

# The musculature of *Squatinella rostrum* (Milne, 1886) (Rotifera: Lepadellidae) as revealed by confocal laser scanning microscopy with additional new data on its trophi and overall morphology

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## Abstract

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The monogonont rotifer *Squatinella rostrum* was investigated with light, scanning electron and confocal laser scanning microscopy to reveal new morphological data on its inner and outer anatomy. In total, the visualized somatic musculature displays five paired longitudinal muscles (musculi longitudinales I–V) and nine circular muscles (musculi circulares I–IX). Compared to other species, *S. rostrum* is characterized by the absence of several longitudinal and circular muscles (e.g. musculus longitudinalis capitis, corona sphincter and pars coronalis). A reconstruction of the mastax musculature revealed a total number of seven paired and two unpaired mastax muscles. Possibly homologous somatic and mastax muscles in other, thus far investigated rotifers are discussed. Moreover, we provide a phylogenetic evaluation of the revealed morphological characters and suggest possible autapomorphic characters supporting *Squatinella* and Lepadellidae. Finally, we refer to some striking similarities in the morphology, ecology and way of movement of *Squatinella* and *Bryceella* that may indicate a closer relationship of both taxa.

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## Introduction

In a series of papers, we try to reveal new phylogenetic relevant morphological characters focusing on the inner and outer morphology of individual rotifer species from different taxa (see Riemann *et al.* 2009b; Wilts *et al.* 2009a,b; Wilts *et al.* 2010) to contribute new data for future phylogenetic analyses. We hope that these contributions will also help to solve taxonomical problems still remaining in several rotiferan taxa.

The ploimid family Lepadellidae Harring 1913, whose monophyletic status was not confirmed yet because of the lack of a detailed phylogenetic investigation, comprises four genera: *Colurella* Bory de St. Vincent, 1924, *Paracolurella* Myers, 1934, *Lepadella* Bory de St. Vincent, 1926 and *Squatinella* Bory de St. Vincent, 1826. In former studies, the family name

Colurellidae Wesenberg-Lund, 1929 was also commonly used until Segers (2002) referred to the priority of the senior synonym Lepadellidae erected by Harring (1913). A specification of characters consulted for the erection of Lepadellidae is not provided by Harring (1913), although Koste and Shiel (1989) provide helpful diagnoses for the family and its genera. Nevertheless, the taxonomy is still incomplete, many species are poorly defined and problems regarding the validity of species still remain, especially regarding *Lepadella* (De Paggi 2001).

The small species of Lepadellidae, which show an average body length of about 100 µm, are traditionally characterized by their lorica morphology solely and its diagnostic characters constitute the basis of previous species descriptions. Inner morphology and trophi morphology have been disregarded to date generally, supposedly because of their small size,

although especially the trophi are the most important character complex for species determination and phylogeny of rotifers (e.g. Markevich and Kutikova 1989; Markevich 1993; Sørensen 2002). Detailed morphological studies, especially with the use of modern techniques like electron or confocal laser scanning microscopy, of the inner and outer morphology of the Lepadellidae are lacking. The result is a poor knowledge of the overall morphology of the representatives. This deficiency even goes so far that two ciliates have been described as species of *Colurella* by mistake, a case discovered by Turner (1995). In fact, secure species identification is extremely difficult for someone who is not very familiar with the species of Lepadellidae, and this bears a high potential for mistakes especially for ecological and molecular studies.

Facing this lack of appropriate morphological studies across Lepadellidae, we here represent a detailed reinvestigation of the internal and external morphology of the lepadellid species *Squatinella rostrum* (Schmarda, 1846) based on light, electron and confocal laser scanning microscopic (CLSM) observations. In so doing, we provide the first description of the somatic musculature and trophi musculature in a species of Lepadellidae. The subject matter belongs to the small lepadellid genus *Squatinella* actually containing ten, mostly acidophilic species (Segers 2007) that are common in the limnopsammon of small ponds, pools, moors and in *Sphagnum* (Koste 1978; Koste and Shiel 1989).

## Materials and Methods

Specimens of *S. rostrum* were obtained from the benthos of a small pond in Leer, north-west Germany (53°15'30"N, 7°31'12"E) at the beginning of October 2008. Specimens of *Bryceella stylata* and *Bryceella* sp. nov. were found in wet mosses collected from a forest in Leer, north-west Germany in January 2007 and 2008. Individuals of *S. rostrum* were isolated from the sample water under a stereomicroscope and studied by differential interference light microscopy (Leica DMLB, Leica Microsystems GmbH, Wetzlar, Germany), scanning electron microscopy and confocal laser scanning microscopy. Light microscopic images were taken with a digital documentation camera (Olympus ColorView, Olympus Europa Holding GmbH, Hamburg, Germany). Single rotifer specimens were narcotized in an aqueous solution of 0.25% bupivacaine (Bucain®, Actavis Deutschland GmbH & Co. KG, Langenfeld, Germany) and subsequently fixed with 4% OsO<sub>4</sub> solution in 0.1 M sodium cacodylate buffer and picric acid formaldehyde at 240 mOsm (following Melone and Ricci 1995). Fixed specimens were dehydrated in a graded ethanol series followed by critical-point drying. Dried rotifers were mounted on stubs and coated with platinum. Trophi were prepared under a stereomicroscope (Leica MZ12<sub>5</sub>) generally following the procedure of De Smet (1998) but using SDS/DTT (modified after Kleinow et al. 1990) as the dissolving agent. Specimens and trophi were studied by scanning electron microscopy (Hitachi S-3200N, Hitachi High-Technologies Corporation, Tokyo, Japan). For

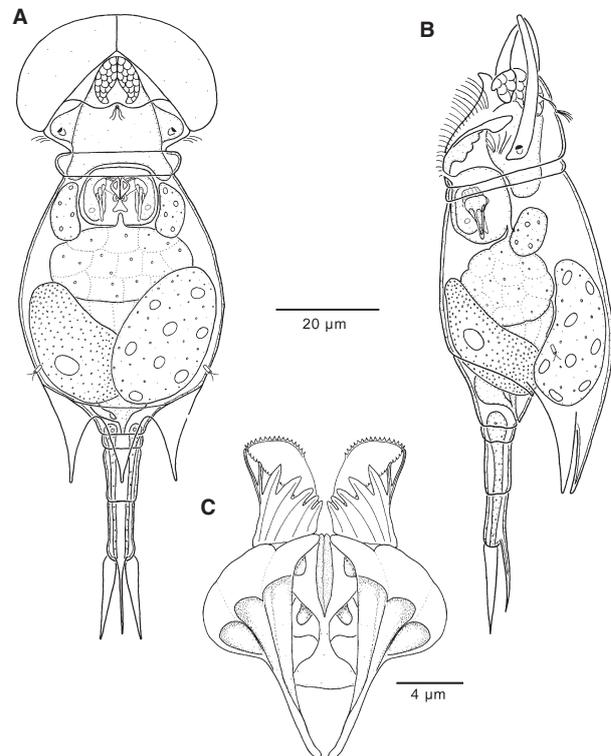
CLSM, specimens were placed in a drop of freshwater and relaxed in a 0.25% solution of bupivacaine at 8 °C. The anaesthetised specimens were fixed for 1 h in phosphate-buffered 4% paraformaldehyde and rinsed in 0.1 M phosphate-buffered saline (PBS) and then made permeable by exposure to 0.1% Triton X-100 buffered in 0.1 M PBS for 1 h. For staining, 2 µL of 38 µM methanolic tetramethylrhodamine isothiocyanate-labelled phalloidin solution were added to 100 µL of Triton X-100 buffered in 0.1 M PBS. Several specimens were stained for 3 h and mounted in Citifluor® (Citifluor Ltd., Leicester, United Kingdom) on a cover slip. Totally, six specimens were analysed. The images were taken under a wavelength of 488 nm using a Leica TCS SP 5 confocal laser scanning microscope. We used ImageJ 1.37v (National Institutes of Health, Bethesda, Maryland, USA) and Leica LASF 1.7.0 for the analysis of the image stacks. Line drawings were prepared with Adobe Photoshop® CS2, whereas diagrammatic drawings were created with Adobe Illustrator® CS2.

## Results

*Squatinella rostrum* (Schmarda, 1846)

*Listrion rostrum* Schmarda, 1846

Syn. *Squatinella lamellata* auct.



**Fig. 1**—General body organization of *Squatinella rostrum*. —**A**. Specimen in dorsal view. —**B**. Specimen in lateral view. —**C**. Mastax hard parts (trophi) in dorsal view.

