

Anatomical and Tissue Study of the Organs of the Marine Gastropod *Phorcus lineatus* (Da Costa, 1778) from El Jadida Coastline (Atlantic coast, Morocco)

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ABSTRACT

This study was part of the monitoring of the state of health of Morocco's Atlantic coasts through bio-indicator species of pollution. The marine gastropod *Phorcus lineatus* (Da Costa, 1778) was the biological material of choice, given its wide geographical distribution, its abundance even in polluted areas, its reduced mobility and its important role in the food chain. The objective of this work was to describe for the first time the anatomy of the marine gastropod *Phorcus lineatus* from the coast of El Jadida with a view to initiating a short-term bioaccumulation study in this same species. Specimens of the gastropod were collected at low tide in the intertidal zone from June 2018 to May 2019. Both macroscopic and microscopic observations were made at the level of the various organs and histological sections made. The organization and layout of the various devices were well analyzed and reported in illustrations in the results section. In addition, the general structure and the tissue and cytological constitution of the different organs were described on the basis of the good quality of the histological sections. The data obtained provided a better knowledge about the internal and external morphology on the one hand and the cellular organization in different devices of *P. lineatus* from the coast of El Jadida on the other hand.

INTRODUCTION

Phorcus lineatus is a mollusk of the class Gastropoda, subclass Vetigastropoda and family Trochidae (Da Costa, 1778). Its two former nomenclatures are *Osilinus lineatus* and *Monodonta lineata* (Shurlock *et al.*, 2017; Little *et al.*, 2020). This gastropod has a wide geographic distribution extending from the Moroccan Atlantic coasts to the south of Great Britain and Ireland (Da Costa, 2015; García-Escárzaga *et al.*, 2019). This species colonizes rocky crevices and foreshore ponds in intertidal zone. On the Moroccan coasts, and more particularly on the coastline of El Jadida region, *P. lineatus* is present despite the pollution level of some of its sites (Merzouki *et al.*, 2009; Rouhi *et al.*, 2012; Sif *et*

al., 2016). This situation aroused our scientific curiosity. The present study was therefore engaged to acquire anatomical and physiological knowledge for a future ecotoxicological study. The absence of these data on *P. lineatus* living in El Jadida coast makes the originality of this work.

MATERIALS AND METHODS

1. Study sites and animal collection

The study site was located at the Moroccan Atlantic coast about 1km from the center of the city of El Jadida (Fig. 1). Adult individuals of *P. lineatus* (shell height 18-25 mm) were sampled at low tide at the rocky part of the intertidal zone. The animals were transported to the laboratory in jars and then placed in oxygenated seawater until their dissection.

