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RESEARCH ARTICLE

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Ecomorphological, space, and mineral relations of dermal denticles in angular angel shark (*Squatina guggenheim*)

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Abstract

Shark skin is predominantly specialized for swimming and protection, with the dermal denticle being the main structure associated with these abilities. The dermal denticle is a mineral structure with a unique morphology for each species, which allows its use as a taxonomic tool. Few studies have investigated the microscopy aspects of skin and dermal denticles, considering the high diversity of sharks. Here, we investigated the three-dimensional morphoquantitative aspects and mineral composition of dermal denticles in different regions of the angular angel shark, *Squatina guggenheim*, using scanning electron microscopy and dispersive energy system. With the microscopy, we were able to observe that the dermal denticle morphology changes according to the area it is located. It was possible to describe the dermal denticles individually, from root to the crown, highlighting all of their individualities. Through the dispersive energy system, we showed the proportions of each mineral found in the denticle, by area, demonstrating the composition and the particularities of crown, body, and root, where whitlockite was described for the first time in elasmobranchs. In this way, the present study presented the specificities of the dermal denticles of *S. guggenheim*, as well sought to understand the different structure functions for the animal, thus assisting future research in animal morphology.

KEYWORDS

anatomy, elasmobranch, morphology, placoid scale, skin, structure

1 | INTRODUCTION

Some sharks have an excellent swimming ability, as well as various shapes that are consistent with their habits, making them highly diverse (Dillon, O'dea, & Norris, 2017). Their skin plays a fundamental role in locomotor activity as its texture and structural complexity play a direct role in hydrodynamics, fitting to each body area (Lauder et al., 2016; Motta, Habegger, Lang, Hueter, & Davis, 2012). The structures responsible for such complexity, the dermal denticles, are composed of a mineralized base and a dentine protrusion covered by an enamel cap with varied sizes and functions (Ankhelyi, Wainwright, & Lauder, 2018; Gillis, Alsema, & Criswell, 2017; Gravendeel, Van Neer, & Brinkhuizen, 2002). Dermal denticles are essential structures for sharks, as they are anchored throughout the dermis by collagen fibers (Sharpey fibers) (Meyer & Seegers, 2012). As these structures are related to ecological function, it is essential to understand how their variation occurs through the shark body (Dillon et al., 2017; Mello, De Carvalho, & Brito, 2013; Peach & Marshal, 2000; Raschi & Tabit, 1992).

Dermal denticles are the subject of several studies due to their functionality in sharks, including protection against predators and ectoparasites, hydrodynamic drag reduction, and accommodation of bioluminescent structures (Dillon et al., 2017; Kemp, 1999; Raschi & Tabit, 1992; Reif, 1985). Due to their superb preservation during the fossilization process, the denticles are commonly used as a tool in taxonomic identifications (Allentoft et al., 2012; Mello et al., 2013), and recently used in paleoecological studies to identify and reconstruct shark communities (Dillon et al., 2017, 2020). Although recent studies have assessed the morphology, taxonomy, and function of dermal denticles in active pelagic sharks, sedentary demersal species have been little studied (Ankhelyi et al., 2018; Dillon et al., 2017; Rangel, Amorim, Kfoury Jr, & Rici, 2019).

To better understand the diversity and morphological aspects in demersal sharks, here we investigated the angular angel shark, *Squatina guggenheim* (Marini, 1936). The angular angel shark is a small bodied size, demersal shark found in tropical and temperate waters, ranging from inshore down to 1,000 m (Compagno, 1984) and distributed from Rio de Janeiro, Brazil to Rawson, Argentina (Gomes, Signori, Gadig, & Santos, 2010; Vaz & Carvalho, 2013). Its population is currently declining, assessed as Endangered according to the World Conservation Union (IUCN) Red List (Oddone et al., 2019). Additionally, the fossil record of the primitive genus *Squatina* goes back to the Jurassic period (Cappetta, 1987), demonstrating the importance of this genus to morphological studies.

Given that the morphological aspects of dermal denticles (i.e., shape, size, and arrangement) are highly variable intra and inter-specifically, as well as correlated with shark lifestyle (Dillon et al., 2017), the present study investigated the three-dimensional morphoquantitative description and mineral composition of dermal denticles in different regions of angular angel sharks. Our findings will contribute descriptive and quantitative information on the dermal denticles of angel sharks, which can be used in future functional, ecological, and taxonomic studies.

