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Remodeling of the nervous system of the indirectly developing rotifer *Cupelopagis vorax* (Gnesiotrocha, Collothecaceae)

Elizabeth Preza[1] | Elizabeth J. Walsh[1] | Rick Hochberg[2]

[1]Department of Biological Sciences, University of Texas, El Paso, TX, USA

[2]Department of Biological Sciences, University of Massachusetts Lowell, Lowell, MA, USA

Correspondence
Elizabeth J. Walsh, Department of Biological Sciences, 500 W. University Ave., University of Texas, El Paso, TX 79968, USA.
Email: ewalsh@utep.edu

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Abstract

Cupelopagis vorax is a sessile, predatory rotifer with indirect development. The topology of its nervous system is partly known through histological examination of the female adult. However, there is no information on the larval stage, and so, no understanding of how metamorphosis might affect the configuration of neurites. Here, we use immunohistochemistry and confocal laser scanning microscopy to map the position of serotonin-like immunoreactive (SLIR) neurites, which are hypothesized to be important in sensory innervation and stimulating locomotory activity. We found that the relative position and number of SLIR neurites were similar between larvae and adults despite differences in their ecologies and the drastic changes that occur at metamorphosis. Both life stages possess at least four pairs of perikarya in the cerebral ganglion, a pair of lateral nerve cords, and a pair of neurites that appear to innervate a portion of the digestive tract. The larval stage also possesses an SLIR neurite ring at the base of the corona that is postulated to function in stimulating ciliary activity and receiving sensory information from the apical field. Although the adult did not appear to possess this ring, we cannot rule out its presence, because immunoreactive signals in the anterior end were weak. In contrast to the larvae, the adult possessed a pair of SLIR neurites that appeared to innervate the neck region. We hypothesize that these neurites form a circuit that functions in prey detection and capture. Based on these results, it appears that despite their overall similarities, the two life stages show some unique neural patterns that correspond to their ecologies; neurites that function in the planktonic environment of larvae and neurites that likely function in prey detection in the sessile adults.

KEYWORDS
larval nervous system, metamorphosis, Rotifera, serotonin-like nervous system

1 | INTRODUCTION

Phylum Rotifera originally consisted of three clades of microscopic aquatic invertebrates, Seisonidea, Bdelloidea, and Monogononta, which comprise over 2,000 marine, freshwater, and semi-terrestrial species (Segers, 2007); more recently, the parasitic acanthocephalans have been subsumed within the phylum (Herlyn et al., 2003; Sielaff et al., 2015). The traditionally recognized rotifers are characterized by a ciliated corona, a syncytial body wall, and a mastax, which is a specialized pharyngeal organ with sclerotized jaws called trophi. Most species are planktonic, benthic, or epiphytic and use their ciliated corona as the primary means of locomotion and food collection. As ciliary feeders, many rotifers consume suspended microalgae, bacteria, or detritus, whereas others are occasional or

obligate predators, and a few are parasitic (Wallace et al., 2006). Food is processed by the mastax, where the trophi masticate it before being moved through the esophagus and into the stomach (Starkweather, 1996; Wallace et al., 2015). Variations in feeding behavior and digestive organization are present in specialized taxa (e.g., parasitic rotifers: May, 1989; *Cupelopagis vorax* (LEIDY 1857): Bevington et al., 1995; Hochberg et al., 2017).

Among the three aquatic clades, the class Monogononta is the most speciose and contains the widest variety of body forms and lifestyles, including a variety of species that are obligately sessile as adults. Sessile rotifers are present in three families of superorder Gnesiotrocha: Flosculariidae, Collothecidae, and Atrochidae. Sessile females are permanently attached to submerged plants and reproduce via cyclical parthenogenesis (Wallace & Edmondson, 1986). The asexual phase is dominant in their life cycle. It leads to the production of amictic (asexual) embryos that develop into nonfeeding, free-swimming, female larvae (Fontaneto et al., 2003). Historically, the larval stage was not considered a true larva because many adult organs are already present (Wallace, 1980; Wallace et al., 2015). However, observations of several species of Collothecidae and Atrochidae, together forming the order Collothecaceae, have shown that this life stage goes through a dramatic metamorphosis. This transformation leads to a complete replacement of the larval head with a new adult head, called the infundibulum (Hochberg & Hochberg, 2015, 2017; Hochberg et al., 2017, 2019; Kutikova, 1995). Larvae do not feed but appear to survive on limited maternal reserves (Wallace, 1993; Young et al., 2019) and must, therefore, find a suitable substrate before expending their energy (Wallace, 1980). Because attachment is permanent, substrate selection is a critical factor in feeding, the survival of adults, and reproductive success (Butler, 1983; Fontaneto et al., 2003).

A sessile lifestyle is hypothesized to entail lower metabolic costs and predation risks; yet, a significant trade-off is lower feeding efficiency (Kiørboe, 2011). An evolutionary adaptation to counteract lower feeding efficiency in the sessile flosculariid rotifers may be the larger, more ornate corona compared to their planktonic and benthic counterparts (reviewed in Wallace et al., 2006). By contrast, in collothecid rotifers the head has taken on an entirely unique shape (Hochberg et al., 2019). In these species, the larval head is replaced by the adult infundibulum at metamorphosis. This new head lacks locomotory (or current-generating) cilia, but instead, it has a funnel or bowl shape that functions to trap prey. Some collothecids, such as *Stephanoceros fimbriatus* (GOLDFUSS 1820), have long tentacles and setae (modified cilia) on their infundibulum that trap suspended organisms (e.g., phytoplankton, protists). By contrast, species of Atrochidae, such as *C. vorax*, have a large, bowl-shaped mouth that allows them to envelop live benthic prey, including gastrotrichs and other rotifers (Wallace et al., 2015). Both of these species are generally considered ambush predators (Wallace et al., 2015).

To date, little is known about how rotifer larvae select their substrata for settlement (Edmondson, 1945; Wallace, 1978, 1980), even though adults often show distinct patterns of distribution on submerged vegetation (Edmondson, 1944; Wallace, 1980; Wallace & Edmondson, 1986). In the case of sessile ambush predators, it is imperative to have a good understanding of both life stages to appreciate the factors that determine substrate selection, which likely govern prey availability and ultimately successful adult reproduction. One method to help understand this process is to study the nervous system.

According to Vasisht and Dawar (1969), the nervous system of adult females of *C. vorax* consists of a cerebral ganglion, three pairs of fine nerves (i.e., neurites) that innervate a dorsal sense organ, and eight pairs of lateral nerves. The largest of the lateral nerves form the paired nerve cords that extend posteriorly, and the remaining nerves appear to innervate various regions of the body, including the viscera and sensory organs of the trunk. Specifically, the fifth lateral nerve innervates two sensory organs: the lateral and coronal antennae. Lateral antennae are located at the junction of the infundibulum and trunk, and coronal antennae are present within the infundibulum. Mechanoreceptive cilia within these sensory organs are proposed to aid individuals of *C. vorax* in detecting the position of potential prey (Bevington et al., 1995). Whether these sensory organs are also present in larvae remains unknown. This lack of data also means that sensory devices likely to play a significant role in larval substrate selection also remain unknown.

