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# THE BYSSUS SYSTEM OF THE MOLLUSCAN BIVALVE LITHOPHAGA LITHOPHAGA FROM ABOU-QIR COAST (MEDITERRANEAN SEA)

BY

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## ABSTRACT

*The byssus system of Lithophaga Lithophaga, is more or less divided into three parts; the foot, the byssus duct and the byssus threads. On the basis of structural appearance, the foot could be easily divided into four recognizable parts namely; the external ciliated epithelium, the posterior groove and canals, the foot musc ulature and nerves and the foot glands.*

*Particular attention has been given to foot glands. Six glands have been recognized and can be divided into two main groups; the pedal and stem glands. The pedal glands can be divided further into four groups $P_1$, $P_2$, $P_3$ and $P_4$, while the stem glands can be divided into two groups $S_1$ and $S_2$. A specific role for each type of these glands has been given depending on their positions, sites of secretion together with information derived from the histochemical tests.*

# INTRODUCTION

The investigation of the byssus system in a wide range of lamellibranchs has intrigued authors over a long period from the earliest time (Muller, 1837; Tullberg, 1877; Carriere, 1879; Jobert, 1882; Coupin, 1892; Boutan, 1895; Frierson, 1903). All these authors came to the conclusion that the byssus is an attachment organ sevreted by special glands of the foot. The available literature dealing with such system were absent in the following years until Yonge (1962) who suggested a possible universal occurrence of byssal apparatus in the post-larval spat of bivalves and indicated that the formation of byssus is a larval characteristic which may or may not be retained in the adult. Subsequent recent studies on the byssus and other foot glands have included early stages of the bivalvians; Ostrea edulis (Cranfield, 1973 a, b, c), Mytilus edulis (Lane and Nott, 1975), Pecten maximus (Gruffydd et al., 1975 and Lane et al., 1982), Chlamys varia (Gruffydd et al., 1979) and Cezastodezma glaucum as well as Cerastodezma edule (Yankson, 1986).

From above it is obvious that many details of the byssus system, particularly the pedal glands of adult bivalves remains unresolved and very far from complete. Thus, the present investigation was undertaken to give a comprehensive histological and histochemical understanding of the byssus system of the boring bivalvian Lithophaga lithophaga. Particular attention has been given in this respect to the pedal glands in an attempt to detect the positions of the glands and to characterize histochemically the secretion of each gland

cell type to provide more information on a specific role for each type of glands during animal like.

## MATERIAL AND METHODS

Specimens of Lithophaga lithophaga were found in abundance inhabiting burrows of a wide range of limestones in depth from 1-5 m in Abou-Qir. Therefore, the investigated samples were collected by carefully broken open using a hammer and chisel.

For anatomical study, the process of fixation is essential for relaxation the animals and preventing their autotomy during dissection. In this respect the method adopted by Morse (1968) was followed. In this method the animals relaxed in 8% magnesium chloride, fixed 4 hours in Baker's formaline and stored in 1% propylene phenoxetol in distilled water (V/V). This mothod preserved some of the original colours of the organs and caused less shrinkage and distortion.

For microscopical examination, it is necessary to narcotize the samples to prevent their distortion and rupture on fixation. Narcotization was carried out by warming the samples up in a limited amount of sea water, at 40°C. After narcotization individuals were dissected out of their shells and transferred to the suitable fixative.

Several fixatives were tried such as 70% alcohol, 10% buffered formaline in sea water and Bouin's fixative. Howevor, Bouin's fixative proved to be the most useful one, because it gave less distortion of the tissue than other fixatives. Fixed

samples were then stored in 70% ethanol. The standard procedures for dehydration, clearing and embedding in paraffin wax were followed. Transverse, longitudinal and sagittal sections were cut to a thickness of 4-6 U. The different histological and histochemical tests employed during this work are summarized in table (1).

**Table (1):** The different histological and histochemical tests employed during this work.

| Demonstrated components | Histochemical tests | Groups identified | References |
|---|---|---|---|
| Carbohydrates | Periodic-acid Schif (PAS) | MPS. | Pears (1968) |
| | Alcian blue PH 1.0, 2.5 (AB 1.0, 2.5) | Selective staining for acidic MPS. | Pears (1968) |
| Proteins | Alcain blue PH 2.5-PAS Mercuric bromophenol blue (BPB) | Differentiation of neutral and acidic MPS. | Mowry (1963) |
| Phenol | Malachite green. | Protein amino groups | Bonhag (1955) |
| For general histological characters | Ehrlich's haematoxylin and eosin. Mallory's triple stain Heidenhain's azan | Phenol group. | Gretchen (1971) |

## RESULTS

Like all bivalves the byssus system, in the species under consideration is more or less divided into three parts: the foot, the byssus duct and the byssus threads.

