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Musculature of the primitive gastrotrich *Neodasys* (Chaetonotida): functional adaptations to the interstitial environment and phylogenetic significance

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Abstract Gastrotrichs are acoelomate micrometazoans common to marine interstitial environments of sublittoral sediments and exposed sandy beaches. The genus *Neodasys* (Chaetonotida) contains three marine species known from oceans and seas worldwide, and figures prominently in discussions of gastrotrich origins. To gain insight into the phylogenetic position of *Neodasys* and to understand the adaptive significance of muscle anatomy in marine interstitial gastrotrichs, a fluorescent phalloidin-linked marker was used to view the organization of muscles in two species from North America and Australia. Muscular topography of *Neodasys cirritus* from Florida, USA, and *Neodasys* cf. *uchidai* from Queensland, Australia, was found to be similar between species and to basal species of Macrodasyida; muscles were present in circular, longitudinal and helicoidal patterns. Musculature of the midgut region was partially reduced relative to basal macrodasyidan gastrotrichs, but well developed relative to most other chaetonotidan gastrotrichs. In general, muscle patterns in species of *Neodasys* closely correspond with those of other gastrotrichs of similar size and body type, and may therefore reflect a common adaptive solution to the physical demands of the interstitial environment. Results also suggest that reductions in midgut musculature may be functionally related to oviposition and, as such, are probably not homologous with similar reductions of circular muscles in other species of Chaetonotida.

Introduction

Gastrotrichs of the genus *Neodasys* are microscopic (400–600 μm long) and slender worms that inhabit the pore spaces of fine- and medium-grained coastal marine sediments worldwide. The slender body form and multiple adhesive organs equips these interstitial worms for littoral regions with various degrees of water flow, including both low- and high-energy beaches. On sandy, exposed beaches, wave impact and surge produce pulsatile, high laminar flows that percolate through the porous substrate and create “stormy interstices” (Riedl 1971). While little is known of the effects of these interstitial laminar flows on animal behavior (Crenshaw 1980), the morphology of the various species that inhabit the interstitial realm are recognized to display a variety of convergent adaptations to their physical environment (Swedmark 1964).

In general, marine gastrotrichs inhabit a variety of sediment types across a spectrum of environments, from the deep sea and sublittoral fringe to the supralittoral zone and even estuaries. Species of *Neodasys* are present across much of this range, and known from oceans and seas worldwide, including the northeast Atlantic Ocean, Baltic Sea and Mediterranean Sea (Hummon 2001), and most recently from the southeast Pacific Ocean around Australia (present study). This vast geographic range is covered by only three described species that exhibit minimal morphological variation. Adding to this mystery is the long and controversial history that species of *Neodasys* have claimed relative to other marine gastrotrichs.

Since their first discovery, species of *Neodasys* have held a curious taxonomic and phylogenetic position within the Gastrotricha. The peculiarity of *Neodasys* was recognized early on by Remane (1927), who originally classified the genus within the order Macrodasyida based on slender body form and presence of multiple lateral adhesive tubes. *Neodasys* was later transferred to the order Chaetonotida based on the Y-shaped

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orientation of the pharyngeal lumen, an ordinal-level character (Remane 1936). Two suborders were subsequently erected to reflect the anatomical differences between *Neodasys* and other chaetonotidan gastrotrichs (D'Hondt 1971). The suborder Multitubulatina contained species of *Neodasys*, defined by the elongate body, papilla-like lateral adhesive tubes, paired posterior adhesive organs constructed of multiply-merged adhesive glands, and anterior to posterior egg development. Paucitubulatina contained all other chaetonotidans, generally defined by an elaborate cuticle and a single pair of posterior adhesive tubes (D'Hondt 1971). Since then, several ultrastructural studies have contributed information confirming the systematic placement of *Neodasys* within the Chaetonotida (Teuchert and Lappe 1980; Ruppert 1982; Travis 1983).

Early uncertainty over the taxonomic placement of the genus was also reflected in its curious phylogenetic position. Several researchers have considered the genus to contain the most primitive species in the phylum (Remane 1961; Ruppert 1982; Tyler et al. 1980); indeed, characteristics of the adhesive system argue for its placement as a perfect intermediate between the two orders, Macrodasyida and Chaetonotida (see Tyler et al. 1980). Subsequent studies using morphological and molecular evidence have placed the genus at the base of the Chaetonotida (Ruppert 1982; Travis 1983; Hochberg and Litvaitis 2000b) or as a derived clade within the Macrodasyida (Todaro et al. 2003). Morphological characters that argue for a potentially basal placement include the apparently primitive topology of the nervous system (Travis 1983) and aspects of muscle ultrastructure (Ruppert 1991). A recent molecular analysis of Gastrotricha using partial SSU (small subunit) 18S rRNA and constrained to reflect traditional morphological phylogenies placed *Neodasys* at the base of the Chaetonotida, with no statistically significant difference from unconstrained trees (Todaro et al. 2003). This latter confirmation of the early divergent nature of *Neodasys* has significance toward a greater understanding of gastrotrich evolution, particularly the plesiomorphic condition of the muscular system and the evolution of body form in the interstitial environment.

