Behavior, metamorphosis, and muscular organization of the predatory rotifer Acyclus inquietus (Rotifera, Monogononta)

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Abstract. The atrochid rotifer, Acyclus inquietus, is a sedentary predator that lives within the colonies of its prey, the rotifer Sinantherina socialis. After larvae infiltrate and become associated with the colony, they secrete a permanent gelatinous tube and undergo metamorphosis to the adult stage. We followed settlement and metamorphosis using bright-field microscopy to document specific larval behaviors after eclosion, and used epifluorescence and confocal microscopy of phalloidin-labeled specimens to visualize some of the morphological changes that occur during metamorphosis. Upon eclosion, larvae possess paired eyespots and a ciliated corona that functions strictly in locomotion. After leaving the parent’s gelatinous tube, larvae eventually settle on unoccupied colonies of S. socialis or on other substrates if colonies are unavailable. Settlement involves a period of gliding among colony members before attachment with the foot and the secretion of a gelatinous tube. After settlement, there is a drastic reconfiguration of the corona that involves loss of the eyespots, loss of the coronal cilia, and the formation of the cup-shaped infundibulum, a deep depression in the anterior of the head that leads to the mouth. The development of the infundibulum involves the expansion of tissues around the mouth and is accompanied by a reorientation of the underlying musculature that supplies the infundibulum and allows its use in prey capture. The arrangement of the muscles in the trunk and foot regions, which contain outer circular (complete and incomplete) and inner longitudinal bands, remains unchanged between ontogenetic stages, and reflects the condition characteristic of other rotifers.

Additional key words: Gnesiotrocha, metamorphosis, larva, freshwater, muscles

The movements of the animal are somewhat of a grotesque character and reminded the author of a zealous demagogue addressing a crowd, obsequiously bowing, and greedily accepting contributions.
Leidy 1883

Rotifera is a familiar group of micrometazoans that inhabit a wide range of aquatic environments, but are especially well known from the planktonic realm of limnetic systems. Planktonic rotifers rely on their corona—a ciliated region of the head—for both locomotion and feeding. The structure of the corona is highly variable within the Rotifera, but generally consists of two concentric rings of cilia that sit atop the head and give the appearance of a rotating wheel, hence their Latin name (rota, wheel, ferre, to bear). The variability in the coronal structure among species is due to organizational differences in the individual coronal rings, known as the trochus and cingulum, and in the presence of sensory structures (both ciliated and non-ciliated) in the apical region anterior to the rings. Even non-planktonic species have a ciliated corona, although its structure often differs considerably from that of planktonic species, presumably as an adaptation to benthic or interstitial locomotion and feeding (Wallace et al. 2006).

Rotifers from each of the three traditional taxonomic groups—Seiosonidea, Bdelloidea, and Monogononta—have a corona at some stage of their development, as a larva or as an adult. However, several species of monogononts in the Atrochidae and Collothecidae bear a highly modified corona that does not resemble the corona of other species, at least among the female forms (Wallace et al. 2006). In general, adults of these sessile rotifers lack the characteristic ciliary rings, and instead possess a naked, bowl-shaped corona. This bowl-shaped corona, called the infundibulum, is highly variable.
in structure, most often taking the form of a wide, depressed mouth surrounded by a flap-like hood (e.g., some species of Collotheca and Cupelopagis) or as a wide, depressed mouth surrounded by tentacles (e.g., species of Stephanoceros and some species of Collotheca). In the few species that have been examined throughout their ontogeny, only the adult stage bears the infundibulum, while the larva possesses a typical head with coronal cilia (Leidy 1882, 1883; Wright 1959; Vasisht & Dawar 1969; Kutikova 1995). The differences in the head shape between these two life stages probably reflect their different functional roles during ontogeny: locomotion and settlement in the larva, and feeding in the adult. To date, the metamorphic events that occur during ontogeny to generate the infundibulum have not been well characterized, although Kutikova (1995) documents some aspects of metamorphosis in two species of Collothecidae.