To date, serotonin-like immunoreactive (SLIR) neurites are known from both the central nervous system (cerebral ganglion, nerve cords) and peripheral nervous system (e.g., neurites that innervate morphologically identifiable sensory organs, coronal region, mastax) of several rotifer species (Gąsiorowski et al., 2019; Hochberg, 2006, 2007, 2009; Hochberg & Hochberg, 2015; Hochberg & Lilley, 2010; Kotikova et al., 2005; Leasi et al., 2009). These taxa include a wide variety of planktonic and sessile forms. In these species, the SLIR neurites are hypothesized to modulate both ciliary activity and feeding behavior, and so, in fact, might have cilioexcitatory and sensory functions. Here, we study the nervous system of the rotifer *C. vorax* to determine whether there are differences in the distribution of SLIR neurites between the nonfeeding planktonic larva, and the sessile adult female. We hypothesize that differences in the SLIR nervous system of larval and adult stages may potentially reveal neurites associated strictly with mobility in larvae or feeding behavior in adults. *Cupelopagis vorax* also provides a unique model among rotifers because it is the only species known to respond to vibrations produced by potential prey (Bevington et al., 1995).

2 | METHODS

Specimens of *C. vorax* were collected from Minto Pond, Marion Co., OR (44.9204 N, 123.0613 W), in May 2014, and Moon (Birch) Lake, Marquette Co., WI (43.8026 N, 89.3698 W), in September 2015. Adults were cultured under laboratory conditions in modified Marine Biological Laboratory (MBL) medium (Stemberger, 1981)

and fed once a week with a mixture of *Chlamydomonas reinhardtii* DANGEARD 1899 (Culture Collection of Algae at The University of Texas at Austin [UTEX] #90), *Chlorella vulgaris* BEYERINCK [BEIJERINCK] 1890 (UTEX #26), *Rhodomonas minuta* SKUJA 1948, and metazoans *Lepadella triba* MYERS 1934 (in culture, Walsh laboratory) and *Lepidodermella squamata* (DUJARDIN 1841) (Carolina Biological Supply Co.). Larvae were isolated by allowing them to settle on coverslips placed in the culture dishes, or while swimming.

Fluorescent staining was performed to visualize serotonin-like immunoreactivity (SLIR) and complementary structures (i.e., nuclei) using methods modified from Hochberg (2009). Antibodies were diluted to their desired concentrations using 0.5% PBT, a solution of 0.1M phosphate-buffered saline (PBS; Bio-Rad Laboratories), and Triton X-100 (Bio-Rad Laboratories). All steps were conducted in 1.5-ml microcentrifuge tubes on an orbital shaker at 4°C. Prior to staining, whole animals were relaxed in 0.5% bupivacaine, fixed in 4% paraformaldehyde for 2 hr, and rinsed in 0.1M PBS for 1 hr. Animals were placed in a blocking solution consisting of 1% bovine serum albumin (Sigma–Aldrich) and 0.1M PBS overnight. Animals were rinsed in 0.1M PBS for 1 hr and transferred into polyclonal primary antibody solution (Sigma-Aldrich #S5545, rabbit anti-5HT whole antiserum, 1:2000). The antibody is designed for immunohistochemistry and labels serotonin in formalin-fixed sections and whole-mount invertebrate tissues (Haynes et al., 2015). As rotifers have been demonstrated to possess serotonin through the use of dot blot immunoassays and HPLC (Gallardo et al., 2000), we inferred that the anti-5HT antibody would successfully label serotonin in *C. vorax*. This same antibody has been used to demonstrate serotonin in a wide variety of other invertebrates (Bekkouche & Worsaae, 2016; Gąsiorowski et al., 2017; Martín-Durán et al., 2016) including rotifers (Gąsiorowski et al., 2019). Negative controls were employed by omitting the primary antibody, which is a way to control for nonspecific binding of the secondary antibody (Hewitt et al., 2014). Subsequently, all animals were rinsed in 0.5% PBT for 24 hr and incubated in polyclonal secondary antibody solution (Invitrogen #A-11010, goat anti-rabbit IgG, 1:200, Alexa 546 nm). Following removal from the secondary solution, animals were rinsed in 0.5% PBT for 24 hr and incubated in DAPI (Invitrogen #D1306). Microcentrifuge tubes were wrapped with foil during secondary and fluorophore incubations to preserve fluorescence. Specimens were mounted in Fluoromount-G (Thermo Fisher Scientific) on glass slides and stored at 4°C before imaging.

Fluorescent labeling was observed using a Zeiss LSM 700 confocal laser-scanning microscope. Confocal z-stacks were generated at 0.1-μm intervals and processed as TIFF images using ZEN 2.3 software. Three-dimensional images and QTVR videos were produced using VOLOCITY 6.3 software. SLIR was labeled according to structure and topology rather than function (Richter et al., 2010). Labels were consistent with Hochberg and Hochberg (2015). Measurements of SLIR cell bodies (perikarya; mean ± *SD*) were performed on confocal images imported into ZEN 2.3 software.

3 | RESULTS

Serotonin-like immunoreactivity (SLIR) was successfully visualized in the nervous system of larvae ($n = 8$) and adults ($n = 8$) of *C. vorax* (Figures 1 and 2). Although this study focuses on the results of SLIR, DAPI staining was also employed to determine whether specific SLIR regions (potential perikarya) were actual cells, based on nuclear staining, and not the result of artifacts. There was no

