INTRODUCTION

Sthenelanella is a small, unusual clade within Sigalionidae with only six nominal species. The most characteristic feature of these tube-dwelling scale worms is silky threads that emerge from the mid-body and posterior notopodia. These fine fibers are used by the animals to build the felt-like material of their tube. In his original description of the genus, Moore (1910) does not mention these silky threads. However, they were noted in subsequent papers (Berkeley & Berkeley, 1941; Hartman, 1939, 1961) and also in Pettibone's (1969) redescription of Sthenelanella uniformis Moore 1910. In that paper, she also establishes these structures as a diagnostic character for the genus.

Within Aphroditiformia, a felt-like material also occurs in Aphroditidae and Acoetidae. In Aphroditida species, a chaetal felt covers the dorsal side of the animal, and in Acoetidae, notopodial threads similar to those of Sthenelanella contribute to tube formation. The terminology used for these fibrous structures dates back to Eisig's (1887) study in which he described the iridescent green-gold "spinning fibers" of the acoetid Polyodontes maxillosus (Ranzani 1817) that emerge from parapodial "spinning glands." Pflugfelder (1934) later showed that the products of these "spinning glands" in Acoetidae are in fact annelid chaetae. Three or four dynamic microvilli of a basal chaetoblast form each of these feltage chaetae, making them the thinnest known annelid bristles. Our results show that the spinning glands of Sthenelanella uniformis are additional, highly modified notopodial chaetal sacs. We also show that the follicle cells, by their secretion of the enamel layer, play an active role in shaping the final chaeta. These findings not only increase the known morphological diversity of chaetae but also demonstrate the apparent plasticity of the machinery that form these chitinous structures. Our results are compared with chaetae in other annelids, with a particular focus on similar fibrous chaetae in Aphroditiformia.

KEYWORDS
Aphroditiformia, chaetogenesis, feltage chaetae, tube formation, ultrastructure
glands" in Acoetidae suggest that they also might be homologous to chaetae. In order to test this hypothesis, we studied the histology and ultrastructure of the "spinning glands" in Sthenelanella and their secretory products.

Chaetae are, without a doubt, one of the most significant and well-studied features of annelids. Previous papers have characterized the process of chaetogenesis and the ultrastructure of chaetae and chaetal sacs in many annelid species in great detail (Bouligand, 1967; Hausen, 2005a; O’Clair & Cloney, 1974). These chitinous extracellular structures appear in a plethora of strikingly diverse shapes and forms: the thick hooks of Thalassematidae (=Echiura) (Tilic et al., 2015); the calcareous stinging chaetae of Amphinomidae (Tilic et al., 2017); the hooks of Sabellaridae with their incredibly long "internalized shafts" (Tilic & Bartolomaeus, 2016); and the golden, petal-like paleae of Chrysopetalidae (Tilic et al., 2019). Regardless of the final product, the overall structure and process of chaetal formation are always the same. A chaeta develops inside a chaetal follicle, which is an epidermal invagination. The basal-most cell of this follicle, the chaetoblast, has dynamic apical microvilli. Through a controlled and intricate process that involves continuous modification of the microvillar template and secretion of chitin, a chaeta is formed. This mechanism is comparable to a tiny biological 3D printer (Warren, 2015). The chaetoblasts and the remaining follicle cells are epidermal cells that all lie above a shared basal lamina. Each chaeta has its chaetal follicle, and many chaetal follicles form a chaetal sac. In most annelids, there is a single chaetal sac in each notopodium and another one in each neuropodium (Bouligand, 1967; Hausen, 2005a).

In this article, we describe the ultrastructure of the notopodial "spinning glands" in S. uniformis. Our results confirm the hypothesis that these structures are in fact highly modified chaetal sacs. We further compare the feltage chaetae of Sthenelanella and their unique chaetogenesis with the chaetae of other annelids and argue that the term "spinning glands" should be avoided when referring to these structures.

