

On the absence of circular muscle elements in the body wall of *Dysponetus pygmaeus* (Chrysopetalidae, 'Polychaeta', Annelida)

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Abstract

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The annelid body wall generally comprises an outer layer of circular muscle fibres and an inner layer of longitudinal muscle fibres as well as parapodial and chaetal muscles. An investigation of *Dysponetus pygmaeus* (Chrysopetalidae) with confocal laser scanning microscopy showed that circular muscles are entirely absent. Further studies indicate that this feature is characteristic for all Chrysopetalidae. A scrutiny of the literature showed a similar situation in many other polychaetes. This lack of circular muscle fibres may either be due to convergence or represent a plesiomorphic character. Since circular muscles are very likely important for burrowing forms but not necessary for animals which proceed by movements of their parapodial appendages or cilia, this problem is also related to the question of whether the ancestral polychaete was epi- or endobenthic.

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Introduction

A body wall musculature comprising an outer layer of circular fibres and an inner layer of longitudinal fibres is generally seen as a key character in the Annelida (Lanzavecchia *et al.* 1988; Gardiner 1992). Additional muscles may be present, such as parapodial, chaetal, oblique and dorsoventral muscles. Especially in polychaetes the structure of the muscle system is highly diverse and is only sufficiently known in a few species (Storch 1968; Pilato 1981; Lanzavecchia *et al.* 1988). In species possessing parapodia the circular muscle fibres are confined to the intrapapodial regions and the longitudinal musculature is restricted to the dorsal and ventral parts of the body. Although it is well-known that circular fibres are usually less developed than the longitudinal fibres in most polychaetes, absence of the former appears to be a rare exception (Lanzavecchia *et al.* 1988; Gardiner 1992).

During transmission electron microscopic investigations in two taxa of the Chrysopetalidae, *Chrysopetalum* spp. and *Dysponetus* spp., Tzetlin *et al.* (2002) could not find circular

muscle fibres in any of the species examined. To determine whether the transverse muscle elements observed are in fact parapodial muscles or reduced circular muscles, a reconstruction of the entire muscle system was carried out in the smaller species *Dysponetus pygmaeus* Levinsen, 1897 (Fig. 1) by labelling muscle fibres and confocal laser scanning microscopy. Proof of absence of circular fibres in the Chrysopetalidae would be of special interest because this taxon is considered to possess several plesiomorphic characters and to be close to the annelid stem species by some authors (Westheide and Watson Russel 1992; see Dahlgren 2000). This would necessitate thorough reinvestigations of polychaete muscle systems and show that this feature has to be taken into account in the discussion of the phylogeny and evolution of the Annelida.

Materials and Methods

Specimens of *Dysponetus pygmaeus* (Fig. 1) were collected from subtidal mud in the White Sea (Kandalaksha Bay,

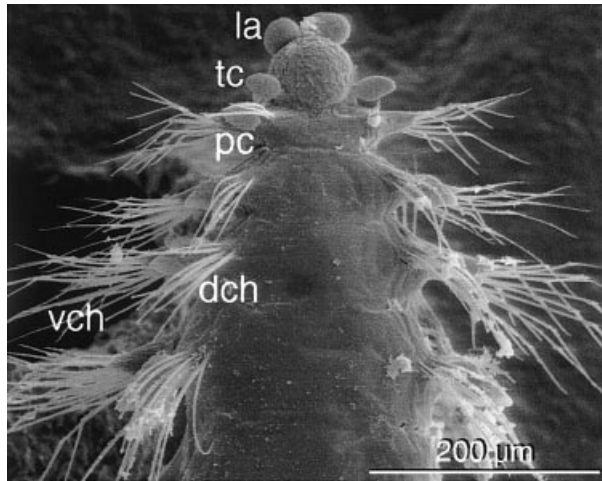


Fig. 1—Dorsal view of anterior end of *D. pygmaeus*; prostomium with paired lateral antennae (*la*), reduced peristomium with tentacular cirri (*tc*) and first four chaetigers with parapodia comprising dorsal and ventral bundles of chaetae (*dch*, *vch*) and parapodial cirri (*pc*); palps situated ventrally and not visible, median antenna broken off. SEM.

