

## Details on the histomorphological characteristics of marine sea pen, *Virgularia gustaviana*, from the Persian Gulf

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Received: 26.06.2020 • Accepted/Published Online: 25.04.2021 • Final Version: 25.08.2021

**Abstract:** Sea pens or pennatulaceans (Phylum Cnidaria, Order Pennatulacea) are colonial marine octocorals that typically anchor themselves into soft sediment. They make large colonies from the intertidal zone down to the abyssal plain that provide an important structural habitat to other organisms. In the present study, the morphological and histological characteristics of the dominant species of *Virgularia gustaviana*, the dominant sea pen species of the Persian Gulf, were surveyed. Samples of *V. gustaviana* were collected along the coastal intertidal zone of the Soru and Bandar Abbas beaches, located at the northeast of the Persian Gulf. The sea pens were then fixed in Bouin's solution. Histological sections were prepared using routine histological techniques and stained with hematoxylin and eosin. *V. gustaviana* morphologically consisted of two major parts, including the rachis and peduncle, originated from the early polyp. Rachis with leaf-like plates consisted of autozooids and siphonozooids that were located at the ventral and dorsal surfaces of the rachis, respectively. The peduncle was the lower part of the sea pen with a smooth structure and the bubble-shaped end. The body wall (coenenchyme) of *V. gustaviana* consisted of three layers including the epithelium, mesogloea, and gastrodermis. There was a calcareous rod, with the dark and light concentric circles, called the axis at the center of the body of the sea pen. A layer of calcified sponge-like tissue called sclerite was observed around the axis. Sclerite was connected to the body wall of the sea pen by four thin tissue septa that divide the coelom into four water canals. Another water canal was found between the axis and the sclerite, which is considered the fifth water canal of the sea pen. In conclusion, *V. gustaviana* have the basic histological structure like other studied species.

**Key words:** Sea pen, *Virgularia gustaviana*, histology, Persian Gulf

### 1. Introduction

Sea pens are colonial marine cnidarians with multiple polyps that owned by the class Anthozoa and order Pennatulacea. These animals are widely distributed and can be found in tropical and temperate waters from the intertidal sand or mud flats to depths of more than 6100 m all over the world [1]. However, sea pens are usually found at depths below 10 m where the turbulence of water is not so high that they root out.

The polyps of sea pens are specialized for certain functions: a single polyp turns into a stiff, vertical stalk (the rachis), losing its tentacles, and producing a globular root named peduncle at its base that usually anchors into sandy or muddy substrate [2]. The other polyps arise from this central stem, developing to water intake (*siphonozooids*), feeding (*autozooids*), and reproductive components [3]. The spicules and a central axial rod of calcium carbonate fortify the entire colony.

Although sea pens are generally sticky, they are able to displace and re-attach if necessary [4]. They place in the flow paths to ensure a constant current of plankton (their main source of food). Nudibranchs and sea stars are the major hunters of sea pens. Some sea pens produce a bright greenish light when they are touched (bioluminescence) [1].

Sea pens may either be single or cover wide zones, creating fields that make a valuable structural habitat to other organisms [5]. Due to the negative effect of human activities on sea pens [6,7,8,9], these animals have been classified as vulnerable species in both shallow and deep ecosystems [10]. According to Gates and Jones [11], although sea pens were the dominant species on the soft sediment in Norwegian Sea (380 m), they were less likely to be found in regions disturbed by drilling. On the other hand, sea pens are slowly growing and then they need a long time to recover [12].

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Sea pens, such as other anthozoans, reproduce seasonally or all over the year through the simultaneous release of sperm and eggs in the water. Fertilized eggs develop into larvae called *planulae* that float freely for almost a week before settling down on the substrate [13].

In spite of the worldwide distribution of sea pens, little is known about their biology. Williams [1] studied the global diversity of sea pens (Cnidaria: Octocorallia: Pennatulacea). In his study, he investigated the morphology of some sea pens, such as *Pennatula inflata* and genera *Renilla*, *Echinoptilum* and *Actinoptilum*. Williams et al. [14] studied the morphology of polyps in pennatulacean Octocorals, with emphasis on polyp polymorphism in the genera *Pennatula* and *Pteroeides* (Anthozoa: Pennatulidae). Morphometry and growth of sea pen species from dense habitats in the Gulf of St. Lawrence, eastern Canada, was studied by Murillo et al. [15].

Only four sea pen species have been identified in the Iranian waters of the Persian Gulf and Gulf of Oman including *Cavernularia Valenciennes*, *Pteroeides spp.*, *Veretillum cynomorium* and *Virgularia gustaviana* [16,17]. *V. gustaviana*, which is one of the native sea hare species of the Persian Gulf, is colonial marine cnidarians in the family Virgulariidae. The species is reported to be distributed in the Indian Ocean and parts of South Africa [1]. It lives in both shallow, sheltered locations, as well as up to depths of 400 m. *V. gustaviana* typically feed on zooplankton and other organic particles. This species has the capacity of both passive predation of small animals and suspension feeding of particles [1].

There is very limited information about it. In this regard, the goal of the present investigation was to make a detailed examination including measurements and descriptions of histological characteristics of this species from the northwest of the Persian Gulf.

## 2. Materials and methods

### 2.1. Study area and sample collection

Three transects were selected in the intertidal areas of Bandar Abbas (the northeast of the Persian Gulf), including South Golshahr transect (fishery pier), behind the city transect, Soru transect (Table 1). Sampling in these three transects took place on July 2017, at the time of the

highest tide in the intertidal zone. For convenience, each transect was approximately divided into 8 high, middle, and low intertidal stations, and a quadrat of  $0.5 \times 0.5 \text{ m}^2$  with 9 replications was randomly dropped in each of these three transects.

A total of 25 marine sea pens detected in terms of the morphological characters of *V. gustaviana* (Figures 1A, 1B) were collected from three transects (Figure 2). The samples were initially fixed in Bouin's solution for a week and then moved to 70% ethanol until histological analysis.