2 | METHODS

2.1 | Material examined

A specimen of S. uniformis was found in its tube buried in an empty gastropod shell collected by SCUBA in June 2018, near the vicinity of Scripps Institution of Oceanography (SIO), La Jolla, in Southern California. The animal was dissected out of its tube, relaxed with MgCl2, and photographed using a Leica DMR HC compound microscope and a Canon EOS Rebel T6s camera. The posterior end of the animal was preserved in 100% ethanol for DNA sequencing. Cytochrome oxidase I (COI) and 18S rRNA barcode sequences are deposited in GenBank (MW173680 and MW173358, respectively). See Tilic et al., (2019) for DNA extraction and PCR protocols. The anterior end was fixed in seawater formalin and is vouchered in the Benthic Invertebrate Collection of SIO (SIO-BIC A12941). Mid-body segments were fixed for transmission electron microscopy (TEM; see below). A second formalin-fixed specimen of S. uniformis from the Benthic Invertebrate Collection (SIO-BIC A174) and its tube were also photographed, and parts of the animal were prepared for scanning electron microscopy (SEM).

2.2 | Scanning electron microscopy

Mid-body segments were dissected and dehydrated through an ascending, graded series of ethanol and hexamethyldisilazane (HMDS). Specimens were allowed to air dry overnight under the fume hood before being mounted on aluminum stubs, sputter-coated with gold-palladium (Au-Pd), and examined under a Zeiss EVO 10 scanning electron microscope at the Scripps Institution of Oceanography, UCSD.

2.3 | Transmission electron microscopy

Mid-body segments were directly dissected into 2.5% glutaraldehyde buffered in 0.05 M phosphate 0.3 M NaCl saline (PBS) for ~12 h at 4°C. Ruthenium red was added to the fixative to improve contrast (Minuth & Denk, 2015). The specimens were rinsed several times in buffer and postfixed with 1% OsO4 for 30 min at 4°C. The material was subsequently dehydrated in an ascending acetone series and embedded in Spurr’s resin.

Semi-thin transverse sections (1 µm in thickness) of complete segments, as well as sagittal sections of multiple segments (Segments XIII–XVI), were prepared using a Diatome Histo 45° diamond knife. Sections were stained with toluidine blue (1% toluidine, 1% sodium tetraborate, and 20% sucrose), mounted in Araldite, and cover-slipped. The sections were then imaged using an Olympus BX-51 microscope equipped with an Olympus CC12 camera, at the Institute of Evolutionary Biology, University of Bonn.

Series of silver interference-colored, ultrathin (70–75 nm) sections of chaetal sacs were prepared with a Diatome Ultra 45° diamond knife. These were placed on Formvar-covered, single-slot copper grids and stained with 2% uranyl acetate (E22400, Science Services) and 2.6% lead citrate (E17810, Science Services) using an automated TEM stainer (QG-3100, Boeckeler Instruments). The sections were examined and imaged using a ZEISS EM 10CS microscope equipped with digital imaging plates (Ditabis), at the Institute of Evolutionary Biology, University of Bonn.

3 | RESULTS

3.1 | Parapodial structure

The studied specimens of S. uniformis were found living in a robust tube consisting of sand and sediment particles woven into a felt-like material (Figure 1A,B,E). The animal was about 2 cm long and 2 mm wide. An eversible pharynx with jaws was present (Figure 1A,B). Dorsally, the body was covered with elytra (Figures 2A,B and 3A). Parapodia were biramous. Neurochaetae were all compound, with
both short and long blades. Notochaetae were coarsely spinous capillaries arranged in a loose bundle (Figure 2B,C). The number of notochaetae was much higher than the neurochaetae, with more than 50 spinous chaetae in each bundle. Starting from Segment XIV, an additional bundle of long (~2 mm), silky threads emerged from the notopodia. These bundles were located dorsally and extended well beyond the longest notochaetae. SEM images of the tube show a densely woven meshwork of the same thin fibrous threads (Figure 2D). The sectioned specimen was a male with spermatozoa in the coelomic cavity (Figure 3A).

