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The ultrastructure of the mastax of *Filinia longiseta* (Flosculariaceae, Rotifera): Informational value of the trophi structure and mastax musculature

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ABSTRACT

The study contributes to the discussion of mastax evolution within Rotifera by giving an insight into the ultrastructure of the mastax in the rotifer species *Filinia longiseta* (Flosculariaceae) and additionally into the bdelloid rotifer species *Adineta vaga* and *Zelinkiella synaptae*. The existence of cuticularized jaw elements (trophi) in the mastax, a muscular pharynx, is one of the defining rotiferan characters and the basis on which the monophyletic taxon Gnathifera Ahlrichs 1995a, comprising Rotifera, Gnathostomulida, Micrognathozoa and Acanthocephala, was erected. By means of SEM observations of the trophi and ultrathin serial sections (TEM) of the mastax, the internal and external organization of the jaw elements of *F. longiseta* is reconstructed. TEM sections of the incus of *Filinia* demonstrate that the fulcrum and the rami are built up by multitudes of tiny cuticular tubes. While tubular substructures in the rotiferan fulcrum have been described previously, distinct cuticular tubes as a substructure of the ramus have only been described for species belonging to the taxa Seisonidea and Bdelloidea so far (Koehler and Hayes, 1969; Ahlrichs, 1995b). By comparing the appearance and arrangement of the cuticular tubes in the rami of *F. longiseta* to those found in species of Seisonidea and Bdelloidea, a higher degree of resemblance between the structures in *F. longiseta* and Bdelloidea can be reported. The occurrence of the ramus substructures in species of Seisonidea (*Paraseison annulatus* and *Seison nebaliae*) is given consideration to represent an intermediate between the ramus substructure of Bdelloidea/Flosculariaceae and Ploima. Additionally, the mastax musculature of *F. longiseta*, being associated with the trophi, is described: A total of seven muscles are found that directly insert the jaw elements or are indirectly associated with them via muscle-to-muscle connections.

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1. Introduction

The Rotifera traditionally comprise cosmopolitan aquatic micrometazoans with body lengths of up to one millimeter, in rare cases even larger. To date, about 2000 species have been described (Wallace et al., 2006; Segers, 2007), most of which live in freshwater environments but also in marine waters and semiaquatic habitats such as damp mosses. Recent molecular studies (see Sørensen and Giribet, 2006 and Garcia-Varela and Nadler, 2006) also recognize the parasitic Acanthocephala as a rotiferan class.

One of the most conspicuous rotiferan characters is the muscular pharynx, referred to as the mastax, which contains a set of cuticularized jaw elements (trophi). The basic trophi set comprises paired manubria and unci (together referred to as malleus), an unpaired fulcrum and paired rami (together referred to as incus). Specific muscles serve to move these elements against one thereby allowing the trophi to penetrate, crush and/or scrape food items.

The basic arrangement of jaw elements is modified across different families and species, reflecting their modes of life and feeding strategies. According to the diverging morphology and function of the trophi, different basic mastax types can be distinguished (malleate, modified malleate, ramate, malleoramate, fulcrate, incudate, cardate, uncinata, virgate and forcipate; compare De Beauchamp, 1909; Remane, 1929–1933; Wallace et al., 2006). Within Rotifera the trophi are used for species identification and are one of the most important characters in rotifer systematics and phylogeny.

Based on the pharyngeal jaw elements existing in the mastax, Ahlrichs (1995a) erected the monophyletic taxon Gnathifera, including Rotifera, Gnathostomulida, *Limnognathia maerski* (Micrognathozoa), and the jaw-less Acanthocephala (for which a close relationship to Rotifera has been suggested, mainly based on a similar ultrastructure of the syncytial epidermis).

In a phylogenetic analysis of rotifer families primarily based on morphological characters of the trophi elements, Sørensen (2002) presents a strict consensus tree in which the large rotifer taxon Monogononta splits into Ploima and Gnesiotrocha (Flosculariaceae and Collothecaceae) (Fig. 1A). While the different families of Ploima comprise species with malleate, cardate, virgate, incudate, and

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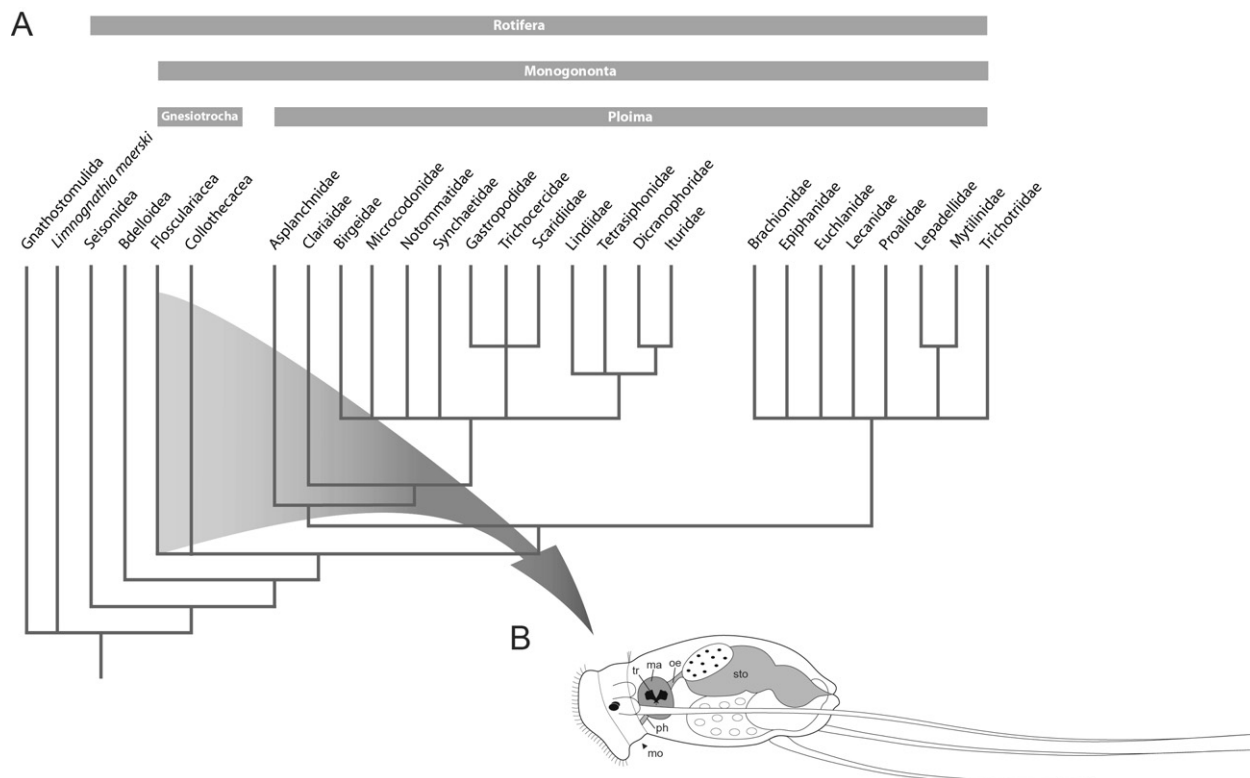


