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Morphology and ultrastructure of the anterior end of *Diplocirrus longisetosus* Marenzeller, 1890 (Flabelligeridae, Polychaeta, Annelida)

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Abstract

The morphology and ultrastructure of the sedentary polychaete *Diplocirrus longisetosus* Marenzeller, 1890, collected from the White Sea, were studied using dissection, histological methods, light microscopy, and both scanning and transmission electron microscopy. The prostomium and peristomium carry a pair of palps, eight branchiae, a pair of nuchal organs and two nephridiopores, ciliated folds and the mouth. The prostomium, peristomium and the first chaetigerous segment with all appendages comprise the so-called siphon complex. The mouth leads to a pharyngeal organ that is closed ventrally and composed of a ventral muscle bulb adjoined dorsally by two folds projecting into the pharyngeal lumen. These parts are connected and enveloped by the longitudinal investing muscle. No tongue-like organ is present. The nervous system of the siphonal part comprises the brain, the circum-oesophageal connectives and the ganglia of the peristomium and first chaetigerous segment.

Introduction

The most important peculiarity of the Flabelligeridae is their ability to retract the anterior end, including pro- and peristomium, and some of the first chaetigers into the body, forming an introvert (specially arranged anterior part of the body). This part of the flabelligerid body has no special name in the literature. Analogous to other invertebrate phyla having retractable anterior end (Cephalorhyncha, Malakchov & Andreanov, 1995; Sipunculida, Cutler, 1994) we call this part of the body as introvert. Corresponding structures can also be found in other polychaete families, such as Poeobiidae, Fauveliopsidae and Sternaspidae (Fauchald & Rouse, 1997). Flabelligeridae is referred to the taxon Terebellida, which includes also Terebellidae, Ampharetidae, Alvinellidae, Trichobranchidae, Poeobidae, Cirratulidae and Acrocirridae (Rouse & Pleijel, 2001). Rouse & Fauchald (1997) found Flabelligeridae to be the sistergroup of Acrocirridae or to be part of a clade comprising Acrocirridae, Fauveliopsidae, Poeobiidae, Sternaspidae and Flabelligeridae.

The histology and fine structure of the introvert, including the chaetigers contributing to this organ in Flabelligeridae, have not previously been studied in detail. The present paper describes the structure of the anterior part of the body of *Diplocirrus longisetosus* Marenzeller, 1890, as an initial example of an introvert occurring in Flabelligeridae.

Materials and methods

The material was collected from Kandalaksha Bay, White Sea, Russia (subtidal mud, between 10 and 200 m depth, collected by A. Tzetlin and A. Filippova during scuba diving or with an Ockelmann sledg). The worms were anaesthetized in a solution of menthol prior to fixation.

Specimens for electron microscopy were fixed in 2.5% glutaraldehyde, buffered with 0.2 M sodium cacodylate buffer containing 0.3–0.36 m sucrose (pH 7.2–7.4). After rinsing in buffer specimens were post-fixed with 1% osmium tetroxide in the same buffer and dehydrated in a graded ethanol series followed by acet-

one. For transmission electron microscopy specimens were embedded in Epon resin. Semithin and ultrathin sections were cut on RMC 6000 or Reichert Ultracut E ultramicrotomes. Semithin sections were stained with 1% toluidine blue. Ultrathin sections were collected on single slot grids and stained with uranyl acetate (Ultrostain I, Leica, Bensheim, Germany) and lead citrate (Ultrostain II, Leica) in a Leica Ultrostainer. Two series of sagittal sections and two series of transverse sections were obtained. The sections were examined in JEOL 1200 C, ZEISS EM 109 and EM 902A transmission electron microscopes. Similarly fixed and dehydrated body fragments were critical point dried, sputter coated with either platinum-palladium or gold and examined with HITACHI 400 400A and ZEISS DSM 962 scanning electron microscopes.

For histological work adult specimens were fixed in 4% formaldehyde solution. After paraffin embedding and serial sagittal-sectioning (5 μm), sections were stained with Heidenhain's iron haemotoxylin and examined with an OPTON MC 63s light microscope.

Results

Structure of introvert

The introvert of *D. longisetosus* is formed by the wall of the introvert, the prostomium, the peristomium and by the first chaetigerous segment (Figs 1A–B and 2A). The border between pro- and peristomium is indistinct and cannot be defined. Pro- and peristomium carry the palps, branchiae and nephridiopores. This complex of structures can be retracted into the body and protracted with the help of a complex system of retractor- and protractor muscles (Figs 4 and 5).

