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Cytochemical Analysis of the Body Wall of the Flounder Parasite *Procamallanus* (*Spirocamallanus*) *halitrophus* (Nematoda: Camallanidae)

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ABSTRACT: *Procamallanus* (*Spirocamallanus*) *halitrophus* (Fusco and Overstreet, 1978) is an intestinal parasite of the flounders *Syacium papillosum* and *Citharichthys macrops*, both of which are native to waters off the coast of the state of Rio de Janeiro, Brazil. With transmission electron microscopy, we observed the body wall which is composed of the cuticle, hypodermis, and somatic musculature. The cuticle of *P.* (*S.*) *halitrophus* is composed of 5 layers: the epicuticle, cortical, median, fibrous, and basal layers. Underlying the cuticle is the hypodermis, a syncytium that contains mitochondria, glycogen granules, vesicles, inclusion bodies, and an endoplasmic reticulum. The region of hypodermal chords contains a nucleus in addition to the other organelles and there is a basal lamina surrounding each muscle cell. The use of imidazole-buffered osmium tetroxide solution revealed the presence of lipids in the epicuticle, the membrane that surrounds each muscle cell, the inclusion bodies, and the endoplasmic reticulum. The phosphotungstic-acid technique revealed basic proteins in the epicuticle and dense bodies. The use of periodic acid-thiosemicarbazide-silver proteinate for carbohydrate detection did not show any reaction products in the cuticle. However, glycogen particles were evident in the hypodermis and muscle cells.

KEY WORDS: *Procamallanus* (*S.*) *halitrophus*, TEM, ultrastructure, cytochemistry.

Nematodes are among the most frequent and most important parasites of fishes, constituting a significant part of the parasite fauna of these hosts in freshwater, brackish, and marine environments throughout the world. They attack most body organs of fishes and some species are known as agents of serious fish diseases, causing considerable losses to fish farming operations (Moravec, 2007).

Camallanids are considered a significant problem for fish that are maintained in a closed environment. Larvae of these nematodes are found in zooplankton and can be introduced into pisciculture when live plankton is used as food for fish (Rychlinski and Deardorff, 1982). Species of Camallanidae can parasitize flounders, which are economically important, and depreciate their commercial value. Species of *Procamallanus* Baylis, 1923, subgenus *Spirocamallanus* Olsen, 1952, have been reported in a variety of piscine hosts in different geographical zones and in both freshwater and marine systems (for a review, see Andrade-Salas et al., 1994).

Recently, Ruhela et al. (2008), using experimental infections, demonstrated that species of *Procamallanus* infecting the kidney of *Clarias batrachus* (Philippine catfish) promote pathological changes such as cloudy swelling, variable-sized glomeruli, degenerative changes in proximal convoluted tubules, distal convoluted tubules, and Bowman's capsule—all necrotic changes similar to those previously observed by Chinabut et al. (1991) with *Procamallanus planolatus* in the stomach and intestine of *Clarias macrocephalus*.

Nematodes are surrounded by a cuticle, an extremely flexible and resilient exoskeleton that permits locomotion via attachment to muscles, confers environmental protection (in free-living species) or immune-attack protection (in parasitic species), and allows growth by molting. This cuticle is synthesized in the outermost tissue layer of the organism, the epidermis or hypodermis (Wright, 1987; Page and Johnstone, 2007). The cuticle is a highly structured extra-cellular matrix composed predominantly of cross-linked collagens, additional insoluble proteins termed cuticlins, associated glycoproteins, and lipids (Page and Johnstone, 2007).

This cuticle is a multilayered structure with well-defined epicuticle, cortical, median, and basal regions that vary between species and between stages of the same species (Wright et al., 1987). The epicuticle of many nematodes is covered by a thin layer having a net negative charge, recognizable by electron microscopy

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as a fuzzy coating. This layer has been called the "surface coat" (Wright, 1987).

In animal parasites, the surface coat is believed to play an important role in both adaptation and survival in paratenic hosts because recognition of exposed surface molecules by the host immune system can contribute to the development of an effective immune response (Kennedy, 1991). Although there are some reports on the fine structure of the cuticle (Peixoto et al., 2000; Moraes Neto et al.; 2001; Oliveira-Menezes et al., 2010), few researchers have attempted to correlate morphology with chemical composition. Knowledge of the structure and composition of the cuticle is important for a better understanding of the host–parasite relationship.

Very few studies exist describing the surface of fish nematodes by electron microscopy (Cárdenas et al., 2005; Frantová et al., 2005). The present work represents the first cytochemical characterization of fish nematodes.

MATERIAL AND METHODS

Parasites

Flounders of the species *Syacium papillosum* (Linnaeus, 1758) and *Citharichthys macrops* (Dresel, 1889) were captured by fishermen off the coast of Rio de Janeiro, RJ, Brazil. They were dissected shortly after capture, usually having been freshly killed. Male and female worms were obtained from the intestines, rinsed in 0.7% NaCl solution, and fixed in 2.5% glutaraldehyde in sodium cacodylate buffer with pH 7.2.