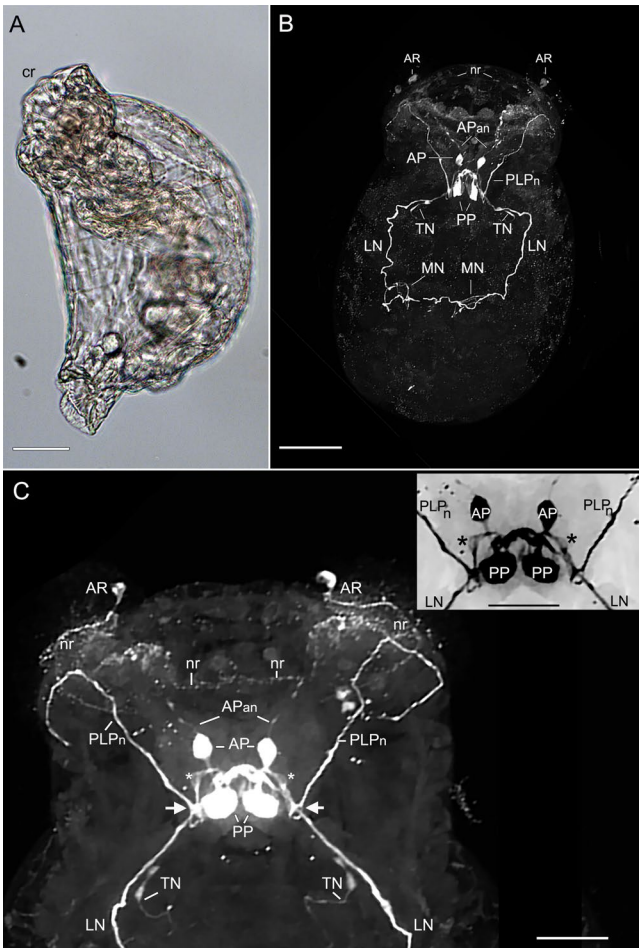

FIGURE 1 The larva of *Cupelopagis vorax*. **A.** Lateral view of larva with corona (top) contracted. B,C. Confocal images of the larva showing serotonin-like immunoreactivity (SLIR). **B.** Neurites and perikarya of the larva, anterior is up. **C.** Close-up of SLIR in the anterior end of a larva, dorsal view. Inset: Inverted gray-scale image of the perikarya in the cerebral ganglion to more clearly show the potential anterolateral perikarya. Scale bars: A = 30 μm; B,C = 34 μm (includes inset). *, position of potential anterolateral perikarya (ALP, see text); short arrows, regions of posterolateral perikarya of the cerebral ganglion (PLP); AP, anterior perikarya of the cerebral ganglion; AP_{an}, anterior-directed neurites of the anterior perikarya; AR, potential anterior receptors of the ventral margin of the corona; cr, corona; LN, lateral nerve cords; MN, medial neurites that extend from the lateral nerve cords in the trunk region; nr, neurite ring; PLP_n, posterolateral neurites of the posterolateral perikarya (PLP; data not shown); PP, large posterior perikarya of the cerebral ganglion; TN, trunk neurites that appear to extend from the lateral nerve cords

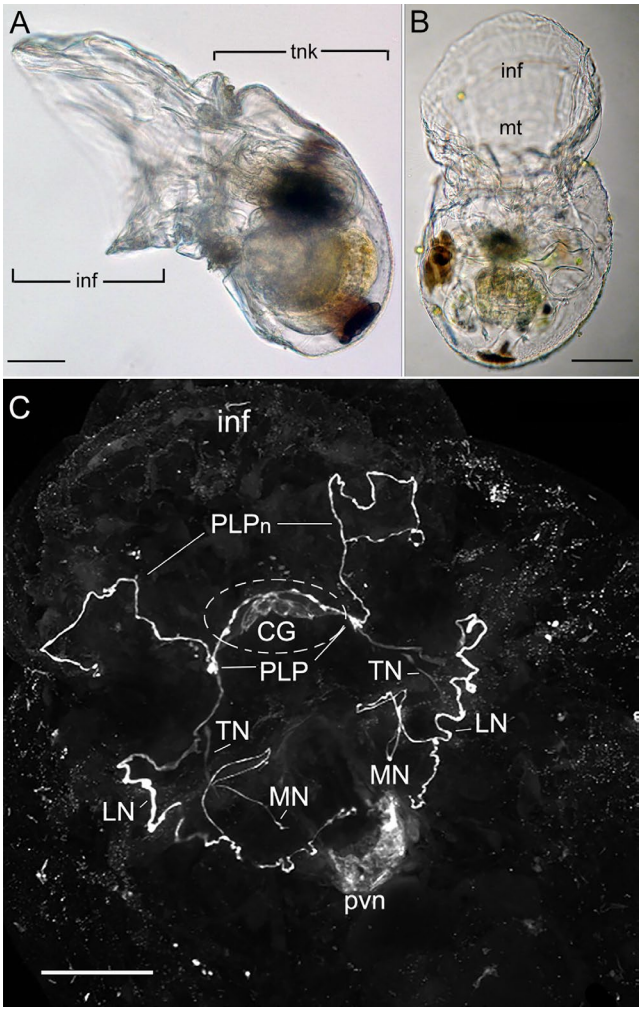

FIGURE 2 Photomicrographs of adult females of *Cupelopagis vorax*. **A.** Lateral view of live specimen. **B.** Ventral view of live specimen. **C.** Serotonin-like immunoreactivity (SLIR) in neurites and perikarya in a contracted specimen; the infundibulum (inf) denotes the anterior end. Dashed circle indicates region of cerebral ganglion. Scale bars: A = 140 μm; B = 150 μm; C = 60 μm. CG, cerebral ganglion; inf, region of infundibulum; LN, lateral nerve cords; MN, medial neurites that extend from the lateral nerve cords in the trunk region; mt, mouth; PLP, posterolateral perikarya appear as swollen immunoreactive regions (see text); PLP_n, posterolateral neurites of the posterolateral perikarya; pvn, region of proventriculus (showing autofluorescent contents); TN, trunk neurites that appear to extend from the lateral nerve cords; tnk, trunk region

labeling of SLIR in the negative controls. We determined that SLIR was present in the cerebral ganglion, paired lateral nerve cords, and a small number of additional neurites in the anterior and posterior body regions. The cerebral ganglion was outlined by a large cluster of nuclei at the anterior end and verified by colocalization between serotonin-like immunoreactivity and DAPI staining. In both larvae and adults, two pairs of neurites innervated the region around the ciliated corona, and three pairs of neurites were present in the trunk region (Figure 3).

The cerebral ganglion of both life stages contained at least four bilateral pairs of SLIR perikarya in similar positions, although the state of contraction of the specimens and their orientation during visualization often made direct comparisons difficult. The most anterior pair of SLIR perikarya (AP) was bipolar, medium-sized (7.03 ± 1.47 μm long; 4.85 ± 1.06 μm wide) and positioned in the dorsal brain region (Figures 1 and 2). In larvae, one neurite extended anteriorly toward the corona and the other extended posteriorly to the neuropile (Figure 1c). The anterior pair of neurites (AP_{an}) projected frontally and appeared to innervate an SLIR neurite ring (nr) that encircled the coronae in larvae (Figure 1B,C). Ventrally, there were some light SLIR regions proximal to the neurite ring (data not shown), but these regions lacked DAPI staining and so appeared to correspond to the ciliary cushions of rotifer coronae (e.g., see Hochberg et al., 2019). Neither the anterior pair of neurites (AP_{an}) nor the ring were observed in adults, although some small patches of immunoreactivity (IR) were present around the infundibulum that may indicate their presence (Figure 2). A pair of small protrusions on the ventral side of the larval corona had weak IR and appeared to be innervated by the neurite ring (AR; Figure 1B,C). These protrusions were not consistently stained but may represent sensory receptors in larvae; they were not observed in adults. The posterior neurite (AP_{pn}) extended into the neuropile, but whether it innervated other regions (e.g., nerve cords) remains unknown.

