

Functional morphology of somatic muscles and anterolateral setae in *Filinia novaezealandiae* Shiel and Sanoamuang, 1993 (Rotifera)

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Received 8 August 2006; received in revised form 20 October 2006; accepted 21 October 2006

Corresponding editor: M.V. Sørensen

Abstract

The freshwater rotifer, *Filinia novaezealandiae*, is a planktonic species in tropical limnetic ecosystems. Like other species in the genus, *F. novaezealandiae* has two mobile, anterolateral setae that supposedly facilitate saltation through the water column as a means of escape from predators. However, very little is known about setal structure or the control of setal movement. Here, we use a combination of behavioral observations, scanning and transmission electron microscopy, phalloidin staining of F-actin, conventional fluorescence and confocal laser scanning microscopy, and three-dimensional (3D) analysis software to investigate saltation in *F. novaezealandiae* and gain insight into the contribution that both setae and muscles play in locomotion. Results from EM reveal that the paired anterolateral setae are smooth, hollow extensions of the syncytial integument and constructed of two layers: a thin internal and thick external lamina. The setal cavity is continuous with the body cavity of the trunk, and the setae articulate with the trunk at a series of flexible folds. A lack of direct muscular supply to the setae indicates that their movement is likely to result from a combination of: (1) contractions of specific muscle sets (e.g., anterior transverse muscle, post coronal ring muscle) that insert on the body wall around the setae; and (2) changes in hydrostatic pressure within the body cavity that accompany coronal withdrawal (via contraction of retractor muscles) and extension. These descriptions, together with behavioral observations, indicate that the primary function of the setae is likely to be predator deterrence and not predator evasion. Likewise, saltation is probably a result of heightened coronal activity that immediately follows cycles of setal abduction/adduction. These insights, together with new descriptions of the muscular system in *F. novaezealandiae*, may be of utility in understanding the evolution of locomotory mechanisms among rotifers.

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Keywords: Rotifer; Muscle; Phalloidin; CLSM

1. Introduction

In an attempt to draw increased attention to the behavior and physiology of planktonic invertebrates, the neurobiologist Theodore H. Bullock (1997) once stated:

“Zooplankton, in its marvelous variety, faces a set of problems in everyday living different from those of benthic, littoral and other faunas and not at all uniform or uneventful....” Indeed, the lives of zooplankton have captured the attention of scientists for more than a century, but for most species, we still remain ignorant about many details of their lives, including the physics of locomotion at low Reynolds numbers, the mechanisms of food selection and capture, and most aspects of

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sensory physiology (Bullock 1997). These subjects, among others, are of particular importance for understanding the ecology of freshwater rotifers, a numerically dominant group of microinvertebrates that have been studied since the late 17th century. Wallace (2002) notes that even today, 300 years after their initial discovery by van Leeuwenhoek, many basic questions remain, not the least of which concerns the structure and function of many organ systems.

One question that preoccupies zooplankton biologists is related to the function and variety of appendages carried by planktonic animals (Dodson 1974; Kerfoot 1988). In some species, these appendages are spine- or bristle-like in appearance and have an unambiguous function in predator defense (Williamson 1987), while in others, the appendages may have a more obvious locomotory function (Gilbert 1985b, 1987), or in some cases, be both anti-predator and pro-locomotion (Williamson 1987). Among rotifers, planktonic species are well known to carry a variety of unusual and structurally diverse appendages. In species of *Brachionus* and *Keratella*, where appendages are normally absent, parthenogenetic mothers may produce heavily spined offspring while in the presence of cladocerans, copepods and predatory rotifers (e.g., Gilbert and Waage 1967; Gilbert and Stemberger 1984; Stemberger and Gilbert 1987; Gilbert 2001). In general, these spines are unarticulated extensions of the thickened skeletal integument (lorica) that function as defensive armament against larger predatory zooplankton (e.g., Gilbert and Stemberger 1984) or prevent incidental handling by filter feeding cladocerans (e.g., Gilbert 1985a). Still, other rotifers such as species of *Polyarthra* possess movable paddle-like appendages that function more as a tool for evasion than structural refuge (Gilbert 1985b, 1987). Such appendages are adapted for locomotion at low Reynolds numbers—being ornate flat paddles with broad surfaces—and act as hydrofoils during both power and recovery strokes that lead to a three-dimensional (3D) tumbling through the water and away from the predator (Gilbert 1985b).

In an apparent turn from the usual, species of *Filinia* possess locomotory appendages that appear more like classic defensive structures—two elongate setae (spines) project from the anterior trunk and one from the posterior trunk—that in most species are movable and thought to function in saltational “jumps” through the water column (Wallace and Snell 2001; Wallace et al. 2006). Unfortunately, cinematographic analysis of locomotion in species of *Filinia* has yet to be performed, unlike that in species of *Polyarthra* (Gilbert 1985b, 1987), and so it remains to be determined exactly how these setae function to create bursts of movement. Moreover, setal length appears to be highly plastic and correlated with specific environmental factors, the significance of which remains to be ascertained

(Ruttner-Kolisko 1980, 1989; Schaber and Schrimpf 1984; Sanoamuang 1993a, b). In this paper, we examine the ventrolateral setae and the associated musculature of *Filinia novaezealandiae* to gain insight into the structure of these locomotory appendages and determine how they might function in saltation. In addition, we provide new information on the general patterns of somatic musculature in the species to improve understanding of muscle diversity in the Rotifera.

2. Material and methods

2.1. Collection and identification

Specimens of *Filinia* Bory de St. Vincent, 1824 were collected with a 64 µm plankton net from a pond at the Smithsonian Marine Station in Fort Pierce, Florida (27° 27.627'N, 80° 18.682'W) in June and July 2005. Rotifers were observed in a Petri dish for interactions with other zooplankton, and several specimens were anaesthetized in 1% MgCl₂ and photographed alive as whole mounts on glass slides. Specimens were identified under bright-field optics using the taxonomic key of Sanoamuang (2002) and Jersabek et al. (2003). The current specimens fit well within the *Filinia longiseta-terminalis* group, but as stated previously (see Ruttner-Kolisko 1974, 1989; Sanoamuang 1993a, b; Shiel and Sanoamuang 1993), this group has a convoluted taxonomy that likely requires revision. The current specimens are identified as *Filinia novaezealandiae* Shiel and Sanoamuang, 1993 based on general body size and morphology, the position and immobility of the caudal seta, and trophi with approximately 19–21 teeth. This is also a warm water species, which according to current literature, in part differentiates *F. novaezealandiae* from the cold stenotherm *F. terminalis* (Plate, 1886) (Sanoamuang 1993b, 2002). However, as evidenced below, egg sculpture in these specimens differs from the type description (see Figs. 10 and 20 in Sanoamuang 2002).

