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Ultrastructure of the integument of parasitic females in entomogenous tylenchids. II. *Howardula phyllotretae, Parasitylenchus dispar, Contortylenchus* sp., and two allantonematid species

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Summary. The structure of the body walls of parasitic females in five species of entomogenous nematodes was studied using transmission and scanning electron microscopy. The body surface of parasitic females in *H. phyllotretae* is covered by short microvilli. Numerous ampullae, invaginations of the outer hypodermal membrane, are situated beneath the surface of parasitic females of all studied species except those of *Contortylenchus* sp., which were characterized by a thick covering layer of extracellular material. It is suggested that the network of ampullae and vacuoles are involved in the assimilation and subsequent transmission of nutrients from the nematode surface to underlying tissues.
Key words: hypodermis, entomogenous nematodes, *Howardula phyllotretae, Parasitylenchus dispar, Contortylenchus* sp., Allantonematidae, microvilli, ultrastructure.

Approximately two hundred species of tylenchid nematodes have been reported as parasites of insects. Entomogenous tylenchid parasitic female nematodes assimilate nutrients from the host haemocoel by direct transport through their body surface. Significant structural changes of the cuticle and hypodermis occur when infective females develop the parasitic mode of their life-cycle. However, the mechanisms involved in these processes have not been fully investigated, partly as a result of insufficient information being available of the body wall ultrastructure. This paper is one of a series (Subbotin *et al.*, 1993, 1994) providing results from investigations of the cuticles of entomogenous Tylenchida and the parasitic female integument ultrastructure in five species of tylenchid nematodes are reported here.

MATERIALS AND METHODS

Gamogenetic parasitic females were extracted from insect-hosts in the Moscow and Jaroslavl regions during the summer and autumn of 1992-1995. The nematodes collected were: *Parasitylenchus dispar* (Fuchs, 1915) Micoletzky, 1922 and *Contortylenchus* sp. from *Ips typographus* (L.); *Howardula phyllotretae* Oldham, 1933 from flea beetles *Phyllotreta* spp.; and an unidentified allantonematid species (sp. 1) from *Stenus* sp.; and a second unidentified allantonematid species (sp. 2) from *Blastophagus minor* Hart.

Female nematodes were fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH=7.2) at 22° C for 1 h, washed in buffer, post-fixed in 1% osmium tetroxide for 2 h, dehydrated in an ethanol series, and embedded in Epon resin. Ultrathin sections, cut with an LKB ultramicrotome IV, were stained with uranyl acetate and lead citrate and examined in a Jeol JEM-100 B at 80 kV. Other specimens of *H. phyllotretae* were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated, processed through critical point drying, coated with gold and examined with a Hitachi S 450 A scanning electron microscope at 15 kV.

RESULTS

Parasitic females of *H. phyllotretae* have cylindrical, elongated, 1.42-2.08 mm long bodies. The cuticle is absent and instead the nematodes are covered only with a well developed hypodermis (Fig. 1A). A thin dark extracellular layer with short microvilli on its surface is present on the external surface of the hypodermis. Saccular ampullae, ap-

