



Microscopic aspects of electrosensory system on the partially euryhaline lesser guitarfish

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ABSTRACT

The electrosensory system on elasmobranchs consists of subcutaneous electroreceptor organs known as ampullae of Lorenzini. The present study investigated the ampullae of Lorenzini morphology of the lesser guitarfish *Zapteryx brevirostris*, using light microscopy and scanning electron microscopy. The pore number found in the ventral skin surface is much higher than that found in the dorsal portion, characteristic of species that inhabit the euphotic zone. Under light microscopy it was possible to observe that the wall canal consists of a single layer of squamous epithelial cells. The canal features distal expansion, where the ampullae are located with up to six alveoli. The sensory epithelium of ampullae is composed by cubic cells, with oval nucleus, restricted to the interior of the alveoli. With analysis the clusters under scanning electron microscopy, it was possible to observe the structure and the random arrangement of individual ampullae, canals and nerves. The distribution of dorsal and ventral pores and ampullae in *Z. brevirostris* resembled those of the same family. The number of alveoli per ampullae was similar to that found in euryhaline elasmobranchs species, suggesting that the morphological organization in *Z. brevirostris* is linked to its possible evolutionary transitory position among batoids.

Key words: elasmobranch, ampullae of Lorenzini, electroreceptors, light microscopy, scanning electron microscopy.

INTRODUCTION

Elasmobranchs (sharks and rays) play a vital role in marine ecosystems since they occupy the

apical portion of the food chain, being important regulators, especially in tropical and subtropical environments (Garrick 1982, Camhi et al. 1998, Stevens et al. 2000, Ferretti et al. 2010). All sharks and rays present an elaborate electrosensory system composed by groups of subcutaneous

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electroreceptors organs, known as “ampullae of Lorenzini” (aL). The system is composed by a complex web of nerve fibers, canals and external pores ranging in size, shape and composition, related to particular needs of each species (Murray 1960, Kajiura 2001, Wueringer and Tibbetts 2008, Kajiura et al. 2010, Wueringer et al. 2011, 2012, Kempster et al. 2012, Bedore et al. 2014).

The ability to detect weak bioelectric fields evolved independently in several lines of vertebrates, emphasizing the importance of this sense in a variety of behaviors (Bullock et al. 1983, Collin 2010, 2012). Phylogenetically, it is considered an ancient sensory modality (Wilkins and Hofmann 2005). The role of the electrosensory system can be multifunctional, facilitating social and reproductive behaviors (Tricas et al. 1995, Sisneros and Tricas 2002), predator detection (Sisneros et al. 1998) and navigation/guidance through the Earth’s magnetic field (Kalmijn 1971, Paulin 1995, Montgomery and Walker 2001, Krylov et al. 2014). Its main role however, is prey detection (Kalmijn 1971, Kalmijn and Weinger 1981, Raschi 1986, Tricas 1982, Kajiura 2001, Bedore et al. 2014).

The canal length and pore distribution are the two main characteristics that determine ampullae sensitivity in a uniform field (Kalmijn 1971, Tricas 2001). The distribution and density on the body surface is influenced by environment, diet composition, predatory behavior and phylogenetic position (Wueringer et al. 2009, Kempster et al. 2012). In batoids, the canals extend over the ventral surface to the end of the dorsal fins, with the highest density around the mouth (Jordan 2008).

Rhinobatidae species are popularly known as “guitarfishes” being the earliest representatives of current skates and rays, with fossil records dating back to the Jurassic period (McEachran et al. 1996, Shirai 1996). The family is comprised of four genera (*Aptychotrema*; *Rhinobatos*; *Trygonorrhina* and *Zapteryx*) and 45 species (Compagno 2005). In

Brazil, two genera and four species can be found: *Rhinobatos horkelii* (Müller and Henle 1841); *R. lentiginosus* (Garman 1880); *R. percellens* (Walbaum 1792), and *Zapteryx brevirostris* (Müller and Henle 1841) (Figueiredo 1977).

