

Body musculature of *Stylochaeta scirtetica* Brunson, 1950 and *Dasydytes (Setodytes) tongiorgii* (Balsamo, 1982) (Gastrotricha: Dasydytidae): A functional approach

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Abstract

Species of the freshwater gastrotrich taxon Dasydytidae show a set of conspicuous structural and behavioural adaptations to a semi-planktonic life. Conspicuously, most dasydytids have several groups of strong, moveable spines that can actively be abducted to perform saltatory movements, change the overall direction of locomotion, or enable the animals to rest in a defensive position. So far, there are only vague ideas of how these spine movements are achieved in dasydytid species. In order to gain insight into the possible morpho-functional coupling of body musculature and motile spines, we have carried out a study targeting the muscular system in two species of Dasydytidae by means of phalloidin staining and confocal laser scanning microscopy.

For spine movements in both species studied, *Stylochaeta scirtetica* and *Dasydytes (Setodytes) tongiorgii*, we have identified an antagonistic system of segmented longitudinal and oblique somatic muscle pairs. In both species, contraction of the musculi obliqua abduct the paired groups of ventro-lateral spines; contraction of the segments of musculi laterales causes their adduction.

A comparison of the muscular system of the studied species to that of other gastrotrichs reveals several homologous muscle pairs, visceral as well as somatic, that might be features of the stem species of a clade comprising all Paucitubulatina exclusive of Xenotrichulidae. The pairs of oblique somatic muscles are most probably an autapomorphy of Dasydytidae.

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1. Introduction

Gastrotricha is a diverse group of microscopic, aquatic metazoans that inhabit a wide range of both

marine and freshwater environments. While the majority of gastrotrich species are obligatory inhabitants of the benthos, mesopsammon as well as epipelon, there are, especially in the freshwater biotopes, several species that have a periphytic or even semi-pelagic lifestyle such as members of the taxa Dasydytidae and Neogosseidae (Balsamo and Todaro 2002).

The study of muscular systems by means of phalloidin staining and confocal laser scanning microscopy (cLSM)

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has become a powerful tool for discovering new phylogenetically informative characters revealing phylogenetic relationships in almost all bilaterian taxa. A fairly large number of studies on musculature in Gastrotricha covering both traditional major sister taxa, the Macrodasyida (Hochberg and Litvaitis 2001a, c, d; Leasi et al. 2006) and the Chaetonotida (Hochberg 2005; Hochberg and Litvaitis 2001b, 2003; Leasi et al. 2006; Leasi and Todaro 2008) have been carried out. Phylogenetic analyses of the muscular patterns of many gastrotrichs have shown to be of considerable information in terms of systematic questions and can be used to reveal the internal relationships of Gastrotricha (e.g. Hochberg and Litvaitis 2001c; Leasi and Todaro 2008).

Derived representatives of a certain taxonomic group of organisms are often of little use for phylogenetic considerations, e.g. for reconstructing the character pattern of the stem species of the whole superordinated taxon. However, data on these derived species may give further support to a suspected evolutionary trend such as secondary simplifications or, on the other hand, diversifications of organ systems. Such diversifications can often be correlated with special adaptations to a new habitat and lifestyle. Members of the freshwater gastrotrich taxon Dasydytidae show such changes in that they all have lost the obligatory benthic lifestyle. Correlated with this changed lifestyle, dasydytids show several aberrant features such as, for example, a modified locomotory ciliation, reduction of all adhesive tubes and possession of conspicuously strong and movable cuticular spines. These movable spines are, in some taxa, used for jumping movements in the water column (Schwank 1990). In other species, as for example in *Stylochaeta scirtetica*, the movable spines can also be used for defense against predators (see Schwank 1990; Fig. 5b). So far, there is only a limited amount of data on the muscular system of two dasydytid gastrotrichs, *Dasydytes ornatus* and *Dasydytes goniathrix*, for which spine movement mediated by a system of special longitudinal and circular muscles was hypothesized (Remane 1936). In the present study, we elucidate the general muscular pattern of two species of the Dasydytidae in order to get an idea of how the process of abduction and subsequent adduction of the cuticular spines is realised.

2. Materials and methods

2.1. Preparation of specimens

Specimens of both species investigated in this study, *Stylochaeta scirtetica* Brunson, 1950 and *Dasydytes (Setodytes) tongiorgii* (Balsamo, 1982) were collected in Oldenburg, North-West Germany. (We here follow the

systematisation given by Schwank (1990), who erected the subgenus *Setodytes*. Alternatively, *Dasydytes tongiorgii* would be placed to the taxon *Setopus*, which Kisielewski (1991) has upgraded from subgenus to genus level.) In May 2007, they were found in a small shaded ditch that was completely filled with submerged macrophytes (e.g. *Ceratophyllum* sp.) and covered with *Lemna* spp. Individual specimens of both species were picked out from the raw samples (sieved and filtered water from rinsed plant material) using a dissecting microscope and a capillary pipette. Live observations and documentation of both species were carried out on a Leica DMLB compound microscope equipped with a Olympus Color View I digital camera. For fluorescence studies, animals were fixed for 1 h (4 °C) with freshly prepared 4% formaldehyde buffered in 0.1 M PBS (pH 7.4). After rinsing in 0.1 M PBS, specimens were incubated over night in a 0.1% solution of Triton X-100 (buffered in 0.1 M PBS) to make the integument permeable. For staining, 2 µl of 38 µM methanolic TRITC phalloidin solution were added to 100 µl of 0.1% solution of Triton X-100 (buffered in 0.1 M PBS). Specimens were stained herein at 4 °C. After 3 h, staining was stopped by rinsing the specimens several times with fresh 0.1 M PBS. Individual specimens were mounted on microscopic slides with Cityfluor[®] and observed under a Leica TCS SP 5 confocal laser scanning microscope. The excitation wavelength for the fluorochrome (TRITC) was 488 nm. We have investigated three specimens of *S. scirtetica* and four specimens of *D. (S.) tongiorgii*. This relatively low N for both species is due to the very low abundances of these rare species. However, we could use all specimens prepared for reconstructing the muscular patterns. The programmes ImageJ 1.37v (Abramoff et al. 2004; Rasband 1997–2007) and Voxel 2.09d (Clendenon et al. 2002) were used to perform different projections, digital sectioning and 3D renderings for the analysis of the image stacks. Digital drawings for this study were made with Adobe Illustrator[®] 10.0.