Efforts to explore gastrotrich anatomy and reveal functional and evolutionary insights have utilized classical histology, electron microscopy (EM), and, most recently, fluorescence microscopy. In particular, fluorescent phalloidin stains have revealed a great diversity in muscle organization that was not evident at the ultrastructural level (Hochberg and Litvaitis 2001a, 2001b, 2001c, 2001d). In general, the topography of the muscular system is considered to be very conservative in phylogenetic terms and, therefore, provides characters for exploring phylogenetic relationships (Ruppert 1991; Hochberg and Litvaitis 2001a). The aim of this research is to provide additional insights on the muscle anatomy of *Neodasys* beyond EM descriptions, using both conventional epifluorescence microscopy and confocal laser scanning microscopy (CLSM), and to explore the pos-

sibility that the muscle patterns of *Neodasys* are consistent with our understanding of gastrotrich phylogeny. In addition, knowledge of the musculature in species of *Neodasys* may provide clues about the adaptive significance of particular muscle orientations in gastrotrichs and the effects of natural selection on locomotion and, hence, muscle organization in interstitial animals.

Materials and methods

Collection

Two species of *Neodasys* were examined. Specimens of *Neodasys* cf. *uchidai* ($n = 14$) were collected from Cylinder Beach, a high-energy, exposed beach located on North Stradbroke Island, Queensland, Australia ($27^{\circ}26'S$; $153^{\circ}32'E$). Type material is deposited at the Queensland Museum, Australia. Animals were common in medium grain sand at the low tide level. Specimens of *Neodasys cirritus* Evans, 1992 ($n = 21$) were collected from the low tide region of Green Turtle Beach, a low-to medium-energy beach on South Hutchinson Island, Fort Pierce, Fla., USA ($27^{\circ}25.177'N$; $80^{\circ}16.368'W$). Gastrotrichs were extracted from the sediment using an anesthetization–decantation technique with 7.5% magnesium chloride (Pfannkuche and Thiel 1988).

Muscle staining and observation

For whole-mount muscle staining, gastrotrichs were relaxed for 15 min in 7% magnesium chloride solution prior to fixation in 5% formaldehyde in 0.1 M PBS (1 h). Animals were rinsed in 0.1 M PBS, permeabilized for 1 h in 0.2% Triton X-100 in PBS, stained 50 min with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, Ore.) and rinsed in PBS before mounting with Gel/Mount (Biomed). Australian specimens were viewed on an Olympus BH2 conventional epifluorescence microscope equipped with a SPOT digital camera at the Centre for Microscopy and Microanalysis, University of Queensland. American specimens were examined using a Nikon Eclipse E800 compound microscope equipped with a Biorad Radiance 2000 laser system at the Smithsonian Marine Station at Fort Pierce. Series of 0.05- μ m optical sections with maximum intensity projection along the z -axis were made into one two-dimensional image with greater focal depth. Measurements of gastrotrichs were performed with an ocular micrometer, and the positions of particular organs are expressed in reference to percentage body units (total body length being 100 U measured from tip of head to tip of posterior adhesive tubes).

Additional specimens of *N. cirritus* were prepared for scanning electron microscopy (SEM) following the protocol of Hochberg and Litvaitis (2000a) using hexamethyldisilazane and viewed on a JEOL 6400 V at the Smithsonian Marine Station at Fort Pierce, Florida.

Results

Species of *Neodasys cirritus* and *N. cf. uchidai* are 450–600 μm long and readily distinguishable by the morphology of the head and caudal regions and the presence of red-pigmented Y-cells in *N. cirritus* (Figs. 1, 2C; Remane 1961; Evans 1992). Both species possess very small lateral adhesive papillae that, when attached to the substratum, can prevent unintentional movement due to water flow (Fig. 1B, C). The compact, acoelomate body plan of these animals means that all muscles are in close association with each other, the outer epidermis, inner pharyngeal epithelium and midgut epithelium (see Ruppert and Travis 1983); only a thin basal lamina separates the muscles from other organs (Ruppert 1991). Phalloidin staining viewed with epifluorescence microscopy and CLSM revealed muscles in three orientations: circular, helicoidal and longitudinal. Cross-striations were evident in all longitudinal muscles. Myoepithelial radial muscles were visualized within the pharynx of *N. cirritus* (Fig. 2A, D). Due to differences in resolution

using CLSM and conventional epifluorescence microscopes, the muscles of *N. cirritus* (examined with CLSM) will be presented in greater detail than those of *N. cf. uchidai* (examined with conventional epifluorescence), and only differences from the former will be noted.

Splanchnic circular muscles represent the innermost muscle layer lining the pharynx, followed exteriorly by longitudinal muscles and helicoidal muscles (Figs. 2A, B, E, 3C). This sequence of layers is characteristic of the pharynx only. Along the midgut (i.e. central body region), the sequence from midgut to body wall is longitudinal, circular and helicoidal (Fig. 2B, E). In the lateral body regions of the midgut, only longitudinal muscles surrounded by somatic circulars are present. In these regions, the somatic circular muscles represent lateral branches of the splanchnic component.

Splanchnic circular muscles line the entire pharynx and appear as a series of individual, compact hoop-like rings of approximately 1 μm diameter (Figs. 2E, 3B, C). Splanchnic circular muscles line most of the midgut from the pharyngeointestinal junction to the anus, though in some regions of the midgut the muscles are

Fig. 1A–D Two species of *Neodasys*. **A** Whole-mount specimen of *N. cf. uchidai*; **B** magnified view of anterior end in dorsal view; **C** magnified view of posterior end displaying elasticity of body wall because of movement during adhesion to the substratum. **D** SEM of *N. cirritus* in ventral view

