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Secretory Cells in *Halla parthenopeia* (Oenonidae): Potential Implications for the Feeding and Defence Strategies of a Carnivorous Burrowing Polychaete

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ABSTRACT

Carnivorous polychaetes are known to bear diversified and often unique anatomical and behavioural adaptations for predation and defence. *Halla parthenopeia*, a species known to be a specialized predator of clams, thrives in the soft bottoms of the Mediterranean Sea, holding potential for polyculture and biotechnology due to the secretion of bioactive compounds. Our objective was to provide a comprehensive description of *H. parthenopeia*'s anatomy and microanatomy, shedding light on the relation between morphology and habitat, chemical defences, and feeding behaviour. The pharynx, housing maxillae and mandibles connected to an extensive mucus gland, occupies a considerable portion of the worm's length, reaching beyond the oesophagus. This unique gland is responsible for secreting the feeding mucus, which immobilizes and aids in the digestion of clams probably acting as a vehicle of bioactive compounds synthesized by specialized serous cells in the mouth. Moreover, *H. parthenopeia* combines behavioural tactics, such as burrowing, and anatomical defences to evade predators. Examination of its epidermis revealed a thick cuticle layer and abundant mucocytes secreting locomotion mucus, both of which save the worm from mechanical harm during movement. When it is preyed upon, the worm can release a substantial amount of Hallachrome, a toxic anthraquinone produced by specific cells in its distal region. This pigment, with its known antimicrobial properties, likely acts as a chemical shield in case of injury. The results suggest that the ability of *H. parthenopeia* to prey on bivalves and to provide mechanical protection plus defence against pathogens rely on its ability to secrete distinct types of mucus. The interplay between highly specialized microanatomical features and complex behaviours underscores its adaptation as a predator in marine benthic environments.

1 | Introduction

Polychaetes have intrigued scientists with their multifaceted ecological roles, exhibiting remarkable adaptations in both offensive and defensive strategies. In fact, within marine ecosystems, polychaetes play roles as prey, predators, filter-feeders, scavengers and bioturbators, contributing to the complex dynamics of aquatic environments (Hutchings 1998; Glasby and Timm 2008). The remarkable diversity among polychaetes suggests that they have developed distinct adaptations to suit various habitats and ecological niches. These adaptations include behavioural strategies (e.g., burrowing in the sediment) and morpho-functional traits (e.g., producing toxins) that protect the worms from potential enemies (such as predators, parasites, bacteria), and enable their feeding (Kicklighter and Hay 2006; Livermore, Perreault, and Rivers 2018; Righi et al. 2021).

In many cases, the defence of worms is mediated by the epidermis, which harbours specialized features to ensure protective and sensory capabilities in both mechanical and molecular ways (Hausen 2005). The integument is equipped with specialized cells secreting a wide range of compounds, from mucins to bioactive pigments with a wide variety of roles including protection against mechanical and biological agents (Rodrigo et al. 2018; Bandaranayake 2006; von Reumont et al. 2014; Coutinho, Teixeira, and Santos 2018). For example, the epidermis of the phyllodocid *Eulalia viridis*, an intertidal predator, contains porphyrinoid pigments stored in cellular granules. These pigments play a crucial role in shielding against UV light, sensory perception and even serve as chemical defence against both fouling organisms and predators (Martins et al. 2019). The sabellid *Sabella spallanzanii* utilizes mucus to defend against bacteria, while the terebellid *Thelepus setosus* produce specific compounds (Thelepin) as antibacterial protection in case of wound healing (Stabili et al. 2009; Higa and Scheuer 1975; Goerke et al. 1991). Additionally, certain compounds within the epidermis act as deterrents against predation; for instance, the cirratulid *Cirriformia tentaculata* and the terebellid *Eupolymnia crassicornis* are chemically defended by the presence of alkylpyrrole sulphamates (Kicklighter et al. 2003).

Feeding in Polychaeta also involves a wide diversity of strategies, from symbiosis with bacteria to active predation and nutrient recycling in sediments and adaptation (Jumars, Dorgan, and Lindsay 2015). Particularly, predatory polychaetes evolved to bear an array of strategies to feed on their prey, whether through ambush predation, active hunting, or burrowing techniques. Some predatory polychaeta rely also on toxin secretion to attack their prey. For example, the active predator *E. viridis* immobilizes the prey (such as mussels and other polychaetes) and extract portion of soft tissue thanks to the toxins secreted by muscular proboscis (Rodrigo et al. 2018), while bloodworms (*Glycera* spp.) have an eversible pharynx tipped with hollow fangs that inject a complex proteinaceous venom into their invertebrate prey (von Reumont et al. 2014; Gonçalves, Alves de Matos, and Costa 2023).

The tube dwelling polychaete *Halla parthenopeia* is a well-known voracious predator. It is a large infaunal worm (up to 1 m long, 30 g weight) that lives buried within coastal muddy-sandy sediments in coastal temperate and subtropical marine habitats (Osman, Gabr, and El-Etreby 2010; Mikac 2015; Ferri, Righi, et al. 2024). *H. parthenopeia* is a selective consumer of bivalves, searching and choosing the most suitable and profitable prey (Ferri, Righi, et al. 2024). Its ability as a predator is based on behavioural strategies and bioactive secretions. Once in contact with the prey, the worm covers it with mucus until the bivalve shell slightly opens. It was hypothesized that this feeding mucus, which is secreted in high quantity during the handling phase by unknown structures, could contain bioactive compound exerting both paralytic and digestive activity (Kawai et al. 1999). Besides the feeding mucus, *H. parthenopeia* also produced a locomotion mucus and a defensive purple mucus. The former is secreted by the epidermis, facilitating the worm's movement into sand galleries to avoid mechanical stress (Kawai et al. 1999). The defensive purple mucus contains a toxic anthraquinone called Hallachrome, and its hypothesized defensive role includes protection against potential competitors, parasites, and/or pathogens in the galleries where the worms live. This compound, easily purifiable from purple mucus, is therefore also of potential biotechnological interest (Simonini et al. 2019).

Despite the available information on the mucous secretion and feeding behaviour of *H. parthenopeia*, there remains a gap in our understanding of the intricate relationship between these secretions, their role for the worm and the anatomical structures responsible for their production. To our knowledge, there is just a preliminary histological essay of the body wall evidenced the presence of thin ducts filled with Hallachrome granules, which dates to the 1950s (Bielig and Möllinger 1960) and was mainly focused on the chemical characterization of Hallachrome. We hypothesize that worms' offensive and defensive abilities are strongly linked to its morphology and anatomy. We aim to expand the state-of-the-art about general morphology of *H. parthenopeia* and particularly to shed light on the anatomy and micromorphology of the feeding apparatus and epidermis of *H. parthenopeia* in correlation with its defensive strategy and peculiar feeding ability of bivalve predator. To achieve this, histological analyses, using both paraffin and resin sections stained with various techniques, were conducted on the epidermis and pharynx where we expected to find cells responsible for the secretion of mucus that assists locomotion, feeding and the pigment, Hallachrome. To investigate the potential role of the red pigment, regenerating segments were also analysed.

