

Chambered chaetae in nereidiform polychaetes (Annelida)

Fredrik Pleijel · Lena Gustavsson

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Abstract The nereidiform polychaete taxa Chrysopetalidae, Hesionidae and Nereididae are characterized by the presence of chambered chaetae. The medullae (inner part) of all examined annelid chaetae are provided with internal longitudinal canals, but in these taxa there are additional thin, transverse walls (diaphragms), giving the chaetae a barred or chambered appearance in light microscopy. We investigate this structure in chrysopetalids, hesionids, nereidids, with light, scanning and transmission electron microscopy and compare it to phyllodocids and syllids, which are outside this clade. We conclude that chambered chaetae likely constitute a synapomorphy for chrysopetalids, hesionids and nereidids, although further study are required of some aphroditids and nephtyids.

Keywords Chambered chaetae · Diaphragms · Nereidiformia · Microscopy

Introduction

Chaetae as taxonomic characters have long been emphasized in polychaete systematics; they provide a wealth of characters, both for species identifications and for higher

taxa, and they can often be studied also on specimens where the soft tissue is in poor condition. The chitinous chaetae are formed in annelids in a strictly regulated process, where each chaeta is produced in a multi-cellular epidermal follicle (Bouligand 1966; Bouligand 1967; O'Clair and Cloney 1974; Specht 1988; Meyer and Bartolomaeus 1996; Schweigkofler et al. 1998; Hausam and Bartolomaeus 2001; Hausen 2005). The basalmost cell in the follicle, the chaetoblast, secretes chaetal material and shapes the morphology of the chaetae. The surface of the chaetoblast cell is provided with microvilli. Chaetal material is secreted between the bases of the microvilli and causes the chaeta to elongate, whereas internal, empty canals are formed at the positions of the microvilli. The inner part of the chaetae is generally provided with wider canals and is referred to as medulla and is surrounded by an outer, more homogenous cortex with finer canals. The transition between medulla and cortex may be gradual or more well-defined.

As indicated by light microscopy (LM) studies, the medullae of the chaetae of some taxa are provided, not only with the longitudinal striation caused by the canals, but also by a dense transverse striation caused by so-called diaphragms or trabeculae. Diaphragms have been observed in species of the three taxa Chrysopetalidae (e.g., Perkins 1985), Hesionidae (e.g., Pleijel 1998) and Nereididae (e.g., Glasby 1993). Views on the relationships between the three taxa have varied in the literature, and the absence or presence of diaphragms is therefore of special interest. Judging from the habit to list Chrysopetalidae and Aphroditiformia adjacent to each other in classifications, earlier authors usually considered chrysopetalids to be closely related to scale-worms (e.g., Fauvel 1923; Hartman 1959; Day 1963). Fauchald (1977) included them as a “superfamily” within a “suborder” Aphroditiformia, but later (Rouse and Fauchald 1997, Fig. 73) instead placed them as the basalmost branching

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F. Pleijel (✉)
Department of Marine Ecology, Tjärnö,
University of Gothenburg,
SE-452 96 Strömstad, Sweden
e-mail: fredrik.pleijel@marecol.gu.se

L. Gustavsson
Department of Invertebrate Zoology,
Swedish Museum of Natural History,
Box 50007, SE-104 05 Stockholm, Sweden

polychaete taxon within Phyllodocida. Perkins (1985) proposed that internally chambered chaetae are present in chrysopetalids, hesionids, nephtyids, nereidids, and some aphroditids and sigalionids and that chrysopetalids are related to hesionids and nereidids “although the relationship might not be especially close”. In a study of chrysopetalid chaetae, Westheide and Watson Russell (1992) provided a detailed description of the ultrastructure of the paleae and compared them to the Burgess Shale fossils *Canadia spinosa* Walcott, 1911 and *Wiwaxia corrugata* (Matthews, 1899), but did not address the relationships of chrysopetalids with hesionids and nereidids. A close relationship between hesionids and nereidids is less controversial, and several authors have used hesionids as outgroups in studies of nereidid relationships (Fitzhugh 1987; Glasby 1991; Santos et al. 2005). Glasby (1993), in a phylogenetic analysis of the nereidiform taxa, was the first to show that chrysopetalids, hesionids and nereidids form a monophyletic group, and these results were subsequently corroborated by Pleijel and Dahlgren (1998) and Dahlgren et al. (2000).

