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Musculature of two bdelloid rotifers, *Adineta ricciae* and *Macrotrachela quadricornifera*: organization in a functional and evolutionary perspective

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Abstract

Organization of muscles in microinvertebrates has often been studied to answer functional questions and understand phylogenetic relationships among taxa. In this study, the musculature of two bdelloid species, *Adineta ricciae* and *Macrotrachela quadricornifera*, was illustrated, and their organization was compared with other rotifer taxa to generate possible hypotheses of evolutionary relationships among Rotifera. The two species share a common habitat but differ from each other in feeding and locomotion. *A. ricciae* feeds on the biofilm by scraping it, is unable to swim, and slides on the head cilia using the foot to propel over the substratum. *M. quadricornifera* feeds by filtration, can swim, and advances by looping in a leech-like motion. Their musculature, stained with TRITC-phalloidin, was observed using confocal laser scanning microscopy. Major differences between the two species were observed in the muscles of head and foot, possibly reflecting differences in their life style. Muscles of the trunk were similarly arranged: circular muscles surrounded longitudinal bands, which were inserted at different points on the body wall. In both bdelloids, circular muscles of the trunk were incomplete ventrally, a condition also present in *Seison* and in soft-bodied monogononts from benthos. Within rotifers, circular muscles in the form of complete rings are present in acanthocephalans and in soft-bodied planktonic monogononts but are absent in loricate monogononts, which generally possess dorsoventral bands. The diversity of muscle organization among rotifers was interpreted and discussed.

Key words: Rotifera – muscular system – evolution – confocal microscopy

Introduction

The phylum Rotifera is currently divided into four major groups: Acanthocephala, Bdelloidea, Monogononta and Seisonidea, whose the phylogenetic relationships are still under investigation, and the results vary with the traits analyzed (e.g. Garcia-Varela et al. 2002; Giribet et al. 2004; Sørensen and Giribet 2006). Among the rotifer taxa, Bdelloidea and Monogononta, with more than 2000 species from marine and freshwater habitats, are the more abundant and diverse groups (Segers 2007). All bdelloids are exclusively female, obligate parthenogens, and their morphology is rather uniform. They possess an elongated soft body, they are mostly filter feeders, live in the benthos, swim with cilia, and crawl over a substrate moving in a leech-like motion which involves adhesive secretion, muscles, and nervous system (Melone and Ricci 1995; Wallace et al. 2006; Leasi et al. 2009). In contrast, monogononts comprise morphologically diverse species that occupy different habitats; their bodies are either soft or stiffened by a lorica, their shape is either elongated, roundish, or conical and move by swimming or either crawling; some are either sedentary or sessile (see Wallace et al. 2006). Monogonont populations comprise females, but punctuate thelytokous parthenogenetic generations with the occasional production of males.

The diversity in external morphology of rotifers, combined with our restricted knowledge of their ultrastructure and biochemistry, and their extremely varied ecology, are all factors that complicate our understanding of evolutionary relationships and diversification in the phylum (Wallace et al. 2006). Among the morphological traits, the muscular system, especially when viewed using whole-mount fluorescent staining, has recently revealed informative details useful for functional, phylogenetic and evolutionary studies in several

microinvertebrates (e.g. Leasi and Todaro 2009; Neves et al. 2009). In this context, investigations using wide-field or confocal microscopy (CLSM) have been performed on the muscular system of several rotifer species (Hochberg and Litvaitis 2000; Kotikova et al. 2001, 2004, 2006; Sørensen et al. 2003; Santo et al. 2005; Sørensen 2005a,b; Hochberg and Gurbuz 2007, 2008; Riemann et al. 2008), adding new details from those obtained in previous studies using bright field microscopy (Zelinka 1886; Remane 1929–1933; Brakenhoff 1937). The muscular system of rotifers generally consists of somatic and splanchnic (visceral) fibers (Remane 1929–1933). Somatic musculature is composed of two layers: an external layer made of separate circular rings and an internal layer of longitudinal muscles. Circular muscle rings surround the trunk either completely or incompletely, if the rings are interrupted ventrally. The latter condition is common in all bdelloids and in some monogononts (Remane 1929–1933; Hochberg and Litvaitis 2000). Circular muscles may also be incomplete both ventrally and dorsally or modified in dorsoventral or either in dorsolateral and ventrolateral muscles (Kotikova et al. 2004; Sørensen 2005a; Riemann et al. 2008).

