ULTRASTRUCTURE OF THE EGGS OF *POLYMORPHUS MAGNUS* (ACANTHOCEPHALA, POLYMORPHIDAE)

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Summary:

The ultrastructure of *Polymorphus magnus* acanthocephalan eggs has been studied. The eggshell consists of four envelopes that are morphologically similar to those of the eggs of other Palaeacanthocephala representatives. The studied acanthors are formed by the cortical syncytium and "central nuclear mass". In the anterior part of acanthors there is a penetration gland. Its secret may provide the larvae migration into intermediate host's organism. "Central nuclear mass" consists of several germinative nuclei and numerous fibrillar bodies, formed as a result of germinative nuclei degradation.

KEY WORDS : *Polymorphus magnus*, egg, embryonal envelope, acanthor, ultrastructure.

Résumé : ULTRASTRUCTURE DES ŒUFS DE *POLYMORPHUS MAGNUS* (ACANTHOCEPHALA, POLYMORPHIDAE)

On a étudié l'ultrastructure des œufs d'un acanthocephale Polymorphus magnus. *La coquille de l'œuf est composée de quatre enveloppes qui ne se distinguent pas de celles des autres Palaeacanthocephala. Les acanthors étudiés sont formés d'un syncytium cortical et d'un "amas nucléaire central". La partie frontale des acanthors contient une glande de pénétration, dont le produit de sécrétion contribue probablement à la migration des larves dans l'organisme de l'hôte intermédiaire. L'amas nucléaire central est composé de noyaux germinatifs peu nombreux et de nombreux corpuscules fibrillaires qui se forment par dégradation des noyaux germinatifs.*

MOTS CLÉS : Polymorphus magnus, *œuf, enveloppe embryonnaire, acanthor, ultrastructure.*

INTRODUCTION

The ultrastructure of acanthocephalans embryonal larvae (acanthors) has been the least studied among all development stages of these helminthes. The study of *Moniliformis moniliformis* body surface, completed by Wright & Lumsden (1970), was the first work to describe the fine morphology of acanthors. This research is the only one made in the hatched larvae. Later on the fine organization of "central nuclear mass", the covers, and penetration gland of *Polymorphus magnus* acanthors was studied (Nikishin & Krasnoshchekov, 1986, 1990). Albrecht *et al.* (1997) found in his detailed study of *Polymorphus magnus, Neoechinorhynchus rutili,* and *Moniliformis moniliformis* acanthors, that their bodies are constituted by three syncytia: frontal, central, and epidermal.

Acanthocephalans eggshell fine morphology has been much better studied. Wright (1971), Peters *et al.* (1991), and Marchand (1994b) demonstrated that Archiacanthocephala specimens have four embryonal envelopes of complicated organization. Whitfield (1973) and Stranack (1972) found that *Polymorphus minutus* and *Pomphorhynchus laevis,* respectively, have only three such envelopes, however, later all Palaeacanthocephala representatives were proved to have four envelopes (Nikishin, 1988, 1994; Taraschewski & Peters, 1992; Marchand, 1994b). The egg shell of the majority of Eoacanthocephala species is also constituted by four envelopes (Marchand, 1994a; Taraschewski *et al.*, 1992), and only *Neoechinorhynchus rutili* have five such envelopes (Taraschewski *et al.*, 1992). With the help of comparative analysis and data generalization we demonstrated that the third embryonal envelope is the most variable. Its constitution conforms to ecological conditions under which the eggs live after they are excreted from the final host (Nikishin, 2001).

The present article generalizes the results of electron microscopic research of *Polymorphus magnus's* eggs. This work is the first attempt to reconstruct the ultrastructure of the whole egg.

MATERIALS AND METHODS

The objects of our study were acanthocephalans females of *Polymorphus magnus,* containing mature eggs, obtained from naturally invaded hags *Somateria fischery*. Living worms were fixed in a 2 % solution of glutaraldehyde in 0.1 M phosphate buffered saline (PBS) for two days, where 10-15 min after their fixation they were cut in segments, 2-5 mm

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long each. Then they were washed twice (for 30 min each time) in PBS before being treated for 2-4 hr in 1 % solution of osmium tetroxide in PBS. After being dehydrated in a graded series of ethanol, the specimens were embedded in a mixture of Epon and Araldite. Contrast was made with uranylacetate in 70 % alcohol during dehydration and lead citrate on sections. Specimens were studied in Tesla BS-500 electron microscope.

Marchand's (1984a, b) nomenclature is used to indicate embryonic covers.

RESULTS

Schematic picture of *Polymorphus magnus* egg, completed in accordance with the results of our research, is given at Figure 1.

THE EGG SHELL

Embryos of the studied acanthocephalans are surrounded with four egg envelopes (Fig. 2). The general shell thickness at the lateral areas of an egg is 3-4 μm and at its poles it's 10 times as thicker because of the growths of two outer envelopes.

The outer envelope (E 1) is 30-40 nm thick. Two electron dense layers, separated with an electron light space constitute it (Figs 3A, B). All these elements are of approximately the same thickness.

The second envelope is a plate (E2b) 0.65-0.85 μm thick at the lateral areas of an egg and 0.15-0.40 μm thick at its poles (Figs 3A, B). This blade is constituted by a layer material; each layer, separated from the nearest ones with a 5-6 nm space, is 7-8 nm thick. Between the first and the second envelopes (space G1) there are thick and thin fibers. Thick fibers at the lateral areas of an egg are oriented mostly in the circular direction; they are placed in one-two layers and their diameter is 0.50-0.75 μm (Fig. 3A). At the egg poles they have a smaller diameter (0.2-0.4 μm); they are oriented mostly in a longitudinal direction and placed in three-four rows (Fig. 4D). Thin fibers have a diameter much smaller than 0.1 μm and are characterized by a chaotic position and orientation (Figs 3A, B). Both thick and thin fibers are connected with the plate E2b and constituted by the same layer material; therefore, they are the elements of E2a. Besides fibers, there is a softly organized finely fibrillar and granular material within the G1 space. The same material is found in the space between the second and the third envelopes (G2).

The third envelope (E3) consists of three layers (Fig. 3B). Its thickness varies from 0.2-1.0 μm in the central part of the egg to 5 μm and more at its poles. The outer layer (E3a) is represented by a wavy membrane, 25 nm thick; the central layer (E3b) is filled with the contents similar to those surrounding the elements of E2a, but firmer, and the inner layer (E3c) is constituted by a dense membrane-like plate, 20 nm thick.