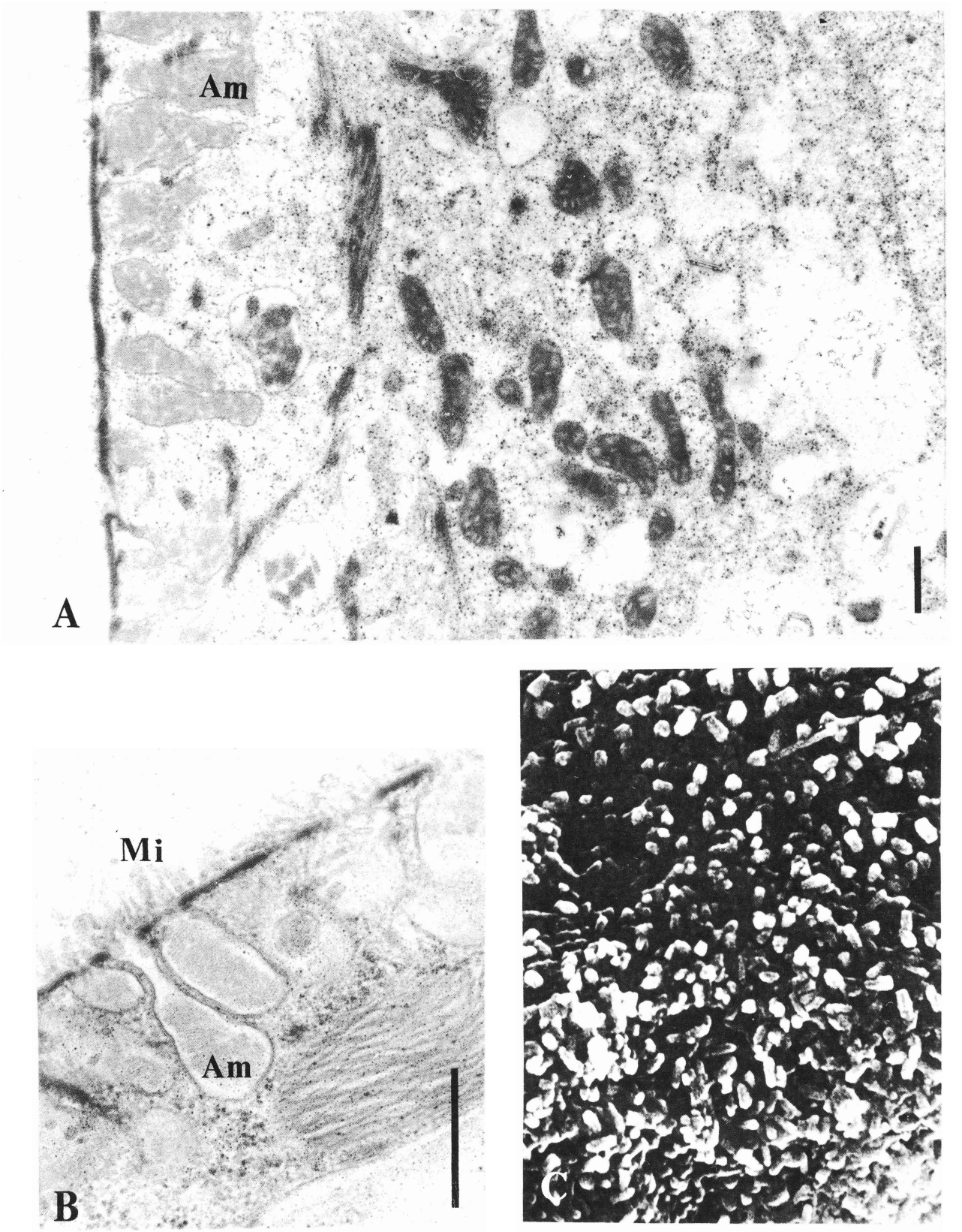

Fig. 1. *Howardula phyllotretae.* The integument of the parasitic female. A: Total view; B: The hypodermis covered by short microvilli and ampullae; C: Microvilli on the hypodermis. Mi - microvilli, Am - ampulla. Scale bars: A, B - 0.5 μm.