Cytochemical labeling

Lipid detection: Fixed nematodes were washed twice in 0.1 M cacodylate buffer and three times in 0.1 M imidazole buffer, pH 7.5, and then fixed in 2% osmium tetraoxide in 0.1 M imidazole buffer (Angermüller and Fahimi, 1982). Nematodes were washed again in the last buffer, dehydrated in an acetone series, and embedded in Epoxi resin. Thin sections, 60 nm thick, were placed on copper grids, counterstained with lead citrate, and observed with a Zeiss 900 transmission electron microscope (TEM) at 80 kV.

Basic protein detection: Fixed nematodes were washed three times in 0.1 M cacodylate buffer, pH 7.2, dehydrated in an ethanol series, and incubated in 2% phosphotungstic acid (PTA) dissolved in ethanol for 24 hr and embedded in Epoxi resin (Gordon and Bensch, 1968). Thin sections, 85 nm thick, were placed on copper grids and observed with a Zeiss 900 TEM with no counterstain. The control samples were incubated in pyridine for 90 min at 37°C after the fixation and washed with 0.1 M cacodylate buffer.

Detection of polysaccharides and glycoproteins: Fixed nematodes were prepared as described for TEM. Thin sections, 85 nm thick, were placed on 300-mesh gold grids and incubated in a 1% periodic acid solution for 30 min at room temperature. The grids were rinsed four times in distilled water (twice under shaking) for 10 min, incubated in 1% thiosemicarbazide solution in 10% acetic acid (40 min to detect glycogen and 24 hr to 72 hr for detection of glycosaminoglycans and glycoproteins, respectively), rinsed in 10%, 5%, and 2% acetic acid series and distilled water, and floated in 1% silver proteinate for 30 min at room temperature in darkness (Thiery, 1967). The grids were rinsed again in distilled water and observed with a Zeiss 900 TEM at 80 kV with no counterstain. The control samples were processed as described above without previous incubation in periodic acid.

RESULTS

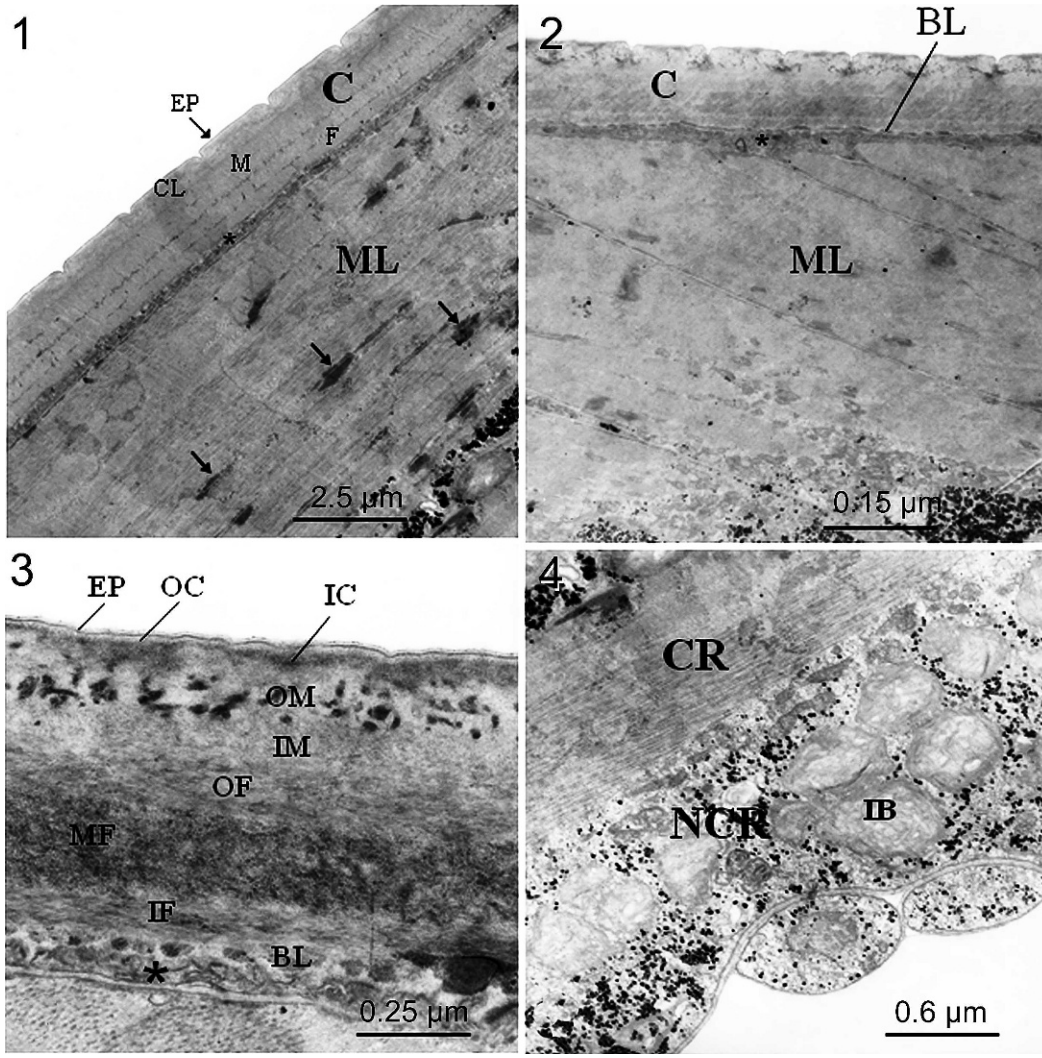
The body wall of *Procamallanus* (*Spirocamallanus*) *halitrophus* is composed of a cuticle, a hypodermis, and a muscular layer (Figs. 1, 2). The cuticle possesses transverse cuticular striations (Figs. 1, 2) and is composed of an epicuticle, cortical, median, fibrous, and basal layers (Fig. 3). The epicuticle is the outer layer and appears as a trilaminar structure that is easily visible in longitudinal and transverse sections (Fig. 3).