### 2.2. Histological study

Tissue samples from different parts of *V. gustaviana* were dehydrated in ascending concentrations of ethanol and embedded in paraffin using a tissue processor (Tissue Tek Rotary, Rx-11B). Tissue samples were then thin sectioned (6–7  $\mu\text{m}$ ) by the rotary microtome (RMZZ45 -Leica, Wetzlar, Germany). The tissue sections were mounted on slides and stained with hematoxylin and eosin (H & E). Microscopic analysis was performed by light microscope (Olympus-CH4O, Tokyo, Japan) and digital images were taken using Dino lit lens (with Dinocapture software, FDP2, New Taipei City, Taiwan).

## 3. Results

Morphologically, *V. gustaviana* consists of two major parts, including the rachis and peduncle, originated from the early polyp (Figures 1A, 1B). Rachis with leaf-like plates forms the upper part of the sea pen, extending from the early buds to the tip of the polyp. Autozooids are located on the ventral surface and the siphonozooids are at the dorsal surface of the rachis (Figures 3A, 3B, 3C, 3D). The peduncle is the lower part of the sea pen with a smooth structure and the bubble-shaped ends (Figure 1A).

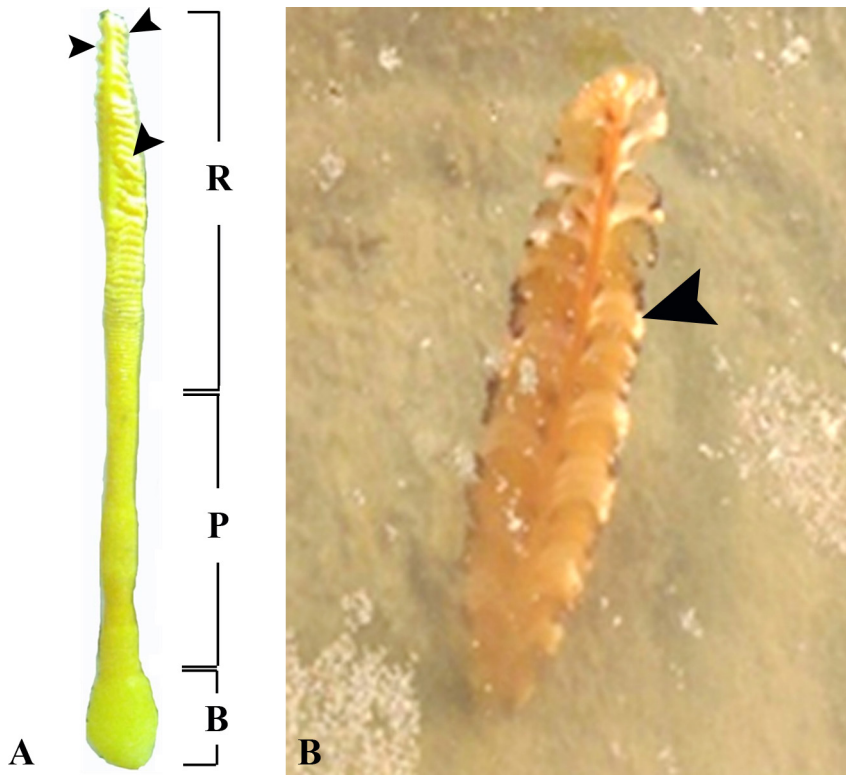
The body wall of *V. gustaviana*, the coenenchyme, consists of three layers including the epithelium, mesogloea (mesenchymal connective tissue) and gastrodermis (Figures 4A, 4B, 4C, 4D). There was a calcareous rod called the axis at the center of the body of the sea pen, around which the body cavity (coelom) was observed (Figures 4A, 4C).

### 3-1. The axis and surrounded tissues

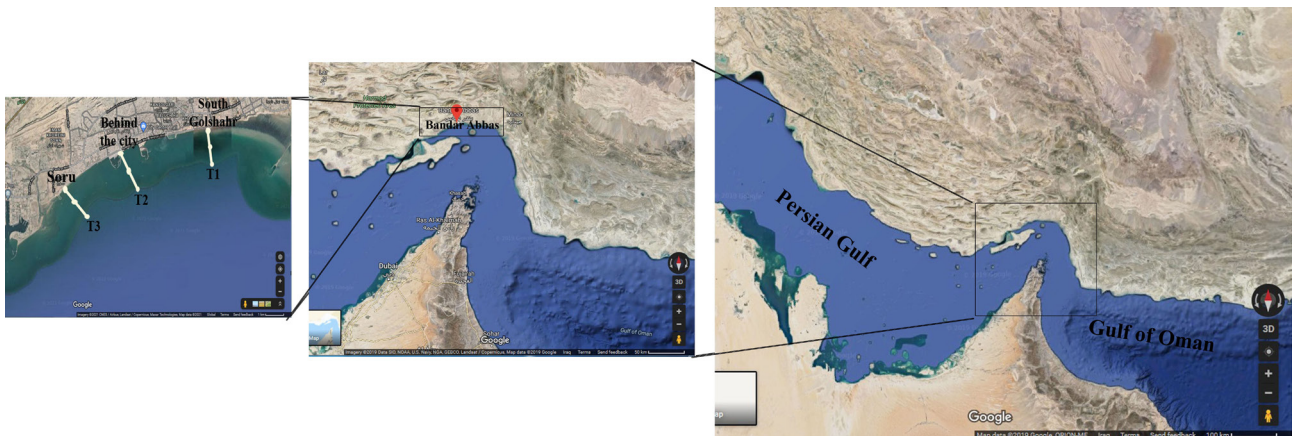
The axis was often cylindrical, but in some cases, it was rectangular (Figures 5A, 5B). There were the dark and

**Table 1.** Details and geographical location of sampling stations on the coast of Bandar Abbas.

Transects	geographical location of sampling stations		Substrate structure	Number of collected <i>Virgularia gustaviana</i>
South Golshahr	27°11'56.9"N	56°19'29.4"E	Sandy mud	9
Behind the city	27°10'16.3"N	56°15'38.8"E	Sandy mud	5
Soro	27°09'18.0"N	56°13'54.4"E	Sandy mud	11



**Figure 1.** Samples of *Virgularia gustaviana* from the intertidal zone of Iranian coasts of the Persian Gulf: A. Fixed sample; B. Live sample. R: rachis, P: peduncle, B: bulb, autozooids (black arrowheads).