2 | MATERIAL AND METHODS

Skin samples were obtained from four male adult angular angel sharks *S. guggenheim* (65–70 cm total length). The individuals were obtained by shrimp trawl fishing bycatch in Southern Brazil (23°–26°S and 44°–48°W, respectively), with the consent of IBAMA—SISBIO (research permit No. 46878-2). The specimens were frozen on board and subsequently fixed in a 2.5% paraformaldehyde solution until analysis. There were individuals donated from *Instituto de Pesca* to the Laboratory of Morphology, UNESP. Samples were obtained from four different body regions: (a) rostro, (b) medial, (c) caudal, and (d) pectoral fin (Figure 1).

2.1 | Scanning electron microscopy

Forty epithelial samples (2 cm^2) were used from the skin's dorsal and ventral surfaces (region divisions; Figure 1). Samples were fixed by modified Karnovsky (Reginato, Bolina, Watanabe, & Ciena, 2014). After fixation, half of the samples were washed in distilled water for

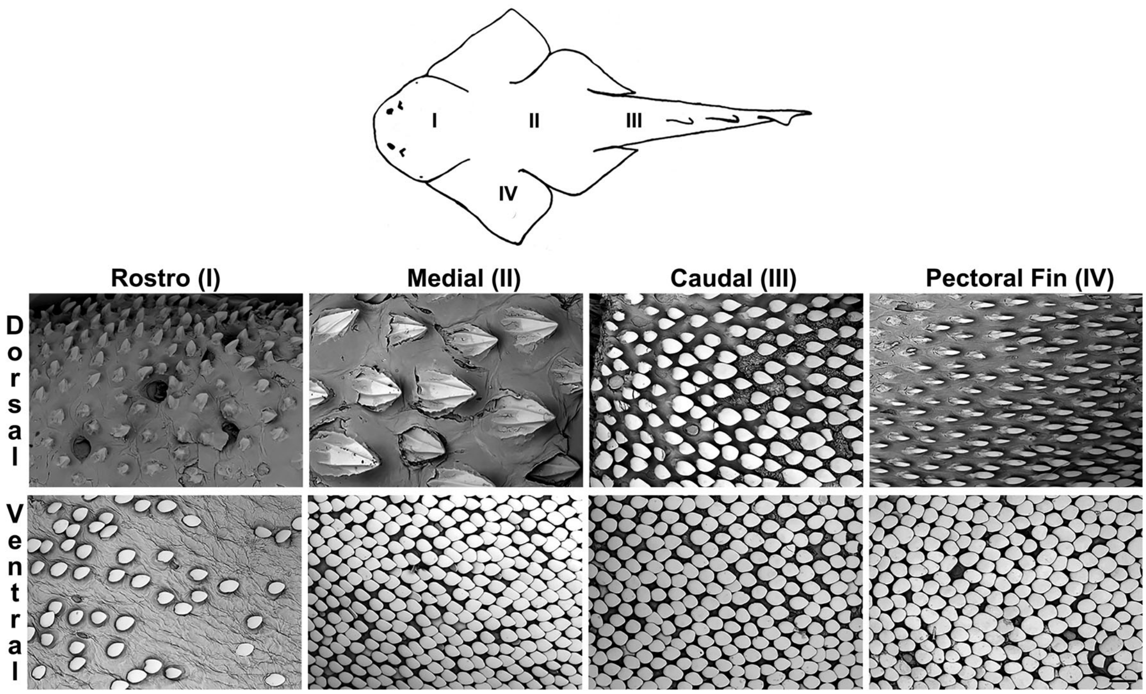

FIGURE 1 Body regions of angular angel sharks *Squatina guggenheim*. I—rostro, II—medial, III—caudal and IV—pectoral fin. Top row—dorsal region, down row—ventral region. Macro view of the skin, with dermal denticles presented in varied distribution and density according to each region. Bar—400 μm

4 hr for epithelial surface analysis and morphometric analysis. The other samples were submitted to the maceration technique which consisted of immersion in 10% aqueous sodium hydroxide (NaOH) solution for 4 days at room temperature (21°C) for the total removal of the superficial epithelium (Ciena et al., 2013) followed by energy dispersive system analysis of dentin mineral composition. After these procedures, all samples were dehydrated in an increasing series of alcohols, then dried in a critical point apparatus with liquid CO_2 (Balzers CPD-030). The samples were metalized with gold ions (Balzers—040 SDC) then examined by Scanning Electron Microscopy Kitochi—UNESP—Rio Claro.