2.2. Muscle staining and observation

Rotifers ($n = 18$) were anaesthetized in 1% MgCl₂ and fixed in 4% paraformaldehyde in 0.1 M PBS for 10 h at 4 °C. Specimens were next rinsed ($3 \times$) over the course of 2 h in 0.1 M PBS and transferred to Alexa Fluor 488 Phalloidin (Invitrogen) for 2 h at 4 °C. Specimens were rinsed, mounted in Fluoromount G (Electron Microscopy Sciences) on glass slides, and refrigerated at 4 °C for at least 24 h before examination.

Wholemound specimens were examined on two microscopes: (1) a Nikon Eclipse E800 compound microscope equipped with a Biorad Radiance 2000 laser system at the Smithsonian Marine Station in Fort

Pierce, Florida; and (2) a Zeiss Axioimager equipped with epifluorescence, digital Axiocam, and Axiovision software at the University of Massachusetts, Lowell. The specimens observed on the Zeiss microscope were kept refrigerated for 4 months prior to examination. The specimens observed on the confocal microscope were examined within 48 h of staining. Lasersharpe software was used to collect a series of 0.1 μm optical sections with maximum intensity projection along the z-axis. Confocal images were imported into Confocal Assistant and made into TIF files. Additional digital files were imported into Volocity (Improvision) to render 3D images and create X–Y–Z rotations in TIF and AVI formats. Movie files (AVI) are available upon request. No manipulations of the original images were made other than changes of color (false coloring or grayscale) or cropping. The program Carnoy V 2.0 (© 2001 Peter Schols) was used to make measurements in some digital images.

2.3. Electron microscopy

Rotifers were prepared for scanning electron microscopy (SEM, $n = 8$) and transmission electron microscopy (TEM, $n = 4$). For SEM and TEM, rotifers were relaxed in 1% MgCl_2 for 20 m, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 24 h, followed by four buffer rinses (15 m each) and post fixation in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h. Following four more buffer rinses (15 m each), rotifers were dehydrated in a graded ethanol series and prepared for either SEM or TEM. For SEM, specimens were transferred to modified BEEM capsules and dehydrated in a critical point dryer. Dehydrated specimens were sputter coated with gold and examined on a JEOL 6400 SEM at 10 kV. For TEM, specimens were transferred to propylene oxide and embedded in an Araldite/Epon mixture. Resin blocks for TEM were sectioned at 60–70 nm with a Diatome diamond knife on a Reichert or Sorvall ultramicrotome, collected on coated grids, and stained with uranyl acetate and lead citrate. Grids were examined with a JEOL 100 CX II TEM at the Smithsonian Marine Station in Fort Pierce, Florida.

3. Results

3.1. Live observations

Live specimens of *F. novaezealandiae* were examined in a Petri dish with pond water and various zooplankton (e.g., copepods, cladocerans, rotifers). While swimming, the rotifers glide gracefully through the water column with their setae in a relaxed (prone) position at their sides (Fig. 1E). Upon encountering another animal (e.g., a cladoceran or copepod), the rotifers quickly abduct

(raise) their anterolateral setae to an extended position above their head; this is always followed by an equally quick adduction (lowering) of the setae back to a prone position. The corona appears to be withdrawn into the trunk during abduction and may be extended during adduction. The sequential movements of the setae and the corona are almost always accompanied by a quick glide through the water column; coronal cilia continue to beat. Setae do not move independent of each other. A few specimens were observed to partially abduct/adduct their setae during gliding; however, this behavior did not appear to be related to the presence of other zooplankton nor did it make a noticeable contribution to locomotion.

3.2. General morphology

Measurements of rotifers were made from specimens examined with SEM ($n = 8$). Body length from the corona to the posterior end (minus the caudal seta) is 68–99 μm . Body width at 50% body length is 34–43 μm . The trunk has two transverse folds that occur at approximately one-fourth and one-third body length (Figs. 1A, 2A and B). These folds may be a result of longitudinal body contraction despite the use of anaesthesia. Anteriorly, the coronal cilia are 6–8 μm long and the buccal cilia around the mouth (mo, Fig. 1A) are 4–6 μm long. Three setae project off the body (Figs. 1 and 2). Two bilateral setae up to 304 μm long (range 260–304 μm) insert in a slightly ventrolateral position approximately 15 μm from the top of the corona. Both setae are narrow in width (ca. 3–4 μm) at their site of insertion and widen to approximately 7 μm (see arrows, Fig. 2A and B) before tapering to 2 μm at their tip. A very slight dimpling is present along the length of the setae (Fig. 2B). A single caudal seta projects off the ventral body wall approximately 10–15 μm from the posterior end (Fig. 1A–C). This seta is approximately 5 μm wide at its point of insertion and tapers to approximately 2 μm . The length of the caudal seta is 185–210 μm . A cloacal pore is terminal (Fig. 1C). Some specimens possess a single egg that is attached to a site close to the cloacal pore on the dorsal side of the trunk (Fig. 1C–E). Eggs are smooth in appearance and 25–32 μm in diameter.

