MEIOFAUNA MARINA

Biodiversity, morphology and ecology
of small benthic organisms

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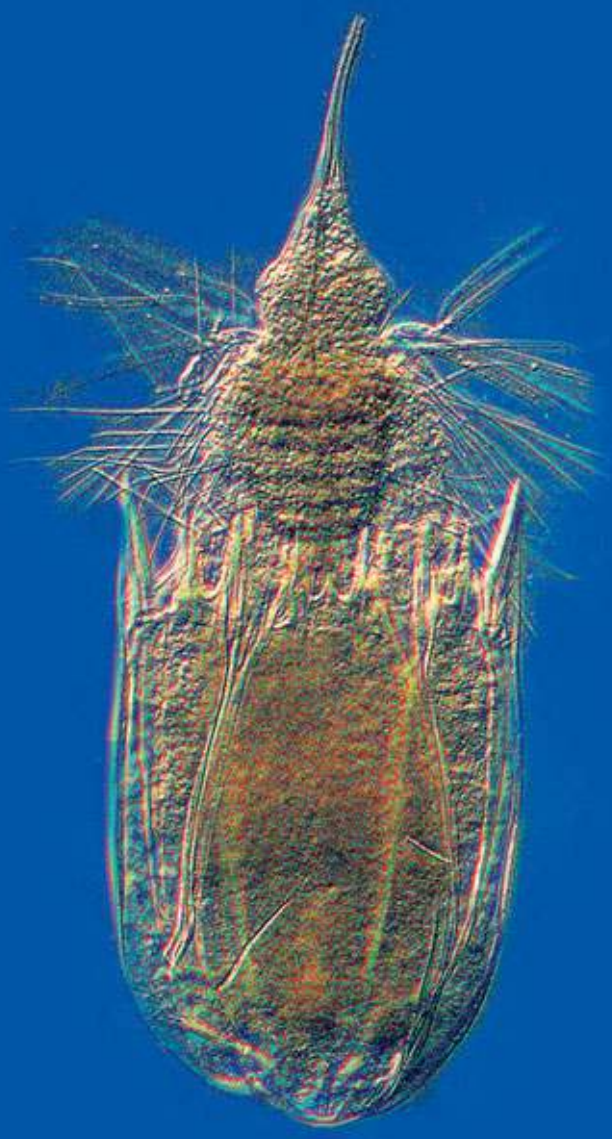

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Variation in the nervous system in three species of the genus *Turbanella* (Gastrotricha, Macrodasyida)

Birgen H. Rothe*, and Andreas Schmidt-Rhaesa***

Abstract

The nervous system of three species of the genus *Turbanella* (Gastrotricha, Macrodasyida), *T. ambronensis*, *T. cornuta* and *T. hyalina* from the German North Sea coast, was investigated using antibodies against serotonin and acetylated α-tubulin and visualized with confocal laser-scanning microscopy. Several specimens collected on the island of Sylt could not unequivocally be assigned to either *T. hyalina* or *T. cornuta*, a problem known from the literature. We found that there is a distinct neuronal pattern for each of the three species. They differ in the number and shape of cell somata associated with the dorsal commissure in the brain and in the presence/absence and position of a ventral commissure. Based on these differences, specimens can be assigned to a particular species unequivocally, even when external morphological features vary. This is particularly the case in *T. cornuta*, which can exhibit a transition of external characters towards *T. hyalina*, while the neuronal characters remain constant.

Keywords: Gastrotricha, nervous system, brain, immunocytochemistry, tubulin, serotonin

Introduction

Gastrotrichs are microscopic aquatic invertebrates. They are found throughout the world both in marine and freshwater environments. Traditionally the gastrotrichs are divided into the two orders, Macrodasyida and Chaetonotida. The Macrodasyida are with a few exceptions marine or brackish (for the exceptions see Kisielewski 1987), whereas the Chaetonotida populate both habitats. Most of the marine representatives live within the interstitial ecosystem, ranging from brackish coastal groundwater (e.g. Remane 1952) to the deep sea (e.g. Gutzmann et al. 2004, Kieneke & Zekely 2007).

Knowledge about the morphology of many organ systems of gastrotrichs is limited. Following light microscopical studies at the beginning of the 20th century (e.g. Remane 1936) and ultrastructural investigations (e.g. Ruppert 1991), advanced microscopical methods (confocal laser scanning microscopy) in combination with cytochemical and immunocytochemical staining techniques have been applied to visualize complex organ

* Zoological Museum and Institute, University Hamburg, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany
** Current address: Evolutionary Biology, University of Bielefeld, Morgenbreede 45, 33615 Bielefeld, Germany; e-mail: birgen_holger.rothe1@uni-bielefeld.de

systems such as the musculature (e. g. Hochberg & Litvaitis 2001, Leasi et al. 2006) and nervous system.

