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Morpho-anatomical Studies of Intertidal Bivalve *Glauconome chinensis* (Gray, 1828) from West Bengal-Odisha Coast, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

West Bengal-Odisha coastal mangrove ecosystem represent a productive and dynamic ecosystem enriched with diversified habitat and niches. The intertidal bivalve *Glauconome chinensis* has been reported presently in the intertidal zone of Subarnarekha estuary of West Bengal-Odisha coast of Bay of Bengal. The studied bivalve characterised by unique morphological and microanatomical features. The present paper deals with morphological (shape and sizes of the shell, siphon, mantle, foot, adductor muscle, gill, digestive gland, reproductive system) and also microanatomical characteristics of different body tissues of the intertidal bivalve *Glauconome chinensis.*

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Keywords: Bivalve; Glauconome chinensis; coastal ecosystem; subarnarekha estuary; morphology; microanatomy.

1. INTRODUCTION

Mangrove ecosystem is a highly productive and dynamic ecosystem with innumerable number of flora and fauna. This is a typical forest ecosystem which are growing around the coast and estuaries. Mangrove forests are rich in organic matter and nutrients. Different faunal species, including arthropods, molluscs, fish, amphibians, birds, mammals, and reptiles, are recorded from the mangrove coastal region [1]. They prefer mangroves for spawning, nursery and feeding grounds. Molluscs are a large, important invertebrate phylum that crucially plays a role in coastal ecosystem health, maintenance, and stability [2]. They are soft-bodied animals and have different habitats, like freshwater, terrestrial, arboreal, marine, and estuarine. In the food web, molluscs behave as herbivores, predators, detritus (deposit or pedal feeding), and filter feeders [3]. They consume nutrients from bacteria, microzooplankton, mesozooplankton, and resuspended benthic microalgae [4,5]. In the global mollusc's diversity, 0.2 million species were recorded [6]. Bivalves are the dominant molluscs in the coastal mangrove ecosystem with two hinged shells [1,7]. They show a diverse life history and perform important ecological functions [2]. Bivalves those are infaunal in nature hide themselves in the benthic region. Phytoplankton is the primary food source for bivalves [8]. They are largely filter feeders and some species feed on detritus (i.e., deposit or pedal feeding) [3,8]. By filtering particles, they deposit nutrients in the pelagic and benthic Systems also. They intake food by syphon and forward it to the stomach by the movement of cilia and mucus. Bivalves act as biofilters for different chemicals, pathogens, and metallic substances. They accumulate contaminants and help in biomonitoring programs. For sustainable water remediation, bioaccumulated contaminant arrestation bivalves is an important component of ecosystem managers. They are able to accumulate environmental pollutants and act as bioindicators. Bivalves have a role in nutrient translocation, storage, and transformation. Suspension and deposit feeding, such as biofiltration of the water column and sediment consumption, pass nutrients from benthic regions into bivalves. Different elements that are stored in bivalve soft tissues affect nutrient sequestration and nutrient stoichiometry. Climate, site, growth stage, and reproductive status are the important factors for nutrient storage in bivalve tissues and shells [9]. Biodeposites and nutrient stoichiometry have a great role in nutrient dynamics. Bivalves also play a key role in biogeochemical transformations under redox conditions in the sediment. Sediment oxygen increases with the accumulation of biodeposites and bioturbation. They create a new habitat for different organisms, such as microbes, protozoa, and larval stages of different invertebrates. Bivalves also allow different symbionts inside their tissues and body cavities as habitats. They were recognised as the first reef builders, too. In an aquatic ecosystem, bivalves help in the bottom-up sequence by supplying energy and nutrients [10]. Bivalves can act as an imperative biological indicator to assess environmental conditions and monitor future environmental change [8]. They are an important edible source for humans also. Modern human culture also uses them as building materials, ornamentation, and currency.