Below the epicuticle is a cortical layer that is subdivided into two regions. The median layer, formed by an electron-lucent matrix, can be subdivided into two layers: an outer layer rich in electron-dense fibrils and an inner layer that does not contain fibrils (Fig. 3). Underlying the median layer is the fibrous layer, which consists of three sublayers (Fig. 3).

The basal layer is composed of a homogeneous matrix which presents electron-dense structures that appear as a continuous band or as rounded or elongated segments (Figs. 2, 3).

The hypodermis is a syncytial layer where vesicles, glycogen granules, and inclusion bodies were observed (Figs. 1–3). The musculature is composed of muscle cells that consist of individualized contractile and noncontractile regions. The contractile region consists of myofilaments organized into muscular fibers (Figs. 2, 4). In the noncontractile region, there are cellular organelles such as the nucleus, endoplasmic reticulum, mitochondria, vesicles, glycogen particles, and inclusion bodies (Fig. 4).

The postfixation process using imidazole-buffered osmium tetroxide solution intensifies the affinity of the osmium with unsaturated fatty acids. Ultrathin sections showed a highly contrasted material on the epicuticle (Fig. 5) and in other membranous regions of the body wall such as the membrane that surrounds each muscle fiber (Fig. 6), the inclusion bodies (Figs. 6–8), and the endoplasmic reticulum present in the sarcoplasm (Figs. 7, 8). The various lamellar processes that surround each fiber do not present markings (Fig. 6).



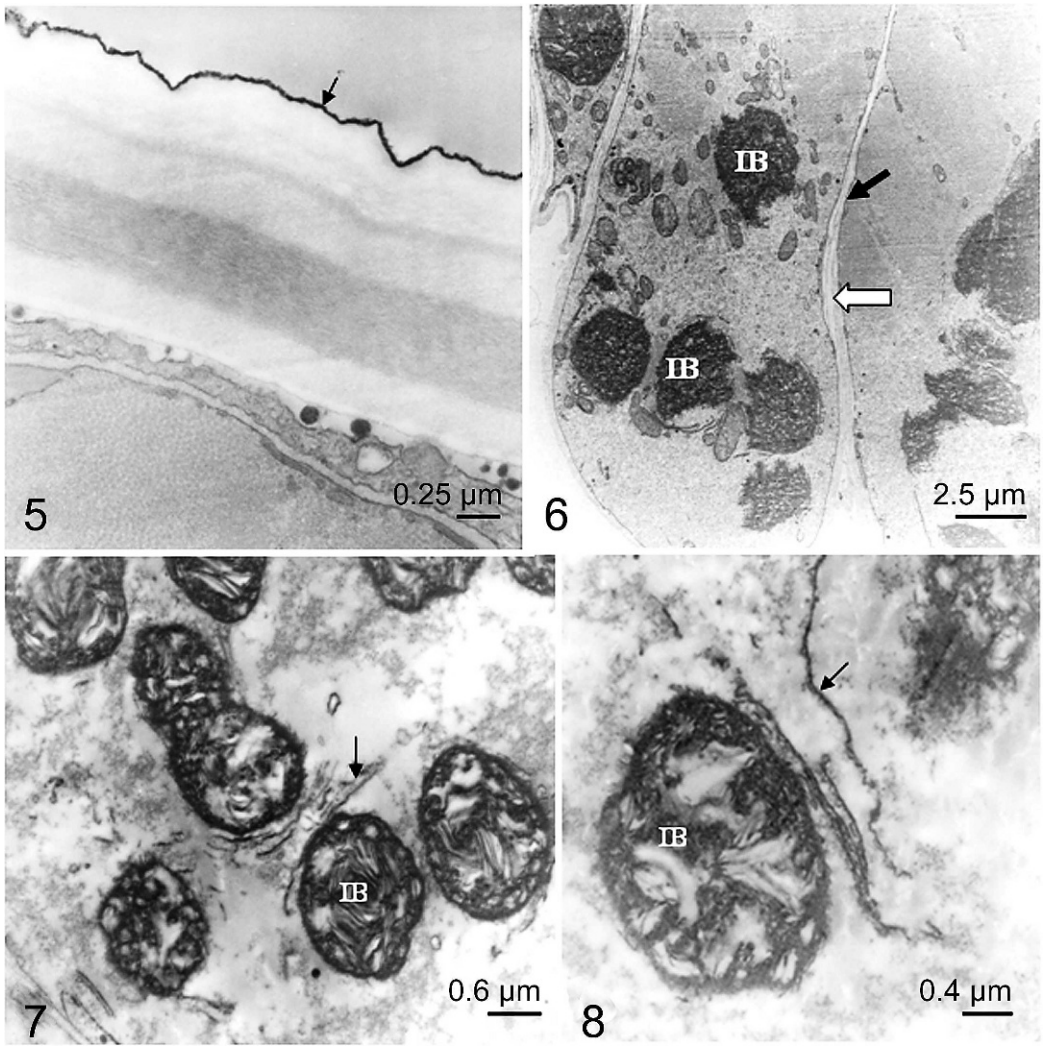
Figures 1–4. Sections of the body wall of *P.* (*S.*) *halitrophus* by transmission electron microscopy (Control). **1.** Epicuticle (EP), cortical layer (CL), medium layer (M), fibrous layer (F). Underlying the cuticle (C) is the hypodermis (asterisk) and the muscular layer (ML) with dense bodies (thin arrow). **2.** Cuticle (C), basal layer (BL), muscular layer (ML). **3.** Trilaminated epicuticle (EP), outer cortical layer (OC), inner cortical layer (IC), outer medium layer (OM), inner median layer (IM), outer fibrous layer (OF), median fibrous layer (MF), inner fibrous layer (IF), basal layer (BL), and hypodermis (asterisk). **4.** Muscular region with contractile region and inclusion bodies (IB) in the noncontractile region (NCR).