## 1. The foot:

The foot is normally short and bilaterally symmetrical. It is heavily pigmented organ that is capable of stretching up to nearly three times its resting length. It is situated in the posterior region of the mantle cavity (PL. 1, A). The outer surface of the foot is completely covered with simple epithelium of ciliated cells. On its ventral surface the foot has a groove extending from the base, where the byssus duct is attached to the animal, to the tip where it ends in a distal depression. From the pedal depression towards the tip the groove takes the form of shallow V shaped concavity along the sole (PL. 1, B). The cells of pedal groove have short cilia.

The foot could be easily divided into four recognizable parts on the basis of its structural appearance: the external ciliated epithelium, the posterior groove and canals, the foot musculature and nerves, and the foot glands.

### 1.1. The external ciliated opithelium:

The external ciliated epithelium covers the whole foot and is composed of very dense tall ciliated columnar cells arranged in furrows (PL. 1, E) which increase in the ventral surface of the foot than the dorsal. However, it was noticed that the ciliation of the pedal epithelium coincides with the position of the underlying pedal mucous glands. Cilia are few dorsally (PL. 1, C) and are longer and more dense at the apex of the foot and on the central and ventrolateral surface (PL. 1, D). As stated by Bayne (1971) in Mytilus edulis, these cilia may be

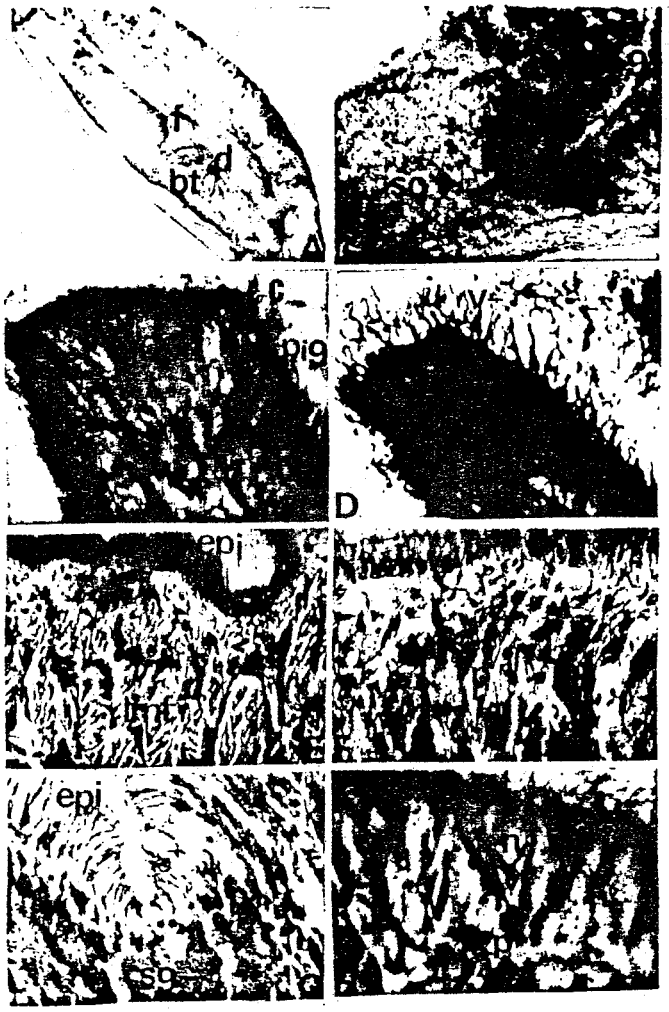



Plate 1:

A-B: Transverse section through the $P_4$ gland.

A : Stained with haematoxylin and eosin, x 1000.

B : Stained with mercuric bromophenol blue, x 1000.

C-D: Transverse section through the foot in the region of the duct to show $S_1$ & $S_2$ glands.

C : Stained with haematoxylin and eosin, x250.

D : Stained with Azan, x400.

E : Transverse section through the foot in the duct region to show $S_1$ gland stained with PAS, x250.

F : Transverse section through the foot in the groove region to show $S_1$ gland stained with PAS, x250.