2.2. Terminological considerations

The schematic drawings in this paper are an approximation to the system of body musculature of the species investigated. They are based on careful analysis of the image stacks of both species. Our aim is to discover the general pattern of body musculature in the two species. This is why ambiguous signals have not been included in the reconstructions. It needs to be pointed out that, whenever we speak of distinct muscles, we are fully aware of the fact that phalloidin staining can only visualise f-actin filaments and not complete muscle cells as such. For ease of communication, we have nevertheless decided to use the term “muscle” throughout this paper. Certain muscles in the two

species investigated are probably composed of several cells arranged consecutively or parallel. However, we will not include cell borders and the exact number of actin filament bundles in our reconstructions, because they could not be verified unambiguously in all specimens investigated. The method of phalloidin staining and fluorescence microscopy cannot resolve the actual insertion of a muscle ending. Nevertheless, when a certain muscle terminates close to the body wall (e.g. the frontal end of the musculi ventrales viscerales), we propose an attachment of this muscle to the integument (cuticle plus epidermis).

In the descriptions of the individual muscles, we follow the terminology for musculature of paucitubulatan gastrotrichs introduced earlier (Hochberg and Litvaitis 2003). However, we add the Latin word “visceralis” to some of the longitudinal muscles to distinguish between their visceral and somatic position which seems to be highly conserved among the different species of the taxon Paucitubulata. Hochberg and Litvaitis (2003) state that identical names of muscles used for species of Xenotrichulidae and Chaetonotidae are not meant to denote homology. However, we discuss homology of the muscles found in the investigated species of Dasydytidae and the muscles of Chaetonotidae. Identical names of muscles in Dasydytidae and Chaetonotidae do indicate homology!

3. Results

3.1. External features and locomotion

Both species investigated have a comparable gross body organisation (Fig. 1A and B). They possess a distinct head region bearing the subterminal mouth opening, a dorsal, hat-shaped cuticular plate, the cephalion, and several rings of long locomotory cilia. The neck constriction is caudally followed by the cigar-shaped (*S. scirtetica*) or spindle-shaped (*D. (S.) tongiorgii*) trunk region, which terminates in a pair of short appendages (styli) in *S. scirtetica* (Fig. 1A).

Most remarkably in almost all members of the family Dasydytidae are the groups of motile cuticular spines which are present in three groups of extremely long spines plus an additional fourth pair in *S. scirtetica* (Figs. 1C and 3A–C) and in six groups of shorter ones in *D. (S.) tongiorgii* (Figs. 1D and 5A–C). They are inserted ventro-laterally in the flanks of both species. In *S. scirtetica*, all motile spines like a coat are kept close to the trunk in their resting position.

Stylochaeta scirtetica is a steady swimmer performing wide screws on its way through the water column. From time to time, the motile spines are raised to initiate slight changes in the direction of locomotion. When the spines

are raised rapidly, *S. scirtetica* can abruptly stop its swimming to continue it in a completely different direction. *Dasydytes (S.) tongiorgii* is a quick swimmer that slowly rotates around itself. Changes in the direction of locomotion in the horizontal plane are achieved by slightly lateral movements of the head. When *D. (S.) tongiorgii* changes its direction in the median plane, it is probable that the motile spines are quickly raised and subsequently abducted (observations through a dissecting microscope).

When disturbed, both species are able to abduct their motile spines permanently to perform a defensive position (see Fig. 1E).

3.2. Body musculature of *Stylochaeta scirtetica*

There are muscles of six different orientations in both species: Radial and complete circular muscles (belonging to the myoepithelium and subepithelial muscle rings of the sucking pharynx, not considered in this study), longitudinal muscles (in a visceral as well as in a somatic position), visceral helicoidal muscles, visceral dorso-ventral muscles and somatic oblique muscles (Fig. 2A–C).

3.2.1. Visceral musculature (Fig. 3A–C)

3.2.1.1. Longitudinal muscles. In *S. scirtetica*, there are three pairs of longitudinal muscles associated with the intestinal tube (sucking pharynx and midgut). The most ventrally positioned musculi ventrales viscerales are frontally inserted in the body wall near the mouth tube and span the whole length of the specimen along the gut tube to the caudal end, where they are inserted in the body wall (Fig. 4B–H). Closely related to these ventral-most longitudinal muscles and nearly parallel throughout their whole length are the musculi ventrolaterales viscerales (Fig. 4B, G and H). We could detect this second pair of ventral visceral muscles from the region of the pharyngo-intestinal junction up to the caudal end of the animal, where they are inserted, as are the musculi ventrales viscerales, in the body wall close to the region of the anus. It is difficult to decide where this pair of muscles is inserted at its frontal end. Most probably, they are anchored in the ventral integument at the level of the pharyngo-intestinal junction. A third pair of visceral longitudinal muscles runs dorsal to the whole gut tube (pharynx and midgut), the musculi dorsales viscerales (Fig. 4A and C–H). As the musculi ventrales viscerales, they are frontally inserted in the integument near the mouth tube and terminate caudally in the anus region. The musculi dorsales viscerales show a striking difference to both other pairs of visceral longitudinal muscles: they branch a short distance caudal to the pharyngo-intestinal junction.