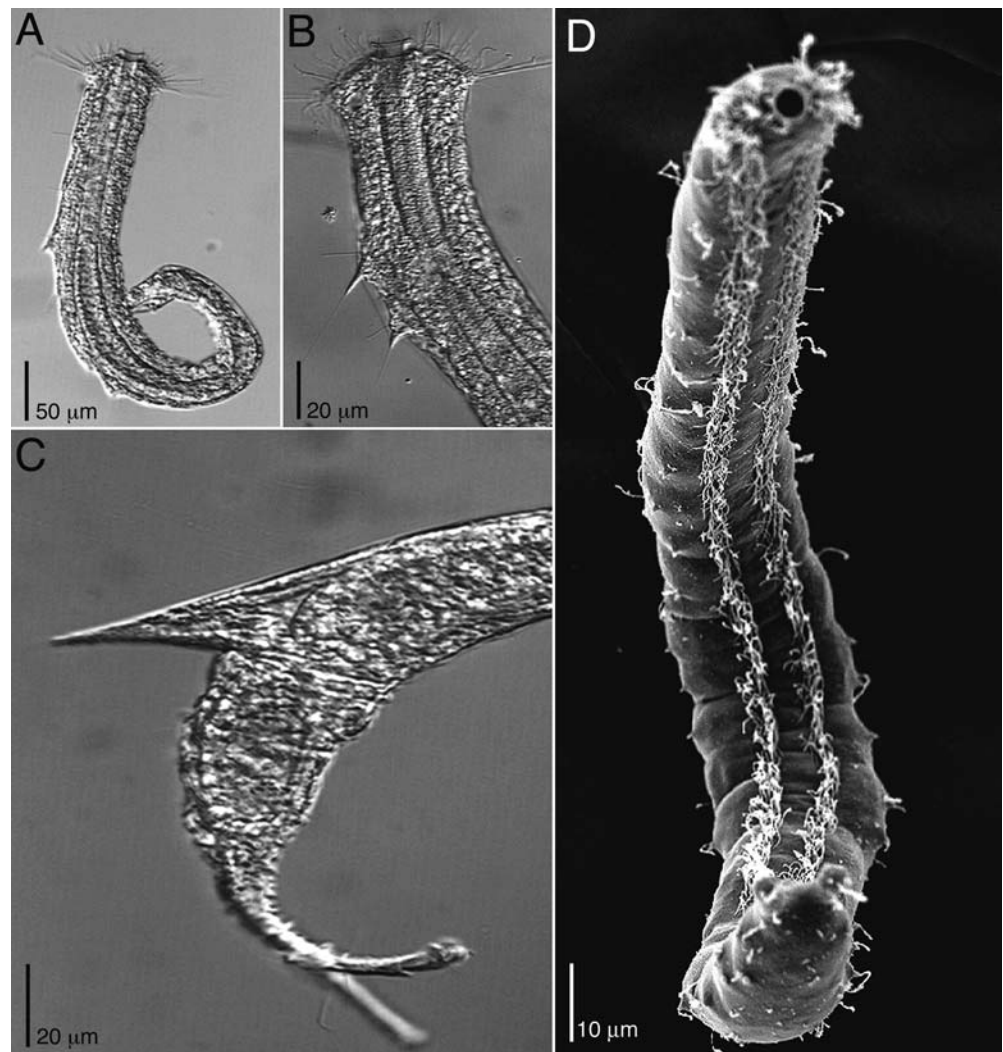
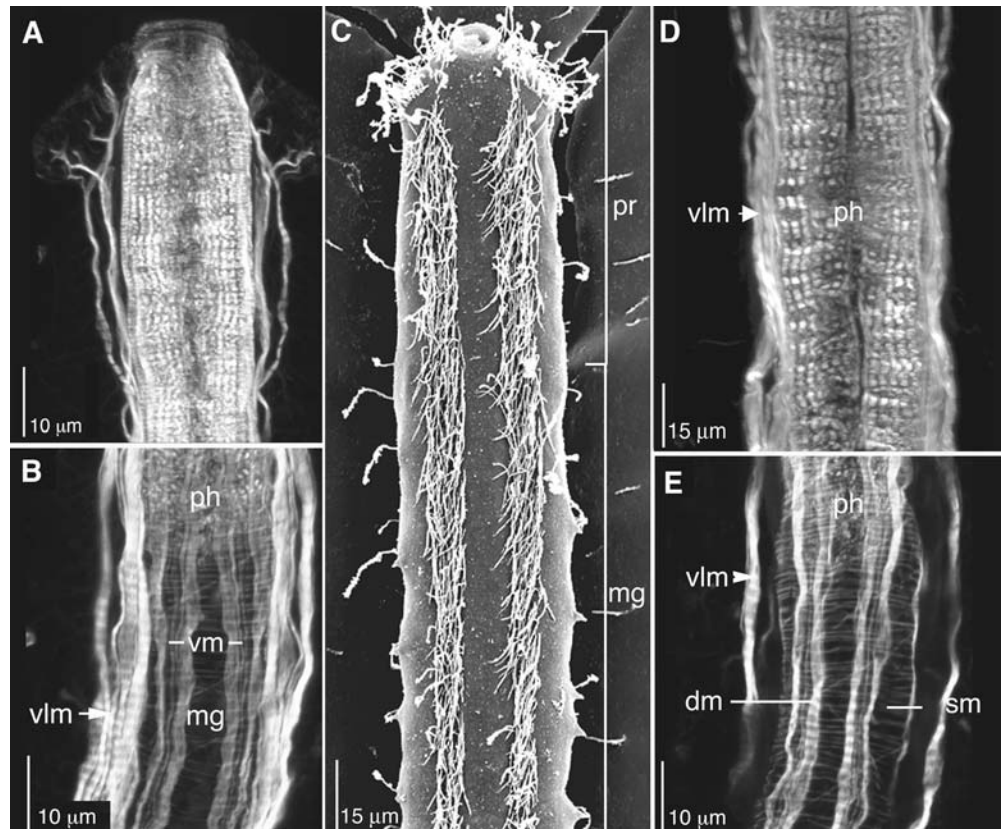


