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Hairy and iridescent chaetae of the sea mouse *Aphrodita* (Annelida, Errantia)

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Abstract

The bright iridescent chaetae of *Aphrodita* species have fascinated scientists for decades. The particular nanoscale substructure of these chitinous bristles resembles photonic crystals and manipulates the incident light in a way that results in spectacular structural coloration. Even though *Aphrodita's* chaetae have garnered great attention from the field of biomimetics, the few previous ultrastructural studies primarily focused on acicular chaetae that are much less iridescent than the thin capillary notochaetae. In this paper, we present new ultrastructural data on the chaetae of two species of *Aphrodita* and illustrate their formation process. Our results allow us to specify the prerequisite for iridescent chaetae. *Aphrodita* species are dorsally covered with a matted felt of intertwined chaetae, and we further discuss the evolution of feltage chaetae in Aphroditiformia. In addition to iridescent feltage chaetae, we also provide new insights into the chaeotogenesis of pilose chaetae. For the first time, our study shows that the hairy tips and ornamentation of the chaetae is formed by follicle cells alone and thus attribute a further role to this type of cells.

KEYWORDS

Aphrodita aculeata, Aphroditiformia, chaetogenesis, feltage chaetae, pilose chaetae, ultrastructure

1 | INTRODUCTION

Aphrodita aculeata Linnaeus, 1767 is one of the best-known annelid species, with a species description even predating Linnaeus (as *Eruca echinata* Barrelier (1714)). The most characteristic feature of these animals is the feltage that covers them dorsally. The furry appearance of the animals has been eponymous for their common name sea mouse, a name that was possibly first used by some imaginative and libidinous fishermen, which later might have inspired Linnaeus's choice in referring to the Ancient Greek goddess of love.

The characteristic dorsal feltage of these animals is made up of thin chaetae interwoven and entangled together with sand and sediment particles. Annelid chaetae have a striking morphological diversity. With assorted shapes and forms, various types of chaetae can occur, not only amongst different taxa but even on a single segment of the same species (Hausen, 2005, Rouse et al. in press, Tilic et al., 2016). In *Aphrodita* species, stout acicular chaetae, distally hairy pilose chaetae and bipinnate chaetae occur alongside feltage chaetae (Barnich & Fiege, 2000). These are extracellular chitinous structures that develop inside a chaetal follicle, which is an epidermal invagination. The basalmost cell of this follicle is called a chaetoblast, and with dynamic apical microvilli, the chaetoblast forms the template of a developing chaeta (O'Clair & Cloney, 1974). The intricate machinery

of chaetogenesis has been likened to a biological 3D printer (Warren, 2015), and it was characterized in great detail for various chaetal forms (Bartolomaeus, 1998; Hausen, 2005; Kolbasova et al., 2014; Tilic et al., 2014; Tilic et al., 2015; Tilic & Bartolomaeus, 2016).

The main goal of this study was to investigate the ultrastructure of feltage chaetae in *Aphrodita,* which is compelling for multiple reasons. The striking iridescence of these fine capillary chaetae has garnered attention in the field of biomimetics, and the chaetae have been likened to natural photonic crystal fibres (McPhedran et al., 2001; Parker et al., 2001; Parker & Townley, 2007). However, these studies were primarily based on thick acicular chaetae, which only have moderate iridescent effects, and ultrastructural characterization of thin capillary chaetae with the most extraordinary iridescence was still lacking. A better understanding of the biological and developmental processes that form such structures is pivotal for advances in engineering and fabrication processes of bioinspired materials (McDougal et al., 2019).

Furthermore, feltage chaetae, similar to those of *Aphrodita,* also occur in two other clades within Aphroditiformia: *Sthenelanella* (Sigalionidae) and Acoetidae. Both of these construct tubes out of notopodial feltage chaetae. The ultrastructure and formation of feltage chaetae in *Sthenelanella* were recently described by Tilic et al., (2021), and there is only a single histological study of feltage chaetae in Acoetidae by Pflugfelder (1934). Our results add to the necessary foundation to understand the evolution of feltage chaetae in Aphroditiformia.

Last but not the least, we also investigated the formation of pilose chaetae. Their chaetogenesis revealed an unexpected involvement of follicle cells in forming the thin hairs that distally cover each chaeta. The follicle cells secrete the outer enamel coating and structurally modify the secreted chaetal material, thereby directly influencing the chaetal morphology. For the first time, we can show that not just the chaetoblast but also the follicle cells play an active role in shaping a developing chaeta.

2 | MATERIAL AND METHODS

2.1 | Specimens

Small, juvenile individuals of *A. aculeata* (1–2 mm in body length) were found in the fluffy layer and mud sampled with a Van Veen grab sampler from the "Phoronis-Grund" off the isle of Heligoland in April 1990 and fixed for transmission electron microscopy. Adult live and formalin-fixed specimens of *A. aculeata* were ordered from the AWI Heligoland, Biota Service, photographed, subsampled for DNA sequencing, and processed (see below) for scanning electron microscopy (SEM). Several small individuals (ca. 3 cm in body length) of *Aphrodita* cf. *longipalpa* Essenberg, 1917 were collected in May 2019, from 500 m depth in San Diego, California. The animals were fixed for transmission electron microscopy (TEM) and directly dissected into the fixative (see below) in the field.

A COI barcode sequence for the studied *A. aculeata* specimen from Heligoland is deposited to GenBank: MZ008275. A voucher specimen for *A.* cf. *longipalpa* was deposited to the Benthic Invertebrate Collection of the Scripps Institution of Oceanography (SIO-BIC A10919).

2.2 | Scanning electron microscopy

Specimens of *A. aculeata* fixed in seawater–formalin were rinsed and dehydrated in an ascending ethanol series and preserved in 75% ethanol. Segments and parts of the dorsal feltage were dissected before additional processing. The dissected pieces were dehydrated up to 100% ethanol with additional incubation in 0.5% phosphothungstic acid in 90% ethanol (5 hr at room temperature). Parapodia and segment tissue were dried using a critical point dryer (Critical Point Dryer CPD 030, Bal-Tec). Pieces of the dorsal feltage were dried using HMDS following Nation (1983). Dried samples were sputter-coated with gold and platinum/palladium using an SEM Coating Unit E5100 (Polaron Equipment LTD) and Cressington Sputter Coater, Modell: 208 HR, respectively. The samples were imaged and analysed using a Verios 460L FEI scanning electron microscope.

2.3 | Transmission electron microscopy

Specimens used for transmission electron microscopy (TEM) were fixed in 2.5% glutaraldehyde buffered in 0.05 M phosphate 0.3 M NaCl saline (PBS). After approximately 24 hr at 4°C, the samples were rinsed and stored in PBS (with NaN_4). For preparing *A. aculeata* for TEM studies, 0.1 M sodium cacodylate (pH 7.2, 4°C) was used for buffering the fixative. Before embedding, the samples were postfixed with 1% OsO_4, buffered in the same way as the glutaraldehyde, for 40 min at 4°C. The material was subsequently dehydrated in an acetone series and embedded in Araldite. Series of silver interference coloured ultra-thin sections (60–70 nm) were prepared with a Diatome Ultra 45° diamond knife mounted on a Leica Ultracut S ultramicrotome and placed on formvar-covered single-slot copper grids. The sections were stained with 2% uranyl acetate and 2.6% lead citrate (E17810, Science Services) after Reynolds in an automated TEM stainer (QG-3100, Boeckeler Instruments). Sections were examined and imaged using a ZEISS EM10CR with phosphate imaging plates (Ditabis).