3.3. Ultrastructure of anterolateral setae

Cross sections through the anterior trunk and buccal regions reveal an electron dense hypodermis and muscles in various orientations; muscles never enter individual setae (Fig. 2C–F). Each individual seta represents a hollow and thickened extension of the body wall and is constructed of a thin internal lamina and thick external lamina (following the descriptions of

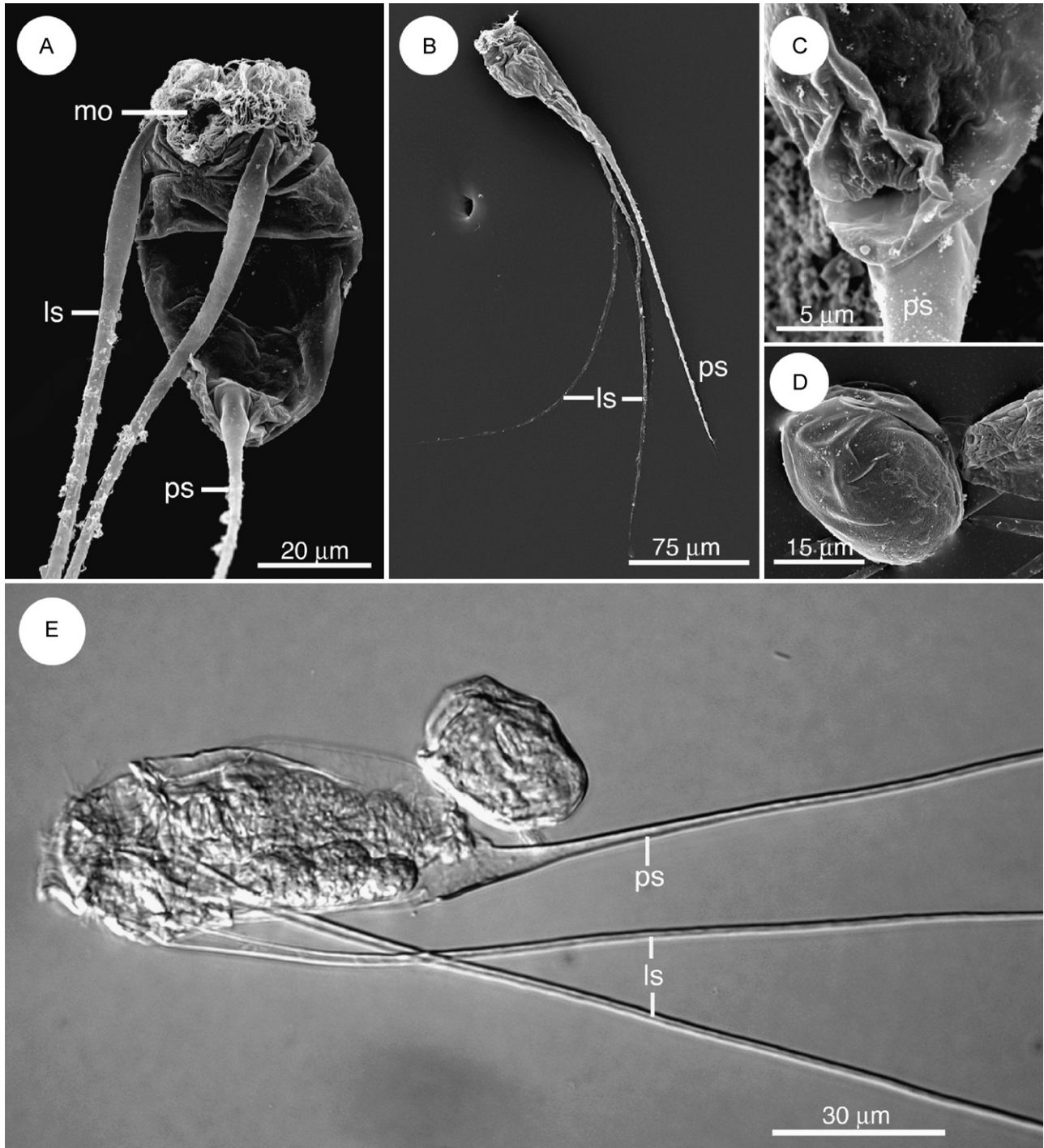


Fig. 1. *Filinia novaezealandiae*: (A) SEM of specimen in ventral view; (B) SEM of whole specimen in lateral view; (C) SEM of posterior trunk showing the terminal cloacal pore; (D) SEM of attached egg; (E) live specimen in lateral view with a single embryo attached to the posterior dorsal margin. ls = lateral seta, mo = mouth, ps = posterior seta.

Clément and Wurdak 1991). The internal lamina is electron dense and reaches a thickness of 800 nm at the shoulder (origin) of each seta (Fig. 2D). The internal lamina thins out to approximately 200 nm or less along the length of each seta. The external lamina is thin (ca. 300–400 nm) and highly folded at the shoulder of each seta but thickens significantly along its length. The

external lamina is always electron lucent and easily distinguishable from the internal lamina (Fig. 2C–F). The external lamina is approximately 900–1200 nm thick along the body of each seta. The setal cavity is confluent with the trunk's body cavity and decreases in diameter along the length of the seta. In cross section, a single transverse line on the medial side of each seta defines the