Concerning the nervous system, there are few targeted ultrastructural investigations (Teuchert 1976, 1977; Gagné 1980; Ruppert 1991; Wiedermann 1995). Joffe & Kotikova (1987) and Joffe & Wikgren (1995) initiated the use of (immuno-) cytochemistry for the investigation of the catecholaminergic (Joffe & Kotikova 1987) and serotonergic nervous systems (Joffe & Wikgren 1995) of *Turbanella* sp. and *T. cornuta* (Macrodasyida), respectively. This was continued by Hochberg & Litvaitis (2003) for the serotonergic nervous system in three other macrodasyidan gastrotrichs *(Dactylopodola baltica*, *Macrodasys caudatus*, *Dolichodasys elongus)*. Liesenjohann et al. (2006) combined TEM and CLSM to investigate the head organs of *Dactylopodola baltica* and used tubulin as a maker for the visualization of the whole cerebral nervous system, which has been done before only for *Dolichodasys elongatus* in the previous study. More recently, Hochberg (2007) demonstrated the widespread occurrence of FMRFamid-like immunoreactivity within the nervous system of *Turbanella* cf. *hyalina*, *Xenodasys riedli* (both Macrodasyida) and *Neodasys cirritus* (Chaetonotida). In comparison, the pattern of distribution of FMRFamide is much more complex than the pattern of serotonin.

So far, all the results of nervous system studies deal only with single species from particular genera of the Gastrotricha. There is no information about possible variation of nervous system patterns among closely related species within a genus based on single traits. Here, we start the first approach to compare the nervous system of different members of a single genus within the Gastrotricha. We have chosen species of the genus *Turbanella* and the neuronal marker serotonin (5HT), because there are previous data on the serotonergic nervous system (*T. cornuta*, Joffe & Wikgren 1995) and because of the simplicity of the 5HT pattern. These data are combined with data from acetylated α-tubulin immunoreactivity for a more holistic view of the nervous system.

Twenty four species are described so far in the genus *Turbanella* (Hummon, this volume). Here, we compare the nervous systems of three species occurring along the German marine coast: *T. hyalina* Schultze, 1853, *T. cornuta* Remane, 1925 and *T. ambronensis* Remane, 1943. These species are of particular interest because of the large amount of morphological variation documented within their populations – this variation has caused considerable taxonomic confusion (e.g. Schmidt & Teuchert 1969, Wieser 1957, Maguire 1976).

Material and methods

The specimens of *T. cornuta* were collected from subtidal sediment near the island of Borkum (54°29.979'N/6°29.957'E) during the MEIONORD cruise with the MS Heinke in May 2006. Individuals tentatively assigned to *Turbanella hyalina* and to *T. cornuta* (from here on indicated as *T.* cf. *hyalina* and *T.* cf. *cornuta*) were collected from intertidal sediment on the island of Sylt near List (55°00'56"N/8°25'17"E) in the spring 2006 and 2007. The material of *T. ambronensis* was collected from intertidal sediments near the HWL on the island of Sylt close to Braderup (54°56'05"N/18°21'37"W) in the autumn 2007. All specimens were extracted with the seawater-ice method (Uhlig 1964) or by the decantation technique after relaxation with a solution of 7% $MgCl_2$ for 10 minutes (Rieger & Ruppert 1978). Better quantitative results were obtained by using the relaxation technique.

Relaxed specimens were incubated overnight in 4 % paraformaldehyde in 0.1 M PBS (pH 7.3) on ice. After fixation, the samples were washed several times in 0.1 M PBS (pH 7.3) and stored in 0.1 M PBS (pH 7.3) containing 0.05 % NaN_3 at 4 °C for several weeks. Prior to immunostaining, the samples were pretreated with 0.1 M PBS containing 0.5 % Triton X-100 (Sigma), 0.25 % BSA and 6 % goat serum overnight at 4 °C. The preparations were incubated for 24-48 h in a solution of the primary antibody (anti acetylated α-tubulin [Sigma] diluted 1:500; anti-5-Ht [Sigma] diluted 1:1000-4000) in 0.1 M PBS containing 1-2 % Triton X-100 at 4 °C and rinsed subsequently several time in 0.1 M PBS.

Specimens were incubated in the secondary antibody solution, anti-rabbit and/or anti-mouse immunoglobulin goat serum conjugated with TRITC (Sigma), FITC (Sigma) or Cy5 (Jackson ImmunoReseach) diluted 1:100 in 0.1 M PBS containing 1-2 % Triton X-100 at 4 °C. The staining was stopped by rinsing again several times in 0.1 M PBS. Better results were made by the use of 2 % Triton X-100.

In some cases, an additional counterstain with propidium iodide (Sigma) and phalloidin TRITC-

conjugate (Sigma) was used. A concentration of 2 µl of 3.8 µM phalloidin or 2 µl (1 mg/ml Aqua dest.) of propidium iodide was added to 100 µl of the solution of secondary antibodies. Depending on the fluorescence of these counterstains, FITC-labelled or Cy5-labelled secondary antibodies were used.

After washing, the gastrotrichs were embedded in Citiflour (Plano) on microscope slides. The microscopic investigation took place with a cLSM Leica TCS 2. The postprocessing of the data and the projections with greater focal depth (maximum intensity projections (mp) and colour coded (by depth) projections (ccd-projection) were made by using LCS Simulator SP software version 2.61 (Leica) and measurements were made by using Zeiss LSM Image Browser version 3.2.0.70.