2.2 | Energy dispersive system

The qualitative and semi-quantitative composition of the dermal denticle samples from the respective regions was determined from the characteristic x-ray emission, performed by scanning electron microscopy, allowing for structural identification at specific points of the image. The x-rays were emitted by an electron beam, and the bombarded regions were measured. Each element has a particular number of atoms so, it was possible to identify them. A conventional spectrometer detected x-rays. The model used was Zeiss DSM 940 with an Oxford Instrument system, coupled with the scanning electron microscopy, allowing for x-ray microanalysis.

2.3 | Morphometric analysis

The ImageJ software was used to generate a random delimitation of squares with 1 μm^2 (Figure 2c) in scanning electron microscopy images (Figure 2c). All dentures within the square were counted, including all those found at the edges (i.e., denticles touching the border and those were outside for their large part). The angle of denticles were stipulated using the pattern 0°–45° if the denticle were parallel to the skin, 45°–90° if the apex of the denticle were not parallel to skin, and 90° if the apex of the denticle pointed to the top. Using GraphPad Prism 7.0 Software, the mean and *SD* for length and density were made. The measure of maximal length was done after set the scale, with the command analyze > measure, it was measured the maximal length from the crown (CL) (Figure 2c). The student's *t*-test was performed to compare the dorsal and ventral regions, both in density and length ($p < .05$).

3 | RESULTS

The dermal denticles differed morphologically according to body region, mainly when compared between the ventral and dorsal regions (Figure 1). Dorsal denticles presented a spear-shaped crown with 3–5 faces that join in cusps, showing ridges, which consolidate in a single point. Denticles in the ventral region were leaf-shaped, with only a smooth face. In both dorsal and ventral denticles, there were variations in slope, size and density (Figure 2), and mineral composition according to the body region localization (Figure 3). Still, all of them presented a rostrocaudal direction.

In the dorsal rostral region, the denticles had an angle close to 90° of inclination, with 0.225 μm in length and 5.6 denticles per mm^2, not overlapping each other. It was possible to observe the delimitation between the denticles without being able to identify the root. In the ventral rostral region, the denticles have a 45° inclination, 0.265 μm long and 3.8 denticles per mm^2, with random distribution and not overlapping each other. In comparison, the ventral denticle length was higher than the dorsal ($p < .001$), while the density was higher in the dorsal region ($p < .05$).

The dorsal denticles had a spear shape, with four faces, an angulation between 45° and 90°, an average size of 0.709 μm, and a density of 3.2 denticles per mm^2. Although they did not overlap, they were closer, and it was not possible to observe clearly defined areas. The ventral denticles had a circular leaf shape, with an angle that makes them close to the epidermis. In the ventral region, the denticles overlapped each other. It was not possible to see the denticular body as this region presented the highest concentrations of denticles on the animal, with 22 denticles per mm^2 and an average size of 0.259 μm in length. Dorsal and ventral areas showed statistical differences; the

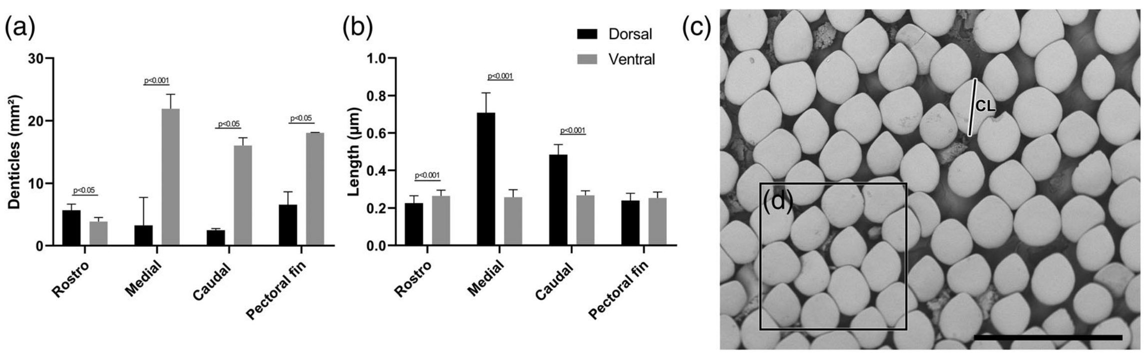

FIGURE 2 (a) Mean ± *SD* of denticular density graph (mm^2) in the angel shark *Squatina guggenheim*, from dorsal and ventral regions. (b) Mean ± *SD* of denticular length (μm) from dorsal and ventral regions. (c) Image of ventral denticle showing methodology of measures. CL—crown length; (d)—density square with 1 mm^2. Bar—1 mm