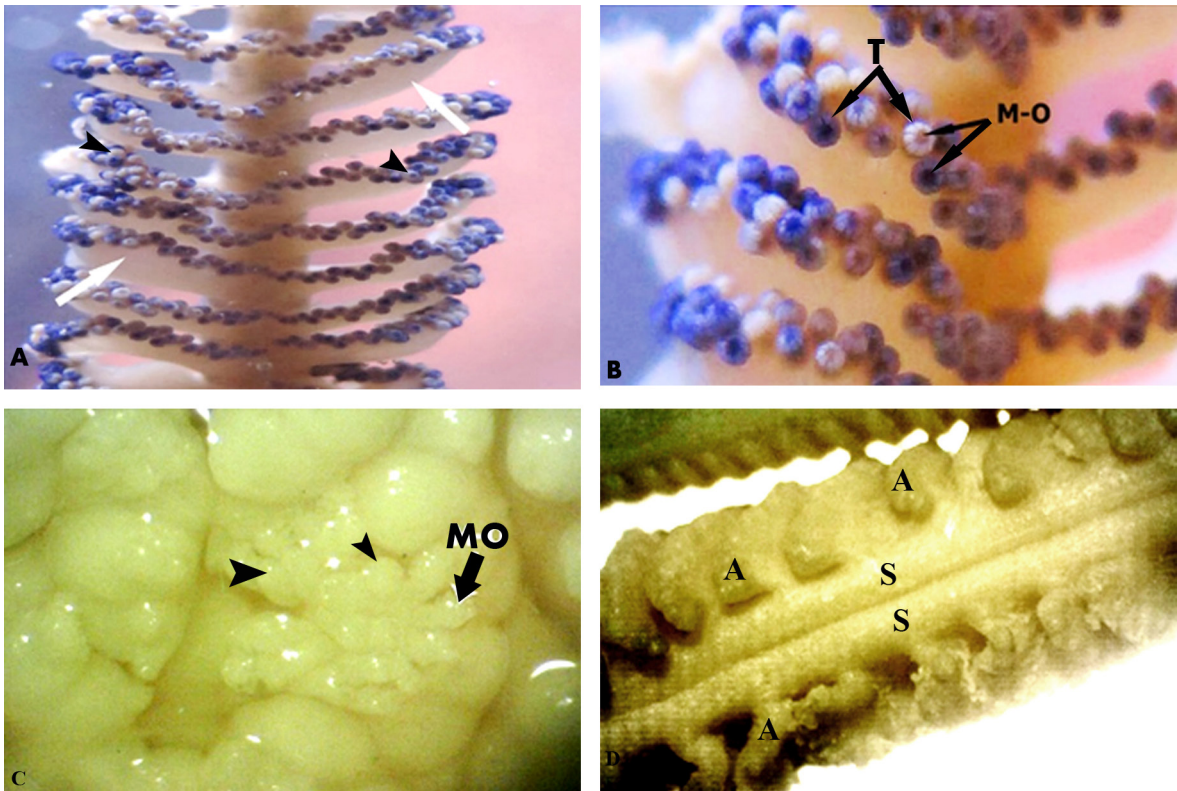


**Figure 2.** Geographical location of sampling stations on the coast of the Soru and Bandar Abbas beaches, located at the northeast of the Persian Gulf.

light concentric circles on the cross-section of the axis that could be used for determining the age of the sea pen (Figure 5C). The surface of the axis was coated by a columnar cell layer called sclerocytes with a basal nucleus and basophilic cytoplasm (Figure 5D). These cells play an important role in calcifying the axis and other calcareous parts of the body of the sea pen. Sclerocytes form a layer

of calcified sponge-like tissue called sclerite around the axis (Figure 5D). These cells are enclosed within the cavities (called lacuna) in sponge tissue after the mineral secretion and calcification of this tissue, and their shape changes from columnar to spherical or elliptical with the heterochromatin nucleus and acidophilic cytoplasm (Figure 5D). Sclerite was connected to the body wall of the





**Figure 3.** Stereomicroscopic images of rachis of *Virgularia gustaviana*: A. Autozooids (white arrows), tentacles (black arrowheads); B. tentacles (T), mouth opening (MO); C. tentacles (black arrowheads), mouth opening (MO); D. autozooids (A), siphonozooids (S).

sea pen by four thin tissue septa called membranous septa (Figures 5B, 5E, 5F). These septa divide the coelom into four water canals (Figure 5). These canals were smaller in the Rachis than the Peduncle and merged and integrated at the dorsal end of the sea pen (Figures 5A, 5B, 5E, 5F). Moreover, another water canal was found between the axis and the sclerite, which is considered the fifth water canal of the sea pen (Figure 5D).

### 3.2. Rachis

The siphonozooids were observed as two narrow strips at the dorsal surface of the rachis (Figures 3D, 4A). These strips had many invaginations with several canal cavities in the center of each one that eventually connected to the central canal of the body. The inner surface of the canals was coated by gastrodermic cuboid cells (Figure 6A).