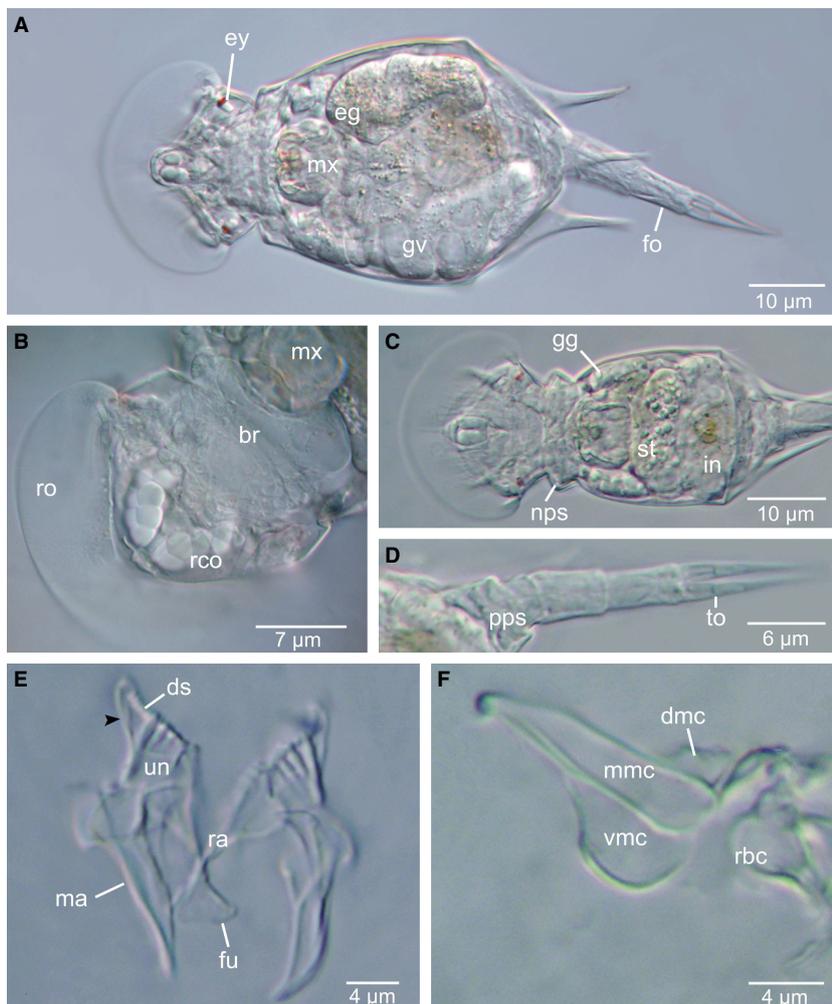
**Diagnosis**

Small, dorsoventrally flattened body: head large, neck region narrow, trunk bulbous, foot slender; planar corona triangular, restricted caudolaterally by sail-like epidermal projections; semicircular rostrum large; trunk with three caudal spines; foot composed of three pseudosegments and long, lanceolate toes; terminal foot pseudosegment with long dorsal spine; rami with well-developed molar surface; unci with seven teeth; distal subuncus with denticulate margin beneath the unci; fulcrum with broad, hoof-shaped terminal end; manubria club-shaped with voluminous, domed clavae and distally incurved caudae; dorsal manubrial chamber drawn out to a flat fin.

**General body organization of parthenogenetic females**

*Squatinnella rostrum* has a hyaline, dorsoventrally flattened body separated into a large head with a narrow neck, a bulbous trunk and a slender foot with toes (Figs 1A,B, 2A–D and 3A–C). The epidermis surface is reseeded with little burls being

visible at higher magnification (Fig. 3D). The head is not retractable into the trunk and consists of three pseudosegments separated from each other and the trunk by distinct transverse folds (Figs 1B and 3A, D). The anteriormost pseudosegment is triangular. A dorsal antenna that is partly covered by an epidermal projection (arrow Fig. 3D) inserts at the intersection to the following subsquare pseudosegment. The narrower third pseudosegment (neck pseudosegment) adjoins the trunk (Figs 1B, 2C and 3A). The two anteriormost pseudosegments carry the large, semicircular and strongly stiffened rostrum (Figs 1B, 2B and 3D), which displays a straight, median, longitudinal seam. The margin of the rostrum is all around arcuate ventrally (Fig. 3E). A retrocerebral organ with two kidney-shaped glands is located in the anteriormost pseudosegment in front of the brain (Figs 1A,B and 2B). Ventrally, it shows a saccate structure located in front of the corona (Fig. 3E). The corona is reduced to a homogeneous, triangular ciliary field on the ventral side of the head. A pair of triangular epidermal projections borders the corona laterally in a funnel-shaped manner. These projections



**Fig. 2**—Internal organization of *Squatinnella rostrum*. Light micrographs. —**A**. Adult specimen in dorsal view. —**B**. Detail of head in dorsal view. Note large glands of retrocerebral organ. —**C**. Juvenile specimen in dorsal view. —**D**. Foot in dorsal view. —**E**. Complete trophi system in dorsal view. Arrow head indicates elongated unci tooth. —**F**. Manubrium and ramus in dorsal view. br, brain; dmc, dorsal manubrial chamber; ds, distal subuncus; eg, egg; ey, eye; fo, foot; fu, fulcrum; gg, gastric glands; gv, germovittellarium; in, intestine; ma, manubrium; mmc, median manubrial chamber; mx, mastax; nps, neck pseudosegment; pps, preanus pseudosegment; ra, ramus; rbc, ramus basal chamber; rco, retrocerebral organ; ro, rostrum; st, stomach; to, toe; un, unci; vmc, ventral manubrial chamber.

decrease gradually in size during their caudal course until they merge together behind the mouth opening (Fig. 3E).

The ovoid trunk is wider than the neck and consists of two pseudosegments separated by transverse folds. The large, anterior pseudosegment bears the lateral antennae and is strongly stiffened in the dorsocaudal region where three spines of equal length are developed (Figs 1B, 2A and 3A). Centrally on the ventral side lies a pair of epidermal lobes in form of a doubled keel (detail Fig. 3B) of unknown function. Caudal to the insertion of the dorsal spines follows a narrow and short preanal pseudosegment (Figs 1A,B and 2D).

The slender foot is divided into one short basal pseudosegment and two longer terminal ones (Figs 1A,B, 2D and 3C). The caudalmost pseudosegment is rounded and carries a dorsal spine having the same size as the long, pointed, lanceolate toes (Figs 1A,B and 3B,C). During swimming, the toes normally lie close together. Two pedal glands stretch through the foot and extend to the preanal pseudosegment.

#### *Digestive tract*

The gastric tract of *S. rostrum* consists of the mouth opening, the spheroidal mastax, the narrow oesophagus, the stomach and the intestine (Figs 1B and 2C). The ventral mouth opening is positioned in the posterior region of the corona in the middle of the neck pseudosegment and leads to a short buccal tube and the cavity of the mastax. The bilaterally symmetrical masticatory apparatus is located in the ventral region of the trunk and extends only slightly into the neck (Figs 1A,B and 2C). A pair of salivary glands is integrated in the mastax complex. Each gland is located between the ramus and the manubrium. The narrow oesophagus is dorsally attached to the mastax and leads over to the small, multicellular stomach. Two longish, rounded gastric glands derive anteriorly from the stomach wall and recline laterally to the mastax (Figs 1A and 2C). The intestine is set off from the ciliated stomach by a transverse constriction and leads to the cloaca that opens dorsally below the preanal pseudosegment (Fig. 1A,B).

