Available online at www.sciencedirect.com
SCIENCE DIRECT
Zoologischer Anzeiger 245 (2006) 53–62
ZOOLOGISCHER ANZEIGER
www.elsevier.de/jcz

On the serotonergic nervous system of two planktonic rotifers, *Conochilus coenobasis* and *C. dossuarius* (Monogononta, Flosculariacea, Conochilidae)

Rick Hochberg*

Department of Biological Sciences, University of Massachusetts, One University Avenue, Lowell, MA 01854, USA

Received 21 November 2005; received in revised form 4 April 2006; accepted 10 April 2006
Corresponding editor: M. Schmitt

Abstract

The serotonergic nervous systems of two non-colonial species of *Conochilus* were examined to obtain the first immunohistochemical insights into the neuroanatomy of species of Flosculariacea (Rotifera, Monogononta). Species of *Conochilus*, subgenus *Conochiloides*, were examined using serotonin (5-HT) immunohistochemistry, epifluorescence and confocal laser scanning microscopy, and 3D computer imaging software. In specimens of *C. coenobasis* and *C. dossuarius*, the serotonergic nervous system is defined by a dorsal cerebral ganglion, apically directed cerebral neurites, and paired nerve cords. The cerebral ganglion contains approximately four pairs of small 5-HT-immunoreactive perikarya; one pair innervates the posterior nerve cords and three pairs innervate the apical field. The most dorsal pair innervates a coronal nerve ring that encircles the apical field. Within the apical field is a second nerve ring that outlines the inner border of the coronal cilia. Together, both the inner and outer nerve rings may function to modulate ciliary activity of the corona. The other two pairs of perikarya innervate a region around the mouth. Specific differences in the distribution of serotonergic neurons between species of *Conochilus* and previously examined ploimate rotifers include the following: (a) a lack of immunoreactivity in the mastax; (b) a greater number of apically directed serotonergic neurites; and (c) a complete innervation of the corona in both species of *Conochilus*. These differences in nervous system immunohistochemistry are discussed in reference to the phylogeny of the Monogononta.

Keywords: Rotifer; Nervous system; Immunohistochemistry; Serotonin

1. Introduction

Rotifers are some of the smallest free-living animals in freshwater environments, and yet account for a large proportion of the taxonomic diversity of zooplankton in lentic and lotic systems (Nogrady et al. 1993; Ricci and Balsamo 2000). Most planktonic rotifers are solitary animals of the order Ploima, which contribute substantially to the trophic dynamics of freshwater communities (Wallace and Snell 2001). Ploima is the largest and most diverse monophyletic assemblage of monogonont rotifers, yet does not contain any colonial species. All colonial rotifers are restricted to the order Flosculariacea, a clade defined by the structure of their pharyngeal hardparts, the jaw-like malleoramate trophi (Nogrady et al. 1993). The clade has questionable relations to the Ploima, and to the

*Tel.: +1 978 934 2885; fax: +1 978 934 3044.
E-mail address: Rick_Hochberg@uml.edu.

0044-5231/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jcz.2006.04.001

order Collothecacea with which it is traditionally aligned (Sørensen 2002).

Within the Flosculariacea are approximately 25 species of rotifers that form colonies (Wallace 1987). Colony formation is generally described as autorecruitive or allorecruitive, depending on whether parthenogenetic embryos develop alongside adults, often within a gelatinous matrix, or settle on adults, arriving from conspecifics outside of the colony (Nogrady et al. 1993). Since colonies are essentially aggregations of solitary individuals, they lack any anatomical interconnections, and so do not share any metabolic demands or trophic resources. However, the organic matrix that binds a colony together, whether it be made of a soft gelatinous medium or hardened secretions, may serve as protection from predators, and large colony size may itself be a deterrent (Stemberger and Gilbert 1987; also reviewed in Wallace 1987). The number of individuals that make up a colony is highly variable and sometimes species-specific. Species of *Lacinularia* have been reported to comprise colonies of greater than 1000 individuals, while some species of *Conochilus* form colonies of only a few individuals (Wallace 1987). Many of these colonies are sessile and highly selective of their substratum, but other colonies form in the plankton, where they grow presumably by autorecruitment. There is apparently little morphological difference between congeners that form sessile and planktonic colonies (Nogrady et al. 1993).

The planktonic lifestyle is prevalent among species of Conochilidae, but coloniality is found in less than half of the known species. The genus *Conochilus* contains both colonial and solitary animals, the former designated to the subgenus *Conochilus* and the latter to the subgenus *Concohiloides*. As noted in the cladistic analysis of Segers and Wallace (2000), the colonial lifestyle is hypothesized as the derived condition within the Flosculariacea, and probably arose several times independently. While the embryos of solitary species may develop alongside their parents within a gelatinous matrix, as is present in all species of Conochilidae and Flosculariidae, adults generally lead a solitary lifestyle.

To date, our knowledge of the anatomical differences between solitary and colonial species, or planktonic and benthic species, is generally limited to differences in optically visible characteristics such as body shape and the organization of the corona. However, many such characters are often highly malleable and therefore ambiguous from a phylogenetic standpoint. Alternatively, pharyngeal hardparts have provided a solid foundation for making phylogenetic inferences because of their conserved structure (Segers and Wallace 2000; Sørensen 2002). Still, mastax structure alone provides limited information on the evolution of lifestyle changes, although it can presumably provide insights into habitat partitioning through trophic specialization. With this in mind, recent attention has been directed at other organ systems, namely the muscular system (Hochberg and Litvaitis 2000; Kotikova et al. 2001, 2004; Sørensen et al. 2003; Santo et al. 2005; Sørensen 2005a, b) and nervous system (Kotikova 1995, 1997, 1998; Kotikova et al. 2005), the latter of which generally shows a high degree of conservation and may therefore provide clues to the origins and evolution of different lifestyles. More specifically, increasing attention has been directed at the nervous systems of the most heterogeneous rotifers, the ploimates (Kotikova, 1988, 1995, 1997, 1998; Kotikova et al., 2005). While no ploimates are colonial, their diverse lifestyles and body morphologies provide an index against which to measure neuroanatomical variation – indeed, conservation within such diversity suggests that a *Bauplan* for the construction of the nervous system arose prior to the divergence in lifestyles. Whether non-ploimate rotifers possess a fundamentally different *Bauplan* or simply modifications to a common pattern will require greater knowledge of species from outside the Ploima.