The second pair of strong IR perikarya (PP) was dorsal in position, located posterior of the neuropile, and large (9.72 ± 2.20 μm long; 6.62 ± 1.35 μm wide). Each perikaryon appeared to be unipolar with an anterior neurite (AP_{an}) that projected frontally from each cell body toward the neuropile (Figures 1C, 3). The neurites appeared to either unite medially or were close enough to each other to appear to form a singular, arch-shaped structure. In either case, two neurites extended ventrally from the center of the arch and projected beneath each pair of perikarya toward a swollen region interpreted as a perikaryon (PLP) based on nuclear staining (data not shown). Each PLP was ~2 μm in diameter and positioned at the posterolateral border of each pair of perikarya (Figure 3). It was undetermined whether the neurites innervated these perikarya. Each PLP appeared to give rise to a single wavy neurite (PLP_n) that extended anteriorly into the head region and always curved posterior before terminating somewhere in the side of the head (neck region in larvae, near the base of the infundibulum in adults; Figures 1–3). A second pair of perikaryon-like swellings (ALP, ~2 μm long) was also present, though in most specimens, their immunoreactivity was weak and it was difficult to determine whether the DAPI staining was colocalized to these or cells just beneath them. The perikaryon-like swellings (ALP) appeared bipolar and positioned on the anterolateral border of the neuropile (asterisks, Figure 1C, inset). One neurite (ALP_{mn}) projected medially into the neuropile, but the posterior neurite (ALP_{pn}) could not be easily followed to termination.

In two adult specimens, there was a pair of weakly stained SLIR neurites on either side of the body projecting from beneath the

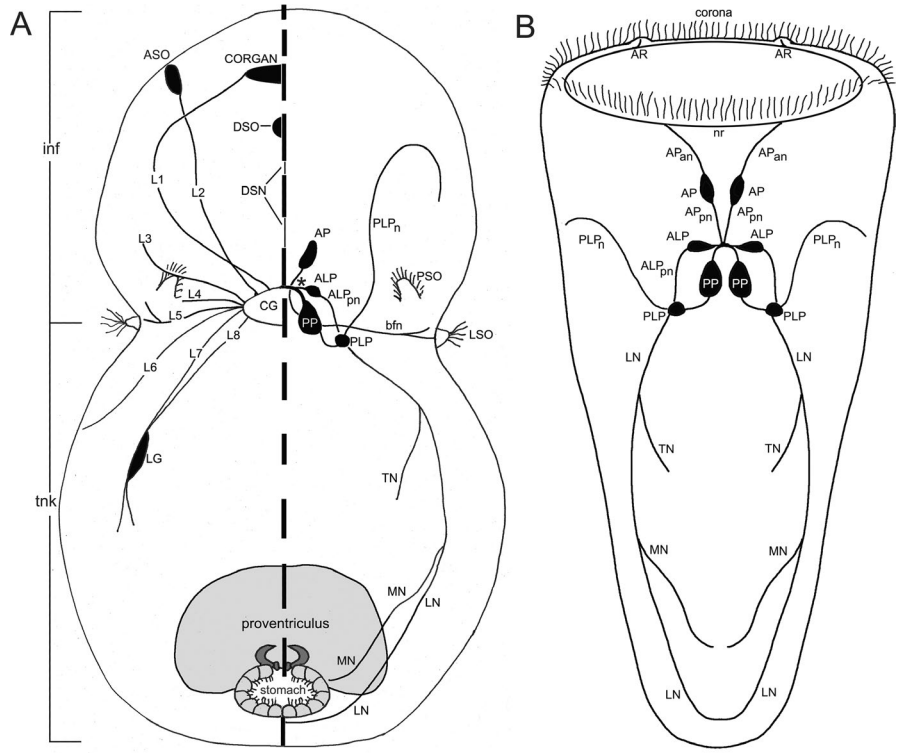

FIGURE 3 Schematic of the nervous system of adults and larvae of *Cupelopagis vorax*. Illustrations are not to scale. **A.** Topology of all neurites and sensory organs in an adult female as described by Vasisht and Dawar (1969) (left side) and position of serotonin-like immunoreactivity (SLIR) in neurites and perikarya of the current study (right side). **B.** Topology of SLIR neurites in the female larva; organ systems not shown because they are still developing. *, anterior SLIR neurites of the ALP; ALP, anterolateral SLIR perikarya; ALP_{pn}, posterior SLIR neurites of the ALP; AP, anterior SLIR perikarya; AP_{an}, anterior SLIR neurites of the AP; AP_{pn}, posterior SLIR neurites of the AP; AR, small SLIR neurite protrusions from the nerve ring. ASO, anterior sensory organ; bfn, bifurcating SLIR neurite with potential homology to lateral nerve #5 of Vashist and Dawar (1969); CG, cerebral ganglion; CORGAN, coronal sensory organ; DSN, dorsal sensory neurite; DSO, dorsal sensory organ; inf, adult infundibular region; L1–8, lateral nerves; LG, ganglion innervated by the seventh and eighth lateral nerves; LN, lateral SLIR nerve cords; LSO, lateral sensory organ; MN, medial SLIR neurites that extend from the lateral nerve cords in the trunk region; nr, SLIR neurite ring; PLP, posterolateral SLIR perikarya; PLP_n, posterolateral SLIR neurites of the PLP; PP, large posterior SLIR perikarya; PSO, pharyngeal sensory organ; TN, trunk SLIR neurites that appear to extend from the lateral nerve cords; tnk adult trunk region

perikarya. The neurites appeared to branch before innervating a lateral region around the neck (bfn, Figure 3). Their cell bodies were not identified.

Three pairs of neurites were always present in the trunk region of all specimens (Figures 1–3). One pair formed the lateral nerve cords (LN), which may either be an extension of the perikaryon-like swellings or an undetermined neurite in the neuropile. The lateral nerve cords appeared to anastomose close to the posterior end of the trunk; it was not determined whether they innervated the foot, because many larvae were contracted. A second trunk neurite (TN) paralleled the lateral nerve cords for approximately one-third of their length and was often undetectable until it branched from the lateral nerve cords (Figures 1C and 2C). This neurite always extended medially into the body around the digestive organs, but its specific site of innervation was never determined. The third pair of neurites (MN) branched off the lateral nerve cords approximately three-quarters of the way down the length of the trunk. In adult specimens, the neurites appeared to terminate around the proventriculus or perhaps the stomach; in larvae, their site of innervation could not be followed with accuracy.

4 | DISCUSSION

In many invertebrates, the activity and connectivity of the nervous system are reorganized during metamorphosis (Carvalho & Mirth, 2015; Kaul-Strehlow et al., 2015; Tissot & Stocker, 2000). However, in the Rotifera (sensu *lato*), only species of Acanthocephala and sessile species of Gnesiotrocha (Monogononta) undergo indirect development, and so, these rotifers provide the only examples of metamorphosis in the phylum. In sessile monogononts, the larval and adult stages are morphologically distinct: larvae are minute (≤200 μm), vermiform, and possess a ciliated corona, whereas adults are large (≤1,000 μm), often encased in a secreted tube, and may possess a large, ornate corona (Flosculariidae; e.g., Fontaneto et al., 2003) or an elaborate infundibulum that develops from the larval digestive tract (Collothecacea; Hochberg & Hochberg, 2017). Details of larval metamorphosis remain vague for most species (e.g., Hochberg & Hochberg, 2015, 2017; Kutikova, 1995, 2007) because larvae are small, highly contractile, and are usually produced in low numbers. Therefore, their morphologies are rarely documented with the

same detail as the adults, and so, changes that occur between life stages are generally unknown.

