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REGULAR ARTICLE

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Ultrastructural description of the musculature, the intraepidermal nervous system, and their interrelation in *Pseudochordodes bedriagae* (Nematomorpha)

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Abstract The ultrastructure of the body wall muscles and the intraepidermal nervous system of the Gordiida *Pseudochordodes bedriagae* are described. The body wall muscles are of the circomyarian type, since the sarcomeres constitute a system of continuous peripheral helices. The organisation of the sarcomeres follows a pattern that resembles that of the striated muscles. The muscle fibres are separated into areas by invaginations formed exclusively by the plasma membrane (T component), while the sarcoplasmic reticulum lies at the sides of the Z granules forming subsarcolemmal cisternae, and in the zone near the nucleus, like flattened vesicles, contributing with the T component to the formation of dyads and triads. The muscle fibres present two types of adaptations for their innervation: (1) cytoplasmic projections towards the epidermis, and (2) invaginations of the plasmalemma. The motor peripheral nervous system is conformed by the nerve fibres that run within the epidermis and their projections towards the basal membrane in order to contact the adaptations of the muscle fibres in a basi-epidermal synapsis. The presence of an intraepithelial peripheral nervous system in Gordiida confirms a structural pattern common to other taxa of Nemathelminthes.

Keywords Gordiida · Ultrastructure · Muscle · Peripheral nerves · Synapsis · *Pseudochordodes bedriagae* (Nemathelminthes)

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Introduction

Nematomorpha are a well-defined monophyletic group represented by two sister taxa, Gordiida and Nectonema. Gordiida are known as horsehair worms, and in their adult phase they live in fresh water. Their minute larvae are obligate parasites from different groups of animals, especially arthropods. Nematomorpha, together with Nematoda, Gastrotricha, Priapulida, Kinorhyncha and Loricifera constitute a taxon named Nemathelminthes in the majority of phylogenetic analyses (Neuhaus 1994; Ahlrichs 1995; Nielsen 1995; Ehlers et al. 1996; Schmidt-Rhaesa 1998b).

Recently, the older theory of Rauther (1909), hypothesising a relationship between arthropods and some groups of Aschelminthes has gained support, primarily based on the analysis of 18 (ribosomal ribonucleic acid) rRNA gene sequences. Comparisons of these molecular data suggest that all taxa with ecdysis, Nemathelminthes and Panarthropoda (onychophorans, tardigrades and euarthropods), should be included in the monophyletic taxon Ecdysozoa (Aguinaldo et al. 1997; Garey and Schmidt-Rhaesa 1998). The sister group, Nematomorpha + Nematoda, was named Nematoida by Schmidt-Rhaesa (1996a), because they share some similarities in their general organisation of the body, especially of the body wall.

In Gordiida the cuticle is very thick and includes an outer epicuticle, which has a variable structure depending on the genus and species. This external layer can be either smooth or sculptured by elevations of different sizes and shapes known as areoles (Schmidt-Rhaesa 1997; de Villalobos and Voglino 2000; de Villalobos and Zanca 2001) and often bears apical spines, bristles or pores. In Gordiida and Nematoda, the main layer of the cuticle is fibrillar and is composed of a variable number of overlapping layers of collagen fibres (Eakin and Brandenburger 1974; Bresciani 1991; de Villalobos and Restelli 2001).

The epidermis of nematomorphs, in the dorsal area of the body, is formed by a single layer of cells. Cell borders are irregular, with numerous interdigitations and desmosomes (de Villalobos and Restelli 2001). In Nematoda the epidermis is thicker and in longitudinal bands, the epidermal cords (Wright 1991; Nielsen et al. 1996).

Nematomorpha share with Nematoda the characteristic of having only longitudinal muscles. A body wall containing longitudinal and circular musculature is a plesiomorphic condition of Nemathelminthes and is present in all nemathelminth taxa other than Nematomorpha and Nematoda (Schmidt-Rhaesa 1998b). However, nematomorphs and nematodes differ in the organisation of the muscle fibres. All nematodes studied thus far show uniformity in muscle fibre arrangement, although the various fibres may differ in structure. There is a simple type of organisation, the platymyarian type (plesiomorphic condition), found in some simpler nematodes, presenting relatively few fibres with a flattened contractile zone at the edge of the fibre (Lanzavecchia 1977; Malakhov 1994). A more complex type, the coelomyarian type, which is typical of higher nematodes (Chitwood and Chitwood 1950), has more fibres in the cytoplasmic region, where the nucleus extends outside the contractile area and the sarcomeres do not form a continuous helical system at the periphery of the fibre (Lanzavecchia 1977).

The muscle structure in Gordiida has already been reported, both under light microscopy (LM; Camerano 1888; Montgomery 1903; Rauther 1904; May 1919) and transmission electron microscopy (TEM; Swanson 1971; Eakin and Brandenburger 1974; Lanzavecchia 1977; Lanzavecchia et. al. 1979; Bresciani 1991; Schmidt-Rhaesa 1996a, 1997, 1998a). This musculature is unusual, with ribbon-like muscle fibres consisting of long, thick filaments of paramyosin and thin filaments (Nielsen et al. 1996). Lanzavecchia et al. (1979) and Bresciani (1991) have pointed out that the muscle cells of nematomorphs belong to the circomyarian type, because they show the typical helical fibre pattern.