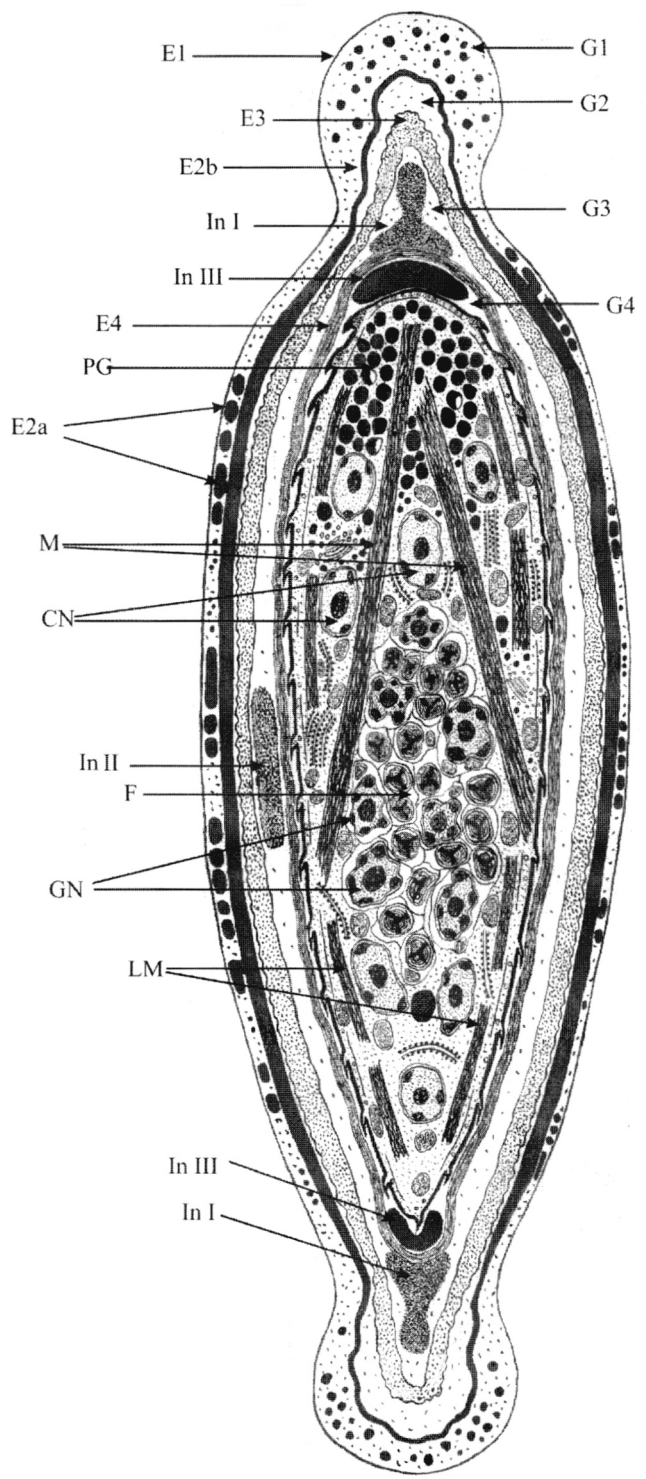

Fig. 1. – Generalized scheme of *Polymorhpus magnus* acanthocephalan invasion egg structure (relative dimensions are not observed for illustration convenience). CN, cortical nuclei; E1-E4, embryonal envelopes; F, fibrillar bodies; G1-G4, spaces between embryonal envelopes; GN, germinative nuclei; In I-In III, inclusions of the I, II and III types between embryonal envelopes; LM, longitudinal muscles; M, anterior-lateral muscles; PG, penetration gland.

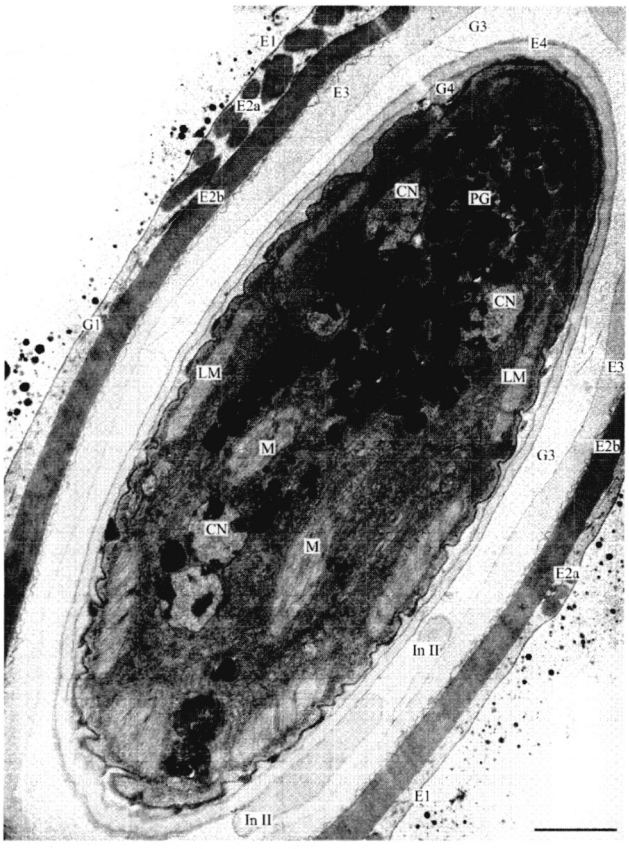

Fig. 2. – Tangential section of the *Polymorphus magnus* acanthocephalan egg in the acanthor foretrunk region. The acanthor is surrounded by four embryonal envelopes (E1, E2, E3, E4), separated with the spaces (G1, G3, G4); inclusions (in II) are seen in the space G3. In the front pole of the acanthor the penetration gland (PG) a concentration of electron dense granules is seen. CN, cortical nuclei; LM, longitudinal muscles; M, anterior-lateral muscles. Bar: 2 µm.