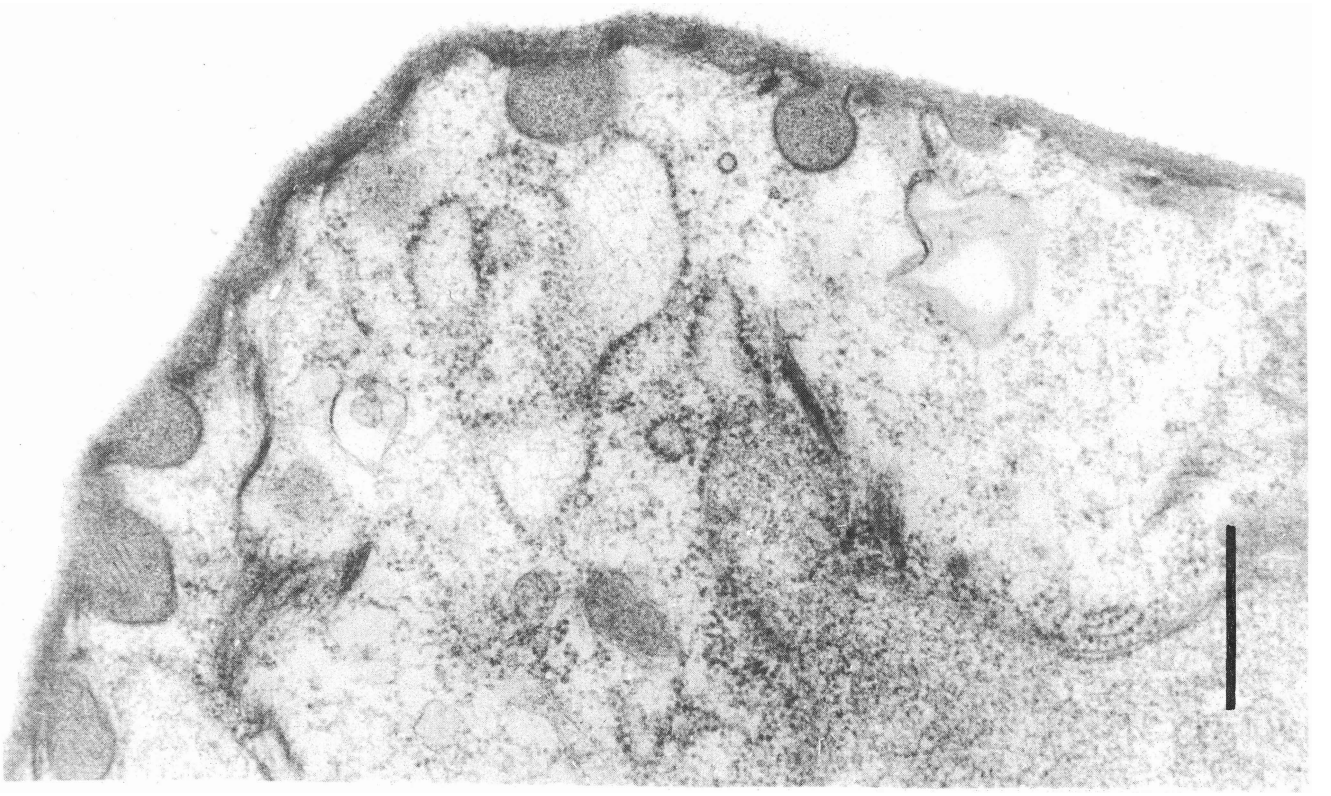

Fig. 2. *Parasitylenchus dispar.* The hypodermis with ampullae. Scale bar - 1 μm.

pearing as large invaginations of hypodermal membrane beneath the dense surface layer, open onto the surface through well defined pores which have numerous microvilli on their margin. The microvilli extend up to 0.3 μm in length. An amorphous deposit is discernible inside the ampullae (Fig. 1B). The hypodermis under the ampullae is strongly vacuolized and filled with mitochondria, fibrils and nuclei. Scanning electron microscopy revealed irregular distribution of dense clusters of microvilli along the nematode hypodermis (Fig. 1C).

First generation gamogenetic parasitic females of *P. dispar* are cylindrical, 0.6-3.2 mm long and 0.05-0.2 mm wide. The integument consists of a thin, dense extracellular layer and ampullae containing a dense material (Fig. 2). The hypodermal cytoplasm contains numerous membranes of rough endoplasmic reticulum, vacuoles and nuclei.

Parasitic females of unidentified allantonematid species 1, recovered from *Stenus* sp., have a sausage-shaped body, 1.3-3.7 mm long and 0.1-0.3 mm wide. Numerous ampullae are present which open on the hypodermis surface and are connected by a network-like structure (Fig. 3A & B). The hypodermal cytoplasm contains mitochondria, fibrils, lipid globules, numerous vacuoles and large nuclei. Occasionally, fibrils subdivide the hypodermal cytoplasm into several layers. Small vesicles are present in the hypodermal ampullae of this species.