Zapteryx brevirostris, popularly known as the lesser guitarfish, distinguish itself from other guitarfish by presenting a shorter rostrum. The species is endemic to the Southwest Atlantic, with a relatively restricted distribution, occurring from Espírito Santo (Brazil) to Argentina (Figueiredo 1977, Vooren et al. 2006). It is commonly found at depths of 60 m (Figueiredo 1977) and feed on small animals such as Polychaeta and decapod crustaceans (Bornatowski et al. 2014). Tons of individuals are accidentally caught and discarded annually (Vooren et al. 2006). Classified as vulnerable by the IUCN Red List, the species is currently unprotected, fact that can lead an irreversibly reduction in stock in the next years (Vooren et al. 2006).

Currently, studies with *Z. brevirostris* are focused on reproduction (Abilhoa et al. 2007), distribution (Santos et al. 2006) and food chain position (Bornatowski et al. 2014), without any analysis of sensory systems, even with the relatively high predatory position featured in marine environments (Bornatowski et al. 2014). In this context, the aim of this study was to analyze the morphology of ampullae of Lorenzini in *Z. brevirostris* using light microscopy (LM) and scanning electron microscopy (SEM), providing detailed information about this important predation system.

MATERIALS AND METHODS

ANIMALS

Shrimp trailers operating in Southeastern Brazil, between depths of 15-70 m, accidentally caught ten adults. The specimens were donated to the Department of Surgery of Veterinary Medicine and Animal Science of the University of São Paulo, under permission from SISBIO 48271-1. The

project was approved by the Ethics Committee on Animal Use (CEUA) (Protocol N° 2330141113-FMVZ/USP).

MACROSCOPY

For detection and analysis of pore distribution, the animals were placed dorsally. For hyoid cluster detection, the dissection of the ventral region was performed just above the first gill pair, guided by morphological descriptions of guitarfish published by Wueringer and Tibbetts (2008). The images were documented with a digital camera (Nikon D3100).

LIGHT MICROSCOPY (LM)

For light microscopy, hyoid cluster samples from five adult specimens were used. The samples were fixed in 4% paraformaldehyde. After complete fixation, the samples were dehydrated in ascending ethanol series (from 70% to 100%) and cleared in xylene for subsequent embedding in paraplast. Paraplast blocks were sectioned (5µm) using a microtome (Leica-German), and then stained with Hematoxylin-Eosin (HE) and Masson Trichrome (MT). Analysis was performed using a light microscope (Nikon Eclipse E-800).

SCANNING ELECTRON MICROSCOPY (SEM)

Samples from the skin of ventral region, the rostrum and the hyoid cluster of five adult specimens were fixed in 10% formaldehyde solution, dehydrated in series of increasing ethanol concentration (70% to 100%). 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. Finally, the samples were photographed in a LEO 435VP scanning electron microscope.

RESULTS

The aL pores were easily identified in the ventral part of the body due to color difference (lighter in

this region) (Fig. 1a). The ventral portion present a higher number of pores, especially close to the mouth and in the rostral cartilage, extending to the anterior half of the pectoral fins (Fig. 1a). Both free and clustered ampullae were detected, surrounded by connective tissue, being also possible to observe the emergence of a nerve that extends to the anterior lateral line nerve (branch of cranial nerve VII) (Fig. 1b and 1c). The hyoid cluster is the largest, found laterally to the jaw and close to the gills on the ventral side and laterally to the eye in the dorsal region (Fig. 1a and 1b).

In the analysis of the dorsal region skin under SEM, the arrangement and shape of dermal denticles were evidenced, being different in both size and position according to their distance to the pores of the ampullae (Fig. 1d). Dermal denticles feature a smooth crown, with irregularly shaped borders, varying in major and minor asymmetrical lobes, resembling morphology of leaves from an oak tree.

In the cross section of the rostrum, under SEM, the location and arrangement of the longitudinal canals (in relation to the surface of the ventral region were detected) (Fig. 1e). The three-dimensional aspect of the hyoid cluster reveals the aL arrangement and the canals entrances parallel to each other (Fig. 2a and 2b). Through the hyoid cluster analysis it was possible to observe the covering structure of the capsule (Fig. 2b). In the base of the canal, the aL was detected (Fig. 2c) as an ovoid capsule with a nerve originating from its base (Fig. 2a).

