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RESEARCH ARTICLE

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Thorns and dermal denticles of skates *Atlantoraja cyclophora* and *A. castelnaui*: Microscopic features and functional implications

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Abstract

Some batoid species are covered with dermal denticles (or placoid scales) that occasionally develop into thorns. In sexually mature males, sharp teeth and alar thorns found on the apex of the lateral disc are used to hold the female during copulation. This study set out to analyze microscopic features of modified dermal denticles and thorns and to investigate sexual dimorphism in *Atlantoraja cyclophora* and *A. castelnaui* species. Skin samples collected from areas covered with thorns were fixed in 10% formaldehyde, processed and analyzed using scanning electron microscopy. Alar thorn morphology varied within species, while caudal thorn, rostral and caudal dermal denticle morphology varied within and between species. These structures play an important role in the protection and reproduction of the species studied and constitute important taxonomic information, given they are often the only elements preserved in archaeological sites and fossil records.

KEYWORDS

eyespot skate, scanning electron microscopy, sexual dimorphism, spotback skate

1 | INTRODUCTION

Elasmobranchs (subclass Elasmobranchii; superorders Selachii and Batoidei) have evolved from placoderm ancestor about 350 million years ago, during the Devonian period. However, batoids (rays, skates, guitarfishes and sawfishes) have diverged from their shark relatives almost 180 million ago, during the Jurassic period (Lutton, George, Murrin, Fileti, & Callard, 2005).

Most batoids are benthic, feeding on crustaceans, polychaetes, clams and fish (Bigelow & Schroeder, 1953; Dean, Bizzarro, &