Parasitic females of unidentified allantonematid species 2, recovered from *Blastophagus minor*, also have sausage-shaped bodies, 0.8-2.4 mm long and 0.1-0.5 mm wide. The structure of the integument is similar to that of allantonematid species 1 and numerous ampullae with dark contents were observed on the hypoderm surface (Fig. 4A & B). Ampullae are connected to large, more transparent, vacuoles which are situated outside the ampullae and these were observed throughout the hypoderm (Fig. 4B). The body surface in some specimens was covered with a dark layer of extracellular material. The hypodermal cytoplasm also contains rough endoplasmatic reticulum, mitochondria and nuclei.

Gamogenetic parasitic females of the *Contortylenchus* sp. examined have cylindrical bodies, 1.3-2.9 mm long and 0.06-0.18 mm wide. Ampullae and microvilli were not present on the body surface which is covered with a layer of extracellular material, 0.5-1.1 μm thick (Fig. 5). Numerous vacuoles, fibrils, mitochondria and nuclei are present in the well

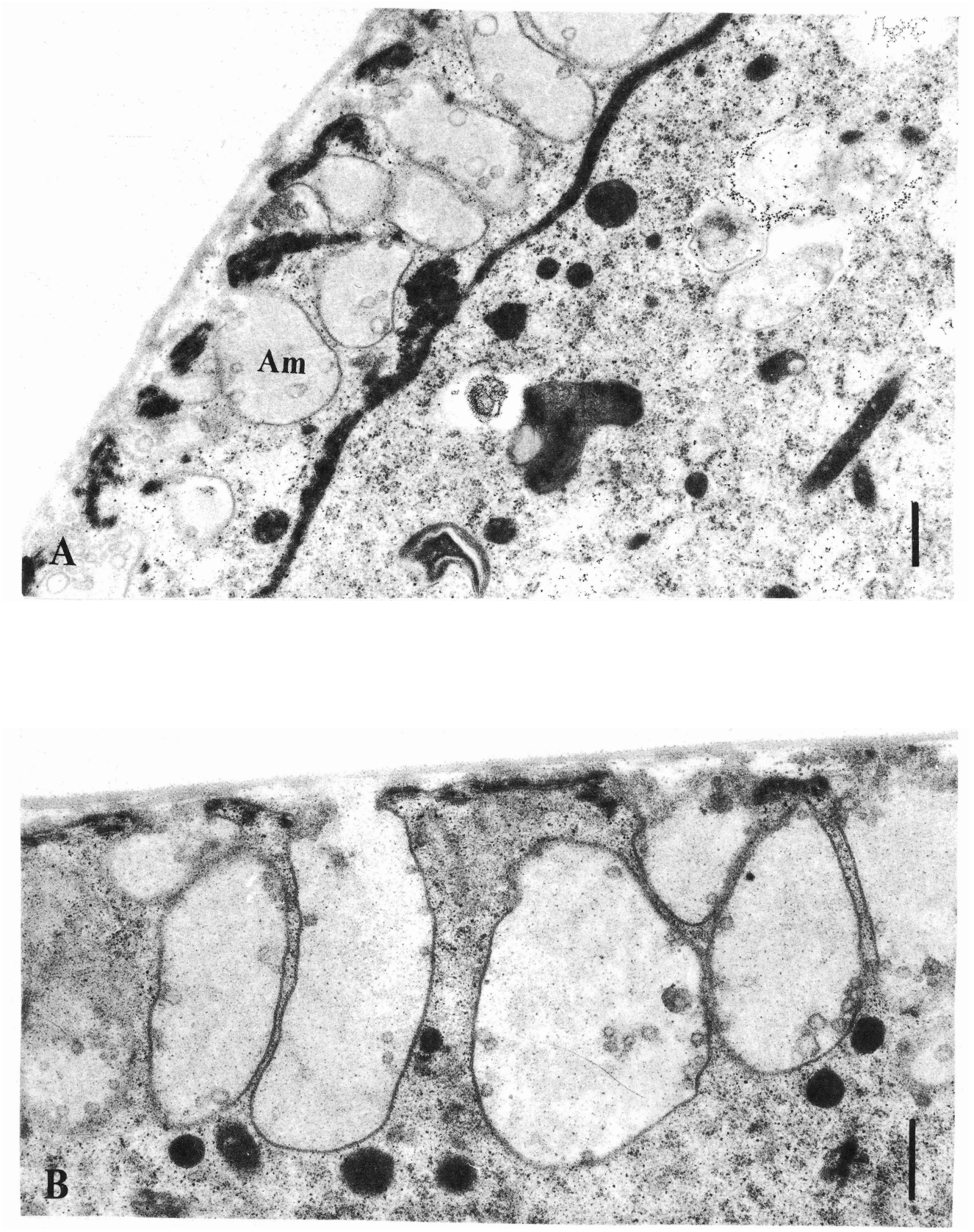

Fig. 3. Unidentified allantonematid species 1 from *Stenus* sp. A: Total view of the integument in the parasitic female; B: Ampullae in the hypodermis. Scale bar: A, B - 0.5 μm.

