

# Ultrastructure and Function of the Mastax in *Dicranophorus forcipatus* (Rotifera: Monogononta)

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**ABSTRACT** Rotifers are characterized by a complex set of cuticularized jaw elements in the pharynx. The fine structure of the jaw elements has been the subject of SEM studies for some time, but only very limited information exists on the ultrastructure of the jaw elements and their function beyond taxonomic considerations. Drawing on SEM and TEM techniques, the present study presents a detailed analysis of the mastax in *Dicranophorus forcipatus*, a carnivorous monogonont rotifer species from freshwater habitats characterized by an extrusible, grasping jaw apparatus. Based on ultrathin serial sections, the jaw elements are reconstructed and, in total, nine paired and two unpaired muscles identified. Possibly homologous muscles in other rotifer species are discussed and functional considerations of the forcipate mastax are suggested. *J. Morphol.* 269: 698–712, 2008. © 2008 Wiley-Liss, Inc.

**KEY WORDS:** forcipate mastax; ultrastructure; feeding mode

Rotifers are a remarkably diverse group of aquatic micrometazoans with about 2,000 species described to date (Wallace et al., 2006; Segers, 2007). They are most abundant, both in terms of species diversity and biomass, in freshwater habitats. A number of species also occur in marine environments and in moist terrestrial habitats such as wet moss cushions and damp soil. One of the most characteristic features of rotifers is a complex set of pharyngeal, cuticularized jaw elements used in various ways for food uptake and processing. The pharyngeal bulb together with the jaw elements is called the mastax; the jaw elements as such are referred to as trophi. The ultrastructure of the trophi is remarkably similar to jaw elements in Gnathostomulida and *Limnognathia maerski*. Based on this common ultrastructural feature and other shared characters, rotifers together with gnathostomulids, *Limnognathia maerski*, *Seison* and the parasitic acanthocephalans have been suggested to comprise a monophyletic taxon Gnathifera (Ahlrichs, 1995; Rieger and Tyler, 1995). Studies relying on molecular data do not produce unambiguous results regarding the monophyly of Gnathifera: While some support its monophyly including also Cyclophora (Giribet et al., 2000), it is questioned by others (Giribet et al., 2004).

The morphology of the jaw apparatus in rotifers has always played an important role in rotifer research. Together with features of the rotatory apparatus, the mastax has been the most important character in traditional rotifer systematics (Remane, 1933; De Beauchamp, 1965). Different mastax types and feeding modes have been identified. Correct species identification very often is impossible without careful analysis of the fine structure of the trophi elements. In contemporary rotifer taxonomy, scanning electron microscopy (SEM) of isolated trophi elements has become a standard procedure (Kleinow et al., 1990; De Smet, 1998). Based primarily on such preparations, cladistic analyses of the taxon Rotifera have been carried out (Sørensen, 2002).

However, while our understanding of the fine structure of the hard elements based on SEM studies is fairly good, we know only very little about how these jaw elements function in living specimens and how they interact with the surrounding musculature. Hardly anything is known about the structural and functional basis of the different mastax types and different modes of feeding they make possible. So far, only very few investigations into the histology and ultrastructure of the rotifer mastax have been carried out. Most of them date back to the early part of the 20th century and are restricted to traditional light microscopic histological techniques (De Beauchamp, 1909; Martini, 1912; Seehaus, 1930; Stoßberg, 1932).

Recent advances in confocal laser scanning microscopy investigations of the muscular system of rotifers have produced superb results for body musculature, but, due to limited resolution, failed to unravel the complex arrangement of pharyngeal

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muscles and jaw elements (Sørensen et al., 2003; Santo et al., 2005; Sørensen, 2005). Transmission electron microscopic (TEM) investigations based on ultrathin serial sections and careful reconstructions seem to be indispensable for a detailed understanding of the complex mastax. Unfortunately, however, there are very few such studies (Koehler and Hayes, 1969a,b; Clément and Amsellem, 1989; Ahlrichs, 1995).

The present study is intended to present a more comprehensive understanding of the forcipate (grasping) mastax type. It is based on SEM preparations and TEM studies of complete ultrathin serial sections of the forcipate mastax in *Dicranophorus forcipatus*, a cosmopolitan, predatory rotifer species commonly occurring in the periphyton of freshwater habitats. For the first time our approach reveals the complex interplay of mastax hard elements, epithelial cells, and individual muscles responsible for the coordinated movement of the jaw apparatus in a representative of Dicranophoridae. Based on these reconstructions and supported by observations of living specimens, we suggest functions of individual muscles in the overall grasping movement of the jaw apparatus. Identifying individual muscles, we expect that future studies may reveal potentially homologous muscles in other species and that in such a way, the musculature of the rotifer mastax may become another tool in refining our knowledge of rotifer phylogeny.

## MATERIALS AND METHODS

Specimens of *Dicranophorus forcipatus* (O. F. Müller, 1786) were sampled in shallow ditches covered with *Lemna* sp. near Oldenburg, North-West Germany. For SEM preparations, individual specimens were dissolved following the protocol given by Kleinow et al. (1990) leaving only the cuticularized jaw apparatus. These jaws were carefully rinsed with distilled water, air-dried, and coated with gold. SEM was carried out on a Zeiss DSM 940 scanning electron microscope. In total, four specimens were prepared for SEM. For TEM studies, specimens were anaesthetized for 5 min in an aqueous solution of 0.25% bupivacaine (Bucain<sup>®</sup>) and subsequently fixed with 1% OsO<sub>4</sub> in 0.1 M NaCaCodylate buffer at 0°C. After fixation, specimens were dehydrated in an increasing acetone series, subsequently embedded in Araldite hardened at 60°C for 72 h and ultrasectioned (70 nm) on a Reichert ultracut followed by automatic staining with uranyl acetate and lead citrate (Leica EM Stain). The resulting TEM preparations were observed on a Zeiss 902 TEM at 80 kV. In total, two complete series of cross sections and one series of horizontal sections through the mastax were analyzed. Photographs of the sections were taken with a Dual Scan CCD camera and subsequently assembled digitally using the MIA (multiple image alignment) function of iTEM<sup>®</sup> software (soft imaging systems). The chief advantage of composite images is that imaging of larger structures at higher magnifications and better resolution is possible. Such digitally assembled images provided the basis for the reconstructions of the mastax. Observations of living specimens under a Leica DM-LB light microscope were carried out at lower to medium magnifications using both bright field (BF) and differential interference contrast (DIC). For light microscopic observations of the trophi, specimens were dissolved on a microscopic slide covered by a

coverslip. The position of the jaws was manipulated by carefully touching the edges of the coverslip with a fine needle. Digital images were taken with an Olympus color view I digital camera.

## Terminology

To avoid terminological confusion, the terms defined in the following are used consistently throughout this article (see also Fig. 2A).

Both the unpaired incus (paired rami, unpaired fulcrum) and the paired malleus (manubrium, unci) consist of distinct jaw elements that are connected at one point. This connection serves as a point of reference for the terms proximal–distal denoting relative position. For a description of the position of the jaw apparatus relative to the specimen, the terms frontal–caudal, dorsal–ventral and median–lateral are used.

Besides the fulcrum, rami, manubria and unci constituting the basic jaw elements that can be found in almost every rotifer species, there are a number of accessory jaw elements characteristic only of certain subtaxa of Rotifera. In *Dicranophorus forcipatus*, a pair of rod-shaped accessory jaw elements ventral to the rami can be found. Traditionally, these are called epipharynges. However, given their position ventral to the rami and considering the fact that in certain species accessory jaw elements dorsal to the rami exist as well, the term hypopharynges (sg. hypopharynx) is suggested and is introduced here for the first time.

## RESULTS

### The Cuticularized Jaw Elements

**General organization.** The mastax with its characteristic, grasping jaw apparatus is very conspicuous in living specimens observed under the light microscope (Fig. 1A). When retracted, it is positioned caudal to the mouth opening, through which it can be extruded to grasp various items of food. In vivo, the longitudinal axis of the jaw apparatus is slightly tilted against the body axis.

