

The musculature of three species of gastrotrichs surveyed with confocal laser scanning microscopy (CLSM)

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Abstract

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The muscular system of gastrotrichs consists of circular, longitudinal and helicoidal bands that when analysed with confocal laser scanning microscopy, provide new insights into their functional organization and phylogenetic importance. We therefore undertook a comparative study of the muscle organization in three species of Gastrotricha from the orders Macrodasyida (*Paradasys* sp., Lepidodasyidae; *Turbanella* sp., Turbanellidae) and Chaetonotida (*Polymerurus nodicaudus*, Chaetonotidae). The general muscle organization of the marine interstitial macrodasyidans, *Paradasys* and *Turbanella*, not only confirms earlier observation on other species but also adds new details concerning the organization and number of helicoidal, longitudinal and other muscle bands (e.g. semicircular band). The freshwater, epibenthic–epiphytic chaetonotid, *Polymerurus nodicaudus*, has a similar muscular organization to other species of Chaetonotidae, especially species of *Chaetonotus*, *Halichaetonotus* and *Lepidodermella*. Perhaps unique to *Polymerurus* is the combined presence of an unbranched *Rückenhautmuskel* (also in *Halichaetonotus* and *Lepidodermella*) and a specialized dorsoventral caudal muscle, which flank the splanchnic component of the longitudinal muscles (only in *Chaetonotus* and *Lepidodermella*). This combination, together with the presence of splanchnic dorsoventral muscles, known only in Xenotrichulidae, implies a unique phylogenetic position for *Polymerurus*, and indicates a potential basal position of this taxon among the Chaetonotidae studied so far (i.e. *Aspidiophorus*, *Chaetonotus*, *Halichaetonotus* and *Lepidodermella*).

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Introduction

Gastrotrichs are microscopic animals living epi- or endobenthically in marine and freshwater environments. The subtaxon Macrodasyida is almost entirely marine whereas the subtaxon Chaetonotida occurs in the sea and in fresh water. Gastrotrichs move primarily using the locomotory cilia on their ventral surface, but can aid movements by muscular action. The muscular system of gastrotrichs, under examination by conventional light microscopy (Remane 1929, 1935–36) and transmission electron microscopy (Teuchert 1974,

1977; Teuchert and Lappe 1980; Ruppert 1982, 1991; Travis 1983), appears to be composed, from the outside to the inside, of somatic circular muscles, four to 12 pairs of longitudinal bands that are distinctly separate from each other, and a series of circular (splanchnic) bands that surround the digestive tract. One or more of these muscles may be missing in some species (Remane 1935–36; Travis 1983). The macrodasyidans have an additional pair of longitudinal ventrolateral bands in a somatic position that aid in specific body movements such as bending, changes in direction, and backward or forward creeping (Teuchert 1978; Hochberg

and Litvaitis 2001a,b). The chaetonotidans possess dorsoventral bands, the specific function of which is unknown (Ruppert 1975; Hochberg and Litvaitis 2001c, 2003).

The recent application of fluorochrome-labelled phalloidin, which interacts with the intercellular F-actin (Wulf *et al.* 1979), not only allows an overall view of the muscular system, but also of some details that escape traditional microscopical techniques, reconstructing the course of muscles from serial sections. In particular, the use of fluorescently labelled phalloidin in wholemount specimens prepared for epifluorescence has revealed the complete muscular organization of gastrotrichs and provided important information on their phylogenetic relationships (Hochberg and Litvaitis 2001a,b,c,d, 2003; Hochberg 2005). Furthermore, epifluorescence has allowed the detection of an autoapomorphic trait for the entire phylum, the so-called ‘muscular double helix’: muscular bands with a peculiar, right- and left-hand helicoidal orientation located around the pharynx and intestine (Hochberg and Litvaitis 2001d). As a result of their extremely thin diameter (< 1 µm) and their unusual orientation along the digestive tract, these muscles have been overlooked in both conventional light and transmission electron microscopy. Nevertheless, conventional epifluorescence microscopy has some restrictions, such as limitations of the visual field, photobleaching and low resolution along the *z*-axis, that can be overcome using confocal laser scanning microscopy (CLSM) (see Paddock 1999; Hochberg 2005).

In this context, the musculature of two marine interstitial macrodasyidans (*Paradasys* sp., Lepidodasyidae and *Turbanella* sp., Turbanellidae) and a freshwater epibenthic-epiphytic chaetonotidan (*Polymerurus nodicaudus*, Paucitubulatina, Chaetonotidae) is described using CLSM. The choice of macrodasyidans is based on the need to gain clues about inter- and intrageneric variation (e.g. *Turbanella*) and the need for new information where knowledge of the muscular system is totally lacking (e.g. *Paradasys*). The choice of chaetonotid gastrotrich (*Polymerurus*) is based on the unique structure of the caudal furca in this genus, for which no information is currently available.

Materials and Methods

Paradasys sp. and *Turbanella* sp., both probably new to science, were collected in March 2005 in the Persian Gulf, along the coast of Kuwait (Todaro *et al.* 2005a) whereas

Polymerurus nodicaudus was found in May 2005 in a small pond near Modena (Italy). Marine taxa were extracted from the sediment using the seawater-ice extraction method and relaxed for 10 min in a 7% MgCl₂ solution (Uhlir *et al.* 1973; Pfannkuche and Thiel 1988); freshwater gastrotrichs were extracted and relaxed using a 1% MgCl₂ solution. Relaxed specimens were incubated on ice overnight in freshly made 4% formaldehyde in 0.1M phosphate-buffered saline (PBS; pH 7.4); subsequently they were washed several times with 0.1 M PBS and permeabilized for 1 h in 0.2% Triton X-100, 0.25% bovine serum albumin and 0.05% NaN₃ in PBS and incubated in tetramethylrhodamine B isothiocyanate (TRITC)-phalloidin (Sigma) 2 µL of a 38-µM solution in 50 µL preincubation solution for 1 h. Specimens were then rinsed in PBS and embedded in Citifluor (Plano, Wetzlar) on microscopic slides and surveyed using a Leica TCS SP2 (Bielefeld) or a Leica DM IRE 2 (Modena) confocal laser scanning microscope. Maximum intensities from the images in each series of optical sections were projected into single images. The positions of muscles are expressed in reference to percentage body units; total body (without furca in *Polymerurus nodicaudus*) = 100 units (U).