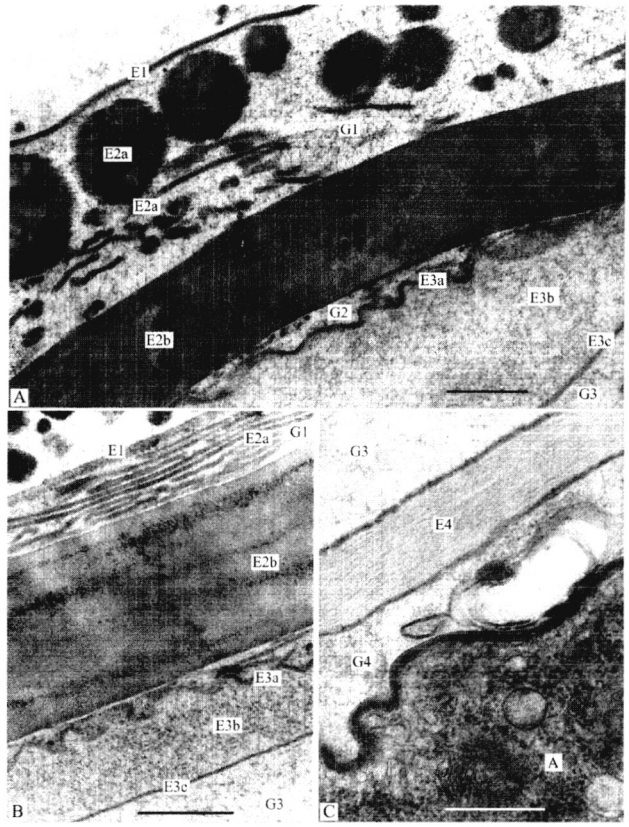

Fig. 3. – Embryonal envelopes of the *Polymorphus magnus* acanthocephalan egg. A, B: the general view of the first, second, and the third embryonal envelopes. C: the fourth envelope. A, acanthor; E1-E4, embryonal envelopes; G1-G4, spaces between the embryonal envelopes. Bars: 0.5 µm.

The space between the third and the fourth envelopes (G3) is filled with a crumbly flaky material and pierced with numerous microfilaments oriented parallel to the acanthor's surface. Here are big bodies, different in form and location. The bodies of the first type are single; they are located at the egg poles and are 10 µm and above long and 6-7 µm wide (Figs 4A, B). Their shape resembles an arrow, and a moderately dense homogenous substance, in which thick granules are chaotically dispersed, constitutes them. From the outside these bodies are limited with a thin layer of small dense granules; this layer falls into the fourth envelope in the places of contact with the one. The bodies of the second type (Fig. 4C) are also single and are located freely in the lateral areas of the egg, they are

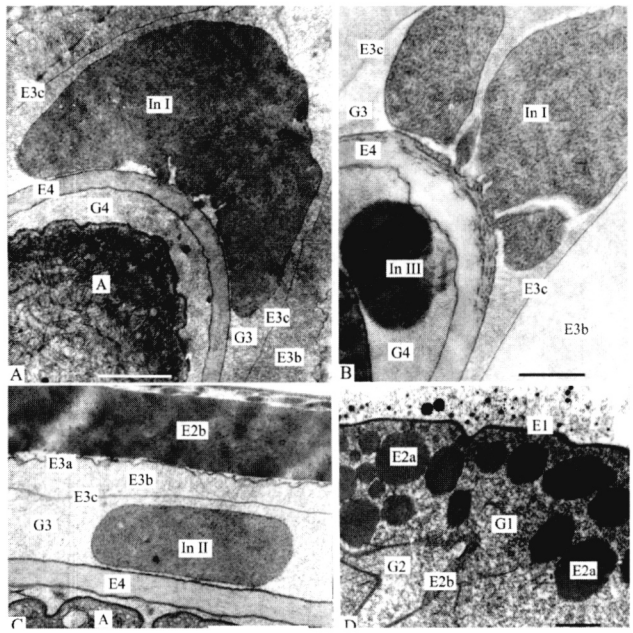

Fig. 4. – Embryonal envelopes ofthe *Polymorphus magnus* acanthocephalan eggs and inclusions between them. A: inclusion of the I type (In I) in the space (G3) between the third and the fourth embryonal envelopes at the back egg pole. B: inclusions of the I and III (In III) types at the front egg pole. C: inclusion of the II type (In II) in the space G3. D: the elements ofthe second embryonal envelope (E2a) at the front egg pole. A, acanthor, E1, E2, E3, E4, embryonal envelopes, G1, G2, G3, G4, the spaces between embryonal envelopes. Bars: 1 µm.

oval-shaped, 7 μm long, and 0.8-1.3 μm wide. They do not differ from the first type bodies in the character of the material constituting them.

The most inner fourth envelope (E4) is of an almost regular spindle-like shape; it doesn't produce polar growths (Fig. 3C). It is 0.25-0.35 μm thick and consists of 10-15 layers of a moderately dense material; each layer is 15-18 nm thick, the space between the adjoining layers is about 10 nm. This layer material is apparently similar to the element E3c, according to Marchand's terminology (1984a, b). From the outside this material is limited with a thin layer of dense granules (E4a) and a light space (E4b). A similar granule layer constitutes the inner boundary of the fourth envelope. The space between the fourth envelope and acanthor's surface is filled with crumbly granule contents including several vesicles and lamellar bodies. At the poles of the egg, where this space gets bigger, there are single bodies of the third type in it, connected neither with acanthor's surface, nor with the fourth envelope. They are bean-shaped and are constituted by a homogenous electron dense material; their dimensions are 0.5-0.9 × 1.2-2.7 μm (Fig. 4B).

THE ACANTHOR

Embryonal larvae of *Polymorphus magnus* are spindle-shaped, with a wavy surface, armed with embryonal hooks and spines (Fig. 5). Outwardly acanthor's body is covered with the coverage complex, 30-40 nm thick. It consists of the surface cytoplasmatic membrane, a layer of a dense homogenous material tightly adjacent to the inner side of the membrane, and a membrane-like plate separated form the material with a narrow light space. All over acanthor's surface we see infrequent narrow pores which are connected by means of short canals with the small light bubbles, located in the surface layer of the cytoplasm.

Embryonal hooks are located on the surface of the front part of the acanthor (Figs 5, 6, 7A). They have the shape of a round-topped cone, crooked toward the back end of the larva. Each hook consists of proximal and distal parts, separated by a narrow light space. The amorphous material of moderate density constitutes the proximal part of hooks; the distal one is a narrow stripe of an electron dense substance. The rest surface of the acanthor is covered with spines, 1.0-1.2 μm long. In comparison with embryonal hooks the spines have a longer and thinner distal part, constituted with the material of a high electron density (Figs 5, 7B). Proximal parts of the spines are represented by short growths of cytoplasm and do not contain amorphous material as distinct from those of the hooks. Outwardly both the hooks and the spines are covered with the coverage complex proceeding to the larva body surface.