In this investigation, we record some of the metamorphic events that take place during the ontogeny of the atrochid rotifer, Acyclus inquietus Leidy (1882). Larvae of this species are widely known to settle within colonies of their main rotifer prey, Sinantherina socialis Linnaeus (1758), and as adults, feed on newly hatched juveniles of that species (Leidy 1882; Edmonson 1940, 1944; May 1989). While Leidy (1882) provided an extensive description of the adult, there are few details on the morphology or the behavior of the larva, as is the case with many rotifer larvae (see Wallace 1980). Here, we use bright-field microscopy to follow the behavior and settlement of larvae and document changes in their morphology. We also use epifluorescence and confocal laser scanning microscopy (CLSM) of phalloidin-labeled specimens to describe the changes in musculature that accompany the metamorphic transformation in members of this species.

Methods

Specimens of Acyclus inquietus were collected from colonies of Sinantherina socialis on submerged vegetation at the edges of Mascuppic pond in Tyngsboro, MA (42°40.703′N, 71°24.032′W). Living colonies of S. socialis were placed in small bowls of unfiltered pond water and observed for the presence of A. inquietus using a Zeiss stereomicroscope (×5–10) (Carl Zeiss Microimaging, Göttingen, Germany). When adult specimens with parthenogenetic eggs were identified, the adults and eggs were gently removed with “000” insect pins and placed in finger bowls (∼2 mL) of filtered pond water. Individual eggs were then separated from the adult with insect pins and placed in their own fingerbowls of filtered pond water. In some cases, small pieces of submerged, defaunated grass were also placed in the finger bowls to serve as a potential substratum for settlement. All bowls were kept at room temperature. Eggs (>20) were observed over a 24-h period and swimming larvae were removed with a micropipette. Larvae were placed in filtered pond water on glass microscope slides and observed using a Zeiss Axioskop equipped with a Nikon Coolpix 8400 digital camera (Melville, NY, USA). A digital video of live larvae was captured for several specimens. Larvae were returned to separate finger bowls until metamorphosis, at which time they were again photographed.

Individual larvae were also added to small colonies (<50 individuals) of S. socialis in bowls of filtered pond water and observed using a Zeiss stereo microscope equipped with a Nikon Coolpix 8400 digital camera. Larval settlement and movement around colonies was captured via digital video. Colonies of S. socialis were observed for 24–48 h, at which time the juveniles of A. inquietus were removed with insect pins and mounted on glass slides for photography.

Several larvae, juveniles, and adult rotifers were relaxed in either 1% MgCl₂ or 0.5% bupivacaine for 15–30 min on ice before fixation in 5% paraformaldehyde in 0.1 mol L⁻¹ Sorensen’s phosphate buffer (PB) for 24 h at 4°C. Individual specimens were then rinsed in 0.1 mol L⁻¹ PB for 30 min and placed in 0.1 mol L⁻¹ PB with 0.5% Triton X-100 (PBT) for 24 h. All rotifers were stained with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR, USA) for 1 h before mounting in Fluoromount G (Electron Microscopy Sciences, Hatfield, PA, USA) on glass slides. Mounts were allowed to harden at 0°C for ≥48 h before examination. These rotifers were examined using two microscopes: (1) a Zeiss Axioimager equipped with epifluorescence, a digital AxioCam, and AxioVision software; and (2) an Olympus FV300 confocal laser scanning microscope (Olympus, Center Valley, PA, USA). For CLSM, Olympus software was used to collect a series of optical sections with a step size of 0.10–0.30 µm. Digital files from CLSM were imported into Volocity (Perkin Elmer, Waltham, MA, USA) to render 3-D images and generate X–Y–Z rotations (TIF, AVI files). No manipulations of any of the original digital images were made other than changes of color (gray-scale, background coloring) or cropping.

Results

Adult behavior

Specimens of Acyclus inquietus were present as larvae, juveniles, and mature adults with eggs in
colonies of *Sinantherina socialis*. Adults reached lengths of 1400–1600 μm when fully extended, with a broad trunk and a tapering foot embedded in a gel-like secretion (see Leidy [1882] for a description of adult morphology). Adult specimens carried ≤20 parthenogenic eggs embedded in the secretion around their foot. No more than two adults were ever present in a single colony, and most colonies (>50) contained one adult or none. Juveniles and mature adults were sessile within their respective colonies but regularly engaged in bending and other contractile motions. These movements were generally in the form of slow, ventral, and dorsal flexions of the longitudinal body axis. A provoked specimen could contract the entire trunk and foot in <1 s, resulting in a completely hidden adult. Individuals were observed to regularly flex their infundibulum when in contact with eggs and adult specimens of *S. socialis*. This flexion involved an apparent contraction of the entire infundibulum, bringing the dorsal, hood-like portion of the infundibulum into contact with the ventral mouth margin.

