Comparative analysis of the mastax musculature of the rotifer species *Pleurotrocha petromyzon* (Notommatidae) and *Proales tillyensis* (Proalidae) with notes on the virgate mastax type

Diana Wulfken[a,*], Eike F. Wilts[a], Pedro Martínez-Arbizu[b], Wilko H. Ahlrichs[a]

[a]*Systematics and Evolutionary Biology, Department of Biology and Environmental Sciences, Carl von Ossietzky University Oldenburg, 26111 Oldenburg, Germany*
[b]*Senckenberg Research Institute, German Centre for Marine Biodiversity Research DZMB, 26382 Wilhelmshaven, Germany*

Abstract

One defining rotiferan character is the mastax, a muscular pharynx that contains a set of cuticularized jaw elements, referred to as trophi. According to different arrangements and functions of the trophi, different basic mastax types are distinguished in literature. There have always been cases in which a distinct classification of a certain species' mastax has turned out to be difficult, since its trophi display characteristics of different mastax types. In our study we analyze the mastaxes of *Pleurotrocha petromyzon* (Ehrenberg, 1830) and the recently described species *Proales tillyensis* (Wilts and Ahlrichs, 2010) by means of TEM, CLSM and SEM to reconstruct their mastax musculature. Due to the strong agreement in its trophi morphology with *Proales fallaciosa*, whose mastax was designated as both, modified malleate and virgate, the mastax musculature of *P. tillyensis* was compared to the one of *P. petromyzon*. In total, we found eight muscles being associated with the pharyngeal jaw elements in *P. petromyzon* and nine such muscles in *P. tillyensis*. Both species share several muscles that previously have been delineated for other rotifer species. Among these muscles, *P. petromyzon* shows mastax receptor retractors that present the most important characteristic of the virgate mastax type and have been described for different species in earlier literature; by evoking a negative pressure in the mastax cavity when contracting, the muscles bring about the pumping function of this mastax type. Contrary to our expectations, *P. tillyensis* lacks mastax receptor retractor musculature, what let us draw the conclusion that this species' mastax cannot be categorized as distinct virgate, since the mastax receptor retractors present the most significant character of the virgate mastax.

1. Introduction

Rotifers are a diverse group of cosmopolitan aquatic micrometazoans with body length up to 1 mm, seldom larger. With about 2000 described species (Wallace et al., 2006; Segers, 2007) rotifers are most abundant in freshwater environments but are also present in marine waters as well as semiaquatic habitats. One of the defining rotiferan characters is the muscular pharynx, referred to as mastax, containing a set of cuticularized jaw elements (trophi). The trophi elements are used for food uptake and can be moved against each other by means of muscles attached to them. The pharyngeal jaw elements of the mastax are the most important character for erecting the monophyletic taxon Gnathifera Ahlrichs,

*Corresponding author.
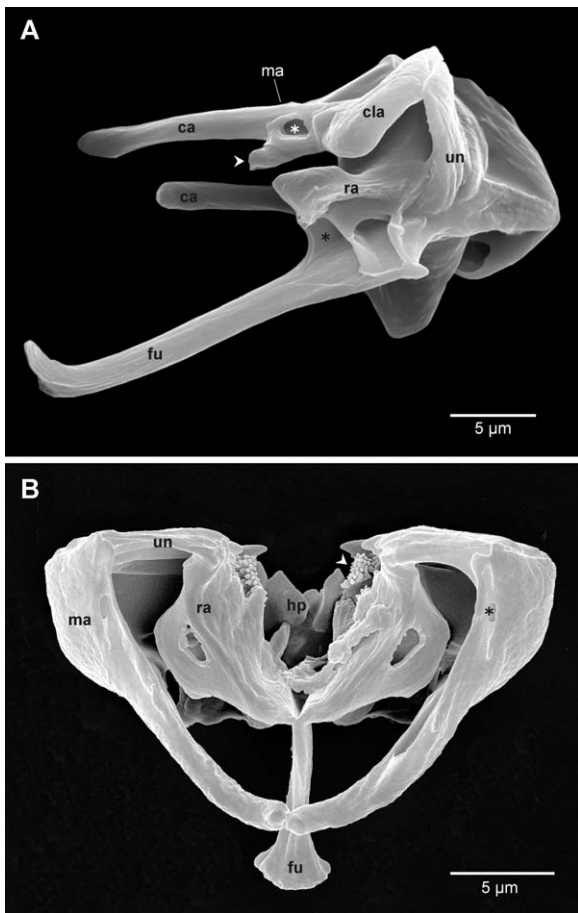E-mail address:* Diana.Wulfken@uni-oldenburg.de (D. Wulfken).

Fig. 1. The cuticularized jaw elements of *Pleurotrocha petromyzon* and *Proales tillyensis*. (A) Trophi of *P. petromyzon* in ventrolateral view, manubrial projection indicated by arrowhead, manubrium foramen medialis indicated by white asterisk, ramus foramen sub-basalis indicated by black asterisk; (B) trophi of *Proales tillyensis* in dorsal view, manubrium foramen dorsalis indicated by black asterisk, subuncus indicated by white arrowhead. ca, cauda; cla, clava; fu, fulcrum; hp, hypopharynx; ma, manubrium; ra, ramus; un, uncus.

Fig. 2. TEM images of cross-sections through the mastax of *Pleurotrocha petromyzon* at two different levels. (A) Distal mastax region with mastax ganglion and salivary glands, ventral manubrium projection indicated by asterisk, ligaments of the mastax indicated by arrowheads; (B) median mastax region, manubrium foramen ventralis indicated by arrowhead, ramus foramen basalis indicated by asterisk. fu, fulcrum; ma, manubrium; mcg, musculus circumglandis; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mg, mastax ganglion; mmc, median manubrial chamber; mmp, musculus manubrico-perioralis; mrm, musculus ramo-manubricus; mrre, mastax receptor retractor externus; mrri, mastax receptor retractor internus; mrrm, mastax receptor retractor medius; oe, oesophagus; ra, ramus; sg, salivary gland; vmc, ventral manubrial chamber.