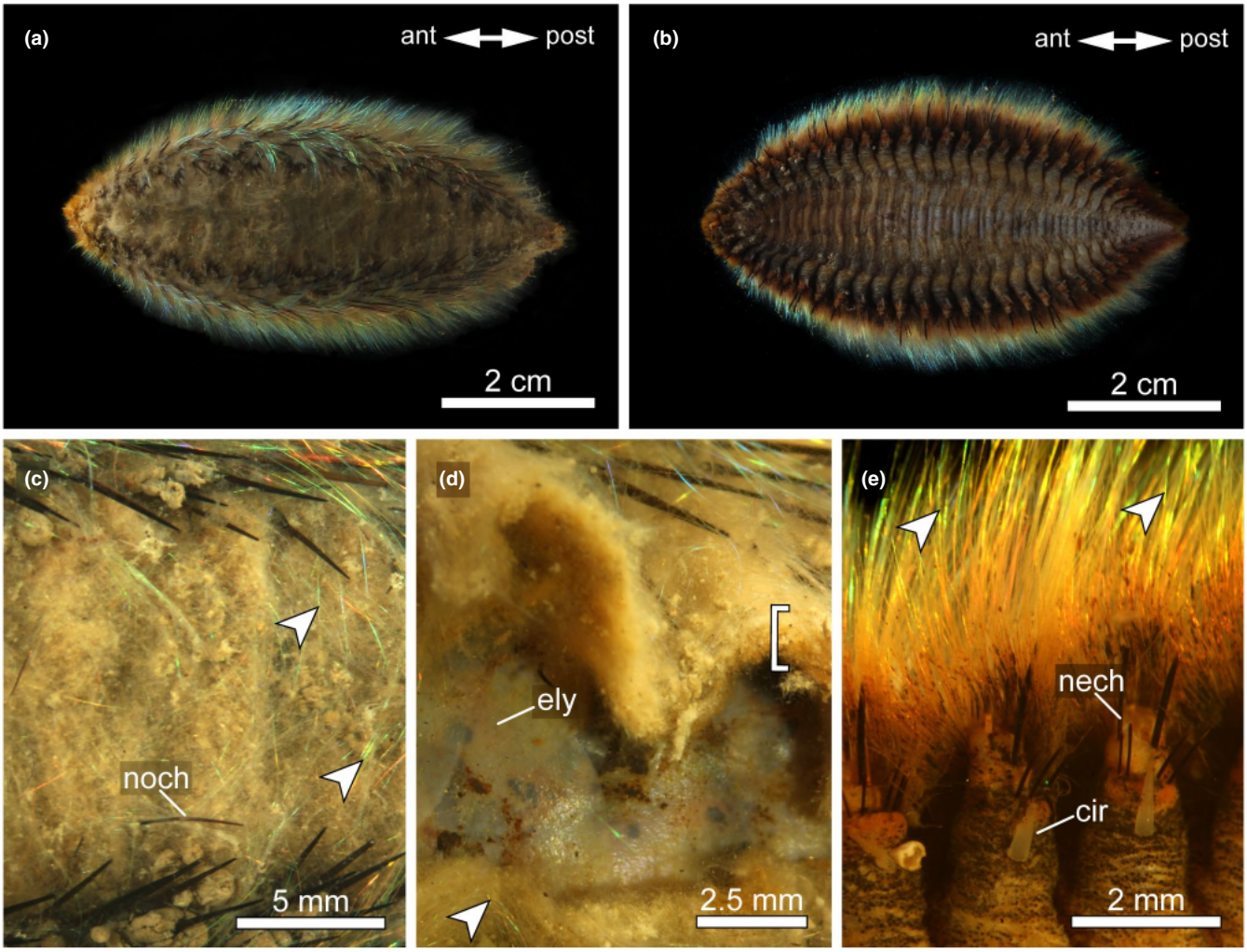


FIGURE 1 Live photographs of *Aphrodita aculeata* (a) dorsal view (b) ventral view. (c and d) show details of the dorsal view showing notochaetae, iridescent capillary feltage chaetae are marked with *arrowheads* in all images. Thick, darkly coloured acicular notochaetae (*noch*) partially penetrating through the matted dorsal feltage. (d) shows the feltage (*square brackets*) cut open to reveal the hidden elytra (*ely*). (e) shows details of the ventral side and neuropodia, neurochaetae (*nech*) are arranged in three rows above the short ventral cirrus (*cir*)

3 | RESULTS

3.1 | Parapodial structure and types of chaetae

The body of both *Aphrodita* species is dorsoventrally flattened and has an elliptical shape (Figure 1a,b). The adult specimens of *A. aculeata* we examined were about 6 cm long with about 30 chaetigers (Figure 1b). Dorsally the animals were covered with the characteristic matted felt, which concealed the smooth and oval elytra (Figure 1a,d). The ventral side of the animal was densely covered with darkly pigmented papillae (Figure 1b,e). Parapodia were biramous, with a ventral neuropodium bearing a short cirrus in all body segments (Figure 1e) and a dorsal notopodium with a longer dorsal cirrus in those segments that lacked elytra. Like the elytra, the dorsal cirrus was largely covered by the feltage chaetae of the notopodium.

3.1.1 | Neurochaetae

The total number of neurochaetae in fully grown *A. aculeata* was about 6–8 per neuropodium. These protruded laterally and were arranged in three successive, vertical rows (Figure 1e). The neurochaetae were dark brown/black in coloration, sometimes with a reddish hue when exposed to light. The neurochaetae were mostly thick with blunt abraded ends and can therefore be described as acicular. However, some neurochaetae were pilose, that is with a hairy tip (Figure 2a-c). These fine hairs covered only the distalmost ±150 μm of a pilose chaeta (Figure 2a-c). They were abraded and worn off to varying degrees in different chaetae (Figure 3a,b). The central core of the chaeta was smooth and tapered to a pointed end, visible only in the light microscope and only when the correct focal plane was observed (Figure 3b). The hairy tips of the pilose chaetae, gave them their stout and blunt appearance (Figure 3a,c). The hairs were longest distally, and