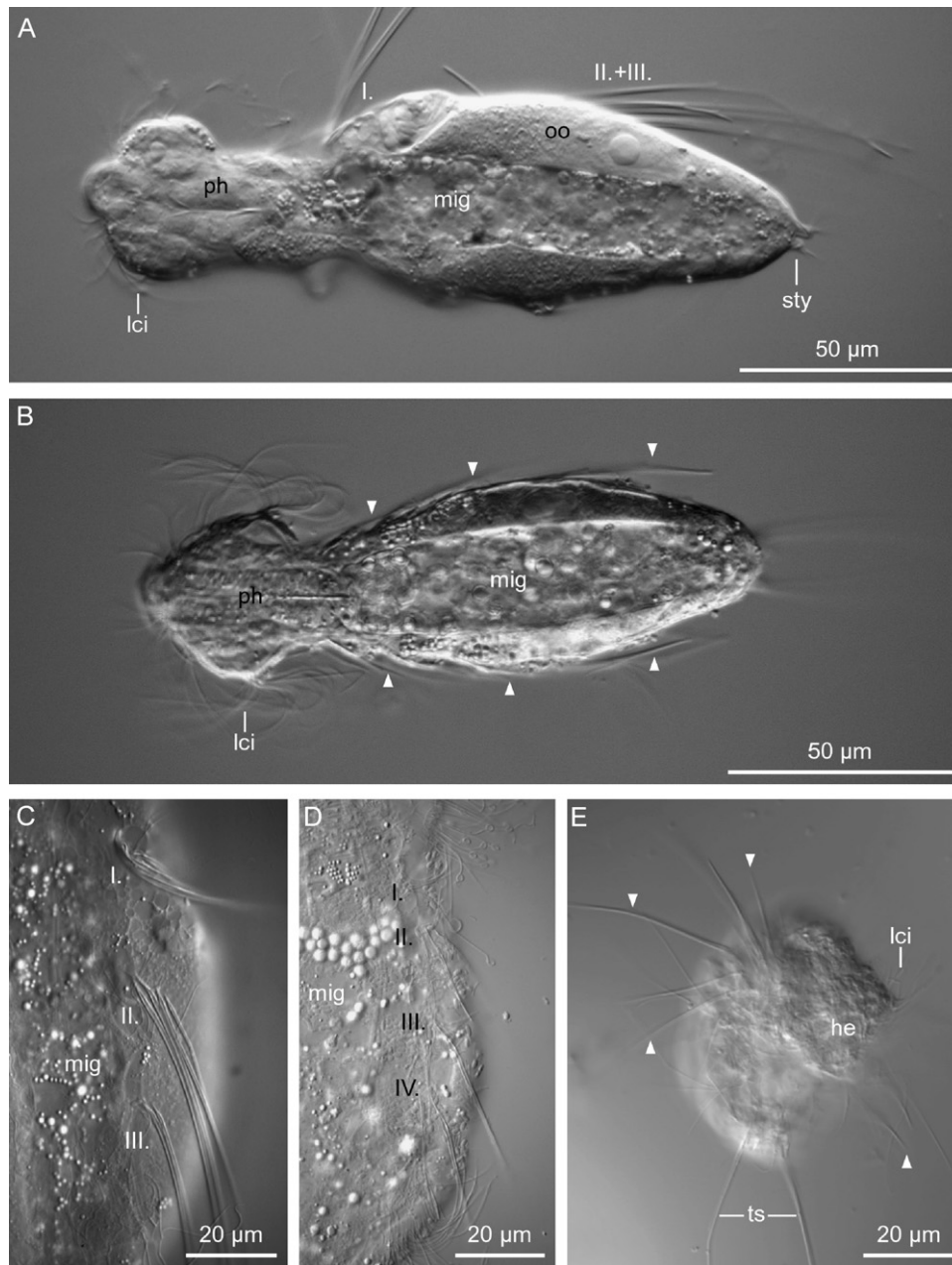


Fig. 1. Light microscopic images (differential interference contrast) of the studied species. (A) Habitus of *Stylochaeta scirtetica*. Note the abducted spines of the first group (I). (B) Habitus of *Dasydytes (Setodytes) tongiorgii*. Note the adducted spines (arrowheads). (C) Close-up of the insertions of the motile spines of *S. scirtetica*, ventral view. (D) Close-up of the insertions of the motile spines of *D. (S.) tongiorgii*, ventral view. Spine groups V and VI out of focal plane. (E) Frontal view of a disturbed *D. (S.) tongiorgii*. Note the abducted spines (arrowheads). he: head, lci: locomotory cilia of the head, mig: midgut, oo: mature oocyte, ph: pharynx, sty: styli, ts: terminal spines, I–IV: motile spine groups I–IV.

This branch-off runs parallel to the unbranched section of the musculus dorsalis visceralis (a little bit dorsolateral to it) and reunites with it some micrometers from the caudal end of the animal (Fig. 4A and E–H). Since we consider this muscular branch-off homologous to the equivalent muscle in species of Paucitubulatina (see Section 4), we term it the r1 (Rückenhautmuskel 1).

3.2.1.2. Helicoidal muscles. Exterior to the visceral longitudinal muscles, exclusive of the r1, there is a muscular helix twisting around the midgut up to the middle of the trunk region (Fig. 4A and B). There are approximately two crossings of muscle fibres on the ventral gut wall and two crossings on the dorsal gut wall. The angle between two crossing fibres varies from