Results

Some notes on the determination of species. Species-level determinations were made by light microscopy using the original descriptions. The differentiation between *T. hyalina* and *T. cornuta* is based primarily on the presence or absence of a palpar organ (also known as head cone) at the sides of the "head" ("co" in Fig. 1D, 2H, I). *Turbanella hyalina* is lacking such an organ; in the same region, there is only a very flat protrusion with a group of sensory cilia on the top ("sci" in Fig. 1B). The differentiation between *T. ambronensis* and the two other species is based on general varieties in the shape of the body of this species (a trilobed head, a very wide buccal cavity) and the loss of lateral adhesive tubes, except for one pair directly in front of the paired group of posterior adhesive tubes (Fig. 1C). The lateral adhesive tubes are not distinctly tube-shaped, but have the form of cone-shaped protrusions of the body with a sensory cilium.

The material of *T. cornuta* from the subtidal sediments (Island of Borkum) showed very prominent lateral palpar organs (Fig. 1D). In this case, the determination was possible without a doubt. The material of *T.* cf. *hyalina* and *T.* cf. *cornuta*, which was collected on the Island of Sylt, was much more complicated, because individuals showed a gradient in the length of the palpar organs. Besides lacking in some specimens (as in *T. hyalina*), some animals have only slightly pronounced protrusions lateral of the head, while the palpar organ is more pronounced in others, although it was never so definitive as we saw it in the sublitoral form (Fig. 2H, I).

General architecture of the serotonergic nervous system of the three species. Within all three species, a strong positive signal for anti-5HT is visible in the brain (br) and in the longitudinal nerve cords (sln, Fig. 1E). The main serotonergic components of the brain are a dorsal commissural neuropil and corresponding lateral cell somata (= pericarya). The presence of a ventral commissure is species-specific.

The dorsal commissure is located slightly anterior of the constriction that separates the "head" and the body. The immunopositive fibres of the commissure form an arc along the dorsal part of the pharynx and are linked to the longitudinal nerve cords of both sides (Fig. 1F, I, J, 2C). Posterolateral of the dorsal commissure are the serotonergic pericarya of the brain (Fig. 1E-L, 2C). The number of immunopositive cells is low, between one and two pairs, depending on the species. The serotonergic fibres of the ventral cords extend from the anterior end to the posterior end and coalesce (Fig. 1E, J). The position of the nerve cords relative to the internal organs and body wall shifts along the length of the body. Along the pharynx, the serotonergic fibres are close to the pharynx in a lateroventral position. The remaining part of the longitudinal nerve runs in a more peripheral position (Fig. 2E). The shift between these two positions is at the level of the pharynx-intestinal border and is relatively abrupt (Fig. 2E).

General architecture of the tubulinergic nervous system (acetylated α-tubulin) of the three species. The anti acetylated α-tubulin antibody clearly stains the ciliary components and the neurotubuli in all three *Turbanella* species, including both the motile cilia at the ventral surface (vcm) and the ciliated sensory devices (Fig. 2A). The immunopositive signal of the ventral motile cilia (vmc) is restricted to the axonemata; the corresponding somata do not give a positive staining (Fig. 2A-C, E-G). The presumed sensory cells give different results; for example, the sensory cells projecting into the pharyngeal lumen (sec_p) and the lateral (sec_l) and dorsolateral (sec_d) sensory cells of the body show a strong immunoreaction in the cilium and the soma (Fig. 2B, F). On the other hand, the presumed sensory cells around the mouth opening and the dorsal belt of cilia at the anterior region of the head show only the cilium