In both parapodial rami, the aciculae were the chaetae that reached deepest into the animal's body (Figure 3B). The neuroaciculae were much greater in diameter (average 37.5 µm) than the notoaciculae (12.5 µm). The aciculae had the characteristic chaetal ultrastructure with numerous longitudinal hollow channels, a remnant from the apical microvilli of the chaetoblast. These empty channels were smaller, and denser in the periphery, resulting in a lighter toluidine staining in the center of each acicula (Figure 3D). The neurochaetae and notochaetae both were arranged in a semicircle around the central acicula (Figure 3A,D). Aciculae were located anterior to the other chaetae (Figure 3B). Both neurochaetae and notochaetae showed a homogenous toluidine staining, indicating that microvilli of equal size formed the template of the shaft (Figure 3). The diameter of the chaetal shafts was ~7 µm in the neuropodia and even smaller (~400 nm) in the notopodia. Parapodial musculature attached to aciculae and was more prominent in the neuropodia. Parapodial and chaetal muscles shaped the outline and structure of the neuropodial chaetal sac (Figure 3A). Developing neurochaetae

![Image](https://via.placeholder.com/150)

**FIGURE 1** Habitus of *Sthenelanella uniformis*. A,B. Formalin-fixed voucher (SIO-BIC A173), inside its tube. The proboscis is everted. C,D. Live photographs of the sectioned individual (SIO-BIC A12941). Arrowheads mark the long threads of feltage chaetae emerging from the notopodia. E. Photograph showing the felt-like material of the tube. dors, dorsal; ne, neurochaetae; no, notochaetae; post, posterior; pr, proboscis; tu, tube
and active chaetogenesis can be seen in a ventrally located pouch of the neuropodial chaetal sac in Figure 3A. Regardless of the difference in size, shape, and number of chaetae in neuropodia and notopodia, all chaetae of a parapodial ramus originated from a single chaetal sac (Figure 3A).

The silky threads that can be seen externally starting at Segment XIV emerged from an epidermal invagination located dorsal to the notopodial chaetal sac (Figure 3A). This invagination has been called “spinning gland” by many authors (Aungtonya & Eibye-Jacobsen, 2013; Gonzalez et al., 2018; Jimi et al., 2019; Palmero et al., 2008). Histological and ultrastructural details described in detail below showed that this invagination is a highly modified chaetal sac. Therefore, we use the appropriate chaetal terminology when describing the second notopodial chaetal sac (previously referred to as "spinning gland").

3.2 | Histology of the second notopodial chaetal sac

In Figure 3B, a series of sagittal sections through Segments XIII–XVI can be seen. As mentioned above, bundles of silky threads, or feltage chaetae, only emerged from the notopodia starting at Segment XIV. Interestingly, an epidermal invagination and an empty sac were present in Segment XIII. This additional chaetal sac remained empty even in deeper tissue layers, indicating that it is either rudimentary or nonfunctional (Figure 3B). In contrast to the neuro- and notopodial chaetal sacs, this additional or second notopodial chaetal sac lacked an acicula (Figure 3B).