The bivalve's shell is constituted with calcium compound and covered the mantle and protect the organ system of mantle cavity. The bivalve mantle is a membranous organ united dorsomedially by an isthmus and the edge of the mantle is divided into three main lobes (inner, middle and outer lobe). The mantle helps in the formation of shell [11]. It conveys external stimuli to internal organs. Underneath the mantle on each end, adductor muscles are present. In bivalves the muscles act as a energy supplier. Infaunal bivalves burrow at various depths in extreme environmental changes and to escape from biological predators. Through the siphons bivalve keep contact with the water above the substrate [12]. During burrowing bivalves extend siphons to intake water and also to discharge it. It helps in intake of food, exchange of gas, pseudofeces and process of excretion. Siphons also has functions in germ cell ejection, photoreception, and mechanosensation [13]. Fusion of the mantle margins formed siphons which partly to completely fused and sometimes separated from each other, known as inhalant and exhalent siphon. For evolutionary studies siphons is an important organ in bivalves. Large leaf-like prominent gills are a major characteristic of bivalves. Gills partly help in gas exchange during respiration. It also helps in filtering food from water. Through the labial palp gills transport the food particles. Bivalves have well developed foot enabling to move and burrow by the digging of surrounding sediment. In unfavourable environmental conditions and presence of predator's bivalves burrow themselves in mud or sand. Bivalve digestive gland plays a great role in digestion and nutrient absorption. Digestive gland consists with anterior mouth, buccal cavity, oesophagus, stomach, intestine and posterior anus. Bivalve digestive gland is also responsible for accumulation as well as detoxification of toxic substances in the aquatic medium [14].

The West Bengal-Odisha intertidal shoreline including the district of Midnapore (east) encompasses a large number of floral and faunal components. It also accommodates a good number of molluscs including bivalves- *Glauconome chinensis* (Grey 1828). It is the most important molluscan species in the Talsari intertidal zone of Subarnarekha estuary of West Bengal-Odisha coast, India. The intertidal bivalve plays a key role in estuarine food chain [4]. Their population decline, nutritional values and their possible application as bioindicators in different ecosystems are the most valuable causes for studying also. The present study and investigation are aimed to describe the morpho-micro anatomical details of *Glauconome chinensis.* The study also offers basic data that will allow species with varied environments to be compared, as well as biological information for further studies on how environmental changes affect the structure of various organs.

2. MATERIALS AND METHODS

2.1 Animal

Glauconome chinenensis was used for the experiments. In 1828, G.E Gray recorded intertidal marine bivalve *Glauconome chinenensis* (Chinese glauconome) from subtropical region of Western Pacific. It is belonging from Phylum- Mollusca (Linnaeus, 1758), Class- Bivalvia (Linnaeus, 1758) and Order- Venerida (H. & A. Adams, 1856). It is a burrowing animal in the intertidal zone of coastal ecosystem. The specimen buried themselves in coastal benthic region along with the soft muddy to sandy sediments dotted with mangrove vegetation. The genus is characterised by two hinged shells with two long siphons as well as a large prominent foot. The studied specimens are mostly gonochoric but some are protandric hermaphrodites. The embryo of the *Glauconome chinenensis* develop into free-swimming trochophore larvae. Then the trochophore succeeded by the veliger, resembling a minute calm (Gray 1828).

Glauconome chinenensis is recorded for the first time from Talsari, the intertidal zone of Subarnarekha estuary of West Bengal-Odisha coast, India. The intertidal regions of the siliciclastic Talsari (situated between 21°35'48" Northern Latitude and 87°27'17" Eastern Longitude) coastline exhibit distinctive features, with sedimentary layers arranged in a linear pattern running from east to west, parallel to the shore. This coastal area experiences moderate tides influenced by tropical oceanic conditions, with a slight daily variation and three distinct seasons: Winter (November to February), Summer (March to June), and Monsoon (July to October)**.** The sampled specimen *Glauconome chinensis* are mostly found in muddy area carrying high sandy loam content with mangrove associated plant species. They showed clumped distribution pattern. The shell of the specimen contains two similar valves. The valves are semi-convex, ovate, elongate and with a pointed umbo. Valves are mostly blackish-brown in colour but some are looks like golden brown. The outer surface of the valves is mostly smooth but indicating growth lines, and Different ornamental features are designed in the inner surfaces of the valves [15]. They are 2.18-6.23 cm in length, 1.67-2.18cm in width. *Glauconome chinensis* is deep burrowing species with long, highly mobile siphons and burrows nearly about 10-40 cm below the surface. They can extend the siphon two to three times longer of the shell to reach the substratum [16**,**17].

