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Organisation of body musculature in *Encentrum mucronatum* Wulfert, 1936, *Dicranophorus forcipatus* (O. F. Müller, 1786) and in the ground pattern of Ploima (Rotifera: Monogononta)

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Abstract

Fluorescence-labelled phalloidin in combination with confocal laser scanning microscopy (cLSM) has been used to reconstruct the body musculature in *Encentrum mucronatum* and *Dicranophorus forcipatus* in order to gain insight into the architecture of body musculature in representatives of the hitherto uninvestigated Dicranophoridae.

In both species, a system of outer circular and inner longitudinal muscles has been found. In *E. mucronatum*, seven circular muscles (musculi circulares I–VII) and six paired longitudinal muscles (musculi longitudinales I–VI) have been identified. In *D. forcipatus*, eight circular muscles (musculi circulares I–VIII) and nine paired longitudinal muscles (musculi longitudinales I–IX) are present. In both species, some of the longitudinal muscles span the whole specimen, while others are shorter and connect head and trunk or foot and trunk. Differences in shape and extension of the circular muscles in both species are related to differences in structure of the trunk integument.

Surveying the literature on rotifer musculature, muscles identified in this study are homologised across Rotifera and given individual names. Based on the study of *E. mucronatum* and *D. forcipatus* and previous studies on other rotifers, a system of musculature in the ground pattern of Ploima comprising at least three circular muscles (pars coronalis, corona sphincter, musculus circumpedalis) and three pairs of longitudinal muscles (musculi longitudinales ventrales, musculi longitudinales dorsales and musculi longitudinales capitum) is suggested.
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1. Introduction

Rotifers are a highly diverse group of aquatic micrometazoans, ubiquitously distributed in both freshwater and marine habitats. Their most characteristic features are the rotatory organ, a frontally positioned ciliary apparatus used for food gathering and locomotion, and a set of cuticularised jaw elements in a bulbous pharynx called the mastax. About 2030 rotifer species have been described so far (Segers 2007). Together with Gnathostomulida, *Limnognathia maerski* and the parasitic Acanthocephala, rotifers constitute a monophyletic taxon Gnathifera (Ahlrichs 1995), well supported not

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only by morphological findings (Ahlrichs 1995; Rieger and Tyler 1995) but also by studies based on DNA sequence data (Giribet et al. 2000). According to the currently best supported topology, Rotifera comprises monophyletic Hemirotifera (Bdelloida, *Seison* and Acanthocephala) and Monogononta as sister taxa of equal rank (Sørensen and Giribet 2006).

In an attempt to find new phylogenetically informative characters and, moreover, address functional questions, epifluorescence and confocal laser scanning microscopy (cLSM) have successfully been applied to the study of phalloidin-labelled body musculature in rotifers (Hochberg and Litvaitis 2000; Kotikova et al. 2001; Sørensen et al. 2003; Santo et al. 2005; Sørensen 2005a, b; Hochberg and Gurbuz 2007). These studies have considerably furthered our knowledge of rotifer body musculature and add to older results obtained by means of serial sections and traditional light microscopic techniques (summarised in Remane 1929–1933; Hyman 1951).

However, only few selected rotifer species, primarily monogononts, have so far been investigated. Taken together, they represent only a small fraction of rotiferan subtaxa. A well-supported assessment of the evolution of rotifer musculature can only be made on the basis of a broad taxon sampling covering as many of the traditional rotifer families as possible. Hence, more information on the muscular system in different rotifer species needs to be accumulated (Sørensen 2005b). The present study contributes to this goal. It reveals for the first time the organisation of body musculature in two representatives of Dicranophoridae, a large and primarily freshwater group of carnivorous or herbivorous rotifers characterised by a protrusible jaw apparatus, a fairly uniform overall morphology and occurrence in benthic-periphytic or interstitial habitats (De Smet 1997).

Going beyond this, we assess data on rotifer body musculature obtained in this and previous studies and propose a set of homologous muscles in the ground pattern of the large rotiferan subtaxon Ploima.

2. Materials and methods

Specimens for this study were collected near Oldenburg, North-West Germany. *Dicranophorus forcipatus* (O. F. Müller, 1786) was obtained from a pH-neutral ditch covered with *Lemna* sp. by careful rinsing of submerged macrophytes and filtering of the water through 41 μm plankton gauze. *Encentrum mucronatum* Wulfert, 1936 was found in wet moss cushions in a pine forest. A handful of moss was collected and squeezed out into petri dishes. Individual specimens of both species were picked out from the raw samples under a stereomicroscope. Observations of living specimens under a Leica DM-LB light microscope were carried out at lower to medium magnifications using bright field. Digital images were taken with an Olympus color view I digital camera. For SEM preparations of *D. forcipatus*, specimens were anaesthetised in a 0.25% solution of bupivacaine (Bucain®), fixed in PAF (picric acid–formaldehyde, 239 mOsm), dehydrated in an increasing ethanol series, critical point-dried, coated with gold and observed under a Zeiss DSM 940 scanning electron microscope. For cLSM preparations, specimens of both species were anaesthetised in a 0.25% solution of bupivacaine (Bucain®). Fixation for 1 h (4 °C) was carried out with freshly prepared 4% formaldehyde buffered in 0.1 M PBS. After rinsing in 0.1 M PBS, specimens over night were made permeable by incubation in a 0.1% solution of Triton X-100 buffered in 0.1 M PBS. For staining, 2 μl of 38 μM methanolic TRITC phalloidin solution were added to 100 μl of Triton X-100 buffered in 0.1 M PBS. Specimens were stained for 3 h. After staining, individual specimens were embedded in Cityfluor® and observed under a Leica TCS SP 5 confocal laser scanning microscope at an excitation wavelength of 488 nm. In total, seven specimens of *E. mucronatum* and nine specimens of *D. forcipatus* were analysed. For analysis of the image stacks, the programmes ImageJ 1.37v (Abramoff et al. 2004; Rasband 1997–2007) and Voxx 2.09d (Clendenon et al. 2002) were used. The digital drawings for this study were made with Adobe Illustrator® 10.0.

3. 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 several image stacks of both species. The overall aim of our study is to discover the general pattern of body musculature in the two species. Only unambiguous signals have 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 multiple cells arranged consecutively along the axis of muscle extension. For some muscles, individual cells are recognisable by distinct gaps between stained actin filaments (see Fig. 4A). Moreover, some longitudinal muscles appear to consist of more than one strand of stained actin filament bundles lying parallel to each other (see Fig. 6E). However, we do 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.

In order to communicate new findings, new structures have to be labelled. We have decided to label muscles according to their general orientation in the specimen (longitudinal vs. circular) and simply number those similarly oriented. As for the circular muscles, numbering proceeds from head to foot. Longitudinal muscles are numbered from ventral to dorsal side. We have refrained from labelling muscles according to assumed functions however obvious the function of some muscles might appear. Functional considerations are given in the appropriate place in the discussion section. Individual names have exclusively been assigned to muscles that, on the basis of our data and data obtained in previous studies on other rotifers, are likely to be homologous in different species (see discussion). Future studies on further dicranophorid rotifers and other rotifer species are expected to improve our knowledge of rotifer body musculature and successively replace preliminary numbers by individual names.

4. Results

4.1. Body organisation of *Encentrum mucronatum* and *Dicranophorus forcipatus*

Both *D. forcipatus* (Figs. 1A, 2A) and *E. mucronatum* (Figs. 1B, 2B) are monogonont rotifers with a characteristic body organisation comprising head with the frontal rotatory organ, trunk, foot and toes. In both species the rotatory organ, consisting of a ciliated buccal field, is tilted ventrally, to a lesser degree in *E. mucronatum* and to a stronger in *D. forcipatus*. Differences between the two species lie in the relative degree of flexibility of the trunk integument: While in *E. mucronatum*, the trunk integument is soft and very flexible with transverse folds depending on the degree of contraction of the specimen, in *D. forcipatus* it is slightly stiffened. In total, there are four integumentary plates (one on the dorsal, one on the ventral and one on each lateral side), each delimited by infoldings of the integument (sulci, Fig. 1C and D). When specimens of *D. forcipatus* contract, the sulci can be seen to slightly widen up. Protraction of specimens is accompanied by a narrowing of the sulci.