Among these taxa, only the nereidid *Nereis vexillosa* Grube, 1851 and two closely related species of chrysopetalids, *Chrysopetalum* spp., have been examined in transmission electron microscopy (TEM) by Gustus and Cloney (1973) and Westheide and Watson Russell (1992), respectively. In order to shed further light on this character and to evaluate the hypothesis that chrysopetalids, hesionids and nereidids form a clade, we here add new TEM studies of four species, accompanied by LM studies of further taxa. We provide the first TEM demonstration of diaphragms in a hesionid, *Ophiodromus flexuosus*, and add another nereidid species, *Hediste diversicolor*. With TEM, we also investigate two other and putative outgroup members of Phyllodocida, the phyllodocid *Sige fusigera* and the syllid *Typosyllis armillaris*, both of which lack diaphragms as observed with LM.

There is some confusion regarding the terminology of this and related chaetal features in annelids. Perkins (1985), in a study on chrysopetalids, used the term “chambered chaetae”, whereas Glasby (1993) used the term “compartmentalized (ladder-like) channels” for the presence of diaphragms in the canals. Pleijel and Dahlgren (1998) applied both terms “internally chambered” and “compartmentalized”. The choice between these two is largely arbitrary, but we use chambered since it is simpler. The term “camerated chaetae” was also used by Westheide and Watson Russell (1992) for chrysopetalids. However, together with “crenulate chaetae” (Hausam and Bartolomaeus 2001), “camerated chaetae” has later been used also for orbiniids (including questids) (Bleidorn 2005) for chaetae that have an external sculpture of protrusions arranged in rings. In order to avoid confusion of distinct features, we prefer not

to use the term “camerated chaetae” for nereidiform polychaetes.

Materials and methods

Specimens of *Hediste diversicolor* (O.F. Müller, 1776) (Nereididae), *Nereis pelagica* Linnaeus, 1758 (Nereididae), *Ophiodromus flexuosus* (delle Chiaje, 1825) (Hesionidae), *Sige fusigera* Malmgren, 1865 (Phyllodocidae) and *Typosyllis armillaris* (O.F. Müller, 1776) (Syllidae) were collected in the Koster area at the Swedish west coast, *Trypanosyllis zebra* (Grube, 1860) (Syllidae) in Plymouth, *Phyllodoce laminosa* Lamarck, 1818 (Phyllodocidae) at the Scilly Islands, SW England, *Chrysopetalum occidentale* Johnson, 1897 (Chrysopetalidae) in La Jolla, California, the unidentified subadult nereidid near Fort Pierce, Florida and *Leocrates chinensis* Kinberg, 1866 (Hesionidae) on New Britain, Papua New Guinea. Specimens used for chaetal studies in light microscopy (LM) were preserved in 10% formaldehyde, cleaned in distilled water and transferred to 70% ethanol. The chaetae were examined and photographed in an Olympus BX 51 equipped with interference contrast and a Canon EOS 5D. The SEM specimen was preserved for 1 h in 1% osmium tetroxide in 0.2 M sodium cacodylate buffer, rinsed in distilled water, stored in 70% alcohol, critical point dried and sputter coated with gold, and examined with a Hitachi S-520. For transmission electron microscopy (TEM), neuropodia of *Ophiodromus flexuosus*, *Hediste diversicolor*, *Sige fusigera*, and *Typosyllis armillaris* were dissected from the specimens and preserved in SPAFG (a mixture of sucrose, picric acid, paraformaldehyde, and glutaraldehyde; Ermak and Eakin 1976) for several days and then rinsed overnight in cacodylate buffer. They were postfixed in 1% osmium tetroxide dissolved in the same buffer for 1 h, washed in buffer, dehydrated in a graded ethanol series, and embedded via propylene oxide in Epon 812. Ultrathin (60 nm) and semithin (1 µm) sections were obtained with a Leica Ultracut. Semithin sections were stained with 1% toluidine blue and studied using a light microscope to get an overview. Ultrathin sections were collected on copper grids, triple-stained with lead citrate and uranyl acetate (Daddow 1983), and observed with a LEO 912 AB electron microscope at 80 kV.