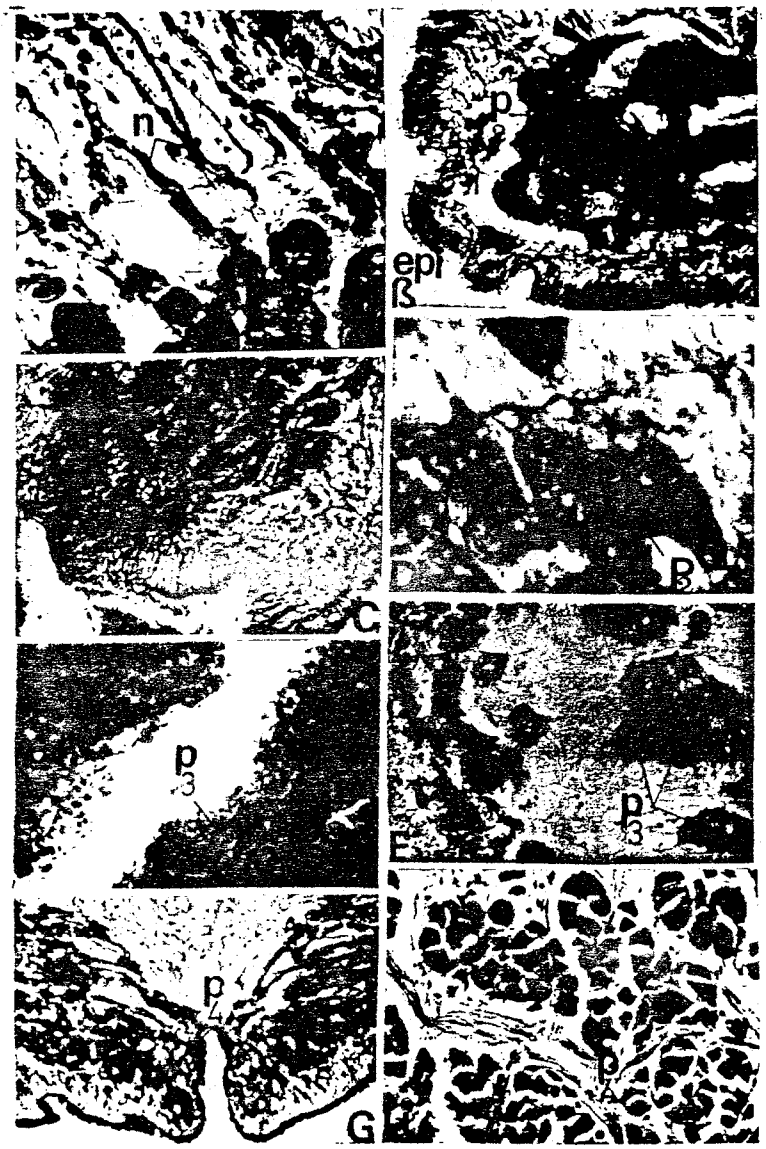
G : Transverse section through the foot in the duct region showing $S_1$ gland stained with alcian blue H 2.5, x100.

H : Longitudinal section through the $S_1$ gland stained with alcian blue pH 1.0, x 250.

responsible for creating the inhalant water current into the mantle cavity. The borders of these cells have a dark line, beneath this line there are dark brown to orange pigment granules filling the majority of the cells spaces (PL. 1, C). The nuclei of these cells are oval with obvious chromatin granules and occupy the basal portions of the cells. There are also occasional epithelial cells discharging their diffuse reticulate contents of spherical colourless vacuoles on the outer surface of the foot (PL. 1, D).

### 1.2. The posterior groove and canals:

The posterior groove widens into the suckers like depression just before the distal end of the foot and run up into the opening of byssus duct at the junction of the foot and the visseral mass (Fig. 1). From the pedal depression towards the tip the groove takes the form of a shallow V shaped concavity along the sole (PL. 1, B). The groove varies in cross section along its length, being much deeper and more slit-like just below the byssus gland than nearer the distal end. The lining epithelium of the groove is composed of tall columnar cells through which the phenol and protein glands discharge their secretions into the groove directly. The two lateral canals in the foot drain that portion of the secretion of the gland which lies deeply embedded in the foot anterior to the protein gland. These canals discharge their secretions into the distal depression of the groove probably by contraction of the longitudinal muscle layer which surrounds the orific of the canal.




## Plate 2:

A : Longitudinal section through the $S_1$ gland cells stained with aldehyde fuchsin, x250.

B : Transverse section through the foot in the duct region showing $S_1$ & $S_2$ glands stained with mercuric bromophenol blue, x100.

C-D: Longitudinal section of the foot showing $S_2$ gland cells.

C : Stained with haematoxylin and eosin, x100.

D : Stained with mercuric bromophenol blue, x1000.

E-G: Transverse section of the byssus duct stained with haematoxylin and eosin.

E : Root and stem, x40.

F : Laminae and crypts, x1000

G : Root and stem, x 400.

H : Isolated byssus thread showing the adhesive disc, x40.

### 1.3. The foot musculature and nerves:

Like some representatives of family Mytilidae, previously described by Bayne (1971) and Lane & Nott (1975), the pedal musculature of the present species consists of 2 pairs of byssal retractor muscle and a little developed pair of retratctor foot muscle (Fig. 2). In addition, the foot is filled with muscle fibres (PL. 1, E). The anterior byssal retractor muscles are inserted close to the dorsal margin of each shell valve, anterior to the hinge. These muscles pass around either side of the alimentary canal and extend in the foot dorsally around the pedal ganglia. The muscle fibres then diverge, some extending throughout the dorsal part of the foot, others passing between the pair of anterior pedal nerve. On the other hand, the posterior pair of byssal retractors insert on the shell valves adjacent to and above the insertion of the posterior adductor muscle. The pedal ganglia are fused and lie in a dorsal position at the base of the foot. Each ganglion gives rise to an anterior pedal nerve, these nerves extend through the dorsal part of the foot towards the tip, and may transmit sensory impulses.