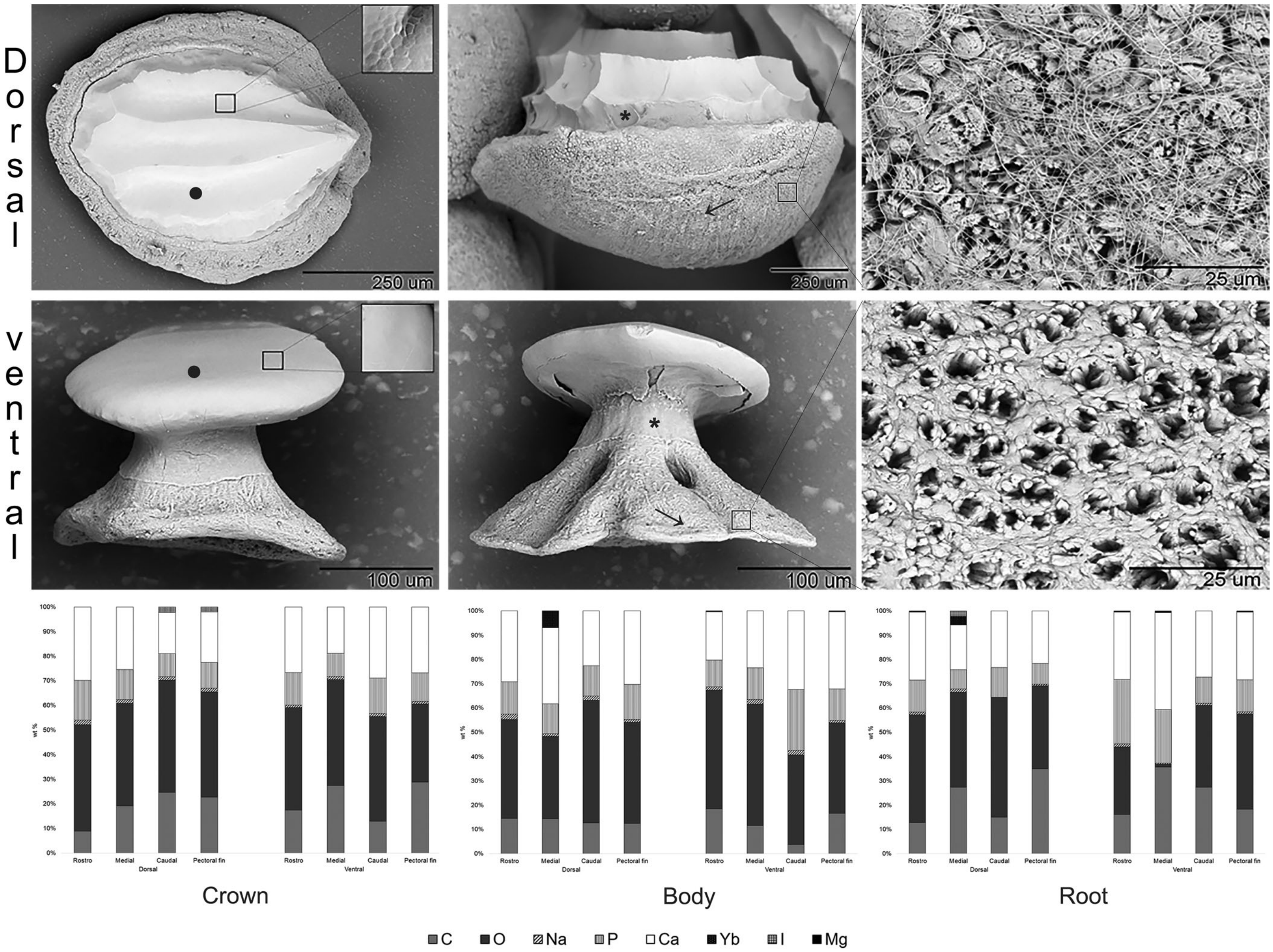

FIGURE 3 Macerated dermal denticles, presenting their diversity of shape and structures in angel shark *Squatina guggenheim*. From dorsal region: upper crown view, with emphasis on the surface texture; in lateral body view, showing from the root to the apex of denticle; and the denticular base (root) showing the entangled mineralized formed for fixation. From ventral region: upper crown view, we observed the denticle with emphasis in the texture; in lateral body view, showing from the root to the apex of the ventral dermal denticle; and the denticular base (root) showing the irregular surface. ●—crown; *—body; ↑—root; □—demonstrates root area represented beside. Means of the mass fraction, expressed as percentage by weight (Wt%), from dorsal and ventral of each area. (C) Carbon; (O) Oxygen; (Na) Sodium; (P) Phosphorus; (Ca) Calcium; (Yb) Ytterbium; (I) Iodine; (Mg) Magnesium

dorsal length was bigger ($p < .001$), whereas the ventral area was denser ($p < .001$).

In the caudal region, dorsal view, there was a morphological similarity with the dorsal region in both inclination and positioning. There was no overlapping of denticles and their delimitation; however, it was possible to observe the denticular body partially. They had an average size of 0.484 μm and a density of 2.49 denticles per mm^2. In the ventral view, it was possible to observe a small denticular overlap, with leaf-shaped denticles with a rounded base and only a smooth face; however, there was a high density, 16 denticles per mm^2, with a size of 0.268 μm. Ventral denticle density was higher ($p < .05$), whereas dorsal denticles were larger ($p < .001$).

The denticles in the dorsal region of pectoral fin were spear-shaped, with 2–3 faces, an average length of 0.24 μm and a density of 6.58 denticles per mm^2. As in the region of the rostro, the denticles did not overlap, making it possible to observe the delimitation between the denticles without exposing the root. In contrast, the denticles of the pectoral region in the ventral view were similar in size, 0.25 μm, but with a bigger concentration, 18 mm^2, with denticular overlap. It was not possible to observe delimited areas of the root or the denticular body. Ventral denticles had higher density than dorsal denticles ($p < .05$).