The purpose of the current analysis is to provide the first details on the organization of the nervous system in planktonic species of *Conochilus*. Specifically, solitary species of the subgenus *Conochiloides* are examined using anti-serotonin (5-HT) immunohistochemistry, epifluorescence and confocal laser scanning microscopy, and 3D imaging software. The distribution of serotonergic neurons is currently known for three species of Ploima (Kotikova et al. 2005) and provides a basis for comparing neural topologies and the innnervation of specific organ systems.

2. Material and methods

2.1. Collection and identification

Specimens of *Conochilus* were collected with a 64 μm plankton net from small lakes at the West Palm Beach Airport, Florida in February and March 2005. Rotifers were anaesthetized in 1% $MgCl_2$ and photographed alive as whole mounts on glass slides. Specimens were identified according to the taxonomic atlas of the Frank J. Meyers Rotifera Collection CD (The Academy of Natural Sciences).

2.2. Immunohistochemistry

Twenty-three rotifers (*Conochilus coenobasis* Skorikov, 1914, $N = 15$; *C. dossuarius* (Hudson, 1885), $N = 8$) were anaesthetized in 1% $MgCl_2$ and fixed in 4% paraformaldehye in 0.1 M PBS for 24 h at 4 °C. Specimens were next rinsed (3 ×) over the course of 12 h in 0.1 M PBS and then placed in IT Signal Enhancer

(Ingenta) for 2 h to minimize non-specific staining. Twenty specimens were transferred to anti-rabbit serotonin antibody (dilution 1:200, Sigma-Aldrich) in 1.5 ml centrifuge tubes at 4 °C on an orbital shaker for 48 h. The antibody was diluted with PBT (0.1 M PBS plus 1% Triton X-100). Three specimens were used as controls and omitted from the primary antibody. Specimens were then rinsed (3 ×) in 0.1 M PBS over 48 h and transferred to goat anti-rabbit Alexa Fluor 546 (dilution 1:500, Sigma-Aldrich). Specimens were stained in the dark at 4 °C on an orbital shaker for 48 h and then rinsed in 0.1 M PBT for 24 h. Specimens were mounted in Fluoromount G (Electron Microscopy Sciences) on glass slides and refrigerated at 4 °C for 24 h before examination.

2.3. Microscopical examination

Wholemount specimens were examined on two microscopes: (1) a Zeiss Axioimager equipped with epifluorescence, digital Axiocam MRm, and Axiovision software (Rel. 4.4) at the University of Massachusetts Lowell; and (2) a Nikon Eclipse E800 compound microscope equipped with a Biorad Radiance 2000 laser system at the Smithsonian Marine Station in Fort Pierce, Florida. The specimens observed on the Zeiss microscope were kept refrigerated for 2 months prior to examination. The specimens observed on the confocal system were examined within 1 week of staining. Lasersharp software was used to collect a series of 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 3-D 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 of neurons in some digital images.

3. Results

Adults of *Conochilus coenobasis* Skorikov, 1914 are enclosed in a gel-like matrix that often contains developing embryos and juveniles (Fig. 1A and C). No more than three developing young are present in any one "colony" and adults are often solitary. Most specimens are 170–250 μm long. Adults of *C. dossuarius* (Hudson, 1885) are always solitary and enclosed in a gel-like matrix. Maximum length of adults is 300 μm.

Anti-serotonin immunofluorescence is prevalent throughout the central and peripheral nervous systems of adult *C. coenobasis* and *C. dossuarius*. Immunofluorescence was never detected in any embryos. Omission of the primary antibody did not produce any immunoreactivity, although secondary antibody staining is present in small amounts on the coronal cilia and the gel-like matrix that surrounds each individual rotifer (not shown). Some autofluorescence is also present in the gut. The nervous systems of both species are similar and are therefore described together.

The cerebral ganglion is located dorsally above the mastax and contains at least four bilateral pairs of serotonin-immunoreactive perikarya (Figs. 2A–C and 3–5). The cell bodies form a cluster that measures approximately 10 μm wide by 10 μm long (Range: 8–12 μm long by 9–10 μm wide). Average perikaryon diameter is 4.5 μm (range: 3.8–5.5 μm). Three pairs of neurites project from the cerebral ganglion toward the corona: a dorsal pair (dsn), a medial pair (msn), a ventral pair (vsn) (Figs. 2–5). The dorsal pair of neurons are unipolar and have neurites (0.6 μm wide by 20 μm long) that project toward the coronal nerve ring (cnr) (Figs. 4A–C and 5A). The cnr forms a strongly immunoreactive loop perpendicular to the body axis, encircling the entire apical region on the periphery of the coronal ciliature (Figs. 3D–E, 4A–B and 5). The diameter of the individual neurites that form the cnr is 0.7–0.9 μm. Average diameter of the entire cnr is 49 μm (range: 47–51 μm). Within the borders of the cnr is an inner nerve ring (inr) in the shape of a horseshoe (inr, Fig. 4B). The inr is parallel to the cnr, perpendicular to the longitudinal body axis, and abuts the inner margin of the coronal ciliature (Figs. 4B and 5). There were no obvious perikarya associated with the inr, nor did it receive any detectable innervation from the cnr or any apically directed neurites from the cerebral ganglion. Projecting within the cnr are two pairs of neurites. One pair of neurites (0.4 μm diameter) originates from the base of the cerebral ganglion and projects towards the center of the corona and through the cnr (vsn, Figs. 2A–B, 4A–B and 5). Each neurite follows a slight ventral angle until reaching the cnr where it bends medially. The site of innervation is unknown but may be close to the mouth. A second pair of weakly immunoreactive neurites (msn, Fig. 4A and B) originates from the middle of the cerebral ganglion and innervates the apical region, but the site of innervation was not determined.