The mastax in *Dicranophorus forcipatus*, comprising the trophi, individual muscles, epithelial cells, paired salivary glands and sensory cilia, is organized in a strictly bilaterally symmetrical way. The cuticularized jaw apparatus consists of the unpaired fulcrum and the paired rami (together referred to as the incus), paired manubria with proximally attached unci (together referred to as the malleus) and paired hypopharyngeal rods positioned ventral to the rami (Figs. 1B,C and 2A). The cuticularized jaw elements are extracellular and surrounded by epithelial cells.

**Fulcrum.** The fulcrum is narrow in dorsal and elongate-triangular in lateral view with the tip projecting caudally (Fig. 3A–D). In cross section, it appears to be composed of narrow, electron-lucent rods with an electron-dense core (Figs. 4A, 7E,F). Both its ventral and its dorsal edge are slightly expanded. Proximally, the fulcrum splits into two parts divided by a complete dorso-ventral cleft (Fig. 7B). The two parts of the fulcrum can be followed in their course alongside the basal chamber of the rami (see below) up to the middle of the rami.

**Rami.** The paired rami are each constituted by two hollow cavities called the subbasal and the

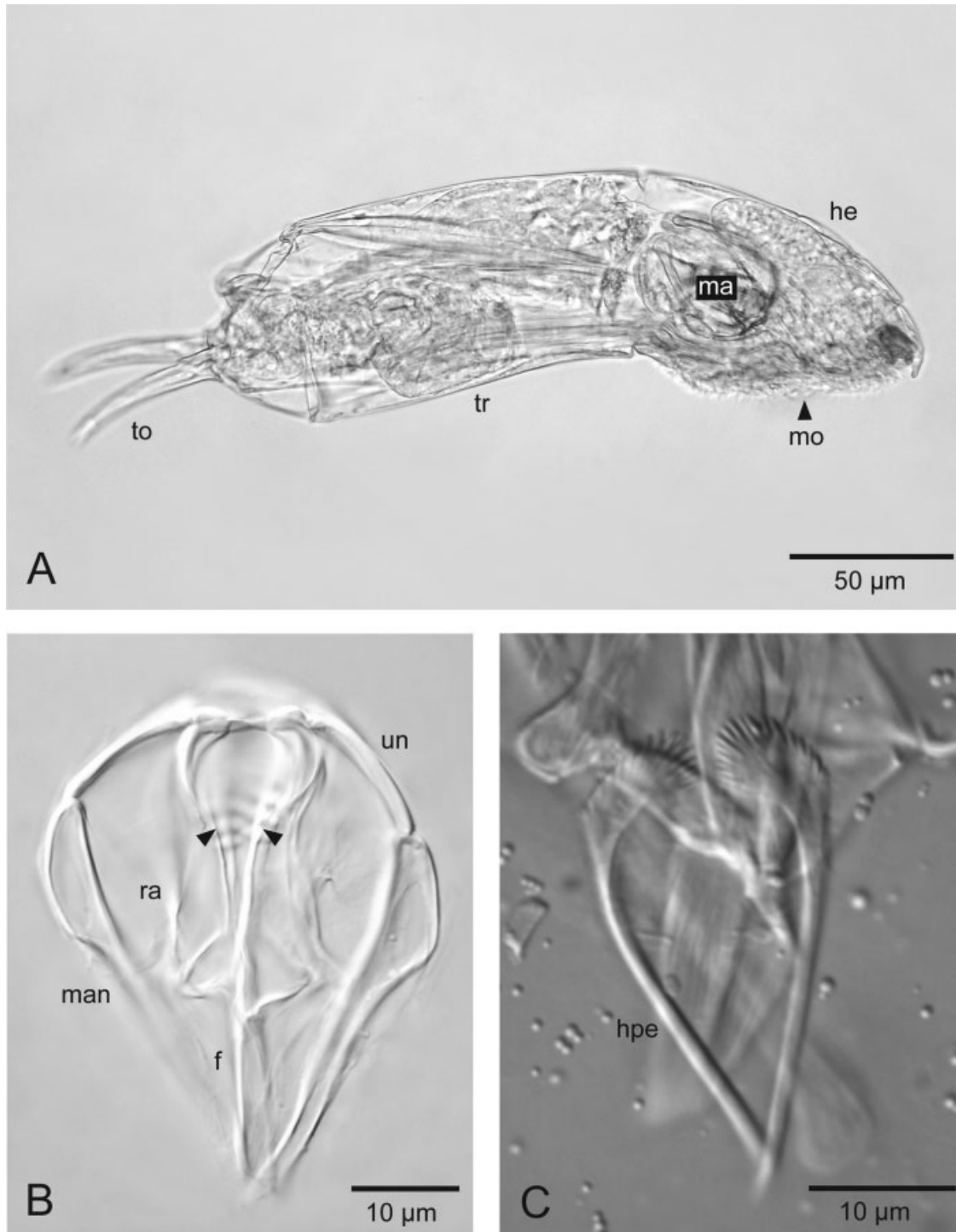


Fig. 1. *Dicranophorus forcipatus*. **A:** *Dicranophorus forcipatus*, habitus, lateral view. BF. Note the position of the jaw apparatus. **B:** Jaw apparatus. Arrowheads indicate triangular teeth on the inner margins of both rami. DIC. **C:** Hypopharyngeal rods with spatulate, serrated endings. DIC. f, fulcrum; he, head; hpe, hypopharyngeal element; ma, mastax; man, manubrium; mo, mouth opening; ra, ramus; to, toes; tr, trunk; un, uncus.

basal chamber, whose walls are fused. Both chambers are characterized by distinct openings visible in both SEM preparations (Fig. 3A,D) and in TEM cross sections (Fig. 4B). The opening of the more proximal subbasal chamber is oriented ventrally,

the opening of the distal basal chamber laterally. The internal cavities of these two chambers are partly filled with epithelial cell material communicating with the surrounding epithelial cells via the openings in the chambers of the rami (Fig. 4B).

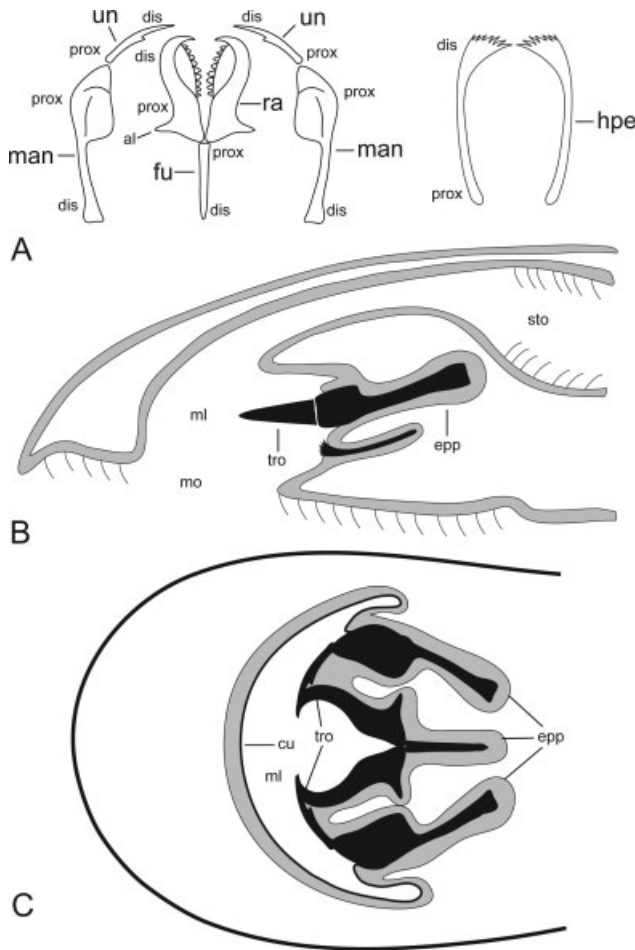


Fig. 2. The jaw apparatus of *Dicranophorus forcipatus*. **A:** The jaw apparatus and terms denoting relative position. **B:** Position of jaw apparatus in specimen, lateral view. **C:** Position of jaw apparatus, dorsal view. al, alula; cu, cuticle; dis, distal; epp, epithelial pouch; fu, fulcrum; hpe, hypopharyngeal element; man, manubrium; ml, mastax lumen; mo, mouth opening; prox, proximal; ra, ramus; sto, stomach; tro, trophi; un, unci.