Fig. 1. Phylogenetic relationships within Rotifera and the species *Filinia longiseta* (Flosculariacea). (A) Strict consensus tree of a maximum parsimony analysis of Rotifera based on morphological characters according to Sørensen (2002). Seisonidea, Bdelloidea and Monogononta together form the phylum Rotifera; (B) the rotifer species *Filinia longiseta* (Flosculariacea) and its digestive tract (modified after Sanoamuang, 1993). ma, mastax; mo, mouth opening; oe, oesophagus; sto, stomach; tr, trophi.

forcipate mastax types, flosculariacean species display only one type: the malleoramate mastax. The trophi of *Filinia longiseta*, which is the flosculariacean focused on in this study, are characterized, among others, by a multitude of differentiated unci teeth, a short fulcrum and the lack of a manubrial cauda (Fig. 2A–D).

With regard to several morphological similarities of the trophi (sickle-shaped manubria; differentiated unci teeth; see Fig. 2A–D), the jaw elements of Flosculariacea and Bdelloidea, the latter being an outgroup to Monogononta sensu Sørensen (2002) (Fig. 1A), were compared in several studies before (Markevich, 1985, 1989; Melone et al., 1998).

In this study, we contribute to the discussion of mastax evolution within Rotifera by providing detailed insights into the ultrastructure of the mastax of species from three lineages: *F. longiseta* (Flosculariacea), *Paraseison annulatus* (Seisonidea), *Adineta vaga* and *Zelinkiella synaptae* (Bdelloidea). While the mastax of species from Seisonidea and Bdelloidea have been the subject of former TEM-studies (Koehler and Hayes, 1969; Ahlrichs, 1995b), our analysis focuses on the malleoramate mastax of *F. longiseta*, which never was the subject of an ultrastructural study before. *Filinia longiseta* (Fig. 1B) reaches body-lengths of 130–250 μm (Sanoamuang, 2002) and can be found in lakes and ponds where it often occurs in large numbers during warmer seasons. The genus *Filinia* feeds upon small algae, flagellates, bacteria and detritus. Their trophi, reaching lengths of 24–30 μm (Nogrady and Segers, 2002), perform a constant opening and closing movement during ingestion.

2. Material and methods

All individuals of *F. longiseta* were collected in a private pond in Logabirum in northwest Germany.

Individual specimens were isolated from samples under a stereomicroscope and studied by differential interference contrast light microscopy (Leica DMLB) and transmission electron microscopy (Zeiss 902 TEM). The isolated trophi were examined under a scanning electron microscope (Zeiss DSM 940 and Hitachi S-3200N).

For scanning electron microscopy (SEM) living specimens were treated with SDS/DTT (SDS=sodium dodecyl sulfate, DTT=dithiothreitol) following the protocol given by Kleinow et al. (1990) to dissolve the body and to isolate the trophi. Trophi were rinsed with distilled water and afterwards pipetted onto a stub. After air-drying, the material was sputter coated with platinum and then examined by SEM.

For transmission electron microscopy (TEM), specimens were anesthetized with carbonated water and then fixed with 1% OsO_4 buffered in 0.1M sodiumcacodylate at 4 °C for 1 h. Afterwards, specimens were dehydrated through an increasing acetone series, embedded in araldite and hardened at 60 °C for 72 h. Ultrathin serial sections (horizontal- and cross-sections) of 80 nm of two female individuals for each species were made with a Reichert Ultracut followed by automatic staining with uranyl acetate (25 °C for 25 min) and lead citrate (30 °C for 30 min) in a Leica EM Stain. Stained sections were examined and photographed in a TEM at 80 kV.

The reconstruction of the trophi is based on observations made by means of SEM and TEM: Complete series of cross- and horizontal sections through the mastax region of specimens were photographed at regular intervals using the MIA (multiple image alignment) function of iTEM® software. The composites show larger structures at higher magnifications and better resolution than would be obtained from single images. Serial sections were examined and compared to SEM photographs to obtain information on the three-dimensional structure of the trophi. Line drawings were handled with Adobe Illustrator® CS2.