Under light microscopy, it was possible to observe that the ampullae are distributed randomly in the cluster. The cross section (Fig. 2d and 2e) and sagittal section (Fig. 2f and 2h) facilitated structural visibility of ampullae (canal, bulb and nerve). The aL observed are multialveolar, according to the classification proposed by Andres and von Düring (1988).

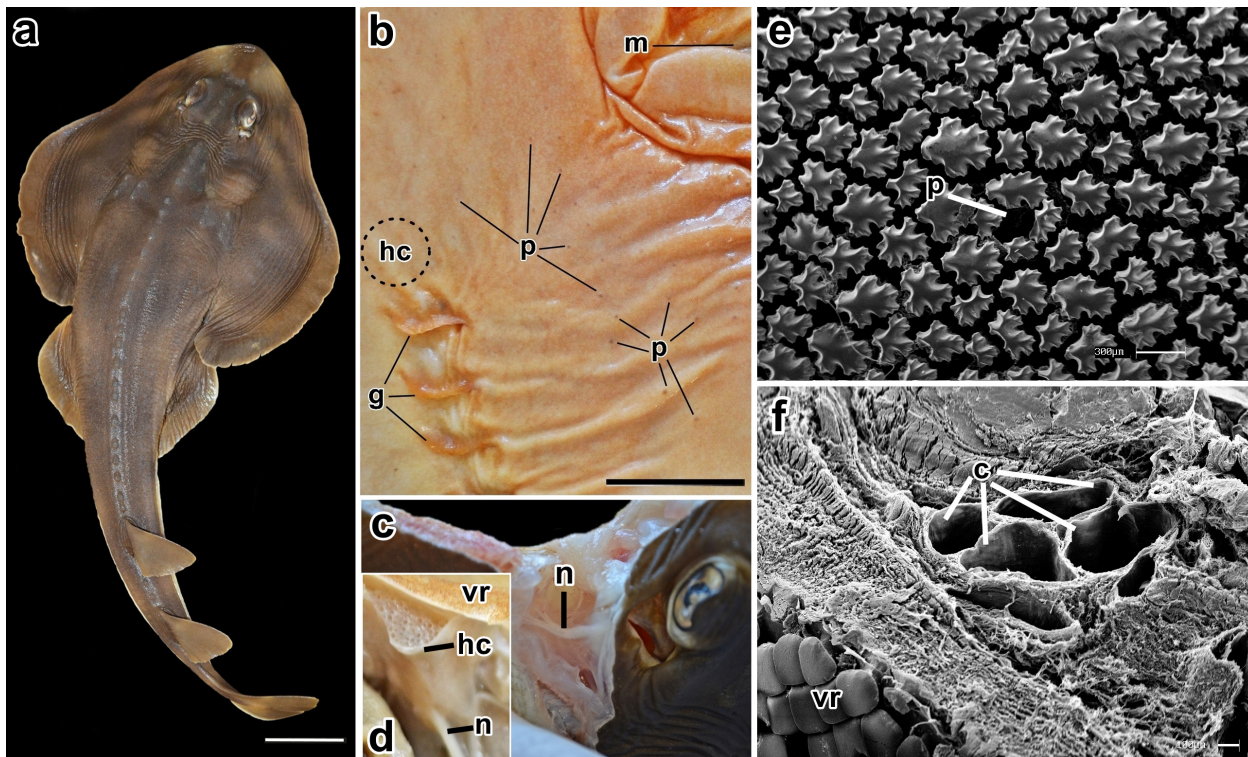


Figure 1 - (a) macrograph of the ray *Zapteryx brevirostris*. (b) the ventral region, showing the location and disposition of some pores (p) located near the gills (g) and mouth (m); dashed circulated region, represents the location of hyoid cluster (hc). (c) nerve (n) of hyoid cluster. (d) hyoid cluster (hc) located next to the gills and nerve (n); ventral region (vr). (e) disposition of the denticles and pores (p) in the skin of the rostral dorsal region, where the denticles around the pores are smaller and differentiated. (f) cross section of the rostrum, location in relation to the region ventral skin (vr) and longitudinal arrangement of the ampullae canals (c). Scale bars: (a) 5 cm; (b) 2 cm; (e) 300µm; (f) 100µm.