### 1.4. The foot glands:

Gland cells of six different types have been recoginzed in the foot of the investigated species (Fig. 3). Many of these cells have very long secretory processes extending forward from cell bodies located backward in the foot. These glands can be divided into two main groups: the pedal glands designated by the letter P and the stem glands associated

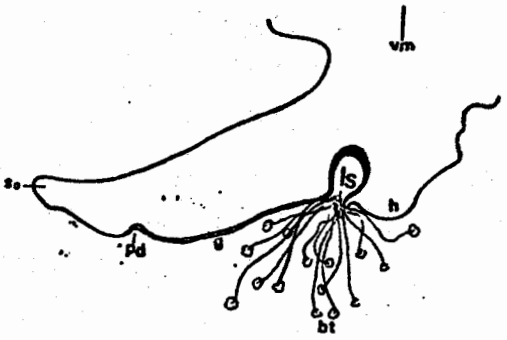



Fig.(1): Morphology of the foot

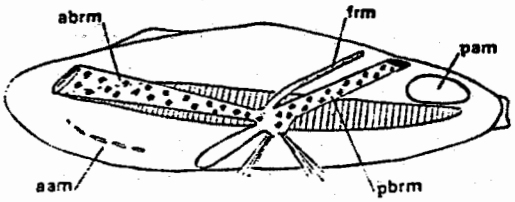



Fig.(2): The foot muscles

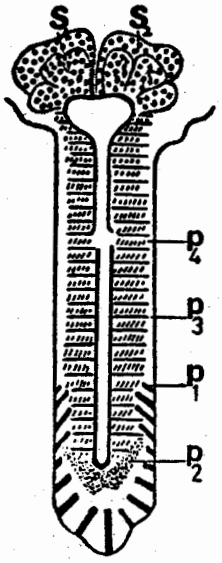



Fig.(3): Schematic view of different types of glands in the foot.

mainly with the duct designated by the letter S. The pedal glands can be further divided into four groups namely: $P_1$, $P_2$, $P_3$ and $P_4$. Also the stem glands can be subdivided into two groups namely: $S_1$ and $S_2$.

### 1.4.1. Pedal glands:

#### 1.4.1.1. Gland $P_1$ (Fig. 3 PL. 1, F, G., H):

It begins at the tip of the foot located distal to the pedal depression, extends forwards and leads by way of pores into the depression. This gland consists of flask-shaped cells which obviously within the foot. The long necks of the gland cells open out between the epithelial cells of the sole. The nucleus of each cell is rounded and has non fixed place. The histochemical tests (Table 1) showed that, the secretion of this gland is composed of mucopolysaccharides. This secretion is alcinophilis at PH 2.5 and 1.0 for most of the fixatives used and small amount of sulfated acid mucopolysaccharides. The other histochemical tests showed that cells of this gland gave negative reaction with protein test as well as phenol test and gave blue colour with Mallory's stain. This means that the cells of this gland gave acid mucopolysaccharides only. The suggested function of the $P_1$ gland is to facilitate the ciliary locomotion and gliding.

#### 1.4.1.2. Gland $P_2$ (Fig. 3, PL. 2 A, B, C, D):

This gland is situtated just above and around the distal depression. This gland contains numerous secretory cells filled

with spherical granules discharging their secretions into the groove directly. Each cell forming this gland is somewhat rounded with long neck and its nucleus is not obvious. The histochemical reactions revealed a strong reaction of these cells to malachite green indicating the presence of phenol group. Also these cells gave positive reaction with bromophenol blue but negative reaction with PAS and gave red colour with Mallory's stain.

### 1.4.1.3. Gland $P_3$ (Fig. 3, PL. 2 E, F):

This is a small gland present around the pedal groove and gave a bright red colour with Mallory's stain and similar in histological appearance to the gland $P_2$. The cells of these glands gave strong positive reaction to malachite green, small amount of protein and acid mucopolysaccharides can be detected.

### 1.4.1.4. Gland P4 (Fig. 3, PL. 2 G, H, PL. 3, A, B):

The cells of this gland form two main groups, each of them extends forward and converge at the mid ventral depression. Cell bodies and cell extensions are filled with spherical globules. The nucleus of each cell is rounded, generally located in the centre and stains darkly with haematoxylin. The gland $P_4$ cells gave positive protein reaction. No reactions were observed to tests for carbohydrates while Mallory's stain gave mauvish colour.