Results

Through the years, we have examined large numbers of chaetae by light microscopy (LM) of both nereidiform and other polychaetes, and these examinations provide information regarding the presence and absence of internal chaetal diaphragms. Specific for this study, we further examined

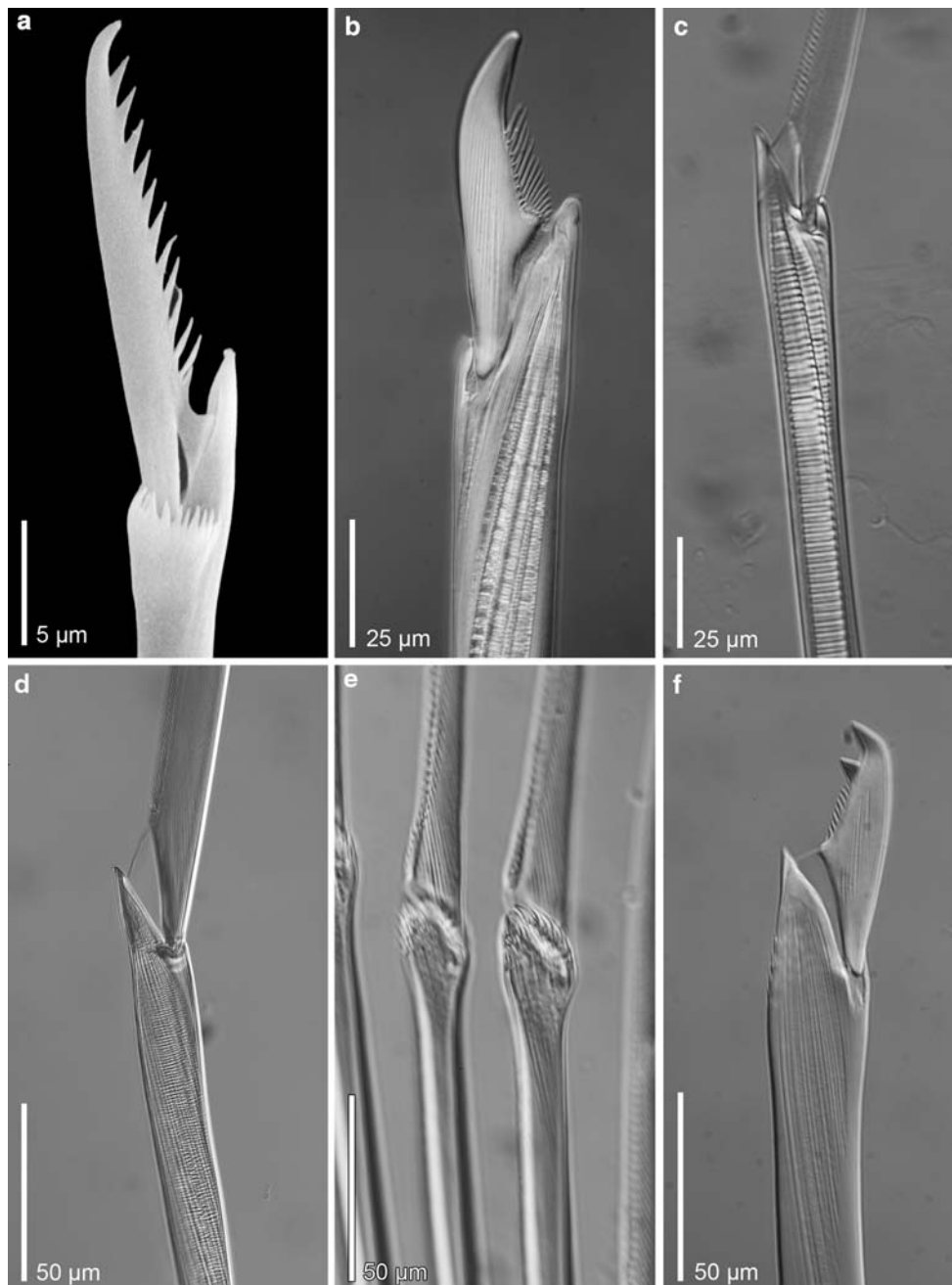


Fig. 1 SEM and LM pictures of neurochaetae. **a** SEM picture of neurochaeta of subadult Nereidinae. **b** LM picture of neurochaeta of *Nereis pelagica* (Nereididae). **c** LM picture of neurochaeta of *Chrysopetalum occidentale* (Chrysopetalidae). **d** LM picture of neurochaeta

of *Leocrates chinensis* (Hesionidae) **e** LM picture of neurochaeta of *Phyllodoce laminosa* (Phyllodocidae). **f** LM picture of neurochaeta of *Trypanosyllis zebra* (Syllidae)

compound chaetae from representatives of hesionids (*Ophiodromus flexuosus*) and nereidids (*Hediste diversicolor*) by transmission electron microscopy (TEM), together with phyllodocids (*Sige fusigera*) and syllids (*Typosyllis armillaris*), which according to the LM studies lack diaphragms. The ultrastructure of chrysopetalid chaetae was previously described by Westheide and Watson Russell (1992). For LM imaging, we also studied *Nereis*

pelagica, *Chrysopetalum occidentale*, *Leocrates chinensis*, and *Phyllodoce laminosa*. The scanning electron microscopy (SEM) picture of compound nereidid chaetae (Fig. 1a) demonstrates that the compartments are not associated with external sculpture.

The chambered structure with diaphragms is present in both the noto- and neurochaeta and in the aciculae in chrysopetalids, hesionids, and nereidids. In compound

chaetae, however, canals are present but no diaphragms observed in the blades (Fig. 1b–d).