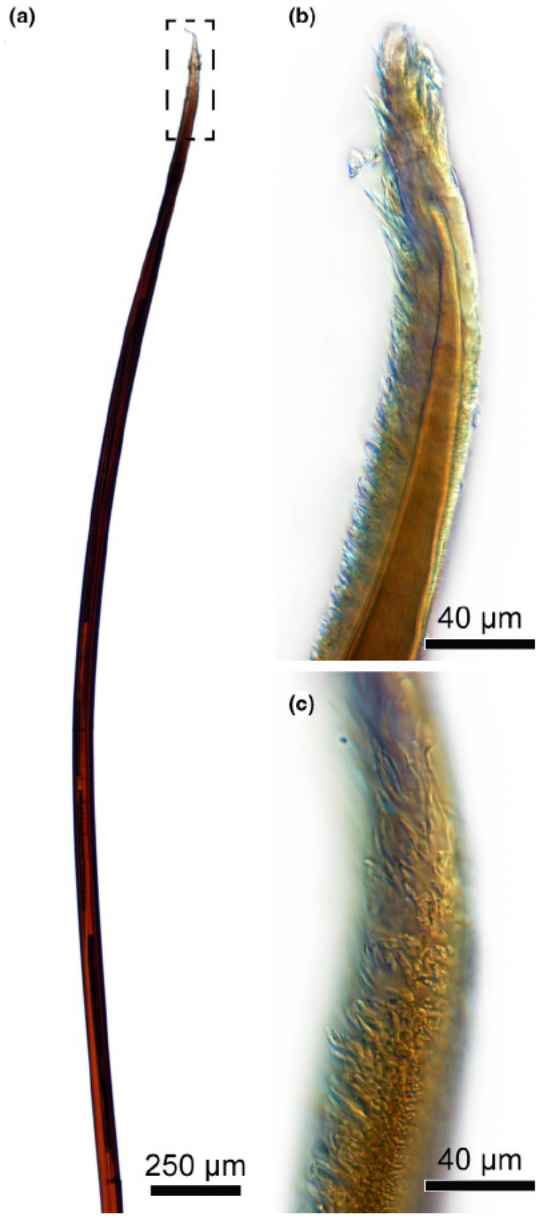


FIGURE 2 (a) Light microscopic image of a pilose neurochaeta of *Aphrodita aculeata*. The distal part of the chaeta, covered with short hairs is marked. (b and c) show the marked region in a higher magnification and in different focal planes

they became shorter and disappeared towards the base of the chaeta, leaving a bare and smooth shaft (Figures 2 and 3a).

Bipinnate neurochaetae were only observed in the posterior segments (the last 11 segments of an approximately 4 cm specimen of *A. aculeata*; Figure 3e,f). These bipinnate chaetae had alternating spurs at the distalmost ±130 µm of a chaeta (Figure 3e). The distances between the spurs become smaller and smaller towards the tip of the bristle (Figure 3f-g). The more proximal parts of the bipinnate chaetae were again smooth (Figure 3f,g). The neuroaciculae were similar in diameter to the pilose chaetae. They pierced through the cuticle but still reached furthest down into the tissue, serving as an attachment site for parapodial musculature.

3.1.2 | Notochaetae

Two kinds of notochaetae were observed: (a) feltage chaetae or capillary notochaetae. These very thin chaetae had an average diameter of about 1.5 µm and were present in very large numbers. Some projected laterally and when exposed to light, they had the characteristic iridescent shine, chaetae of *Aphrodita* species are known for (Figure 1a,b,d). Others were directed dorsally, getting tangled and matted forming the dorsal feltage (Figure 1c,e; Figure 4a,d). This felt-like material accumulates sediment and organic material in addition to the fibrous feltage chaetae (Figure 1c,e); (b) acicular notochaetae, with dark brown coloration and stout abraded tips. These protruded markedly from the dorsal feltage (Figure 1c) and had an approximate diameter of about 30 µm (Figure 3c). In SEM images of damaged acicular notochaetae, the internal fine structure of the chaetal shaft became visible, exposing the characteristic honeycomb pattern (Figure 4c,d). These hollow channels are the typical remnants of the chaetal formation process, which we describe in the sections below.

3.2 | Ultrastructure of chaetae

There were no ultrastructural differences of the chaetae amongst *A. aculeata* and *A.* cf. *longipalpa*. Each chaeta emerges from its own chaetal follicle, composed of follicle cells and a basal chaetoblast with apical dynamic microvilli. Both the follicle cells and the chaetoblasts are epidermal. Multiple chaetal follicles form a chaetal sac, and there is a single chaetal sac in each neuropodium and another one in each notopodium. Transmission electron micrographs show details of the chaetal ultrastructure, which is a direct reflection of the complex formation process that shapes the final chaetal morphology.

3.2.1 | Pilose chaetae

The smooth core or shaft of a pilose chaeta had the typical chaetal ultrastructure composed of numerous hollow channels of varying sizes (Figure 5a). These channels were much larger in the centre, becoming gradually smaller towards the periphery (Figure 5a). The lamellae between these channels, formed by polymerized chitin, were much thinner in the middle. The chitinous wall of the large central channels was about 35 nm in thickness, whereas the chitinous component of the chaeta was much thicker in the peripheral regions

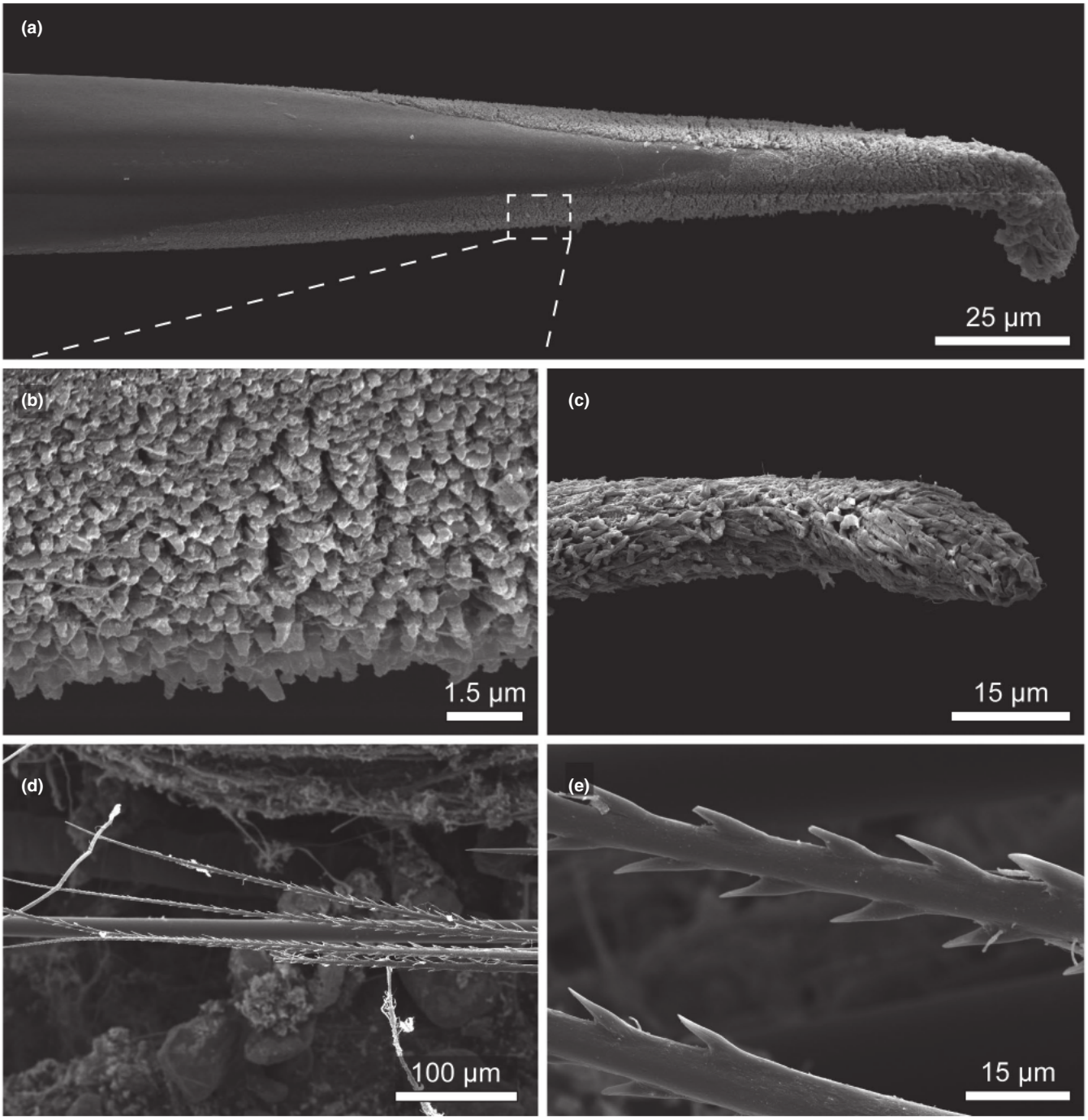


FIGURE 3 SEM images showing the neurochaetae of *Aphrodita aculeata*. (a-c) pilose neurochaeta. (b) shows the marked region in (a) at a higher magnification with shorter hairs. (c) shows the tip of the chaeta with longer hairs. (d and e) show the bipinnate neurochaetae, only present in the anteriormost segments