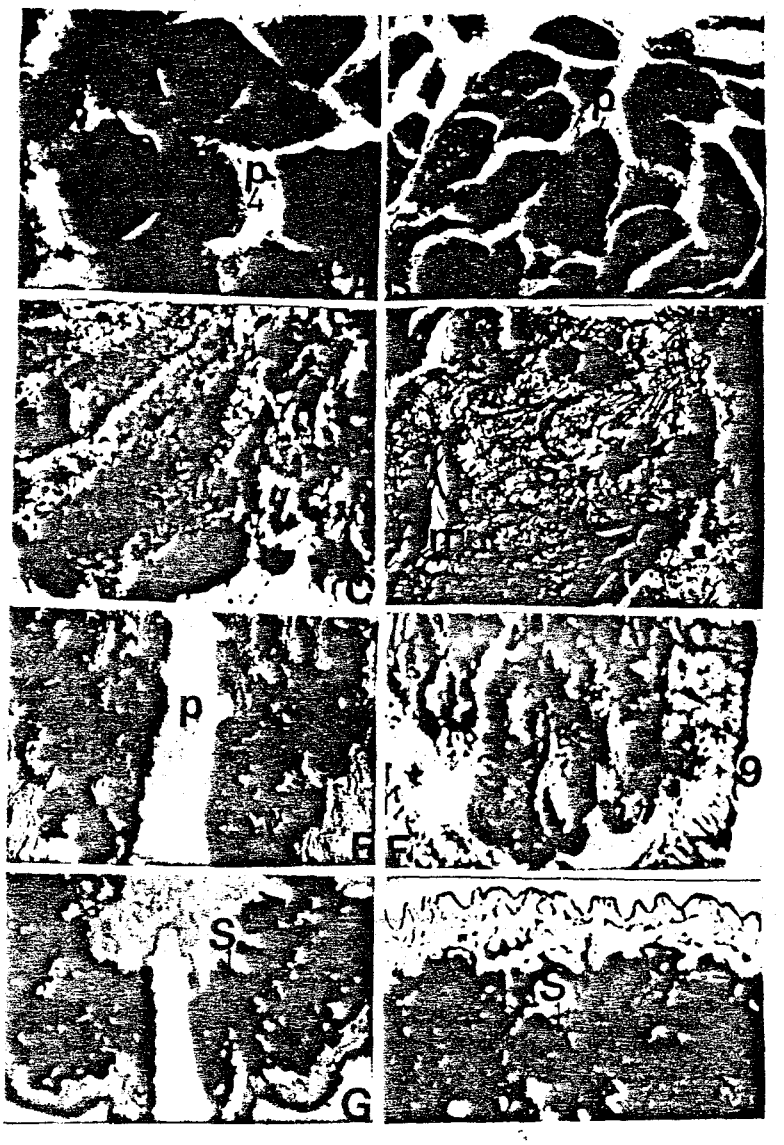
### 1.4.2. Stem glands:

#### 1.4.2.1. Gland $S_1$ (Fig. 3 PL. 3 C, D, E, F, G, H, PL. 4A,B):

Gland $S_1$ is a large gland which consists of large cells lying dorsally at the blind end of the duct and it opens into the upper part of the duct and into the pedal groove. In section stained with Azan the cells have uniform light blue colour with little or no evidence of granular content. However, with bromophenol blue small tightly packed dark purple granules are evident with no background staining. The cells of this gland gave positive PAS and also positive alcian blue at PH 2.5 and 1.0 which indicate the presence of sulphated acid mucopolysaccharids which also give positive reaction with aldehyde fuchsin. Thus, the results of the histochemical reactions revealed that the secretion of this gland composed of highly sulphated acid mucopolysaccharides with small amount of protein.

#### 1.4.2.2. Gland S2 (Fig. 3. PL. 3 c, D, PL. 4 B, C, D):

It is also large bilateral gland which opens into the posterior duct. The cell bodies of this gland filled with spherical globules. The cells forming this gland stained blue with Mallory's stain and gave positive reaction with general test for protein, but gave negative reaction for carbohydrate and phenol tests.




## Plate 3:

A : Dissected adult animal showing the position of the foot and byssus threads. x10.

B : The exposed Lithophaga foot showing the sole, the pedal depression and the groove. x 40.

C-D: Transverse section through lining epithelium of the foot.

C : The brown pigment stainedwwith merrcuric bromophenol blue x1000.

D : The spherical colourless vacuoles stained with Mallory's stain. x 1000.

E : Transverse section of the foot showing the dorsal lining epithelium and complex fibres within the foot stained with haematoxylin and eosin. x100.

F : Transverse section through the $P_1$ gland stained with haematoxylin and eosin. x 250.

G-H: Transverse section of the sole of the foot, showing secretory gland cells ($P_1$) discharging between ciliated epithelial cells.

G : Stained with PAS. x 400.

H : Stained with alcian blue PH 2.5. x400.