1995 including Rotifera, Gnathostomulida, the non-jaw possessing, parasitic Acanthocephala as well as *Limnognathia maerski*. Within the group of rotifers the trophi are used for species identification and are one of the most important characters in rotifer systematics and phylogeny. The mastax and its trophi have been subject to several scanning electron microscopical (SEM) investigations during the last decades (e.g. Markevich and Kutikova, 1989; Kleinow et al., 1990; Sørensen, 2002; Fontaneto et al., 2007), whereas only few studies dealt with the rotiferan mastax based on histological sections (De Beauchamp, 1909; Martini, 1912; Seehaus, 1930; Stoßberg, 1932; Riemann and Ahlrichs, 2008; Wilts et al., 2010). The basic trophi set consists of paired manubria and unci (together referred to as the mallei), an unpaired fulcrum and paired rami (together referred to as the incus) (Figs. 1A and B, 6B and 8B). This arrangement of jaw elements is modified across the different families or species, reflecting their mode of life and feeding strategy.
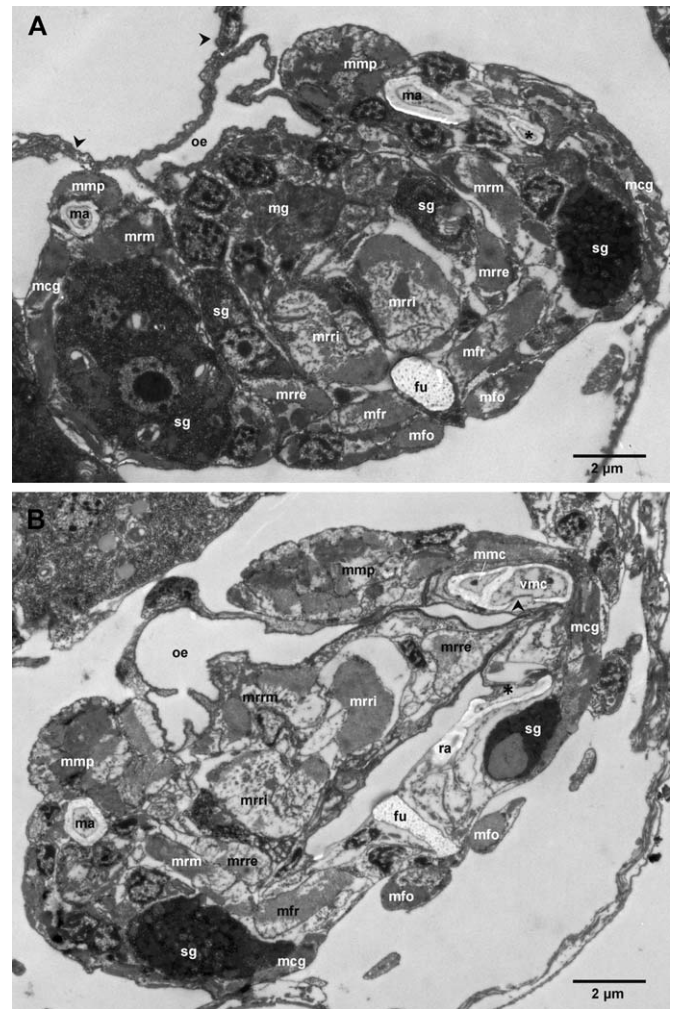
According to different arrangements and functions of the jaw elements, different basic mastax types can be distinguished in rotifers as following: malleate, modified malleate, ramate, malleoramate, fulcrate, incudate, cardate, uncinate, virgate and forcipate (compare De Beauchamp, 1909; Remane, 1929–1933; Nogrady et al., 1993). In this study, the mastax of *Pleurotrocha petromyzon* (Ehrenberg, 1830), categorized as virgate (De Smet, 2006), is investigated by means of transmission electron and confocal laser scanning microscopy, whereas the results are basically compared to series of CLSM
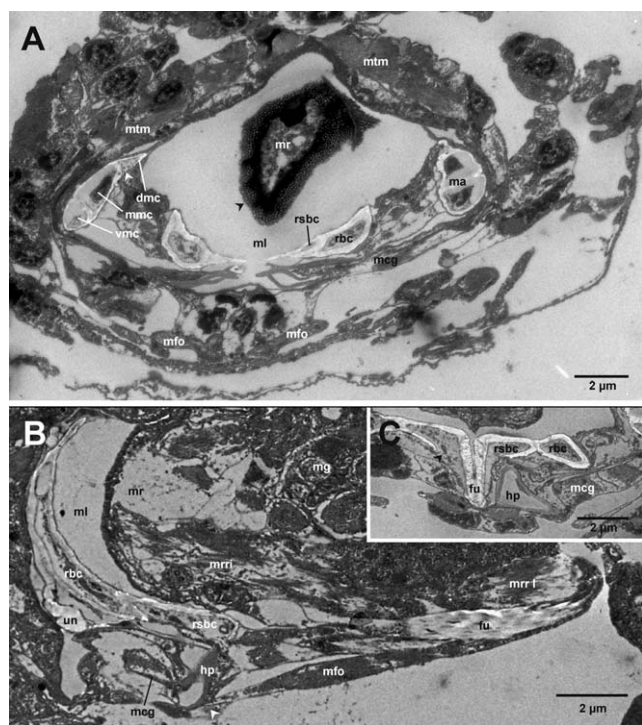
Fig. 3. TEM images of the mastax region of *Pleurotrocha petromyzon*. (A) Cross-section through the frontal mastax region, manubrium foramen dorsalis indicated by white arrowhead, villi of mastax receptor indicated by black arrowhead; (B) lateral section through the incus and hypopharynx with parts of the uncus. Note the attachment of the musculus fulcro-oralis to the hypopharynx as indicated by arrowhead; (C) basal incus region with hypopharynx attached by musculus circumglandis, ramus foramen subbasalis indicated by arrowhead. dmc, dorsal manubrial chamber; fu, fulcrum; hp, hypopharynx; ma, manubrium; mcg, musculus circumglandis; mfo, musculus fulcro-oralis; mg, mastax ganglion; ml, mastax lumen; mmc, median manubrial chamber; mr, mastax receptor; mrri, mastax receptor retractor internus; mtm, musculus transversus manubrii; rbc, ramus basal chamber; rsbc, ramus subbasal chamber; un, uncus; vmc, ventral manubrial chamber.

images of the mastax musculature of the recently described species *Proales tillyensis* Wilts and Ahlrichs, 2010. Due to the strong agreement in its trophi morphology with *Proales fallaciosa*, whose trophi system was originally designated as modified malleate (Voigt, 1957) and subsequently as virgate (see De Smet, 1996), *P. tillyensis* was chosen to compare it to the virgate mastax of *P. petromyzon*. The virgate mastax type is primarily characterized by a pumping action for food uptake by producing a negative pressure in the mastax cavity (Remane, 1929–1933).

On the example of *P. petromyzon* and *P. tillyensis* we like to gain information about how similar or different the mastaxes of the two species are. Are there just differences in the morphology of the jaw elements? Or do these different species with assumed same mastax types display a completely different organization of the mastax musculature? And if so, what func-

tional disparity does different muscle arrangements bring about?

Using a combination of CLSM and TEM on one hand, gives a picture of the relative position of the muscle strands to each other (CLSM). On the other hand the relative position of the muscles towards the trophi, as well as their points of insertion on the jaw apparatus can be determined (ultrathin sections). Using CLSM and TEM to unravel mastax musculature was lately used by Wilts et al. (2010) on the example of *Bryceella stylata*, introducing a helpful complementary method.

The free-living, predatory and necrophagic notommatid *P. petromyzon* is a cosmopolitan species, occurring in running water as well as in lakes and ponds with acidic or brackish conditions and can be found in warmer and in colder seasons. The species reaches a body length of 220–480 μm, whereas the symmetrical trophi (Fig. 1A) have a length of 30–37 μm (Nogrady and Pourriot, 1995). The warm temperature adapted proalid species *P. tillyensis* was found in floating plant material in Lake Tilly in Oldenburg, North-West Germany. The species reaches body lengths of up to 170 μm, the trophi (Fig. 1B) show lengths of 16–19 μm (Wilts and Ahlrichs, 2010).

2. Materials and methods

P. petromyzon and *P. tillyensis* were collected in two different lakes in Oldenburg/Northern Germany. Individual specimens were isolated from the sample under a stereomicroscope and studied by differential interference light microscopy (Leica DMLB), transmission electron microscopy (Zeiss 902 TEM) as well as confocal laser scanning microscopy (Leica TCS SP 5). The excavated 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 excavate the trophi. Trophi were rinsed with distilled water and afterwards pipetted onto a stub. After air-drying, the material was sputtered with platinum and then examined by SEM. In total, twelve specimens were investigated (two specimen of *P. petromyzon* and ten specimens of *P. tillyensis*).

For transmission electron microscopy, specimens were anesthetized with CO_2 and then fixed with 1% OsO_4 buffered in 0.1 M sodiumcacodylate at 4 °C for 1 h. Afterwards, specimens were dehydrated in an increasing acetone series, embedded in araldite and hardened at 60 °C for 72 h. Ultrathin serial sections (horizontal-, cross- and lateral-sections) of 80 nm of five female individuals (three specimen of *P. petromyzon* and two of *P. tillyensis*) 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
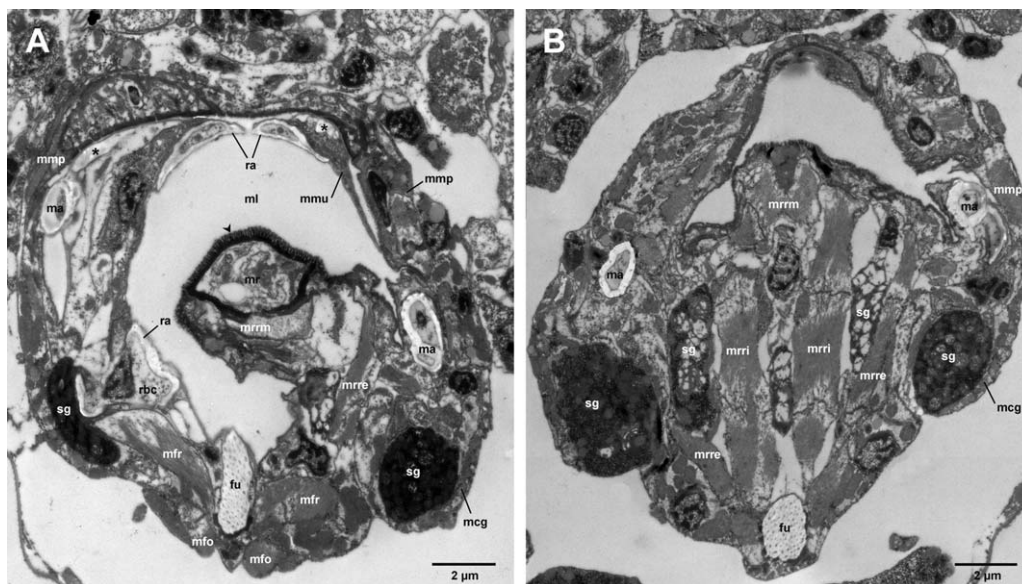
Fig. 4. TEM images of the mastax of *Pleurotrocha petromyzon*. (A) Horizontal section through the basal incus region of the mastax, unci indicated by asterisks, villi of mastax receptor indicated by arrowhead; (B) horizontal section through the dorsal mastax region. fu, fulcrum; ma, manubrium; mcg, musculus circumglandis; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; ml, mastax lumen; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mr, mastax receptor; mrre, mastax receptor retractor externus; mrri, mastax receptor retractor internus; mrrm, mastax receptor retractor medialis; ra, ramus; rbc, ramus basal chamber; sg, salivary gland.

examined and photographed in a Zeiss 902A TEM at 80 kV.