Unlike the fulcrum, the walls of the rami chambers are composed of homogeneous, electron-lucent material (Fig. 7B). The inner margins of the rami are studded with triangular teeth (Figs. 1B and 3B). Each ramus terminates in two distinct teeth. In the proximal section of the rami, the basal chambers each bear a blunt, triangular alula (Fig. 3A).

**Manubria.** The paired manubria are composed of the elongate, narrow, distal cauda and the wider, proximal clava (Fig. 3A). In a manner similar to the rami, the manubrial caudae are characterized by an internal cavity partly filled with epithelial cells and a wall composed of homogeneous, electron-lucent material (Fig. 4A,B). The manubrium has a distinct opening projecting ventromedially (Fig. 4B). The distal end of the elongate manubrial cauda is slightly expanded (Fig. 3B). The widened clava bears a dorso-medially projecting lamella (Fig. 3B) which, in cross section,

appears as an electron-lucent, slightly curved arc (Figs. 4B and 7C).

**Unci.** The paired unci are loosely attached to the manubrial clavae through flexible, cuticularized material (Fig. 3A,B). In frontal view, they terminate in a long and acutely pointed, incurved tooth interlocking with the terminal rami teeth (Fig. 3C).

As opposed to the rami and manubria, the unci lack internal cavities and are composed of homogeneous, electron-lucent material (Fig. 7A).

**Hypopharyngeal elements.** Ventral to the rami, there is a pair of rod-shaped hypopharyngeal elements, each with a strongly elongate proximal part and an incurved, widened distal end with a serrated margin (Figs. 1C and 3A). In cross section, the hypopharyngeal elements, like the fulcrum, are made up of electron-lucent rods with an electron-dense core (Fig. 4C).

### The Muscular System of the Mastax

Between the jaw elements and adjacent epithelial cells, there is a bilaterally symmetrical system of individual muscles. In total, nine paired and two unpaired muscles could be identified. Generally, the muscles span individual jaw elements or connect jaw elements and the body wall. The attachment of muscles to jaw elements is in all cases mediated by epithelial cells connected to muscle cells and extracellular jaw elements through hemidesmosomes and electron-dense filaments (Fig. 7E,F).

Except for the unpaired *Musculus transversus manubrii* (see below), all muscles identified are single celled with z-dots connecting the individual sarcomeres. No regular pattern of striation has been observed. The complete set of mastax muscles consists of the following muscles, which, for ease of communication, we have individually labelled with reference to the jaw elements to which they attach (Fig. 8A-J).

***Musculus fulcro-ramicus* (Fig. 8A).** This very conspicuous, paired muscle connects the unpaired fulcrum and the paired rami. Its broad proximal ends are attached to the distal half of the fulcrum over a length of  $\sim 10 \mu\text{m}$  (Fig. 5). Its distal ends contact the alulae of the rami. In cross section, the muscle can be seen to almost completely envelop the narrow fulcrum (Fig. 4A). In total, this muscle in its relaxed state has a length of  $\sim 20 \mu\text{m}$  and, where it is attached to the fulcrum, a width of  $12 \mu\text{m}$  in cross section.

***Musculus transversus manubrii* (Fig. 8B).** This unpaired muscle interconnects the clavae of both manubria. It is positioned dorsal to the rami and is anchored to the edges of the manubrial lamellae (Fig. 7D). The *Musculus transversus manubrii* is fairly flat with a length of  $\sim 10 \mu\text{m}$  and a maximum width of  $4 \mu\text{m}$  in cross section. Towards the points of attachment to the clavae,

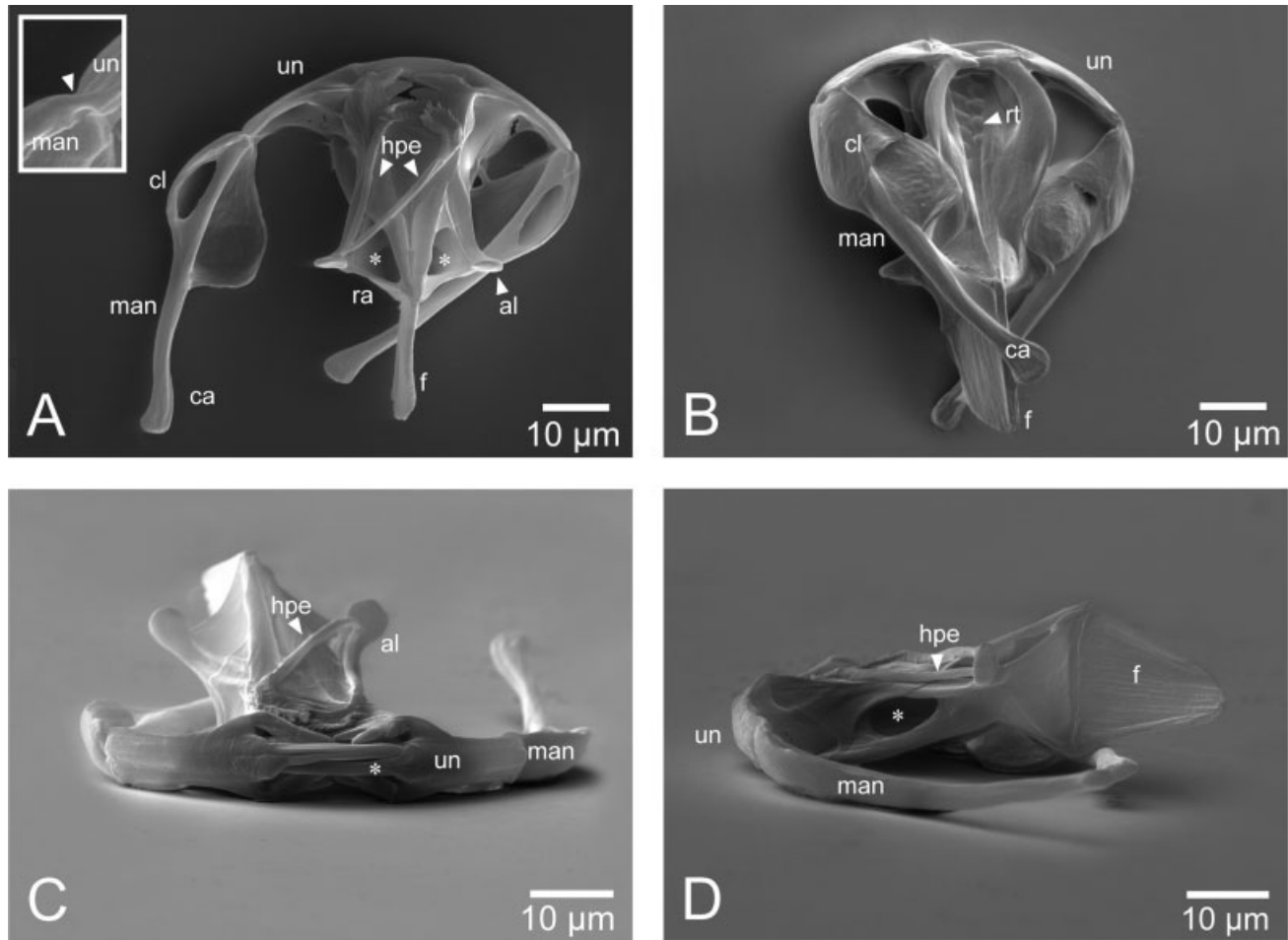


Fig. 3. *Dicranophorus forcipatus*. The cuticularized elements of the jaw apparatus. SEM. **A:** Ventral view. Asterisks (\*) indicate openings of ramus subbasal chambers. Inset: Close-up of A, showing flexible cuticularized material connecting manubrium and unci. **B:** Dorsal view. **C:** Frontal view. Asterisk (\*) indicates incurved unci tooth. **D:** Lateral view. Asterisk (\*) indicates opening of ramus basal chamber. al, alula; ca, cauda; cl, clava; f, fulcrum; hpe, hypopharyngeal element; man, manubrium; ra, ramus; rt, ramus teeth; un, unci.

the muscle tapers considerably. Unlike all other muscles identified, the *Musculus transversus manubrii* is composed of two cells attached to one another in the medio-sagittal plane.