The analyzed ampullae consisted in a group at maximum six alveoli (Fig. 2d), covered with connective tissue. At the distal end of the alveoli, it was possible to observe the aL nerve (Fig. 2f and 2h). The receptor epithelium of the ampullae (restricted to the alveoli) is formed by a monolayer of simple cuboidal cells with oval nuclei (Fig. 2i). The canals observed in transverse and sagittal sections are randomly distributed in the hyoid cluster, separated by connective tissue (Fig. 2g). The walls of canals are composed by squamous epithelial cells.

DISCUSSION

The present study brings the identification and description of morphological characteristics of the electrosensory system in *Z. brevirostris*.

The analysis are relevant and important since this sensory system is fundamental for animal's survival and hunting adaptation. Our results showed a higher number of pores on the ventral surface of the skin compared to the dorsally portion. The observed distribution is advantageous not only because most of *Z. brevirostris* preys are found buried, but also because other senses are not as well developed as in pelagic animals. Batoids are flattened dorsoventrally with mouth positioned ventrally while the eyes occupy a dorsal position. That way, batoids little depend on vision during hunting, since most of the preys are buried (Raschi 1986). According to Kempster et al. (2012), the benthic zone is a two-dimensional environment, and the majority of the species live on the bottom or very near to the substrate. Under these conditions,