## 2. The byssus duct:

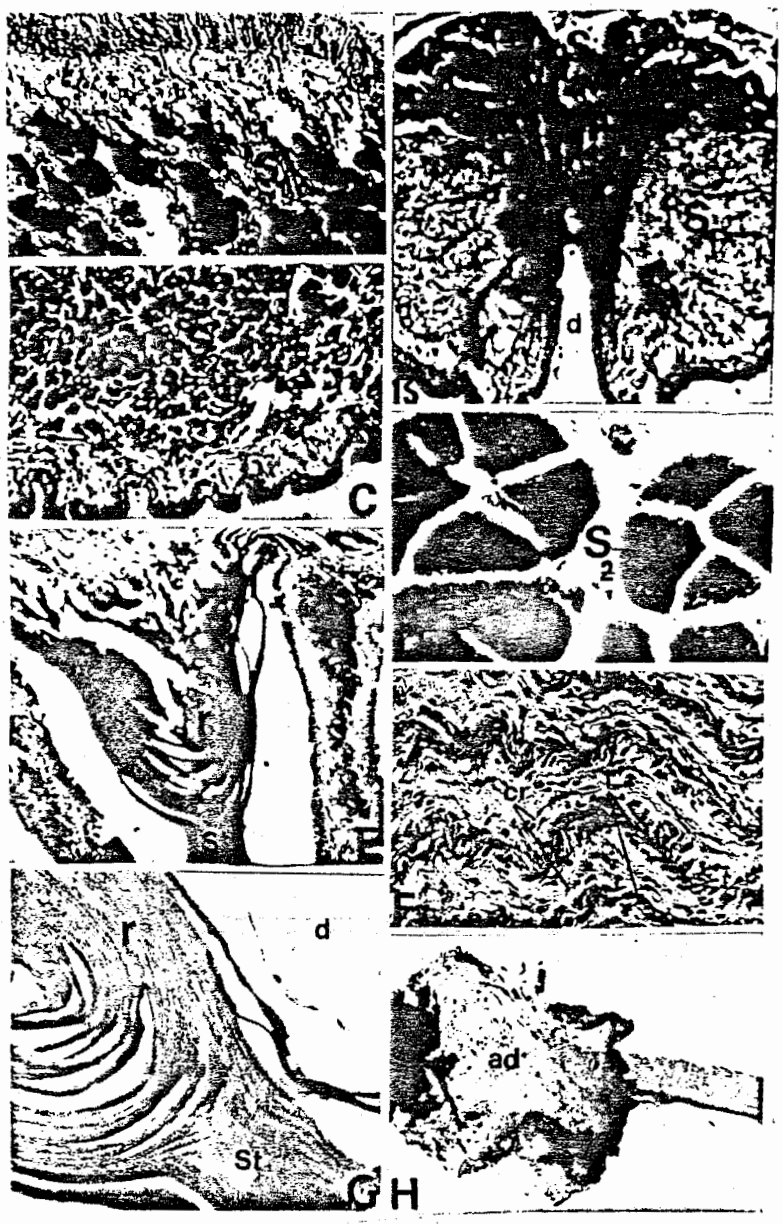
The gland which secrete the byssus are located in the foot. Some glands discharge into a ventral groove and other discharge into the posterior duct. The blind end of this duct appears as a flask-shaped cavity partitioned by numerous lamellae (PL. 4, E, F) plane of which is defined by the antero-posterior and dorso-ventral axis of the animal and called byssus. Like other bivalves (Brown, 1952) the root and stem of byssus formed by the glands which surround the duct. The secretions of these glands are formed into flat sheets or ribbons as a result of being dircharged between the numerous lamellae which occupy the byssus duct.

## 3. The byssus:

The byssus of the investigated species is an attachment organ secreted by special glands of the foot. It consists of numerous discrete ribbons which are continuous from the root, which is held in the byssus duct, to the attachment points at the substratum. Between the root and the substratum, the structure of the ribbons changes gradually. Like Mytilus edulis (Brown 1952), the byssus complex consists of three portions (Fig. 4) as follows.

a) The yellow untanned root made up of thin, fibrous sheets originating and lying in the antero-posteriorly laminated byssus gland.

b) The root continuous with external byssus stem. (PL. 4 G).




## Plate 4:

A-D: Transverse section of the foot in the region of the pedal depression to show $P_2$ gland and its secretion into the groove.

A : Stained with haematoxylin and eosin. x400.

B : Stained with malachite green. x250.

C : Stained with Mallory's triple stain. x400

D : Stained with mercuric bromophenol blue. x1000

E-F: Transverse section of the foot in the region of the pedal groove showing the gland $P_3$ cells.

E : Gland cells stained with Mallory's triple stain. x1000

F : Gland cells stained with malachite green. x250

G : Transverse section of the foot showing the distribution of $P_1$ gland stained with haematoxylin and eosin. x100.

H : Transverse section through the $P_4$ gland stained with haematoxylin and eosin. x400.