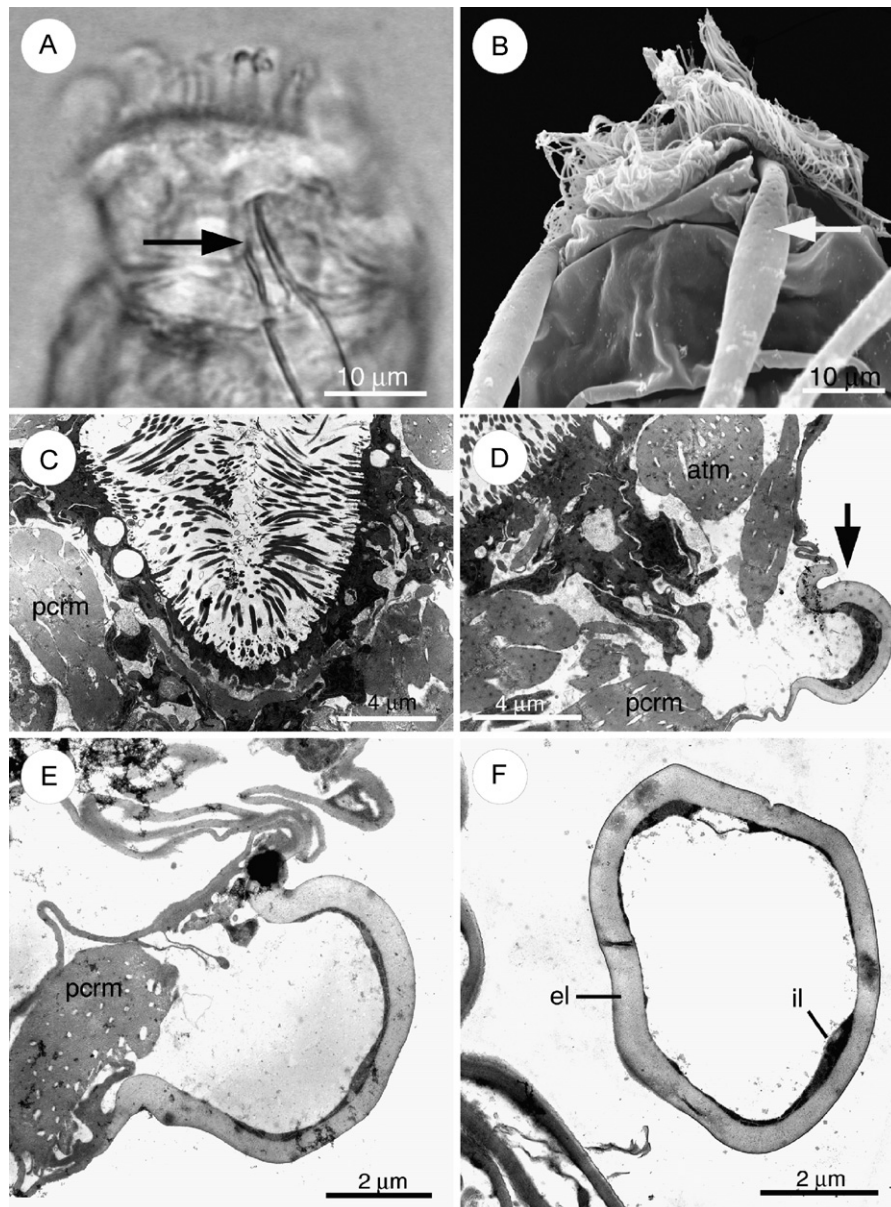


Fig. 2. *Filinia novaezealandiae*: (A) light micrograph of fixed specimen in lateral view, ventral to the right; (B) SEM of anterior end, ventral view; (C) TEM section through the buccal region, oblique angle; (D) TEM section through the shoulder of an anterolateral seta, slightly oblique cross section; (E) closeup of TEM section through the shoulder region of an anterolateral seta; (F) TEM cross section through an anterolateral seta, at approximately 50% seta length. atm = anterior transverse muscle, el = external lamina of body wall, il = internal lamina of body wall, pcm = post coronal ring muscle, black arrow = thin shoulder region of anterolateral seta, white arrow = external dimpling of anterolateral seta.

region where the external lamina fuses with itself to close off the seta from the outside (Fig. 2F). The ultrastructure of the caudal seta was not examined.

3.4. Muscle orientation

Somatic muscles are present in a variety of orientations including longitudinal, transverse, and circular. Visceral and cloacal muscles are also present but not

described here. The current terminology for rotifer muscles is based largely on their orientations and presumed functions; however, the homology of muscles in similar orientations (and with presumably similar functions) among phylogenetically distant species is far from certain. For these reasons, the terminology applied to different species is often different (compare species examined by Hochberg and Litvaitis 2000; Kotikova et al. 2001, 2004, 2006; Sørensen et al. 2003; Santo et al. 2005; Sørensen 2005a, b). While we wish to avoid adding

excess terminology, we note that there are no obvious conventions in place. Therefore, we apply a terminology based on muscle position, orientation, and function that fits within the scheme of previous research and utilizes similar names for potentially homologous muscles.

The somatic muscles can be divided into three general orientations: circular, transverse, and longitudinal. All muscles are noticeably cross-striated. The circular muscles are only present at the anterior end and consist of the pars coronalis (pc) and a post coronal ring muscle (pcrm). Both muscles encircle all longitudinal muscles that supply the coronal region (described below). The pc is a complete circular muscle that surrounds the apical region and makes a slight posterior bend to encompass the mouth and buccal field (Fig. 3A and E). A fine fiber-form muscle (cf) is present in the middle of the corona

(Fig. 4A), but its origin is undetermined. The pcm is incomplete and divided into four components: one short dorsal muscle, two lateral semicircles, and one ventral semicircle. Each division consists of multiple (>5) muscle fibers. The lateral ends of the ventral semicircle appear to insert close to the posterior origin of the anterolateral setae (observed in partially contracted and laterally oriented specimens) (see Fig. 6).

Ventrally, there are four pairs of muscles in slightly transverse orientations. The anterior transverse muscle (atm) forms an u-shaped arc that is directed towards the anterior, with each end of the muscle inserting on the ventral body wall and close to the anterolateral setae (Figs. 3A and E, 4–6). The atm may be an extension of the ventrolateral retractor muscles (described below). A short separate muscle lies close to the anterior end of