2 | Materials and Methods

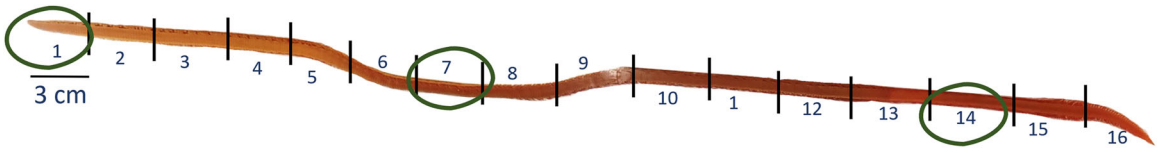
Live *H. parthenopeia* (Delle Chiaje, 1828) specimens were purchased from a retailer as in the previous studies (Iori et al. 2014; Simonini et al. 2019; Ferri, Righi, et al. 2024). Once in the laboratory, they were acclimatized, selected and maintained according to Ferri, Righi, et al. (2024) for at least 6 months. Worms were removed from the sediment monthly to check them and substitute the sediment. During these occasions we observed worms' behaviour focusing on the modality Hallachrome secretion. The 12 worms selected for histological analysis (length up to 80 cm) were isolated in a plastic tank with

only cleaned artificial seawater (ASW) and fasted for 1 week. Then, they were anaesthetized for 1 h at 4°C in 164 mg/L MS222 and cut with a scalpel, obtaining several 2-cm long cylindrical pieces (Figure 1). The pieces were treated with different fixatives: Carnoy's (6:3:1 absolute ethanol, chloroform, acetic acid), Bouin's (aqueous 24% formaldehyde; 5% acetic acid and picric acid to saturation), Davidson's fixatives (aqueous 30% absolute ethanol, 10% formalin, 10% acetic acid) or 4% m/v paraformaldehyde (PFA) 4% in phosphate-buffered saline (PBS). For each fixative we processed pieces derived from three worms. Time and temperature for fixation, washing and storage solution are reported in Figure 1. Sample were dehydrated in a progressive series of ethanol (30%–100%), intermediately infiltrated with xylene, embedded in paraffin (Paraplast) and sectioned (5 μm thickness) with a Jung RM2035 model rotary microtome (Leica Microsystems). In addition, some fixed samples were dissected under the stereomicroscope to recover organs and tissues: these materials were embedded in Epon (Sigma Aldrich, St. Louis, MO, USA) resin, following Luft's mixture (Luft 1961), after being dehydrated in acetone. Intermediate infiltration was done with Epon:polypropylene oxide 1:2, 1:1 and 2:1 (30 min each). Histological and histochemical analyses, both in paraffin and in resin sections, involved haematoxylin–eosin staining (HE) and a tetrachrome (TC) technique based on Alcian Blue (AB) for acidic sugars, periodic acid/Schiff's (PAS) for neutral polysaccharides, Weigert's iron haematoxylin (WH) for chromatin and picric acid (PA) for muscle and cytoplasm, following Rodrigo et al. (2018). Other staining procedures were performed to highlight specific details, such as Coomassie Blue for proteins staining. Details of the procedures can be found in Costa (2018). Some unstained slides were also mounted looking for the presence of granules of Hallachrome in the body wall, which should appear as dark spots (Bielig and Möllinger 1960). For each type of staining procedure, more than 25 slides with 10–12 transversal and longitudinal sections were analysed. Observations were done with a DMLB model microscope adapted for epifluorescence with an EL6000 light source for mercury short-arc reflector lamps. The microscope was equipped with A, N2.1 and I3 filters (corresponding to blue, red and green channels, respectively). All equipment was supplied by Leica Microsystems. Additional observations were made using a WHX-7000 Keyence stereoscope. The nomenclature of anatomical and histological structures proposed by Tzetlin, Vortsepneva and Zhadan (2023) was adopted when appropriate.

3 | Results

3.1 | General Anatomy

Dorsally, the cone-shaped prostomium was characterized by three antennae, which could fold into a V-shaped sulcus extending up to the second chaetiger (Figure 2a). Two pairs of simple eyes, one larger and one smaller, were located on the postero-lateral margin of the prostomium. Each eye had a lens and was deeply embedded within the head, being covered with cuticle. The eyes were internally lined with a layer of melanocyte-like cells holding brown-blackish pigments (Supporting Information S2: Figure I). The epithelium of the most anterior part of the prostomium, over the mouth, was rich in mucocytes. The body included hundreds of homonomous segments. The major structures (i.e., from ventral to dorsal part: nervous system, ventral vessel, nephridia, digestive system and dorsal vessel) were identified in the transversal sections (Figure 3b,c and Supporting Information S2: Figures II and III). All these structures were surrounded by four longitudinal muscle bundles and circular muscles. Between the longitudinal bundles, departed the transverse parapodial muscles which were strictly connected with the chaetigers. The latter were similar throughout the body and showed a well-developed flattened dorsal cirrus, two chaetal lobes and capillary chaetae. The large ventral nerve cord ran along all body length and was characterized by giant axons, up to 70 μm in diameter. Furthermore, the peripheral nervous system consisted of segmental nerves branching off from the connectives and longitudinal nerves branching off from the brain (Supporting Information S2: Figure IV). The vascular system included a ventral and a dorsal vessel, which were connected by lateral segmental vessels. An extensive capillary network supplied blood to the body wall and the parapodia (Supporting Information S2: Figure V). Symmetrical pairs of nephridia were present throughout the entire body length, although these structures exhibited variations from the anterior to the posterior sections of the worms. Posteriorly, they were smaller, characterized by ciliated cells, and lacked blood but were closely associated with a medium-sized blood vessel



Fixative	Time of fixation (h)	Temperature of fixation (°C)	Washes after fixation	Storage solution
Carnoys	2 + 2 + 2	RT	3x30'EtOH100% 3x30'Xylol	Xylol
Bouin	24 + 12	RT	1x15'EtOH70%	EtOH70%
Davidson	24 + 12	RT	1x15'EtOH70%	EtOH70%
PFA 4% in PBS 1X	Overnight	4	1x15'EtOH30% 1x15'EtOH50%	EtOH70%

FIGURE 1 | Sectioning method of *Halla parthenopeia*. Each specimen was divided into 16 pieces of more or less 2 cm in lenght the main processed portions are highlighted with a green circle. Time and temperature for fixation, washing and storage solution for each fixative.