To date, there is little information on the lifecycle dynamics or larvae of *C. vorax* (Evans, 1981; Koste, 1978), but several details exist on the morphology of the adult female (Cori, 1925; Gast, 1900; Hochberg et al., 2017; Koste, 1978; Leidy, 1857; Montgomery, 1903; Vasisht & Dawar, 1969). In particular, the study by Vasisht and Dawar (1969) provided comprehensive information on the adult female's nervous system, ultimately concluding that despite the species' unique appearance, it was very similar to that of other rotifers. In the current study, our goals were to supplement this information by providing the first data on the larval nervous system, determine whether serotonin was present in specific subsets of nervous cells, and decide whether metamorphosis had a significant effect on the organization of the nervous system.

Our major finding is that despite the dramatic changes that take place during metamorphosis of the larvae of *C. vorax* (e.g., loss of corona, loss of pigmented eyespots, development of infundibulum, elongation of body, and maturation of organ systems), there are few major changes in the number or position of SLIR perikarya or neurites in the central or peripheral nervous systems. In general, the nervous systems of larval and adult females are similar: they possess a cerebral ganglion with four pairs of SLIR perikarya that innervate the head and trunk regions; and in the trunk, a pair of lateral nerve cords that innervate the posterior body region, likely the foot. Neurites also extend from the nerve cords to innervate portions of the digestive tract. While specific sites of innervation are extremely difficult to determine in both life stages, which limits our understanding of their specific roles, evidence suggests that serotonin is probably important in modulating the activity of different organ systems. For example, both larvae and adults possess SLIR neurites that appear to innervate sensory receptors in the head region, and both possess neurites that likely innervate the proventriculus or stomach (in adults) and perhaps their ontogenetic precursors (which are undergoing development) in larvae. Both the sensory organs and stomach are ciliated, while the proventriculus is not ciliated but rather a large postoral cavity for retaining prey prior to mastication by the mastax. The fact that SLIR neurites innervate ciliated organs is not surprising because many other invertebrates show similar patterns of innervation (e.g., larval phoronids, ctenophores, molluscs; Hay-Schmidt, 1990, 2000) including other rotifers (e.g., Gąsiorowski et al., 2019; Hochberg & Hochberg, 2015; Hochberg, 2007, 2009; Hochberg & Lilley, 2010; Kotikova et al., 2005; Leasi et al., 2009). In some cases, these SLIR neurites are presumed to be sensory, whereas in others, they may be cilioexcitatory and therefore function as motor neurons. For example, innervation of the anterior sense organs, which may be the case for perikaryon, would indicate a sensory function; but potential innervation of the ciliated stomach wall by the third pair of neurites in the trunk region may indicate a motor function, unless there are undetermined sensory cells in the stomach. Likewise, SLIR innervation of the proventriculus might indicate the presence of sensory receptors that function to detect the presence of prey in the cavity (i.e., fullness; see more below).

While similarities between larvae and adults are obvious (see Figure 3), we do note some potential differences in neuronal patterns between the life stages. For example, only larvae appear to possess a distinct pair of neurites, the anterior neurites (AP_{an}), that project from the AP perikarya to an SLIR neurite ring. This ring is closely affiliated with a pair of (presumed) sensory receptors in the ventral apical field of the corona (Figure 1). Unfortunately, these receptors could not be verified with light microscopy, so we are uncertain of their true identity. Still, presence of a circular neurite that is adjacent to the ciliated corona, and its proximity to receptors, underscores the likelihood of the importance of serotonin in both locomotory activity and sensory reception. The pair of AP_{an} neurites, the SLIR nerve ring, and ventral receptor innervation were not observed in adult females, suggesting three possibilities: (a) they are lost at metamorphosis; (b) their signal is too diffuse to verify; or (c) their neurotransmitter phenotype changes at metamorphosis. Distinguishing among these choices is difficult without further evidence, but we currently think the latter two possibilities are the most intriguing for exploration. A loss of the anterior perikarya and neurite ring may certainly occur considering the dramatic changes that happen at metamorphosis, but when we compare our results with those of other studies, the other possibilities seem a better choice. For example, another gnesiotrochan rotifer with indirect development, *S. fimbriatus*, also possesses a neurite ring in the adult stage despite losing its coronal field of cilia at metamorphosis (Hochberg & Hochberg, 2015). Also, many direct developing rotifers possess such a ring (Hochberg, 2006; Kotikova et al., 2005). The study of *C. vorax* by Vasisht and Dawar (1969) also provides compelling evidence that the ring may still be present in adults. They describe lateral nerve #1 as a pair of neurites that extend anteriorly from the brain, but they could not follow it to innervation. However, they think the neurites may bend around to unite at the coronal ganglion (we did not see such a ganglion), effectively producing a neurite ring. Whether this is accurate will require further study. However, if the ring is in fact present in adults of *C. vorax*, it seems likely to be a homolog of the neurite (coronal) rings in other taxa based on position.

The possible retention of the neural ring in adults that lack a corona is difficult to explain considering the absence of locomotory cilia. However, we speculate that the ring may have more than one function that varies with ontogeny. For example, gnesiotrochan larvae require innervation of the apical field for both locomotion and sensation, as do most adult ploimate rotifers (Hochberg, 2009; Kotikova et al., 2005). The only difference is that adult rotifers also feed while swimming, while gnesiotrochan larvae appear to be lecithotrophic (Yang et al., 2019; Young et al., 2019). However, a lack of feeding should not imply that larvae do not require apical sensory receptors, only that their receptors are not important for locating prey. We hypothesize the neurite ring may, therefore, function as part of a circuit to relay motor information (to the locomotory cilia) *and* receive sensory information (from the apical field), regardless of taxon or age. The major differences will be found in the types of receptors present in the apical field. For example, large predatory ploimates such as *Asplanchna brightwellii* Gosse 1850 have a wide diversity of

receptors that function in chemical and mechanical reception for feeding and reproduction (Joanidopoulos & Marwan, 1998) and at least some of these receive SLIR innervation (Hochberg, 2009). In larvae, the receptor fields are largely unknown, but we suspect there are receptors that receive substrate-specific cues prior to settlement and metamorphosis, whether those cues be chemical (e.g., Clément, 1987: *Collotheca*; Wallace, 1978: *Ptygura beauchampi* EDMONDSON 1940) or perhaps related to substrate morphology (e.g., Butler, 1983; Edmondson, 1949). In sessile adult gnesiotrochans, the ring may also play a role in sensation and movement: different sensory receptors on the infundibulum might detect prey and relay information to the cerebral ganglion that leads to closure of the infundibulum around the prey; receptors may also sense chemicals or mechanical vibrations of potential predators, leading to the withdrawal of the body away from the source of the disturbance. Why the neurite ring fails to have significant immunoreactivity in adults of *C. vorax* is unknown, but it could be related to the third possibility mentioned above, that the neurotransmitter phenotype changes after metamorphosis. If this scenario is plausible, then the absence of SLIR in adults of *C. vorax* merely indicates a switch in modality after metamorphosis, which may be related to a necessary change in sensory reception as an adaptation to living on hydrophytes.