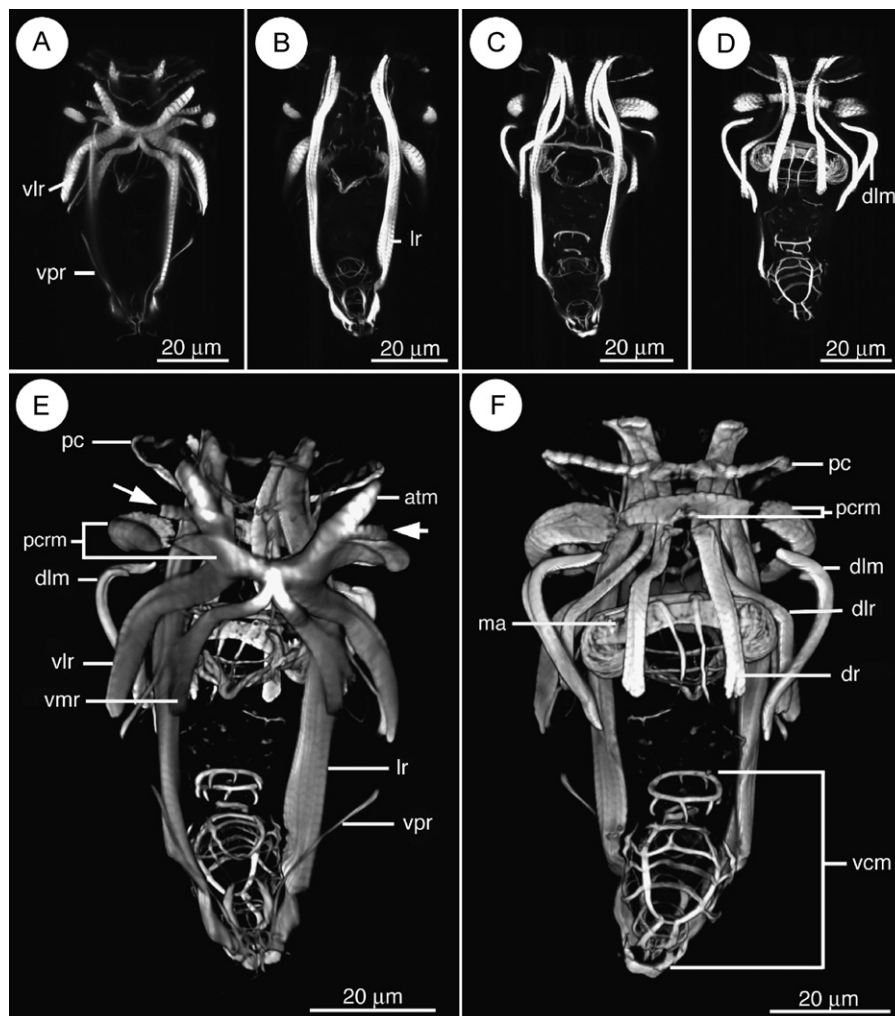


Fig. 3. *Filinia novaezealandiae*: (A–D) series of confocal images through the musculature of an entire specimen, ventral to dorsal; (E) computer-rendered view of the musculature in ventral view; (F) computer-rendered view of the musculature in dorsal view. atm = anterior transverse muscle, dlm = dorsolateral muscle, dlr = dorsolateral retractor, dr = dorsal retractor, lr = lateral retractor, ma = mastax muscles, pc = pars coronalis, pcm = post coronal ring muscle, vcm = viscero-cloacal musculature, vlr = ventrolateral retractor, vmr = ventromedial retractor, vpr = ventroposterior retractor, white arrows = separate small muscle on the atm.

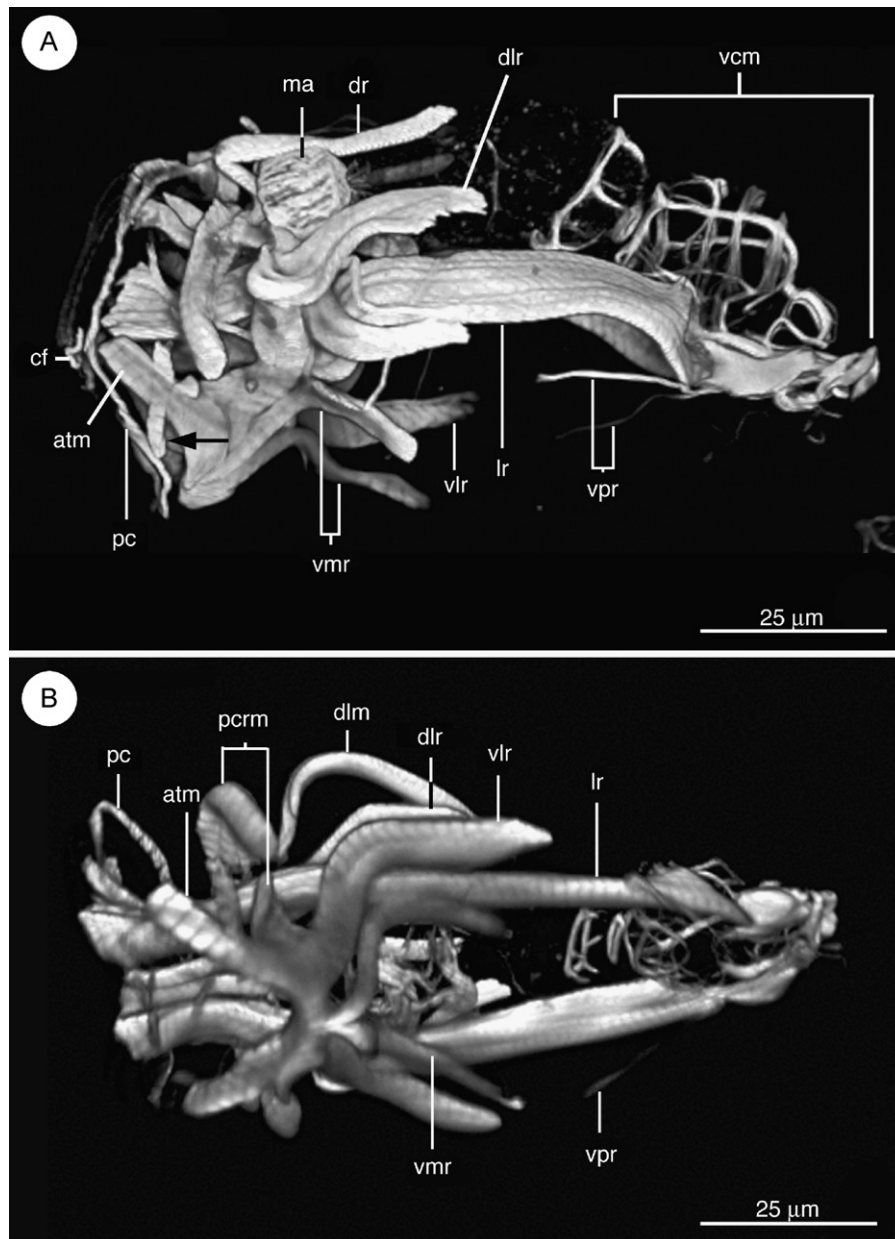


Fig. 4. *Filinia novaezealandiae*: (A) computer-rendered view of the musculature in lateral view, ventral is downward; (B) computer-rendered view of the musculature in ventrolateral view, animal is dorsoventrally compressed. atm = anterior transverse muscle, cf = fiber-form muscle of the corona, dlm = dorsolateral muscle, dlr = dorsolateral retractor, dr = dorsal retractor, lr = lateral retractor, ma = mastax muscles, pc = pars coronalis, pcr = post coronal ring muscle, vcm = viscero-cloacal musculature, vlr = ventrolateral retractor, vmr = ventromedial retractor, vpr = ventroposterior retractor, black arrow = separate small muscle on the atm.

each arm of the atm and inserts close to the anterolateral setae (arrow; see Figs. 3E and 5A). Extending from the mid-point of the atm and projecting posteriorly are two pairs of transverse, arc-shaped muscles: the ventromedial retractors (vmr) and the ventrolateral retractors (vlr). The vmr extends to the ventral body wall and the vlr extends to the ventrolateral body wall (Figs. 3E and 4). Various other muscles are present in and around the transverse muscles. Some of these muscles are highly

elongate fibers that wind around the larger somatic muscles. One pair of fibers, the ventral posterior retractors (vpr), extend from a region around the cloaca to about mid-body length (Figs. 3A and E, 4A and B).

