Handbook of Zoology
Gastrotricha, Cycloneuralia and Gnathifera

Volume 3:
Gastrotricha and Gnathifera

Handbook of Zoology

Founded by Willy Kükenthal
Editor-in-chief Andreas Schmidt-Rhaesa

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Introduction

This third subvolume of the volume "Gastrotricha, Cycloneuralia and Gnathifera" deals with the taxa Gastrotricha and Gnathifera. Gastrotrichs have become a phylogenetically mysterious group because despite some corresponding characters between gastrotrichs and cycloneuralian taxa, their DNA tells a different story. According to DNA comparisons so far, gastrotrichs cluster within Spiralia, often close to platyzoan groups (see Schmidt-Rhaesa 2013). Therefore, gastrotrichs are presented here in a more or less isolated way.

Gnathiferans include animals that are morphologically and ecologically very disparate at first view. Specimens are small or large, free-living or parasitic, and display a number of morphological differences. This has made it quite difficult to recognize relationships among these taxa, until the right characters were put into focus. It is especially the jaw apparatus and the structure of the epidermis that provide the key to the understanding of gnathiferan relationships. Rotifers and acanthocephalans, so different they might appear, share a corresponding structure of the (syncytial) epidermis, which has led to the common name Syndermata. Gnathostomulida are related to Syndermata based on the fine structure of the jaw apparatus, and the discovery of Micrognathozoa supported these hypotheses (see Schmidt-Rhaesa 2012 for details and literature).

Most open questions concern the relationship between rotifers and acanthocephalans. Rotifers include three subtaxa (Bdelloidea, Monogononta, and Seisonacea), and it is still under debate whether acanthocephalans are related to Rotifera as a whole, to a part of them, or to one of its subgroups. We decided here to present rotifers (Bdelloidea and Monogononta) and Seisonacea in two different chapters. Unfortunately, the Seisonacea chapter could not be completed in time and will occur in a later volume of the *Handbook of Zoology* series. Acanthocephala are split into three single chapters on morphology, systematic, and ecology.

With this third subvolume, we finish the volume "Gastrotricha, Cycloneuralia and Gnathifera" and hope that we could present an up-to-date review and overview on these often neglected but nevertheless important and fascinating animal groups. I thank all the authors for their contribution and De Gruyter for the enthusiasm to realize these volumes.

Andreas Schmidt-Rhaesa

Literature

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1 Gastrotricha

1.1 Introduction

The microscopically small gastrotrichs are abundant in diverse aquatic habitats. Gastrotrichs must have been among the first small animals studied with early microscopes. Remane (1936) lists some reports from the 18th century, which likely represent gastrotrich specimens. At first, gastrotrichs were treated, together with protozoans, rotifers, and other tiny animals, as “infusorians”, until Ehrenberg (1830) separated gastrotrichs and rotifers from protozoans. Ehrenberg treated gastrotrichs as part of rotifers and only subsequently researchers recognized the differences between these two groups. Mečnikow (1865) introduced the name Gastrotricha.

Originally, gastrotrichs were only found in freshwater, until Schultze (1853) found the first marine species, *Turbanella hyalina*, in sandy samples from the island Neuwerk (North Sea). Soon after, Claparéde (1867) described *Hemidasys agaso* from the harbour in Naples (Mediterranean Sea). The main era of the discovery of marine species started with Remane’s intensive investigations of marine sediments in the Kiel Bight (Baltic Sea) (Remane 1924, 1925a, 1926a, b, 1927a, b, see also 1927c, 1929, 1936). Today, we know about 780 species, a number that is constantly growing. Still, the gastrotrich fauna of many places is unknown or has been sampled only superficially. Gastrotrichs occur in a variety of freshwater habitats and in the sea, from the littoral region to the deep sea. In the marine environment, gastrotrichs are part of the mesopsammon, the community of animals living in the pore system between sand grains. In freshwater, gastrotrichs are either benthic (mesopsammic or epipelic, respectively) or live among vegetation, some species are swimming in the free water.

The first broad account to gastrotrich morphology was made by Zelinka (1889). Because of the minute size of gastrotrichs, transmission electron microscopy (TEM) played an important role to understand their internal anatomy (see, e.g., Ruppert 1991). The position of gastrotrichs in the phylogenetic system has changed several times and still is not solved convincingly.

1.2 Morphology

1.2.1 General and external morphology

Most gastrotrich species are microscopically small animals in the size range of a few hundred micrometers. Especially among Paucitubulatina, there are very small species with about 70 µm length. The longest gastrotrichs belong to the macrodasyid genus *Megadasys* and reach up to 3.5 mm in length (Schmidt 1974). The body form varies quite a bit. Almost all species in the taxon Paucitubulatina are more or less tenpin-shaped with a clearly defined head region, a narrowing neck, and a slightly bulbous trunk (Fig. 1.1 A). Species of Macrodasyida vary much more in shape. A head and neck region is present in several species, but often not very distinct. The trunk is usually of equal diameter throughout, giving the animals a shape that is often called “strap-shaped” (Fig. 1.1 B). Some species are short and broad and can best be termed “tongue-shaped”. The chaetonotid genus *Neodasys* resembles the strap-shaped macrodasyids in body outline.

In the posterior end, many gastrotrichs have paired extensions often called feet or, in paucitubulatinans, furca (Fig. 1.1 A, B). The feet carry one or more pairs of adhesive tubes (see below). In Paucitubulatina, only one pair of adhesive tubes is present on the furca. The exceptions are the absence of feet in swimming chaetonotids in the taxa Neogosseidae and Dasydytidae as well as reported two pairs in the genera *Dichaetura* and *Diuronotus* (see, e.g., Schwank 1990, Todaro et al. 2005). A recent description of *Dichaetura filispina* (Suzuki et al. 2013) found one adhesive tube and a solid spine on each foot, making a reinvestigation of the other species of the genus desirable. In macrodasyidan species, there is usually more than one adhesive tube on each foot (Fig. 1.1 B). When feet are absent, the posterior end is rounded or an unpaired tail is present. This tail is most conspicuous and several times as long as the trunk in species of the genus *Urodasys* (e.g., Wilke 1954, Schoepfer-Sterrer 1974). Based on their parsimonious character optimization, Kieneke et al. (2008a) reconstructed the stem species of Gastrotricha as an elongate (worm-shaped), dorsoventrally flattened, benthic-marine animal with a rounded frontal and a bilobed caudal trunk end.

Further appendage-like extensions of the body wall occur in some species, such extensions either act as a basal structure for adhesive tubes or as sensory structures in the head region (see chapter Sensory Structures and descriptions of genera).

Adhesive tubes are slender extensions, often accompanied by a cilium. They have a secretory function (see below). In chaetonotids of the subtaxon Paucitubulatina, they are restricted to the feet, but in macrodasyids and in *Neodasys*, adhesive tubes occur on various body regions (Fig. 1.1 A, B). Usually, adhesive tubes are divided into three major groups (see Fig. 1.1 B). Anterior adhesive tubes (usually abbreviated TbA) are the tubes in the head region. Often, they are concentrated in paired clusters on the ventral side; in some cases, they originate from a common basis. This basis is sometimes called “hand” or “fleshy base”. The lateral adhesive tubes (TbL) are positioned along the body in a lateral position; they may be few (e.g., in *Dactylopodola*) or very many (e.g., in different species of *Turbanella*). Posterior adhesive tubes (TbP) are those tubes present on feet or, when feet are absent, on the posterior end of the animals. In addition to the TbL, there may also be dorsal (TbD), dorsolateral (TbDL), ventrolateral (TbVL), or even ventral (TbV) adhesive tubes arranged along the entire body or restricted to certain regions of the trunk. The stem species of Gastrotricha at least had adhesive tubes in the lateral (TbL) and posterior (TbP) arrangements (Kieneke et al. 2008a).

The mouth opening is terminal or subterminal on the anterior tip of the animal. In some species, it leads to a funnel- or barrel-shaped buccal cavity. In some species (e.g., from the genera *Diplodasys, Oregodasys, Ptychostomella*, and *Tetranchyroderma*), the mouth opens very broad and occupies almost the entire anterior end, dorsally shielded by the so-called oral hood.

Very characteristic and important for determination is the covering of the body by cilia and cuticular structures. The restriction of locomotory cilia to the ventral side of the animals is the name-giving feature of gastrotrichs (Fig. 1.2 D–H). Cilia occur as a broad field, as transverse rows, as isolated paired patches, or as paired longitudinal bands in the trunk region (Remane 1936). In the head region, they usually cover the entire ventral surface. An exception occurs in species of the Xenotrichulidae; here, cilia in the anterior region and in the midtrunk region are tightly packed together and form so-called cirri (Fig. 1.1 A). Further, isolated and often stiff

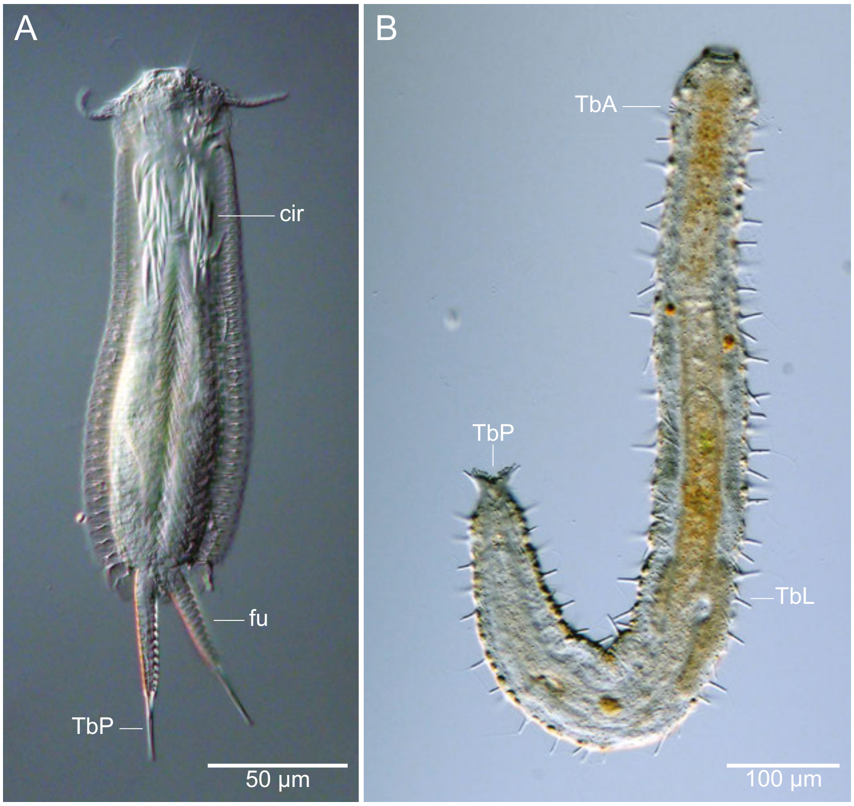

Fig. 1.1: Gross body organization of Gastrotricha. (A) *Xenotrichula velox* (Paucitubulatina) from Tulip Beach, Lee Stocking Island (Bahamas), ventral view. (B) *Turbanella hyalina* (Macrodasyida) from the intertidal at Schillig, Northern Germany, ventral view. (A and B) differential interference contrast (DIC). Abbreviations: cir, ventral locomotorc cirri; fu, furca; TbA, anterior adhesive tubes; TbL, lateral adhesive tubes; TbP, posterior adhesive tubes.

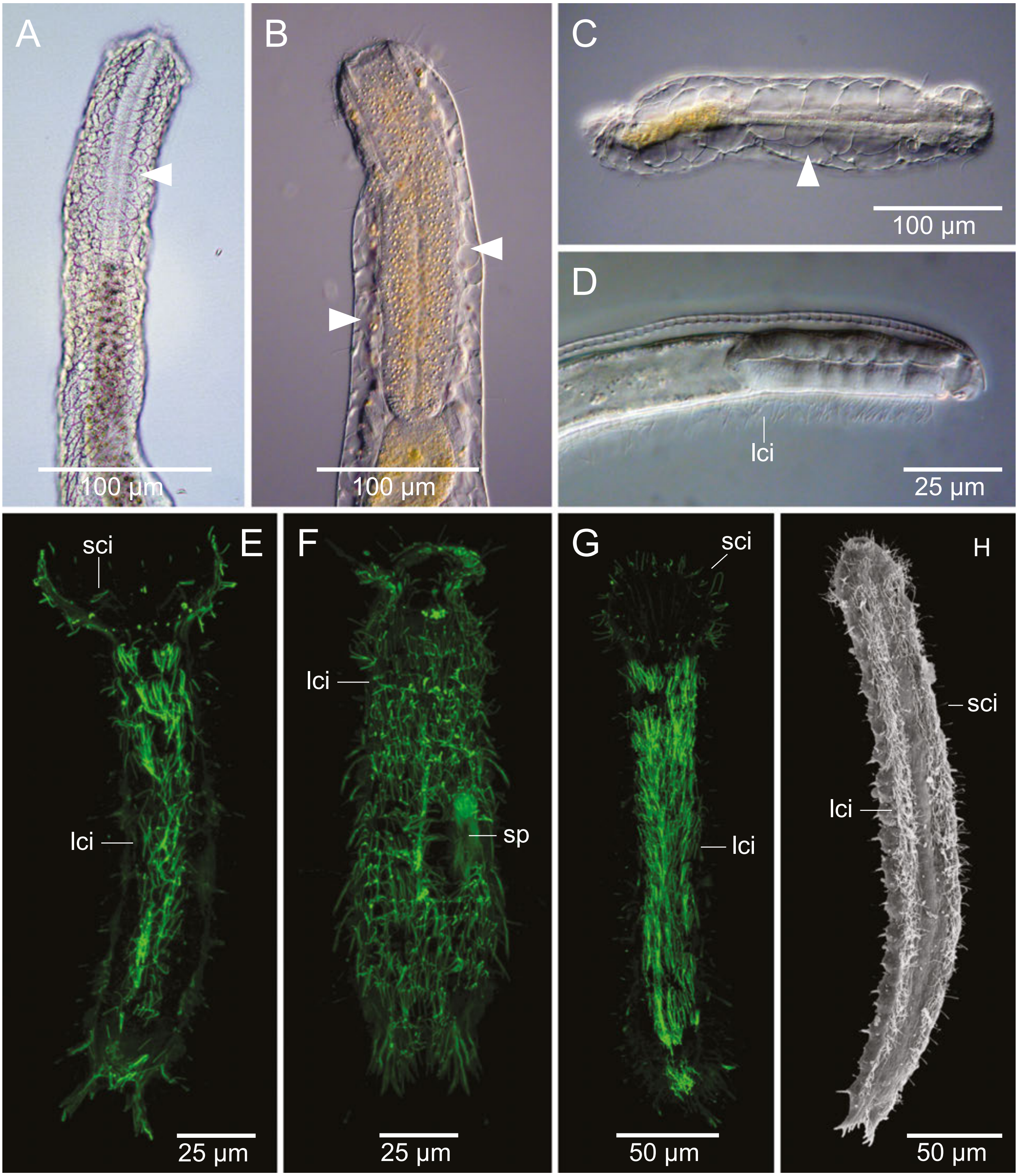

Fig. 1.2: Epidermis and external cilia of Gastrotricha. (A) *Neodasys uchidai* (Multitubulatina). Dorsal view of anterior end. Light microscopic bright field (BF) image. (B) *Macrodasys* sp. Horizontal view of anterior end. (C) Dorsal view of a juvenile *Macrodasys*. Note the vacuolated epidermis cells in A–C (white triangles). (D) Anterior end of a marine *Aspidiophorus* sp., lateral view. Cilia are restricted to the ventral side. (B–D) DIC. (E–G) Maximum projections of confocal image stacks. In all 3 species, α-tubulin was stained with fluorescence-labeled antibodies thereby making visible locomotor and sensory cilia. (E) *Pseudostomella roscovita*. (F) *Thaumastoderma ramuliferum*. (G) *Tetranchyroderma* sp. (H) Scanning electron microscopic (SEM) image of the ventral surface of a *Turbanella subterranea*. Note the paired columns of locomotory cilia. Abbreviations: lci, locomotory cilia; sci, sensory cilia; sp, cilia of spermatozoa.

cilia may be seen in different locations, particularly in the anterior end. Such cilia are assumed to have sensory functions (see chapter Sensory Structures).