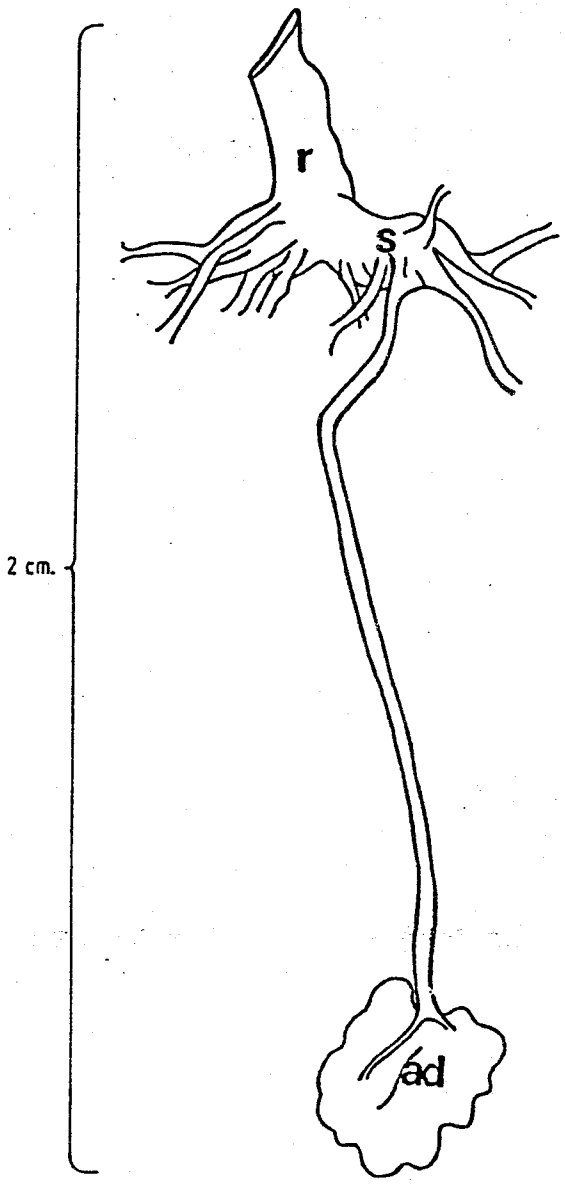



Fig.(4): The byssus complex.

c) The stem attached to the walls of the burrows by two bundles of flattened byssal threads ending in adhesive discs adhering to the substratum (PL. 4 H).

In this respect, it can be safely said that the presence of two bundles of flattened byssal thread attached to the wall of the burrows may represent unique character of the present species because all known filibranchiates possess only one bundle byssal threads (e.g. Mytilus edulis, Arcazebra chlamys islandica and Pinna nobilis).

Each individual thread is composed of four recognizable regions:

a) An adhesive disc by which the thread is attached to the substratum.

b) Hard cylindrical portion joined to the disc. This part is stiff, smooth and amounts to proximately one third of the length of the thread.

c) Soft flattened portion representing the remaining part of the thread.

d) A ring representing the most specific proximal portion of the thread and composed of specific material by which it is attached to the stem.

Abbreviations of figures and plates

aam, anterior adductor muscle; abrm, anterior byssal retractor muscle; ad, adhesive disc; bt, byssus threads; c, cilia; cr, crypts; d, byssus duct; epi, epithelial cells; f, foot; frm, foot retractor muscle g, pedal groove; l, lamellae; lmf, longitudinal muscle fibers; ls, lamellae space; mu, muscle; n, neck; p, pedal gland; pam, posterior adductor muscle; pbrm, posterior byssal retractor muscle; pd, pedal depression; pig, pigmented granules; r, root; S, stem gland; sg, secretory glands; so, sole; st, stem; v, vacuole; vm, visceral mass.

## DISCUSSION

It is well known that the byssus is a structure produced by some marine bivalvian molluscs to adhere, usually permanently, to submerged structures. A survey proposed by Yonge (1962) on the byssal apparatus in bivalves indicated that the formation of byssus is a larval characteristic which may or may not be retained in the adult. Therefore, a comparison of the present adult system with that have been described is, in most cases, more difficult. Furthermore, this also seems difficult because of the incomplete histological descriptions and lack of uniformity in the techniques, particularly staining technique, used in al known investigations.

In conclusion, the predominance of gland cells in the foot is a striking histological and histochemical feature of the foot in the mussel. On the other hand, the disposition of the six gland cell types identified in the foot of the herein mussel demonstrated a greater complexity of organization than was observed by authors in other mussels.

The strong indication of the probable functions of the foot glands in the present species obtained from their morphology and also by identifying homologous structures in other described species. The primary byssus gland $S_1$ is similar in histology and position to that previously described in Mytilus edulis (Tullberg, 1877; Williamson, 1907; Seydel, 1909; Turchini & Broussy, 1934; Brown, 1952; Smyth, 1954; Pujol, 1967; Mahéo, 1969), Pecten irradians (Belding, 1910), Arca Tetragona (Boutan, 1895)., Margritifera oulgaris (Herdman,

1904). As indicated from the investigation carried out during this work, this gland contains highly sulphated acid mucopolysaccharides together with small amount of protein seems to contribute in the formation of the primary byssus filaments which emerge from the duct. Cranfield (1973c) concluded that the function of this gland is restricted in the secretion of the twin cores of the primary byssus threads. On the other hand, Yonge (1962) postulated that if a functional byssus apparatus is retained in the adultitis used either for direct attachment of secondary byssus threads which are produced by the collagen gland. Also the secondary byssus gland $S_2$ is homologous to the secondary byssus gland of adult Mytilus calitornianus (Tamarin & Keller, 1972), Mytilus edulis (Pujol, 1967 and Mahéo, 1969) and the larval foot of Pecten maximus (Gruffydd, et al., 1975). The histochemical evidences from the present work lead to the conclusion that the protein contained in this gland is a type of collagen which may contributes to the byssus forming a collagenous or secretory byssus. In this respect it is closely agreed with the conclusion given by Land & Nott (1975). It is worthy to mention that, the $P_4$ gland resembles $S_2$ in most histological and histochemical appearance.