The second notopodial chaetal sac, which gave rise to the feltage chaetae, was pear-shaped, with a narrow canal opening dorsally, right above the notochaetae of the first notopodial...
chaetal sac. The proximal part of this structure was wider than the distal part and reached deep inside the body, all the way down to the intestine (Figure 3A). It penetrated ~500 μm into the animal's body and had a cross section of about 140 μm at its widest part (Figure 3A). The structure was neither curved nor branched. Histologically, two very distinct layers could be seen in the bulbous part of the sac (Figure 3C,D). The first layer marked the outline of the second notopodial chaetal sac and was characterized
Figure 4. TEM images showing the apical portion of the modified, second notopodial chaetal sac. These correspond to cell layer 2 (cl2) in Figure 3. A. Bundle of feltage chaetae inside the large epidermal canal lined with cuticle. B, C. Details of fully developed feltage chaetae in transverse and longitudinal sections. D–J. Details of the third follicle cell. Electron-dense chaetal material is synthesized by the large number of Golgi stacks. G, H. Formation of the enamel barb. Arrowheads mark the apical adherens junctions, where both ends of a single third follicle cell surrounding a chaeta meet. Asterisks (*) mark the electron-dense bars of the enamel layer. fc3, third follicle cell; cu, cuticle; dors, dorsal; f, feltage chaetae; gs, Golgi stack; n, nucleus; vent, ventral.
by large, loosely arranged nuclei. Toluidine blue stained some of them relatively dark, accentuating the outer border of the sac (Figure 3D). There was a large gap of ~10 μm between the first and second distinct layer of nuclei. This gap consisted of very lightly stained cell membranes and cytoplasm (Figure 3D). Furthermore, thin feltage chaetae could be seen in between the cells of the sac, indicating that this is the site of their formation. The second layer of cells was more compact than the first, with more darkly stained, smaller nuclei (Figure 3D).

### 3.3 Ultrastructure of the second notopodial chaetal sac

The narrow epidermal canal that surrounded the bundle containing hundreds of feltage chaetae emerging from the pear-shaped base was lined with a cuticle and was nothing else but an invagination of the body wall (Figures 3C and 4A). As such, it was underlain by the basal lamina or basal extracellular matrix (ecm). Several interdigitating flat cells formed the epidermal canal, each of them resting on the ecm and apically interconnected by adherens junctions. The cuticle these cells secreted became thinner down its course to the origin of the feltage chaetae. Chaetae within the bundle differed in arrangement and were more dense in ventral parts of the bundle than in dorsal or central parts (Figure 4A). The fully differentiated feltage chaetae did not show the typical ultrastructure of annelid chaetae. There were no hollow channels, as one would expect to see in a transverse section of a chaeta (Figure 4B,C). The diameter of a single feltage chaeta was very small, 400 nm. Each chaeta had an electron-dense core and an electron-dense outer layer, giving it a bullseye appearance in cross section (Figure 4B). The sections also revealed that the outline of feltage chaetae was not smooth, but ornamented with small barbs (Figure 4B,C). These barbs were always electron dense and at their widest point almost as wide as the diameter of the central chaetal axis.

When following the course of a single chaeta, it became clear that each chaeta emerged from a chaetal follicle that consisted of three follicle cells and a basal chaetoblast. The chaetoblasts rested on the basal ecm underlying the entire second chaetal sac (Figure 5A–F). Apically, the chaetoblast had three or four short microvilli that extended into the chaeta. Adherens junctions connected the chaetoblast to two follicle cells that wrapped the chaeta base (Figures 5D–F and 6) and connected the apical margins of the follicle cells where they faced the chaeta. A third follicle cell completely surrounded the following section of the chaeta. This cell was long and slender, with electron-lucent cytoplasm and an apically located nucleus. It contained several Golgi stacks that released vesicles with electron-dense content at their cis face (Figure 4D–F). Its lateral margins wrapped the chaeta and were connected by apical adherens junctions followed by septate junctions. These adluminal adherens junctions linked an inner subapical net of actin filaments (Figures 4D,E and 6). Whereas the nuclei of the third follicle cells of all feltage chaetae formed a single layer in the semi-thin sections, those of the first and second follicle cells and those of the chaetoblasts were next to each other and formed a thick basal layer of loosely arranged nuclei (Figure 3C,D).