4.2. Somatic musculature of *Encentrum mucronatum*

4.2.1. Circular muscles (Figs. 2b, 3, 4)

In total, seven circular muscles directly underlying the integument have been identified (musculi circulares I–VII). Two of these circulars are complete with no interruptions (musculi circulares I and VII). The others are incomplete to different degrees. While the musculus circularis I (pars coronalis) is wide and conspicuous, the other circular muscles are fine and not easy to detect.

4.2.1.1. Musculus circularis I (pars coronalis). This frontalmost circular muscle is an uninterrupted ring in the frontal section of the head. In its course, it roughly follows the outline of the ciliated buccal field of the rotatory apparatus.

4.2.1.2. Musculus circularis II. This circular muscle is incomplete and interrupted both ventrally and dorsally. Seen from lateral, it is positioned in the neck region and appears to be associated with a transverse fold in the integument.

4.2.1.3. Musculus circularis III. This circular muscle is interrupted both ventrally and dorsally as well. It is positioned in the trunk caudal to the border of neck and trunk.

4.2.1.4. Musculi circulares IV–VI. These muscles are incomplete rings with a ventral interruption. The musculus circularis IV is located in the frontal section and the musculus circularis V in the middle section of the trunk associated with a fold in the integument. The musculus circularis VI is positioned in the caudal third of the trunk, similarly associated with an integumentary fold. Compared to the musculi circulares IV and V, the musculus circularis VI extends over only a short distance across the lateral sides of the specimen.

4.2.1.5. Musculus circularis VII (m. circumpedalis). The musculus circularis VII is the most caudally positioned circular muscle in *E. mucronatum*. Unlike the incomplete trunk circulars, it forms a complete ring at the caudal end of the foot.

4.2.2. Longitudinal muscles (Figs. 2b, 3, 4)

In total, six longitudinal muscles have been identified (musculi longitudinales I–VI). All of these muscles are paired. Some muscles extend from the head all the way through the trunk to the foot (musculi longitudinales I, III, VI), while others are shorter and connect head and trunk or are restricted to the trunk region (musculi longitudinales II, IV, V). The strongest and most conspicuous longitudinal muscle is the paired musculus longitudinalis I (m. longitudinalis ventralis).

4.2.2.1. Musculus longitudinalis I (m. longitudinalis ventralis). This muscle is the ventralmost longitudinal muscle. Frontally in the head, it is anchored to the pars coronalis, caudally in the foot to the musculus circumpedalis.
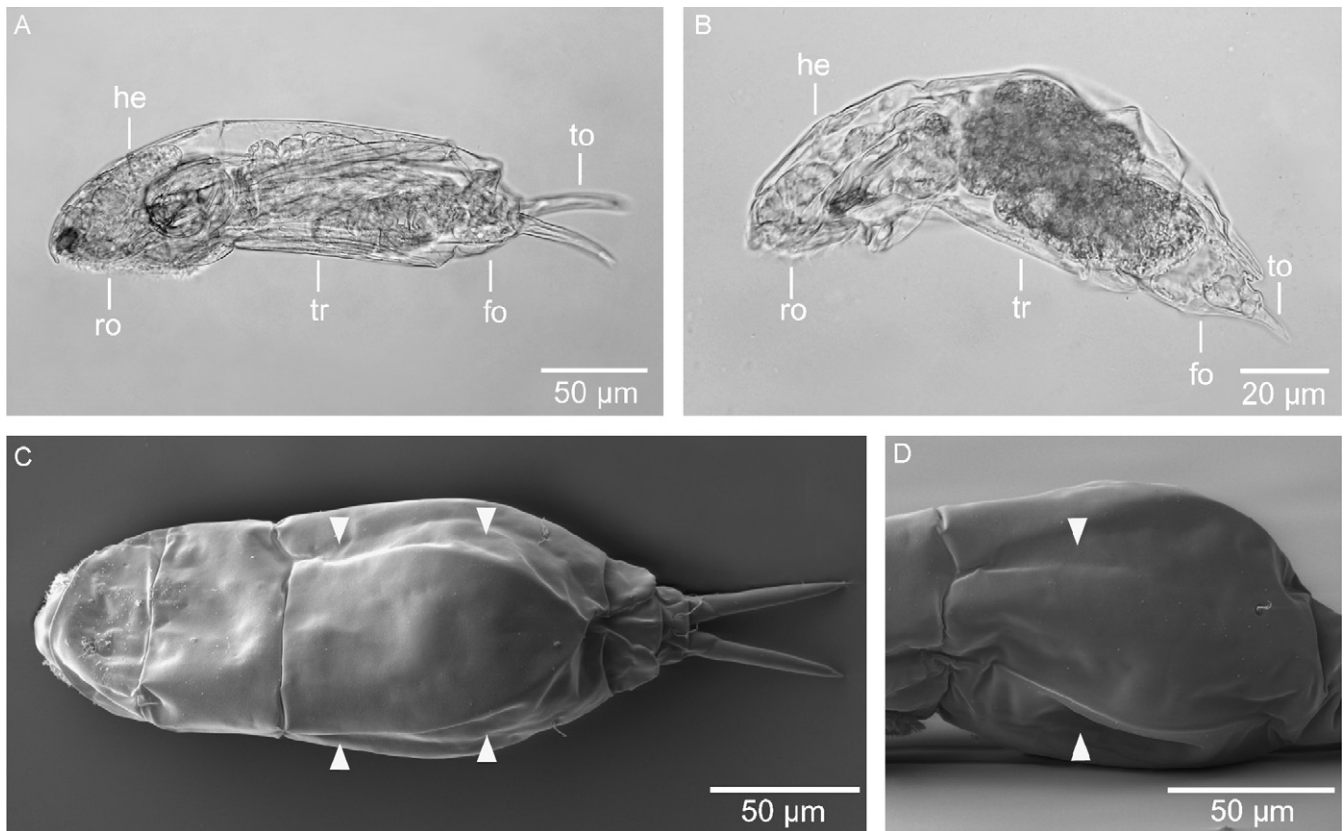
Fig. 1. *Dicranophorus forcipatus* (A, C, D), *Encentrum mucronatum* (B): (A) light micrograph (brightfield) of a living specimen in lateral view; (B) light micrograph (brightfield) of a living specimen in lateral view; (C) SEM preparation of specimen in dorsal view, arrowheads indicate infoldings of integument (sulci); (D) SEM preparation of specimen, showing trunk in lateral view; arrowheads indicate lateral sulci. fo = foot, he = head, ro = rotatory organ, to = toes, tr = trunk.

4.2.2.2. Musculus longitudinalis II. This muscle extends from the frontal section of the head (pars coronalis) down to the caudal end of the trunk, where it converges with the musculus longitudinalis ventralis. It is not quite clear whether the two muscles actually fuse or whether the musculus longitudinalis II attaches to the body wall.

4.2.2.3. Musculus longitudinalis III. Similar to the musculus longitudinalis ventralis, the musculus longitudinalis III traverses the whole length of the specimen. Frontally in the head, it splits off into numerous fine filaments inserted on the lateral body wall. Caudally, it attaches to the musculus circumpedalis.

4.2.2.4. Musculus longitudinalis IV. The musculus longitudinalis IV differs from all other longitudinal muscles by its relative shortness. It is restricted to the trunk where it extends from the musculus circularis IV to the musculus circularis VI.