The majority of our TEM sections are oblique to near longitudinal and from the distal part of the chaetal shaft. The number of canals varies as seen from the oblique sections, from a few in the nereidid to more than 15 in the hesionid species. The distance between two diaphragms in the same canal is usually 0.5–1 μm . The width of the canals is about 1–2 μm , and the canal walls are much thicker than the diaphragms. These observations are also in full agreement with the TEM pictures of the simple chrysopetalid notochaetae in Westheide and Watson Russell (1992). Both diaphragms and canal walls are much stouter in the examined nereidid species than in the hesionid.

The diaphragms only appear to be present within the canals and do not traverse the canal walls. This can also be seen from the situation that a diaphragm in one canal is often not situated at exactly the same level as the ones in the neighbouring canals (Fig. 2a–d). The diaphragms are not stretched between the canal walls but tend to have distinct folds (Fig. 2a, b, d).

In the hesionid *Ophiodromus flexuosus*, there are electron-dense granules, usually with a diameter of about 50 nm (Fig. 2a, b). These granules do not appear to represent fixation artefacts and are always situated in association with the walls of the canals or the diaphragms. Granules are absent from the other examined species and also from the chrysopetalid species studied by Westheide and Watson Russell (1992).

The TEM examinations of the phyllodocid *Sige fusigera* and of the syllid *Typosyllis armillaris* corroborate the LM observations that they have medullary canals without diaphragms (Fig. 2e, f). The observed canals in these two taxa were thinner than in the nereidiforms, about 0.5 μm .