A pair of neurites (nc) project from the posterior region of the cerebral ganglion and are presumably part of the nerve cords. Each neurite appears to originate from a single perikaryon close to the posterior midline of the cerebral ganglion (Fig. 4D). After exiting the cerebral ganglion, the neurites bend ventrally for approximately 20–25 μm before curving posteriorly (Figs. 2–5). A pair of cell bodies is present along the length of the nerve cords at approximately 50% body length (Fig. 2D). The nerve cords coalesce in the foot region.

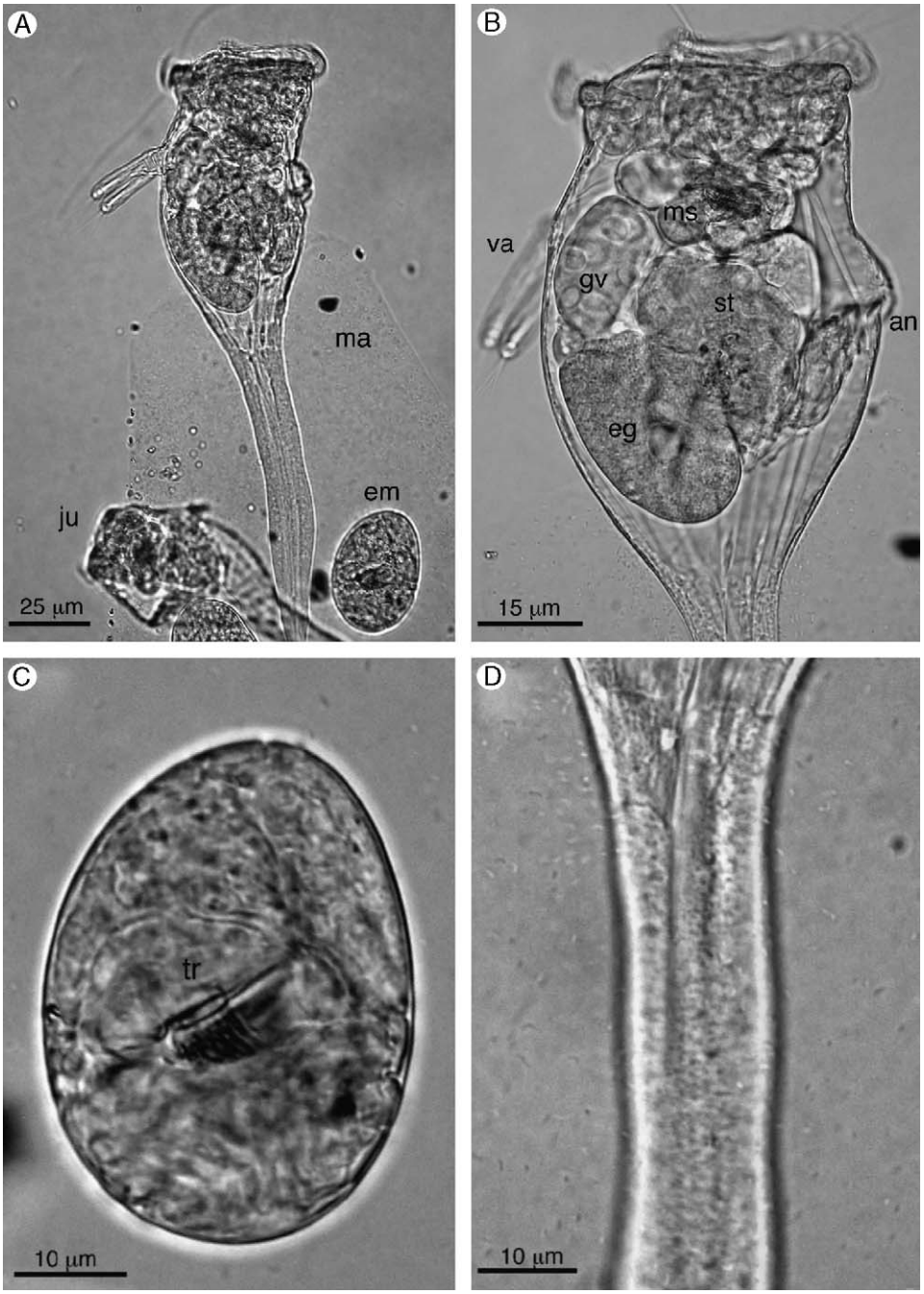

Fig. 1. Live specimens of *Conochilus coenobasis*: (A) a colony consisting of one adult, one juvenile, and one embryo in a gelatinous matrix; and (B) anterior end of an adult specimen. Compared to Fig. 5A; (C) developing embryo; and (D) stalk-like foot region of an adult specimen. Inset: cell bodies at mid-length of nerve cords. an = anus, eg = egg, em = embryo, gv = germovitellarium, ju = juvenile, ma = gelatinous matrix, ms = mastax, st = stomach, tr = trophi, va = ventral antennae.

Very few peripheral neurites are present. In *C. dossuarius*, very fine neurites appear to innervate the ventral antennae, while there is no such innervation present in *C. coenobasis*. In the former species, the ventral antennae are partially fused along their length for approximately 20 μm. Very thin immunoreactive fibers are present along the ventral side of the fused portion, but do not enter either of the individual antennae (Fig. 3A).

4. Discussion

The rotifer central nervous system can be broadly described as comprising a dorsal cerebral ganglion that sits atop the mastax and paired ventrolateral nerve cords that innervate the trunk region (Remane 1929–1933). Neurites from the cerebral ganglion project toward the apical region within the coronal field and innervate the ciliature and

