Fine Structure of the Male Reproductive System In Two Species of *Haplognathia* Sterrer (Gnathostomulida, Filospermoidea) *

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Summary. The structure of the male reproductive systems of two species of *Haplognathia* cf. *lyra* and *H.* cf. *rosacea* was described. The structure of the testes and the anterior portions of the sperm ducts in both species was found to be similar. However, considerable species differences were found between the structures of the glands and muscles associated with the reproductive systems. These were more elaborate in *H.* cf. *lyra* than in *H.* cf. *rosacea*. The former species possessed an H-shaped sperm duct gland, three distinct groups of penis muscles and a penis with two cell types and with a lumen. The latter species had paired sperm duct glands, no specialized penis muscles and a penis with only one cell type and without a detectable lumen. No open gonopore was observed in either species. The sperm presumably exit through a ventral tissue connection observed connecting the penis and the ventral epidermis. These findings were discussed in the light of Mainitz's (1977) theory concerning the primitive penis type within the Gnathostomulida.

A. Introduction

One of the characteristics of the Gnathostomulida, which first caused them to be classified as an order of the Turbellaria, is the presence of a cuticular penis stylet in the order Scleroperalia (Ax, 1956; Sterrer, 1972, 1974). In recent investigations, however, Mainitz (1977, 1979) has found that the basic structure of many of the scleroperalian penis stylets is remarkably uniform and that there appears to be no direct relationship between the structure of turbellarian and gnathostomulid stylets.

Sterrer (1972) has recognized several evolutionary trends within the Gnathostomulida, especially the Scleroperalia; for example, the progressive shortening

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of the body length and rostrum proportions, concentration and increasing complexity of jaws and pharynx structures, and differentiation of paired sensory structures. Mainitz (1977) has seen a parallel trend to these in the increasing complexity of the scleroperalian penis stylet. She has used these observations in trying to determine how the scleroperalian penis stylet could have evolved. She has devised an evolutionary scheme (Mainitz, 1977, Fig. 10) deriving the stylet from a simple rosette of epidermal gland cells lined internally with a thin muscle layer derived from the body wall musculature. This glandular rosette surrounds the opening of the sperm duct to the exterior.

This type of penis has been described by light microscope techniques from various members of the class Filospermoidea (Sterrer, 1969, 1970), generally regarded as the most primitive class of the Gnathostomulida (Sterrer, 1972). However, this description is based mainly upon the appearance of the penis in squeezed specimens, supplemented by serial sections of a few specimens of *Haplognathia ruberrima*, *H. simplex* and *Pterognathia swedmarki* (Sterrer, 1969). Unfortunately, the sectioned specimens show an internal glandular penis, not swollen epidermal glands, making it difficult to tell whether the penis is truly epidermal in origin. The other filospermoidean reproductive structures are equally difficult to interpret by light microscope techniques, and therefore are largely unknown. Filospermoidean reproductive anatomy plays an important role as the presumed primitive character state within the Gnathostomulida and is of importance in determining the phylogenetic position of the Gnathostomulida within the lower worms. Therefore, it was decided to use transmission electron microscopy to examine the male reproductive systems of two species of filospermoidean gnathostomulids, concentrating on the penis structures.

B. Material and Methods

Two species of *Haplognathia* were collected from the upper 5 cm of fine sand near the mid tide level of a flat of the White Oak River near Swansboro, N.C. Live specimens were examined in squeeze preparation and identified as *Haplognathia* cf. *rosacea* and *H.* cf. *lyra* (Sterrer, 1970). The former corresponds well to the characteristics of the original description except for some differences in the basal plate. Because of a paucity of material, only one specimen of *H.* cf. *lyra* was squeezed, and its identification is less certain. The animals were extracted from the sediment by decantation with $MgCl_2$ isotonic to sea water. For electron microscopy, the animals were relaxed with $MgCl_2$ and then fixed for 1 h in 2% glutaraldehyde in a 0.1 M phosphate buffer with 10% sucrose and trace $CaCl_2$, pH 7.3. After rinsing in buffer, the specimens were dehydrated in a standard acetone series and embedded in an Epon-Araldite mixture. The posterior ends of four specimens of *Haplognathia* cf. *rosacea* and two specimens of *H.* cf. *lyra* were serially sectioned with a Reichert OMU2 ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined with Zeiss EM9S2 or a Hitachi HU 11B transmission electron microscope. Reconstructions of medial sagittal and frontal sections of the posterior 40 μm of a specimen of each species were made from the best cross section series of both species (Figs. 1, 2, 11, 12).