For confocal laser scanning microscopy (CLSM) specimens were placed in a drop of freshwater and relaxed in a 0.25% solution of bupivacine at 8 °C. The anaesthetised specimens were fixed for 1 h in phosphate-buffered 4% paraformaldehyde, rinsed in PBS and then made permeable by exposure to 0.1% Triton X-100 buffered in 0.1 M PBS for 1 h. For staining, 2 µl of 38 µM methanolic TRITC-labelled phalloidin solution (Sigma®) were added to 100 µl of Triton X-100 buffered in 0.1 M PBS. Specimens were stained for 3 h and mounted in Citifluor® on a cover slip. The images were taken under wavelength of 488 nm, obtained with a Leica TCS SP 5 confocal laser scanning microscope. We used Leica LAS AF 1.7.0 and Amira 5.1 for analysis of the image stacks. Line drawings were handled with Adobe Illustrator® CS2. In total we analyzed four specimen for *P. petromyzon* and seven specimen for *P. tillyensis*.

3. Results

3.1. The trophi of *P. petromyzon*

P. petromyzon displays bilaterally symmetrical trophi, consisting of paired manubria, unci and rami, an unpaired fulcrum and a ventrally located paired hypopharynx (Fig. 1A). The manubrium, consisting of the dorsal, the median and the ventral manubrial chamber, is slightly curved showing a short, knob-like projection ventrally (Figs. 1 and 2A). Dorsal, median and ventral manubrial chamber together
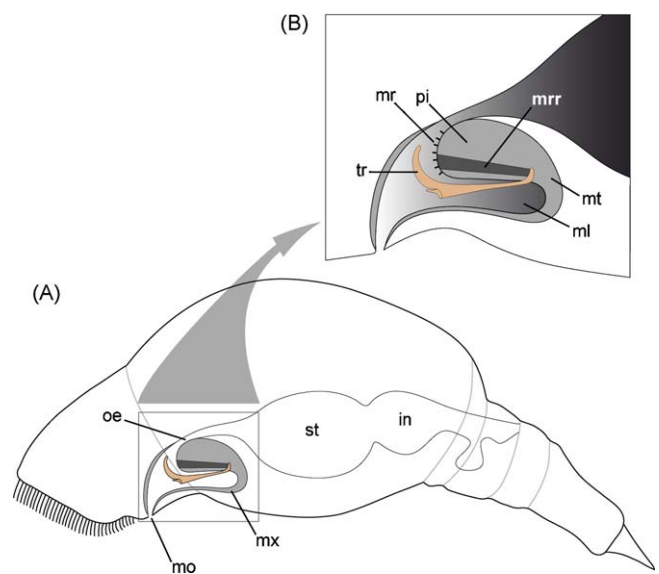
Fig. 5. Mastax and piston of *Pleurotrocha petromyzon*. Diagrammatic view. (A) Habitus of *P. petromyzon* with mastax and digestive tract; (B) detailed view of the mastax of *P. petromyzon* with mastax receptor retractor musculature and piston. in, intestine; ml, mastax lumen; mo, mouth opening; mr, mastax receptor; mrr, mastax receptor retractors; mt, mastax tissue; mx, mastax; oe, oesophagus; pi, piston; st, stomach; tr, trophi.

form the broad anterior clava (Figs. 1A, 2B and 3A), whereas the median manubrial chamber distally tapers into the slender cauda (Fig. 1A). The projection of the manubrium is formed by the ventral manubrial chamber that possesses a foramen ventralis on the interior lateral

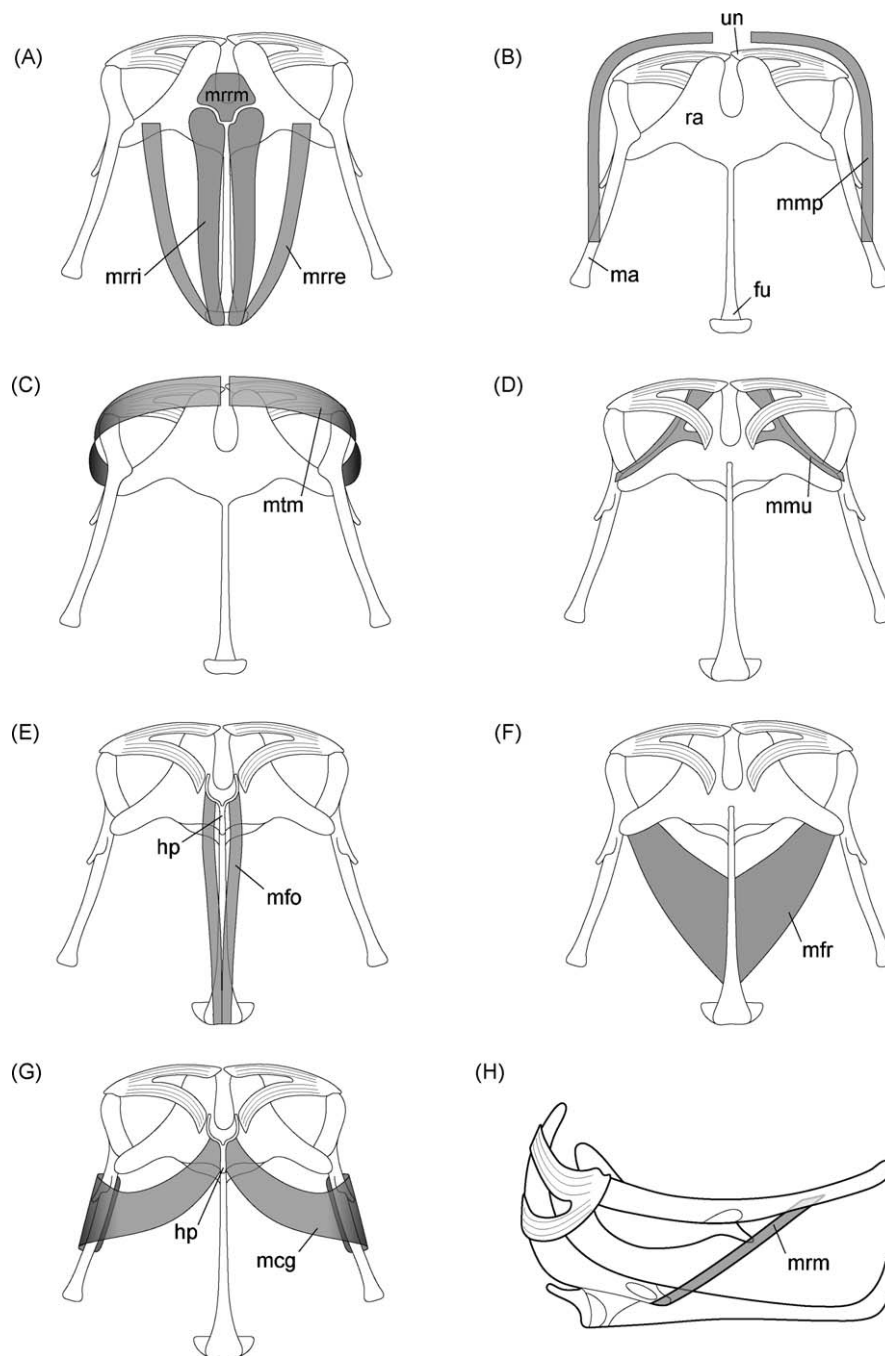**Fig. 6.** Mastax musculature of *Pleurotrocha petromyzon*. Diagrammatic view. The order of the different muscles from (A–G) reflects their appearance in the mastax from dorsal to ventral side. (A–C) Dorsal view, (D–G) ventral view, and (H) lateral view. (A) Mastax receptor retractor internus, externus and medius; (B) musculus manubrico-perioralis, (C) musculus transversus manubrii, (D) musculus manubrico-uncus, (E) musculus fulcro-oralis, (F) musculus fulcro-ramicus, (G) musculus circumglandis, and (H) musculus ramo-manubricus. fu, fulcrum; ma, manubrium; hp, hypopharynx; mcg, musculus circumglandis; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mrm, musculus ramo-manubricus; mrre, mastax receptor retractor externus; mrri, mastax receptor retractor internus; mrrm, mastax receptor retractor medius; mtm, musculus transversus manubrii; ra, ramus; un, uncus.

side (Fig. 2B). The median manubrial chamber shows a wide foramen medialis on the exterior lateral margin (Fig. 1A), whereas the inconspicuous foramen dorsalis is situated laterally on the interior margin of the manubrium (Fig. 3A). The uncus consists of five slightly curved teeth that do not show any kind of lumen in histological section (Figs. 1A, 3B and 4A).