**Larval morphology and behavior**

A brief description of larval morphology can be found in Leidy (1882:247). In the current study, larvae hatched from eggs that were embedded in a gel-like secretion at the base of the adult’s foot (Fig. 1A). Embryos were contained within a thin, sculptureless (using bright-field optics) shell (Fig. 1B). Embryos in different stages of development were present in all egg masses: some embryos appeared to be undergoing early cleavages, while others were in an early post-gastrula stage, or were nearly fully developed larvae (Fig. 1C). Shelled larvae were always in a curled position, and the anterior end was visible by the presence of two red eyespots and coronal cilia (Fig. 1C). While most eggs hatched while unmonitored, several also hatched during periods of observation. Larval activity within the eggshell increased for several minutes before eclosion and release of the larva. Larvae wriggled away from any attached eggshell material before making their way out of the adult’s gelatinous tube (Fig. 1D). Escape from the adult’s tube often took several minutes.

Fully extended larvae reached a length of 360 μm, and had a broad trunk and a tapering foot (Fig. 1E). At the anterior end were two parallel rings of cilia that encircled the corona; individual cilia were ≤19 μm long (Figs. 1E, 2A,B). A depression (possibly a mouth) was observed in the naked, apical region of the corona (Fig. 2A). Two red eyespots were present just posterior to the rings of cilia (Figs. 1C,E, 2A). A mastax with hardparts (trophi) was not evident in any specimens, but a cellular stomach with a birefringent body (an anisotropic crystalline structure, *sensu* Wallace 1993) was present in all the specimens (Figs. 1E, 2C). The foot region was delineated from the trunk by the presence of a fold in the body wall ~70% of the body length from the anterior end; this fold gave the foot a segmented appearance (Figs. 1E, 2D). A tuft of cilia was present in a cavity at the posterior end of the foot (Fig. 2D). These posterior cilia did not appear to contribute to any locomotion in the larvae, which swam through the water using their coronal cilia. Most larvae swam in a helical manner, exploring submerged vegetation by gliding over the substratum and appearing to touch it with their corona. Larvae would intermittently contract their corona inside their trunk while exploring a surface.

When in the presence of adult colonies of *S. socialis*, larvae often glide in between or above colony members before settlement. The exact movements of larvae during settlement were difficult to document because of the constant movement of colony members. In general, most larvae appeared to settle in a region between colony members and creep around the members before permanent attachment. It was undetermined whether the larvae relied on their coronal cilia for this creeping behavior, but all larvae did bend and twist their bodies during this activity. The larvae were often lost from view for several minutes or even hours before reemergence as juveniles with an adult morphology.

**Metamorphosis**

To gain more insight into the metamorphosis from a larva to an adult, we examined larvae in the absence of colonies of *S. socialis*. Larvae would actively swim in Petri dishes for several hours before settlement on the bottom of the dish, on a glass slide in the dish, or on plant material (grass) in the dish. Larvae would creep along to their eventual site of settlement and then contract into a sphere. After an undetermined period of time, the body became mostly immobile, but coronal cilia continued to beat. Eventually, the head began to contract into the trunk and would sometimes remain hidden from view for several minutes or longer. Individuals eventually reemerged with the adult morphology (Fig. 3A,B).

Juveniles were similar in morphology to adults, except that they possessed a thinner, more translucent, and somewhat segmented trunk (Fig. 3A,B) and a more pronounced infundibulum (Fig. 3C). The red eyespots characteristic of the larva were either totally...
absent from newly metamorphosed individuals or were displaced posteriorly on the head, with one eyespot lower than the other (not shown). In some specimens, the red pigment appeared to have broken up and was scattered across several cells of the head. The infundibulum became a pentagon-shaped depression that opened to the mouth and had a prominent dorsal hood (Fig. 3). The hood was much more pronounced in juveniles compared with adults (compare Fig. 3A,E), where it often appeared more rounded, wrinkled, and smaller relative to the size of the head. The hood was highly contractile and could be retracted without closing the infundibulum.