Appendages and structures of the anterior end

The palps originate from an elevation of the prostomium (Fig. 1A–C). They are rather long (about 270 μm). The ventral side of the palps is densely ciliated, but in the center of the ventral side there is a large unciliated groove. On the dorsal side there are cilia only at the end of the palps, while the other parts have small papillae (Figs 1C and 2A), which are probably additional sensory structures. Branchiae are located on the branchial membrane behind the prostomium, which consists of two parts (Figs 1A–B and 2A), each of which carries 4 branchiae. The branchiae are equipped with long and abundant cilia. Close to the second branchiae on the right and left sides are the nephridiopores (26 μm long), symmetrically disposed along the midline (Figs 1A, D and 2A). They have the shape of a truncated cone. Near the nephridiopores the cilia of the nuchal organs can be seen. The nuchal organs of *Diplocirrus* are narrow pits extended transversally in between the branchia and palps (Figs 1B and 2A). The organs are mainly composed of densely ciliated columnar cells.

The mouth opening is surrounded by heavily ciliated lips (Figs 1A–B and 2A). The upper lip is small and only distinct when the mouth is widely opened. One lower and two lateral lips are well developed. The ciliated epithelial cells are large (9.6–14.3 μm high). The cuticle is thin. The large nuclei (about 7 μm in diameter) are situated at the distal end of the multiciliated cells. The cilia rest on well-developed basal bodies (0.2 μm high) with rootlets about 2.4 μm long. Between the rootlets there are numerous mitochondria. Several microvilli arise between the cilia. A multitude of gland cells (app. 9.5 μm high) are situated among the ciliated cells. In these cells there are numerous densely packed secretory vesicles and many mitochondria.

Modified cilia of the lips

Several cilia of the lips have an unusual sheath (Fig. 1E–G); it consists of 2–30 electron-dense layers, which appear to be as though they are wound around the cilia. The maximal diameter of such cilia is 0.42 μm (Fig. 1E). Among the cilia, fibrous cord-like structures can be seen, which wrap the cilia or lie between them without any contact (Fig. 1G). Usually the cords consist of 3 layers, but often the number of layers is not divisible by three. Cilia can be wrapped only partially. A few vacuoles may lie underneath the membrane of the wrapped cilia.

Similar electron-dense cords were also found between the bodies of the ciliated cells of the lip epithelium (Fig. 1H). The images show that such fibrous structures have become detached from the surface of the ciliated cells.

Ventral pharyngeal organ of juveniles

The ventral pharyngeal organ of *D. longisetosus* comprises only a bulb (Figs 2B, 4 and 5). The bulb lies behind the base of the lower lip, deep in the mouth lumen, and is separated from the body cavity by a thin coelothelium (70 nm).