The follicle cells of the feltage chaetae were epithelial in having apical adherens junctions that interconnected their apices. In contrast to epithelial cells such as those forming the epidermal canal, however, the follicle cells were not resting on the basal ecm. Accordingly, the follicle cells of the second notopodial chaetal sac did not contain any intermediate filaments. These crossed the follicle cells and connected chaetae to the ecm in the other notopodial chaetal sac and in the neuropodial chaetal sacs. Hundreds of chaetae were formed continuously and next to each other; only the chaetoblasts and the outer layer of follicle cells had direct contact with the ecm. The majority of the chaetal follicles were directly adjacent to each other (Figures 4D,I,J and 5G), but junctions were not found connecting the cells of two neighboring follicles.

### 3.4 Chaetogenesis of the feltage chaetae

Sections of feltage chaetae from outside or inside the body did not show the typical honeycomb pattern of other noto- and neuropodial chaetae (Figure 4). However, when feltage chaetae were sectioned at their base, it became evident that they were formed by the same process as other notopodial and neuropodial chaetae (Figure 5A–C). The site of formation, however, was not restricted to a specific portion of the second notopodial chaetal sac as it was in the other chaetal sacs.

The basal-most cells located in the second notopodial chaetal sac were all chaetoblasts, fixed in their position while forming the chaeta. Chaetogenesis started after a cluster of three (rarely four) microvilli appeared on the apical surface of a chaetoblast (Figure 5A–C). These microvilli were all relatively small and round, each measuring ~150 nm in diameter. They extended into a narrow extracellular compartment formed and surrounded by the follicle cells (Figures 5D–F and 6). This cluster of microvilli was clover shaped in cross section. As chaetal material that was released between the bases of the microvilli polymerized, this cluster became circular in cross section and formed the template of the chaeta (Figure 5F). The chaeta grew longer as chitin was added to its base. During this process, the microvilli kept their original length, which caused the hollow channels visible in Figure 5G and H. During the further course of development, these channels were filled with electron-dense material. The chaetoblasts always contained several apically located mitochondria, indicative of high metabolic activity (Figure 5A,B).

During elongation, the chaeta grew into the compartment formed by the follicle cells. The main axis of the chaeta was then completed once chitin appeared to be fully polymerized and no hollow channels within the chaetae were observed (Figure 4F–I). The electron-dense material stored in cis Golgi vesicles of the follicle cells, each with a diameter ~100 nm (Figure 4F,I), released their content into the extracellular space surrounding the chaeta (Figure 4G,H). There, the chaeta became coated by this material, forming the electron-dense outer sheath or the enamel layer (Figures 4H and 6). The third follicle cells,
however, not only synthesized the enamel layer but also appeared to be making the outer barbs of the chaeta. These barbs formed a continuous and homogeneous layer with the enamel layer (Figure 4B,C,G,I). The vast number of chaetae that were formed, and the active involvement of the third follicle cells in the process of chaetogenesis, may explain the distal position of the third cell nuclei. TEM images of this region only showed densely packed nuclei and fully differentiated chaetae that were tightly squeezed within the chaetal follicle (Figure 4J). Here, the chaetae had a distinct electron-dense enamel layer (Figure 4I), which was still being formed in proximal sections.

## DISCUSSION

### 4.1 “Spinning glands” versus chaetal sacs

As already pointed out in the Introduction, feltage chaetae in Acoetidae had initially been regarded by Eisig (1887) as thread-like secretions of a spinning gland. Pflugfelder’s (1934) histological study showed that the fine threads formed by the acoetid *P. maxillosus* are annelid chaetae. This led Pettibone (1989) to point out that the term “spinning gland” is likely to be an inadequate designation for these chaetae.
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Chaetae also consist of β-chitin released by the basal-most cell of a multicellular chaetal follicle (Hausen, 2005a). A chaetal follicle is an epidermal invagination and several of these chaetal follicles are grouped within a chaetal sac. All follicle cells, including the basal chaetoblast, are epidermal cells that rest on top of a shared basal lamina (ecm). During the formation of chaetae (chaetogenesis), chitin polymerizes between the microvilli of the chaetoblast, and the structure and arrangement of the microvilli change simultaneously, thereby influencing the pattern of chitin polymerization (O’Clair & Cloney, 1974). These dynamic microvilli do not release material from their tips; they exclusively contain actin filaments responsible for their dynamics. The microvilli, thus, provide a template for chitin deposition and leave hollow canals inside the chaeta during chaetogenesis. This important functional difference of the microvillar function allows distinguishing between glands and chaetal follicles. The formation process of feltage chaetae described in this study is largely in accordance with this general pattern of chaetogenesis.