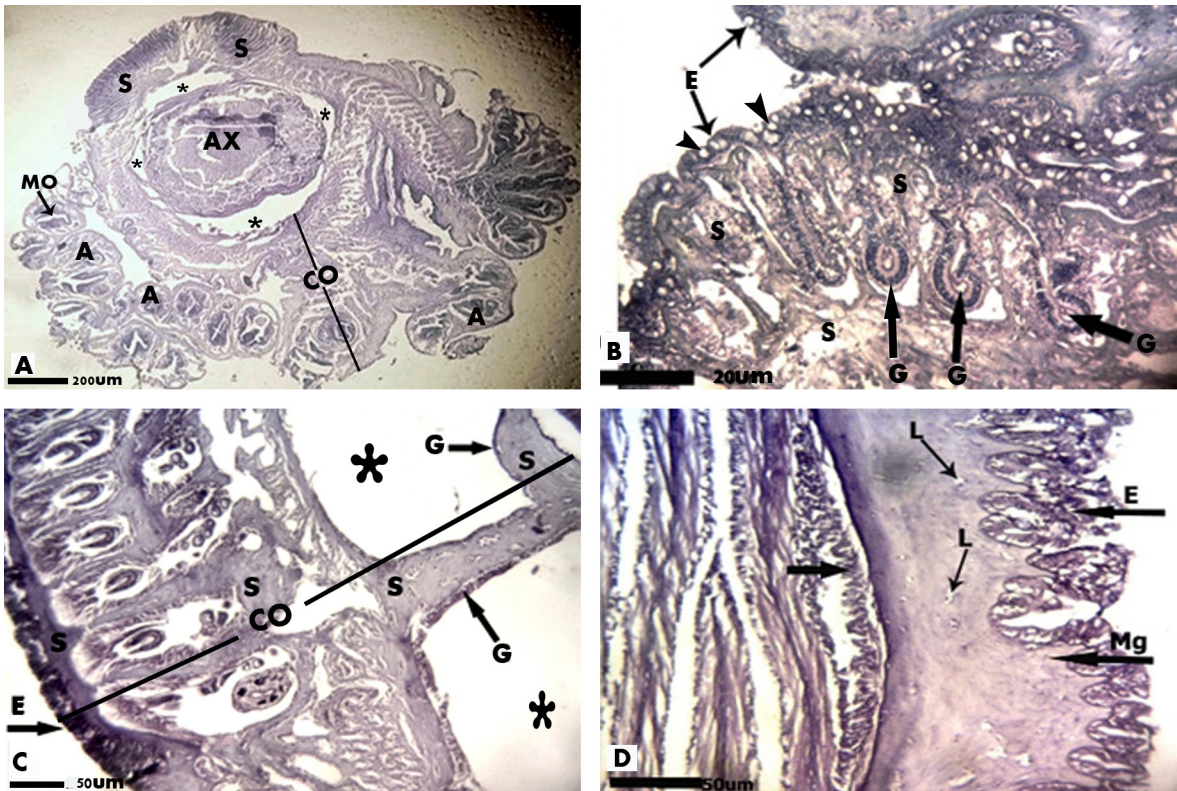
The ventral surface of the rachis was coated with the leaf-like plates called autozooids, which are involved in feeding (Figures 3B, 3C, 4A). Each autozooid had eight hollow finger-like eminences called tentacles, which were covered with a layer of gastrodermic columnar cells and a smooth muscular layer around it (Figure 6B, 6C, 6D). There was a central cavity within each of the autozooids called gastric cavity, which is divided by eight mesenteric septa into eight mesenteric canals (Figures 6B, 6D, 6E).

Each mesenteric canal was associated with a tentacle, all of which ultimately led to an opening mouth (Figures 6B, 6C, 6D, 6E).

The outer surface of the rachis was coated by an epidermis, comprising a layer of columnar cells, with the glandular cells between them (Figure 6F). Several invaginations from the epidermis into the mesogloea were found whose walls were covered by a layer of gastrodermic columnar cells (Figure 6F). On the other hand, sclerite calcareous plates progressed from the mesogloea to the epidermis and formed a layer around the epidermal invaginations (Figure 6G).

### 3.3. Peduncle

In the peduncle, like the rachis, sclerite tended to extend toward the epidermis, and, thus, many calcareous plates were visible in the body wall (Figures 7A, 7B, 7C). The inner surface of the body wall was coated by a layer of gastrodermic columnar cells with a euchromatin nucleus in the base of the cell and basophilic cytoplasm (mean thickness of  $77.22 \pm 3.5 \mu\text{m}$ ) (Figures 7B, 7D, 7E, 7F). The outer surface of the body wall was covered by the epidermis layer (mean thickness of  $46.3 \pm 13.19 \mu\text{m}$ ). The epidermis consists of two types of cells including columnar cells with a euchromatin nucleus in the base and acidophilic



**Figure 4.** Representative photomicrographs of the body wall tissue structures in rachis (A and B) and peduncle (C and D) of *Virgularia gustaviana*: A. coenenchyme (CO), autozooids (A), siphonozooids (S), mouth opening (MO), axis (AX), mesenteric canals (black stars); B. epidermis (E) with glandular cells (black arrowheads), gastrodermis (G), sclerite (S); C. coenenchyme (CO), epidermis (E), gastrodermis (G), sclerite (S), mesenteric canals (black stars); D. epidermis (E), mesogloea (MG), gastrodermis (black arrows), lacuna (L); A (H&E;  $\times 290$ ), B (H&E;  $\times 2900$ ), C and D (H&E;  $\times 725$ ).

cytoplasm and glandular cells with mucosal secretions (Figure 7D).

There were a lot of cavities inside the body wall that could be divided into three classes: 1. Gamete-containing canals: These cavities were large, within which gametes were seen at different stages of maturation depending on the colony growth stage; 2. Sclerite cavities: These cavities were very small in size; and 3. Water canals: The size of these cavities was between the Gamete-containing canals and the sclerite cavities (Figures 7A, 7B, 7C).

The extension of the sclerite calcareous tissue to below the epidermis led to the formation of calcified folds coated with the gastrodermis in the wall of the peduncle. Between the folds, there were water canals, in which gastric tubules were found with a wall composed of a layer of smooth muscle cells coated with a gastrodermic columnar cells and a gastric cavity in the center (Figure 7A).