Subbasal and basal ramus chamber together form the large, triangular shaped rami (Figs. 1A and 3A–C). The subbasal ramus chamber displays a wide ramus foramen subbasalis
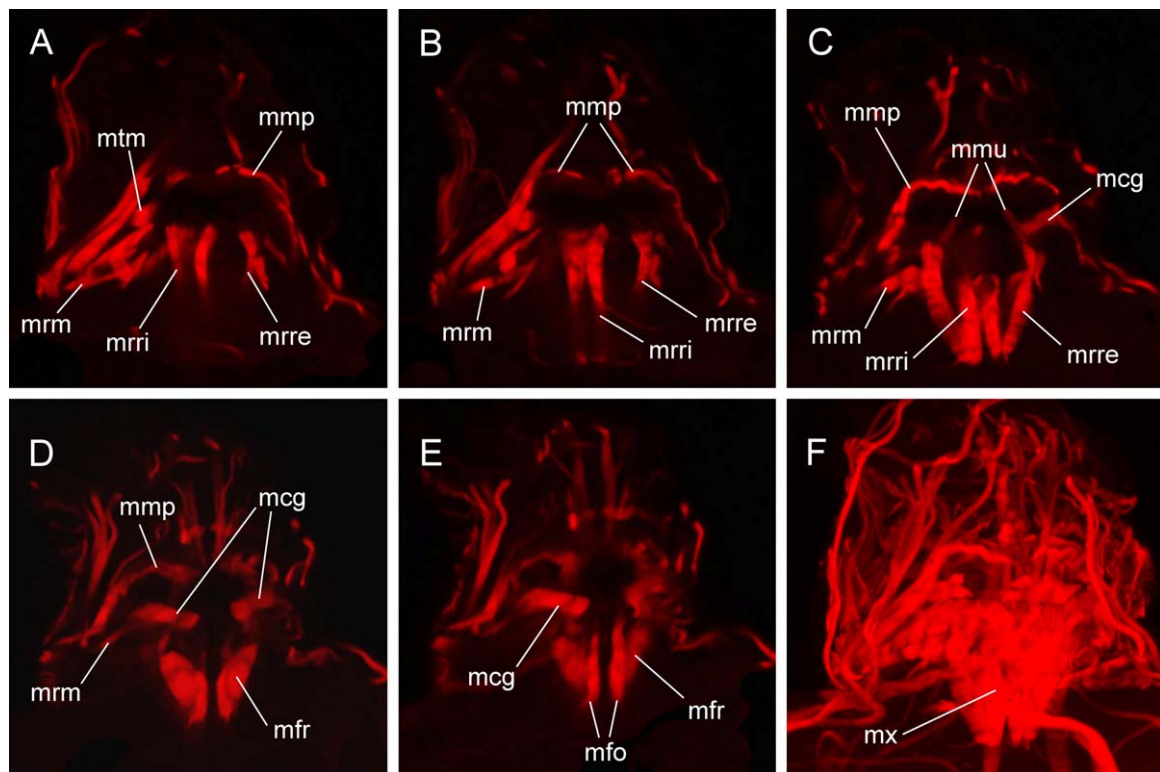
Fig. 7. CLSM images of the mastax region of *Pleurotrocha petromyzon*. The images from (A–E) show the different muscles in their appearance from dorsal to ventral side; (F) maximum projection of head musculature with mastax. mcg, musculus circumglandis; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mrm, musculus ramo-manubricus; mrre, mastax receptor retractor externus; mrri, mastax receptor retractor internus; mtm, musculus transversus manubrii; mx, mastax.

ventrally (Figs. 1A and 3C), whereas the basal ramus chamber, that forms the alula and stretches out into the ramus tip, shows a ramus foramen basalis dorsolaterally (Fig. 2B). The long and slim unpaired fulcrum is formed by a multitude of cuticular rods that are merged in their longitudinal axis (Figs. 1A, 2A and B, 3B and C and 4A and B). Distally, the fulcrum broadens and is curved dorsally.

3.2. The ultrastructural organization of the mastax of *P. petromyzon*

The mastax is ventrally located in the trunk region, right behind the mouth opening, showing a complex structure of muscles, nerves, glands and epithelial tissue associated with the cuticular jaw elements. The sack-like mastax is hung up on the dorsal side of the animal via two thin ligaments inserting the exterior margin of the mastax dorsally (Fig. 2A).

The mastax can be divided into an internal and two external sections; the unpaired median section containing incus, mastax ganglion (Remane, 1929–1933; Seehaus, 1930) and mastax receptor (Clément et al., 1980; Wurdak et al., 1983; Clément, 1987) and paired lateral sections comprising manubria, unci and one pair of salivary glands (Remane, 1929–1933) (Fig. 2A). The first pair of salivary glands is situated ventrally below the alulae of the rami

from where they stretch to the lateral margins of the mastax (Figs. 2 and 4A and B). A second pair is located in the median mastax section, between the mastax receptor retractor muscles (Fig. 4B).

The mastax receptor is located in the median mastax section, freely projecting into the mastax lumen (Figs. 3A and B, 4A and 5B). The receptor is characterized by a conspicuous electron dense surface with long villi (Figs. 3 and 4A).

3.3. The mastax musculature of *P. petromyzon*

In the following, the muscles are described in order of their appearance in the mastax from dorsal to ventral, except for the musculus ramo-manubricus that interconnects malleus and incus in dorsoventral axis.

All muscles in this study are named related to their points of insertion on the trophi and/or their course in the mastax. In cases where muscle names in earlier literature correspond to this principle of nomenclature we here inherit these names (see Table 1).

Mastax receptor retractors (*mrr*) (Figs. 2A and B, 3B, 4A and B, 5B, 6A and 7A–C). The conspicuous, broad mastax receptor retractor muscles consist of two symmetrical muscle pairs and one unpaired muscle. Cross-sections

Table 1. Overview of the appearance of different mastax muscles in following species with virgate mastaxes: *Pleurotrocha petromyzon*, *Proales tillyensis*, *Notommata copeus*, *Synchaeta pectinata*, *Trichocerca rattus* and characteristical muscles of the virgate mastax. Homologous muscles of formerly investigated species are listed with the name of the muscle stated in the literature.

	Mastax receptor retractor	M. manubrico-perioralis	M. transversus manubrii	M. manubrico-uncus	M. fulcro-oralis	M. fulcro-ramicus	M. circumglandis	M. ramo-manubricus	M. manubrico-frontalis	M. fulcro-manubricus	M. caudo-ramicus
Position of the muscle	Connects dorsocaudal end of fulcrum with opposite pharyngeal wall/mastax receptor	Stretches frontally from manubrial caudae, encompassing mastax cavity	Stretches dorsally from manubrial clavae, encompassing mastax cavity	Connects unci and manubrial clavae on interior malleus sides	Connects ventrocaudal fulcrum end with mouth opening or hypopharynx	Interconnects fulcrum and rami	Stretches ventrally from margins of manubrial caudae towards salivary glands; contact with hypopharynx	connects alulae of the rami and the manubrial caudae	stretches frontally from the dorsal manubrial caudae	interconnects fulcrum and interior manubrial caudae	interconnects manubrial caudae and rami, encompassing salivary glands
Pleurotrocha petromyzon	Mastax receptor retractors	M. manubrico-perioralis	M. transversus manubrii	M. manubrico-uncus	M. fulcro-oralis	M. fulcro-ramicus	M. circumglandis	M. ramo-manubricus			
Proales tillyensis		M. manubrico-perioralis	M. transversus manubrii	M. manubrico-uncus	M. fulcro-oralis	M. fulcro-ramicus	M. circumglandis		M. manubrico-frontalis	M. fulcro-manubricus	M. caudo-ramicus
Notommata copeus (De Beauchamp, 1909; Remane, 1929–1933)		Hypopharynx muscle	Not named			Horizontal abductor	Not named	Vertical abductor			
Synchaeta pectinata (De Beauchamp, 1909; Remane, 1929–1933)		Hypopharynx muscle	Hind adductors			Abductor rami			Hind adductors		
Trichocerca bicristata (De Beauchamp, 1909; Remane, 1929–1933)		Hypopharynx muscle				Abductor rami		Vertical abductor			
Trichocerca rattus (Clément, 1987)	Not named	Not named	Not named	Not named	Not named	Not named	Not named	Not named	Not named	Not named	
Characteristical muscles of the virgate mastax (De Beauchamp, 1909; Remane, 1929–1933)	Hypopharynx muscle	Dorsal adductor	Dorsal adductor	Not named		Horizontal abductor		Vertical abductor		Not named	