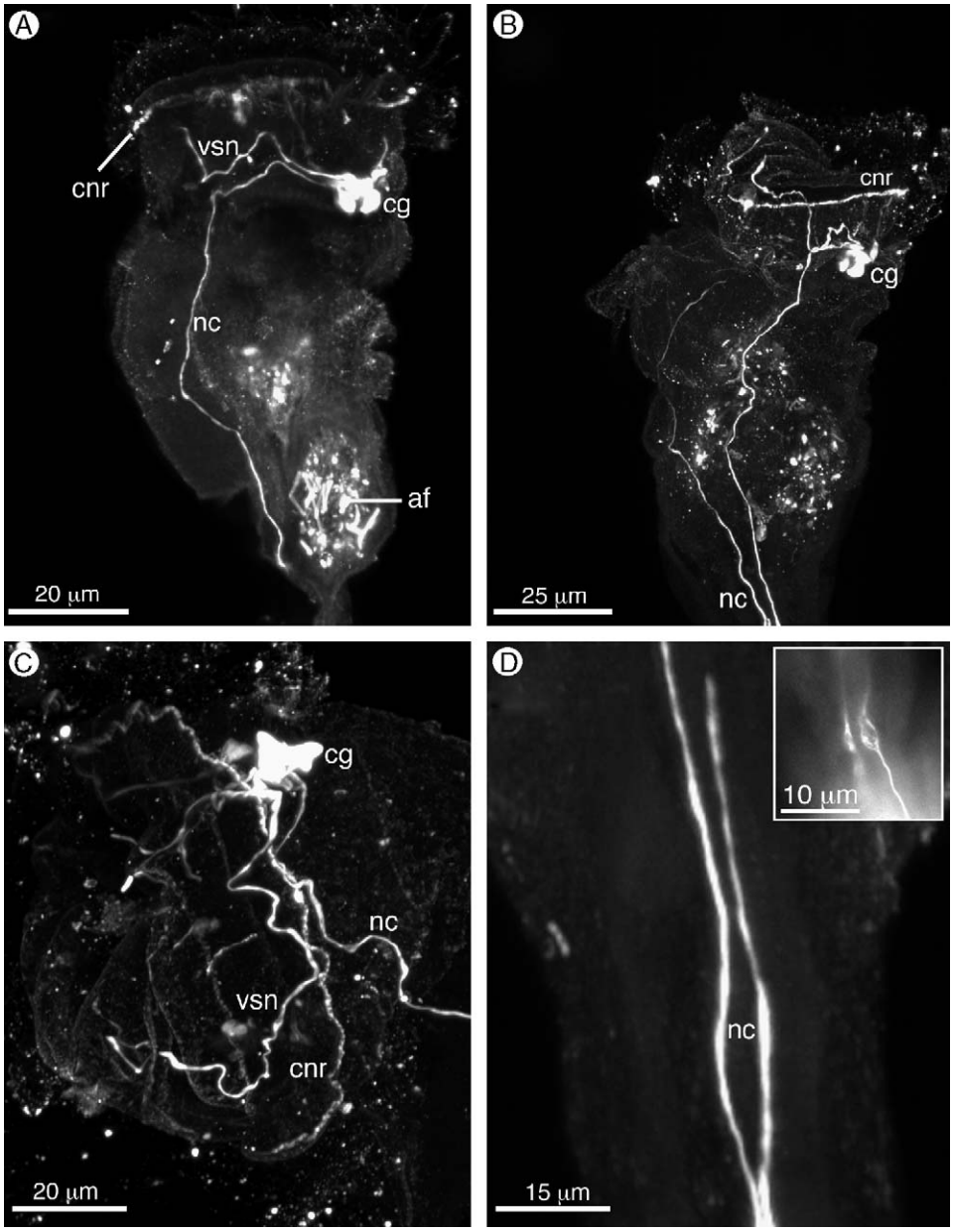

Fig. 2. Anti-serotonin immunohistochemistry in specimens of *Conochilus coenobasis*: (A) epifluorescence view of the lateral trunk region of an adult specimen; ventral is to the left; (B) *Z*-projection of a different adult specimen; ventral is to the left. 0.1 um × 150 optical sections; (C) *Z*-projection of the anterior end of a juvenile specimen revealing part of the cerebral ganglion and some of the cerebral neurons. 0.05 μm × 100 optical sections; and (D) epifluorescence view of the nerve cords in the foot. Inset: Serotonergic cell bodies on the nerve cords. af = autofluorescent material in gut, cg = cerebral ganglion, vsn = ventral cerebral innervation of the apical field.

various sensory devices (Clément and Wurdak 1991). Peripheral neurites innervate the mastax and sometimes form a complex neural network (Kotikova et al. 2005). In the posterior trunk, neurites may arise off the major nerve cords and innervate the toes (Kotikova 1995). While some variation in this organization is present, the extent of our knowledge is based almost exclusively on species of Ploima, and to a large degree, the results of chemical histofluorescence (Nogrady and Alai 1983; Raineri 1984; Keshmirian and Nogrady 1987, 1988; Kotikova 1988, 1995, 1997, 1998).

To date, only three ploimates have been studied using anti-serotonin immunohistochemistry: *Plationus patulus* (Müller, 1786), *Euchlanis dilatata* (Ehrenberg, 1832), and *Asplanchna herricki* (Guerne, 1888) (Kotikova et al. 2005). The distribution of serotonergic perikarya and neurites in these species is similar despite their distant evolutionary relationships within the Ploima