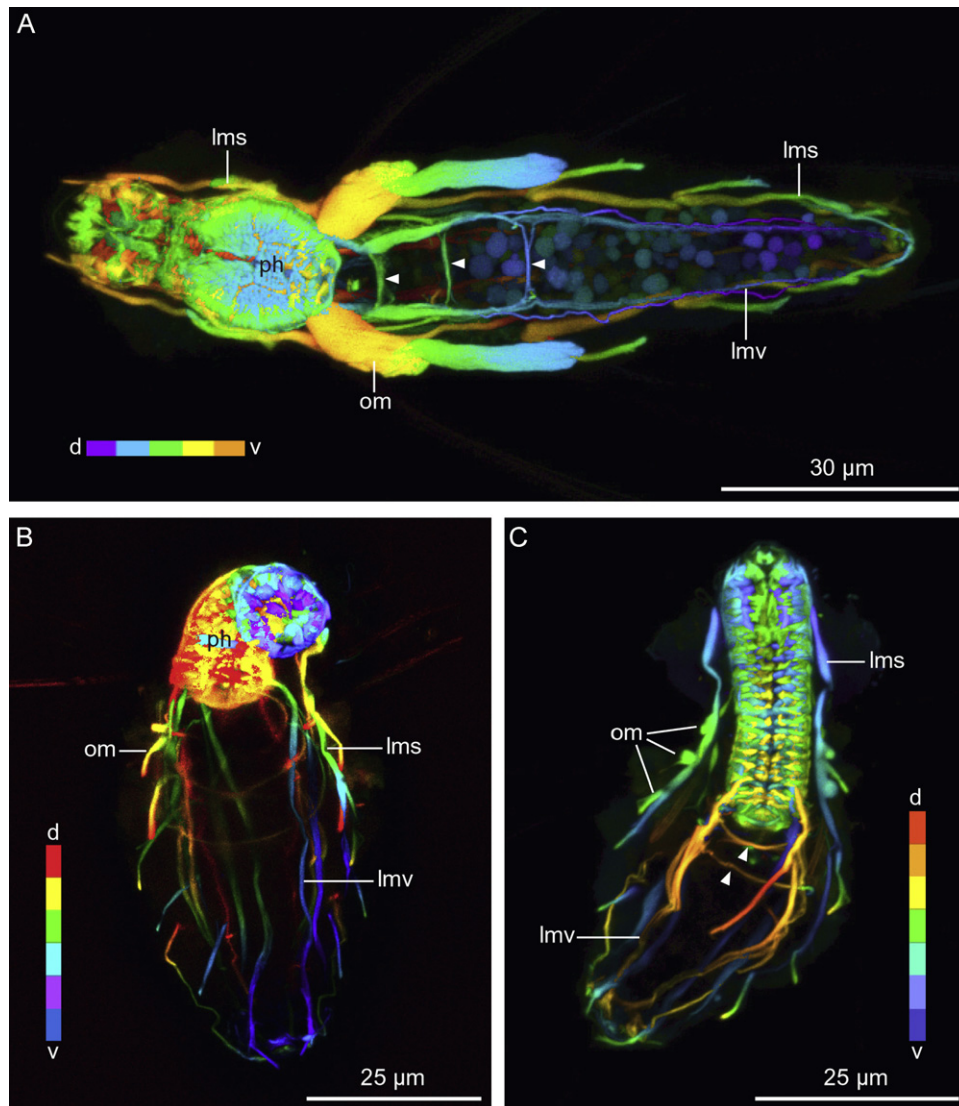


Fig. 2. Depth coded maximum projections of the investigated species demonstrating the three-dimensional arrangement of the muscular systems: (A) *Stylochaeta scirtetica*; (B and C) *Dasydites (Setodytes) tongiorgii*. For a detailed labelling of individual muscles, see Figs. 4 and 6. d: dorsal, lms: somatic longitudinal muscles, lmv: visceral longitudinal muscles, om: oblique muscles, ph: pharynx, v: ventral, arrowheads: helicoidal muscles.

fairly acute angles up to 45° . While the muscular helix encloses the three pairs of visceral longitudinal muscles, r1 lies outside the helix. The muscular helix does not continue to embrace the pharynx.

3.2.1.3. Other muscle arrangements. Close to the terminations of the muscoli ventrales viscerales, muscoli ventrolaterales viscerales and muscoli laterales (see below), there is a signal that we interpret as a pair of small and inconspicuous dorso-ventral muscles that encloses or probably connects the aforementioned longitudinal muscles (Fig. 4B). In the region of the anus, we have detected a small muscular ring that represents an anal sphincter (Fig. 4B: inset).

3.2.2. Somatic musculature (Fig. 3A–C)

3.2.2.1. Longitudinal muscles. In *S. scirtetica*, there is only one pair of ventro-lateral, somatic longitudinal muscles, the muscoli laterales (Fig. 4B–J). This bilateral muscle does not represent a pair of uniform muscle strands spanning the whole length of the animal! Each musculus lateralis is separated into five segments per side (musculus lateralis segmentum I–V, Fig. 4B). The four gaps between the muscular segments are closely related to the ventro-lateral insertions of the three groups of movable cuticular spines and to that of the fourth pair of spines (Fig. 3A–C). Musculus lateralis segmentum I is completely situated in the head region and spans alongside the pharynx, whereas all others lie in the trunk region lateral to the midgut

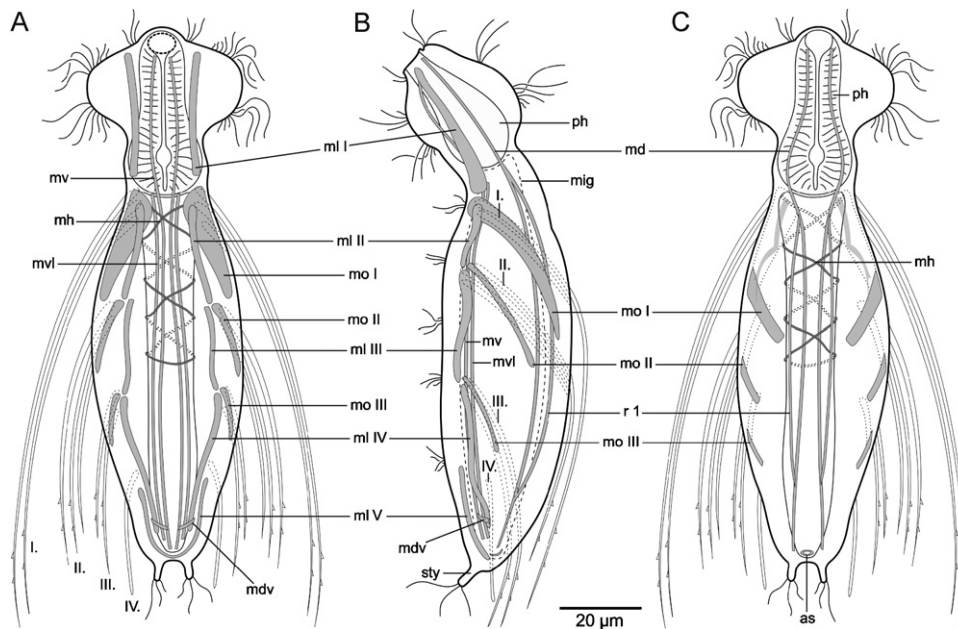


Fig. 3. Schematic reconstruction of the body musculature in *Stylochaeta scirtetica*: (A) ventral view; (B) lateral view, helicoidal muscles not displayed; (C) dorsal view. as: anal sphincter, md: muscoli dorsales viscerales, mdv: muscoli dorso-ventrales, mh: muscular helix, mig: midgut, ml I–V: muscoli laterales segmentum I–V, mo I–III: muscoli obliqua I–III, mv: muscoli ventrales viscerales, mvl: muscoli ventrolaterales viscerales, ph: pharynx, r1: Rückenhautmuskel 1, sty: styli, I–IV: groups I–IV of motile spines.