A second difference between the life stages is in the presence of some weak IR neurites in the lateral neck region of adults compared to larvae. In some adult specimens, we noted a neurite (bfn) that appeared to originate in the cerebral ganglion and innervate a site near the base of the infundibulum (Figure 3). Interestingly, this neurite bifurcated prior to innervation, though its termini were never ascertained. Its position and structure correspond to lateral nerve #5 of adult females examined by Vasisht and Dawar (1969), who described a neuron that innervates both the pharyngeal sense organ and a posterolateral sense organ (Figure 3; Table 1). Both organs are ciliated, and so, their innervation by an SLIR neurite is not surprising. Significantly, behavioral studies provide indirect evidence for the presence of mechanoreceptors within the infundibulum—likely to be the pharyngeal sense organ—which is expected to be ciliated. These mechanoreceptors are probably involved in the detection and capture of prey items (Bevington et al., 1995). An individual of *C. vorax* initiates an attack by lunging forward on its foot, capturing the prey in the hood-like infundibulum, and then pushing the prey back to the esophagus for storage in the proventriculus. Eventually, prey are macerated by the mastax and transferred to the stomach (Wallace et al., 2015). We think it is possible that this behavior—from sensory reception, to lunging, to storage within the proventriculus—may be controlled in part by SLIR neurites. We propose that these neurites may form a circuit that the adult uses to know when to attack or not attack prey given the storage capacity of the proventriculus. The presence of sensory cells in the proventriculus would provide evidence for this possibility.

To date, the most complete description of the nervous system in *C. vorax* comes from the study of Vasisht and Dawar (1969), who identified 11 cerebral nerves (neurites) and seven individual sense organs. Three of the 11 neurites innervated the dorsal sense organ, and the remaining eight neurites are proposed to innerve other regions of the body, including a pair of anterolateral sense organs, a pair of lateral sense organs, and a pair of pharyngeal sense organs (see Figure 3). We feel confident that some of our SLIR neurites are likely to be homologous with those described by Vasisht and Dawar (1969), including a few already mentioned (Table 1). The remainder we interpret with more caution because, in many cases, our specimens were

TABLE 1 The nerves of *Cupelopagis vorax* as described by Vasisht and Dawar (1969), their proposed sites of innervation, and their proposed homologies with serotonin-like immunoreactivity (SLIR) based on position in the current study

Nerves of Vasisht and Dawar (1969)	Sites of innervation	Homology with detected SLIR neurite(s)
DSN	Dorsal sense organ	No known homology
L #1	Coronal ganglion	AP, AP_{pn}, AP_{an}, and coronal neurite ring
L #2	Anterior sense organ	PLP, PLP_n
L #3	First coronal sphincter muscle	No known homology
L #4	Posterior portion of the corona	No known homology
L #5	Lateral and pharyngeal sense organs	bfn
L #6	Lateral side of the trunk body wall	No known homology
L #7	Lateral ganglion and trunk viscera	LN or TN
L #8	Lateral ganglion and trunk viscera	LN or TN

AP, anterior perikarya of the cerebral ganglion; AP_{an}, anterior-directed SLIR neurites of the anterior perikarya; AP_{pn}, posterior-directed SLIR neurites of the anterior perikarya; bfn, bifurcating SLIR neurite; DSN, dorsal sensory SLIR neurite; L1–8, lateral nerves; LN, lateral SLIR nerve cord; PLP, posterolateral SLIR perikarya; PLP_n, anterior-directed posterolateral SLIR neurites; TN, trunk SLIR neuritis.

contracted and the sites of innervation were obscure. For example, we observed three pairs of SLIR neurites that might correspond with neurites described in the former study: PLPn (=lateral nerve #2), LN, and TN (=lateral nerves #7 and #8). The perikarya PLP are positioned posterior of the largest IR perikarya in the cerebral ganglion and appear to send a neurite into the head region of both larvae and adults. These neurites are often quite curvy and their position can vary based on the extent to which the specimens were contracted. Nonetheless, they always terminated somewhere near the side of the infundibulum in adults, often close to its base. Our initial interpretation was that these neurites innervated the lateral sense organs, but considering the correspondence of bfn and lateral nerve #5 in position and structure, we offer an alternate homology. We think PLP might innervate the anterior sense organs (as lateral nerve #2), but the contraction of the infundibulum in the adults makes this difficult to verify. These SLIR neurites are also present in larvae, but without a better description of the morphology and position of larval sensory organs, it remains challenging to know whether our interpretation is accurate. The other potential homologies are seen in the elongate neurites that innervate the trunk. Here, a single neurite (LN) appears to extend from the cerebral ganglion and then branch (MN and possibly TN) in the trunk region; an alternative to branching is that two neurites extend parallel to each other but cannot be optically resolved. In either case, lateral nerves #7 and #8 also parallel each other into the trunk, but instead of separating, they unite at a ganglion (LG), after which, they independently innervate different regions in the viscera (Figure 3; Vasisht & Dawar, 1969). Such a ganglion was not observed in our specimens based on DAPI staining, so their homologies still remain in question.

At present we lack a comprehensive and well-resolved phylogeny of the Rotifera, as well within the Monogononta (Sørensen, 2002; Sørensen & Giribet, 2006), and so remain uncertain about the origins of indirect development (larvae), the sessile lifestyle, and the ancestral body plans of Monogononta, Ploima, and Gnesiotrocha. While our studies have revealed the presence of SLIR cells in the larval and adult nervous systems, we note that these SLIR cells are a small subset of what is likely to be present in both life stages. The distribution of catecholamines, acetylcholine, FMRFamide, and small cardioactive peptide b (SCPb) has been successfully investigated in both bdelloid and monogonont rotifers (Gąsiorowski et al., 2019; Hochberg & Hochberg, 2015; Hochberg, 2006, 2007, 2009; Kotikova et al., 2005; Leasi et al., 2009). Ultimately, a better understanding of neuronal homology is going to depend on our abilities to analyze more taxa—both closely and distantly related species—from a wide ecological spectrum that includes both direct and indirectly developing rotifers. Additionally, details of larval anatomy are extremely rare, and our inability to follow a species through metamorphosis limits our understanding of how much anatomical change takes place after settlement (e.g., Hochberg & Hochberg, 2015, 2017). Our efforts to understand the evolution of rotifer morphology, including patterns of neural innervation, are therefore predicated in future efforts to resolve rotifer phylogeny.