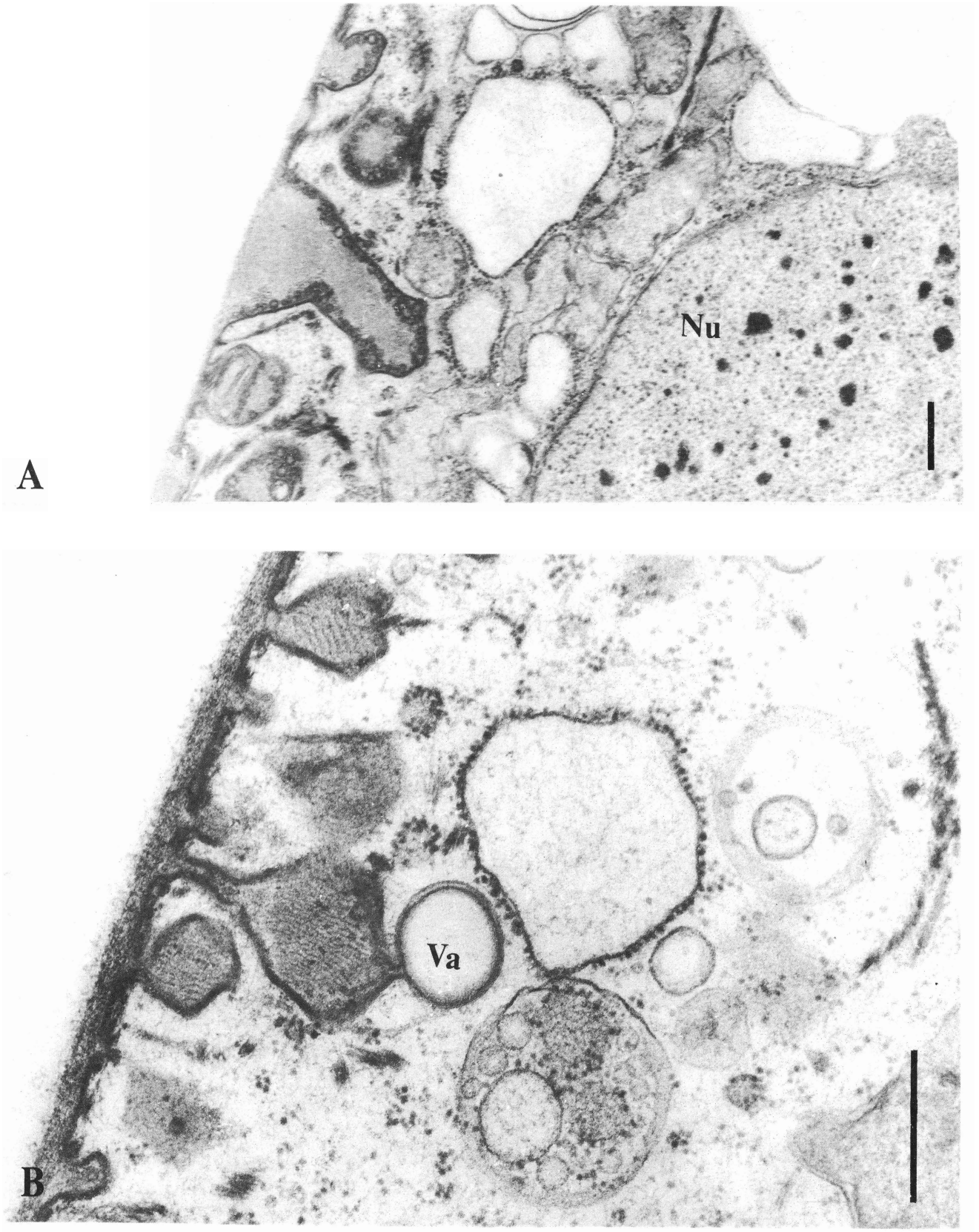

Fig. 4. Unidentified allantonematid species 2 from *Blastophagus minor*. A: Numerous ampullae with dark contents in the hypodermis; B: The network of ampullae and vacuoles. Nu - nucleus, Va - vacuole. Scale bars: A, B - 0.5 μm.