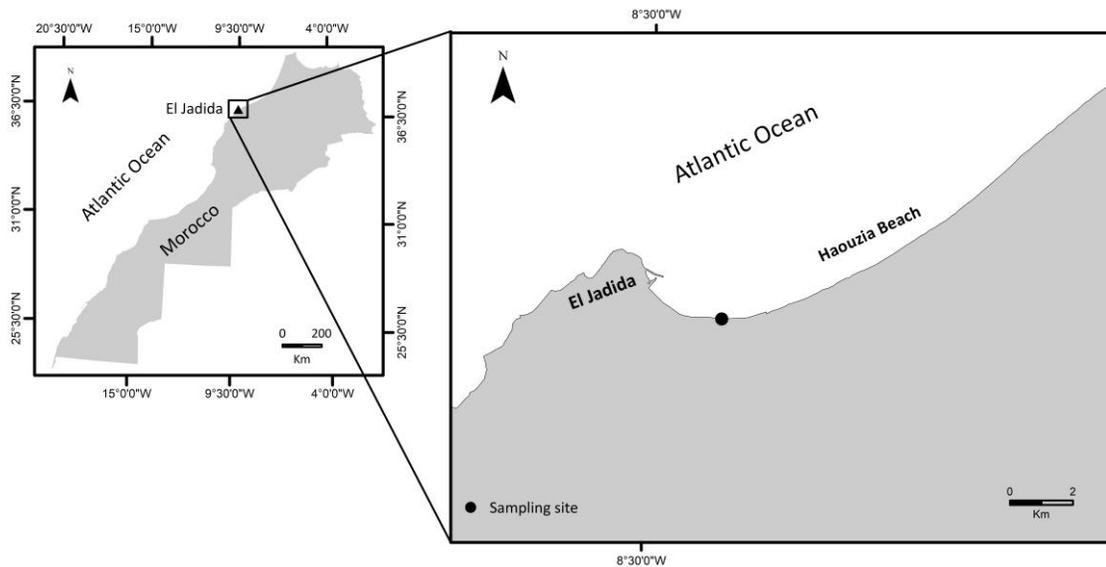


Fig. 1. Geographical location of the study site

2. Anatomical study

It was based on macroscopic and microscopic observations of the animals. These observations which concern both the shell and the soft part are made with the naked eye or under binocular magnifying glass and/or with light microscope.

2.1. Dissection technique

After shelling, the specimens were dissected to identify and note the organization of various apparatuses: respiratory, digestive, excretory, reproductive and nervous. The dissection is very delicate and was carried out in two stages (Fig. 2), allowing the observation of the organs contained in the visceral cavity.

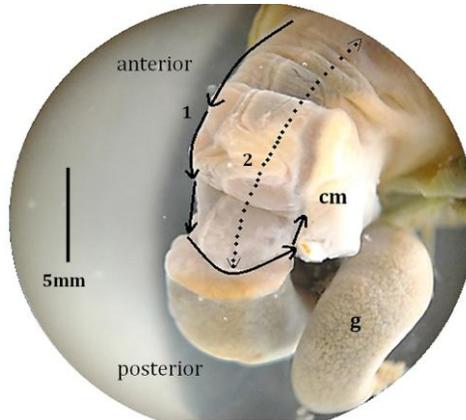


Fig. 2. Dissection of *P. lineatus* in dorsal view (1: first stage; 2: second stage). cm: columellar muscle; g: gonad.

2.2. Realization of histological sections

After the dissection of the samples, various organs were removed and fixed in Gendre's solution for five days. The samples were then dehydrated into a series of ethanol solutions (80, 95 and 100%). Then, they were clarified in toluene and incorporated into paraffin. Sections 6 to 7 μ m thick were made with the microtome (YD-335). The sections were mounted on glass slides, bathed in toluidine blue and covered by cover slips. Observations were made using an optical microscope with different magnifications.

RESULTS

1. Shell morphology

The shell of *P. lineatus* is conical, with bulging base, solid in appearance and with four to five turns of spires. It is ornamented with numerous zigzag bands that characterize the species (Fig. 3). The aperture called peristome is pearly. It is closed by a circular operculum, carried by the foot when the animal is retracted inside the shell.

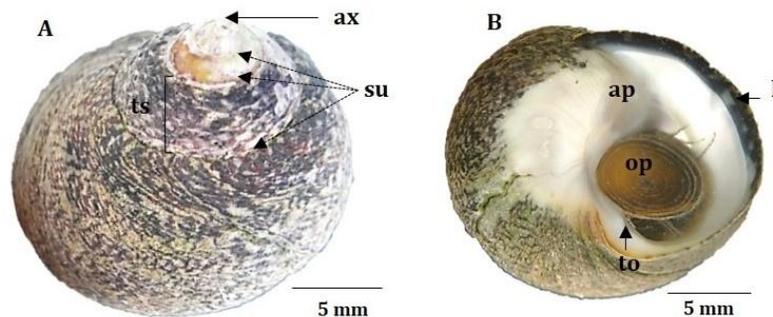


Fig. 3. Shell morphology of *P. lineatus* in dorsal (A) and ventral (B) view. ax: apex; su: sutures; ts: turn of spire; ap: aperture; op: operculum; to: tooth; l: lip.

2. Soft tissue

Delicate extraction of the gastropod from its shell allowed us to have an overview of the different organs and their position within the soft part of the animal (Fig. 4).