Although the large extracellular cavity, from which the fine threads originate, superficially resembles a multicellular gland, ultrastructure and formation process of these threads show that they are very thin feltage chaetae. The terms chaetal follicles and sacs are much more specific and complex than “spinning gland.” By definition, it encompasses the secretion of N-acetylglucosamine, its controlled deposition, subsequent polymerization into hard chitin, and final attachment to the cell surface. Therefore, our findings provide striking evidence that what was previously termed a “spinning gland” in Sthenelanella is clearly a modified chaetal sac.

FIGURE 6 Schematic drawing of the chaetal follicle of a single feltage chaeta. Note the large number of Golgi stacks in fc3, forming the barbed outer layer of the chaeta. fc1 and fc2 envelop the base of the chaeta from either site. The chaetoblast (star) forms the chaetal template with three or four dynamic apical microvilli (mv), aj, apical adherens junction; star, chaetoblast; ecm, extracellular matrix; fc1, first follicle cell; fc2, second follicle cell; fc3, third follicle cell; gs, Golgi stack; mv, microvilli; n, nucleus

structures. Epidermal glands have a distinct ultrastructure. These secretory cells produce and store secretions, if unicellular, or store secretions in a commonly formed extracellular space, if multicellular (Hausen, 2005b; Rößger et al., 2015).

Certain parapodial glands are known to secrete β-chitin. They are involved in tube formation in several other annelids including some Spionidae (Guggolz et al., 2015; Rößger et al., 2015), Oweniidae (Guggolz et al., 2015), and tube-dwelling Siboglinidae (Gaill et al., 1992; Gaill et al., 1992; Shillito et al., 1993, 1995). However, in all of the above-mentioned cases there are cup-shaped microvilli that release tiny fibers as microfibrils (Meißner et al., 2012). Guggolz et al. (2015) assume that these fibers consist of β-chitin fibrils; they are stored with other albuminous material in the gland’s reservoir.

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4.2 Comparison with other annelid chaetae

The feltage chaetae of S. uniformis is the newest addition to the high structural diversity of annelid chaetae. Besides simple capillary chaetae, a vast array of chaetal types occur in Annelida (Tilic et al., 2016). They are mostly used by the animals for defense or locomotion, but also for foraging, drilling into hard substrates, or anchoring to the tube (Merz & Woodin, 2006; Pernet, 2000; Tilic et al., 2016, 2017). Now, the use of chaetae as construction material for tubes can be added to this list.

The thinnest chaetal elements described thus far are the long adrostral rods of abdominal uncini in Sabellaria alveolata (Linneaus 1767) in the Sabellariidae (Tilic & Bartolomaeus, 2016). The feltage chaetae of S. uniformis, however, are even thinner (400 nm in S. uniformis vs. 1.5 µm in S. alveolata), and also are formed by fewer template microvilli during their chaetogenesis (3 or 4 in S. uniformis vs >20 in S. alveolata).

Compared to other annelids, the most significant difference, however, is the presence of a second modified notopodial chaetal sac. Annelid parapodia normally possess two chaetal sacs, one in each parapodial lobe (Hausen, 2005). Each chaetal sac consists of several follicles, and each follicle consists of a basal chaetoblast and at least three follicle cells that serve in connecting the chaeta and the muscular system. The chaetal sac is surrounded by a basal ecm that is continuous with the basal lamina of the epidermis. All cells of the chaetoblast adhere to this basal matrix. In the second notopodial chaetal sacs of S. uniformis, only the chaetoblasts are connected to the basal matrix. At least the basal part of the chaetal sac thus resembles a multilayered epithelium rather than a collection of monolayered follicles, as in the other notopodial sac and in the neuropodial sac.