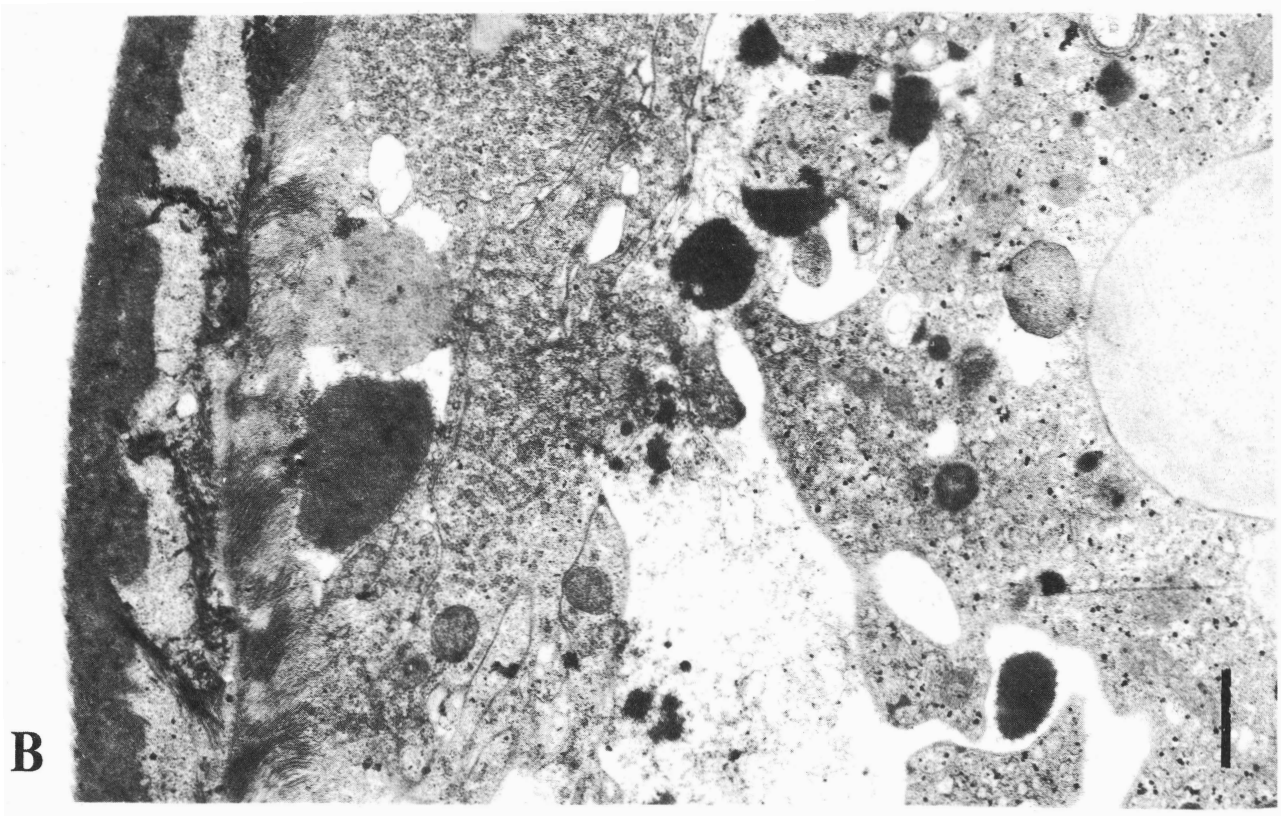

Fig. 5. *Contortylenchus* sp. The integument of the parasitic female. Fb - fibriles. Scale bars: A, B - 1 μm.

developed hypodermis.

DISCUSSION

Short microvilli present on the hypodermis surface of *H. phyllotretae* parasitic females occur in several other species of entomogenous tylenchid nematodes. Formation of these structures in parasitic females probably represents adaptations to increase the body surface area resulting in more efficient nutrient assimilation from the insect's haemocoel. Although microvilli and corresponding hypodermal structures have been previously described for several species of entomogenous parasitic tylenchids (Riding, 1970; Subbotin *et al.*, 1993, 1994, Chizhov *et al*, 1995), it is only in *H. phyllotretae* that a combination of microvilli with hypodermal ampullae has been observed.

Ampullae formed from invagination of the hypodermal membrane also serve to substantially increase the surface area of the hypodermis. These ampullae can contain dark amorphous or structural deposits or small vesicles. It is possible that numerous ampullae are associated with younger stages of parasitic females and that their number decreases with the development of the nematode. The association of intracellular vacuoles with ampullae provides further evidence of the likely involvement of this system in the assimilation and subsequent transmission of nutrients from the nematode surface.

A similar system is present in the uterus of the entomogenous tylenchid, *Sphaerularia bombi*. The entire surface of the uterus of this nematode is normally exposed to the host's hemolymph and was modified into a network of saccular indentations comprised of fine cytoplasmic extensions. The presence of pinocytotic vacuoles in the terminal portions of the cytoplasmic extensions and intracellular vacuoles at the base of the saccular indentations strongly suggested that the assimilation of nutrients by *S. bombi* is achieved through pinocytosis (Poinar & Hess, 1972). Numerous finger-like structures penetrating into the integument has also been observed in several Acanthocephala (Dunagan & Miller, 1991).