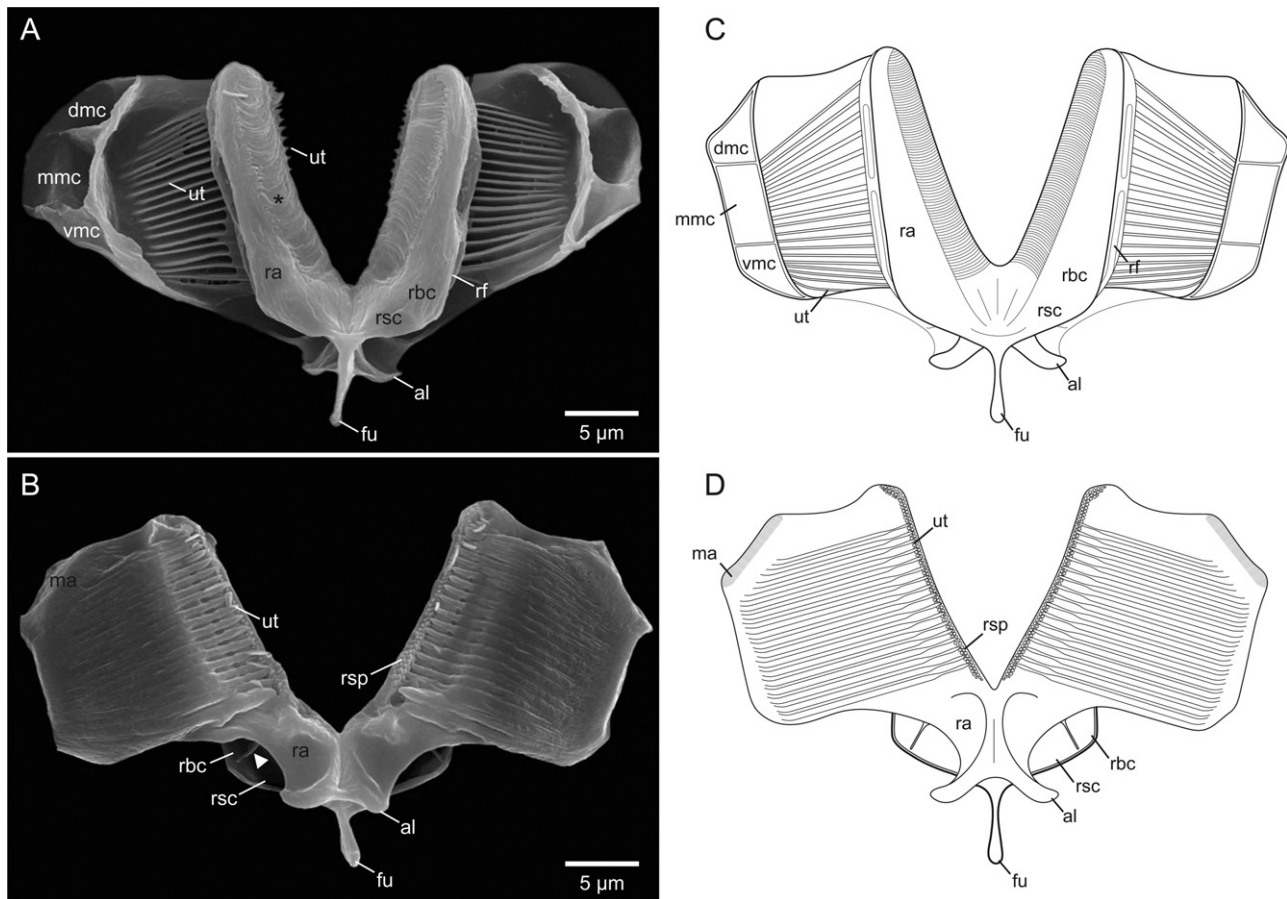


Fig. 2. The jaw elements of *Filinia longiseta*. (A) SEM photo of the trophi in dorsal view; serrated rami grooves indicated by black asterisk; (B) SEM photo of the trophi in ventral view; wall between ramus basal and subbasal chambers indicated with white arrowhead; (C) diagrammatic drawing of the trophi in dorsal view; (D) diagrammatic drawing of the trophi in ventral view. al, alula; dmc, dorsal manubrial chamber; fu, fulcrum; ma, manubrium; mmc, median manubrial chamber; ra, ramus; rbc, ramus basal chamber; rf, ramus foramen; rsc, ramus subbasal chamber; rsp, ramus scleropili; ut, unci tooth; vmc, ventral manubrial chamber.

Additionally, individuals of the species *A. vaga* were collected from wet mosses on the campus of Oldenburg University and individuals of *Z. synaptae* and *P. annulatus* were collected in Concarneau and Roscoff, France. Samples for TEM were treated and processed as described above.

In this study we distinguish between the terms 'tube' and 'rod', since the first term is defined as a hollow and the latter as a massive cylindrical body. Due to the fact that the trophi substructures in free-living, microscopic Rotifera (as well as Gnathostomulida and Micrognathozoa) can be described as cylindrical bodies, (partly) filled with cellular tissue, we term them 'cuticular tubes' and not 'cuticular rods'.

Moreover, please note that Acanthocephala is excluded from our figures (Figs. 1A and 7) and discussion, because species of this taxon are jawless.

3. Results

3.1. The trophi of *F. longiseta*

The trophi of *F. longiseta* are bilateral symmetrical, consisting of paired manubria, unci and rami, and an unpaired fulcrum (Fig. 2A–D). These jaw elements are found lying almost perpendicular to the main body axis in the mastax with the fulcrum pointing ventrally (Fig. 1B).

The crescent-shaped manubrium (ma) is subdivided into three distinct chambers, the dorsal (dmc), the median (mmc), and the ventral manubrial chamber (vmc), all of which are completely open

to the frontal side (Figs. 2A, C and 3A and B). These chambers together form the so-called clava which displays a very delicate cuticle (Fig. 3A). A manubrial cauda, which is commonly present in most trophi types, is entirely lacking. As visible in ultrathin sections, the three manubrial chambers are completely filled with epithelial tissue (Fig. 3A and B).

The prominent unci attach to the internal rounded boundary of the manubria at the level of the median and the ventral chambers (Fig. 2A and C). The unci consist of 17–19 solid teeth (ut) that are situated adjacent to each other without any internal cavities. Together, the unci teeth form an almost quadrangular plate that is slightly bent ventrally (Figs. 2B, D and 3A). The proximal regions of the teeth are merged, while the distal parts are clearly separated from each other, making close contact to the rami ventrally, with the lance-like tips protruding beyond the internal ramus margin (Fig. 2A and B). The teeth are triangular in cross-section, with a flattened ventral side (Fig. 2B) and a narrow ridge on the dorsal side (Fig. 2A).

The broad rami (ra) display blunt rounded distal tips and two large external openings laterally (rf) (Fig. 2A and C). In ventral view, the ramus basally gives an insight into its interior, where a narrow inner dividing wall is visible (Fig. 2B and D). As visible on ultrathin sections, this wall separates the subbasal ramus chamber (rsc) from the basal ramus chamber (rbc) (Fig. 3C). A third anterior ramus chamber would be expected due to the anterior ramus opening, but is not visible in TEM section.

As apparent on ultrathin sections, the rami consist of multitudes of tiny cuticular tubes (Fig. 3C). Dorsally, both rami show deep