#### *Mastax hard parts (trophi)*

The cuticularized, bilaterally symmetrical trophi system resembles the malleate type but shows modified malleate tendencies. The individual trophi elements are embedded in epithelial tissue, from which cells extend into the cuticular cavities of the rami (ramus basal and ramus subbasal chambers) and manubria (dorsal, median and ventral manubrial chambers). The short, unpaired fulcrum usually lies in the longitudinal axis of the body with its terminal end directing somewhat caudally. The base of the fulcrum obliquely attaches the rami and appears slender and short viewed dorsally. Caudally, the fulcrum rapidly broadens in a hoof-shaped manner with its margin curved downwards (Figs 1C, 2E, 4A,B and 5A). In dorsal view, the individual ramus basal chambers are triangular and appear almost rhombic in

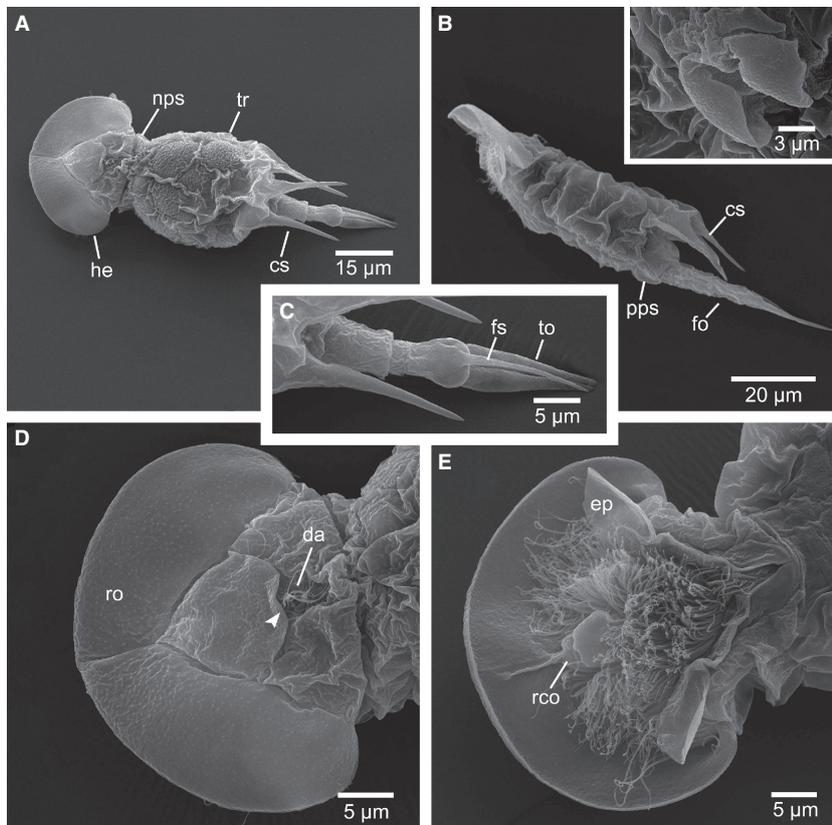
combination, whereas the ramus subbasal chambers protrude below them lateral to the fulcrum (Figs 1C, 4A and 5E). The anterior margins of the rami present molar surfaces consisting of consecutively arranged cross rows of tubercles with small teeth-like projections (Figs 4B and 5B). Ventral to the rami lies an unpaired hypopharynx that stretches between rami and manubria (Fig. 5B,E). Both the ramus basal and subbasal chambers display distinct openings with the large, nearly semi-circular ramus foramen basalis and the small circular ramus foramen sub-basalis facing dorsally (Figs 1C, 4A and 5E). The paired unci are built on planar plates, bearing seven unci teeth each, decreasing gradually in length from the most ventral to the most dorsal tooth. The five most dorsal teeth are arranged parallel; the two longer, most ventral teeth are bent and stick out forwardly (Figs 1C, 2E, 4D and 5A,F). A large and lobate distal subuncus with a denticulate margin is located ventral to the five dorsalmost unci teeth and is carried frontally by the two long ventralmost teeth. (Fig. 2E, 4D and 5A,C,F). In live specimens, the small denticles of the distal subuncus are in close contact with the anterior margin of the ventral rami surface. The club-shaped manubria attach to the unci proximally by fine ligaments and are divided into a broad, voluminous proximal clava and a distal, gradually tapering cauda whose terminal ends are curved inwardly (Figs 2E,F, 4C and 5A,C). The openings of the three manubrial chambers are clearly discernable: the ventral manubrial chamber is voluminous and presents the largest part of the clava with the manubrium foramen ventralis facing caudally (Fig. 5D). The median manubrial chamber presents the largest part of the manubrium with the manubrium foramen medius extending almost to the tip of the cauda (Figs 4C and 5C,D). The dorsal manubrial chamber is drawn out in a blunt thorn-shaped manner with the small manubrium foramen dorsalis facing dorsocaudally (Fig. 5A,D).

#### *Nervous system and sensory organs*

The cerebral ganglion is positioned in the anterior part of the head in front of the mastax (Figs 1A,B and 2B). Two red-pigmented lateral eyes display a distinct lens and lie in the second head pseudosegment, associated with the epidermis (Fig. 2A). The dorsal antenna is located centrally on the head and is partially covered by an epidermal projection deriving from the first head pseudosegment (Fig. 3D). The lateral antennae present several cilia protruding from small pedestals positioned in the last third of the anterior trunk pseudosegment (Fig. 1B).

#### *Excretory system*

The protonephridial system presents distinct terminal organs distributed laterally in the body cavity. The collecting tubules open into a contractile bladder that is positioned ventrocaudally in the trunk. The fluid of the bladder is emptied into the terminal part of the intestine (cloaca) (Fig. 1A).



**Fig. 3**—Scanning electron microscopic (SEM) images of *Squatinnella rostrum*. —**A**. Specimen in dorsal view. —**B**. Specimen in lateral view. Detail shows ventral epidermal projection. —**C**. Closer view of foot. —**D**. Head in dorsal view. Note surface of epidermis. Arrow head indicates epidermal projection shielding the dorsal antenna. —**E**. Head in ventral view. cs, caudal spine; da, dorsal antenna; ep, epidermal projection; fo, foot; fs, foot spine; he, head; nps, neck pseudosegment; pps, preanus pseudosegment; rco, retrocerebral organ; ro, rostrum; to, toe; tr, trunk.

### Reproductive organs

*Squatinnella rostrum* is an oviparous species. The parthenogenetic females have a syncytial germovitellarium situated dorso-laterally in the posterior part of the trunk (Figs 1A, B and 2A). The vitellarium contains eight nuclei. Most observed amictic females bore one dark pigmented, ovoid egg (Fig. 2A).

### Measurements

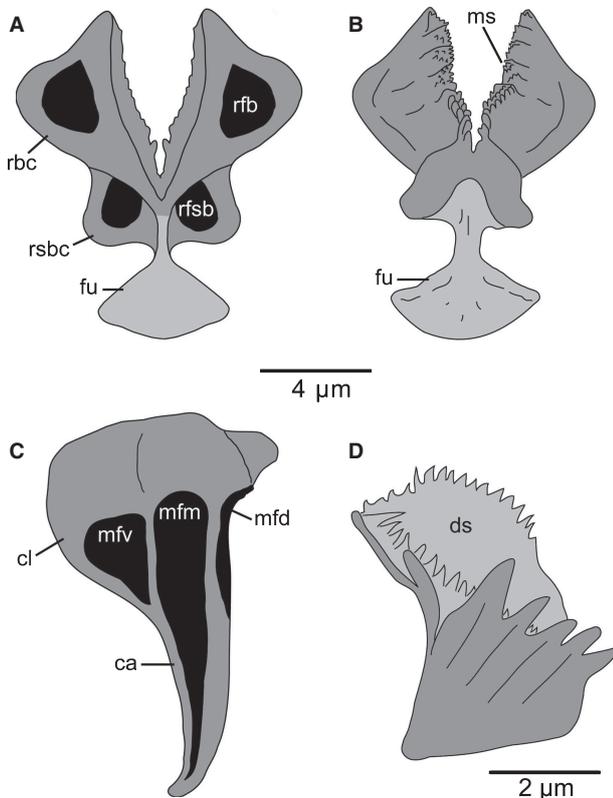
Total length 90–130 µm, maximum dorsoventral dimension 22 µm, maximum width 30 µm, foot length 34–38 µm, toe length 15–20 µm, trophi length 19–20 µm, trophi width 15 µm, ramus length 10 µm, manubrium length 14 µm, cauda width 9–10 µm and fulcrum length 4 µm.

### Somatic musculature

In total, the body musculature of six specimens in dorsoventral position and one specimen in lateral orientation was visualized by CLSM. For a reliable reconstruction, the examination of multiple specimens was necessary, because of the fact that all muscles often could not be recognized in each specimen. *Squatinnella rostrum* displays longitudinal and circular muscles that occur paired, bilateral and symmetrical. All muscles, including the circular ones, show a conspicuous pattern of cross-striation.