### 3.4. Bulb

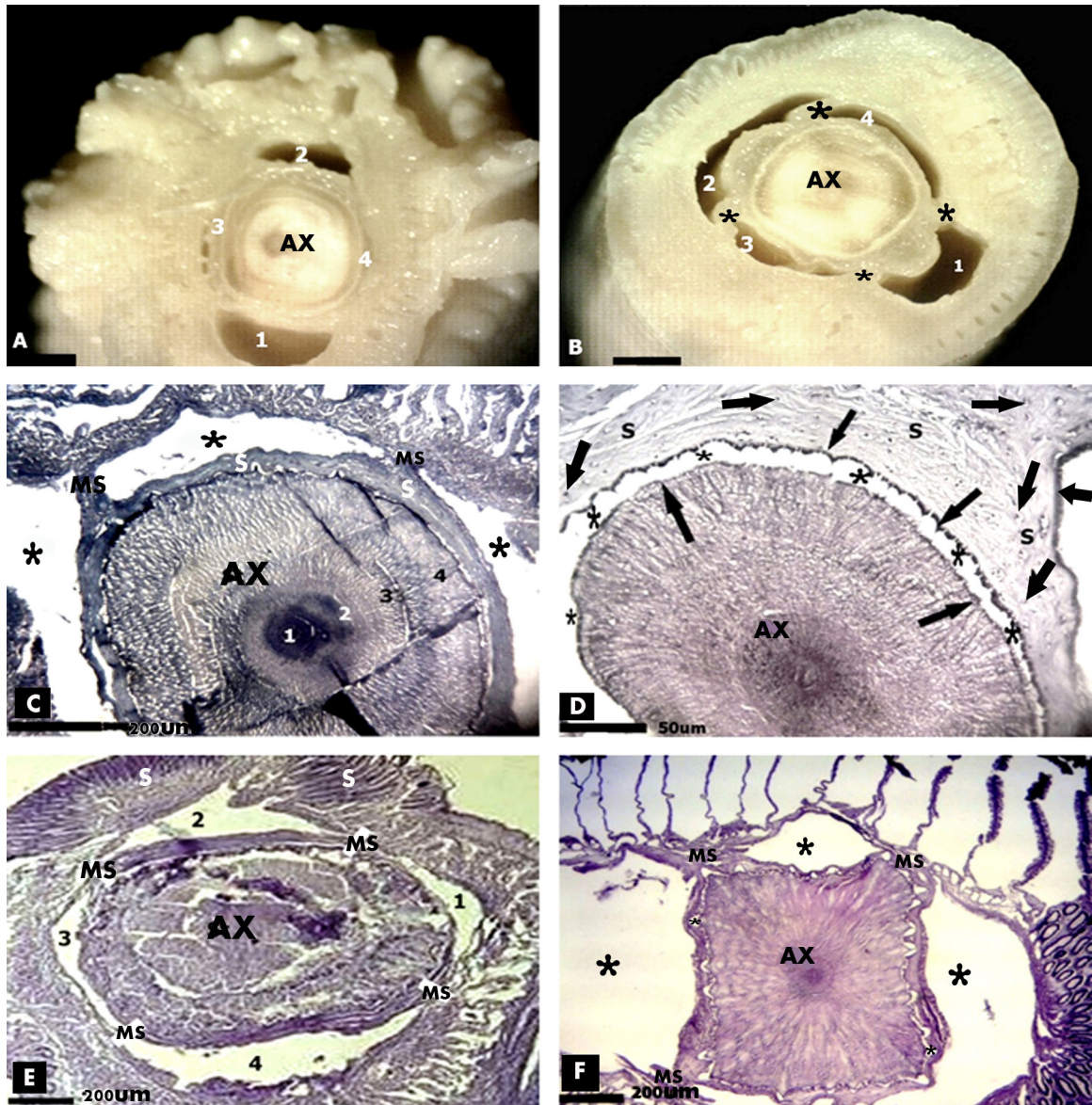
The underside part of the peduncle, which is the point of attachment to the sea bed, is flat and bubble-shaped with an anchor-like function (Figure 8A). The body wall of

bulb in the *V. gustaviana*, like the other parts, consisted of three layers: the outer epithelium, the middle mesogloea, and the inner gastrodermis. Unlike other parts, the bubble lacked the axis (Figures 8B, 8C, 8D). But in some cases, the hook like axis was visible in the transverse section (Figure 8C). Sclerite, coated on both sides by columnar sclerocytes, extended from the body wall to the other side and divided the coelom into two canals (Figure 8C). The inner surface of the body wall in the bulb was covered by columnar cells of gastrodermis (mean thickness of  $22.14 \pm 7.5 \mu\text{m}$ ) with a euchromatin nucleus and basophilic cytoplasm (Figures 8C, 8E). The sclerite cavities were visible in the bulb mesogloea, but unlike other parts of the peduncle, gamete-containing canals were not observed in this area (Figures 8C, 8F). In the bulb, like other parts of the peduncle, the sclerite septa extended down under the epidermis, leading to the formation of gastric canals (Figure 8F).

### 4. Discussion

Octocorals are considered as an important part of the benthic ecosystem in the Persian Gulf. Sea pens are among