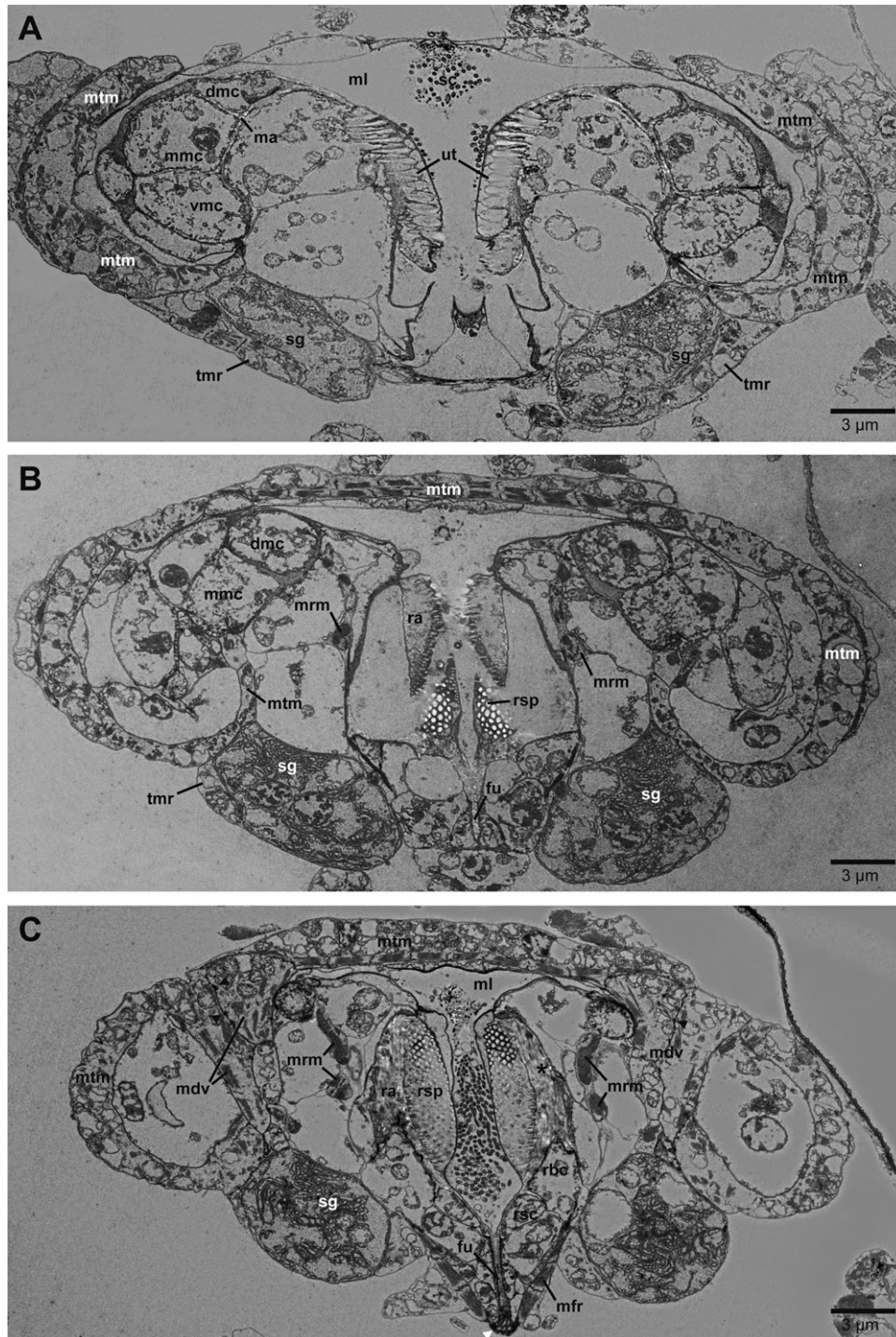


Fig. 3. TEM images of cross-sections through the mastax of *Filinia longiseta* at three different levels. (A) Frontal mastax region with the three manubrial chambers and unci teeth; (B) median mastax region with manubria, rami, fulcrum and salivary glands, hemidesmosomes interconnecting muscle cells are indicated by black arrowheads, tubular fulcrum structure indicated by white asterisk, ramus tubes indicated by black asterisk. dmc, dorsal manubrial chamber; fu, fulcrum; ma, manubrium; mdv, musculus dorsoventralis; mfr, musculus fulcro-ramicus; ml, mastax lumen; mmc, median manubrial chamber; mrm, musculus ramo-manubricus; mtm, musculus transversus manubrii; ra, ramus; rbc, ramus basal chamber; rsc, ramus subbasal chamber; rsp, ramus scleropili; sg, salivary gland; tmr, transversus manubrii retractor; ut, unci tooth; vmc, ventral manubrial chamber.

rounded, serrated grooves that enclose a cavity when the ramus is closed (Fig. 2A and C). Ventrally, just below the unci teeth, the internal ramus margin is beset with a multitude of scleropili (rsp) (Fig. 2B and D), that are visible as cuticular tubes on ultrathin sections (Fig. 3B and C): these tubes are not only superficial structures but also continue deep inside the trophus element (Fig. 3C).

The distinctly curved alulae (al) are located on the basis of the ramus chamber (Fig. 2A–D). The short and slender fulcrum (fu) is

situated below the alulae (Fig. 2A–D). The cuticle of the fulcrum is constituted of a multitude of cuticular tubes (Fig. 3C, white arrowhead).

3.2. The mastax musculature of *F. longiseta*

In the following, the muscles are described in the order of their appearance in the mastax from dorsal to ventral.

All muscles in this study are named related to their points of insertion on the trophi and/or their course in the mastax. If muscle names in the earlier literature correspond to this principle of nomenclature, those names are adopted.

Musculus transversus manubrii (mtm) (Figs. 3A–C and 4A). The unpaired musculus transversus manubrii interconnects the manubria by forming a long and slim muscle that encompasses the trophi on the dorsal mastax side (Fig. 3A–C). As visible on TEM