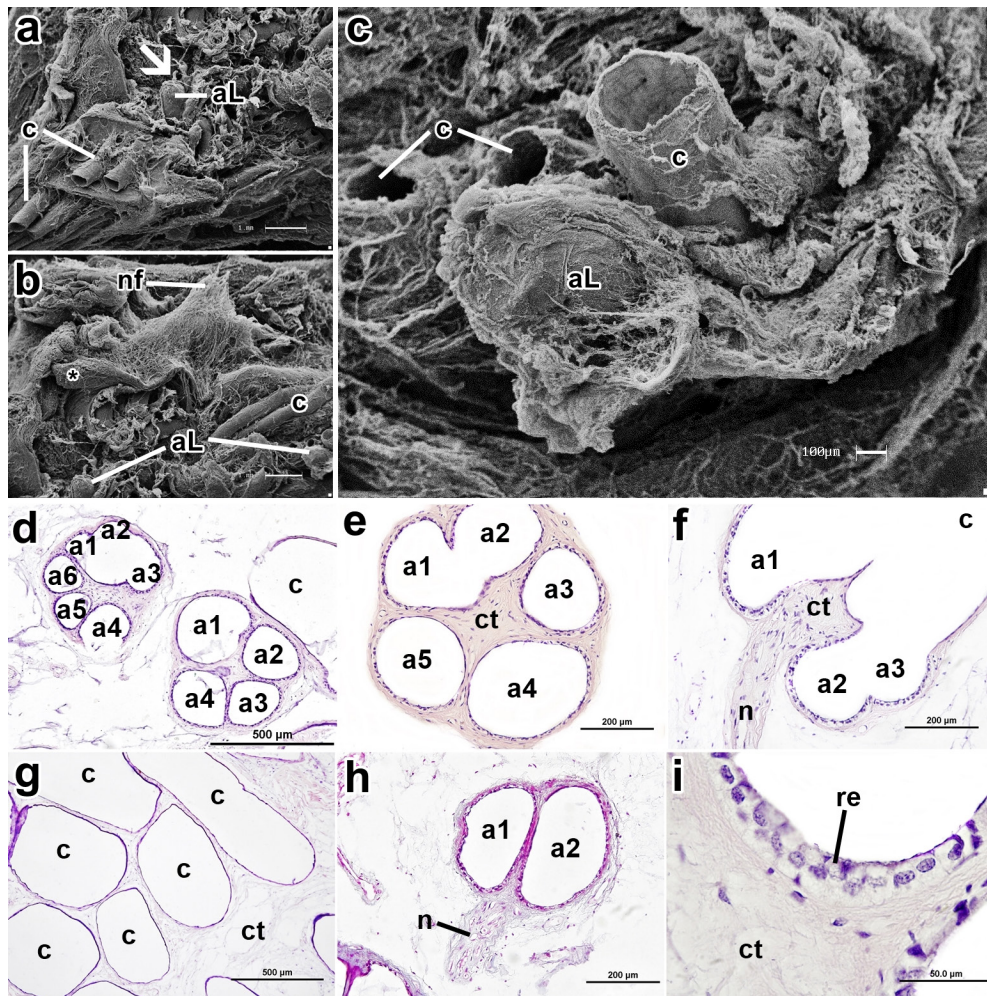


Figure 2 - Hyoid cluster microscopy. (a, b and c) disposition and parallel of input canals (c) and location of the ampullae of Lorenzini (aL) in the hyoid cluster; (*) capsule coating the hyoid cluster. (d, e and f) in HE staining the vials in cross section, covered with connective tissue (ct), with up to six alveoli (a1-a6) and canal (c). (g) canals at random in sagittal and transverse sections, separated by connective tissue (ct). (h) in TM staining, ampullae in sagittal section with visible nerve (n) (i) in HE staining the receptor epithelium (re) in largest magnification formed by cubic cells and oval nucleus. Scale bars: (a) 500 µm; (b-f) 200µm; (g) 100µm; (h and i) 50µm.

animals are generally slow, with limited range, often displaying cryptic camouflage. To compensate visual inefficiency, benthic predators have become quite dependent of the electrosensory system, with a higher proportion of pores in the ventral portion of the body. Pores are more common around the mouth, position probably related to the need of orientation for animal's mouth during capture (Raschi 1986, Jordan 2008, Wueringer et al. 2011, Kempster et al. 2012).

For sawfish (*Pristis* sp.) (Wueringer et al. 2011) it was suggested that the high density of pores over the rostrum and low around the mouth may be an indication of the use of rostrum to stun prey before ingesting. This may be an indication of pore distribution plasticity due to hunting behavior and feeding habits. The elongated rostrum found in guitarfish may be related to the need to increase sensory area/information, with numerous ampullary clusters observed in the rostral region

in Rhinobatidae (Wueringer and Tibbetts 2008). Therefore, it is possible that the spatial distribution for guitarfishes have the same function/importance of that observed in sawfishes. Based on this hypothesis, we can infer that *Z. brevirostris* has a lower electrosensory sensitivity compared with other species of guitarfishes, due to the absence of the elongated rostrum. Nevertheless, more studies that characterize the distribution and density are needed, since it is possible that the rostrum shortening is compensated in other ways.

For Kajiura et al. (2010), the importance of electroreception decreases with increased water clarity. *Rhinobatos tyus* and *Aptychotrema rostrata*, both guitarfishes, live deeper environments (100 m) with lower luminosity, while *Z. brevirostris* live in lesser depth (up to 60 m) and lighter environments. *R. tyus* and *A. rostrate* feature longer rostrum, possibly related to the need to increase electrosensory sensitivity due to the reduced vision ability, while *Z. brevirostris* does not depend that much on electrosensory system since the species lives in environments with higher visibility. The species also possible to observe a large number of pores surrounding the gills. This distribution may be related to the use of water expelled through the slits to move the substrate, thus facilitating the perception of buried prey. The behavior of using spiracle movements for other purposes besides the respiratory function has already been described (Tricas et al. 1995), thus being reasonable to infer that *Z. brevirostris* can also use the expulsion of water through the slits for other purposes.

The distribution described for *Z. brevirostris* resembles that seen in *Dasyatis* sp. known as active swimmers and spending brief periods buried, presenting little or no dependence on the presence of a rostrum for predatory purposes (Bedore et al. 2014). This leads us to believe that the rostral region of *Z. brevirostris* lost its importance during evolutionary process, which could be indicative of a transitional adjustment between guitarfish with

elongated rostrum and skates and rays without rostral portion.