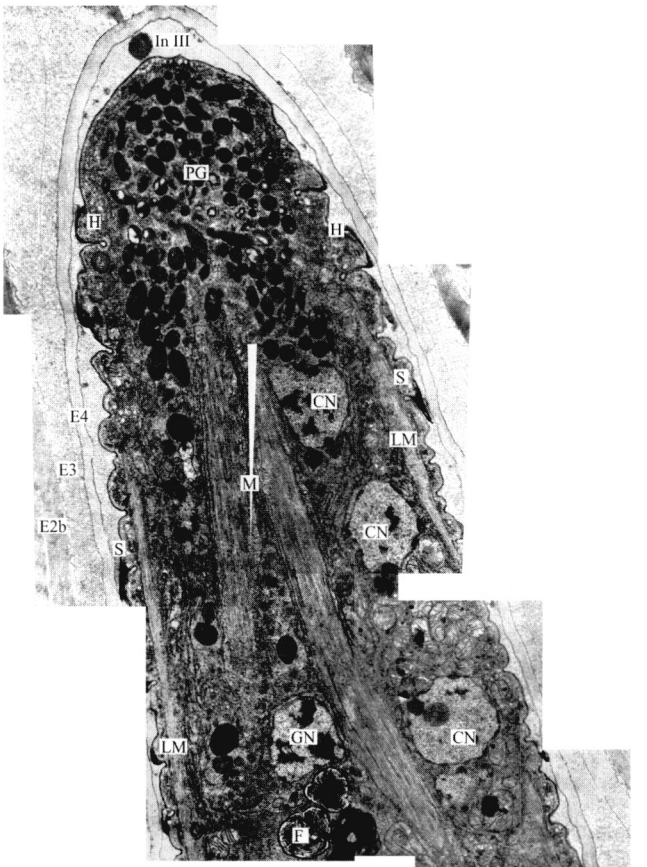

Fig. 5. – The acanthor of *Polymorphus magnus* acanthocephalae. The foretrunk of the larva is represented by aclid organ, armed with hooks (H) and spines (S), and containing the "penetration gland" (PG) and strong anterior-lateral muscles (M). The fourth embryonal envelope and a polar inclusion (In III) between it and the acanthor are noticeable at the front egg pole. CN, cortical nuclei; E2b, E3, E4, embryonal envelopes; F, bibrillar bodies; GN, germinative nucleus; LM, longitudinal muscles (bar: 2 μm).

The cytoplasm surface layer of the studied larvae is inconstantly limited with the cytoplasm membranes and, except for the foretrunk, with bands of longitudinal miofilaments from the inside. However, in the fore-, mid- and hindtrunk of the acanthors there are extended arrears lacking of these cytoplasmatic membranes; in theses arrears the surface cytoplasm and the one located deeper merge (Fig. 7A). The surface layer of the cytoplasm contains ribosomes, small concentrations of short canals of rough endoplasmic reticulum (RER), single mitochondrions and light vesicles, connected with pores on the larva surface. No inclusions containing electron dense material, similar to that constituting distal parts of hooks and spines have been found. There is no basal plate or any other non-cellar elements.

Under the surface layer of the cytoplasm there are 10 bands of miofilaments, oriented longitudinally

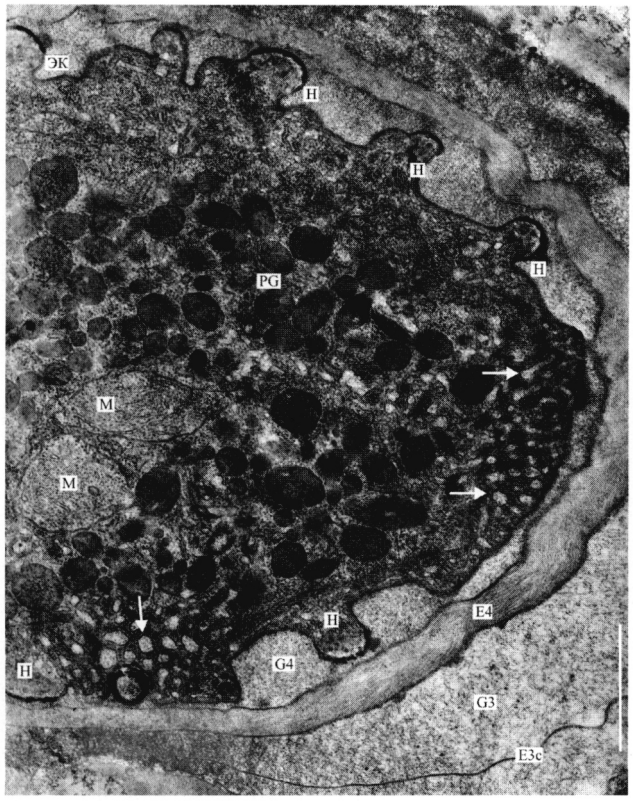

Fig. 6. – The fragment of the cross-section through acanthor's foretrunk of the *Polymorphus magnus* acanthocephalae. The "penetration gland" (PG) occupies the main bulk of the larva foretrunk. The light vesicles (arrows) are, apparently, the canals opening into the body surface. Embryonal hooks (H) are constituted by cytoplasm growths with apical stripes of a dense material. G3, G4, the spaces between the embryonal envelopes; E3c, E4, embryonal envelopes; M, anterior-lateral muscles (bar: 1 μm).

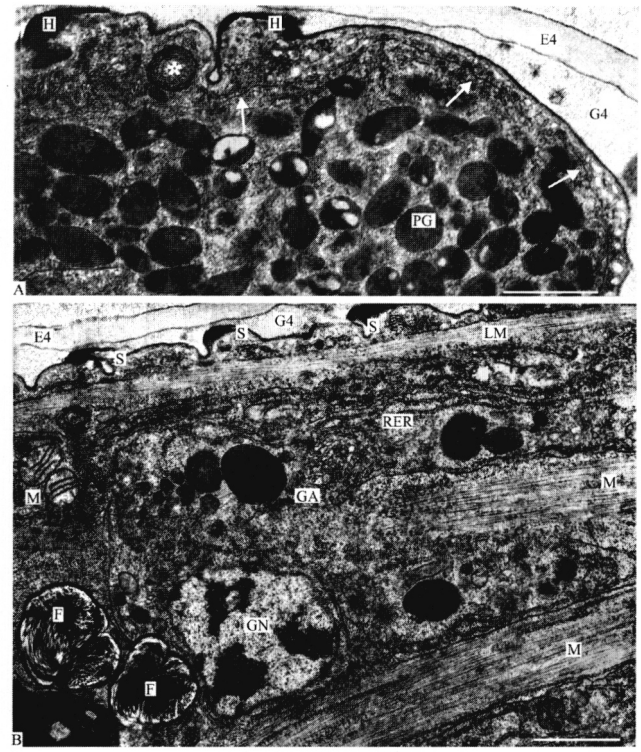

Fig. 7. – The "penetration gland" of the *Polymorphus magnus* acanthocephalae acanthor (fragments of Fig. 4). A - a concentration of secret granules (PG), some of which are partially filled with the contents. This concentration is separated from the surface layer of the cytoplasm with a membrane arrows; B - the secret granules formation in the Golgi apparatus (GA). IN the surrounding cytoplasm rough endoplasmic reticulum (RER) canals and mitochondrions (Mi) are noticeable. The surface layer of the cytoplasm is separated from longitudinal miofilaments (LM) with a cytoplasmic membrane; short RER canals are seen in this layer. E4, fourth embryonal envelope; G4, space between E4 and acanthor; GN, germinative nucleus; F, fibrillar bodies; H: embryonal hooks; M, antero-lateral muscles, S, embryonal spines. bars: 1 μm.