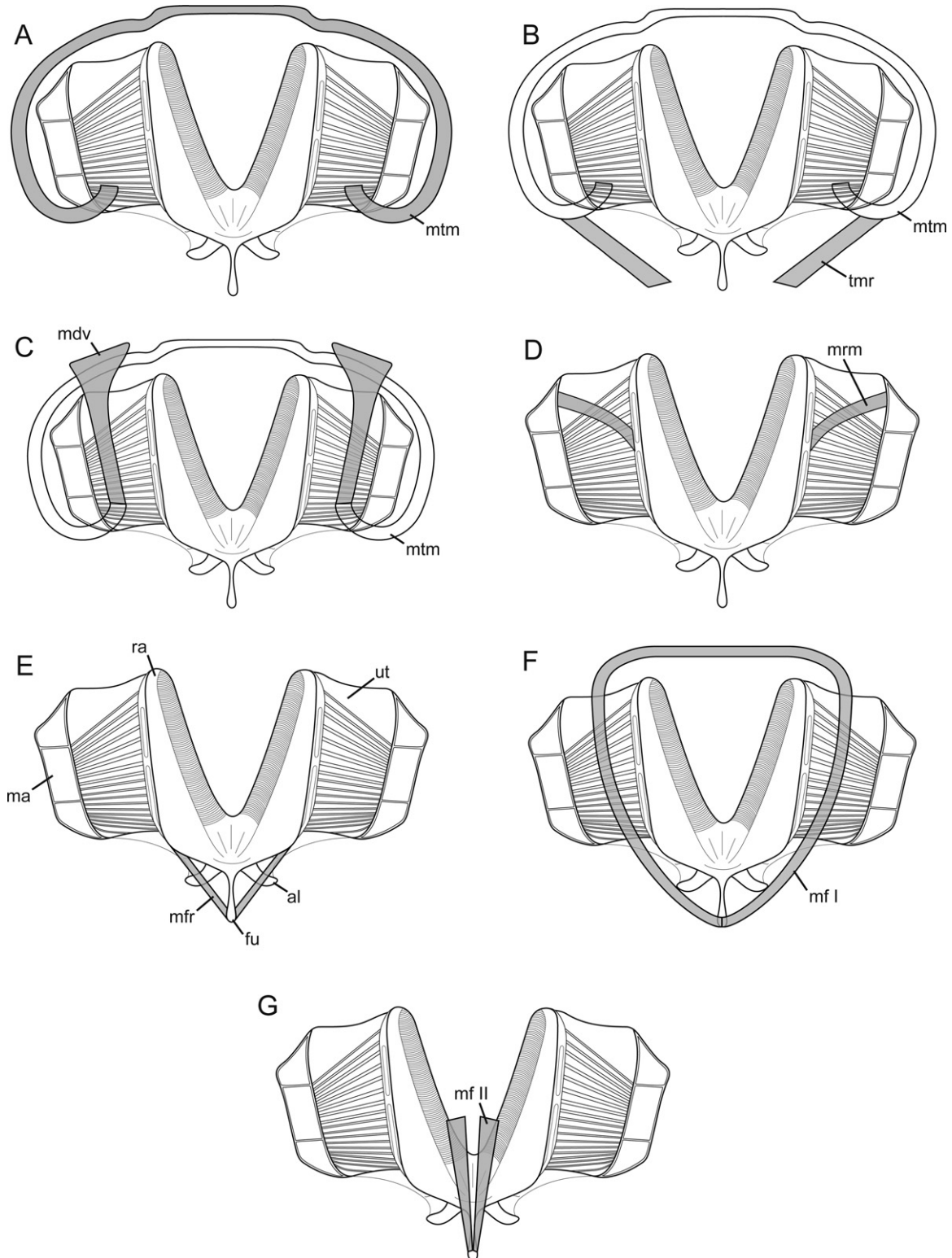


Fig. 4. Mastax musculature of *Filinia longiseta*. Diagrammatic view. The order of the different muscles from (A–G) reflects their appearance in the mastax from frontal to caudal. (A) Musculus transversus manubrii; (B) transversus manubrii retractor; (C) musculus dorsoventralis; (D) musculus ramo-manubricus; (E) musculus fulcro-ramicus; (F) musculus fulcralis I; (G) musculus fulcralis II. al, alula; fu, fulcrum; ma, manubrium; mdv, musculus dorsoventralis; mf, musculus fulcralis; mfr, musculus fulcro-ramicus; mrm, musculus ramo-manubricus; mtm, musculus transversus manubrii; ra, ramus; tmr, transversus manubrii retractor; ut, uncus tooth.

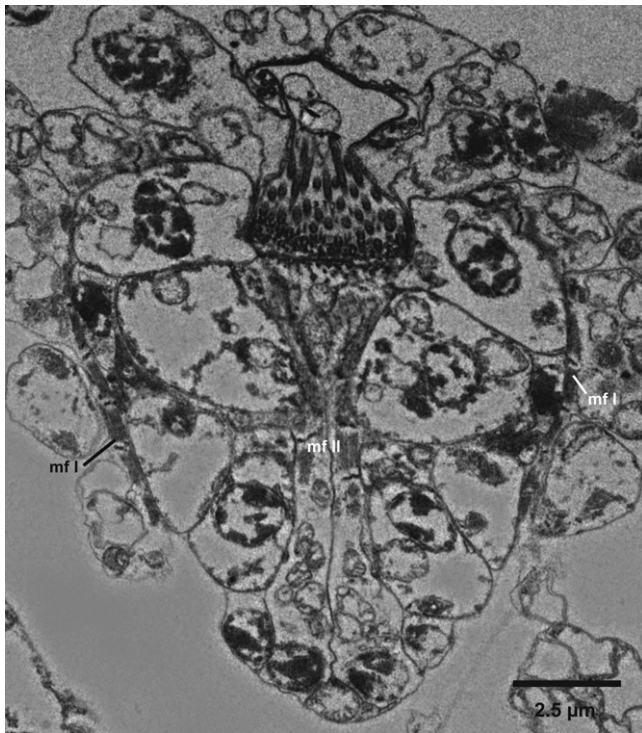


Fig. 5. TEM image of cross-section through the caudal mastax region with musculus fulcralis I and II. mf, musculus fulcralis.

sections, the muscle is made up of at least three interconnected cells. Two paired cells are attached to the manubrium and lie against it on the lateral mastax sides (Fig. 3A), whereas a single, unpaired cell interconnects the two former cells dorsally (Fig. 3B and C). The musculus transversus manubrii does not terminate in the region where it is attached to the manubria but continues beyond it in the dorsal direction (Figs. 3B and 4A).

Transversus manubrii retractor (tmr) (Figs. 3A and 4B). The paired transversus manubrii retractor is one of the two mastax muscles that are not in direct contact with the pharyngeal hard parts: It attaches to the musculus transversus manubrii ventrally, from where it stretches toward the fulcrum without touching it (Fig. 4B).

Musculus dorsoventralis (mdv) (Figs. 3C and 4C). The paired musculus dorsoventralis does not come into direct contact with the trophi elements, but is inserted into the musculus transversus manubrii in the region where it is attached to the manubrium (Fig. 4C). The musculus dorsoventralis presents the elongation of the musculus transversus manubrii and is orientated in the mastax along the dorsoventral axis.

Musculus ramo-manubricus (mrm) (Figs. 3B, C and 4D). The inconspicuous, paired musculus ramo-manubricus attaches to the external lateral ramus side, from where it stretches toward the dorsal manubrial chamber, attaching it laterally.

Musculus fulcro-ramicus (mfr) (Figs. 3C and 4E). The paired musculus fulcro-ramicus attaches to the distal end of the fulcrum laterally, from where it runs toward the ramus, inserted into it in the region of the subbasal ramus chamber.

Musculus fulcralis I (mf I) (Figs. 4F and 5). The unpaired musculus fulcralis I inserts to the fulcrum ventrodorsally, stretching out to the dorsal mastax side in an oval circle.