Fig. 2A–E *Neodasys cirritus*. **A** Z-projection of anterior end displaying pharynx and mouth (0.05 μm \times 75 optical sections); **B** z-projection of pharyngeointestinal junction in ventral view. Note the absence of somatic circular muscles around the ventrolateral longitudinal muscles (0.05 μm \times 60 optical sections); **C** SEM of anterior end in ventral view; **D** z-projection of pharynx around midlength (0.05 μm \times 10 optical sections); **E** z-projection of pharyngeointestinal junction in dorsal view (0.05 μm \times 55 optical sections) (*dm* dorsal longitudinal muscles; *mg* midgut region; *ph* pharynx; *pr* pharyngeal region; *sm* splanchnic circular muscles; *vlm* ventrolateral longitudinal muscles; *vm* ventral longitudinal muscles)



considerably more spaced apart than present on the pharynx. In general, splanchnic circular muscles of the midgut are extremely thin (0.5–1 μm diameter). From approximately U40 to U55, splanchnic circular muscles are entirely absent from the midgut (Figs. 4A, B, 5B, see also Fig. 7B). Circular muscles begin again around U50–U55, consisting first of splanchnic circular muscles on the midgut and several microns posterior, somatic circular muscles in the lateral body regions (Figs. 4A, B, 5). Splanchnic circular muscles enclose both maturing eggs and the frontal organ (seminal receptacle) (Figs. 4C, D, 5, see also Fig. 7B). The somatic circular muscles enclose the large ventrolateral longitudinal muscle blocks on either side of the midgut, and are distinctly thicker (2–3 μm) than the splanchnic muscles of the midgut (Figs. 4C, D, 5B).

Longitudinal muscles are arranged around the circumference of the digestive tract, inserting anteriorly on the pharynx or body wall and posteriorly in the caudal furca or along the body midline. Four principal sets of longitudinal muscles are present in dorsal, lateral, ventrolateral and ventral positions (Figs. 2, 3, 4). Dorsally, two to three pairs of longitudinal muscles span the length of the body, inserting anteriorly on the pharynx and posteriorly at the body midline or in the caudal furca. Only two pairs of dorsal longitudinal muscles were observed in *N. cf. uchidai*, though this may have been the result of poor resolution and staining in some specimens. Along the midgut of

N. cirritus, the middle and lateral muscles of the three-part set were thicker (2–3 μm) than the central pair (1 μm), in close proximity to the ventrolateral longitudinal muscles, and often indistinguishable from the latter muscles if whole-mount specimens were not oriented in the dorsal–ventral plane. A single pair of lateral longitudinal muscles extends from the pharynx to the caudal end, but is difficult to follow due to the overlapping ventrolateral longitudinal muscles. The ventrolateral longitudinal muscle bands actually represent a block of several muscle fibers (five to eight muscles) that run in parallel along the length of the body. As the principal locomotory muscles in the body, the bilateral muscle blocks are also the largest diameter muscle units (6–8 μm). In several regions along the length of the body, individual muscle fibers of the ventrolateral muscle block often deviate from the anterior–posterior orientation of the muscle unit. For example, at the anterior end of the pharynx, individual muscle fibers depart from the muscle block and follow the contour of the head, inserting on the lateral body wall or mouth margin (Figs. 2A, 3B, C, 6). Slight, but consistent, differences between *N. cirritus* and *N. cf. uchidai* were noted, though individual variation is also present within each species (Fig. 6). Along the midgut, individual muscle fibers often diverge when the animal is in a contracted position, and may bend and twist independently of other muscle fibers in the ventrolateral muscle block (Fig. 4C).

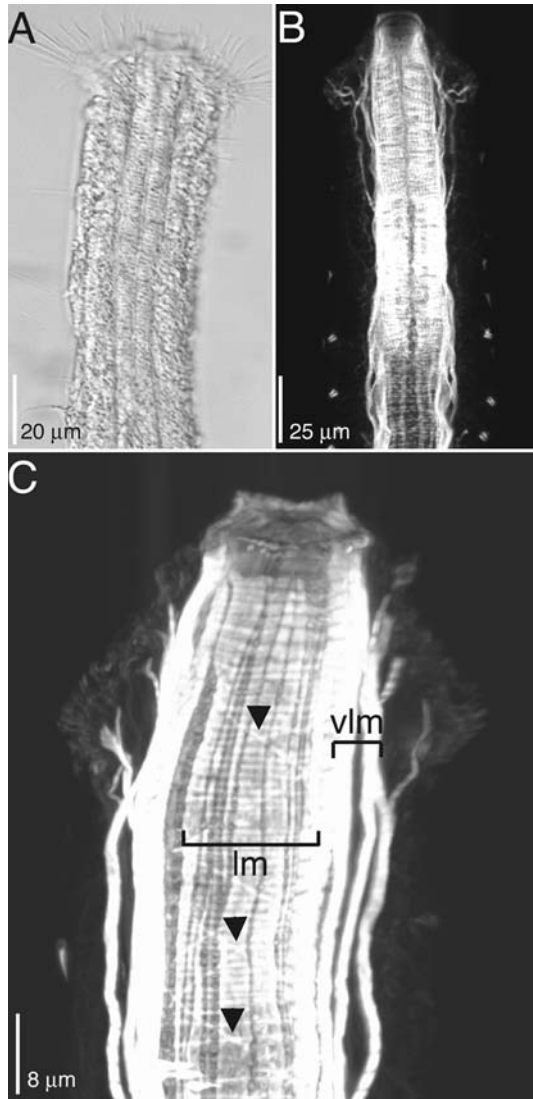


Fig. 3A–C Two species of *Neodasys*. **A** Light micrograph of anterior end of *N. cf. uchidai*; **B** epifluorescent view of stained specimen of *N. cf. uchidai*, ventral view of pharynx; **C** z-projection of anterior end of *N. cirritus*, dorsal view (0.05 $\mu\text{m} \times 43$ optical sections) (arrowheads point to helices; *lm* longitudinal muscles; *vlm* ventrolateral longitudinal muscles)

