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Ultrastructure of the body wall of parasitic and infective females of *Skarbilovinema laumondi* (Tylenchida : Iotonchiidae)

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Summary — The body wall of mature parasitic and preadult infective females of *Skarbilovinema laumondi* parasitizing flower flies were examined by scanning and transmission electron microscopy. The cuticle of parasitic females was completely reduced. Numerous interwoven microvilli as processes of hypodermal cytoplasm covered the whole body and formed a " spongy layer ". The hypodermis contained lipid globules and nuclei with large nucleoli. The infective vermiform preadult females of *S. laumondi* had a cuticle consisting of three distinct layers and resembled that of plant parasitic nematodes. The " spongy layer " of the hypodermis of parisitic females is considered to act as a special adaptation to increase the nematode surface for a more effective uptake of nutrients from the haemocoel of the host insects. The significance of the body wall structure for systematics and phylogeny of entomoparasitic tylenchid nematodes is discussed.

Résumé — ***Ultrastructure de la paroi des femelles de* Skarbilovinema laumondi *(Tylenchida : Iotonchiidae)*** — La paroi des femelles préadultes infestantes de *Skarbilovinema laumondi*, parasite des mouches *Syrphida*, a été étudiée en microscopie optique et électronique (TEM et MEB). La cuticule de ces femelles est réduite. De nombreux microvilli ramifiés — prolongements du cytoplasme de l'hypoderme — couvrent tout le corps et forment une couche spongieuse. L'hypoderme est composé de mitochondries, des membranes du reticulum endoplasmique, de gouttes lipidiques et de noyaux avec nucléotides. Les femelles infestantes de *S. laumondi* ont une cuticule comportant trois couches, ce qui est caractéristique des nématodes phytoparasites. La couche spongieuse de l'hypoderme des femelles parasites est considérée comme une adaptation particulière augmentant la surface du corps du nématode et contribuant ainsi à une absorption beaucoup plus efficace des substances nutritives provenant de l'hémocoele de l'insecte. Il est discuté du rôle de la structure de la paroi du corps dans la systématique et la phylogénie des nématodes entomoparasites.

Key-words : Nematodes, insect parasites, *Skarbilovinema*.

In 1989 Chizhov and Zakharenkova found an unknown species of an entomoparasitic nematode parasitizing the flower flies of *Helophilus* spp. and *Eristalis* spp. (Syrphidae : Diptera). This nematode was described by Chizhov and Zakharenkova (1991) as a new species of a new genus, *Skarbilovinema* (Iotonchiidae).

Skarbilovinema laumondi Chizhov & Zakharenkova, 1991 is widespread in the European part of the USSR within the bounds of the host's natural habitat. The nematode has a single annual gametogenetic generation. The infective preadult females penetrate the host's larva and later develop into mature parasitic females in the haemocoel of the adult insect, where they produce juveniles that grow, moult and copulate. The infective preadult females leave the host via the rectum or oviduct of the fly (Chizhov & Zakharenkova, 1991).

Little information exists on the body wall structures of entomoparasitic nematodes of Tylenchida. Such information would be useful for a more complete understanding of homologies among families and genera.

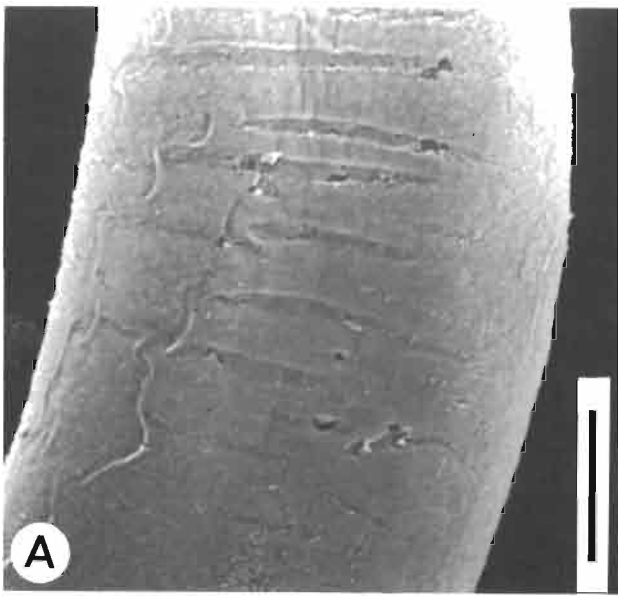
The present study describes the structure of the body wall of parasitic and infective females of *S. laumondi*, using scanning and transmission electron microscopy.