According to Remane (1929–1933) and Riemann et al. (2008), the thickening of the intracytoplasmic lamina (lorica) determines the development of either circular or dorsoventral muscles, but the wide diversity in the muscle organization suggests that other ecological and/or phylogenetic factors may also affect their structure, and other investigations on diverse species should be required. For instance, most of the investigations on muscular system of rotifers regarded monogonont species but only few bdelloid taxa, such as *Mniobia* and *Rotaria*, which muscles have been surveyed at bright field microscopy (Zelinka 1886; Brakenhoff 1937; Remane 1929–1933). Recently, Hochberg and Litvaitis (2000) surveyed the musculature of an additional bdelloid taxon, *Philodina*, using wide-field epifluorescence microscopy. The three genera, *Mniobia*, *Philodina*, and *Rotaria*, belong to a same family, Philodinidae; all of

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them feed by filtration, swim with cilia, crawl with leech-like movements, and their musculature is similar. To understand the extent to which life style affects the muscle organization of bdelloids, species with different behaviors should be compared. Among bdelloid families, Adinetidae exhibits a diverse behavior and life style. It comprises animals that do not swim, but slide with the cilia over a substrate, do not exhibit leechlike locomotion, do not feed by filtering, but scrape the substrate with a peculiar cuticular structure, the 'rake' (Melone and Ricci 1995). The musculature of only one Adinetidae species (*Adineta barbata*) has been documented long ago by Brakenhoff (1937) who reconstructed its muscular arrangement in an excellent drawing. However, the bright field microscopy used by the author cannot sometimes revealed detailed comparable with data obtained using the modern techniques.

In this study, using fluorescent-labelled phalloidin and high-resolution microscopy (CLSM), the musculature of two bdelloid

species belonging to Adinetidae and Philodinidae families was described. The two species were compared in the attempt to find relationships, if present, between muscle organization and animal life style (e.g. locomotion and/or feeding behavior). The obtained results, added to the data on other rotifer species, might allow tracking a possible evolutionary pathway connecting the different muscular organizations found within Rotifera.

Materials and Methods

The species investigated were *Adineta ricciae* Segers and Shiel, 2005 (Fig. 1a), collected from dry sediment in Australia, and *Macrotrachela quadricornifera* Milne, 1886 (Fig. 2a), isolated from mosses around Milan, Italy. Since years, both species were cultivated under laboratory conditions at the Department of Biology, University of Milan, Italy (Ricci 1991; Ricci et al. 2003). *A. ricciae* (Adinetidae) is 200–250 µm long, and like all species of Adinetidae, cephalically presents a ventral

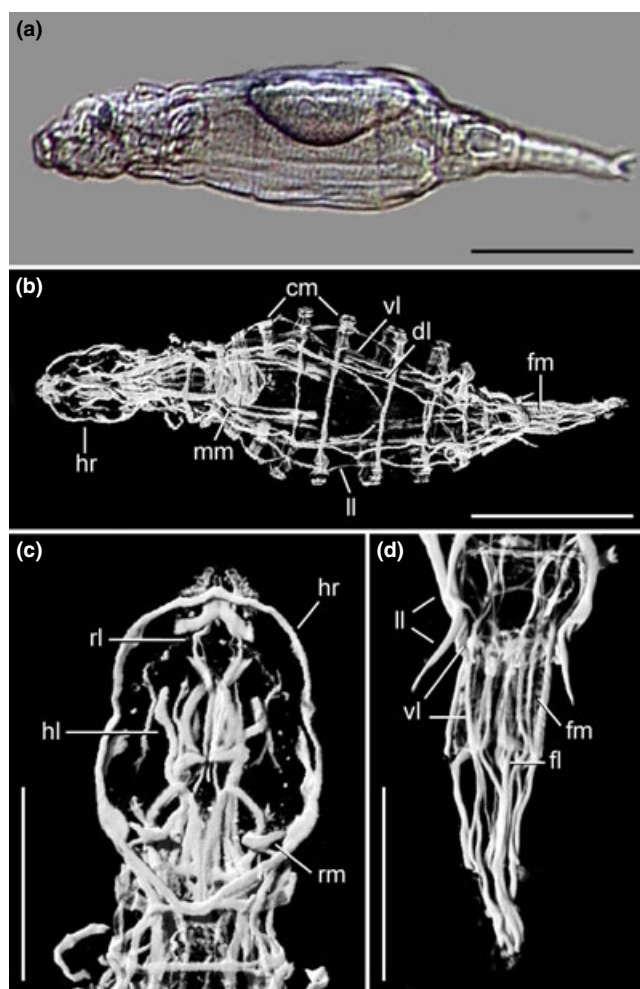


Fig. 1. *Adineta ricciae* at light microscopy (a), and its muscular system at CLSM in fluorescence simulation projection (b–d). (b) Whole-mount showing the general muscular arrangements. (c) Close-up of the cephalic region showing the musculature of the head. (d) Close-up of the posterior region showing the musculature of the foot. cm, circular muscles; dl, dorsal longitudinal muscles; fl, longitudinal muscles of the foot; fm, transversal muscles of the foot; hl, dorsal longitudinal muscles of the head; hr, ring-muscle of the head; ll, lateral longitudinal muscles; mm, musculature of mastax; rm, musculature of the rake; rl, muscles of the rostrum; vl, ventral longitudinal muscles. Scale bars: (a), (b), 50 µm; (c), (d), 25 µm.