Shaded parts mark the here investigated species and their mastax musculature.
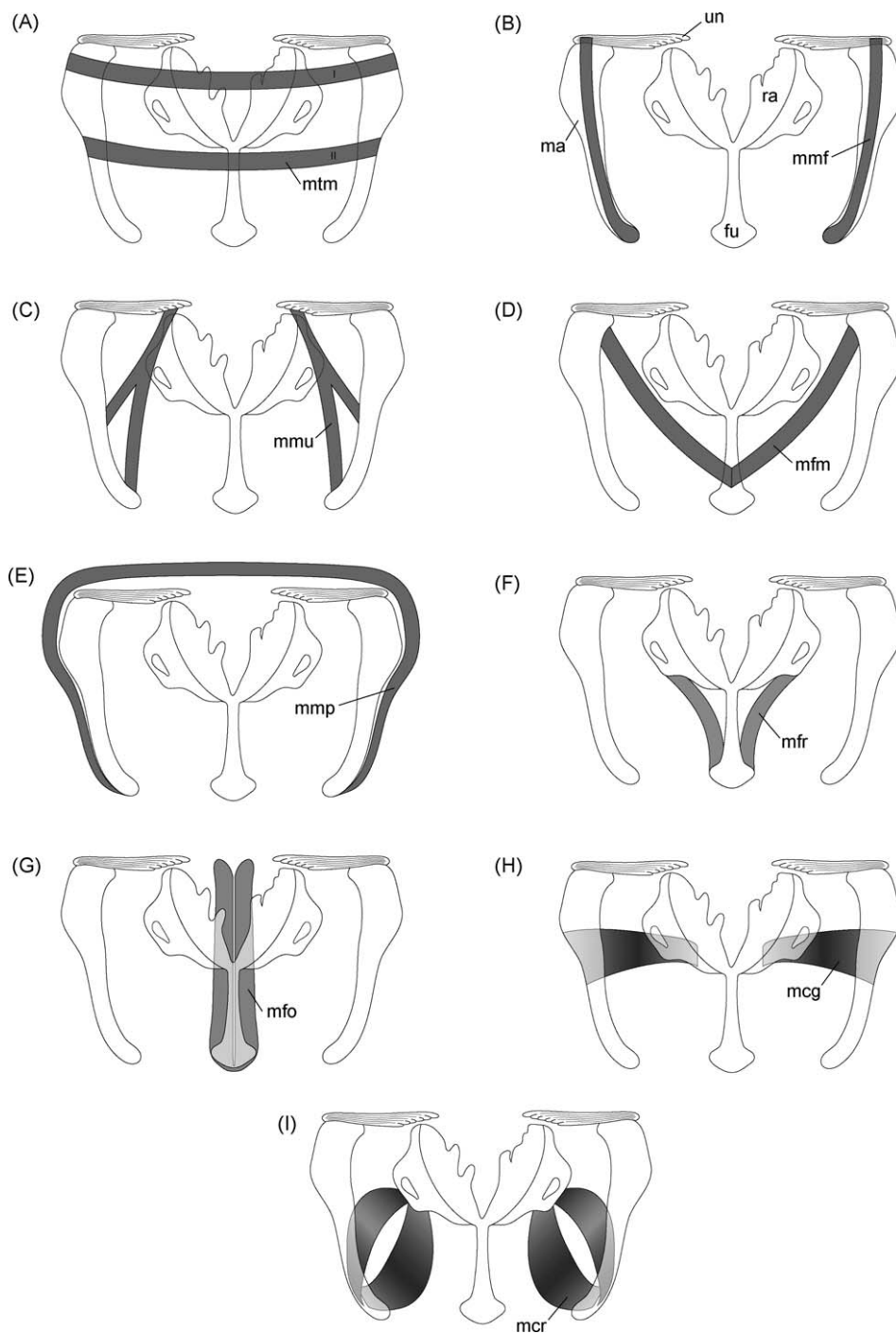
Fig. 8. Mastax musculature of *Proales tillyiensis* in dorsal view, diagrammatic. The order of the different muscles from (A–I) reflects their appearance in the mastax from dorsal to ventral side. (A) Musculus transversus manubrii I and II; (B) musculus manubrico-frontalis; (C) musculus manubrico-uncus; (D) musculus fulcro-manubricus; (E) musculus manubrico-perioralis; (F) musculus fulcro-ramicus; (G) musculus fulcro-oralis; (H) musculus circumglandis; (I) musculus caudo-ramicus. fu, fulcrum; ma, manubrium; mcg, musculus circumglandis; mcr, musculus caudo-ramicus; mfm, musculus fulcro-manubricus; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mmf, musculus manubrico-frontalis; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mtm, musculus transversus manubrii; ra, ramus; un, uncus.

show that the mastax receptor retractor muscles stretch ventrally below the mastax ganglion (Fig. 2A). The interior muscle pair (mrri) is dorsally attached to the caudal end of the fulcrum from where it stretches dorsofrontally towards the mastax receptor which projects into the mastax cavity (Figs. 2A and B, 3B, 4B, 5B, 6A and 7A–C). The exterior muscle pair (mrre) interconnects the caudolateral fulcrum end with the frontal part of the piston

(Figs. 2A and B, 4A and B, 6A and 7A–C). Frontally, these two muscles terminate in close proximity of the manubrial clavae not showing any points of attachment (Fig. 4B). The apical part of the piston shows a third muscle that is positioned in front of the interior muscle pair, the median mastax receptor retractor (mrrm) (Figs. 4A and B and 6A).

Musculus manubrico-perioralis (*mmp*) (Figs. 2A and B, 4A and B, 6B and 7A–D). This paired muscle is attached to the dorsointerior margins of the manubrial caudae from where it extends frontally, terminating in the mastax tissue (Figs. 2 and 4A and B). In its course the muscle strands are inwardly curved and encompass the mastax cavity (Figs. 6B and 7A–D).

Musculus transversus manubrii (*mtm*) (Figs. 3A, 6C and 7A). The delicate, paired musculus transversus manubrii inserts on the lateral exterior margins of the manubrial clavae from where it stretches dorsally, encompassing the mastax cavity (Figs. 3A, 6C and 7A).

Musculus manubrico-uncus (*mmu*) (Figs. 4A, 6D and 7C). This inconspicuous paired muscle spans between the interior uncus tips and the interior margins of the manubrial clavae (Figs. 4A, 6D and 7C). Possibly the musculus manubrico-uncus is also attached to the exterior margins of the ramus basal chamber. The muscle displays a very disordered and loose myofibril structure and can easily be overlooked on TEM-sections.

Musculus fulcro-oralis (*mfo*) (Figs. 2A and B, 3A and B, 4A, 6E and 7E). This paired muscle is ventrocaudally attached to the very end of the fulcrum. From this point it stretches frontally towards the mouth opening, whereas both muscle strands diverge in a slight curve and terminate being attached to the hypopharynx that is situated ventrally to the incus (Figs. 2A and B, 3A and B, 6E and 7E).

Musculus fulcro-ramicus (*mfr*) (Figs. 2A and B, 4A, 6F and 7D and E). The strong, paired musculus fulcro-ramicus connects the mediolateral sides of the fulcrum with the basal ramus chamber inserting on the alulae (Figs. 2A and B, 4A, 6F and 7D and E).

Musculus circumglandis (*mcg*) (Figs. 2A and B, 3B and C, 4A and B, 6G and 7C–E). The paired musculus circumglandis enfolds the trophi and salivary glands from dorsal to ventral, consisting of several muscle bundles (Figs. 2A and B, 3A, 4A and B, 6G and 7C–E). Being attached to the lateral margin of the manubrial caudae this muscle stretches ventrally towards the fulcrum and hypopharynx and dorsally projects above the cauda. On the ventral side the muscle makes contact with the hypopharynx that is situated ventrally to the incus (Fig. 3B and C).