***Musculus fulcro-manubricus* (Fig. 8C).** Unlike the broad *Musculus fulcro-ramicus*, the paired *Musculus fulcro-manubricus* in horizontal section appears long, narrow and slightly curved (Figs. 6 and 7C). It spans the distal end of the fulcrum and the clava of the manubrium. In cross section, this muscle is fairly inconspicuous and can be seen to run parallel to the dorsal edge of the fulcrum (Fig. 4A). In its course towards the clava, the *Musculus fulcro-manubricus* slightly bends dorsally. In its relaxed state, this muscle reaches a total length of 45 µm. In cross section, it is narrow and does not exceed a width of 3 µm.

***Musculus manubrico-uncus* (Fig. 8D).** The paired *Musculus manubrico-uncus* is one of the two pairs of prominent longitudinal muscles. It

interconnects the distal ends of the manubria and the unci (Figs. 5 and 7A,C). In cross section, this muscle is the most massive of all muscles in the mastax of *Dicranophorus forcipatus* (Fig. 4B). In its course, it runs just ventral to the arched lamellae of the clava. Where the *Musculus manubrico-uncus* attaches to the manubrium, it envelopes half of the manubrium, forming a muscular sheath (Fig. 4A). The total length of the *Musculus manubrico-uncus* in its relaxed state is 40 µm. In cross sections, it reaches a width of up to 8 µm.

***Musculus caudo-ramicus* (Fig. 8E).** This paired muscle differs from the other muscles, since it is not attached to jaw elements on both sides. Although its origin could not be determined with certainty, it is probably connected to the *Musculus circumglandis* (see below) caudally. More frontally, the *Musculus caudo-ramicus* continues alongside the rami (Fig. 5). Just prior to the terminal teeth of the rami, it attaches to the basal chamber of the

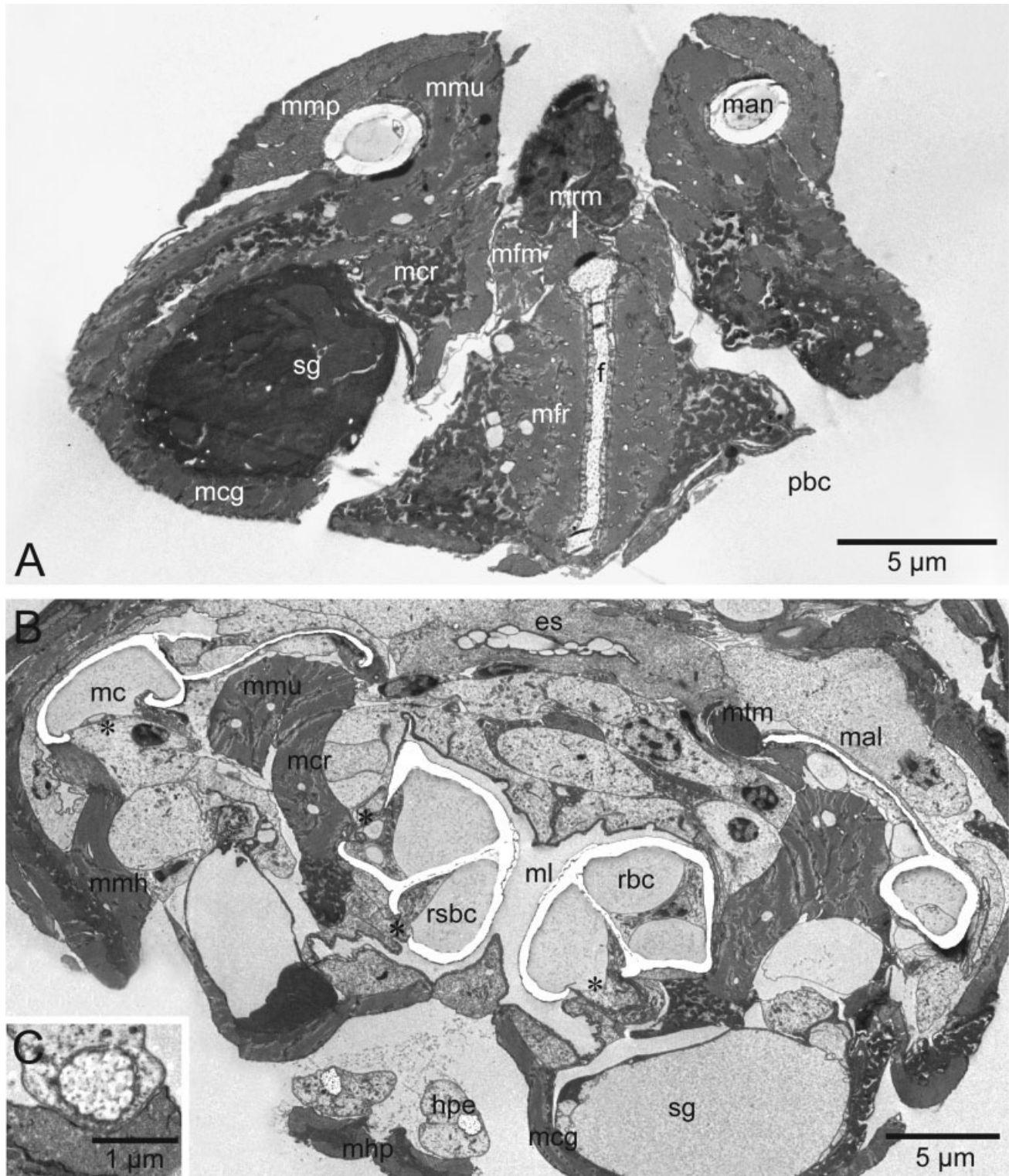


Fig. 4. Cross sections through the jaw apparatus of *Dicranophorus forcipatus* at two different levels in fronto-caudal axis. TEM. **A:** Section through caudal region. **B:** Section through middle region. Note the openings of the trophi chambers indicated by an asterisk (\*). **C:** Cross section through hypopharyngeal element. Note electron-lucent rods and electron-dense cores. es, esophagus; f, fulcrum; hpe, hypopharyngeal element; mal, manubrial lamella; man, manubrium; mc, manubrial chamber; mcp, Musculus circumglandis; mcr, Musculus caudo-ramicus; mfm, Musculus fulcro-manubricus; mfr, Musculus fulcro-ramicus; mhp, Musculus hypopharyngeus; ml, mastax lumen; mmh, Musculus manubrico-hypopharyngeus; mmp, Musculus manubricus perioralis; mmu, Musculus manubrico-uncus; mrm, retractor of mastax receptor; mtm, Musculus transversus manubrii; pbc, primary body cavity; rbc, ramus basal chamber; rsbc, ramus subbasal chamber; sg, salivary gland.