The samples incubated with PTA presented a positive reaction in the epicuticle and in the dense bodies present in the contractile region of the muscular fiber, showing basic protein detection on these sites (Figs. 9–11).

The periodic acid–thiosemicarbazide–silver proteinate technique was used for location of carbohydrate residue in thin sections of Epoxi-embedded organisms. The presence of large amounts of glycogen granules was evidenced in the sarcoplasm region and in lower amounts in the hypodermis (Figs. 12, 13). However, there were no reactions to glycosaminoglycans and glycoproteins (Figs. 14, 15) and no reaction of any type was found in the cuticle (Figs. 12–15).

DISCUSSION

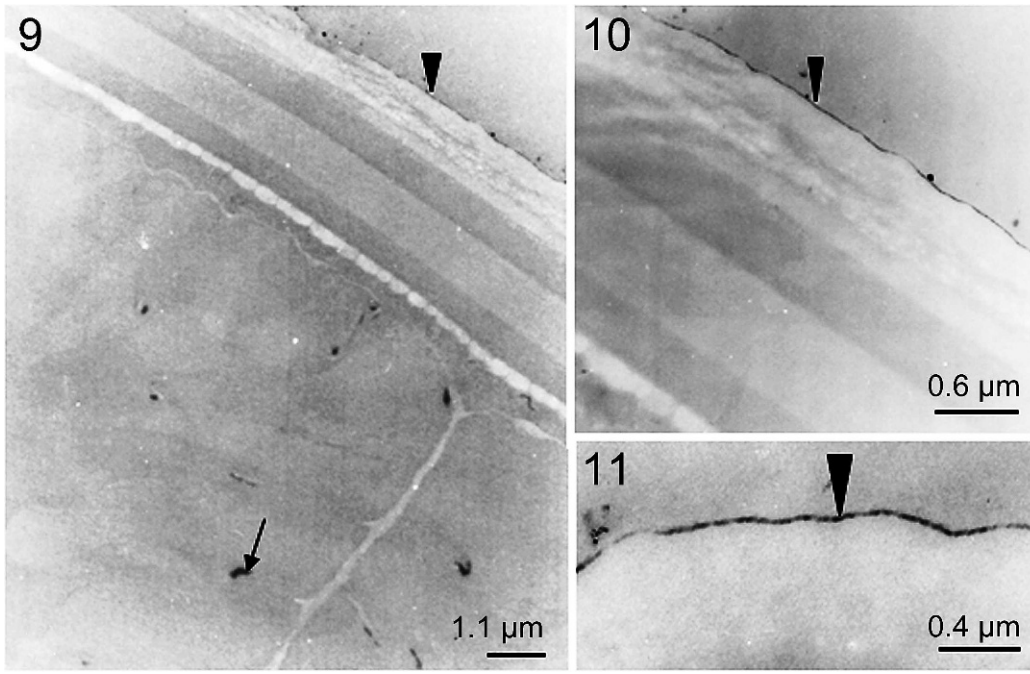
The successful establishment of nematode parasites within their host depends on the host–parasite interactions at their interface. Therefore, the biochemical composition of the cuticle is of great interest.



Figures 5–8. Cytochemical labeling of longitudinal thin sections of *P*. (*S*.) *halitrophus* for detection of lipids. **5.** Transversal sections of cuticle showing strong reaction in the epicuticle (arrow). **6.** Muscular layer showing intense reaction in the inclusion bodies (IB), muscular fiber membrane (black arrow), and basal layer of each fiber (white arrow). **7–8.** Details of reactions in the inclusion bodies (IB) and endoplasmic reticulum (thin arrow) of muscular, noncontractile region.

A notable characteristic of *P*. (*S*.) *halitrophus* is the presence of inclusion bodies in the muscle fibers, hypodermis, hypodermal chords, and intestine of this nematode. These structures have varied sizes and often are near the endoplasmic reticulum and vesicles. Through the osmium–imidazole technique, we detected the presence of lipids (unsaturated fatty acids). However, the periodic acid–thiosemicarbazide–silver proteinases and phosphotungstic-acid techniques were negative for the presence of carbohydrates and basic proteins in this structure, respectively.