In nematodes the muscle innervation takes place through the contact among the cytoplasmic projections of the muscle cells and the dorsal and ventral nerve cords (Wright 1991). In Gordiida the mechanism of muscle innervation has not been clearly explained yet. Schmidt-Rhaesa (1998a) has described in Gordiida, for the first time, the presence of small projections of the muscle fibres towards the epidermis and presumes that they are involved in muscle innervation. These projections, unlike those observed in the nematodes, do not contact the nerve cord.

Villot (1874, 1889, 1891), Montgomery (1903) and May (1919) describe the nervous system in Gordiida as being formed by a circumpharyngeal brain and a ventral nerve cord that contacts the epidermis through a ventral neural lamella and a peripheral nervous system. The latter has not been observed in any of the subsequent studies, not even in those using TEM (Bresciani 1991; Schmidt-Rhaesa 1996b).

The aim of this investigation is to describe, for the first time, in *Pseudochordodes bedriagae* the ultrastructural characteristics of musculature, to confirm the presence of a peripheral nervous system and to analyse its intervention in muscle contraction.

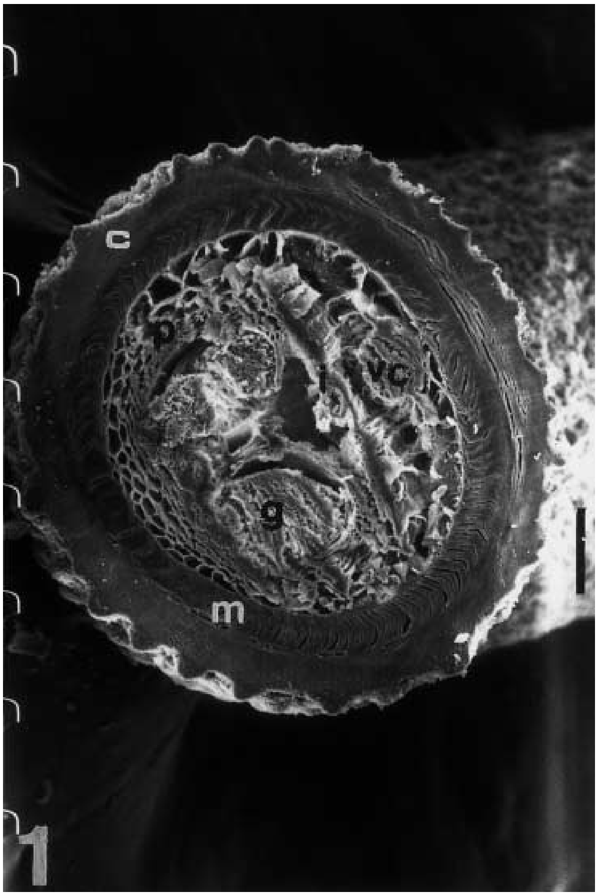

Fig. 1 Cross section of the soma (*c* cuticle, *g* gonads, *i* gut, *m* muscle, *p* parenchyma, *vc* ventral cord). Scanning electron microscopy (SEM). *Bar* 100 μm

Materials and methods

Six male *Pseudochordodes bedriagae* specimens were studied. They were collected during the spring and summer seasons of 1998 and 1999, from the stream El Negro in Sierra de la Ventana, Province of Buenos Aires, Argentina, and from an irrigation channel in General Conesa, Province of Rio Negro, Argentina. They were maintained alive in aerated, non-chlorinated water, at a temperature ranging between 16 and 20°C.

For analyses by TEM, we obtained tangential and cross sections of 0.5–1.0 mm in thickness from the anterior, medial and posterior ends of the body of three specimens. These pieces were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.1–7.3) at 4°C. They were washed twice every 30 min with cacodylate-phosphate buffer in 0.1 M sucrose and maintained under refrigeration for later processing.

For analyses under a scanning electron microscope (SEM), three specimens were fixed in 95% ethanol and dehydrated in 100% ethanol, critically point dried, gold-argon plasma-sputtered and viewed under SEM (JEOL SLM 1000).

Results

Muscle tissue

In a body cross section analysed under SEM (Fig. 1), we observed beneath the epidermis the layer of longitudinal

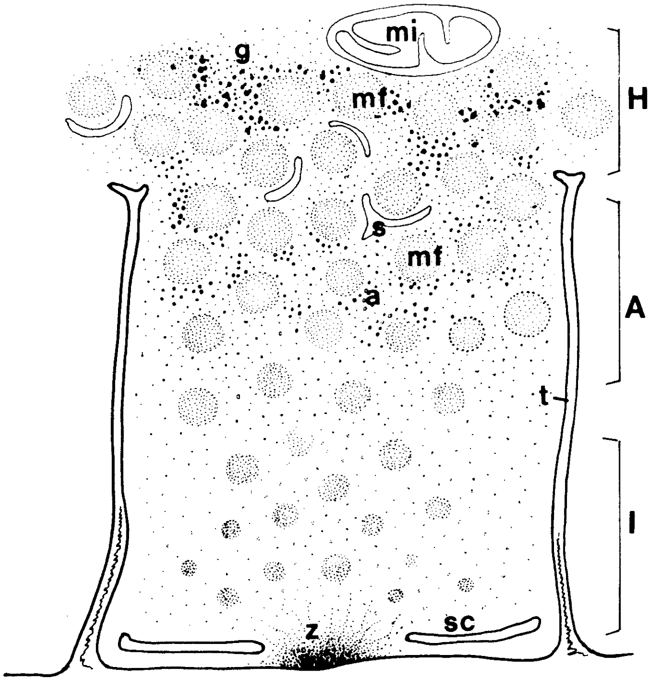

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Fig. 2 Schematic diagram of the sarcomere (*a* actin filament, *g* glycogen granules, *mf* myosin filament, *mi* mitochondria, *s* cisternal profile of the sarcoplasmic reticulum, SR, *sc* subsarcolemmal cisterna of the SR, *t* T component, *z* Z granule, *H*, *A*, *I* sarcomere bands)