Results

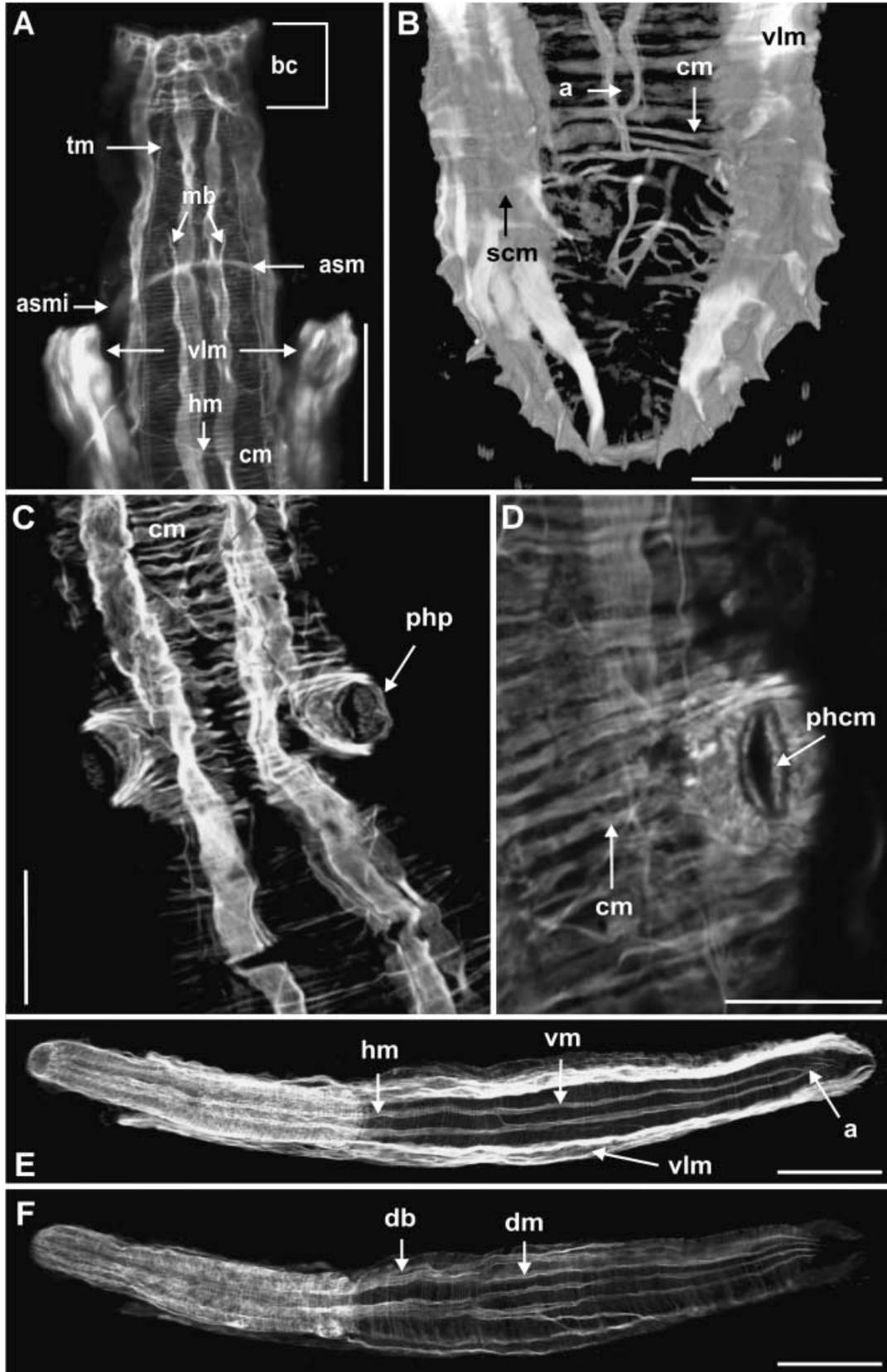
Musculature of *Paradasys* sp.

Circular muscles. Circular bands, 0.3–0.5 µm wide, are present as hoop-like rings in a splanchnic position (cm), around the pharynx and intestine, and in a somatic position (scm) surrounding the ventrolateral muscles of the lateral trunk region (Fig. 1A–D). The number of circular rings differs between pharynx (125–150 rings) and intestine (150–170 rings) and also varies among specimens of different body lengths. Circular muscles are internal to all longitudinal muscles on the pharynx but external to them on the intestine. Somatic circular muscles are external to the ventrolateral compartments (Fig. 1B).

Longitudinal muscles. Longitudinal, obliquely striated, muscles are present in splanchnic (dorsal, lateral, ventral) and somatic (ventrolateral) positions. Dorsal muscles (dm), each 4 µm wide, insert anteriorly at the mouth rim and posteriorly above the anus or body mid-line close to the anus (Fig. 1F). Of the six muscles inserting at the mouth rim, four (two on

Fig. 1—Musculature of *Paradasys* sp. stained with TRITC-phalloidin. —**A.** Ventral view of anterior end showing the buccal cavity, semicircular band and insertion of the ventrolateral muscles. —**B.** Ventral view of posterior end showing the anal region and insertion of the ventrolateral muscles. —**C.** Dorsolateral view showing the pharyngeal pores. —**D.** Close-up of the pharyngeal pore. —**E.** Wholemount, ventral view showing the longitudinal muscles. —**F.** Wholemount, dorsal view showing the branching of the longitudinal muscles. Scale bars.