4.2.2.5. Musculus longitudinalis V (musculus longitudinalis capitis). The musculus longitudinalis V is again a fairly short muscle connecting head and trunk. Fron-

tally, it is attached to the pars coronalis. It extends caudally up to the musculus circularis IV. In lateral and dorsal view, the musculus longitudinalis V crosses the musculus longitudinalis dorsalis at an acute angle.

4.2.2.6. Musculus longitudinalis VI (m. longitudinalis dorsalis). The musculus longitudinalis VI stretches through the whole length of the specimen. Frontally, it is attached to the pars coronalis. Caudally, it is anchored to the musculus circumpedalis.

4.3. Somatic musculature of *Dicranophorus forcipatus*

4.3.1. Circular muscles (Figs. 2a, 5, 6)

In total, eight circular muscles associated with the body wall have been identified (musculi circulares I–VIII). With the exception of one (musculus circularis VIII), all of them are incomplete and composed either of two or more subunits. The circular muscles in *D. forcipatus* markedly differ in width with some of them appearing as narrow strands (musculi circulares II and
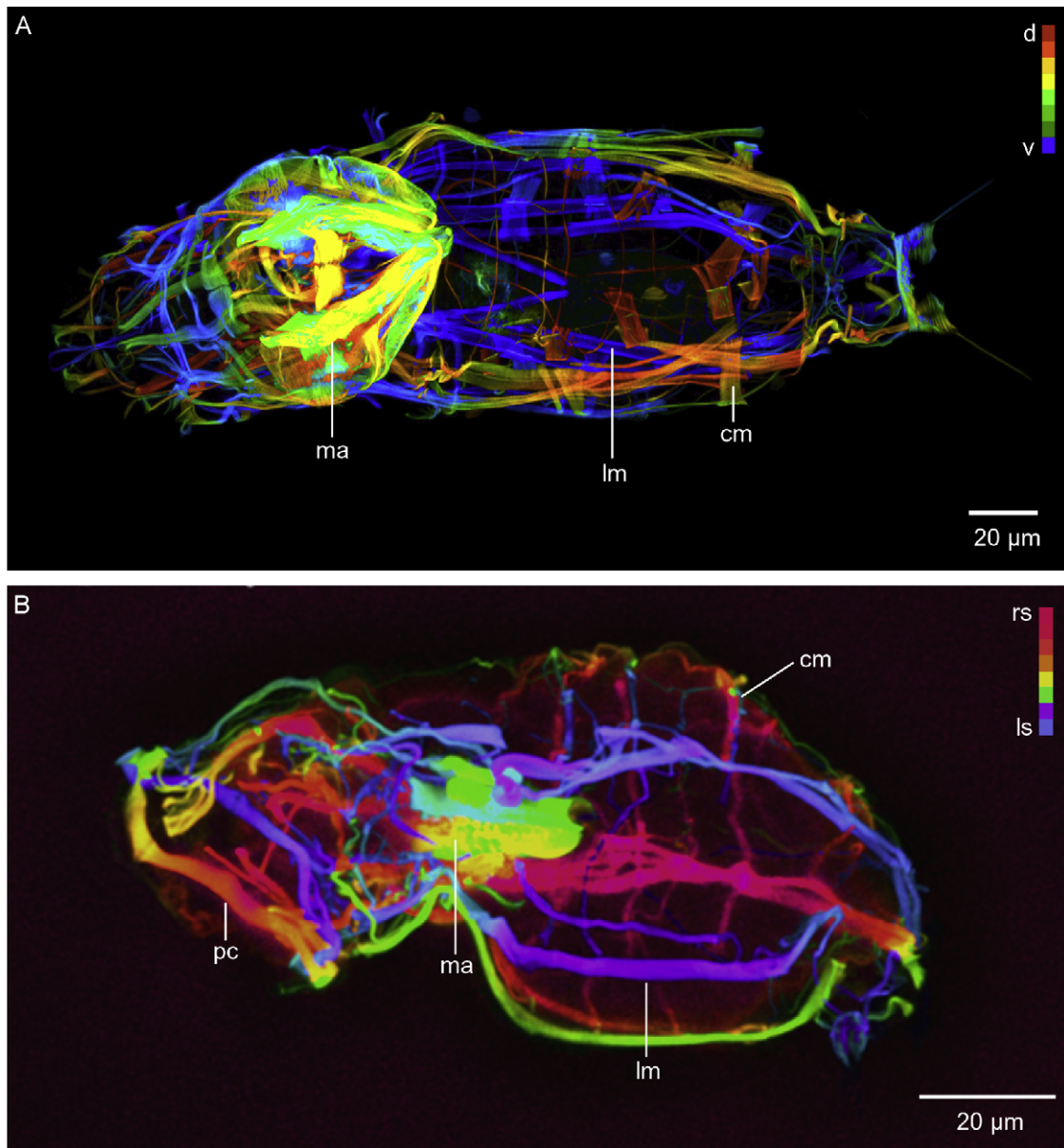
Fig. 2. Depth-coded maximum projections of *Dicranophorus forcipatus* (A) and *Encentrum mucronatum* (B). For detailed labelling of individual muscles see Figs. 4 and 6. cm = circular muscle, d = dorsal, lm = longitudinal muscle, ls = left side of specimen, ma = mastax, pc = pars coronalis, rs = right side of specimen, v = ventral.

III) and others as broad muscular layers (musculi circulares I, IV–VII).

4.3.1.1. Musculus circularis I. This frontalmost circular muscle is the only circular muscle in the head. It comprises two short, dorso-lateral subunits divided by wide ventral and dorsal interruptions.

4.3.1.2. Musculus circularis II. This ventro-lateral circular muscle is positioned in the frontal region of the trunk. It consists of two narrow subunits ventrally divided by a wide median interruption. Laterally, the two subunits on either side run up to the middle of the trunk.

4.3.1.3. Musculus circularis III. This circular muscle follows the musculus circularis II caudally. It also consists of two narrow subunits divided by a ventral and dorsal interruption, but, compared to the musculus circularis II, the two subunits each stretch along a greater distance towards the dorsal side of the specimen.