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We propose that the peculiar ultrastructure of the second notopodial chaetal sac results from functional constraints on the feltage chaetae. Since a vast number of these chaetae need to be produced, it seems reasonable to assume that a significant multiplication of chaetal follicles occurred in order to produce a bundle of fibers consisting of hundreds of single feltage chaetae. A specific site of chaetal formation such as found in most annelids studied thus far (Hausen, 2005) was not seen. In annelids, chaetogenesis ends with replacing the actin filaments inside the microvilli with intermediate filaments that serve in attaching the chaeta to the follicle (Bartolomeus, 1995). None of the feltage chaetae showed such a condition, and instead all chaetae were fixed while still undergoing chaetogenesis. This, and the fact that there were no restricted formation sites, leads us to conclude that chaetogenesis occurs continuously and permanently within the second notopodial chaetal sac of *S. uniformis*. This may be a prerequisite for the extraordinary length of the feltage chaetae, which protrude far beyond other notopodial chaetae. Taking the function of feltage chaetae in tube formation into consideration, continuous growth of chaetae is not unexpected. The construction and maintenance of the tube most likely requires a large and continuous supply of threads. The electron-dense barbs of the feltage chaetae possibly play a role in constructing the felt, as the fibers are more likely to be tangled up together. Furthermore, the feltage chaetae are not anchored to the follicles with intermediate filaments, as with the chaetae in most annelids. They are loosely held inside the chaetal follicle, which makes it much easier for them to get plucked out and incorporated into the tube. The exact process of tube formation by *Sthenelanella* is not known. Therefore, experiments in which live animals are observed building new tubes would be highly interesting.

The series of sagittal sections shown in Figure 3B revealed that a rudimentary or nonfunctional chaetal sac is present in Segment XIII. The lack of feltage chaetae in the anterior segments is in accordance with previous observations. The specimens we studied had feltage chaetae starting from Segment XIV. In Pettibone’s (1969) specimen they also start at Segment XIV, in Hartman’s (1939) at Segment XVI, and in Berkeley and Berkeley’s (1941) at Segment XV. This slight variation, and the presence of a vestigial chaetal sac in Segment XIII of our specimen, suggests that there might be some ontological differences, a hypothesis that might be tested by observing the development of *S. uniformis*.

4.3 | Feltage chaetae within Aphroditiformia

The position and structure of the chaetal sac that gives rise to the feltage chaetae in species of *Sthenelanella* allow us to hypothesize that it evolved by duplication and modification of a notopodial chaetal sac. Within Aphroditiformia, similar fibrous chaetae that serve as construction material for feltage occur in Acoetidae and Aphroditidae. In the following paragraphs, we compare our findings with the available data on the feltage chaetae of these two clades. Claparède (1870) described the feltage chaetae of Acoetidae, formerly Polyodontidae, as golden threads emerging from body segments. Eisig (1887) followed up on this observation, describing dense clusters of thin threads, especially in the pharyngeal region, decreasing in length toward the middle of the body. Eisig (1887) also established the idea that notopodial spinning glands, located within an ectodermal invagination, secrete these bundled fibers. In contrast to the condition we observed in *S. uniformis*, there are no notopodial chaetae other than the feltage chaetae in the acoetid *P. maxillosus* (Eisig, 1887; Pflugfelder, 1934). The bundle of notopodial feltage chaetae in *P. maxillosus* is also directly associated with a small notopodial acicula (Eisig, 1887; Pflugfelder, 1934). Pflugfelder’s (1934) study provides a histological description of the notopodial chaetal sac in *P. maxillosus*. Although he still refers to these paired notopodial structures as “spinning glands,” he also points out that the formation process giving rise to the fibers is typical for chaetae. In his figures, Pflugfelder (1934) illustrates numerous empty channels within the feltage chaetae of *P. maxillosus*. The feltage chaetae in *S. uniformis* differ with respect to formation site, lack of an associated acicula and chaetal structure. In contrast to the situation in Acoetidae, there are no visible hollow channels within fully differentiated chaetae, and the microvillar template that forms the chaetae is much smaller in *S. uniformis*. Pflugfelder (1934) further postulated that feltage chaetae in *P. maxillosus* have an excretory function and that excretory products get permanently stored in the chaetae, giving them their characteristic golden-green coloration. He describes the paired “spinning glands” as directly associated with segmental nephridia. The exact nature of this association, however, cannot be inferred from his illustrations and is at odds with our current understanding of annelid chaetae and nephridia. An ultrastructural investigation of feltage chaetae and chaetal sacs, as well as nephridia in Acoetidae, is warranted to further characterize these structures.