Musculus fulcralis II (mf II) (Figs. 4G and 5). The paired musculus fulcralis II attaches dorsally to the caudal end of the fulcrum, from where it stretches out in dorsofrontal direction, terminating in the epithelial tissue.

3.3. The ultrastructure of the trophi of *A. vaga*, *Z. synaptae*, and *P. annulatus*

As visible on ultrathin sections, the rami and the manubria of *A. vaga* (Bdelloidea) are composed of multitudes of tiny cuticular tubes. The manubrial tubes are assembled in two rows (Fig. 6A and B).

TEM-images of the trophi of *Z. synaptae* (Bdelloidea) show that the manubria as well as the rami are composed of inordinately arranged, tiny cuticular tubes (Fig. 6C and Ahlrichs, 1995b).

The rami of *P. annulatus* (Seisonidea) show at least three large ramus chambers (see Ahlrichs, 1995b). In addition to these chambers, several smaller cuticular tubes are visible in ultrathin sections (Fig. 6D). There are no cuticular tubes visible in the cuticle of the manubria (see Ahlrichs, 1995b).

4. Discussion

4.1. Informational value of the trophi ultrastructure

As stated by Sørensen (2002), the polarity of the mastax types can only be determined via a comparison of particular trophi elements in taxa that are supposed to have diverged earlier, such as the Seisonidea (see Fig. 1A). Following Sørensen's approach and having a look at the ultrastructure of the jaw elements of *P. annulatus* and *Seison nebaliae* (see Ahlrichs, 1995b), we would like to highlight one important character in these species concerning the occurrence of their ramus cuticle: As in *F. longiseta*, the cuticle of the ramus displays small cuticular tubes in addition to the large ramus chambers (Fig. 6D and Ahlrichs, 1995b). However, these tubes differ from those we found in *F. longiseta* regarding their quantity as well as their appearance. *Filinia* shows multitudes of distinct tiny tubes in the ramus cuticle (Fig. 3C), whereas *P. annulatus* displays fewer small tubes.

In all of our rotifer species formerly investigated – as well as in *F. longiseta* (Fig. 3C; white arrowhead) – we found cuticular tubes in the ultrastructure of the fulcrum (*Pleurotrocha petromyzon* and *Proales tillyensis* (see Wulfken et al., 2010), *Bryceella stylata* (see Wilts et al., 2010), *Dicranophorus forcipatus* (see Riemann and Ahlrichs, 2008), *Paraseison annulatus*, and *S. nebaliae* (see Ahlrichs, 1995b)). In all of these species, except for *F. longiseta*, *P. annulatus* and *S. nebaliae*, the cuticular tubes are restricted to the fulcrum region. Concerning Bdelloidea, Kristensen and Funch (2000) remark that cuticular tubes are difficult to recognize due to the lack of a fulcrum. Nevertheless, the authors believe that these subunits were present in all sclerites in the stem species of Rotifera. Indeed, these cuticular tubes are visible in Bdelloidea, too. Both *Adineta vaga* and *Zelinkiella synaptae* possess cuticular tubes as ultrastructural subunits of the trophi in the rami and manubria (Fig. 6A–C). Moreover, Koehler and Hayes (1969) demonstrate the presence of cuticular tubes in the rami and manubria of one additional bdelloid rotifer, *Philodina acuticornis odiosa*. These structures in Bdelloidea strongly resemble the ones found in *F. longiseta* in their appearance.

To sum up it can be said that distinct cuticular tubes are a common substructure of the rami in species of Bdelloidea, Seisonidea and *Filinia*. There are, however, differences in the size and packing of these cuticular tubes that may have systematic value (see below). For example, the tubes in species of Seisonidea are larger and less densely packed than those of Bdelloidea and *Filinia* (Fig. 7, boxes 4 and 5). Also, Bdelloidea is the only taxon with multitudes of tubular structures that are not limited to the incus, but can also be demonstrated in the manubrium (Fig. 7, box 7). In all of our formerly investigated ploimid species (*D. forcipatus*, Riemann and Ahlrichs, 2008; *B. stylata*, Wilts et al., 2010; *P. petromyzon*, *P. tillyensis*, Wulfken et al., 2010; *Encentrum mustela*, *Itura aurita*, *Lindia tecusa*, *Asplanchna priodonta*, unpublished data), cuticular tubes