A–C = 20 µm, D = 10 µm, E, F = 40 µm. a – anus, asm – anterior semicircular muscle, asmi – insertion of the anterior semicircular muscle, bc – buccal cavity, cm – splanchnic circular muscle, db – dorsal muscle branches, dm – dorsal longitudinal muscle, phcm – circular muscle around the pharyngeal pore, hm – helicoidal muscle, mb – muscle branches of the ventral longitudinal muscles, php – pharyngeal pore, scm – somatic circular muscle, tm – thin muscle which inserts into posterior ring of buccal cavity, vlm – ventrolateral muscle, vm – ventral longitudinal muscle.



each side) run laterally along the entire intestine and terminate posteriorly at U90 lateral to the anus; two muscles, more dorsal in position, run each as a single branch until U50 where they split into three branches, each of which subdivides again at about U70 into several units (Fig. 1F). The exact number of branches deriving from the second splitting could not be determined; however, four of these branches end abruptly in the anal region in a position dorsal to the anus. Most of the other branches insert lateral to the anus together with the lateral muscles (Fig. 1F). Six longitudinal muscles are present in a ventral position (vm) inserting anteriorly on the mouth rim; the two muscles in a median position insert posteriorly on the anus (Fig. 1B,E). From each of the two muscles in a median position originates, at U9, a thin accessory muscular branch (mb; 1.5 μm wide), which runs lateral to the main muscle bands in an anterior direction until U7 (Fig. 1A). The bands more lateral in position merge with the ventrolateral muscle near the anus (U90). A pair of longitudinal muscles occur in the ventrolateral position (vlm) and are the largest in diameter (10 μm wide; Fig. 1A,B,E). These muscles insert anteriorly at the position of the anterior adhesive tubes behind the mouth (Fig. 1A,E). Posteriorly, the ventrolateral muscles end in the caudal lobe, inserting at the body mid-line (Fig. 1B,E). Each muscle appears to be composed of at least six to eight individual fibres. Except for the dorsomedial muscles, the ventrolateral muscles are the only longitudinal units that branch extensively along their length. In this case however, the branches do not appear to be bifurcations of individual myocytes; rather, the branches are reoriented myocytes within the larger muscle unit.

Helicoidal muscles. Two easily discernible, 0.5- μm wide, helicoidal muscles (hm) are arranged in left- and right-handed spirals. Anteriorly, each fibre inserts dorsally on the mouth rim and terminates in the intestinal region, on the dorsal side, at U43. At least six crossings are present (five pharyngeal and one postpharyngeal); where helices crossed, they form an angle between 50° and 60° with respect to the longitudinal axis of the body. Helices are continuous from anterior to posterior, looping completely around the digestive tract and associated musculature, i.e. circular and longitudinal; helicoidal muscles however, do not surround the large ventrolateral bands (Fig. 1A).

Other muscle arrangements. A single semicircular muscle band (asm), 1 μm wide, is present in the pharyngeal region

at U8 (Fig. 1A). The band enwraps the ventral pharynx wall and its associated muscles (i.e. longitudinal, circular and helicoidal), while being dorsally open. The semicircular band appears to originate at offshoots of the ventrolateral bands, which then unite medially (Fig. 1A).

At the anterior end is a set of six circular muscles (2 μm wide); these muscles form a sphincter around the buccal cavity (Fig. 1A). Two thin ventral longitudinal muscles insert medially on the most posterior sphincter muscle. These longitudinal bands run the length of the pharynx. Two additional muscles originate at U2, and run from the sides to mid-line, flanking the median dorsal muscles, from U8 to the pharyngeal base.

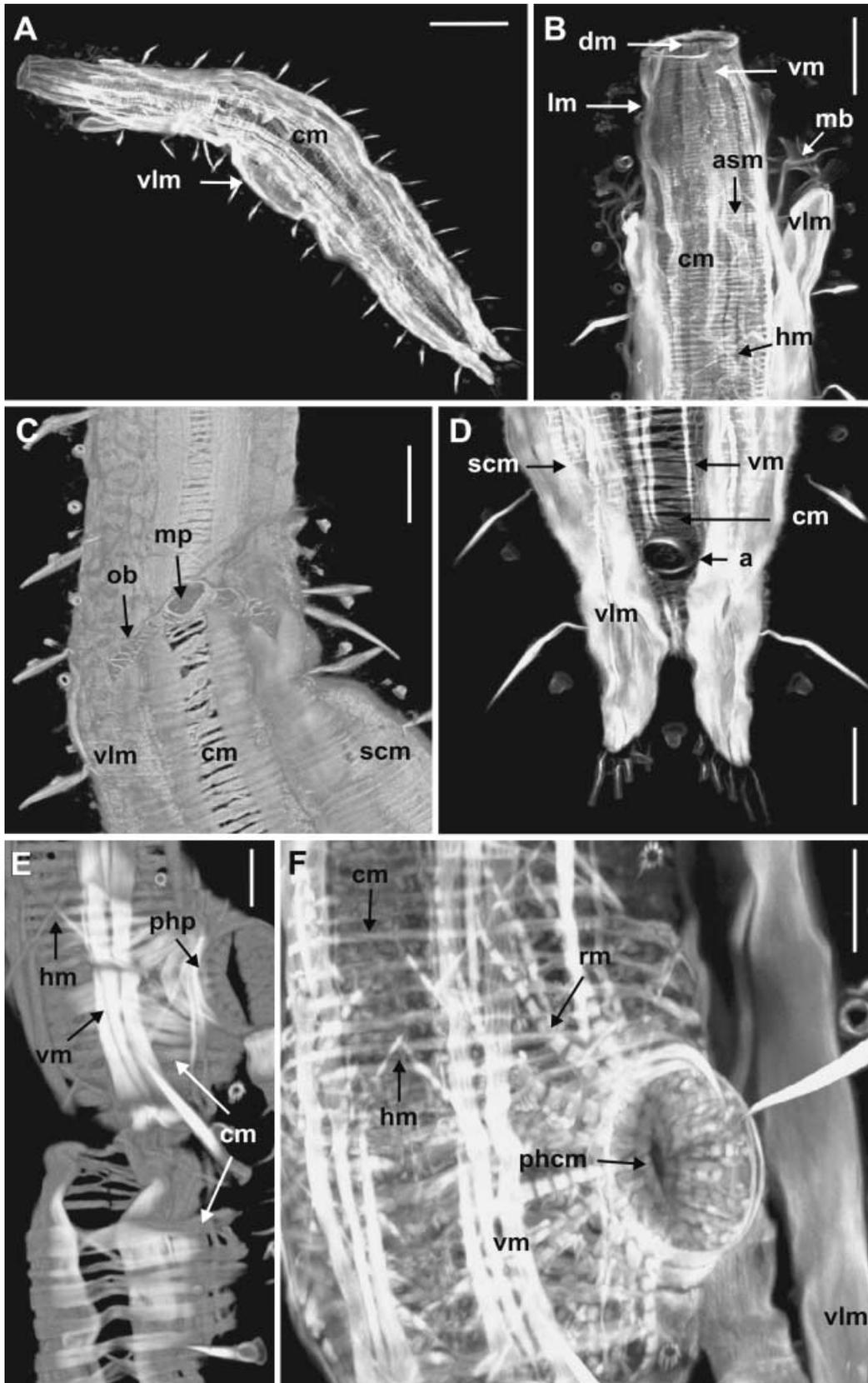
The pharyngeal pores, located at U40 (Fig. 1C), are supplied with splanchnic circular muscles and a further muscular band (phcm), 0.7 μm wide, around the external perimeter (Fig. 1D).