### Longitudinal muscles

The somatic musculature in *S. rostrum* comprises five pairs of longitudinal muscles (musculus longitudinalis I–V). These longitudinal muscles differ in length, strength and number of subunits (Figs 6A,B and 7A–D). The paired musculus longitudinalis I (musculus longitudinalis ventralis) is the ventralmost longitudinal muscle. Its two strands consist of four subunits and continue from the second head pseudosegment to the second foot pseudosegment narrowing each other in the midbody region (Figs 6B and 7B). In the second foot pseudosegment, both muscle strands bifurcate caudally showing a lateral enlargement following the main strands to their caudal end (Fig. 6B). The short, ventral, paired musculus longitudinalis II is located in the posterior half of the second foot pseudosegment between the caudal ends of the musculus longitudinalis I (Figs 6B and 7C). The paired musculus longitudinalis III is the dorsalmost longitudinal muscle and consists of three subunits. The two muscles of this pair anchor bifurcate in the head and run dorsally through the body up to the second foot pseudosegment where they again terminate bifurcate (Figs 6A and 7A, D). The musculus longitudinalis IV comprises a pair of short and triangular appearing muscle strands that are located dorsolaterally in the caudalmost foot pseudosegment (Figs 6A and 7A,B,D). The short and slender musculi longitudinales V are situated dorsomedially in the caudalmost foot



**Fig. 4**—Diagrammatic drawings of the trophi delimited by different grey scales. —**A.** Incus in dorsal view. —**B.** Incus in ventral view. —**C.** Manubrium in lateral view. —**D.** Uncus in dorsal view. ca, cauda; cl, clava; ds, distal subuncus; fu, fulcrum; mfd, manubrium foramen dorsalis; mfm, manubrium foramen medius; mfv, manubrium foramen ventralis; ms, molar surface; rbc, ramus basal chamber; rfb, ramus foramen basalis; rfsb, ramus foramen subbasalis; rsbc, ramus subbasal chamber.

pseudosegment and anchor in a point between the toe bases (Figs 6A and 7A,B,D).

#### Circular muscles

In total, we have identified nine circular muscles (musculi circulares I–IX) directly underlying the integument. Except for musculus circularis VIII and IX, all circular muscles present incomplete muscular rings that are developed only ventrally and laterally and show various degrees of incompleteness (Figs 6A,B and 7A–D). Except for musculus circularis VIII, all other circular muscles in the trunk cross the musculus longitudinalis I ventrally (Figs 6B and 7B). While musculus circularis I is difficult to detect because of its concinity, the other circular muscles are more easily recognizable but vary in strength (Figs 6A,B and 7A,B).

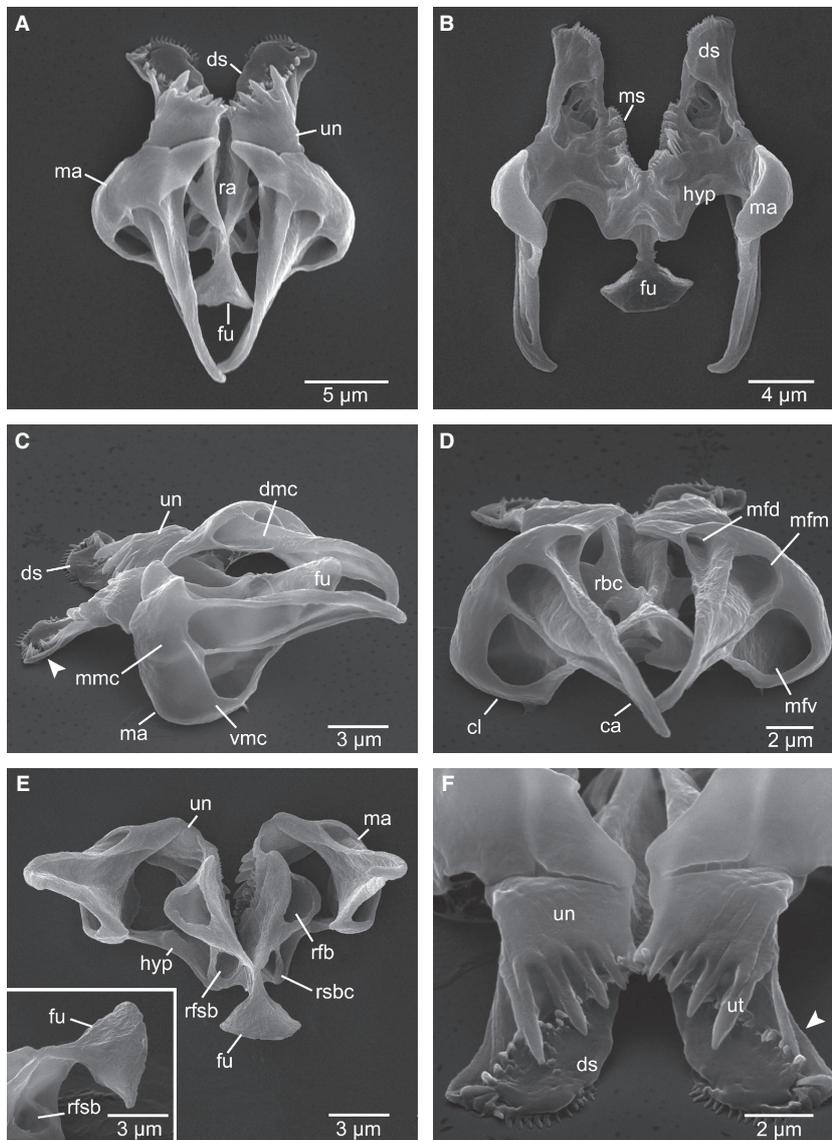
The musculus circularis I is a very delicate, highly incomplete and inconspicuous circular muscle that is located ventrolaterally in the anterior head region. Musculus circularis

II runs ventrolaterally in the neck pseudosegment. The musculus circularis III is situated in the foremost region of the anteriormost trunk pseudosegment and bifurcates laterally with both endings crossing the musculus longitudinalis I. Musculus circularis IV is situated directly behind the mastax and bifurcates ventrally as well. Musculus circularis V follows caudally presenting a trifurcate ending on the ventral side. Dorsolaterally, the muscle strongly broadens and reaches the musculus longitudinalis III. The following circular muscles, musculus circularis VI and musculus circularis VII, originate in a lateral position and terminate ventrally near the musculus longitudinalis I. The broad, conspicuous musculus circularis VIII only runs dorsolaterally in the trunk in front of the caudal spines. The musculus circularis IX (musculus circumpedalis) forms, unlike the remaining circular muscles, a u-shaped ring, running from the lateral to the ventral side in the caudalmost region of the last foot pseudosegment in front of the toe bases (Figs 6A,B and detail in 7A).

#### Visceral muscles

The species shows a complex network of visceral musculature characterized by delicate circular, longitudinal and transverse fibres associated with the corona, stomach, gut and cloaca (Fig. 7A,B). Especially the head and the preanus pseudosegment show several ramified muscles that could not be determined in detail because of their convoluted or unusual course. Two of these muscles are short, paired and distinctly recognizable in all investigated specimens (arrow heads Figs 6B, 7A–C): One pair is located centrally in the head with its two subunits appearing v-shaped in combination; a second pair is located ventrally in the preanus pseudosegment presenting a somewhat kidney-shaped form.

The distinct cross-striated, bilaterally symmetrical mastax muscles represent an antagonistic muscle system displaying an intense fluorescence signal, clearly visible in all analysed specimens. Closest to the ventral side of the species spans the fine, paired musculus fulcro-oralis (mfo). Frontally, the muscle apparently anchors close to the mouth opening at the inner caudal margin of the triangular epidermal projections. From there, it runs frontolaterally before it stretches to the anterior ventral margin of the expanded fulcrum end (Fig. 8B). The musculus fulcro-ramicus (mfr) inserts more laterally on the fulcrum end interconnecting the fulcrum and the rami. This muscle is hardly recognizable because it is overlaying by the musculus fulcro-manubricus (mfm). The musculus fulcro-manubricus is a conspicuous paired muscle that attaches the caudolateral margin of the expanded fulcrum end running frontally above the rami to the dorsal chamber of the manubrium (Fig. 8D,E). The short, paired mastax receptor retractor (mrr) attaches the basal fulcrum region dorsally and runs frontally along the median axis of the mastax through the rami and terminates at a point where the mastax receptor is