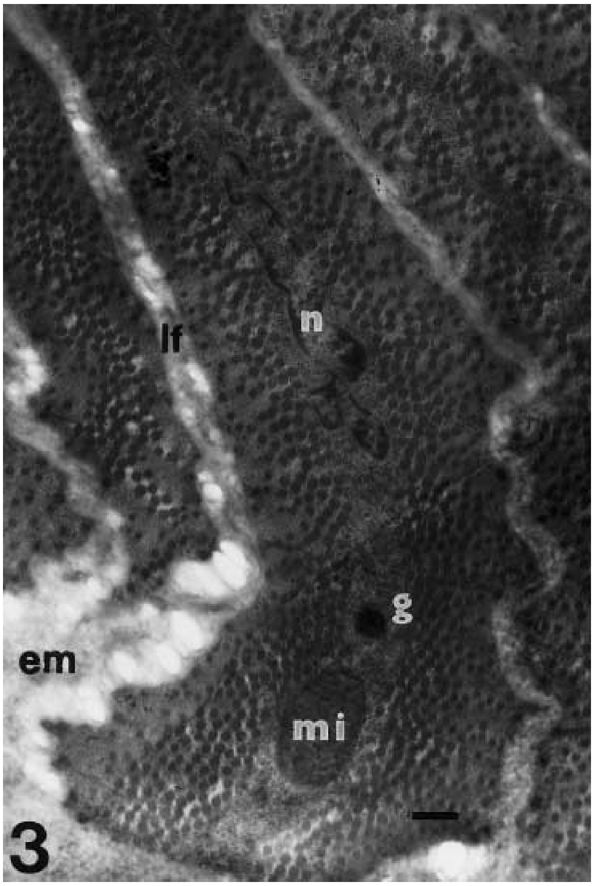

Fig. 3 Cross section of muscular fibre at the end neighbouring the parenchyma (*em* extracellular matrix, ECM, *g* glycogen granules, *lf* fibroreticular lamina of the extracellular matrix, *mi* mitochondrion, *n* nucleus). Transmission electron microscopy (TEM). *Bar* 500 nm

muscle fibres interrupted in the midventral line by the nervous lamella. These muscle fibres were radially disposed and presented a width of 3.5°µm and a thickness of 6.6 µm.

In a cross section analysed under TEM (Figs. 2, 3) the fibres, showing a ribbon-like aspect, extended from the conjunctive basal membrane to the parenchyma and were separated from each other by a thin layer (300 nm) of fibro-reticular extracellular matrix (ECM). The fibres were seen contacting the basal membrane through hemidesmosomes, presenting at this level thin cytoplasmic projections (Fig. 12). The sarcolemma quite regularly presents invaginations (T component) which extend until almost reaching the central zone of the cell (Figs. 2, 4, 5, 7) These septum-like invaginations incompletely divide the contractile peripheral zone into regular areas. The central zone of the fibre was occupied by a cytoplasmic axis, where an irregular nucleus with perinuclear dense chromatin, mitochondria and rosette-shaped beta granules (Figs. 3, 6) are basally located.

In the areas limited by the invaginations contacting the plasma membrane, an electron-dense granule (Z granule) was observed, from which thin actin filaments (6 –7 nm in diameter) extended radially (Figs. 2, 4, 5). The sarcoplasmic reticulum (SR) was represented on each side of the Z granule by free saccular profiles, sometimes with electron-dense content, which constitute the subsarcolemmal cisternae of the SR (Figs. 3, 4); and in the central area, it was represented by flattened and anastomosed vesicles surrounding the thick filaments (Figs. 4, 6). The SR, together with the T component, formed structures similar to dyads and triads (Figs. 4, 7). In the peripheral zone of the fibre, there appeared only thin myofilaments of actin coming out of the Z granule (Figs. 2, 4, 5); the thick filaments, with a diameter ranging between 40 and 60 nm, appeared surrounded by an undefined number of thin filaments (Figs. 2, 4, 5, 6). Both types of filaments were interrelated and formed bridges. In the paracentral zone, only thick filaments (Figs. 2, 4, 6) with a larger diameter (70–90 nm) were observed, surrounded by SR vesicles and abundant beta granules (Fig. 6).

In a sagittal section (Fig. 7), it was observed that the muscle fibres were separated by the ECM (55 µm) and had very irregular ends. Z granules in contact with the plasma membrane were presented in the form of a band, and the profiles of the SR could be seen neighbouring them. The T component could be clearly observed next to the thin filaments and distant from the thick filaments, the latter possessing a periodic striation of approximately 69 nm (Fig. 8).