Musculature of *Turbanella* sp.

Circular muscles. Circular muscles are present in the form of isolated ring-like bands in a splanchnic position (cm) on the digestive tract (pharynx + intestine), and in a somatic position (scm), surrounding the ventrolateral longitudinal muscles on either side of the intestine (Fig. 2). Splanchnic circular muscles (1–1.5 μm) in the pharyngeal region are internal to all longitudinal muscles whereas along the intestinal region they are external to all longitudinal bands (Fig. 2E). The number of circular rings differs between pharynx (80–100 rings) and intestine (65–80 rings) and varies among specimens of different body lengths.

Longitudinal muscles. All specimens possess dorsal (dm), ventral (vm), and lateral (lm) longitudinal muscles in a splanchnic position on the pharynx and intestine, and ventrolateral (vlm) longitudinal muscles in a somatic position (Fig. 2). Overall, longitudinal muscles on the intestine appear to be continuations of muscles from the pharynx. Moreover, all longitudinal muscles, with the exception of the ventrolateral bands, are bound by helicoidal muscles. Splanchnic longitudinal muscles insert anteriorly on the mouth rim as 12 bands (six dorsal and six ventral), all 4 μm wide (Fig. 2A,B). The dorsal muscles, insert posteriorly above the anus (at U89) or body mid-line posterior to the anus at U90. More precisely, the two most lateral muscular bands run singly along the whole gut and insert lateral to the

Fig. 2—Musculature of *Turbanella* sp. stained with TRITC-phalloidin. —**A**. Wholemound, ventral view showing the general muscular pattern. —**B**. Ventral view of the anterior end showing the buccal cavity, semicircular band and insertion of the ventrolateral muscles. —**C**. Ventral view showing the male pore and associated musculature. —**D**. Ventral view of the posterior end showing the anal region and insertion of the ventrolateral muscles. —**E**. Ventrolateral view of the pharyngo-intestinal junction. —**F**. Close-up of the pharyngeal pore. Scale bars: A = 40 μm ; B, C, D = 20 μm , E, F = 10 μm . a – anus, asm – anterior semicircular muscle, cm – splanchnic circular muscle, dm – dorsal longitudinal muscle, ob – oblique band, phcm – circular muscle around the pharyngeal pore, hm – helicoidal muscle, lm – lateral longitudinal muscle, mb – branches of the ventrolateral muscles, mp – male pore, php – pharyngeal pore, rm – radial muscle, scm – somatic circular muscle, vlm – ventrolateral muscle, vm – ventral longitudinal muscle.



anus while the four central bands split at U50 in two branches. Of the eight resulting offshoots, the four central branches insert singly at U89, whereas the four lateral ones unite each with a counterpart in the mid-line, in the region above the anus or at the caudal lobe indentation. The ventral longitudinal muscles insert posteriorly close to the anus (Fig. 2D). The two central bands run from the mouth rim to the pharyngo-intestinal junction (U30) where they split into three branches each; these then run posteriorly and fuse with the muscular sphincter of the anus at U89 (Fig. 2D). The lateral muscles span the entire body length, flanking the somatic ventrolateral muscles (see below); the intermediate bands run very close to the ventrolateral band and coalesce with it at about U80. The paired ventrolateral longitudinal muscles have the largest diameter ($\geq 10 \mu\text{m}$). Each muscle appears to be composed of at least six individual fibres joined side by side. Anteriorly, the ventrolateral muscles insert at the position of the anterior adhesive tubes behind the mouth (Fig. 2A,B). Posteriorly, the muscles insert in the caudal lobes (Fig. 2A,B). The ventrolateral muscles branch extensively along their length. As in *Paradasys* sp., branches do not appear to be bifurcations of individual myocytes but rather reoriented fibres within the larger muscle unit.

Helicoidal muscles. Helicoidal muscles (hm), $0.5 \mu\text{m}$, are arranged in left- and right-handed spirals, beginning at approximately U10, and ending at U60 (Fig. 2B,E,F). Where helices cross, they form an angle between 50° and 60° with respect to the longitudinal axis of the body. Helices are continuous from anterior to posterior, looping completely around the digestive tract and associated musculature. In a mature specimen (total length $500 \mu\text{m}$), approximately four helices are observed on the pharynx and four on the intestine. Helicoidal muscles do not surround the large ventrolateral bands (Fig. 2B,E,F).

Other muscle arrangements. Ventrally, a single semicircular muscle band (asm), $2 \mu\text{m}$ wide, is present on the pharynx at U10 in between the ventrolateral muscle bands (Fig. 2B). The semicircular muscle does not extend to the dorsal side of the pharynx and connects to the ventrolateral muscles from which it probably originates (Fig. 2B).

At the anterior end of each ventrolateral band (at about U10) is a single muscle ($1 \mu\text{m}$ wide) that supplies the lateral portion of the head; this muscle runs from U10 to U7 (Fig. 2B).

Ventrally, two obliquely orientated (ob) muscles, $2 \mu\text{m}$ wide, extend medially from the paired ventrolateral longitudinal muscles on the side of the intestine and meet the body mid-line (U38.5) where a pore exists. This pore is probably the male genital opening that functions in sperm transfer between individuals (Fig. 2C). Mature specimens do not show muscular accessory organs.

Pharyngeal pores (at U37.5) are ventrolateral ($14 \mu\text{m}$ wide); they are supplied by thin cross-striated radial muscles (rm), $1 \mu\text{m}$ wide, that probably insert on the longitudinal

muscles. A separate muscular band (Phcm), $0.5 \mu\text{m}$ wide, is located around the external perimeter of each pore (Fig. 2F).