C. Results

1. *Haplognathia* cf. *lyra*

In *Haplognathia* cf. *lyra* the paired testes (Figs. 3–5) lie dorsolaterally between the gut and the body wall, beginning in the posterior third of the body. In a
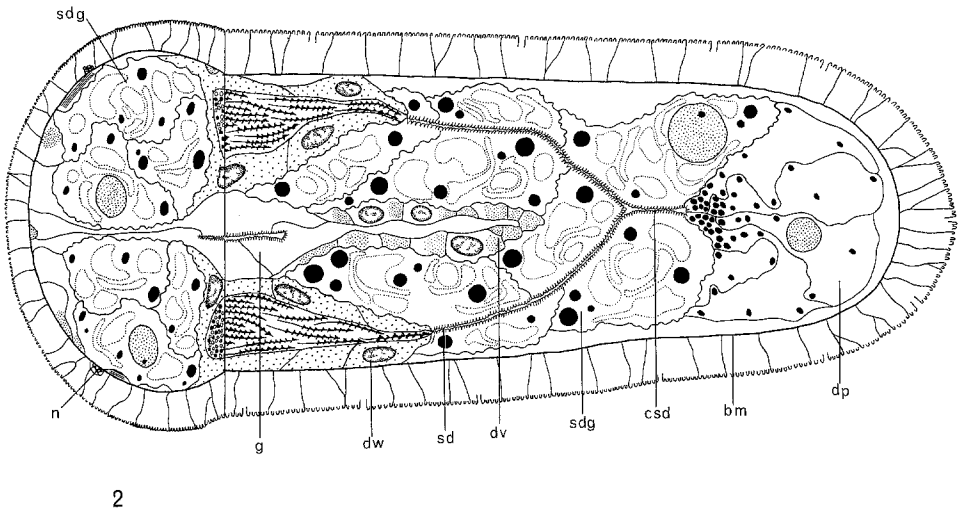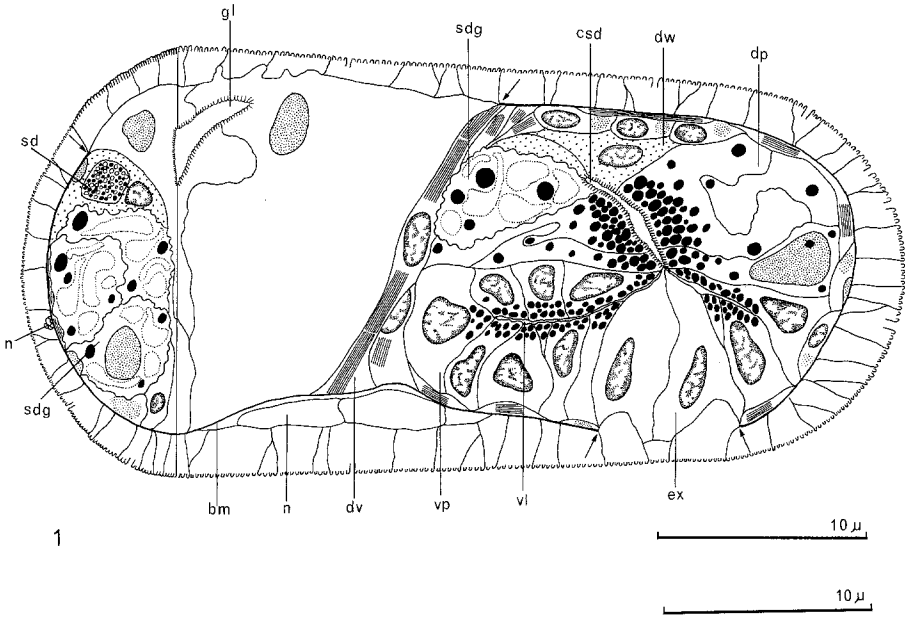
Fig. 1. Reconstruction of a medial sagittal section through the caudal end of *Haplognathia* cf. *lyra*. The arrows indicate the edges of gaps in the basement membrane bordering the anal tissue connection (dorsal) and the tissue connection gonopore (ventral). See the text for explanations of the penis structure

Fig. 2. Reconstruction of a frontal section through the caudal end of *Haplognathia* cf. *lyra* showing the structure of the sperm ducts. The areas in the posterior end of the animal where body wall muscles can be found have been left blank
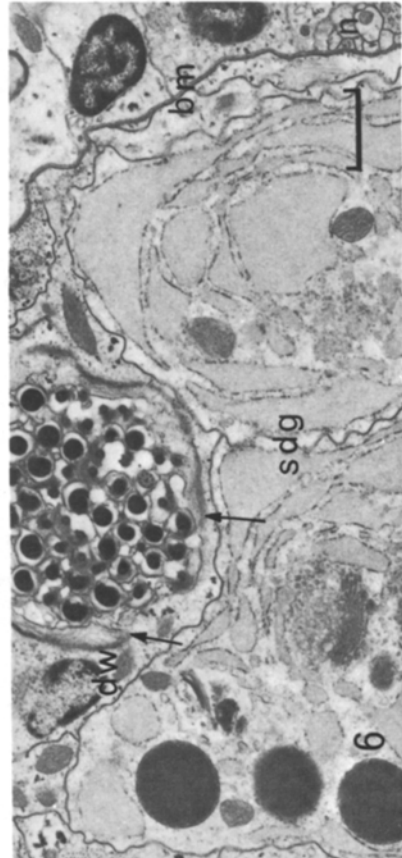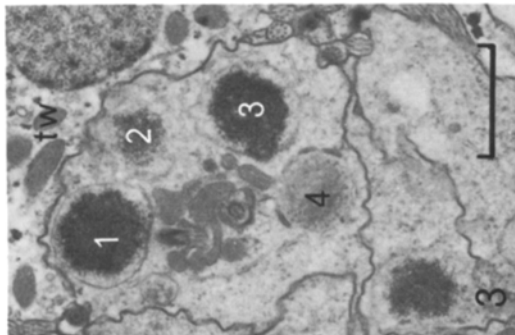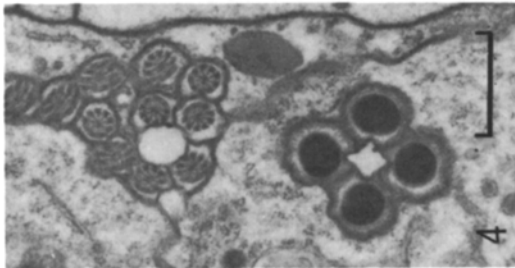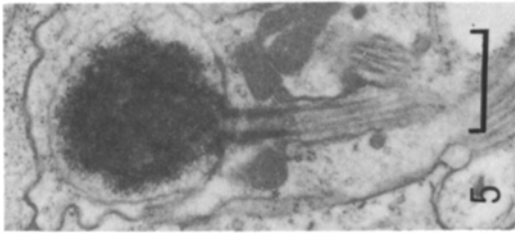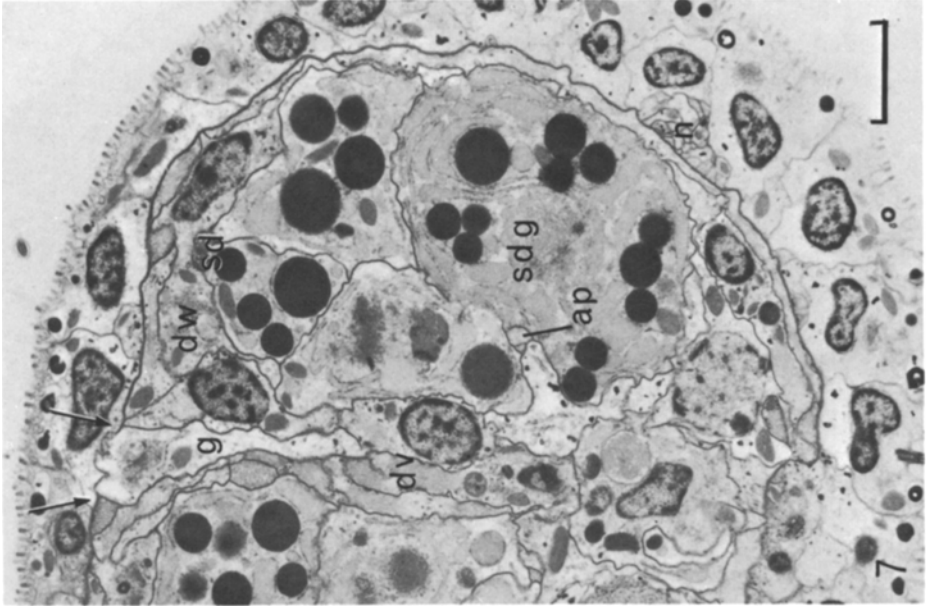
cross section of an animal measuring 30 μm in diameter, the testes range from 5 to 9 μm wide (frontal plane) by about 10 to 13 μm tall (sagittal plane). The cytoplasm of the germ cells has a fine granular appearance throughout spermiogenesis. In spermatogonia, the nucleus is very large compared to the size of the cell and has a loosely granular chromatin structure. Some rough endoplasmic reticulum is present, as is a well-developed Golgi body. A number of electron transparent lipid droplets measuring 0.7 μm in diameter are present. Their function in spermiogenesis is not known. Mitochondria normally measure 0.6 μm long by 0.3 μm in diameter but become elongated when incorporated into the sperm. Large cytoplasmic bridges can be seen between spermatogonia, and these are maintained throughout spermiogenesis.