Musculus ramo-manubricus (*mrm*) (Figs. 2A and B, 6H and 7A–D). This paired muscle spans between the malleus and the incus (Figs. 6H and 7A–D). Ventrally the muscle pair is attached to the alulae of the rami from where it runs in caudal direction towards the manubrial caudae inserting ventrolaterally on their interior margins (Fig. 2A and B). The musculus ramo-manubricus lies adjacent to the salivary glands.
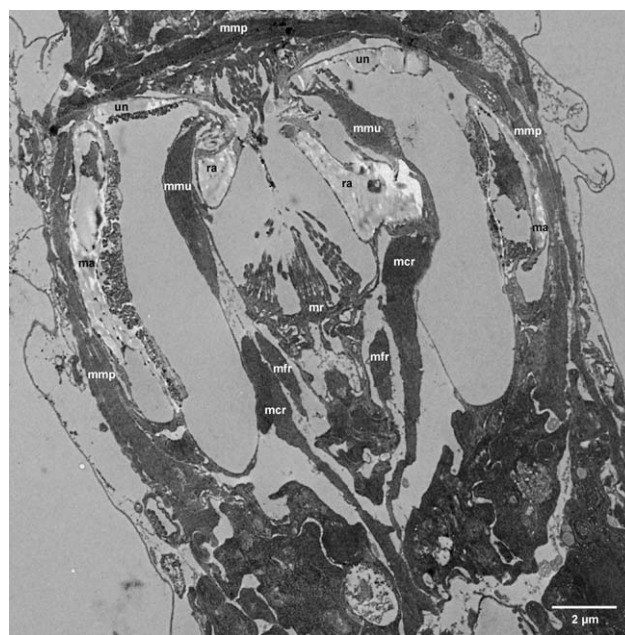

Fig. 9. Horizontal section through the median mastax region of *P. tillyiensis*. ma, manubrium; mcr, musculus caudo-ramicus; mfr, musculus fulcro-ramicus; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mr, mastax receptor; ra, ramus; un, uncus.

3.4. The trophi of *P. tillyensis*

The trophi of *P. tillyensis* are bilaterally symmetrical, consisting of the unpaired incus, the paired mallei and the unpaired hypopharynx (Figs. 1B and 8A). The manubria present a broad, voluminous clava and a long, slender cauda with inwardly curved endings. The dorsal manubrial chamber is small and has a caudally directed, rip-shaped manubrium foramen dorsalis (Fig. 1B). The large ventral manubrial chamber shows a rounded, ventrocaudally directing manubrium foramen ventralis. A distinct manubrium foramen medius is not recognizable. The paired unci consist of domed plates, each carrying six unci teeth that gradually decrease in size from dorsal to ventral (Fig. 1B). Each bent principal tooth carries an additional, small accessory toothlet. A subuncus with short, scleropilar structures is located beneath the uncus (Fig. 1B).

The rami appear truncate triangular in dorsal view. The ramus subbasal chambers present large, rounded apophyses located on the ventral side of the rami. Thorn-shaped projections as well as tubercle-like swellings are recognizable on the inner margins of the rami (Fig. 1B). Both, the ramus basal and the ramus subbasal chamber, display distinct openings; the small, circular ramus foramen subbasalis pointing caudally and the centrally located, oval ramus foramen basalis facing caudodorsally (Fig. 1B). A large, unpaired hypopharynx is located ventral to the rami (Fig. 1B). It carries two denticulated platelets anteriorely and shows somewhat
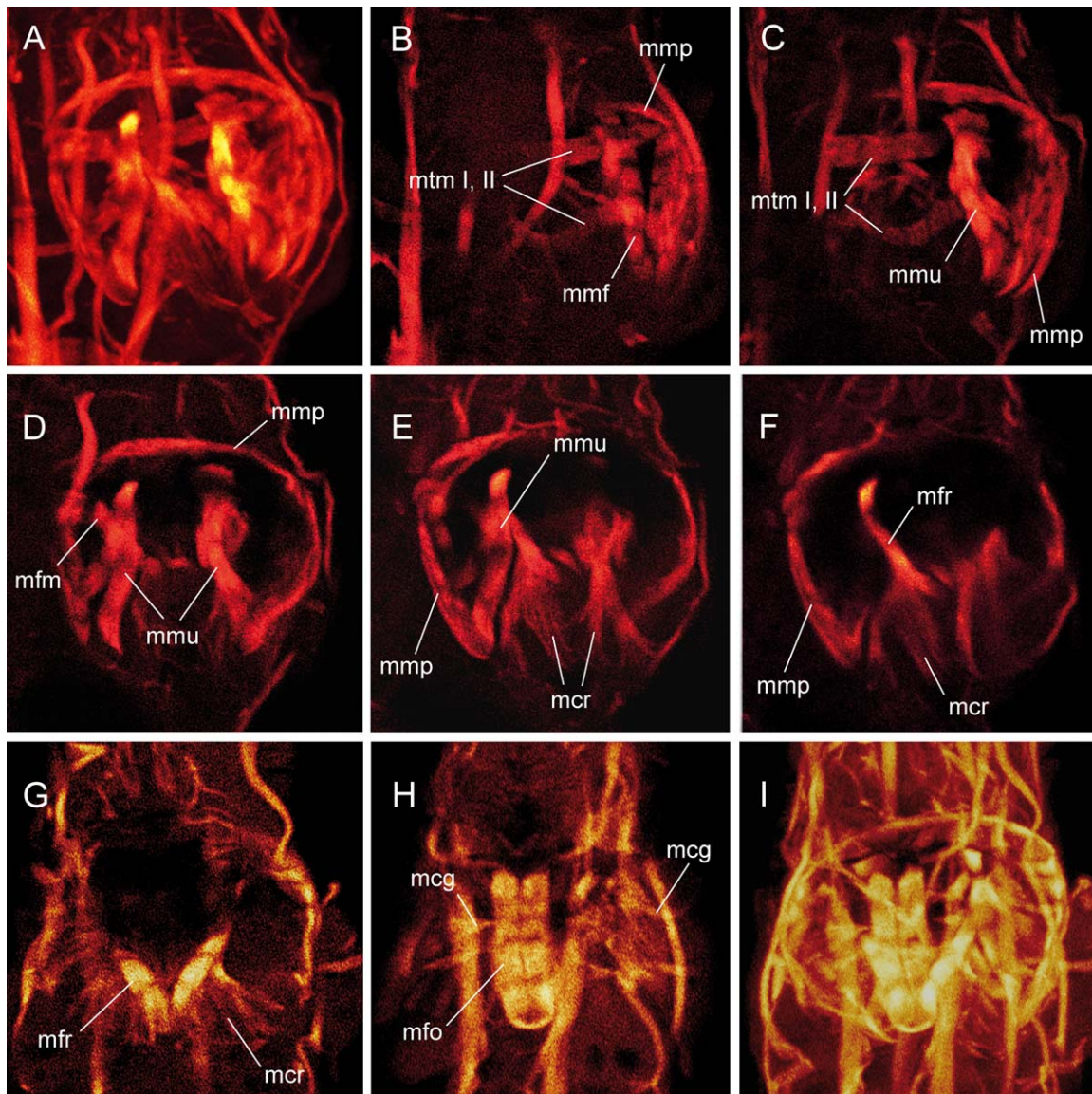
Fig. 10. CLSM images of the mastax region of *Proales tillyensis*. (A–F) Fluorescence signals of specimen 1, (G–I) fluorescence signals of specimen 2. (A) Maximum projection of mastax musculature; (B–H) optical sections dorsal to ventral; (I) maximum projection of mastax musculature. mcg, musculus circumglandis; mcr, musculus caudo-ramicus; mfm, musculus fulco-manubricus; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mmf, musculus manubrico-frontalis; mmp, musculus manubrico-perioralis; mmu, musculus manubrico-uncus; mtm I and II, musculus transversus manubrii I and II.

more strongly cuticularized pockets laterally. The fulcrum is oriented in the longitudinal axis of the body, with its prominent fan-shaped terminal end directing caudally (Fig. 1B). In ventral view it is slender and rod-shaped but broadens terminally. Seen from lateral, the fulcrum exposes a high base, tapering ventrally more than dorsally to its terminal end.

3.5. The ultrastructural organization of the mastax of *P. tillyensis*

The mastax is located ventrally in the head region, right behind the ventral mouth opening, displaying a complex structure of muscles being associated with the cuticular jaw elements (Fig. 9). As well as in *P. petromyzon*, the mastax can be divided into three sections; the internal median section with incus, mastax ganglion and mastax receptor and the external lateral sections with manubria, unci and salivary glands. The conspicuous salivary glands are situated ventrally in the space between manubrial caudae and fulcrum.

The mastax receptor, which displays several cilia projecting into the mastax lumen, is located in the median mastax region, above the rami (Fig. 9). Another receptor with cilia is located in front of the uncus, at the inner side of the musculus manubrico-perioralis (Fig. 9).

3.6. The mastax musculature of *P. tillyensis*

In the following the muscles are described in order of their appearance in the mastax from dorsal to ventral. As a matter of clarity the hypopharynx is excluded in the diagrammatic overview of the mastax musculature, since no definite points of insertion were found on this jaw element (Fig. 8A–I).

Musculus transversus manubrii (*mtm*) (Figs. 8A and 10B and C). The unpaired musculus transversus manubrii comprises two bundles of contractile fibers that interconnect both manubria dorsally (Fig. 8A). The first bundle (mtm I) interconnects the clavae, the second one (mtm II) interconnects both caudae (Figs. 8A and 10B and C).

Musculus manubrico-frontalis (*mmf*) (Figs. 8B and 10A). The paired musculus manubrico-frontalis dorsally inserts on the distal part of the cauda from where it stretches along the dorsal margin of the manubrium terminating at the manubrium–uncus joint (Figs. 8B and 10A).