Fig. 5. Horizontal section through the jaw apparatus of *Dicanophorus forcipatus*. TEM. Arrowheads indicate cilia of mastax receptor. ep, epithelial cell; f, fulcrum; man, manubrium; mcg, Musculus circumglandis; mcr, Musculus caudo-ramicus; mfr, Musculus fulcro-ramicus; ml, mastax lumen; mmu, Musculus manubrico-uncus; mr, mastax receptor; ra, ramus; sg, salivary gland.



Fig. 6. Horizontal section through the jaw apparatus of *Dicranophorus forcipatus*. TEM. Plane of section more dorsal to that in Figure 5. gg, gastric glands; man, manubrium; mcg, Musculus circumglandis; mcr, Musculus caudo-ramicus; mfm, Musculus fulcro-manubricus; mmp, Musculus manubricus perioralis; mmu, Musculus manubrico-uncus; sg, salivary gland.



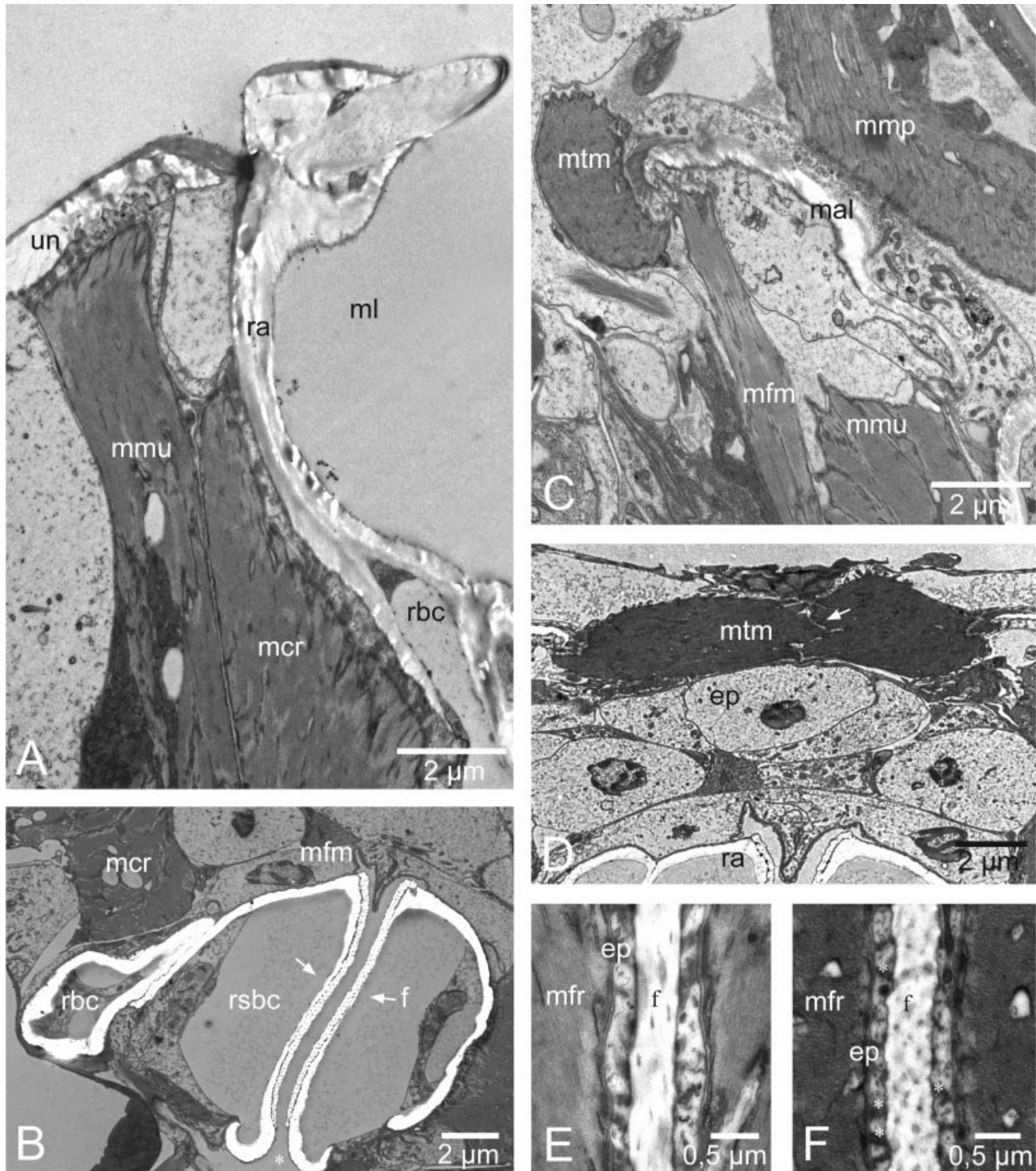


Fig. 7. Selected details of the jaw apparatus of *Dicranophorus forcipatus*. TEM. **A:** Horizontal section through ramus terminal tooth, uncus and adjacent musculature. **B:** Cross section through rami chambers. Note the ultrastructural differences between the wall of rami subbasal chamber and fulcrum. Fulcrum indicated by arrows. Ventral opening to dorso-ventral cleft in fulcrum indicated by asterisk (\*) **C:** Horizontal section through manubrial lamella and adjacent musculature. **D:** Epithelial cells dorsal to rami, cross section. Arrow indicates dividing line between the two cells forming the Musculus transversus manubrii. **E:** Epithelial cell enveloping fulcrum, adjacent musculature, horizontal section. **F:** Epithelial cell enveloping fulcrum, adjacent musculature, cross section. Asterisks (\*) indicate electron-dense filaments traversing epithelial cell. ep, epithelial cell; f, fulcrum; mal, manubrial lamella; mcr, Musculus caudo-ramicus; mfm, Musculus fulcro-manubricus; mfr, Musculus fulcro-ramicus; ml, mastax lumen; mmp, Musculus manubrico-uncus; mmu, Musculus manubrico-uncus; mtm, Musculus transversus manubrii; ra, ramus; rbc, ramus basal chamber; rsbc, ramus subbasal chamber; un, uncus.