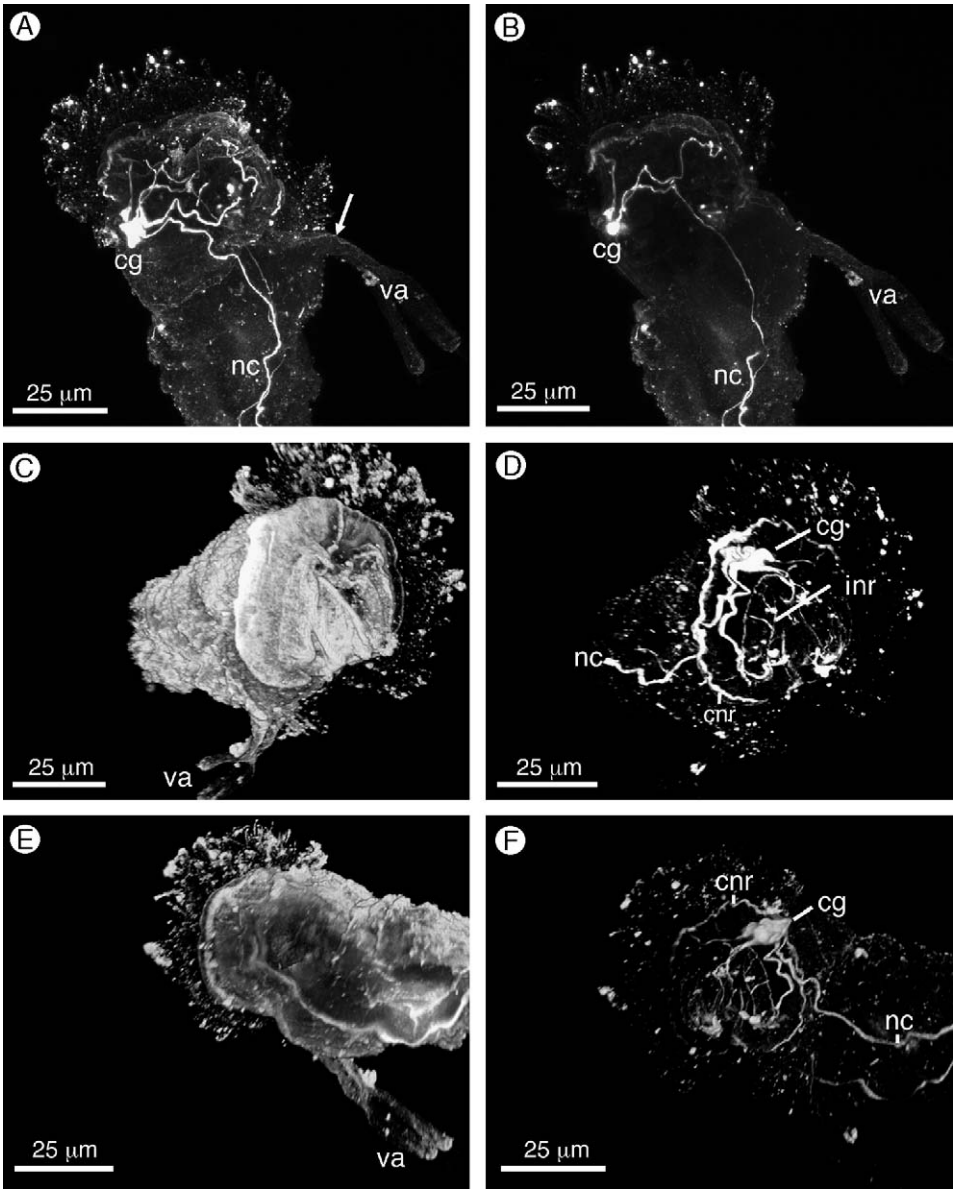

Fig. 3. Anti-serotonin immunohistochemistry in specimens of *Conochilus dossuarius*: (A) *Z*-projection of the anterior end of an adult specimen. 0.1 μm × 452 optical sections; (B) *Z*-projection of same specimen, different focal plane. 0.35 μm × 206 optical sections; (C–E) 3D images rendered using Volocity software and confocal imaging; (C) gray-scale transmission image of the anterior end of an adult specimen; (D) Same specimen viewed with fluorescence; (E) gray-scale transmission image of the same specimen from a postero-lateral view; (F) Same specimen viewed with fluorescence. arrow = thin immunoreactive fibers at the base of the ventral antenna, cg = cerebral ganglion, cnr = coronal nerve ring, inr = inner nerve ring (horseshoe shape) of the apical field, nc = cerebral innervation of the nerve cords, va = ventral antennae, vsn = ventral cerebral innervation of the apical field.

(see Sørensen 2002). In general, serotonergic perikarya make up only a small proportion of the cell bodies in the cerebral ganglion (compared to FMRFamidergic perikarya). Cerebral innervation is limited to the coronal tufts of cilia and the nerve cords. The mastax is innervated by serotonergic neurons, but a direct association with the cerebral ganglion has not been demonstrated; an indirect association, by way of the nerve cords, is known in *A. herricki* (Kotikova et al. 2005). A similar condition to that described above has now been demonstrated for species of *Conochilus* (subgenus *Conochiloides*). Both the cerebral ganglion and neurites that presumably innervate the nerve cords display strong anti-serotonin immunoreactivity. The cerebral ganglion is composed of only a few serotonergic perikarya, all of which produce neurites that innervate the apical region. However, specific differences exist between species of *Conochilus* and the ploimates, namely in cerebral