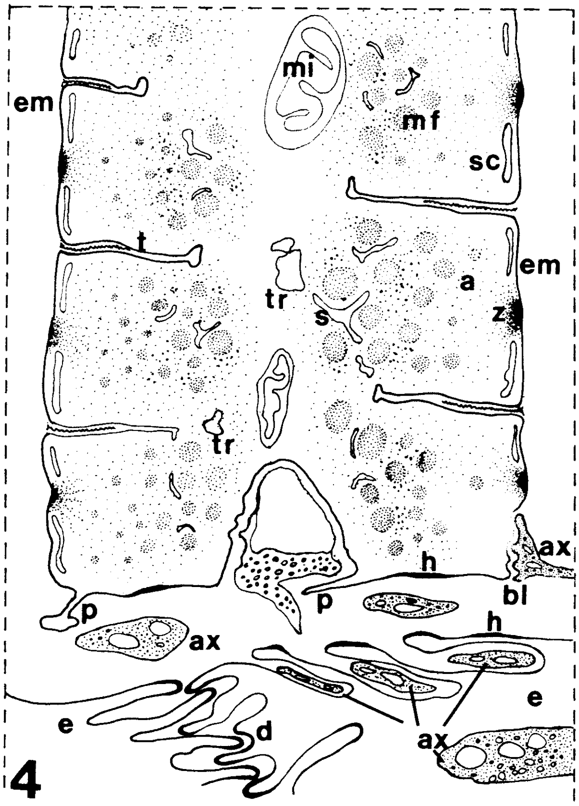

Fig. 4 Schematic diagram of the interrelation between the intraepidermal nervous system and the muscular fibre (*a* actin filament, *ax* axons, *bl* basal lamina, *d* desmosome, *e* epidermis, *em* ECM, *g* glycogen granules, *h* hemidesmosomes, *mf* myosin filament, *mi* mitochondrion, *p* projection of muscular fibre, *s* cisternal profile of SR, *t* T-component, *tr* triad-like, *z* Z granule)

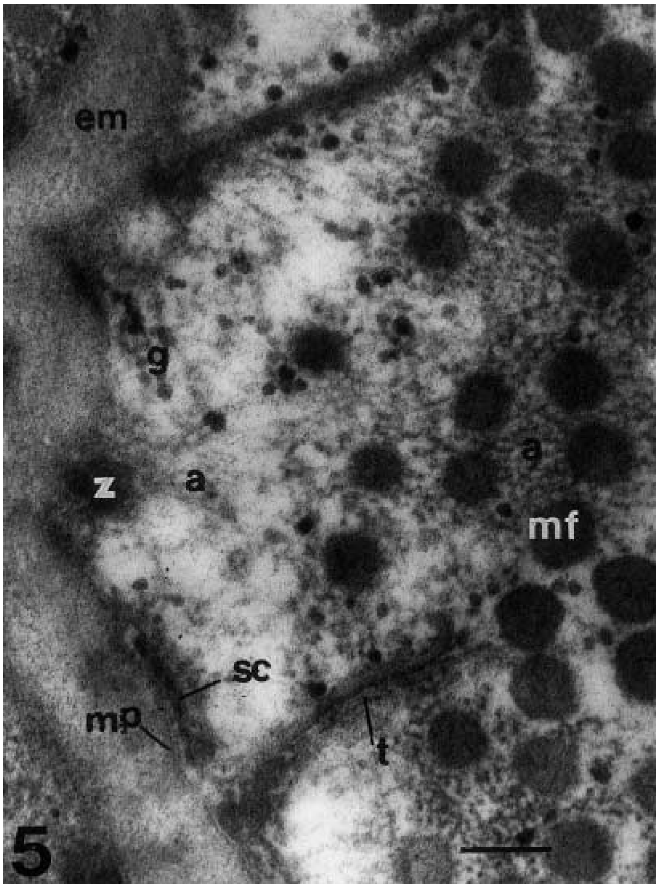

Fig. 5 Cross section of the sarcomere, peripheral zone (bands I and A) (*a* actin filament, *em* ECM, *g* glycogen granules, *mf* myosin filament, *mp* plasma membrane, *sc* subsarcolemmal cisterna of the SR, *t* T component, *z* Z granule). TEM. *Bar* 100 nm

Intraepidermal nervous system

By TEM it could be observed that the epidermal cells from the dorsal zone of the body formed a single layer. Cell borders (Figs. 4, 10, 11, 12) are irregular, with numerous interdigitations, and the nuclei are large and ovoid (de Villalobos and Restelli 2001). We observed nerve fibres among the interdigitations of the epidermal cells, mainly in the ventral and lateral zones of the body, which constitute a peripheral intraepidermal nervous system. This nervous system is formed by two types of cellular projections following a longitudinal course: those that are electron dense, which we interpreted as glial cells, and those that are electron lucent, presenting, in their interior, clear vesicles and tubules, and which correspond to nerve cells (neurones).

Nerve fibres that contact the muscle fibres or their projections extend from the epidermis towards the basal membrane. The ventral neural lamella, which extends from the epidermis to the ventral cord (Fig. 9), is formed by nervous elements similar to those described in the intraepidermal nervous system. These nervous elements are easy to identify, since in this region epidermal cells do not intercalate. In addition, the limit between the epidermis and the lamella could not be observed with precision (Fig. 10).