The largest diameter muscles are the lateral retractors (lr), which reside between the ventral transverse muscles and the dorsal longitudinal muscles (described below). The lr inserts anteriorly in the corona and extends to the posterior end (Figs. 3–6). Each lr muscle consists of

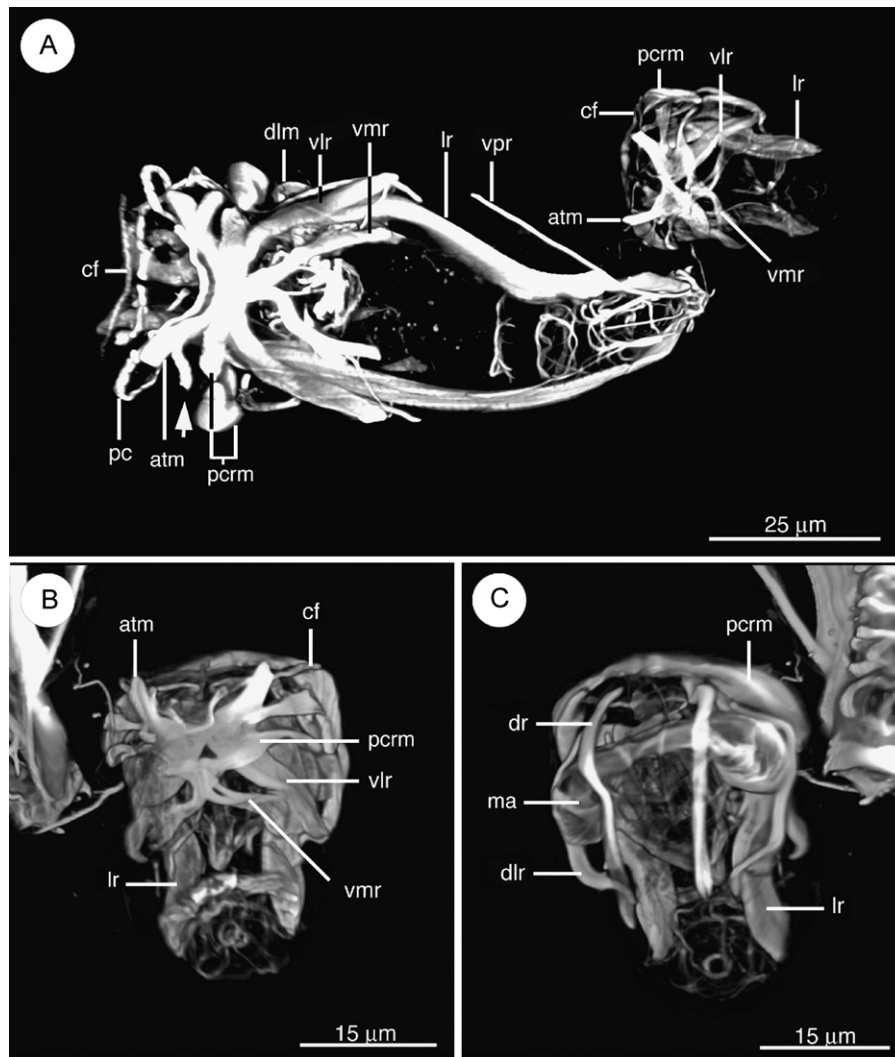


Fig. 5. *Filinia novaezealandiae*: (A) Epifluorescence view of the musculature of a single adult specimen with encapsulated embryo at the posterior end. (B) Computer-rendered view of the embryo, ventral view. (C) Computer-rendered view of the embryo, dorsal view. atm = anterior transverse muscle, cf = fiber-form muscle of the corona, dlm = dorsolateral muscle, dlr = dorsolateral retractor, dr = dorsal retractor, lr = lateral retractor, ma = mastax muscles; pc = pars coronalis, pcr = post coronal ring muscle, vlr = ventrolateral retractor, vmr = ventromedial retractor, vpr = ventroposterior retractor, white arrow = separate small muscle on the atm.

at least six fibers (Fig. 4A). The large size of these muscles makes them distinguishable in embryonic rotifers that are still encapsulated; however, the number of individual muscle fibers cannot be determined in embryonic rotifers (see Fig. 5).

Dorsally, there are two pairs of longitudinal muscles that originate in the middle of the body and insert in the corona: the dorsal retractors (dr) and the dorsolateral retractors (dlr). Each dr consists of two muscle fibers. Both the dr and dlr can be distinguished in encapsulated embryonic rotifers (Fig. 5). A pair of dorsolateral muscles (dlm), each consisting of two muscle fibers, is oriented in a slightly oblique position and curve from the dorsal body wall to the lateral

body wall. These muscles bifurcate at both ends (Figs. 3F, 4B, 6).

4. Discussion

Free-living rotifers employ a variety of devices with which they move about. Benthic species use a combination of coronal cilia to glide above the benthos and adhesive toes to gain hold of the substrate. Benthic bdelloids also make use of antagonistic muscles to stretch, creep and crawl their way through interstitial voids (Hochberg and Litvaitis 2000). Planktonic rotifers rely heavily on their coronal cilia to swim a helical path