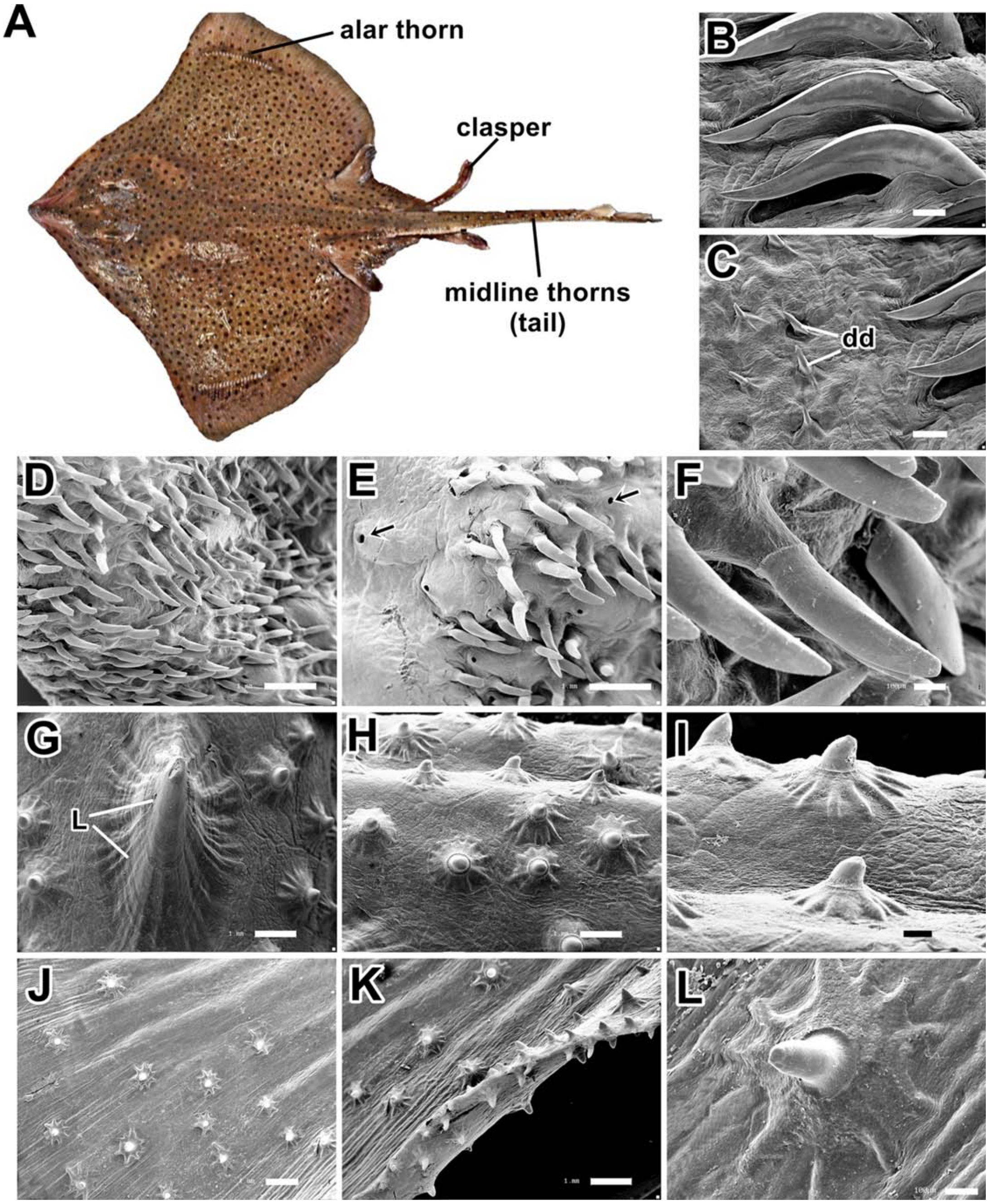

FIGURE 1 *Atlantoraja castelnaui*. (a) Macrograph showing different body regions. (b,c) Alar thorns and dermal denticles (dd) of sexually mature males. (d–f) Thorns (rostroventral area); note pore corresponding to Lorenzini ampulla (arrow). (g) Thorn (tail). Note growth rings (L) surrounding dermal denticles. (h,i) Dermal denticles (tail). (j–l) Dermal denticles (dorsal fin). Bar—(b, c, d, g, h, j, k): 1 mm; (E, I): 300 μm; (f, l): 100 μm. [Color figure can be viewed at wileyonlinelibrary.com.]

Summers, 2007; McEachran & Dunn, 1998). These fish have adapted to a large variety of habitats and live in oceans throughout the world, although they are more commonly found in shallow estuarine and coastal waters and in shelf seas up to 3000-m deep (Bigelow & Schroeder, 1953; Compagno, 1990; Mceachran & Aschliman, 2004). Batoid species are covered with dermal denticles (or placoid scales) which occasionally develop into thorns, while others are totally devoid of scales (Bigelow & Schroeder, 1953; Mceachran & Carvalho, 2002).

The typical composition and toughness of dermal denticles and thorns make them more resistant to decay than the cartilaginous skeleton; therefore, these structures may constitute important and historical records for identification of fossil species. However, comparative morphological studies applicable to the identification of such structures are lacking (Gravendeel, Neer, & Brinkhuizen, 2002).

Sexual dimorphism has been reported in elasmobranchs and include differences in skin thickness (Kajiura, Sebastian, & Tricas, 2000) and mouth/teeth morphology (Ellis & Shackley, 1995; Kajiura & Tricas, 1996; Nordell, 1994). In sexually mature males, alar thorns are found dorsally at the lateral apices of pectoral fins; along with sharp teeth, these structures are thought to aid in holding the female during copulation (Braccini & Chiaramonte, 2002; Mceachran & Konstantinou, 1996).

The genus *Atlantoraja* (family Rajidae, subfamily Arhynchobatinae) consists of three species of skates endemic to the western Atlantic, along the coast of South America (Compagno, 2005). Spotback skates *Atlantoraja castelnaui* (Ribeiro, 1907) occur from the State of Rio de Janeiro, Brazil, all the way to Argentina (Figueiredo, 1977); wider depth distribution has been reported in southern Brazil, where the species is found in depths ranging from 20 to 220 m in most of the continental shelf (Hozbor, Massa, & Vooren, 2004). The large size and high commercial value of this species translates into high commercial fishing pressures, which led to a 75% biomass decrease between 1994 and 1999 (Hozbor et al., 2004). The eyespot skate *A. cyclophora* (Regan, 1903) is found from coastal waters to water depths up to 220 m; species distribution extends from Cabo Frio, in the state of Rio de Janeiro, Brazil, to Argentina (Figueiredo, 1977; Hozbor et al., 2004; Oddone & Amorim, 2007). Overexploitation has led to classification of this species under IUCN (International union for Conservation of Nature) "endangered" category, with a downward trend in population abundance (Hozbor et al., 2004).

This study set out to describe microscopic features of thorns and modified dermal denticles, and to investigate sexual dimorphism of external morphological traits of skates *A. cyclophora* and *A. castelnaui* using scanning electron microscopy (SEM). It is hoped the data presented will add to the existing body of information on comparative morphology of the two species.