Ventromedial to the main muscle block are two pairs of thin ventral muscles that line the pharynx and midgut. In *N. cf. uchidai*, only a single pair of ventral longitudinal muscles was observed.

Helicoidal muscles wrap around the circular and longitudinal muscles of the pharynx and midgut (Figs. 2B, E, 3C). Helicoidal muscles do not line the lateral body regions (i.e. somatic circular muscles or ventrolateral longitudinal muscles). Helicoidal muscles are extremely thin (ca. 1 μm) and difficult to visualize in the Australian *N. cf. uchidai*. Crossed helices are present as a quadruple helix made up of four individual muscle bands. Helicoidal muscles appear to begin close to the mouth and end at approximately U50–U60.

Discussion

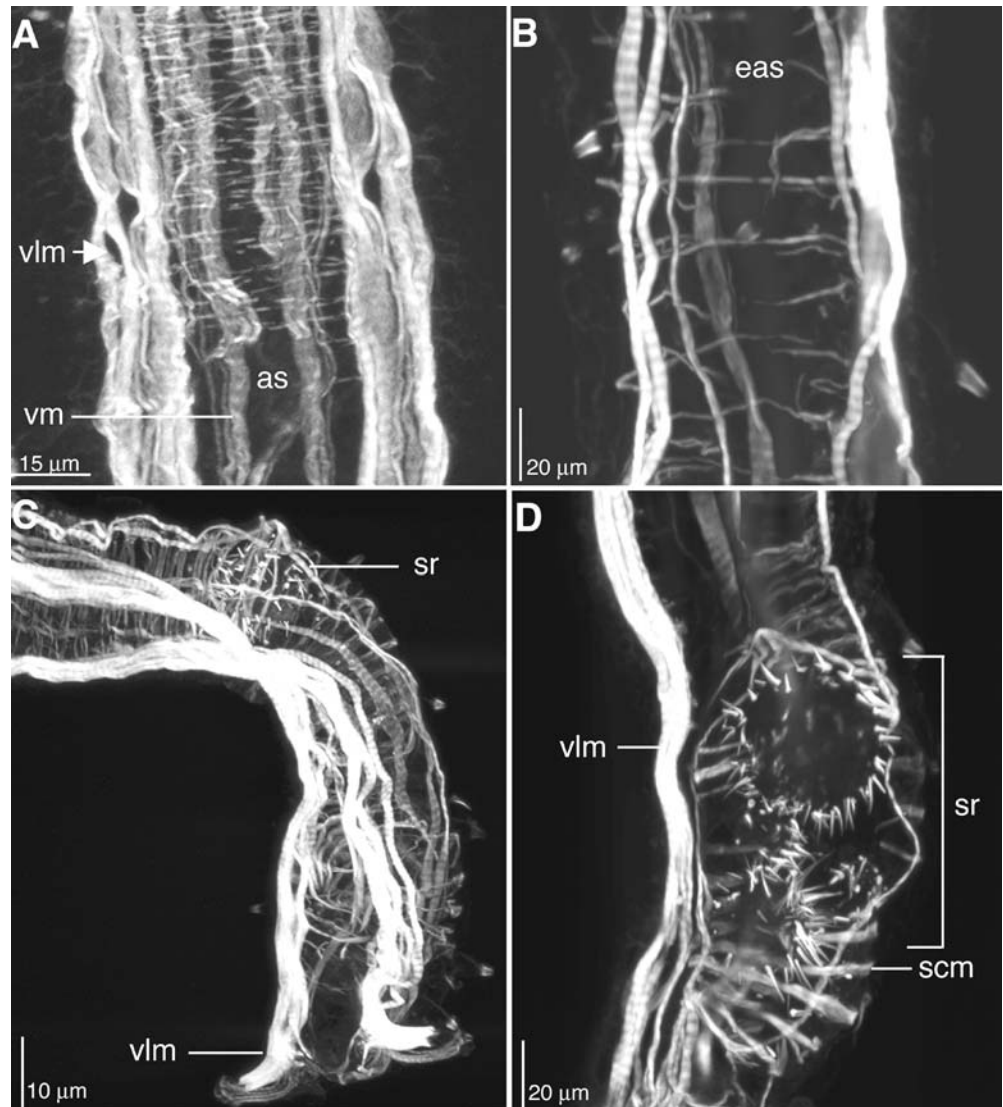
Morphological observations

The interstitial pore system of exposed sandy beaches is recognized as a dynamic environment and one regulated by such physical factors as water flow and granularity (e.g. grain size, porosity, permeability), among other factors (see Giere et al. 1988). The force and regularity of water movement within the three-dimensional pore system will, in part, determine the dynamism of other variables such as the chemistry of pore water and the depth of the redox-potential discontinuity layer. Together, these characteristics determine the size and movement patterns of organisms that can inhabit this unique, but globally expansive environment.