Materials and methods

The parasitic and infective females were dissected from the flower flies of *Helophilus* spp. collected in the Moscow region. For transmission electron microscopy the nematodes were fixed in 2.5 % glutaraldehyde in 0.05 M phosphate buffer (pH = 7.2) at 22 °C for 1 h. Specimens were then washed in buffer, postfixed in 1 % osmium tetroxide for 2 h, dehydrated in an ethanol series and embedded in Epon resin. Ultrathin sections were cut with an LKB ultramicrotome IV, stained with uranyl acetate and lead citrate and then examined in a Tesla BS-500 transmission electron microscope operated at 60 kV.

Parasitic females were also processed for examination of the body surface by scanning electron microscopy. Nematodes were fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated, processed through criti-

cal point drying, coated with gold and examined with a Hitachi S 450 A scanning electron microscope at 15 kV.



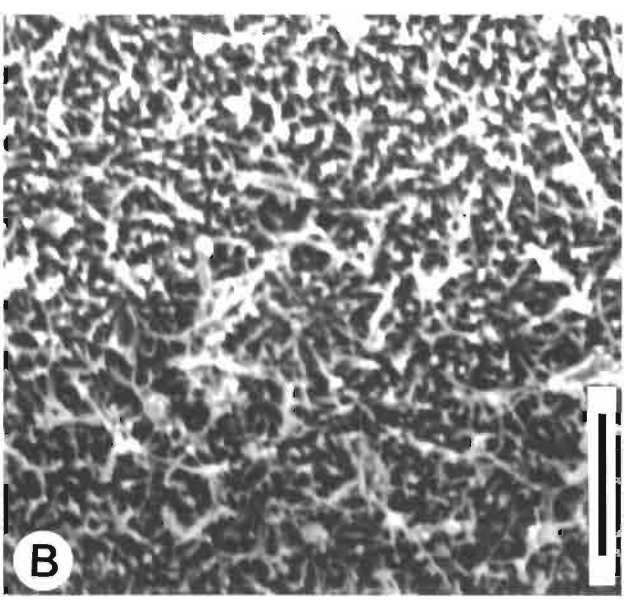


Fig. 1. *Skarbilovinema laumondi* Chizhov & Zakharenkova, 1991. Scanning electron micrographs of the parasitic female. A : Part of the female body; B : Surface of the body wall with numerous microvilli (N : nucleus; Mi : microvilli; Cu : cuticule; H : hypodermis; Lg : lipid globule; SM : somatic muscle). *(Bars : A = 0.5 mm; B = 10 µm).*