The entire body is covered by a cuticle, and this cuticle may form various structures covering the body, especially on the dorsal side. Such coverings are spines, scales, or mixtures of these forms. In macrodasyids, scales occur, for example, in genera such as *Lepidodasys* and *Diplodasys* and spines occur in *Acanthodasys*. However, species of *Diplodasys* additionally possess spines at the lateral margins while species of *Acanthodasys* may display a spiny surface interspersed with tiny scales. Very conspicuous are cuticular structures in which three to five curved and pointed branches originate from a common base. Such structures are called triancres, tetrancres, and pentancres, and occur in the genera *Pseudostomella, Thaumastoderma*, and *Tetranchyroderma*. In chaetonotids, the variability of spines and scales is more diverse and characteristic for the genera. Spines can originate from scales (spined scales) or scales can be stalked, i.e., they rest on a cuticular rod that is basally connected to the cuticle that directly lines the epidermis. There are, however, also many species with a rather thin cuticle without any of the above-mentioned differentiations. This condition was probably also present in the last common ancestor of Gastrotricha (Kieneke et al. 2008a).

1.2.2 Integument

The integument of gastrotrichs is composed of a layer of epidermal cells, the cuticle, and the basal extracellular matrix (ECM). Also described here are glandular structures associated with the epidermis, in particular the adhesive tubes and epidermal glands.

The epidermis is cellular, and the cells differ in their structure between the dorsal and the ventral sides of the animal. The ventral cells are usually cuboidal ciliated cells; the dorsal cells are flatter, contain less cytoplasm, and lack cilia (Ruppert 1991). Epidermal cells are connected to each other by adhaerens junctions and septate junctions (Ruppert 1991). Adhaerens junctions are mechanical cell-cell connections, and they occur in various types, of which desmosomes and hemidesmosomes are the most well-known ones (see Schmidt-Rhaesa 2007). At least in species of *Macrodasys*, the dorsal epidermal cells are large and contain a vacuole (Teuchert 1978, Ruppert 1991; Fig. 1.2 A–C), this condition suggests either a skeletal function during locomotion (Teuchert 1978) or possibly an adaptation to the interstitial habitat (Ax 1966). To our knowledge, gap junctions have not been shown in gastrotrichs but should be present because they are broadly distributed among eumetazoans (see, e.g., Schmidt-Rhaesa 2007). The basal lamina (ECM) is very thin or may even be absent in gastrotrichs (Ruppert 1991).

The name-giving feature of gastrotrichs is the presence of locomotory cilia on the ventral side of the animals (Fig. 1.2 D). Their action allows ciliary gliding, which is the most important form of locomotion (see chapter Musculature for muscle-aided locomotion). The ventral cells have either one cilium per cell (monociliated; Fig. 1.3 A) or more cilia per cell (few up to about 40; multiciliated; Fig. 1.3 B). Monociliated cells occur in several macrodasyids and in *Neodasys*, multiciliated cells in all Paucitubulatina and several macrodasyids (Rieger 1976, Boaden 1985, Ruppert 1991).

Cilia have the usual axonemal pattern of internal microtubules (nine peripheral duplets and two single central ones) and the usual basal structure of nine peripheral triplets of microtubuli (Fig. 1.3 E). An accessory centriole is present and a pair of ciliary rootlets anchors the cilia in the epidermal cells (Rieger 1976, Ruppert 1991; Fig. 1.3 A, B). In most species, a rostral and a caudal rootlet are present (see Rieger 1976 for length measurements), only in the investigated species of *Lepidodasys* and *Xenotrichula* is the rostral rootlet absent (Rieger 1976). Species of Xenotrichulidae are peculiar in possessing cirri, which are bundles of cilia that act as a functional unit (Ruppert 1979; Fig. 1.3 B). In *Xenotrichula*, each cirrus as a whole is surrounded by epicuticle (Rieger 1976, Ruppert 1991; Fig. 1.3 B), wheraes this is not the case in the xenotrichulid genus *Draculiciteria* (Ruppert 1991). The rootlets of all cilia from one cirrus form a bundle that is anchored in the epidermal cell (Rieger 1976, Ruppert 1991; Fig. 1.3 B). Cirri also occur in species of the genus *Oregodasys*, but these have not been investigated ultrastructurally to date.

The epidermis is covered by a cuticle, and the pharynx also has a cuticular lining. Further cuticular structures are rare; in some species of *Urodasys*, a cuticular stylet is present in the reproductive system (Schoepfer-Sterrer 1974). A further presumably cuticular hard part of the reproductive system may be the recently discovered sclerotic canal inside the caudal organ of *Tetranchyroderma bronchostylus* (Atherton & Hochberg 2012). The body cuticle is composed of two layers, the endocuticle and the epicuticle. Please note that the outer layer is often called “exocuticle”, but for comparative reasons explained below, we prefer the

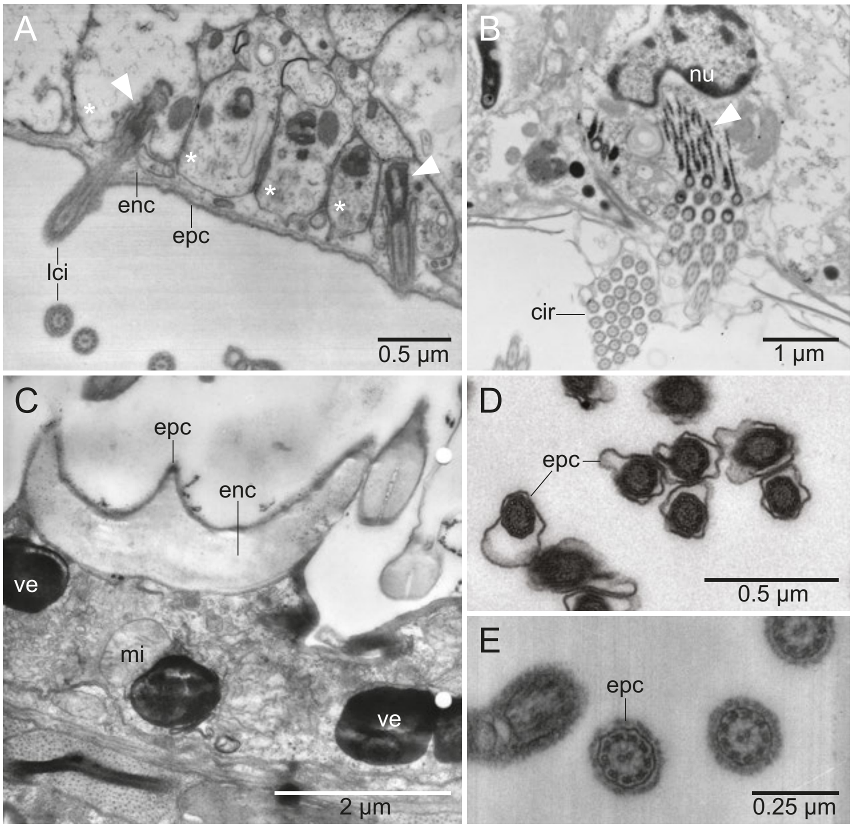

Fig. 1.3: Ultrastructure of epidermis, external cilia and cuticle of Gastrotricha. (A) *Neodasys chaetonotoideus* (Multitubulatina), ventral epidermis with monociliated epithelial cells. Note the cellular junctions/belt desmosomes (asterisks) and the basal bodies of 2 cilia (white triangles). (B) *Xenotrichula carolinensis* (Paucitubulatina), multiciliary ventral epidermis cell forming a locomotory cirrus. Note the bundle of ciliary rootlets (white triangle). (C) Dorsal epidermis of *Tetranchyroderma* sp. (Macrodasyida) with a pentancre formed by endocuticle and epicuticle. (D and E) Cross sections of locomotory cilia covered by epicuticle: (D) *Chaetonotus maximus* and (E) *Neodasys chaetonotoideus*. (A–E) TEM images of cross-sectioned specimens. Abbreviations: cir, locomotory cirrus (compound cilium); enc, endocuticle; epc, epicuticle; lci, locomotory cilium; mi, mitochondrium; nu, nucleus of epidermis cell; ve, electron-dark vesicles.

term “epicuticle”. The epicuticle covers the entire body, including the sensory and locomotory cilia (Fig. 1.3 D, E), which is a very peculiar feature among animals and much likely an autapomorphy of the Gastrotricha (Kieneke et al. 2008a). Sensory cilia in *Tetranchyroderma adelae* are more complex; in these case, both cuticular layers, endocuticle and epicuticle, surround each cilium (Hochberg 2008). Within the endocuticular layer, 10 microvilli are embedded (Hochberg 2008).

Among gastrotrich species, the cuticle varies in thickness and may be smooth or sculptured. The fine structure of the cuticle was extensively investigated by Rieger & Rieger (1977) and Ruppert (1991); if not otherwise indicated, the following data refer to these sources.

The thickness of the cuticle ranges from 100 nm up to 4 µm. The two layers, epicuticle and endocuticle, can always be distinguished. The epicuticle is composed of a varying number of layers. Each such layer is usually trilaminate, which means that it is composed of an electron-dark outer and inner sublayer and an electron-lucent middle sublayer. In some species, this trilaminate substructure of the individual layers has not been recognized and appears to be a thin monolayer (observed in representatives of *Crasiella*, *Dactylopodola*, and *Urodasys*) or a thicker monolayer (observed in *Neodasys*, Fig. 1.3 A, E). The number of layers ranges from 1 to 25, species with an unsculptured (smooth) cuticle have 2 to 25 layers, species with a sculptured cuticle have 1–18 layers (see, e.g., Balsamo et al. 2010a for one layer of epicuticle in *Diuronotus aspetos* and *Musellifer delamarei*). The endocuticle is granular or fibrous in fine structure, and sometimes, a subdivision is observed. In this case, there is an outer striated, a middle finely fibrous, and an inner loosely fibrous substructure.

Especially in species of Paucitubulatina, there are local thickenings of the cuticle in the head region that have the appearance of cuticular plates (cephalion, pleura, hypostomium) (Fig. 1.4 J, K). The presence and shape of these plates is of taxonomic importance. Ultrastructural sections through these regions are not available, but there is a peculiar surface structure of curved ridges on the cuticular plates on the head of *Lepidodermella squamata* observable with the scanning electron microscope (SEM) (Hochberg 2001).

When cuticular structures such as spines or scales are present (Fig. 1.4 A–I), these are formations of the endocuticle. Rieger & Rieger (1977) and Ruppert (1991) recognized three different types of such structures. In *Xenodasys*, the cuticular structures are hollow and include processes of the epidermis. In all other cases, structures are only made of cuticular material, and no epidermal components extend into them. In macrodasyids (*Lepidodasys* and Thaumastodermatidae), cuticular structures are solid local thickenings in the endocuticle (see Hochberg 2008 as one example; Fig. 1.3 C). Sometimes a substructure such as a fine striation or a honeycomb pattern can be observed. In representatives of Paucitubulatina, cuticular structures are derivates of the outer sublayer of the endocuticle; they have a homogeneous or finely striated substructure. In *Diuronotus aspetos* and *Musellifer delamarei*, the solid scales are made up of two electron-dense, homogeneous layers (Balsamo et al. 2010a). Sometimes scales and spines of Paucitubulatina are hollow structures, but in contrast to *Xenodasys*, they never include epidermal extensions.

The gastrotrich cuticle does not contain chitin (Neuhaus et al. 1996), and is not molted during development (Ruppert 1991). Despite the fact that molecular analyses do not favor a close relationship between gastrotrichs and cycloneuralians (nematodes and related groups, see Schmidt-Rhaesa 2013), the structure of the cuticle appears comparable to some extent. Cycloneuralians probably have an ancestral cuticular structure composed of three layers, a proteinaceous endocuticle, a chitinous exocuticle, and a trilaminate epicuticle (see Schmidt-Rhaesa et al. 1998). The trilaminate epicuticle and the proteinaceous endocuticle appear comparable and could argue for a common ancestor of Cycloneuralia and Gastrotricha. During gastrotrich evolution, the epicuticle becomes multiplied; during cycloneuralian evolution, an additional layer, the chitinous exocuticle, occurs (Schmidt-Rhaesa 2002).

In macrodasyids, the epidermis often contains glandular cells (glandulocytes), the so-called epidermal glands (Fig. 1.5 A–D). Epidermal glands may be arranged in paired longitudinal rows along the dorsal side of the animals. Each epidermal gland is composed of a single, flask-shaped glandulocyte and acts as an individual unit. Beside the sparsely arranged organelles and a rather big,

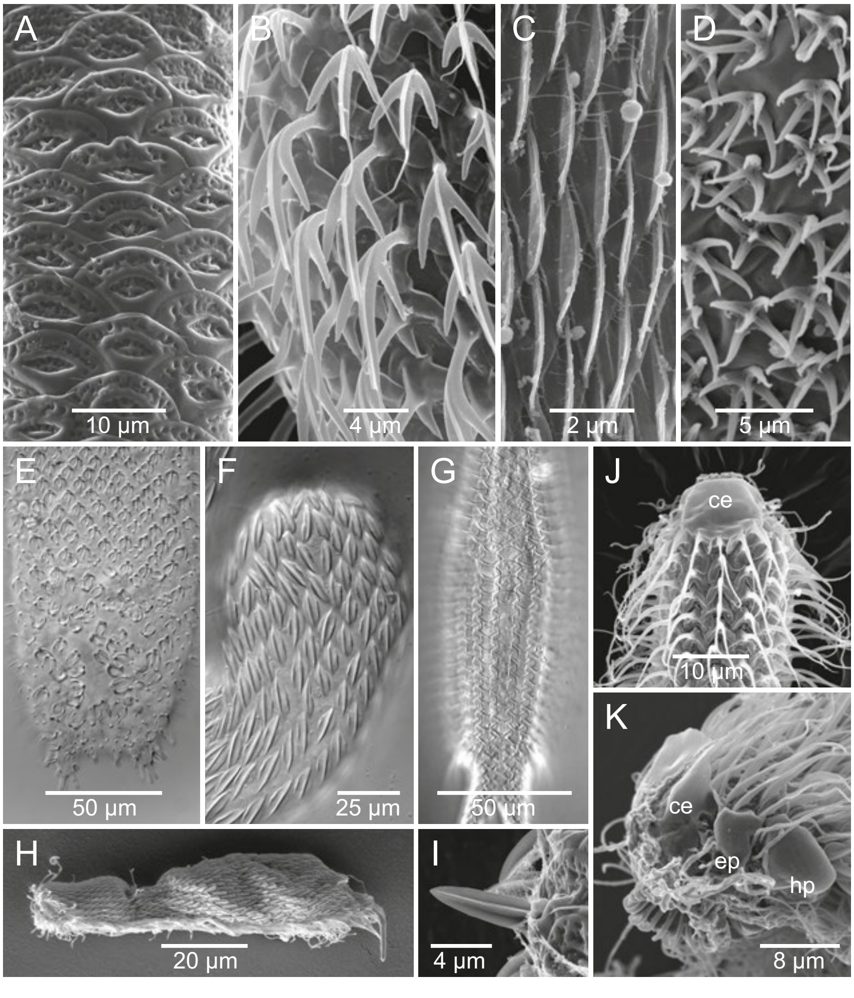

Fig. 1.4: Cuticular differentiations of Gastrotricha. (A) Dorsal tile-like scales of *Diplodasys rothei* (Macrodasyida). (B) Spined scales of *Chaetonotus schulzei* (Paucitubulatina). Note the pair of denticles slightly proximal to the tip of each spine. (C) Dorsal keeled scales of *Aspidiophorus* sp. (Paucitubulatina) (D) Pentancres of *Tetranchyroderma* sp. (Macrodasyida). (E) Another species of *Tetranchyroderma* with tetrancres. (F) Keeled scales of *Lepidodasys* sp. (Macrodasyida). (G) Slightly polygonal scales of *Draculiciteria tesselata* (Paucitubulatina). (H) *Heterolepidoderma* sp. (Paucilubulatina) with keeled scales, lateral view. (I) Lateral thorn of *Diplodasys rothei*. (J) Head region of *Chaetonotus maximus* (Paucitubulatina), dorsal view. (K) Head region of another species of *Chaetonotus*, lateral view. Note the “mouth basket” around the mouth opening. (A–D and H–K) SEM images. (E–G) DIC images. Abbreviations: ce, cephalion; ep, epipleurion; hp, hypopleurion.