2.2 Sample Preparation

For research work, the intertidal bivalve *Glauconome chinensis* were collected (20 specimen) from three different study sites viz. site-I, site-II and site- III of West Bengal-Odhisha coastal region near Subarnarekha estuary with Bay of Bengal. Random samplings of studied specimen were done on intertidal belts of Talsari in three seasons premonsoon, monsoon and post monsoon. For the collection of the sample specimens' quadrates measuring 1m^2 areas were placed randomly. The specimens were unearthed and collected. Then shell lengths, width and length of the siphon were measured by slide calipers and also with a centimeter scale. The specimen was 3.92 ±0.393 cm (mean ±SD) in shell length, 1.45 ±0.351cm (mean ±SD) in shell width and 3.89 ±0.382 gm (mean ±SD) in shell biomass.

The collected specimens were dissected and tissues were fixed in 4% formalin solution. The fixation procedure was done for a minimum of 24 hours. Next tissues were processed via 70%, 90%, and 100% alcoholic grades. Then the tissues were embedded in wax. All these processes were done utilizing the standard methods of. Sections were cut from paraffin blocks within 4-5µm with the help of a rotary microtome followed by dewaxing.

2.3 Image Analysis

All the observation of the specimens was done with Nikon D7500. Preliminary study of the specimens was done under Binocular microscope, Model CH20iBIMF. All the drawing were completed under Camera lucida and collected data were compared with various literature. For the correct identification, the studied specimens were deposited at the Ministry of Environment, Forest and Climate Change; Zoological Survey of India, Prani Vigyan Bhavan, Kolkata, West Bengal.

3. RESULTS

3.1 Morpho-anatomical and Micro-anatomical Study

3.1.1 Shell

The shell of *Glauconome chinensis* contain two similar valves. The valves are ovate, elongate, bilaterally symmetrical and blackish-brown in colour. They have lateral margins and also with straight edges. The outer surface of the species is mostly smooth but indicating growth lines. Different ornamental features are designed in the inner surfaces of the valves. Both left and right valves are joined by a ligament. The valves of the studied animal are equal in size and consists with calcium carbonate (Fig. 1).

Fig. 1. Specimen of *Glauconome chinensis*

3.1.2 Mantle

Mantle of *Glauconome chinensis* is a thin membrane and covered the soft parts. It is formed with two thick tissue sheaths. Mantle tissue is strongly attached to the bivalve shell. mainly 3 parts in the mantle known as marginal zone, middle zone, and apical zone. Marginal mantle is composed with three folds as well: inner, middle, and outside folds. The inner fold is strongly developed region. Mantle is covered by inner and outer epidermis layer also. Mucous cells are also visible in the bivalve mantle which mainly developed from epidermis to connective tissue. Mantle is composed with 3 layers-outer, middle and inner layers. Inner fold of the mantle is consisting with epithelial cells. There are present mainly columnar cells in the inner fold and cuboidal cells in the outer fold. On the free surface of inner epidermis layer a well-developed striated border is observed. But the striated border on the outer epidermis layer is weak. In the inner epidermis of middle and apical zones, cuboidal cell layers are visible. But outer epidermis layer of the same zone is consisting with columnar cell layer. At the marginal zone, mantle thickness is thickest. On the other hand, the outer epidermis of the marginal region is comparatively thicker than the inner epidermis. There are present mucous cells which developed from connective tissue. High number of mucous cells present in the marginal mantle (Fig. 2).

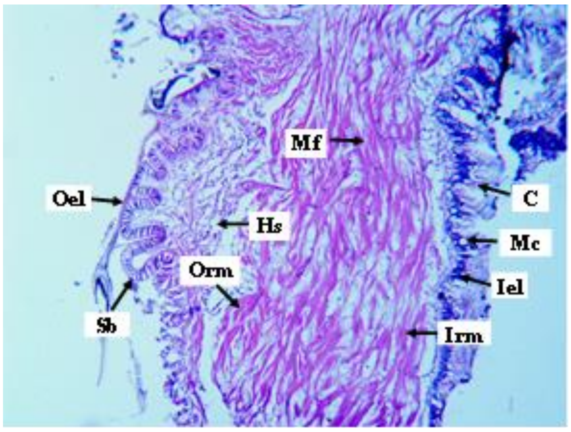

Fig. 2. Transverse section of Mantle (40x×10x). Oel-Outer epidermis layer, Iel-Inner epidermis layer, Orm- Outer radial muscle, Irm- Inner radial muscle, C- Cilia, Mf-Muscle fibre, Sb- Striated border, Mc Mucous cell, Hs- Hemolymph sinus