4.3.1.4. Musculi circulares IV–VI. Each of these circular trunk muscles consists of four short, massive subunits separated from each other by wide interrup-
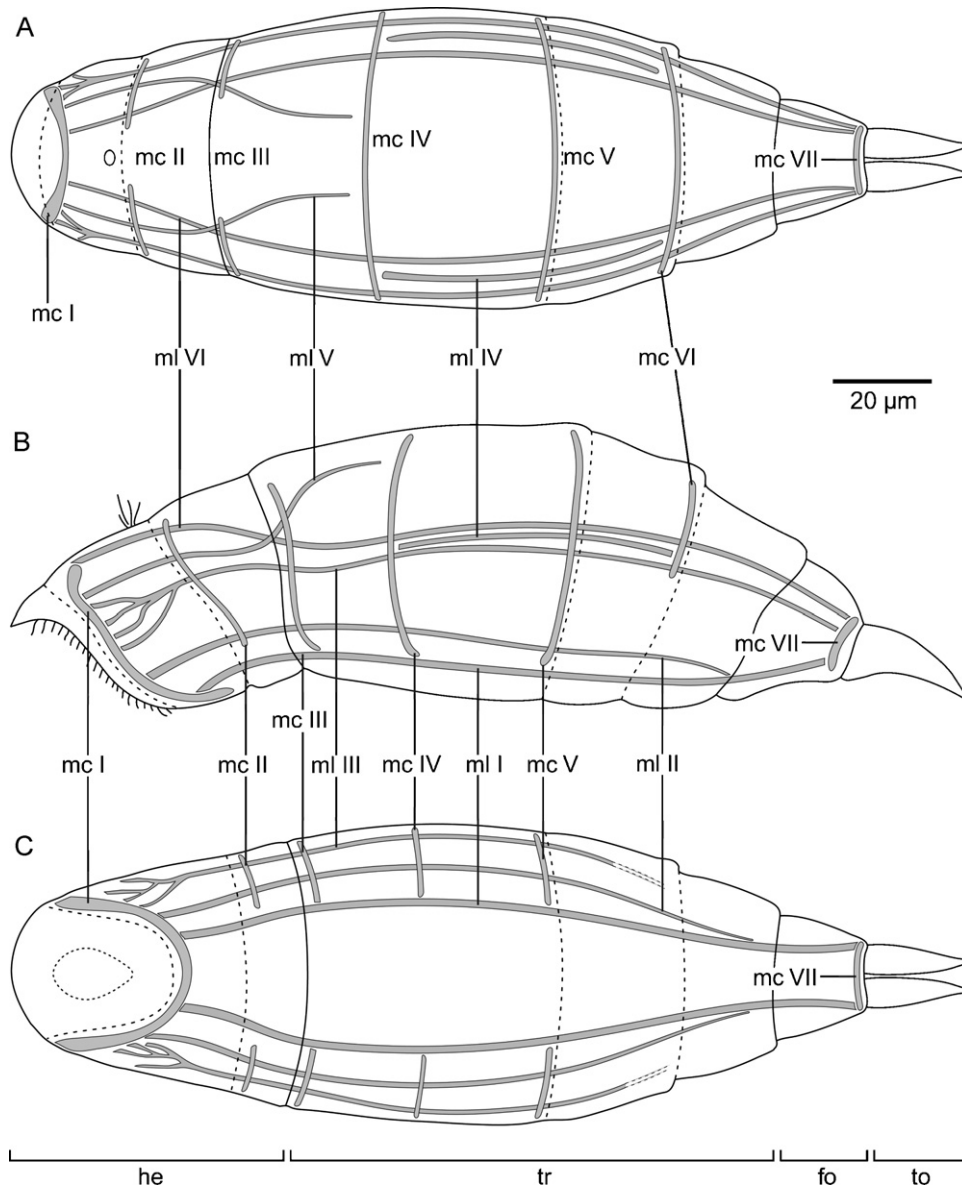
Fig. 3. Diagrammatic drawings of somatic musculature in *Encentrum mucronatum*: (A) dorsal view; (B) lateral view; (C) ventral view. fo = foot, he = head, mc I–VII = musculi circulares I–VII, ml I–VI = musculi longitudinales I–VI, to = toes, tr = trunk.

tions. Two of the subunits are positioned ventro-laterally in the trunk, the others dorso-laterally. They appear to be associated with the ventro-lateral and dorso-lateral sulci (see discussion).

4.3.1.5. Musculus circularis VII. This circular muscle, positioned caudally in the trunk, differs from the other trunk circulars in consisting of five subunits in total. Two of them are located ventro-laterally, two dorso-laterally, where they appear to be associated with the sulci of the trunk. Additionally, there is a fifth, dorsally positioned, unpaired muscle segment between the two dorso-lateral ones.

4.3.1.6. Musculus circularis VIII (m. circumpedalis). The musculus circularis VIII is the most caudally positioned circular muscle in *D. forcipatus*. It is a complete, massive muscular ring situated at the junction of foot and toes.

4.3.2. Longitudinal muscles (Figs. 2a, 5, 6)

In total, nine longitudinal muscles could be identified (musculi longitudinales I–IX). All are paired. The longitudinal muscles widely differ in length ranging from extremely long muscle strands running all the way through the trunk from head to foot (musculus longitudinalis VIII) to short, massive muscles restricted to the foot (musculus longitudinalis VI). Some of the longitudinal muscle strands conspicuously split off into a multitude of fine filaments in the head.
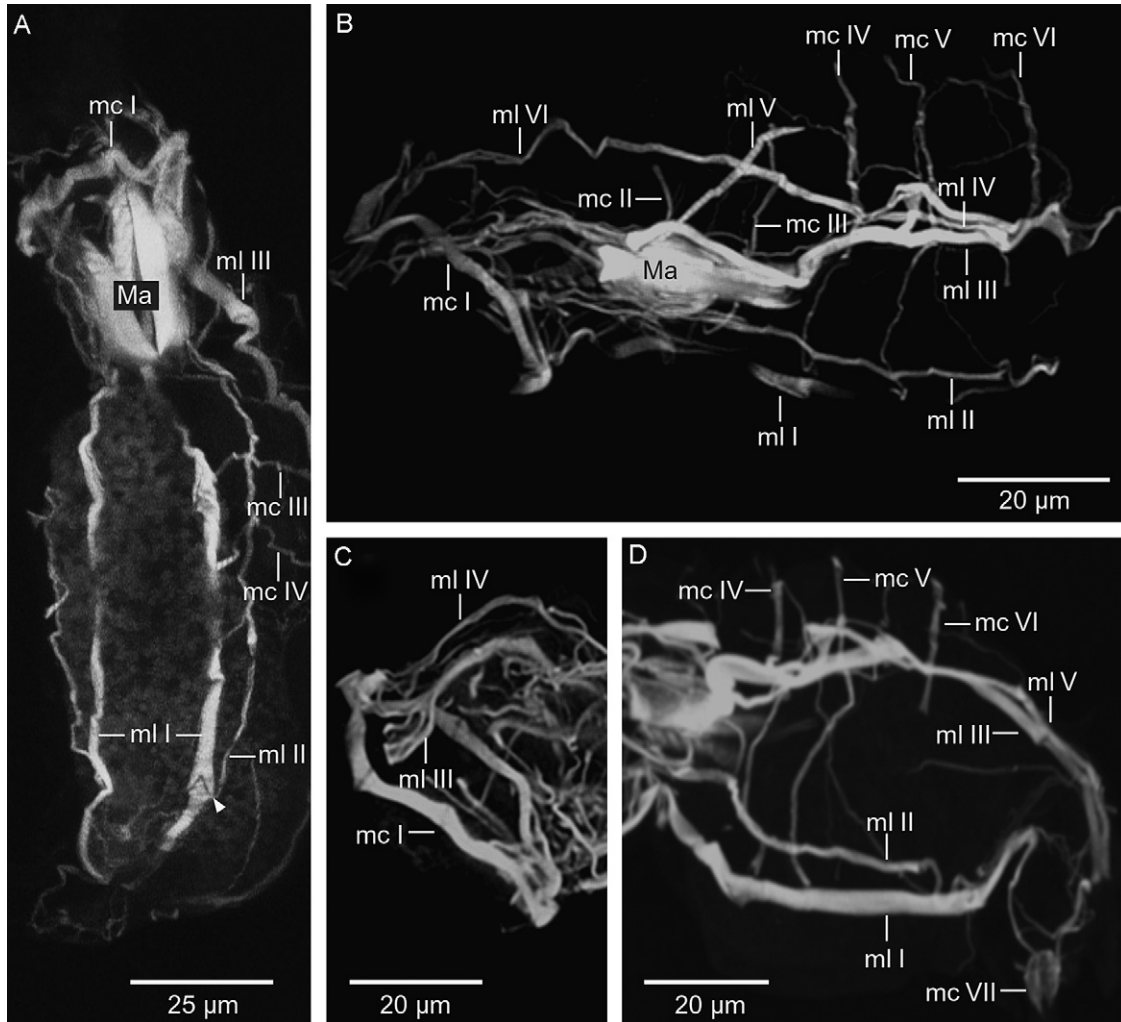
Fig. 4. Fluorescence signals of phalloidin-stained actin filaments in *Encentrum mucronatum*. Projections of selected sections along *z*-axis: (A) specimen in ventro-lateral view. Arrowhead indicates borderline between distinct bundles of stained actin filaments; (B) specimen in lateral view; (C) head of specimen in lateral view; (D) trunk of specimen in ventro-lateral view. Ma = mastax, mc I–VII = musculi circulares I–VII, ml I–VI = musculi longitudinales I–VII.