After the maceration process, it was possible to identify three layers that make up the denticle: crown, body, and denticular root (Figure 3). The dorsal and ventral denticles had several anatomical differences. The crown of the dorsal denticles consisted of 3 to 5 faces, which joined in cusps that converged to a single point in the anterior region of the denticle, evidencing the denticle's spear shape. The surface of the faces was smooth but had a geometric formation. The crown was polished in the ventral denticles; therefore, it was smooth with a leaf shape, presenting a single face. It may show variation in format, oscillating between angular leaf and round leaf.

Below the crown, there was the region of the denticular body. The denticular body has different thicknesses, varying with the body region and their crown exposure. In dorsal denticles, the denticular body presented several faces supporting the crown, which had the same shape, the thickness also varying with the region, leaving the crown more or less exposed, as well as assisting in the angle of fixation. In the ventral region, the denticular body presented narrowing structures, and angle variation, influencing the projection of the crown according to the localized region.

The root of the denticle was below the epithelium, which had a convex shape. In the dorsal region, we observed cylindrical projections surrounded by irregular saccules, vesicles, and empty spaces and the presence of a tangled cylindrical mineral fiber structure overlapping these projections. In the ventral region, the surface was irregular, with grooves and deep empty places with a rounded shape arranged without a defined organization. In the region closest to the epidermis, there was the formation of a plateau that gave origin and support to the denticular body. In its structure, it was possible to observe foramina of different sizes located anterolaterally, usually found in pairs; however, the one at the base varies its position but communicates with the others.

In the mineral analysis (Figure 3), the found compounds were compatible with dentin's presence; therefore, hydroxyapatites, just like enameloid tissue, formed by bioapatite a group of phosphate minerals. Thus, in different concentrations for dorsal and ventral, carbon, oxygen, sodium, phosphorus, calcium, ytterbium, iodine, and magnesium were observed.