Species of the genus *Neodasys* are restricted to the marine interstitial sand environment. As in most other gastrotrichs, the locomotory behavior of these species is dependent on the size of the interstitial spaces. Tightly packed sand grains with little pore space, such as mud, restrict movement to those animals that are either extremely narrow (e.g. nematodes) or can burrow using appendages (e.g. copepods). Both types of animals possess thick cuticles that prevent damage by abrasion of tightly packed sediments. Alternatively, very porous sand with high permeability, such as sediments characteristic of some mid- to high-energy exposed sandy beaches, contains species with a range of body sizes and body-wall structures; many of these species rely on cilia and adhesive organs to move around and maintain their position within the pore system. Gastrotrichs utilize both cilia and adhesive organs; however, it is the complex system of muscles that enables gastrotrichs to feed, change directions during ciliary gliding, escape from harmful stimuli (e.g. noxious chemicals, predators, etc.), and engage in the various positions characteristic of precopulatory searching and mating behavior (see Ruppert 1978).

Species of *Neodasys* are relatively slow-moving gastrotrichs compared to other marine species, but can rapidly contract their body to nearly one-third original size when provoked (Ruppert and Travis 1983). Maintenance of a disturbing stimulus might also elicit a rearward escape response involving an inchworm-like progression of posterior extension followed by longitudinal contraction of the anterior body region. Based on the speed and extent of body contraction, the muscles implicated in these behaviors are likely the large ventrolateral longitudinal muscles. These paired muscles are, in fact, blocks of multiple, cross-striated muscle bands. When partially or fully contracted, the individual bands can be seen to separate from the muscle block, indicating that the blocks are not bound by an external lamina (Fig. 4C). Interestingly, reextension of the anterior end after disturbance is comparatively slow, and Ruppert and Travis (1983) speculate that this is likely due to the general lack of circular muscles in the trunk

Fig. 4A–D *Neodasys cirritus*. Z-projections of f-actin stained specimens: **A** beginning of midgut region, where circular muscles are absent ($0.05\ \mu\text{m}\times 20$ optical sections); **B** midgut region slightly more posterior, where circular muscles begin again ($0.10\ \mu\text{m}\times 50$ optical sections); **C** ventrolateral view of posterior end revealing presence of seminal receptacle (frontal organ) ($0.10\ \mu\text{m}\times 65$ optical sections); **D** lateral view showing seminal receptacle with numerous spermatophores containing non-specifically stained sperm ($0.05\ \mu\text{m}\times 60$ optical sections) (*as* midgut region lacking circular muscles; *eas* posterior end of region where circular muscles are absent; *scm* somatic circular muscles; *sr* seminal receptacle; *vlm* ventrolateral longitudinal muscles; *vm* ventral longitudinal muscles)



region [based on transmission electron microscopy (TEM) observations] to provide an antagonistic force to longitudinal contraction. In the absence of circular muscles, alternative modes of reextension, such as ciliary movement or elastic rebound of the cuticle and hydrostatic internal organs (e.g. Y-cells), would be necessary. The current findings in this study show that somatic circular muscles are indeed absent from the anterior midgut region and may therefore explain the relatively slow mode of reextension in these species (Fig. 2B, E).

Despite an absence of somatic circular muscles from the anterior midgut region, somatic circular muscles are still present on the posterior midgut (Fig. 4C), and splanchnic circular muscles line most of the digestive tract (Fig. 4A). These findings contradict previous observations that circular muscles are entirely absent from the trunk of *Neodasys* (Ruppert and Travis 1983; Travis 1983). As in most macrodasyidan gastrotrichs, the circular muscles surround the longitudinal muscles and are in turn enclosed by the helicoidal muscle bands. Only the ventrolateral longitudinal muscles, which are

surrounded by the somatic circular muscles, are free of helicoidal muscles. The helices, which appear to be similarly arranged in all gastrotrichs investigated (Hochberg and Litvaitis 2001a, 2001b, 2001c, 2001d, 2003), might also have similar functions among species, though these functions are not yet known. Inferring function from form is particularly difficult with this group of muscles because of their small size ($1\ \mu\text{m}$ diameter) and layering, and, to date, there is no direct evidence that these helices play a role in gastrotrich movement (but see speculations of Hochberg and Litvaitis 2001b, 2001d).

Twisting and bending movements, characteristic of both stationary and gliding gastrotrichs, likely involve the same muscles used for rapid shortening, in addition to other muscles of the trunk region (see also Hochberg and Litvaitis 2001b). Unilateral longitudinal muscle contraction will lead to generalized bends in the dorsal, lateral and ventral planes, but may be made more localized due to antagonistic contractions of particular sets of organs (e.g. gut tube, Y-cells) or muscles (e.g.

opposing longitudinal muscles, splanchnic and/or somatic circular muscles). Axial twisting, often observed while an animal is stationary, may be produced by combined lateral and dorsal bending resulting from contractions of lateral, ventrolateral and dorsal longitudinal muscles. In addition, regional subdivisions of the circular musculature may also account for differences in movement patterns relative to other species. For example, the absence of anterior somatic circular muscles may explain the relatively slow reextension of the body during longitudinal contraction, while dorsoventral flexion may be comparatively rapid, because both sets of longitudinal muscles are bound by splanchnic circular muscles that may aid in antagonism. Splanchnic circular muscles may also function in the digestive processing of prey items, namely diatoms. In the absence of a ciliated midgut epithelium, circular muscles are required to generate a propulsive force to move food items down the digestive tract and dispose of fecal material such as diatom shells. This might explain the presence of splanchnic circular muscles in all macrodasyidans and *Neodasys*, and the general absence of circular muscles from chaetonotidan gastrotrichs, which feed on smaller prey such as bacteria and may therefore rely on general body movements to propel the food posteriorly.