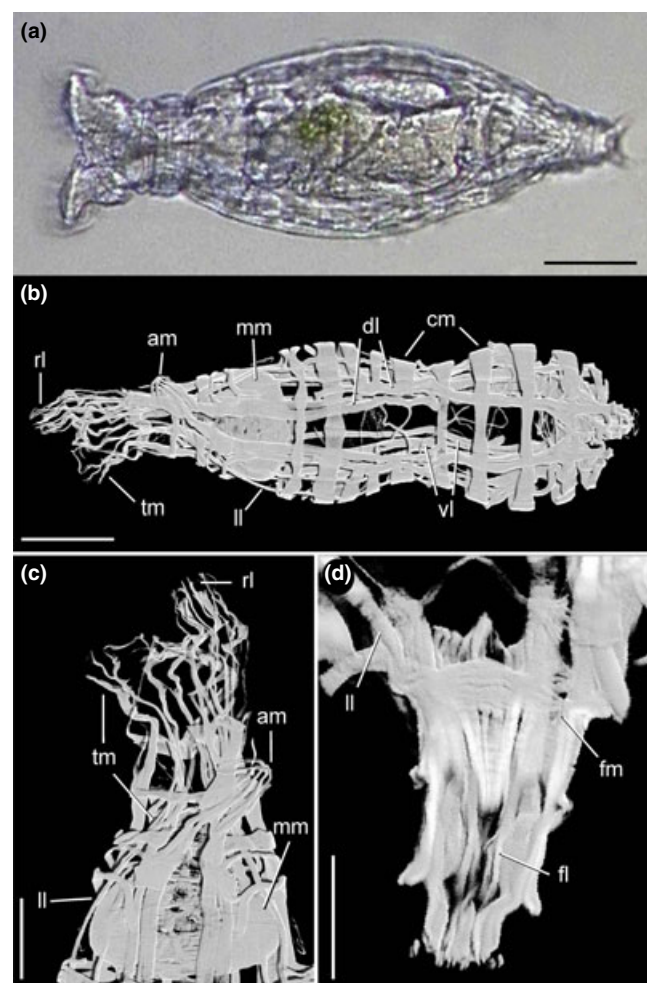


Fig. 2. *Macrotrachela quadricornifera* at light microscopy (a), and muscular system at CLSM in fluorescence simulation projection (b–d). (b) Whole-mount showing the general muscular arrangements. (c) Close-up of the cephalic region showing the musculature of the head. (d) Close-up of the foot. am, musculature of the antenna; cm, circular muscles; dl, dorsal longitudinal muscles; fl, longitudinal muscles of the foot; fm, transversal muscles of the foot; ll, lateral longitudinal muscles; mm, musculature of mastax; rl, longitudinal muscles of the rostrum; tm, musculature of the trochi; vl, ventral longitudinal muscles. Scale bars: (a), (b), 50 µm; (c), (d), 25 µm.

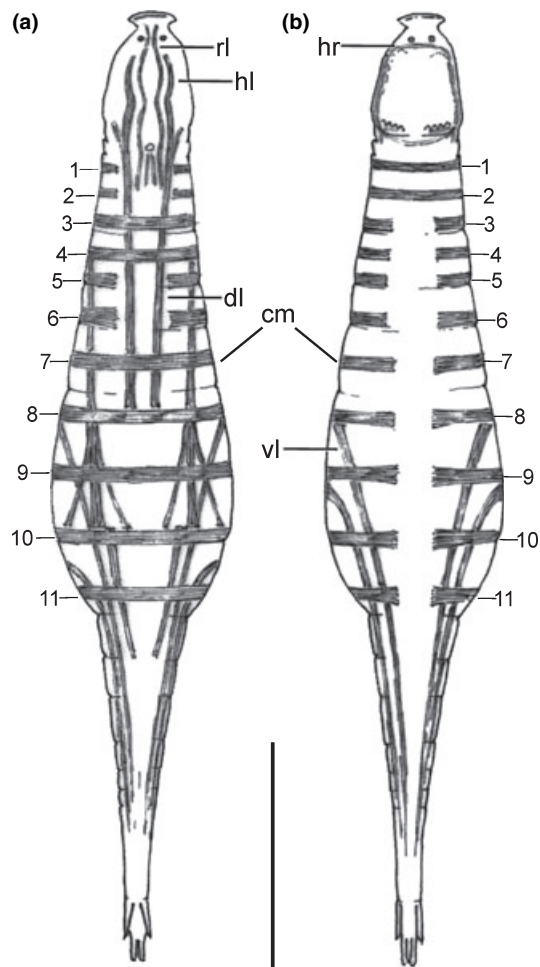


Fig. 3. Diagrammatic drawings of somatic musculature in *Adineta ricciae*: (a) dorsal view; (b) ventral view. cm, circular muscles (1–11); dl, dorsal longitudinal muscles; hl, dorsal longitudinal muscles of the head; hr, ring-muscle of the head; rl, muscles of the rostrum; vl, ventral longitudinal muscles. Scale bar: 50 μ m.

ciliated field. *M. quadricornifera* (Philodinidae) is about 350–380 μ m long and possesses ciliated trochi on pedicels and corona, typical of most bdelloids (Melone and Ricci 1995).