4.3.2.1. Musculus longitudinalis I. This muscle consists of a ventral pair of longitudinal muscles. Frontally, their signal could be detected up to the mastax. Caudally, the two muscles of this pair converge without, however, complete fusion and terminate in the frontal section of the trunk.

4.3.2.2. Musculus longitudinalis II (m. longitudinalis ventralis). The musculus longitudinalis II is a ventral pair of massive longitudinal muscles running from the border of neck and trunk through the trunk up to the caudal section of the foot, where they attach to the musculus circumpedalis.

4.3.2.3. Musculus longitudinalis III. The musculus longitudinalis III is a paired, ventro-lateral longitudinal muscle connecting head and trunk. It frontally terminates on the body wall at the border of the antenna bearing pseudosegment and the neck. Its caudal end is just frontal to the musculus circularis VII in the trunk.

4.3.2.4. Musculus longitudinalis IV. This is a ventro-lateral pair of longitudinal muscles restricted to the trunk, where they insert between the musculus circularis V and VI and continue up to the borderline separating trunk and foot.

4.3.2.5. Musculus longitudinalis V. The musculus longitudinalis V is a paired, lateral longitudinal muscle positioned in the frontal half of the trunk. Frontally, it is anchored to the body wall in the head. More caudally, the paired musculus longitudinalis V bifurcates with both branches ending at the body wall between musculus circularis V and VI.
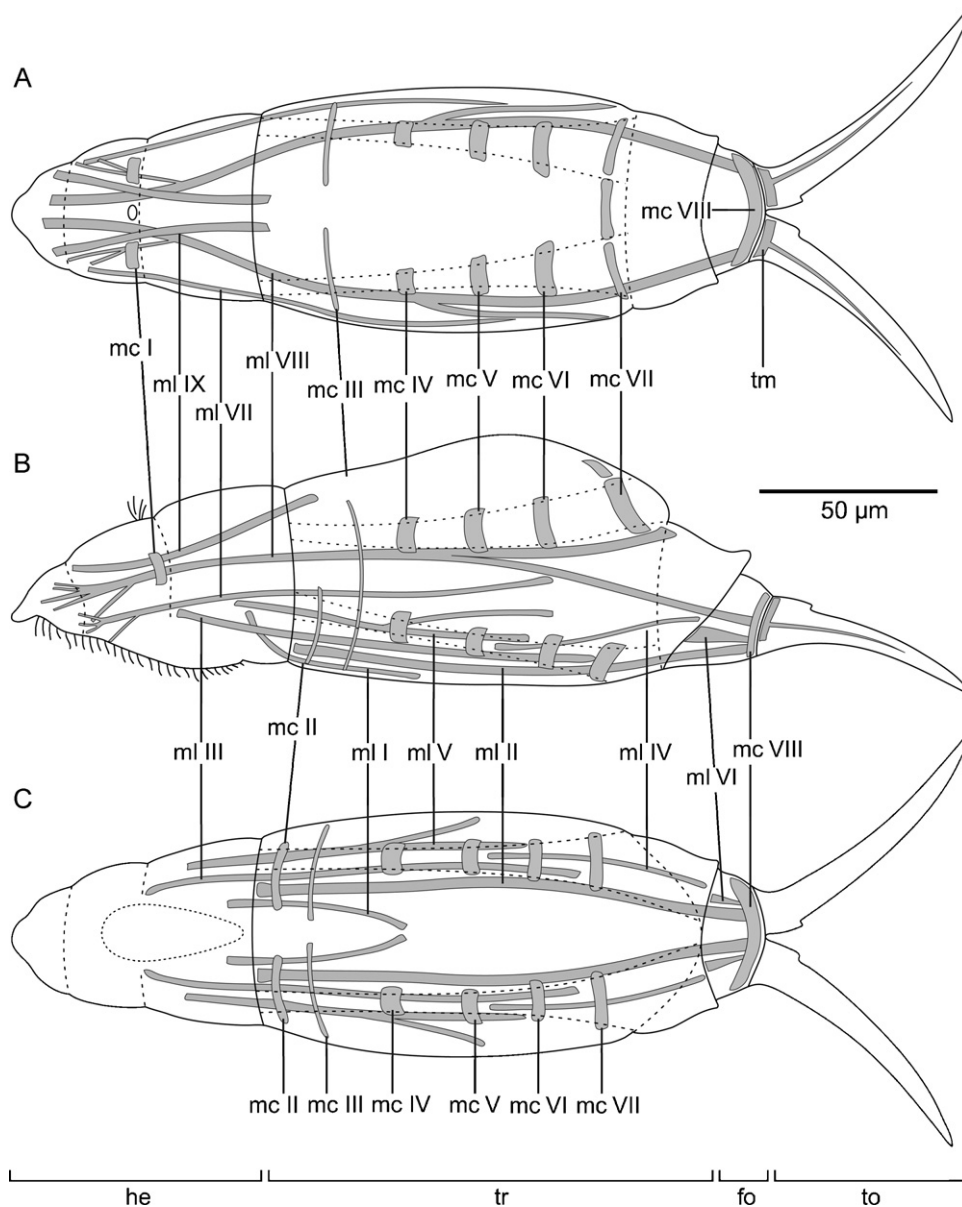
Fig. 5. Diagrammatic drawings of somatic musculature in *Dicranophorus forcipatus*: (A) dorsal view; (B) lateral view; (C) ventral view. Sulci delimited by dotted lines. fo = foot, he = head, mc I–VIII = musculi circulares I–VIII, ml I–IX = musculi longitudinales I–IX, tm = toe musculature, to = toes, tr = trunk.

4.3.2.6. Musculus longitudinalis VI. Unlike the other longitudinal muscles, the musculus longitudinalis VI is remarkably short. With its frontal end on the body wall at the junction of trunk and foot and its caudal attachment to the musculus circumpedalis, it traverses only the foot.

4.3.2.7. Musculus longitudinalis VII. The musculus longitudinalis VII is a paired, lateral muscle connecting head and trunk. Frontally, the two muscles of this pair branch off into numerous fine filaments. Traversing the border between head and trunk, they caudally terminate on the body wall between musculus circularis V and VI.