(Fig. 5). Each band is limited by cytoplasmatic membranes from the outside; from the inside these limits are not always seen (Figs 7B, 8A). Contractile elements are represented with thick and thin miofilaments.

According to the peculiarities of inner constitution acanthor's body can be conventionally divided into the foretrunk, midtrunk, and hindtrunk. The front and middle larva parts are crossed by the two thick bands of miofilaments, extending from the front pole of the acanthor to its surface at the borderline of its midtrunk and hindtrunk (Figs 5, 6, 7B). These muscles are similar to retractor ones, reported from the acanthors of other species (Albrecht *et al.*, 1997; etc.). They are limited by cytoplasmatic membranes from all sides and constituted by thin and thick miofilaments like subsurface muscle elements. Cortical nuclei located between the central nuclear mass and acanthor surface are connected with these muscles (Fig. 8A). The cytoplasm surrounding these nuclei is different from that of the central nuclear mass by lesser electron density and includes bigger mitochondrions.

The foretrunk of the larva is occupied by a vast concentration of oval granules, located under the longitudinal miofilaments (Fig. 5). In the front pole area where there are no such miofilaments the concentration of granules is separated from the surface layer of the cytoplasm with the intermittent cytoplasmatic membrane (Figs 6, 7A). Maximum dimensions of granules are 0.8-1.0 × 0.5-0.7 μm. In the front and central parts of the concentration granules are located closely; this results in the change of their shape, however no features of their merging have been found. Each granule is limited with the cytoplasmatic membrane, and the majority of granules are filled with homogenous electron dense material. In some granules dense contents fill their volume only in part, whereas the other part includes finely granulated material of moderate electron density or looks "empty". The cytoplasm surrounding granules contains few small mitochondrions and concentrations of ribosomes.

Acanthor's body part located behind the concentration contains "central nuclear mass" and is characterized by a great number of organelles and inclusions. Two kinds of nuclei nave been found: cortical and non-differentiated. Cortical nuclei are located in a row under the longitudinal miofilaments, non-differentiated ones are found only in the central nuclear mass (Fig. 5). Cortical nuclei are of a round or oval shape with a slightly wavy edge, their dimensions being 2.3-4.5 × 1.5-2.3 μm. They are characterized by a light nucleoplasm, small nucleolus, and several small chromatin concentrations, located along the nuclear cover and in the bulk of nucleoplasm as well. Some cortical nuclei together with the portions of surrounding cytoplasm are inconstantly separated from the rest of the body with cytoplasmatic membranes, however in none of the cases completely formed cells were found. Among the organelles the most numerous are oval or round mitochondrions with light matrix and long narrow cristae. Some mitochondrions have some signs of destruction: rarefied matrix, cristae getting destructed, and inclusion of lamellar bodies and small dense granules. RER is represented by numerous long non-ramified canals or, more seldom, by concentric ones. Golgi zones are not numerous and look like concentrations of small light vesicles and short cisterns (Fig. 7B). Granules of various sizes with electron dense contents are seen nearby; these are similar to those described in the foretrunk of the larva. Evidently, these granules are formed in Golgi apparatus in larva's central part and then transported to its foretrunk. Concerning other inclusions lamellar bodies are found alongside with formations of tightly packed small vesicles, embedded in electron dense matrix and surrounded with a membrane. The presence of lamellar bodies and concentric canals RER indicates the processes of autophagia (Bogitsh, 1975).

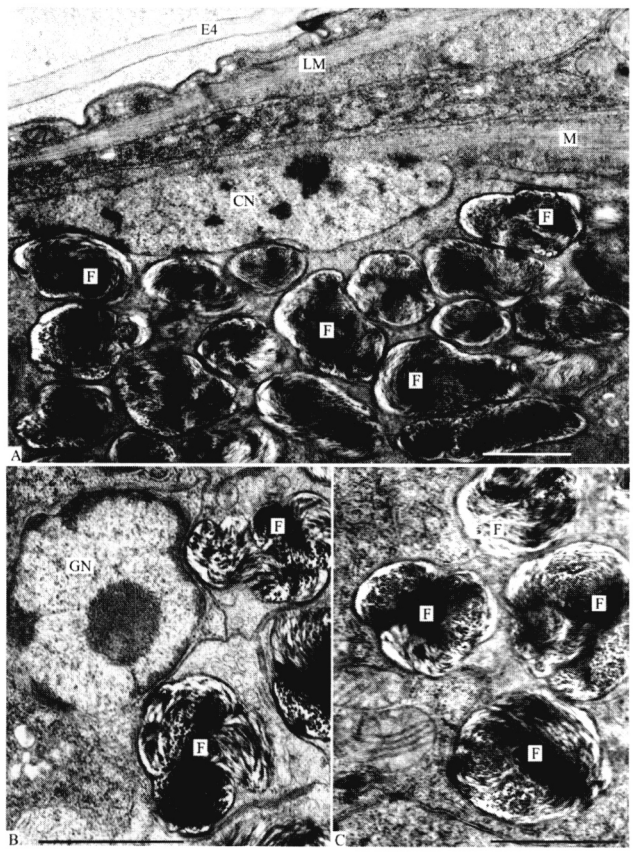

Fig. 8. – The central nuclear mass of the *Polymorphus magnus* acanthocephalae acanthor. A: fibrillar bodies (F) and acanthor body area adjoining to them. The cortical nucleus (CN) connected with the aclid organ muscle (M) is seen. B: the germinative nucleus (GN) with a small amount of the cytoplasm is surrounded with a membrane and resembles a non-differentiated cell. Each fibrillar body (F) together with a portion ofthe cytoplasm is also limited with a membrane. C: the fibrillar bodies concentration fragment. E4, inner embryonal envelope; LM, longitudinal muscles. Bars: 1 μm.