(Fig. 4B–H). However, musculus lateralis segmentum IV and V overlap for a certain distance and both parts (from either side of the specimen) of musculus lateralis segmentum V appear to be fused caudally, thus forming a single unpaired, horseshoe-shaped element of the body musculature in *S. scirtetica* (Fig. 4B).

3.2.2.2. Oblique muscles. There is a conspicuous system of three pairs of strong oblique muscles in *S. scirtetica* (Figs. 2A and 4A, B, D–F). These muscoli obliqua I–III run obliquely from the ventro-lateral integument alongside the body wall to the dorso-lateral integument, where they are attached (Fig. 4J). Ventro-lateral insertion points of muscoli obliqua I–III are the caudal ends of the muscular segments I–III of the muscoli laterales (Fig. 4B). These insertions are closely related to the ventro-lateral insertions of the movable cuticular spines of *S. scirtetica* (Fig. 3A–C). The muscoli obliqua I are by far the strongest muscles of *S. scirtetica*, with a diameter of more than 5 µm (Figs. 2A and 4A, B, E). The caudally following muscoli obliqua II and III are much smaller and thinner (around 1 µm) than muscoli obliqua I (Figs. 2A and 4A, B, G, H). Additionally, the length of these oblique somatic muscles decrease from muscoli obliqua I to muscoli obliqua III (Fig. 4J).

3.3. Body musculature of *Dasydytes (Setodytes) tongiorgii*

3.3.1. Visceral musculature (Fig. 5A–C)

3.3.1.1. Longitudinal muscles. As in the species described above, *D. (S.) tongiorgii* has three pairs of visceral longitudinal muscles: muscoli ventrales viscerales, muscoli ventrolaterales viscerales and muscoli dorsales viscerales (Fig. 6A–C, F and J–L). As opposed to these muscle pairs in *S. scirtetica*, all visceral longitudinal muscles in *D. (S.) tongiorgii* are frontally attached to the integument near the caudal end of the pharynx and terminate caudally in the body wall in the region of the anus. Another difference between both species investigated is in the r1 branch-offs of muscoli dorsales viscerales in *D. (S.) tongiorgii*. Whereas the split-offs are identically situated only a few micrometers caudal to the pharyngo-intestinal junction, the reunion of both muscle strands of each musculus dorsalis visceralis occurs just close to the caudal termination of this visceral longitudinal muscle pair (Fig. 6A and B). Dorso-lateral to the r1, there is another short, isolated pair of muscle strands that start around half the distance of muscoli dorsales viscerales and run to the dorsal integument. This muscle pair could represent a modified second branch-off of each musculus dorsalis visceralis, which is present in several species of Paucitubulatina (see Section 4). Therefore, we have preliminary termed this muscle pair the “r2?” (Fig. 6B).

