure 02V) the denticles have an irregular and well-spaced distribution, with a clear visualization of the epidermis and denticles with a crown without keels and shorter expansion in relation to the regions analyzed previously, thus Giving the triangular shape of an arrowhead, with presence of a pointed cusp facing the animal's caudal region (Figure 02 VABC).

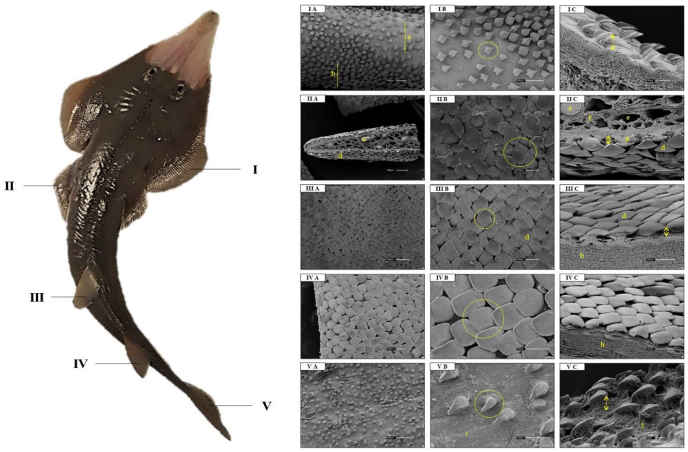


**Figure 2:** Photomacrography of P. horkelli in dorsal view, indicating the regions where the samples were collected and their respective photomicrographs by scanning electron microscopy showing in circular highlight the morphologies of the denticles in each region. In I ABC pectoral fin, II ABC pelvic fin, III ABC first dorsal fin, IV ABC second dorsal fin and V ABC caudal fin. The dorsal aspect of the fin **(a)**, ventral aspect of the fin **(b)**, circular muscle bundle **(c)**, dermal denticle **(d)**, spaces belonging to the bundles of muscle fibers **(e)**, extracellular matrix **(f)** are evident. central cavity and pulp of the denticle **(g)**, muscle layer with rectangular fiber bundles **(h)**, epidermis *(i)*, protective mucus surrounding the animal **(j)**, height of the angles of the denticles (dashed arrows).

Rangel et al. [15], describe the rostrum region of the Heptranchias perlo shark with denticles presenting a smooth crown, with rounded edges, being compact and overlapping, similar the findings found in three of the five fins of P. horkelli, differing only in the two ends, pectoral, and caudal fin, while Bueno [24] describes the presence of keels and cusps in the denticles in all fins of Rhizoprionodon lalandii. Although they present different topographies between the species, both sharks and the ray viola have overlapping denticles, without exposing the epithelium, which is facilitated by the lateral laterally expansion of the anchor, leaving the denticle wider and with a rounded shape, which helps in protecting these animals from abrasion and fixation of ectoparasites as well as predation [11].

Furthermore, it can be stated that as their orientations are rostro-caudal, the denticles are adjusted to the hydrodynamic flow, as described by Reif [25], Raschi and Musick [26].

When analyzing the rostral region of the viola streak (Figure 03I), both on the dorsal and ventral sides, it was possible to observe the presence of spaced denticles with exposure of the epidermis and crown without the presence of keels, with a pointed cusp facing the tail region of the animal and little lateral laterally expansion of the crown, giving the shape of an arrowhead to the denticle (Figure 03I ABC). In the dorsal cephalothoracic region (Figure 03 II) the denticles appear in greater quantity, in relation to its more rostral portion, but still with exposure of the epidermis. The crown is turned caudally with an elevation of 70° in relation to the epithelium, with the presence of keels all over the surface, with variations from 3 to 5, with the medial being the most developed, with a single pointed cusp ending, giving the shape from leaf to denticle (Figure 03 ABC).