At the level of the anus is a ring-like muscle, which probably acts as anal sphincter (Fig. 2D). The more ventral longitudinal muscles insert in this ring-like muscle (Fig. 2D).

Musculature of *Polymerurus nodicaudus*

Circular muscles. Circular muscles (cm) are present as complete individual bands that line the pharynx from the mouth rim to the pharyngo-intestinal junction (Fig. 3A,B). Circular muscles are approximately $0.5 \mu\text{m}$ in diameter and evenly spaced along the pharynx. We counted 50 circular bands on the pharynx ($75 \mu\text{m}$ length) of a single specimen (body length $275 \mu\text{m}$, furca excluded). Incomplete, splanchnic dorsoventrally orientated circular muscles (dv) are present along the first half of the intestinal region from U35 to U60 (Fig. 3B,C). No somatic circular muscles were observed.

Longitudinal muscles. Longitudinal muscles are found in ventral (vm), ventrolateral (vlm), lateral (lm) and dorsal (dm) positions (Fig. 3A). Most longitudinal muscles are $1\text{--}2 \mu\text{m}$ wide. Dorsally, two thin longitudinal muscles ($1 \mu\text{m}$) span the length of the entire gut tube (Fig. 3A). This muscle pair inserts close to the mouth rim and extends posteriorly, remaining in contact with the pharynx and intestine. Each dorsal muscle branched at U30 to produce the thicker ($2 \mu\text{m}$) *Rückenhautmuskel* (terminology after Zelinka 1889). The paired *Rückenhautmuskel* (R; Fig. 3A,C) is not bound to the intestine by helicoidal muscles as are the dorsal muscles. Instead, they are often external to a maturing egg that is present dorsal to the intestine (Fig. 3A). The *Rückenhautmuskel* bows laterally and is often in contact with the dorsolateral body wall of the trunk. When a fully grown egg is present, the muscles wrap tightly around the dorsolateral margin of the egg. Dorsal muscles and the *Rückenhautmuskel* join again at the furcal base (Fig. 3A). As in other chaetonotids e.g. *Halichaetonotus* and *Lepidodermella*, the *Rückenhautmuskel* does not branch to form the R2 bands found in other taxa such as *Aspidiophorus* and *Chaetonotus*.

Ventrally, there are two pairs of longitudinal muscles that extend over most of the length of *P. nodicaudus* (Fig. 3A). The most ventral muscle pair remains in contact with the gut along its entire length. These muscles insert anteriorly at the mouth rim and posteriorly enter each branch of the caudal rami (Fig. 3A). The ventrolateral pair runs laterally along the entire gut tube and inserts posteriorly on the dorsoventral muscle (Fig. 3A). Slightly lateral to the ventrolateral muscles are the lateral muscles, which may not be encircled by the helicoidal muscles. This muscle pair ($1.5 \mu\text{m}$ wide) inserts anteriorly on the body wall lateral to the pharynx, runs along the pharynx and intestine, and posteriorly joins the ventral muscles at U90 before inserting into each branch of the caudal furca (Fig. 3A,B).