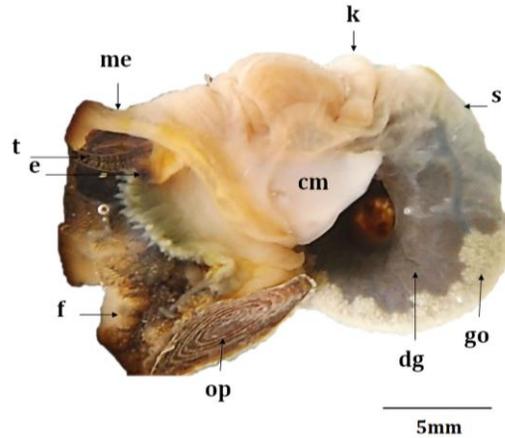


Fig. 4. Left external lateral view of *P. lineatus* extracted from its shell. me: mantle edge; t: tentacle; e: eye; f: foot; op: operculum; dg: digestive gland; g: gonad; cm: columellar muscle; s: stomach.

2.1. Respiratory apparatus

Respiration in *P. lineatus* is achieved by a gill located in the mantle cavity on the left side of the animal (Fig. 5A). It is formed by an axis and filamentous lamellae (Fig. 5B). The gill lamellae have central hemolymphatic vessels and are surrounded by several epithelial cells. Their tips are supported by skeletal rods (Fig. 5C, D).

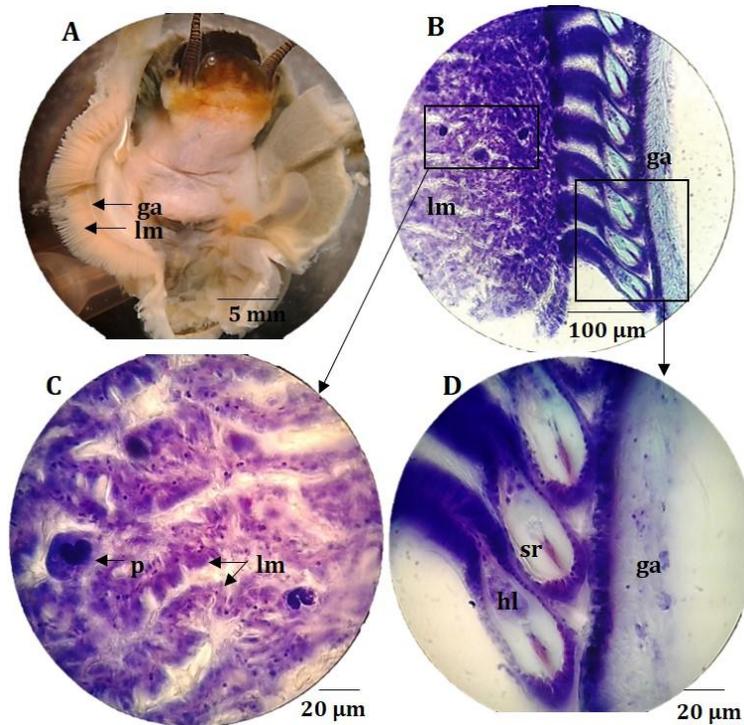


Fig. 5. Observation under binocular magnifying glass (A) and microscope (B, C, D) of the gill of *P. lineatus* from El Jadida coastline. ga: gill axis; lm: lamellae; sr: skeletal rods; ec: epithelial cells; hl: hemolymphatic vessels; p: parasite.

2.2. Excretory apparatus

In this species, it is constituted by two kidneys, which differ in their position and size. The left kidney is located in the pallial cavity, while the right kidney is located between the heart and the gonad. The right kidney is presented as a band that elongates through a very thin and transparent tube (a kind of conduit) opening through an orifice in the pallial cavity just beside the left kidney orifice (Fig. 6).