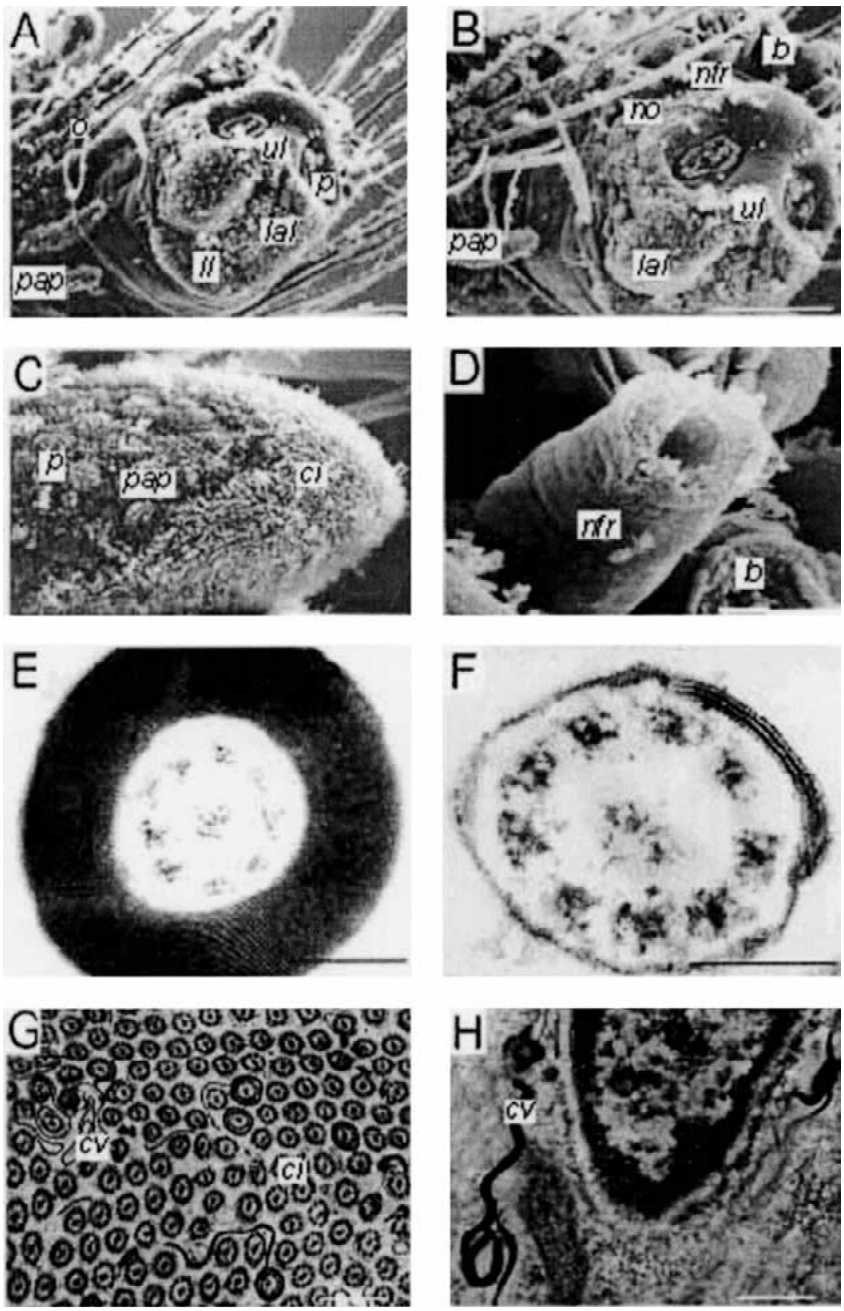

Figure 1. Diplocirrus longisetosus. Anterior end of the body. (A) Frontal view of introvert. SEM. Scale: 100 μm. (B) Enlargement of prostomium. Palps removed. SEM. Scale: 100 μm. (C) Dorsal side of palp. SEM. Scale: 10 μm. (D) Papilla with nephridiopore. SEM. Scale: 10 μm. (E–G) Modified cilia of lips. TEM. (E–F) High magnification of covered cilia. Scales. 0.1 μm. (G) Cross sections of cilia. TEM. Scale: 1 μm. (H) Cover of cilia between ciliated cells. TEM Scale: 1 μm. - b – branchiae, ci – cilia, cv – cover, lal – lateral lip, ll – lower lip, nfr – nephridiopore, o – fan of setae, p – base of palp, pap – papilla, ul - upper lip.

There are no tongue-like organs and no jaws. Just behind the bulb is the beginning of the oesophagus. In juveniles the bulb consists of an epithelium and muscle layers (Figs 3A–D and 4). In these specimens the bulb is about 50 μm long and 17 μm wide. The epithelium is covered with a thin cuticle (1 μm thick), which is penetrated by microvilli. (Fig. 3A). A distinct epicuticle is not developed. The epithelium is made