Central nuclear mass is a concentration of not numerous non-differentiated (germinate) nuclei and peculiar bodies prevailing in numbers, and containing electron dense fibrillar material (Fig. 8A). Their shape, dimensions, and structure vary greatly. The largest ones can be 2 μm in diameter and are commensurate in their dimensions to germinate nuclei contained by the central nuclear mass. Each body together with adjoining narrow layer of the cytoplasm is surrounded with the cytoplasmatic membrane. The majority of them are constituted by a cavity filled with the fibrils, tightly adjoining to one another, S-curved or resembling a spiral (Figs 8A, B, C, 9C, D). These bodies resemble a fingerprint, to some degree. In some bodies, usually in the central part, fibrils merge into a continuous electron dark mass. On other bodies there is no cavity; the space limited with membranes is filled with granular matrix similar in the way it looks to heterochromatin of non-differentiated nuclei located nearby. These kinds of fibrillar bodies, evidently, represent the successive stages of their development, during which their fibrils are formed and get thicker, and density of their location in the bunches and that of the bunches in the body cavity increases.

Non-differentiated nuclei are located in periphery areas of the central concentration. Their dimensions are 1.1-1.8 × 0.8-1.6 μm; these nuclei are characterized by a great amount of heterochromatin occupying the most part of the nucleus section (Fig. 8B). In all the cases nuclei, as well as fibrillar bodies, together with the surrounding cytoplasm are surrounded with a membrane and resemble quite formed cells. Among organelles only small mitochondria with minimal number of cristae are found in these "cells". Germinative nuclei are often seen; their chromatin has a raised electron density and occupies almost all the bulk of the egg (Figs 9A, C, D). In some cases fibrillar material is found among the chromatin in the nucleoplasm of these nuclei (Fig. 9B).

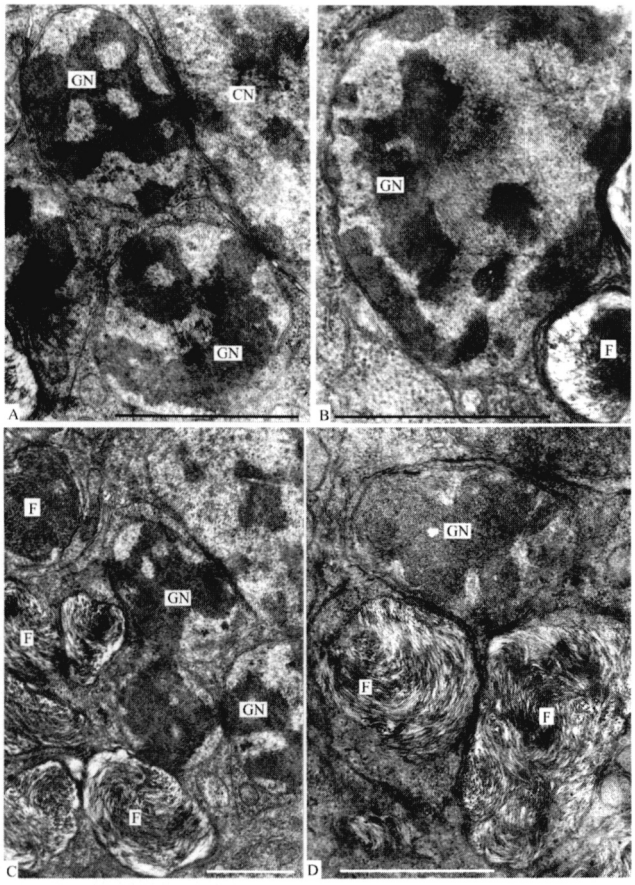

Fig. 9. – The central nuclear mass elements of the *Polymorphus magnus* acanthocephalae acanthor. A: germinative nuclel (GN) with decondensed chromatin and nucleolus. Each nucleus together with a small amount of the cytoplasm is surrounded with a membrane. B: germinative nucleus (GN) transformation into a fibrillar bodies. C: fibrillar bodies (F) and germinative nuclei (GN) with modified chromatin. D: the structure of the typical fibrillar bodies. CN, cortical nucleus. bars: 1 μm.

Fewer organelles and inclusions characterize the hindtrunk of the studied acanthors. Besides several somatic nuclei, mitochondria, RER elements, and single lipid drops are found here.

DISCUSSION

THE EGG SHELL

The egg shell organization of *P. magnus* corresponds to the general pattern of its constitution for Palaeacanthocephala class representatives (see Marchand, 1984b; Nikishin, 2001). Each of the four embryonal envelopes has characteristic morphological peculiarities, owing to which each one is easily distinguished from the others. The outer envelope of all studied acanthocephalans looks like a very thick three-layered membrane and varies for different species only in thickness. This envelope is easily penetrable for many chemical reagents (Anantaraman & Ravindranath, 1976). The envelope of some species whose eggs are excreted into the water environment (for example, Leptorhynchoides thecatus) is shown to get easily destructed, setting free E2a elements (Uznanski & Nickol, 1976). E1b elements of *P. magnus* as well the majority of other Palaeacanthocephala representatives have not been found.

The second envelope is represented by two components: a plate (E2b) and numerous fibers (E2a) connected with it. It's remarkable that all the second envelope elements of all the studied Palaeacanthocephala have a layer constitution, thickness of each layer being 7-8 nm (Whitfield, 1973; Marchand, 1984b; Nikishin, 1988). Among the contents of this envelope keratin-like protein prevails (Monnè & Hönig, 1954; Monnè, 1964). Such constitution provides high mechanical and chemical firmness of the second envelope elements corresponding to its biological role. E2b plate can protect acanthor from mechanical and chemical effects at the anterior part of the intermediate host digestion tract. E2a elements, sometimes called a fibrillar coat, presumably provide the invasion of an intermediate host (Uznanski & Nickol, 1976; Barger & Nickol, 1998).

The central component of the third envelope (E3b) is hardly morphologically different from the contents of spaces G2 and G3. Consequently, the legitimacy of its being called a self-sufficient structure can be doubtful. However, a thorough analysis of electronograms (Figs 2, 3B) demonstrates that the material constituting it is located closer than in nearby spaces and, consequently, is electron denser. Membrane-like layers E3a and E3c characterize this envelope. The former looks wavy in all studied acanthocephalans (Marchand, 1984b; Nikishin, 2001).

The fourth envelope is the least variable and is of the similar structure for the majority of species. Chitin has been found in its constitution (Edmonds, 1966; Peters *et al.*, 1991).

THE ACANTHOR

The obtained results do not prove that the body of studied acanthors consists of frontal, epidermal, and central syncytia, as it was described for acanthors of other species (Albrecht *et al.*, 1997). Acanthors of *P. magnus* demonstrate only somatic syncytium (Nikishin & Krasnoshchekov, 1986), apparently similar to epidermal syncytium, described by Albrecht and his co-authors. Epidermal and possible frontal syncytia of the studied larvae were not fully separated with cytoplasmatic membranes, though in some places these limits were seen. We didn't manage to find out to what particular syncytium the subsurface muscle elements can be related because outwardly they were always restricted

with membranes. However, there reference to the central syncytium, showed by Albrecht and his co-authors, is still doubtful, because morphology of organelles, in particular, mitochondrions, found in the central nuclear mass and near longitudinal miofilaments, is quite different. This is also related to retractor muscles. Though cortical nuclei related to them are located near the central nuclear mass morphologically they are different from the nuclei of the latter. Besides, the features of the cytoplasm surrounding nuclei of both kinds are also different.