reaching up to a thickness of 200 nm (Figure 5a,c). The size of the channels was similar to one another near the tip of the chaeta (Figure 6a). However, one could still see a marked difference between the periphery and the core of the chaeta. The chaetal material becomes gradually more electron-dense near the periphery (Figure 5b; Figure 6a) and has a distinct dark enamel layer that surrounds the outer rim of a chaeta (Figure 6a).

The ultrastructure and modification of this enamel layer revealed interesting details relating to the formation of the fine hairs that cover the distal portion of pilose chaetae. In cross section, these hairs appear as stellate electron-dense structures that surround the chaeta (Figure 5a,b). Each of these stellate structures was about 40 nm in diameter and was composed of four to thirteen round, electron-dense lumps surrounding an electron-dense core (Figure 5b). In sections where the

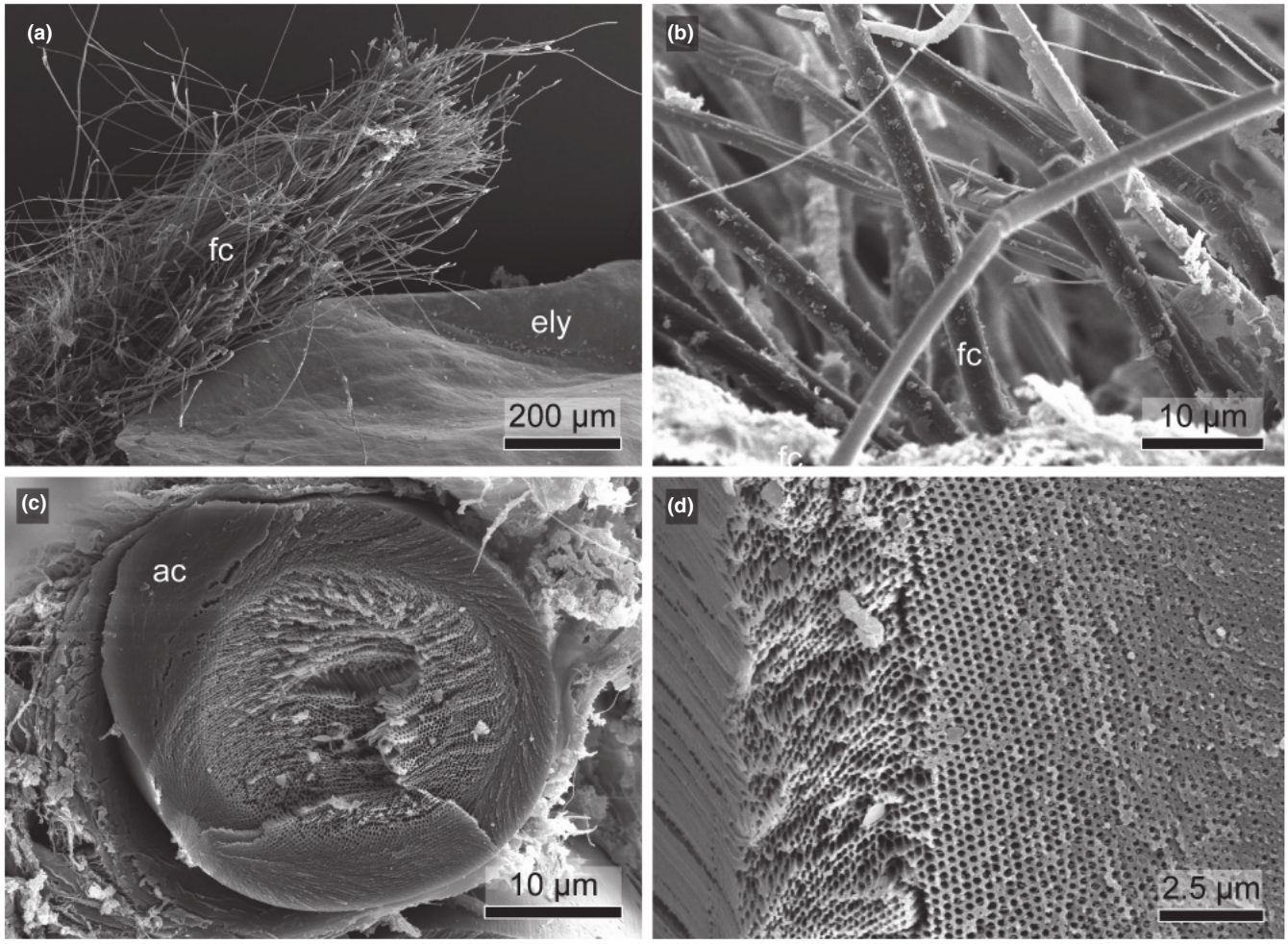


FIGURE 4 SEM images showing the notochaetae of *Aphrodita aculeata*. (a and b) show the densely matted capillary feltage chaetae (*fc*) dorsally covering the elytra (*ely*). (c and d) show a broken acicular notochaetae (*ac*), where the characteristic honeycomb pattern of the chaetal fine structure has become visible. Note that the size of the hollow channels becomes larger towards the inner of the chaeta and that they are more tightly arranged in the periphery

chaetae emerge out of the tissue and the cuticle, it becomes clear that these stellate structures form long continuous fibres (Figure 5c-e). At higher magnification, it became evident that these hairs merge into the enamel coating (Figure 6d).

Sections of the developing chaetal tip showed that the hairs of the pilose chaetae were being formed after completion of the chaetal core (Figure 6a-c). There were no microvilli of the chaetoblast visible inside the chaetae and the chaetal material appeared fully polymerized (Figure 6a). The chaeta was surrounded by the follicle cells, which were interdigitating with each other (Figure 6a,b) and the adluminal surface of these follicle cells was covered with a large number of microvilli radiating towards the chaeta (Figure 6a,c). Apical adherens junctions ("belt desmosomes") and intercellular septate junctions connect the follicle cells with each other; these junctions can be seen between the meandering cell membranes in Figure 6b.

The electron-dense enamel layer that coats the chaeta is secreted by the follicle cells, as this layer only appears later in chaetogenesis and is not visible in early stages, when there is still activity of the chaetoblast. Vesicles carrying electron-dense material could be observed within the follicle cells near the adluminal microvilli fringe (Figure 6b). This material is then most likely released and assembled to form the stellate structure of the fine hairs.