Musculus manubrico-uncus (*mmu*) (Figs. 8C, 9 and 10C–E). The conspicuous, paired musculus manubrico-uncus spans between the manubrium and the interior dents of the unci (Figs. 8C, 9 and 10C–E). The caudal part of that muscle bifurcates and inserts at two different positions on the manubrial cauda.

Musculus fulcro-manubricus (*mfm*) (Figs. 8D and 10D). The paired musculus fulcro-manubricus dorsally inserts on the fulcrum stretching to the dorsal part of the interior surface of the cauda (Figs. 8D and 10D).

Musculus manubrico-perioralis (*mmp*) (Figs. 8E, 9 and 10B–F). The musculus manubrico-perioralis is a prominent, unpaired, semicircular muscle that interconnects both manubrial caudae forming a ring in front of the trophi (Figs. 8E, 9 and 10B–F).

Musculus fulcro-ramicus (*mfr*) (Figs. 8F, 9 and 10F and G). The paired musculus fulcro-ramicus interconnects the fulcrum and the rami. It is attached to the anterior margin of the broad fulcrum end from where it stretches to the subbasal chambers of the rami (Figs. 8F, 9 and 10F and G).

Musculus fulcro-oralis (*mfo*) (Figs. 8G and 10H). The paired, massive musculus fulcro-oralis inserts ventrally on the fan-shaped ending of the fulcrum and stretches frontally towards the hypopharynx. It could not be figured out clearly if this muscle is attached to the hypopharynx (Figs. 8G and 10H).

Musculus circumglandis (*mcg*) (Figs. 8H and 10H). The musculus circumglandis is a paired muscle that is attached to the lateral side of the manubrial clavae, stretching out to the salivary glands and lying against them ventrally in a net-like manner (Figs. 8H and 10H).

Musculus caudo-ramicus (*mcr*) (Figs. 8I, 9 and 10E–G). The paired musculus caudo-ramicus surrounds the salivary glands in a net-like manner (Figs. 8I and 10E–G). Frontally, this muscle is attached to the basal ramus chamber from where it stretches towards the manubrial cauda, enveloping the salivary gland dorsally and ventrally. Caudally, several delicate bundles branch off the musculus circumglandis and pass by the fulcrum and manubrium, ending in the mastax tissue (Fig. 9).

4. Discussion

4.1. *P. petromyzon* and *P. tillyensis* – two species with a virgate mastax type?

Remane (1929–1933) depicts the virgate mastax type as a category that rather embraces mastaxes showing the same sucking/pumping function than it pools mastaxes with resembling trophi. Characteristically for the virgate mastax, as De Beauchamp (1909) and Remane (1929–1933) say, is the mastax receptor retractor musculature (hypopharynx muscle *sensu* De Beauchamp and Remane) that was found in *Notommata copeus*, *Synchaeta pectinata* and *Trichocerca bicristata* (De Beauchamp, 1909) (see Table 1). Remane (1929–1933) describes the mastax receptor retractors to connect the dorsal end of the fulcrum with the opposite pharyngeal wall. In our study we found such musculature in *P. petromyzon* and named them accordingly mastax receptor retractors (Figs. 5B, 6A and 7A–C) after Riemann and Ahlrichs (2008) who state this name for *Dicranophorus forcipatus*. We preferred using the name mastax receptor retractors because it very well describes the location of the musculature in the mastax; from the distal end of the fulcrum it reaches frontally towards the mastax receptor (Clément et al., 1980), pulling it back towards the fulcrum when contracting (Fig. 5A and B). We decided against using the name "hypopharynx muscle" because it might implicate that the muscle is located on the ventral side of the trophi. Remane (1929–1933) states the mastax receptor retractor musculature to be responsible for evoking a negative pressure in the mastax cavity by pulling down the pharyngeal wall of the mastax, upon which the musculature inserts frontally, consequently enlarging the mastax lumen.

One additional term that De Beauchamp (1909) and Remane (1929–1933) use describing the virgate mastax of the Synchaetidae is "piston". The piston depicts a horizontal protuberance of the mastax wall that delineates the mastax dorsally towards the esophagus, comprising the mastax receptor retractor musculature. The mastax morphology, as described by De Beauchamp (1909) and Remane (1929–1933), was confirmed for *P. petromyzon* (Fig. 5A and B). The piston here protrudes like a tongue into the mastax lumen and contains the mastax receptor retractors (Figs. 2A and B, 3B and 4A and B). The location of these three muscles (mastax receptor retractor internus, externus and medius) in the piston, was the reason why we collectively named them mastax receptor retractors. Together with the surrounding mastax tissue, the musculature builds up a strong piston (Fig. 2B) that, by contraction, produces a negative pressure in the mastax lumen and therefore causes the pumping/sucking function. In *P. petromyzon* the piston frontally

terminates with the mastax receptor that freely protrudes into the mastax lumen (Figs. 4A and 5B).

Stoßberg (1932) describes a similar muscle in *Euchlanis* and *Brachionus*; the musculus fulcro-mucosus. The musculus fulcro-mucosus can be homologized with the mastax receptor retractor (hypopharynx muscle *sensu* Remane) and reaches from the dorsal end of the fulcrum up to the pharyngeal wall where it inserts under a sensory organ that is located in the middle between the rami (Stoßberg, 1932). Muscles that can be homologized with the mastax receptor retractors in *P. petromyzon* were found in several other species, across diverse rotifer families, possessing virgate, forcipate, fulcrate and (modified) malleate mastaxes: *B. stylata*: mastax receptor retractor (Wilts et al., 2010); *D. forcipatus*: mastax receptor retractor (Riemann and Ahlrichs, 2008); *Seison annulatus* and *Seison nebaliae*: musculus hypopharyngeus (Ahlrichs, 1995); *Trichocerca rattus*: muscle not named (Clément, 1987); *Euchlanis* and *Brachionus*: musculus fulcro-mucosus (Stoßberg, 1932); *Epiphanes senta*: musculus fulcro-mucosus brevis (Martini, 1912); *E. senta*, *N. copeus*, *S. pectinata* and *T. bicristata:* dépresseure de piston (De Beauchamp, 1909). Although this muscle is characteristic for the virgate mastax type, the mastax receptor retractor musculature does not only appear in species having a virgate mastax. Without having a piston and mastax receptor retractor musculature respectively, a strong pumping function (see Remane, 1929–1933) would not be possible. Stoßberg (1932) notices that in species possessing mastax receptor retractors without having a virgate mastax, like *Euchlanis* and *Brachionus*, the piston is circumstantial because the malleate mastax is even more empowered to grasp than the virgate mastax. Against our expectations, we did not find mastax receptor retractor musculature in *P. tillyensis*. Since these muscles are always easy to recognize in CLSM data sheets (compare Fig. 7A–C) as well as in TEM-sections (compare Fig. 4B), and as we know from earlier investigation (Wilts et al., 2010 and further unpublished data of Wulfken and Wilts), we can be confident that *P. tillyensis* lacks mastax receptor retractor musculature (Fig. 10A–I). Raising the hypothesis that not every species that displays mastax receptor retractors necessarily has to have a virgate mastax but every species having a virgate mastax has to have mastax receptor retractors, *P. tillyensis* consequently would not have a distinct virgate mastax.

4.2. Muscles shared by both species

Comparing the mastaxes of *P. petromyzon* and *P. tillyensis*, we find six muscles that both species have in common (musculus transversus manubrii, musculus manubrico-uncus, musculus manubrico-perioralis, musculus fulcro-ramicus, musculus fulcro-oralis and musculus circumglandis) (Figs. 6B–G and 8A, C, and E–H and Table 1). These muscles have been described in previous investigations of different rotifer species. Muscles that can be homolo-

gized with the musculus transversus manubrii have been found in the following species possessing a virgate mastax: *T. rattus* (Clément, 1987), *N. copeus* and *S. pectinata* (De Beauchamp, 1909; Remane, 1929–1933) (Table 1). A muscle that interconnects manubrium and uncus (here: musculus manubrico-uncus; Figs. 6D and 8C) has been described for a virgate mastax in *T. rattus* by Clément (1987) and certainly it causes a flexion of the manubrium–uncus joint.

Regarding the criterion of relative position, the musculus manubrico-perioralis of *P. petromyzon* and *P. tillyensis* (Figs. 6B and 8E) was named following the musculus manubricus perioralis that Riemann and Ahlrichs (2008) found in *D. forcipatus*, although the name of the muscle might implicate that it surrounds the mouth opening what in fact is wrong here; in our species it surrounds the mastax lumen and buccal tube rather than the mouth opening.