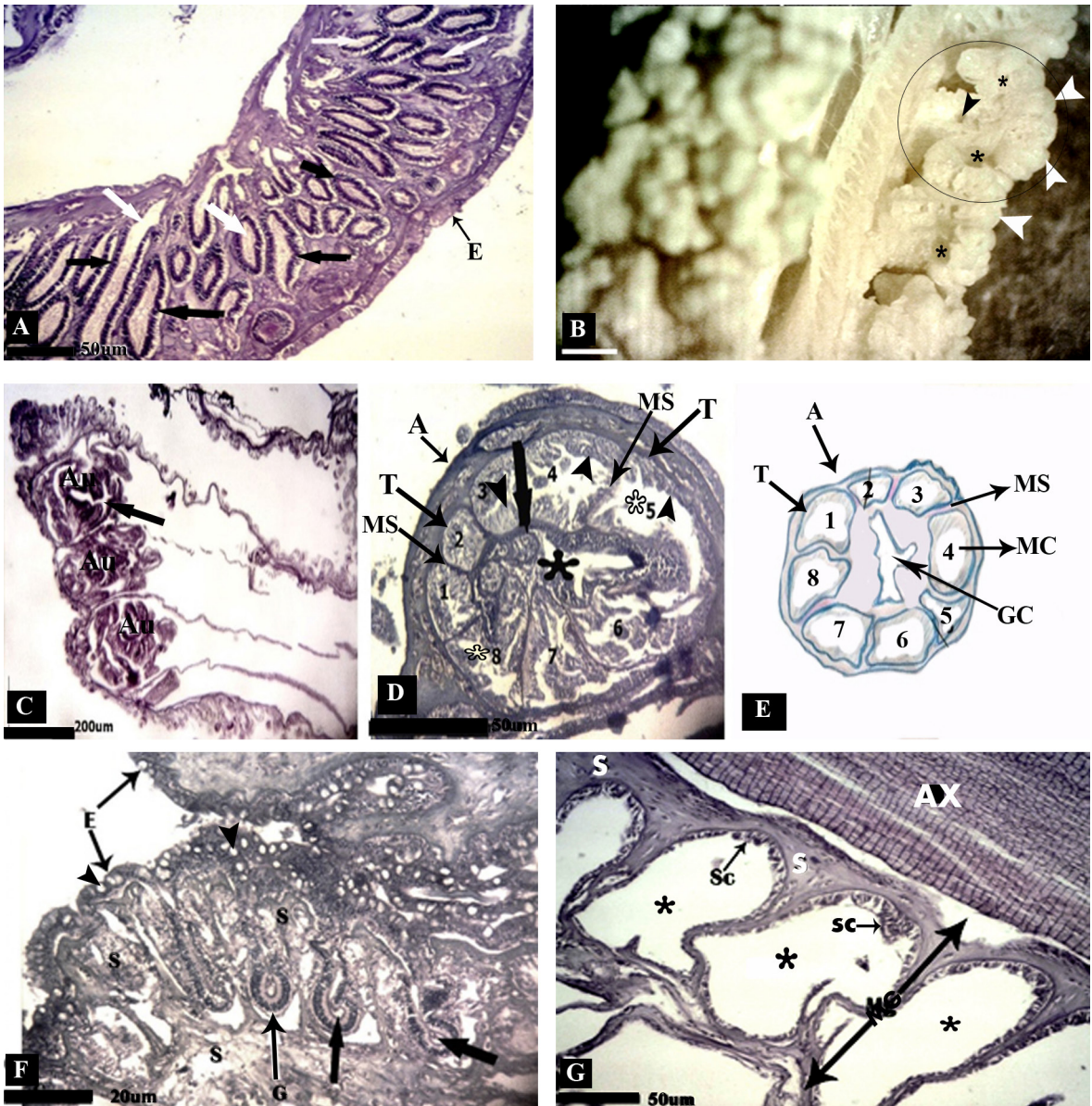


**Figure 5.** Representative stereomicroscopic and microscopic images of the axis and surrounded tissues in the rachis (A,C,E) and peduncle (B,D,F) of *Virgularia gustaviana*: A. axis (AX), dorsal mesenteric canal (1), ventral mesenteric canal (2), lateral mesenteric canals (3,4); B. axis (AX), lateral mesenteric canals (1,2), dorsal mesenteric canal (3), ventral mesenteric canal (4), membranous septa (MS); C. axis (AX), dark and light concentric circles (1, 2, 3, 4), sclerite (S), membranous septa (MS), mesenteric canals (black stars); D. axis (AX), sclerite (S), sclerocytes (black arrows), the fifth water canal (black stars); E. axis (AX), siphonozooids (S), dorsal mesenteric canal (2), lateral mesenteric canals (1, 3), ventral mesenteric canal (4), membranous septa (MS); F. axis (AX), mesenteric canals (black stars), membranous septa (MS); C,E,F (H&E;  $\times 290$ ), D (H&E;  $\times 725$ ).

the most important octocorals. According to available reports, the *Virgularia gustaviana* is the dominant species of the Iranian waters of the Persian Gulf [17,18,19]. In general, few studies have been conducted on the morphological and histological description of different species of sea pens, most of which have only investigated their reproductive system [20,21,22, 23]. However, there is

no report of histological examination of the Persian Gulf sea pens. In the present study, we used morphological characteristics and spicule shape to identify *Virgularia gustaviana*. Three-edged and brass-like spicules were found in autozooids and peduncle of *V. gustaviana*, respectively. Spicules contain color pigments so that the color of the colonies is affected by these colored pigments.





**Figure 6.** Representative stereomicroscopic and microscopic images of the rachis in *Virgularia gustaviana*: A. siphonozooid canals (white arrows) coated by gastrodermic cuboid cells (black arrows), epidermis (E); B. autozooid (black circle), tentacles (white arrowheads), gastric cavity (black arrowhead), mesenteric canal (black stars); C. autozooid (Au), gastric cavity (black arrow); D. autozooid (A), tentacles (T, 1-8) coated by gastrodermic cells (black arrows), gastric cavity (black star), mesenteric canal (white stars), mesenteric septum (MS); E. autozooid (A), tentacles (T, 1-8), gastric cavity (GC), mesenteric canal (MC), mesenteric septum (MS); F. sclerite (S), gastrodermic cells (G), gastric cavity (black arrows), epidermis (E) with glandular cells (black arrowheads); G. axis (AX), sclerite (S), sclerite canals (black stars), sclerocytes (SC), mesogloea (MG); A,D,G (H&E;  $\times 725$ ), C (H&E;  $\times 290$ ), F (H&E;  $\times 2900$ ).