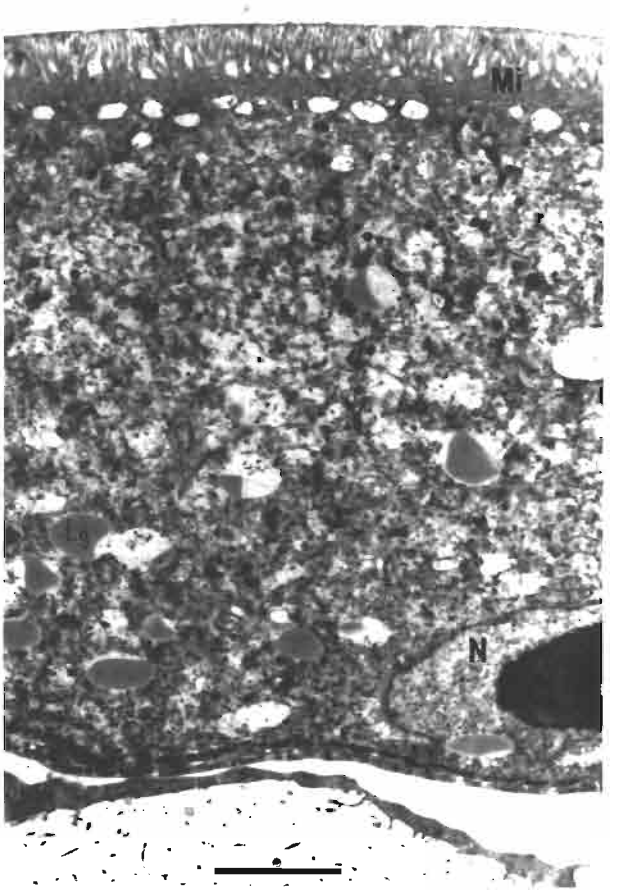


Fig. 2. *Skarbilovinema laumondi* Chizhov & Zakharenkova, 1991. Cross-section through the body wall of an old parasitic female. The hypodermis has still a well-marked " spongy layer ". Hypodermal cytoplasm contains numerous vacuoles, lipid globules and nuclei with a hypertrophied nucleolus. *(Bar = 40 µm).*

Results

The parasitic females of *S. laumondi* had an obese and sausage-shaped body, about 2-15 mm long (Fig. 1 A). Young females were first white and then turned yellow during parasitism. Their oesophageal lumen was deformed and all other parts of the oesophagus were reduced. Different stage juveniles were located inside the female body.

Analysis of the body surface of parasitic females with the scanning electron microscope (at high magnification) showed numerous interwoven microvilli covering the whole nematode body (Fig. 1 B). The cuticle was completely reduced. The body wall was composed mainly of the well developed hypodermis.

The hypodermis (Fig. 2) had a complex structure. Its external layer consisted of tightly arranged microvilli,