The obtained results do not allow us to state the central syncytium existence either, because on microscopic sections all the central nuclear mass elements with the adjoining cytoplasm were isolated from one another. The "central nuclear mass" nuclei, as different from cortical ones, are smaller and have a relatively large nucleolus and great amount of chromatin. There are few organelles in the cytoplasm surrounding them; the organelles are represented mostly by small oval mitochondrions with minimal number of cristae. According to all their features the "cells" of the "central nuclear mass" are similar to non-differentiated or germinative cells of many other invertebrates like, for instance, tapeworms (Collin, 1970; etc.) or Turbellaria (Hay & Coward, 1975).

One of the most characteristic peculiarities of studied *P. magnus* acanthors is the frontal concentration of electron dense granules. They are formed in Golgi apparatus on the border of larva fore- and midtrunk and then are transported to the larva front pole. We named this structure a "penetration gland" (Nikishin & Krasnoshchekov, 1990) similarly to analogous formations in embryos of flat parasitic worms (Erasmus, 1972; Lethbridge, 1980). The evidence for this version is the fact, that these granules are found only at the final embryo genesis stage and are not found in the larvae that have penetrated through the intermediate host's intestine wall (Taraschewski, 2000; Nikishin, unpublished data). Apparently, the granule contents are realized during the process of penetration through the intermediate host's intestine wall. It is also possible that the "gland" secret provides the acanthor release of embryonal envelopes in intermediate host's intestine, though, as it has been shown in the experiments on *Moniliformis moniliformis*, the dominating role in this process belongs to embryonal hooks and larva body moves (Edmonds, 1966; Wright, 1971). It's interesting that acanthors of some species have a small invagination in the front body end during several hours after their release of embryonal envelopes (De Giusti, 1949; Hopp, 1954; Schmidt, 1973; Uglem & Larsen, 1969). This defect is possibly connected just with the penetration gland secret realization by activated larvae.

Concentrations of similar granules (authors call them vesicles of moderate electron density) are found also in acanthors of other species – the representatives of all the three classes of Acanthocephala (Albrecht *et al.*, 1997; our unpublished data). Authors point out granules (vesicles) shape and dimensions variability possibly connected with specialization to some intermediate hosts group. We can suppose that the penetration gland is a necessary attribute of Acanthocephala embryonic larvae and can be viewed as one of the characteristics for the ground pattern of a monophylum Acanthocephala.

Another remarkable and unexpected peculiarity of the studied acanthors is the structure of "central nuclear mass". According to the obtained results, peculiar fibrillar bodies prevail in its contents, and the number of typical nuclei (germinative and non-differentiated) is small. Apparently, fibrillar bodies are similar to the condensed nuclei, pointed out by other authors (Albrecht *et al.*, 1997). On the photos presented by them and related to *Polymorphus minutus* and *Neoechinorhynchus rutili* the fibrillar contents of "condensed nuclei" is clearly seen. However the likeness between the typical nuclei and fibrillar bodies is restricted only to the existence of two cytoplasmatic membranes, surrounding both structures. At the same time in some nuclei chromatin was at the transformation stage, but in other ones the fibrillar contents formation was observed. That's why we consider these corpuscles to be the result of the central nuclei degradation.

The mechanism and functional meaning of this degradation remain unknown. The embryonal development of acanthocephalans has been studied on all the three classes representatives (Meyer, 1931a; Nicholas & Hynes, 1963; Schmidt, 1973, 1985), however in none of the cases the appearance of structures, that could be evaluated as fibrillar bodies, was pointed out. It's apparently caused by the high density of elements location in the central nuclear mass and close dimensional characteristics of fibrillar bodies and non-differentiated nuclei. Owing to this it's problematic to notice them with the help of light-microscopic technique. In connection with this the work by A. Meyer (1931b) is remarkable. Though he didn't describe the central nuclear mass structure of *Macracanthorhynchus hirudinaceus* acanthors, he, nevertheless, illustrated it with a splendid figure, showing its dense bodies of significantly smaller dimensions than those of periphery (somatic) nuclei. According to our unpublished data the first fibrillar bodies in the central nuclear mass of *Arhythmorhynchus petrochenkoi* are observed before the second embryonic envelope formation.

Earlier we supposed the possibility of fibrillar bodies appearing in the process of heterochromatin transformation, the latter being discharged out of non-differentiated nuclei into the cytoplasm (Nikishin & Krasnoshchekov, 1986). The nematodes at the embryonal development stage are well known for the chromatin

diminution phenomenon, which is the indicator of differentiation of germinative cells into somatic ones (Walton, 1959; Müller & Tobler, 2000). Unfortunately we haven't found electronic-microscopic description of this process for nematodes, and it prevents us from completing a comparative analysis of both cases. We can only state that both nematodes and acanthocephalans have nuclear material amount reduction mechanisms, realized during the embryonic development. However the chromatin diminution of nematodes takes place only in the period of the first five divisions and is characteristics only of presomatic cells (Müller *et al.*, 1996). Fibrillar bodies appearing of acanthocephalans is connected with the nuclei of central nuclear mass, which is the primordia of internal organs, and lasts for rather a long period, even till the final stages of embryonal development.

ACKNOWLEDGEMENTS

The author thanks Dr. G.P. Krasnoshchekov for his assistance in research work and results interpretation and Ms A. Nikishina for her help in manuscript translation into English. The research was supported with grant of Far Eastern Department of Russian Academy of Sciences (Project N° A-36).

REFERENCES

ALBRECHT H., EHLERS U. & TARASCHEWSKI H. Syncytial organization of acanthors of *Polymorphus minutus* (Palaeacanthocephala), *Neoechinorhynchus rutili* (Eoacanthocephala), and *Moniliformis moniliformis* (Archiacanthocephala) (Acanthocephala). *Parasitology Research*, 1997, *83*, 326-338.

ANANTARAMAN S. & RAVINDRANATH M.H. Histochemical characteristics of the egg envelopes of *Acanthosentis oligospinus* (Acanthocephala). *Zeitschrift für Parasitenkunde*, 1976, *48*, 227-238.

BARGER M.A. & NICKOL B.B. Structure of *Leptorhynchoides thecatus* and *Pomphorhynchus bulbocolli* (Acanthocephala) eggs in habitat partitioning and transmission. *Journal of Parasitology*, 1998, *84*, 534-537.