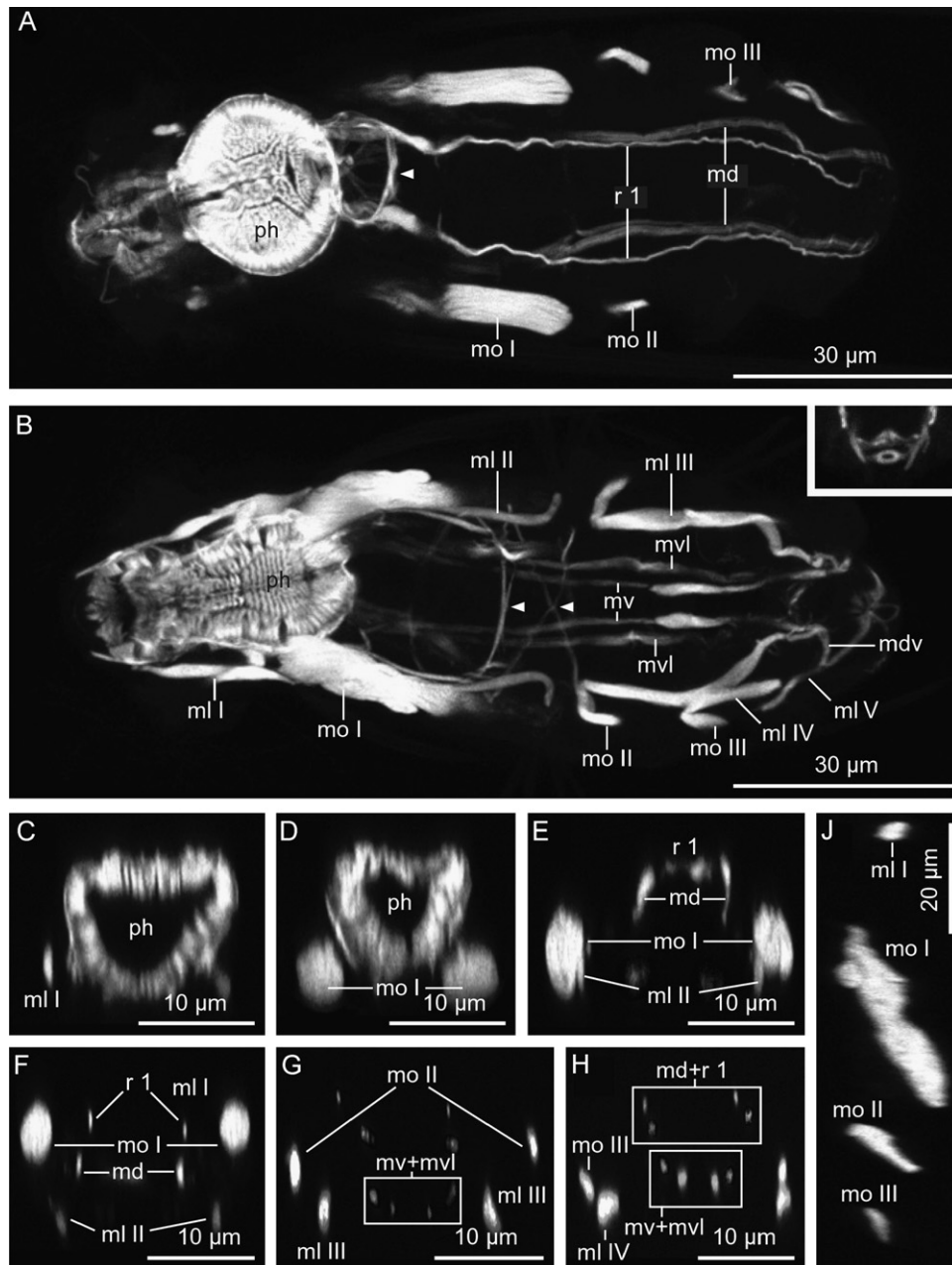


Fig. 4. Fluorescence signals of phalloidin stained actin filaments in *Stylochaeta scirtetica*. (A) Z-projection of a selection of some dorsal confocal images. (B) Z-projection of a selection of some ventral confocal images. Inset: Close-up of the anal sphincter. (C–H) Fronto-caudal series of digital y - z sections showing visceral and somatic muscle components. (J) Digital x - z sections demonstrating the oblique muscles. md: musculi dorsales viscerales, mdv: musculi dorso-ventrales, ml I–V: musculi laterales segmentum I–V, mo I–III: musculi obliqua I–III, mv: musculi ventrales viscerales, mvl: musculi ventrolaterales viscerales, ph: pharynx, r1: Rückenhautmuskel 1, arrowheads: muscular helix.

3.3.1.2. Helicoidal muscles. As in *S. scirtetica*, there is a muscular helix exterior to the visceral longitudinal muscles in *D. (S.) tongiorgii*. The muscular helix is twisting around the midgut to the middle of the trunk region (Fig. 6B and C). There is approximately one crossing of muscle fibres on the ventral gut wall and two crossings on the dorsal gut wall. The angle between two crossing fibres is around 35°. As in the species described

above, there are no helicoidal muscles embracing the pharynx. The muscular helix encloses the three pairs of visceral longitudinal muscles. However, the branch-offs of musculi dorsales viscerales (r1) lie outside the helix.

3.3.1.3. Other muscle arrangements. There is a signal in *D. (S.) tongiorgii* as well that probably represents a pair of small caudal dorsoventral muscles (Fig. 6E

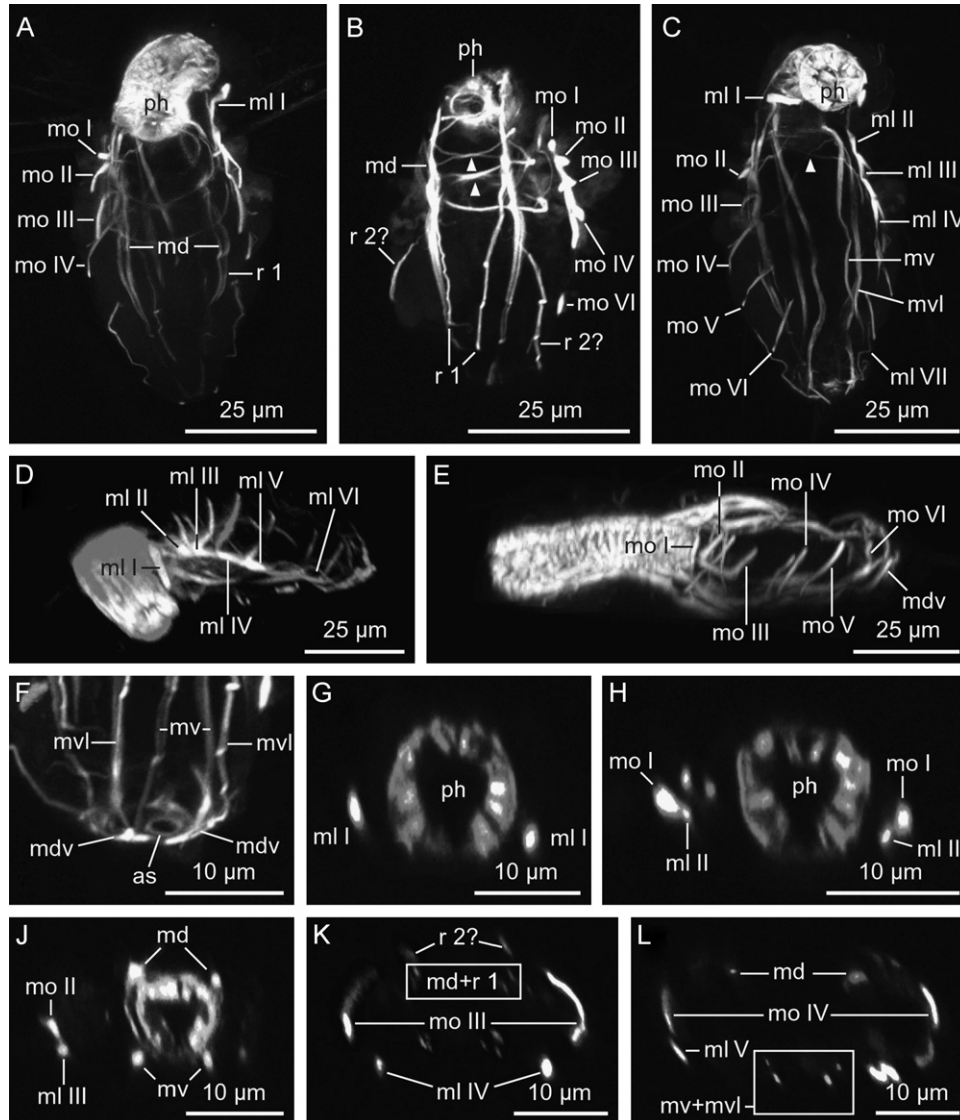


Fig. 6. Fluorescence signals of phalloidin stained actin filaments in *Dasydytes (Setodytes) tongiorgii*. (A and B) Z-projection of a selection of some dorsal confocal images. (C) Z-projection of a selection of some ventral confocal images. (D and E) Snapshots of volume rendered confocal image stacks, specimens seen from the left side. (F) Detail of the rear trunk region demonstrating the anal sphincter and the dorsoventral muscles. (G–L) Fronto-caudal series of digital y - z sections showing visceral and somatic muscle components. as: anal sphincter, md: muscoli dorsales viscerales, mdv: muscoli dorso-ventrales, ml I–VII: muscoli laterales segmentum I–VII, mo I–VI: muscoli obliqua I–VI, mv: muscoli ventrales viscerales, mvl: muscoli ventrolaterales viscerales, ph: pharynx, r1: Rückenhautmuskel 1, r2?: putative Rückenhautmuskel 2, arrowheads: muscular helix.