The presence of inclusion bodies in nematodes is not common, but it has been observed in some species. In *Brugia malayi*, these bodies are present in lateral chords (Vincent et al., 1975). According to Dick and Wright (1973), the inclusion bodies of *Syphacia obvelata* can be responsible for the disposition of materials in the cuticle of mature females. In turn, Weber (1984) reported that *Wuchereria bancrofti* contains an abundance of inclusion bodies near the cuticle during the formation and development of the third larval stage. However,

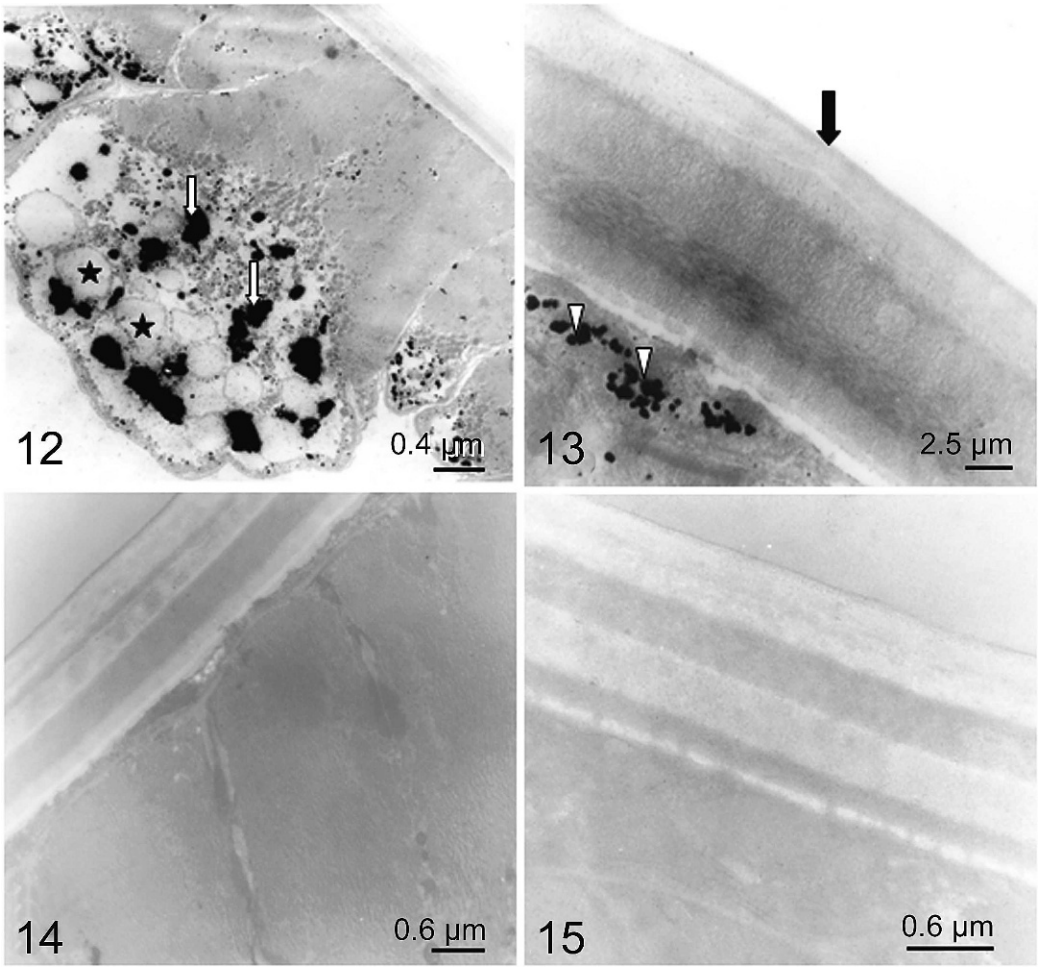


Figures 9–11. Cytochemical labeling of longitudinal thin sections of *P.* (*S.*) *halitrophus* for detection of basic protein. Transverse sections of cuticle showing a positive reaction to basic proteins in the epicuticle (head arrow) and dense bodies (thin arrows).

the morphology of these bodies appears to vary from one species to another. Females of *Meloidogyne javanica* carry cells responsible for producing a gelatinous matrix surrounding the eggs. At their peak production, these cells contain a dense cytoplasm with many mitochondrial profiles, a Golgi complex, and multivesicular lamellar bodies together with a channel that leads to the organism's exterior. Histochemical tests indicate that these structures contain glycosaminoglycans, glycoproteins, or both. In turn, alkaline phosphatase, basic phosphatase, and lipids were found in small quantities (Bird and Rogers, 1965). The inclusion bodies examined during the peak activity of the matrix appear to secrete a substance that is histochemically and morphologically similar to the material contained in this channel. Additionally, this secretion is also similar to the gelatinous matrix that surrounds the eggs (Bird and Rogers, 1965). These inclusion bodies are morphologically similar to those observed in *P.* (*S.*) *halitrophus*. However, the presence of glycosaminoglycans, glycoproteins, or both in *M. javanica* differs from the findings in the present work.