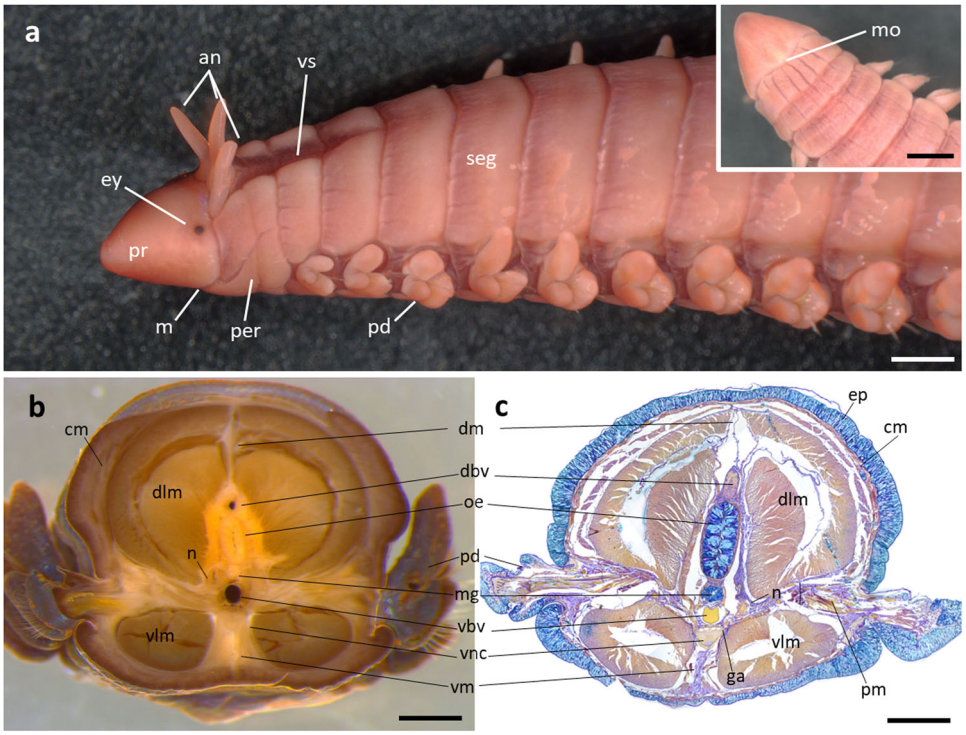


FIGURE 2 | Overview of the internal anatomy of *Halla parthenopeia*. Head lateral (a) and ventral (a inset) view at a stereoscope. Transverse section of the 30th chaetiger before sectioning (b) and after TC2 staining (c). an, antenna; cm, circular musculature; dbv, dorsal blood vessel; dlm, dorsal longitudinal musculature; dm, dorsal mesentery; ep, epithelium; ey, eye; ga, giant axon; m, mouth; mg, mucous gland; mo, mouth opening; n, nephridia; oe, oesophagus; pd, parapodia; per, peristomium; pm, parapodial muscle; pr, prostomium; seg, segment; vbv, ventral blood vessel; vlm, ventral longitudinal musculature; VM, ventral mesentery; vnc, ventral nervous cord; vs, V-shaped sulcus. Scale bar = 1 mm.

(Supporting Information S2: Figure V). The digestive system consisted of a feeding apparatus with a distinct pharynx (described in detail in the next paragraph) followed by a tubular intestine characterized by a thin muscle layer surrounding a stratified ciliated endothelium. Moving posteriorly, the volume of longitudinal muscles and intestine reduced progressively, leaving space for the coelomic cavity and the gonadic tissues (Supporting Information S2: Figure III).

3.2 | Feeding Apparatus

3.2.1 | Feeding Apparatus: Mouth and Pharynx

The mouth was located on the ventral side of the peristomium: the area surrounding the mouth opening showed six epithelial folds which favoured its extensions during feeding (Figure 2a, inset). The pharynx appeared as an oblong eversible muscular structure (extending from the mouth up to the 9th–10th chaetiger when the worm was at rest), which contained a paired maxillae carrier supporting five pairs of maxillae and a pair of mandibles (Figure 3a,b). The muscular structure of the pharynx included the longitudinal mandibular and maxillary muscles, which extend ventrally to mandible and maxillae respectively. It was connected to the body wall by the external longitudinal muscles, which were placed laterally to the masticatory apparatus. Two canals met in the mouth: the oesophagus (the first part of the intestine, placed dorsally, larger) and a tight duct running through the pharynx and connected to the maxillae. The latter structure is a glandular duct because it connected the mouth to a massive 'mucous gland' reported here for the first time (Figures 3c and 5d). The mouth (Figures 4c and 6a) was found to be lined with a pseudostratified epithelium mostly comprised of mucocytes, AB-positive cells recognizable for the presence of sacculi in the cytoplasm and nuclei located at the cell's periphery near the lumen that opened directly into the mouth cavity. Additionally, some PAS-positive cells with cytoplasm rich in granules were present (Figure 6a,a′). These cells, unlike mucocytes, were also Coomassie blue positive and here named 'Serous-like cells' (Figure 6b,b′,b″). In transverse sections, the distinction between the first portion of the oesophagus (dorsal to the pharynx) and the glandular duct became evident starting from the third chaetiger (Figures 4d and 5a), and in the fifth chaetiger, the two structures were completely separated by a thick layer of muscles (Figure 5c). The first portion of the oesophagus had, in cross-section, a swallow shape and contained mainly AB-positive cells and was innervated by somatogastric nerves (Figure 4d). The oesophagus extended to approximately one-third of the worm length (Figure 3c). It exhibited a spiral organization and was surrounded by a thin layer of muscle, with the internal lumen delimited by a cuticle layer (Supporting Information S2: Figure VIa–d). The walls were divided into septa that gave rise to the spiral shape and showed a pseudostratified epithelium consisting of various cell types. Transverse sections revealed an accumulation of strongly AB-positive (mucus secretion) and PAS-positive (cuticle secretion) cells at the septa levels (Figure 7a). Notably, these cells were smaller in both nuclei and cytoplasm compared to those in the protuberance. Mucous cells were the most frequent cell type (Supporting Information S2: Figure VId). In longitudinal sections, TC staining highlighted the different chemical composition of cells in different