4.1. Phylogeny of Chaetonotida

Many morphological-systematic studies (e.g. Ruppert 1982, 1991; Travis 1983; Kieneke et al. 2007, 2008) and cladistic analyses (e.g. Hochberg and Litvaitis 2000; Zrzavý 2003; Todaro et al. 2006) support a well defined monophyletic taxon Paucitubulatina comprising the four major traditional families Xenotrichulidae, Chaetonotidae, Neogosseidae and Dasydytidae. According to several studies, *Neodasys* and Paucitubulatina are sister taxa forming the monophyletic subgroup Chaetonotida (d'Hondt 1971; Hochberg and Litvaitis 2000; Zrzavý

2003). Since we have investigated two dasydytid species, we in our comparison of the muscular system focus on members of Paucitubulatina. Apart from confirming the monophyly of Chaetonotida and Paucitubulatina, there are several studies based on morphological traits dealing with internal relationships of these groups (Ruppert 1982; Travis 1983; Hochberg and Litvaitis 2000; Marotta et al. 2005). According to one of these studies (Hochberg and Litvaitis 2000), the most basal taxon of Paucitubulatina might be the genus *Musellifer*. Within the remaining Paucitubulatina, Xenotrichulidae, together with partial Chaetonotidae, is the sister group

to a clade comprising the other Chaetonotidae, Neogosseidae and Dasydytidae (Hochberg and Litvaitis 2000). Whereas the internal relationships of this group, especially of the putative paraphyletic Chaetonotidae, are quite unclear, it is certain that the monophyletic Dasydytidae represent the most strongly derived taxon within Paucitubulatina (Hochberg and Litvaitis 2000).

4.2. Body musculature in Paucitubulatina

These phylogenetic relationships coincide with muscular data of Paucitubulatina species investigated so far. While there is a relatively constant pattern of longitudinal muscles (see below) and a visceral muscular helix reaching up to the middle of the intestine in all species of Paucitubulatina, the putative basal Xenotrichulidae additionally possess a combination of complete and incomplete visceral circular muscles, incomplete somatic circular muscles and somatic dorso-ventral muscles (*Xenotrichula intermedia*; Hochberg and Litvaitis 2003) or a combination of visceral- and somatic dorso-ventral muscles (*Draculiciteria tessellata*; Hochberg and Litvaitis 2001b). Within the family Chaetonotidae, the putative basal species *Polymerurus nodicaudus* also has visceral dorso-ventral muscles alongside the midgut (Leasi et al. 2006). Other species of Chaetonotidae only have a single caudal pair of dorso-ventral muscles, probably in a visceral position (*Chaetonotus lacunosus*, *C. cf. murrayi*, *C. cf. maximus*, *C. cf. minimus*, *Chaetonotus* sp.1, *Halichaetonotus aculifer*, *Halichaetonotus* sp.1, *Lepidodermella squamata*, *Lepidodermella* sp.1; Hochberg and Litvaitis 2003). Some species lack such muscle components altogether (*Aspidiophorus marinus*; Hochberg and Litvaitis 2003). The two investigated dasydytid species, *S. scirtetica* and *D. (S.) tongiorgii* also have only a single pair of caudal, visceral dorso-ventral muscles. Our data of these derived species strongly support a scenario of successive reduction from complete visceral and somatic muscle rings as they are present in species of *Neodasys* (see Hochberg 2005), possibly the sister taxon to Paucitubulatina (Hochberg and Litvaitis 2000), over incomplete circular muscles (or dorso-ventrals, respectively) towards a single pair or a complete loss of this muscular component in the stem lineage of Chaetonotida (= *Neodasys* + Paucitubulatina; d'Hondt 1971). This evolutionary scenario is further supported by a recent study on the muscular system of several Paucitubulatina species including *Musellifer delamarei*, which has, beside the longitudinal muscle strands, incomplete muscular rings in a visceral and somatic position (Leasi and Todaro 2008).

While the xenotrichulids *X. intermedia* and *D. tessellata* have five pairs of longitudinal muscles (Hochberg and Litvaitis 2001b, 2003), three in a visceral position (musculi ventrales, musculi ventrolaterales,

musculi dorsales) and two in a somatic position (musculi laterales and musculi ventromediales/paralaterales), all investigated chaetonotids (exclusive of *H. aculifer*) have four pairs of longitudinal muscles (Hochberg and Litvaitis 2003; Leasi et al. 2006), again three in a visceral position (musculi ventrales, musculi ventrolaterales, musculi dorsales) and a single pair in a somatic position (musculi laterales). We have undoubtedly identified four corresponding pairs of longitudinal muscles with comparable positions (musculi ventrales, musculi ventrolaterales, musculi laterales, musculi dorsales) for which we suggest homology to the longitudinal muscles described in species of Chaetonotidae investigated so far. An evolutionary modification of the musculi laterales of *S. scirtetica* and *D. (S.) tongiorgii* is their subdivision into several segments.

As in other Paucitubulatina species (e.g. see Hochberg and Litvaitis 2003), both dasydytids investigated in this study possess a pair of muscular branch-offs of the musculi dorsales viscerales, the r1. Some species, such as *A. marinus* and some *Chaetonotus* species (Hochberg and Litvaitis 2003), have a second split of the musculi dorsales viscerales, the r2. Since they have an identical dorso-lateral position, the unidentified pair of short longitudinal muscle strands in *D. (S.) tongiorgii* might be the equivalent to the r2.

A unique set of muscles, certainly apomorphic for Dasydytidae, are the bilateral pairs of oblique muscles, three pairs in *S. scirtetica* and six pairs in *D. (S.) tongiorgii*. We do not consider these conspicuous elements of the body musculature in Dasydytidae derived from somatic circular muscles. As they are closely associated with the segments of musculi laterales, we suggest that they might have been developed from lateral muscle branches of each musculus lateralis. Interestingly, a *Halichaetonotus* species shows such short lateral branches of the musculi laterales in the rear trunk region (Hochberg and Litvaitis 2003).