In each species, 10 specimens were isolated, relaxed for 10 min with marcain, and fixed at 4°C overnight in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Subsequently, they were rinsed several times in 0.1 PBS, permeabilized for 2 h in 0.2% Triton X-100, 0.25% bovine serum albumin, and 0.05% NaN₃ PBS, and stained by adding 2 μ l of 38 mM methanolic TRITC-phalloidin solution to 100 μ l of Triton X-100 buffered in 0.1M PBS.

Specimens were subsequently rinsed in PBS, mounted on microscopic slides in DABCO (Aldrich, Milan, Italy), and observed with Leica TCS SP2 confocal laser scanning microscope (Leica Microsystems GmbH, Wetzlar, Germany). In parallel, five animals of each species were processed as described, but not stained. These were used to check autofluorescence, which was never found. Series of optical sections were projected as a fluorescence simulation projection (SFPJ) for a three-dimensional reconstruction.

Results

The general pattern of musculature followed the layout of a sub-epidermal layer typical of worm-like animals, composed of outer circular and inner longitudinal muscles. Circular muscles form complete or incomplete rings, and longitudinal muscles connect head to trunk or trunk to foot or are extended along

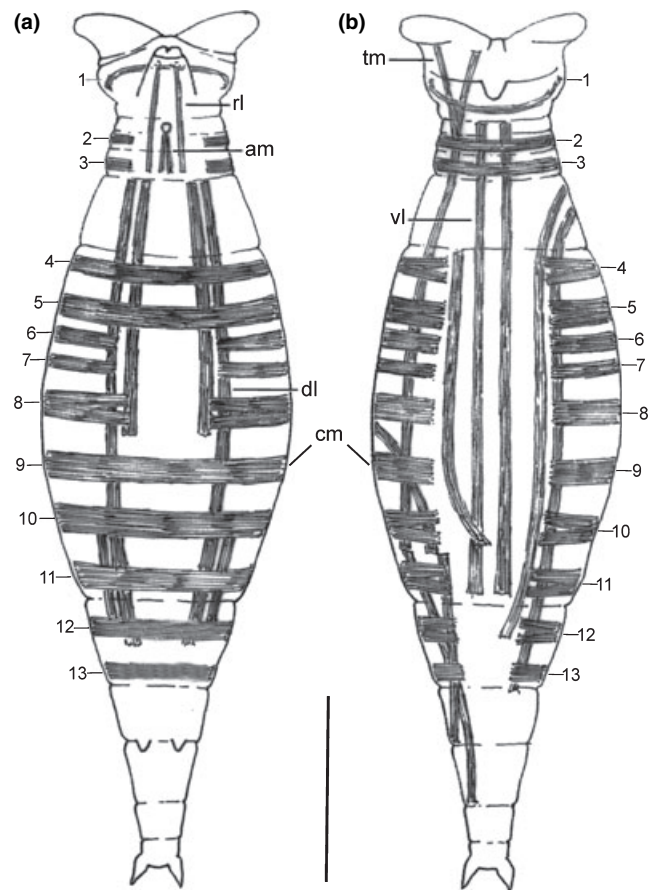


Fig. 4. Diagrammatic drawings of somatic musculature in *Macrotachela quadricornifera*: (a) dorsal view; (b) ventral view. am, musculature of the antenna; cm, circular muscles (1–13); dl, dorsal longitudinal muscles; rl, longitudinal muscles of the rostrum; tm, musculature of the trochi; vl, ventral longitudinal muscles. Scale bar: 50 μ m.

the whole body length (Figs 1 and 2). This schematic pattern fits both species, nevertheless, differences are present. The musculature of each species is described separately and is illustrated in two schematic drawings (Figs 3 and 4).

Adineta ricciae

Circular muscles

Eleven rings of cross-striated muscles were present from head to foot (cm; Fig. 1b). The rings were thicker ventrally (about 7 μ m) than dorsally (about 4 μ m), and the distance between contiguous rings varied from 10 μ m ventrally to 17 μ m dorsally. All the circular muscles were not complete. Thus, the arrangement of the circular muscles, from cephalic to caudal end, was of two incomplete rings, dorsally open, in the cephalic region followed by a series of four rings: two of which were open ventrally, and the other two muscles consisted of two C-shaped paired muscle arcs. In the trunk, five muscles in the form of ventrally interrupted rings were present (Figs 1b and 3).