basally positioned nucleus, there are few big secretion granules with irregularly staining content (Fig. 1.6). Secretion products are released through an apical pore within the cuticle (Teuchert 1977a, Ruppert 1991). In *Turbanella cornuta*, the apical neck of the glandulocyte is formed by a cellular protrusion that is surrounded by up to 50 microvilli, which do not penetrate the cuticle. Further proximal, 10 rings of short microvilli surround the neck of the epidermal gland (Fig. 1.6). It is hypothesized that these microvilli serve as a mechanic protection against pressure from the surrounding epidermis cells (Teuchert 1977a). In *Oregodasys katharinae*, the glandular system is more complex, it comprises at least three types of papillae beneath the cuticle, blunt, triangle-shaped and sensory ones (Hochberg 2010a). Furthermore, insunk glandulocytes are present. Until now, there is no convincing hypothesis for the functional role of the epidermal gland system in macrodasyidan gastrotrichs (Ruppert 1991, but see Hochberg 2010b).

Conspicuous glandular structures of the Gastrotricha are the adhesive tubes (Fig. 1.7), which are present in different body regions in macrodasyids and on the posterior end in paucitubulatinan chaetonotids. These structures are tube-like extensions of the body cuticle containing two types of glandulocytes (all information in this section from Tyler & Rieger 1980 and Ruppert 1991. These glandulocytes are basal to the tubes and extend through the tube to open at its apical end where the cuticle is broken by one or more pores. Two different types of glandulocytes are distinguished (Fig. 1.8 A–B). One produces a secretion made up of larger, electron-dense vesicles, and this is called "viscid gland cell" and is assumed to have an adhesive function. The other glandulocyte produces smaller vesicles, this is called "releasing gland cell" and is assumed to dissolve the adhesive secretion and release the attachment. In the investigated cases, one releasing gland cell and one to few viscid gland cells have been observed. They usually open through one apical pore, but in *Tetranchyroderma* sp., each of the two viscid cells has its own pore. The structure of the adhesive tubes corresponds to the definition of the "duo-gland adhesive system" (see, e.g., Tyler 1988). Further, glandulocytes (epidermal glands) or

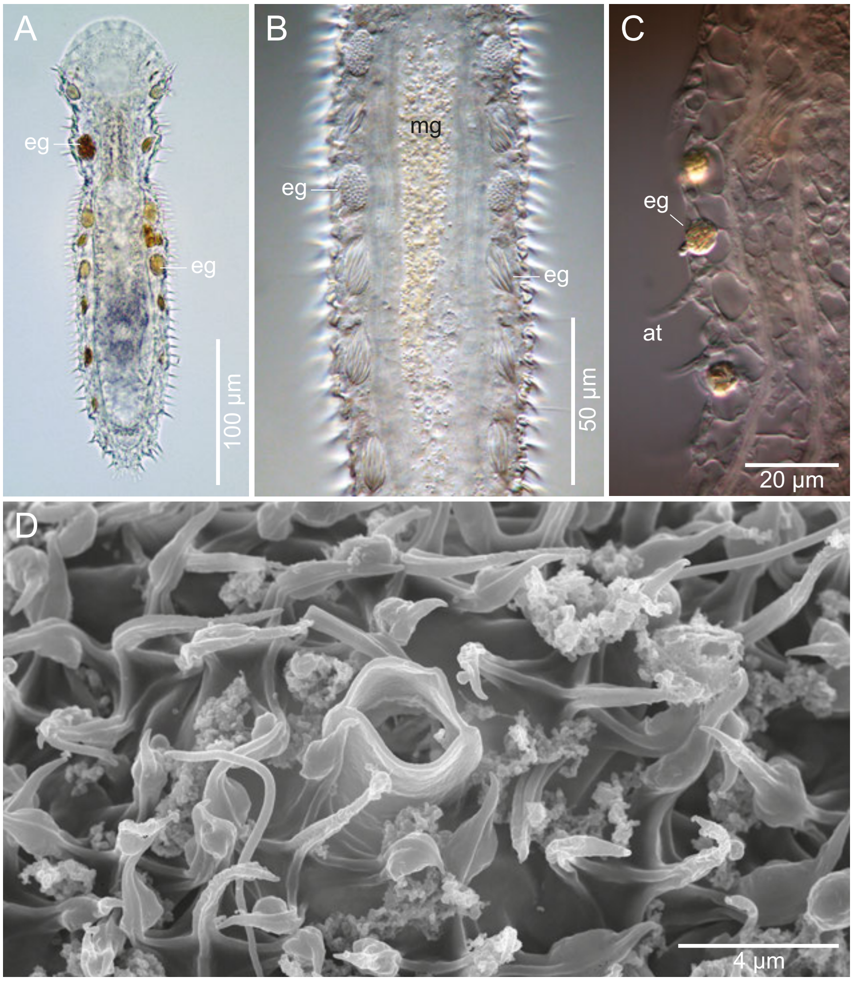

Fig. 1.5: Epidermal glands of Macrodasyida. (A) *Diplodasys* sp., habitus. Note the slightly colored epidermal glands. (B) Different types of epidermal glands of *Diplodasys* cf. *meloriae*. (C) Epidermal glands and adhesive tubes within the vacuolated epidermis of *Turbanella bocqueti*. (D) Elevated pore of an epidermal gland of *Tetranchyroderma* sp. (A) BF-image (B and C) DIC Images. (D) SEM image. Abbreviations: at, adhesive tube; eg, epidermal gland; mg, midgut.

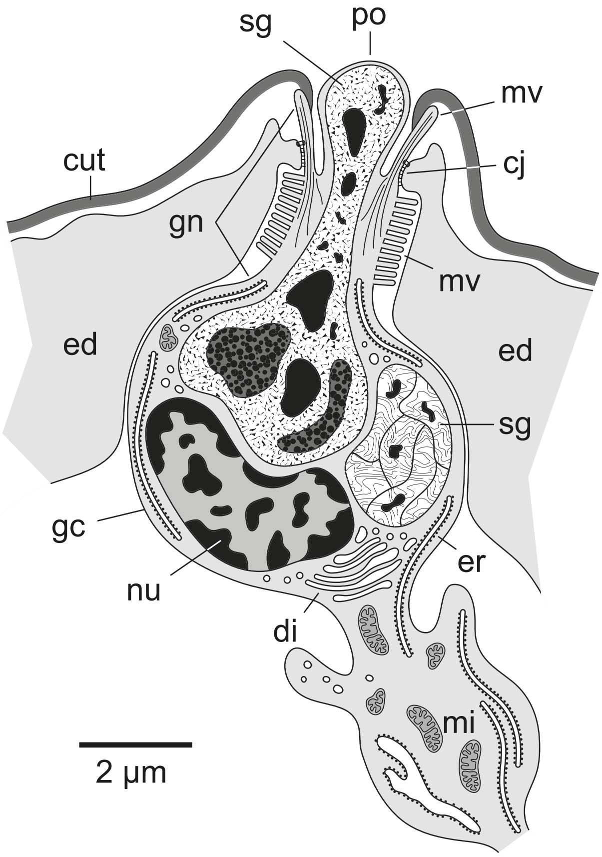

Fig. 1.6: Epidermal gland of *Turbanella cornuta*, schematic. Note the circularly arranged microvilli on the surface of the neck of the gland cell. The proximal extension of the gland cell leads to the basal matrix. Abbreviations: cj, cellular junction (adhaerens junction and septate junction); cut, cuticle; di, dictyosome; ed, epidermis; er, endoplasmic reticulum; gc, gland cell; gn, neck of the gland cell; mi, mitochondria; mv, microvilli; nu, nucleus; po, pore of the epidermal gland; sg, secretory granule. (According to figure 5 of Teuchert 1977).

monociliated sensory cells can be associated with the adhesive tubes (see chapter Sensory Structures).

A peculiar exception to the structure described above occurs in the genus *Neodasys* (Tyler et al. 1980, see also Ruppert 1991; Fig. 1.7 E). *Neodasys* has, from a general appearance, a distribution of adhesive tubes comparable to macrodasyidan species (however, anterior adhesive tubes are absent in *Neodasys*), but the fine structure of these tubes is not comparable. The papilliform lateral tubes in *Neodasys* contain only one glandulocyte per tube, this cell has a rudimentary cilium. A ciliated sensory cell is closely associated with this glandulocyte (Fig. 1.8 C). Tyler et al. (1980) interpret this structure as a kind of forerunner of the duo-gland adhesive tubes of other gastrotrichs. According to this model, the viscid gland cell(s) of the adhesive tubes are derived from ciliated epidermal cells, and the releasing gland cell is derived from the associated sensory cell. Such a scenario also supports the hypothesis of *Neodasys* as the earliest branch within the phylogenetic system of Gastrotricha as proposed by Kieneke et al. (2008a). The posterior adhesive organs of *Neodasys* are also of peculiar structure. The paired caudal feet include adhesive tubes similar in ultrastructure to the lateral ones but additionally possess a distal pore lined by a microvillar border of a specialized myoepithelial cell (Tyler et al. 1980, Ruppert 1991).

1.2.3 Musculature

Solely based on light microscopic investigations, Remane (1936) already provided a quite detailed understanding of the muscular system in different species of the Gastrotricha, for instance, in *Macrodasys* sp., *Turbanella cornuta, Dactylopodola baltica, Chaetonotus* sp., *Chaetonotus simrothi, Aspidiophorus paradoxus*, and *Dasydytes ornatus*. Although he was not able to present any data on circular muscles in Gastrotricha in his slightly earlier monograph (Remane 1929), the occurrence of this muscle component could be demonstrated a few years later in taxa such as *Polymerurus, Dactylopodola, Pleurodasys*, and *Oregodasys* (Remane 1936). He concluded that Gastrotricha are characterized by a musculature consisting of a system of separate strands of longitudinal and circular muscles in contrast to the nearly closed muscular sheath below the epidermis composed of outer circular muscle layer and inner longitudinal muscle layer in many other vermiform taxa of the Bilateria, a consideration that is currently up-to-date. However, some of Remane's (1936) findings had to be revised or complemented as well, as new techniques and methods provided a much more detailed view on muscle arrangement of microscopic animals. The resolving power of electron microscopy, primarily TEM, gave a first insight to the ultrastructure and cytomorphology of single muscle cells of Gastrotricha. Muscle ultrastructure was intensely studied in *Turbanella cornuta* by Teuchert (1974) and will be reviewed below. Then, the combination of specific fluorescence staining methods (e.g., staining of f-actin with fluorochrome-labeled phalloidin) with three dimensionally resolving confocal laser scanning microscopy (or initially conventional epifluorescence microscopy) provided a holistic look on the myoanatomy of many microscopic invertebrate taxa including the Gastrotricha. Richard Hochberg and Marian K. Litvaitis were the first to demonstrate the diversity of muscle arrangement among several gastrotrich species

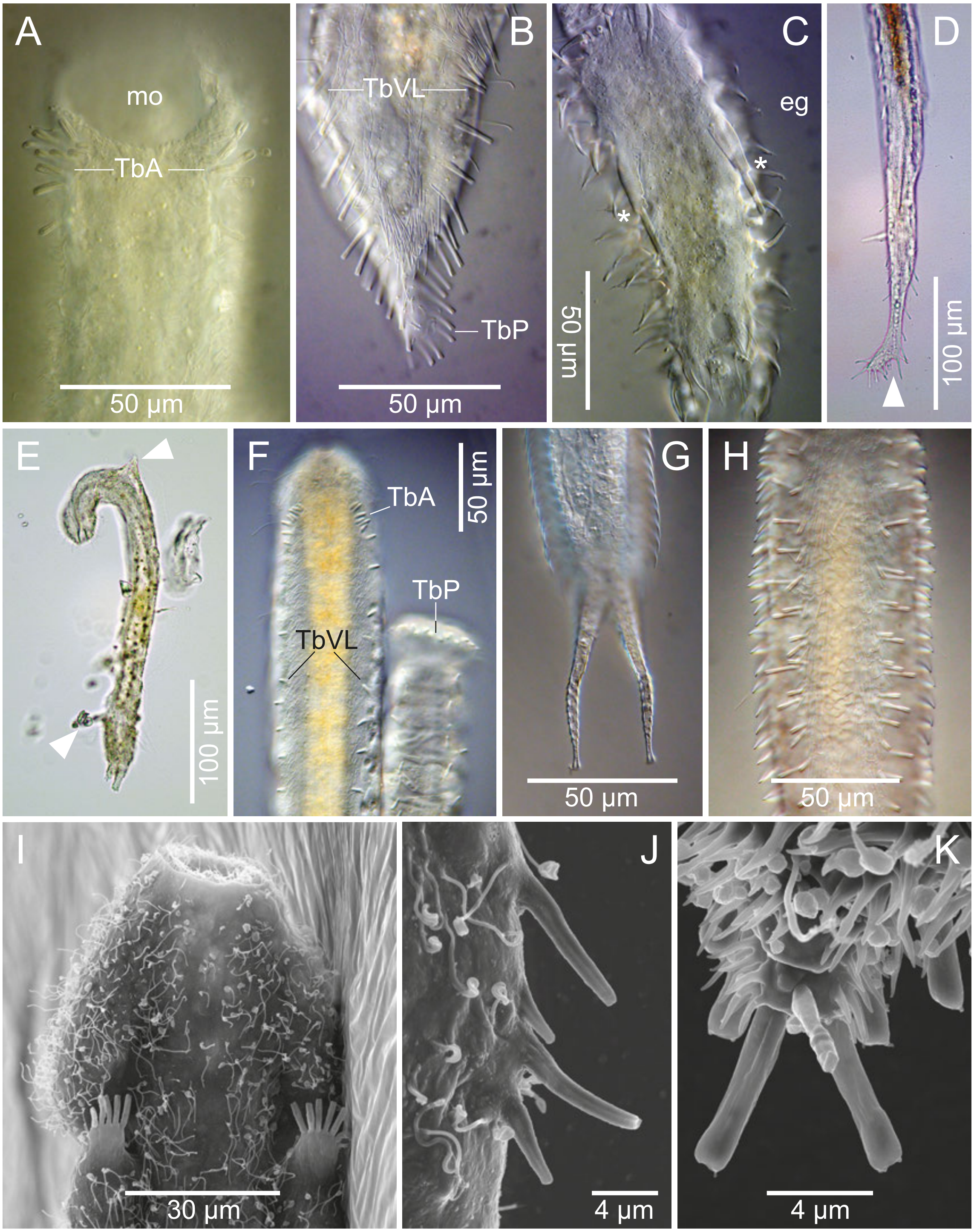

Fig. 1.7: Different arrangements of adhesive tubes in Gastrotricha. (A) *Tetranchyroderma* sp., ventral view of the head. (B) Posterior trunk end of *Macrodasys* sp., ventral view. (C) Ventral view of *Xenodasys riedli* showing the peculiar adhesive organs or pedicles (asterisks). (D) Rear trunk end of *Macrodasys caudatus* that is tightly glued to the microscopic slide (white triangle). (E) *Neodasys chaetonotoideus* that vigorously adheres to the glass slide and to sediment particles (triangles). (F) Anterior and posterior end of *Megadasys* sp., ventral view. (G) Caudal furca of *Draculiciteria tesselata* (Paucitubulatina), ventral view. (H) Underside of *Diplodasys* sp. with ventrolateral adhesive tubes. (I) Ventral side of the head of *Cephalodasys maximus* with hand-like arranged anterior adhesive tubes on a “fleshy base”. (J) Posterior lateral adhesive tubes of *Dactylopodola baltica*. (K) Posterior adhesive tubes of *Tetranchyroderma* sp. on a pedicle. (A–C and F–H) DIC images. (D and E) BF images. (I–K) SEM images. Abbreviations: mo, mouth opening; TbA, anterior adhesive tubes; TbP, posterior adhesive tubes; TbVL, ventrolateral adhesive tubes.