Another, and arguably the more commonly known, occurrence of a felt-like chaetal mat in annelids, is the felt layer that covers the dorsal side of *Aphroditida* species (Barnich & Fiege, 2003; Gonzalez et al., 2018). Eisig (1887) also compared his observations in *P. maxillosus* with the felt of *Aphroditida* aculeata Linnaeus 1758 but only in terms of function, either as construction material for a tube or as a dorsal covering for the body. Feltage chaetae in species of *Aphroditida* arise from a single notopodial chaetal sac, have a diameter of ~1.5 μm, and are formed by ~25-30 equally sized microvilli (E. Tilić, unpubl. data). Thus, they are clearly different from what is described here for *Sthenelanella* and also from what is known from Eisig’s (1887) and Pflugfelder’s (1934) descriptions of acoetid feltage chaetae. Acoetidae, Aphroditidae, and Sigalionidae (to which *S. uniformis* belongs) represent three distinct lineages within Aphroditiformia (Gonzalez et al., 2018; Zhang et al., 2018). Gonzalez et al. (2018) published a phylogeny of the group, combining molecular and morphological data to revise the systematics of Aphroditiformia. In their study Aphroditidae, including *Aphroditida*, was the sister group of remaining Aphroditiformia. Acoetidae grouped together with Iphionidae and Polynoidae, whereas *Sthenelanella* was part of the monotypic *Sthenelanellinae* within Sigalionidae (Gonzalez et al.,...
2018). Zhang et al.,’s (2018) phylogeny is mostly congruent. The relationships within Sigalionidae still remain largely unresolved. According to Gonzalez et al. (2018), Sthenelanella is likely to be an independent and highly divergent lineage, potentially representing one of the earliest splits of Sigalionidae. At this juncture, it is important to point out that both Gonzalez et al. (2018) and Zhang et al. (2018) only used previously published 18S rRNA and 28S rRNA sequences of S. uniformis (GenBank accession numbers AY894322 and DQ790064; Struck et al., 2005, 2007). The remaining Sthenelanella terminals in Gonzalez et al. (2018) were only represented in the morphology matrix. Our 18S rRNA (MW173358) and COI (MW173680) sequences of S. uniformis clearly differ from those published by Struck et al. (2005). The COI sequences were 21.8% divergent and the 18S rRNA sequences were 10% divergent. Given that our specimen was collected near the type locality of S. uniformis and that the morphology matches the descriptions of Moore (1910) and Pettibone (1969), we conclude that the sequences used in both published Aphroditiformia phylogenies stem from a misidentified specimen that may or may not be a Sthenelanella. Therefore, a thorough phylogenetic analysis of Sigalionidae is still warranted in order to resolve the position of Sthenelanella within the group. Whatever the exact position of Sthenelanella within Sigalionidae might be, the ultrastructure of feltage chaetae described herein, and the apparent differences from other superficially similar chaetae highlighted above, presently suggest that feltage chaetae evolved repeatedly and independently within the diversity of Aphroditiformia.

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