In *A.* cf. *longipalpa* in addition to developing chaetae, chaetal degeneration was also observed within the same chaetal sac. The degenerating chaeta can be easily recognized and distinguished from developing chaetae as the surrounding microvilli of the follicle cells were already filled with intermediate filaments which only appear once chaetogenesis is complete (Figure 6e). These are static components of the cytoskeleton and fix the final product of chaetal formation by attaching a chaeata to its follicle. In addition to this, numerous vesicles and multivesiculated bodies with electron-lucent content appeared within and amongst the degrading chaetal material, which gets dismantled and reabsorbed by the chaetoblast (Figure 6e).

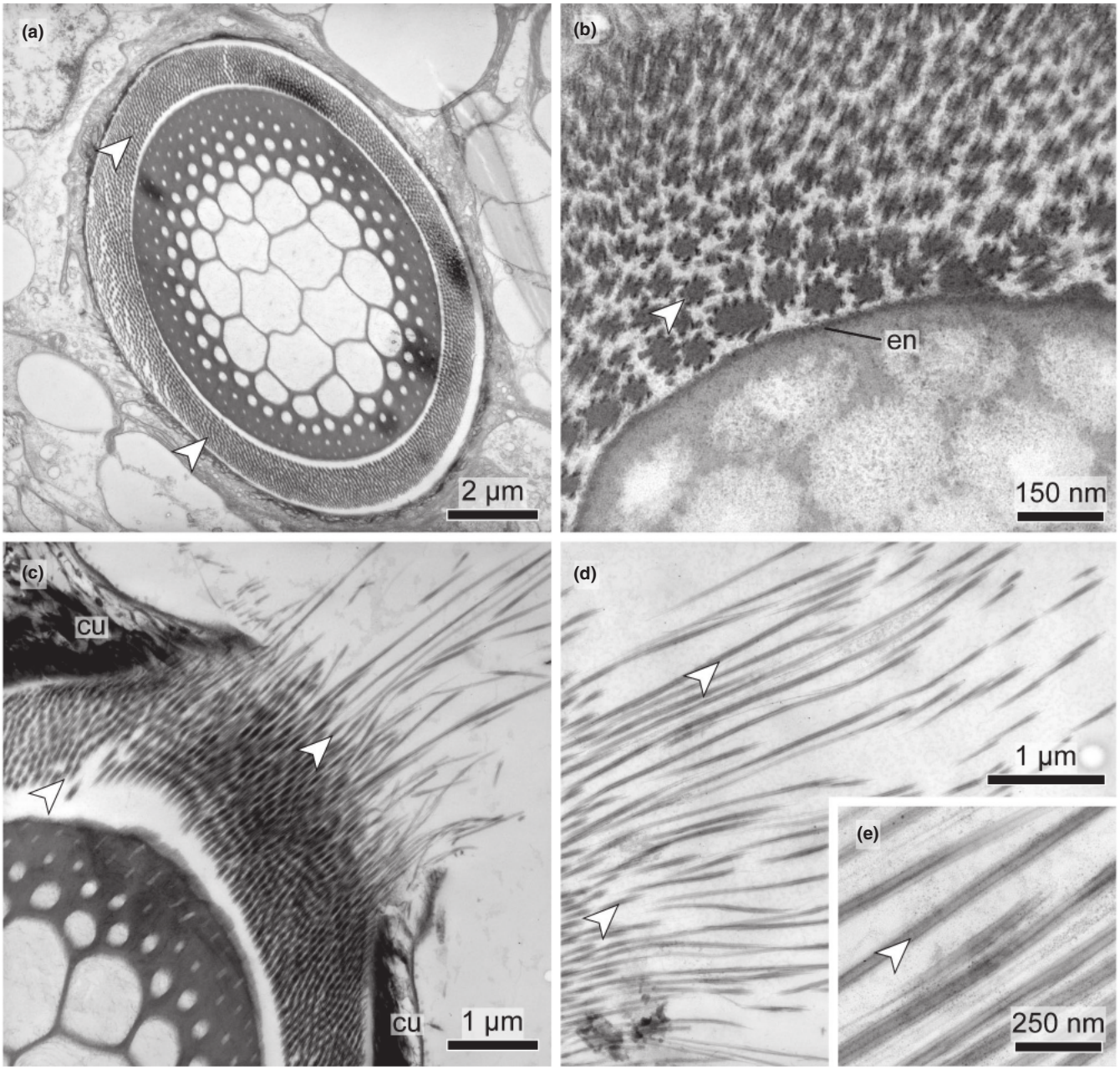


FIGURE 5 TEM images of pilose neurochaetae in *Aphrodita aculeata* (a, c–e) and *Aphrodita* cf. *longipalpa* (b). (a) Cross section of a pilose chaetae showing the empty channels at the core of a chaeta is many times larger than those in the periphery. Surrounding the bristles, hairs can be seen as stellate structures. (b) Detail of a pilose chaetae, showing the hairs in cross section and their stellate form, with a large circular core surrounded by smaller electron-dense lumps. A thin enamel layer (*en*) surrounds the periphery of the chaeta. (c) A fully differentiated pilose chaetae piercing through the cuticle (*cu*). (d and e) show longitudinally sectioned hairs in higher magnification. *Arrow heads*, mark in all images the hairs of the pilose chaetae

3.2.2 | Iridescent feltage chaetae

Chaetogenesis starts with the formation of a microvillar template on the apical surface of the chaetoblast. This can be seen in transmission electron micrographs as regularly arranged cross sections of dynamic microvilli that start forming the typical honeycomb pattern of a chaeta (Figure 7a). In these early stages of chaetogenesis, only the chaetoblast partakes in chaetal formation by forming delicate chitinous lamellae deposited between the microvilli (Figure 7g). The chaetoblast also had numerous apical mitochondria, in close proximity to the microvillar template, indicative of the cell high metabolic activity (Figure 7a). As chaetal development continues, the first two follicle cells reach between the chaeta and the chaetoblast, enveloping the developing chaeta from either side. This can be seen in transverse sections (Figure 7e), where the