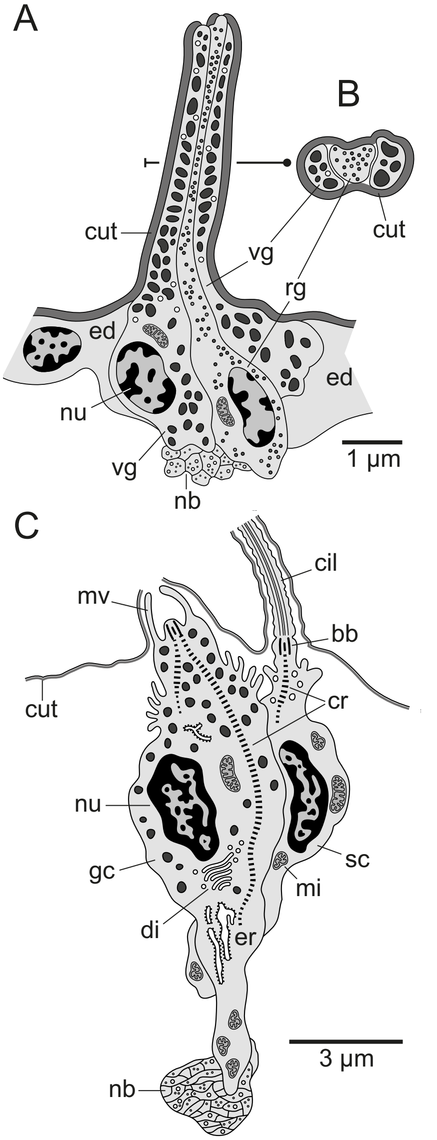

Fig. 1.8: Adhesive organs (adhesive tubes) of Gastrotricha. (A) Longitudinal section (schematic) of a dorsolateral adhesive tube of *Tetranchyroderma* sp. consisting of 2 viscid glands and 1 releasing gland. (B) Cross section of that tube (level indicated by bold line in A). Note the larger and electron-dark vesicles of the viscid glands and the smaller and lighter vesicles of the releasing gland. In *Tetranchyroderma* sp., each gland cell has its own cuticular pore. (C) Longitudinal section (schematic) of a lateral adhesive tube of *Neodasys* sp. A sensory cell, probably mechanoreceptive in function, is closely associated with the single gland cell. A releasing gland is missing in *Neodasys*. Abbreviations: bb, basal body; cil, cilium of the sensory cell; cr, ciliary rootlet; cut, cuticle; di, dictyosome; ed, epidermis; er, endoplasmic reticulum; gc, gland cell; mi, mitochondria; mv, microvilli; nb, neurite bundle; nu, nucleus; rg, releasing gland; sc, sensory cell; vg, viscid gland. (A and B, According to a TEM micrograph of Tyler & Rieger 1980; C, modified from Tyler et al. 1980.)

representing various taxa (Hochberg & Litvaitis 2001a–d, 2003a,Hochberg 2005). Among other findings, these studies comprise the discovery of helicoidally arranged muscles that partially enwrap the gut tube (pharynx plus midgut) and represent a unique character (autapomorphy) of the Gastrotricha (Hochberg & Litvaitis 2001a). Furthermore, quite a high abundance of circular muscles (in visceral as well as somatic positions) was observed in macrodasyidan species and in *Neodasys* (e.g., Hochberg & Litvaitis 2001b, Hochberg 2005), unlike what was anticipated before by Remane (1936). Hochberg & Litvaitis (2001b) also tested and demonstrated the phylogenetic value of muscle characters of Gastrotricha and established a species-character-matrix. This matrix, which has been expanded by current data since, provides a thorough survey of general muscle patterns of the Macrodasyida (Tab. 1.1).

Taking the muscular characters of several species of Macrodasyida and Paucitubulatina and those of two investigated species of the phylogenetically important taxon *Neodasys* (see chapter Phylogeny) into account, Hochberg (2005) suggests a “primitive organization” of the gastrotrich musculature, i.e., the character pattern that was probably present in the last common ancestor (stem species) of Gastrotricha. According to this scenario, the stem species was provided with muscle strands in three different orientations, longitudinal, circular, and helicoidal (Figs. 1.9 and 1.10). The myoepithelial sucking pharynx (for details of the contractile elements of the pharynx see below) is surrounded by numerous consecutive visceral muscle rings followed by visceral longitudinal muscle fibers that accompany the whole gut tube from the terminal mouth opening to the ventral anus. These visceral longitudinal muscles are located dorsal, lateral, and ventral to the gut tube. In the intestinal region posterior to the pharynx, the visceral longitudinal muscle fibers are surrounded by aligned visceral muscle rings. Hence, the spatial arrangement of visceral longitudinal and visceral circular muscles is inverted form the pharynx to the intestine (inner circulars and outer longitudinals *versus* inner longitudinals and outer circulars, see Figs. 1.9 B–C, 1.11 C–D). Both circular and longitudinal muscle components of the gut tube are enwrapped with a muscular double helix. Such fibers are crossing on the dorsal, ventral, and lateral sides of the gut tube (Figs. 1.9 A, 1.10, 1.11 A–B, 1.12 B). The helicoidally arranged muscles do not span the whole intestine down to the anus but only reach the midtrunk region in most species (e.g., Hochberg & Litvaitis 2001b, 2003a). Such a pattern can also be assumed for the stem species of Gastrotricha. In a somatic, ventrolateral position, there is a pair of massive longitudinally arranged muscle bands composed of several closely arran-

Tab. 1.1: Characters related with musculature and muscle arrangement in Macrodasyida.

	Character-number according to Hochberg & Litvaitis (2001b)														**Source**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	
	Somatic circular muscles	**Visceral circular muscles on pharynx**	**Semicircular muscle band on ventral side of pharynx**	**Visceral circular muscles on intestine (midgut)**	**Visceral longitudinal muscles on dorsal side of gut tube**	**Visceral longitudinal muscles on ventral side of gut tube**	**Somatic longitudinal muscle bands in ventrolateral position (*musculus principalis* according to Remane 1936)**	**Splitting of *musculus principalis* in midtrunk region**	**Anterior insertion of *musculus principalis***	**Cross-over muscles in caudal region**	**Branches of *musculus principalis* supply head region**	**Helicoidal muscles enwrap pharynx**	**Helicoidal muscles enwrap intestine (midgut)**	**Muscle striation pattern (see also Tab. 1.3)**	
Dactylopodola baltica	1	1	1	1	1	1	1	1	At level of TbA	1	1	1	1	Cross-striated	Hochberg & Litvaitis (2001b)
Dolichodasys elongatus	1	1	0	1	1	1	1	1	At level of TbA	0	0	1	0	Oblique striation	Hochberg & Litvaitis (2001b)
Paradasys sp.	1	1	1	1	1	1	1	1	At level of TbA	0	0	1	1[a]	Oblique striation	Leasi et al. (2006)
Lepidodasys ligni	1	1	0	?	1	1	1	0	On mouth rim	0	0	1	1	Oblique striation	Hochberg et al. (2013)
Macrodasys caudatus	1	1	0	1	1	1	1	?	On mouth rim	0	0	1	0	Oblique striation	Hochberg & Litvaitis (2001b)
Turbanella ambronensis	1	1	1	1	1	1	1	1	At level of TbA	1[b]	1	1	0	Oblique striation	Hochberg & Litvaitis (2001b)
Turbanella sp.	1	1	1	1	1	1	1	1	At level of TbA	0	1	1	1	Oblique striation	Leasi et al. (2006)
Acanthodasys aculeatus	0	1	0	1	1	1	1	1	On mouth rim	0	0	1	1	Oblique striation	Hochberg & Litvaitis (2001b)
Pseudostomella roscovita	0	1	0	1	1	1	1	0	On mouth rim	1	0	1	1	Oblique striation	Hochberg & Litvaitis (2001b)
Tetranchyroderma papii	0	1	0	1	1	1	1	0	On mouth rim	1	0	1	1	Oblique striation	Hochberg & Litvaitis (2001b)

Tab. 1.1 (*Continued*)

	Character-number according to Hochberg & Litvaitis (2001b)														**Source**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	
	Somatic circular muscles	**Visceral circular muscles on pharynx**	**Semicircular muscle band on ventral side of pharynx**	**Visceral circular muscles on intestine (midgut)**	**Visceral longitudinal muscles on dorsal side of gut tube**	**Visceral longitudinal muscles on ventral side of gut tube**	**Somatic longitudinal muscle bands in ventrolateral position (*musculus principalis* according to Remane 1936)**	**Splitting of *musculus principalis* in midtrunk region**	**Anterior insertion of *musculus principalis***	**Crossover muscles in caudal region**	**Branches of *musculus principalis* supply head region**	**Helicoidal muscles enwrap pharynx**	**Helicoidal muscles enwrap intestine (midgut)**	**Muscle striation pattern (see also Tab. 13.2.3.3)**	
Tetranchyroderma megastoma	0	1	0	1	1	1	1	0	On mouth rim	1	0	1	1	Oblique striation	Hochberg & Litvaitis (2001b)
Thaumastoderma heideri	0	1	0	1	1	1	1	0	On mouth rim	1	0	1	1	Oblique striation	Hochberg & Litvaitis (2001b)
Oregodasys cirratus	1	?	0	?	1	1	1	1	On mouth rim	0	0	1	1	Oblique striation	Rothe & Schmidt-Rhaesa (2010)
Neodasys cirritus	1	1	0	1	1	1	1	?	On mouth rim	0	1	1	1	Cross-striated	Hochberg (2005)

Modified and amended from Hochberg & Litvaitis (2001b). A question mark (?) indicates an unknown character state; 0, absence; 1, presence; TbA, anterior adhesive tubes.

[a] There is only one crossing of the helicoidal muscle on the intestine in *Paradasys* sp. (see Leasi et al. 2006).

[b] Hochberg & Litvaitis (2001b) code the crossover muscle as “present” for *T. ambronensis*, although in the text, they report its absence.

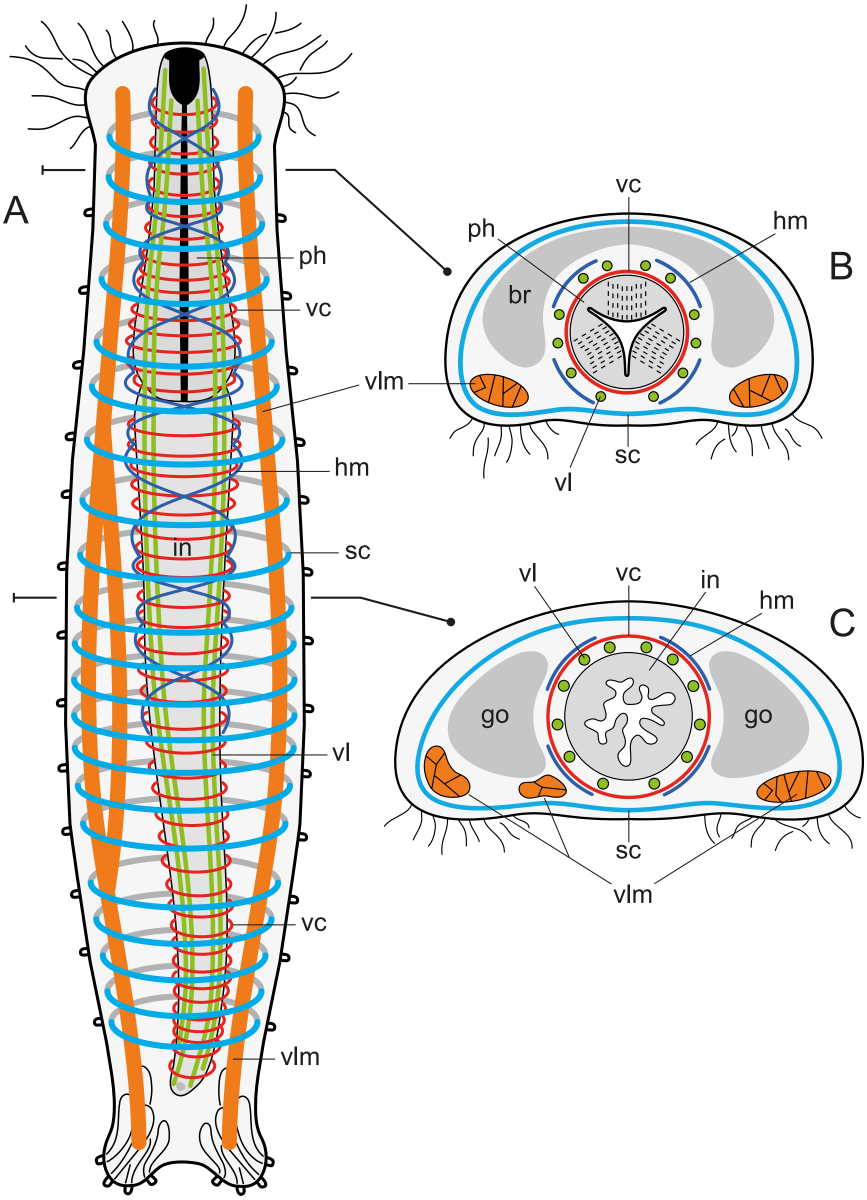

Fig. 1.9: Muscular system (schematic) of the last common ancestor of Gastrotricha. (A) Myoanatomy, dorsal view. (B and C) Trunk cross sections at different levels (indicated by bold lines). Note the reversal of the sequence of visceral circular and longitudinal muscles from pharyngeal (B) to intestinal (C) region. It is not sure if the stem species, like many extant gastrotrichs, possessed a splitting of the ventrolateral muscle bands in the midtrunk region (left body side in A and C). Abbreviations: br, brain; go, gonads; hm, helicoidal muscle; in, intestine; ph, pharynx; sc, somatic circular muscle; vc, visceral circular muscle; vl, visceral longitudinal muscles; vlm, ventrolateral muscle bands (*musculi principales*).