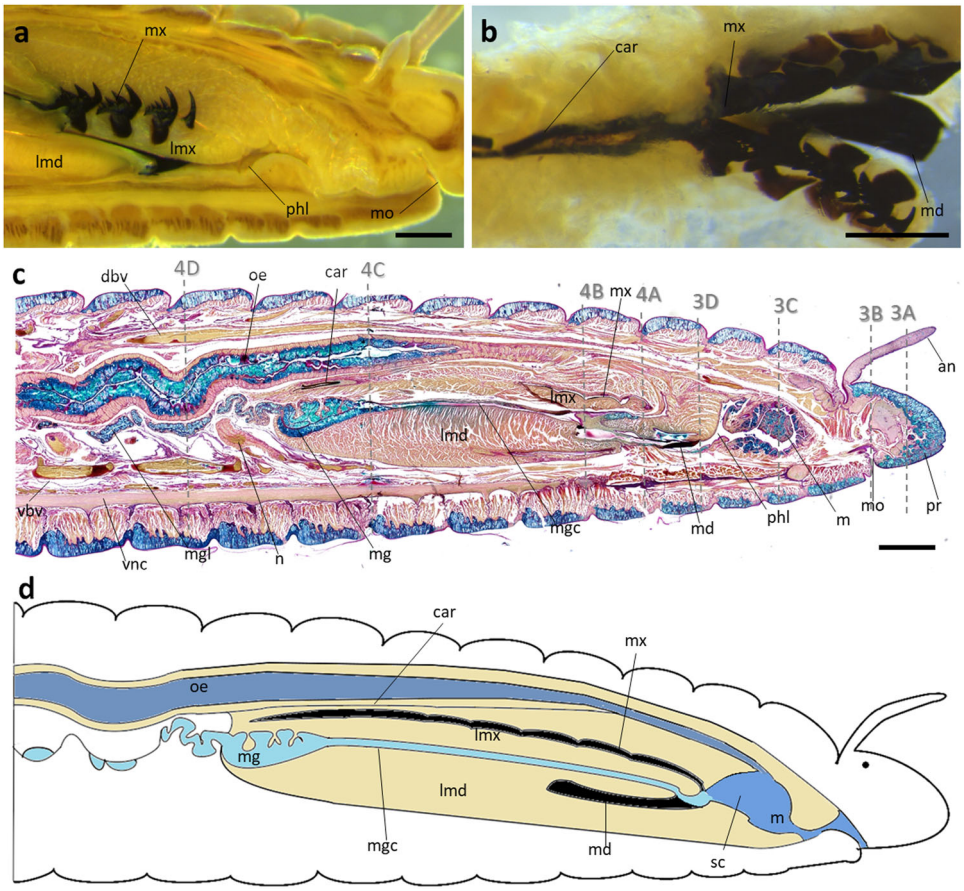


FIGURE 3 | Overview of *Halla parthenopeia* feeding structures. (a, b) Longitudinal unstained section through the feeding apparatus (a) and particular of the jaws after dissection (b). The feeding apparatus of *H. parthenopeia* is schematized in (d). Longitudinal TC-stained section of *H. parthenopeia* from the prostomium to the 10th segment of the worms (c). an, antenna; car, carriers; dbv, dorsal blood vessel; lmd, longitudinal mandibular muscle; lmx, longitudinal maxillary muscle; m, mouth; md, mandibula; mg, mucous gland; mgc, mucous gland canal; mgl, mucous gland lumen; mo, mouth opening; mx, maxillae; n, nephridia; oe, oesophagus; phl, pharyngeal lumen; pr, prostomium; sc, serous cells; vbv, ventral blood vessel; vnc, ventral nervous cord. Scale bar = 1 mm.

regions of the canal. Strongly AB-positive cells were placed mainly near the basal lamina. Moving toward the cuticle, some regions exhibited lightly PAS-positive mucocytes (basic mucins), while the others were stained AB-positive (acid sugar mucins; Figure 7a). Blue Coomassie did not reveal positive cells in the oesophagus, indicating the absence of protein accumulation by these cells (Figure 7c).

3.2.2 | Feeding Apparatus: Mucous Gland

The longitudinal mucous gland originated in the maxillary area and extended parallel to the oesophagus from the fourth to fifth chaetiger (glandular duct) up to one-third of the worm length (up to the 200th chaetiger in the examined specimens, Figures 3d and 5b,c). This gland, which was wrapped by a thin muscle layer, was organized as a tubular gland with sacculi opening into a common central lumen (Supporting Information S2: Figure VIa,b). The lumen was filled with mucus and did not present a cuticle layer, except in the portion proximal to the buccal apparatus (Figure 7d). The gland walls consisted of a monolayer of cells that produced acid mucopolysaccharides (AB-positive) (Figure 7e). The cytoplasmic sacculi were challenging to preserve during histological techniques due to the delicacy of the gland. Coomassie blue staining revealed no cells capable of protein accumulation (Figure 7f).

3.3 | Epidermis and the Secretion of Locomotion and Defensive Mucus

The epidermis covered the entire body and consisted of a pseudostratified epithelium with various types of cells. Externally, this epithelium was consistently lined by a relatively thick layer (10 μm) of collagenous PAS-positive cuticle and a thin AB-positive epicuticle (Figure 8b). The thickness and complexity of the epidermis varied within each segment. In anterior margin of the intersegmental area the epithelium was thin (60 μm), while in the central portions and parapodia was well-developed (about 140 μm). Histochemical staining (TC) enabled the identification of different cell types.

3.3.1 | Supportive Cells

Supportive cells exhibited an elongated shape, cytoplasm rich in small granules and are connected to the cuticle through a small channel (Figure 8). These cells were stained PAS-positive when samples were fixed in Bouin's, while exhibited a green colour

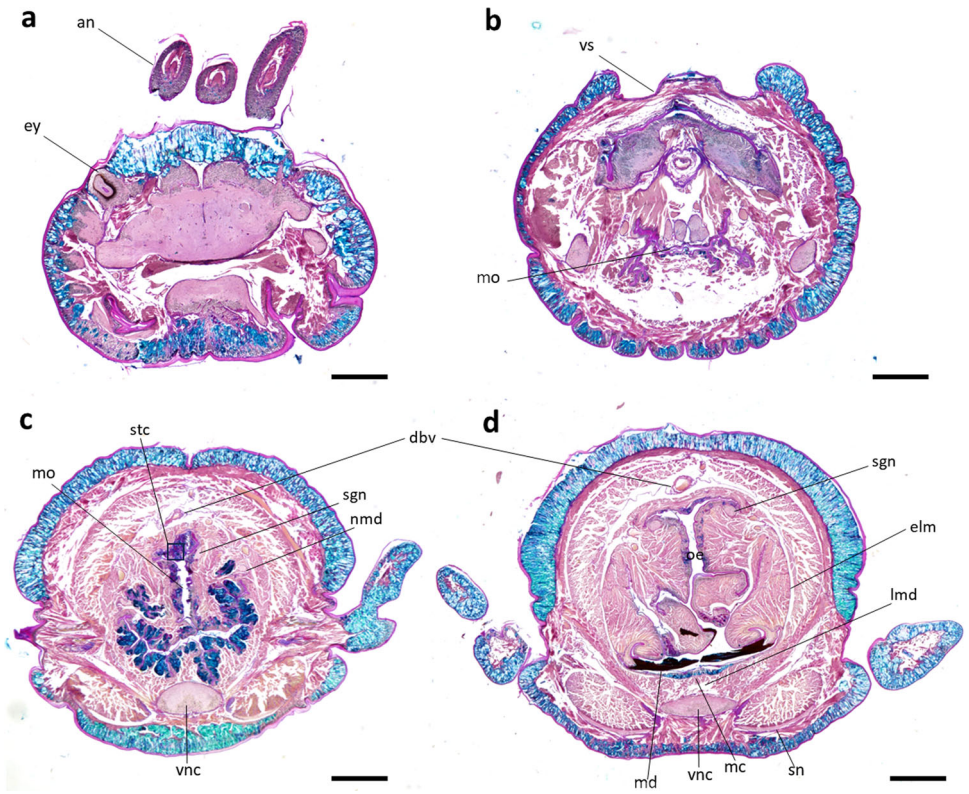


FIGURE 4 | Four sequential TC-stained sections of *H. parthenopeia* head: at prostomium (a), at mouth opening (b) at peristomium (c) and at third segment (d) level. an, antenna; dbv, dorsal blood vessel; elm, external lateral muscle; ey, eye; lmd, longitudinal mandibular muscle; mc, mucus cell; md, mandibula; mo, mouth opening; nmd, mandibular nerve; sgn, somatogastric nerve; stc, serous toxin cell; vnc, ventral nervous cord. Scale bar = 500 μm.

when samples were fixed in 4% PFA in 1× PBS (Figure 8b, showing the same staining that the cuticle presents with the respective fixatives. Non-stained sections effectively revealed the shape of the cells due to the presence of small black granules filling the cytoplasm (Figure 8c).