Longitudinal muscles

Several longitudinal bands, in dorsal (dl), lateral (ll), and ventral (vl) positions, were inserted at different points of the body tegument (Fig. 1b, d). A number of longitudinal muscles encompassed the whole rotifer body, from head to foot; others

were shorter and arrested at 1/3, 1/2, or inserted at 2/3 of the body and entered in the foot. Cephalically, dorsal longitudinal muscles encompassed the length of the head (hl; 1–1.5 μm wide; Figs 1c and 3). Among these, a pair of muscles inserted at the base of the head and ran into the short rostrum (rl; 1 μm wide; Fig. 1c). In the foot, six pairs of longitudinal muscles were visible; of these, three pairs were extensions of the ventral longitudinal muscles (vl), and the others were restricted to the foot (fl; 0.3–0.5 μm ; Fig. 1c–d).

Other muscular arrangements

Somatic muscles that cannot be classified as either circular or longitudinal were present in the head and foot. The head was ventrally surrounded by a muscle forming a complete ring along the rim of the ciliated field; it was referred to as 'head ring' (hr; 1 μm wide; Figs 1b,c and 3). Other muscles running in various directions were present ventrally of the head and were connected to hr (Fig. 1c). Among these, short muscles were present at either sides of the mouth; these were likely associated with rakes (rm; 6 μm long, 1 μm wide; Fig. 1c). In the foot, along the ventral side, about ten transversal bands were present external to the longitudinal muscles (fm; 0.2 μm wide; Fig. 1b,d). Visceral muscles of the mastax comprised numerous transversely and obliquely-oriented muscles that connected the different hard parts of the trophi (mm; Fig. 1b). These were not described in this study.

Macrotrachela quadricornifera

Circular muscles

Thirteen cross-striated muscles in form of rings surrounded the body from the corona to the foot, most of them are incomplete and ventrally open (cm; 10–13 μm wide; Figs 2b and 4). From cephalic to caudal end, the circular muscles were arranged as one complete ring around the head, followed by two rings dorsally open and two circular muscles incomplete ventrally. Then, three pairs of C-shaped paired muscles were present and they were followed by five circular muscles ventrally incomplete (Figs 2b and 4).

Longitudinal muscles

Longitudinal muscles, in dorsal (dl), lateral (ll), and ventral (vl) positions, were present underneath the muscle rings of the body wall (Figs 2b and 4). Some of them appeared separately in cephalic or caudal muscles, others extended along the whole body from corona to the foot. Several longitudinal muscles run in the rostrum (more than 20 fibers; rl; 0.5–1 μm ; Figs 2b,c and 4) and in the trochi discs (tm; 0.5–1 μm ; Figs 2b,c and 4). In the foot, about 20 longitudinal muscles were present (fl; 0.5–1 μm ; Fig. 2d). A number (about 6 paired muscles) of these were the lateral and ventral muscles already mentioned, and 4 paired muscles connected the junction between trunk and foot to toes.

Other muscular arrangements

Additional somatic muscles were present in the dorsal antenna and in the foot. The antenna contained at least three paired thin muscles (am; 1–1.5 μm wide; Figs 2b,c and 4). In the foot, about 30 transversal muscles surrounded the longitudinal bands in ventral position (fm; 0.5 μm ; Fig. 2d). Splanchnic muscles were associated to the mastax; thin muscles connected trophi parts either transversally or obliquely (mm; Fig. 2b,c). A detailed description of the muscles of the mastax is not given in this study.

Discussion

The muscle organization of the trunk in *A. ricciae* and *M. quadricornifera* consists of outer circular and inner longitudinal muscles and follows the scheme of somatic muscular system described for other bdelloid species from genera *Mniobia*, *Rotaria*, *Philodina*, and *Adineta* (Zelinka 1886; Remane 1929–1933; Brakenhoff 1937; Hochberg and Litvaitis 2000). Circular muscles of both species are cross-striated and run externally to the longitudinal muscles that encompass body length and insert at different points on the body wall. Longitudinal and circular muscles work as antagonists against the fluid of the body cavity (hydroskeleton): longitudinal muscles are retractors and circular muscles, when contract, increase the pressure of body fluid causing the extension of the animal body (Remane 1929–1933; Hyman 1951; Hochberg and Litvaitis 2000; Ricci et al. 2007; Riemann et al. 2008). The alternate contraction of the longitudinal and circular muscles, modulated by the pseudosegmentation, is responsible for the telescopic retraction of head and foot and for the locomotion by sliding (*Adineta*) or crawling (*Macrotrachela*) over a substrate.