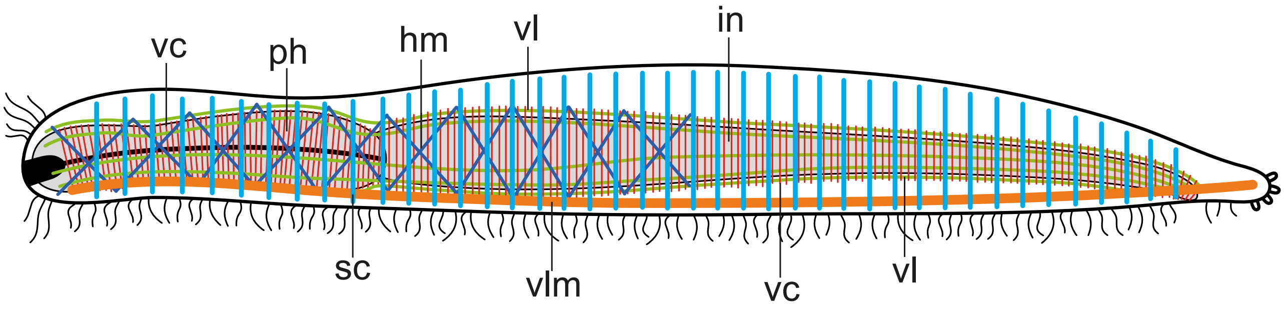

Fig. 1.10: Muscular system (schematic) of the last common ancestor of Gastrotricha, lateral view. Note the reversal of the sequence of visceral circular and longitudinal muscles from pharyngeal to intestinal region. Abbreviations: hm, helicoidal muscle; in, intestine; ph, pharynx; sc, somatic circular muscles; vc, visceral circular muscles; vl, visceral longitudinal muscles; vlm, ventrolateral muscle band (*musculus principalis*).

ged muscle fibers (Fig. 1.12 A–D). These paired *musculi principales* (according to Remane 1936, but see the notes on this terminology in Teuchert 1974) or ventrolateral muscle bands insert anteriorly close to the mouth rim and reach the caudal lobes that bear the posterior adhesive tubes (the stem species of Gastrotricha was characterized by a bilobed caudal trunk end according to Kieneke et al. 2008a). Along the whole body, there are separate somatic circular muscles that surround the ventrolateral muscle bands and probably all other muscular components (Figs. 1.9 and 1.10). Yet, it is not clear if these somatic circular muscles of the stem species of Gastrotricha represent closed rings. In many species of the Macrodasyida, the somatic circular muscles are reported to enclose the ventrolateral muscle bands "on either side of the midgut". Such a description, in addition with the presented data, indicates the presence of incomplete muscle rings at least in these species (e.g., Hochberg & Litvaitis 2001b, Leasi et al. 2006). In *Neodasys*, the somatic circular muscles are branches of the visceral circular muscles that line the gut tube (Hochberg 2005). Such a condition is generally regarded to demonstrate the evolutionary origin of the somatic circular muscles (see Leasi & Todaro 2008 and references therein). As for the stem species, there are not data on the exact numbers concerning different muscular components such as the number of visceral and somatic circular muscles, of visceral longitudinal muscles, of crossings of the helicoidal muscles, and of muscle fibers per ventrolateral muscle band. It remains to mention that there are diverse modifications (e.g., reductions, losses, branching patterns, additional muscle components) from the previously described ancestral muscular character pattern in extant species of Gastrotricha (see Tab. 1.1). These include, for instance, the presence of a peculiar semicircular muscle band on the ventral side of the pharynx in taxa such as *Dactylopodola baltica*, *Paradasys* sp., or *Turbanella* (Hochberg & Litvaitis 2001b, Leasi et al. 2006). When present, this muscle connects both ventrolateral muscle bands (*musculi principales*) close to the level of the anterior adhesive tubes (see Fig. 1.24 C). A comparable (homologous?) muscle is also present in the basal paucitubulatinan species *Musellifer delamarei* (Leasi & Todaro 2008, see Tab. 1.2). Additionally, there might be one or two so-called crossover muscles connecting both parts of *musculi principales* in the caudal region of many but not all species with a bilobed caudal end (Hochberg & Litvaitis 2001b, c, Tab. 1.1). The crossover muscle results from a splitting of one or few fibers of the ventrolateral muscle band passing over to the other side and *vice versa* (Hochberg & Litvaitis 2001c). There is a splitting of each ventrolateral muscle band in the midtrunk region in several species with paired testes: Some fibers run more laterally (*pars lateralis* according to Remane 1936), while others are located more medially (*pars ventrolateralis* according to Remane 1936). Further posterior, all fibers converge forming a common muscle band (e.g., in *Dactylopodola baltica, Paradasys* sp., *Turbanella* sp., or in *Crasiella fonseci*, see Hochberg & Litvaitis 2001b, Leasi et al. 2006, Hochberg 2014). Such kind of splitting is mostly absent in members of the Thaumastodermatinae that only possess one single testis (Hochberg & Litvaitis 2001b, but see Rothe & Schmidt-Rhaesa 2010 for the situation in *Oregodasys cirratus*). This taxon is further characterized by complete absence of somatic circular muscles (Hochberg & Litvaitis 2001b, c). An exception to this pattern is *Oregodasys cirratus* (Rothe & Schmidt-Rhaesa 2010, Tab. 1.1). A peculiarity of whole Thaumastodermatidae is the formation of anterior branches of dorsal visceral longitudinal muscles that spread out into the oral hood (Remane 1936, Hochberg & Litvaitis 2001b, c). These muscle branches facilitate the withdrawal of the oral hood (Remane 1936). Additional variation in muscle organization concerns the anterior insertion of the ventrolateral muscle bands. In species that possess distinct, frequently hand-like anterior adhesive organs composed of several closely arranged adhesive tubes (e.g., taxa *Turbanella* and *Paradasys*), the *musculi principales* terminate in close proximity to these organs and hence a certain distance posterior to the anterior end. In species that do not possess those anterior adhesive organs but instead have separately arranged adhesive tubes close to the anterior trunk end (e.g., *Lepidodasys*, *Macrodasys*, Thaumastodermatidae), the ventrolateral muscle bands insert close to the mouth rim (Tab. 1.1). Species such as *Dactylopodola baltica* and species of *Turbanella* and *Neodasys* have thin branches of the ventrolateral muscle bands in the anterior region that supply the head (Hochberg & Litvaitis 2001b, Hochberg 2005, Tab. 1.1). These are different from the muscle branches within the head region (oral hood) of the Thaumastodermatidae, which are formations of the dorsal visceral longitudinal muscles (see above).

Severe evolutionary modifications of the muscular system occurred both along the stem lineage of the gastrotrich subtaxon Paucitubulatina and within this clade. Through fluorescence staining and confocal or epifluorescence microscopy, the musculature of several species of this group has been studied so far (Hochberg & Litvaitis 2001d, 2003a, Leasi et al. 2006, Kieneke et al. 2008b, Kieneke & Ostmann 2012, Leasi & Todaro 2008, 2009). In contrast to the Macrodasyida and *Neodasys*, the number and arrangement of longitudinal muscles, somatic and visceral, is much more determined in Paucitubulatina.

Pairs of potentially homologous longitudinal muscles that have been detected in all hitherto investigated species of the Paucitubulatina are the *musculi ventrales, m. ventrolaterales, m. laterales, m. dorsales*, and one or two pairs of dorsodermal muscles that are branches of *musculi dorsales* (Hochberg & Litvaitis 2001d, 2003a, Leasi & Todaro 2008, Tab. 1.2, Figs. 1.11, 1.12 E, F). Although this is not based on a thorough phylogenetic reconstruction, we conclude that these five to six longitudinal muscle pairs probably belong to the character pattern of the stem species of Paucitubulatina (this clade is most likely a monophyletic group, see chapter Phylogeny). As most investigated basal species of Paucitubulatina (*Musellifer delamarei, Draculiciteria tesselata*, and most species of Xenotrichulinae) possess only one dorsodermal branch of *musculi dorsales* ("Rückenhautmuskel" according to Zelinka 1889, see Hochberg & Litvaitis 2003a), we suspect that this represents the ancestral condition (Figs. 1.11 and 1.12 F). Owing to their more peripheral position in the body, *musculi laterales* and dorsodermal muscles are considered to represent somatic components, whereas all other longitudinal muscles are visceral components (Hochberg & Litvaitis 2001d, 2003a). However, the assignment to one or the other group of musculature – visceral or somatic – is not always that explicit, and it seems that in different members of the Xenotrichulidae, the *musculi ventrolaterales* rather belong to the somatic musculature (see results of Leasi & Todaro 2008). Similar names imply a homology of these muscles, which may not always be the case. For example, it is likely that the *musculi ventrolaterales* of Paucitubulatina are not homologous to the ventrolateral muscle bands (*musculi principales*) in Macrodasyida and *Neodasys*. It is more likely that the somatic *musculi laterales* of Paucitubulatina are homologous to the ventrolateral muscle bands of Macrodasyida and *Neodasys* or at least to one or two of their fibers.

In addition to the aforementioned five to six pairs of longitudinal muscles, there may be further pairs. In Xenotrichulinae, for instance, there is always a visceral pair of *musculi ventromediales* that spans between *m. ventrales* and *m. ventrolaterales* for most of its course (Hochberg & Litvaitis 2003a, Leasi & Todaro 2008, 2009). In *Draculiciteria tesselata*, there is another somatic (?) longitudinal muscle pair, the *musculi paralaterales*. This muscle spans between *m. laterales* and *m. ventrolaterales* (Hochberg & Litvaitis 2001d). The basal paucitubulatinan species *Musellifer delamarei* has additional somatic longitudinal muscles: There are two pairs of broad muscle bands running along the body wall in dorsal and ventral positions (Leasi & Todaro 2008). Further differences between species can be found in the specific course of certain longitudinal muscles. Especially the *musculi*

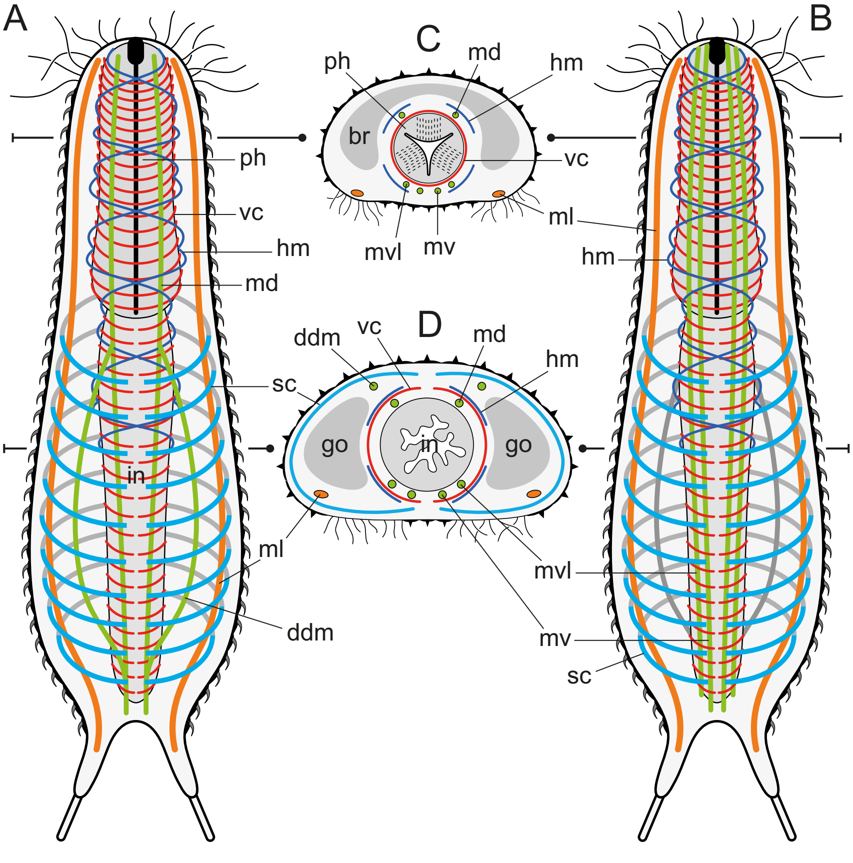

Fig. 1.11: Muscular system (schematic) of the last common ancestor of Paucitubulatina. Myoanatomy in (A) dorsal and (B) ventral views. (C and D) Trunk cross sections at different levels (sectional plains indicated by bold lines). Note the reversal of the sequence of visceral circular and longitudinal muscles from pharyngeal (C) to intestinal (D) region. Abbreviations: br, brain; ddm, dorsodermal muscle; go, gonads; hm, helicoidal muscle; in, intestine; md, *musculus dorsalis*; ml, *musculus lateralis*; mv, *musculus ventralis*; mvl, *musculus ventrolateralis*; ph, pharynx; sc, somatic circular muscle; vc, visceral circular muscle.