3.1.3 Adductor muscle

Glauconome chinensis have anterior and posterior adductor muscles located underneath the mantle of the body. When the muscles are contract, the valves come to close very tightly. At the low water levels, presence of predators, adductor muscles are contract and the shell valves automatically pulled open when adductor muscles relax. Through the contracting and reluxing process the species can move in water. At the anterior and posterior margin of the valves of the studied specimen, two adductor muscles are present. Adductor myocytes are filamentous. Adductor nadductor muscles. Anterior adductor muscles contain a large number of red eosinophelic fibres. Posterior adductor muscles also contain long myofibers. eosinophilic filaments, heterochromatic nuclei and a distinct nucleolus are also visible in adductor muscles (Fig. 3).

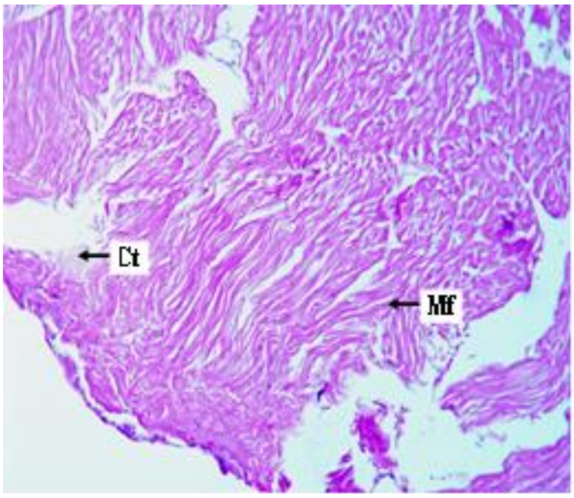

Fig. 3. Transverse section of Adductor muscle (40x×10x). Mf- Muscle fibre, Ct-Connective tissue

3.1.4 Siphon

Glauconome chinensis is deep burrowing species with long, highly mobile siphons and burrows nearly about 20-30 cm below the surface. At the posterior position of *Glauconome chinensis,* two siphons are observed known as inhalant siphon (incurrent siphon) and exhalant siphon (excurrent siphon) originate from the mantle cavity. Both the siphons are white in colour but slightly blackish in anterior tip. Through the inhalant siphon water enters into the mantle cavity. Then the water transferred into the gills, circulated into the body and finally came out from the body by exhalent siphon. On the margin of the inhalant siphon rows of tentacles are found which are directed inward. Siphon of the *Glauconome chinensis* are composed of the matrix, siphonal sheath, exterior epithelial layer, muscle layer, connective tissue layer, and inner epithelial layer. During present investigation a series of muscle layers are visible in the siphon walls. The outer (Eo) and inner unistratified epithelia contain muscle layers which are cubic or columnar in shape. A fully formed nucleus and nucleolus present in the sampled specimen. Muscle layers constitute with numerous fibres which are are oriented as longitudinally (L), circularly (c) and radially (R). The longitudinal muscle fibres are located on the tip of the siphons. Both outer and inner epithelia contain radially arranged muscle fibres. The primary longitudinal layer situated in the median portion (Lm) of the epithelia. Near the epithelia one inner peripheral circular layers (Ci) and one outer peripheral circular layer (Co) layer are distributed. In the siphon the most advanced layer is always the Lm. There present a series of median circular layer in between the inner (Lmi) and outer (Lmo) median longitudinal layer. The ci is very thin. Lo and Coo are arranged in a single row but Coi is thick.

The radial muscles are branched and muscle, epithelia and nerve tissues are modified in the inhalant and exhalent siphons. Both the siphons are structurally very similar. The wall of the inhalant siphon is about 58 μm and exhalant siphon is 42 μm in thickness. It is also observed that in inhalant siphon Lmi and Lmo are equal in size Whereas, Lmo is more developed layer than the Lmi in exhalent siphon. The Coi is also very thick in exhalent siphon (Fig. 4).