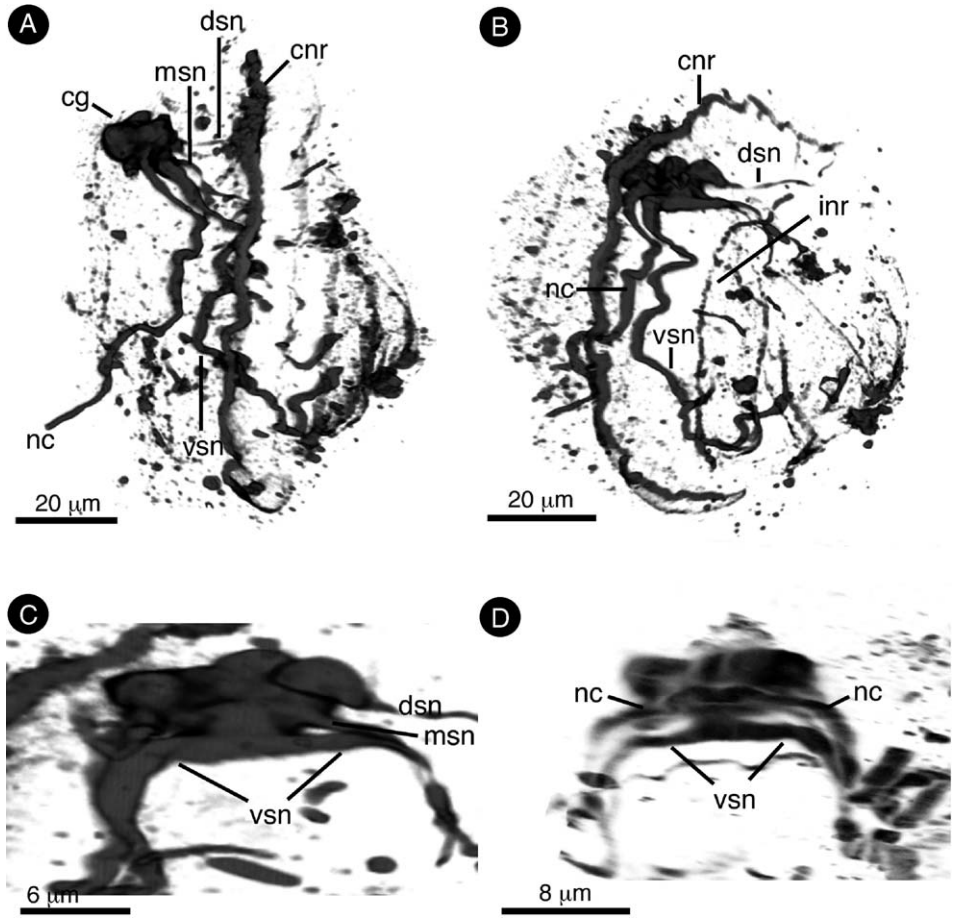

Fig. 4. Three-dimensional images of the serotonergic nervous system of *Conochilus dossuarius*. Volocity images are collected in gray scale and inverted for contrast: (A) lateral view of head region showing major neurons innervating the apical field; (B) same specimen rotated to the left; (C) frontal view of the cerebral ganglion; and (D) posterior view of the cerebral ganglion. cg = cerebral ganglion, inr = inner nerve ring (horseshoe shape) of the apical field, msn = medial cerebral innervation of the apical field, nc = cerebral innervation of the nerve cords, dsn = dorsal cerebral innervation of the coronal nerve ring, vsn = ventral cerebral innervation of the apical field.

innervation of the apical field and the distribution of immunoreactive cell bodies in the mastax.

The cerebral ganglion of both *C. coenobasis* and *C. dossuarius*, as delineated by anti-serotonin immunoreactivity, is relatively small and weakly defined. Specific pathways between cerebral perikarya are therefore difficult to map out. Still, pathways from perikarya to regions outside the cerebral ganglion can be traced, making comparative studies of innervation possible. Specifically, there are at least three pairs of neurites that innervate the apical field. A pair of thin dorsal neurites (dsn) innervate the coronal nerve ring (cnr) and may function to control ciliary beat and therefore swimming velocity. In the ploimates *P. patulus* and *E. dilatata*, a pair of serotonergic neurites also innervates the corona, but in neither species is there any neural structure equivalent to the coronal nerve ring; instead, there is an "anterior dorsal semi-ring" close to the corona. Kotikova (1998) also documents a catecholaminergic ring in species of *Philodina*, *Brachionus*, and *Dicranophorus*, but whether such rings innervate the coronal ciliature, and whether the chemical histofluorescence is due to serotonin (see Lindvall and Björklund 1974; De La Torre and Surgeon 1976), is unknown.

Based on studies of serotonin distribution in other invertebrates (Hay-Schmidt 2000) and its function in modulating ciliary beat (reviewed in Weiger 1997), it is not unexpected to find direct innervation of the coronal ciliature in rotifers. Most invertebrate larvae, of either protostomian or deuterostomian ancestry, have brains that innervate ciliary organs by way of serotonergic neurons (Hay-Schmidt 2000). The same is true of adult invertebrates without larval stages (Hochberg and Litvaitis 2003). Perhaps what is most surprising is that so few rotifers possess a similar innervation of the corona as in species of *Conochilus*. All rotifers, despite their specific form of locomotion (e.g., creeping or swimming) or lifestyle (e.g., planktonic or sessile), rely