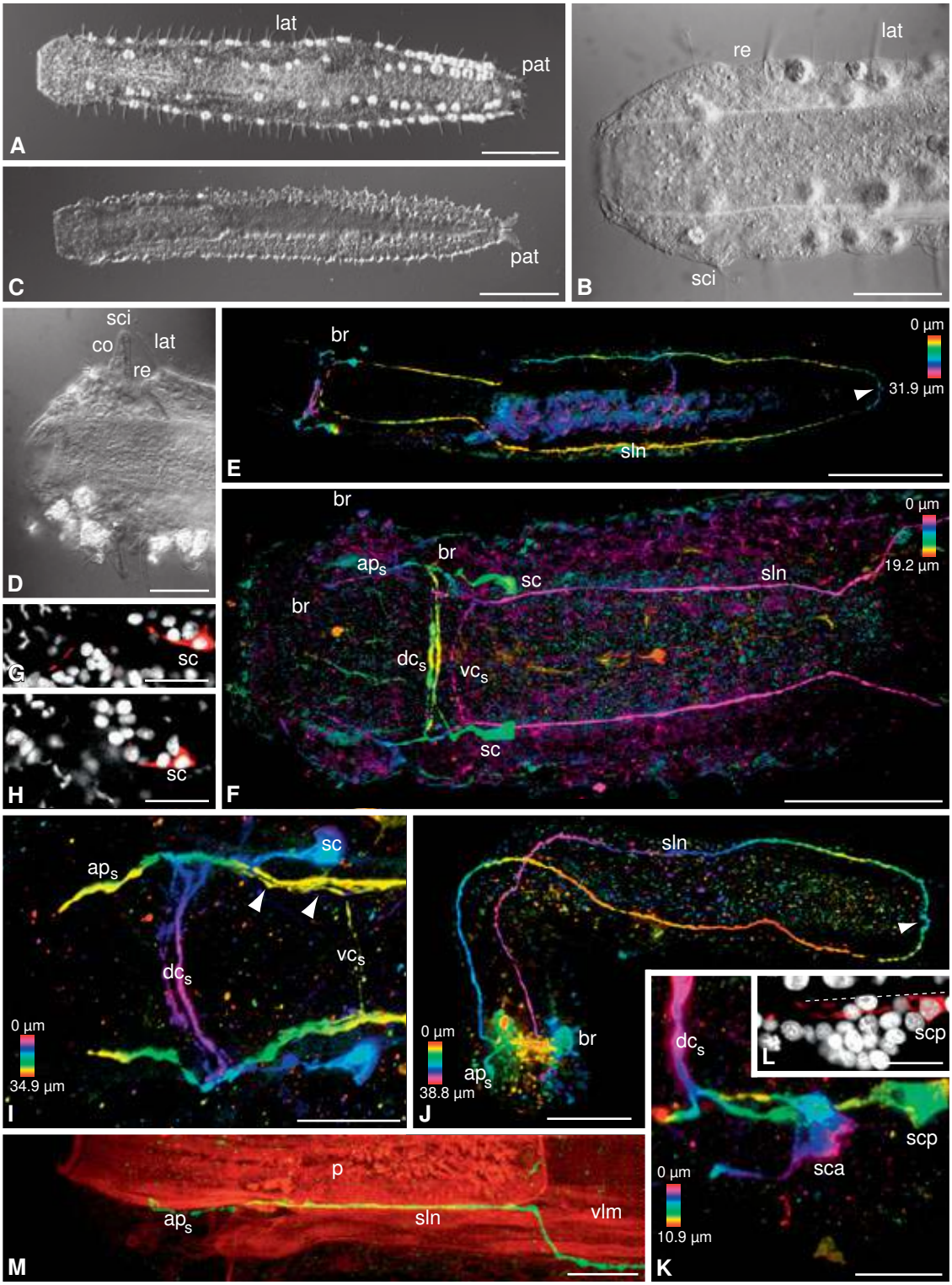
being stained. Additionally, the adhesive tubes (anterior [aat], posterior [pat] and lateral [lat]) give a very strong signal (Fig. 2A). Where testes (te) are present, the axonemata of the spermatozoa give a strong signal, too (Fig. 2A).

The neurotubuli are clearly stained in the dorsal commissure (dc) and in the main lateral longitudinal nerves (mln) (Fig. 2C, E-G). Anterior of the brain, the two sensory head organs are visible. It is possible to distinguish between the anterior sensory head organ (aso) and the posterior one (pso) not only from the position, but also by clear structural differences. The anterior sense organ of the head (aso) gives a very dense signal and is roundish in shape; it is clearly separated from the tissue of the proximity (Fig. 2B, C, F, G). In all three species, it is located in a dorsolateral position in front of the dorsal commissure. The posterior sense organ is located frontolateral of the brain and it looks like an aggregation of cilia. In contrast to the anterior sense organ, single cilia are visible. The ciliary areas of both organs do not contain nuclei (Fig. 2D). The innervation of these two organs comes directly from the neuropil of the brain.

Nervous system of *Turbanella ambronensis* (n = 10). The brain contains only two serotonergic fibers, an anterior and posterior one, that form a dorsal commissure, with one cell soma on each posterolateral side of the dorsal commissure (Fig. 1F). These dorsolateral cells (sc) are unipolar, their shape is slender, and the somata grade into a single anteriomedially-directed fibre that connects the cell with the posterior serotonergic fibre of the dorsal commissure (Fig. 1F). In combination with propidium iodide, it is obvious that only one pair of cells is present (Fig. 1G, H). As described, the dorsal commissure itself is composed of two densely adjoining immunoreactive fibres, an anterior and a posterior one. The fibres are separated throughout the entire length, from their origin at the longitudinal cord at the ventral base of the dorsal commissure. The longitudinal nerve cords (sln) extend from the dorsal commissure in an anterior direction approximately 24 to 26 µm (serotonergic anterior projection [ap_s], measured right and left from the base of the dorsal commissure). Posterior of the dorsal commissure is a ventral fiber that connects the paired nerve cords, the serotonergic ventral commissure (measurement of the distance in the median region) (Fig. 1F). This commissure consists of a very narrow fibre, approximately 0.5 µm in diameter. The origin of the fibre is directly at the longitudinal cords, 10 µm behind the dorsal commissure (measurement of the length of the serotonergic longitudinal fibre between the base of the dorsal and the ventral commissure).

