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Histological characterization of skin and radial bodies of two species of genus *Isostichopus* (Echinodermata: Holothuroidea)

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ABSTRACT

Surface morphological structure of the body, and radial organs in sea cucumbers *Isostichopus* sp. aff *badionotus*, *Isostichopus badionotus* morphotype I and *I. badionotus* morphotype II were characterized by histological techniques. The three morphotypes studied demonstrated differences between them in gross morphology and in the distribution of body fleshy projections in the dorsal region. The body wall consists of a layer of epidermal cells covered by a thin cuticle, a superficial dermis formed by a compact layer of cells with large cytoplasmic contents, slack dermis or delimited connective tissue into the body by a layer of muscle of circular lined by mesothelial cells. Radial bodies are made up of a nerve cord, two channels systems, a channel of water vascular system and five pairs of longitudinal muscles in the ambulacral region. Integument tissue structure showed no differences in the ventral or dorsal region. However, vascular channels through the integument give rise in the dorsal region to different fleshy protrusions wart-like, or fleshy papillae distinctive of each species while in the ventral region ends in podiums. Caribbean sea *Isostichopus* genus shows good quality skin structure and are able to be commercialized as sandfish or *Bêche de mer* in international markets.

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Introduction

The holothurians or sea cucumbers inhabit worldwide seabed (Brusca and Brusca, 2005) and are considered important due to their environmental and economical role since they are nutrient recyclers and highly demanded in the world trade as a food (*Bêche de mer* or sandfish) as well as their importance in medicine in many cultures of the Middle East and Asia. The integument of these organisms is shown as the most developed within the group and unlike other echinoderms their calcareous skeleton has been reduced to microscopic ossicles dispersed and embedded in the body wall. This particular organ represents much of the body weight of sea cucumber, has prodigious regenerative capacity (Candia-Carnevali, 2005), a high nutritional value protein, essential amino acids and low fat contents (Kerr and Zen, 1995, Vergara and Rodríguez, 2016; Arias et al., 2017). *Bêche de mer* or dried muscle preparing after being gutted, cooked, salted and dried is considered a delicacy for most Asian countries and is the most traded product worldwide (Bordbar et al., 2011, Purcell, 2014).

During long time, it was stated that in the Colombian Caribbean Sea there was only *Isostichopus badionotus*, easily identified due to its chocolate spots. However, within this species was included *Isostichopus* sp. aff *badionotus*, which presents large variations at the morphological and genetic level. Colombian *Isostichopus* species can be distinguished easily through the macroscopic morphology of their skin, by their pigmentation patterns and fleshy protrusions, since each one has its own characteristics and are unique to their own species. In spite of this, the species has been currently relegated into the synonymy of *I. badionotus*. *Isostichopus* sp. aff *badionotus* shows a beige pigmentation with dark lines and finlets. On the other hand, there are two patterns of coloration for *I. badionotus*, hence the nomenclature of morphotype I and II is proposed herein. *I. badionotus* morphotype I shows beige coloring spots and warts while *I. badionotus* morphotype II shows two types of coloration: coffee dark with clear spots at the ends of its papillae or coffee- white with dark spots at the ends of its papillae which are small conspicuous and sometimes imperceptible. Moreover, preliminary results of phylogenetic analyses supporting our hypothesis suggest that *Isostichopus* sp. aff *badionotus* that lives on rocky substrates is genetically isolated from the two other morphotypes of *I. badionotus* living in seagrass beds.

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Nevertheless, there are few studies related to the tissue wall of the sea cucumber. Most of them are generally linked to their regenerative capacity (Dolmatov et al., 1996, Byrne, 2001, Odintsova et al., 2005, Miguel-Ruiz and Garcia-Arrarás, 2007). Also there are those that describe the ultrastructure of podias (Vander-Spiegel et al., 1995) and oral tentacles (Bouland et al., 1982). In Colombia studies on holothurians are increasing (Borrero-Pérez et al., 2002; Ortiz, 2011; Borrero-Pérez et al., 2012; Rodríguez et al., 2013; Agudelo and Rodríguez, 2013, 2015, 2017; Vergara and Rodríguez, 2015, 2016; Arias et al., 2017). To date, the information about skin and radial organs of holothurians are limited, mostly concerning to variations between species while the skin is the most commercially important product in the Asian Far East markets. Therefore, in this paper we analyze and compare the morphology of the body wall and radial body tissues of native wild sea cucumber species: *Isostichopus* sp. aff *badionotus*, *Isostichopus badionotus* morphotype I, and *Isostichopus badionotus* morphotype II, as a contribution to the understanding of their basic biology. The aim of this study was to describe and to compare the difference in structure of the integument between three morphotypes of sea cucumbers as an input to elucidate that *Isostichopus* genus comprises other species different to *Isostichopus badionotus* in the Caribbean sea which have not been described so far. In addition and in accordance, we report which species might show better conditions for its commercialization as *Bêche de mer*.