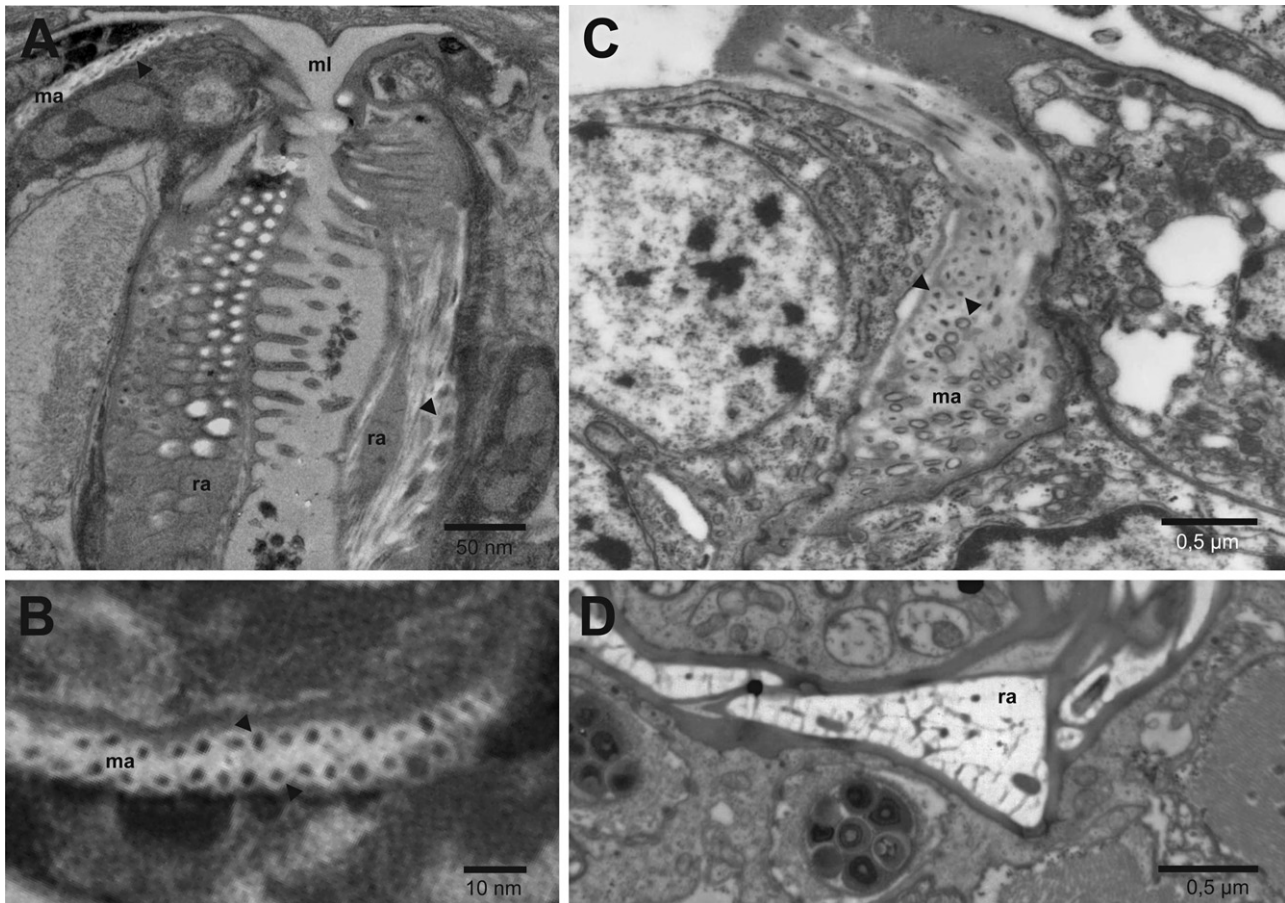


Fig. 6. TEM sections of the rami and manubria of the rotifer species *Adineta vaga*, *Zelinkiella synaptae* and *Paraseison annulatus*. (A) *Adineta vaga*. Horizontal section through the rami and the manubrium, cuticular tubes as substructures of the trophi elements indicated by arrowheads; (B) *Adineta vaga*. Tubular substructure of the manubrium in detail, cuticular tubes indicated by arrowheads; (C) *Zelinkiella synaptae*. Cross-section through the manubrium with tubular substructure, cuticular tubes indicated by arrowheads. (D) *Paraseison annulatus*. Cross-section through the rami with several cuticular tubes. ma, manubrium; ml, mastax lumen; ra, ramus.

are restricted to the fulcrum region. The cuticle of the rami and manubria appears mostly homogeneous and electron lucent, in one case (see the incus of *Notommata copeus*, Clément and Wurdak, 1991) mottled but without distinct cuticular tubes.

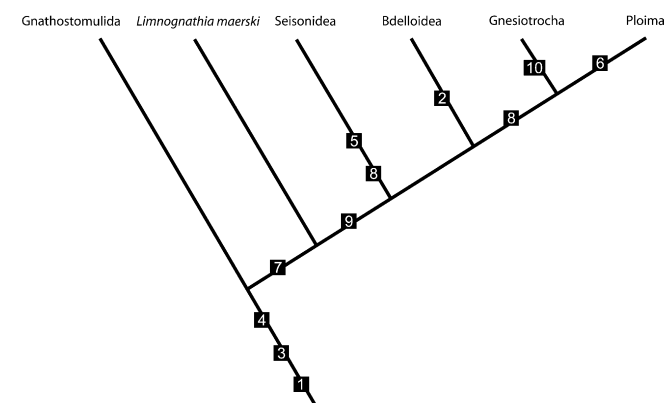


Fig. 7. Hypothetical character transformations within Rotifera mapped to the phylogenetic relationships according to Sørensen (2002). Character transformations based on the data given in Table 1. Outgroups represented by Gnathostomulida and *Limnognathia maerski* (Micrognathozoa). Numbers in boxes propose single transformation steps. (1) Fulcrum constituted of multitude of cuticular tubes; (2) loss of fulcrum; (3) ramus constituted of cuticular tubes; (4) ramus substructure: Multitude of tiny cuticular tubes; (5) ramus substructure: Fewer small cuticular tubes; (6) loss of multitude of cuticular tubes in ramus; (7) manubrium constituted of multitude of cuticular tubes; (8) loss of multitude of cuticular tubes in manubrium; (9) mastax receptor retractor musculature; (10) musculus fulcralis.

What can we propose from this information? Beginning with the fulcrum, we suggest, like Melone et al. (1998), that this trophus element – built up of cuticular tubes – is part of the ground pattern of Gnathifera (Fig. 7, box 1) since homologous jaw elements (with tubular substructures) are present in Gnathostomulida (compare with symphysis/pseudofulcrum, Sørensen and Sterrer, 2002), *L. maerski* (compare with articularium; Kristensen and Funch, 2000) and in all rotiferan taxa except for Bdelloidea. As opposed to this, the absence of the fulcrum in Bdelloidea is considered to be a secondary loss (Fig. 7, box 2).

The next fact to be addressed is the presence of cuticular tubes in the ramus region in species of Seisonoidea, Bdelloidea and *Filinia*. To answer the question of what can be concluded from this characteristic, we have a look at the gnathiferan sister taxa Micrognathozoa and Gnathostomulida. *L. maerski* (Micrognathozoa) shows cuticular tubes in the main jaw, which is supposed to be homologous to the rotiferan incus (Kristensen and Funch, 2000). Comparing the tubular substructures of the main jaw with the rotiferan incus, it should be noted that the degree of resemblance is higher between *L. maerski* and species of Bdelloidea/*F. longiseta* than between *L. maerski* and species of Seisonoidea. For Gnathostomulida, Rieger and Tyler (1995) describe the cuticular tubes of a scleroperalian gnathostomulid as ‘striking similar to that in the manubrium of [...] the bdelloid rotifer *P. acuticornis odiosa*’.

According to this, it is plausible to suggest that cuticular tubes as substructures of the ramus (compare with lamellae symphysis in Gnathostomulida and dentarium in Micrognathozoa) are a ground pattern feature for Gnathifera (Fig. 7, box 3). Since distinct

Table 1
Matrix of different character states of the gnathiferan jaw elements.