Anterior of the brain are two paired tubulinergic = sensory organs, the posterior (pso) and the anterior sensory organ (aso). The posterior sensory organ is anterolateral of the dorsal commissure (Fig. 2F, G). The anterior sensory organ is in an almost dorsal position in front of the brain (Fig. 2F). The distance between the pair is 3-4 µm; this is equivalent to approximately 3 % of the width of the head. The innervation comes

Fig. 1. Lightmicroscopy and visualisation of 5-HT (serotonin) in *Turbanella*. **A-D.** DIC. **E-M.** cLSM. **E, F, I-K.** ▷ Colour coded by depth (CCD-Projections) of the serotonergic nervous system. **G, H, J**. Single optical section of a double-labelling of 5-HT (red) and nuclei (white). **M.** Maximum intensity projection of a double-labelling of actin with phalloidin (musculature-red) and serotonin (green). **A.** Overview of *T.* cf. *hyalina*. **B.** Detail of the anterior region of *T.* cf. *hyalina*. **C.** Overview of *T. ambroniensis*. **D.** Detail of the head of *T. cornuta* from Borkum with distinct lateral cones of the head. **E-H.** Serotonergic nervous system of *T. ambronensis*. **E.** Whole mount with the brain and serotonergic longitudinal fibers. **F.** Detail of the brain with the two serotonergic cells and the dorsal and ventral commissure. **G, H.** Detail of the serotonergic somata (red) and the nuclei (white). **I.** Detail of the brain of *T.* cf. *hyalina* and the ventral commissure (orange arrows). Note the presence of the fibers (white arrowheads) that innervate the ventral commissure. **J-M.** Serotonergic nervous system of *T. cornuta*. **J.** Overview of *T. cornuta* with the brain and the serotonergic longitudinal nerves; the posterior connection of the nerves is highlighted with a white arrowhead. **K.** Detail of the right half of the brain with the anterior and posterior serotonergic cell. **L.** Detail of the posterior serotonergic cell on the left side, dashed line marks the border of the pharynx. **M.** Localisation of the ventral serotonergic longitudinal nerve in the pharyngeal region, only the underpart of the left side shown. Scale bar in A, C, E 100 µm, B, D, F, J 50 µm, I, K-M 20 µm, H 10 µm. **br**, brain; **co**, lateral cone of the "head"; **dcs**, serotonergic dorsal commissure; **lat**, lateral adhesive tubes; **re**, restriction behind the "head"; **sc**, serotonergic cell; **sca**, anterior serotonergic cell; **sci**, sensory cilia; **scp**, posterior serotonergic cell; **sln**, serotonergic longitudinal nerve; **p**, pharynx; **pat**, posterior adhesive tubes; **vcs**, serotonergic ventral commissure; **vlm**, ventral longitudinal musculature.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from a more dorsolateral position of the dorsal commissure (Fig. 2F, G). The innervation process is short, approximately 5 µm in length. The anterior sensory organ has a more or less roundish form, about 5 µm in diameter.