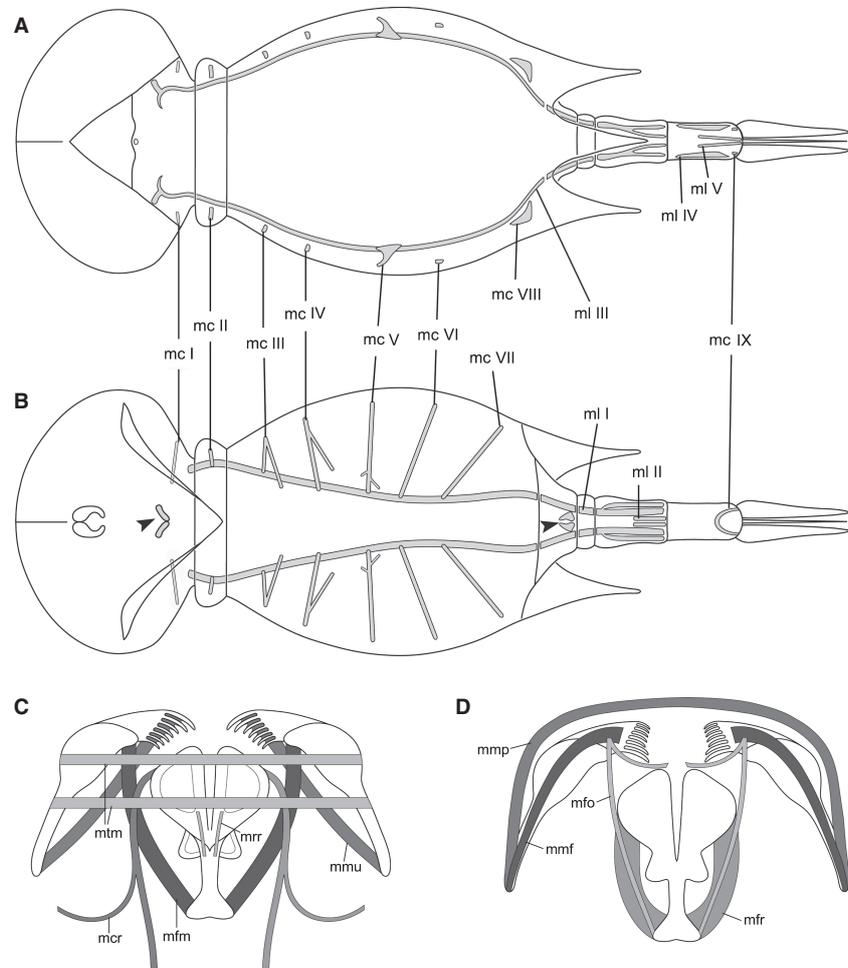
**Fig. 5**—Scanning electron microscopic (SEM) images of trophi. —**A.** Dorsal view. —**B.** Ventral view. —**C.** Lateral view. Detail shows fulcrum in lateral view. —**D.** Caudal view. —**E.** Dorsal view with clear sight at the rami. —**F.** Unci in frontodorsal view. Arrow heads point out elongated uncs teeth. ca, cauda; cl, clava; dmc, dorsal manubrial chamber; ds, distal subuncus; fu, fulcrum; hyp, hypopharynx; ma, manubrium; mfd, manubrium foramen dorsalis; mfm, manubrium foramen medius; mvf, manubrium foramen ventralis; mmc, median manubrial chamber; ms, molar surface; ra, ramus; rbc, ramus basal chamber; rfb, ramus foramen basalis; rfsb, ramus foramen subbasalis; rsbc, ramus subbasal chamber; un, uncus; ut, uncus tooth; vmc, ventral manubrial chamber.

assumed to be located (Fig. 8D). Another fine paired muscle, *musculus caudo-ramicus* (*mcr*), inserts anteriolaterally on the ramus basal chamber and splits caudally. One branch adapts on the caudal margin of the salivary gland and terminates in the near of the manubrial cauda, and the other branch terminates in the near of the fulcrum. The *musculus manubrico-uncus* (*mmu*) is a paired, prominent longitudinal muscle interconnecting the inner, distal part of the manubrial cauda with the inner surface of the uncus (Fig. 8B,D,E). The *musculus manubrico-perioralis* (*mmp*) is a prominent, unpaired, semicircular muscle that interconnects of both manubrial caudae. The muscle anchors dorsolateral on the tips of both caudae and stretches along the lateral side of the manubria forming a ring in front of the trophi (Fig. 8B,D). The strong, paired *musculus manubrico-frontalis* (*mmf*) runs

along the dorsal surface of the manubrium and terminates at the manubrium-uncus joint (Fig. 8B,F). The unpaired *musculus transversus manubrii* (*mtm*) comprises two bundles of contractile fibres that are distinctly separated from each other interconnecting the manubrial clavae dorsally (Fig. 8A,F).

#### *Ecology and distribution*

We found *S. rostrum* in large numbers in an herb-infested pond in the late summer and the early autumn. The species usually glide on the ground or on submersed macrophytes, moving in a jerky manner that resembles the movement of species of *Bryceella*. The species was also found gliding upside down on the water surface. We also observed the species in cultured moist *Sphagnum*.



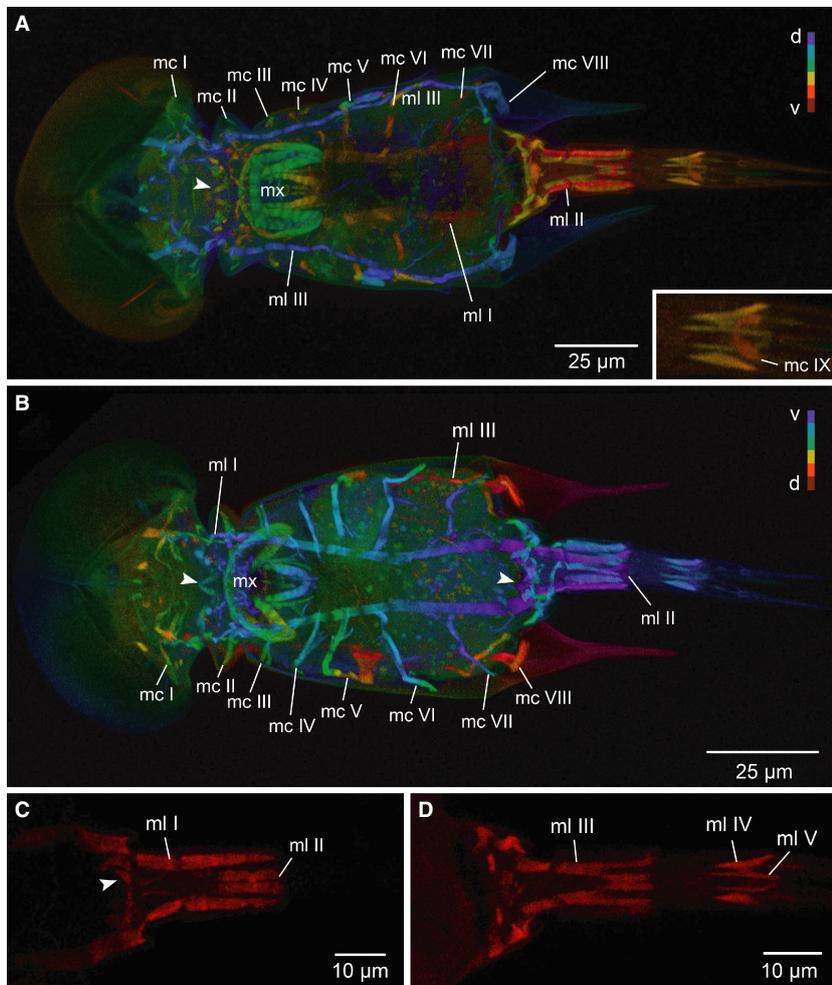
**Fig. 6**—Musculature of *Squatinnella rostrum*, schematic. —**A** and **B**. Somatic musculature, —**C** and **D**. Mastax musculature. —**A**. Dorsal view. —**B**. Ventral view. Arrow heads indicate undetermined, conspicuous muscles. —**C**. Dorsal view. —**D**. Ventral view. mc I–IX, musculi circulares I–IX; mcr, musculus caudo-ramicus; ml I–V, musculi longitudinales I–V; mfm, musculus fulco-manubricus; mfo, musculus fulcro-oralis; mfr, musculus fulcro-ramicus; mmf, musculus manubrico-frontalis; mmp, musculus manubrico-perioralis; mmmu, musculus manubrico-uncus; mrr, mastax receptor retractor; mtm, musculus transversus manubrii.