The muscles of head and foot differ between the two species, reflecting their differences in feeding and locomotion. The foot of *A. ricciae* has longer and thinner longitudinal muscles than *M. quadricornifera*, however, major differences concern their head musculature. Like all Adinetidae, *A. ricciae* slides over its uniform ciliated field that, together with the entire head, can be retracted into the trunk. Peculiar of *Adineta*, a complete circular muscle shaped as a ring (hr) extends along the rim of the head ciliated field. The ring has been observed in other *Adineta* species using CLSM (see Wallace et al. 2006; N. Santo, personal communication), but was not reported by Brakenhoff (1937) for *A. barbata*. During locomotion, *Adineta* maintains contact with the substrate using its head while pushing forward its body by extending and retracting its foot, and feeds by scraping the biofilm with the rakes, moved by paired muscles at either side of the mouth. Food particles removed from substrate are thus collected and brought to the mouth by the head cilia. The head ring muscle can help by keeping the contact with substrate during locomotion and feeding. In the filter feeder *M. quadricornifera*, like in all Philodinidae, the corona consists of three distinct ciliated fields (two on trochi discs and one on rostrum). Each of the ciliated fields contains several longitudinal muscles that are responsible for the retraction of the trochi, the rostrum, and the entire head, as well (see also Hochberg and Litvaitis 2000). Alternating extension of rostrum and foot, *M. quadricornifera*, can progress along the substrate with the characteristic leech-like movement.

Head and foot muscle disposition is likely affected by life style, but trunk muscles seem to be constant across different taxa. This is true in bdelloids, on the other hand, monogononts possess diverse conditions of circular muscles in the trunk (Remane 1929–1933; Sørensen 2005b; Riemann et al. 2008). Species of rotifers investigated so far using fluorescent techniques, with the respective circular muscle condition in the trunk, are listed in the Table 1, while the Figure 5 summarized the presence and distribution of each trunk circular muscle form in the major Rotifera taxa. Circular muscles in the trunk are most interrupted ventrally (incomplete-V) not only in bdelloids but also in *Seison* and in some monogonont species (e.g. Ahlrichs 1995; Kotikova et al. 2001; Sørensen et al. 2003; Tab. 1; Fig. 5). All these rotifers have soft elongated bodies,

Table 1. Species of rotifers stained with fluorescent phalloidin and their respective habitat, lorica (–, absent; +, present; W, weak) and circular muscle states along the trunk (complete; DL–VL, dorsolateral–ventrolateral; dorsoventral; incomplete-DV, both ventrally and dorsally open; incomplete-V, incomplete ventrally open; –, absent)

| Taxa | Habitat | Lorica | Muscle state | References |
|--|----------|--------|---------------|-------------------------------------|
| BDELLOIDEA | | | | |
| Philodinidae | | | | |
| <i>Philodina</i> sp. | Benthos | – | Incomplete-V | Hochberg and Litvaitis 2000 |
| <i>Macrotrachela quadricornifera</i> Milne 1886 | Benthos | – | Incomplete-V | Present study |
| Adinetidae | | | | |
| <i>Adineta ricciae</i> Segers & Shiel 2003 | Benthos | – | Incomplete-V | Present study |
| MONOGONONTA | | | | |
| GNESIOTROCHA | | | | |
| Flosculariidae | | | | |
| <i>Floscularia ringens</i> (Linnaeus 1758) | Benthos | – | Incomplete-V | Santo et al. 2005 |
| Hexarthridae | | | | |
| <i>Hexarthra mira</i> (Hudson 1871) | Plankton | – | — | Santo et al. 2005 |
| <i>Hexarthra</i> cf. <i>mira</i> | Plankton | – | — | Hochberg and Gurbuz 2008 |
| Testudinellidae | | | | |
| <i>Filinia novaezealandiae</i> Shiel & Sanoamuang 1993 | Plankton | – | — | Hochberg and Gurbuz 2007 |
| <i>Testudinella patina</i> (Hermann 1783) | Plankton | + | Dorsoventral | Kotikova et al. 2006 Sørensen 2005a |
| PLOIMA | | | | |
| Asplanchnidae | | | | |
| <i>Asplanchnopus multiceps</i> (Schrank 1793) | Plankton | – | Complete | Kotikova et al. 2004 |
| Brachionidae | | | | |
| <i>Brachionus quadridentatus</i> Hermann 1783 | Plankton | + | Dorsoventral | Kotikova et al. 2001 |
| <i>Brachionus urceolaris</i> (Müller 1773) | Plankton | + | Dorsoventral | Santo et al. 2005 |
| <i>Notholca acuminata</i> (Ehrenberg 1832) | Plankton | + | Dorsoventral | Sørensen et al. 2003 |
| <i>Platylabus patulus</i> (Müller 1786) | Plankton | + | Dorsoventral | Kotikova et al. 2006 |
| Dicranophoridae | | | | |
| <i>Dicranophorus forcipatus</i> (Müller 1786) | Plankton | W | DL–VL | Riemann et al. 2008 |
| <i>Encentrum mucronatum</i> Wulfert 1936 | Benthos | – | Incomplete-V | Riemann et al. 2008 |
| Euchlanidae | | | | |
| <i>Euchlanis dilatata unisetata</i> (Leydig 1854) | Plankton | + | Dorsoventral | Kotikova et al. 2001 |
| Proalidae | | | | |
| <i>Proales daphnicola</i> Thompson 1892 | Epizoic | – | Incomplete-V | Sørensen 2005b |
| <i>Proales fallaciosa</i> Wulfert 1937 | Benthos | – | Incomplete-V | Sørensen 2005b |
| <i>Proales reinhardti</i> (Ehrenberg 1834) | Benthos | W | Incomplete-DV | Sørensen 2005b |
| Notommatidae | | | | |
| <i>Notommata glyphura</i> Wulfert 1935 | Plankton | – | Complete | Santo et al. 2005 |
| Synchaetidae | | | | |
| <i>Polyarthra major</i> Burckhardt 1900 | Plankton | – | — | Hochberg and Gurbuz 2008 |
| <i>Polyarthra</i> cf. <i>vulgaris</i> | Plankton | – | — | Hochberg and Gurbuz 2008 |