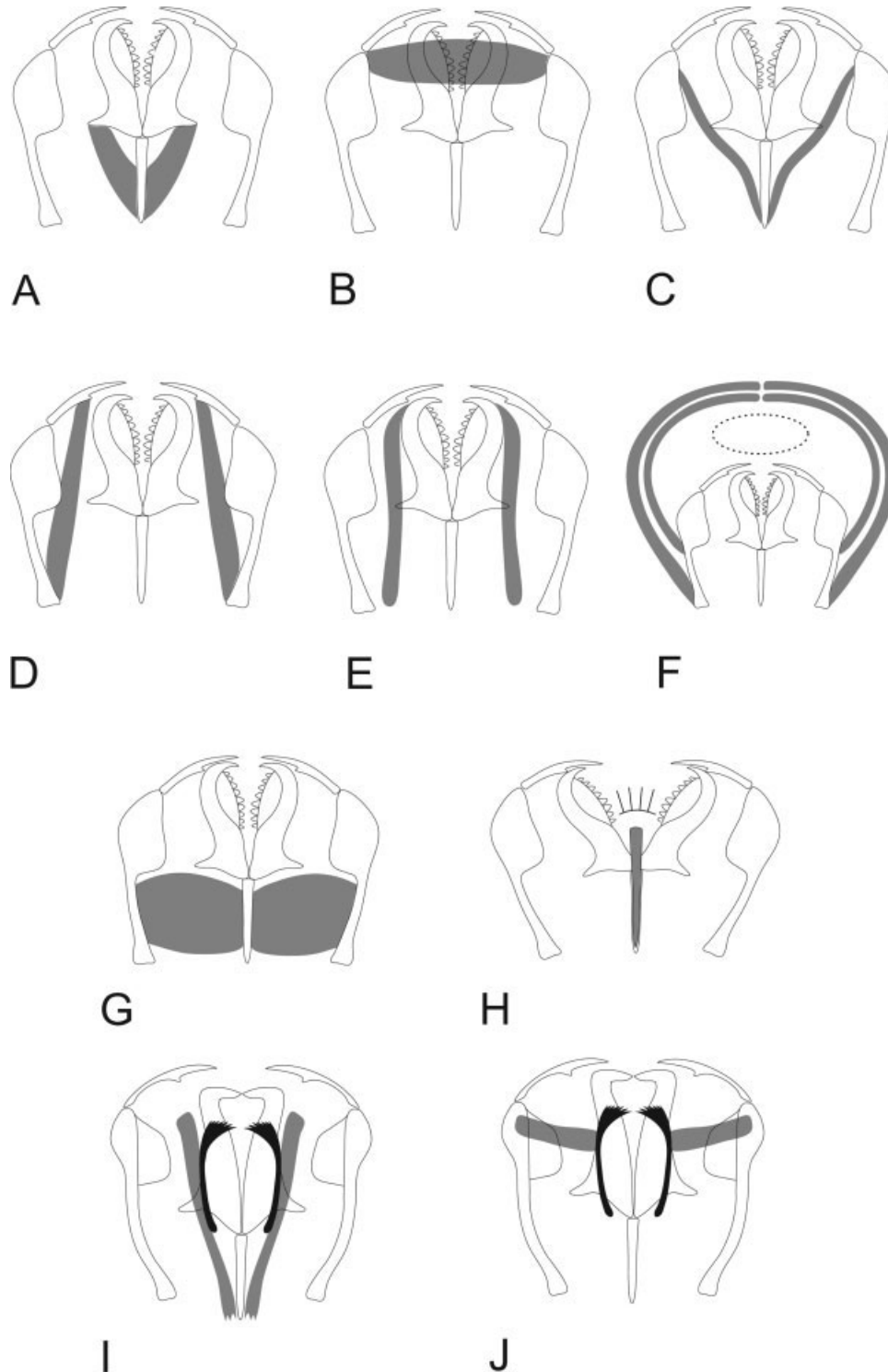


Fig. 8. Musculature of the jaw apparatus of *Dicranophorus forcipatus*, diagrammatic. Dorsal view of jaw elements in **A–H**, ventral view in **I** and **J**. **A**: Musculus fulcro-ramicus. **B**: Musculus transversus manubrii. **C**: Musculus fulcro-manubricus. **D**: Musculus manubrico-uncus. **E**: Musculus caudo-ramicus. **F**: Musculus manubricus perioralis. Dotted line indicates mouth opening. **G**: Musculus circumglandis. **H**: Mastax receptor retractor. **I**: Musculus hypopharyngeus. **J**: Musculus manubrico-hypopharyngeus.

rami (Fig. 7A). The Musculus caudo-ramicus in its relaxed state has a length of  $\sim 35 \mu\text{m}$  with a maximum width of  $6 \mu\text{m}$ .

**Musculus manubricus perioralis (Fig. 8F).** Two pairs of muscles attached to the manubria surround the mouth opening (Figs. 4A and 6).

They are attached to the distal end of the manubria and the middle region of the manubria, respectively. Frontal to the mouth opening, the two muscles of each pair are in close contact via cell-cell connections. Both pairs of muscles have a total length of about 60  $\mu\text{m}$ . Frontally, they are flattened, forming thin muscular layers up to 15  $\mu\text{m}$  wide, but only 3  $\mu\text{m}$  strong.

**Musculus circumglandis (Fig. 8G).** The salivary glands (see below) are surrounded by the paired *Musculus circumglandis* (Figs. 4A and 5). This muscle is a conspicuously thin, muscular sheath enveloping the salivary glands. Over a considerable length ( $\sim 15 \mu\text{m}$ ), it is attached to the ventral edge of the manubrial caudae. Enveloping the salivary glands, it connects to the ventral edge of the fulcrum.

**Mastax receptor retractor (Fig. 8H).** This inconspicuous, unpaired muscle is positioned above the dorsal edge of the fulcrum and terminates in the mastax receptor (see below) between the rami (Fig. 4A). This muscle could only be followed over a distance of  $\sim 30 \mu\text{m}$ ; it originates distally at the dorsal edge of the fulcrum.

**Musculus hypopharyngeus (Fig. 8I).** This is a paired longitudinal muscle accompanying the hypopharyngeal rods. In cross sections, it appears to run freely through the primary body cavity ventral to the rami (Fig. 4B). Frontally, the *Musculus hypopharyngeus* is anchored in the ventral body wall. Since it was not possible to determine exactly where this muscle has its origin caudally, no measurements are given.

**Musculus manubrico-hypopharyngeus (Fig. 8J).** This is a paired, slightly curved muscle connecting the manubria and the hypopharyngeal rods (Fig. 4B). It is attached to the manubria ventral to the opening of the manubrial chamber and to the hypopharyngeal rods caudal to their serrated distal endings. The total length of this muscle is  $\sim 15 \mu\text{m}$ .

### Epithelial Cells, Salivary Glands and Sensory Cilia

Besides the elements of the jaw apparatus and the muscular system, the most conspicuous feature of the mastax in TEM sections is some of the epithelial cells. Especially the voluminous epithelial cells dorsal to the rami are characterized by a large, polymorphic nucleus and homogeneous, electron-lucent cytoplasm. Longitudinal muscle strands (*Musculus fulcro-ramicus*, *Musculus caudo-ramicus*) can be seen in close contact to them (Figs. 5 and 7D).

On either side of the fulcrum, slightly displaced ventrally, a salivary gland is positioned (Figs. 4A and 5). The salivary glands discharge their contents into the mastax lumen through openings just below the subbasal chambers of the rami. In TEM sections, the salivary glands are characterized by

stacks of membranes and electron-dense membrane-bound inclusions. More distally towards the opening of the duct into the mastax lumen, the salivary glands are apparently filled with electron-lucent material (Fig. 4B). It was not possible to determine with certainty, whether only one or several cells form the salivary glands.

In the space between the rami, the mastax receptor projects its sensory cilia into the mastax lumen. The cilia are arranged in two distinct groups, forming ciliary tufts (Fig. 5).

### Mastax Lumen and Position of Jaw Elements

The spacious mouth cavity is continued by the narrower lumen of the mastax (Fig. 2B,C). The mastax lumen leads into the esophagus running dorsal to the jaw apparatus and opening into the ciliated stomach. The jaw elements are positioned in a pouch caudal to the mastax lumen and only partly project into this lumen. The epithelial wall of the mastax lumen is covered by a thin cuticular layer. As the most caudal jaw element, the distal part of the fulcrum is completely surrounded by epithelial cells and muscles. Frontal to where the fulcrum splits into two parts, the two parts are in direct contact to the mastax lumen. The elongate caudae of the manubria are completely surrounded by epithelial cells and muscles. Only the lateral surfaces of the manubrial clavae are in direct contact with the mastax lumen. For the greatest part of their length, the inner surfaces of the rami project freely into the lumen of the mastax (Fig. 4B). The frontally projecting surface of the uncus is in direct contact with the mastax lumen as well (Fig. 7A). For the greater part of their length, the hypopharyngeal rods are positioned slightly ventral to the mastax with only their serrated endings projecting into the mastax lumen.

### Observations of Living Specimens

In the following, a brief description is given of how the individual jaw elements move in the overall coordination of the grasping action of the forcipate mastax in *Dicranophorus forcipatus*. The description based on light microscopy of living specimens is complemented by functional hypotheses regarding the role of individual muscles (see Discussion). For a summary of the movements of the jaw elements, the reader is referred to the diagrammatic Figure 9. Arrows indicate movement of jaw elements relative to each other and to the mouth opening.