Neuro-muscular union

The muscle fibres present two types of adaptations for their innervation: (1) thin expansions of cytoplasm, and (2) invaginations of the plasma membrane of the muscle cell. The synapses are set by the union of the adaptations of the muscle fibre with a nerve fibre (Figs. 4, 11, 12). This starts at the epidermis and ends in dilatations containing synaptic vesicles, some of them clear and others with a dense core, surrounded by scarce cytoplasm.

Discussion

Ps. bedriagae posses a musculature similar to that of other species of Gordiida described as *Gordius aquaticus* (Swanson 1971; Lanzavecchia et al. 1979; Bresciani 1991; Schmidt-Rhaesa 1998a), *Gordius panighettensis* (Lanzavecchia et al. 1979), *Paragordius varius* Leidy, 1851 (Montgomery 1903; May 1919), *Gordius* sp.

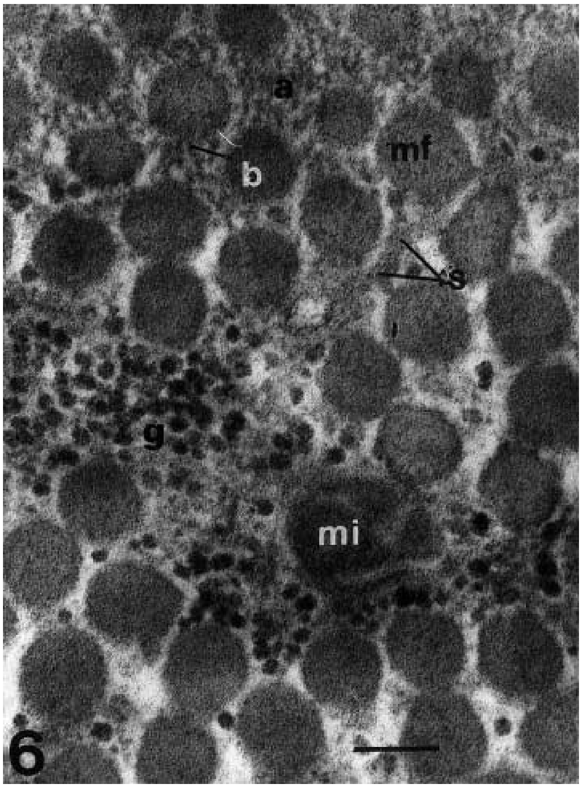

Fig. 6 Cross section of the sarcomere, paracentral zone (bands A and H) (*a* actin filament, *b* actomyosin bridges, *g* glycogen granules, *mf* myosin filament, *mi* mitochondrion, *s* cisternal profile of the SR). TEM. *Bar* 100 nm

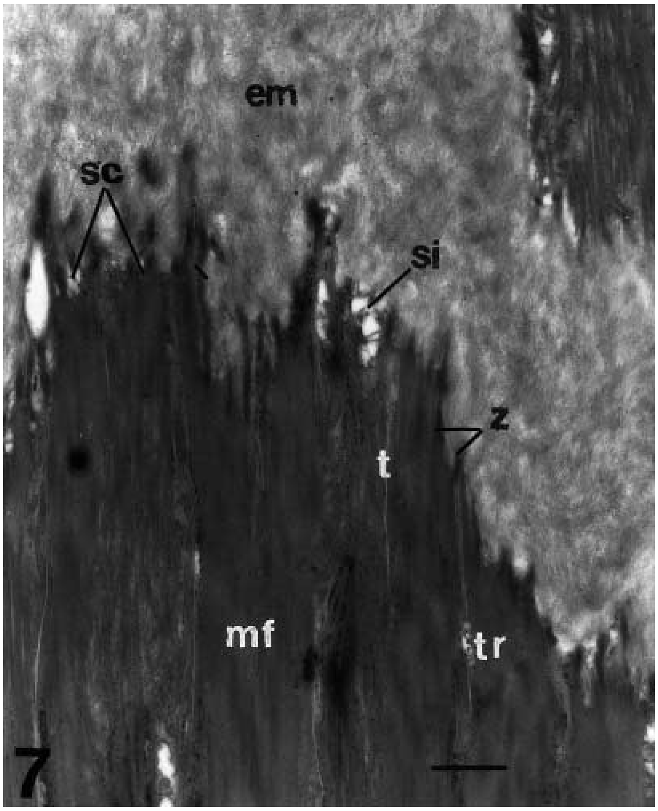

Fig. 7 Longitudinal section of the muscular fibre (*em* ECM, *mf* myosin filament, *sc* subsarcolemmal cisterna of the SR, *si* synapsis, *t* plasma membrane invagination (T component), *tr* triad of the SR, *z* Z granule). TEM. *Bar* 1 µm

(Eakin and Brandenburger 1974) and *Gordius robustus* (May 1919). We consider it a musculature of the circomyarian type, in agreement with Lanzavecchia et al. (1979) and Bresciani (1991), since the sarcomeres constitute a system of continuous peripheral helices, forming an obliquely striated muscle. In *Ps. bedriagae* as well as in *Paragordius varius*, the nucleus is displaced towards the fibre base, and it is not central (Swanson 1971; Montgomery 1903) as in the other species studied.