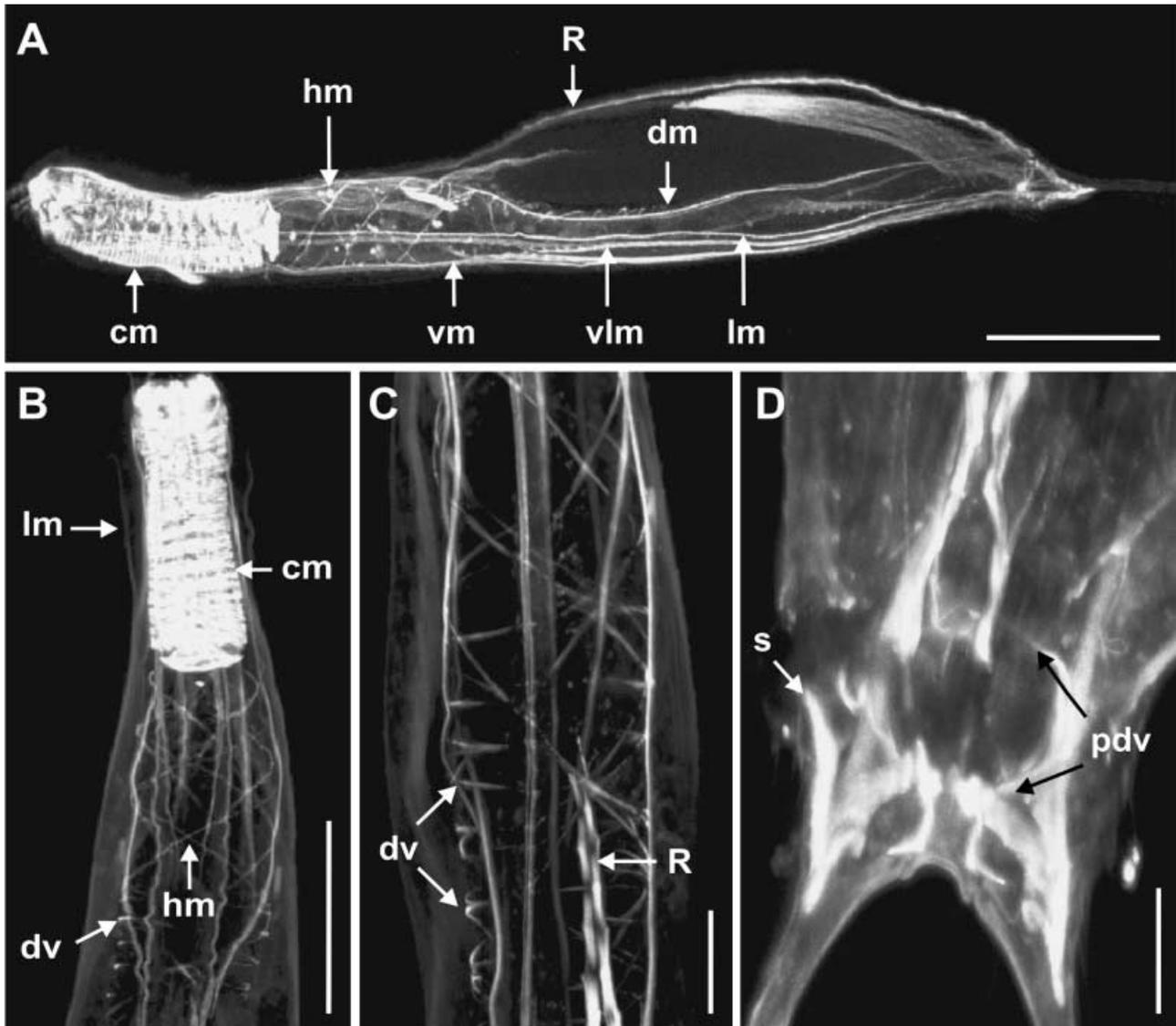


Fig. 3—Musculature of *Polymerurus nodicaudus* stained with TRITC–phalloidin. —**A**. Wholemount, lateral view. —**B**. Anterior view, showing the pharynx, helicoidal muscles and dorsoventral muscles. —**C**. Mid intestinal region showing details of the dorsoventral muscles. —**D**. Caudal end showing the muscle spike and posterior dorsoventral muscles. Scale bars A, B = 50 μ m; C, D = 10 μ m cm – splanchnic circular muscle, dm – dorsal longitudinal muscle, dv – splanchnic dorsoventral muscle, hm – helicoidal muscle, lm – lateral longitudinal muscle, pdv – posterior dorsoventral muscle, R – *Rückenhautmuskel* branch off dorsal longitudinal muscles, s – posterior muscle spike, vlm – ventrolateral longitudinal muscle, vm – ventral longitudinal muscle.

Helicoidal muscles. Helicoidal muscles (hm), beginning at U8, line the entire pharynx and approximately one-third of the length of the intestine (Fig. 3A,B). These very thin muscles (< 0.5 μ m) spiral around the gut tube forming crosses with angles of approximately 50–55° to the longitudinal body axis. Helicoidal muscles (three crosses on the pharynx, three crosses on the intestine) are external to the circular muscles and splanchnic longitudinal muscles, but internal to the lateral muscles (Fig. 3B).

Other muscle arrangements. A bilateral pair of dorsoventral muscles is located at the posterior end just before the caudal furca (s; Fig. 3D). This pair of muscle ‘spikes’ (1.5 μ m wide) is present at the base of the body just before the caudal furca and is directed anteriorly branching off the lateral muscles (Fig. 3D).

A dorsoventral muscle, 1 μ m in diameter, is present at the posterior end, forming connections between the splanchnic component of the longitudinal muscles (dorsal and ventral

muscles), but not the somatic component, such as the lateral muscles (pdv; Fig. 3D). No muscles are present inside the furcal branches.

Discussion

The recent use of epifluorescence microscopy and fluorescent phalloidin for the study of muscular organization in gastrotrichs has dramatically increased our knowledge of whole body muscle patterns compared to the traditional techniques of optical microscopy and transmission electron microscopy. Moreover, the use of confocal microscopy provides even greater resolution than widefield epifluorescence, thereby overcoming some of the restrictions of the standard epifluorescence and providing even more detailed images.

The organization of the musculature in *Paradasys* sp. is generally similar to that of the other macrodasyidans but with some differences. The somatic musculature is composed only of a pair of ventrolateral longitudinal muscles and the circular bands that surround them. Anteriorly, the somatic ventrolateral longitudinal muscles insert at the position of the anterior adhesive tubes behind the mouth as in species of *Dactylopodola* (Dactylopodolidae), *Turbanella* (Turbanellidae) and *Dolichodasys* (Lepidodasyidae); a different condition is present in species of *Macrodasys* (Macrodasyidae) and Thaumastodermatidae, where the ventrolateral longitudinal muscles insert on the pharynx along the mouth rim. Posteriorly, the ventrolateral longitudinal muscles of *Paradasys* insert at the body mid-line (as in *Dolichodasys* and *Macrodasys*) and not in the caudal lobes (as in *Turbanella* and Thaumastodermatidae; cfr. Hochberg and Litvaitis 2001a,b). A further difference is present at the anterior end, where a semicircular muscle band straddles the ventral pharynx wall; a similar muscle band is absent in *Dolichodasys elongatus* (the only other Lepidodasyidae studied so far) but present in species of *Turbanella* and *Dactylopodola* (Hochberg and Litvaitis 2001b). Additionally, the ventrolateral longitudinal muscles bifurcate in the trunk region in *Dolichodasys* but not in *Paradasys*. These differences between *Paradasys* and *Dolichodasys* lend further support to the hypothesis that the Lepidodasyidae has a polyphyletic origin (cf. Hochberg and Litvaitis 2000, 2001e; Todaro et al. 2003; Guidi et al. 2004). Substantial differences in musculature (e.g. occurrence of anterior semicircular band, insertion of the somatic ventrolateral muscles, arrangement of the musculature of the pharyngeal pores) among lepidodasyid taxa also emerge from preliminary CLSM observations on representatives of the genera *Lepidodasys*, *Megadasys*, *Mesodasys*, *Pleurodasys* and *Cephalodasys* (Leasi & Todaro, unpublished data).

Perhaps surprisingly, there were no muscles associated with the reproductive organs of *Paradasys* as there are in species of Macrodasyidae, some Lepidodasyidae, and all species of Thaumastodermatidae. The absence of muscles associated with the reproductive organs could be attributed to the fact that the individuals studied, even though of different sizes,

may not have been sexually mature. In this regard, it should be pointed out that hermaphroditic specimens observed under Differential Interference Contrast (DIC) optics apparently do not show muscular accessory reproductive organs. Nevertheless, it should be investigated further whether *Paradasys* has muscular reproductive organs, and if not, then it represents the plesiomorphic condition of the order, in which the musculature of the reproductive organs became progressively more complex in more derived species (e.g. *Dactylopodola baltica* in Hochberg and Litvaitis 2001b; versus macrodasyidan species in Ruppert and Shaw 1977; Ruppert 1978a,b).