4.3.2.8. Musculus longitudinalis VIII (musculus longitudinalis dorsalis). The musculus longitudinalis VIII is a paired, dorso-lateral longitudinal muscle connecting head and trunk. In a similar manner to the musculus longitudinalis VII, the two muscles of this pair frontally divide up into smaller filaments. In their course through the trunk, each of the two muscles splits off into two branches with one branch anchored in the body wall slightly caudal to the musculus circularis VII and the
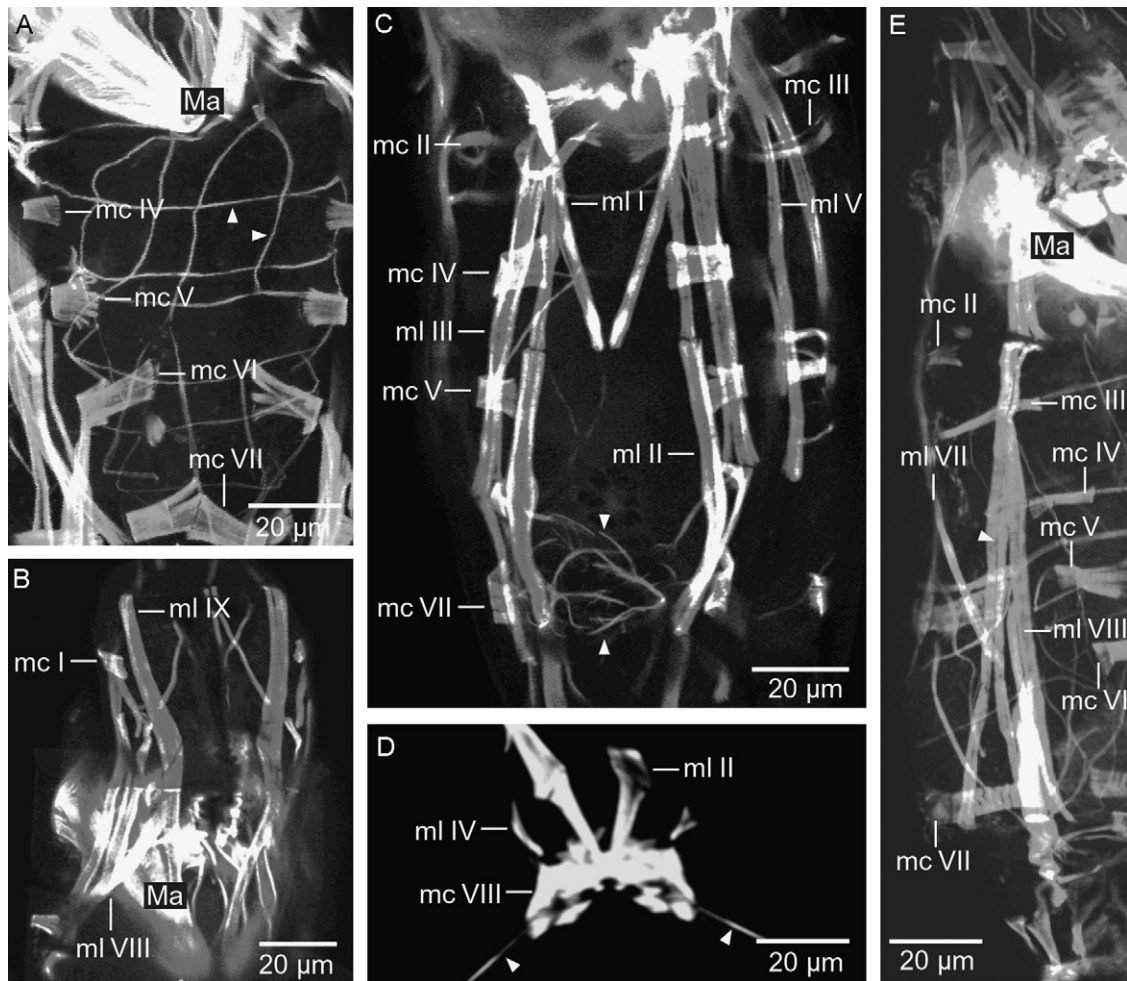
Fig. 6. Fluorescence signals of phalloidin-stained actin filaments in *Dicranophorus forcipatus*. Projections of selected sections along *z*-axis: (A) detail of trunk in dorsal view. Arrowheads indicate circular and longitudinal muscle fibers of visceral musculature surrounding the gastric tract; (B) detail of head in dorsal view; (C) detail of trunk in ventral view. Arrowheads indicate fine muscular filaments associated with protonephridial bladder; (D) detail of foot in ventral view. Arrowheads indicate fluorescence signal in toes; (E) detail of left side of specimen in dorsal view. Arrowhead indicates parallel actin filaments. Ma = mastax, mc I–VIII = musculi circulares I–VIII, ml I–IX = musculi longitudinales I–IX.

other continuing up to the foot, where it attaches to the musculus circumpedalis.

4.3.2.9. Musculus longitudinalis IX (musculus longitudinalis capitis).
This paired muscle is one of the shortest longitudinal muscle strands spanning head and trunk. The two muscles of this pair run from the frontal section of the head up to the musculus circularis II in the trunk, where they converge dorsally and insert on the body wall. In dorsal view, the musculus longitudinalis IX crosses the musculus longitudinalis dorsalis at an acute angle.

4.3.2.10. Musculature associated with base of toes.
At the base of both toes, a fluorescent signal consisting of a basal bundle of stained actin and a fine filament drawing distally could be detected. These elements of muscula-

ture seem to be in connection with the musculus circumpedalis.

4.4. Visceral musculature and musculature associated with the protonephridial bladder

Apart from somatic circular and longitudinal muscles, both *D. forcipatus* and *E. mucronatum* display a system of circular and longitudinal visceral musculature. In *D. forcipatus*, about six fine longitudinal and six circular muscle strands could be detected (Fig. 6A). In *E. mucronatum*, the signal of the individual muscles around the gastro-intestinal tract was too weak to be verified unequivocally across all specimens investigated. In both species investigated, the mastax musculature gives a very strong fluorescence signal (Figs. 4A, B, 6A, B, E). However, this specific musculature is not the focus of our study and is hence omitted in the reconstructions

(for details of mastax musculature in *D. forcipatus*, see Riemann and Ahlrichs, 2008). Moreover, in *D. forcipatus* an intricate net of fine muscle strands has been found associated with the protonephridial bladder (Fig. 6C).

5. Discussion

5.1. Somatic musculature in *Dicranophorus forcipatus*, *Encentrum mucronatum* and across Rotifera

Both *D. forcipatus* and *E. mucronatum* have a system of body musculature consisting of complete and incomplete circular and longitudinal muscles. The circular muscles constitute the outer and the longitudinal muscles the inner system. Studies of body musculature in representatives of the major clades within Rotifera (Hemirotifera and Monogononta *sensu* Sørensen and Giribet 2006) have demonstrated a universal distribution of a similar system of outer, incomplete circular and inner longitudinal muscles (Martini 1912: *Epiphanes senta*; Stoßberg 1932: *Euchlanis, Rhinoglena frontalis*; Ahlrichs 1995: *Seison nebaliae, Seison annulatus*; Hochberg and Litvaitis 2000: *Philodina* sp.; Kotikova et al. 2001: *Euchlanis dilatata unisetata, Brachionus quadridentatus*; Sørensen et al. 2003: *Notholca acuminata*; Santo et al. 2005: *Brachionus urceolaris, Floscularia ringens, Hexarthra mira, Notommata glyphura*; Sørensen 2005a: *Proales* spp.; Sørensen 2005b: *Testudinella patina*; Hochberg and Gurbuz 2007: *Filinia novaezealandiae*). Hence, this general organisation of body musculature must be considered a ground pattern feature of Rotifera. Although different in detail, the systems of circular and longitudinal muscles can be assumed to be homologous in all rotifer species.

According to traditional classifications (summarised in Hyman 1951), rotifer somatic musculature consists of ventral, dorsal, central and lateral longitudinal muscles functioning as corona and foot retractors and complete or incomplete, subepidermal muscular rings. The central head retractors insert on the dorsal body wall and run frontally, where they are anchored in the head. The dorsal retractors are either continuous and run the whole length of the specimen from head to foot or are broken up into distinct head and foot retractors. The lateral retractors are usually only developed as head retractors and the ventral retractors as continuous bands running the whole length of the specimen.

5.2. Ventral longitudinal muscles

Paired ventral longitudinal muscles have been identified in *Proales daphnicola*, *Proales fallaciosa* and *Proales*

reinhardti (ventral trunk retractors, Sørensen 2005a). *N. acuminata* was also shown to have a pair of ventral longitudinal muscles which, however, do not extend through the whole trunk, but terminate in the caudal third of the trunk (ventral trunk retractor, Sørensen et al. 2003). In *N. glyphura*, several ventral longitudinal muscles were demonstrated (ventral retractor of corona, median retractor of corona, Santo et al. 2005). *B. urceolaris* was also shown to have a pair of ventral longitudinal muscles (ventral retractor of corona, Santo et al. 2005) as was the sessile species *F. ringens* with ventral longitudinal muscles running from the four-lobed corona through the trunk and into the foot (ventral muscle pair, Santo et al. 2005). *Philodina* sp. was demonstrated to have several longitudinal muscles (Hochberg and Litvaitis 2000). While some function as head and foot retractors and do not extend from the head through the trunk down to the foot, others span the whole length of the specimen. Ventrally, a single pair of longitudinal muscles spanning the whole specimen exists.