Lanzavecchia et al. (1979) emphasise that it is difficult to determine whether the membranes limiting the areas in the muscle fibres are composed by the plasma membrane or by the SR. We were able to determine clearly that the fibres are separated into areas through invaginations formed exclusively by the plasma membrane (T component), and not by the SR (Schmidt-Rhaesa 1998a; Lanzavecchia et al. 1979). This can be explained by the fact that the SR forms a structure independent of the plasmalemma. It lies at the sides of the Z granules forming subsarcolemmal cisternae, and in the zone near the nucleus, like flattened vesicles, which are like those described by Wright (1964) in the nematode *Capillaria hepatica*, contributing with the plasmalemma in the formation of dyads and triads similar to those observed in the muscles of vertebrates (Fawcett 1994).

Eakin and Brandenburger (1974) and Schmidt-Rhaesa (1998a) have described hemidesmosomes present throughout the whole plasma membrane of the muscular fibre. On the contrary, we found them confined to the contact zone between this plasma membrane and the basal membrane of the epidermis. This, by definition, corresponds with the fact that hemidesmososmes interrelate the proteins of the cytoskeleton with those of the extracellular matrices of the basal laminae of the epithelia (Ponzio 1996). The dense bodies that Eakin and Brandenburger (1974) consider to be hemidesmosomes are the Z granules, and the long fibres, which they consider to be monofilaments, are actually the fine filaments of actin (6–8 nm).

In most invertebrates the basic physiology and biochemistry of muscle contraction is constituted by a double system of myofilaments (actin and myosin) sliding over each other. However, a variation of the basic model of muscular organisation has evolved in, among other taxa (see Lanzavecchia 1977), Nematomorpha. The contractile mechanism of the longitudinal body wall musculature of *Paragordius varius* and *Gordius robustus* (Swanson 1970) is made up of two kinds of filaments: thin (actin) filaments and thick filaments that consist of a central axis of paramyosin, formed by a helical layer surrounded by a sheath of mysoin (Eliot and Lowy 1970; Lanzavecchia 1972).

Our analysis allows us to confirm that, among the species of Gordiida studied, also in *Ps. bedriagae*, the

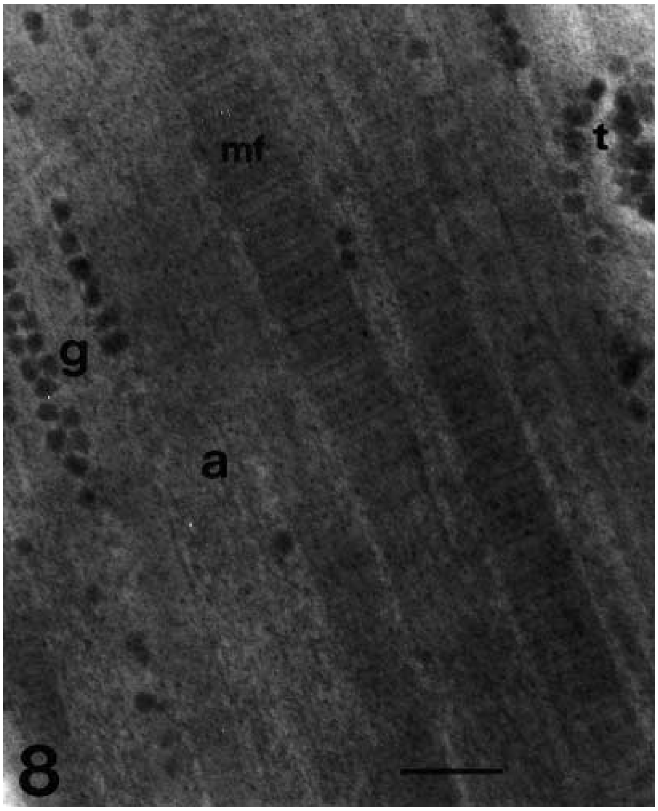

Fig. 8 Longitudinal section of the muscular fibre (*a* actin filament, *g* glycogen granules, *mf* myosin filament, *t* T component). TEM. *Bar* 100 nm