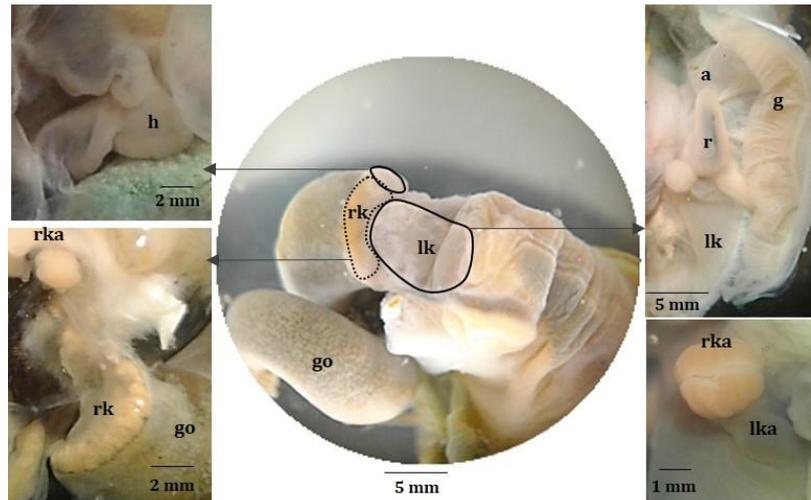


Fig. 6. Organization of the excretory apparatus of *P. lineatus* from El Jadida coastline observed under a binocular magnifying glass. lk: left kidney; rk: right kidney; rka: right kidney aperture; lka: left kidney aperture; h: heart; g: gill; r: rectum; a: anus; go: gonad.

2.3. Digestive apparatus

The digestive apparatus is not rectilinear because of the twist characteristic of gastropods (Fig. 7). It begins with the buccal cavity, the esophagus, the stomach and ends with the intestine, which continues with a short rectum. The latter opens into the pallial cavity through the anus.

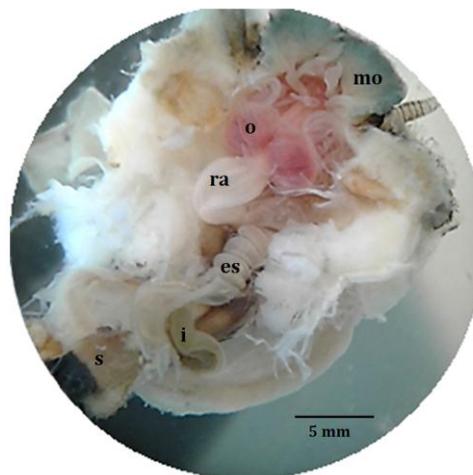


Fig. 7. Ventral view of the digestive apparatus of *P. lineatus* from El Jadida coastline observed under a binocular magnifying glass. mo: mouth; o: odontophore; ra: radula; es: esophagus; i: intestine; s: stomach.

The buccal cavity contains the radula carried by a cartilaginous tongue called odontophore (Fig. 8A). The latter is formed by two pairs of cartilages: anterior and posterior. The radula is a whitish band with an orange colored tip. It is composed of three types of teeth that allow the animal to graze on the algae attached to the rocks: central, lateral and marginal teeth (Fig. 8B). Microscopic observation of odontophoral cartilage at high magnification revealed the cytological organization. It is thus composed of juxtaposed cells of geometrically hexagonal shape (Fig. 8C). The tissue has a reduced extracellular matrix.