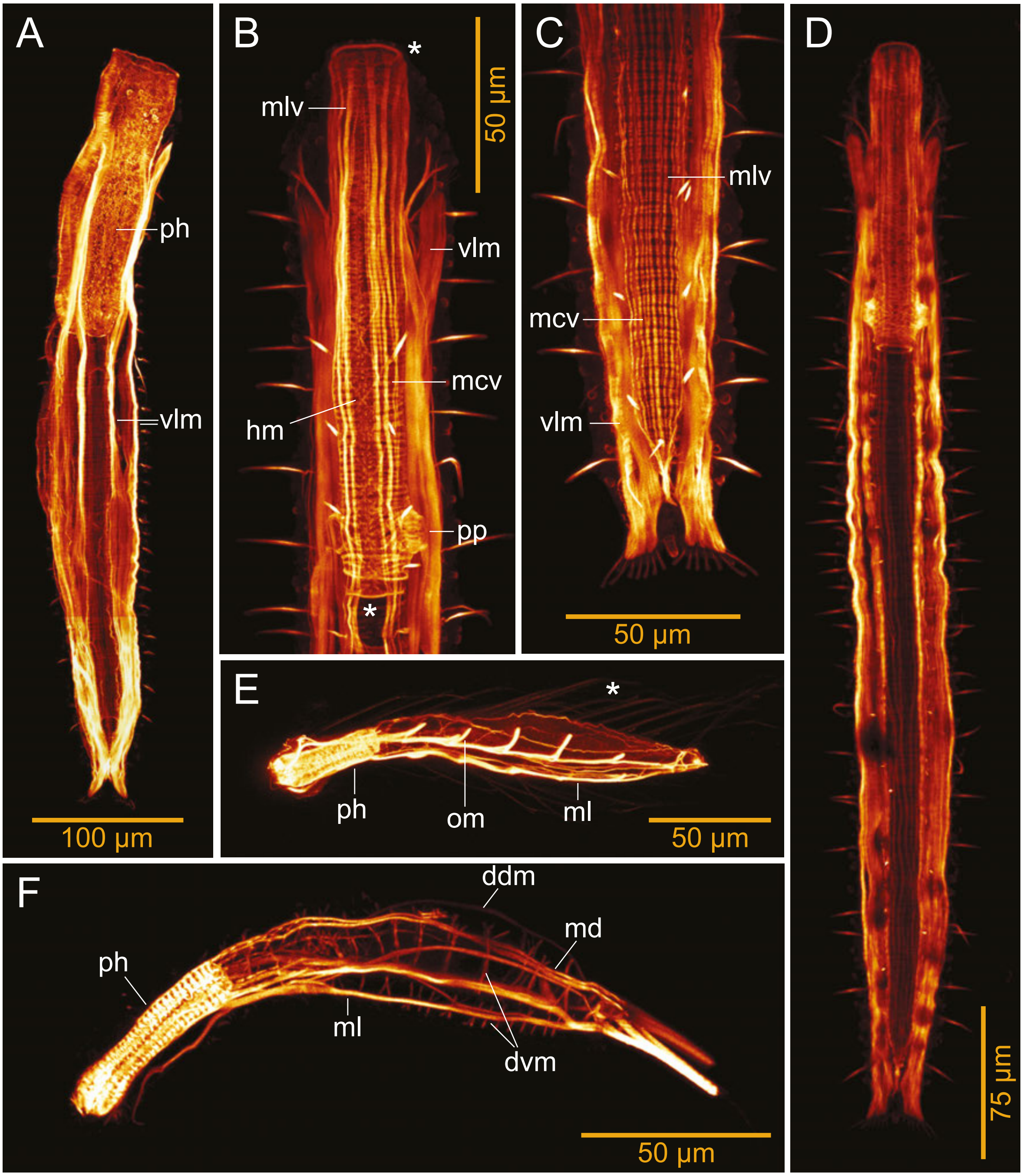

Fig. 1.12: Muscular system of Gastrotricha. Maximum projections of confocal image stacks. F-actin was stained with fluorescence-labeled phalloidin. (A) *Turbanella ambronensis* (Macrodasyida), horizontal view. (B–D) *Turbanella hyalina*, horizontal views. Note the stained filaments inside the adhesive tubes. (B) Anterior end. Note the sphincter-like circular muscles at the anterior and posterior end of the pharynx (asterisks). (C) Rear trunk end. (D) Whole specimen. (E) *Dasydytes goniathrix* (Paucitubulatina) with a highly derived somatic musculature consisting of oblique and segmented longitudinal muscles that are used for moving the long cuticular spines (asterisk, autofluorescence). Lateral view, specimen slightly tilted. (F) *Xenotrichula velox*, a rather primitive species of the Paucitubulatina that still has dorsoventral muscles in a visceral and somatic position, lateral view. Abbreviations: ddm, dorsodermal muscle; dvm, dorsoventral muscles; hm, helicoidal muscles; mcv, visceral circular muscles; md, *musculus dorsalis*; ml, *musculus lateralis* (of Paucitubulatina); mlv, visceral longitudinal muscles; om, oblique muscles; ph, myoepithelial pharynx; pp, pharyngeal pore; vlm, ventrolateral muscle blocks of Macrodasyida (*musculi principales*).

Tab. 1.2: Characters related with musculature and muscle arrangement in Paucitubulatina.

	Character-number according to Leasi & Todaro (2008)							
	1	2	3					
	Number of myocytes (fibers) per somatic longitudinal muscle[a]	**Anterior insertion of somatic longitudinal muscles**[a]	**Muscles in the furca/caudal lobes**	**Visceral *musculi paralaterales***	**Visceral *musculi ventrolaterales***	**Visceral *musculi ventromediales***	**Visceral *musculi ventrales***	**Visceral *musculus dorsalis***
Dactylopodola baltica	>2	Distant to mouth	1	–	–	–	–	–
Neodasys cirritus	>2	Close to mouth[b]	1	–	–	–	–	–
Draculiciteria tesselata	1–2	Close to mouth	1	1	1	0	1	1
Heteroxenotrichula squamosa	1–2	Close to mouth	1	0	1	1	1	1
Xenotrichula intermedia	1–2	Close to mouth	1	0	1	1	1	1
Xenotrichula punctata	1–2	Close to mouth	1	0	1	1	1	1
Aspidiophorus marinus	1–2	Close to mouth	0	0	1	0	1	1
Chaetonotus sp.	1–2	Close to mouth	0	0	1	0	1	1
Halichaetonotus sp. 1/*H. aculifer*	1–2	Close to mouth	0	0	1	0	1	1
Lepidodermella squamata	1–2	Close to mouth	0	0	1	0	1	1
Musellifer delamarei	>2	Distant to mouth	1	0	1	0	1	1
Polymerurus nodicaudus	1–2	Close to mouth	0	0	1	0	1	1

	Character-number according to Leasi & Todaro (2008)				
	4	5	6	7	8 + 9
	Dorsodermal muscle (branch of visceral *musculus dorsalis*)	**Posterior branching of *musculus dorsalis* with crossing**	**Striation pattern**	**Extent of helicoidal muscles**	**Visceral muscles in the intestinal region**
Dactylopodola baltica	0	0	Cross-striation	Longer than one third of intestine	Complete circular
Neodasys cirritus	0	0	Atypical cross-striation	To one third of intestine	Complete circular
Draculiciteria tesselata	1	1	Atypical cross-striation	Longer than one third of intestine	Complete dorsoventral
Heteroxenotrichula squamosa	1	1	Oblique striation	Longer than one third of intestine	Complete dorsoventral
Xenotrichula intermedia	1	1	Oblique striation	Longer than one third of intestine	Incomplete circular
Xenotrichula punctata	1	1	Oblique striation	To one third of intestine	Incomplete dorsoventral
Aspidiophorus marinus	1	0	Oblique striation	To one third of intestine	Absent
Chaetonotus sp.	1	0	Oblique striation	To one third of intestine	Absent
Halichaetonotus sp. 1/*H. aculifer*	1	0	Oblique striation	To one third of intestine	Absent
Lepidodermella squamata	1	0	Oblique striation	To one third of intestine	Absent
Musellifer delamarei	1	0	Atypical cross-striation	To the base of pharynx	Incomplete circular
Polymerurus nodicaudus	1	0	Oblique striation	To one third of intestine	Complete dorsoventral

(*Continued*)

Tab. 1.2: (*Continued*)

	Character-number according to Leasi & Todaro (2008)				**Source**
	10	**11**	**12 + 13**	**14**	
	Termination of circular or dorsoventral muscles in the intestinal region	**Semicircular muscle band(s) on ventral side of pharynx**	**Somatic muscles in the intestinal region**	**Somatic dorsoventral musclee pairs close to the pharyngeointestinal junction**	
Dactylopodola baltica	–	1	Circular	0	Hochberg & Litvaitis (2001b)
Neodasys cirritus	–	0	Circular	0	Hochberg (2005)
Draculiciteria tesselata	Branched	0	Complete dorsoventral	1	Hochberg & Litvaitis (2001d)
Heteroxenotrichula squamosa	Branched	0	Complete dorsoventral	1	Leasi & Todaro (2008)
Xenotrichula intermedia	Branched	0	Incomplete dorsoventral	1	Hochberg & Litvaitis (2003a), Leasi & Todaro (2008)
Xenotrichula punctata	Branched	0	Incomplete dorsoventral	1	Leasi & Todaro (2008)
Aspidiophorus marinus	–	0	Absent	0	Hochberg & Litvaitis (2003a)
Chaetonotus sp.	–	0	Absent	0	Leasi & Todaro (2008)
Halichaetonotus sp. 1/*H. aculifer*	–	0	Absent	0	Hochberg & Litvaitis (2003a)
Lepidodermella squamata	–	0	Absent	0	Hochberg & Litvaitis (2003a)
Musellifer delamarei	Not branched	1	Incomplete dorso-ventral[c]	0	Leasi & Todaro (2008)
Polymerurus nodicaudus	Branched	0	Absent	0	Leasi et al. (2006)

Modified and amended from Leasi & Todaro (2008). *Dactylopodola baltica* and *Neodasys cirritus* were used for the outgroup comparison. A question mark (?) indicates an unknown character state; dash (–), inapplicable character state; 0, absence; 1, presence.

[a] The “somatic longitudinal muscles” are the ventrolateral muscle bands (*musculus principalis*) in Macrodasyida and *Neodasys*, called *musculi laterales* in Paucitubulatina according to Hochberg & Litvaitis (2003a).

[b] Leasi & Todaro (2008) coded the longitudinal muscle insertion in *N. cirritus* distant to the mouth, although original data (Hochberg 2005) demonstrate a different pattern.

[c] Leasi & Todaro (2008) coded the somatic muscles in the intestinal region of *M. delamarei* as “incomplete dorsoventral”, although they are later treated as “incomplete circular” (see, e.g., their figure 7).

laterales and *m. ventrolaterales* may run in close proximity to the pharynx in certain species, whereas in others, they follow the contours of the anterior body or continuously diverge toward the anterior end (compare reconstructions of *Aspidiophorus marinus, Chaetonotus* spp., *Halichaetonotus* spp., and *Lepidodermella* spp. in Hochberg & Litvaitis 2003a). In *Xenotrichula intermedia, musculi ventrales* are reported to cross over in the region of the anus (Hochberg & Litvaitis 2003a). However, such a crossover was not confirmed for *X. intermedia* and other members of the Xenotrichulinae (Leasi & Todaro 2008). Meanwhile, a peculiar x-shaped connection between the paired *musculi dorsales* in the posterior trunk region was detected in all species of Xenotrichulidae (Xenotrichulinae plus *Draculiciteria tesselata*) studied so far (Leasi & Todaro 2008, Tab. 1.2).

In the pharyngeal region of Paucitubulatina, like in Macrodasyida and *Neodasys*, there are densely piled complete circular muscles that line the pharynx inward the visceral longitudinal muscle components. However, such muscle rings could not be detected with certainty in species of the derived paucitubulatinan taxon Dasydytidae (see Kieneke et al. 2008b, Kieneke & Ostmann 2012). In *Musellifer delamarei*, a semicircular muscle band on the ventral side of the pharynx was found, which is comparable to those muscles discovered in *Dactylopodola baltica* and other species of Macrodasyida (Leasi & Todaro 2008, compare Tab. 1.1 with Tab. 1.2). Even more structural diversity can be observed among the “circular” muscle components in the intestinal region of Paucitubulatina (Hochberg & Litvaitis 2001d, 2003a, Leasi & Todaro 2008, 2009, Tab. 1.2). In the assumed basal species *M. delamarei*, there are incomplete visceral circular muscles plus somatic dorsoventral muscles. “Incomplete” refers to the fact that these circular muscles do not represent closed rings but have a median gap dorsally and ventrally (Leasi & Todaro 2008). Some species of the Xenotrichulinae show a comparable muscle arrangement: The visceral component is represented by incomplete circular muscles, whereas the somatic component consists of dorsoventral muscle fibers that attach to the dorsal and ventral integument. In *Draculiciteria tesselata* and *Heteroxenotrichula squamosa*, the somatic and visceral components consist of such dorsoventral muscles. In the lineage that leads to the predominantly freshwater inhabiting Chaetonotidae (much likely this family does not represent a monophyletic group, see chapter Phylogeny), only assumed basal species such as *Polymerurus nodicaudus* possess comparable muscle components: This species still has dorsoventral muscles in a visceral position in its intestinal region, whereas any circular or dorsoventral components are absent in the somatic position (Leasi et al. 2006, Leasi & Todaro 2008, Tab. 1.2). Other taxa of the Chaetonotidae like *Aspidiophorus, Chaetonotus, Halichaetonotus*, and *Lepidodermella* completely lack circular or dorsoventral muscles apart from a tiny single pair close to the anus (Hochberg & Litvaitis 2003a, Leasi & Todaro 2008). Also, species of the exclusively freshwater-dwelling Dasydytidae lack any circular muscle components in the intestinal region (Kieneke et al. 2008b, Kieneke & Ostmann 2012), a result that partially revises the muscular description of *Dasydytes ornatus* by Remane (1936), who suspected the presence of incomplete circular muscles in that species. The Paucitubulatina are another example for muscular diversity: The muscle endings are simple in *M. delamarei*, whereas all investigated Xenotrichulidae and *P. nodicaudus* have branched muscle endings (Tab. 1.2). We can deduce from the evolutionary scenario of circular muscle evolution in Paucitubulatina developed by Leasi & Todaro (2008), that the last common ancestor of Paucitubulatina could have had a system of incomplete circular muscles in visceral and somatic positions in its intestinal region in addition to the longitudinal muscle pairs discussed above (Fig. 1.11). The presence of incomplete circular muscles in a somatic position, however, is purely hypothetical and has never been observed in any extant species of the Paucitubulatina (Leasi & Todaro 2008). Because a visceral helicoidal musculature is present in all investigated species of Paucitubulatina studied so far (Hochberg & Litvaitis 2001d, 2003a, Leasi et al. 2006, Kieneke et al. 2008b, Kieneke & Ostmann 2012, Leasi & Todaro 2008, 2009), this muscular component is an ancestral paucitubulatinan character, too.

The taxon Dasydytidae is a highly derived group of freshwater-dwelling planktonic gastrotrichs (almost all other species of Paucitubulatina have an endobenthic, epibenthic, or periphytic lifestyle, see chapter Ecology), which have paired groups of motile cuticular spines. These spines are actively movable and can be abducted and adducted serving either for supporting locomotion (species such as *Haltidytes crassus* may perform short “jumps” in the water column) or for performing defensive positions at which the animals take a strong ventral flexion of the trunk and abduct their spines to a maximum. Spine movement is brought about by a highly specialized musculature consisting of serially arranged somatic oblique muscles and segments of the partitioned *musculi laterales* (Kieneke et al. 2008b, Kieneke & Ostmann 2012; Fig. 1.12 E). Although it was initially supposed that oblique and segmented longitudinal muscles represent elements of an antagonistically working system (Kieneke et al. 2008b), it is more likely that both muscle components work synergistically to facilitate the spine abduction-adduction cycle (Kieneke & Ostmann 2012). It is not likely that the oblique musculature of Dasydytidae is a derivate of ancestral somatic circular muscles because not a single investigated species of the Chaetonotidae, among them putative relatives of the Dasydytidae (see, e.g., Kånneby et al. 2013), possesses a somatic circular musculature. Somatic circular (or dorsoventral) musculature, still present in Muselliferidae and Xenotrichulidae (see above), hence must have been lost within the lineage that leads to Chaetonotidae, Dasydytidae, and some other minor taxa of freshwater-dwelling gastrotrichs. Oblique musculature therefore represents a new evolutionary formation, an autapomorphy of the Dasydytidae (Kieneke & Ostmann 2012).