and live either in benthos, or are attached to a substrate. Other soft-bodied monogononts, within Ploima, have roundish shape and live in the plankton: these possess ring-shaped circular muscles in the form of complete rings; a trait shared by acanthocephalans (e.g., Miller and Dunagan 1985; Kajihara et al. 2001; Kotikova et al. 2004; Santo et al. 2005; Tab. 1; Fig. 5). Other species do not have circular muscles, but have shorter bands that can be present in either (1) ventrally and dorsally incomplete C-shaped muscles (incomplete-DV), or (2) paired muscle strands that connect dorsal to ventral side and are inserted on the body wall (dorsoventral) or (3) two paired muscle bands that are present dorsolaterally and ventrolaterally (DL–VL) (e.g., Sørensen 2005a,b; Riemann et al. 2008; Table 1; Fig. 5). Conditions from (1) to (3) are present in ‘weakly loricate’ (*Dicranophorus*) or ‘loricate’ (*Testudinella*) monogononts, in which the presence of a hard structure, i.e. lorica, provides support to muscle bands, and circular muscles around the body would not be effective. Other monogonont species lack any circular or dorsoventral muscle conditions; such rotifers are minute and possess articulated movable appendages, like the paddles of *Polyarthra*, or the long spines of *Filinia*. In this case, appendages are controlled by ‘ad hoc’

muscles connected to the trunk (Santo et al. 2005; Hochberg and Gurbuz 2007, 2008; Table 1; Fig. 5). In sum, except for the species with appendages, soft-bodied rotifers share muscle rings, either complete or ventrally open, while loricate or weakly loricate species possess shorter muscular strands that may insert on the body wall.

Sørensen (2005a,b) suggested that all the muscle organizations listed above are homologous structures for rotifers, and that dorsoventral muscles might be seen as modified incomplete-V muscles. Furthermore, Hochberg and Gurbuz (2007, 2008) suggested that some muscles of appendages might likely be derived from circular bands. Following this line of reasoning, it may be hypothesized that all the muscle conditions derive from modifications of an ancestral circular form, probably through a gradual reduction, concomitance with a progressive insertion into the body wall, in case of dorsoventral bands. An analogous hypothesis has been proposed in a related taxon, the platyzoan gastrotrichs (Dunn et al. 2008), in which circular muscles are present as complete, incomplete-DV, and dorsoventral muscles (Ruppert 1975; Leasi et al. 2006). Complete circular muscles have been hypothesized to represent the ancestral state while all the other conditions are