In spermatid formation, meiosis apparently proceeds without cytokinesis, resulting in four spermatid nuclei within a single cell (Fig. 3). These nuclei condense to form the sperm heads. As the nuclei condense, they become attached to the centriole, which then organizes the flagellum (Fig. 5). At least one, and probably more than one, mitochondrion wraps around the neck of the sperm. The nucleus then elongates and takes its typically spiraled shape. It is not entirely clear how the four sperm then separate from one another (Fig. 4).

The testes are enclosed by cells which are continuous with and identical to the cells forming part of the wall of the sperm ducts (see below). There is never a basement membrane surrounding any of the reproductive structures.

In the posterior part of the testes, mature sperm can be seen lying between the testes wall and the undifferentiated germ cells. Farther posterior, the undifferentiated cells gradually disappear and are entirely absent from the sperm ducts.

The paired dorsolateral sperm ducts conduct sperm from the testes to the penis. Through most of their length they are recognizable by the presence of the sperm that they enclose (Fig. 6). About 25–30 μm anterior to the caudal end of the animal, the sperm bundles end and the sperm duct lumen decreases in size and becomes lined with microvilli (Fig. 7). The sperm ducts continue for around 10–12 μm (Fig. 8) and then unite to form a short (2–3 μm) common sperm duct which then descends into the penis (Fig. 9).

The anterior portions of the sperm ducts are only formed by epithelial wall cells which are identical to the testis wall cells. These cells are very thin

Figs. 3–5. Sections through the testes of *Haplognathia* cf. *lyra*. **3** Section showing four condensing spermatid nuclei within a single cell. Scale = 1 μm. **4** Cross sections through developing sperm. Ventrally a tetrad of sperm heads in the process of separating from one another can be seen. Dorsally sperm tails with a 9+2 flagellar axoneme can be seen. Scale = 0.5 μm. **5** Enlargement of a condensing sperm head. Scale = 0.5 μm

Fig. 6. Cross section through the anterior portion of a sperm duct in *Haplognathia* cf. *lyra*. Wall cells united by zonulae adhaerentes (*arrows*) enclose the sperm. The sperm duct gland does not form part of the lumen wall in this region. Scale = 1 μm

Fig. 7. Cross section of *Haplognathia* cf. *lyra* near the posterior end of the anal tissue connection (lateral edges indicated by *arrows*). Sperm are no longer present in the sperm duct lumen, and the ventral wall of the lumen is formed by the sperm duct gland. The dorsoventral penis muscles can be clearly seen in the center of the section. Scale = 2 μm
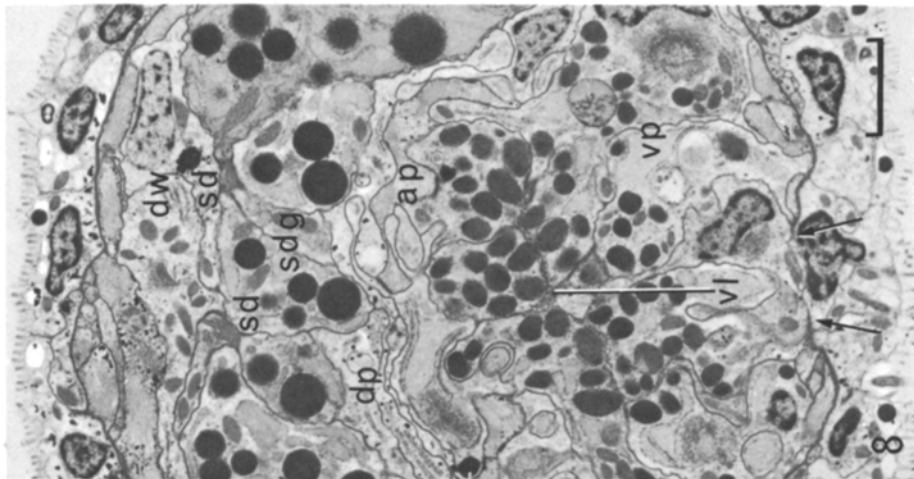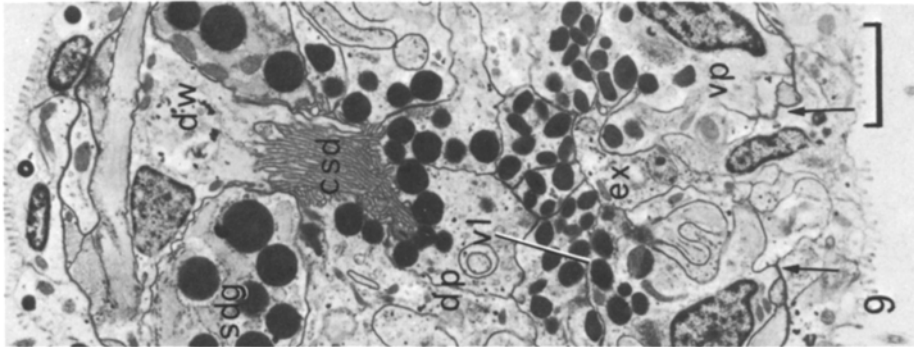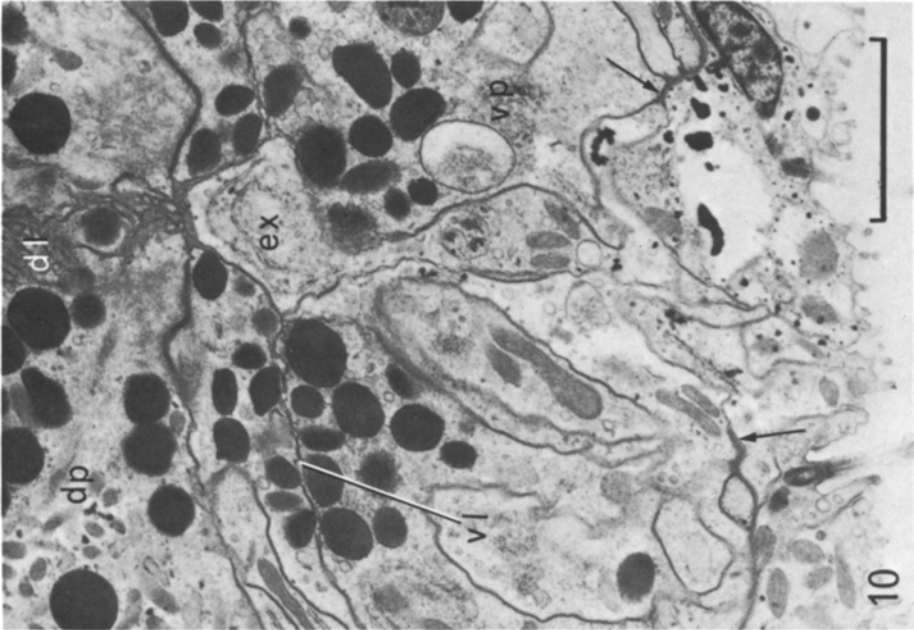
and flattened to wrap around the sperm bundles. The wall cells are joined to one another by zonulae adhaerentes (Fig. 6). The sperm duct lumen can reach up to 10 μm in diameter when filled with sperm. The sperm duct wall can be from 0.1–0.2 μm wide to 2–3 μm wide in the region of the nucleus. The nucleus itself is usually irregular in shape, sometimes lobed, with large areas of electron dense heterochromatin.