Russia, 20–200 m depth). They were fixed on ice overnight in 4% paraformaldehyde in 0.15 M phosphate-buffered saline (PBS; pH 7.4) containing 8% sucrose. After rinsing in PBS, specimens were preincubated with 0.1% Triton X-100 in PBS (1 h) and then incubated in phalloidin-fluorescein isothiocyanate-labelled solution (5 μ L 3.3 μ M solution in 100 μ L PBS). They were investigated with a Zeiss LSM 410 confocal laser scanning microscope.

Results

The musculature of the body wall in *D. pygmaeus* consists of longitudinal fibres only (Fig. 2A,B). They lie directly beneath the epidermis and are arranged in four bundles, two dorsal and two ventral. The dorsal muscle fibres cover the entire width of the segments and are only interrupted by a small gap where the mesentery reaches the epidermis (Fig. 2A). The ventral bundles are more intensely labelled, thicker and separated by a larger space (Fig. 2B) which is occupied by the ventral nerve cord. There are neither transverse nor circular muscle fibres between the epidermis and these longitudinal muscles.

Transverse muscle elements are present in the trunk of each segment, but these belong to the parapodial musculature (Fig. 2C,D). The most prominent fibres form the ventral oblique muscles, running from the midventral line into the parapodia (Fig. 2C). These muscles branch into two bundles of fibres, the smaller and anterior of which enters the parapodium of the preceding segment and attaches to the epidermis on the posterior part of the parapodial lobe. The larger bundle enters the anterior part of the same segment

and is attached to various parts of the parapodium (Fig. 2C). Dorsally similar oblique muscle fibres are present in a corresponding arrangement (Fig. 2D). However, in the trunk these fibres penetrate the layer of longitudinal muscle fibres. These oblique fibres are of different lengths and are attached to the epidermis over the entire width of the dorsum. As a result the dorsal musculature of the trunk has a lattice-like appearance and the existence of circular fibres is simulated (Fig. 2D). The parapodial muscle system comprises acicular chaetal and intrinsic muscle fibres as further elements (Fig. 2C,D). The former serve as protractor muscles and run from the chaetal bases to the parapodial epidermis, whereas the latter comprise more or less longitudinal fibres inside the parapodial lobes. This system of parapodial muscles is responsible for the complex movements displayed by the parapodia.

Discussion

The present data show that labelling of F-actin and subsequent confocal laser scanning microscopy is an excellent and accurate method by which to investigate the arrangement of muscle fibres. The results are more quickly obtained than in conventional histological or electron microscopical investigations, provided that the specimens do not exceed an appropriate size. Each muscle cell is labelled individually and can easily be followed for its entire length. Thus, absence or presence of a certain type of muscle fibre can be investigated with a greater degree of certainty, especially if such muscles are weakly developed and hardly visible in histological sections.

Lack of circular muscle fibres is not only demonstrated in the meiobenthic interstitial or mud-dwelling species of *Dysponetus* but also in the larger epibenthic species of *Chrysopetalum* spp. (Müller, unpublished observation). This indicates that this feature is, most likely, characteristic of the entire taxon Chrysopetalidae.

Although it is known that many variations of muscle arrangements occur in polychaetes, the presence of an outer layer of circular fibres and an inner layer of longitudinal fibres is considered to represent the ground pattern in Annelida. Circular muscles are less developed in parapodia-bearing taxa and may be restricted to the intrapapodial spaces, but absence of circular muscles appears to be a rare exception and is only seldom reported in reviews and textbooks (e.g. Lanzavecchia *et al.* 1988; Gardiner 1992). However, absence of circular muscle fibres occurs more often than is generally thought and several examples have been found in polychaetes including macrobenthic, meiobenthic, parapodia-bearing as well as sedentary species of the following taxa: Opheliidae, Polygordiidae, Protodrilidae, Spionidae, Oweniidae, Aphroditidae, Acoetidae (= Polyodontidae), Polynoidae, Sigalionidae, Phyllodocidae, Nephtyidae, Pisionidae and Nerillidae (McIntosh 1917; Hartmann-Schröder 1958; Orrhage 1964; Jouin and Swedmark