Apart from the visceral and somatic musculature of Gastrotricha that is present as longitudinal, circular/dorsoventral, and helicoidal muscles (see above), there may be further muscle arrangements. Most prominent is a specialized musculature as part of the reproductive system. Taxa that possess a distinct caudal organ as a sperm-transferring device (see chapter Reproductive Organs) may have a strong circularly or slightly helically arranged musculature that surrounds this accessory reproductive organ as, for example, in *Macrodasys* sp. (Ruppert 1978a), *Tetranchyroderma papii* (Hochberg & Litvaitis 2001c), or in *Lepidodasys ligni* (Hochberg et al. 2013). Muscle contractions of the caudal organ are used to support the release of spermatozoa from the caudal organ lumen or, as in *Macrodasys* sp., to evert the copulatory tube (Ruppert 1978a, see also chapters Reproductive Organs and Reproductive Biology). However, there are also species that contain a caudal organ but obviously lack a specialized musculature like *Crasiella fonseci* (Hochberg 2014), although congeneric species, e.g., *C. diplura*, possess such a circular muscle sheath (Guidi et al. 2011). It is hypothesized that caudal organ musculature of *C. fonseci* may be formed rather late during an individual's development (Hochberg 2014). A sheath of circular muscles also surrounds the distal parts of the vas deferens in species of Thaumastodermatidae (Ruppert 1978b). A comparable musculature on the distal section of the sperm ducts may be present in species of *Turbanella* (see figure 2c of Leasi et al. 2006). A narrow, ring-shaped muscle at the level of the anus was reported from different species. This single circular muscle probably represents an anal sphincter (see, e.g., Leasi et al. 2006, Kieneke et al. 2008b). A *sphincter oris* surrounding the mouth opening is reported for different species of the Paucitubulatina (Remane 1936). A strong, sphincter-like circular muscle is also present around the mouth opening of the macrodasyid *Crasiella fonseci* (Hochberg 2014) and in species of *Turbanella* (Fig. 1.12 A–B).

The ultrastructure of musculature and muscle cells was intensively studied in the marine gastrotrich *Turbanella cornuta* (Teuchert 1974). These findings were later complemented by ultrastructural data of several other species from all of the three major gastrotrich subtaxa Macrodasyida, *Neodasys* (Multitubulatina), and Paucitubulatina (Ruppert 1991, see Tab. 1.3). Longitudinal muscle cells of *T. cornuta* are to 40 µm long, spindle-shaped, and with an axially situated cytoplasmic compartment at one end that houses the nucleus (see Fig. 1.13 A for longitudinal muscles in *Polymerurus*). In cross section, such longitudinal muscle cells are somehow leaf-shaped with a coelomyarian to ribbon-like arrangement of contractile elements. The ventrolateral longitudinal muscle bands of *T. cornuta* (*musculi principales*, but see Teuchert 1974 for concerns about this terminology) consist each, in cross section, of nine fibers; each fiber is composed of 8–12 consecutive mononuclear cells (Teuchert 1974). Circular muscle cells of *T. cornuta* are as well spindle-shaped (approximately 10 µm long) but with an abaxially positioned nucleus (Teuchert 1974). An abaxial position of the nucleus, however, was also found in longitudinal muscle cells of *Neodasys* sp. (Ruppert 1991; Fig. 1.13 B–C). Variation among the ultrastructure and cytomorphology of gastrotrich muscle cells can be found, for instance, in the striation pattern, type of z-material, and the absence/presence of a t-system for the excitation-contraction-coupling (Ruppert 1991, Tab. 1.3). A peripheral coupling of the muscle cell membrane (sarcolemma) with the sarcoplasmic reticulum was confirmed for almost all species investigated so far. Interestingly, taxa that are generally regarded to occupy rather basal positions within the phylogenetic tree of the Gastrotricha such as *Neodasys, Dactylopodola, Xenodasys, Chordodasiopsis, Draculiciteria*, and *Musellifer* (see chapter Phylogeny) possess a cross-striated musculature, whereas most remaining taxa show an oblique striation pattern (Ruppert 1991, Tab. 1.3). Because *Neodasys, Musellifer*, and *Draculiciteria* have rods instead of dense bodies as z-material, their striation type is regarded as "atypical cross-striation". If basal positions of the aforementioned taxa with cross-striated musculature will be supported (but see chapter Phylogeny for differing phylogenetic scenarios), this striation pattern would be part of the character pattern of the stem species of Gastrotricha as the most parsimonious reconstruction (Ruppert 1991). So far, *Lepidodasys* is the only known gastrotrich taxon with a smooth muscle organization (Ruppert 1991). Except *Lepidodasys* all gastrotrichs have radially arranged, cross-striated myofibrils in the myoepithelial pharynx (Fig. 1.13 D). Regarding the sarcomeres per contractile element of the pharynx, the number varies from one in *Lepidodermella squamata* up to 12 sarcomeres in *Turbanella cornuta* (Ruppert 1982). The mechanic coupling between neighboring muscle cells or a muscle cell and a non-muscle cell is by adhaerens

Tab. 1.3: Characters related with the ultrastructure and cytomorphology of longitudinal muscle cells of various species of the Gastrotricha.

	Diameter of single muscle fiber	**Arrangement of sarcomeres within fiber (as seen when cross-sectioned)**	**Cross-striated myofibrils**	**Oblique-striated myofibrils**	**Smooth myofibrils**	**Length of sarcomere**	**Diameter of myosin**	**Dense bodies as Z-material**	**Rods as Z-material**	**Peripheral couplings of sarcolemma and sarcoplasmic reticulum**	**T-system**
Chordodasiopsis antennatus (former *Xenodasys antennatus*)	7 × 3 μm	Circomyarian to polygonal	1	0	0	0.8 μm	?	1	0	?	1 (?)
Xenodasys riedli	6.0 μm	Circomyarian to polygonal	1	0	0	2.0 μm	30 nm	1	0	1	1
Dactylopodola sp.	7.5 μm	Circomyarian to polygonal	1	0	0	1.0 μm	?	1	0	1	1
Cephalodasys sp.	?	?	0	1	0	?	?	0	1	1	0
Cephalodasys littoralis	3 × 1 μm	Coelomyarian/ribbon-like	0	1	0	?	33 nm	0	1	1	0
Crasiella diplura	4.5 × 1.3 μm	Coelomyarian/ribbon-like	0	1	0	?	33 nm	0	1	1	0
Macrodasys sp.	3.4 × 1 μm	Coelomyarian/ribbon-like	0	1	0	?	28 nm	0	1	1	0
Mesodasys sp.	3.2 × 1.6 μm	Coelomyarian/ribbon-like	0	1	0	?	28 nm	0	1	1	0
Paraturbanella sp.	12 × 1.7 μm	Coelomyarian/ribbon-like	0	1	0	?	27 nm	0	1	1	0
Turbanella cornuta	6 × 1 μm	Coelomyarian/ribbon-like	0	1	0	1.8 μm	32 nm	0	1	1 (?)	0
Dolichodasys carolinensis	4 × 2.5 μm	Polygonal	0	1	0	?	?	0	1	1	0
Acanthodasys sp.	7 × 1.3 μm	Polygonal to coelomyarian	0	1	0	?	39 nm	0	1	1	0
Thaumastoderma sp.	5.3 × 1.5 μm	Polygonal to coelomyarian	0	1	0	?	?	0	1	1	0
Oregodasys sp. (former *Platydasys*)	?	?	0	1	0	?	?	0	1	1	0
Lepidodasys sp.	7 × 1.4 μm	Circomyarian to polygonal	0	1	1	?	17–100 nm	0	1	1	0
Neodasys sp.	1.4 μm	Circomyarian to polygonal	1	0	0	2.5 μm	32 nm	0	1	1	0
Diuronotus sp.[a]	3.2 μm	Circomyarian to polygonal	1	0	0	?	26 nm	1	0	1	1
Draculiciteria tesselata	3 × 1 μm	Circomyarian to polygonal	1	0	0	?	21 nm	?	?	1	1
Musellifer sublittoralis	8.0 μm	Circomyarian to polygonal	1	0	0	?	?	?	?	1	1
Chaetonotus sp.	3.0 μm	Polygonal to coelomyarian	0	1	0	?	?	0	1	1	0
Lepidodermella squamata	1.5 × 1.0 μm	Coelomyarian/ribbon-like	0	1	0	?	?	0	1	1	0
Xenotrichula carolinensis	4 × 1.3 μm	Circomyarian to coelomyarian	0	1	0	?	31 nm	0	1	1	0
Aspidiophorus sp.	4.0 × 1.0 μm	Coelomyarian/ribbon-like	0	1	0	?	23 nm	0	1	1	0

Modified from Ruppert (1991). A question mark (?) refers to unknown data; 0, absence; 1, presence.

[a] Ruppert (1991) presented muscular characters of an undescribed species of an undescribed genus referring to a drawing of his gastrotrich chapter in Ruppert (1988). Todaro et al. (2005) identified this animal as belonging to the taxon *Diuronotus*.

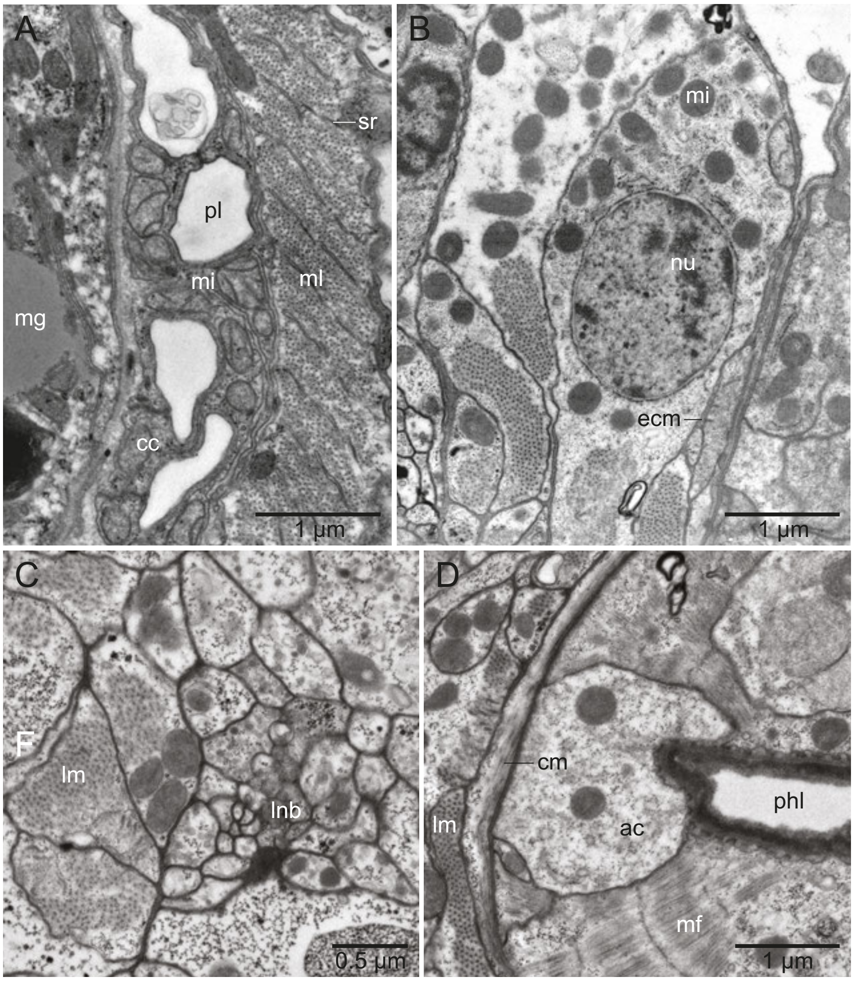

Fig. 1.13: Muscle ultrastructure (TEM-cross sections) of Gastrotricha. (A) *Polymerurus nodicaudus* (Paucitubulatina). Somatic longitudinal musculature in close proximity to the protonephridium. (TEM micrograph from Kieneke & Hochberg 2012, with permission by Wiley.) (B–D) *Neodasys chaetonotoideus* (Multitubulatina). (B) Cell body of a longitudinal muscle cell with nucleus. (C) Longitudinal muscle cells and nervous system in close proximity. (D) Detail of the pharynx showing myoepithelial cells and subpharyngeal visceral musculature. Abbreviations: ac, apical cell; cc, canal cell of the protonephridium; cm, circular muscles; ecm, extracellular matrix; lm, longitudinal muscles; lnb, longitudinal neurite bundle; mf, cross-striated myofilaments; mg, midgut; mi, mitochondria; ml, musculus lateralis; nu, nucleus; phl, pharyngeal lumen; pl, protonephridial lumen; sr, sarcoplasmic reticulum.

junctions, most probably desmosomes. If a muscle inserts on the body wall, muscle cells do not attach directly to the cuticle, but always to an epidermal cell. Tension between muscle and cuticle is provided by microfilaments that span through the epidermis cell from adhaerens junctions between muscle and epidermal cell to hemidesmosomes between epidermal cell and cuticle (Ruppert 1991). A comparable situation is assumed for the mechanic coupling between the sections of partitioned *musculi laterales*/ oblique muscles and the movable spines in Dasydytidae (Kieneke et al. 2008b). This, however, has to be supported by ultrastructural studies in the future.

Mechanic coupling of contractile elements (myofibrils) and the cuticle is slightly different in the pharynx (see also chapter Intestinal System for this issue). Here, the myofibrils of the myoepithelial cells are directly attached to the apical pharyngeal cuticle *via* specialized, plaque-like hemidesmosomes. Hemidesmosomes also attach the myofibrils basally to the basal lamina. There is quite a high ultrastructural diversity among hemidesmosomes of the pharynges of different species and groups of the Gastrotricha (see Ruppert 1982 for details). Some of the basal attachments of the pharyngeal myofibrils are furthermore mechanically coupled to the body cuticle *via* intracellular fibers that may span through different layers of cells before reaching the cuticle (Ruppert 1991). The ultrastructure of nerve-to-muscle connections for signal transduction was investigated in *Turbanella cornuta* by Teuchert (1977a). A peculiarity of these myoneural synapses is that the muscle cell itself forms one or few short processes that project into the neighboring nerve cell (Teuchert 1977a). Whether this represents a general pattern of the Gastrotricha, however, needs to be confirmed by further investigations (see Fig. 1.13 C for a close proximity between nervous and muscular cells).

Somatic circular and longitudinal muscles in Gastrotricha are generally regarded as reciprocal antagonists (Ruppert 1991). Contractions of the longitudinal muscles cause a shortening of the body, whereas its diameter has to increase because of the unchanged volume of the animal and incompressibility of liquid (Fig. 1.14 A–E). Owing to the increasing diameter, the circular muscles will relax. If circular muscles contract, the diameter will decrease again that leads to an elongation of the trunk and simultaneously to a relaxation of longitudinal muscles. Body movements of Gastrotricha that are brought about by muscle action include, for example, longitudinal elongation and shortening of the trunk (see above, Fig. 1.14 A–D), ventral and lateral flexion (Fig. 1.14 F), nodding and slightly turning the head, flexion of appendages (Fig. 1.14 G), or spreading of cuticular spines (e.g., Remane 1936, Ruppert 1991, Hochberg & Litvaitis 2001c, Kieneke et al. 2008b). The main mode of locomotion in Gastrotricha is of course the cilia-mediated gliding or, in some taxa, swimming. Muscle action, however, severely aids ciliary gliding and swimming when, for instance, a lateral flexion of the whole trunk or lateral plus ventral/dorsal flexion of the head is used for controlling the direction of locomotion. Furthermore, appendages such as the caudal lobes or toes of many species of the Paucitubulatina or the already mentioned motile spines of Dasydytidae (see above) can actively be moved by muscle action and hence may be used as a "rudders" (Remane 1936, Kieneke & Ostmann 2012). Waving movements with the anterior trunk end are known from different species, e.g., *Turbanella cornuta* or *Tetranchyroderma papii*. These movements probably involve alternating contractions and relaxations of the ventrolateral muscle bands and the visceral longitudinal muscles (Remane 1936, Hochberg & Litvaitis 2001c). The waving of the head and anterior trunk is interpreted as a kind of "searching behavior". Thus, the animal tries to optimize sensory perception (Remane 1936). A peculiar way of locomotion in many species of the Macrodasyida is shown during an escape behavior: Taxa such as *Macrodasys, Turbanella, Paradasys*, or *Tetranchyroderma papii*, just to mention some, may perform a leech-like or inchworm-like creeping, at which animals successively attach their posterior (or anterior) adhesive tubes to the substratum, then strongly flex or shorten their body, attach the adhesive tubes of the opposite body end (anterior or posterior tubes, respectively), and stretch the trunk again. Several quick repetitions of such actions allow a fast escape from a potential harmful stimulus, e.g., a collision with a predator or just with a scientist's micropipette (Remane 1936, Hochberg & Litvaitis 2001c). There are interspecific differences in this special behavior. *Macrodasys*, for instance, escapes in a frontal direction, whereas taxa like *Turbanella* or *Paradasys* escape backward. *Tetranchyroderma papii* may engage in both, backward or forward directed inchworm-like creeping (Hochberg & Litvaitis 2001c). A similar escape behavior can be observed in *Neodasys* sp. However, these animals stay attached to a sand grain by means of their posterior adhesive organs and quickly contract the whole trunk accordion-like (Ruppert & Travis 1983).