Posterior to the sperm bundles, these wall cells form only the dorsal parts of the lumen walls of the sperm ducts. The ventral walls are formed by the cells of the sperm duct gland (Figs. 7–9).

The lumen of the sperm ducts is only 0.4–0.8 μm wide in this region and is lined with short microvilli (Figs. 7, 8). The lumen of the common sperm duct is larger, 1.5–2 μm wide and the microvilli are longer (close to 1 μm) and more closely packed. The common sperm duct lumen opens into the lumen of the penis (Figs. 1, 9).

Two types of glands are found in the male system of *Haplognathia* cf. *lyra*, an anterior sperm duct gland mentioned above and the posterior penis glands. The sperm duct gland consists of a number of large gland cells which form the ventral walls of the sperm ducts posterior to the sperm bundles. There were 9 of these cells in one specimen, 8 in another. Each cell is spindle shaped, extending thin processes posteriorly and anteriorly. Some sperm duct gland cell bodies extend rostrally and underline the anterior region of the sperm duct (Fig. 6). The gland cells are at least 30 μm long and are irregular in cross-section. The sperm duct gland appears paired in cross-section for all but the caudal 5 μm of its 45–50 μm length (Figs. 7–9), where it forms the ventral wall of the common sperm duct. Anteriorly, the halves of the gland are separated by the gut and some muscle (Fig. 7).

The cytoplasm of the sperm duct gland cells is finely granular but is mostly obscured by the large number of inclusions. Numerous large spherical electron dense secretion droplets can be seen. They are very regular in shape, with a 1–1.3 μm diameter, and stages of their formation by Golgi vesicles may be observed. Occasional electron transparent lipid droplets (diameter 0.8 μm) are present. A large amount of rough endoplasmic reticulum is present. The cisternae are usually enlarged, being filled with a very finely granular gray substance.

Fig. 8. Cross section of *Haplognathia* cf. *lyra* at the anterior end of the tissue connection gonopore (lateral edges indicated by *arrows*). The two halves of the sperm duct gland are united in this region. The penis may be seen ventral to the sperm duct gland. The anterior-posterior penis muscles are especially distinct in this specimen. Scale = 2 μm

Fig. 9. Cross section of *Haplognathia* cf. *lyra* at the union of the common sperm duct and the dorsal lumen of the penis. Note the differences in the secretion granules in the three types of gland cells in the section. The orientation of the body wall muscles is reversed in this region, with the circular muscles interior to the longitudinal. The tissue connection gonopore can be seen ventrally. Its lateral edges are indicated by *arrows*. Scale = 2 μm

Fig. 10. Enlargement of the union of the dorsal and ventral penis lumina in *Haplognathia* cf. *lyra*. Scale = 2 μm

The nucleus is evenly granular in appearance. Rarely, small electron dense areas of heterochromatin can be seen closely applied to the nuclear membrane.

The penis is a large gland filling most of the posterior end of the animal behind the gut. It measures about 20 µm long, 13 µm wide and 13 µm tall. Several distinct groups of muscles surround it. The lumen of the penis is shaped like an upside down mushroom. The dorsal lumen (the mushroom stem) is a continuation of the common sperm duct, and is lined with microvilli (Figs. 1, 9). It unites with a very thin and flattened ventral lumen (the mushroom cap) near the center of the penis. The walls of the two lumina are formed by two separate cell types.

The cells forming the walls of the dorsal lumen are rather large and are so convoluted that it is difficult to find a meaningful way to describe their size. In one animal there were six of these cells, occupying almost half the total volume of the penis. The nuclei of these cells are large and irregular in shape, having a homogeneous fine granular appearance. Many electron dense secretion granules can be seen. These are especially concentrated around the lumen (Figs. 9, 10). These granules are smaller and more irregular in shape than those of the sperm duct glands, having a diameter of 0.6–0.8 µm. Golgi bodies are present and also a large amount of rough ER. Sometimes the ER cisternae appear slightly swollen. Numerous mitochondria are present which are particularly narrow and elongated (0.1 by 0.8 µm) with a dense matrix. Desmosomes unite the cells around the lumen.