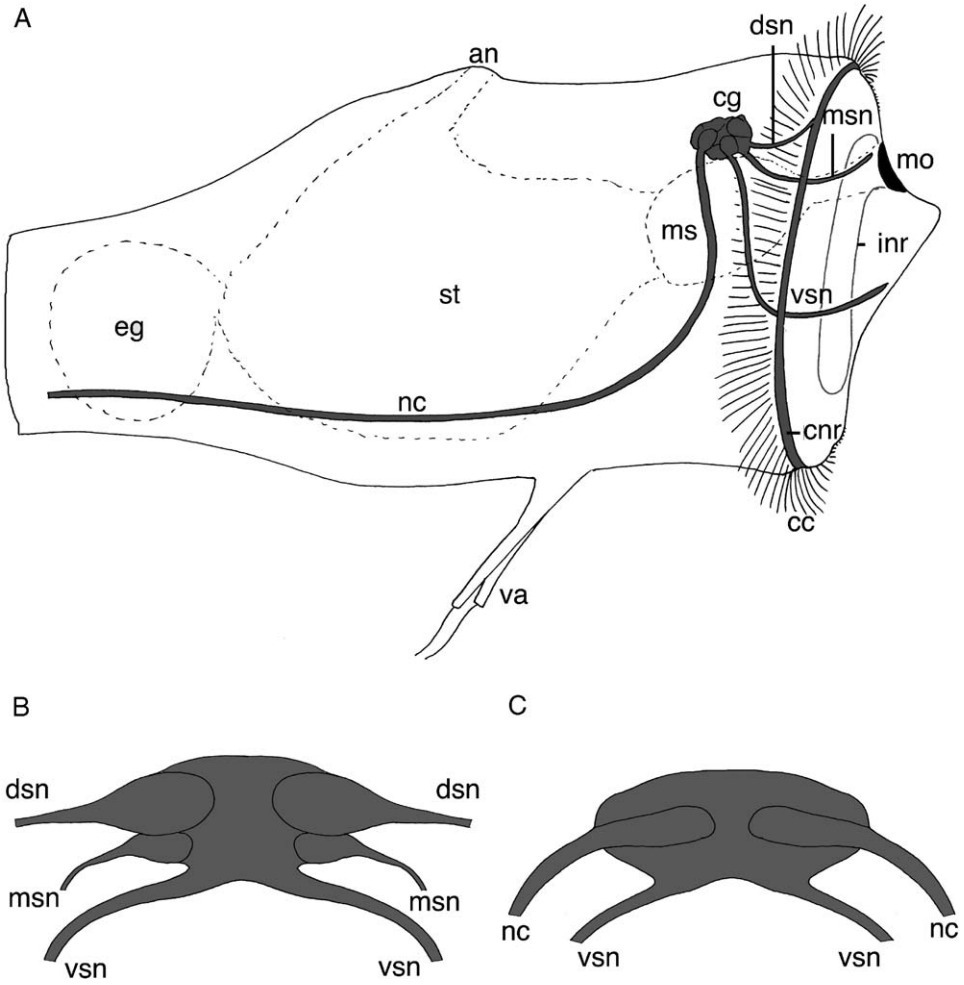

Fig. 5. Schematic illustrations of the serotonergic nervous system of species of *Conochilus*: (A) lateral view of the anterior end showing the general nervous system architecture; (B) frontal view of the cerebral ganglion showing the general distribution of perikarya and axons; and (C) posterior view of the cerebral ganglion. an = anus, cc = coronal cilia, cg = cerebral ganglion, cnr = coronal nerve ring, dsn = dorsal cerebral innervation of the coronal nerve ring, eg = egg, inr = inner nerve ring (horseshoe shape) of the apical field, mo = mouth, ms = mastax, msn = medial cerebral innervation of the apical field, nc = cerebral innervation of the nerve cords, st = stomach, va = ventral antennae, vsn = ventral cerebral innervation of the apical field.

on the coronal ciliature to capture food. In the absence of complete serotonergic innervation, there may be other neurotransmitters that have taken over the stimulatory role (e.g., FMRFamide, see Kotikova et al. 2005). Alternatively, the neural stimulus may be propagated along the ciliated epithelium by other means such as gap junctions, although such junctions are unknown from the rotatory apparatus of other species (see Clément and Wurdak 1991).

Two additional pairs of cerebral perikarya send neurites to a region around the corona in species of *Conochilus*, although these neurites do not appear to innervate the coronal ciliature. The largest diameter neurites (vsn) innervate a region around the ventral mouth margin, and a pair of extremely thin neurites (msn) innervate a region around the dorsal apical field. It is unknown whether either of these neurites contribute directly or indirectly to the inr in the apical region. This ring is especially interesting because it outlines the horseshoe shape of the corona, but is located along the corona's inner margin. Unfortunately, immunoreactivity in the nerve ring was extremely weak making it difficult to locate the source perikarya. It seems likely that this nerve ring may also function in the control of the coronal cilia, but without a more detailed knowledge of its precise location and innervation, this remains speculative.

Aside from direct innervation of the apical field, serotonin is often detected in the nerve cords and pharynges of rotifers (Kotikova et al. 2005). In species of *Conochilus*, a single pair of serotonergic perikarya in the posterior region of the cerebral ganglion send neurites into the presumptive nerve cords. Serotonergic perikarya are not present in a similar position in any of

the previously examined ploimate rotifers (Kotikova et al. 2005), nor in the rotifer *Notommata copeus* (Hochberg, personal observations). However, in most ploimates there is always a pair of cells in the pathway that connects the nerve cords to the cerebral ganglion (Kotikova et al. 2005). Anti-serotonin immunoreactivity is present along the entire length of the nerve cords in species of *Conochilus*, as well as *N. copeus* (Hochberg, personal observation), but in other ploimates, immunoreactivity is lost in the posterior region of the nerve cords (Kotikova et al. 2005).

Different neural patterns are also present in the pharynges of *P. patulus*, *E. dilatata*, and *A. herricki*, although no serotonergic innervation is detected in species of *Conochilus*. Serotonergic control of muscles associated with feeding structures is well documented in other invertebrates such as *Caenorhabditis elegans* (Horvitz et al. 1982) and *Aplysia californica* (Kupfermann and Weiss 1981), where serotonin stimulates contractions of the myoepithelial pharynx and buccal mass, respectively. Similar control of mastax musculature has not been analyzed in rotifers, although neuropharmacological analysis is certainly possible as has been demonstrated previously using neurotransmitters, hormones, and neurotransmitter antagonists (Nogrady and Keshmirian 1986a, b; Nogrady 1987; Gallardo et al. 1999, 2000). The different levels of serotonergic innervation in rotifers may be a function of the number of different muscles and/or hardparts under neural control. How this relates to the lack of immunoreactivity in species of *Conochilus* is unknown.

The present study demonstrates that there are common patterns in the distribution of serotonergic neurons among rotifers that may prove useful in future studies of rotifer phylogeny and evolution. Specifically, species of Flosculariacea as demonstrated herein, as well as species of Collothecacea, occupy phylogenetic positions outside of the Ploima and are therefore uniquely suited to help evaluate trends in nervous system organization within the most heterogeneous clade (see also Sørensen 2002). While some similarities in serotonergic innervation are present between ploimates and species of *Conochilus*, the differences among all species (even within Ploima) are large enough to require further examination of increased rotifer biodiversity.

Acknowledgements

I thank Dr. Martin V. Sørensen and two anonymous reviewers for their constructive criticism of this manuscript. I am 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 through a postdoctoral fellowship to the Smithsonian Marine Station at Fort Pierce, Florida. This is Smithsonian Marine Station at Fort Pierce contribution # 638.

References

Clément, P., Wurdak, E., 1991. Rotifera. In: Harrison, F.W., Ruppert, E.E. (Eds.), Microscopic Anatomy of Invertebrates, Vol. 4. Aschelminthes. Wiley-Liss, Inc., New York, pp. 219–297.

De La Torre, J.C., Surgeon, J.W., 1976. Histochemical fluorescence of tissue and brain monoamines: results in 18 min using the sucrose-phosphate-glyoxylic acid (SPG) method. Neuroscience 1, 451–453.

Gallardo, W.G., Hagiwara, A., Tomita, Y., Snell, T.W., 1999. Effect of growth hormone and γ-aminobutyric acid on *Brachionus plicatilis* (Rotifera) reproduction at low food or high ammonia levels. J. Exp. Mar. Biol. Ecol. 240, 179–191.

Gallardo, W.G., Hagiwara, A., Snell, T.W., 2000. Effect of juvenile hormone and serotonin (5-HT) on mixis induction of the rotifer *Brachionus plicatilis* Müller. J. Exp. Mar. Biol. Ecol. 252, 97–107.

Hay-Schmidt, A., 2000. The evolution of the serotoninergic nervous system. Proc. R. Soc. London B 267, 1071–1079.

Hochberg, R., Litvaitis, M.K., 2000. Functional morphology of the muscles in *Philodina* sp. (Rotifera: Bdelloidea). Hydrobiologia 432, 57–64.