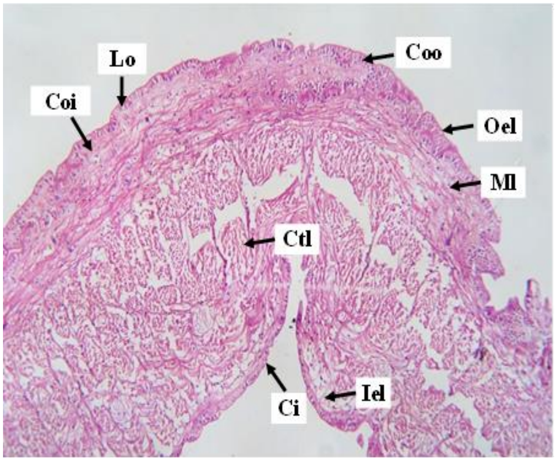

Fig. 4. Transverse section of siphon (40x×10x). Oel- Outer epithelium layer, Iel-Inner epithelium layer, Mi- Muscle layer, Ctl-Connective tissue layer, Ci- Inner circular layer, Lo- Outer Longitudinal layer, Coo-Outer parts of the outer circular layer, Coi-Inner parts of the outer circular layer

3.1.5 Gill

In *Glauconome chinensis* one pair of gills present behind the siphon. Gill is formed by outer and inner demibranch lined by two lamellae known as ctenidium. They are large and arranged in a V shape and greyish white in colour. There are present inter-lamellar junctions between the lamellae which have also pneumerous blood vessels. Ctenidium contain highly efficient surface for filter feeding. The gill filaments formed a longitudinal array and adjacent filaments. Those filements are lined by an epithelium which contains cuboidal adiphase and columner cell layer. The entire frontal surface of the gill filament of the studied specimen is covered in cilia known as frontal, latero-frontal, and lateral cilia. The frontal cilia are about 3 µm in length. Latero-frontal cilia are located on either side of the filament, directly beneath the frontal cilia which are 14 µm in length. The lateral cilia are numerous and have an approximate length of 20 µm. Different ducts have been observed in the lateral surface of cilia at regular intervals. The lateral surface is not ciliated. Mucus haemocytes, ciliated and nonciliated cells are observed in gill filaments. There are present the interlamellar space between two lamellae of demibranch. The interlamellar space lined by endothelium contains haemolymph vessels. and the interlamellar space is connected to external environment by ostia (Fig. 5).

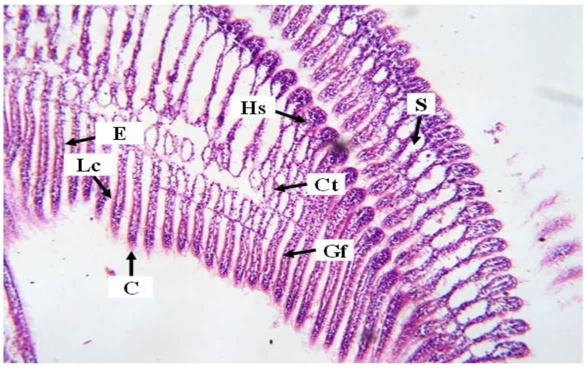

Fig. 5. Transverse section of Gill (40x×10x). C- Cilia, Gf- Gill filament, Lc- Lateral cilia, S- Septum, Ct- Connective tissue, Hs- Hemolymph sinus, E- Epithelium

3.1.6 Foot

During the present investigation it was observed that the studied specimen has a retractable, muscular foot. Foot is large, laterally flattened and used as the locomotory organ of the bivalve species**.** The foot is white in colour, wedge-shaped. It is capable for digging in soft sediment. The foot has lot of muscle and composed with large number of smooth muscle fibre. The foot of the organism under study is composed of three layers: muscle, connective tissue, and epithelium. The epithelial layer is made up of mucous cells and ciliated columnar epithelia. The epithelial layer is 14µm thick. On the free surface of the foot, a well-developed striated border formed. Apical region of the epithelial cell is mostly columnar. There were mucous cells seen in the muscle and epithelial layers, with a larger concentration in the anterior and posterior tips compared to the ventral region. Collagen fibres predominate in the connective tissue layer seems to be extremely thin and loose. and bundles of muscle fibres, with haemolymph sinus present (Fig. 6).