In the animal's dorsal abdominal region (Figure 03 III) the denticles had the same distribution as the cephalothoracic region, also with the exposition of the epidermis, but with a smooth surface crown without the presence of keels, with a single and pointed cusp, with an angle of 45° in relation to the epidermis and variation of the lateral laterally expansion of the crown, giving the denticles cordiform and leaf shapes (Figure 03III ABC). In the dorsal caudal region (Figure 03IV), the denticles appear in greater quantity, with little exposure of the epithelium, crown with a smooth surface without the presence of keels, greater lateral laterally expansion of the crown and with only a few denticles showing cusp, giving the circular shape, aiding in the lining of the epithelium, with overlapping denticles (Figure IVABC).

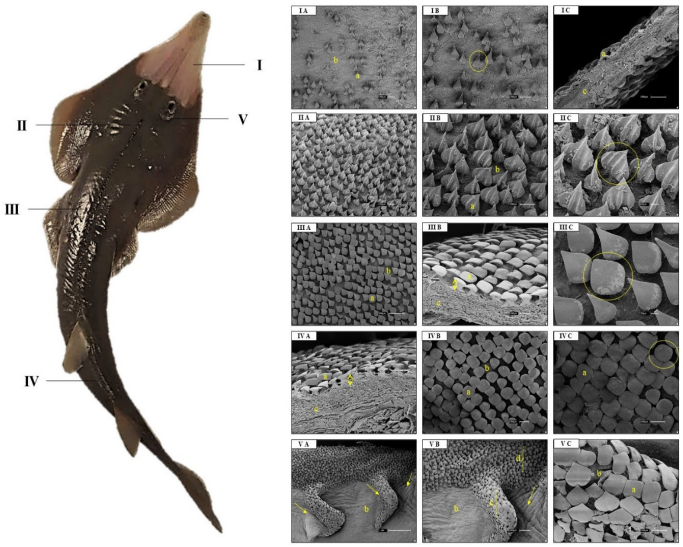


**Figure 3:** Photomacrography of P. horkelli in dorsal view, indicating the regions where the samples were collected and their respective photomicrographs by scanning electron microscopy, highlighting the morphology of the denticles in each region in circular highlights. In I ABC rostral region, II ABC cephalothoracic region on dorsal side, III ABC abdominal region on dorsal side, IV ABC caudal region on dorsal side and V ABC spiracle. The dermal denticle **(a)**, epithelium **(b)**, muscular layer with rectangular bundles **(c)**, margin of the spiracle **(d)**, height of the angles of the denticles (dashed arrows) and peduncle of the spiracle (arrow) are evident.

The spiracle, also known as the respiratory orifice, is positioned laterally to the eyes, with the function of entering water up to the gill chamber, for gas exchange carried out by the gills, which is, breathing. Thus, the denticles were similar those

found in the cephalothoracic region on its margin, with changes in its topography as they approached the spiracle, with its interior completely smooth, without the presence of denticles, and with the presence of spiracle peduncle. With surface covered by denticles with smooth crown, single cusp and cordiform shape (Figure 03V ABC).

The morphology found in the dorsal denticles is in accordance with their function. With greater prominence of the cusp and narrowing of the expansion of the crown, it allows the rapid passage of water. With greater dimension laterally-laterally, dorsoventrally and crown with cusps, it allows current breakage, propelling the animal forward, thus allowing greater speed, as described by Raschi and Musick [10] and Reif [25], corroborating works carried out with sharks, which are described in the animal's dorsal region with the presence of a crown with three keels and three cusps [24] and five keels and a single cusp [15]. However, Bueno [24] describes the dorsal surface of striated crowns that connect in a pattern of hexagonal shapes that was not visualized in our findings. The presence of fins contributes to greater stability of movement, increasing its hydrodynamic efficiency [27].

In the other regions of the back of the ray, the denticles have no cusps on their surface, thus providing a less functional aspect to the hydrodynamics, and greater protection function [22].

When analyzing the animal ventrally, a white color and smoother appearance to the touch and the presence of dermal denticles can be seen. In the nasal cavity, which includes the olfactory rosette, there is a nasal peduncle (Figure 04 I), with an external surface covered by cordiform denticles, without the presence of keels on the crown surface, some have cusp and rounded edges facing the interior of the cavity. Nasal, crown with lateral laterally expansion filling the epithelium and overlapping between the denticles. On the inner surface of the nasal peduncle, the presence of dermal denticle was not observed (Figure 04 IABC).