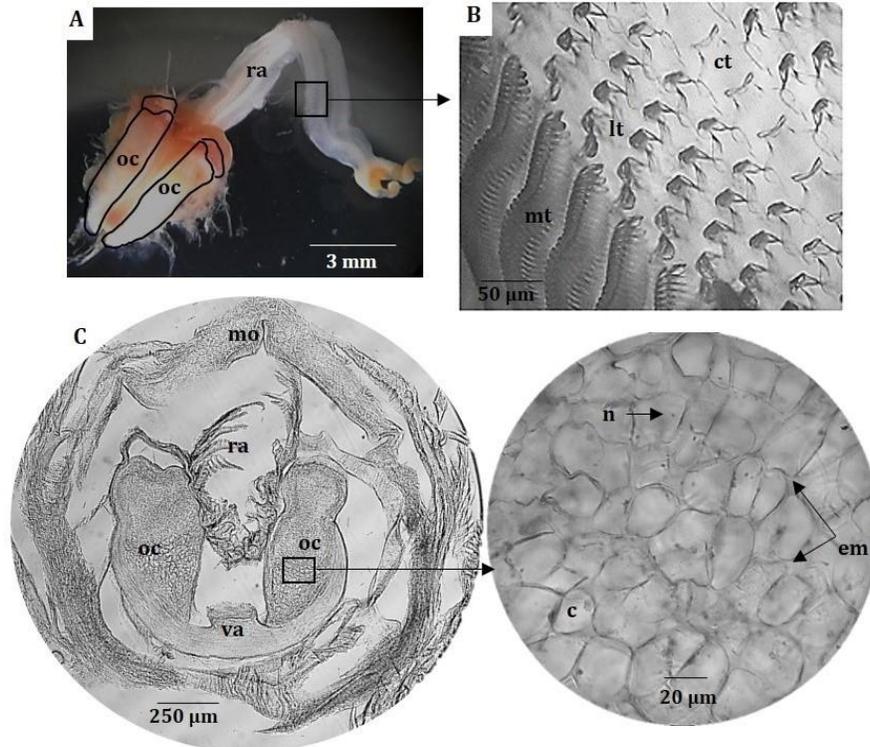


Fig. 8. Observation under binocular magnifying glass (A) and microscope (B, C and D) of the odontophore-radula complex of *P. lineatus* from El Jadida coastline. ra: radula; mt: marginal teeth; lt: lateral teeth; ct: central teeth; oc: odontophoral cartilage; mo: mouth; va: ventral muscle; n: nucleus; c: cell; em: extracellular matrix.

The esophagus is a long, thin cylindrical tube. It has folds that are clearly visible externally, allowing it to extend in length (Fig. 7). The stomach is located in the dorsal part, right next to the digestive gland. It is characterized by the presence of a spiral caecum and represents the junction where the esophagus, the digestive gland and the start of the intestine emerge. Microscopic observation of the cross section of the stomach wall allowed the identification of three layers: the mucosa, submucosa and muscularis (Fig. 9A). The cytologic organization of the mucosa appears to be lined by columnar epithelial cells with an elongated nucleus in a basal position and cilia that are erect toward the stomach lumen. The surface of the mucosa is covered by a thin pellicle (Fig. 9B). The submucosa consists of connective tissue containing many granular cells.

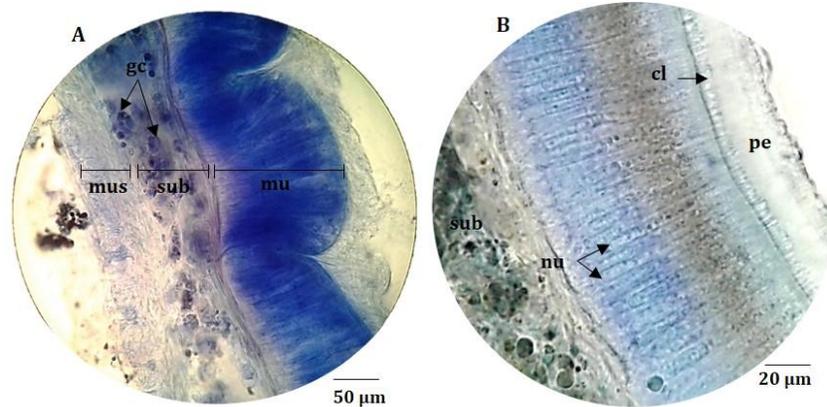


Fig. 9. Cross section of the stomach of *P. lineatus* from El Jadida coastline seen under a light microscope. mu: mucosa; sub: submucosa; mus: muscularis; cl: cilia; pe: pellicle; gc: granular cells; nu: nuclei.

The intestine, which measures about 4.5 cm, is connected to the stomach and ends in the rectum, which opens through the anus into the pallial cavity. Microscopic observation of the cross section of the intestinal wall shows the presence of villi lining the inner surface of the intestine. The intestinal epithelium is particular and is composed of several ciliated cells with a basal nucleus. It is surrounded by a less thick muscular layer than that of the stomach.

The digestive gland, the annex gland of the digestive tract, is greenish to brown and is located on the inner side of the spiral part of the gonad. The cross section shows that this gland consists of several digestive tubules of different sizes, which are hollowed out with a lumen (Fig. 10A). The tubule epithelium is composed of two cells types:

- Digestive cells that are columnar in shape and characterized by a cytoplasm filled with vesicles.
- Secretory cells that are pyramidal in shape and have an enlarged base (Fig. 10B).