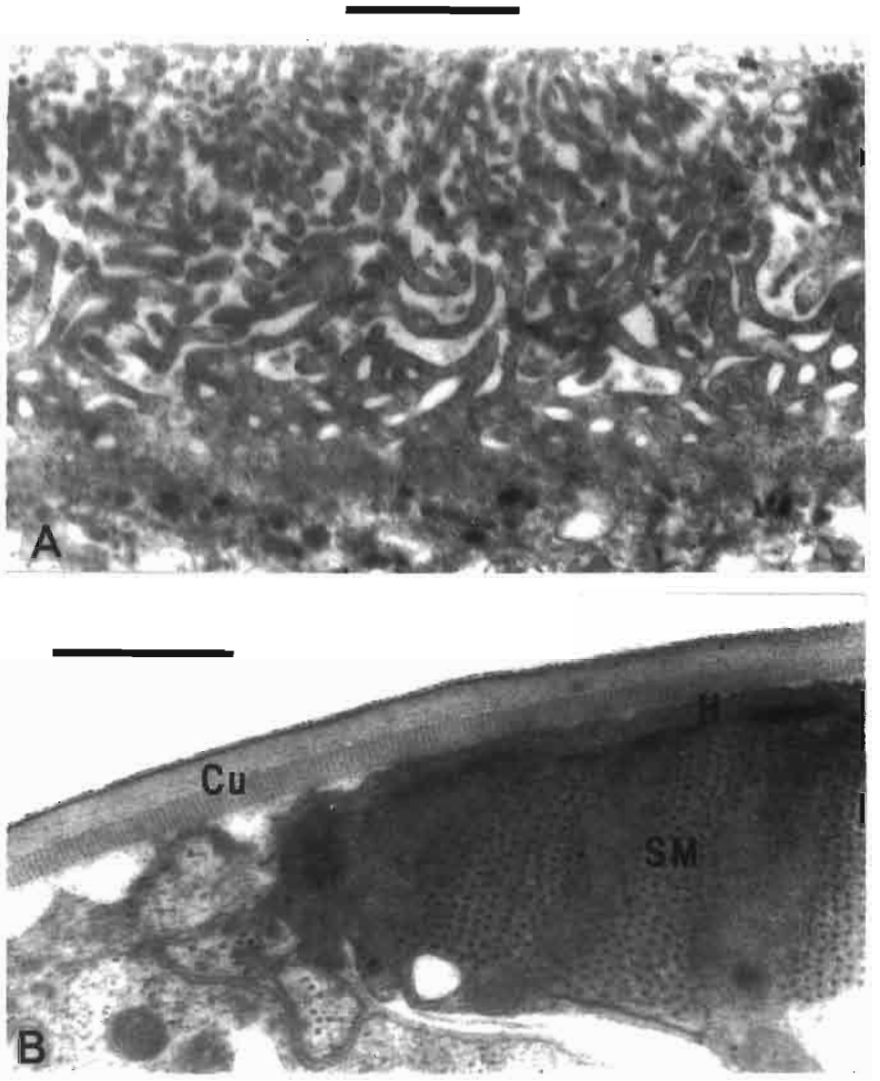


Fig. 3. *Skarbilovinema laumondi* Chizhov & Zakharenkova, 1991. A : Cross-section through the " spongy layer " of hypodermis of the parasitic female. It consists of numerous interwoven microvilli from cytoplasm processes; B : Cross-section through the body wall of an infective preadult female. Note cuticule consisting of three layers. *(Bars : A = 5 µm; B = 10 µm).*

forming a labyrinth, which may be termed " spongy layer ". The microvilli were processes of the hypodermal cytoplasm, whose diameter decreased towards the surface (Fig. 3 A). The thickness of the " spongy layer " varied from 1.7 to 2.3 µm. Older females had cavities in the " spongy layer " and contained fewer processes than young ones.

The hypodermal thickness of parasitic females varied from 14 to 23 µm. The hypodermal cytoplasm of young females contained numerous organelles. The hypodermis of old yellow females contained only few organelles and exhibited features of degradation (Fig. 2). Cytoplasm also contained large lipid globules and vacuoles. The nuclei with large nucleoli occurred towards the base of the hypodermis.

The cuticle of infective vermiform preadult females resembled that of plant parasitic nematodes. It consisted of a thin intensively stained epicuticle, a homogeneous exocuticle and an endocuticle, with striated structure when viewed in cross-section (Fig. 3 B). The infective females had a well-marked lateral field with numerous distinct incisures.

Discussion

Numerous cytoplasmic hypodermal processes in the form of microvilli cover the whole body of parasitic female of *S. laumondi* and form a " spongy layer ". The cuticle of entomoparasitic mermithid nematodes is known to become modified or completely reduced in the transition to the parasitic stage inside the haemocoel of the host. Microvilli of the hypodermis serve for transcuticular nutrient uptake directly through the body wall (Poinar *et al.* 1981). There is only one report that describes a similar structure in tylenchids : different shapes and sizes of microvilli on the hypodermal surface of parasitic females *Howardula hysseyi* were observed by Riding (1970) however, these microvilli were not arranged closely to each other as in *S. laumondi.*

The microvillar cytoplasmic processes on the nematode body are connected with parasitism and may be considered as powerful absorptive organs for highly specialized parasites. Due to these processes the surface of absorption increases and promotes nematode growth in the host's haemocoel. The " spongy layer " may also be an original catalyst, taking part in the primary digestion of nutrients.

Cliff and Baldwin (1985) used the fine structure of body wall cuticle of females Heteroderidae for taxonomy and analysis of their phylogeny. In our opinion surface structures of the body wall may also be useful in the systematics of entomoparasitic tylenchid nematodes. Although there is still very little information about these structures, well-known facts indicate that the body wall becomes subjected to different modifications in the transition to parasitism. Riding (1970) observed that the females of *H. husseyi* had no cuticle and that the hypodermal membrane covered the nematode surface and formed numerous microvilli. The parasitic heterosexual female of *Paraiotonchium nicholasi* has a well developed cuticle with numerous ramified canals running from the hypodermis to the outer surface (Nicholas, 1972). According to our observations the parasitic female of *S. laumondi* is covered by microvilli, forming a " spongy layer " of the hypodermis. The obvious variety in body wall structure may be useful for the systematics of entomoparasitic tylenchid nematodes.

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