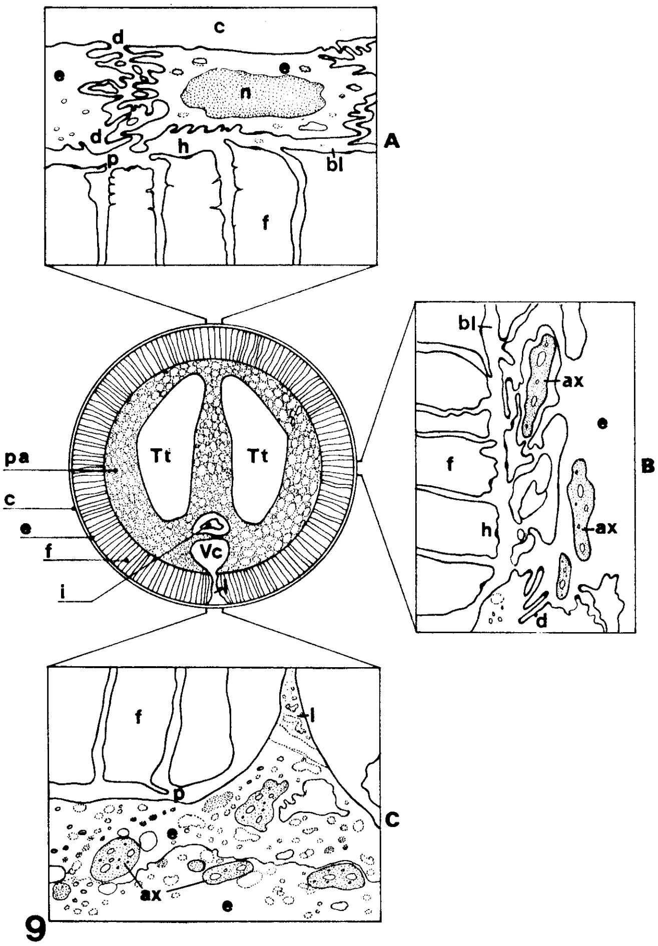

Fig. 9A–C Schematic diagram of a cross section of the intraepidermal nervous system. **A** Epidermis of the dorsal region of the body; **B** intraepidermal nerve fibres in the paramedial region; **C** Epidermis in the ventral region, neuroepidermal elements and origin of the nervous lamella. (*ax* Axon, *bl* basal lamina, *c* cuticle, *d* desmosomes, *e* epidermis, *f* muscular fibre, *h* hemidesmosomes, *i* intestine, *l* nervous lamella, *n* nucleus, *pa* parenchyma, *p* projection of muscular fibre, *Tt* testis tubes, *Vc* ventral nerve cord)

thick filaments are formed by a central axis of paramyosin surrounded by myosin, since a periodic striation of 690–700 nm is clearly manifested to be coincident with the asymmetrical polarisation of paramyosin (720 nm; Swanson 1971; Lanzavecchia 1972, 1977). Likewise, our observation of bridges of actomyosin in the A band would straightforwardly prove the presence of peripheral myosin, since paramyosin does not form bridges with actin (Lanzavecchia 1972), but acts as a support for the outer surface of myosin (Cooke 1986; Pollard and Cooper 1986). The presence of paramyosin in Gordiida would allow us to find an explanation for our observations about the behaviour of these worms, who can bend their cylindrical bodies, twining themselves around the stems of plants (“catch contraction”), keeping a high level of tension for weeks without signs of fatigue.

The initiation of a catch contraction requires the traditional actomyosin system. However, maintenance of the catch state appears to require a paramyosin that has a “sticky” end. This end may facilitate the binding or “catching” of one paramyosin molecule to a neighbouring molecule. When this happens repeatedly with large numbers of paramyosin molecules, the muscle fibres become “locked” in the catch state. Thus, little or no energy is needed to sustain the contraction (Radlick and Koretz 1992).

The Nematomorpha ultrastructural muscle organisation is a good taxonomic diagnostic tool and suggests that all of these worms may have descended from a common ancestor, who, independently of the other Nemathelminthes, developed a paramyosin system peculiar to it.

As we have already pointed out, in Gordiida, the studies on the peripheral nervous system and the mechanism of muscle innervation are scarce and confusing. Villot (1889, 1891) in *Gordius preslii*, considered that the peripheral nervous system was formed by nerve fibres coming from circumpharyngeal brain, from which some fibres passed through the epidermis until they reached the cuticular papillae, while others penetrated the “perimysium” (basal lamina) and innervated the muscle fibres. Montgomery (1903) observed in *P. varius* that the peripheral nervous system is formed by plexuses of intraepidermal nervous fibres and he supposed that they

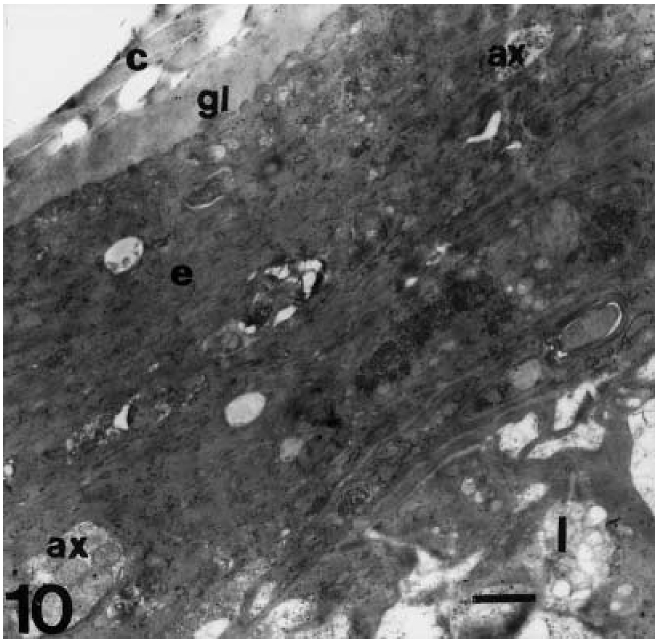

Fig. 10 Cross section of the tegument (ventro-medial region) (*ax* axons, *c* cuticle, *e* epidermis, *gl* germinal layer, *l* nervous lamella, origin). TEM. *Bar* 1 µm