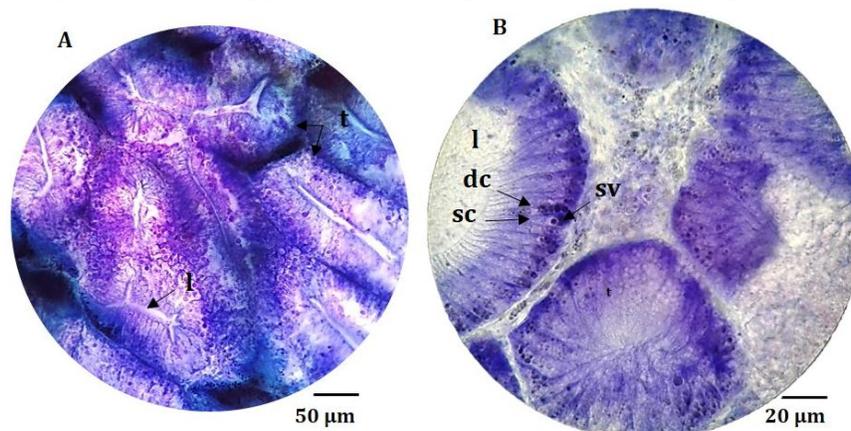


Fig. 10. Low (A) and high (B) magnification microscopic observation of digestive gland of *P. lineatus* from El Jadida coastline. dc: digestive cells; sc: secretory cells; t: tubules; l: lumen; sv: secretory vesicle.

2.4. Nervous system

The nervous system of *P. lineatus* is composed of several orange ganglia connected by whitish commissures (Fig. 11A). Microscopic observation on the sections made at ganglia shows that they are composed of two areas: the cortex and the medulla (Fig. 11B). The cortical part is composed of cell bodies of nerve cells (Fig. 11C). The medulla contains both the axons of neurons and the neuroglia (Fig. 11D).

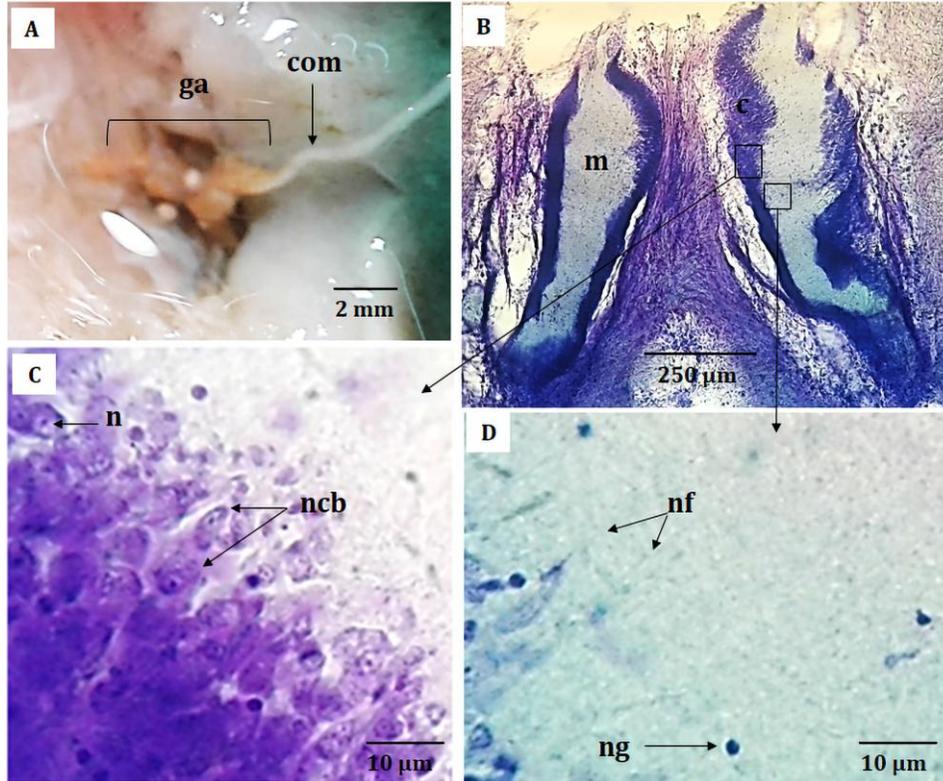


Fig. 11. Organization of the nervous system of *P. lineatus* from El Jadida coastline seen under binocular magnifying glass (A) and by microscopy (B, C, D). ga: ganglia; com: commissure; m: medulla; ncb: neuronal cell bodies; n: nucleus; nf: neuronal fibers; ng: neuroglia.