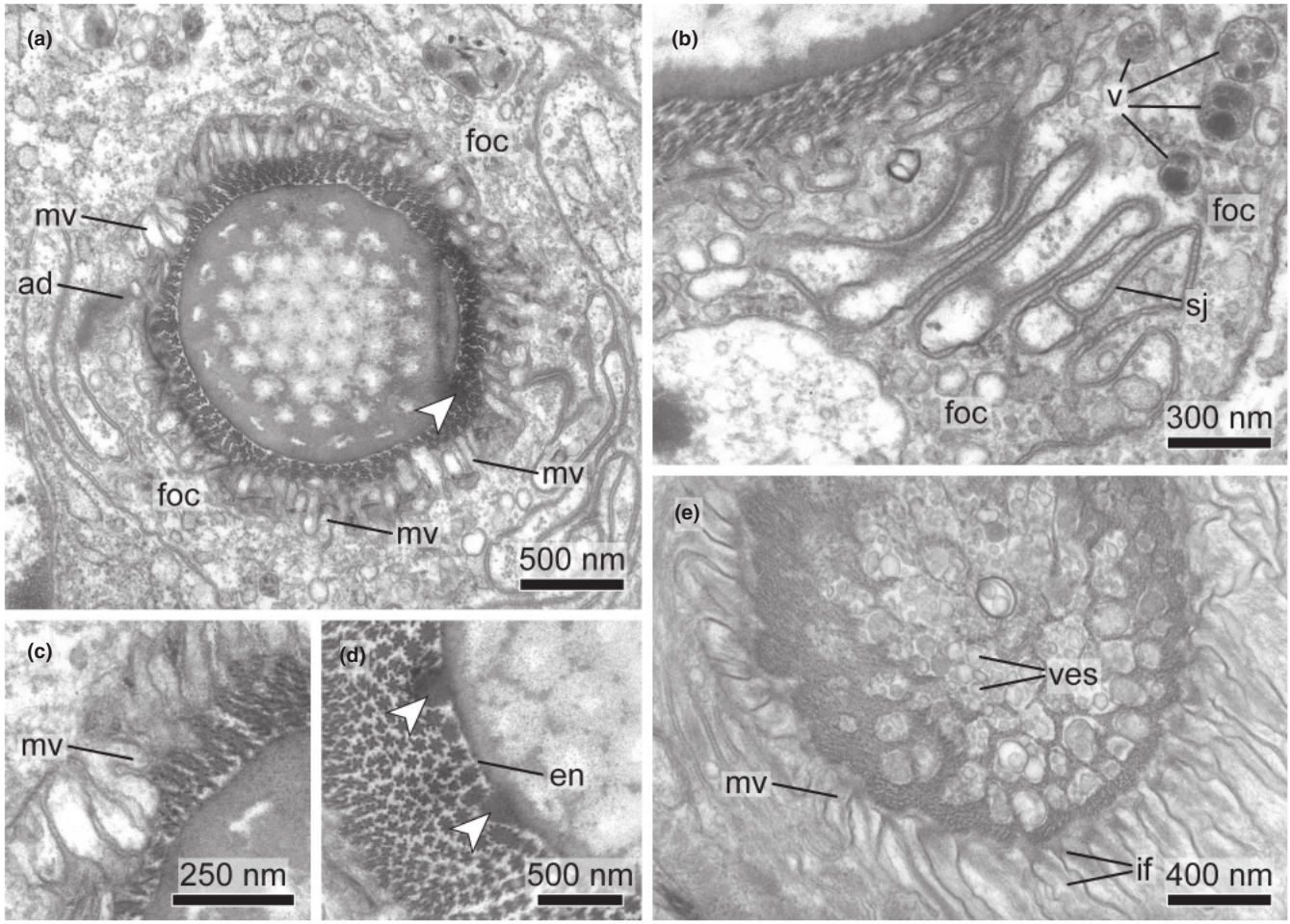
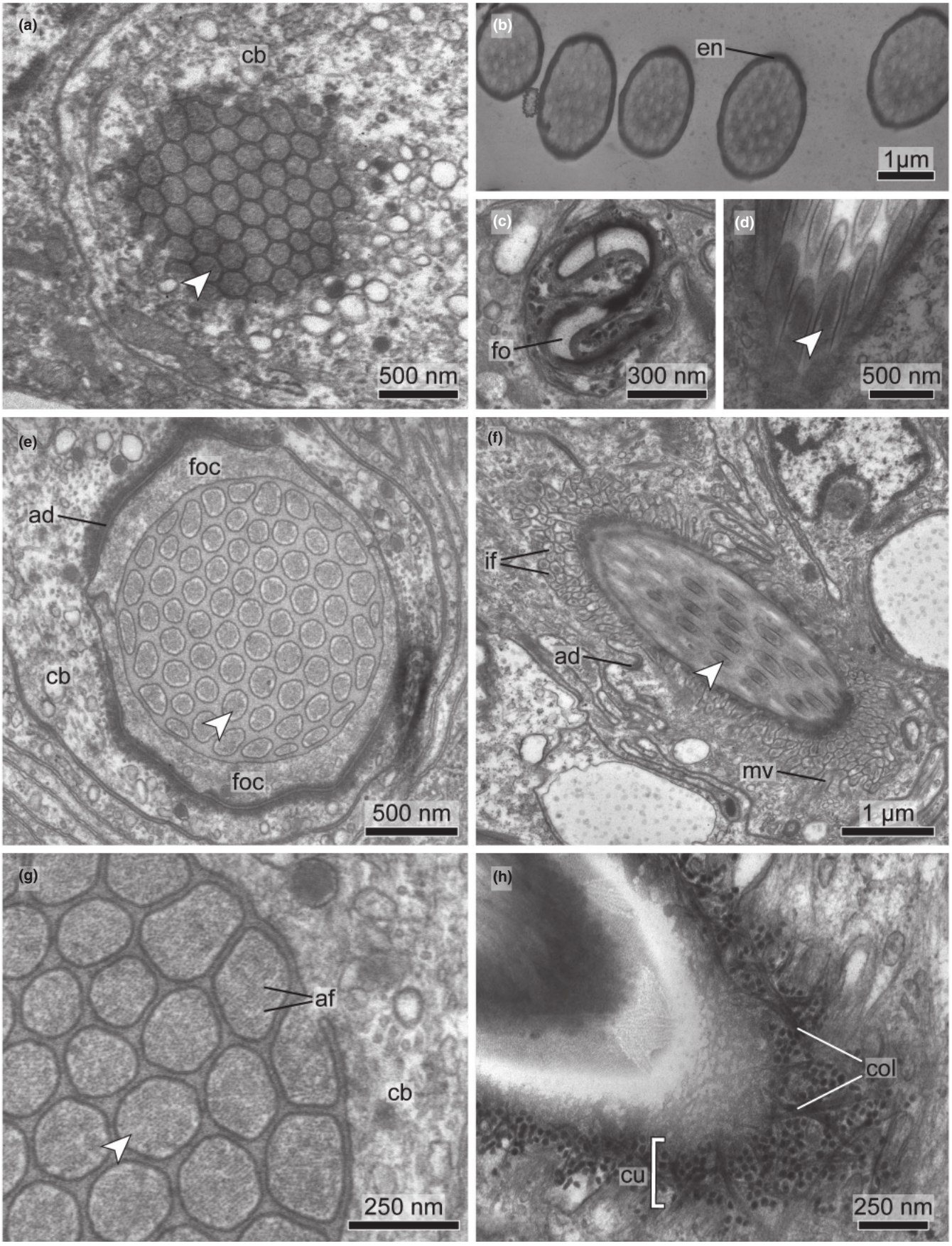


FIGURE 6 TEM images of developing pilose chaetae (a–e) and a degenerating chaeta (e) in *Aphrodita* cf. *longipalpa*. (a) complete cross section of the tip of a pilose notochaeta. The hairs can be seen as stellate structures that surround the chaeta. Two follicle cells (*foc*) envelop the chaeta, their microvilli (*mv*) protrude into the follicular space towards the developing chaeta. The two follicle cells are connected to each other via adluminal adherens junctions (*ad*). (b) Detail of the juncture of follicle cells, the meandering cell membranes are connected to one another via septate junctions (*sj*) that follow the adluminal adherens junctions (*ad*). Vesicles (*v*) within the follicle cells transport electron-dense chaetal material towards the developing hairs of a pilose chaeta. (c) Microvilli (*mv*) of the follicle cells shown in a higher magnification. (d) *Arrow heads* mark the hairs merging to the enamel layer (*en*) that surrounds the chaeta. (e) A degenerating chaeta, note the static microvilli of follicle cells anchoring the chaeta to the surrounding tissue via intermediate filaments (*if*). Numerous vesicles (*ves*) and multivesiculated bodies dismantle the chaeta from within

chaetoblast is still visible as an outer cytoplasmic ring, connected to both follicle cells through apical adherens junctions. With the involvement of the follicle cells, chitin is secreted around the microvillar template, filling up the follicular space and more importantly the spaces between the apically protruding microvilli of the chaetoblast. As chitin polymerizes, the shape of a chaeta becomes more defined and circular in cross section (Figure 7e,f). The capillary feltage chaetae