The specific position as well as the nature of secretrons of the phenolic glands ($P_2$ & $P_3$) lead to the conclusion that these glands may contribute to the formation of the terminal attachment plaques of the byssus which are would in the pedal depression. This statement comes in accordance with that given by Brown (1952) and Cranfield (1973c) in Mytilus edulis

and Ostrea edulis respectively. The detection of aromatic protein in the secretion of gland P3 and its similarity in appearance to that in the phenol gland $P_2$ suggests the presence of a phenolic tanning mechanism and points to a cementing role for the pedal depression secretions. Moreover, these phenolic glands may play an important role in cementing the borehole in the investigated adult stage. The previous suggestion is supported by Nelson, (1924) who noted that cementing oyster larvae extended the mantle margin over the substratum and he considered that, this action spread the cement discharged by the foot and that the folds themselves did not secrete more material. Also Stafford, (1913) has dismissed or ignored any part that, the mantle folds may play in cementing. However what increase the probablity of the present suggestion is the presence of gland cells packed with quantities of mature secretion in both the inner mantle fold and gland cells of the foot in the investigated species. The additional function is further supported by the fact that the pedal depression is closely applied to the substratum during the muscular contraction phase of crawling.

The distribution of gland $P_1$ openings over the sole of the foot implies that its secretion has a function in relation to progression over the substratum. The presence of weakly acidic mucopolysaccharides in the secretion indicated its low viscosity. Thus, it must be realized that the presence of this gland appears to be characteristic to the sole of the adult Lithophaga and not absent as mentioned by Seydel, (1909) who postulated that the pedal mucous gland in this species occurs

only in the larva stage to facilitate their locomotion. In this respect, it should not be regected the notable work given by Tamarin et al., (1976) on the role of the mucosubstance in the secretions of foot gland. This author noted that, the mucous is discontinous phase colloidally dispersed polyphenolic protein and suggested that it may provide points of tackiness with the surface before the polyphenolic adhesive has set. It was also speculated that, the mucosubstance functions as a temporary adhesive or prepares the substrate surface in some way for adhesion. Thus, the mucosubstance may be a coupling agent applied as a monolayer to the surface, a dispersing agent to form a fine water-insoluble colloid of the polyphenolic protein and for a temporary adhesive to keep the foot glued to the substratum during the byssus secretion. The previous statement appears correct in the larval stages. In our opinion the mucous of the adult may play a role in lining the barehole or temporary adhesive the tip of the foot which aid the shell to make mechanical boring.

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# جهاز الرسن فى احدى ذوات المصراعين الرخو : ليثوفاجا ليثوفاجا من شاطىء أبـــو قير ( البحر الأبيض المتوسط ) ٠

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## ملخص البحـــث

يهدف هذا البحث إلى دراسه جهاز الرسن فى احدى ذوات المصراعين الناخرة للصخور ( ليثوفاجا ليثوفاجا ) من الناحية الهستولوچيه والهستوكيميائيه ، ومن المعلوم ان جهاز الرسن يعمل علـــى الالتصاق الشديد بالصخور مما يساعد الحيوان على عمليه النخر الميكانيكية بواسطة الصدفة ٠

وأوضحت الدراسة تكون الجهاز الرسنى من ثلاثة أجزاء هى القدم والقناة الرسنيه والخيوط الرسنيه وأمكن تقسيم القدم اعتماداً على الناحية التركيبية إلى أربعة أجزاء هى : الطلائية المهدبة الخارجية الميزاب الخلفى والقنوات ، عضلات القدم والأعصاب والغدد القدمية ٠

ولقد تم التركيز خلال هذه الدراسة على الغدد القدمية وتبين من خلالها وجود سته أنواع مـــن الخلايا الغدية فى القدم ٠ وقد أمكن تقسيم هذه الخلايا الغدية إلـــى تحت مجموعتين رئيستـــين هى الغدد القدمية والغدد الساقية ٠ وقسمت الغدد القدمية إلى أربعة مجموعات هى ( $P_1$ , $P_2$ , $P_3$ , $P_4$ ). بينما الغدد الساقيه فقد قسمت إلى مجموعتين هى ( $S_1$ , $S_2$ ) ٠ كما تم توضيح تركيب هذه الغدد وأماكن انتشارها على القدم ومناقشة الدور الذى يقوم به كل نوع فى بيولوچيا الحيوان ٠