The intestinal cells of *Metastrongylus* spp. and *Ascaris suum* contain structures called lamellar bodies (Sheffield, 1964; Jenkins and Erasmus, 1969) which, although smaller in size, resemble the inclusion bodies of *P.* (*S.*) *halitrophus*. Jenkins and Erasmus (1969) suggested that these bodies play a role similar to that of lysosomes. These inclusion bodies have also been found in the intestinal cells of *P.* (*S.*) *halitrophus* located near the vesicles and endoplasmic reticulum. Although many authors have suggested different functions for these structures, we believe there is still not enough evidence to determine any type of function for the inclusion bodies observed in the present study.

Phosphotungstic acid reveals proteins rich in histidines (De Souza, 1998). Basic proteins were described by Anya (1966) in a histochemical analysis of the cuticle of Oxyuridae and *Ascaris lumbricoides* using bromophenol blue, who noted markings in all the cuticular strata of these nematodes. In the samples of *P.* (*S.*) *halitrophus* treated with PTA, we detected the presence of basic proteins in the epicuticle and the dense bodies present in the muscle layer. Peixoto and De Souza (1992) also found a positive reaction in these regions in *Caenorhabditis elegans*, but there were markings in the external cortical sub-layer and in the struts present in the intermediate layer. Araújo et al. (1995) observed markings in the blocks present in the middle layer and external cortical sublayer of



Figures 12–15. Cytochemical labeling of longitudinal thin sections of *P.* (*S.*) *halitrophus* for detection of polysaccharides and glycoprotein. **12.** Transversal sections of cuticle presented a negative reaction after 72 hr of the periodic acid–thiosemicarbazide–silver proteinate incubation (large arrow) and a positive and intense reaction in the hypodermis, indicating the presence of glycogen (head arrow). **13.** Positive reaction in muscular, noncontractile region indicating the presence of glycogen after 40 min of the periodic acid–thiosemicarbazide–silver proteinate incubation (white arrow) and a negative reaction in the inclusion bodies (star). **14.** Control showing a negative reaction in the body wall when periodic acid was suppressed. **15.** Control showing a negative reaction in the body wall when silver proteinate was suppressed.

W. bancrofti. Despite the fact that these compounds are involved with a series of cellular functions, such as cell differentiation and gene function regulation in eukaryotic cells (Cavalcanti et al., 2009), we can conclude that their distribution varies and can be present in different regions of the nematode body wall depending on the species.

Nematode cuticle is composed of different classes of lipids. The cuticle is present in the external surface of nematodes and is formed by a protective lipid coat that is synthesized by specific cells or groups of cells in the outermost layers of the nematode (Mangold, 1984). Cuticular lipids constitute the initial passive barrier to desiccation as well as to bacterial and fungal infection; they may be involved in chemical communication and may reduce the penetration of chemicals and toxins. Cuticles are also important in nutrient distribution and, in parasitic nematode roundworms, play a major role in protecting them from host immune systems (Blaxter, 1993; Mika et al. 2010).

The specimens of *P.* (*S.*) *halitrophus* submitted to the osmium–imidazole technique indicated the presence of

unsaturated fatty acids in the epicuticle, the membrane surrounding the muscle fibers, the membrane between the cuticle and hypoderm, the vesicles, and the endoplasmic reticulum present in the sarcoplasm. The inclusion bodies also had markings, indicating they have a membranous composition. The various lamellar processes that surround each fiber do not show any markings, demonstrating the probable absence of unsaturated fatty acids in this region. Therefore, it can be surmised that the composition of the membrane that surrounds each fiber is different from the composition of those lamellar processes. Peixoto et al. (1994) reported that adult specimens of *C. elegans* and *Strongyloides venezuelensis*, on being analyzed by the osmium–imidazole technique, did not present any reaction in the cuticle, while the dauer larval stage of *C. elegans* and the third larval stage of *S. venezuelensis* presented intense markings in the cortical layer and surface of the cuticle. These findings suggest there is variation in the lipid distribution among the different larval stages and different nematode species, as observed by Proudfoot (1990).

The periodic acid–thiosemicarbazide–silver proteinate technique (Thiéry, 1967) has been utilized by several authors to verify the presence and distribution of glycosaminoglycans, glycoproteins, and glycogen in different nematode species. The presence of glycosaminoglycans and glycoproteins was not verified in the cuticle of *P.* (*S.*) *halitrophus*, even after 72 hr of incubation in thiosemicarbazide. The absence of markings was also observed in the cuticle of *Trichinella spiralis* (Wright and Hong, 1988), *C. elegans* (Peixoto and De Souza, 1992), in microfilariae of *W. bancrofti* (De Souza et al., 1989), and in various adults of *W. bancrofti* (Araújo et al., 1995). Nevertheless, the surface coat has been described in various nematodes (Cherian et al., 1980; Hulinska and Shaihenov, 1982; Wright and Hong, 1988; Page et al., 1992; Lee et al., 1993; Martinez and De Souza, 1995, 1997). According to Blaxter et al. (1992), the absence of a surface layer in a particular stage or species of nematode does not necessarily mean this layer does not exist, as it may have been lost during processing of the material.