FIGURE 7 TEM images of capillary notochaetae of *Aphrodita* cf. *longipalpa*. (a) Early developmental stage, visible only as a field of dynamic microvilli (*arrow head*) that apically emerge from the chaetoblast (*cb*). (b) Cross sections of fully differentiated capillary feltage chaetae, sectioned outside of the tissue. Note the thin enamel layer (*en*) that coats each chaeta. (c) The apical pore of a chaetal follicle (*fo*), the developing chaetae still remains in deeper tissue layers and is yet to pierce through. (d) An oblique section of a developing chaetae showing the apical dynamic microvilli (*arrow heads*) of the chaetoblast (*cb*) still inside the chaeta. (e) A developing chaeta surrounded by two follicle cells (*foc*) connected via adluminal adherens junctions (*ad*). The chaeta is still penetrated by the dynamic microvilli (*arrow heads*) of the chaetoblast (*cb*). (f) A late developmental stage, where the dynamic microvilli of the chaetoblast are only visible in the centre of the chaeta and the follicle cells connect to chaeta via static microvilli (*mv*) and intermediate filaments (*if*). (g) Detail of the dynamic microvilli of a chaetoblast (*cb*). Note that in contrast to the static microvilli, these are filled with actin filaments (*af*). (h) Detail of apically sectioned chaetal follicle, showing the cuticular (*cu*) lining of the epidermal follicle cells. Collagen fibres (*col*) of the cuticle are clearly visible



only had a diameter of about 1.5 μm, and the chaetal template consisted of at least 25 equally sized dynamic microvilli (Figure 7b,e,f). As the chaeta grows within its chaetal follicle continuous deposition of chitin and elongation of chaeta causes hollow channels, which is characteristic for the chaetal ultrastructure (Figure 4d, Figure 5a, Figure 7b). In sections of early developmental stages, the dynamic microvilli are either still completely inside the chaeta (Figure 7d,e) or in the process of retracting (Figure 7f). In these stages, distal section of the chaetal follicle is lined by epithelial cells characterized by their apical collagenous cuticle (Figure 7c,h). As long as the developing chaeta does not protrude from the follicle to the exterior, the lumen of the follicle is narrow (Figure 7c) and only a small pore in the cuticle indicates the future position of the chaeta.

The follicle cells are not only actively involved in chaetogenesis by secreting chaetal material and the enamel layer but they also anchor the chaeta inside the follicle. They form static microvilli that retain and stabilize the chaetae during and after its formation and attach these chitinous structures via intermediate filaments to the musculature (Figure 6e; Figure 7f). These intermediate filaments originate in hemidesmosomes in the apical cell membrane of static microvilli, pass the follicle cell and adhere to hemidesmosomes of the basal cell membrane. The hemidesmosomes apically connect the follicle cell to the chaeta and basally to the ECM, to which the muscles cells adhere by dense plaques. The static microvilli of follicle cells differ from the dynamic microvilli of the chaetoblast. Whereas the dynamic microvilli of the chaetoblast contain actin filaments that extend into the cytoplasm (Figure 7g), the static microvilli of the follicle cells possess a core of intermediate filaments that run from the hemidesmosome in the tip of the microvillus down to the basal face of the follicle cell (Figure 6e; Figure 7f). Here, the intermediate filaments adhere to the cytoplasmic face of hemidesmosomes that connect them to the basal lamina.

4 | DISCUSSION

4.1 | Feltage chaetae in Aphroditiformia

Within Aphroditiformia, thin notopodial feltage chaetae, similar to those that make up the dorsal covering of *Aphrodita* species, occur in two other clades, in Acoetidae and in *Sthenelanella* (Sigalionidae). In both of these taxa, the animals utilize feltage chaetae to construct the tubes they dwell in. Also within Aphroditidae, forming feltage is not restricted to *Aphrodita* alone. Although not all species of Aphroditidae form a dense feltage that covers the dorsum, several others including some species of the genera *Laetmonice* Kinberg, 1856, *Heteraphrodita* Pettibone, 1966, and *Pontogenia* Claparède, 1868 also construct a matted dorsal feltage (Barnich et al., 2013; Ebbs, 1966; Pettibone, 1966). The density and thickness of the feltage varies amongst the different species of these genera. *Laetmonice tunicata* Barnich et al., 2013, for example, was even named for its very dense dorsal feltage, while *Laetmonice filicornis* Kinberg, 1856 only forms shorter capillary notochaetae and has a less pronounced feltage (Barnich et al., 2013); Pettibone, 1966).

Recently, Tilic et al., (2021) described the ultrastructure of feltage chaetae in *Sthenelanella* and a more detailed discussion of feltage chaetae of Aphroditiformia is provided therein. In Acoetidae and *Sthenelanella,* the chaetal sacs that give rise to feltage chaetae are sometimes inadequately referred to as spinning glands (Tilic et al., 2021). In *Sthenelanella,* the feltage chaetae develop within a second notopodial chaetal sac, completely independent from the remaining notochaetae. The presence of a second notopodial chaetal sac is unparalleled within Annelida. Taking also its unique ultrastructure into account, Tilic et al., (2021) have argued for a convergent evolution of feltage chaetae within Aphroditiformia. An ultrastructural investigation of chaetal sacs in Acoetidae is missing and still warranted. Pflugfelder's (1934) and Eisig's (1887) description of notopodial chaetal sacs in *Polyodontes maxillosus* (Ranzani, 1817) show that these are simple capillary chaetae, emerging from a single notopodial chaetal sac, together with other notochaetae, that is acicula.

The feltage chaetae in *Aphrodita* are more similar to those of Acoetidae in that they also arise from a single notopodial chaetal sac, are associated with aciculae and have the typical chaetal ultrastructure with hollow channels, remnant from the microvillar template. In most recent phylogenetic analyses of Aphroditiformia by Gonzalez et al., (2018) and Zhang et al., (2018), Aphroditidae (incl. *Aphrodita*) was recovered as the sister group to all remaining Aphroditiformia. Acoetidae and *Sthenelanella* represent two independent lineages within the diversity of the group. Our results on *Aphrodita's* feltage chaetae corroborate Tilic et al., (2021) hypothesis on a convergent evolution of feltage chaetae in *Stenelanella*. The feltage chaetae of Aphroditidae and Acoetidae, however, are homologous as notochaeta, because in both groups they are simple notopodial capillary chaetae, emerging from a single notopodial chaetal sac associated with aciculae. The fact that both form a feltage, however, certainly evolved convergently.