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ORCID

Elizabeth Preza https://orcid.org/0000-0002-6670-0945
Elizabeth J. Walsh https://orcid.org/0000-0002-6719-6883
Rick Hochberg https://orcid.org/0000-0002-5567-5393

REFERENCES

Bekkouche, N., & Worsaae, K. (2016). Nervous system and ciliary structures of Micrognathozoa (Gnathifera): Evolutionary insight from an early branch in Spiralia. *Royal Society Open Science*, *3*. https://doi.org/10.1098/rsos.160289

Bevington, D. J., White, C., & Wallace, R. L. (1995). Predatory behavior of *Cupelopagis vorax* (Rotifera; Collothecacea; Atrochidae) on protozoan prey. *Hydrobiologia*, *313*(314), 213–217. https://doi.org/10.1007/BF00025953

Butler, N. M. (1983). Substrate selection and larval settlement by *Cupelopagis vorax*. *Hydrobiologia*, *104*, 317–323. https://doi.org/10.1007/BF00045984

Carvalho, M. J. A., & Mirth, C. K. (2015). Coordinating morphology with behavior during development: An integrative approach from a fly perspective. *Frontiers in Ecology & Evolution*, *3*, 1–13. https://doi.org/10.3389/fevo.2015.00005

Clément, P. (1987). Movements in rotifers: Correlations of ultrastructure and behavior. *Hydrobiologia*, *147*, 339–359. https://doi.org/10.1007/BF00025764

Cori, C. I. (1925). Zur Morphologie und Biologie von *Apsilus vorax* Leidy. *Sonderdruck aus Zeitschrift f. wissensh. Zoologie*, *125*, 557–584.

Edmondson, W. T. (1944). Ecological studies of sessile Rotatoria: Part I. Factors affecting distribution. *Ecological Monographs*, *14*, 31–66. https://doi.org/10.2307/1961631

Edmondson, W. T. (1945). Ecological studies of sessile Rotatoria: Part II. Dynamics of populations and social structure. *Ecological Monographs*, *15*, 141–172. https://doi.org/10.2307/1948601

Edmondson, W. T. (1949). A formula key to the Rotatorian genus *Ptygura*. *Transactions of the American Microscopical Society*, *68*, 127–135. https://doi.org/10.2307/3223262

Evans, W. A. (1981). Spatial-dispersion, prey selection and fecundity in the sessile rotifer *Cupelopagis vorax* (Leidy). *American Zoologist*, *21*, 932.

Fontaneto, D., Melone, G., & Wallace, R. L. (2003). Morphology of *Floscularia ringens* (Rotifera, Monogononta) from egg to adult. *Invertebrate Biology*, *122*, 231–240. https://doi.org/10.1111/j.1744-7410.2003.tb00087.x

Gallardo, W. G., Hagiwara, A., Hara, K., Soyano, K., & Snell, T. W. (2000). GABA, 5-HT and amino acids in the rotifers *Brachionus plicatilis* and *Brachionus rotundiformis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *127*, 301–307. https://doi.org/10.1016/s1095-6433(00)00266-x

Gąsiorowski, L., Bekkouche, N., & Worsaae, K. (2017). Morphology and evolution of the nervous system in Gnathostomulida (Gnathifera, Spiralia). *Organisms Diversity & Evolution*, *17*, 447–475. https://doi.org/10.1007/s13127-017-0324-8

Gąsiorowski, L., Furu, A., & Hejnol, A. (2019). Morphology of the nervous system of monogonont rotifer *Epiphanes senta* with a focus on sexual dimorphism between feeding females and dwarf males. *Frontiers in Zoology*, *16*, 1–13. https://doi.org/10.1186/s12983-019-0334-9

Gast, R. (1900). Beiträge zur kenntniss von *Apsilus vorax* (Leidy). *Zeitschrift Für Wissenschaftliche Zoologie*, *67*, 167–214.

Haynes, P. R., Christmann, B. L., & Griffith, L. C. (2015). A single pair of neurons links sleep to memory consolidation in drosophila melanogaster. *eLife*, *4*. https://doi.org/10.7554/eLife.03868.001

Hay-Schmidt, A. (1990). Catecholamine-containing, serotonin-like, and FMRFamide-like immunoreactive neurons and processes in the nervous system of the early actinotroch larva of *Phoronis vancouverensis* (Phoronida): Distribution and development. *Canadian Journal of Zoology*, *68*, 1525–1536. https://doi.org/10.1139/z90-226

Hay-Schmidt, A. (2000). The evolution of the serotonergic nervous system. *Proceedings of the Royal Society B: Biological Sciences*, *267*, 1071–1079. https://doi.org/10.1098/rspb.2000.1111

Herlyn, H., Piskurek, O., Schmitz, J., Ehlers, U., & Zischler, H. (2003). The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, *26*, 155–164. https://doi.org/10.1016/s1055-7903(02)00309-3

Hewitt, S. M., Baskin, D. G., Frevert, C. W., Stahl, W. L., & Rosa-Molinar, E. (2014). Control for immunohistochemistry: The histochemical society's standards of practice for validation of immunohistochemical assays. *Journal of Histochemistry and Cytochemistry*, *62*, 693–697. https://doi.org/10.1369/0022155414545224

Hochberg, A., & Hochberg, R. (2015). Serotonin immunoreactivity in the nervous system of the free-swimming larvae and sessile adult females of *Stephanoceros fimbriatus* (Rotifera: Gnesiotrocha). *Invertebrate Biology*, *134*, 261–270. https://doi.org/10.1111/ivb.12102

Hochberg, A., & Hochberg, R. (2017). Musculature of the sessile rotifer *Stephanoceros fimbriatus* (Rotifera: Gnesiotrocha: Collothecaceae) with details on larval metamorphosis and development of the infundibulum. *Zoologischer Anzeiger*, *268*, 84–95. https://doi.org/10.1016/j.jcz.2016.09.002

Hochberg, R. (2006). On the serotonergic nervous system of two planktonic rotifers, *Conochilus coenobasis* and *C. dossuarius* (Monogononta, Flosculariacea, Conochilidae). *Zoologischer Anzeiger*, *245*, 53–62. https://doi.org/10.1016/j.jcz.2006.04.001

Hochberg, R. (2007). Topology of the nervous system of *Notommata copeus* (Rotifera: Monogononta) revealed with anti-FMRFamide, -SCPb, and -serotonin (5-HT) immunohistochemistry. *Invertebrate Biology*, *126*, 247–256. https://doi.org/10.1111/j.1744-7410.2007.00094.x

Hochberg, R. (2009). Three-dimensional reconstruction and neural map of the serotonergic brain of *Asplanchna brightwellii* (Rotifera, Monogononta). *Journal of Morphology*, *270*, 430–441. https://doi.org/10.1002/jmor.10689

Hochberg, R., & Lilley, G. (2010). Neuromuscular organization of the freshwater colonial rotifer, *Sinantherina socialis*, and its implications for understanding the evolution of coloniality in Rotifera. *Zoomorphology*, *129*, 153–162. https://doi.org/10.1007/s00435-010-0108-6

Hochberg, R., Walsh, E. J., & Wallace, R. L. (2017). The ultrastructure of the integument and proventriculus in the raptorial rotifer *Cupelopagis vorax* (Monogononta: Collothecacea: Atrochidae). *Invertebrate Biology*, *136*, 50–61. https://doi.org/10.1111/ivb.12161

Hochberg, R., Yang, H., Hochberg, A., Walsh, E. J., & Wallace, R. L. (2019). When heads are not homologous: The coronae of larval and adult collothecid rotifers (Rotifera: Monogononta: Collothecaceae). *Hydrobiologia*, *844*, 191–207. https://doi.org/10.1007/s10750-018-3760-3

Joanidopoulos, K. D., & Marwan, W. (1998). Specific behavioural responses triggered by identified mechanosensory receptor cells in the apical field of the giant rotifer *Asplanchna sieboldi*. *Journal of Experimental Biology*, *201*, 169–177.