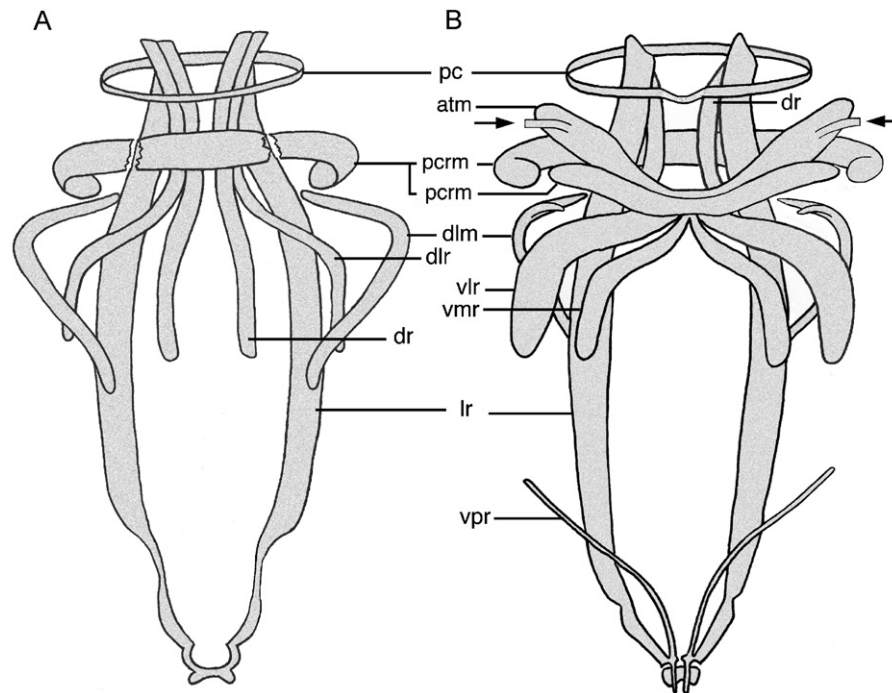


Fig. 6. *Filinia novaezealandiae*: Schematics of the somatic musculature in (A) dorsal and (B) ventral views. atm = anterior transverse muscle, dlm = dorsolateral muscle, dlr, dorsolateral retractor, dr = dorsal retractor, lr = lateral retractor, pc = pars coronalis, pcrm = post coronal ring muscle, vlr = ventrolateral retractor, vmr = ventromedial retractor, vpr = ventroposterior retractor, black arrow = separate small muscle on the atm.

through the water column (Starkweather 1987). Several species of Monogononta also possess a variety of appendages that aid in rapid locomotion to escape predators (Gilbert 1985b, 1987; Gilbert and Williamson 1978), and at least in part, prevent predation (Gilbert and Waage 1967; Gilbert and Stemberger 1984; Stemberger and Gilbert 1987; Gilbert 2001), and avoid rapid sinking (Starkweather 1987). In several species, the appendages are likely to serve multiple functions.

4.1. Structure and function of rotifer appendages

The structural and functional diversity of rotifer appendages serves as a testament to the selective pressures placed on microscopic animals in a low Reynolds number environment. Species of *Filinia*, *Hexarthra* Schmarda, 1854, and *Polyarthra* Ehrenberg, 1834 possess a variety of structurally diverse appendages, from elongate setae to arm-like limbs and ornate paddles that presumably evolved as a means of predator avoidance. However, only species of *Hexarthra* and *Polyarthra* possess appendages that, at least superficially, resemble hydrofoils that function to produce lift. For example, the arm-like appendages of *H. mira* (Hudson, 1971) are covered in an assortment of bristles that may function to increase the surface area and therefore add thrust during a power stroke; if flexible,

these same bristles might also lie back against the appendages during the recovery stroke to minimize drag (analogous to beetle bristles; see Nachtigall 1980). The arms also receive a direct muscular supply (Santo et al. 2005), which may lead to greater force of movement compared to appendages without muscles. In contrast, species of *Polyarthra* have flattened, paddle-like appendages with an indirect muscle supply, meaning that the muscles attach on the integument around the paddles, e.g., at apodemes (“teeth” and “hooks” of Allen 1968). In this case, the integument serves as a linkage for an assortment of large longitudinal muscles—contraction of these muscles pulls the integument above the paddles inward—leading to paddle abduction and a broad arc of motion. Similarly, species of *Filinia* have appendages with an indirect muscular supply; however, their appendages do not fit the shape of traditional hydrofoils.

In *F. novaezealandiae*, the anterolateral setae are relatively smooth, tapering cylinders up to four times the body length but less than 10% body width, making them appear delicate. Moreover, they lack bristles that might otherwise increase their surface area and add thrust during a power stroke. Internally, the setae are hollow extensions of the body wall, containing only a thin internal lamina and thick external lamina; the external lamina probably serves as the main source of structural rigidity. In the absence of a direct muscular

contribution, the setal cavity is devoid of any anatomical structures, and is instead completely open to the body cavity of the trunk. Several sets of muscles insert close to the shoulder of each seta, but only the atm appears to fit the shape and position of a muscle built for setal abduction. The atm is a thick, cross-striated u-shaped muscle that inserts just dorsal to the shoulder of each seta (see Figs. 3A and 6B). By virtue of its structure and orientation, contraction of the atm would appear to pull on the shoulder integument of each seta simultaneously, and therefore lift both setae towards the corona. Observational data indicates that neither seta moves independent of the opposite appendage, signifying that both setae are probably under the control of the same muscle. In contrast, species of *Polyarthra* can move their paddles independently of one another, and in fact, each are controlled by an independent muscle (Allen 1968).

During ciliary gliding, *F. novaezealandiae* appears to rely exclusively on the beat of the coronal cilia to propel its body through the water column. Occasionally, slow and partial movements of the setae occur during gliding, generally as a single cycle of abduction (the initial “recovery stroke” to raise setae above the head) and adduction (the “power stroke”), but these movements do not add any obvious contribution to locomotion. Moreover, quick cycles of complete abduction/adduction that occur when an animal is relatively stationary do not always accompany leaps through the water column. In fact, rapid bursts of movement are usually initiated directly after a full cycle of setal extension and flexion. If these cycles were the primary source of rapid locomotion, then abduction should involve some form of setal rotation or bending to minimize surface area effects, because any drag caused by abduction would be negated by an equal and opposite lift produced by adduction. Hence, the rotifer would return unintentionally to the same position from where it started. Also, setal movement appears to be dependent on coronal withdrawal (via contraction of lateral retractors and dr; see Fig. 6), so abduction should be accompanied by a drop in the ciliary contribution to locomotion; thus, the rotifer would stop moving. As an alternative, the rapid bursts of locomotion are more likely to be the result of heightened coronal activity. Rotifers are well known to control the beat frequency of the coronal cilia, and in fact do so during regular swimming activity (Clément 1987) and especially during escape from predators (Gilbert and Kirk 1988). High-speed cinematographic analysis should help resolve this question in species of *Filinia*.