Each cycle of prey uptake is initiated by a forward thrust of the incus (Fig. 9A). The rami are extruded through the mouth opening. Having been thrust forward, the rami are subsequently spread out (Fig. 9B). The unci, interlocking with the rami by the uncinial apophyses, follow the spreading of the rami. Subsequently, the whole malleus is thrust forward

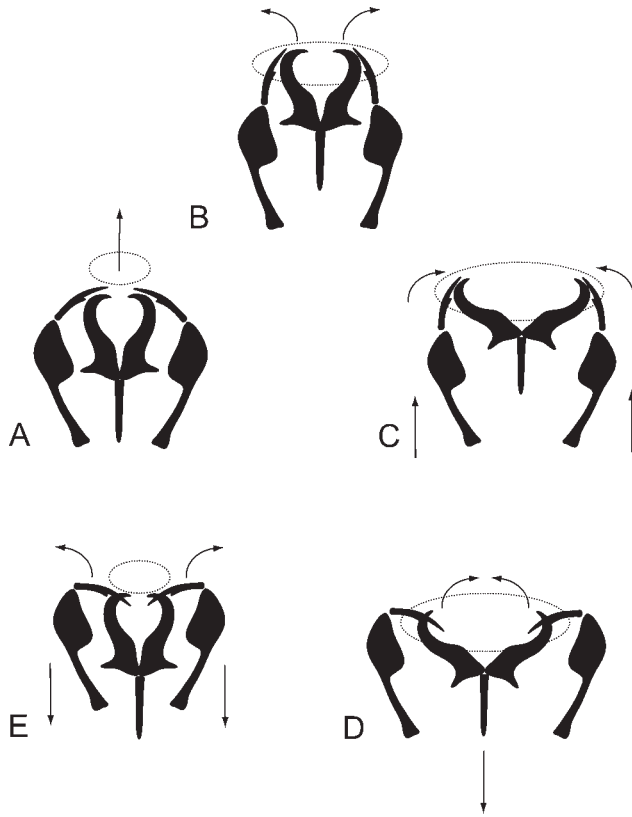


Fig. 9. Jaw apparatus of *Dicanophorus forcipatus* and movement of individual jaw elements relative to each other and to mouth opening in food uptake (A–E). Arrows indicate direction of movement of jaw elements; mouth opening is symbolized by dotted line.

(Fig. 9C). Hinged on the rami tips, the unci are tilted inwards. As a result, items of prey are pushed into a position suitable for the rami to draw them into the mouth opening. Prey items are grasped by the closing rami (Fig. 9D). With the rami closed, the incus is retracted, drawing the prey into the mouth opening. Finally, the manubria are retracted and the unci, hinged on the rami, resume their original position relative to the manubria (Fig. 9E).

Analyzing the contents of the stomach reveals the predatory nature of *Dicanophorus forcipatus*. The presence of trophi of *Cephalodella* and *Lecane* species demonstrates that *D. forcipatus* feeds on other rotifer species. Apart from ingested rotifer trophi, nematodes and the remains of gastrotrichs could be found as well.

## DISCUSSION

### The Cuticularized Jaw Elements Within Rotifera

Confirming the results of previous studies on other rotifers species (Koehler and Hayes, 1969a,b; Ahlrichs, 1995), the cuticularized elements of the

jaw apparatus in *Dicanophorus forcipatus* are clearly extracellular.

An ultrastructural composition of densely packed, electron-lucent rods with electron-dense cores as demonstrated here for the fulcrum and the hypopharyngeal rods in *Dicanophorus forcipatus* has also been reported for the fulcrum in *Polyarthra vulgaris*, *Taphrocampa selenura*, *Encentrum marinum*, *Seison nebaliae*, *S. annulatus* (Ahlrichs, 1995), *Brachionus calyciflorus*, *Notomata copeus* (Clément and Wurdak, 1991) and the manubrium of *Philodina acuticornis* (Koehler and Hayes, 1969a). In *S. annulatus* and *S. nebaliae*, the ultrastructure of the jaw elements other than the fulcrum is not completely clear. Judging by the images provided (Ahlrichs, 1995), it may very well be that they are made up of modified electron-lucent rods with electron-dense cores as well.

The ultrastructure of the rami and manubria in *Dicanophorus forcipatus* (wall composed of homogeneous, electron-lucent material, cavities filled with cellular material) is similar to the rami and manubria of the monogonont rotifers *Asplanchna sieboldi* (Koehler and Hayes, 1969b), *B. calyciflorus*, *Asplanchna brightwelli*, *N. copeus* (Clément and Wurdak, 1991), *E. marinum* and *Colurella colurus* (Ahlrichs, 1995).

A solid uncus made up of homogeneous cuticle as in *Dicanophorus forcipatus* has not been expressly mentioned for the other monogonont rotifers investigated so far, but, given the similarity in ultrastructural composition of the different jaw elements across different species, can be presumed to be a universal condition in monogonont rotifers.

The differences in ultrastructure of the jaw elements may be a reflection of different developmental modes. It seems probable that the cuticular walls of the rami and the manubria are secretion products of epithelial cells that, after secretion of the hard material, persist within the cavities and at least partly fill them. The electron-lucent rods with electron-dense cores comprising the fulcrum and the hypopharyngeal rods may be interpreted as a product of microvilli secreting cuticular material to the outside, possibly in an analogous manner to the formation of setae in certain annelids (for a discussion of seta formation, see Bartolomaeus, 1995).

In its plesiomorphic condition, the manubrium in rotifers is assumed to be composed of three distinct chambers (Markevich and Kutikova, 1989; Sørensen, 2002). The present investigation demonstrates a high degree of modification of the three chambers in *Dicanophorus forcipatus*. Only one of the three chambers is a hollow cavity with an oval opening and a lumen filled with epithelial cell material. Following De Smet's (1997) terminological suggestion, this chamber is the elongate anterior chamber forming the cauda of the manubrium. Both the median and the posterior chambers sensu De Smet are strongly modified. The median

chamber displays a slit-shaped, lateral opening and the posterior chamber is a flattened lamella with no internal cavity whatsoever.

### The Jaw Apparatus and its Homology Within Gnathifera

The jaws of the gnathiferan subtaxa Gnathostomulida, *Limnognathia maerski* and Rotifera are composed of electron-lucent rods with an electron-dense core (Ahlrichs, 1995; Rieger and Tyler, 1995; Kristensen and Funch, 2000). This shared ultrastructural character is considered the most important link between the jaws in Gnathostomulida, *L. maerski* and Rotifera (Sørensen, 2003). While the rods as basic building blocks can consistently be identified in the jaws of Gnathifera, it is more difficult to homologize individual jaw elements across the gnathiferan subtaxa. It has been suggested that the main jaw in *L. maerski*, the gnathostomulid articularium and the rotifer incus are homologous and apomorphic for Gnathifera. Moreover, the pseudophalangium and associated sclerites in *L. maerski* are considered homologous to the rotifer malleus (Sørensen, 2003).

Our study of the jaw apparatus in *Dicranophorus forcipatus* provides a further piece of evidence supporting the homology of the main jaw in *Limnognathia maerski*, the gnathostomulid articularium and the rotifer incus. While in SEM preparations of the jaw apparatus in *D. forcipatus* the fulcrum appears to be unpaired over its whole length, in TEM cross sections it can be seen to split where it connects to the rami. The fulcrum may thus be assumed to be a product of fusion of two, formerly distinct elements. Given the paired nature of the cauda in the main jaw of *L. maerski* (Kristensen and Funch, 2000) and in some species of Gnathostomulida, for instance *Gnathostomula paradoxa* (Sørensen and Sterrer, 2002), this observation suggests that the rotifer fulcrum, the gnathostomulid symphysis with its cauda and the symphysis and cauda in *L. maerski* are homologous. If the rotifer fulcrum, the gnathostomulid symphysis and cauda and the symphysis and cauda in *L. maerski* are homologous, this probably also holds true for the main jaws in *L. maerski*, the gnathostomulid articularium and the rotifer incus as a whole.