The musculature in species of the genus *Turbanella* (e.g. *T. ambronensis*) had already been observed using epifluorescence by Hochberg and Litvaitis (2001b). However, our research with CLSM has highlighted some species-specific differences. In *Turbanella* sp., the helicoidal bands cross four times posterior of the pharyngo-intestinal junction, instead of once as in *T. ambronensis*. In *Turbanella* sp. there is, like in *T. ambronensis*, a ventral semicircular muscular band. However, according to our observations, it connects with the ventrolateral bands; the latter then does not bifurcate beyond the base of the pharynx. Confocal microscopy also provided new insights into muscle organization by revealing the musculature of the anal sphincter, and the radial muscles of the pharyngeal pores and male genital opening. In particular, the male genital pore is also supported by a circular band that outlines the perimeter of the pore itself, in addition to two oblique bands that extend laterally. These bands may have functional significance during reproduction. For example species of Turbanellidae are hermaphrodites that transfer sperm from the male genital pore to their partner's frontal (dorsal) organ via whole body bending movements (cf. Balsamo et al. 2002). Contraction of the oblique muscle bands may presumably dilate the male pore and, via their connection with the ventrolateral muscles, aid the transmission of sperm.

The musculature of *Polymerurus nodicaudus* has the general characteristics of the musculature possessed by other Chaetonotida Paucitubulatina: bands are present in circular, dorsoventral, helicoidal and longitudinal orientations in ventral, ventrolateral, lateral and dorsal positions. The dorsal bands are divided and produce the *Rückenhautmuskel* that functions in egg retention in gravid animals.

It should be noted that in *Polymerurus*, as in *Halichaetonotus*, *Lepidodermella* (Chaetonotidae) and in species of Xenotrichulidae, the *Rückenhautmuskel* (R) is not branched, in contrast with what has been observed in species of the genera *Aspidiophorus* and *Chaetonotus* (Chaetonotidae), where this is subdivided into the two components R1 and R2 (Hochberg and Litvaitis 2003).

As in all the other Chaetonotidae investigated up to now, and in contrast with Xenotrichulidae, *Polymerurus* lacks penetrating muscles in the furca. Other similarities with members of the Chaetonotidae include the presence of a pair

of dorsoventral, hook-shaped muscles (= 'spikes' found in *Chaetonotus*) at the base of the furca and dorsoventral bands that surround only the splanchnic component of the longitudinal muscles (condition shared with *Chaetonotus* and *Lepidodermella*). So overall, it appears that the musculature of *Polymerurus* follows the general scheme of that of other species of Chaetonotidae. However, the muscular system of *Polymerurus nodicaudus* differs from all the other representatives of the Chaetonotidae described until now (*Aspidiophorus*, *Chaetonotus*, *Halichaetonotus*, *Lepidodermella*; Hochberg and Litvaitis 2003), in the presence of dorsoventrally orientated bands in a splanchnic position halfway along the intestine. Since splanchnic dorsoventral muscles have thus far only been found in members of the Xenotrichulidae, they were thought to be an apomorphy of this family (Ruppert 1975; Hochberg and Litvaitis 2001c, 2003). However, the presence of these muscles in *Polymerurus* indicates that within Chaetonotida Paucitubulatina, they might be regarded also as a plesiomorphy retained in only a few taxa (Xenotrichulidae and *Polymerurus*). Consequently, in a hypothetical evolutionary scenario of the Paucitubulatina, inferred from this and other muscular traits, *Polymerurus* should occupy a basal position within the family Chaetonotidae.

According to Ruppert (1975) and Hochberg and Litvaitis (2001c, 2003) the main function of the dorsoventral muscles is to antagonize movement of the longitudinal bands, facilitating defecation and expulsion/deposition of parthenogenetic eggs (see also Ruppert 1979). However, a primarily adaptive role for interstitial life and sperm transfer facilitation cannot be excluded, because dorsoventral muscles are found mostly in the very flat, hermaphroditic taxa that live in the sand (i.e. Xenotrichulidae), while they are missing in the flask-shaped epibenthic-periphytic gastrotrichs such as species of Chaetonotidae. The presence of these muscles also in the marine, interstitial, parthenogenetic *Draculiciteria* and in the freshwater, epibenthic *Polymerurus* supports a working hypothesis that dorsoventral muscles originate in marine interstitial hermaphroditic gastrotrichs and were reduced and finally lost during changes in life style (interstitial versus epibenthic-periphytic) and reproduction modality (hermaphroditic versus parthenogenetic) that took place with the invasion of the freshwater environment. The presence of dorsoventral muscles in marine, interstitial, hermaphroditic species of Chaetonotidae, such as *Musellifer*, considered basal among Chaetonotida (see Hochberg and Litvaitis 2000; Guidi et al. 2003) and in the recently described *Diuronotus* (see Todaro et al. 2005b) would strengthen this vision.