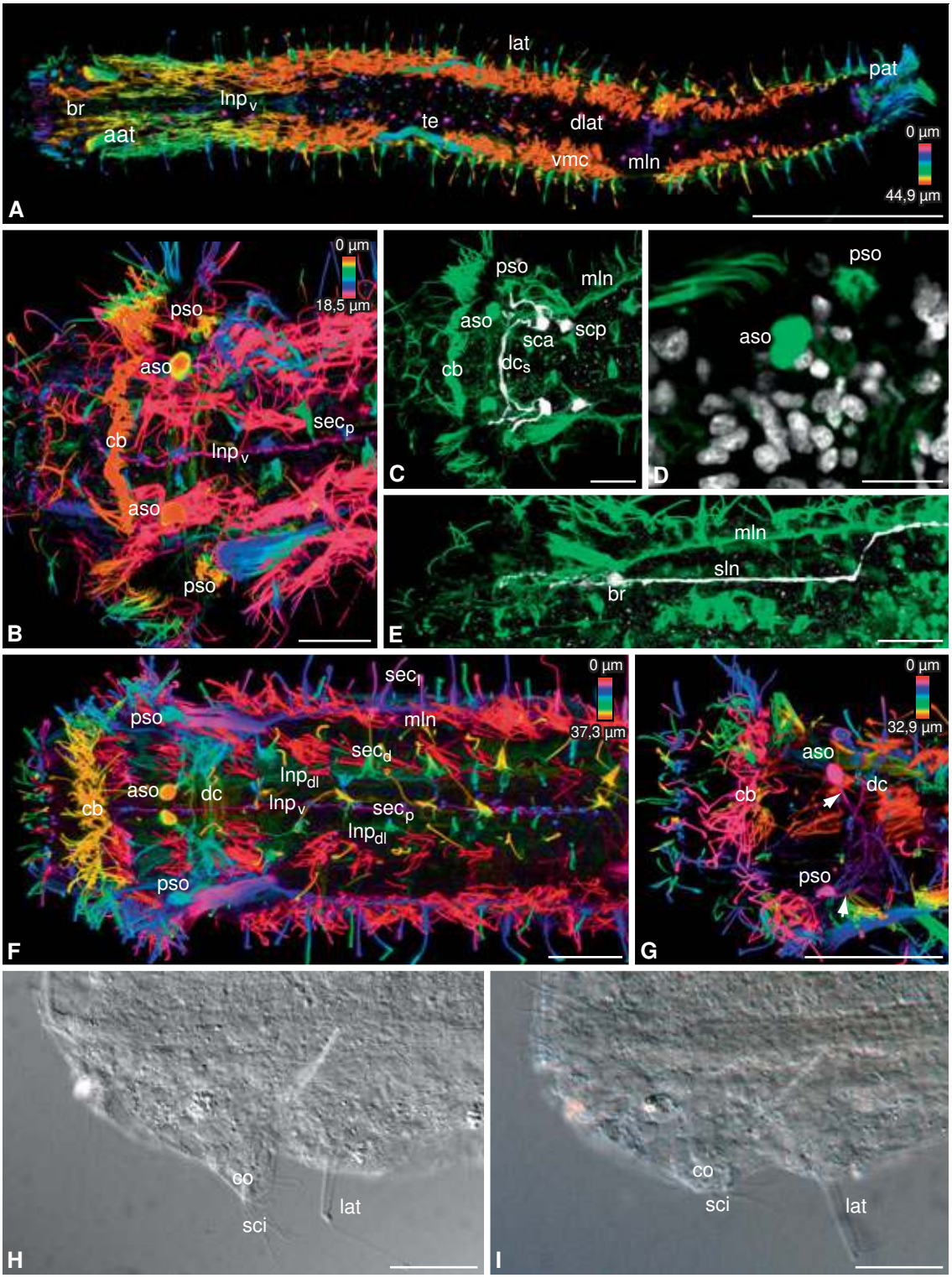
Nervous system of *Turbanella cornuta* (sample from Borkum n = 4). All of these animals bear distinct lateral cones at the head. The size of the cones can be approximately 25 % of the width of the head (Fig. 1D). The serotonergic components within *T. cornuta* differ in some respects from those in *T. ambronensis*. The dorsal commissure is composed of one fibres. Two serotonergic cells are located posterolateral to the dorsal commissure, an anterior serotonergic cell (sca) and a posterior one (scp). The anterior serotonergic cell is bipolar and the soma is irregularly shaped. The inner process connects the soma with the dorsal commissure (Fig. 1K), the outer process is located at the anterodistal region of the soma and runs anteriorly toward the posterior sense organ of the head (pso, Fig. 2C). The length of this process is approximately 16 to19 µm. The process joins the sensory organ of the head in a medioposterior position.

The posterior serotonergic cell is unipolar (scp, Fig. 1K) and has a shape comparable to the serotonergic cell in *T. hyalina*, with a "tip" at the distal region of the soma. This cell is located directly at the border to the pharyngeal bulb (Fig. 1L). The immunoreactive fiber from the posterior serotonergic cell runs parallel to the fiber from anterior serotonergic cell prior to their fusion and eventual connection to the dorsal commissure. A ventral commissure was not observed.

The sensory organs of the head are clearly observable (Fig. 2B-D). The anterior sensory organ (aso) is directly in front of the dorsal commissure in a dorsolateral position, but behind the dorsal belt of cilia (cb). The distance between them is about 33 µm (approximately 30 % of the width of the head). The innervation comes directly from the neuropil of the dorsal commissure. The innervating process runs from a more medial position directly to the organ; the length is about 4 µm. The posterior sensory organ is anterolateral to the base of the dorsal commissure. The innervation of the organ comes directly from the neuropil of this area; additionally, the anterior serotonergic cell (sca) seems to be involved (see above) (Fig. 2C).

Nervous system of *Turbanella* cf. *hyalina* (n = 11) and *Turbanella* cf. *cornuta* (n = 11), material from the island of Sylt. The serotonergic nervous system of *T.* cf. *hyalina* is roughly comparable to *T. ambronensis*. The main differences are present in the shape of the serotonergic cells (sc) in the brain (br) and the position of the ventral serotonergic commissure (VCs, Fig. 1I). The somata of the pair of cells associated with the brain is not slender as in *T. ambronensis*; instead, the soma are more or less sickle-shaped and expand in the dorsolateral direction to form an anteriorly directed tip (Fig. 1I). Two additional differences between the species are seen where the ventral commissure crosses the body. In *T.* cf. *hyalina*, the ventral commissure is more posterior (approximately 35 µm behind the dorsal commissure) when compared to *T. ambronensis*, but the origin is in approximately the same region (10 µm behind the base of the dorsal commissure). Unlike in *T. ambronensis*, the