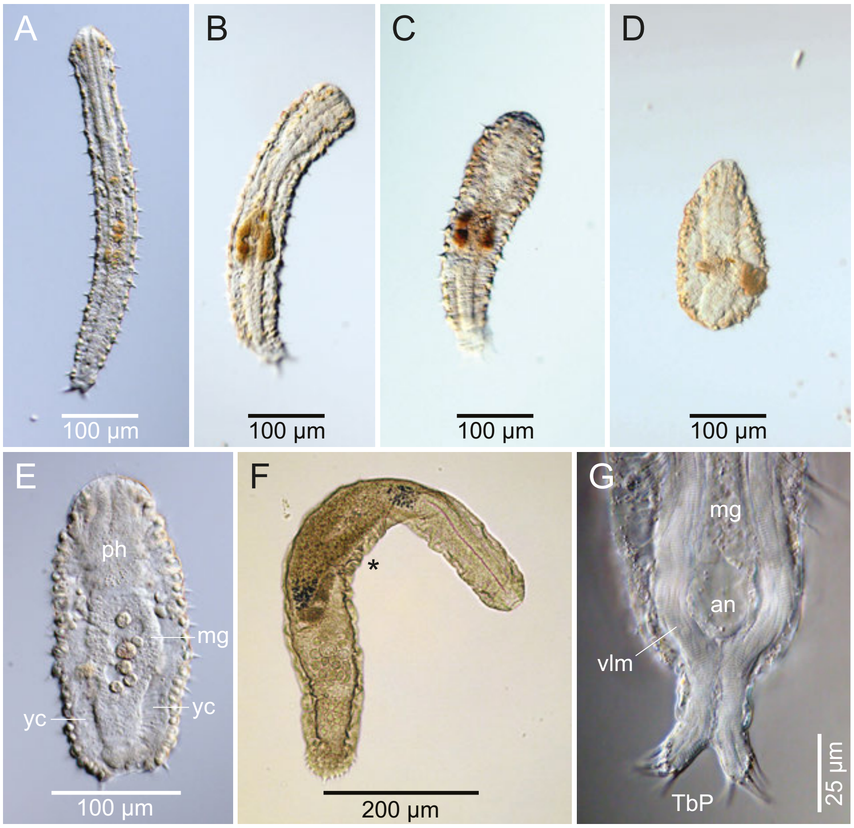

Fig. 1.14: Muscle-mediated movements of Gastrotricha. (A–D) *Turbanella subterranea* (Macrodasyida), dorsal views. The animal was consciously disturbed and showed a quick retraction of the trunk as a kind of escape response. (E) Fully retracted *Turbanella subterranea* with compressed organs such as the pharynx, midgut, and Y-cells. Due to incompressibility of liquids, the animal is much broader than usual. (F) A slipping *Paradasys subterraneus* (Macrodasyida) that takes a sharp right curve by contracting longitudinal muscles of its right side (asterisk). (G) Rear trunk of *Dactylopodola deminuitubulata* (Macrodasyida) with caudal pedicles. With the strong and cross-striated longitudinal musculature, species of *Dactylopodola* can perform rapidly repeated ventral flexions of the caudal pedicles and thereby escape with a "hopping" movement. Abbreviations: an, anus; mg, midgut; ph, pharynx; vlm, ventrolateral longitudinal muscle blocks; yc, Y-cells.

The escape response in *Dactylopodola* and *Chordodasiopsis* is different to the aforementioned mode. In these taxa, rapidly repeated ventral flexions of the caudal lobes (Fig. 1.14 G) cause a rearward "hopping". A correlation between such fast movements and the occurrence of cross-striated body musculature in *Dactylopodola* and *Chordodasiopsis* is obvious and can be functionally explained because cross-striated muscles are better suited for short but quickly repeating contractions than is obliquely striated musculature. The latter, meanwhile, is hypothesized to be the optimal muscle type for soft-bodied, vermiform animals where hyperextensions are followed by strong contractions of the whole body (Ruppert 1991). A predominant occurrence of obliquely striated longitudinal musculature among Gastrotricha supports this hypothesis (see Tab. 1.3). In addition to the already mentioned defensive behavior of Dasydytidae (see above), taxa like *Thaumastoderma*, *Lepidodasys martini*, or *Kijanebalola* are able to partially retract their anterior body end as a defensive response (Remane 1936). We have observed a comparable behavior in some specimens of *Turbanella* cf. *subterranea* that where consciously disturbed. First, the animals partially retracted the head and/or the caudal lobes bearing the posterior adhesive tubes. Shortly after, an extreme contraction of the whole body may follow (see Fig. 1.14 A–E). The main role of the visceral circular and helicoidal muscles is regarded to antagonize dilations of the pharynx and the midgut (Ruppert 1982, 1991, Hochberg & Litvaitis 2001c). Further roles of these muscles in the intestinal region could be the allocation of propulsive force to shift big food items (e.g., diatoms) through the midgut and to stiffen the whole gut tube (Hochberg & Litvaitis 2001c). Egg deposition in Macrodasyida often involves strong contractions of the trunk of spawning animals (Teuchert 1968, see chapter Reproductive Biology). These contractions are obviously brought about by longitudinal muscles. In freshwater Chaetonotidae, ripe eggs leave the trunk on the ventral side, possibly through a still unknown pore, pushed by muscular contractions (Hummon & Hummon 1983a). One or two branches of *musculi dorsales*, the dorsodermal muscles, form an arch above the developing egg in paucitubulatinan gastrotrichs. These muscles are hypothesized to stabilize the position of the egg and during egg deposition they may lead it ventrally and out of the body (Hochberg & Litvaitis 2003a). The functional role of yet other muscle components like, e.g., the crossover muscle(s) in the posterior trunk of many species of the Macrodasyida is still not satisfactorily understood (Hochberg & Litvaitis 2001c).

1.2.4 Nervous system

The nervous system of the stem species of Gastrotricha includes a dorsal to dorsolateral, bilateral symmetric brain in the anterior part of the body (head region) and a pair of lateroventral longitudinal cords (neurite bundles) as main components (Rothe et al. 2011a, Figs. 1.15 and 1.16 A–D). The brain has been reconstructed in different ways. Its basic structure has in principle already been described by Ludwig (1875) and Bütschli (1876), in more detail by Zelinka (1889) and Remane (1936). According to these authors, the brain consists of a bridge of neurons (the dorsal commissure) dorsal of the pharynx and cells (=somata) on both sides of this "bridge". Ultrastructural investigations by Teuchert (1977a) and Wiedermann (1995) reconstructed the brain as circumpharyngeal, but with a stronger dorsal part. Both investigations found a different distribution of somata, which cover a broader area, either more or less homogeneous over the brain (Wiedermann 1995) or separated in an anterior and a posterior part (Teuchert 1977a). Immunohistochemical investigations on *Dactylopodola baltica, Macrodasys caudatus* and *Dolichodasys elongatus* (Hochberg & Litvaitis 2003b), on *Neodasys cirritus, Xenodasys riedli*, and *Turbanella* cf. *hyalina* (Hochberg 2007), on three *Turbanella* species (Rothe & Schmidt-Rhaesa 2008), two *Dactylopodola* species (Rothe & Schmidt-Rhaesa 2009), *Oregodasys cirratus* (Rothe & Schmidt-Rhaesa 2010), two *Xenotrichula* species (Rothe et al. 2011b), *Lepidodasys worsaae* (Hochberg & Atherton 2011), and *Neodasys chaetonotoideus* (Rothe et al. 2011a) confirmed the description by Zelinka and Remane. There is a broad commissure composed only of neurites dorsal of the pharynx (Figs. 1.16 A–C and 1.17 A). This commissure is well shown with immunoreactivity (IR) against tubulin. IR against other neuronal components may show only subsets, as is shown exemplary in the IR against serotonin, histamine, and FMRFamides in *Dactylopodola* species, which all stain only some fibers within the dorsal commissure (Rothe & Schmidt-Rhaesa 2009). This observation also accounts for the immunohistochemical investigations in the other species. Nuclear staining shows a number of cells in the region lateral of the dorsal commissure (approximately 20 per side in *Dactylopodola*; Rothe & Schmidt-Rhaesa 2008), but the neural markers used (anti-serotonin, anti-FMRFamides, anti-histamine) all stain only subsets of the nerve cells of the brain (compare Fig. 1.16 C with 1.16 D, see also Fig. 1.17 B). Although there are at maximum few pairs of anti-serotonin IR cells (one to five pairs) in the studied species (e.g. Fig. 1.16 A–C), a count of anti-FMRF amide IR cells in the brain of *Nesodasys chaetonotoideus* and *Xenodasys riedli* yielded a mean number of 24 cells per hemisphere of the brain (Hochberg 2007, Rothe et al. 2011a). Therefore, it cannot be said with certainty how many neurons in total constitute the brain of Gastrotricha. All somata stained with any neuronal marker are positioned lateral of the dorsal commissure, a result that gets support by ultrastructural data (e.g., Rothe et al. 2011a).

The lateral ends of the dorsal commissure are the origin of the longitudinal nerve cords or neurite bundles (Fig. 1.16 A–D). Ultrastructural cross sections show one pair of longitudinal nerve cords in a lateroventral position in the animals (Teuchert 1977a, Ruppert 1991, Rothe et al. 2011a; Figs. 1.13 C, 1.17 C–D). In species such as *Turbanella cornuta*, a basiepithelial and intraepidermal position of the nerve cords is reported (e.g. Ruppert 1991). However, in putative basal taxa such as *Neodasys*, the nervous system is most likely subepidermal (Fig. 1.17 B–C). Immunohistochemical investigations show some additional aspects. The distance between the two longitudinal nerve cords is quite wide in most species, but in *Xenotrichula* species, the cords are closer together (Rothe et al. 2011b). In the broad species *Oregodasys cirratus*, the distance between the longitudinal cords is wide, but not as wide as possible. The cords run in a distance of about 50 µm from the lateral margin and fine neurites run from the cords into the lateral regions of the animal (Rothe & Schmidt-Rhaesa 2009). In this species, anti-serotonin IR reveals a second pair of longitudinal neurites or neurite bundles median of the longitudinal cords (Rothe & Schmidt-Rhaesa 2009), this structure is unknown from other species so far. Sometimes it appears that two longitudinal neurite bundles per body side are present in close proximity (see, e.g., for *Dactylopodola*; Rothe & Schmidt-Rhaesa 2009); in this case, it is likely that two fibers within the entire broader nerve cord were stained. In *Turbanella* species, the longitudinal cord first runs close to the pharynx and then turns laterally to proceed in a more lateral position. In the posterior end, both longitudinal cords merge in a loop. In *Xenodasys riedli*, there is a strong posterior commissure, from which a pair of additional neurites runs further posterior (Hochberg 2007). In the two *Xenotrichula* species and in *Neodasys chaetonotoideus*, a pair of anti-serotonin IR somata is present in the posterior part of the longitudinal nerve cords (Rothe et al. 2011a, b; Fig. 1.16 A, B). The presence of such an "anal ganglion" (*sensu* Remane 1936) is possibly also a character of the last common ancestor of all extant Gastrotricha (see Rothe et al. 2011a, Fig. 1.15). In some species, putative nerve cell somata where observed along the ventrolateral neurite bundles (Hochberg 2007, Rothe et al. 2011a).

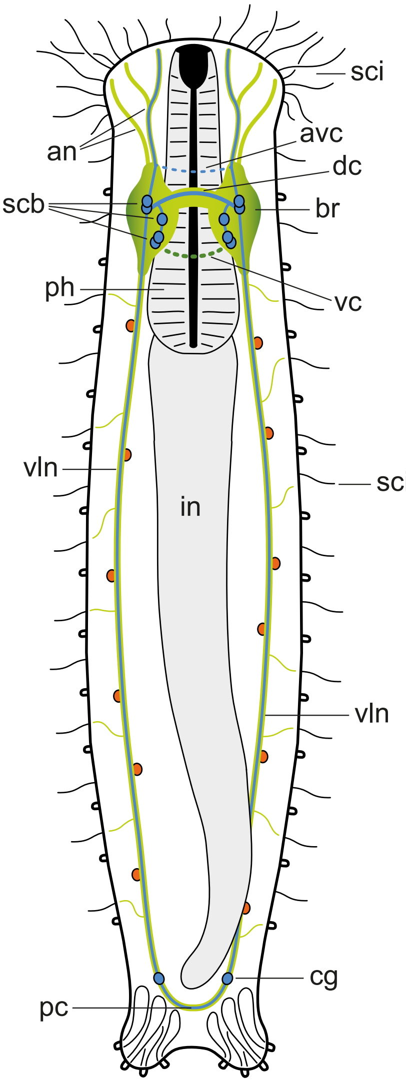

Fig. 1.15: Nervous system (schematic) of the last common ancestor of Gastrotricha. Green color indicates general nervous patterns, blue color indicates serotonin expressing components. The orange ovals are serially arranged FMRF-immunoreactive cells alongside the ventrolateral neurite bundles that were possibly also present in the stem species of Gastrotricha. Abbreviations: an, anterior longitudinal neurite bundles; avc, anterior ventral commissure of the brain; br, brain; cg, caudal ganglion/anal ganglion; dc, dorsal commissure of the brain; in, intestine; pc, posterior commissure; ph, pharynx; scb serotonin-expressing nerve cells of the brain; sci, sensory cilia; vc, ventral commissure of the brain; vln, ventrolateral longitudinal neurite bundles. Drawing according to the reconstructed character pattern of Rothe et al. (2011a).

Very fine ventral commissures between the longitudinal nerve cords are present, but these are detected only by a particular IR. In *Turbanella* species, one serotonin-IR ventral commissure is present close to the level of the dorsal commissure (Rothe & Schmidt-Rhaesa 2008). In *Dactylopodola* species, four commissures are present in different positions along the body, all four are stained by anti-tubulin, two of them by anti-RF amide, and none by anti-serotonin or anti-histamine (Rothe & Schmidt-Rhaesa 2009). Two ventral commissures are present in *Neodasys chaetonotoideus*, one is stained by anti-RF amide, and the other by anti-serotonin (Rothe et al. 2011b; Fig. 1.16 A).

Several neurons are observed to run from the brain region into the anterior end, these neurons likely innervate the sensory structures in the head region. The anterior and posterior sensory organs can usually be seen in immunohistochemical investigations quite well. Some neurites also innervate the pharynx and its ciliated sensory cells (see chapters Intestinal System and Sensory Structures).