Hochberg, R., Litvaitis, M.K., 2003. Ultrastructural and immunohistochemical observations on the nervous system of three macrodasyidan gastrotrichs. Acta Zool. 84, 171–178.

Horvitz, H.R., Chalfie, M., Trent, C., Sulston, J.E., Evans, P.D., 1982. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. Science 216, 1012–1014.

Keshmirian, J., Nogrady, T., 1987. Histofluorescent labelling of catecholaminergic structures in rotifer (Aschelminthes) in whole mount animals. Histochemistry 87, 351–357.

Keshmirian, J., Nogrady, T., 1988. Histofluorescent labelling of catecholaminergic structures in rotifers (Aschelminthes). II. Males of *Brachionus plicatilis* and structures from sectioned females. Histochemistry 89, 189–192.

Kotikova, E.A., 1995. Localization and neuroanatomy of catecholaminergic neurons in some rotifer species. Hydrobiologia 313/314, 123–127.

Kotikova, E.A., 1997. Localization of catecholamines in the nervous system of Transversiramida. Dokl. Russ. Acad. Sci. 353, 841–843.

Kotikova, E.A., 1998. Catecholaminergic neurons in the brain of rotifers. Hydrobiologia 387/388, 135–140.

Kotikova, E.A., Raikova, O.I., Flyatchinskaya, L.P., Reuter, M., Gustafsson, M.K.S., 2001. Rotifer muscles as revealed by phalloidin-TRITC staining and confocal scanning laser microscopy. Acta Zool. 82, 1–9.

Kotikova, E.A., Raikova, O.I., Reuter, M., Gustafsson, M.K.S., 2004. Musculature of an illoricate rotifer *Asplanchnopus multiceps* as revealed by phalloidin fluroescence and confocal microscopy. Tissue Cell 36, 189–195.

Kotikova, E.A., Raikova, O.I., Reuter, M., Gustafsson, M.K.S., 2005. Rotifer nervous system visualized by FMRFamide and 5-HT immunohistochemistry and

confocal laser scanning microscopy. Hydrobiologia 546, 239–248.

Kupfermann, I., Weiss, K.R., 1981. The role of serotonin in arousal of feeding behavior of *Aplysia*. In: Jacobs, B.L., Gelperin, A. (Eds.), Serotonin Neurotransmission and Behavior. MIT Press, Cambridge, MA, pp. 255–287.

Lindvall, O., Björklund, A., 1974. The gyloxylic acid fluorescence histochemical method: a detailed account of the methodology for the visualization of central catecholamine neurons. Histochemistry 39, 97–127.

Nogrady, T., 1987. Neuropharmacology of rotifer feeding, oviposition and anesthesia. Hydrobiologia 147, 373.

Nogrady, T., Alai, M., 1983. Cholinergic neurotransmission in rotifers. Hydrobiologia 104, 149–153.

Nogrady, T., Keshmirian, J., 1986a. Rotifer neuropharmacology I. Cholinergic drug effects on oviposition of *Philodina acuticornis* (Rotifera, Aschelminthes). Comp. Biochem. Physiol. 83C, 335–338.

Nogrady, T., Keshmirian, J., 1986b. Rotifer neuropharmacology II. Synergistic effect of acetylcholine on local anesthetic activity in *Brachionus calyciflorus* (Rotifera, Aschelminthes). Comp. Biochem. Physiol. 83C, 339–344.

Nogrady, T., Wallace, R.L., Snell, T.W., 1993. Rotifera Biology, Ecology and Systematics. In: Dumont, H.J.F. (Ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, vol. 1. SPB Academic Publishing, The Netherlands, 142pp.

Raineri, M., 1984. Histochemical investigations of Rotifera Bdelloidea. I. Localization of cholinesterase activity. Histol. J. 16, 601–616.

Remane, A., 1929–1933. Rotatoria. In: Bronn, H.G. (Ed.), Klassen und Ordnung des Tierreichs, vol. 4, sect II. Book 1, part 3. Akademische Verlagsgesellschaft, Leipzig.

Ricci, C., Balsamo, M., 2000. The biology and ecology of lotic rotifers and gastrotrichs. Freshwater Biol. 44, 15–28.

Santo, N., Fontaneto, D., Fascio, U., Melone, G., Caprioli, M., 2005. External morphology and muscle arrangement of *Brachionus urceolaris*, *Floscularia ringens*, *Hexarthra mira* and *Notommata glyphura* (Rotifera, Monogononta). Hydrobiologia 546, 223–229.

Segers, H.H., Wallace, R.L., 2000. Phylogeny and classification of Conochilidae (Rotifera, Monogononta, Flosculariacea). Zool. Scr. 30, 37–48.

Sørensen, M.V., 2002. On the evolution and morphology of the rotiferan trophi, with a cladistic analysis of Rotifera. J. Zool. Syst. Evol. Res. 40, 129–154.

Sørensen, M.V., 2005a. Musculature in three species of *Proales* (Monogononta, Rotifera) stained with phalloidin-linked fluorescent dye. Zoomorphology 124, 45–47.

Sørensen, M.V., 2005b. Musculature of *Testudinella patina* (Rotifera: Flosculariacea), revealed with CLSM. Hydrobiologia 546, 231–238.

Sørensen, M.V., Funch, P., Hooge, M., Tyler, S., 2003. Musculature of *Notholca acuminata* (Rotifera: Ploima: Brachionidae) revealed by confocal scanning laser microscopy. Invertebr. Biol. 122, 223–230.

Stemberger, R.S., Gilbert, J.J., 1987. Defenses of planktonic rotifers against predators. In: Kerfoot, W.C., Sih, A. (Eds.), Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, New Hampshire, pp. 227–239.

Wallace, R.L., 1987. Coloniality in the phylum Rotifera. Hydrobiologia 147, 141–155.

Wallace, R.L., Snell, T.W., 2001. Phylum Rotifera. In: Thorp, J.H., Covich, A.P. (Eds.), Ecology and Classification of North American Freshwater Invertebrates, second ed. Academic Press, New York, pp. 195–254.

Weiger, W., 1997. Serotonergic modulation of behaviour: a phylogenetic overview. Biol. Rev. 72, 61–95.