Material and methods

Ten specimens per morphotypes (*Isostichopus* sp. aff *badionotus*, *Isostichopus badionotus* morphotype I and *Isostichopus badionotus* morphotype II), were captured in the Rodadero Bay (11° 13'22.73"–32.59 74° 13'N") and transferred to the Aquaculture Laboratory of the Universidad del Magdalena. Individuals were killed by hypothermia and body wall segments were examined in the ventral and dorsal regions (anterior ventral and dorsal; mid-ventral and dorsal; and posterior ventral and dorsal, to evaluate skin and describe radial organs). These were fixed in Bouin solution and decalcified overnight (Dietrich and Fontaine, 1975). The samples were treated by histology with cuts of 5–6 µm thick and stained with hematoxylin-eosin (H-E), trichrome stain (TRI), and Periodic Acid Schiff (PAS), to be observed under a microscope equipped with a Carl Zeiss camera with



Fig. 1. Sections of the body of *Isostichopus* sp. aff *badionotus* from which skin samples were taken for histology: dorsal and ventral – anterior, media and posterior.

AxioVision 4.8.2 program. For all species the same protocol was followed (Fig 1.).

Results

Macroscopic characteristics

Native sea cucumber species were characterized by having a trapezoidal-shaped body: the dorsal region is slightly arched and the sides of the body are nearly vertical. The margin of the lateral wall and the ventral surface is thick and is provided with fleshy extensions called papillae or warts-like (Fig. 2).

Isostichopus badionotus has an elongated body, cylindrical, completed in rounded ends with a chocolate chips spots over their body wall, while *Isostichopus aff badionotus* has an elongated body, cylindrical, completed in blunt ends which have varieties in colorations from cream-yellow with irregular spots: coffee, orange or reddish. Ventral region and podia are beige. *I. badionotus* presents varieties in skin-colors, size and in the ending of the edge. This species has at least two color variants: morphotype I, which showed diverse brown coloration and numerous conical warts coloring brown and morphotype II which showed elongated body, cylindrical or fusiform, robust, with blunt edges. This species has dark brown coloration with tiny conical pinnules, which coloring is reddish-brown and whose endings are yellow in color.

Skin and radial organs histology

The integumentary tissue of the sea cucumber has the following layers: cuticle, epidermis, dermis and superficial dermis consisting of connective tissue which forms the bulk of the integument of the sea cucumber. Then there was a circular smooth muscle that delimits the dermis (Fig. 3). Radial organs were present in the five ambulacral areas: a pair of longitudinal muscles, radial nerve cord, two channels of the perihemal system and a channel of the vascular water system, which branches and gives rise to long channels running through the tissues of the body wall. Those end in podia or in fleshy protrusions above the dorsal region (Fig. 4). Circular and longitudinal muscle as water vascular system channels are lined by mesothelial cells that contact the coelom.

Cuticle and epidermis

The body surface of the three morphotypes was covered by a thin acellular cuticle, lacking pigments, granules or other structures. A basal membrane was not evidenced. Epidermal cells presented apical processes, which appear long and conical (Fig. 4).

Dermis

The superficial dermis is a layer of sub-epidermal tissue, consisting of cells with large cytoplasmic contents, and limited fundamental substance. The deep dermis is composed of connective tissue, with small fibroblasts, and long oval bipolar cytoplasmic developments, fundamental substance and collagen fibers are arranged in regular or irregular nuclei. This tissue is divided into two groups according to the amount and organization of its components: 1) compact dermis and, 2) lax dermis (deeper). The compact dermis is followed by a lax dermis. The former is present only in *Isostichopus* sp. aff *badionotus*. The second layer is the only type of connective tissue exhibited in the dermis of *I. badionotus* morphotypes I and II (Figs. 5 and 6).

In the skin and podia wall of the three morphotypes cell aggregates denominated brown bodies, were observed distributed throughout their integument. They form circular clusters and look

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