Fig. 2. Visualisation of acetylated α-tubulin and 5-HT (serotonin) in *Turbanella* and lightmicroscopy of *T.* cf *cornuta* from Sylt. **A-G.** cLSM. **H-I**. DIC. **A, B, F, G**. Colour coded by depth (CCD-Projections) of acetylated α-tubulin. **C, E.** Maximum intensity projection of a double-labelling of acetylated α-tubulin (green) and serotonin (white). **D.** Single optical section of a double-labelling of acetylated tubulin (green) and nuclei (white). **A.** Overview of *T.* cf. *hyalina,* all tubulin-containing structures are stained: motile cilia, adhesive tubes, sperms in the testes and neurotubuli. **B.** Detail of the head of *T.* cf. *cornuta* with the brain. **C.** Detail of the head of *T.* cf. *cornuta* with the connection of the anterior serotonergic cell and the area of the posterior sensory organ of the head. The arrowheads mark the lateral process of the anterior serotonergic cell. **E.** Right anterior half of *T.* cf. *cornuta* only the ventral sector shown, with the main and serotonergic longitudinal nerves. **D.** Detail of the head sensory organs in *T.* cf *cornuta*. **F.** Anterior region of *T. ambronensis*. **G**. Detail of the head of *T. ambronensis*. **H, I.** Details of the left half of the head of *T.* cf. *cornuta* with varying prominent lateral head cones. Scale bar in A, 150 µm, F 50 µm, B, C, F, H, I 20 µm, D 10 µm. **aso**, anterior sensory organ; **br**, brain; **cb**, ciliary beld of the head; **co**, lateral cone of the "head"; **dc_s**, serotonergic dorsal commissure; **lat**, lateral adhesive tubes; **lnp_{dl}**, dorsolateral longitudinal nerve of the pharynx; **lnp_v**, vental longitudinal nerve of the pharynx; **mln**, main longitudinal nerve; **re**, restriction behind the "head"; **sca**, anterior serotonergic cell; **sci**, sensory cilia; **scp**, posterior serotonergic cell; **sec_d**, dorsal sensory cell; **sec_l**, lateral sensory cell; **sec_p**, sensory cell of the pharynx; **sln**, serotonergic longitudinal nerve; **te**, testes; **pat**, posterior adhesive tubes; **pso**, posterior sensory organ.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immunoreactive fibres of the ventral commissure run parallel of the ventral nerve cord for 22 µm before they form the commissure (Fig. 1I). The serotonergic fibres of the longitudinal nerve extend in anterior direction (anterior projections ap_s) for approximately 28 µm from the dorsal commissure. The position of the sensory head organs is identical to *T. cornuta*, but the anterior head sensory organ (aso) lacks a serotonergic innervation from the anterior serotonergic cell (sca).

The specimens of *T.* cf. *cornuta* from Sylt have the same serotonergic nervous pattern as the individuals from the subtidal sediment of Borkum (see above), regardless of the extent to which lateral cones on the head are formed. Some individuals bear short cones comparable to the material from Borkum, but most show only minor protrusions of the head (Fig. 2H, I). In two cases, not even this was observable; externally, the animals looked like *T. hyalina*, but the nervous system was identical to *T. cornuta* (not shown).

Discussion

In comparison with the previous data on the serotonergic nervous system of *Turbanella cornuta* by Joffe & Wikgren (1995), we can extend this work in some points. (i) The two serotonergic cells on each side of the head are each individually connected to the brain. (ii) The target organ of the anterior serotonergic cell is probably the posterior sensory organ. (iii) Joffe & Wikgren (1995) pointed out that there is no hint for a serotonergic innervation of the pharynx, because no serotonergic neurons or processes are observable within the pharynx, but the course of the longitudinal nerve cord in close association with the pharynx suggests such an innervation. The absence of serotonergic innervation of the pharynx in gastrotrichs would be exceptional, as this is present in many invertebrates, for example in Plathelminthes (Joffe & Reuter 1993), Annelida (Lent et al. 1989) and Nematoda (Niacaris & Avery 2003). The present data do not show the exact position of the pharyngeal fibres (basiepithelial within the pharynx or subepithelial outside of the pharynx). There is the possibility that innervation by the serotonergic fibres happens en passant, as descriped by Teuchert (1977) for interneural synapses. The exact position of the pharyngeal nerves should be cleared in future ultrastructural investigations. Additionally, the immunohistochemistry of synaptic-specific proteins, like synapsin, could highlight the distribution of regions rich in synapses.

Another postulated function of serotonin in many invertebrates is to control the locomotory cilia; this has been investigated in planktonic free-swimming larval forms (Hay-Schmidt 2000). In gastrotrichs, a correlation between the distribution of the cilia and the serotonergic nerves is not so clear. In *Turbanella* species, there are two ventrolateral bands of cilia, which are densest in the pharynx region. As the serotonergic nerve runs more medial than the main longitudinal nerve cord in this region of the body (see Fig. 2E), it is unclear whether this course and the denser ciliation are correlated. Hochberg & Livaitis (2003) figured for *Dactylopodola baltica* (their Fig. 6) that the serotonergic ventral longitudinal fibres run in the region of the head directly beneath the pharyngeal bulb. The distribution of the motile cilia in *D. baltica* is not so ordered as in *Turbanella* species. The motile cilia are here more evenly distributed across the ventral side as in *Turbanella*.