D. forcipatus and *E. mucronatum* also have a ventral pair of longitudinal muscles (musculus longitudinalis I in *E. mucronatum*; musculus longitudinalis II in *D. forcipatus*). These longitudinal muscles traverse the whole length of the trunk and are caudally attached to the musculus circumpedalis at the caudal end of the foot.

The presence of an uninterrupted pair of ventral longitudinal muscles running from head to foot has thus been demonstrated for Monogononta (Ploima and Gnesiotrocha) and Hemirotifera, the two sister taxa of highest rank within Rotifera and can reasonably be assumed to be a ground pattern feature of Rotifera. This given, the paired ventral longitudinal muscles are most probably homologous in all rotifers. We have decided to term them musculi longitudinales ventrales (sg. musculus longitudinalis ventralis). For *D. forcipatus* and *E. mucronatum*, the presence of paired ventral longitudinal muscles has to be considered plesiomorphic.

5.3. Dorsal longitudinal muscles

P. fallaciosa, *P. reinhardti* and *P. daphnicola* possess dorso-lateral longitudinal muscles (dorsal trunk retractors, Sørensen 2005a). In *P. daphnicola*, they do not extend down to the foot but terminate in a bifurcation in the caudal third of the trunk. A dorsal pair of longitudinal muscles is also present in *N. acuminata* (dorsal retractor, Sørensen et al. 2003). Like the ventral pair of longitudinal muscles in this species, the muscles of the dorsal pair terminate in the middle of the trunk. In *N. glyphura*, different systems of paired longitudinal muscles have been found (median retractor of corona, ventral retractor of corona, lateral retractor of corona,

Santo et al. 2005). Given the dorsalmost position of the paired lateral retractor of the corona, it might correspond to the pair of dorso-lateral longitudinal muscles demonstrated for other rotifer species. *B. urceolaris* has a pair of dorsal longitudinal muscles terminating in the middle of the trunk (dorsal retractor of corona, Santo et al. 2005). The sessile species *F. ringens* has a pair of dorsal longitudinal muscles running from the corona through the trunk and into the foot (dorsal muscle pair, Santo et al. 2005). *Philodina* sp. was also demonstrated to have several longitudinal muscles (Hochberg and Litvaitis 2000). Dorsally, a single pair of longitudinal muscles spanning the whole specimen has been shown to exist.

In *D. forcipatus* and *E. mucronatum* a pair of dorso-lateral longitudinal muscles (musculus longitudinalis VI in *E. mucronatum*, musculus longitudinalis VIII in *D. forcipatus*) is present as well. In both species, these dorso-lateral muscles run all the way from head to trunk and are caudally attached to the musculus circumpedalis.

Thus, the presence of a pair of dorsal or dorso-lateral longitudinal muscles has been demonstrated for Monogononta (Ploima and Gnesiotrocha) and Hemirotifera. Similar to the paired ventral longitudinal muscles, the paired dorso-lateral longitudinal muscles are a ground pattern feature of Rotifera. We treat these paired dorso-lateral muscles as homologues and call them musculi longitudinales dorsales (sg. musculus longitudinalis dorsalis). The presence of the musculi longitudinales dorsales in *D. forcipatus* and *E. mucronatum* has to be considered a plesiomorphic character.

5.4. Central longitudinal muscles

Paired dorsal muscles connecting head and trunk have been identified in *P. daphnicola*, *P. fallaciosa* and *P. reinhardti* (dorsal head retractors, Sørensen 2005a) and, possibly equivalent, in *N. acuminata* (Sørensen et al. 2003). They are reported in *E. senta* as well (Martini 1912). *E. mucronatum* and *D. forcipatus* also display a pair of fairly short, dorso-laterally positioned longitudinal muscles connecting head and trunk. They terminate in the frontal section of the trunk (musculus longitudinalis V in *E. mucronatum*; musculus longitudinalis IX in *D. forcipatus*). In both species, these muscles cross the musculi longitudinales dorsales at an acute angle. Such a crossing of dorsal longitudinal muscles appears to be present in *P. daphnicola* and *P. fallaciosa* as well (Sørensen 2005a). We argue for a homology of these paired dorsal longitudinal muscles and call them musculi longitudinales capitum (sg. musculus longitudinalis capitis). Data on this pair of muscles in other rotifer species is uncertain. It may have evolved in the stem lineage of Monogononta, but,

without more complete data, phylogenetic evaluations have to be treated with caution.

5.5. Lateral longitudinal muscles

Lateral longitudinal muscles have been identified in almost all rotifer species thus far investigated, including *D. forcipatus* and *E. mucronatum* as representatives of Dicranophoridae. Other than in the case of the dorsal, ventral and central systems of longitudinal muscles, where assumptions of homology are reasonably well supported, no clear patterns are discernible in the system of lateral longitudinal muscles. Attempting to find muscles in other rotifer species equivalent to the musculi longitudinales III–VII in *D. forcipatus* and musculi longitudinales II–IV in *E. mucronatum* would be highly speculative at the moment. Possibly, a larger taxon sampling might help tackle this problem.

5.6. Circular muscles

While paired ventral and dorsal longitudinal muscles have been identified in all rotifer species thus far studied, it is much more difficult to find a corresponding pattern in the incomplete circular muscles. The number of circular muscles varies widely across the species investigated from only 5 (including pars coronalis and corona sphincter, see below) in *N. acuminata* (Sørensen et al. 2003) to up to 14–16 in *Philodina* sp. (Hochberg and Litvaitis 2000). Differences in trunk circulars between *D. forcipatus* and *E. mucronatum* lie in their relative width and length, position and degree of completeness. While in *E. mucronatum*, the trunk circulars are fairly slender and uninterrupted over a considerable length, they are massive muscular belts in *D. forcipatus* with wide gaps between the individual segments of each belt (except for musculi circulares II and III).

Several species, among them *E. mucronatum* (this study), share the presence of a circular muscle associated with the margin of the rotatory organ [Pars coronalis: *E. senta* (Martini 1912); *N. acuminata* (Sørensen et al. 2003); *P. daphnicola*, *P. fallaciosa*, *P. reinhardti* (Sørensen 2005a, b); pars coronaria oralis: *R. frontalis*, *Brachionus pala*, *Euchlanis pellucida* (Stoßberg 1932)]. The distribution of this muscle across the species thus far investigated suggests that it evolved either at the base of Monogononta or Ploima depending on whether or not a pars coronalis is present in representatives of Gnesiotrocha.

Within Ploima, the presence of a broad circular muscle caudal to the rotatory organ has been demonstrated [Sphincter coronalis: *E. senta* (Martini 1912); *B. pala*, *R. frontalis* (Stoßberg 1932); *B. quadridentatus* (Kotikova et al. 2001); *N. acuminata* (Sørensen et al.

2003); *F. ringens* (Santo et al. 2005); *P. daphnicola*, *P. reinhardti* (Sørensen 2005a)]. The corona sphincter is apparently absent in *D. forcipatus* and *E. mucronatum*. Given the distribution of the corona sphincter in representatives of Ploima and Gnesiotrocha, it is plausible to assume that it evolved in the stem lineage of Monogononta and may have been lost secondarily at the base of Dicranophoridae.