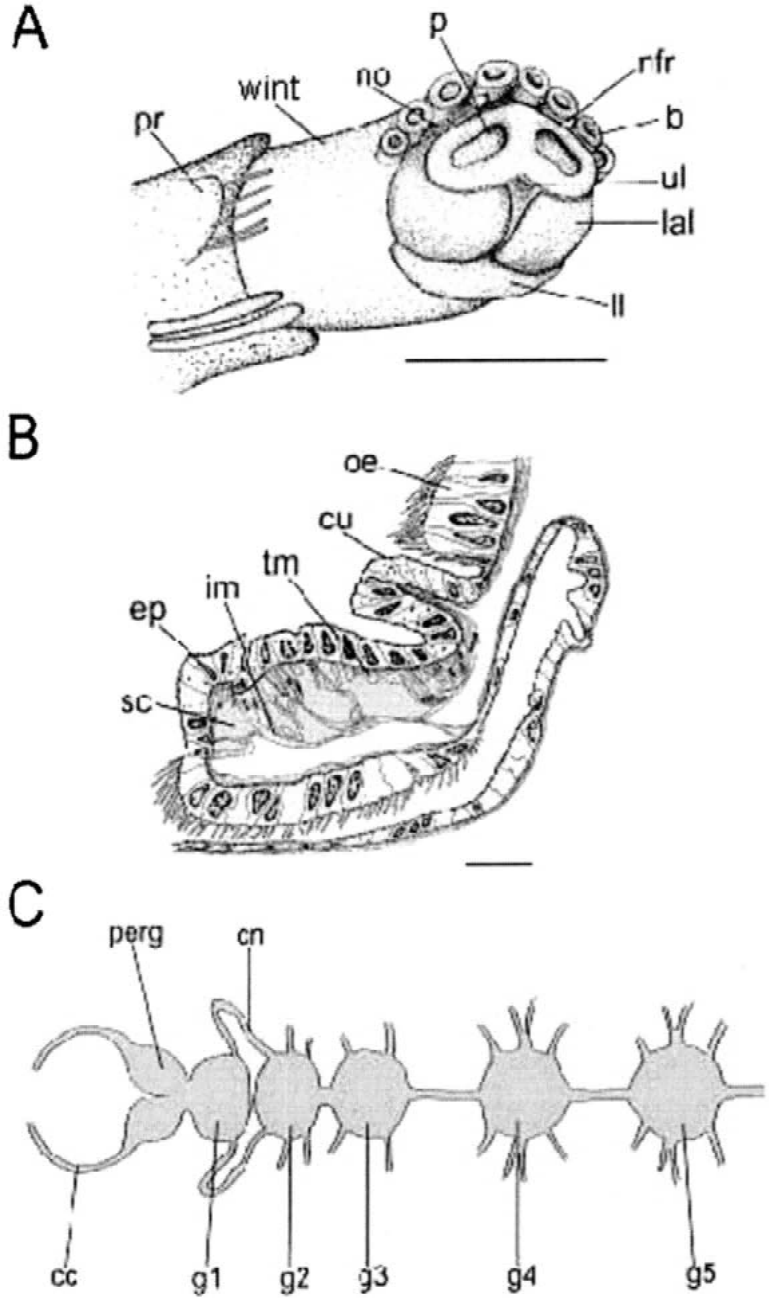

Figure 2. Diplocirrus longisetosus. (A) Drawing ofextended anterior end. Scale: 100 μm. (B) Sagittal reconstruction of ventral pharyngeal organ of adult. Scale: 20 μm. (C) Schematic representation of central nervous system. Brain cut off due to dorsal position above the loop of circumoesophageal connectives. - b – branchiae, cc – circumoesophageal connectives, cn – connective between g1 and g2, cu – cuticle, ep – epithelium, g1, g2, g3, g4, g5 - ganglion of the 1st, 2nd, 3rd, 4th, 5th chaetiger, im – investing muscle, lal – lateral lip, ll – lower lip, nfr – nephridiopore, no – nuchal organs, oe – oesophagus, p – base of palp, perg – peristomial ganglion, pr – parapodium of the first chaetiger, sc – supporting cell, wint –wall of introvert, tm – transverse muscle, ul – upper lip.

up by a single cell layer, about 3.5 μm high. The cells contain large nuclei (about 3 μm), Golgi stacks, well developed rough endoplasmic reticulum, mitochondria, tonofilaments and vacuoles (0.11–0.26 μm) with electron-dense contents. Glandular cells were not found (Fig. 3A).