2 | MATERIAL AND METHODS

Skin samples collected from areas covered with thorns and dermal denticles were harvested from male and female of the family Rajidae, subfamily Arhynchobatinae (*A. castelnaui*, $N = 4$; *A. cyclophora*, $N = 4$). Skate specimens were obtained from shrimp trawl fishing bycatch in southern and southeastern Brazil (23°–26°S and 42°–47°W, respectively), with the consent of IBAMA – SISBIO (research permit No. 35614-3). Specimens were donated to the *Instituto de Pesca*, Santos, São Paulo, where identification (Gomes, Signori, Gadig, & Santos, 2010) and sampling were carried out. Samples were then sent to the Department of Anatomy of the School of Veterinary Medicine and Animal Sciences, University of São Paulo (FMVZ-USP), as part of a partnership agreement. This study was approved by the FMVZ-USP Ethics Committee for the Use of Animals (CEUA), protocol No. 4245050214.

Skin samples destined for scanning electron microscopy were fixed in 10% formaldehyde, washed in running water, dehydrated in increasing alcohol concentrations and dried in a hothouse at 37°C. Processed samples were then attached to aluminum bases with carbon adhesive and submitted to sputter coating with gold in EMITECH-K550 coater. Sample analyses and photographic documentation were carried out using electron scanning microscope LEO 435VP (FMVZ-USP).

3 | RESULTS

Mature males of *A. castelnaui* ($n = 2$) were identified by the presence of a clasper and alar thorns near the tips of pectoral fins (disc) (Figure 1a). Total length (TL) of male and female *A. castelnaui* specimens in this study ranged from 71 to 92.8 cm and 48 to 67.5 cm, respectively.

Alar thorn features and distribution were analyzed using SEM (Figure 1b,c). Up to 21 parallel alar thorns, obliquely oriented and pointing to the center of the disk, with smooth crowns and sharp apices, were documented in this species (Figure 1b). Smaller, randomly distributed thorns with more upright apex and partially exposed base were observed near alar thorn apices (Figure 1c).

Thorns found in other areas of the body of *A. castelnaui* did not differ between males and females. Large numbers of cylindrical thorn-like dermal denticles with slightly rounded apices were observed rostroventrally (Figure 1d–f). Pores corresponding to the opening of Ampullae of Lorenzini were visible between denticles (Figure 1e).

Sharp thorns were documented along the midline of the tail and other regions of the body (Figure 1g). These thorns are larger than those found on other body areas and have a broad base with clearly visible growth rings, which tapers into a smooth cylindrical area with rounded apex (Figure 1g). Dermal denticles covering the tail skin surface have a broad base from which projections arise; the cylindrical portion is short and has a slightly sloped smooth crown with the rounded apex (Figure 1h,i).

Dermal denticles covering the dorsal fins are similar to those found on the tail, except for a slightly more tapered cylindrical portion (Figure 1j–l). These denticles are randomly distributed, with higher density in some areas (Figure 1k).

Some morphological differences between *A. cyclophora* and *A. castelnaui* were documented in this study. Alar thorns were also present dorsally, near the tips of pectoral fins (disc) of sexually mature male *A. cyclophora* (Figure 2a). The total length of male and female *A. cyclophora* specimens in this study ranged from 52 to 55 cm and 58 to 62 cm, respectively.

Atlantoraja cyclophora alar thorns are arranged in three rows of 4–16 thorns each (Figure 2a). Scanning electron microscopy revealed obliquely oriented thorns with crown apices pointing to the center of the disc (Figure 2d,e). These thorns have a smooth crown and sharp apex (Figure 2d).

Large numbers of cylindrical thorn-like dermal denticles with slightly sharp apices were observed rostrally (Figure 2b,c) in this species. Pores corresponding to the openings of ampullae of Lorenzini were visible between denticles. Different from other sharp midline thorns, tail thorns were triangular in shape, with a broader base tapering into a rounded apex and clearly visible growth rings (Figure 2f).

Dermal denticles lateral to midline thorns are sparsely distributed. Three projections arise from their base; the cylindrical portion is slightly sloped and sharp, with a smooth crown (Figure 2g).