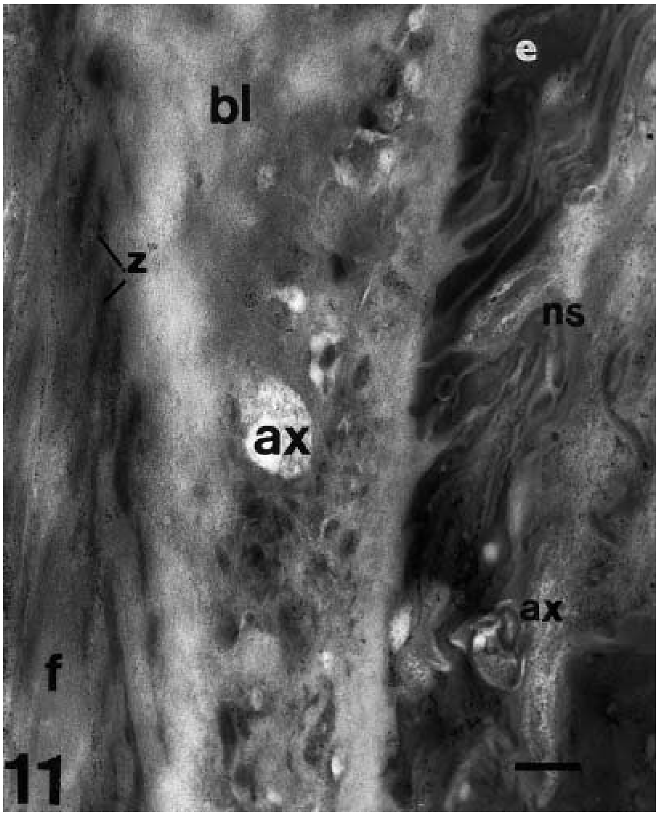

Fig. 11 Longitudinal section showing epidermal cells intraepidermal axons and their projections to basal lamina (*ax* axons, *bl* basal lamina, *e* epidermis, *f* muscular fibre, *ns* intraepidermal nervous system, *z* Z granule). TEM. *Bar* 500 nm

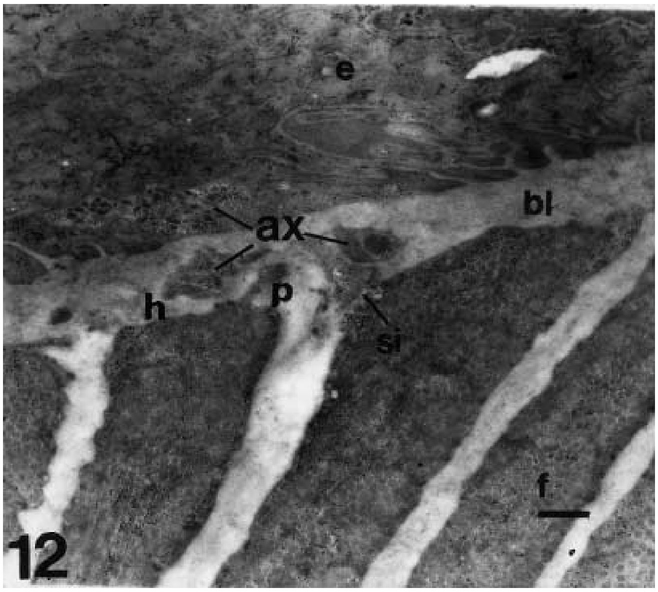

Fig. 12 Cross section of the tegument (*ax* axons and their projections, *bl* basal lamina, *e* epidermis, *f* muscular fibre, *h* hemidesmosomes, *p* cytoplasmic projections, *si* synapsis). TEM. *Bar* 500 nm

come from the chromophilic cells (neuronal) of the ventral nerve cord. Since no nervous projections from this peripheral nervous system to the cuticle or musculature could be distinguished, May (1919), in *G. robustus* and *P. varius*, considered that the peripheral nervous system consists of peripheral fibres and nerve cells located in the epidermis and fibres passing outward from the nerve cells to the surface of the body. Montgomery (1903) and May (1919) considered that the muscular innervation was likely to take place through the nerve projections from the ventral nerve cord.

The finding of an intraepidermal peripheral nervous system in *Ps. bedriagae* would allow us to explain that the nerve impulse carried by the projections of the intraepidermal plexuses of nerve fibres to the motor plate (synapsis) would be transmitted to the sarcomeres through the T component. This motor plate is located (1) between the nerve fibres and the cytoplasmic expansions of the muscular fibres, mentioned by Schmidt-Rhaesa (1998a), or (2) within the invaginations of the muscular fibre. The presence of a basi-epidermal peripheral plexus-like nervous system in *N. munidae* (Schmidt-Rhaesa 1996b) and intraepidermal in *Ps. bedriagae* has to be considered another characteristic of the ground pattern of Nematomorpha.

The presence of peripheral nerves included in the epidermis (plesiomorphic condition) has also been described for other taxa of Nemathelminthes (Neuhaus 1994; Schmidt-Rhaesa 1998b) such as in the Loricifera, by Kristensen (1991), in the Kinorhyncha, by Kristensen and Higgins (1991), and in the Priapulida, by Storch (1991). Likewise, Adrianov and Malakhov (1995) considered that, among other characteristics, the presence of an intraepithelial peripheral nervous system would constitute a common structural pattern within Cephalorhyncha.

Future research into the ontogenesis of the central and peripheral nervous system in Nematomorpha may reveal new information about the phylogenetic relationships of this taxon.

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