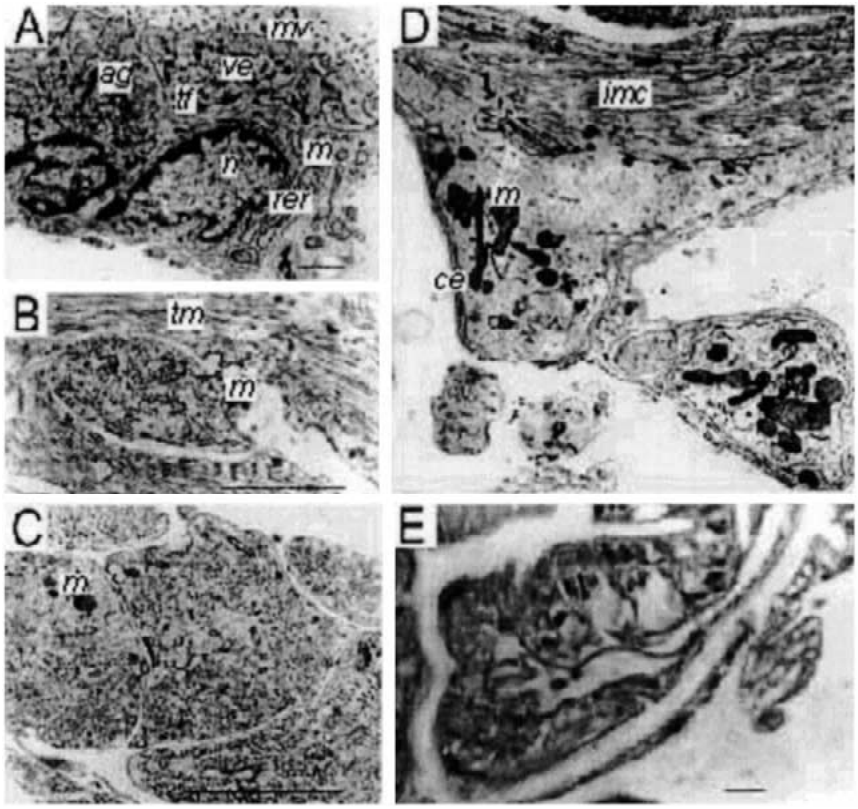

Figure 3. Diplocirrus longisetosus. (A–D). Ventral pharyngeal organ of juvenile (sagittal section, transmission electron microscopy). (A) Epithelium of bulb. Scale: 1 μm. (B–C) Muscle bulb composed of transverse muscle fibers. Scales: 1 μm. (D) Investing muscle. Scale: 1 μm. (E) Bulb of adult. Sagittal section, light microscopy. Scale: 10 μm. ag – Golgi Apparatus, bm- basal membrane, imc – investing muscle, m –mitochondrion, mv – microvilli, n – nucleus, rer – rough endoplasmic reticulum, tf – tonofilaments, ve – vesicle, imc -investing muscle, m – mitochondrion.

The epithelium rests on a basal lamina. Below this lamina are several layers of muscle cells forming the bulb. These cells are characterized by the presence of a number of myofilaments, oriented in various directions, although most myofilaments are transversal. In the middle part, which is more massive, the thickness of the muscle bulb component increases to 5 μm. In the muscle cells, no tonofilaments or non-contractile cytoplasmic extensions were observed. Supporting or interstitial cells were also not found.

The muscle bulb is enclosed by an investing muscle (about 4.9 μm), which is formed by single layer of muscle cells. The nuclei of these cells are situated in cytoplasmic extensions on the outer surface of the muscle (Fig. 3D).

Ventral pharyngeal organ of adults

The adult bulb is about 130 μm long and 44 μm wide (Figs 2B and 3E). Hence the proportions of the bulb do not change during growth. The epithelium is thicker (about 6.7 μm) and contains bigger nuclei (about 6 μm). The epithelium is covered by a cuticle (about 0.8 μm), similar in structure and thickness to the cuticle in juveniles. The number of muscle cells comprising the bulb is also comparable to that of juveniles, but their size is increased proportionally. The nuclei of muscle cells lie in cytoplasmic extensions. Between the muscle cells there are big voids, which occupy the main part of the interior volume of the organ. The investing muscle lies below the bulb.

The wall of the introvert

The wall of the introvert is rather thin (about 4.6 μm). It consists of the cuticle, an epithelium occupying 1.3 μm and a layer of circular muscle cells (appr. 1.6 μm). Muscle and epithelial layers are separated by a basal membrane. The cuticle is penetrated by a few microvilli. On the inner side of the epithelium

there are 0.3 μm long appendages, which extend into corresponding pits on the upper side of the muscle cells. Thus epithelium and muscle cells interdigate with each other. The appendages of both cell types include tonofilaments. Between muscle cells there are desmosomes. A coelothelium was not observed.

Nervous system

The central nervous system of *D. longisetosus* consists of a ventral nerve cord with well-developed segmental ganglia, circumoesophageal connectives and a brain (Figs 2C, 4 and 5). In general, three pairs of peripheral nerves run from each segmental ganglion, the middle one then branches in two.

The intraepithelial brain is located in the prostomium and divided into a right and a left part, which are connected to each other. The outer cells of the ganglion are covered by the cuticle. The cuticle is represented by an amorphous collagen matrix and penetrated by microvilli (Fig. 4). An epicuticular layer is not visible. Between the nerve cell bodies, right under the cuticle, numerous gland cells are situated. The circumoesophageal connectives originate from the brain and run to the peristomial ganglia.

The paired ganglion of the first chaetiger lies just behind the peristomial ganglia and is connected with the peristomial pair by very short connectives. The ganglion of the first chaetiger is smaller than the other segmental ganglia. Two pairs of transversal segmental nerves come from this ganglion. From the ganglion of the third chaetiger also two pairs of segmental nerves arise. The nerve cord is subepithelial.