3.3.2 | Mucocytes

The mucocytes were the main type of cells present in the epithelium and are probably responsible for the secretion of locomotory mucus. These large cells stained positive for Alcian Blue (AB) due to their rich content of acid sugars, characteristic of mucins. Mucocytes were present in various stages of maturation, which can be distinguished by their size and histochemical signature of mucous sacculi within the cytoplasm (Figure 8a). In the initial stage, mucocytes were located near the basal lamina: here, they were filled with small and densely packed sacculi and were strongly positive for AB staining. In the second stage, sacculi were larger but remained easily identifiable. Finally, in the third stage, the cytoplasm appeared uncompartmentalized, and cells exhibited a light blue AB staining. The second and third stage cells extend outward through channels crossing the cuticle for mucus secretion.

3.3.3 | Hallachrome Secreting Cells

Hallachrome secreting cells exhibited a basophilic cytoplasm, with an extensive circular soma (area: 300 μm^2) located in the central part of the epithelium that branches towards the cuticle and basal lamina (Figure 9g,h). The branching toward the basal lamina is associated with pigment accumulations, where is full of granules of pigment with brownish natural staining (Figure 9g,h). The other branching crosses the epithelium and ends on the surface of the cuticle, suggesting its involvement in the release of Hallachrome when the animal is disturbed. Non-stained sections unveil the inherent excitability of these cells under UV light, with the cells emitting fluorescent blue light and Hallachrome granules emitting in red-brownish light (Supporting Information S2: Figure VII). The number, shape and size of the cells responsible for producing the toxic pigment Hallachrome differed between the anterior (Figure 9a,e) and the posterior portion of the body (Figure 9b,f). The posterior segments, which presented cells as described above, exhibited a central line in each segment, rich in intradermal pigment, which can be recognized under a stereomicroscope (Figure 9c,d) and also in histological section (Figure VIIa). We named this area rich in cells capable of accumulating and secreting Hallachrome 'Hallachrome secreting' (Figure 9f,g). Cells resembling those which secrete Hallachrome, likely in an inactive form, are present in the body wall also in the anterior portion of the worm. Here, these cells exhibit an extremely reduced soma (50–100 μm^2) and lack the pigment storage at the basal part of the epidermal cell, that is, near the basal lamina (Figure 9e).

3.4 | Histology of Regenerated Body Portion

Analyses of body portions that underwent autotomy over a 6-month period before fixation and subsequently regenerated, particularly when fixed in Carnoy's, revealed distinct pigmentation differences

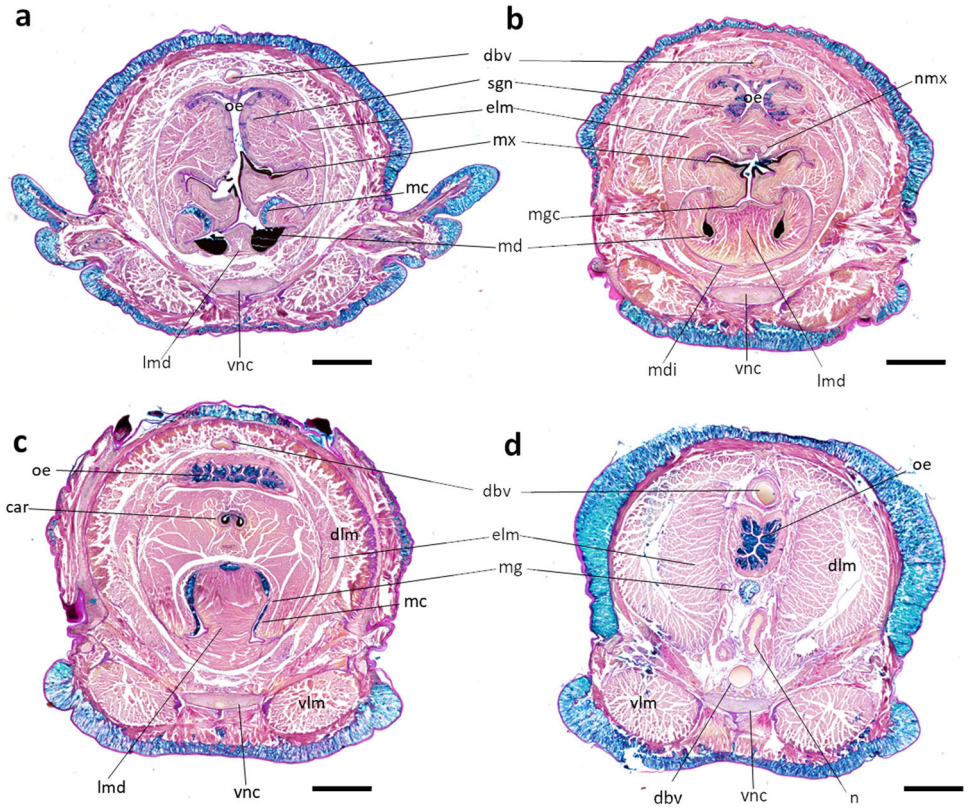


FIGURE 5 | Four sequential TC-stained sections of *Halla parthenopeia* head: at fourth (a), fifth (b), seventh (c) and ninth segment (d) level. car, carriers; dbv, dorsal blood vessel; dlm, dorsal longitudinal musculature; elm, external lateral muscle; lmd, longitudinal mandibular muscle; mc, mucus cell; md, mandibula; mdi, mandibles invagination; mg, mucous gland; mgc, mucous gland canal; mx, maxillae; n, nephridia; oe, oesophagus; vlm, ventral longitudinal musculature; vnc, ventral nervous cord. Scale bar = 500 μm.

between the segment anterior to the break and regenerated portions under stereomicroscope observation (Figure 10b). At the site of autotomy, the segments of the non-renewed portion exhibited significantly more pigmentation than the adjacent regenerated segments. Transverse sections further revealed intense Hallachrome accumulation in certain tissues, particularly within all tissues beneath the epidermis and surrounding the intestine (Figure 10c,d).

3.5 | Hallachrome Secretion

During worm extraction from sediment, we did not observe the presence of Hallachrome in the sand or in the galleries. However, when handling the worms outside the sediment, we observed the secretion of defensive purple mucus from the posterior body portion of the worms. This secretion occurred when the worm experienced mechanical stress, especially when pressure was applied to the epidermis (File S1). Sometimes, the manipulation induced the loss of posterior segments through autotomy (Figure 10a). In these cases, there was a simultaneous massive release of Hallachrome near the wound.

4 | Discussion

4.1 | Feeding Apparatus

Given the broad spectrum of food sources exploited by polychaetes, feeding structures exhibit numerous adaptations. In general, the alimentary canal of polychaetes exhibits a tripartite structure comprising the foregut, midgut and hindgut. The foregut gives rise to the mouth, pharynx and oesophagus, while the midgut can be further subdivided into a stomach and the intestine proper. The foregut and hindgut originate from the ectoderm, forming stomatodeal and proctodeal invaginations, typically covered by a cuticle (Tzetlin and Purschke 2005). Even among homonomously segmented polychaetes, the foregut displays several specializations: dorsolateral folds, ventral pharynx, axial muscular pharynx, axial non-muscular proboscis and dorsal pharynx (Saulnier-Michel 1992).