2.5. Reproductive apparatus

The reproductive apparatus of *P. lineatus* is formed by a gonad, a duct and a genital orifice. The latter opens with the urinary orifice in the pallial cavity, constituting the urogenital orifice. The identification of the sex is based on gonad color. In males, it is creamy white, while in females, it is grayish green (Fig. 12). Variation in the color of the urogenital opening lips is also identified and is a function of sex. They are small and white in males, while they are more developed and orange in females (Fig. 12).

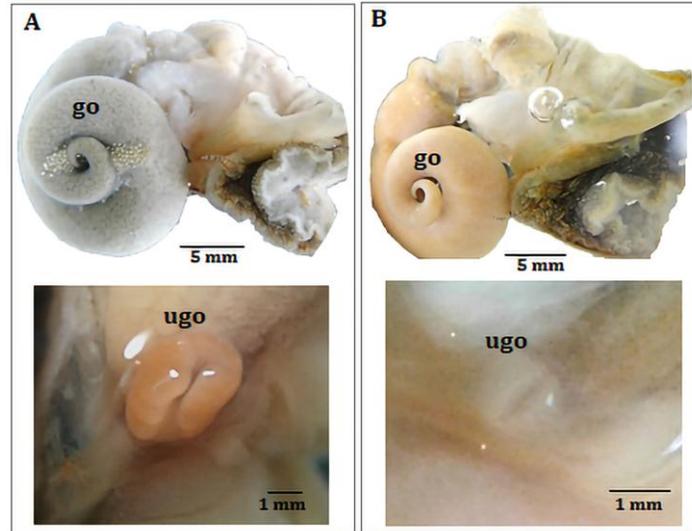


Fig. 12. Female (A) and male (B) reproductive apparatus of *P. lineatus* from El Jadida coastline. go: gonad; ugo: urogenital orifice.

Microscopic observations on histological sections of the gonads revealed the internal structure and organization of the gametes. In the mature phase, the ovaries contain ripe oocytes, with a kind of thick membrane surrounding the cytoplasm (Fig. 13A). The cross section through the testes shows all stages of spermatogenesis: spermatogonia; spermatocytes; spermatids and spermatozoa (Fig. 13B).

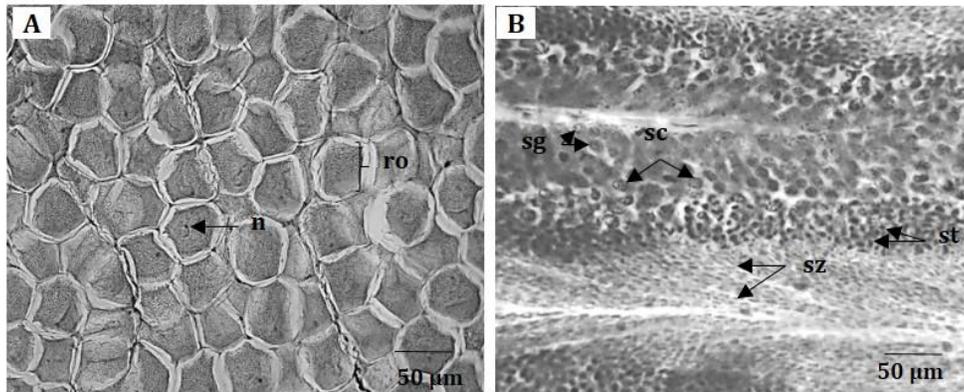


Fig. 13. Cross section of female (A) and male (B) gonad of *P. lineatus* from El Jadida coastline. ro: ripe oocyte; n: nucleus; sg: spermatogonia; sc: spermatocytes; st: spermatids; sz: spermatozoa.