In the region close to the oral cavity of the ray, pre-oral grooves can be observed on both sides (Figure 04 II), with the presence of denticles, without changes in the crown, without keels and cusps on its margin, thus giving the circular shape (Figure 04 IIABC). In the region of the branchial clefts, the denticles have a smooth surface crown, single and prominent cusp facing the interior of the branchial chamber, spaced distribution (Figure 04 III ABC).

In the animal's ventral abdominal region, the distribution of denticles was observed, with a smooth surface crown without the presence of keels, some denticles with cusp, and with rounded margins, a crown with large lateral laterally expansion, rounded in shape, overlapping each other. Some denticles in this region show crown fusion (Figure 04 IVABC). In the caudal - ventral region, the denticles presented with a disarrangement, without uniformity, with a smooth surface crown without keels and a wide base with taper to the cusp, closely adhered to the epidermis, with a leaf shape (Figure 04 VABC).

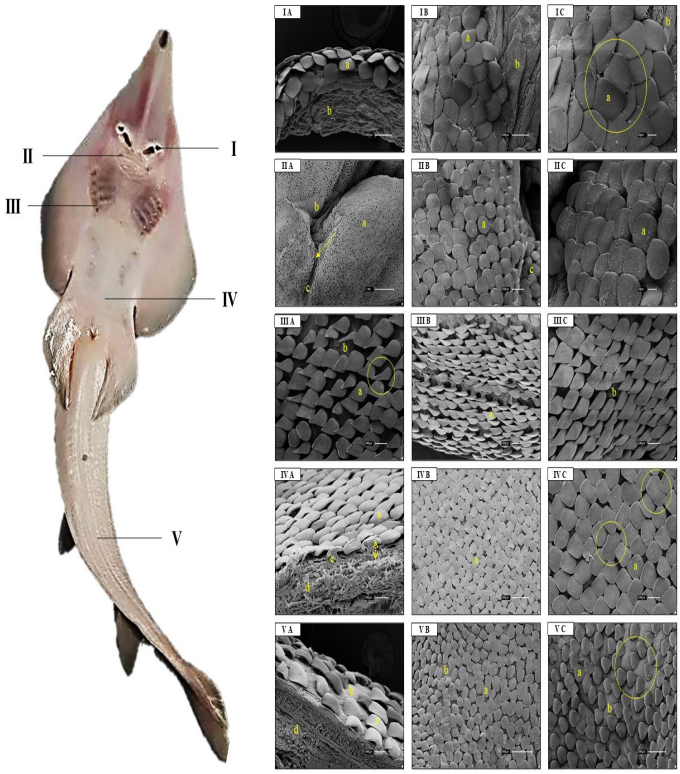


**Figure 4:** Photomacrography of P. horkelli in ventral view, indicating the regions where the samples were collected and their respective photomicrographs by scanning electron microscopy, highlighting the circular morphology of the denticles in each region. In I ABC nasal cavity, II ABC preoral region, III ABC region of the branchial clefts, IV ABC abdominal region ventral side and V ABC caudal region ventral side. The dermal denticle **(a)**, epithelium **(b)**, preoral sulcus **(c)**, muscular layer with rectangular bundles **(d)**, central cavity and pulp of the denticle **(e)** are evident.

Even the presence of cusps in the animal's ventral region are more related to the direction of water, the entry of water into the nasal cavity to bathe the olfactory rosette where the chemical receptors are located, during foraging, helping to capture food and during breathing, ingress and egress of water through the gill slits. With a more coastal life habit, in sandy to muddy substrates, the dermal denticles present a greater protective function than in hydrodynamic ones, in the same way as district in Nebrius and Ginglymostoma sharks, they present mostly the surface of the crown. smooth without fins[22].

## Conclusion

The SEM technique used provided a precise and clear analysis of the shape of the denticles, thus allowing to correlate the shape with its function. After this thorough analysis, we can suggest that the denticles can help in the identification of species. In guitar ray, the denticles of the regions analyzed by their shape showed to have a greater protection function in relation to the hydrodynamic function, characteristic of a species with coastal and benthic behavior of sandy and muddy substrate.

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