Among Eunicida, the ventral pharynx lies under the oesophagus, and is characterized by the presence of ventral mandibles and dorsal maxillae in a ventral muscularized pharynx (Clemo and Dorgan 2017; Zanol et al. 2021). Within this group, the Oenonidae stands as the unique extant family characterized by a priognath-type (Kielan-Jaworowska 1966) jaw apparatus. *Drilonereis* cf. *filum* is the only oenonid, for which the movement of the pharynx and the muscles responsible for it were characterized (Tzetlin, Vortsepneva, and Zhadan 2023).

The configuration of jaws and pharynx that we found in *H. parthenopeia* closely resembles that of *Drilonereis* cf. *filum*.

Such a masticatory system is adequate for 'grasping' relatively soft fed such as detritus (in *D.* cf. *filum*, Tzetlin, Vortsepneva and Zhadan 2023) or bivalve flesh by *H. parthenopeia* (Ferri, Righi, et al. 2024). It is well known that predatory

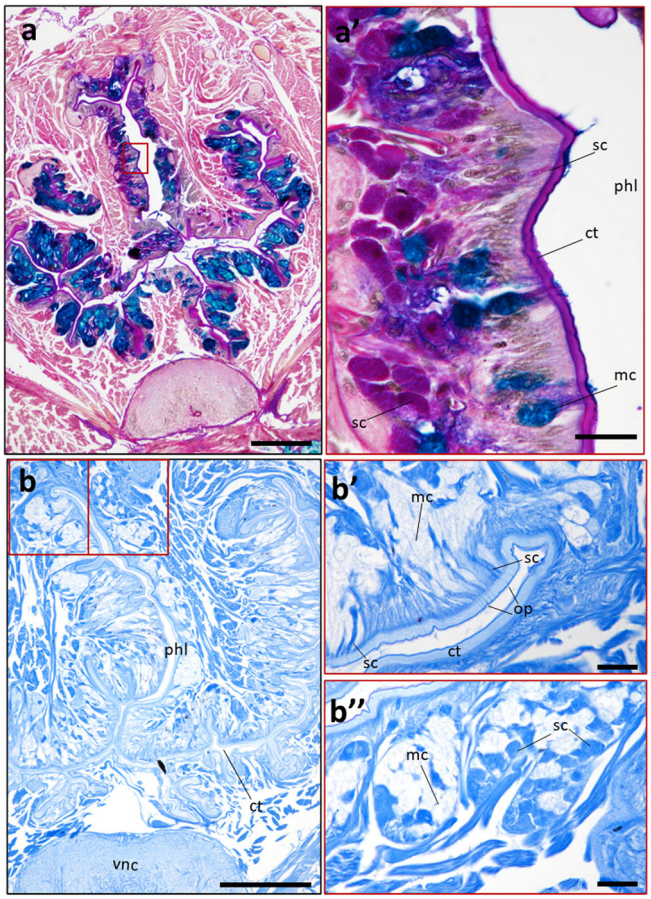


FIGURE 6 | Histochemical characterization of cells at mouth level. Transverse section at mouth level stained with TC2 (a and a′ detail) and with Coomassie Blue (b and b′ and b″ details). Mucocytes and PAS/CB positive protein secreting cells are evidenced in photo detail. ct, cuticle; mc, mucus cell; op, secretory cell opening; phl, pharyngeal lumen; sc, serous cell; vnc, ventral nervous cord. Scale bars a,b = 200 μm; a',b′,b″ = 25 μm.

effectiveness of *H. parthenopeia* relies on the production of a specific feeding mucus and a peculiar behaviour. When the worm intends to prey on a clam, it envelops it with a mucus bolus through repeated movements along the valve junction (Ferri, Righi, et al. 2024). The alternation of contraction and relaxation of the mouth led to the secretion of a bioactive mucus that caused the opening of clam valves after a few hours due to a paralytic effect of the substance and the pre-digestion of soft tissue (Ferri, Righi, et al. 2024). The most striking peculiarity of the feeding apparatus of *H. parthenopeia* consists in its unique longitudinal mucous gland and the massive development of mucocytes in oesophagus epithelia. The novel mucous gland described here appears to be responsible for producing the mucus emitted by the worm during prey manipulation. This unpaired fragile structure allocates within the pharynx and is mainly constituted by mucocytes and directly connected to the mouth.

In the mouth, serous cells (SCs) with granule reactive to Coomassie Blue suggested active secretion of proteinaceous materials (e.g., enzymes or toxins). Their localization, morphology and cytochemical characteristics closely resemble that of specialized cells secreting toxins and enzymes of the carnivorous polychaete *E. viridis* (Rodrigo et al. 2018). We thus hypothesized that SCs are involved in the secretion of enzymes or toxins which can be responsible for the digestive and paralytic effect of the feeding mucus on the prey. In addition, the oesophagus has a spiral structure that increases its secretory/absorptive surface and perhaps protects the epithelial germinal cells at the base of the septa. The oesophagus is characterized by a thick cuticle layer and an high number of mucocytes and seems to have a functional specialization in the secretion of acidic and basic mucins. The cuticle protects the oesophagus walls from possible mechanical damage due to the rubbing of undigested pieces, while the mucus ensures the smooth mobility of the bolus towards the intestine.

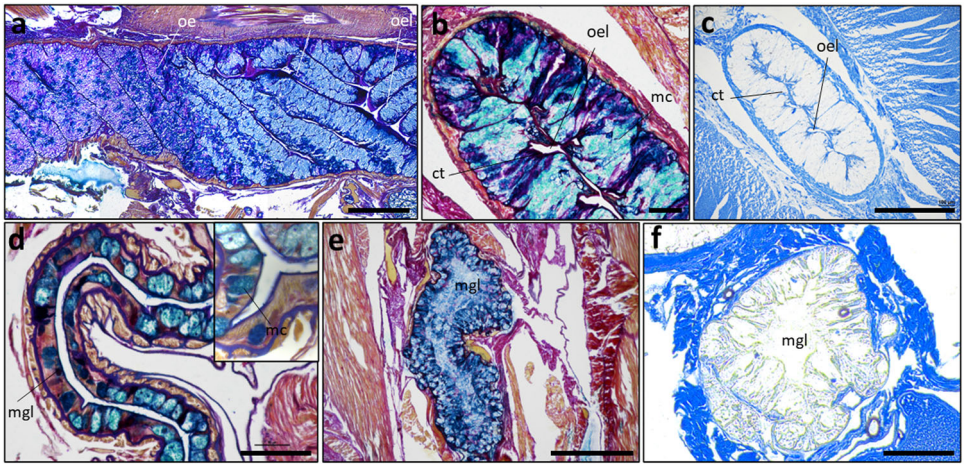


FIGURE 7 | Histochemical characterization of oesophagus and mucus gland in the feeding apparatus. Longitudinal (a) and transverse (b, c) section of oesophagus TC stained (a, b) and Coomassie blue stained (c). Longitudinal (a at seventh segment and b at 15 cm from the prostomium) and transverse (c) section of mucus gland TC stained (a, b) and Coomassie blue stained (c). ct, cuticle; mg, mucous gland; mgl, mucous gland lumen; oe, oesophagus; oel, oesophagus lumen. Scale bars: a,e,c = 500 μm; b,d,f = 100 μm.