## Discussion

### *Comparison of the musculature of Squatinnella rostrum and other rotifers*

Compared to other ploimid rotifer species, *S. rostrum* is conspicuous for the absence of a number of longitudinal trunk muscles and some specific circular muscles and matches in this regard most closely the muscular pattern found in *B. stylata* and *Beauchampiella eudactylota*. *Beauchampiella eudactylota* presents three longitudinal muscles stretching from the head to the foot: a ventral, a lateral and a dorsal longitudinal muscle pair (see Riemann *et al.* 2009b). *Bryceella stylata* also features three longitudinal muscle pairs admittedly a ventral, a ventrolateral and a dorsal longitudinal muscle pair (see Wilts *et al.* 2009b). *Squatinnella rostrum* displays a paired ventral and a paired dorsal longitudinal muscle (musculus longitudinalis ventralis and musculus longitudinalis dorsalis) that can reasonably be assumed as homologous with the dorsal and ventral longitudinal muscle pairs in *Be. eudactylota* and *B. stylata* because these muscles have been considered to be ground pattern features of Ploima (see Riemann *et al.* 2009a

and Wilts *et al.* 2009b). It is noteworthy that in the foot of all three species, the musculus longitudinalis ventralis increases in width and splits into two filament bundles separated by a narrow gap. Like in *Be. eudactylota*, a longitudinal muscle that causes a retraction of the head (musculus longitudinalis capitis) is lacking in *S. rostrum*, although it has been reported for several species of Ploima (e.g. *Epiphanes senta*, see Martini 1912; *Proales daphnicola*, *Proales fallaciosa*, *Proales reinhardti*, see Sørensen 2005; *Dicranophorus forcipatus*, *Encentrum mucronatum*, see Riemann *et al.* 2008; *B. stylata*, Wilts *et al.* 2009b). The lack of a musculus longitudinalis capitis in *S. rostrum* appears plausible because of the large rostrum and its correlated disability of head and corona retraction. Furthermore, like *B. stylata*, *S. rostrum* displays neither a distinct pars coronalis (observed in *E. senta*, see Martini 1912; *Brachionus pala*, *Euchlanis pellucida* and *Rhinoglena frontalis*, see Stoßberg 1932; *Notholca acuminata*, see Sørensen *et al.* 2003; *Brachionus urceolaris* and *Notommata glyphura*, Santo *et al.* 2005; *P. daphnicola*, *P. fallaciosa* and *P. reinhardti*, see Sørensen 2005; *Filinia novaezealandiae*, Hochberg and Gurbuz 2007; *Encentrum mucronatum*, see Riemann *et al.* 2008; *Hexarthra* sp., Hochberg and Gurbuz 2008; *Pleurotrocha petromyzon* and *Pleurotrocha*

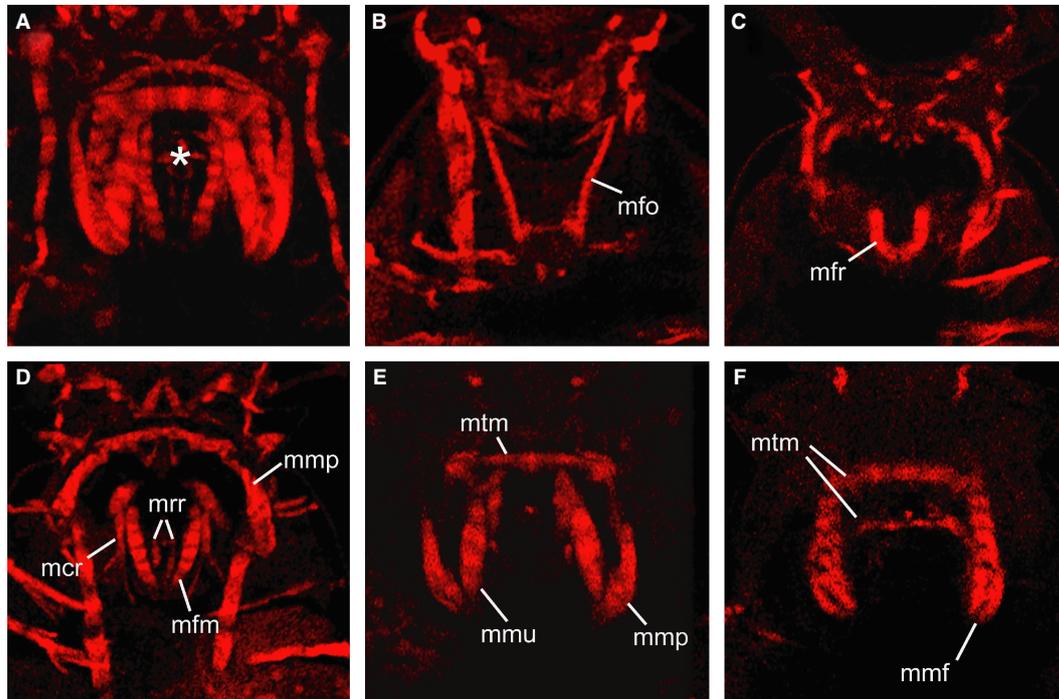


**Fig. 7**—Somatic musculature of *Squatinella rostrum*. Confocal laser scanning microscopic images of phalloidin-staining. —**A**. Depth-coded maximum projection of fluorescence signals (specimen1). Detail shows musculature in caudalmost foot pseudosegment. —**B**. Depth-coded maximum projection of fluorescence signals (specimen2). —**C**. Detail of ventral foot muscles. —**D**. Detail of dorsal foot muscles. Arrow heads indicate undetermined, conspicuous muscles. mc I–IX, musculi circulares I–IX; ml I–V, musculi longitudinales I–V; mx, mastax musculature.

*robusta*, Wilts and Wulfken unpublished data) nor a corona sphincter (documented in *E. senta*, see Martini 1912; *Br. pala* and *R. frontalis*, see Stoßberg 1932; *Brachionus quadridentatus*, see Kotikova et al. 2001; *N. acuminata*, see Sørensen et al. 2003; *Floscularia ringens*, see Santo et al. 2005; *P. daphnicola* and *P. reinhardti*, see Sørensen (2005); males of *Brachionus manjavacas* Leasi et al. in press.; *Pleurotrocha petromyzon*, *Pleurotrocha robusta* and *Trichocerca* sp., Wilts and Wulfken unpublished data). The absence of these two circular coronal muscles in *S. rostrum* is certainly also correlated with the presence of a large, stiffened and non-retractile rostrum and has to be evaluated as a secondary loss because the pars coronalis and the coronal sphincter have reasonably been assumed to be ground pattern features of Ploima (see Riemann et al. 2008). Anyhow, *S. rostrum* exhibits overall seven circular muscles in the neck and trunk. This number agrees with the number of circular muscles found in *B. stylata*. By contrast, *Be. eudactylo* possesses only two circular muscles in the trunk. Finally, *S. rostrum* displays a distinct circular muscle in front of the toe bases (musculus circumpedalis) that has been reported for several other rotifer species (for example in *Euchlanis dilatata*

*unisetata* and *Br. quadridentatus*, see Kotikova et al. 2001; *P. reinhardti*, see Sørensen 2005; *D. forcipatus* and *Enicentrum mucronatum*, see Riemann et al. 2008; *Be. eudactylo* Riemann et al. 2009b; *B. stylata*, Wilts et al. 2009b) and has been assumed to be a component of the musculature system in the ground pattern of Ploima (see Riemann et al. 2008).

In the early 20th century, a few investigations dealing with the mastax musculature of different rotifer species have been carried out on the basis of histological sections (De Beauchamp 1909; Martini 1912; Seehaus 1930; Stoßberg 1932). As recently as in the last years, fresh new data regarding this subject matter have been revealed by the use of confocal laser scanning microscopy (*N. acuminata*, Sørensen et al. 2003), transmission electron microscopy (*D. forcipatus*, Riemann and Ahlrichs 2008) and a combination of both microscopical techniques (*B. stylata*, Wilts et al. 2010). Although the high resolution of transmission electron microscopy is indispensable for recognizing the precise regions of attachment of mastax muscles to the trophi elements realized by hemidesmosomes and tonofilaments, this technique is very time-consuming regarding to preparation, analysis and reconstruction of such large



**Fig. 8**—Confocal laser scanning microscopic images of the mastax musculature in *Squatinella rostrum*. —**A**. Maximum projection of fluorescence signals. Asterisk indicates position of mastax receptor. —**B–F**. Optical sections from ventral to dorsal. 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; mrr, mastax receptor retractor; mtm, musculus transversus manubrii.

and complex structures as the mastax. However, with some background knowledge and qualitatively good image stacks of several specimens, CLSM provides an advantageous alternative technique for reconstructing the individual mastax muscles. The three-dimensional impression of the image stacks and the possibility of comparing and overlaying transmission light and fluorescence laser channel provide a good overview of the relative position of the muscles to each other and the trophi elements as well. In surveying the older literature, we attempt to identify muscles possibly homologous to those in the jaw apparatus of *S. rostrum*.