Kaul-Strehlow, S., Urata, M., Minokawa, T., Stach, T., & Wanninger, A. (2015). Neurogenesis in directly and indirectly developing enteropneusts: Of nets and cords. *Organisms, Diversity, & Evolution*, *15*, 405–422. https://doi.org/10.1007/s13127-015-0201-2

Kiørboe, T. (2011). How zooplankton feed: Mechanisms, traits and trade-offs. *Biological Reviews*, *86*, 311–339. https://doi.org/10.1111/j.1469-185X.2010.00148.x

Koste, W. (1978). *Rotatoria. Die Rädertiere Mitteleuropas*, Vol. 2. : Gebrüder Borntraeger.

Kotikova, E. A., Raikova, O. I., Reuter, M., & Gustafsson, M. K. S. (2005). Rotifer nervous system visualized by FMRFamide and 5-HT immunocytochemistry and confocal laser scanning microscopy. *Hydrobiologia*, *546*, 239–248. https://doi.org/10.1007/s10750-005-4203-5

Kutikova, L. A. (1995). Larval metamorphosis in sessile rotifers. *Hydrobiologia*, *313*, 133–138. https://doi.org/10.1007/BF00025942

Kutikova, L. A. (2007). Five species of rotifers of the family Flosculariidae (Rotifera), their larvae and metamorphosis. *Invertebrate Zoology*, *4*, 161–172. (in Russian).

Leasi, F., Pennati, R., & Ricci, C. (2009). First description of the serotonergic nervous system in a bdelloid rotifer: *Macrotrachela quadricornifera* Milne 1886 (Philodinidae). *Zoologischer Anzeiger*, *248*, 47–55. https://doi.org/10.1016/j.jcz.2008.10.002

Leidy, J. (1857). Note on *Dictyophora vorax*. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *9*, 204–205.

Martín-Durán, J. M., Wolff, G. H., Strausfeld, N. J., & Hejnol, A. (2016). The larval nervous system of the penis worm *Priapulus caudatus* (Ecdysozoa). *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*. https://doi.org/10.1098/rstb.2015.0050

May, L. (1989). Epizoic and parasitic rotifers. *Hydrobiologia*, *186*(187), 59–67. https://doi.org/10.1007/BF00048897

Montgomery, T. H. (1903). On the morphology of the rotatorian family Flosculariidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *55*, 363–395.

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., Vogt, L., Wanninger, A., Brenneis, G., Döring, C., Faller, S., Fritsch, M., Grobe, P., Heuer, C. M., Kaul, S., Møller, O. S., Müller, C. H., Rieger, V., Rothe, B. H., … Harzsch, S. (2010). Invertebrate neurophylogeny: Suggested terms and definitions for a neuroanatomical glossary. *Frontiers in Zoology*, *7*, 1–49. https://doi.org/10.1186/1742-9994-7-29

Segers, H. (2007). Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, *1564*, 1–104. https://doi.org/10.11646/zootaxa.1564.1.1

Sielaff, M., Schmidt, H., Struck, T. H., Rosenkranz, D., Mark Welch, D. B., Hankeln, T., & Herlyn, H. (2015). Phylogeny of Syndermata (syn. Rotifera): Mitochondrial gene order verifies epizoic Seisonidea as sister to endoparasitic Acanthocephala within monophyletic Hemirotifera. *Molecular Phylogenetics and Evolution*, *96*, 79–92. https://doi.org/10.1016/j.ympev.2015.11.017

Sørensen, M. V. (2002). Phylogeny and jaw evolution in Gnathostomulida, with a cladistic analysis of the genera. *Zoologica Scripta*, *31*, 461–480. https://doi.org/10.1046/j.1463-6409.2002.00089.x

Sørensen, M. V., & Giribet, G. (2006). A modern approach to rotiferan phylogeny: Combining morphological and molecular data. *Molecular Phylogenetics and Evolution*, *40*, 585–608. https://doi.org/10.1016/j.ympev.2006.04.001

Starkweather, P. L. (1996). Sensory potential and feeding in rotifers: Structural and behavioral aspects of diet selection in ciliated zooplankton. In P. H. Lenz D. K. Hartline J. E. Purcell & D. L. Macmillan (Eds.), *Zooplankton: sensory ecology and physiology*. (pp.255–266) Amsterdam, Netherlands: Gordon and Breach Publishers.

Stemberger, R. S. (1981). A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 721–724. https://doi.org/10.1139/f81-095

Tissot, M., & Stocker, R. F. (2000). Metamorphosis in *Drosophila* and other insects: The fate of neurons throughout the stages. *Progress in Neurobiology*, *62*, 89–111. https://doi.org/10.1016/S0301-0082(99)00069-6

Vasisht, H. S., & Dawar, B. L. (1969). Anatomy and histology of the rotifer *Cupelopagis vorax* Leidy. *Research Bulletin Panjab University*, *20*, 207–221.

Wallace, R. L. (1978). Substrate selection by larvae of the sessile rotifer *Ptygura beauchampi*. *Ecology*, *59*, 221–227. https://doi.org/10.2307/1936366

Wallace, R. L. (1980). Ecology of sessile rotifers. *Hydrobiologia*, *73*, 181–193. https://doi.org/10.1007/978-94-009-9209-2_31

Wallace, R. L. (1993). Presence of anisotropic (birefringent) crystalline structures in embryonic and juvenile monogonont rotifers. *Hydrobiologia*, *255*(256), 71–76. https://doi.org/10.1007/978-94-011-1606-0_9

Wallace, R. L., & Edmondson, W. T. (1986). Mechanism and adaptive significance of substrate selection by a sessile rotifer. *Ecology*, *67*, 314–323. https://doi.org/10.2307/1938575

Wallace, R. L., Snell, T. W., Ricci, C., & Nogrady, T. (2006). In H. Segers (Ed.), *Rotifera: Biology, ecology and systematics*. Backhuys.

Wallace, R. L., Snell, T. W., & Smith, H. A. (2015). Phylum Rotifera In J. Thorp, & D. C. Rogers (Eds.), *Thorp and Covich's freshwater invertebrates* (pp. 225–271). New York, NY: Academic Press.

Yang, H., Hochberg, R., Walsh, E. J., & Wallace, R. L. (2019). Systematic distribution of birefringent bodies in Rotifera and first evidence of their ultrastructure in *Acyclus inquietus* (Gnesiotrocha: Collothecaceae). *Hydrobiologia*, *844*, 209–219. https://doi.org/10.1007/s10750-018-3784-8

Young, A. N., Hochberg, R., Walsh, E. J., & Wallace, R. L. (2019). Modeling the life history of sessile rotifers: Larval substratum selection through reproduction. *Hydrobiologia*, *844*, 67–82. https://doi.org/10.1007/s10750-018-3802-x

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