The wall of the ventral lumen of the penis is formed by gland cells which are smaller and much more regular in shape than the dorsal cells. These ventral cells have a diameter of around 3.5–4.5 µm. The nuclei are also smaller and are irregular in shape, with large areas of electron dense heterochromatin. Occasionally, the mitochondria appear elongated as in the dorsal penis cells, but usually they are oval in shape, measuring 0.6 µm by 0.3 µm. Golgi bodies and rough ER are present. The rough ER cisternae are sometimes swollen by a very fine granular gray substance. As in the dorsal penis cells, the secretion granules of the ventral penis cells are especially concentrated around the lumen. The granules are even smaller and more irregular in shape than those of the dorsal penis gland cells (0.4–0.5 µm in diameter by 0.7 µm long). The ventral cells are joined around the lumen by desmosomes with very long and distinct microfilaments.

Ventral to the union of the dorsal and ventral penis lumina, in the center of the ventral part of the penis, there are a number of radially arranged exit cells. Three were found in one specimen, and at least four were present in another. These exit cells form a tissue connection with the ventral epidermis similar to the anal tissue connection described in Knauss (1979). There is an interruption in the basement membrane measuring 7 µm long by 3–4 µm wide, and in this region the epidermal cells interdigitate with the exit cells. These radially arranged exit cells form a cone, with the apex of the cone reaching dorsally to where the dorsal penis lumen empties into the ventral lumen, (Fig. 10). The exit cells are joined to the surrounding penis cells by desmosomes. Presumably, the sperm and the various secretion products of the male genital organs pass between the exit cells and then between the epidermis cells to reach the exterior.

The exit cells are not glandular and can therefore be easily distinguished from the surrounding penis cells (Figs. 9, 10). Because they lack secretion granules these cells would appear more transparent in squeeze preparation, and this may account for what appears to be an open gonopore that is sometimes seen in the center of the penis. The exit cell nuclei are irregular, measuring around 1–2 μm in diameter, with large areas of electron dense heterochromatin. The cytoplasm has a fine granular appearance. Rough ER, mitochondria and Golgi bodies are present.

There are three sets of muscles which appear to be connected with the male reproductive system of *Haplognathia* cf. *lyra*. The body wall musculature adjacent to the penis is specialized, especially dorsally and laterally. The muscle cells are concentrated and are larger than elsewhere, especially the circular muscles. Moreover, the normal orientation of the muscles appears to be reversed, with outer longitudinal and inner circular muscles (Figs. 1, 8, 9). The muscles do not insert directly upon the penis, but effectively surround it, inserting upon the basement membrane of the body wall. Their contraction probably protrudes the penis, as described by Sterrer (1969).

Anterior to the penis there are two sets of muscles affecting the functioning of the penis. The first of these is a group of muscle cells originating on or just lateral to the midventral body wall beginning about 10 μm anterior to the union of the two halves of the sperm duct gland. They run between the gut and the sperm ducts and sperm duct glands to insert dorsally on the basement membrane lateral to the edges of the anal tissue connection (Fig. 7). Posterior to the anal tissue connection they insert upon the middorsal basement membrane. These dorsoventral muscles essentially form two sheets of muscle surrounding the posterior portions of the lateral sperm ducts and sperm duct glands, and their function may be to help pump sperm through the sperm ducts.

The third set of muscles involved with the male genital system of *Haplognathia* cf. *lyra* is a small pair of muscles derived from the ventrolateral longitudinal muscle bands. The number and position of cells involved is variable. These muscles probably act as retractors. They run in an anterior-posterior direction, originating from the ventrolateral body wall and inserting directly upon the anterior end of the penis between the dorsal and the ventral gland cells (Fig. 8).

2. *Haplognathia* cf. *rosacea*

The structure of the testes and the anterior portion of the lateral sperm ducts of *Haplognathia* cf. *rosacea* does not differ significantly from that of *H.* cf. *lyra* except that they are positioned ventrolaterally instead of dorsolaterally (Fig. 13). However, there are major differences between the two species in the rest of the male reproductive system.

As in *Haplognathia* cf. *lyra*, there are two types of glands in the male reproductive system of *H.* cf. *rosacea*, anterior paired sperm duct glands (Figs. 12, 13) and posterior penis glands (Fig. 15). The size of these glands appeared to be more variable than in *H.* cf. *lyra*, with the size proportional to body length. All measurements given are from a relatively small specimen, with a
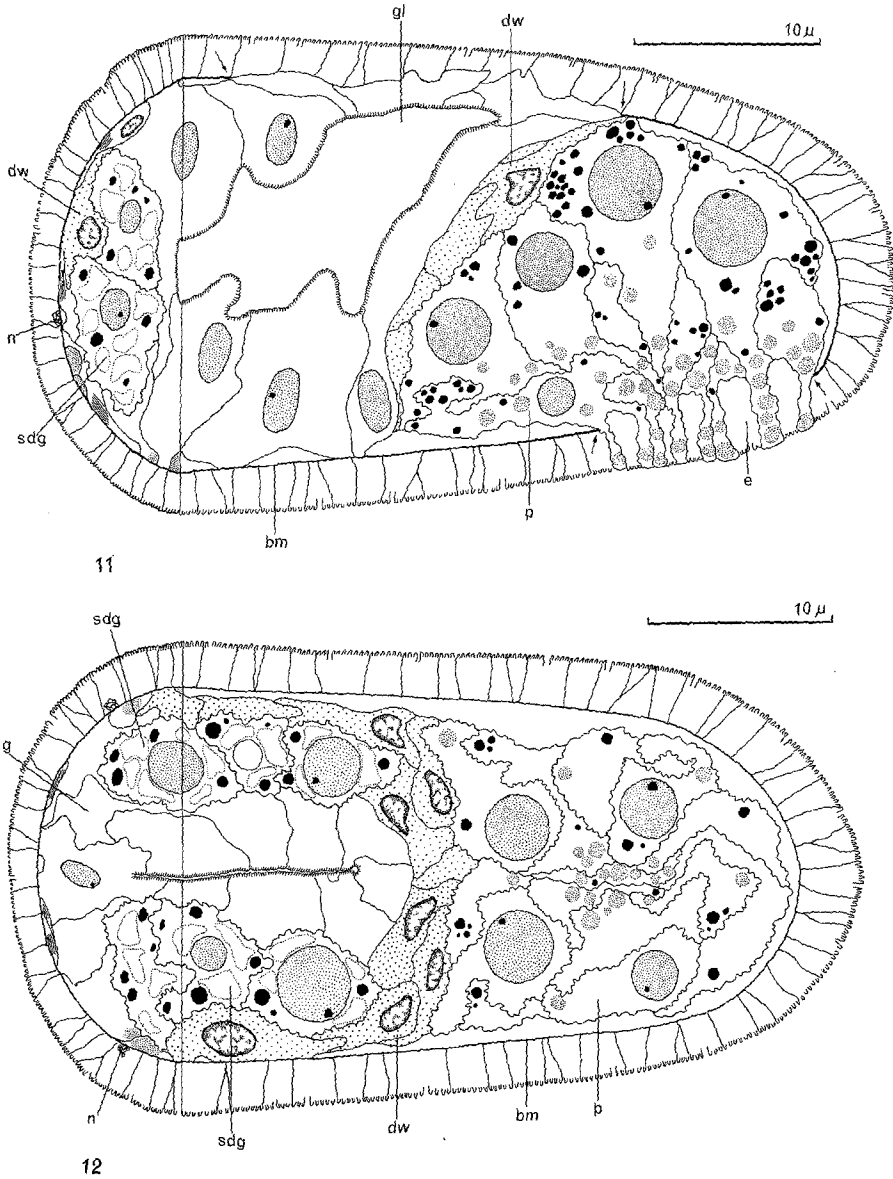
Figs. 11–12. Reconstruction of a medial sagittal section (Fig. 11) and a frontal section (Fig. 12) through the caudal end of *Haplognathia* cf. *rosacea*. The *arrows* (Fig. 11) indicate the edges of the gaps in the basement membrane forming the anal tissue connection (*dorsal*) and the tissue connection gonopore (*ventral*). The areas in the posterior end of the animal where body wall mucles can be found have been left blank.