The presence of glycogen granules in the sarcoplasm and hypodermis, visible even without the use of cytochemical methods, was confirmed after incubation of the material for 40 min in thiosemicarbazide. These glycogen granules were also verified in the muscle cells of *W. bancrofti* microfilariae and adults (De Souza et al., 1989; Araújo et al., 1995) in the hypodermis, muscle, and intestinal cells of *T. spiralis* (Wright and Hong, 1988) and in muscle and intestinal cells of *C. elegans* (Peixoto and De Souza, 1992).

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TRIBUTE

On March 15th, 2009 the parasitology world lost Dra. Reinalda Marisa Lanfredi, an important Brazilian helminthologist who dedicated about 28 years to morphological and ultrastructural analysis of helminths. All of your students and collaborators have a little of you in their hearts, minds, and research. Your contribution to science is immortal. Thanks from the students, teachers, and friends of Laboratorio de Biologia de Helmintos Otto Wucherer, IBCCF-UFRJ, Brazil.

LITERATURE CITED

Andrade-Salas, O., R. F. Pineda-López, and L. García-Magaña. 1994. *Spirocamallanus rebecae* sp. n. (Nematoda: Camallanidae) from freshwater fishes in south-eastern Mexico. Folia Parasitologica 41:259–270.

Angermüler, S. and J. D. Fahime. 1982. Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy. Histochemical Journal 14:823–825.

Anya, A. O. 1966. The structure and chemical composition of the nematode cuticle. Observations on some oxyurids and *Ascaris*. Parasitology 56:179–198.

Araújo, A., T. Souto-Padrón, and W. De Souza. 1995. Ultrastructural and cytochemical aspects of the cuticle of adult *Wuchereria bancrofti*. International Journal for Parasitology 25:569–577.

Bird, A. F., and G. E. Rogers. 1965. Ultrastructural and histochemical studies of the cells producing the gelatinous matrix in *Meloidogyne*. Nematologica 11: 231–238.

Blaxter, M. L. 1993. Cuticle surface proteins of wild type and mutant *Caenorhabditis elegans*. Journal of Biological Chemistry 268:6600–6609.

Blaxter, M. P., A. P. Page, W. Rudin, and R. M. Maizels. 1992. Nematode surface coats: actively evading immunity. Parasitology Today 8:243–247.

Cárdenas, M. Q., W. De Souza, and R. M. Lanfredi. 2005. Ultrastructure of *Procamallanus* (*Spirocamallanus*) *halitrophus* (Nematoda: Camallanidae) parasite of flounder. Parasitology Research 97:478–485.

Cavalcanti, M. G., H. R. Araújo, M. H. Paiva, G. M. Silva, C. C. Barbosa, L. F. Silva, F. A. Brayner, and L. C. Alves. 2009. Ultrastructural and cytochemical aspects of *Schistosoma mansoni* cercaria. Micron 40: 394–400.

Chinabut, S., P. Chanratchakool, and M. Primpol. 1991. Histopathological studies of infected walking catfish, *Clarias macrocephalus* Gunther. Pages 330–340 *in* Proceedings of the Seminar on Fisheries (September 16–18, 1991). Department of Fisheries, Bangkok. 538 pp.

Cherian, P. V., B. E. Stromberg, D. J. Weiner, and E. J. L. Soulsby. 1980. Fine structure and cytochemical evidence for the presence of polysaccharide surface coat of *Dirofilaria immitis* microfilariae. International Journal for Parasitology 10:227–233.

De Souza, W. 1998. Técnicas básicas de microscopia eletrônica aplicada as ciências biológicas. Sociedade Brasileira de Microscopia, p. 179.

De Souza, W., T. Souto-Padrón, G. Dreyer, and L. Dias de Andrade. 1989. Fine structure and localization of anionic sites on the surface of microfilariae *Wuchereria bancrofti*. Journal of Submicroscopic Cytology and Pathology 21:121–129.

Dick, T. A., and K. A. Wright. 1973. The ultrastructure of the cuticle of the nematode *Syphacia obvelata* (Rudolphi, 1802). I. The body cuticle of larvae, males, and females, and observations on its development. Canadian Journal of Zoology 51:187–196.

Frantová, D., M. Brunanská, H. P. Fagerholm, and M. Kihlström. 2005. Ultrastructure of the body wall of female *Philometra obturans* (Nematoda: Dracunculoidea). Parasitology Research 95:327–232.

Fusco, A. C., and R. M. Overstreet. 1978. *Spirocamallanus cricotus* sp. n. and *S. halitrophus* sp. n. (Nematoda: Camallanidae) from fishes in the northern Gulf of Mexico. Journal of Parasitology 64:239–244.

Gordon, M., and K. G. Bensch. 1968. Cytochemical differentiation of guinea pig sperm flagellum with phosphotungtic acid. Journal of Ultrastructural Research 24:35–50.

Hulinska, D., and B. Shaihenov. 1982. Comparative electron microscopic and cytochemical studies on the cuticle and the hypodermis of *Trichinella nativa* (Britov et Boev, 1972) and *T. pseudospiralis* (Garkavi, 1972). Folia Parasitologica 29:159–165.

Jenkins, T., and D. A. Erasmus. 1969. The ultrastructure of the intestinal epithelium of *Metastrongylus* sp. (Nematoda: Strongyloidea). Parasitology 59:335–342.