Another fact seems to support the derived position of Dasydytidae. While the visceral muscular helix embraces the pharynx in many paucitubulatan species (Hochberg and Litvaitis 2001b, 2003; Leasi et al. 2006), *Neodasys* (see Hochberg 2005) and Macrodasysida (Hochberg and Litvaitis 2001c,d; Leasi et al. 2006), both species investigated in this study do not show this pattern. Here, the muscular helix is restricted to the anterior half of the midgut. This investigation may be due to an artefact since the fluorescence signal of the myoepithelial pharynx is very strong. However, we have analysed every single image stack carefully and have not found any signal of the muscular helix around the pharynx.

With regard to the system of Dasydytidae proposed by Kisielewski (1991), we have investigated a putative basal member of Dasydytidae (*S. scirtetica*) as well as a more derived one from Kisielewski's genus *Setopus* (*D. (S.) tongiorgii*). Therefore, we are able to make

assumptions on the character pattern of the stem species of Dasydytidae. Presumably, it had three pairs of visceral longitudinal muscles accompanying the gut tube, one pair of segmented somatic longitudinal muscles in a lateral position and a system of oblique somatic muscles. If the ancestor of Dasydytidae had seven paired groups of lateral spines (Kisielewski 1991), it is likely that it also had seven pairs of muscoli obliqua and eight segments of muscoli laterales. The visceral muscular helix was probably restricted to the anterior half of the midgut.

4.3. Functional considerations

As a recent study on the planktonic rotifer *Filinia novaezealandiae* has shown, it is possible to deduce excellent morpho-functional models from a combination of live observations, external morphology and the study of the musculature by phalloidin staining and cLSM. In this freshwater rotifer, the movement of the two anterolateral setae is facilitated by a combination of contraction of special muscles inserted next to the setal bases (shoulder region) and a hightening of the pressure of the primary body cavity liquid due to corona withdrawal. It is suggested that, rather than a mode of locomotion, setae movement in *F. novaezealandiae* is used as a mechanism to deter predators (Hochberg and Gurbuz 2007).

For *D. ornatus* and *D. goniathrix*, Remane (1936) suggests a combination of special branch-offs of the muscoli laterales and circular muscles associated with the groups of motile spines to facilitate their locomotion. However, a model of how these different muscle elements in the two species of *Dasydytes* interact to realise the process of abduction and adduction of the cuticular spines is lacking. Since *S. scirtetica* and *D. (S.) tongiorgii* do not possess any somatic circular muscles, the mode of spine movement has to be different in these two species than in *D. ornatus* and *D. goniathrix*. *Stylochaeta scirtetica* and *D. (S.) tongiorgii* have quite a comparable set of somatic muscles that only differ in the number of pairs and the strength of some of the muscles. Therefore, we suspect a comparable mode of spine locomotion in the two species. We suppose that the segments of the muscoli laterales and the muscoli obliqua represent pairs of antagonistic muscles which move the cuticular spines in both species. The muscoli obliqua are hypothesized to be the abductors which raise the spines of each group when contracted, while the segments of the muscoli laterales frontal to each group of spines serve as adductors which move the spines to their initial position (Fig. 7). This outlined scheme of spine movement in the two investigated species means that each spine must have a base projecting into the animal to which abductors and adductors must be adhered (mediated via tonofilaments

in the epidermis). In this way the muscles can transfer the force of their contraction activity to the spines. The hypothetical pivot point of the spines must lie near to where the spines “penetrate” the body cuticle (Fig. 7). We have to study specimens at the ultrastructural level to test these hypotheses.

For the musculus lateralis segmentum I present in *S. scirtetica* and *D. (S.) tongiorgii*, we propose a second role. Since both species can slightly move their head region in the horizontal plane to produce changes in the direction of ciliary gliding, we suppose that by contraction of their first segment, the muscoli laterales bring about these head movements.

For the movable spines which are present in most species of the Dasydytidae, a dual role of protection and locomotion support is proposed (Balsamo and Todaro 2002). This double role is confirmed by our findings in both species investigated. Each of them uses spine movements for changing the direction of swimming, *S. scirtetica* can abruptly stop its movements by rapidly raising the motile spines. Additionally, both species can permanently abduct the spines, thus performing a kind of “defense position” (see also Schwank 1990: Fig. 5b).

Our study sheds light on a rare and disregarded, but, from a morpho-functional and evolutionary point of

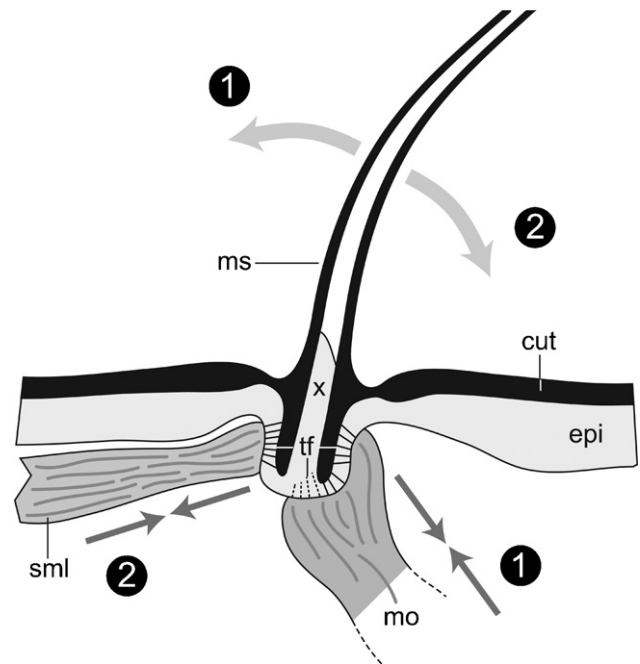


Fig. 7. Model of muscular spine movement in Dasydytidae. Schematic horizontal section of the body wall, seen from ventral. cut: cuticle, epi: epidermis, mo: musculus obliquus, ms: motile spine, sml: segment of musculus lateralis, tf: tonofilaments, x: hypothetical pivot point of motile spine, 1: contraction of oblique muscle causes abduction of spine, 2: contraction of longitudinal muscle segment causes adduction of spine.

view, highly interesting group of freshwater gastrotrichs. Future studies should reveal the exact muscle–spine coupling at the ultrastructural level and test if the model developed for spine movement is also applicable to other species of the Dasydytidae such as species of the subgenera *Dasydytes* and *Setopus* or in the genus *Haltidytes*.

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