25–30 μm diameter. In larger specimens the glands are larger and the sperm duct glands are found farther anterior.

In *Haplognathia* cf. *rosacea* the sperm duct glands never unite as they do in *H.* cf. *lyra*. They are also noticeably longer (60 μm as opposed to 45–50 μm) and are composed of more and smaller cells. The gland cells can first be seen beginning about 85–90 μm anterior to the end of the animal. The cells are irregularly ovoid instead of spindle shaped, measuring a maximum of 7–10 μm long, 5–7 μm wide and 9–11 μm high. The nuclei are spherical (about 4–5 μm in diameter) with a homogeneous fine granular appearance. Aside from size and shape, the sperm duct gland cells appeared very similar to those of *H.* cf. *lyra*. The cytoplasm is granular, with mitochondria, Golgi bodies, some lipid droplets and rough endoplasmic reticulum with the cisternae expanded by a very finely granular gray substance. The electron dense secretion droplets are spherical with a 1–1.3 μm diameter. The sperm duct glands could only be seen in one specimen of this species in squeeze preparation. This specimen was unusually large and mature, possessing a mature egg.

The sperm duct glands end about 30–50 μm anterior to the caudal end of the animal. However, the gut lumen continues posterior to this for 5–20 μm.

In *Haplognathia* cf. *rosacea* the sperm duct is filled with sperm to about the rostral end of the sperm duct glands (Fig. 13). The structure of the sperm duct up to this point is the same as in *H.* cf. *lyra*, 2–3 sperm duct wall cells joined by zonulae adhaerentes forming the cellular tube around the sperm. In the region of the sperm duct glands the sperm duct is empty and its lumen exists only as small spaces between the sperm duct gland cells and a band of sperm duct wall cells which extends posteriorly between the sperm duct glands and the body wall (Figs. 12, 14). As in *H.* cf. *lyra*, the gland cells and wall cells are joined around the collapsed lumen by zonulae adhaerentes. There are two main differences between the posterior regions of the sperm ducts in *H.* cf. *lyra* and *H.* cf. *rosacea*. In the latter, the ducts are not lined with microvilli, and the sperm duct glands form part of the lumen wall along their entire length instead of just in the gland's caudal region as in *H.* cf. *lyra*.

At the posterior ends of the sperm duct glands, the two sperm duct wall cell bands unite to form a layer of cells which separates the penis from the gut and the sperm duct glands (Figs. 11, 12). Thus, the cells of the penis and the cells of the sperm duct glands never touch upon one another in *H.* cf. *rosacea* as they do in *H.* cf. *lyra*. How the sperm pass into the penis and then exit from the body is not entirely clear. There appears to be no detectable lumen within the penis.

The penis of *Haplognathia* cf. *rosacea* is a large ovoid gland filling the interior of the animal posterior to the gut (Figs. 11, 12, 15). It measures about 24 μm long by 13–15 μm wide and 15–17 μm high. Only one type of gland cell appears to be present. They are large, irregular and extremely convoluted. Eighteen cells were present in one specimen. The nuclei are spherical, 3–4 μm in diameter with one or more nucleoli. They have a homogeneous fine granular appearance with occasional small electron dense spots of heterochromatin. Occasionally, there was less densely granular areas within the nucleus. Within the same gland cell, two kinds of secretion droplets are present. One is electron
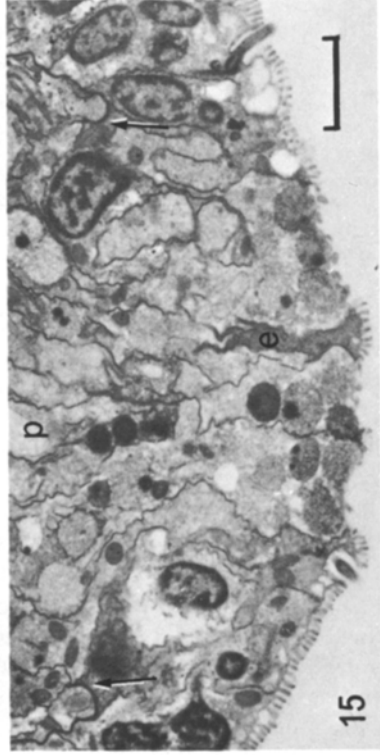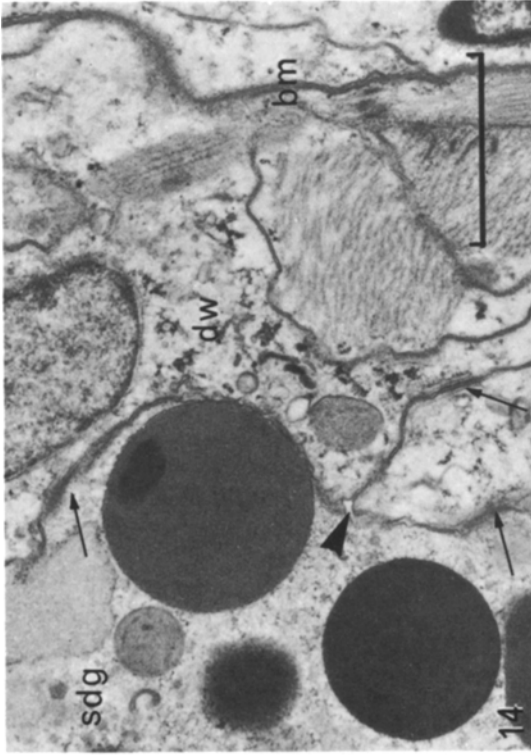
dense and spherical, with a diameter averaging 0.8 μm. The other is finely granular to filamentous in appearance, often with an electron dense core. These measure 1.3–1.5 μm in diameter. The latter are especially numerous at the secretory surfaces of the cells (Fig. 15). It is possible that the first type transforms into the second preparatory to secretion.