A pair of unusually long nerves (connectives) runs from the ganglion of the first chaetiger to the second one. The connectives are clearly separated and curved longer than those between the other segmental ganglia. These long connectives are curved when the introvert is retracted and elongated and straight when it is protruded. These nerves mark the border of the introvert. Sequentially, the introvert of *D. longisetosus* consists of the prostomium, the peristomium and the 1st chaetiger (Fig. 2C).

Discussion

The structure of the introvert

The construction of the central nervous system of the anterior part of the body of *D. longisetosus* shows that the introvert consists of the prostomium and two anterior segments: the peristomium and the first chaetiger. At the same time, the general construction of the introvert of *D. longisetosus*, bearing the pair of palps, the branchiae, the different lips, the nephridiopores and the nuchal organs, is very similar to that of other genera and species of Flabelligeridae (Spies, 1975).

These data provide a basis for the future comparison of the Flabelligeridae with other polychaete taxa. The retractable anterior end of *Laubieriopsys* (Fauveliopsidae) consists of only prostomium and peristomium and does not include an additional segment (Shabanova & Zhadan, 2002).

These data supports the opinion by Buzhinskaya (1977) and Petersen (2000, pers. com.). On the basis of an analysis of external structures these authors concluded that the introvert of Fauveliopsidae consists of the pro- and peristomium only. Unfortunately, at present there is no detailed information available about the segmental structure of the anterior end of Poeobiidae. Robbins (1965) described the structure of the brain of *Poeobius meseres* Heath, 1930 and counted the total number of segmental ganglia, but did not note a segmental content of the introvert.

The introvert of Sternaspidae consists of seven anterior setigers of the body and does not bear any appendage (Fauchald & Rouse, 1997). In view of these differences, the retractable anterior end of Flabelligeridae, Fauveliopsidae and Sternaspidae more likely is convergent character, perhaps with the exception of Poeobidae.

Position of palps and branchiae

According to Spies (1975), paired nuchal organs and palps of Flabelligeridae are located on the prostomium, and branchiae, on the dorsal surface of peristomium. Rouse & Pleijel (2001) define branchiae of Flabelligeridae, Terebellidae, Ampharetidae, Alvinellidae, Pectinariidae, Cirratulaidae and Acrocirridae as peristomal.

Cirratulidae, Sternaspidae, Acrocirridae have paired palps located on the peristomium. Members of the taxa Terebellida *s. stricto* (Terebellidae, Ampharetidae, Alvinellidae, Trichibranchidae, Pectinariidae) have a number of buccal tentacles derived from peristomial palps (Zhadan & Tzetlin, 2002). Our data on *Diplocirrus longisetosus* does not contradict this conclusion, although the border between prostomium, peristomium and 1st segment is indistinct in all flabelligerids, hence the determination of palp and