*Squatinella rostrum* shows a paired muscle that is attached to the distal part of the manubrium and terminates frontally at the manubrium-uncus joint (musculus manubrico-frontalis, Fig 6D and 8A,F). Such a muscle, apparently effecting a spread of the malleus, has also been observed in *N. acuminata* (mallei flexors; Sørensen *et al.* 2003), *B. stylata* (musculus manubrico-frontalis; Wilts *et al.* 2010) and *Be. eudactylota* (Wilts unpublished data). In *B. stylata*, it could not be observed directly, whether this muscle is attached to the uncus or not, although it contacts the manubrico-uncus joint and terminates in its proximity. Because of the position, orientation and distribution of the muscle within rotifers with a malleate and modified malleate trophi system, the muscle is assumed to be homologous in the named species. Considering the literature, we can reject the assumption that the muscle is

homologous with the musculus extensor mallei quoted for *E. senta* by Martini (1912) because this muscle is orientated more ventrolaterally and connects both manubrial caudae by forming a complete band surrounding the mouth opening.

In *S. rostrum*, we observed a muscle spanning the anterior part of both manubria dorsally (musculus transversus manubrii, Fig. 6C and 8A,F). A muscle with the same orientation is also present in *Eu. pellucida* and *Br. pala* (musculus lateralis manubrii; Stoßberg 1932), *Br. urceolaris* (see Fig. 1C,F in Santo *et al.* 2005), *E. dilatata unisetata* (see Fig. 6A in Kotikova *et al.* 2001), *D. forcipatus* (musculus transversus manubrii; Riemann and Ahlrichs 2008), *B. stylata* (musculus transversus manubrii; Wilts *et al.* 2010) and *Be. eudactylota* (Wilts unpublished data). The muscle comprises one or more distinctly separated fibres. A homology of this muscle in the above-mentioned species can be assumed considering the same position, orientation, distribution and assumed function (stabilization of mallei and approaching of manubria with contraction).

A muscle extending from the inner region of the manubrial cauda to the inner side of the uncus is present in *S. rostrum* (musculus manubrico-uncus, Figs 6C and 8E) and several other pliomid rotifers like *E. senta* (musculus flexor mallei; Martini 1912); *Eu. pellucida*, *Br. pala* (musculus flexor mallei; Stoßberg 1932); *D. forcipatus* (musculus manubrico-uncus; Riemann and Ahlrichs 2008); *B. stylata* (see Wilts *et al.* 2010)

and *Be. eudactylota* (Wilts unpublished data). De Beauchamp (1909) quoted such a muscle for the virgate mastax but it could not be determined on which species this assumption is founded. Given the position, distribution and assumable function (deflection of malleus and inward movement of uncus) of this muscle, it is plausible that it is homologous in all species where it has been identified.

Riemann and Ahlrichs (2008) documented a musculus manubrico-perioralis in *D. forcipatus* comprising two muscle pairs being attached to both manubria by forming two interrupted bands surrounding the mouth opening. The contractile filaments of each muscle pair are frontally in close contact via cell–cell connections. A similar muscle, drawing the manubrial caudae frontally, was observed in *E. senta* (musculus extensor mallei; Martini 1912), *Eu. pellucida* and *Br. pala* (musculus adductor mallei; Stoßberg 1932), *E. dilatata unisetata* (see Fig. 6C in Kotikova et al. 2001), *Be. eudactylota* (Wilts unpublished data) and *S. rostrum* (musculus manubrico-perioralis, Figs 6D and 8D,E) but a frontal interruption seems only to be present in *D. forcipatus* and possibly in *E. dilatata unisetata*. Concerning its distribution among different rotiferan taxa, the muscle is considered to be homologous. A homology with the mallei extensor documented in *N. acuminata* by Sørensen et al. (2003) is also conceivable but in this case, the muscle is restricted to the region between the proximal parts of the manubria and the pharyngeal epithelium.

Similar like the musculus fulcro-manubricus in *S. rostrum* (Figs 6D and 8D), a paired muscle, spanning between the laterocaudal fulcrum end and the dorsal border of the manubrial clava, has also been observed in *E. senta* (musculus fulcro-manubricus; Martini 1912), *Eu. pellucida* (musculus fulcro-manubricus; Stoßberg 1932), *N. acuminata* (fulcro-manubricus; Sørensen et al. 2003), *D. forcipatus* (musculus fulcro-manubricus; Riemann and Ahlrichs 2008), *B. stylata* (musculus fulcro-manubricus; Wilts et al. 2010) and *Be. eudactylota* (Wilts unpublished data). Because of its orientation and assumable function (extension of the malleus), we assume a homology of the corresponding muscles in the above listed species.

Furthermore, a paired muscle is attached anteriorly to the ramus basal chamber (musculus caudo-ramicus, Figs 6C and 8D) and bifurcates caudally. One caudal branch terminates dorsolateral to the fulcrum and the other huddles against the caudal border of the salivary gland. A similar muscle was documented in *D. forcipatus* (musculus caudo-ramicus; Riemann and Ahlrichs 2008) where it seems to be attached to a muscle surrounding the salivary glands (musculus circumglandis) but at this point of knowledge, a homology statement is hardly possible.

*Squatinnella rostrum* features a muscle that attaches to the fulcrum and to a region between the rami where the mastax receptor is supposably located (mastax receptor retractor, Figs 6C and 8D). This kind of muscle has previously been noticed in *Synchaeta pectinata* (muscle de'presseure de piston; De Beauchamp 1909), *Trichocerca bicristata* (muscle

de'presseure de piston; De Beauchamp 1909), *Notommata copeus* (muscle de'presseure de piston; De Beauchamp 1909), *E. senta* (musculus fulcro-mucosus; Martini 1912), *Eu. pellucida* (musculus fulcro-mucosus; Stoßberg 1932) and *D. forcipatus* (mastax receptor retractor; Riemann and Ahlrichs 2008) and *B. stylata* (mastax receptor retractor; Wilts et al. 2009b). Except for *Synchaeta pectinata*, *T. bicristata* and *S. rostrum*, this muscle is unpaired in all above-mentioned species. Concerning the orientation and distribution of these muscles, they are considered to be homologous with the mastax receptor retractor in *S. rostrum*.

The musculus fulcro-ramicus (Figs 6D and 8C) is a conspicuous, paired muscle interconnecting fulcrum and rami realizing an opening action of the rami. It has been found in *S. rostrum* as well as in *B. calyciflorus* and *Euclanlis deflexa* (abducteur horizontal; De Beauchamp 1909), *E. senta* (musculus fulcroscapalis; Martini 1912), *Eu. pellucida* and *Br. pala* (musculus fulcroscapalis; Stoßberg 1932), *Testudinella patina* (musculus abductor rami; Seehaus 1930), *N. acuminata* (musculus fulcro-scapalis; Sørensen et al. 2003) and *D. forcipatus* (musculus fulcro-ramicus; Riemann and Ahlrichs 2008). It is considered to be a ground pattern feature in Monogononta (see Riemann and Ahlrichs 2008; Wilts et al. 2010) and can reasonably assumed to be homologous in all species where it has been identified.

Finally, *S. rostrum* shares the presence of a muscle being attached to the terminal fulcrum end stretching frontally below the rami [musculus fulcro-oralis, Figs 6D and 8B]) with several rotiferan taxa including *E. senta* (musculus fulcro-oralis; Martini 1912), *Eu. pellucida*, *Br. pala* and *R. frontalis* (musculus fulcro-oralis; Stoßberg 1932), *B. stylata* (Wilts et al. 2010) and *Trichocerca* sp. (Wilts unpublished data). This muscle usually appears paired except in *Br. pala*. An attachment of the muscle on the pharyngeal wall near the mouth opening was only observed in *E. senta*, *R. frontalis*, *B. stylata* and *Trichocerca* sp. Stoßberg (1932) does not agree with the assumption of Martini (1912) that the musculus fulcro-oralis effects a widening of the mouth opening but favours the hypothesis that the muscle acts as a mastax protractor. However, both possible cases result in an approximation of the trophi and the mouth opening (see also Wilts et al. 2010). The musculus fulcro-oralis is considered to be homologous in *E. senta*, *Eu. pellucida*, *Br. pala*, *R. frontalis*, *B. stylata* and *Trichocerca* sp. because of its course, function and distribution. A ventral coursing muscle connecting the distal end of the fulcrum with the pharyngeal plates was documented in *D. forcipatus* (musculus hypopharyngeus; Riemann and Ahlrichs 2008) but a homology with the musculus fulcro-oralis in the above-listed species remains ambiguous.