Except for the unidentified allantonematid species 1, an extracellular dense layer was present on the trophohypodermal surface of each of the species examined here. This surface layer was most developed in *Controtylenchus* sp. parasitic females, but this observation may result from the use of rather old female nematodes in the study. Older parasitic females are likely to have developed a thicker extracellular dense layer. It seems probable that this thick layer covering the hypodermal surface is secreted from the hypodermal cells and provides a protective layer. The secretion may provide a protective layer but further investigations are require to elucidate the nature and the importance of the layer for entomogenous tylenchids.

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Субботин С. А., Чижов В. Н. Ультраструктура интегумента паразитических самок энтомопатогенных тиленхид. II. *Howardula phyllotretae, Parasitylenchus dispar, Contortylenchus* sp. и два вида аллантонематид.

Резюме. Изучена ультраструктура стенок тела паразитических самок пяти видов энтомопатогенных нематод с помощью сканирующего и трансмиссионного микроскопов. Поверхность тела паразитических самок *Howardula phyllotretae* была покрыта короткими микровиллями. Многочисленные ампулы, являющиеся инвагинациями внешней гиподермальной мембраны, обнаружены у всех паразитических самок, за исключением *Contortylenchus* sp., поверхность которых была покрыта толстым экзоцеллюлярным слоем. Предполагается, что система ампул и вакуолей вовлечена в поглощение и последующий перенос питательных веществ от поверхности тела паразитической самки к ниже лежащим тканям.