BOGITSH B.J. Cytochemistry of gastrodermal autophagy following starvation in *Schistosoma mansoni*. *Journal of Parasitology*, 1975, *61*, 237-248.

COLLIN W.K. Electron microscopy of postembryonic stages of the tapeworm, *Hymenolepis citelli*. *Journal of Parasitology*, 1970, *56*, 1159-1170.

DE GIUSTI D.L. The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. *Journal of Parasitology*, 1949, *35*, 437-460.

EDMONDS S.J. Hatching of the eggs of *Moniliformis dubius*. *Experimental Parasitology*, 1966, *19*, 216-226.

ERASMUS D.A. The biology of trematodes. London, Edward Arnold, 1972, 1-312.

HAY E. & COWARD S.J. Fine structure studies on the planarian *Dugesia*. 1. Nature of the "neoblast" and other cell types in noninjured worms. *Journal of Ultrastructure Research*, 1975, *50*, 1-21.

HOPP W.B. Studies on the morphology and life cycle of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of the map turtle, *Graptemys geographica* (Le Sueur). *Journal of Parasitology*, 1954, *40*, 284-299.

LETHBRIDGE R.C. The biology of the oncosphere of cyclophyllidean cestodes. *Helminthological Abstracts*, 1980, *49*, 59-72.

MARCHAND B. The elaboration of the acanthor shell of *Acanthosentis acanthuri* (Acanthocephala). *Journal of Parasitology*, 1984a, *70*, 712-718.

MARCHAND B. A comparative ultrastructural study of the shell surrounding the mature acanthor larvae of 13 acanthocephalan species. *Journal of Parasitology*. 1984b, *70*, 886-901.

MEYER A. Urhautzelle, Hautbahn und plasmodiale Entwicklung der Larve von *Neoechinorhynchus rutili* (Acanthocephala). *Zoologische Jahrbucher. Abteilung für Anatomie und Ontogenie der Tiere*. 1931a, *53*, 103-126.

MEYER A. Infektion, Entwiklung und Wachstum des Riesenkratzers (*Macracanthorhynchus hirudinaceus*) im Zwischenwirt. *Zoologoscher Anzeiger*, 1931b, *93*, 163-172.

MONNÉ L. Chemie und Bildung der Embryophoren von *Polymorphus botulus* Van Cleave (Acanthocephala). *Zeitschrifft für Parasitenkunde*, 1964, *25*, 148-156.

MONNÉ L. & HÖNIG G. On the embryonic envelopes of *Polymorphus botulus* and *P. minutus* (Acanthocephala). *Archiv für Zoologie*, 1954, *7*, 257-260.

MULLER F., BERNARD V. & TOBLER H. Chromatin diminution in nematodes. *BioEssays*, 1996, *18*, 133-138.

MÜLLER F. & TOBLER H. Chromatin diminution in the parasitic nematodes *Ascaris suum* and *Parascaris univalens*. *International Journal for Parasitology*, 2000, *30*, 391-399.

NICHOLAS W.L. & HYNES H.B.N. The embryology of *Polymorphus minutus* (Acanthocephala). *Proceedings of the Zoological Society of London*. 1963, *141*, 791-801.

NIKISHIN V.P. Ultrastructure of embryonic envelopes of *Polymorphus magnus*. *Parazitologiya*, 1988, *22*, 100-103 (In Russian).

NIKISHIN V.P. Structure and formation of embryonic envelopes in the proboscis worm *Arhythmorhynchus petrochenkoi*. *Parazitologiya*, 1995, *29*, 398-403 (In Russian).

NIKISHIN V.P. The structure and formation of embryonic envelopes in acanthocephalans. *Izvestiya RAN. Seriya biologicheskaya*, 2001, 47-62 (In Russian).

NIKISHIN V.P. & KRASNOSHCHEKOV G.P. Micromorphology of the "central nuclear mass" of acanthors in the acanthocephala *Polymorphus magnus*. *Cytologiya*, 1986, *28*, 1261-1263 (In Russian).

NIKISHIN V.P. & KRASNOSHCHEKOV G.P. Ultrastructure of the acanthors *Polymorphus magnus* (Acanthocephala: Polymorphidae): Covers and "gland of penetration". *Parazitologiya*, 1990, *24*, 135-139 (In Russian).

PETERS W., TARASCHEWSKI H. & LATKA I. Comparative investigations of the morphology and chemical composition of the eggshells of Acanthocephala. I. *Macroacanthorhynchus hirudinaceus* (Archiacanthocephala). *Parasitology Research*, 1991, *77*, 542-549.

SCHMIDT G.D. Early embryology of the acanthocephalan *Mediorhynchus grandis* Van Cleave, 1916. *Transactions of the American Microscopical Society*, 1973, *92*, 512-516.

SCHMIDT G.D. Development and life cycles, *in :* Biology of the Acanthocephala. Cambridge University Press, 1985, 273-305.

STRANACK F.R. The fine structure of the acanthor shell of *Pomphorhynchus laevis* (Acanthocephala). *Parasitology*, 1972, *64*, 187-190.

TARASCHEWSKI H. Host-parasite interactions in Acanthocephala: a morphological approach. *Advances in Parasitology*, 2000, *46*, 1-179.

TARASCHEWSKI H. & PETERS W. Comparative investigations of the morphology and chemical composition of the eggshells of Acanthocephala. II. Palaeacanthocephala. *Parasitology Research*, 1992, *78*, 376-381.

TARASCHEWSKI H., PETERS W. & LATKA I. Comparative investigations of the morphology and chemical composition of the eggshells of Acanthocephala. III. Eoacanthocephala. *Parasitology Research*, 1992, *78*, 382-387.

UGLEM G.L. & LARSON O.R. The life history and larval development of *Neoechinorhynchus saginatus* Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology*, 1969, *55*, 1212-1217.

UZNANSKI R.L. & NICKOL B.B. Structure and function of the fibrillar coat of *Leptorhynchoides thecatus* eggs. *Journal of Parasitology*, 1976, *62*, 569-573.

WALTON A.C. Some parasite and their chromosomes. *Journal of Parasitology*, 1959, *45*, 1-20.

WHITFIELD P.J. The egg envelopes of *Polymorphus minutus* (Acanthocephala). *Parasitology*, 1973, *66*, 387-403.

WRIGHT R.D. The egg envelopes of *Moniliformis dubius*. *Journal of Parasitology*, 1971, *57*, 122-131.

WRIGHT R.D. & LUMSDEN R.D. The acanthor tegument of *Moniliformis dubius*. *Journal of Parasitology*, 1970, *56*, 727-735

Reçu le 11 juillet 2003

Accepté le 28 juillet 2003