4.2 | The iridescence of notopodial chaetae

The brilliant iridescence of *Aphrodita's* chaetae has attracted scientists for a very long time. Earliest studies date back to the beginning of the 20th century (Schepotieff, 1903). In his spectacularly illustrated work, Schepotieff (1903) clearly shows the internal structure and the enamel layer of chaetae based on his light microscopic observations. Around 30 years

later, Bobin and Mazoué (1944) published their paper on the chaetae of *A. aculeata* and referred to their iridescence as pseudo-birefringence. The fascinating coloration of the sea mouse's bristles, which disappear when the chaetae are dried, are clearly the result of so-called structural coloration. This kind of iridescence in nature is the result of optical effects caused by interference of light from nanoscale periodic structures (Johansen et al., 2017; Kinoshita & Yoshioka, 2005). The chaetae change their apparent colour when the viewing angle or angle of light changes.

Lippert and Gentil (1963) published the first ultrastructural study on the chaetae of *Aphrodita* and considered that the longitudinally arranged chitinous fibrils act as diffraction gratings causing the structural coloration. However, they only investigated acicular chaetae which have a lot less iridescence than the thin notopodial capillary chaetae. In 2001, McPhedran et al., (2001) showed that chaetae of *Aphrodita* act as photonic crystals. The uniform substructure of chaetae with hollow cylindrical channels ensures that wavelengths of incident light are diffracted to varying extents. Depending on the thickness and spacing of the channels, light of some wavelengths interferes constructively, resulting in the expression of a higher amplitude and visibly emitting the light of that wavelength. Other wavelengths, however, interfere destructively in so-called band gaps, resulting in the cancellation of light colour (McPhedran et al., 2001; Parker et al., 2001).

The ultrastructure of annelid chaetae with regularly arranged cylindrical channels, resembling a hexagonal grid, is optimal for exploiting optical effects and controlling the colours of light that they reflect. In fact, it is not just the chaetae of *Aphrodita* but all annelid chaetae show that characteristic pattern, which is a result of chaetogenesis (Hausen, 2005). Yet, not all annelid chaetae are iridescent. Similar iridescent effects were also shown in the long, anteriorly directed chaetae of *Pherusa* Oken, 1807 (Flabelligeridae), making up the so-called cephalic cage (Trzeciak & Vukusic, 2009). All previously published TEM images of *Aphrodita's* chaetae only show the ultrastructure of thick acicular chaetae and not that of the highly iridescent capillary chaetae (Lippert & Gentil, 1963; McPhedran et al., 2001).

There is an entire field of optical biomimetics dedicated to structural coloration in nature (Johansen et al., 2017; McDougal et al., 2019; Parker & Townley, 2007), and the iridescence of the capillary bristles of *Aphrodita* is arguably one of the most prominent examples. 3D photonic crystals have been fabricated out of chitosan, inspired by the chitinous bristles of *Aphrodita* (Huang et al., 2014). However, the biomimetics/biophysics literature have either played down or overlooked the fact that all annelid chaetae, not just the iridescent chaetae of *Aphrodita,* are composed of periodically arranged uniform hollow channels. So, this alone cannot be the reason for the spectacular coloration we can observe in *Aphrodita*. However, the iridescent capillary notochaetae of *Aphrodita* differ from the broad mass of annelids in being amongst the thinnest bristles yet recorded (averaging 1.5 μm in diameter). The iridescent effect of the capillary chaetae is likely due to an interplay of several factors: the thickness and composition of the channels, the thickness of the bristle and enamel layer, and the exact patterning of the hollow channels. Future simulation studies are warranted to test and compare the optical properties and iridescent effects of all these factors. For *Aphrodita* species, however, it is evident that only thin chaetae containing hollow channels of almost identical width show the most pronounced iridescence and those with differing channel diameters do not.

4.3 | The role of follicle cells in chaetogenesis

The ultrastructure and formation of annelid chaetae is well characterized in the literature (Hausen, 2005). A chaeta is a chitinous extracellular structure formed within a chaetal follicle. The basalmost cell of this follicle is called the chaetoblast and the apical microvillar template of the chaetoblast determines the final shape and structure of the developing chaetae. The shape, position, and configuration of microvilli are continuously altered during chaetogenesis, which was first described by O'Clair and Cloney (1974), where they also coined the term dynamic microvilli. N-Acetyl-D-glucosamine is secreted between and around these microvilli, and as it polymerizes to hard chitin, the microvillar template becomes frozen in time. The pivotal role of the chaetoblast in shaping the final product of chaetogenesis is therefore clear.

However, first studies on chaetogenesis overlooked the role of the follicle cells during the formation process and suggested that chaetae are secreted by the chaetoblast alone (Bobin, 1944; Fedotov, 1914; Lubischew, 1924; Schepotieff, 1904). Ebling (1945) suggested that the lateral cells (=follicle cells) add an outer coat (=enamel layer) to the chaetae that are otherwise secreted by the chaetoblast. Other authors argued that the chaetoblast secretes the medulla of each bipartite chaeta, while the lateral follicle cells secrete the cortex (Bouligand, 1966, 1967; George & Southward, 1973; Lippert & Gentil, 1963). O'Clair and Cloney (1974) noted "the abundance of highly developed bodies in the lateral follicular cells in their micrographs, and their concomitant paucity in the chaetoblast," suggesting that most of the chaetal material derives from the follicle cells and that the principal function of the chaetoblast is to direct the morphogenesis of the chaeta. Our own investigations of chaetal formation repeatedly corroborated this observation (Bartolomaeus, 1998; Hausen & Bartolomaeus, 1998; Tilic et al., 2015). Gazave et al., (2017) were also able to demonstrate this using a molecular approach and looking at the genes expressed by the follicle cells.

In fact, it is not only the secretion of chaetal material but also the topology and dynamics of the follicle cells that influence the final shape and structure of the chaetae. This was particularly evident in the formation of abdominal uncini in *Sabellaria alveolata* (Linnaeus, 1767), described in Tilic and Bartolomaeus (2016). There, the follicle cells cover parts of the chaetoblast, directly interfering the microvillar template of the chaetoblast and resulting in the elongation of the chaetal rods.

Furthermore, follicle cells function in mechanically attaching the final product of chaetogenesis to the epidermal tissue. Once chaetogenesis is finalized, actin filaments inside the microvilli get replaced with intermediate filaments that connect to the follicle via hemidesmosomes (Bartolomaeus, 1995). In this paper, we also illustrate clearly the difference between the dynamic microvilli of the chaetoblast, containing actin filaments (see Figure 7g), and the static microvilli of the follicle cells (see Figure 6e; Figure 7f).

Lastly, our results on the chaetogenesis of pilose chaetae in *Aphrodita* revealed a thus far undescribed contribution of follicle cells to chaetal structures. The hairs that distally cover each pilose chaeta are not formed by the chaetoblast but appear to be a modification of the enamel layer secreted solely by the follicle cells. These ornamentations that appear as remarkable stellate structures in cross section appeared only after the chaetal core was formed and the chaetoblast was retracted from within the chaetae. No dynamic microvilli were observed in the assembly of these structures; however, the follicle cells had a high number of static microvilli making contact with the hairy portion of the chaetae. This suggests that the static microvilli in some way influenced the formation of the enamel layer and fine hairs, either to increase the secretory surface area of the cell membrane or maybe even physically shaping the hairs. Similarly, the follicle cells of the feltage chaetae in *Sthenelanella* species also secrete an electron-dense enamel layer, and thereby add an outer ornamentation in the form of small barbs (Tilic et al., 2021). This however is neither as complex nor prominent as what was observed in the pilose chaetae of *Aphrodita*.

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