While anterior movement of the setae is clearly under muscular control, it is somewhat unclear which muscles contribute to setal adduction and hence the power stroke. At least two muscles insert on the integument close to the posterior origin of each shoulder; a small

longitudinal muscle and a portion of the pcr. The single longitudinal muscle is present at the anterior end of each arm of the atm (see arrows, Figs. 3E, 4A, 5A, 6B), and alone, appears inadequate to adduct an individual seta. In fact, if the function of these muscles is setal adduction, then both muscles would have to contract simultaneously to maintain synchrony of setal motion. Alternatively, the pcr is a large muscle composed of multiple muscle fibers. The ventral portion of the pcr is transversely oriented (see Figs. 3 and 6) and would require only a single contraction to simultaneously adduct both setae and produce a power stroke. Still, any movement of the setae is dependent on the status of the corona. If observational data are correct and setal adduction is functionally dependent on re-extension of the corona, then it is likely that the entire pcr (dorsal, lateral and ventral components) must contract simultaneously to produce three effects: (1) antagonize the longitudinal muscles that contribute to coronal withdrawal, (2) pressurize the body cavity, and (3) re-extend the corona.

While the structure of the anterolateral setae of *F. novaezealandiae* appear inadequate for rapid bursts or jumps of locomotion, their function in saltation cannot be ruled out until further observations and experiments are performed (e.g., flow chamber analysis). Still, observations on other species of *Filinia* suggest that the anterolateral setae have a function other than locomotion, and that their mobility need not be tied to saltation. For example, in the related species *F. terminalis*, the setae significantly reduce predation by the predatory copepod *Diaptomus pallidus* (Williamson 1987). As noted by Williamson (1987), cycles of setal movement serve as the main deterrent to the predator; no significant bursts of locomotion accompany setal abduction or adduction. Likewise, the paddle-like appendages of *Polyarthra major* also prevent predation by the predatory copepod; however, in this species, the paddles function purely as hydrofoils, leading to movement away from the predator (see also Gilbert 1985b, 1987). These descriptions, in combination with observations of gliding, saltation, and defense in *F. novaezealandiae*, indicate that the function of the anterolateral setae is probably more for predator deterrence than predator evasion.

4.2. Comparison of rotifer muscle systems

Recent studies have revealed that the rotifer muscular system is a complex network of both individual and grouped (as functional blocks) muscle fibers (Hochberg and Litvaitis 2000; Kotikova et al. 2001, 2004; Sørensen et al. 2003; Santo et al. 2005; Sørensen 2005a,b). In most cases, the functions of individual muscles can be ascertained from their position and orientation in the

body; the majority of muscles appear to function in coronal withdrawal and general bending movements. While observations of many species continue to enhance our knowledge of rotifer function and behavior, questions still remain about the homology of even the most basic muscular units.

At first glance, the somatic muscular system of *F. novaezealandiae* appears to be quite different from the muscular systems described in other rotifers. For example, many of the ventral muscles in *F. novaezealandiae* (e.g., atm, vlr, vmr) have a more transverse orientation than those in other monogononts (e.g., see *Asplanchnopus multiceps* (Schränk, 1793) Kotikova et al. 2004; species of *Proales* Gosse, 1886, Sørensen 2005a). The atm appears to be a singularly unique muscle, with no obvious homology to muscles in species of other genera. Examination of additional species of *Filinia* and other closely related taxa might help identify intermediate forms of this muscle and reveal its evolutionary origin. Alternatively, the ventral muscles probably correspond to the ventral retractors in other species, as many of these retractors appear to insert on a ring-like muscle below the corona (pcrm of *F. novaezealandiae*; tm of *Brachionus quadridentatus* (Hermann, 1783) and *Euchlanis dilatata* Ehrenberg, 1832, Kotikova et al. 2001; pcm of *A. multiceps*, Kotikova et al. 2004). However, in *F. novaezealandiae*, the ventral muscles have taken on a new orientation, perhaps signifying their functional link to the anterolateral setae, e.g., by “pulling” on the atm or pcm to aid abduction or adduction, respectively (see Fig. 6B). Most of the other somatic muscles of *F. novaezealandiae* fit into the same general pattern as that found in a variety of monogononts including both ploimates and flosculariaceans. In particular, most species possess a pc, corona sphincter (or equivalent pcm), a pair of lateral retractors, and one or two pair of dr. Aside from differences in muscle size, i.e., the number of muscle fibers that compose each functional muscle block, and differences in muscle origin, e.g., mid-trunk versus posterior trunk, many of these longitudinal muscles appear to share a similar position and function.

As noted by Sørensen (2005a), there is a great deal of variation in muscle patterns among rotifers, and despite some similarities in orientation, position and structure, any attempts to homologize muscles among distantly related taxa must be met with caution. For these reasons, a greater variety of species representing a larger range of habitats and lifestyles needs to be examined to separate cases of homology from homoplasy. In the case of rotifers with movable appendages, a greater in-depth examination of the muscles that supply these appendages would go a long way toward understanding the evolution of different locomotory mechanisms, and hence the planktonic lifestyle. For example, an earlier study of *Polyarthra vulgaris* Carlin, 1934 by Allen (1968)

revealed the presence of a large circular muscle in the anterior trunk region that may supply the locomotory paddles. The size and position of this muscle indicates potential homology with the pcm of *F. novaezealandiae* (and similar muscles noted above). The muscles in both species supply the movable appendages and may even have similar functions in appendage adduction. However, their anatomical and functional links to these vastly different appendages are more likely to be the result of convergence on a similar lifestyle rather than phylogenetic proximity (see Sørensen and Giribet (2006) and Wallace et al. (2006) for latest hypotheses of rotifer relationships). In conclusion, greater attention to species of *Filinia*, *Hexarthra* and *Polyarthra* will vastly improve our knowledge about how rotifers avoid predators, and how life at low Reynolds numbers has influenced their evolution.

Acknowledgments

We thank Dr. R.L. Wallace, two anonymous reviewers, and Dr. M.V. Sørensen for their valuable comments on our manuscript. We are also grateful to the staff at the Smithsonian Marine Station in Fort Pierce, Florida for the use of their facilities. This research received financial support from the University of Massachusetts Lowell and from the Sumner Gerard Foundation at the Smithsonian Marine. This is Smithsonian Marine Station at Fort Pierce contribution 671.

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