The data on the number of serotonergic cells in other members of macrodasyidan gastrotrichs are rare. So far only Hochberg & Litvaitis (2003) have stained for serotonin in *Dactylopodola baltica* (Dactylopodolidae), *Macrodasys caudatus* (Macrodasyidae) and *Dolichodasys elongatus* (Lepidodasyidae). The number of serotonergic cells is restricted to one pair in *M. caudatus* and *D. elongatus* and to two pairs in *D. baltica* (own unpublished data show 8 serotonergic cells, but one to two pairs posterior of the dorsal commissure comparably to Hochberg & Litvaitis 2003). In comparison to hitherto existing data on the peptidergic components of the gastrotrich nervous system. Hochberg (2007) found that FMRFamide-like immunoreactivity is more prevalent and widely distributed in the nervous system than is serotonin-like immunoreactivity. Hochberg (2007) was able to estimate the number of FMRF-like immunoreactive cells in *Turbanella* cf. *hyalina*, *Xenodasys riedli* (both Macrodasyida) and *Neodasys cirritus* (Chaetonotida). The number of cells range from a high of 50+ in *N. ciritus* to a low of 18 cells in *T.* cf. *hyalina*. In the case of *T.* cf. *hyalina*, it is possible to hypothesise a ratio of 1 : 9 for the serotonergic:FMRFamidergic cells. In the future, such comparative studies would benefit greatly from a knowledge of intraspecific variation, allowing for a more accurate assessment of the differences between neurotransmitters within a species. A good model for such a study could be *Dactylopodola baltica*; this species shows limited

morphological variation to create taxonomic confusion, and some data on its nervous system is already at hand (Hochberg & Litvaitis 2003, Liesenjohann et al. 2006).

In this study, we have demonstrated that the serotonergic nervous system shows interspecific variation within the genus *Turbanella*. The number of cells and the occurrence of a ventral commissure form a special pattern in each species. Especially in the context of the *T.* cf. *hyalina* and *T.* cf. *cornuta* material, it was possible to separate the samples due to the number and shape of the serotonergic cells, when the external morphology was not clear (n = 2).

The differentiation between *T. cornuta* and *T. hyalina* is an old taxonomic problem that dates back to the first description of *T. cornuta* (Remane 1925). The main criterion to distinguish the two species from each other is for Remane (1925, 1927, 1936, 1942) the presence or absence of the lateral cones. A restriction behind the head separates another species, *T. plana* Giard, 1904 from *T. cornuta* (determination key of Gastrotricha by Remane 1926). In the case of *T. cornuta* and *T. plana*, Remane (1952) mentioned that he could not exclude that *T. plana* is a synonym for *T. cornuta* and that Giard (1904) was describing a juvenile individual of *T. cornuta*. Similar remarks on the taxonomic confusion within the genus have occurred since then, and several researchers (Karling, 1954; Schrom 1966; Maguire 1976) have commented on the extent to which morphological variation (and ontogenetic variation) should be addressed in the future. Our data in this study suggest that neuronal patterns may represent another data set for addressing this taxonomic confusion. We find that both *T. hyalina* and *T. cornuta* are separate species with specific neuronal patterns (e.g., 1 pair of serotonergic cells plus a ventral commissure in *T. hyalina* and two pairs with no ventral commissure in *T. cornuta*). Both species can occur sympatrically. In some populations, such as on the island of Sylt, *T. cornuta* can bear head cones, but sometimes these are reduced. We exclude that these two groups form a single variable species like *T. varians* (Maguire 1976), in respect of the distinct neuronal pattern of these two groups and in comparison to *T. ambronensis*. This shows how neuronal patterns can help in the resolution of species diversity and motivate future investigators to consider other sources of data for taxonomic investigations.

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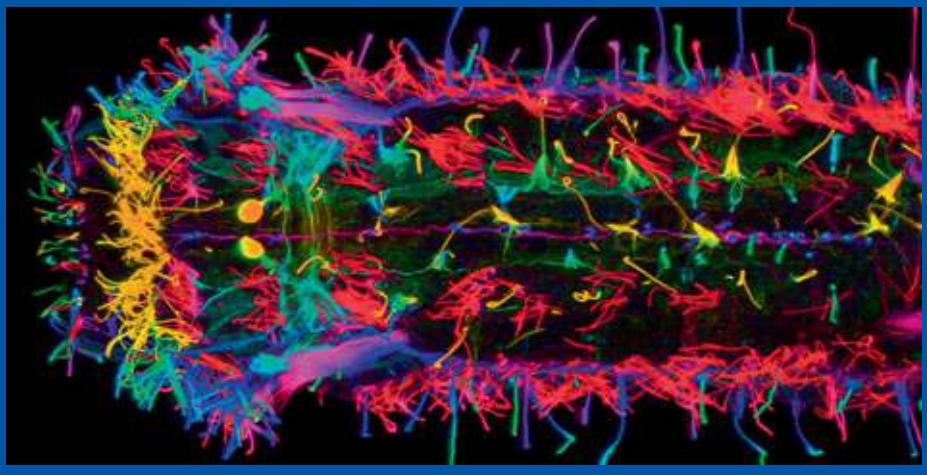

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