The muscle here referred to as musculus fulcro-ramicus (Figs. 6F and 8F) is probably one of the most conspicuous muscles of the rotiferan mastax. It is the only muscle that was found in all formerly investigated species having a virgate mastax: *N. copeus*, *S. pectinata*, *T. bicristata* (De Beauchamp, 1909; Remane, 1929–1933) and *T. rattus* (Clément, 1987) (Table 1). Muscles that can be homologized with the musculus fulcro-ramicus have been described for different mastax types across several rotifer families. As noted by Wilts et al. (2010) this muscle probably is a ground pattern feature for Monogononta, since there is existing data on the presence of such a muscle in Epiphanidae (*E. senta*: De Beauchamp, 1909; Martini, 1912), Euchlanidae (*Euchlanis pellucida*: Stoßberg, 1932), Brachionide (*Brachionus pala*: De Beauchamp, 1909), Dicranophoridae (*D. forcipatus*: Riemann and Ahlrichs, 2008), Proalide (*B. stylata*: Wilts et al., 2010), Asplanchnidae (*Asplanchna priodonta*: De Beauchamp, 1909), Trichocercidae (*T. bicristata*: De Beauchamp, 1909) and Gnesiotrocha (*Testudinella patina*: Seehaus, 1930).

P. petromyzon as well as *P. tillyensis*, show the musculus fulcro-oralis (Figs. 6E and 8G) that is attached to the ventral end of the fulcrum, reaching frontally towards the mouth opening. On the ultrathin sections of *P. petromyzon* it is clearly visible that this muscle is frontally attached to the hypopharynx (Fig. 3B) and therefore probably stabilizes the fulcrum when the mastax receptor retractor muscles contract. Muscles that can be homologized with the musculus fulcro-oralis have been delineated for several species, as *B. stylata* (Wilts et al., 2010), *D. forcipatus* (Riemann and Ahlrichs, 2008), *S. annulatus* (Ahlrichs, 1995), *S. nebaliae* (Ahlrichs, 1995; De Beauchamp, 1909), *E. pellucida* (Stoßberg, 1932) and *E. senta* (De Beauchamp, 1909). A muscle like the musculus fulcro-oralis has not previously been found in a virgate mastax (compare Table 1).

The last muscle that both species, *P. petromyzon* and *P. tillyensis*, share is the musculus circumglandis (Figs. 6G and 8H) that inserts on the manubria and from there reaches ventrally, lying against the salivary glands and certainly squeezing them during contraction. Besides our here

investigated species, this muscle was formerly reported for a virgate mastax in *N. copeus* by De Beauchamp (1909) and Remane (1929–1933) (Table 1).

4.3. Further muscles of *P. tillyensis*

In addition to the muscles that both of our here investigated species display, *P. tillyensis* displays three extra muscles; the musculus manubrico-frontalis, the musculus fulcro-manubricus and the musculus caudo-ramicus (Fig. 8B, D and I). The first two muscles would be in accordance with a grasping function of the trophi elements; the musculus manubrico-frontalis opens the malleus with a deflexion of the manubrium–uncus joint, whereas the musculus fulcro–manubricus would approach the manubria, and unci respectively, to the rami.

The musculus manubrico-frontalis and the musculus fulcro-manubricus were found in a virgate mastax before, as Clément (1987) shows in a transmission electron investigation of the mastax and the mastax receptor of *T. rattus* (Table 1). In addition to that, the musculus manubrico-frontalis was described for *S. pectinata* by De Beauchamp (1909) and Remane (1929–1933) (Table 1). Moreover, the musculus manubrico-frontalis was found in *B. stylata* (Wilts et al., 2010) and *Notholca acuminata* (Sørensen et al., 2003) whose mastaxes were categorized as modified malleate and malleate respectively. Sørensen et al. (2003) refer to this muscle as mallei flexor, which implicates that the muscle causes an abduction of the malleus. Regarding the CLSM images of the mastax musculature of *N. acuminata*, the flexor mallei attaches the manubrium and uncus on the external lateral sides and therefore causes an adduction of the malleus when contracting.

Taking a look at the musculus caudo-ramicus of *P. tillyensis* (Fig. 8I), we cannot be quite sure if the muscle indeed inserts on the caudal manubria or if it just terminates in a position close to the caudae. If this muscle would be attached to the manubria, we would consider comparing it to the musculus ramo-manubricus of *P. petromyzon* (Fig. 6H) but without knowing that, a comparison of the two muscles is not acceptable. The musculus caudo-ramicus of *P. tillyensis* almost completely encompasses the salivary glands in a net-like manner, compressing them during contraction and, if it makes contact with the caudae, approaching them to the rami.

4.4. Further muscles of *P. petromyzon*

Besides the above mentioned muscles that both of our species have in common, *P. petromyzon* shows two extra muscles/muscle complexes; the formerly discussed mastax receptor retractors (Fig. 6A) and the musculus ramo-manubricus (Fig. 6H) that connects the manubrial cauda with the alula of the ramus. Considering the criterion of relative position, the musculus ramo-manubricus can be homologized with the vertical abductor of *N. copeus* and *T. bicristata* (De

Beauchamp, 1909; Remane, 1929–1933) (Table 1). Moreover, Clément (1987) describes a muscle connecting ramus and manubrium for *T. rattus* (Table 1).

In his disquisitions about the different mastax types, Remane (1929–1933) gives, in dependence on the work of De Beauchamp (1909), a schematic overview of a set of muscles that characterizes the virgate mastax type, showing the "hypopharynx muscle" (here: mastax receptor retractors; Fig. 6A), the dorsal adductors (here: musculus manubrico-perioralis and musculus transversus manubrii; Figs. 6B and C and 7A and E), the horizontal abductor (here: musculus fulcro-ramicus; Figs. 6F and 7F) and the vertical abductor (here: musculus ramo-manubricus; Fig. 6H) (Table 1). Furthermore, he describes two muscles that are not named explicitly but can be compared to our musculus manubrico-uncus (Figs. 6D and 8C) and musculus fulcro-manubricus (Fig. 8D) (Table 1). Regarding the table of musculature (Table 1) that we set up for the two investigated species and all species with trophi that formerly have been categorized as virgate in the literature (including the muscles that De Beauchamp (1909) and Remane (1929–1933) give for the virgate mastax type) there is just one muscle/muscle complex that presents a strong characteristic for the virgate mastax type: the mastax receptor retractor musculature. Another muscle that often occurs together with the mastax receptor retractors, is the musculus ramo-manubricus. A reasonable explanation for the two muscles appearing together in different species, could be the fact that the musculus ramo-manubricus (Fig. 6H) again flattens the mastax after the mastax receptor retractor muscles (Fig. 6A) have shortened the piston and therewith increased its diameter.

4.5. Conclusions and future perspectives

Regarding our here investigated species, we confirm that *P. petromyzon* shows a virgate mastax type, recognizable by an elongated fulcrum, dorsally bent rami, reduced unci teeth, long manubrial caudae and a sucking/pumping function realized by the prominent mastax receptor retractor musculature. Although *P. tillyensis'* trophi elements show some characteristics of the virgate mastax (elongated, caudally widened fulcrum, elongated manubrial cauda) it lacks the most important feature; the mastax receptor retractor musculature. The mastax of *P. tillyensis* can therefore rather be categorized as modified malleate, showing characteristics of both, the virgate and the malleate mastax type (stout, lyriform rami with molar surface, rami in same angle with fulcrum, well developed unci with strong unci teeth). Classifying *P. tillyensis'* mastax as modified malleate coincides with the statement of Remane (1929–1933) who says that the Proalinae represent an intermediate between the virgate and the malleate mastax type. Admittedly, it is unclear, if the modified malleate mastax of *P. tillyensis* represents an intermediate state that has evolved in an evolutionary transition towards the virgate mastax or if its virgate characteristics have evolved conver-

gently. In this regard, it would be important to gain more detailed information about species differing in their mastax morphology to draw conclusions about the evolution of the mastax within Rotifera.

The proposed homologies concerning the mastax musculature of *P. petromyzon*, *P. tillyensis* and earlier described muscles of further rotifer species are primary homologies, erected on the criterion of relative position, that should be additionally supported through a phylogenetic tree to exclude a convergent evolution of the muscles. But due to the fact that the mastax muscles of different rotifer species are much less diverse than their trophi elements are, the pharynx musculature cannot only help understanding the function of the mastax, but also is, as Stoßberg (1932) already stated, of basic importance for rotifer systematics.

In general, the classification of a species' mastax relying on the traditional morphological criteria of their hard elements is sufficient. However, in case of mastaxes that do not clearly fit into one of the type categories, it appears to be useful to consult the arrangement of the mastax musculature, since it provides additional information on the function of the mastax.

Future investigations on the mastax musculature would be helpful to find out if there exist specific mastax muscles or muscle sets for different mastax types, making classification more reliable.

Acknowledgements

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