Similar to the ventral tissue connection in *H.* cf. *lyra*, a gap in the basement membrane is present in the ventral epidermis (Fig. 15). It is 11–12 μm long and 13–14 μm wide. The gland cells of the penis extend necks between the epidermis cells to actually form part of the surface of the animal. It is through these necks that secretion takes place. These necks lack microvilli, which helps to distinguish them from the isolated epidermis cells resting within the tissue connection. Sperm most likely also exit through this tissue connection.

There appears to be no special musculature associated with the penis of *Haplognathia* cf. *rosacea*. The dorsoventral and anterior-posterior muscles found in *H.* cf. *lyra* are completely lacking. The body wall musculature dorsal, lateral and posterior to the penis is somewhat more elaborate than elsewhere in the body, however, not nearly as distinct as in *H.* cf. *lyra*. In addition, the muscles maintain their normal orientation, with the longitudinal muscles interior to the circular muscles. This lack of special musculature in *H.* cf. *rosacea* may be related to the fact that the penis cells each have direct access to the external surface for secretion.

D. Discussion

In only one study has any histological investigation of filospermoidean reproductive anatomy been attempted (Sterrer, 1969). Sterrer could only obtain detailed data for three species, *Haplognathia simplex, H. rubberima* and *Pterognathia swedmarki*. These species were examined both in squeeze preparation and in serial section stained with Azocarmine-Pacini. The most complete description of the male reproductive system was made for *H. ruberrima*, which appeared to have a *lyra*-type male system, with short sperm duct glands and a distinct central accumulation of secretion granules within the penis.

Fig. 13. Cross section through the rostral end of the sperm duct glands in *Haplognathia* cf. *rosacea*. In this species the sperm ducts are found ventrolaterally. The sperm bundles end a few microns posterior to this section. Scale = 5 μm

Fig. 14. Cross section through the sperm ducts posterior to the sperm bundles in *Haplognathia* cf. *rosacea*. The medial wall of the duct is formed by the cells of the sperm duct glands. The cells are united around the collapsed lumen (*large arrowhead*) by zonulae adherentes (*arrows*). Scale = 2 μm

Fig. 15. Cross section through *Haplognathia* cf. *rosacea* in the region of the tissue connection gonopore. Note the two kinds of secretion granules present. Epidermal cells with microvilli can be seen between gland cell necks. Arrows indicate the basement membrane at the lateral edges of the tissue connection. Scale = 2 μm

The greater resolution of the electron microscope allows clarification of some uncertainties in Sterrer's work. He describes the testes as a blue tinged web. The web-like appearance probably results from large numbers of dissolved lipid droplets. He also described 2–4 μm acidophilic secretion granules in the testes. These may be condensing sperm nuclei. Sterrer's observation of sperm developing in tetrads is confirmed. Sterrer describes the tissue surrounding the testes and sperm bundles as syncytial strings of parenchyma. However, by TEM these can clearly be seen to be a specialized cellular lining. The present study strongly suggests that the actual presence of mesenchyme and its spatial arrangement need further careful EM investigation. The complex interdigitation of gut cells and the sarcoplasmic portions of muscle cells makes it difficult to prove or disprove the presence of mesenchyme, and this can only be resolved by the use of complete serial sections. Sterrer also described 1 μm large basophilic granules which accompany the entire length of the sperm ducts in *H. simplex* and *P. swedmarki*. There appear to be no corresponding structures in either *H.* cf. *rosacea* or *H.* cf. *lyra*.

The sperm duct glands appear to correspond to a bow-shaped aggregation of granules which Sterrer observed in squeezed specimens of *H. simplex, H. rosea* and fragments of an undescribed species. These granules wrap around the posterior ends of the sperm bundles and touch upon the anterior end of the penis. In *H. ruberrima* these probably appear as the "blue tissue" which wraps around the sperm bundles beginning 50 μm anterior to the male genital pore.

Sterrer described the penis as a rosette of gland cells surrounding the male genital pore. In *H. ruberrima* he saw an accumulation of 1–2 μm basophilic granules in the center of the bulb. He found it impossible to discern any substructure within the penis, no doubt because the cells are so extremely convoluted. It is only possible to trace the borders of these cells with the aid of the electron microscope.

The male reproductive systems of the two *Haplognathia* species studied here differ in several respects. *H.* cf. *rosacea's* type of system appears simpler than that of *H.* cf. *lyra*. In the latter, the posterior portion of the sperm ducts are lined with microvilli. The sperm duct gland is more complex, and the portion of it bordering on the sperm duct lumina is restricted. *Haplognathia* cf. *rosacea* lacks the complex musculature associated with the penis that *H.* cf. *lyra* has. Comparison of the penis structure of the two species is difficult with present data since the exact organization of the connection of the sperm ducts to the penis and the route of passage of sperm through the penis is not clear for *H.* cf. *rosacea*. From the present data the following hypothesis can be proposed for the construction of this region in *H.* cf. *rosacea*. Posterior to the sperm duct glands, the collapsed sperm duct lumen probably unites to form a short common sperm duct where the two wall cell bands unite. Within the penis, the lumen might continue between a group of penis glands which open together in the center of the ventral tissue connection, where gland cell necks have been seen to fill most of the surface. Normally this lumen would not be seen unless filled with sperm; however, traces of it might be seen in frontal sections through the penis. With this interpretation it appears

that the *rosacea*-type of penis is simpler than that of *H.* cf. *lyra*, not only because it has just one type of gland cell but also because it forms a direct connection from the sperm duct lumen to the outside.