Discussion

With transmission electron microscopy (TEM), we here show the presence and fine structure of chaetal diaphragms in the hesionid *Ophiodromus flexuosus* and the nereidid *Hediste diversicolor*, and their absence in the phyllodocid *Sige fusigera* and the syllid *Typosyllis armillaris*. With scanning electron microscopy (SEM) of a nereidid, we also demonstrate that these features are not associated with external sculpture, and we add light microscopical evidence for the presence of diaphragms in the chrysopetalid *Chrysopetalum occidentale*, the nereidid *Nereis pelagica*, the hesionid *Leocrates chinensis*, the phyllodocid *Phyllodoce laminosa*, and the syllid *Trypanosyllis zebra*.

This, together with the evidence from the ultrastructural studies of chaetae in *Nereis vexillosa* (Gustus and Cloney 1973; O'Clair and Cloney 1974) and two species of *Chrys-*

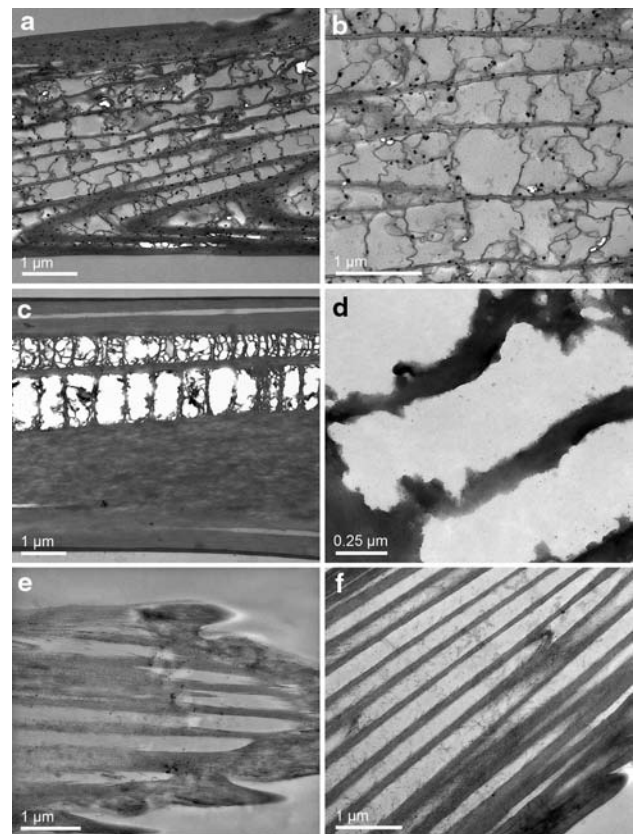


Fig. 2 TEM pictures of neurochaetae. **a** *Ophiodromus flexuosus* (Hesionidae). Neurochaeta, oblique section through distal part of shaft. **b** *Ophiodromus flexuosus*. Close-up of canal walls and diaphragms. **c** *Hediste diversicolor* (Nereididae). Neurochaeta, oblique section through distal part of shaft. **d** *Hediste diversicolor*. Close-up of diaphragms. **e** *Sige fusigera* (Phyllodocidae). Oblique section of neurochaeta at rostral region. Diaphragms absent. **f** *Typosyllis armillaris* (Syllidae). Oblique section of neurochaeta near rostral region. Diaphragms absent

opetalum Westheide and Watson Russell 1992), corroborate the hypothesis that diaphragms constitute a synapomorphy for a clade consisting of chrysopetalids, hesions and nereidids forwarded by Glasby (1993), Pleijel and Dahlgren (1998) and Dahlgren et al. (2000). Two more recent molecular studies on the phylogeny of annelids and incorporating members of these taxa were not able to recover this clade (Rousset et al. 2006; Struck et al. 2007). However, it was not strongly refuted either since alternative solutions also had very weak support. Chrysopetalids have previously by most authors been considered as closely related to or members of Aphroditiformia (e.g., Fauvel 1923; Hartman 1959; Day 1963; Fauchald 1977).