Taxon	Character									
	Fulcrum Absent = 0 Present = 1	Fulcrum built up of distinct cuticular tubes Absent = 0 Present = 1	Ramus Absent = 0 Present = 1	Ramus cuticle built up of distinct cuticular tubes Absent = 0 Present = 1	Multitudes of tiny cuticular ramus tubes = 0 Fewer small cuticular ramus tubes = 1	Manubrium Absent = 0 Present = 1	Manubrium cuticle built up of distinct cuticular tubes Absent = 0 Present = 1	Mastax receptor Retractor = 0 Musculus fulcralis = 1		
Gnathostomulida	1	1	1	1	0	0	1	1	0	0
<i>Limnognathia maerski</i>	1	1	1	1	0	1	1	?	0	0
Seisonidea	1	1	1	1	1	1	1	1	1	1
Bdelloidea	0	-	1	1	0	1	1	1	1	1
<i>Filinia longiseta</i>	1	1	1	1	0	1	1	1	1	1
Ploima	1	1	1	0	-	1	1	1	0	0

cuticular tubes as substructures of the ramus cuticle have not been described so far from species of Ploima, the loss of these structures is suggested to be an autapomorphy for Ploima (Fig. 7, box 6).

As mentioned above, the presence of cuticular tubes as subunits of the rotiferan manubrium could be demonstrated only for bdelloid species so far (Koehler and Hayes, 1969; *P. acuticornis odiosa* and this study; *A. vaga* and *Z. synaptae*). For *L. maerski*, Sørensen (2003) reports that 'most, if not all, sclerites are composed of tubular rods', including the accessory sclerites that are considered to be homologous to the rotiferan manubrium. Based on these facts, the presence of multitudes of cuticular tubes in the manubrium is suggested to be a ground pattern feature for at least Rotifera (Fig. 7, box 7) although this characteristic is absent in species of Seisonidea, Gnesiotrocha and Ploima.

On the basis of different phylogenetic analyses by Garcia-Varela and Nadler (2006; molecular analysis) and Sørensen and Giribet (2006; combination of morphological and molecular data) defining Monogononta as a sister group of Seisonidea + Bdelloidea, character transformations turn out to be different: In this scenario, the presence of large ramus chambers as well as the loss of tiny cuticular tubes in the manubrium must have evolved convergently in Seisonidea and Monogononta (compare with Fig. 7).

4.2. Informational value of the mastax musculature

By observing live individuals of *F. longiseta* under the microscope, we are able to see the pumping mastax through the transparent body. The whole mastax continuously contracts like a pumping heart, while the jaws open and close in a flapping manner.

Taking a look at the set of trophi muscles of *F. longiseta*, it can easily be recognized that five of the seven identified muscles (m. transversus manubrii, transversus manubrii retractor, m. dorsoventralis, m. ramo-manubricus, m. fulcro-ramicus) serve to perform the opening-closing action of the jaws (Fig. 4A–E).

One of the most common rotiferan mastax muscle that is present in several ploimate families, as well as in *P. annulatus* and *S. nebaliae*, is the mastax receptor retractor (syn.: musculus hypopharyngeus, Ahlrichs, 1995b; musculus fulcro mucosus, Dehl, 1934; dépresseure de piston, De Beauchamp, 1909). While the mastax receptor retractor is usually located in the so-called 'piston' which frontally terminates in the mastax receptor (see Riemann and Ahlrichs, 2008; Wulfken et al., 2010), *F. longiseta* displays neither a piston nor a mastax receptor located between the rami (Fig. 3A–C). Nevertheless, *F. longiseta* exhibits a muscular complex, the musculus fulcralis I and II (Figs. 4F, G and 5), that attaches to the caudal end of the fulcrum on its dorsal side and stretches out frontally. The accordance in positions of mastax receptor retractor and musculus fulcralis relative to the trophi leads to the assumption that both muscles are homologous.

While the existence of a mastax receptor retractor is uncertain for *L. maerski*, the muscle is absent in Gnathostomulida (Table 1) (compare character matrix of Sørensen, 2002). On the basis of this knowledge and the phylogenetic relationships proposed by Sørensen (2002), we propose the mastax receptor retractor to be a characteristic of the ground pattern for Rotifera (Fig. 7, box 9). The musculus fulcralis of *F. longiseta* can be considered to be a modification of the mastax receptor retractor (Fig. 7, box 10), whereas the absence of the mastax receptor retractor (as well as the fulcrum) in Bdelloidea is considered to be a secondary loss (Fig. 7, box 2).

5. Conclusion and perspectives

What information is provided by a comparative analysis of mastax morphology and musculature in *F. longiseta* and other rotifer species investigated so far concerning the polarity of characters related to the mastax morphology?

The ramate trophi of Bdelloidea and the malleoramate trophi of Filiniidae show much resemblance to each other in some points: Both of them display differentiated unci teeth as well as sickle-shaped manubria. A close relationship of Flosculariacea and Bdelloidea can be assumed on the basis of these facts. However, there are also differences such as the absence/presence of the fulcrum, aberrances in the shape and ultrastructure of the manubrium (multitudes of cuticular tubes in the manubrium of bdelloid species) as well as the occurrence of the unci teeth. Nevertheless, there is one additional (ultrastructural) characteristic that both taxa share: The presence of multitudes of tiny cuticular tubes in the ramus.

On the basis of the ultrastructural ramus architecture in *P. annulatus* and *S. nebaliae*, the fulcrate mastax of Seisonidea (ramus cuticle with fewer small cuticular tubes besides the ramus chambers) can be assumed to represent an evolutionary transitional stage between the ramate/malleoramate mastax types of Bdelloidea/Gnesiotrocha (ramus cuticle with multitudes of tiny cuticular tubes) and Ploima (ramus cuticle mostly homogeneous or mottled but without distinct small or tiny cuticular tubes besides the ramus chambers). Additionally, the presence of the mastax receptor retractor (see Ahlrichs, 1995b; musculus hypopharyngeus) in *P. annulatus* and *S. nebaliae* would support a closer relationship of Seisonidea and Ploima.

However, the manubrial architecture of *Filinia* (three distinct manubrial chambers – as present in the majority of ploimid species) favors a closer relationship of Gnesiotrocha and Ploima.

Additional morphological studies of further rotifer species are required to gain more information about the phylogenetic relationship of Seisonidea, Bdelloidea, Gnesiotrocha and Ploima. Detailed comparisons of the ultrastructure of the rotiferan trophi and their homologous parts in Micrognathozoa and Gnathostomulida could be carried out (as long as jaw elements can be homologized): By working out minute ultrastructural characteristics of the jaws and their cuticular tube structures, further statements could be made on the primary condition of trophi ultrastructure. More detailed ultrastructural information about the jaw apparatuses of Micrognathozoa and Gnathostomulida would enable a further piece to be contributed to the evolutionary puzzle.

A detailed identification of the pharyngeal musculature in Micrognathozoa and Gnathostomulida may be an additional helpful tool. Furthermore, the identification of similar muscle sets may be consulted to identify homologous jaw elements.

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