It is possible that the type of male system found in *Haplognathia* cf. *rosacea* could be derived from the *lyra* type by reduction. *H.* cf. *rosacea's* penis gland cells with their direct access to the exterior surface could account for the loss of the complicated muscles found in *H.* cf. *lyra*. However, it seems likely that the less elaborate male system of *H.* cf. *rosacea* is the more primitive, especially if the above interpretation of the penis is correct; *H.* cf. *rosacea* then retained a direct connection from the sperm ducts to the outside. This organization appears more primitive if compared to related phyla, such as gastrotrichs (Ruppert, 1978b) and turbellarians (Ax, 1963; Karling, 1974) where the gonoducts commonly connect directly to the outside (however, see Rieger et al., 1974; Ruppert, 1978a and Teuchert, 1968 for different data in other Gastrotricha). In addition, on the basis of present data, it is generally considered that the genus *Pterognathia* is more derived than *Haplognathia* (Sterrer, 1972). Paired ventrolateral testes are considered to be primitive within the Gnathostomulida as these are present in both orders as the norm. It is possible that the paired dorsolateral testes found in *Haplognathia* cf. *lyra* represent an intermediate stage between the primitive condition as found in *H.* cf. *rosacea* and the derived *Pterognathia* unpaired dorsal testis. Sterrer (pers. comm.) has found an as yet undescribed *Pterognathia* species having unpaired testes but with long paried sperm duct glands perhaps like those of *H.* cf. *rosacea*. While the type of male gland system found in an animal may not be linked with the number and position of the testes, the presence of elongated *rosacea*-type sperm duct glands in both filospermoidean genera may indicate that this is a primitive feature of the group.

According to Mainitz (1977) the scleroperalian penis stylet is clearly derived from the epidermis by invagination. It is still surrounded by its basement membrane, and its musculature is derived from the underlying body wall musculature. This is not true for the two penis types described in this study. Therefore, it does not seem possible to derive the scleroperalian penis stylet directly from any known filospermoidean penis type.

However, both the filospermoidean and the scleroperalian penises may have been derived from a common ancestor with a penis formed by a rosette of gland cells resting within the epidermis and surrounding a simple male pore (Mainitz, 1977). The *rosacea*-type of penis may have been derived by an enlargement of these gland cells with the cell bodies and nuclei sinking into the interior yet maintaining necks to the surface extending through the basement membrane. This type of structure is reminiscent of many epidermal glands in the Turbellaria. The *lyra*-type of penis, like the scleroperalian penis stylet, may have originated as the result of invagination of the primitive epidermal rosette penis. The associated epidermal basement membrane would have been lost in the *lyra*-type penis as the cells moved to the interior. The specialized circular muscles surrounding the penis may have been derived from the invaginating body wall muscles. This could explain the reversed orientation of the muscles in this region of the body, with the circular internal to the longitudinal. If the above

evolutionary schemes are correct, both the scleroperalian penis stylet and the filospermoidean penis must be seen as parallel and independent specializations of the primitive type of male reproductive system in Gnathostomulida.

Abbreviations

ap	anterior-posterior penis muscles	g	intestine
bm	basement membrane	gl	gut lumen
csd	common sperm duct	n	nerve
dl	dorsal lumen of the penis	p	penis
dp	dorsal gland cells of the penis	sd	sperm duct
dv	dorsoventral muscles anterior to the penis	sdg	sperm duct gland
dw	sperm duct wall cell	tw	testes wall cell
e	epidermis	vl	ventral lumen of the penis
ex	exit cell	vp	ventral gland cells of the penis

References

Ax, P.: Die Gnathostomulida, eine rätselhafte Wurmgruppe aus dem Meeressand, Abh. math.-naturw. Kl. Akad. Wiss. Mainz **8**, 1–32 (1956)

Ax, P.: Relationships and phylogeny of the Turbellaria. In: The Lower Metazoa (E.C. Dougherty, ed.), pp. 191–224. Berkeley and Los Angeles: University of California Press 1963

Karling, T.G.: On the anatomy and affinities of the Turbellarian orders. In: Biology of the Turbellaria (N.W. Riser and M.P. Morse, ed.), pp. 1–16. New York: Mc Graw-Hill 1974

Knauss, E.B.: Indication of an anal pore in Gnathostomulida. Zool. Scripta **8**, 181–186 (1979)

Mainitz, M.: The fine structure of the stylet apparatus in Gnathostomulida Scleroperalia and its relationship to turbellarian stylets. Acta zool. fenn. **154**, 163–174 (1977)

Mainitz, M.: The fine structure of Gnathostomulid reproductive organs I. New characters in the male copulatory organ of Scleroperalia. Zoomorphologie **92**, 241–272 (1979)

Rieger, R.M., Ruppert, E.E., Rieger, G.A., Schoepfer-Sterrer, C.: On the fine structure of Gastrotrichs with description of *Chordodasys antennatus* sp. n. Zool. Scripta **3**, 219–237 (1974)

Ruppert, E.E.: The reproductive system of Gastrotrichs II. Insemination in *Macrodasys*: a unique mode of sperm transfer in Metazoa. Zoomorphologie **89**, 207–228 (1978a)

Ruppert, E.E.: The reproductive system of Gastrotrichs III. Genital organs of Thaumastodermatinae subfam. n. and Diplodasyinae subfam. n. with discussion of reproduction in Macrodasyida. Zool. Scripta **7**, 93–114 (1978b)

Sterrer, W.: Beiträge zur Kenntnis der Gnathostomulida I. Anatomie und Morphologie des genus *Pterognathia* Sterrer. Ark. for Zool. **22**, 1–125 (1969)

Sterrer, W.: On some species of *Austrognatharia, Pterognathia,* and *Haplognathia* nov. gen. from the North Carolina coast (Gnathostomulida). Int. Rev. Ges. Hydrobiol. **55**, 371–385 (1970)

Sterrer, W.: Systematics and evolution within the Gnathostomulida. Syst. Zool. **21**, 151–173 (1972)

Sterrer, W.: Gnathostomulida. In: Reproduction of Marine Invertebrates (A.C. Giese and J.S. Pearse, ed.), pp. 345–357. New York: Academic Press 1974

Teuchert, G.: Zur Fortpflanzung und Entwicklung der Macrodasyoidea (Gastrotricha). Z. Morphol. Ökol. Tiere **63**, 343–418 (1968)