The two interstitial taxa *Hesionides* Friedrich, 1937 and *Microphthalmus* Meczniow, 1865 lack chaetal diaphragms (Pleijel and Dahlgren 1998; personal observation), but have traditionally been treated as members of Hesionidae. However, in phylogenetic analyses of nereidiform

polychaetes, Pleijel and Dahlgren (1998) and Dahlgren et al. (2000) demonstrated that these two taxa are not members of Hesionidae, and at present they are not referred to any family–group taxon but only as Nereidiformia incertae sedis (e.g., Rouse and Pleijel 2001). The absence of chaetal diaphragms in *Hesionides* and *Microphthalmus* species is therefore not in conflict with diaphragms as a synapomorphy for chrysopetalids, hesionids and nereidids.

Perkins (1985) suggested that internally chambered chaetae occur in chrysopetalids, nereidids, nephtyids, hesionids, and in some species of the Aphroditidae (*Pontogenia chrysocoma* (Baird, 1865)) and Sigalionidae (*Sthenelais simplex* Ehlers, 1887), although he did not consider them as evidence for a close relationships between these taxa. As for *Sthenelais simplex*, those chaetal structures indicating internal diaphragms actually represent external sculpture of the chaetae (Pleijel and Dahlgren 1998). In the absence of suitable study material, we have not been able to assess presence or absence of diaphragms in the chaetae of *Pontogenia chrysocoma*. *Pontogenia* belongs to the Aphroditidae and this taxon is sister group to the remaining aphroditiforms (Struck et al. 2005; Wiklund et al. 2005). If there is a close relationship between aphroditiform and nereidiform polychaetes then, although unlikely, it cannot be excluded that the diaphragms in Chrysopetalidae, Hesionidae, and Nereididae are homologous with those in aphroditids and subsequently reduced in the line leading to the other aphroditiforms. This character could thus be a synapomorphy at a more general level or represent a homoplasy, and the finer details and the taxonomic distribution of this character in aphroditids deserve further study. The same problem occurs with the “barred chaetae” in some nephtyids (e.g., Hartman 1950), which have been interpreted as modified internally chambered chaetae Perkins (1985), and also these merit further investigation.

The position of the enigmatic Burgess Shale fossil *Wiwaxia corrugata* has, and still is, debated (see Eiby-Jacobsen 2004 and references within). Butterfield (1990) compared the putative chaetae with those of chrysopetalids. The external fine structure of the chaetae of these taxa indeed shows high degrees of resemblance, although the internal-chambered structure with diaphragms could not be observed in *Wiwaxia corrugata*, and our study therefore cannot add any further evidence bearing on the position of this taxon.

The diaphragms in our studied specimens are always longer than the distance between the canal walls, and they are therefore also folded. These folds are not randomly distributed, but tend to be situated in same positions in adjacent diaphragms along the same canal. Diaphragms may provide increased bending strength to the chaetae with little cost in terms of additional chaetal material. The folds will then allow for the diaphragm to be stretched to a certain

degree when the chaeta is bent. The situation that the diaphragms do not seem to penetrate the canal walls and that they do not tend to be situated at exactly the same level in the neighbouring canals indicates that they are synthesized individually in each canal. One possibility is that they are secreted from the chaetoblast microvilli with regular intervals, although the present investigation does not provide answers regarding the actual mechanism of synthesis; for further discussion on this issue, see O’Clair and Cloney (1974) and Westheide and Watson Russell (1992).

The diaphragms can often be observed in the illustrations in taxonomic literature of chrysopetalids, hesionids and nereidids, also in many of the older descriptions from the 19th century. However, caution should be used when assessing their absence, since they sometimes are omitted in illustrations, both in older and more recent studies.

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