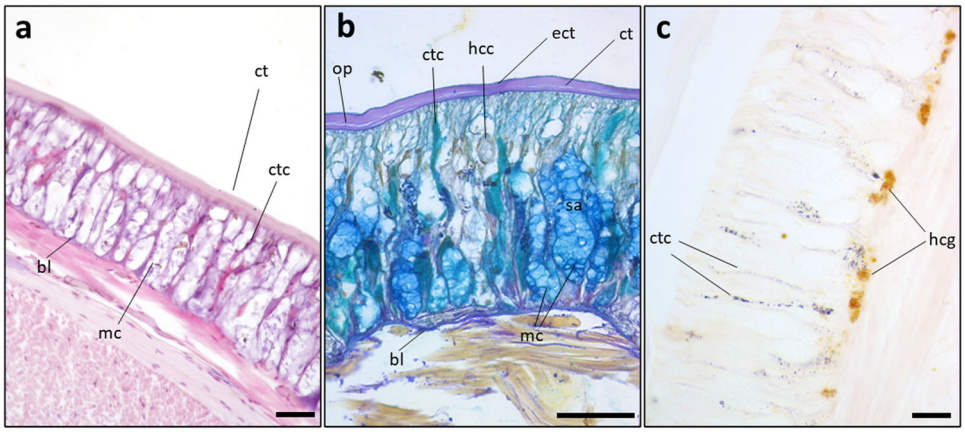


FIGURE 8 | Epidermis of *Halla parthenopeia*. Section stained with HE (a), TC after PFA 4% in PBS 1× fixation (b) and non-stained section (c). bl, basal lamina; ct, cuticle; ctc, cuticle secreting cell; etc, epicuticle; hcc, Hallachrome secreting cell; hcg, Hallachrome granules; mc, mucus cell; op, secretory cell opening; sa, sacculi. Scale bar = 50 μm.

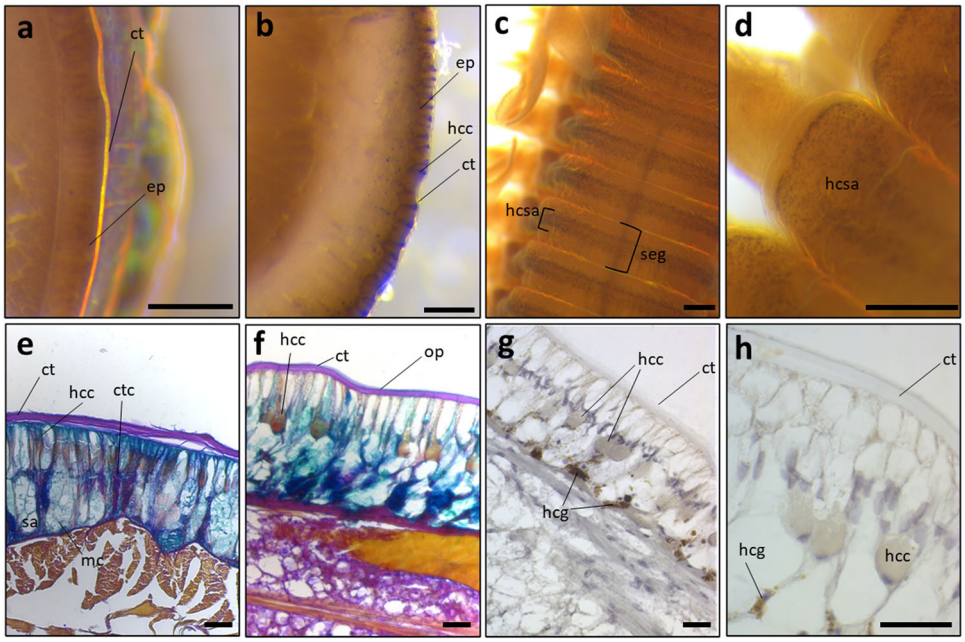


FIGURE 9 | *Halla parthenopeia* epidermis. Anterior (a) and posterior (b–d) epidermis acquired with a stereomicroscope. Anterior section of epidermis stained with TC (e) compared to the posterior (f). Hallachrome is secreted only in the central part of the posterior segment (c, d), where Hallachrome secreting cells, WH-positive (g–h), are present. ct, cuticle; ctc, cuticle secreting cell; ep, epithelium; hcc, Hallachrome secreting cell; hcg, Hallachrome granules; hcsa, Hallachrome secreting area; mc, mucus cell; op, secretory cell opening; as, sacculi. Scale bars a, b = 300 μm; c, d = 1 mm; e–h = 25 μm.

Combining the previous observation on the behaviour of the worm during the prey manipulation (Ferri, Righi, et al. 2024) and the present finding on the anatomy of the feeding apparatus, a mechanism of prey consumption and assimilation can be reconstructed as follows: (1) the contraction of the pharynx squeezes the lumen and canal of the mucous gland; (2) The mucus flows toward the mouth, where it mixed with the secretion of the SC forming the feeding mucus; (3) the worm deposits the feeding mucus just along the junction of the clam valves, where it exerts its paralytic and digestive action in a matter of hours; (4) when valves open, the worm grasps the externally digested meat through the coordinated action of its maxillae and mandibles; (5) The copious secretion of mucus in the oesophagus permits the move of the ingested meat from the mouth to the intestine (Figure 3d).

4.2 | Epidermis and the Secretion of Locomotion and Defensive Mucus

The epidermis of polychaetes is characterized by a pseudostratified epithelium comprising supportive cells, gland cells and sensory cells covered by cuticle (Hausen 2005). In burrowing polychaetes, morphological and functional adaptations of the epithelia like a thick cuticle and copious mucus secretions facilitate the locomotion and protect the body wall from potential mechanical damage due to friction with sediment. Epidermal cells in polychaetes exhibit a remarkable versatility in secretion, contributing to not only structural support but also actively participating in the organism's physiological processes and adaptive responses. Oenonidid polychaetes are distinguished by their highly iridescent cuticle, which typically