In the both dorsal and ventral denticle crowns, the formation of dentin and enamel was predominant. In the dorsal dermal denticles, there was a presence of whitlockite demonstrated by the magnesium, in the regions of the rostrum (0.35%) and pectoral fin (0.38%). In contrast, the medial region of the ventral iterbium was observed. For the root of the denticle, in the dorsal region, there are signs of whitlockite in both the rostrum, medial, and pectoral fin regions, composing the denticular weight at 0.5%, 0.35% and 0.45% of magnesium respectively. In comparison, in the ventral region, only the rostrum presented the proportion of 0.49% of magnesium; however, both ytterbium and iodine were observed in the medial region, at 3.57% and 2.09% respectively.

4 | DISCUSSION

In this study, we demonstrated that the dermal denticles of *S. guggenheim* aimed towards protection and defense, with variations in shape, length, and distribution throughout the body, highlighted by the differences between the dorsal and ventral regions of the body. Through the maceration process, it was possible to clearly observe the dermal denticles from crown to root in its specificities by region and its mineral composition, with elements previously seen only in chimeras, such as whitlockite (Ishiyama, Sasagawa, & Akai, 1984; Smith, Underwood, Goral, Healy, & Johanson, 2019), while observing the different mineral proportions that composes it. Thus, the present study quantified and qualified the dermal denticles, associating them to shark ecology and providing valuable information about their structural and composition.

The denticles found in *S. guggenheim* presented variation in the crown shape according to the region, suggesting that their morphology is related to ecological aspects (e.g., Dillon et al., 2017; Rangel et al., 2019). We found two predominant functional morphotypes, following the denticle's classification proposed by Dillon et al. (2017). The morphology of dorsal denticles indicates a defense mechanism against predators and ectoparasites, while the ventral denticles were predominantly for resistance to abrasion (i.e., large, thick, unridged denticles with a single rounded peak). The dorsal denticles found here were very similar to those previous described by Vaz and Carvalho (2013) in a taxonomic revision of specie. Additionally, as showed in other species of genus *Squatina* (Raschi & Tabit, 1992), *S. guggenheim* presented denticles with primarily protective function. These functional morphotypes are probably related to lifestyle of *S. guggenheim*. Despite being a shark, *S. guggenheim* has many characteristics similar to the batoids, for example, is a sit-and-wait predator, with a demersal lifestyle and demonstrates a sedentary behavior (Gomes et al., 2010; Vaz & Carvalho, 2013).

The different densities and crown sizes observed in the dermal denticles throughout the body seem to corroborate our findings for different functional morphotypes. For example, smaller denticles but in higher densities found in the ventral region may confer a greater protection from abrasion, since these parts of the body are in constant contact with the marine substrate. On the other hand, larger crown sizes but in lower densities maybe confer more resistance (Raschi & Tabit, 1992). Future studies investigating the relationship between morphotypes and denticle density across the shark's body would help test these hypotheses. In addition, as dermal denticle density can be sexually dimorphic, especially in the body regions related with mating events (Crooks, Babey, Haddon, Love, & Waring, 2013), a comparison between males and females could bring additional insights into how the evolutionary process would be related with dermal denticle morphology.

Our results also demonstrated that the denticles' difference was not restricted to the distribution or the morphology of the crown. Still, the root has a significant difference since the dorsal region presents tangled mineralized threads that allow micro-movements, without losing anchorage in the dermis. Several studies have been investigating the hydrodynamics of elasmobranchs (Bechert, Bruse, Hage, & Meyer, 2000; Kemp, 1999; Lauder & Di Santo, 2016), including those describing the aerodynamic properties based on the design of denticles (Ott, Lazalde, & Gu, 2020), with fewer showing the anchoring of the dermal denticles in the dermis. Miyake, Vaglia, Taylor, and Hall (1999) demonstrated that, in rays, denticles are anchored in the deep dermal tissue by fixing the fibers of the denticles and tendons to the dorsal musculature, named an acro-protothercodonty (Gaengler & Metzler, 1992), but none showed the root shape's influence in anchoring. It is necessary to point out that among the dorsal denticles, classified mainly for defense, they present a greater area of contact with the dermis, through the convex shape of the root and the mineral tangle, which may be associated with the more malleable anchoring process to the movement, but without the loss of structure. Whereas, ventral denticles, therefore of protection, have a flatter root, but with greater lateral projection, in addition to the root with spaces and structures that allow it to remain close to the dermis.