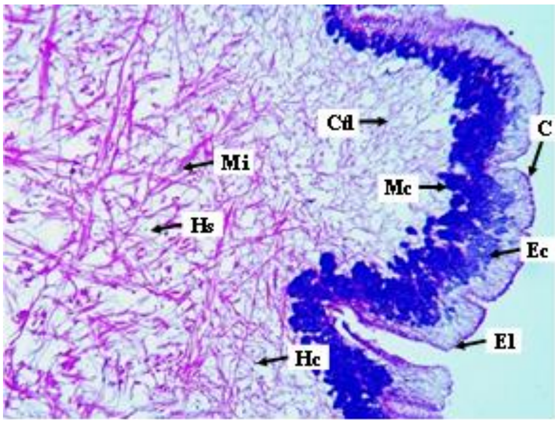

Fig. 6. Transverse section of Foot (40x×10x). C- Cilia, El- Epithelium cell, Ec- Epithelium cell, Ml- Muscle layer, Mc- Mucous cell, Ctl- Connective tissue layer, Hc- Hemocyte, Hs- Himolymph sinus

3.1.7 Digestive gland

The digestive tract of studied specimen, *Glauconome chinensis* consists with oesophagus, stomach and intestine. Stomach is surrounded by tubulo-acinar cells. Digestive tract covered mainly with peritoneum. Different tubules, channels and connective tissue were present in the bivalve mussels. Opening of the stomach contain curled intestine which extends into the foot. The peritoneum appears to be externally covering the gut or digestive tract. Between the layers of epithelial cells and muscular layers are seen a large number of glandular cells, often known as goblet cells. The digestive tract is primarily covered by cilia. A group of digestive glands that surround the stomach are seen during the inspection. Within, such glands take up the majority of the space. Digestive gland acini are composed of lipid droplets, several secretary granules, and a layer of connective tissue (Fig. 7).

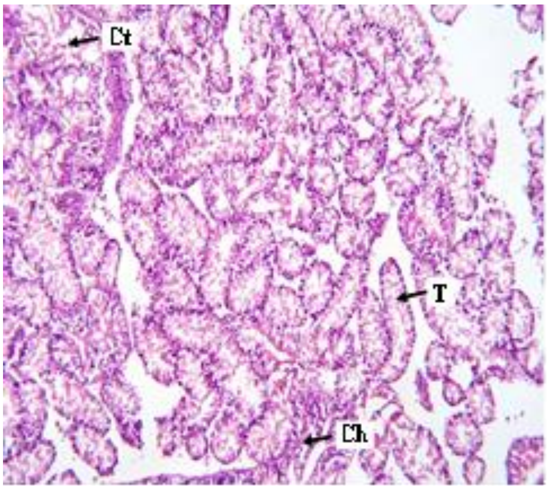

Fig. 7. Transverse section of Digestive gland (40x×10x). T- Tubule, Ch- Channels, Ct- Connective tissue

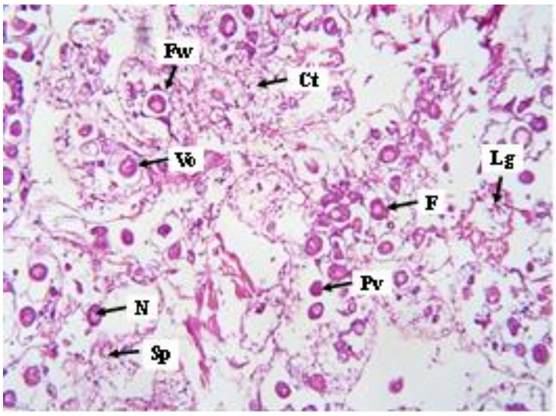

Fig. 8. Transverse section of Gonad (40x×10x). F- Follicle, Fw- Follicular wall, Vo- Vitellogenic oocytes, Pv- Previtellogenic oocytes, N- Nucleus, Ct- Connective tissue, Log- Lipid globule, Sp- Sperms

3.1.8 Gonad

Glauconome chinensis is gonochoric and sometimes protandric hermaphrodites. Gonads are running along the digestive tract. The paired gonads are consisting with branching tubules which unite and form larger ducts. The majority of the cells in female follicles were nutritive cells, lipid globules, and germ cells. Male follicles consist with sperm morulae, spermatids with free spermatozoa. Small follicles with primary and secondary oogonia and oocytes are also visible.In the maturation stage, lumen of the follicles are compacked with Spermatids and matured spermatocytes. In the late maturation stage vitellogenic oocyte enlarges in size. Nucleus of the oocytes becomes rounded. The lumens of male follicles were fulfilled with matured spermatozoa. In the spawning stage follicle's size is reduced and compressed. Spermatids and sperms developed fully. Both male and female gonads show empty follicles in the cytolysis stage. Few gametes are released and support the nourishment to the developing gametes (Fig. 8).