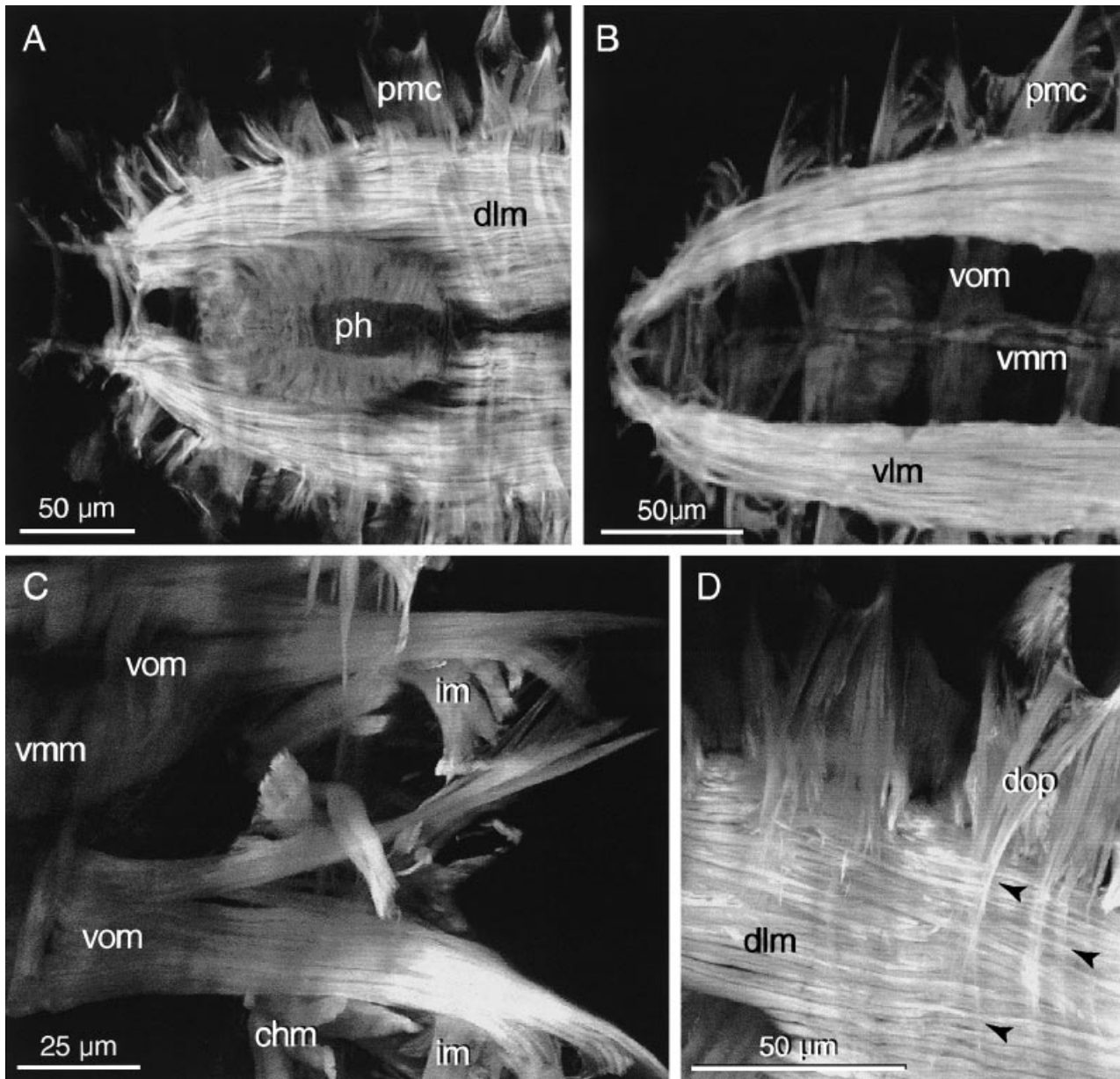


Fig. 2—Arrangement of muscle system of *D. pygmaeus* as seen after phalloidin labelling. CLSM micrographs. —**A**. Anterior end, dorsal view. Dorsal longitudinal muscles (dlm) occupy entire width of the trunk, transverse muscle elements are represented by fibres of the parapodial muscle complex (pmc). In the body the pharynx muscles (ph) are also labelled. —**B**. Anterior end, ventral view. Body wall musculature represented only by two prominent ventral longitudinal muscles (vlm). vmm ventral mesenteric muscle. —**C**. Parapodial

muscle complex, ventral view. Ventral longitudinal muscles not shown. Parapodial muscles comprise ventral oblique muscle fibres (vom), chaetal muscles (chm) and intrinsic muscles (im). —**D**. Dorsal view of parapodial muscle complex comprising dorsal oblique parapodial muscle fibres (dop), some of which extend between the dorsal longitudinal muscle fibres, resulting in a lattice-like appearance (arrowheads) and simulating circular fibres dorsally.

1965; Mettam 1967, 1971; Storch 1968; Hermans 1969; Gardiner and Rieger 1980; Tzetlin 1987; Ivanov and Tzetlin 1997). Absence of these fibres has also been reported for *Jennaria pulchra*, an enigmatic taxon with annelid affinities (Rieger 1991). This suggests that the lack of circular muscles

may not be a rare departure but a common situation in many polychaetes.

It is remarkable that the whole muscular system in the Chrysopetalidae is similar to that of *Aphrodita aculeata* and other scale worms (Storch 1968; Mettam 1971). These

similarities of muscle fibre arrangement, including the lack of circular muscle fibres in the body wall, may either be a plesiomorphic character or a convergent feature. The view that a complete muscular lining comprising circular and longitudinal fibres belongs to the ground pattern of the annelids goes back to Clark's ideas of an oligochaete-like burrowing stem species in this group (Clark 1964, 1981). Since the body cavity is often not segmentally divided by complete septa, the propulsive movements caused by the antagonistic actions of circular and longitudinal muscular fibres that are characteristic of oligochaetes – the 'model' annelids of Clark (1964, 1981) – are only seldom found in polychaetes (Lanzavecchia *et al.