It is known that dermal denticles and teeth of sharks are formed not just by dentin and enamel, but also by minerals (i.e., calcium, phosphorus, and hydroxyl), organic matter, water, collagen and non-collagen (Arnold & Gaengler, 2007; Kemp, 1984). However, we found other additional minerals, such as traces of magnesium and other minerals, consistent with whitlockite, a calcium phosphate so far only reported in the tooth plate of holocephalans (Chondrichthyes) (Johanson et al., 2020; Smith et al., 2019). Whitlockite has a comparable hardness of tooth enamel and has been associated with the hypermineralized portion of the tooth plate in holocephalans (Iijima & Ishiyama, 2020). Additionally, whitlockite is usually found in human kidney stones, and although it presents medium rigidity and porosity, its composition is similar to apatite rocks (Balaji & Menon, 1997). Despite not being found in large proportions, the presence of whitlockite in the dermal denticles of *S. guggenheim* suggests, for the first time, its involvement in the resistance of dermal denticles in a elasmobranch species.

The dermal denticles of *S. guggenheim* showed a chaotic network of dentinal tubules formed by tubules, saccules, vesicles, and mineral granules (visualized at the root only), similar to those found in the teeth of the holocephalan *Harriotta raleighana* (Smith et al., 2019). Also, it was observed the formation of oblique fiber connections, arranged in different ways between the dorsal and ventral regions, which can act as a support for trabecular dentin, just like mineralized dentin. Such conformation makes a dense and resistant structure with varied morphology, to resist wear and tear, preventing loss dentition (Johanson et al., 2020). Most studies are necessary to comprehend its role in dermal denticles and if it is a usual compound.

Marine animals are exposed continuously to metal influences, temperature and pH variation, and other temporal changes, which leads to a constant need for adaptation of these animals (Galvão, Rebelo, Guimarães, Torres, & Malm, 2009; Hofmann et al., 2011). Therefore, understanding the composition of the dermal denticles of sharks will help us understand climate and environmental changes (i.e., natural or human-induced). For example, Dziergwa et al. (2019) demonstrated that when inducing an increase in the water's pH, as a result of the effects of acidification in the ocean, there was corrosion of the denticles, modifying their shape and composition. Such changes would lead to a reduction in ecological efficiency, making the denticles less protective of the epidermis and reducing hydrodynamics due to increased drag given that the mineral structure of the denticles is susceptible to change to avoid loss of function across ontogeny and evolutionary process (Dillon et al., 2017; Marra et al., 2018; Sibert, Cramer, Hastings, & Norris, 2017). Further studies, including other shark species of different lifestyles, would reveled if the mineral composition presented here is characteristic of angel sharks or if it could be present in other sharks. Additionally, the study of fossilized denticles would reveal a temporal trend in mineral composition across the evolutionary process.

Based on our findings, we concluded that, despite being a shark, *S. guggenheim* has many morphological characteristics similar to the batoids, demonstrating the direct relationship between the dermal denticles' morphology and lifestyle. Additionally, dermal denticles presented singularities with dentition mineral composition, in addition to a highly specialized base in all the regions analyzed. The crown also showed specificities for each region, acting in an optimized way to guarantee the performance of all functions, with protection being the main one. The results from this study help us understand the morphological aspects related to the evolution and ecology of this primitive group within the class Chondrichthyes, and it was the first to report the presence of whitlockite in Elasmobranchii.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Adriano Polican Ciena, Marcela Coffacci de Lima Viliod, and Ii-Sei Watanabe, conceptualize and draw the project. Alberto Ferreira de Amorim, donated the samples. Lara Caetano Rocha, Adriano Polican Ciena and Marcela Coffacci de Lima Viliod, performed the tissue preparation (SEM). Adriano Polican Ciena and Lara Caetano Rocha, acquired the images. Adriano Polican Ciena, Bianca de Sousa Rangel, Ii-Sei Watanabe, and Marcela Coffacci de Lima Viliod, analyzed the images/results. Adriano Polican Ciena, and Marcela Coffacci de Lima Viliod, wrote the manuscript. Adriano Polican Ciena, Marcela Coffacci de Lima Viliod, Lara Caetano Rocha, Bianca de Sousa Rangel, Alberto Ferreira de Amorim, Ii-Sei Watanabe, Carlos Eduardo Malavasi Bruno, and Júlia Ferreira dos Santos Domingos discussed the results, conducted a critical review of the manuscript, and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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