4 | DISCUSSION

Dermal denticles are often used as references in shark identification guides (Compagno, 1984; Marshall, 2011) and generic taxonomic and phylogenetic classification of skates (Deynat, 2000; Gravendeel et al., 2002; Mceachran & Konstantinou, 1996). Along with vertebrae, thorns

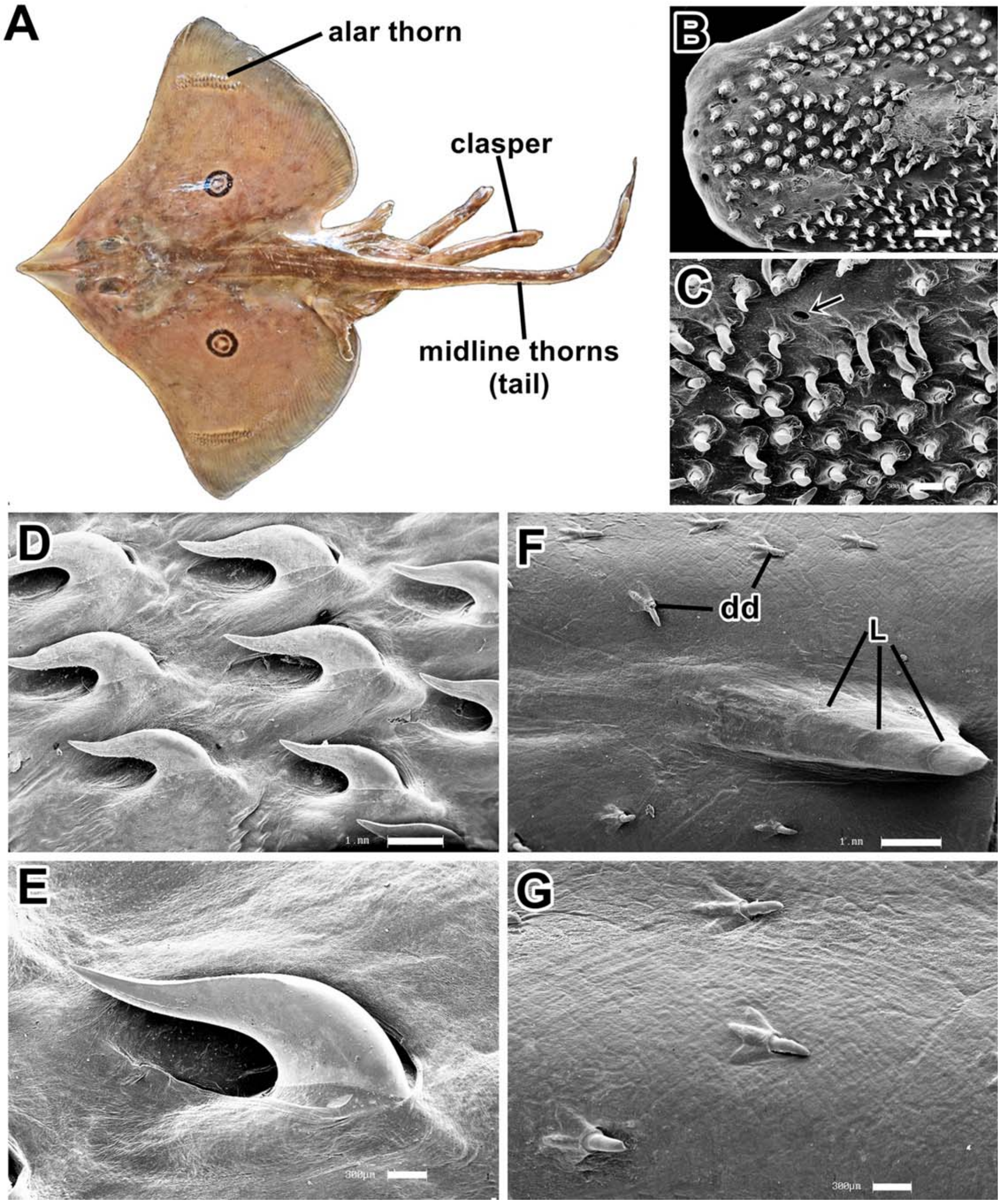

FIGURE 2 *Atlantoraja cyclophora*. (a) Macrograph showing different body regions. (b,c) Dermal denticles (rostroventral area); note pore corresponding to Lorenzini ampulla (arrow). (d) Alar thorns of sexually mature males. (e) Alar thorns of sexually mature males. (f,g) Thorns and dermal denticles (dd) (tail); note growth rings (L). Bar—(b, d, f): 1 mm; (c, e, g): 300 μm. [Color figure can be viewed at wileyonlinelibrary.com.]

and teeth are the most relevant elasmobranch's elements preserved in archaeological sites; species identification at such sites provides insights into their relationship with men and contributes to species distribution and abundance studies (Gonzalez, 2005). The morphological analysis performed brings relevant information about microscopic characteristics of dermal denticles and spines for both species, allowing extrapolation of data presented not only for phylogenetic studies, but also behavioral and ecological inferences.