Muscular system

Muscles were labeled in embryos, larvae, juveniles, and adult specimens. In all stages, only circular and longitudinal muscles were present (Figs. 4–6). Embryos showed selective labeling based on their stage of embryogenesis; early cleaving embryos and gastrulas showed little labeling, as did some later stage embryos with eyespots and coronal cilia. Still, some late-stage embryos did become labeled, revealing ≥ 10 somatic circular muscles surrounding a series of thick and thin longitudinal muscles (not shown). Few larval and adult specimens were fixed in a relaxed position, complicating a comprehensive view of both the number of muscles in each life stage and their origins and insertions. In free-swimming larvae, ≥ 12–13 somatic circular muscles were present from the trunk to the head (Fig. 4A). In the trunk region, all circular muscles were incomplete on the ventral side. The four most anterior circular muscles in the neck and head region appeared to be complete. Circular muscles surrounded at least four pairs of thick longitudinal muscles that extended the length of the body. The longitudinal muscles were present in ventral (two muscles), lateral (four), and dorsal (two) positions (Figs. 4A, 5A). Members of most muscle
pairs remained adjacent to each other in the foot region before separating and inserting individually into the head (Fig. 5A). The insertion points of the muscles in the head could not be determined with accuracy, but appeared to be similar to the position of the muscles in the trunk region, i.e., ventral, lateral, or dorsal. Muscle insertions were on the epidermis and not other muscles. Several extremely thin (1–2 μm) muscles were also present in the body between the major longitudinal bands. These muscles appeared to originate in the foot and extend anteriorly to form a loose meshwork around the developing digestive tract. No mastax musculature was observed (see Fig. 5A).

During metamorphosis, rotifers were in a contracted state, preventing us from making clear observations of their muscular organization. This also prevented us from determining whether the infundibulum is generated internally, i.e., as a “new” head within the larval corona as occurs in species of Stephanoceros (Kutikova 1995) or as a modification (outgrowth) of the existing larval corona. The only obvious difference between the muscle organization of larvae and both juveniles and adults was in the corona. Expansion of the mouth and growth of the dorsal hood during metamorphosis was accompanied by repositioning of already existing muscles; no new somatic muscles appeared to be generated (Fig. 4B). Circular muscles that were once present in the upper trunk region of the larva now contributed to the structure of the infundibulum (Figs. 5, 6B,C). Three of the most anterior circular muscles were not oriented perpendicular to the long axis of the body like other circular muscles of the trunk; instead, the dorsal arch of each muscle was positioned anterior to the ventral arch, thereby contributing to the dorsal hood of the infundibulum (Figs. 4C, 5B, 6C). The longitudinal muscles that once inserted radially around the larval head in dorsal, lateral, and ventral positions retained their relative arrangements, but now inserted beyond the mouth margin and into the extensions of the body wall (e.g., dorsal hood).
that comprised the infundibulum. In addition, several very thin muscle fibers, presumably associated with the mastax, were also present. The mastax was now heavily invested with its own musculature (Fig. 5B).

**Discussion**

*Acyclus inquietus* was first described from specimens collected within a colony of *Sinantherina socialis* in Pennsylvania, USA (Leidy 1882). Since then, this sessile predator has been found in several localities across three biogeographical regions (Segers 2007). Unique to this species is its close association with *S. socialis*, which it feeds upon (Edmonson 1940, 1944; May 1989). In his original description, Leidy (1882) noted the rotifer as “being destitute of wheels, or ciliated disks” and as having an “apparently restless habit.” Leidy (1882) went on to describe the structure of the larva and observed that, in contrast to the adult, it possessed “a soft worm-like body, with a blunt head end and tapering behind to a rounded tail end in the dorsal view.” Since this description, there have been no detailed studies of the anatomy or the ontogeny of *A. inquietus*, although other studies have noted similar ontogenetic changes in other rotifers that lack a conventional corona [e.g., *Collotheca campanulata longicaudata* (Hudson, 1883); Wright 1959; *Stephanoceros fimbriatus* (Goldfuß, 1820); Kutikova 1995]. In these rotifers and other species, the larva is a mostly non-descript, verminiform animal with little similarity to the adult; this is why the term “larva” has often been used to describe this ontogenetic stage (Wallace 1980; Kutikova 1995). Here, we have presented additional observations on *A. inquietus* and some aspects of the metamorphic process that leads to the drastic change in coronal morphology from a larva to an adult.