DISCUSSION

The shell of *P. lineatus* is conical, swollen at the base and very hard in consistency. Its outer face is ornamented with zigzag lines (Cabral, 2020). The organization of the soft parts of *P. lineatus* required several dissection attempts to locate and identify the different apparatuses of this species.

Respiration of *P. lineatus* is carried out by a gill which is housed in the mantle cavity and is formed by an axis from which lamellar filaments leave. The gill lamellae

have central hemolymphatic vessels surrounded by several epithelial cells and supported by skeletal rods. The gill morphology of the gastropod *Austrocochlea constricta* is similar to that of *P. lineatus* (Eertman, 1996).

The excretory apparatus of *P. lineatus* is consisted of a right kidney and a left kidney, which differ in position and size. Delhaye (1976) described in Trochidae two kidneys asymmetrical in shape and their internal structure: the left kidney is entirely contained in the mantle and located on the left of the rectum, while the right kidney is visible only as a narrow band between the pericardium, the stomach and the digestive gland.

The digestive system of *P. lineatus* includes the buccal cavity, esophagus, stomach, intestine and digestive gland. The buccal cavity contains a “radula” supported by an odontophore. The radula consists of a multitude of small teeth to allow scraping the food on the rocks. Odontophore is the cartilaginous part that supports the movement of the radula during feeding (Hall, 2015). The odontophoral cartilage tissue is composed of cartilage cells with small nuclei surrounded by a reduced extracellular matrix. Katsuno *et al.* (2008) showed that the morphology and the number of odontophoral cartilages differ between different groups of gastropods.

The buccal cavity opens into an esophagus, which connects to the stomach. Microscopic observation of the cross section of the stomach wall shows that it is formed of three layers. The first is the mucosa, which is lined by ciliated columnar epithelial cells and covered with a thin pellicle. The second is the submucosa, which is made up of connective tissue containing numerous granular cells, and finally the muscularis which consists of muscle cells. The same layers were described in the gastropod *Lobatus gigas* (Tiley *et al.*, 2019).

The intestine extends from the anterior part of the stomach until the rectum, which opens through the anus into the pallial cavity. The histological section through the intestine shows that the epithelium is composed of elongated cells with nuclei in basal position. The intestinal epithelium is surrounded by a thin muscle layer.

The digestive gland of *P. lineatus* is constituted by a set of digestive tubules. The tubular epithelium is composed of two cells types: digestive and secretory cells. The latter are also known as basophilic cells, calcium cells or crypt cells (Lobo-da-Cunha *et al.*, 2018). Many researchers agree that the epithelium of the digestive gland of prosobranchs gastropods is composed of two cell types: the digestive cells and the excretory or secretory cells (Merdsoy & Farley, 1973; Bogen & Farley, 1974).

The nervous system of *P. lineatus* is composed of pairs of ganglia attached to each other by commissars. The ganglia are surrounded by connective tissue and comprised of two parts: a cortex and a medulla. The cortex is composed of the cell bodies of neurons, and the medulla contains the axons of nerve cells and neuralgia. The histological characteristics are similar to other gastropods (Thaweethamsewee *et al.*, 2011; Tiley *et al.*, 2019).

P. lineatus is a dioeciously gastropod with a single gonad coiled to it forming a spiral. In the adult stage, the female gonad is grayish-green, while the male gonad is creamy white. The data obtained in this work are confirmed by several authors (**Fretter & Graham, 1962; Underwood, 1972**). The gametes are evacuated through a genital canal into the urinary chamber of the right kidney. The lips of the urogenital opening are small and white in males, swollen and orange in females (**Fretter & Graham, 1962**). Histological analysis of cross sections of gonads showed the internal structure and organization of the gametes within the gonads.

CONCLUSION

For the purpose of using the species *Phorcus lineatus* in future ecotoxicological studies for our research team, this study enriched our knowledge about this species. In conclusion, comprehensive information was obtained on the structural and cytological organization of the various organs of this gastropod as well as their positioning in relation to each other.

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