Atlantoraja castelnaui and *A. cyclophora* are phylogenetically close. However, interspecies variations in morphology and color patterns are clearly visible. Interspecies variations in thorn and dermal denticle morphology were observed in this study. Macroscopic analysis revealed a single row of alar thorns in *A. castelnaui*, in contrast with up to three rows in *A. cyclophora*. Species-specific differences in alar thorn size and shape were also described. The higher density of rostral and caudal denticles was observed in *A. castelnaui* compared to *A. cyclophora*.

In elasmobranchs, dermal denticles are associated with the accommodation of bioluminescent and sensory organs, resistance to abrasion, protection from parasites and reduction of frictional drag (Kemp, 1999; Raschi & Tabit, 1992). The presence of thorns and dermal denticles was limited to specific areas of the body in the skate species studied, suggesting a role in mechanical, biological, and protection. Thorns may increase drag, leading to higher energetic demands due to lower swimming efficiency and as consequence a less effective predation. It is possible that changes in body shape, especially in pectoral fins exhibited by skates that have thorns (*Atlantoraja* and *Raja* genus), nullifies the negative effect caused by the reduction in hydrodynamics, by increasing the swimming efficiency. Thorn distribution may, therefore, reflect an evolutionary adaptation potentially determined by the increased need for successful copulation in batoids, given the typical body shape which might mean a greatest difficulty during mating (while animals are swimming) compared to sharks.

Clasper size, rigidity, and the presence of alar thorns rows indicate sexual maturity in males Rajiformes (Mabragaña, Lucifora, & Massa, 2002; Templeman, 1987). Given alar thorns are thought to facilitate copulation in *Raja eglanteria* (Tricas, 1980), it would be reasonable to infer a similar function in *A. castelnaui* and *A. cyclophora*.

Internal fertilization depends upon proper clasper (copulatory organ) insertion into the female's cloaca; hence, the female must be held in proper alignment during the pre-mating phase (Carrier, Pratt, & Castro, 2004). In many shark and batoid species, the male seizes the female by biting her fins. These bites are less vicious than feeding bites and tend not to involve total force or complete closure of the jaw (Carrier et al., 2004). This behavior is thought to reflect a precopulatory releasing mechanism to evoke female cooperation in mating (Springer, 1960). Also, thorns as well known for its function as attaching structures, mainly used during copulation. That way, the presence and location of thorns in male for both species studied here made us believe that the mating behavior in *A. castelnaui* and *A. cyclophorsa* is strictly related not only with bite use but also thorn attachment.

Sexual dimorphism in *A. castelnaui* and *A. cyclophora* dentition has recently been reported; grabbing and crushing type teeth have been described in sexually mature males and females, respectively (Rangel et al., 2014). Such secondary sexual characteristics and the alar thorn dimorphism observed in this study suggest these structures play a role in the copulatory behavior of both species.

Gender-related differences in *A. cyclophora* and *A. castelnaui* thorn morphology have been highlighted in this study, with alar thorns being described in sexually mature males of both species. Other thorns found on the body also differed morphologically between the two species. The presence of a single row of thorns in *A. castelnaui*, as opposed to three rows in *A. cyclophora*, suggests a thinner skin in females of the first species, possibly contributing to successful male attachment. Alar thorn arrangement (i.e., sharp apex pointing to central disk area) suggests the male flexes his pectoral fins (disc) ventrally, then uses the thorns to hold onto the female body in a belly to belly or belly to dorsum (male and female respectively) position.

Knowledge of thorn and dermal denticle morphology is of paramount significance in comparative studies of skates, given the important role of these structures in animal protection and reproduction. Also, those structures can be used in order to elucidate ecological and behavioral patterns; however, links between morphology, ecology and behavior are rarely made. Thorn and dermal denticle morphology are also taxonomically relevant, once these structures are often the only elements preserved in archaeological sites as fossil records (Meyer & Seegers, 2012).

That said basic morphological studies combined with microscopic analysis needs to be taken into account not only for traditional comparative and taxonomic studies, but also as a tool to better understand how animals interact with other and with the environment.

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