Interestingly, in SEM investigations of the jaw apparatuses of *Rastrognathia macrostoma* (Gnathostomulida) and *Encentrum graingeri* (Rotifera), the presence of a cavity in the pseudofulcrum of *R. macrostoma* and the fulcrum of *E. graingeri* has been demonstrated (Sørensen, 2000). It appears as a small hole in the fulcrum at the junction of the rami. Considering the ultrastructure of the fulcrum in *Dicranophorus forcipatus* and our interpretation of it as the product of fusion of two elements, Sørensen's findings corroborate the observations of the present study made on *D. forcipatus*.

### Musculature of the Mastax Within Rotifera

Since the complex set of mastax muscles in different rotifer species is only poorly known, it is very difficult to compare the situation in different species and to identify possibly homologous muscles inherited from a common ancestor. The situation is made even more difficult by the fact that early observations were based on light microscopic investigations, while the present study relies on TEM. Nonetheless, in surveying the older literature an attempt has been made to identify muscles possibly homologous to some of the muscles in the jaw apparatus of *Dicranophorus forcipatus*.

A paired muscle connecting the fulcrum and the rami (in the present study called *Musculus fulcro-ramicus*) has been reported for other taxa as well (*Epiphanes senta*: Martini, 1912, *Musculus fulcro-scaphalis*; *Euchlanis*: Stoßberg, 1932, *Musculus fulcro-scaphalis*; De Beauchamp, 1909, *Abducteur horizontalis*; *Testudinella patina*: Seehaus, 1930, *Musculus abductor rami*). Given the wide distribution of this paired muscle within Rotifera, it is plausible that it was inherited from a common ancestor and is homologous in all species where it has been identified. It can be presumed to have evolved very early in the stem lineage of rotifers, thus probably representing a plesiomorphic condition for *Dicranophorus forcipatus*.

In a species of the genus *Euchlanis* (Stoßberg, 1932) and in *Epiphanes senta* (Martini, 1912), the presence of a *Musculus flexor mallei* has been reported. Given its position and possible function (inward movement of uncus), homology to the *Musculus manubrico-uncus* in *Dicranophorus forcipatus* is plausible. Considering that both *Euchlanis* and *E. senta* are characterized by a grinding malleate mastax, whose grinding function is very different from the forcipate, grasping mastax in *D. forcipatus*, it is probable that the *Musculus manubrico-uncus* in *D. forcipatus* is not a specific adaptation to the grasping feeding mode. Hence, its presence in *D. forcipatus* may be plesiomorphic as well.

Martini (1912) in *Epiphanes senta* identified a paired muscle (*Musculus fulcro-manubricus*), attached to the caudal end of the fulcrum and the dorsal edge of the manubrial clava (processus posterior in Martini's terminology). Drawing on the criterion of relative position, it may be homologous to the *Musculus fulcro-manubricus* in *Dicranophorus forcipatus*.

Finally, Stoßberg (1932) found a paired muscle (*Musculus ramo-manubricus*) interconnecting the distal ends of the manubria and the rami in *Euchlanis*. He assumes its function to be the spreading of the rami. A possible homologue of this muscle in *D. forcipatus* may be the paired *Musculus caudo-ramicus*. However, unlike the *Musculus ramo-manubricus* in *Euchlanis*, the *Musculus caudo-ramicus* in *D. forcipatus* is not directly

attached to the manubrium, but seems to be anchored to the *Musculus circumglandis* in proximity to the distal ends of the manubria.

### Functional Considerations: The Role of Individual Muscles in Prey Uptake

Based on observations of living specimens and on the reconstruction of the muscular system of the jaw apparatus in *Dicranophorus forcipatus*, we suggest the following muscular activities in the overall coordination of prey uptake.

Each cycle of prey uptake is initiated by a forward thrust of the incus (Fig. 9A). The movement is likely to be brought about by a considerable contraction of the *Musculus fulcro-manubricus*. Spanning the fulcrum and the lamellar clava of the manubrium, this muscle is responsible for the movement of the incus relative to the malleus. The rami, having been extruded through the mouth opening, are subsequently opened by the sustained contraction of the *Musculus fulcro-ramicus* and the *Musculus caudo-ramicus* (Fig. 9B). Since the rami and the unci are linked by the uncincl apophyses interlocking with the rami, the unci passively follow the spreading of the rami. Following the opening of the rami, the whole malleus is thrust forward, presumably by contraction of the paired *Musculus manubricus perioralis*. Hinged on the rami tips, the unci are subsequently drawn backwards, brought about by a shortening of the *Musculus manubrico-uncus* (Fig. 9C). As a result, items of prey are pushed into a position suitable for the rami to draw them into the mouth opening. This grasping of prey by the rami is caused by the *Musculus fulcro-ramicus* and the *Musculus caudo-ramicus* relaxing their contraction and the resulting closing of the rami. In the absence of a definite antagonist to these two muscles, the closing movement of the rami is presumed to be brought about by an elastic connection of the proximal sections of the rami. As soon as muscle contraction ceases, the rami revert to their original position. At the same time, the incus moves backward in its original position, thus drawing captured prey into the mouth opening (Fig. 9D). To complete the cycle, the paired *Musculus manubricus perioralis* and the *Musculus manubrico-uncus* relax their contraction and the malleus resumes its original position (Fig. 9E). Since no antagonist to the *Musculus manubrico-uncus* could be identified, the returning of the unci to their original position is suggested to be brought about by an elastic connection of the manubria and unci and the resumption of the original, relative position with muscle contraction relaxing.

Although the overall movement of the jaw apparatus brought about by the mastax musculature is fairly clear, some questions still remain. Which mechanism is responsible for the backward move-

ment of the incus? Based on our TEM reconstructions, no muscular antagonist to the *Musculus fulcro-manubricus* has been identified. The same applies to the withdrawal of the malleus. No antagonist to the paired *Musculus manubricus perioralis* could be identified.

Given the proximity of the mastax muscles and the voluminous mastax epithelial cells, it is likely that these epithelial cells have a role to play as well in the overall coordination of the movements of the jaw elements. Maybe, they have a protective function and serve as cellular cushions for the mastax muscles. Their presence might be important in minimizing the abrasive effects of contracted muscle strands and cuticularized jaw elements moving immediately next to each other.

No clear function can be assigned to the paired hypopharyngeal elements. Since their serrated distal endings project into the mastax lumen, they can also be assumed to have a certain function in capture and uptake of prey. Maybe, they are responsible for securing an additional hold on the prey. Alternatively, their most important function might be to provide a point of attachment for the paired *Musculus manubrico-hypopharyngeus*, in effect stabilizing the whole complex of protrusible jaws and muscles.

### CONCLUSION

To arrive at a better understanding of the function and evolution of the forcipate mastax, studies similar to the present one ought to be carried out on other dicranophorid species as well as outgroup representatives. Possible candidates within *Dicranophoridae* are species of the large *Encentrum* group. Similar in function to *Dicranophorus forcipatus*, species of this genus are also characterized by a protrusible, grasping jaw apparatus. However, the overall shape of the jaw elements is considerably different from that in *D. forcipatus*. Possible outgroup candidates are species of the genera *Itura*, *Notommata*, and *Lindia*. Together with *D. forcipatus*, species of all these genera have a similarly shaped rotatory apparatus, similar body organization, share many characteristic features in the overall shape of the jaw apparatus and have traditionally been considered closely related (Harring and Myers, 1928; Remane, 1933). Recent analyses arrive at similar phylogenetic affinities (Sørensen, 2002; Sørensen and Giribet, 2006). Addressing these species in future studies is likely to further our understanding of the function and evolution of the forcipate mastax. Going beyond that and studying species characterized by jaw apparatuses of other functions (such as grinding, sucking, piercing) and reconstructing the musculature may provide new characters and potential synapomorphies helping us understand the evolution of the whole taxon Rotifera in greater detail.

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