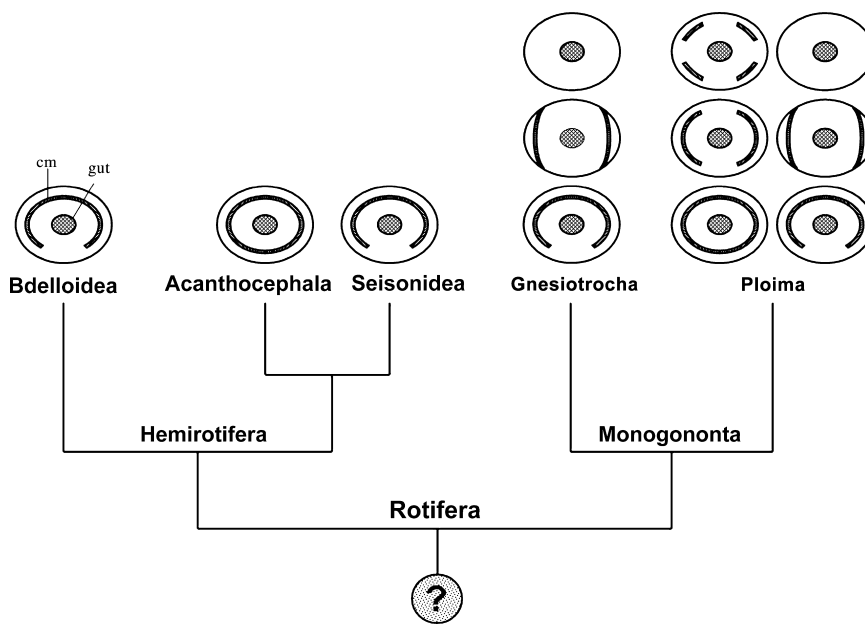


Fig. 5. Scheme showing the condition of somatic circular muscle (cm) seen in the cross section, superimposed to the current phylogeny of the four major Rotifera taxa: Acanthocephala, Bdelloidea, Monogononta, and Seisonidea (after Sørensen and Giribet 2006). Bdelloidea, Seisonidea and within Monogononta, Gnesiotrocha and Ploima bear the putative plesiomorphic condition: incomplete-V circular muscles. In acanthocephalans, the muscular rings are in complete forms, a condition shared by some ploimids. Ploima represents the group more diverse in this regard and possesses all the muscular arrangements found in rotifers: complete, incomplete-V, incomplete-DV, dorsoventral, DL-VL, or none.

derivatives; moreover, the gradual reduction of these muscles seems to follow the evolutionary history of the group (Ruppert 1979; Hochberg and Litvaitis 2001; Leasi and Todaro 2008). If the same interpretation is applied to the rotifers, it might be hypothesized that their basal pattern is represented by complete circular muscles, as it can be transformed by reduction into the other arrangements. Complete circular muscles are present in acanthocephalans and in soft-bodied planktonic ploimids (e.g. *Asplanchnopus* and *Notommata*). Following this assumption, both acanthocephalans and planktonic ploimids should share the ancestral trait for rotifers and, in this context, may be considered basal among the phylum. This hypothesis, however, seems to be strongly in contrast with the current phylogenetic view, that in fact placed bdelloids basal with respect to acanthocephalans and seisonids, even if the exact relationships between these three taxa still needs further examination, while *Asplanchnopus* and *Notommata* are placed in a derivative position among Monogononta (Sørensen and Giribet 2006; Fig. 5).

Alternatively, the incomplete-V muscle could be regarded as the basal trait, as suggested by Sørensen (2005b), because this condition is the widest spread among rotifers (Fig. 5). If this is true, the complete circular muscles of acanthocephalans and planktonic ploimids should be considered a derivative trait, and may only be explained as the result of a reversal process due to the modification from a muscle arc into a complete ring. In any case, other forms, like incomplete-DV, dorsoventral, and DL-VL muscles should be considered as results of transformation from circular muscles, whether complete or incomplete is to be ascertained.

In conclusion, the wide diversity in muscle organization of Rotifera reflects the variety in the body shape, ecology, and/or phylogeny of these animals. This is mainly true for monogononts that have radiated and occupy different habitats, where shape and stiffness of epidermis differ, while much less so for bdelloids, and generally for all hemirotifers, that are linked to a definite habitat (cfr. Tab. 1; Fig. 5). Nevertheless, some bdelloids have partly abandoned the benthic habitat and live a more planktonic life. This is the case of some *Rotaria* species. The comparison of muscular

system in a greater number of taxa with different phylogeny and ecological traits might help to clarify the still enigmatic evolutionary history of Rotifera.

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Riassunto

La muscolatura di due rotiferi bdelloidei, Adineta ricciae e Macrotrachela quadricornifera: organizzazione in prospettiva funzionale ed evolutiva

Lo studio dell'organizzazione muscolare è stato condotto in numerosi microinvertebrati nel tentativo di comprenderne la funzione ma anche i rapporti evolutivi tra i vari taxa. Seguendo la medesima linea investigativa, abbiamo osservato la muscolatura di due rotiferi bdelloidei, *Adineta ricciae* e *Macrotrachela quadricornifera*, quindi confrontato i risultati con i dati di letteratura presenti per altri rotiferi. Le due specie investigate condividono lo stesso habitat ma differiscono per modalità di alimentazione e locomozione. *Adineta ricciae* si nutre grattando il fondo, è incapace di nuotare e si muove strisciando sul substrato utilizzando la forza propulsiva del piede; *M. quadricornifera* è filtratrice, è in grado di nuotare e di spostarsi sul fondo con caratteristico movimento a sanguisuga. La loro muscolatura, evidenziata con falloidina rodaminata, è stata osservata con microscopio confocale. I risultati mostrano differenze nella regione del capo e del piede, differenze che verosimilmente riflettono lo stile di vita delle specie, la muscolatura del tronco invece mostra un'organizzazione simile: muscoli circolari incompleti esterni e muscoli longitudinali interni inseriti in diversi punti del corpo. I muscoli circolari del tronco sono interrotti ventralmente in entrambe le specie investigate, condizione condivisa con i *Seison* e con i monogononti bentonici a corpo molle. Estendendo il confronto ad altre specie di rotiferi, si evince che muscoli circolari completi sono presenti in ploimidi planctonici a corpo molle e negli acantocefali, mentre mancano nei monogononti loricati, i

quali possiedono muscoli dorsoventrali. Differenze nell'organizzazione muscolare vengono interpretate e discusse sulla base dell'attuale visione filogenetica dei rotiferi.

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