Metamorphosis generally occurs after the larva has located a suitable substratum, preferably a colony of *S. socialis*. In the absence of *S. socialis*, larvae are able to metamorphose on other substrata, as indicated by their settlement and metamorphosis in glass fingerbowls this study. Preferences for settlement on aquatic vegetation, which are known for other species of rotifers (e.g., Wallace 1978; Pejlier &
Berzins 1993), were not examined in this study. The major anatomical changes that occur during metamorphosis are as follows: (1) secretion of the gelatinous tube around the base of the foot; (2) elongation of the foot and trunk; (3) change in the position of the somatic circular muscles along the antero-posterior axis; (4) differentiation of the tissues that lead to the formation of the digestive tract and mastax; (5) loss of all coronal cilia; (6) loss or depigmentation of the eyespots; (7) widening of the oral cavity; and (8) development of the body wall around the mouth to form a pentagonal infundibulum. The anisotropic crystal that may serve as a source of energy for non-feeding larvae (Wallace 1993) remains in place after metamorphosis and for at least several hours into the adult stage (see also Beuchamp 1912; Wallace 1993). All of these changes take place while the larva is in a contracted condition and embedded in the colony of its rotifer prey, *S. socialis*. While the exact time course for these events was not established in this study, we did determine that such changes occur over the course of several hours. Such a timeline appears to be typical for rotifer metamorphosis. Kutikova (1995) measured the time for larval metamorphosis in

Fig. 4. Wide-field fluorescence images of phalloidin-labeled specimens of *Acyclus inquietus*. Anterior is to the left; ventral is up. A. Larva. Scale bar = 25 µm. B. Metamorphosing larva. Note the relative size of the infundibulum compared with (A). Scale bar = 40 µm. C. Reproductive adult. The focal view does not show the mastax musculature. Scale bar = 100 µm. Cm, complete circular muscles of the corona/infundibulum; Dh, dorsal hood of the infundibulum; Dl, dorsal longitudinal muscle; Ic, incomplete circular muscles of the trunk; If, infundibulum; Ii, lateral longitudinal muscles; Sm, splanchnic musculature; Vl, ventral longitudinal muscle.
S. fimbriatus at ~3 h, although the precise metamorphic events were not recorded because the rotifer remains contracted in its gelatinous tube. Upon reemergence, the juvenile is still undergoing changes, as the five arms of the infundibulum continue to develop. Similarly, in A. inquietus, the infundibulum continues to expand radially and the dorsal hood of the infundibulum becomes less apparent over time. Kutikova (1995) further states that the infundibulum of S. fimbriatus and another sessile species, Collotheca ornata (EHRENBERG, 1832), actually develops inside the larval body as opposed to originating as an outgrowth of the larval corona. Our unpublished observations confirm this for S. fimbriatus, but we were unable to determine whether a similar process occurs in A. inquietus. Precisely how the infundibulum develops internally within the trunk is unknown, as is the process of its eversion. Major anatomical changes must be taking place, including apoptosis, mitosis, redifferentiation, and dedifferentiation of larval tissues, including larval sensory organs and the nervous system. Future studies with the use of an effective anesthetic to study larval metamorphosis should be able to determine whether the infundibulum develops internally in A. inquietus, and if so, what type of anatomical changes are involved in the process.