4. DISCUSSION

The shell of the studied species is consisting with two calcareous equivalve. Both the valves articulate to each other through the teeth. There are 3 different layers in shell structure known as periostracum, prismatic layer and naceous layer. The outer surface of the body is covered by Periostracum and it is secreted by mantle epithelial cell [15]. In *Glauconome chinensis* exposed soft parts are the foot, mantle edge and siphons. These parts having highly sensory epithelium and connective tissue. Outer mantle epidermis is cubical. From the mantle epidermis a mantle papilla is formed that contain vertical tubules or canals. The cells toward mantle edge are columnar [18]. The shell formation depends on the growth of the marginal position of mantle. Inner epithelium cells of the marginal zone secrete a substance to form periostracum. Epithelial cells in the middle fold of the mantle play a key role in supporting the periostracum [19]. Developed matrix, collagen fibers, muscle fibers and hemolymph sinuses were collectively formed mantle connective tissue layer. Similar structures were reported in many bivalves, although future investigation and discussion are very much necessary on the tissue.

Adductor muscles are the energy storage house of studied specimen. Smooth and obliquely striated components were present in large number in each adductor muscle. All the striated muscle segregated into an anterior opaque and a posterior translucent portion [20]. The Siphons are highly developed and essential part of the bivalve body and help in adaptation in benthic sediment Siphons usually made by outgrowth of mantle and fused together. According to shape, structure and function, siphons are two types, Inhalent siphon and Exhalent siphon. Their various functions were observed during study and mainly have ability to absorb and discharge water. In longitudinal section of tissue, the outer most surface epithelium cells are cuboidal and regular in shape. The inner surfaces of epithelium cells are regular in shape. Siphonal sheath is the outermost structure to protect the siphon from external environment. The tentacles are highly developed and large sized in Inhalent siphon than Exhalent siphon, so large particles cannot enter into the body and helps in filter feeding. Water enters into the body by Inhalent siphon and come outside from the body by Exhalent siphon. All the structures help in the development of filaments and proper filtration. Skeletal rod and skeletal loop present in the gill filaments [21].

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Foot structure strongly depend on bivalve habitat. Foot of the bivalve is simple and degenerated. As a burrowing bivalve, the foot is in wedge shaped. Numerous vertical furrows are found on the foot surface. There are large number of mucous cells in the foot epithelium layer. In this study, foot of *Glauconome chinensis* showed similar shapes and structural features. All those characteristic features would represent advantageous situation for that burrowing bivalve. Histological analysis of the bivalve revealed that foot is composed of epithelial layer with columnar epithelial and secretory cells, a thin connective tissue layer and a muscular layer with collagen and smooth muscle fibres [22]. Analysis of the foot of studied species *Glauconome chinensis* revealed that the foot is possess an epithelial layer, connective tissue layer and a muscular layer. In the studied specimen, *Glauconome chinensis* the presence of a columnar ciliated epithelium enriches the digestive glands. The epithelium contains secretory droplets and with gland cells [23]. The peritoneum and extremely thin connective tissue are to be used to cover the epithelium externally. Only the ducts contain muscle cells in *Glauconome chinensis.*

In present study, it was observed that the gametogenesis of both sexes of *Glauconome chinensis* begins during November to January. Maturation and spawning were observed during summer and early monsoon seasons [24]. During study period, it was noticed that different physicochemical parameters of water such as temperature, dissolved oxygen affects the reproductive activities and gametogenesis [25,26]. The gonad is made up of several ciliated, branching ducts that give rise to multiple sacs known as follicles. They are mostly gonochoric or protandric hermaphrodites [27].

5. CONCLUSION

The study's overall findings indicate that *Glauconome chinensis* exhibits a variety of distinct interspecific traits in its various body segments. The results so far give us a glimpse of how more research on the composition and structure of the shell, mantle, siphon, adductor muscles, foot, gills, and digestive tissues could contribute additional information for a more thorough understanding of the animal's biology. The reproductive features are crucial for the establishment of *Glauconome chinensis* as gonochoric and sometimes protandric hermaphrodites. The identification of *Glauconome chinensis* as a distinct bivalve species is justified by all of these traits. In investigations into the phylogeny of the bivalve *Glauconome chinensis*, this knowledge will help to clarify disputes on the homology of the structures.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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