Despite possessing areas of reduced circular musculature, which might constrain locomotory movements, species of *Neodasys* have retained a well-developed circular musculature in the trunk region that may aid in reproduction and oviposition. For example, basal species of Gastrotricha generally lack a muscular coat around their reproductive organs (e.g. species of *Dactylopodola*, Hochberg and Litvaitis 2001a) as opposed to more derived species (e.g. species of *Macrodasys*; Ruppert 1978). The apparent function of a muscular coat around such organs as the male caudal organ and seminal receptacle (i.e. frontal organ) is to force auto-sperm and allosperm to copulatory partners or maturing eggs, respectively. In the absence of a muscle coat, the mechanism of sperm transfer is unclear, but may, in part, be driven by peristaltic contractions of the splanchnic circular muscles. These muscles may also function in oviposition. Egg development and maturation in species of *Neodasys* occur from anterior to posterior (Fig. 7A). As the most mature egg gradually enters the posterior body region containing circular muscles (Figs. 5, 7), it encounters the non-muscular, sperm-containing seminal receptacle. Once fertilized, the egg can be oviposited through the body wall by contractions of the overlying circular muscles. Whether the egg is squeezed through individual circular muscles as occurs in macrodasyidan gastrotrichs (Fig. 8) or is pushed anteriorly toward the region devoid of circular muscles is unknown. However, the relatively thin cuticle of species of *Neodasys* (see Rieger and Rieger 1977) may in part explain why mature eggs are initially displaced toward the circular muscle zone to provide a stable cellular layer and avoid premature rupture of the body wall.

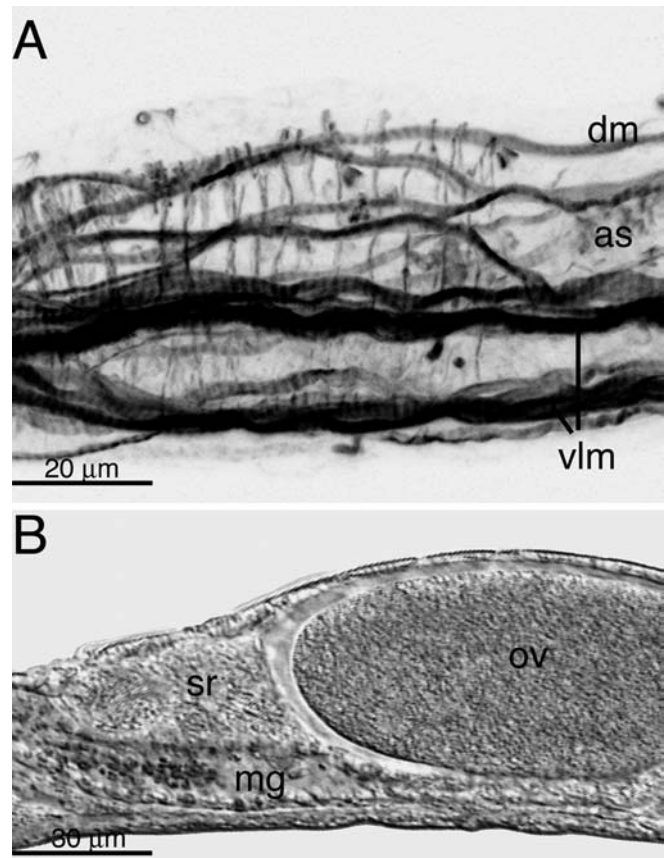


Fig. 5A, B *Neodasys* cf. *uchidai*. **A** Epifluorescent view of stained specimen showing the posterior trunk region containing seminal receptacle and maturing egg; **B** light micrograph of the same region of the trunk (*as* midgut region lacking circular muscles; *dm* dorsal longitudinal muscle; *mg*, midgut; *ov* mature egg; *sr* seminal receptacle; *vlm* ventrolateral longitudinal muscle)

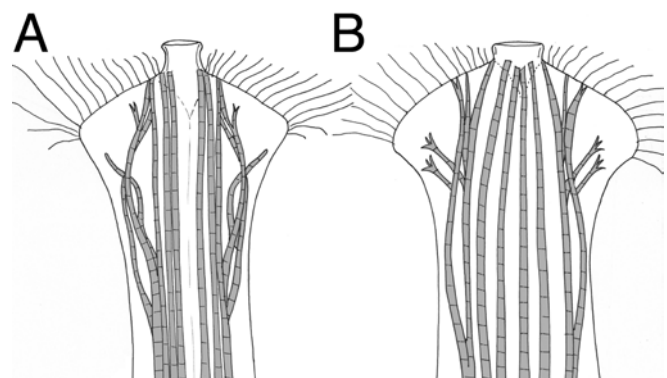
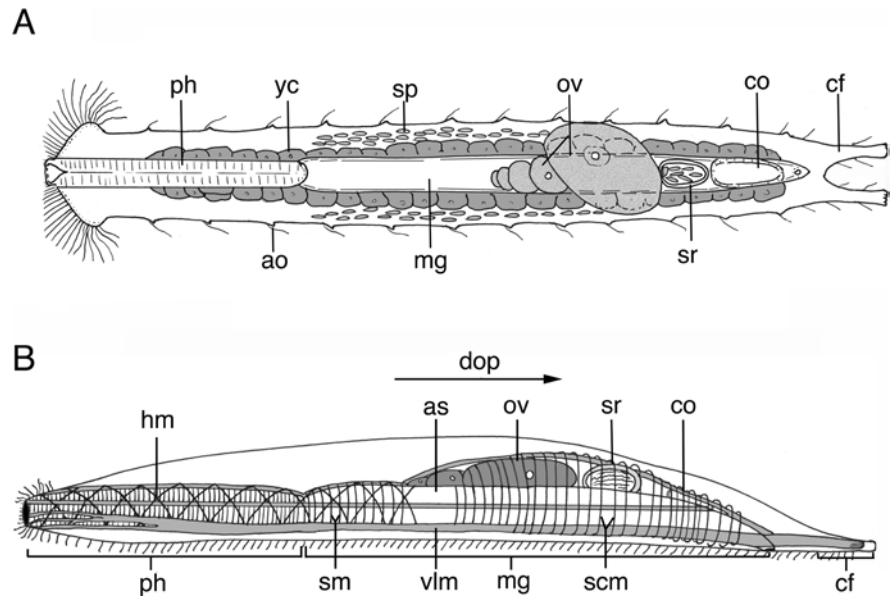