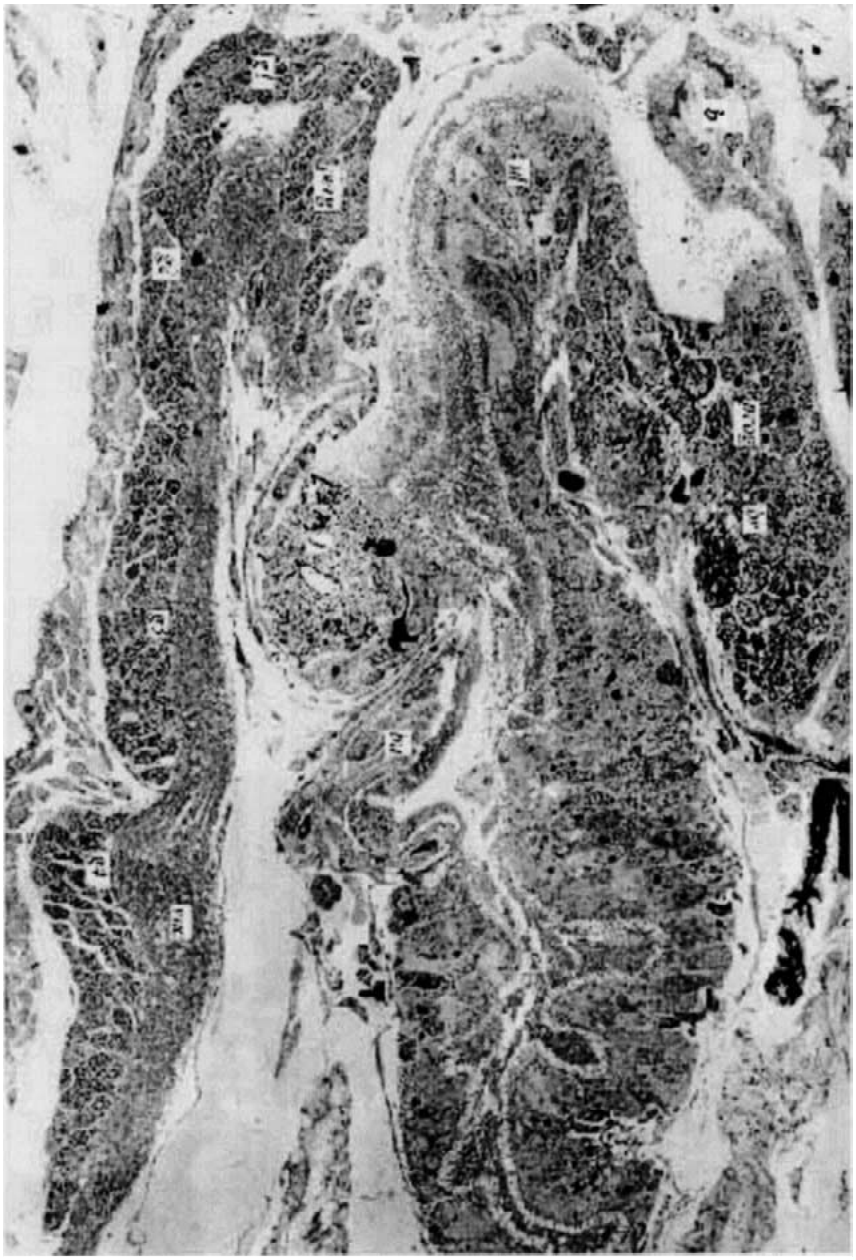

Figure 4. Sagittal section through anterior end of *Diplocirrus longisetosus*. TEM. br – brain, b – branchiae, bu – muscle bulb, f – food, g1, g2, g3, g4, - ganglion of 1st to 4th chaetiger, oe – oeosophagus, perg – peristomial ganglion, prog – prostomial ganglion, ul – upper lip, vnc – ventral nerve cord.

branchiae location needs a special study of development and innervation of these appendages. Although the studies of Orrhage (e.g., 1995, 2001) demonstrated an overall homology of these appendages among various polychaete taxa (see also Purschke, 2002) a distinction between prostomial and peristomial palps has been made by Rouse & Fauchald (1997) and Rouse & Pleijel (2001) due to their definite position in the adults.

The structure of the foregut

The ciliated lips at the mouth of Flabelligeridae correspond to the so-called dorsolateral folds of other polychaetes(Purschke & Tzetlin, 1996). The only dif-