D. forcipatus and *E. mucronatum* share the possession of a complete circular muscle (musculus circularis VII in *E. mucronatum*, musculus circularis VIII in *D. forcipatus*) at the caudal end of the foot. Given the similar position and shape, we consider this muscle to be homologous in both species and term it musculus circumpedalis with reference to the foot. A complete circular muscle at the caudal end of the foot has been detected in *Euchlanis dilatata unisetata* and *B. quadridentatus* as well (Kotikova et al. 2001). *P. reinhardti* also has a circular muscle in the caudalmost section of the foot (transverse toe muscle, Sørensen 2005a). Unlike the musculus circumpedalis in *D. forcipatus* and *E. mucronatum*, the transverse toe muscle does not form a complete muscular ring. However, data on this muscle is at present very incomplete. Although we consider it probable that the musculus circumpedalis is part of the ground pattern of Monogononta, any final phylogenetic assessment would as yet be premature.

5.7. Functional considerations

In rotifers, the longitudinal and the circular muscles generally function as antagonists: While the longitudinal muscles serve as retractors of corona and foot, the circular muscles upon contraction increase the pressure of the body cavity fluid and are responsible for extension of the specimen (Hyman 1951; Hochberg and Litvaitis 2000; Santo et al. 2005). These general functions certainly also apply to the systems of longitudinal and circular muscles in *D. forcipatus* and *E. mucronatum*.

In *E. mucronatum*, the paired musculus longitudinalis ventralis, musculus longitudinalis dorsalis and musculus longitudinalis III probably serve as retractors of both head and foot. Attached to the pars coronalis frontally and the musculus circumpedalis caudally, their contractions withdraw the head and the foot into the trunk. The musculus longitudinalis capitis and the musculus longitudinalis II, attached to the pars coronalis frontally, terminate in the trunk and probably serve as head retractors only.

In *D. forcipatus*, the paired musculus longitudinalis dorsalis, running the whole length of the specimen, can be assumed to function as retractor of both head and foot. Involved in retraction of the foot are the musculus longitudinalis ventralis and the short musculus longitudinalis VI. Head retractors are the musculus long-

itudinalis capitis, the musculus longitudinalis VII and the musculus longitudinalis III.

Differences between *D. forcipatus* and *E. mucronatum* in shape and extension of the circular trunk muscles are probably related to differences in trunk integument. Integumentary plates and sulci present in *D. forcipatus* are absent in *E. mucronatum*. The integumentary plates in *D. forcipatus* confer a certain amount of rigidity to the specimen, which is completely lacking in the soft-bodied *E. mucronatum*. Thus, our observations on the differences in trunk circulars between the two species are in keeping with the general assumption that circular muscles are reduced in length in loricate species compared to the situation in illoricate ones (Hyman 1951). The segments of the muscular belts in *D. forcipatus* run across the sulci and are attached to the margins of each integumentary plate. In a similar manner to species with a distinct lorica (e.g. *Mytilina*, *Brachionus*, *Euchlanis*), the circular muscles in *D. forcipatus* may serve to bring the integumentary plates together, thus in effect contracting the trunk and exerting pressure on the body cavity.

6. Conclusions

Surveying the literature on rotifer body musculature and the results obtained in our study, we suggest that in the stem species of Ploima, the most species rich taxon within Rotifera, at least the following characters in the system of body musculature must have been present:

- Paired ventral longitudinal muscles (musculi longitudinales ventrales).
- Paired dorsal longitudinal muscles (musculi longitudinales dorsales).
- Paired dorsal muscles connecting head and trunk (musculi longitudinales capitum).
- Circular pars coronalis associated with the margin of the rotatory organ.
- Circular corona sphincter.
- Circular muscle in caudal section of foot (musculus circumpedalis).

For a more complete understanding of the evolution of rotifer body musculature, future studies are suggested to focus on the lateral longitudinal muscles. It appears very likely that, with more species investigated, a pattern across Rotifera becomes apparent. Moreover, attention should be paid to the circular muscles in order to identify possible homologues. Such an approach is likely to shed light on transformations of the circular trunk musculature in species with a rigid trunk integument and their correlations with the evolution of a strong lorica.

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References

Abramoff, M.D., Magelhaes, P.J., Ram, S.J., 2004. Image processing with ImageJ. Biophoton. Int. 11 (7), 36–42.

Ahlrichs, W.H., 1995. Ultrastruktur und Phylogenie von *Seison nebaliae* (Grube 1859) und *Seison annulatus* (Claus 1876). Cuvillier Verlag, Göttingen, 310pp.

Clendenon, J.L., Phillips, C.L., Sandoval, R.M., Fang, S., Dunn, K.W., 2002. Voxx: a PC-based, near real-time volume rendering system for biological microscopy. Am. J. Physiol. 282, 213–218.

De Smet, W.H., 1997. Rotifera 5: the Dicranophoridae. In: Dumont, H.J., Nogrady, T. (Eds.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, vol. 12. SPB Academic Publishing BV, The Hague, The Netherlands, pp. 1–325.

Giribet, G., Distel, D.L., Polz, M., Sterrer, W., Wheeler, W.C., 2000. Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. Syst. Biol. 49, 539–562.

Hochberg, R., Gurbuz, O.A., 2007. Functional morphology of somatic muscles and anterolateral setae in *Filinia novaezealandiae* Shiel and Sanoamuang, 1993 (Rotifera). Zool. Anz. 246, 11–22.

Hochberg, R., Litvaitis, M.K., 2000. Functional morphology of the muscles in *Philodina* sp. (Rotifera: Bdelloidea). Hydrobiologia 432, 57–64.

Hyman, L.H., 1951. The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The Pseudocoelomate Bilateria. McGraw-Hill Book Company, New York, USA, pp. 1–572.

Kotikova, E.A., Raikova, O.J., Flyatchinskaya, L.P., Reuter, M., Gustafsson, M.K.S., 2001. Rotifer muscles as revealed by phalloidin-TRITC staining and confocal scanning laser microscopy. Acta Zool. 82, 1–9.

Martini, E., 1912. Studien über die Konstanz histologischer Elemente. III. *Hydatina senta*. Z. wiss. Zool. 102, 425–645.

Rasband, W.S., 1997–2007. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA ⟨http://rsb.info.nih.gov/ij/⟩.

Remane, A., 1929–1933. Rotatoria. In: Bronn's Klassen und Ordnungen des Tier-Reichs Bd. 4, Abt. II/1: 1–577.

Rieger, R.M., Tyler, S., 1995. Sister-group relationship of Gnathostomulida and Rotifera-Acanthocephala. Invertebr. Biol. 114, 186–188.

Riemann, O., Ahlrichs, W.H., 2008. Ultrastructure and Function of the Mastax in *Dicranophorus forcipatus* (Rotifera: Monogononta). J. Morphol., in press.

Santo, N., Fontaneto, D., Fascio, U., Melone, G., Caprioli, M., 2005. External morphology and muscle arrangement of *Brachionus ureolaris*, *Floscularia ringens*, *Hexarthra mira* and *Notommata glyphura* (Rotifera, Monogononta). Hydrobiologia 546, 223–229.

Segers, H., 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. Zootaxa. 1564, 1–104.

Sørensen, M.V., 2005a. Musculature in three species of *Proales* (Monogononta, Rotifera) stained with phalloidin-labelled fluorescent dye. Zoomorphology 124, 47–55.

Sørensen, M.V., 2005b. The musculature of *Testudinella patina* (Rotifera: Flosculariacea), revealed with CLSM. Hydrobiologia 546, 231–238.

Sørensen, M.V., Giribet, G., 2006. A modern approach to rotiferan phylogeny: combining morphological and molecular data. Mol. Phylogenet. Evol. 40, 585–608.

Sørensen, M.V., Funch, P., Hooge, M., Tyler, S., 2003. Musculature of *Notholca acuminata* (Rotifera: Ploima: Brachionidae) revealed by confocal scanning laser microscopy. Invertebr. Biol. 122 (3), 223–230.

Stoßberg, K., 1932. Zur Morphologie der Rädertiergattung *Euchlanis*, *Brachionus* und *Rhinoglena*. Z. wiss. Zool. 142, 313–424.