Kennedy, M. W. 1991. Biophysical properties of the nematode surface. Pages 1–26 *in* M. W. Kennedy, ed. Parasitic nematodes—Antigens, membranes and genes. Wellcome Laboratory for Experimental Parasitology, University of Glasgow, Taylor and Francis, London, U.K. 241 pp.

Lee, D. L., K. A. Wright, and R. R. Shivers. 1993. A freeze-fracture study of the cuticle of adult *Nippostrongylus brasiliensis* (Nematoda). Parasitology 107: 545–552.

Mangold, H. K. (ed.). 1984. Handbook of chromatography. Vol. 1. CRC Press, Boca Raton, Florida. 381 pp.

Martinez, A. M. B., and W. De Souza. 1995. A quick-frozen, freeze-fracture and deep-etch study of cuticle and hypodermis of adults forms of *Strongyloides venezuelensis* (Nematoda). Parasitology 111:523–529.

Martinez, A. M. B., and W. De Souza. 1997. A freeze-fracture and deep-etch study of cuticle and hypodermis of infective larvae of *Strongyloides venezuelensis* (Nematoda). International Journal for Parasitology 27: 289–297.

Mika A., M. Gołebiowski, J. Szafranek, J. Rokicki, and P. Stepnowski. 2010. Identification of lipids in the cuticle of the parasitic nematode *Anisakis simplex* and the somatic tissues of the Atlantic cod *Gadus morhua*. Experimental Parasitology 124:334–340.

Moraes-Neto, A. H. A., R. M. Lanfredi, and W. De Souza. 2001. Fine structure, freeze-fracture and deep-etch views of the sheath and cuticle of microfilariae of *Litomosoides chagasfilhoi* (Nematoda: Flarioidea). Parasitology Research 87:1035–1047.

Moravec, F. 2007. Nematode parasites of fishes: recent advances and problems of their research. Parassitologia 49:155–160.

Oliveira-Menezes A., J. Norões, G. Dreyer, and R. M. Lanfredi. 2010. Ultrastructural analysis of *Wuchereria bancrofti* (Nematoda: Filarioidea) body wall. Micron 41:526–531.

Page, A. P., A. J. Hamilton, and R. M. Maizels. 1992. *Toxocara canis*: monoclonal antibodies to carbohydrate epitopes of secreted (TES) antigens localize to different secretion-related structures in infective larvae. Experimental Parasitology 75:56–71.

Page, A. P., and I. L. Johnstone. 2007. The cuticle. Pages 1–15 *in* A. P. Page and I. L. Johnstone, eds. WormBook. University of Glasgow, Glasgow, Scotland, U.K.

Peixoto, C. A., L. C. Alves, J. V. De Melo, and W. De Souza. 2000. Ultrastructural analyses of the *Caenorhabditis elegans* sqt-1(sc13) left roller mutant. Journal of Parasitology 86:269–274.

Peixoto, C. A., and W. De Souza. 1992. Cytochemical characterization of the cuticle of *Caenorhabditis elegans* (Nematoda: Rhabdtoidea). Journal of Submicroscopic Cytology and Pathology 24:425–435.

Peixoto, C. A., A. M. B. Martinez, M. Farina, and W. De Souza. 1994. *Caenorhabditis elegans* and *Strongyloides venezuelensis*: ultrastructural visualization of lipids in the cuticles of adults and larvae forms (Nematoda: Rhabditoidea). Acta Microscopica 3:107–115.

Proudfoot, L., J. R. Kusel, H. V. Smith, W. Harnett, M. J. Worms, and M. W. Kennedy. 1990. The surface lipid of parasitic nematodes: organization and modifications during transition to mammalian host environment. Acta Tropica 47:323–330.

Ruhela, S., A. K. Pandey, and A. K. Khare. 2008. Histopathological manifestations in kidney of *Clarias batrachus* induced by experimental *Procamallanus* infection. Journal of Environmental Biology 29:739–742.

Rychlinski, R. A., and T. L. Deardorff. 1982. Disease prevention and control. *Spirocamallanus*: a potential fish health problem. Freshwater and Marine Aquarium 5:22–23.

Sheffield, H. G. 1964. Electron microscope studies on the intestinal epithelium of *Ascaris suum*. Journal of Parasitology 50:365–379.

Thiéry, J. P. 1967. Mise en evidence des polysaccharides sur coupes fines en microscopie életronique. Journal of Microscopy 6:987–1018.

Vincent, A. L., L. R. Ash, and S. P. Frommes. 1975. The ultrastructure of adult *Brugia malayi* (Brug, 1927) (Nematoda: Filarioidea). Journal of Parasitology 61:499–512.

Weber, P. 1984. Electron microscope study on the developmental stages of *Wuchereria bancrofti* in the intermediate host: structure of the body wall. Zeitschrift für Parasitenkunde 35:221–230.

Wright, K. A. 1987. The nematode's cuticle—its surface and the epidermis: function, homology, analogy—a current consensus. Journal of Parasitology 73:1077–1083.

Wright, K. A., and H. Hong. 1988. Characterization of the accessory layer of the cuticle of muscle larvae of *Trichinella spiralis*. Journal of Parasitology 74:440–451.