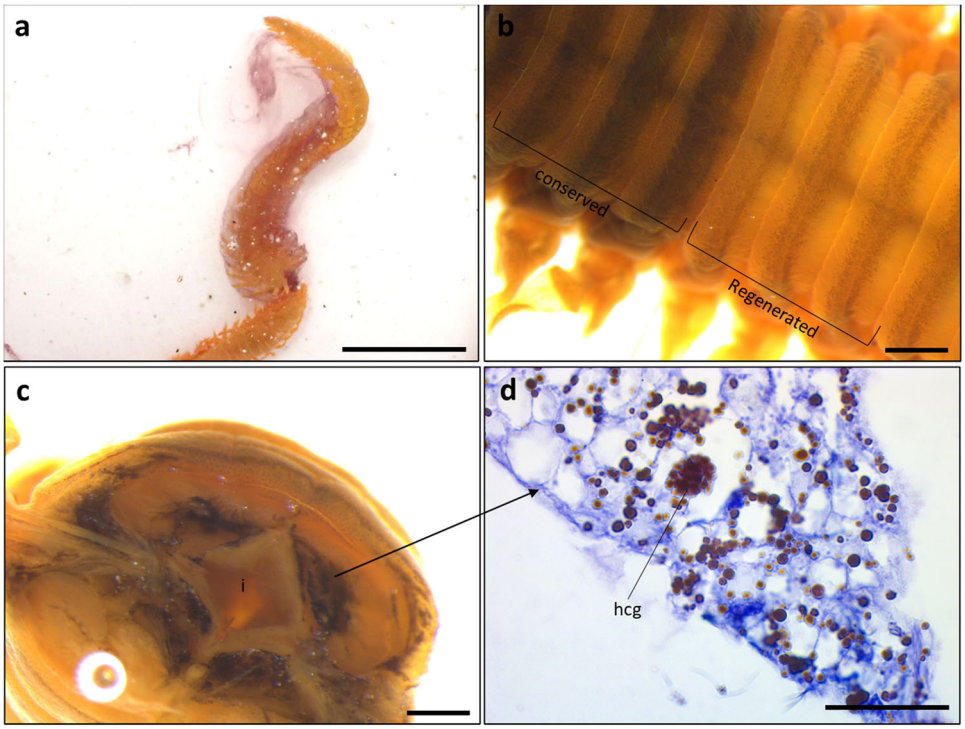


FIGURE 10 | Hallachrome secretion and function. *Halla parthenopeia* undergo autotomy after mechanical stress with massive secretion of purple mucus (a). The conserved portion beside the broken point remains more pigmented than the regenerated one (b). In the conserved portion, the Hallachrome is attached to tissue in granules form (c, d). hcg, Hallachrome granules; I, intestine. Scale bars a = 1 cm, b, c = 1 mm, d = 25 μm.

exhibits greater thickness compared to other eunicid species, particularly those that are free-living species (Menchini Steiner and Oenonidae 2022).

H. parthenopeia is a mobile and behaviourally complex species that could avoid its predators using refuges (burrowing tubes in the sediment) and other defensive behaviours (e.g., feeding preferably during the night and covering the tube entrance with sediment mixed with mucus; Osman, Gabr, and El-Etreby 2010; Simonini et al. 2019, Ferri, Righi, et al. 2024). Our findings reveal that such a strategy relies on the epithelium characteristics and integrates the previous fragmentary information on its morphology (Bielig and Möllinger 1960). In *H. parthenopeia*, most of the epithelial cells are involved in the secretion of the thick cuticle or in the production of the transparent locomotion mucus. The thickness of the cuticle makes it adequate to protect the epidermis from most of mechanical damages. The locomotion mucus creates a tube around the body and facilitates the locomotion and strengthens the tubes in which the worm lives (Osman, Gabr, and El-Etreby 2010).

Few polychaetes are known to be toxic or venomous (Righi et al. 2021; D'Ambrosio et al. 2022), and only three species secrete toxic secondary metabolites: the echiurid *Bonellia viridis*, the lumbrinerid *Kuwaita* (*Lumbriconereis) heteropoda* and the species considered in the present study *H. parthenopeia*. They produce bonellin (de Nicola Giudici 1984), nereistoxin (Hashimoto and Okaichi 1960) and Hallachrome, respectively. To our knowledge, the epithelial structures specialized in the production and accumulation of these toxicants were not subject of investigation, except for *H. parthenopeia*. In this species, cryostat transverse sectioning of the body wall evidenced the presence of thin ducts filled with Hallachrome granules (Bielig and Möllinger 1960). We found that almost all the purple mucus is secreted in the posterior portion of the body, and its secretion is induced by a gentle pressure exerted along the body wall (File S1). According to Rodrigo et al. (2018), the 'pressure by contact' release is associated with an increase of extracellular pressure on the apical part of secreting cells which induce the release of toxins. In the present study, the cells potentially responsible for Hallachrome secretion have been identified. They are concentrated in transverse bands in the middle of each segment; from the anterior to the posterior portion of the body, they differ in shape and size suggesting distinct status of activation. In the epithelium the Hallachrome seems to be present in a stock form (granules) at the basal part of the epidermal cell near the basal lamina and in a different form in cellular soma. Given the low solubility of Hallachrome in seawater, we suggest that the granules contain Hallachrome in the form of precursors with low toxicity (e.g., as precipitates or polymers), which are converted to the active form for the release. The posterior body portion, with active Hallachrome secreting cells, is the one that may undergo autotomy during mechanical stress.

Polychaetes have a well-developed capacity to autotomize their posterior part (Wilkie 2011) and regeneration of the lost part normally occurs. Autotomy represents a specialized defence against environmental stressors (Fleming, Muller, and Bateman 2007) and predators (Livermore, Perreault, and Rivers 2018). Some marine benthic and planktonic invertebrates employ a strategy of activating their defences by chemically transforming inactive molecules stored within their tissues into bioactive analogues when they face threats or injuries. Furthermore, the presence of chemical signals linked

to mechanical damage can trigger the production of more potent compounds (Tan 2023). For instance, Thelepin, an antimicrobial compound, has been exclusively discovered in the distal parts of *T. setosus*, and its potential as an antiseptic agent in wound healing was supposed (Higa and Scheuer 1975). In *H. parthenopeia*, autotomy is concomitant with a massive release of Hallachrome. Histological observations of the segment anterior to the break after autotomy and posterior regeneration have revealed that the pigment remains attached in granular form to the tissues. Given the antimicrobial properties of Hallachrome, we hypothesized that it prevents wound infection in injured worms. Indeed, the lack of Hallachrome in the galleries does not support its involvement in the defence against the microflora colonizing worms' tube and body wall (Simonini et al. 2019; Ferri, Simonini, et al. 2024).

5 | Conclusion

We described the anatomical adaptations in relation to complex behaviour of *H. parthenopeia*, a marine polychaete. Notably, the worm possesses a unique gland composed of cells responsible for secreting feeding mucus enriched with bioactive compounds, facilitating its selective predation on bivalves despite lacking mechanical structures for shell penetration. Additionally, our examination along with the body plan revealed two main types of secretory cells in the epidermis: mucocytes, responsible for locomotion mucus secretion to protect worms during movement, and Hallachrome-secreting cells, predominantly located in the posterior part, potentially serving in defence against bacterial infection post-injury.

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Author Contributions

Anita Ferri: conceptualization, methodology, formal analysis and investigation, writing–original draft preparation. **Pedro M. Costa:** conceptualization, methodology, formal analysis and investigation, writing– review and editing, funding acquisition, resources, supervision. **Roberto Simonini:** conceptualization, methodology, formal analysis and investigation, writing–review and editing, funding acquisition, resources, supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Histological slides are stored at the Life Science Department of the University of Modena and Reggio Emilia. Access will be granted upon request to the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.