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References

- Balsamo, M., Ferraguti, M., Todaro, M. A. and Tongiorgi, P. 2002. Reproductive system and spermatozoa of *Paraturbanella teissieri* (Gastrotricha, Macrodasysida): implications for sperm transfer modality in Turbanellidae. – *Zoomorphology* 121: 235–241.
- Guidi, L., Marotta, R., Pierboni, L., Ferraguti, M., Todaro, M. A. and Balsamo, M. 2003. Comparative sperm ultrastructure of *Neodasys cirtus* and *Musellifer delamarei*, two species considered to be basal among Chaetonotida (Gastrotricha). – *Zoomorphology* 122: 135–143.
- Guidi, L., Pierboni, L., Ferraguti, M., Todaro, M. A. and Balsamo, M. 2004. Spermatology of the genus *Lepidodasys* Remane, 1926 (Gastrotricha, Macrodasysida): towards a revision of the family Lepidodasyidae Remane, 1927. – *Acta Zoologica* 85: 211–221.
- Hochberg, R. 2005. Musculature of the primitive gastrotrich *Neodasys* (Chaetonotida): functional adaptations to the interstitial environment and phylogenetic significance. – *Marine Biology* 146: 315–323.
- Hochberg, R. and Litvaitis, M. K. 2000. Phylogeny of Gastrotricha: a morphology-based framework of gastrotrich relationships. – *Biological Bulletin* 198: 299–305.
- Hochberg, R. and Litvaitis, M. K. 2001a. Functional morphology of muscles in *Tetranchyroderma papii* (Gastrotricha). – *Zoomorphology* 121: 37–43.
- Hochberg, R. and Litvaitis, M. K. 2001b. The musculature of *Dactylopodola baltica* and other macrodasysidan gastrotrichs in a functional and phylogenetic perspective. – *Zoologica Scripta* 30: 325–336.
- Hochberg, R. and Litvaitis, M. K. 2001c. The musculature of *Draculiciteria tessellata*: implications for the evolution of dorso-ventral muscles in Gastrotricha. – *Hydrobiologia* 452: 155–161.
- Hochberg, R. and Litvaitis, M. K. 2001d. A muscular double helix in Gastrotricha. – *Zoologischer Anzeiger* 240: 61–68.
- Hochberg, R. and Litvaitis, M. K. 2001e. Macrodasysida (Gastrotricha): a cladistic analysis of morphology. – *Invertebrate Biology* 120: 124–135.
- Hochberg, R. and Litvaitis, M. K. 2003. Organization of muscles in Chaetonotida Paucitubulatina. – *Meiofauna Marina* 12: 47–58.
- Paddock, S. W. 1999. An introduction to confocal imaging. In Paddock, S. W. (Ed.): *Confocal Microscopy Methods and Protocols*, pp. 1–34. Humana Press, Totowa, NJ.
- Pfannkuche, O. and Thiel, H. 1988. Sample processing. In Higgins, R. P. and Thiel, H. (Eds): *Introduction to the Study of Meiofauna*, pp. 134–145. Smithsonian Institution Press, Washington DC.
- Remane, A. 1929. Gastrotricha. In Kukenthal, W. and Krumbach, T.

- (Eds): *Handbuch der Zoologie* II Band, 1. Hälfte, pp. 121–186. Walter de Gruyter, Berlin.
- Remane, A. 1935–36. Gastrotricha. In Bronn, H.G. (Ed.): *Klassen und Ordnungen*, pp. 1–242. Des Tierreichs Akademische Verlagsgesellschaft, Leipzig.
- Ruppert, E. E. 1975. *Monograph of the Xenotrichulidae (Gastrotricha: Chaetonotida)*. PhD dissertation, University of North Carolina, Chapel Hill: 271 pp.
- Ruppert, E. E. 1978a. The reproductive system of Gastrotrichs. II. Insemination in *Macrodasys*: a unique mode of sperm transfer in Metazoa. – *Zoomorphologie* 89: 207–228.
- Ruppert, E. E. 1978b. The reproductive system of Gastrotrichs. III. Genital organs of Thaumastodermatinae subfam. n. and Diplodasyinae subfam. n. with discussion of reproduction in Macrodasysida. – *Zoologica Scripta* 7: 93–114.
- Ruppert, E. E. 1979. Morphology and systematics of the Xenotrichulidae (Gastrotricha, Chaetonotida). – *Mikrofauna Meeresbodens* 76: 1–56.
- Ruppert, E. E. 1982. Comparative ultrastructure of the gastrotrich pharynx and the evolution of myoepithelial foreguts in Aschelminthes. – *Zoomorphologie* 99: 181–200.
- Ruppert, E. E. 1991. Gastrotricha (Aschelminthes). In Harrison, F. and Ruppert, E. E. (Eds): *Microscopic Anatomy of Invertebrates*, Vol. 4, pp. 41–109. Wiley-Liss, Washington DC.
- Ruppert, E. E. and Shaw, K. 1977. The reproductive system of Gastrotrichs. I. Introduction with morphological data for two new *Dolichodasys* species. – *Zoologica Scripta* 6: 185–195.
- Teuchert, G. 1974. Aufbau und Feinstruktur der Muskelsysteme von *Turbanella cornuta* Remane (Gastrotricha, Macrodasysida). – *Mikrofauna Meeresbodens* 39: 223–246.
- Teuchert, G. 1977. The ultrastructure of the marine gastrotrich *Turbanella cornuta* Remane (Macrodasysida) and its functional and phylogenetic importance. – *Zoomorphologie* 88: 189–246.
- Teuchert, G. 1978. Strukturanalyse von Bewegungsformen bei Gastrotrichen. – *Zoologische Jahrbücher* 103: 424–438.
- Teuchert, G. & Lappe, A. 1980. Zum sogenannten ‘Pseudocoel’ der Nematelminthes. Ein Verleih der Leibes-holen von mehreren Gastrotrichen. – *Zoologische Jahrbücher für Anatomie* 103: 424–438.
- Todaro, M. A., Al-Kady, S. and Faraj, M. N. 2005a. Primi dati sui gastrotrichi marini del Golfo Arabico. 66° Congresso Unione Zoologica Italiana; Rome September 2005 (abstract).
- Todaro, M. A., Balsamo, M. and Kristensen, R. M. 2005b. A new genus of marine chaetonotids (Gastrotricha), with a description of two new species from Greenland and Denmark. – *Journal of the Marine Biology Association of the United Kingdom* 85: 1391–1400.
- Todaro, M. A., Littlewood, D. T. J., Balsamo, M., Herniou, E. A., Cassanelli, S., Manicardi, G., Wirz, A. and Tongiorgi, P. 2003. The interrelationships of the Gastrotricha using nuclear small rRNA subunit sequence data, with an interpretation based on morphology. – *Zoologischer Anzeiger* 242: 145–156.
- Travis, P. B. 1983. Ultrastructural study of body wall organization and Y-cell composition in the Gastrotricha. – *Zeitschrift für Zoologische Systematik und Evolutionsforsch* 21: 52–68.
- Uhlig, G., Thiel, H. and Gray, J. S. 1973. The quantitative separation of Meiofauna. – *Helgoländer Wissenschaftliche Meeresuntersuchungen* 25: 173–195.
- Wulf, E., DeBoben, A., Bautz, F., Faulstich, H. and Wieland, T. 1979. Fluorescent phallotoxin, a tool for the visualization of cellular actin. – *Proceedings of the National Academy of Sciences USA* 76: 4498–4502.
- Zelinka, C. 1889. Die Gastrotrichen. Eine monographische Darstellung ihrer Anatomie, Biologie und Systematic. – *Zeitschrift für Wissenschaftliche Zoologie* 49: 209–384.