Fig. 6 *Neodasys* cf. *uchidai* and *N. cirritus*. Schematic illustration of longitudinal muscle orientations in anterior body region, dorsal view

Phylogenetic interpretation

Phylogenetic analyses of morphological characters place *Neodasys* at the base of the Chaetonotida in recognition of the plesiomorphic characters retained within the lineage: elongate body, simple cuticle, cross-striated

Fig. 7A, B *Neodasys cirritus*. **A** Schematic representation of general morphological features visible under the light microscope; **B** schematic view of muscles in lateral body view (ao adhesive organ; as midgut region lacking circular muscles; co caudal organ; cf caudal furca with adhesive glands; dop direction of egg development; hm helicoidal muscle; mg midgut; ov egg; ph pharynx; sm splanchnic circular muscle; scm somatic circular muscle; sp sperm; sr seminal receptacle; vlm ventrolateral longitudinal muscle block; yc y-cell)



muscles, multisarcomerous myoepithelial pharynx, multiple adhesive organs, and absence of releaser glands within the adhesive organs (see Tyler et al. 1980; Travis 1983; Hochberg and Litvaitis 2000b). In the light of the present work, it is unquestionable that species of *Neodasys* also share several similarities in muscle organization with macrodasyidan gastrotrichs, and display a degree of complexity that was previously thought absent from most chaetonotidan gastrotrichs. In particular, it was assumed in earlier studies that species of *Neodasys*, as evolutionary intermediates between Macrodasida and Chaetonotida–Paucitubulatina, might display an intermediate muscular organization, and when TEM failed to show circular muscles on the midgut (Travis 1983), observations led to the hypothesis that reduced musculature is a synapomorphy of Chaetonotida. However, to date, there is no evidence to suggest that the absence of circular muscles from a particular body region of *Neodasys* (e.g. anterior somatic circular muscles) is homologous with the condition present in any species of Paucitubulatina. For example, *Xenotrichula*

intermedia (Xenotrichulidae) lacks somatic circular muscles in the anterior midgut region, *Draculiciertia tessalata* only possess highly modified somatic circular muscles (Xenotrichulidae), and several species of Chaetonotidae lack circular muscles altogether (Hochberg and Litvaitis 2003). Unfortunately, our knowledge of evolutionary relationships within these taxa is poor, and it would be premature to speculate about the plesiomorphic condition of their muscular systems, let alone the existence of a potential intermediate form linking *Neodasys* with species of either of the other two lineages.

Given the morphological and molecular evidence for the basal position of *Neodasys* (Hochberg and Litvaitis 2000b; Todaro et al. 2003), the present work, coupled with recent phylogenetic analyses, strongly suggests that a fully developed muscular system is plesiomorphic for Gastrotricha. The primitive organization is likely to have existed as follows: pharynx lined with inner splanchnic circular muscles, longitudinal muscles and helicoidal muscles, and midgut lined with inner longitudinal muscles followed by splanchnic circular muscles and helicoidal muscles. The lateral regions of the body contained the ventrolateral longitudinal muscle blocks enclosed by somatic circular muscles. And, while evolutionary changes in muscular organization have occurred in several lineages throughout Macrodasida and Chaetonotida (see Hochberg and Litvaitis 2001a, 2003), it is perhaps surprising that similar changes are not characteristic of species of *Neodasys*, despite peculiarities at the ultrastructural level (Ruppert 1991). Still, investigations such as this only reflect a small amount of the known gastrotrich diversity, and must be considered a first step toward understanding the evolution of form and function in interstitial gastrotrichs.

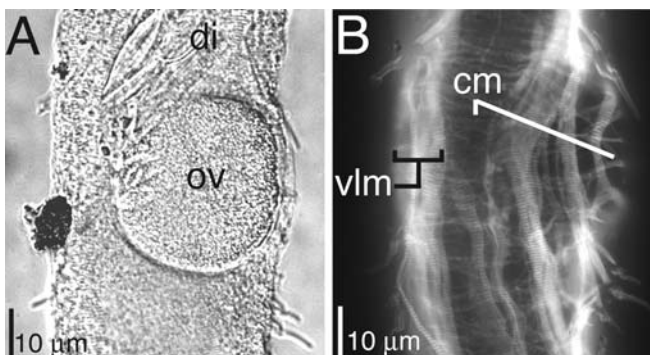


Fig. 8A, B *Dactylopdola baltica* (Macrodasida) with egg. **A** Light micrograph of midgut region and large egg; **B** epifluorescent view of midgut region stained with phalloidin (cm circular musculature; di diatom; vlm ventrolateral longitudinal muscle block; ov egg)

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