Since the sensorial epithelium is sensitive to voltage gradients created between the environment and the interior of the ampullae, sensitivity increases with canal length (Murray 1974). The elongate canals observed in the dorsal fins of guitarfish may facilitate the detection of weak electric fields at longer distance, allowing that way, the initial location of prey. On the other hand, the shorter canals found in the rostrum and near to the mouth may present a lower range of perception, being probably used for detection of the precise position of prey on short range (Tricas and New 1998, Wueringer and Tibbetts 2008).

Few studies emphasizing the microscopic features are available for a very small portion of species, even though great diversity in the system is found in elasmobranchs (Wueringer and Tibbetts 2008, Wueringer et al. 2009, Camilieri-Asch et al. 2013, Whitehead et al. 2014). These studies suggest that the canals that come out of the pores both in ventral and dorsal regions, come together close to cluster areas. The nerve is located laterally to the entrance of the canals in the cluster, same as observed by Wueringer (2012). This is the largest cluster present in batoids (Raschi 1978, Wueringer and Tibbetts 2008, Wueringer et al. 2011) being absent in Carcharhiniformes and Lamniformes sharks (Raschi 1986). Guitarfish and sawfishes features five bilaterally symmetric clusters (Norris 1929 cited Wueringer 2012, Wueringer and Tibbetts 2008, Wueringer et al. 2011).

Zapteryx brevirostris possess macro-multialveolar ampullae, which are commonly found in marine elasmobranchs (Andres and von Düring 1988). Even being classified as marine, *Z. brevirostris* present a number of alveoli per ampullae close to guitarfishes found in estuaries (*A. rostrata*, average of 6 ± 0.7), lower compared with marine guitarfishes (*R. typus* present an average of 17.0 ± 5.8 alveoli per ampullae) (Wueringer and

Tibbetts 2008). The same amount of alveoli it is also observed in *Carcharhinus leucas*, famously known for its high adaptive capacity in freshwater environments (Pillans and Franklin 2004, Pillans et al. 2006). Bull sharks also present other morphological similarities in aL distinct from other elasmobranchs (Whitehead et al. 2014), so it is possible that differences observed are salinity dependents. The same pattern is in *Pristis microdon* during the juvenile stage, spent in lower salinity environments (Wueringer et al. 2011).

A study of two sympatric species of rays revealed that *Dasyatis fluviatorum*, considered euryhaline, presents morphological characteristics in the electrosensory system similar to *Neotrygon kuhlii*, a marine species (Camilieri-Asch et al. 2013), being the morphological differences observed, possibly related to habitat differences (Camilieri-Asch et al. 2013). The number of alveoli found in *Z. brevirostris* also resembles the results found in *D. fluviatorum*, which present 6-10 wells per ampullae.

Therefore, it is possible to infer, based on morphological similarity and the number of alveoli per ampullae between *Z. brevirostris* and *A. rostrata* (Wueringer et al. 2009), *C. leucas* (Whitehead et al. 2014), *P. microdon* (Wueringer et al. 2011) and *D. fluviatorum* (Camilieri-Asch et al. 2013), that the strong link based on electrosensory system is a relevant evidence for at least partial euryhalinity in *Z. brevirostris* currently described as exclusively marine (IUCN, Fishbase).

Studies show that in fact, *Z. brevirostris* has high physiological plasticity facing different salinities in laboratory experiments (Wosnick and Freire 2013). Since the species is not found in estuaries (Chaves and Correa 1998), it is possible that this ability is related to the evolutionary past of the group, possibly in brackish environments (Smith 1936, Lutz 1975, Griffith 1985, Ditrich 2007). That said, *Z. brevirostris* might be an evolutionarily transitional species, not only because

of the reduced importance of the rostrum compared to other benthic species, but also because of the electrosensory system organization.

CONCLUSIONS

The distribution of pores, canals and ampullae for *Z. brevirostris* resembled the pattern observed in other guitarfishes. However, it is possible that the reduction of the rostral region, combined with a higher amount of pores near the mouth and gills are transitional adjustments between benthic and pelagic rays.

The number of alveoli per ampullae was similar to that found in euryhaline species, suggesting that such morphological differences are linked to its possible evolutionary transition position, presenting physiological and morphological characteristics that make us believe that the species represents a mark in the evolutionary past of the group in lower salinity environments.

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