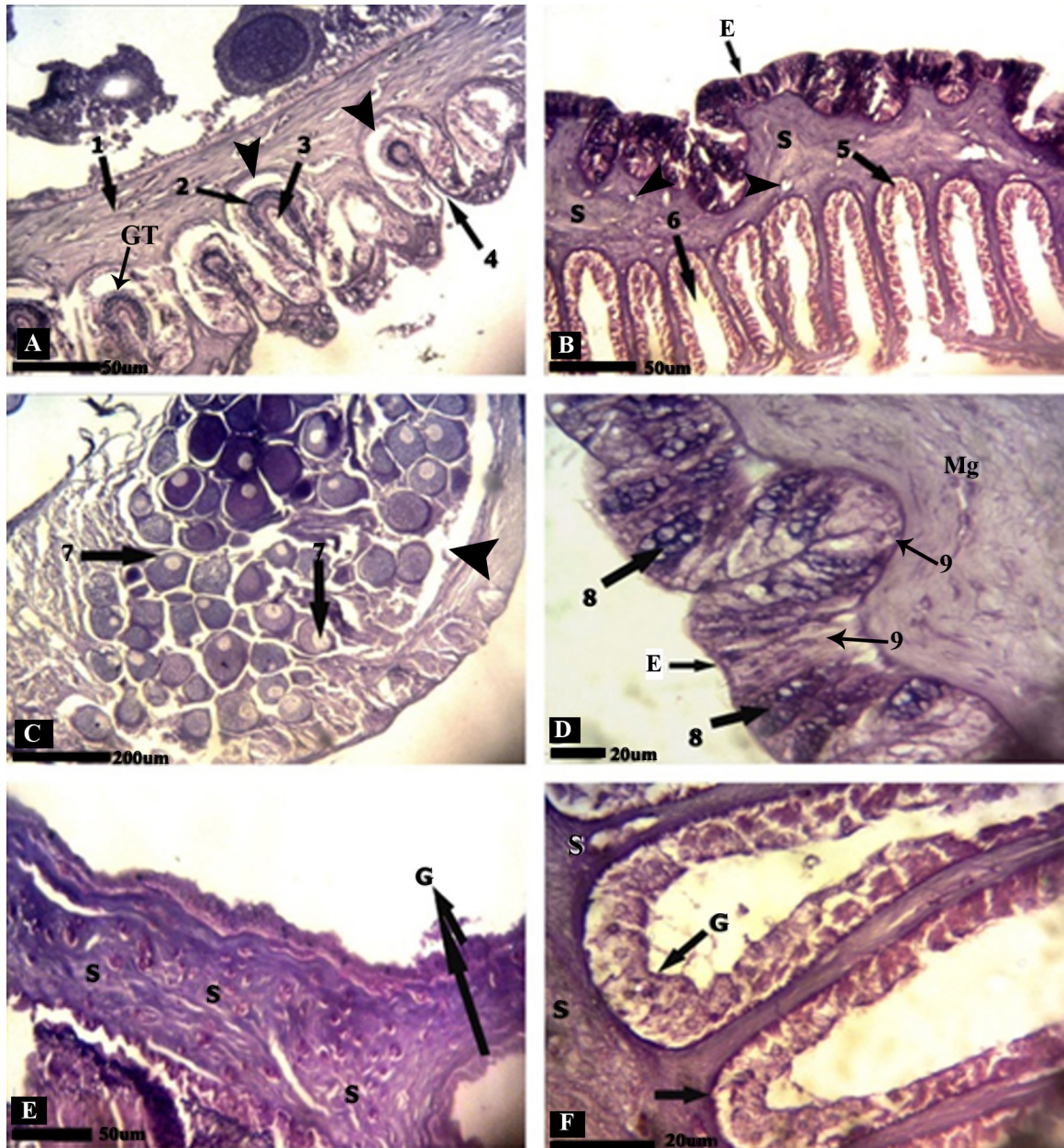
In a study on *V. gustaviana*, William [1] had identified two types of spicules using an electron microscope, in the form of three edges in atosoids and siphonozooids, and rice grains in the peduncle.

Williams [1] stated that the primary polyp is composed of an adult colony and several kinds of secondary polyps including autozooids related to feeding and sexual

reproduction; siphonozooids involved in internal water circulation; mesozooids and Acrozooids (found only in a few species of the genera Pennatula and Pteroeides) implicated in exhalant water circulation and asexual reproduction, respectively.

According to Williams and Alderslade [24], almost all sea pens attach to the soft benthic substrate by an