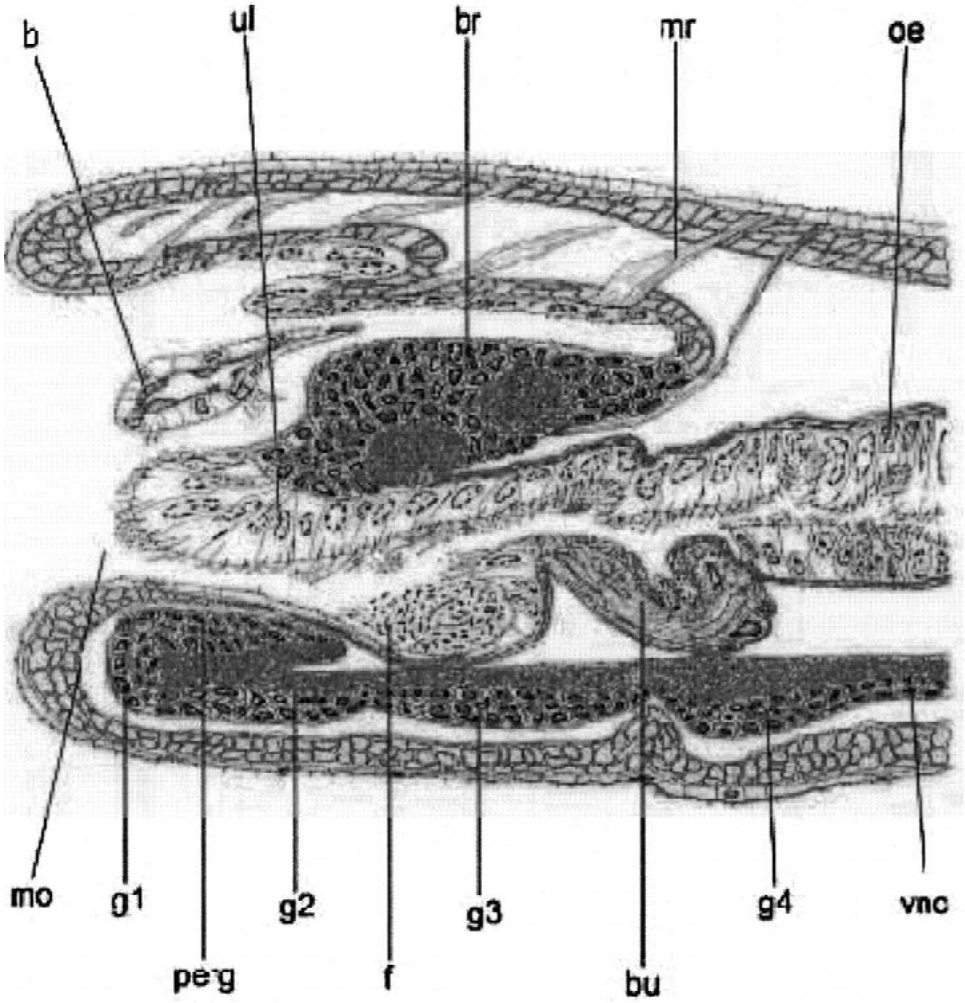

Figure 5. Drawing of *Diplocirrus longisetosus*. Sagittal section of the anterior end. br – brain, b – branchiae, bu – muscle bulb, f – food, g1 – ganglion of the first chaetiger, g2 – ganglion of the second chaetiger, g3 – ganglion of the third chaetiger, g4 – ganglion of the fourth chaetiger, mo – mouth opening, mr – retractor muscle, oe - - oeosophagus, perg – peristomial ganglion, ul – upper lip, vnc – ventral nerve cord.

ference is that they are shifted somewhat to the dorsal part of the body. Moreover, four lips were found rather than three as in other Polychaeta. Their function remains the same: collecting material during feeding. The ventral pharyngeal organ of juvenile Flabelligeridae consists only of a muscle bulb; no accessory structures such as a tonguelike organ are present (see Purschke, 1988a; Saulnier-Michel, 1992). A bulb composed of muscle cells and non-contractile interstitial cells is typical of a number of sedentary polychaete taxa and has, for instance, been described in the Orbiniidae, Ctenodrillidae and Terebellidae (Tzetlin, 1987; Purschke, 1988b; Zhadan & Tzetlin, 2002,). In the bulb of *D. longisetosus* interstitial cells were not observed and in all probability are absent. However, instead of such cells, large empty voids were found between the muscle cells of the bulb. This observation represents a unique peculiarity of the bulb of *D. longisetosus*, as does the weakly developed and very thin coelomic lining that covers the entire bulb. These voids might represent fixation artifacts, although this appears unlikely because of their regular appearance and their presence in every specimen investigated. Moreover, they also occur between the muscle cells of the bulb in the adults and are clearly recognizable in histological sections. Probably these voids have a supporting function, similar to that of interstitial cells in the bulb of other polychaetes (Purschke, 1988b). The ventral pharynx of Cirratulidae (Tzetlin, 1992)

has ventral bulb, lateral folds and investing muscle sheath.

The present data demonstrate the structural relationships among Flabelligeridae, Orbiniidae, Ctenodrillidae and Terebellidae, because in these groups the ventral pharyngeal organ usually consists only of a muscle bulb, whereas other structures, such as a tongue-like organ, are lacking.

The functional significance of the modified cilia observed in the epithelium of the lips remains unknown. It seems likely that such dense sheaths and vacuoles would prevent the pulsation of the cilia. If they are not artifacts, they might have additional secretory or sensory functions.

With respect to the structure of the anterior part of the body and the appendages, Flabelligeridae most closely resemble Poeobiidae: Poeobiidae have 8 tentacles on their anterior body functioning as branchiae, two palps, and a flexible tongue, which functions of as a ciliated lip. Moreover, the first chaetigers can be retracted together with the pro- and peristomium. Therefore, their anatomical structure suggests that Poeobiidae are the taxa most closely related to the Flabelligeridae. To verify this inference additional research is needed, especially in Fauveliopsidae and Poeobiidae.

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