In contrast to the changes in head shape, our observations of the somatic muscular system indicate that major anatomical changes do not take place during metamorphosis. The relative number and position of muscles in larvae is similar to that present in adults, and during metamorphosis, there is only a slight change in the position of both circular and longitudinal muscles as the infundibulum develops. Specifically, the infundibulum develops around the terminal mouth as the tissues expand radially. Allometric growth of the tissues around the mouth leads to greater development of the dorsal body wall relative to the ventral and lateral body walls, thus forming the prominent dorsal hood of the infundibulum, described by Leidy (1882:247) as an “incurved digitiform appendage.” The hood is supplied with at least two to three somatic circular muscles that arch anteriorly. These are positioned outside of the paired longitudinal muscles that insert close to the tip of the hood. Based on their position, both the circular and the dorsal longitudinal muscles appear to function in contraction of the infundibulum, a movement that is essential for prey capture. When adults feed, they continually expand and contract their infundibulum as they move within colonies of their prey, S. socialis. Adults appear to capture small rotifers by contracting the anteriormost circular muscles, thereby closing the infundibulum around the prey and pushing the prey toward the mastax through further circular muscle contraction. The longitudinal muscles do not appear to directly play into the movement of the prey toward the mouth, but lead to constant bending of the adult, which may indirectly force the prey toward the mastax.

Of some significance in this study is the apparent lack of mastax musculature in the larvae of A. inquietus (see Fig. 5). In fact, we did not observe any trophi (mastax hardparts) in our larvae, but this could be attributable to the restless nature of the larvae and the opacity of their tissues; trophi were observed in the adults (not shown). A previous study on other species of rotifer larvae indicated the presence of trophi, and hence the mastax (see Kutikova 1995), but did not examine mastax musculature. The absence of...
any observable mastax musculature in the larvae of *A. inquietus* in this study may indicate that the mastax musculature develops after the trophi and probably during metamorphosis of the larval corona.

No other differences were observed in the position and/or the number of somatic muscles in the trunk and foot of the larva and adult. In fact, the muscular organization of these two regions in *A. inquietus* is similar to that of more mobile (planktonic and benthic) species: the trunk contains a set of ventrally incomplete circular muscles that surround several longitudinal bands, which originate from a common position at the base of the foot. A similar condition is known for species of Bdelloidea and Monogononta alike, including *Philodina* sp. (Bdelloidea: Hochberg & Litvaitis 2000), *Adineta ricciae Segers & Shiel, 2003*, *Macrotrachela qudricornifera Milne, 1886* (Bdelloidea: Leasi & Ricci 2010), *Floscularia ringens* (Linnaeus, 1758) (Monogononta: Santo et al. 2005), *Proales daphnicola Thompson, 1892*, and *Proales fallaciosa Wulfert, 1937* (Monogononta: Sørensen 2005), among others (see Hyman 1951 for a review). As noted by Sørensen (2005) and reiterated by Leasi & Ricci (2010), the pattern of outer, incomplete circular muscles surrounding a series of inner longitudinal fibers is diagnostic of rotifers, with extreme variations in muscle position and location dictated by body morphology and habitat (planktonic vs. benthic), as best represented by the six-armed planktonic species *Hexarthra mira* (Hudson, 1871) (Santo et al. 2005; Hochberg & Gurbuz 2008) and the paddle-bearing planktonic species *Polyarthra major Burckhardt, 1900* (Hochberg & Gurbuz 2008).

The general patterns of somatic musculature in rotifers, while useful in studies of functional morphology, may also be significant in understanding the evolution of unusual coronal types. For example, most species of Atrochidae and Collothecidae possess a pentagonal infundibulum. In many species from two genera, such as *Collotheca* and *Stephanoceros*, the tips of the pentagon have formed body wall extensions that surround the mouth. In *Collotheca*
gracillipes Edmonson 1940, only the dorsal tip of the pentagon is well formed, while in Collotheca coronetta (Cubitt, 1869) and S. fimbriatus, these extensions form prominent, ciliated arms or tentacles that function in prey capture. Other species, such as Acycus trilobus (Lucks, 1911), possess a triangular infundibulum. Unfortunately, a paucity of knowledge on their development, internal anatomy, and evolutionary relationships makes it difficult to evaluate the homologies of these unusual coronal shapes. A detailed comparative study of the musculature that supplies the infundibulum in these species (and species of Cupelopagis with a cup-shaped infundibulum [see Vasisht & Dawar 1969]) would lead to a better understanding of their evolutionary and ecological significance.

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