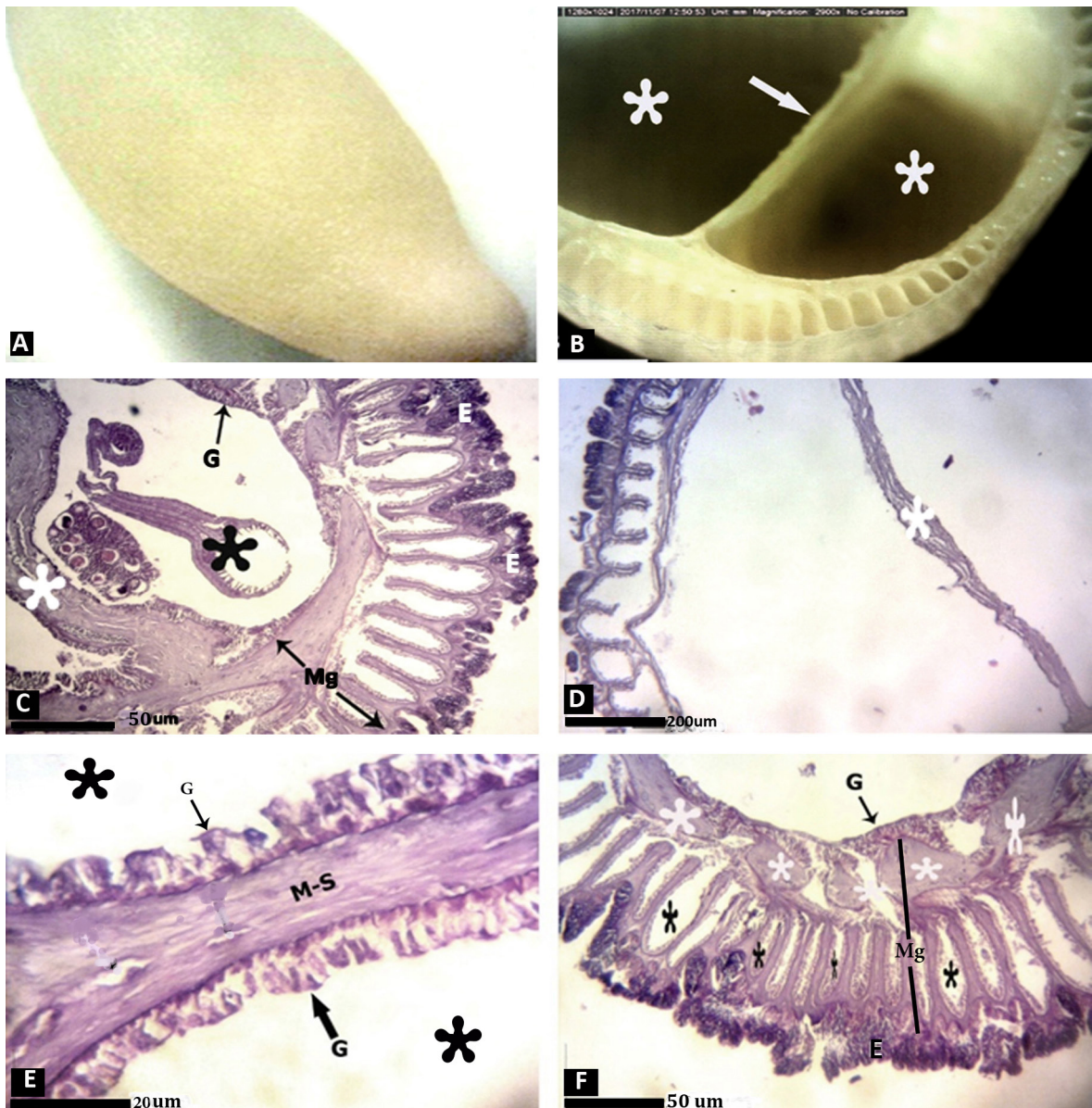


**Figure 7.** Representative photomicrographs of the peduncle in *Virgularia gustaviana*: A. sclerite (1), water canals (black arrowheads), gastric tubule (GT), gastrodermic cells (2), gastric cavity (3), mouth opening (4); B. epidermis (E), sclerite (S), sclerite cavities (black arrowheads), gastrodermic cells (5), gastric cavity (6); C. gamete-containing canals (black arrowhead), gametes (7); D. epidermis (E) consisted of glandular cells (8) and columnar epithelial cells (9), mesogloea (Mg); E. sclerite (S), gastrodermis (G); F. sclerite (S), gastrodermis (G), gastric cavity (black arrow); A,B,E (H&E;  $\times 725$ ), C (H&E;  $\times 2900$ ), D,F (H&E;  $\times 290$ ).

unbranched and root like cylindrical peduncle, which gives them the ability to stand straight. Based on the available reports in many species of the family Veretillidae, the rachis has leaf-like tentacles, and its length is more than half the length of the body [25]. The peduncle, the lower part of the body in the sea pen, is lacking tentacle.

The bubble-shaped bottom of the peduncle drops like an anchor in the bed, helping to keep the animal constant [25]. All of these findings were in agreement with the anatomical structure of *V. gustaviana*. In each autozooids, the internal lumen of the tentacles opens into the mid space of the autozooid, and then into mesenteric canals





**Figure 8.** Representative stereomicroscopic and microscopic images of the bulb in *Virgularia gustaviana*: A. bulb; B. coelom (white stars), median septum (white arrow); C. epidermis (E), gastrodermis (G), mesogloea (Mg), hook like axis (black star), median septum (white star); D. median septum (white star); E. median septum (white star), gastrodermis (G), coelom (black stars); F. epidermis (E), gastrodermis (G), mesogloea (Mg), gastric cavity (black stars), sclerite (white star); C,F (H&E;  $\times 725$ ), D (H&E;  $\times 290$ ), E (H&E;  $\times 2900$ ).

[25]. The beautiful appearance of the sea pens is due to the expansion of autozooids and tentacles to absorb suspended food in the water column. In an entirely contracted sea pen, the water is completely ejected from all mesenteric canals, autozooids, and tentacles [25].

López-González et al. [25] reported that, on the autozooids of *Malacobelemon daytoni*, as in other species of the genus Pennatulacea, there were eight hair-like tentacles. The throughout rachis had a two-way symmetry

with the wider dorsal and thinner ventral surfaces and the end of the siphonozooid was considered as the rachis border with the peduncle in this species. In *M. daytoni*, the size of the polyps increased toward the tip of the rachis, and, therefore, it was difficult to determine the young autozooids from the adult siphonozooid [25].

The peduncle's wall is very porous, which allows it to be completely swollen with water entering the cavities. There are two rows of canals in the peduncle's spongy wall, the

outer canals being in the longitudinal arrangement parallel to the peduncle. At the center of the peduncle, there is a central axis surrounded by spongy sclerite and mesenteric canals [25]. According to Nonaka et al. [26], the central axis with many brown and white spots (probably due to its calcium and protein content) in the sea pens of Virgulariidae family extended throughout the length of the colony. This was in agreement with the present study. Williams [1] also stated that most pennatulaceans have a central axis, mainly composed of calcium carbonate, which extends partially or completely along the colony and has a transverse section of the circle or quadrant. In the present study, the cross sections of the axis in *Virgularia gustaviana* were circular and quadrature respectively in the rachis and the end of the peduncle. Wilson et al. [12] reported that, in the *Halipteris willemoesi*, the thin cone-shaped axis had extended throughout the colony; however, the thickness of it decreased to the end of peduncle (Bulb). The entirely white axis (the completely calcified axis) with smooth and circular cross section was surrounded by a soft tissue attached to the peduncle in this species. In the present study, the axis was observed in a hook-like form in both ends of the polyp.

According to the results of the present study, the sclerite and mesenteric septa in rachis and peduncle were almost in the same position. The sclerite connected to the body wall by four membranous septa in both rachis and the peduncle, and there were four water canals (mesenteric canals) between the septa. The mesenteric channels in

Rachis were smaller than the peduncle so that they were completely integrated into the Roche's tip.

Batie [27] had also stated that four septa divided the central cavity of the primary polyp of *Ptilosarcus gurneyi* into 5 channels: a ventral canal, two lateral canals, a dorsal canal, and a small blinded canal within the axis. In the peduncle, along each of the mesenteric blades, a flat muscular band was obliquely attached from the axis to the body wall in this species. The same findings were obtained in the present study.

## 5. Conclusion

In conclusion, although all sea pens have the basic histological structure as described, the shape of the colony in different species varies considerably in terms of evolution, especially their environmental compatibility. Future research will focus on the comparative morphometric and histologic study of different species of sea pens from the Persian Gulf.

## Acknowledgement/disclaimers/conflict of interest

The authors would like to thank the Marine Fish Research Station in the Hormozgan for practical support. The authors report no declarations of interest.

## Informed consent

This manuscript does not report the results of experimental investigations conducted with humans.

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