* 1988). Antagonists of the longitudinal fibres are either the dorso-ventral, the transverse, the parapodial or the longitudinal fibres themselves in these polychaetes. The ideas of Clark (1964, 1981) have recently been supported by the cladistic analyses of Rouse and Fauchald (1995, 1997), but challenged by McHugh (1997) and Westheide (1997), who among others see an epibenthic parapodia-bearing animal as the stem species in the Annelida. Since circular muscles are especially important for burrowing forms and are not necessary for animals that proceed by movements of their parapodial appendages and chaetae (Mettam 1971, 1985), the absence of such muscles in extant epibenthic polychaetes is related to the question whether these muscles were present in the ancestral annelid. In case this stem species was in fact epibenthic and equipped with parapodia, these circular muscles appear not to be a requisite for the complex movements shown by errant polychaetes, confirming the hypothesis developed by Mettam (1985; p. 306): 'In the primitive annelid, longitudinal muscles may have been the main locomotory agent...'. Unfortunately, the systematic position of the Chrysopetalidae (and Aphroditidae) is still under discussion and not resolved (see Dahlgren *et al.* 2001; and Tzetlin *et al.* 2002). The question whether the lack of circular muscles in this group represents a primary or secondary situation cannot be answered and is hard to evaluate (see Purschke *et al.* 2000). However, this feature should now very seriously be considered in the discussion of the ground pattern of the Annelida. Further studies in other polychaete taxa are definitely required to investigate whether the absence of circular muscles is much more widely distributed within the polychaetes than is currently known.

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