#### Phylogenetic evaluation of morphological characters

Our morphological investigation has revealed a series of other character traits that inhere in some phylogenetic relevance. With the results of our study of *S. rostrum*, we are able to consider the following morphological characters as autapomorphic

traits for the whole genus: (1) the large semicircular rostrum, (2) the sail-like development of the epidermal projections restricting the corona laterally, (3) the paired, ventral, lobate epidermal projection of the trunk, (4) the large retrocerebral glands forming a saccate structure in front of the corona and (5) the elongation of the ventralmost uncus teeth. The conspicuous, large, semicircular rostrum (also referred to as head shield) is the most conspicuous diagnostic character of *Squatinella* (Fig. 3D, E) and truly a unique structure amongst rotifers. Indeed, a rostrum can be found in several other taxa like *Cohurella*, *Lepadella*, *Bryceella*, *Wulfertia*, *Proales* and *Dicranophoridae*; therefore, its presence in *Squatinella* has to be considered either plesiomorphic or the structure has evolved convergently. However, its enormous size and specific shape can reasonably be assumed as autapomorphic character for *Squatinella*. A paired epidermal projection that restricts the corona caudolaterally (Fig. 3E) is present in different species of *Pleurotrocha* (Wilts et al. 2009a), in *B. stylata* (Wilts et al. 2009b), in different species of *Mytilina* and *Lophocharis*, in *P. reinhardti*, *Lecane inermis*, *Euchlanis triquetra* and *Trichotria tetractis* (Wilts unpublished data) as well. This paired projection can also be found in all *Squatinella* species and was previously designated as side plates, triangular lamellae or ears (compare, e.g. Remane 1929–1933; Koste and Shiel 1989). Concerning the wide distribution of this structure within Ploima, its presence seems to be plesiomorphic, although its size and characteristic shape is autapomorphic for *Squatinella*. Its function remains unknown. An active participation in uptaking food particles seems not to be possible because the projections are not associated with musculature as revealed by our CLSM study. Moreover, the paired lobate projection (detail in Fig. 3B) located medially on the ventral side of the trunk is also a characteristic and assumable autapomorphic feature for *Squatinella* because it is shared by all species of the genus (see Wulfert 1939) and does not occur in species of any other genus. Wulfert (1939) referred the structure as ‘Bauchschuppe’ and documented it in *Squatinella mutica* and *S. rostrum* (see Fig. 27B,C and 30B in Wulfert 1939). The function of this structure is unclear and transmission electron microscopic studies on this structure are recommended. The two kidney-shaped glands of the retrocerebral organ forming a large, saccate structure ventrally in front of the corona (Figs 1A,B, 2B and 3B) represent another diagnostic and autapomorphic character for *Squatinella*. These glands are peculiar and distinctly recognizable under light microscopy but their function also remains unclear. Finally, the two elongated most ventral uncus teeth (Figs 4D and 5F) carrying the distal subuncus most likely represent another autapomorphic character trait of *Squatinella* because this specific character can also be recognized in *Squatinella bifurca* (see Fig. 25 and Fig. 26 in De Smet 2007) and do not occur in any other genus within Rotifera.

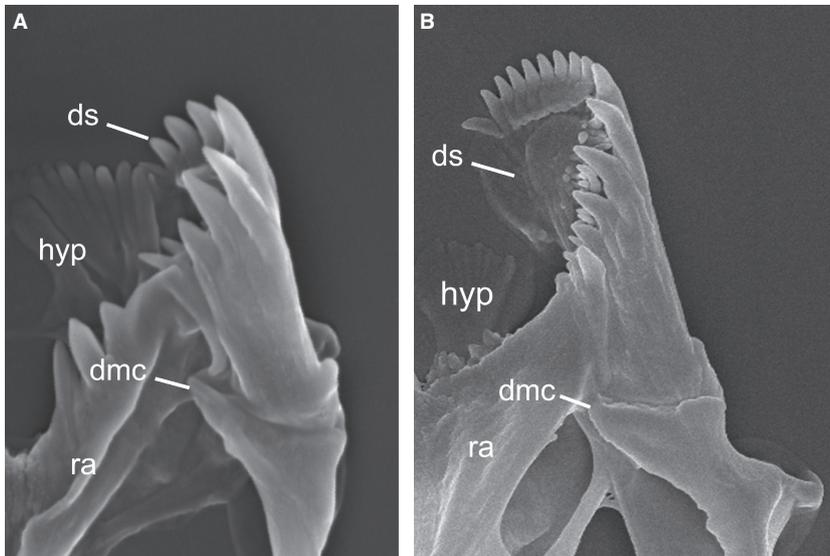
Although, *Squatinella* does not share some characters that are present in all other three genera of Lepadellidae (e.g. a strongly stiffened and closed lorica and alulae on the rami), however, some of the morphological traits observed in

*S. rostrum* may be considered as possible synapomorphies for all genera within Lepadellidae: (1) hoof-shaped distal fulcrum end and (2) dorso-caudal orientation of the ramus foramen sub-basalis. Besides for *S. rostrum*, a hoof-shaped distal fulcrum end was also documented for some other lepadellid species (*Cohurella adriatica*, see De Smet 2006; *Cohurella unicauda*, see Sørensen 2002; *Lepadella patella*, see De Smet 2007). *Squatinella bifurca* also displays this character (see De Smet 2007) but, most likely because of the preparation, the delicate margins of the fulcrum end are more spread. This character was assumed as a possible synapomorphic character shared by Mytilinidae and Lepadellidae by Sørensen (2002), nevertheless species like *Mytilina mucronata* have a more flattened and a riven, plate-shaped terminal fulcrum end. Other mytilinid species like *Lophocharis salpina* have a different and very slender fulcrum end. A dorso-caudal orientation of the ramus subbasal chambers can be recognized in *C. adriatica* (see De Smet 2006), *S. bifurca* (see De Smet 2007) and *S. rostrum*. For *Lepadella*, the situation is unclear because hardly any adequate images or drawings of the trophi of the genus exist in the literature. Therefore, morphological studies on the trophi of *Lepadella* are necessary to verify our assumption that a dorso-caudal orientation of the ramus foramen sub-basalis represents a synapomorphy for all genera of the Lepadellidae. This character can be observed in some species of *Encentrum* as well but both traits seem to have evolved independently because the character is derived within Dicranophoridae (see also Riemann et al. 2009a) and no evidence for a closer relationship of these genera exists.

Several other morphological characters are characteristic for all lepadellid genera like the rostrum, lateral eyespots, long, continuously tapering toes with needle-shaped tips and the inwards curved distal cauda ends. It is difficult to decide if these characters may also represent apomorphic characters of Lepadellidae, because they also occur distributed within other ploimid taxa. Whether these traits are based on convergent developments, symplesiomorphies or autapomorphies can only be evaluated on the basis of further detailed morphological investigations and an elaborated phylogenetic system.

#### Comparison with *Bryceella*

*Squatinella rostrum* shows, like the other species of the genus, a conspicuous similarity with species of *Bryceella* (Proalidae), whose phylogenetic position is unsolved as well. Representatives of both phytophagous genera have a similar habitus characterized by a small, dorsoventrally flattened body (average length between 100 and 200 µm) with a large rostrum, a bulbous trunk, a slender foot and a planar corona. The representatives of both taxa live basically on the ground or on submersed plants of acidic waters or inhabit mosses where they glide on surfaces in a nimble and jerky manner. Like *S. rostrum*, *B. stylata* is characterized by the reduction in the number of longitudinal muscles in the trunk as well as the pars coronalis and coronal sphincter. Furthermore, *B. stylata* and



**Fig. 9**—SEM images of trophi details of *Bryceella*. —**A.** *Bryceella stylata*. —**B.** *Bryceella* n.sp. dmc, dorsal manubrial chamber; ds, distal subuncus; hyp, hypopharynx; ra, ramus.

*S. rostrum* present a similar set of mastax muscles but differ in the presence of a musculus circumglandis (present in *B. stylata*), a musculus caudo-ramicus and musculus manubrico-perioralis (both present in *S. rostrum*) and the fact that mastax receptor retractor in *S. rostrum* is paired. Remarkably is the fact that *Squatinella* presents a distal subuncus and a large, lobate subuncinal element that is attached ventrally to the uncus featuring a denticulate margin. This specific form of subuncus is otherwise only known for *Bryceella* (Fig. 9A,B). The distal subuncus was apparently not recognized in *Squatinella* previously although it can be recognized on drawings and images in the literature (Pl. 33 Fig. 2C, 2D in Voigt 1957; Fig. 25 and Fig. 26 in De Smet 2007). The fact that *Bryceella* and *Squatinella* share the presence of such a unique structure as the subuncus may indicate a closer relationship of both taxa. Especially this morphological character, the conspicuous jerky movement as well as the congruent choice of habitat may represent possible synapomorphies of both taxa. But this hypothesis has to be confirmed by further phylogenetic analysis ideally using molecular methods.

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